Co-introduction of metazoan parasites with an invasive host, *Micropterus salmoides* (Lacépède, 1802) in non-native regions in South Africa

M Truter

orcid.org/0000-0002-0091-1598

Dissertation submitted in fulfilment of the requirements for the *Masters* degree in *Environmental Science* at the North-West University

Supervisor:	Prof NJ Smit
Co-supervisor:	Prof OLF Weyl
Assistant Supervisor:	Dr I Přikrylová

Graduation May 2018

23378123

Table of Contents

List of Figures iv

List of Tables vi

Acknowledgements viii

Abstract x

Keywords xiii

Study Outputs xiv

Chapter 1: General Introduction

1.1. Largemouth bass: a global invader 5
1.2. Associated parasites: native and non-native range 6
1.3. Hypotheses 8
1.4. Aims and Objectives 8

Chapter 2: Materials and Methods

2.1. Selection of localities 10
2.1.1. North West (NW) 10
2.1.2. KwaZulu-Natal (KZN) 14
2.1.3. Eastern Cape (EC) 14
2.1.4. Western Cape (WC) 14
2.2. Sampling of host species 17
2.2.1. *Micropterus salmoides* 17
2.2.2. Other fish species 17
2.3. Necropsy, blood parameters, biometric indices and parasite screening 19

Chapter 3: Parasite communities of South African Largemouth bass *Micropterus salmoides* (Lacépède, 1802) populations: support for enemy release

3.1. Introduction 21
3.2. Materials and Methods 22
3.2.1. General 22

3.2.2. Parasite fixation, morphology and identification 22
3.2.3. Statistical analysis 23
3.3. Results 23
3.3.1. General parasitological data 23
3.3.2. Parasite community: composition, diversity and richness 25
3.3.3. Morphological characterisation 27
3.4. Discussion 36

Chapter 4: Molecular characterisation of ancyrocephalid monogeneans parasitising *Micropterus salmoides* in South Africa

4.1. Introduction 42
4.2. Materials and Methods 43
4.2.1. DNA extraction and Polymerase Chain Reaction 43
4.2.2. Sequence alignment and phylogenetic analysis 44
4.3. Results 47
4.4. Discussion 54

Chapter 5: Host health and parasitic infection

5.1. Introduction 56
5.2. Materials and Methods 58
5.2.1. General 58
5.2.2. Statistical analysis 60
5.3. Results 60
5.3.1. Fish health and organosomatic indices 60
5.3.2. Blood parameters 63
5.4. Discussion 65

Chapter 6: Parasite spill-over from Largemouth bass (*Micropterus salmoides*) to native freshwater fish species: a biological invasion case study

6.1. Introduction 68
6.2. Materials and Methods 69
6.2.1. General 69
6.3. Results 70
6.3.1. General and parasitic infection 70
6.4. Discussion 75

Chapter 7: Concluding remarks and recommendations for future studies

7.1. Introduction 79

7.2. Concluding remarks 79

7.3. Future studies 80

References 82

List of Figures

Chapter 1

Figure 1. 1. Categorisation scheme for the invasion process with introduced species into a novel environment (adapted from Richardson *et al*., 2000, Colautti and MacIsaac, 2004; Blackburn *et al.*, 2011). 2

Figure 1. 2. Schematic representation of co-introduced and co-invasive parasites. Alien host has a parasite that follows process of introduction, establishment and spread with original host. Parasite switches to native host (blue) to become a co-invader (adapted from Lymbery *et al.*, 2014). 7

Chapter 2

Figure 2. 1. Map of the localities throughout South Africa where the field sampling for the present study was carried out. 11

Figure 2. 2. Specific sites where host specimens were collected. A – C: Mooi River, Potchefstroom Dam and Boskop Dam (North West); D – Friedrichkrön Dam (KwaZulu-Natal); E – F: Howison's Poort Dam and Settlers Dam (Eastern Cape); G – H: Groenvlei Lake and Vergenoegd Farm Dam (Western Cape). 16

Figure 2. 3. Methods used to collect host specimens; A – rod and reel; B – fyke nets; C – gill nets; D – seine netting; E – electro-fishing. 18

Figure 2. 4. Typical setup at a field site: A – aerated containers containing fish; B – haematocrit centrifuge; C – stained blood smears air drying; D – weighing station and data sheet; E – measuring of fish on measuring board; F – organs separated in petri dishes; G – internal organs in saline; H – screening of organs using a stereomicroscope; I – presence of Monogenea on the gill filaments.....20

Chapter 3

Figure 3. 1. Composition and abundance of the monogenean communities at each sampling locality. 26

Figure 3. 2. *Clavunculus bursatus* (Mueller, 1936) haptoral hooks (A), male copulatory organ (B); *Onchocleidus dispar* (Mueller, 1936) haptoral hooks (C); male copulatory organ (D).....29

Figure 3. 3. *Onchocleidus furcatus* (Mueller, 1937) haptoral hooks (A), membrane on ventral bar (indicated by white arrow in insert), male copulatory organ (B), unsclerotised vagina (C). 32

Figure 3. 4. *Onchocleidus principalis* (Mizelle, 1936) haptoral hooks (A), membrane on ventral bar (indicated with white arrow in insert); male copulatory organ (B); *Syncleithrium fusiformis* (Mueller, 1934) haptoral hooks (C) male copulatory organ (D).....35

Chapter 4

Figure 4. 1. Bayesian Inference tree from combined 18S-ITS-1 and 28S regions (1121 bp long) positioning ancyrocephalid monogeneans parasitising *Micropterus salmoides* in South Africa within the Dactylogyridae (Dactylogyridea). Numbers along branches indicate bootstrap values of BI on the left and ML on the right. The Gyrodactylidea was used as an outgroup.....52

Figure 4. 2. Maximumlikelihood tree from combined 18S-ITS-1 and 28S regions (1121 bp long) positioning ancyrocephalid monogeneans parasitising *Micropterus salmoides* in South Africa within the Dactylogyridae. Numbers along branches indicate bootstrap values of BI on the left and ML on the right. 53

Chapter 5

Figure 5. 1. Mean values for: A – Condition Factor and B – FHAI scores for all localities, and C – HSI, D – SSI, E – GSI, GSI of females (F) and males (G) from KZN, EC and GV. Mean percentages for H – haematocrit and I – white blood cell counts for KZN and EC…64

Chapter 6

Figure 6. 1. Summary of mechanisms associated with the introduction of alien species into novel environments, referred to as biological invasion mechanisms. 68

Figure 6. 2. Micrographs of *Quadriacanthus* sp. 1 from *Clarias gariepinus*, A – ventral bar, B – dorsal bar, C – male copulatory organ (MCO). Haptoral sclerites of *Dactylogyrus* sp. 1 from *Labeobarbus aeneus*, D – anchors and ventral sclerite (indicated with white arrow), E – dorsal bar (indicated with white arrow), F – MCO and G – vagina. 73

Figure 6. 3. Micrographs of *Cichlidogyrus* sp. 1, A – whole organism, B – anchors, dorsal and ventral bar complex, C – MCO and *Cichlidogyrus* sp. 2, D – anchors, dorsal and ventral sclerite complex, E – MCO and vagina (indicated with white arrow) from *Tilapia sparrmanii*. Differences in anchor, inner roots and hooklet morphology between species encircled with broken line.....74

List of Tables

Chapter 1

Table 1. 1. Categorisation scheme for populations of introduced or translocated species into novel environment (adapted from Richardson *et al.*, 2000, Colautti and MacIsaac, 2004; Blackburn *et al.*, 2011; Lymbery *et al.*, 2014).....3

Chapter 2

Table 2. 1. Potential assemblage of the fish communities in Boskop and Potchefstroom dams (table modified from Skelton, 2001; Jacobs, 2013 and pers. comm. resort manager, Mr. J Wessels, 2017). 13

Chapter 3

Table 3. 1. Fish biometrics and ecological parameters of monogenean parasite load. n – number of fish studied; Mean M – mean mass; Mean SL – mean standard length; Mean IF – mean intensity of infection. 24

Table 3. 2. Summary of parasitic groups and species found from *Micropterus salmoides* from studied localities. 27

Table 3. 3. Morphometrics of *Onchocleidus dispa*r, *O. furcatus* and *O. principalis*, from gills of *Micropterus salmoides* in South Africa.....40

Table 3. 4. Morphometrics of *Clavunculus bursatus* and *Syncleithrium fusiformis* from gills of *Micropterus salmoides* in South Africa.....41

Chapter 4

Table 4. 1. Species details and accession numbers of sequences used in phylogenetic analyses (AUS – Austria, CZ – Czech Republic, RSA – South Africa, UK – United Kingdom). 45

Table 4. 2. Successful sequences obtained of the 18S-ITS-1 and 28S rDNA regions of ancyrocephalid parasites in South African *Micropterus salmoides* populations. 47

Table 4. 3. Uncorrected *p*-distances (in %) between 18S rDNA fragments of 443 bp length of ancyrocephalid parasites included in the analysis. 48

Table 4. 4. Uncorrected *p*-distances (in %) between ITS-1 fragments of 254 bp length of ancyrocephalid parasites included in the analysis. 49

Table 4. 5. Uncorrected *p*-distances (in %) between 28S rDNA fragments of 424 bp length of ancyrocephalid parasites included in the analysis. 50

Chapter 5

Table 5. 1. Description of blood parameter variables in the FHAI (from Adams *et al.*, 1993).....59

Table 5. 2. Summary of fish biometrics and ecological paramaters of monogenean parasite load. n – number of fish studied; Mean SL– mean standard length; Mean IF – mean intensity of infection. 62

Chapter 6

Table 6. 1. Fish biometrics and ecological parameters for infection with Monogenea from hosts collected from Boskop Dam. 70

Table 6. 2. Presence and absence of parasitic groups on hosts from Boskop Dam.....72

Acknowledgements

I would like to express my sincere appreciation to the following persons and institutions that enabled the successful completion of my MSc dissertation.

To my supervisor, **Prof Nico Smit** from North-West University. Thank you for your unwavering support, commitment, motivation, confidence and guidance throughout the past two years.

To **Dr Iva Přikrylová** for your assistance, patience, guidance and the opportunity to visit the Department of Botany and Zoology, Masaryk University, Brno, Czech Republic.

To **Prof Olaf Weyl** from the South African Institute for Aquatic Biodiversity for your guidance and support throughout the project.

To the **Centre of Excellence for Invasion Biology**, that financially supported this study.

Sampling permits were issued by the **Department of Rural, Environmental and Agricultural Development (permit no. HQ 12/09/16-202 NW)** and **CapeNature (permit no. 0056-AAA041-00176)**.

To **Edwin Gewers**, the staff and shareholders of the **Fountain Hill Estate**. Your hospitality, cooperation and support is much appreciated.

To **Gordon O'Brien**, **Wesley Evans** and **Lungelo Madiya** for their contribution towards collection of material for this project.

Vergenoegd Farm owner **Mr Dian Kotze**. For access to your farm and hospitality.

To **Adri Joubert**. Thank you for all your behind the scenes magic and support.

To **Drs Kerry Hadfield Malherbe** and **Wynand Malherbe**. Thank you for your assistance in arrangements for fieldtrips, advice and overall support throughout the past two years.

To **Drs Olena Kudlai** and **Wihan Pheiffer** for assistance in the laboratory, data processing and arrangement of field trips.

To **Dean Impson** from **CapeNature**, for organising a research visit to your facilities and assistance in permit applications, hospitality, knowledge and for liaising with local farmers.

To **Nikol Kmentová**, you are a gem! Thank you for all your patience, for answering all my questions, quick replies and after hour discussions on the molecular aspects of this project.

To **Michael Kriel** from the **Department of Water and Sanitation** for accommodating us, the use of your facilities and your assistance at Boskop Dam.

To **Jaap Wessels**, resort manager at Potchefstroom Dam for support and information on the fish species and history of the Mooi River system.

To my parents, **Danielle**, **Jurgens** and brother **Albertus Truter**. Without your support, prayers, faith, sense of humour, phone calls and messages, the past two years would have been a lonely and uneventful experience. Thank you for believing and supporting me in all my 'missions' in life!

To **Rika** and **Johan Louw**. Thank you for your support, encouragement and invaluable contribuitions from the first day I stepped into the world of academics.

To **Jaydee Beneke**, **Elrika Rossouw**, **Tiaan Clasen**, **Pappabeer (Neil) Pretorius, Richard Barry, Michelle Smith**, **Gerardt Nagel** and **Mariëtta van der Merwe**. Thank you for believing in me and all your prayers over the past two years. Also, the wonderful memories I can cherish, for being the best support system, a family and a home away from home.

To **Hannes Erasmus**, **Anja Greyling**, **Mathys de Beer**, **Marelize Labuschagne**, **Suranie Horn** and **Natasha Voigt**. Your help and support with fieldwork, drawing of maps and contributions in the laboratory and office is invaluable!

To **Anrich Kock**. I cannot express my appreciation in enough words for all your time, patience, assistance, management skills and support that went into this project throughout the past two years, I am forever in your debt. Thank you.

"The most beautiful thing we can experience is the mysterious. It is the source of all true art and all science. He to whom this emotion is a stranger, who can no longer pause to wonder and stand rapt in awe, is as good as dead: his eyes are closed."

— Albert Einstein

Abstract

Since the early 18th century the introduction of non-native fish species occurred into South African freshwater systems. Drivers for these introductions included stocking for sports angling, aquaculture, bio-control and the pet trade. Little attention has been given to the co-introduction of symbionts, especially of introduced alien species, that succeeded in overcoming barriers set by introduction into new environments. A typical example in freshwater systems would be fish and their parasites. The movement and introduction of fish hosts typically result in co-introduction of these accompanying parasites and four possible mechanisms: enemy release, dilution, spillback and spill-over, where the latter results in co-invasion of the introduced parasites (Sheath *et al.*, 2015). An example of such a species is the North American native, largemouth bass *Micropterus salmoides* (Lacépède, 1802) that was introduced into South Africa in 1928 for sport angling and aquaculture. Limited research has been done on the parasites of this fish in South Africa, only including the investigation of mass mortalities of largemouth bass fingerlings (see Du Plessis, 1948) and inclusion in checklists compiled on the helminths of Africa or parasites of freshwater fishes in southern Africa (van As and Basson, 1984; Khalil and Polling, 1999). Other information available include the studies of Barson *et al.* (2008), Tavakol *et al.* (2015) and unpublished data of three co-introduced Monogenea from the ureters and gills (see Matla, 2012).

The present study investigated the parasite diversity of largemouth bass in South Africa, focusing on populations from the North West, KwaZulu-Natal, Eastern Cape and Western Cape provinces. The populations from the Eastern Cape and Western Cape are believed to be of the first largemouth bass introduced into the country, where it was distributed to other impoundments and freshwater systems throughout the country. This is supported by literature (see Harrison, 1936; McCafferty *et al.*, 2012) and the study of Hargrove *et al.* (2017). Parasitic communities, especially of the Monogenea are of interest, as these hosts are parasitised by specialist Ancyrocphalidae, that have been co-introduced. To shed light on the diversity of introduced parasites and the possibility or probability of these specialists to spill-over or spillback is investigated, as previous literature did not note or determine if these mechanisms are at play, or failed to identify these parasites up to generic level. An attempt was made to identify these introduced parasites using morphological as well as molecular approaches. To fill the gaps in our knowledge of these specialist monogenean parasites of *M. salmoides* in the freshwater systems of South Africa, the following hypotheses were proposed: 1) that parasite enemy release did not occur upon the

introduction of *M. salmoides* into South African freshwater systems; 2) that it will be possible to distinguish between parasitic genera and species using three nuclear markers; 3) that there will be a negative correlation between the health of *M. salmoides* and the intensity of infection with these specialist gill parasites and 4) that no parasite spill-over has occurred to native freshwater fish species. To achieve these hypotheses the main aims of the study was to 1) perform a full macro- and microscopic parasite screening of *M. salmoides* to determine the parasite diversity of populations from eight localities throughout South Africa (Mooi River system, North West Province (NW); Eerste River catchment (VD) and an closed natural lake, Groenvlei Lake (GV), in the Western Cape; the uMngeni catchment in KwaZulu-Natal (KZN) and two impoundments in the Kariega River system in the Eastern Cape (EC); 2) identify the monogenean parasitic species using both morphological and molecular approaches; 3) to implement a macroscopic necropsy-based fish health assessment to determine health status of the host species from impoundments with the highest infection levels and 4) to investigate the parasite community of native fishes in an impoundment with a specialist gill parasite known to be less host-specific than the other to their centrarchid host, to determine if parasite spill-over has occurred.

Micropterus salmoides were collected with the aid of angling and electrofishing techniques from seven impoundments, during October 2015, February 2016, April 2016, October 2016 and April 2017. All other fishes were sampled with the use of gill-, fyke and seine nets, in January and April 2017, from the Boskop Dam, North West. Fish were kept in aerated containers until a dissection and necropsy-based health assessment was performed (recommended by Adams *et al.*, 1993; Fouché, 2016) at the field site, and a macro- and microscopic parasite screening was done.

The parasite screening of *M. salmoides* and morphological characterisation confirmed the presence of eight parasite species from all the populations investigated and consisted of a single protozoan (*Trichodina* sp.) from the gills, two nematodes (*Contracaecum* sp. and *Spinitectus* sp.) from the body cavity and stomach and five ancyrocephalid monogenean parasite species from the gills (*Clavunculus bursatus*, *Onchocleidus dispar*, *Onchocleidus furcatus*, *Onchocleidus principalis* and *Syncleithrium fusiformis*). Overall, EC had the highest species richness with five parasite species present, followed by KZN, GV, VD and the lowest in NW. Monogenean species richness was the highest in KZN and the EC, and lower for GV, VD and the lowest in NW with only one species present. The invasive status of the *Trichodina* sp. and two nematodes are uncertain, but all five monogenean parasites

were co-introduced with the invasive *M. salmoides* and provides new locality records for these species. The lower species richness of the monogenean parasites in South Africa also supports the enemy release hypothesis. Molecular characterisation of the five co-introduced monogenean gill parasites were successful and provides the first molecular data available for these monogenean parasites found on largemouth bass in South Africa. The newly obtained data can potentially serve as a good platform for taxonomic revision of these ancyrocephalids and provide support for future studies in revisions of the phylogeny of the Ancyrocephalidae.

The macroscopic and necropsy-based fish health assessment was used and showed that *M. salmoides* from all localities were in good health condition, with the exception in GV where 46% of the fish had discoloured livers. This may not be linked to parasitic infection, but rather water quality or presence of pollutants in the system. Although the intensity of infection (IF) was the highest in EC, there was a very weak correlation (and not of statistical significance) between white blood cell counts and IF. The absence of a correlation between host health and parasitic infection suggest that the loss in parasite diversity may not be related to the fitness of the fish in the novel environment, but rather the co-evolution of the host and its parasites, this also supports the enemy release hypothesis.

All the parasites recorded from the five different native fish species collected from Boskop Dam in the present study represents infection with parasite species known from the specific hosts. The absence of infection with any of the ancyrocephalids from *M. salmoides* confirms that no spill-over occurred. The possibility that no spill-over has occurred within the past 60 years that this host is present in the Mooi River system, suggest that it is unlikely that any of the Ancyrocephalidae will switch hosts. The possibility of host-switching or spill-over should, however, not be disregarded as little is known about the evolutionary relationship of these parasites with its centrarchid hosts.

From the results presented in this study, supplementary knowledge on the invasion status and potential, as well as additional morphological and molecular data is available for these parasite species. The potential for these co-introduced monogeneans to become co-invasive should not be underestimated or assumed from this single study. Future studies should monitor and investigate these parasite species to detect events of spill-over or spillback. Health impacts of these parasites should also be monitored, their presence in South African freshwater systems are still less than a century, and the evolutionary relationship with their hosts are still uncertain.

Keywords

Ancyrocephalidae

Largemouth bass

Co-introduced

Spill-over

Enemy Release Hypothesis

Invasive species

Fish Health Assessment

Study Outputs

Conferences (oral presentations presented at the following conferences):

Three national conferences:

- **Truter, M.**, Přikrylová, I., Weyl, O.L.F., Smit, N.J. 2016. Successful survival of ancyrocephalid monogeneans from alien invasive, *Micropterus salmoides* (Lacépède, 1802) populations in South Africa. Fountain Hill Estate Mini-Research Symposium, Fountain Hill Estate, Wartburg, South Africa, 19 – 20 October 2016.
- **Truter, M.**, Přikrylová, I., Weyl, O.L.F., Smit, N.J. 2016. Testing Enemy Release in largemouth bass in South Africa. Centre of Excellence for Invasion Biology, Annual Research Meeting, Stellenbosch University, Stellenbosch, South Africa, 8 – 10 November 2016.
- **Truter, M.**, Přikrylová, I., Weyl, O.L.F., Smit, N.J. 2017. Largemouth bass: parasite invasion mechanisms. Centre of Excellence for Invasion Biology, Annual Research Meeting, Stellenbosch University, Stellenbosch, South Africa, 9 – 10 November 2017.

One international conference:

- **Truter, M.**, Přikrylová, I., Weyl, O.L.F., Smit, N.J. 2017. Co-introduction of ancyrocephalid monogeneans on their invasive host, the largemouth bass, *Micropterus salmoides* (Lacépède, 1802) in South Africa. 8th International Symposium on Monogenea, Brno, Czech Republic, 9 – 12 August 2017.

Published papers:

One invited article:

- **Truter, M.**, Přikrylová, I., Weyl, O. L.F., Smit, N. J. (2017): Co-introduction of ancyrocephalid monogeneans on their invasive host, largemouth bass, *Micropterus salmoides* (Lacépède, 1802) in South Africa. International Journal for Parasitology: Parasites and Wildlife, 6:420–429 doi: 10.1016/j.ijppaw.2017.06.002.

Chapter 1: General Introduction

Introduction of various species, including plants, livestock and fishes, into new environments is no new concept to man. In particular, distribution of fish species has been a common and increasing practice from the early 18th to the middle 19th centuries (see Welcomme, 1992). Fish species have been translocated and distributed across borders and oceans for aquaculture enhancements, angling, biological control and the ornamental trade (Gozlan, 2008; Savini *et al.*, 2010). These introduced or translocated species are alien species, transported beyond the limits of their native range and can have different invasive status in accordance with the extent to which they overcome barriers of introduction, establish and reproduce within captivity or released populations in the novel environment (see **Fig. 1.1**, **Table 1.1**) (Richardson *et al.*, 2000; Colautti and McIsaac, 2004; Blackburn *et al.*, 2011). These introduced fish species may also bring symbionts and parasites along (see Taraschewski, 2006). When these parasites succeed in overcoming the barriers to introductions, establishment and spread (Blackburn *et al.*, 2011) they are known as co-introduced, while those introduced into a new environment with their alien host and then spill over to native hosts are known as co-invaders (see **Fig. 1.2**) (Lymbery *et al.*, 2014).

With regard to fish parasites, the movement and introduction of their fish hosts typically results in four possible mechanisms: enemy release, dilution, spillback and spill-over (Sheath *et al.*, 2015). Enemy release is attained when, upon introduction into a new environment, the alien host loses some of its natural parasites. The result is that in some cases, introduced fishes may host fewer parasite species than in their native range (Torchin *et al.*, 2003; Roche *et al.*, 2010; Grendron *et al.*, 2012; Petterson *et al.*, 2016). Spillback occurs when parasites from native hosts transfer to the introduced host and there is increase in infection (Kelly *et al.*, 2009). In some cases, spillback may result in dilution, when there is a decrease in the infection of the native hosts as aliens reduce transmission of parasites (Keesing *et al.*, 2006; Poulin *et al.*, 2011). Finally, spill-over, also called pathogen pollution, might occur when an alien introduces new parasites which then parasitise native hosts in the new range (Daszak *et al.*, 2000; Taraschewski, 2006).

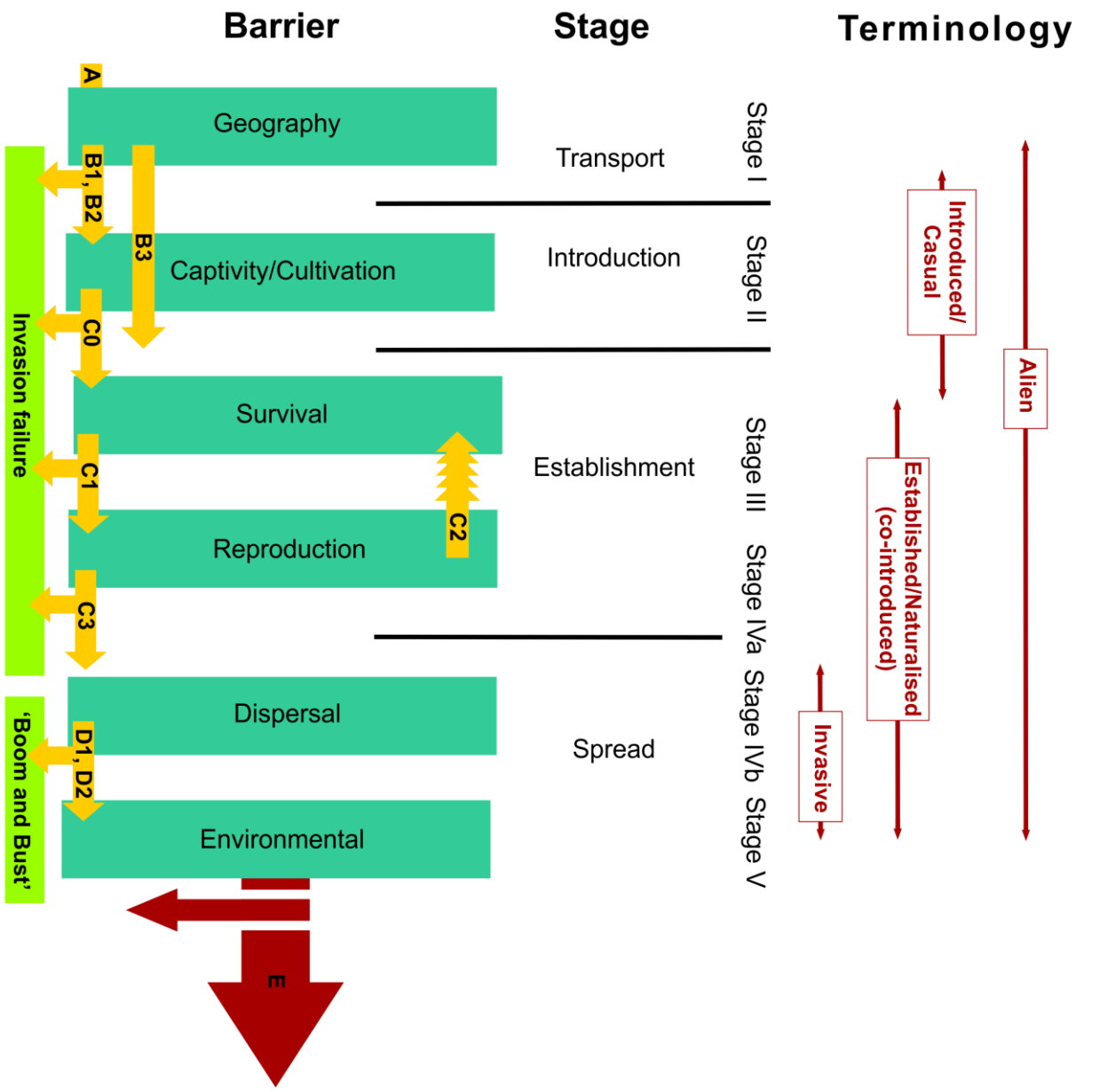

Figure 1. 1. Categorisation scheme for the invasion process with introduced species into a novel environment (adapted from Richardson *et al.*, 2000, Colautti and MacIsaac, 2004; Blackburn *et al.*, 2011).

Table 1. 1. Categorisation scheme for populations of introduced or translocated species into novel environment (adapted from Richardson *et al.*, 2000, Colautti and MacIsaac, 2004; Blackburn *et al.*, 2011; Lymbery *et al.*, 2014).

Stage	Category	Definition
Stage I	**A**	Individuals not transported beyond limits of native or natural range
	B1	Individuals transported beyond limits of native range, and in captivity or quarantine
Stage II	**B2**	Individuals transported beyond limits of native range and in cultivation
	B3	Individuals transported beyond limits of native range and directly released into novel environment
	C0	Individuals released into the wild in location where introduced, incapable of surviving for a significant period
Stage III	**C1**	Individuals surviving in the wild in introduced location, no reproduction
	C2	Individuals surviving in the wild where introduced location, reproduction occurring, but not self-sustaining
	C3	Individuals surviving in the wild where introduced location, reproduction occurring, self-sustaining
Stage IVa	**D1**	Self-sustaining population in the wild, with individuals surviving a significant distance from the original point of introduction
Stage IVb	**D2**	Self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction
Stage V	**E**	Fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence

Examples of co-introduced parasites of fishes are that of the monogeneans *Onchocleidus dispar* (Mueller, 1936) that was introduced with the pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) into Norway (see Sterud and Jørgensen, 2006), several localities along the Danube River Basin (Ondračková *et al.*, 2011), Britain (Hockley *et al.*, 2011) and the Ukraine (see Rubtsova, 2015); and *Onchocleidus principalis* (Mizelle, 1936) introduced with largemouth bass *Micropterus salmoides* (Lacépède, 1802) into the British Isles (see Maitland and Price, 1969). Examples of co-invader spill-over includes the copepod *Lernaea cyprinacea* Linnaeus, 1758 that was introduced with *Cyprinus carpio* Linnaeus, 1758 and *Carassius auratus* (Linnaeus, 1758) into the Kor River Basin, Iran where it now infests native cyprinids (Sayyadzahed *et al.*, 2016).

In South Africa, fishes have been introduced since the 18th century for sports angling, aquaculture, bio-control and as pets, and there are several examples of parasite co-introductions (see Ellender and Weyl, 2014; Smit *et al.*, 2017). Co-introductions are best described for cyprinid species such as *C. carpio* which are thought to have been the vector for the co-introduction of the ciliates *Apiosoma piscicola* (Blanchard, 1885); *Ichthyophthirius multifiliis* Fouquet, 1876; *Chilodonella cyprini* (Moroff, 1902); *Chilodonella hexasticha* (Kiernik, 1909); *Trichodina acuta* Lom, 1961; *Trichodina nigra* Lom, 1960; *Trichodinella epizootica* (Raabe, 1950) and the flagellate, *Ichthyobodo necator* Henneguy, 1883 (see Smit *et al.*, 2017). Grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), are thought to be responsible for the introduction of the Asian tapeworm, *Schyzocotyle* (*Bothriocephalus*) *acheilognathi* (Yamaguti, 1934) and the Japanese fishlouse, *Argulus japonicus* Thiele, 1900 was most likely introduced in association with fishes in the pet trade (reviewed by Ellender and Weyl, 2014). Spill-over to native fishes, with conparthenogenic effects have been observed for five of these species *A. japonicus, C. hexasticha*, *I. multifiliis*, *S. acheilognathi* and *T. acuta* (see Bruton and van As, 1986). Co-introduction of monogenean species into South Africa have been recorded and consist of *Acolpenteron ureterocoetes* Fischtal & Allison, 1940 believed to have been co-introduced with *Micropterus dolomieu* Lacépède, 1802; *Micropterus punctulatus* (Rafinesque, 1819) and *M. salmoides* (see Du Plessis, 1948), *Gyrodactylus kherulensis* Ergens, 1974 with the *C. carpio koi* var. (see Maseng, 2010)

and three *Dactylogyrus* Diesing, 1850 species i.e. *Dactylogyrus extensus* Mueller & Van Cleave, 1932; *Dactylogyrus lamellatus* Achmerow, 1952 and *Dactylogyrus minutus* Kulwiec, 1927, all co-introduced with *C. carpio*—to date no spill-over events have been documented (Crafford *et al.*, 2014; Smit *et al.*, 2017).

1.1. Largemouth bass: a global invader

The largemouth bass *M. salmoides* is native to the eastern regions of North America (Hargrove *et al.*, 2017) and is a popular sport angling and aquaculture species from the sunfish family (Centrarchidae Bleeker, 1859). Its natural distribution range reaches from the lower great lakes of North America, the Mississippi River basins from southern Quebec to Minnesota and south Texas, the Gulf coast, southern Florida, northwards to the Atlantic coast of Virginia, and drainages from North Carolina to northern Mexico (see De Moor and Bruton, 1988; Claussen, 2015). Currently largemouth bass is listed as one of the world's 100 worst invasive species (Lowe *et al.*, 2000) and its detrimental effects has been widely reported in introduced regions (e.g., Iguchi *et al.*, 2004; Takamura, 2007; Cucherousset and Olden, 2011; Ellender and Weyl, 2014).

The first introductions of *M. salmoides* into South Africa occurred in 1928 when 45 largemouth bass fingerlings were imported into the Jonkershoek Inland Fish Hatchery, Western Cape and 43 to the Pirie Hatchery in the Eastern Cape from the Surrey Trout Farm, England (Harrison, 1936; Hargrove *et al.*, 2017). This was followed by the introduction of four other centrarchid species: smallmouth bass *Micropterus dolomieu* in 1937; bluegill *Lepomis macrochirus* Rafinesque, 1819 in 1939; spotted bass *Micropterus punctulatus* in 1940 and Florida bass *Micropterus floridanus* (Lesueur, 1822) in 1984 (Ellender and Weyl, 2014). Following their introduction, *M. salmoides* and the other centrarchid species were widely distributed for sport angling and populations have established throughout South Africa (Ellender *et al.*, 2014; Hargrove *et al.*, 2015). In 1930 the distribution of *M. salmoides* from the Jonkershoek Fish Hatchery to other regions throughout the country began and within 10 years at least five major catchments in the Western Cape and KwaZulu-Natal had established populations of *M. salmoides* (see De Moor, 1996). In 1952 the Umgeni Hatchery opened in KwaZulu-

Natal and later largemouth bass were distributed throughout the province from here (De Moor and Bruton, 1988).

While the ecological impacts, predation on native invertebrates and fishes, habitat destruction, population alterations and competition with native species, are well documented (e.g., Shelton *et al.*, 2008; Weyl *et al.*, 2010; Ellender *et al.*, 2011; Kimberg *et al.*, 2014), their parasite communities have not received much attention (see Ellender and Weyl, 2014).

1.2. Associated parasites: native and non-native range

In its native region, the parasite diversity and communities of largemouth bass has extensively been documented. It is known to be parasitised by at least 150 parasite species from the Protozoa, Monogenea, Trematoda, Cestoda, Nematoda, Acanthocephala, Mollusca and Crustacea (see Beverley-Burton, 1984; Hoffman, 1999). On the African continent, there are only a few published records, mainly from Kenya, of Nematoda and Acantocephala parasitising *M. salmoides* (see Schmidt and Canaris, 1967, 1968; Amin and Dezfuli, 1995; Khalil and Polling, 1997; Aloo and Dezfuli, 1997; Aloo, 1999). In South Africa, current knowledge is limited to: (1) a 1948 report of the presence of the monogenean parasite *A. ureterocoetes* from the ureter of largemouth bass in the Jonkershoek Hatchery in the Western Cape (Du Plessis, 1948); (2) records of *Dactylogyrus* sp., *Gyrodactylus* sp. and *Dolops ranarum* (Stuhlmann, 1892) in the checklist of van As and Basson (1984); (3) an unpublished thesis (Malta, 2012) reporting that *M. salmoides* from Lake Tzaneen, Limpopo were parasitised by monogeneans *A. ureterocoetes* in the the urinary bladder; *Onchocleidus furcatus* (Mueller, 1937) and *Syncleithrium fusiformis* (Mueller, 1934) on the gills; and a nematode (*Contracaecum* sp.) and cestode larvae *Ligula intestinalis* (Linnaeus, 1758) in the intestine and (4) the sampling of *Contracaecum* spp. larvae in specimens collected in the Limpopo and Mpumalanga provinces (Tavakol *et al.*, 2015).

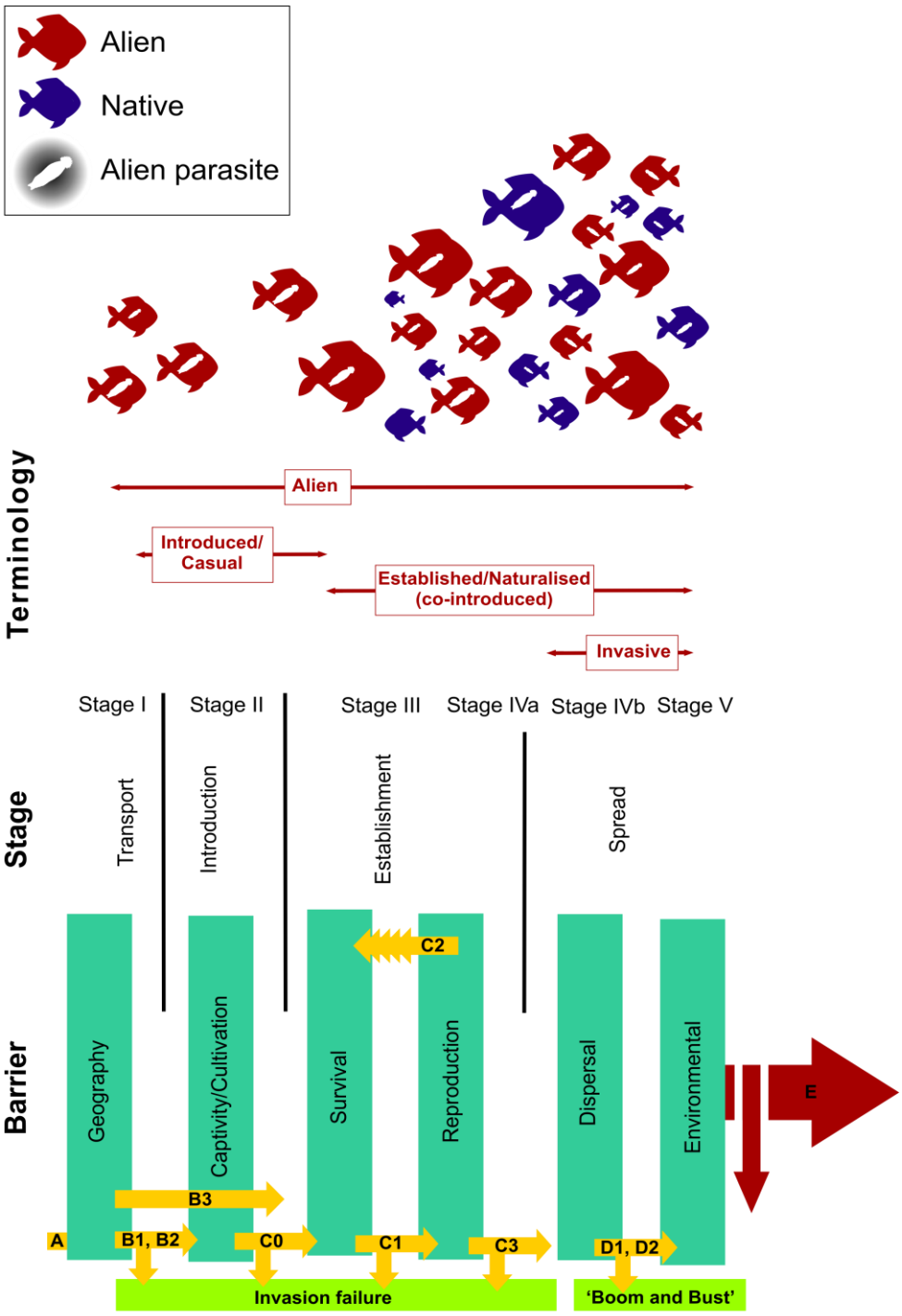

Figure 1. 2. Schematic representation of co-introduced and co-invasive parasites. Alien host has a parasite that follows process of introduction, establishment and spread with original host. Parasite switches to native host (blue) to become a co-invader (adapted from Lymbery *et al.*, 2014).

1.3. Hypotheses

As demonstrated by the short review in the previous section, it is clear there is a paucity of knowledge on the parasite community, diversity and invasion status of parasites from *M. salmoides* in South Africa. The main aim of the present study was therefore to investigate the parasite communities and diversity of *M. salmoides* in South Africa almost 90 years after their initial introduction and to assess for the potential of enemy release, parasite dilution, spillback and spill-over. The dissertation will test the following hypotheses:

Hypothesis 1: Enemy release has not occurred with the introduction of *M. salmoides* into South Africa.

Hypothesis 2: Using three molecular markers, it will be possible to distinguish between, and subsequently identify the monogenean parasite species collected from *M. salmoides*.

Hypothesis 3: There is a negative correlation between host health and monogenean infection.

Hypothesis 4: No spill-over occurred from invasive host to native species.

1.4. Aims and Objectives

To address these hypotheses, the following aims and objectives were set:

Hypothesis 1: Enemy release

Aim 1: To assess the parasite diversity of *M. salmoides* in South Africa through collection and identification (using both, molecular characterisation and the morphology of taxonomical important structures) of parasite specimens from six host populations across the country.

Aim 2: Use the data on parasite richness to determine if enemy release occurred during the introduction of the alien host, by comparing parasite communities of *M. salmoides* in South Africa to that of populations in the native range.

Hypothesis 2: Molecular characterisation

Aim 3: Obtain DNA sequences for the ectoparasites from South African *M. salmoides* targeting three nuclear markers i.e. 18S-ITS-1 rDNA and 28S rDNA;

Hypothesis 3: Parasite infection and host health

Aim 4: To determine health of the host using blood parameters and selected somatic indices, and compare with intensity of parasitic infection.

Hypothesis 4: Spill-over

Aim 5: To determine if parasite spill-over occurred by investigating the parasite communities occurring on native fish species where *M. salmoides* is also present.

Chapter 2: Materials and Methods

2.1. Selection of localities

The study was conducted throughout South Africa, and fish were sampled from eight impoundments in the North West, KwaZulu-Natal, Eastern Cape and Western Cape provinces, representing an overview of *Micropterus salmoides* introduction into and distribution in South Africa (**Fig. 2.1**). As mentioned in **Chapter 1**, **Section 1.1**, introduction of *M. salmoides* into South African freshwater systems occurred at more than one event from different stock populations, and despite legislation, translocation and distribution prolonged. The selection of localities aimed to obtain an overview of the parasite communities of the *M. salmoides* populations throughout the country.

2.1.1. North West (NW)

The Mooi River (26°41'03" S; 27° 5'59" E) (**Fig. 2.2 A**) and two major sub-catchments at the lower end of the Mooi River catchment were sampled in the present study; Potchefstroom (26°40'14" S; 27° 5'46" E) (**Fig. 2.2 B**) and Boskop dams (26°33'34" S; 27° 7'15" E) (**Fig. 2.2 C**). These dams serve as the main water supply reservoirs (DWAF, 2015) for the nearest town, Potchefstroom, and are popular recreational fishing venues. Both dams are fed by local run off, the Mooi River, Wonderfonteinspruit and Gerhard Minnebron eye (Barnard *et al.*, 2013).

The fish fauna of the Mooi River system includes both indigenous and alien fish species (see **Table 2.1**). Both smallmouth and largemouth bass were introduced as angling species. Smallmouth bass was introduced in the 1950's and had established populations in both reservoirs from 1970 to 1990 (pers. comm. resort manager, Mr. J Wessels, 2017). Population declines were noted as droughts during breeding seasons affected fecundity—the last known recorded bass catches were in 2007 and 2008 in Boskop and Potchefstroom dams, respectively (pers. comm. resort manager, Mr. J Wessels, 2017). Established populations of *M. salmoides* are still present in both reservoirs and the Mooi River. Introduction of *M. salmoides* occurred at Boskop Dam in the early 1940's and from time to time during floods or high-flow, recruitment into Boskop Dam from the upstream Klerkskraal Dam (upstream in the Mooi River) is likely.

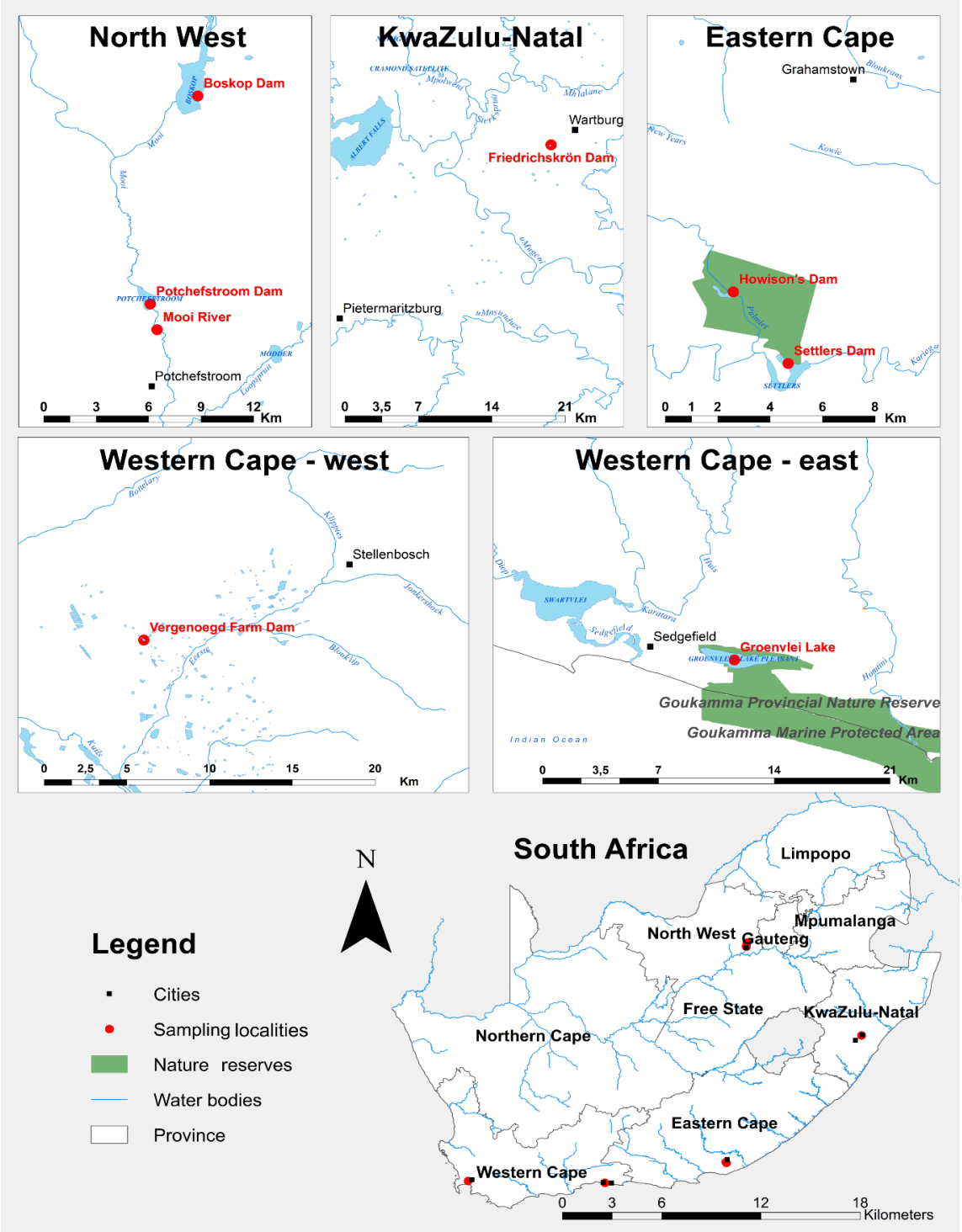

Figure 2. 1. Map of the localities throughout South Africa where the field sampling for the present study was carried out.

The *M. salmoides* population of Potchefstroom Dam is most likely derived from Boskop Dam. Legal stocking of fingerlings into the Potchefstroom Dam was also done with stock purchased from the Hartebeespoort Dam fish breeding station (North West), the Blydepoort Fish and Otters Den Hatcheries in Mpumalanga and private breeders in Vrede, Free State. Stocking has not been necessary over the past eight years (pers. comm. resort manager, Mr. J Wessels, 2017). The Vaal-Orange smallmouth yellowfish *Labeobarbus aeneus* (Burchell, 1822), although native to the region, and the non-native red breasted tilapia *Coptodon rendalli* (Boulenger, 1897) were introduced for angling and biological control purposes, respectively (Ellender and Weyl, 2014). Other alien species introductions include common carp *C. carpio*, grass carp *C. idella* – the latter only in Potchefstroom Dam, introduced for biological control of grass in the dam (pers. comm. resort manager, Mr. J. Wessels).

Table 2. 1. Potential assemblage of the fish communities in Boskop and Potchefstroom dams (table modified from Skelton, 2001; Jacobs, 2013 and pers. comm. resort manager, Mr. J Wessels, 2017).

Species	**Common name**
Enteromius anoplus (Weber, 1897)	Chubbyhead Barb
Enteromius paludinosus (Peters, 1852)	Straightfin barb
Enteromius trimaculatus (Peters, 1852)	Threespot barb
Clarias gariepinus (Burchell, 1822)	Sharptooth catfish
Ctenopharyngodon idella (Valenciennes, 1844)	Grass carp*
Cyprinus carpio Linnaeus, 1758	Common carp*
Gambusia affinis (Baird & Girard, 1853)	Mosquito fish*
Labeo capensis (Smit, 1841)	Orange River mudfish
Labeo cylindricus (Peters, 1852)	Redeye labeo
Labeo umbratus (Smith, 1841)	Moggel
Labeobarbus aeneus (Burchell, 1822)	Vaal-Orange smallmouth yellowfish
Micropterus dolomieu (Lacépède, 1802)	Smallmouth bass*
Micropterus salmoides (Lacépède, 1802)	Largemouth bass*
Pseudocrenilabrus philander (Weber, 1897)	Southern mouthbrooder
Tilapia sparrmanii (Smith, 1940)	Banded tilapia

(*) Alien species present – Introduced into system either for recreational angling or biological control.

2.1.2. KwaZulu-Natal (KZN)

Friedrichskrön Dam (29°26'46" S; 30°33'38" E) (**Fig. 2.2 D**) is a man-made farm dam situated on the Fountain Hill Estate, approximately 24 km north-east of Pietermaritzburg and 3 km West of Wartburg. This dam was constructed in 1967 and has a surface area of 3 km^2. Situated within the uMngeni catchment it is dependent on rainfall and surface run-off. There is no major river system that flows into the dam, the Nhlambamasoka stream drains from the dam into the uMngeni River. Stocking of *M. salmoides* is believed to have occurred shortly after its construction, manually or during flooding of upstream impoundments in the region. No recreational fishing or stocking with other fish species in the dam has occurred or been allowed since the dam's construction (pers. comm. farm manager, Mr. E Gevers).

2.1.3. Eastern Cape (EC)

The selected sampling localities, Howison's Poort (33°23'10" S; 26°29'4" E) (**Fig. 2.2 E**) and Settlers dams (33°24'41" S; 26°30'11" E) (**Fig. 2.2 F**), are situated within the Thomas Baines Nature Reserve. These two impoundments are part of the upper catchment of the Kariega River system. Both impoundments provide water to the nearest town, Grahamstown and the Settlers Dam is used for recreational activities such as swimming, fishing, sailing and canoeing, while Howison's Poort Dam is a restricted access area, with no recreational fishing or fish introduction allowed. Fish species of these two impoundments are the Southern mouthbrooder *Pseudocrenilabrus philander* (Weber, 1897) (extralimital), Banded tilapia *Tilapia sparrmanii* (Smith, 1940) (extralimital), bluegill *L. macrochirus*, and in 1934 largemouth bass *M. salmoides* were introduced (Hargrove *et al.*, 2017).

2.1.4. Western Cape (WC)

The Groenvlei Lake (GV) (34°1'48" S; 22°51'11" E) (**Fig. 2.2 G**) is one of two closed natural freshwater lakes in South Africa (Spencer *et al.*, 2016). This lake has a 2.5 km^2 surface area and is a slightly brackish, mesotrophic lake situated 5 km East of Sedgefield, within the Groenvlei catchment, on the coast of the Southern Cape. As Groenvlei Lake is cut off from the ocean, it is fed from groundwater recharge, direct rainfall and surface runoff (van Ginkel *et al.*, 2001; Parsons, 2009; Spencer *et al.*, 2016;

Whitfield *et al.*, 2017). Two small indigenous fish species, the estuarine round herring *Gilchristella aestuaria* (Gilchrist, 1913) and the Cape silverside *Atherina breviceps* Valenciennes, 1835 occur naturally in the lake. Legal stocking of alien species was initiated by Inland Fisheries in 1934 when *M. salmoides* were introduced (from the Jonkershoek Hatchery) for angling purposes, followed by bluegill *L. macrochirus,* as food source for bass. Other alien species introduced include *Oreochromis mossambicus* (Peters, 1852) and *Gambussia affinis* (Baird & Girard, 1853) for biological control purposes and an illegal introduction of *C. carpio* occurred in the 1990's. All the alien species established and formed reproducing populations in the lake (Harrison, 1936; Spencer *et al.*, 2016; Hargrove *et al.*, 2017; Whitfield *et al.*, 2017).

Vergenoegd Farm Dam (VD) (33°58'28.50" S; 18°44'54.15" E) (**Fig. 2.2 H**) is a small impoundment situated ± 32 km West of Stellenbosch and falls within the Eerste River catchment. This locality was selected as it is in close proximity to where the first *M. salmoides* fingerlings were introduced into South Africa. It is thought that this dam's population was sourced from the initially introduced *M. salmoides* in 1928. The Vergenoegd Farm Dam was constructed in the late 1920's and was initially stocked with a small population of Israeli tilapia *Oreochromis aureus* (Steindachner, 1864). Later *M. salmoides*, obtained from local authorities, were stocked in the dam for recreational purposes (pers. comm. with landowner, Mr. D Kotze and Cape Nature's Mr. D Impson).

Figure 2. 2. Specific sites where host specimens were collected. A – C: Mooi River, Potchefstroom Dam and Boskop Dam (North West); D – Friedrichkrön Dam (KwaZulu-Natal); E – F: Howison's Poort Dam and Settlers Dam (Eastern Cape); G – H: Groenvlei Lake and Vergenoegd Farm Dam (Western Cape).

2.2. Sampling of host species

2.2.1. *Micropterus salmoides*

Fish were collected from seven of the eight impoundments by angling with artificial lures (**Fig. 2.3 A**) and the use of electro-fishing techniques (**Fig. 2.3 E**). Sampling took place in October 2015 (NW – Mooi River and Potchefstroom Dams), February 2016 (EC), April 2016 (KZN), October 2016 (GV) and April 2017 (VD).

2.2.2. Other fish species

To investigate invasion biology mechanisms (see **Chapter 1**, **Section 1**) the following fish species: *Clarias gariepinus*, *Labeobarbus aeneus*, *Labeo umbratus*, *Labeo capensis, Tilapia sparrmanii* were collected from Boskop Dam with the use of rod and reel and fyke (**Fig. 2.3 B**), gill (**Fig. 2.3 C**), and seine nets (**Fig. 2.3 D**) in January and April 2017. Upon collection, all fish species were identified by either experienced researchers or with keys provided in Skelton (2001).

Figure 2. 3. Methods used to collect host specimens; A – rod and reel; B – fyke nets; C – gill nets; D – seine netting; E – electro-fishing.

2.3. Necropsy, blood parameters, biometric indices and parasite screening

Live fish were kept in aerated containers (**Fig. 2.4 A**) at a field site until humanely killed by means of percussive stunning and cervical dislocation (Fouché, 2016). For *M. salmoides* a blood[a] sample from the caudal vein was collected in a heparin vacutainer and centrifuged at 3000 r.min^{-1} for 10 min. to determine plasma proteins and stored at 4°C until processed in laboratory. Total plasma protein prepared per Basic Protocol 6 (Bradford, 1976) and analysed in triplicate using a universal micro-plate reader at 540 nm wave lengths. To determine haematocrit, blood was collected in a capillary tube and centrifuged (**Fig. 2.4 B**) at 3000 r.min^{-1} for 2 min. and measured, with a ruler, and expressed as percentages of the total measurement in millimeters. For leukocrit determination a thin blood smear was prepared, air dried and fixed in methanol for subsequent staining in Giemsa stain (**Fig. 2.4 C**) (Heath *et al.*, 2004). Fish were weighed (total mass) with an electronic balance or digital lip-grip scale (**Fig. 2.4 D**) and measured (**Fig. 2.4 E**) to the nearest millimeter (total, standard and fork length) to calculate condition factor (CF) (Adams and McLean, 1985; Heath *et al.*, 2004). The whole liver, spleen and gonads were weighed (in grams), before a macro- and microscopic parasite screening, to determine the associated organosomatic indices[b]. The following formula: Organ weight (g)/body weigth (g) x 100 was used for calculation of hepatosomatic index (HSI), splenosomatic index (SSI) and the gonadosomatic index (GSI) (Adams and McLean, 1985; Adams *et al.*, 1993). During the fish health assessment (FHAI) abnormalities were noted (Adams *et al.*, 1993; Heath *et al.*, 2004). Each fish was macroscopically screened for parasites on the external body surface. A dissection followed where the eyes, fins and gills (**Fig. 2.4 F**) of the fish were removed and placed in water and all internal organs (**Fig. 2.4 G**) were removed, separated and placed in a saline solution for screening of ecto- and endoparasites with the aid of a Nikon stereomicroscope (**Fig. 2.4 H–I**).

[a] Only collected for *M. salmoides* from the Eastern Cape and KwaZulu-Natal and evaluated as stipulated in Adams *et al.* (1993).
[b] Only calculated for *M. salmoides* from the Eastern Cape, KwaZulu-Natal and Groenvlei Lake.

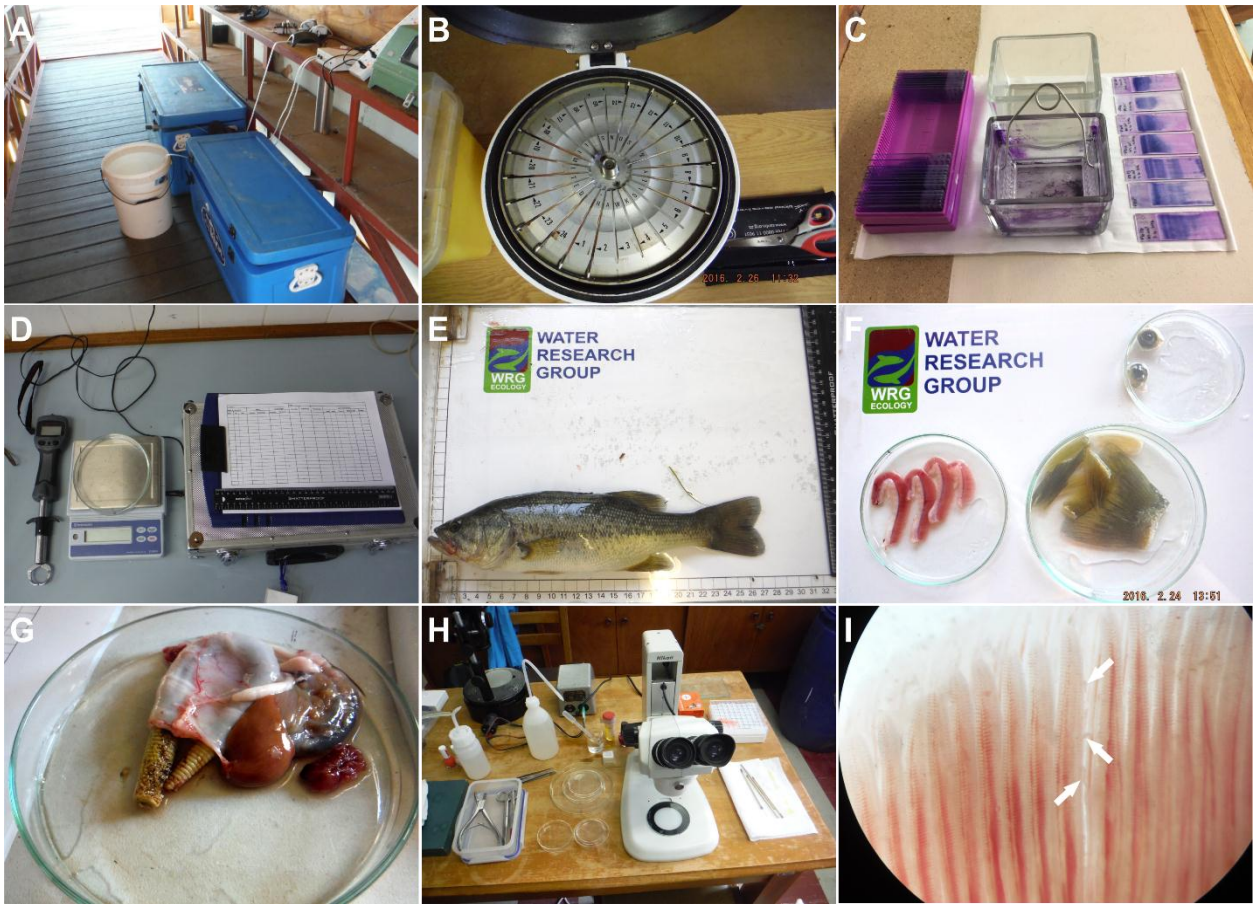

Figure 2. 4. Typical setup at a field site: A – aerated containers containing fish; B – haematocrit centrifuge; C – stained blood smears air drying; D – weighing station and data sheet; E – measuring of fish on measuring board; F – organs separated in petri dishes; G – internal organs in saline; H – screening of organs using a stereomicroscope; I – presence of Monogenea on the gill filaments.

Chapter 3: Parasite communities of South African largemouth bass *Micropterus salmoides* (Lacépède, 1802) populations: support for enemy release

3.1. Introduction

Centrarchid fishes are native to North America and are parasitised by a variety of parasites across several taxa. One of these are Monogenea Van Beneden, 1858, a very diverse parasitic group, primarily infecting bony fishes (Buchmann and Bresciani, 2006) and they can exhibit remarkable host specificity (Whittington, *et al.*, 2000; Öztürk and Özer, 2014). Parasites of the Ancyrocephalidae Bychowsky & Nagibina, 1978 is a known monogenean group containing genera which has a preference to parasitise fishes of the Centrarchidae (Beverley-Burton, 1986). Several parasite species from the genera *Actinocleidus* Mueller, 1937; *Anchoradiscus* Mizelle, 1941; *Clavunculus* Mizelle, Stokely, Jaskoski, Seamster & Monaco, 1956; *Crinicleidus* Beverley-Burton, 1986; *Onchocleidus* Mueller, 1936 and *Syncleithrium* Price, 1967 have been described from centrarchid hosts (Beverley-Burton and Klassen, 1990).

The parasitic communities of centrarchid fishes have been extensively studied in North America, including that of *M. salmoides* (see Mizelle and Crane, 1964; Esch, 1971; Lemly and Esch, 1984; McGee *et al.*, 2001). In a detailed parasite-host checklist on the parasites of the freshwater fishes of North America (Hoffman, 1999) and an additional record by Galaviz-Silva *et al.* (2016) it was reported that *M. salmoides* can be parasitised by almost 150 parasite species across 11 taxa that include the Fungi, Protozoa, Monogenea, Trematoda, Cestoidea, Nematoda, Acanthocephala, Gordiacea, Hirudinea, Mollusca and Crustacea. Thirteen monogenean species are known to occur on this host and eight of these are from the Ancyrocephalidae. These eight monogenean species can also be found on related host species including *Archoplites interruptus* (Girard, 1854); *Micropterus dolomieu*; *M. punctulatus*; *Lepomis cyanellus* Rafinesque, 1819; *L. gibbossus*; *Lepomis gulosus* (Cuvier, 1829); *Lepomis humilis* (Girard, 1858); *L. macrochirus*; *Lepomis marginatus* (Holbrook, 1855); *Lepomis megalotis* (Rafinesque, 1820) and *Lepomis microlophus* (Günther, 1859) (see Beverley-

Burton, 1986; Wheeler and Beverley-Burton, 1989). Some of these parasites exhibit preference for specific centrarchid hosts even in the presence of the other suitable centrarchid hosts (Collins and Janovy, 2003; Hockley, 2011).

With the introduction of centrarchid species such as *M. salmoides, M. punctulatus, M. dolomieu, L. macrochirus* and *L. gibbosus* throughout the world, there is a high probability that the parasites from the native range of these hosts were co-introduced with the host or might have disappeared along the way. The enemy release hypothesis states that introduced fish species lose some of their native parasites that cannot overcome the barriers set by introduction, establishment and reproduction in novel environments. The possibility of these co-introduced parasites switching hosts should also not be ignored. The afore mentioned mechanisms regarding invasion biology are discussed in full in **Chapter 1**, **Section 1**.

This chapter addresses **Hypothesis 1**.

3.2. Materials and Methods

3.2.1. General

The materials and methods used in this chapter are described in **Chapter 2**. For site selection, see **Chapter 2**, **Section 2.1**. For collection methods of *M. salmoides*, see **Chapter 2**, **Section 2.2.1** and the necropsy and parasite screening, see **Chapter 2**, **Section 2.3**.

3.2.2. Parasite fixation, morphology and identification

Ecto-parasites found on the external body surface were collected and preserved in 70% ethanol. Microscopic parasites collected from the gills were placed in a drop of water on a microscope slide and fixed in glycerine-ammonium picrate (GAP) (Malmberg, 1970). Specimens collected for molecular analysis were excised, with the haptor fixed on a slide with GAP and the rest of the tissue in a microtube with 96% molecular ethanol. Nematode specimens were removed from the encapsulating membrane, relaxed with a warm saline solution or 4% Formalin and preserved in either of the former solutions or 96% molecular ethanol. Fixed monogenean specimens were studied with the use of a Nikon Eclipse 80i compound microscope under 40x, 60x and 100x immersion oil

magnification and 60x and 100x phase contrast. Images and morphometrics were obtained with a DS-Fi1 camera mounted on the microscope and NIS-Elements V4 software. Identification of monogenean parasites was done by comparison of taxonomical sclerotised structures (anchors, male copulatory organ (MCO) and hooks) and their morphometrics to published literature (Mizelle, 1940, Beverley-Burton and Suriano, 1980; Beverley-Burton, 1986; Wheeler and Beverley-Burton, 1989). For all structures measured mean and range are given in micrometers, unless otherwise indicated. All other parasite groups were only identified up to genus level.

3.2.3. Statistical analysis

Prevalence (P), mean intensity and intensity of infection (IF) were calculated according to Bush *et al.* (1999). GraphPad Prism 5 software was used to perform statistical analysis and comparisons of data collected from each site. The D'Agostino & Pearson omnibus normality test was used to test for normality of fish size (SL) and intensity of parasite infection of each site in relation to one another. One-way analysis of variance (ANOVA) was performed with Tukey's multiple comparison test as a post test, if data were parametrically distributed. For non-parametric data sets, the Kruskal-Wallis test was performed with Dunn's multiple comparison test as a post-hoc test. A $p < 0.05$ was considered as significant. Spearman's rank correlation analysis was used to determine if there was a correlation between fish size and parasite load.

3.3. Results

3.3.1. General parasitological data

The gills of all *M. salmoides* specimens from all seven localities (NW n = 13; EC n = 30; KZN n = 15; GV n = 15 and VD n = 15), except one specimen from the Vergenoegd Farm Dam (VD), were infected with monogenean parasites. **Table 3.1** summarizes the size and mass of fish and ecological parameters of the Monogenea. The IF varied between localities, with a significant lower IF in the NW, GV and VD localities relative to KZN and the EC ($p < 0.0001$). Fish size across localities were evenly distributed, although NW fish were significantly smaller than EC, KZN, GV and VD ($p < 0.0001$). A correlation analysis to determine if host size affected parasitic infection showed a weak relationship ($r = 0.2314$, $p = 0.0416$) between the host size and IF. Infection with other

parasites included a protozoan species from the gills and a low prevalence (1 – 33 %) and IF (1 – 2) with nematodes from the body cavity, mesenteries and kidneys.

Table 3. 1. Fish biometrics and ecological parameters of monogenean parasite load. n – number of fish studied; Mean M – mean mass; Mean SL – mean standard length; Mean IF – mean intensity of infection.

	n	Mean M ±SD	Mean SL ±SD	Prevalence (%)	Mean IF
NW	13	133.4 ± 128.9	165.8 ± 79.24	100	32 (1 – 86)
KZN	15	377.4 ± 156.2	248.3 ± 46.80	100	399 (60 –736)
EC	30	248.2 ± 131.5	248.2 ± 131.5	100	448 (194 – 1668)
Western Cape					
GV	15	344.7 ± 93.34	262.1 ± 17.27	100	35 (1 – 282)
VD	15	336.5 ± 120.6	246.6 ± 24.12	93	25 (1 – 54)

3.3.2. Parasite community: composition, diversity and richness

A single protozoan, two nematode and five monogenean parasite species were collected. The protozoans consisted of seven individuals of *Trichodina* sp. specimens collected from from the gills of five hosts in the EC populations (P = 33%). Nematode specimens comprised of larval stages of *Contracaecum* sp. in low numbers from the EC (n = 4, P = 13%), KZN (n = 2, P = 13%), GV (n = 2, P = 13%), VD (n = 8, P = 33%) and a larval *Spinitectus* sp. from the NW (n = 1, P = 7%). A total of 20180 monogenean parasites were counted and a sub-sample of 1006 specimens were collected. Among collected samples, five species belonging to three genera of the Ancyrocephalidae were identified. These were as follow: *Clavunculus bursatus* (Mueller, 1936) (**Fig. 3.2 A–B**), *Onchocleidus dispar* (**Fig. 3.2 C–D**), *Onchocleidus furcatus* (**Fig. 3.3 A–C**), *Onchocleidus principalis* (**Fig. 3.4 A–B**) and *Syncleithrium fusiformis* (**Fig. 3.4 C–D**).

Abundance and community composition of monogenean parasites are presented in **Fig. 3.1**. The least abundant species was *C. bursatus* 3% (KZN) and 4% (EC) followed by *Syncleithrium fusiformis* (8%) from KZN, and *O. dispar* 9% (EC), 13% (GV) and 17% (VD). *Onchocleidus furcatus* 100% (NW) and 89% (KZN) and *O. principalis* 86% (EC), 87% (GV) and 83% (VD) were the most abundant species, respectively, and were not found in association with each other, but dominating in their respective geographic region in South Africa. For monogeneans, species richness was highest is KZN (n = 3) and EC (n = 3) and lower for GV (n = 2), JH (n = 2) and the NW (n =1). Overall, EC had the highest species richness with five parasite species present, followed by KZN (n = 4), GV (n = 3), VD (n = 3) and NW (n = 2) (see **Table 3.2**).

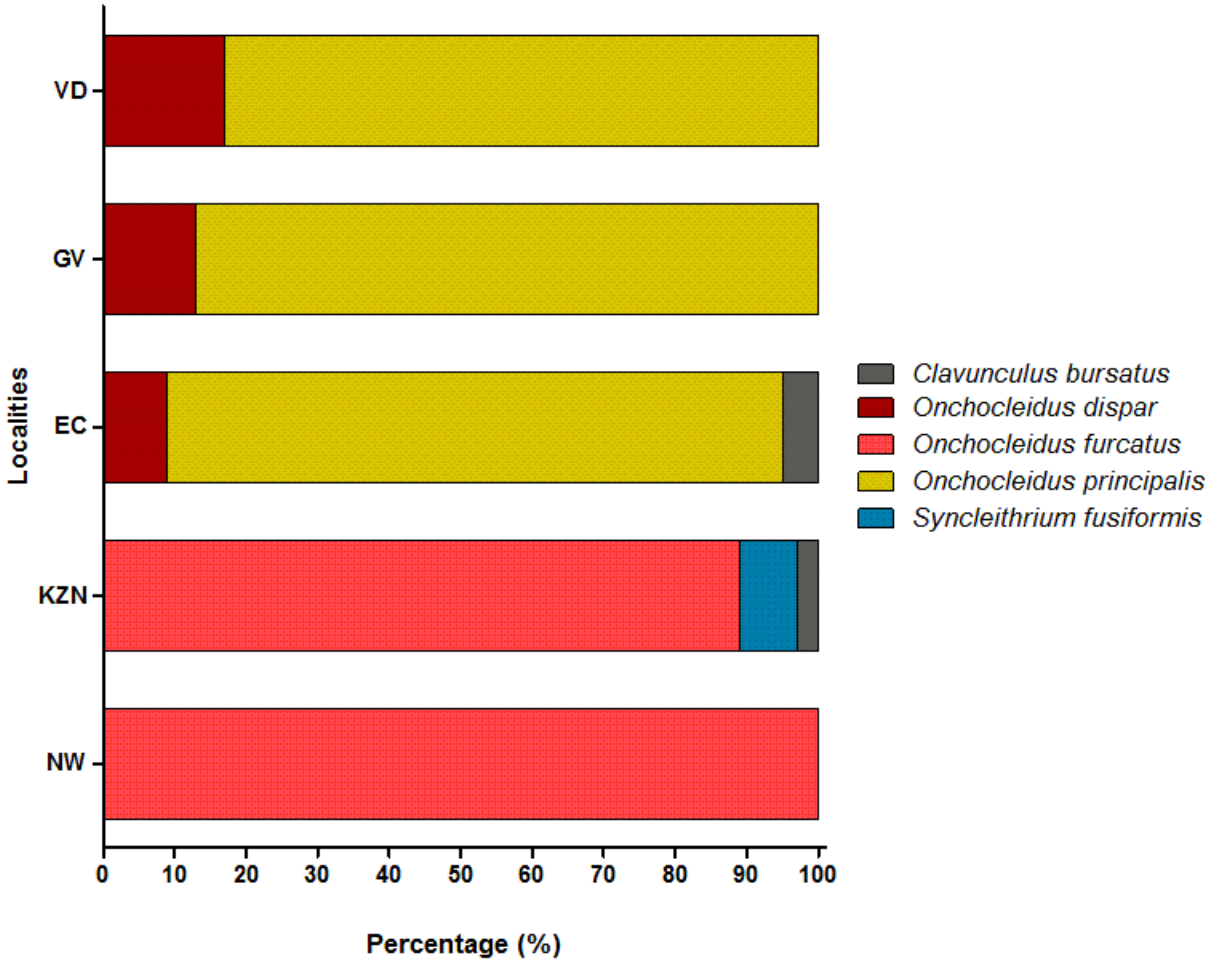

Figure 3. 1. Composition and abundance of the monogenean communities at each sampling locality.

Table 3. 2. Summary of parasitic groups and species found from *Micropterus salmoides* from studied localities.

	Species	NW	EC	KZN	Western Cape	
					GV	VD
Protozoa	*Trichodina* sp.	–	+	–	–	–
Nematoda	*Contracaecum* sp.	–	+	+	+	+
	Spinitectus sp.	+	–	–	–	–
Monogenea	*Clavunculus bursatus*	–	+	+	–	–
	Onchocleidus dispar	–	+	–	+	+
	Onchocleidus furcatus	+	–	+	–	–
	Onchocleidus principalis	–	+	–	+	+
	Syncleithrium fusiformis	–	–	+	–	–

+ parasite species present; – parsite species absent

3.3.3. Morphological characterisation

Family Ancyrocephalidae Bychowsky & Nagibina, 1978

Genus *Clavunculus* Mizelle, Stokely, Jaskoski, Seamster & Monaco, 1956

Clavunculus bursatus (Mueller, 1936) (**Fig. 3.2 A–B**)

Type host: *Micropterus salmoides*

Site of infection: Gill filaments

Other hosts: *Lepomis macrochirus*; *Micropterus dolomieu*; *M*. *punctulatus*

Type locality: London, Ohio, USA

Material examined: A total of 19 specimens from *M. salmoides* were examined. Eleven specimens from five hosts from the Howison's Poort Dam (33°23'10" S; 26°29'4" E), four specimens from three hosts at the Settlers Dam (33°24'41" S; 26°30'11" E) and four specimens from three hosts at the Friedrichskrön Dam (29°26'46" S; 30°33'38" E) were collected and studied. Voucher material of one specimen from EC is deposited in the parasite collection of the National Museum, Bloemfontein (acc. no. NMB P 442).

Description: Large dactylogyrid with characters of genus, dimensions presented in **Table 3.4**. Umbrella-like haptor with typical marginal indentations each accommodating a hook (pairs III – VII), pair I directly anterior to ventral bar, pair V situated between the two pairs of anchors (**Fig. 3.2 A**). Hooks similar in shape and size, with bulbous base, elongate shaft and hook proper. Anchor and bars small relative to haptor, in central region of haptor. Anchor similar in size and shape, with short robust blade and distinctive outer root notch. Transverse bars articulate with each other, dorsal bar with median suture appearing bipartite, ventral bar V-shaped. Male copulatory complex (**Fig. 3.2 B**) well sclerotised tubular penis with distinctive shaft with inflated sclerotised base, accessory piece well sclerotised with fenestrated base attached to proximal region of penis shaft and sharp distal point.

Remarks: Morphometrics of specimens from South Africa were within the same ranges as those parasitising *M. punctulatus* and *M. salmoides* from native regions reported by Mizelle (1940) and Beverley-Burton (1986), except in that the ventral bar is shorter: 18 (17 – 20) present study; 30 (27 – 33) Beverley-Burton (1986) and 31 (26 – 36) Mizelle (1940). The male copulatory complex is larger in size in South African specimens: 69 (58 – 80) present study; 55 (51 – 63) Beverley-Burton (1986) and 41 (39 – 46) Mizelle (1940) (see **Table 3.3**)

.

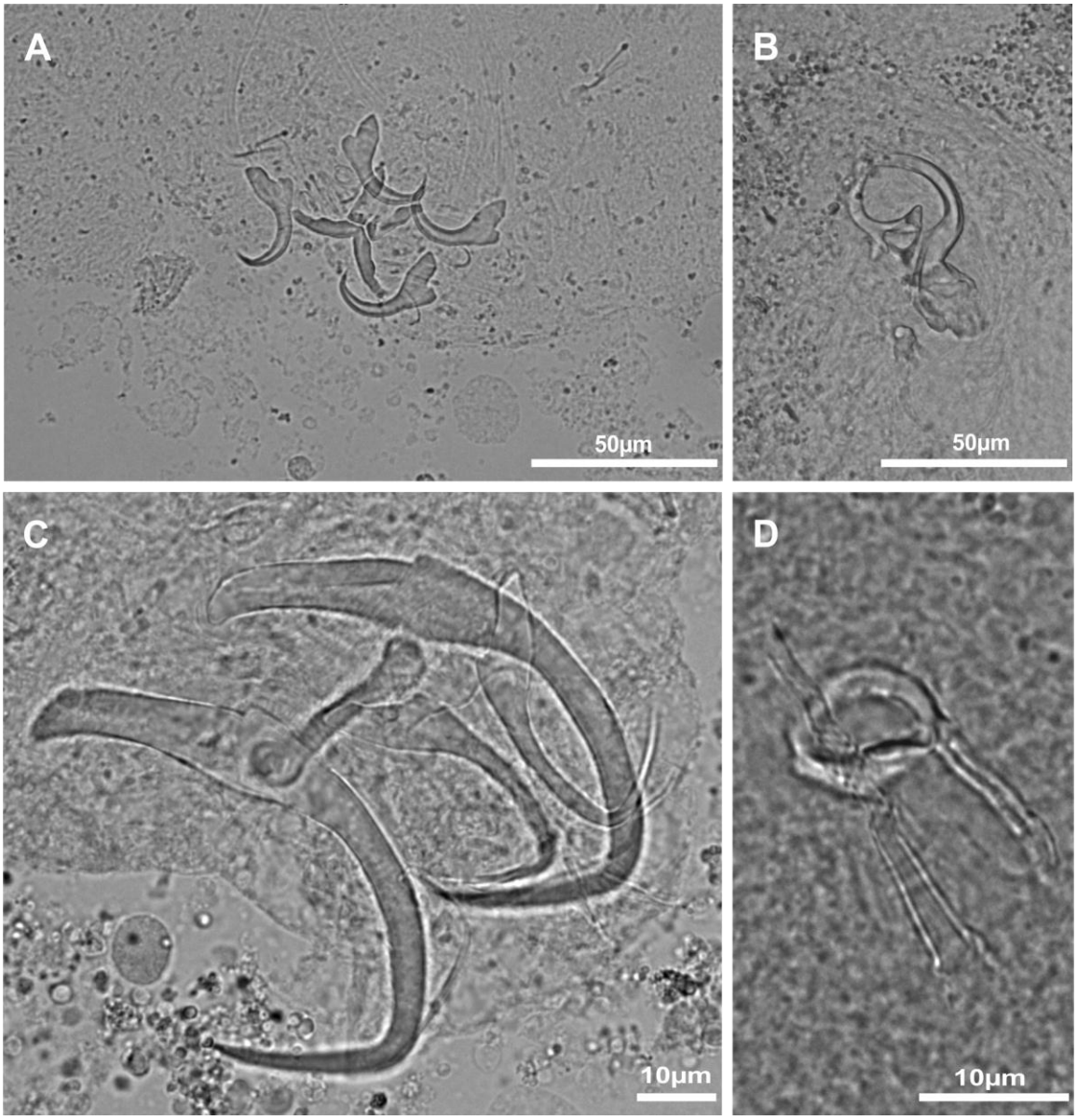

Figure 3. 2. *Clavunculus bursatus* (Mueller, 1936) haptoral hooks (A), male copulatory organ (B); *Onchocleidus dispar* (Mueller, 1936) haptoral hooks (C); male copulatory organ (D).

Genus *Onchocleidus* (Mueller, 1936)

Onchocleidus dispar (Mueller, 1936) (**Fig. 3.2 C–D**)

Type host: *Lepomis gibbosus*

Site of infection: Gill filaments

Other hosts: *Archoplites interruptus*; *Lepomis auritus*; *L. cyanellus*; *L. gulosus*; *L. humulis*; *L. macrochirus*; *L. megalotis*; *Micropterus dolomieu*; *M. salmoides*

Type locality: Constantia, New York, USA

Material examined: A total of 17 specimens from *M. salmoides* were examined. Three specimens collected from one host at the Howison's Poort Dam (33°23'10" S; 26°29'4" E), three specimens collected from two hosts at Settlers Dam (33°24'41" S; 26°30'11" E), nine specimens from five hosts at Groenvlei Lake (34°1'48" S; 22°51'11" E) and two specimens from one host from the Vergenoegd Farm Dam (33°58'28" S; 18°44'54" E) were collected and studied. Voucher material (acc. no. NMB P 443) of three specimens are deposited in the parasite collection of the National Museum, Bloemfontein (NMB).

Description: Two pairs of anchors, dissimilar in shape and size (see **Table 3.2** and **Fig. 3.2 C**); dorsal bar straight with knobbed ends, ventral bar bow shaped. Hooks with ovate elliptical base, slender shaft and sickle shaped hook, similar in shape, pairs I – II similar in size, pairs III – VII slightly longer. Male copulatory complex (**Fig. 3.2 D**) comprise of sclerotised straight penis, thick at base, sclerotised accessory piece with elongate handle and distal ring through which penis passes. Vagina not observed.

Remarks: Compared to *O. dispar* populations from native regions, individuals from non-native region (present study) has a ventral bar similar in size—corresponds to length reported by Hanek and Fernando (1972), but is slightly shorter (present study: 20 (17 – 20)) than reported by Beverely-Burton and Suriano (1980) (21 (17 – 25)), and have smaller hooks (pairs I – II, present study: 15 (14 – 16)) than those reported by Beverley-Burton and Suriano (1980) (18 (15 – 20), but correspond with values provided by Mizelle and Cronan (1943). All other characters are within range of measurements given from the different hosts by various studies (see Mizelle and Cronan, 1943; Hanek and Fernando, 1972; Beverley-Burtonn and Suriano, 1980) (see **Table 3.3**).

Onchocleidus furcatus (Mueller, 1937) (**Fig. 3.3 A–C**)

Type host: *Micropterus salmoides*

Site of infection: Gill filaments

Other hosts: *Lepomis cyanellus*; *L*. *macrochirus*; *L*. *marginatus*; *L*. *megalotis*; *L*, *microlophus*; *Micropterus dolomieu*; *M*. *punctulatus*

Type locality: Florida, USA

Material examined: A total of 18 specimens from *M. salmoides* were examined. Fifteen specimens from three hosts in the Mooi River (26°41'3" S; 27° 5'59" E) and Potchefstroom Dam (26°40'14" S; 27°5'46" E) and three specimens from one host in the Friedrichskrön Dam (29°26'46" S; 30°33'38" E) were collected and studied. Voucher material (acc. no. NMB P444) of two specimens are deposited in the parasite collection of the National Museum, Bloemfontein (NMB).

Description: Two pairs of anchors dissimilar in shape and size (**Fig. 3.3 A**); dorsal bar straight with knobbed ends, ventral bar slightly bowed with or without membrane. Hooks similar in shape with ovate elliptical base, slender shaft and sickle shaped hook with, pair I – II similar in size, positioned directly posterior to dorsal anchor and anterior to ventral anchor, respectively, pairs III – VII longer, distributed along lateral margins of haptor, male copulatory complex, larger than *O. dispar*, comprise of sclerotised straight to slightly curved penis, sclerotised accessory piece with elongate handle and distal ring through which penis passes (**Fig. 3.3 B**). Spiral filament 8 – 9 turns. Vagina unsclerotised (**Fig. 3.3 C**).

Remarks: *Onchocleidus furcatus* collected during the present study is morphologically the same as specimens from native range and measurements fall within ranges reported for this species (Mizelle, 1940; Wheeler and Beverley-Burton, 1989), except penis length is shorter than previously reported, but accessory piece is the same length (see **Table 3.3**). The accessory piece was observed to have a closed distal ring in most of the collected specimens (**Fig. 3.3 B**). A ventral bar is present with membrane absent or present (**Fig. 3.3 A**), which has not been reported from previous studies.

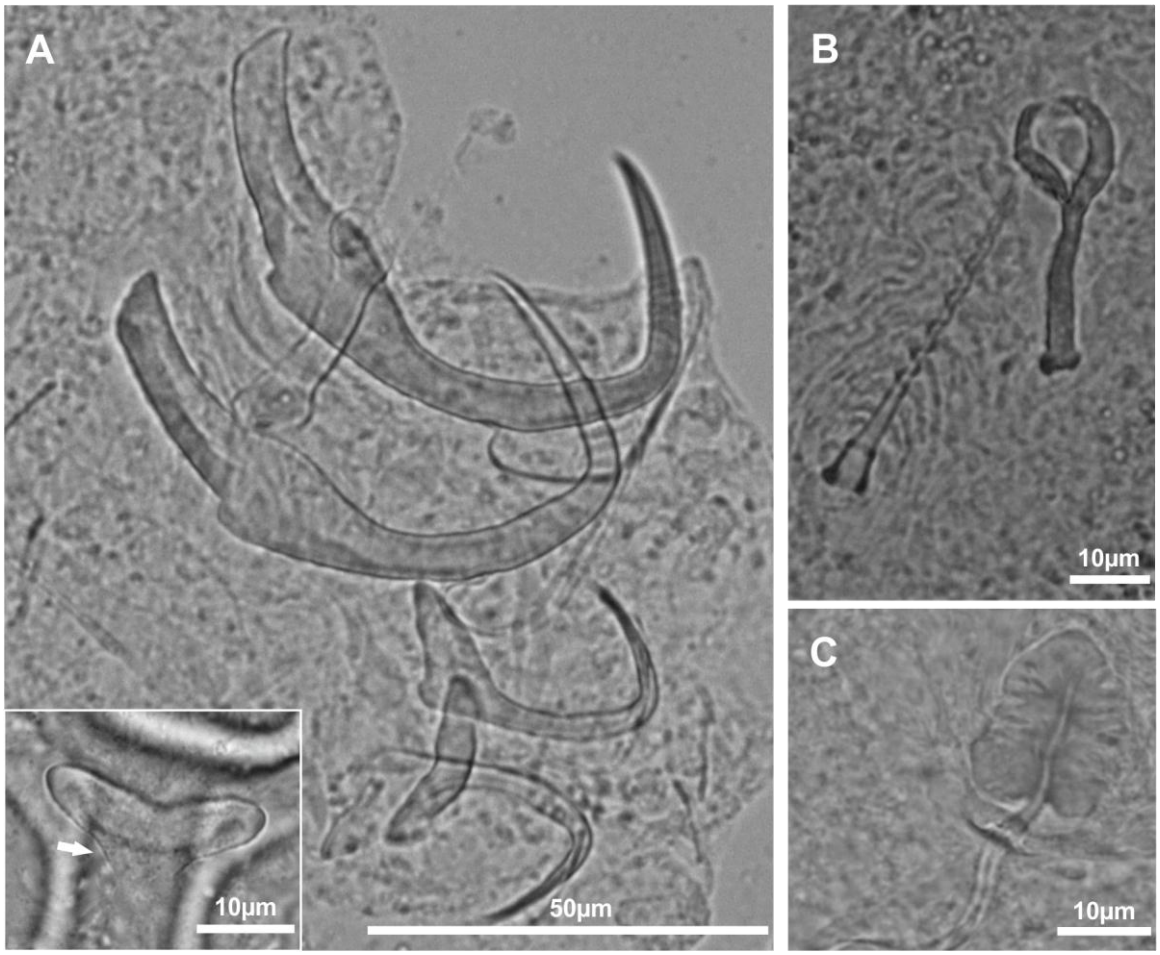

Figure 3. 3. *Onchocleidus furcatus* (Mueller, 1937) haptoral hooks (A), membrane on ventral bar (indicated by white arrow in insert), male copulatory organ (B), unsclerotised vagina (C).

Onchocleidus principalis (Mizelle, 1936) (**Fig. 3.4 A–B**)

Type host: *Micropterus punctulatus*

Site of infection: Gill filaments

Other hosts: *Lepomis cyanellus*; *L*. *macrochirus*; *Micropterus dolomieu*; *M*. *salmoides*

Type locality: Salt Fork of the Big Vermillion River, Homer, Illinois, USA

Material examined: A total of 24 specimens from *M. salmoides* were examined Fifteen specimens from two hosts from Settlers Dam (33°24'41" S; 26°30'11" E), four specimens from one hosts in Groenvlei Lake (34°1'48" S; 22°51'11" E) and five specimens from two hosts from the Vergenoegd Farm Dam (33°58'28" S; 18°44'54" E) were collected and studied. Voucher material (acc. no. NMB P445) of one specimen is deposited in the parasite collection of the National Museum, Bloemfontein (NMB).

Description: Two pairs of anchors similar in shape and size (see **Table 3.3** and **Fig. 3.4 A**); dorsal bar curved with knobbed ends, ventral bar slightly curved with membrane present or absent. Hooks similar in shape, pairs I – II similar in size, positioned directly posterior to dorsal anchor and anterior to ventral anchor, respectively, pairs III – VII slightly longer, distributed along anterolateral margins of haptor. Male copulatory complex comprises of sclerotised helical with 7 – 9 turns, sclerotised accessory piece with elongate handle, bifid distally **(Fig. 3.4 B**). Vagina unsclerotised.

Remarks: *Onchocleidus principalis* specimens found in present study are morphometrically the same to previous descriptions of this species (see **Table 3.2**). The only difference found is the observation of a ventral bar membrane at some specimens from the South African population (**Fig. 3.4 A**) that have not been recorded previously (Wheeler and Beverley-Burton, 1989).

Genus *Syncleithrium* Price, 1967

Syncleithrium fusiformis (Mueller, 1934) (**Fig. 3.4 C–D**)

Type host: *Micropterus dolomieu*

Site of infection: Gill filaments

Other hosts: *Lepomis cyanellus*; *L*. *gulosus*; *L*. *macrochirus*; *L*. *megalotis*; *Micropterus punctulatus*; *M*. *salmoides*

Type locality: Syracuse, New York; London, Ohio, USA

Material examined: Seventeen specimens collected from eight *M. salmoides* caught in Friedrichskrön Dam (29°26'46" S; 30°33'38" E) were studied. Voucher material (acc. no. NMB P 446) of two specimens are deposited in the parasite collection of the National Museum, Bloemfontein (NMB).

Description: Large dactyrogylid with characters of genus. Haptor not wider than body. Hooks distributed in typical ancyrocephalid pattern, as described above. Hooks similar in shape, with base, elongate shaft and hook, slightly dissimilar in size. Anchors and bars in central region of haptor ventral and dorsal bars projecting laterally beyond haptoral margin (**Fig. 3.4 C**). Anchors robust, distinguishable – dorsal anchors long inner root, compared to that of ventral anchors. Transverse bars articulate with each other forming single supporting plate for anchor. Ventral bar centrally horizontal, V-shaped, with oblique distal struts, dorsal bar a solid, shield-like plate, wider than long, central portion may be absent. Male copulatory complex (**Fig. 3.4 D**) comprising of well sclerotised penis with shaft and curved distal point and a lightly sclerotised base, accessory piece sclerotised with distal limb characterized by bifid tip which guides distal extremity of penis. Accessory piece attached to penis by strands of muscle. Vagina sclerotised, sub-marginal, left side of body.

Remarks: The *S. fusiformis* collected from South Africa is morphometrically similar to those previously described (see **Table 3.3**).

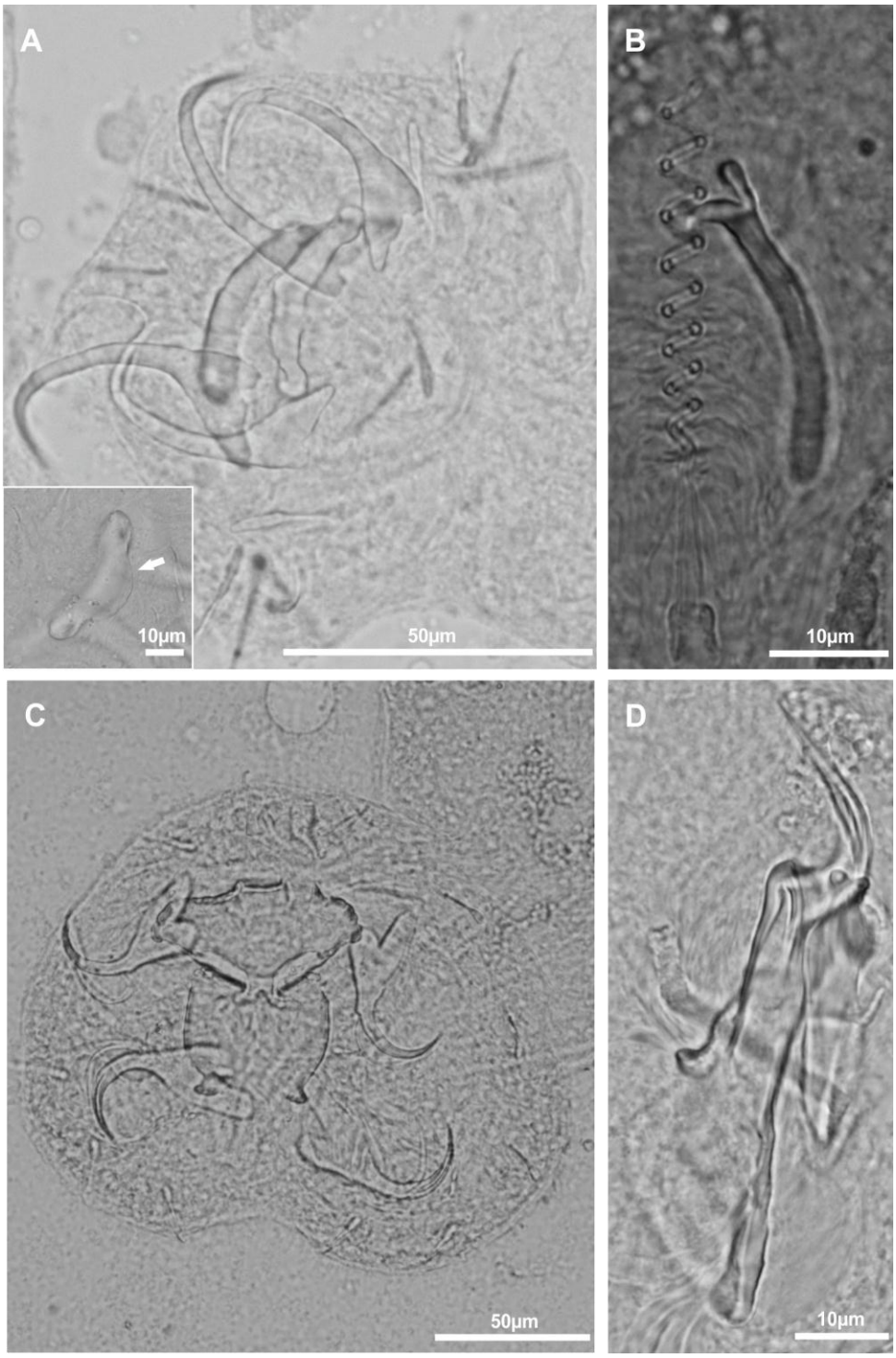

Figure 3. 4. *Onchocleidus principalis* (Mizelle, 1936) haptoral hooks (A), membrane on ventral bar (indicated with white arrow in insert); male copulatory organ (B); *Syncleithrium fusiformis* (Mueller, 1934) haptoral hooks (C) male copulatory organ (D).

3.4. Discussion

Until recently seven ancyrocephalid species: *Actinocleidus fergusoni* Mizelle, 1938; *Clavunculus bursatus*; *Onchocleidus dispar*; O*nchocleidus furcatus*; *Onchocleidus helicis* Mueller, 1936; *Onchocleidus principalis* and *Syncleithrium fusiformis* were known to parasitise *M*. *salmoides* in their native range (Mizelle and Cronan, 1943; Mueller, 1937; Mizelle and Crane, 1964; Hargis, 1953; Rawson and Rogers, 1972; Molnar *et al.*, 1974; Joy, 1984; Hoffman, 1999). Galaviz-Silva *et al.* (2016) increased this to eight, when reporting *M. salmoides* as a new host of *Clavunculus bifurcatus* (Mizelle, 1941). During the present study five of these eight ancyrocephalid species were found on *M. salmoides* in South Africa. As these parasites are common in centrarchid fishes in their native range, they were most likely co-introduced with *M*. *salmoides* or the other centrarchid fishes that were introduced into South Africa between 1928 and 1980 (see **Chapter 1**, **Section 1.1**). Interestingly, the results of the current study also correspond to Mizelle and Crane's (1964) observations in the native range of *M. salmoides* that no more than four of the seven known species occur at any one locality.

Previous studies reporting similar monogenean community structure, as in the present study, include that of Joy (1984) reporting four ancyrocephalids with a low frequency of *C*. *bursatus* and *S*. *fusiformis* and *O*. *furcatus* and *O. helicis* as the dominant species from *M*. *salmoides* collected in Beech Fork Lake, West Virginia. Mizelle & Crane (1964) and Rawson & Rogers (1972) reported a parasite community comprising of all species present in this study with the exclusion of *O. dispar*. The former reported fluctuation in abundance of species in parasite population between summer and autumn, respectively, from the California pond: *C. bursatus* (50% and absent), *O. furcatus* (23% and 32%), *O. principalis* (60% both seasons) and *S. fusiformis* (3% and 7%). Similarly, the latter reported *O*. *furcatus* and *O*. *principalis* dominating but on the same host population in association with low numbers of *C. bursatus* and *S. fusiformis* from the Walter F. George Reservoir, Alabama, USA. Cloutman (1975) also reported the same four monogenean species (excluding *O. dispar*) and *A. ureterocoetes* parasitising this host from Lake Fort Smith, Arkansas. All of the aforementioned studies reflects seasonal differences in the abundance of parasite community from a single location studied. In the study of Galaviz-Silva *et al.* (2016) the parasite community of *M. salmoides* from five localities were investigated.

Of the six monogenean species these authors found to parasitise *M. salmoides*, the presence of four ancyrocephalids correspond with the present study. *Clavunculus bursatus* were found to parasitise between 60% and 86.6% of hosts at all studied locations in relative high abundances. *Onchocleidus furcatus*, *O*. *principalis* and *S*. *fusiformis* occurred only on a few hosts from one locality in relatively low numbers. From these studies, it is clear that changes in season and related changes in the water temperature has an influence on the abundance or reproduction of these ancyrocephalids. Predominantly, the parasite community structure in the present study changed in terms of species abundance and not richness. Although investigation of parasite fauna for the selected localities in the present study were not performed throughout all seasons, it is interesting that such distinct community structures were found from each locality despite the relatively low fluctuation in temperature. This might be an example of interspecific competition (Cloutman, 1975) between species for a specific niche on the host in the novel environment. Species richness for monogeneans was lower in South Africa (n = 5) than in North American *M. salmoides* populations with a total of 13 monogenean species reported from its native range (Hoffman, 1999; Galaviz-Silva *et al.*, 2016). The lower abundance and species richness in South Africa therefore supports the enemy release hypothesis (Sheath *et al.*, 2015). With regard to the ancyrocephalid communities in particular, the species composition on the *M. salmoides* host populations is also less diverse (50%) than in North American populations (n = 10) and is comprised of those species capable of overcoming barriers set by introduction into a novel environment, also supporting enemy release (Lymbery *et al.*, 2014).

Differences between studied localities in South Africa could also be the consequence of the introduction from populations which might simply reflect the parasite community structure in the native region from where hosts were sourced (Mizelle and Crane, 1964). Although evidence is scant, the possibility of multiple *M. salmoides* introduction or cross-infection from other centrarchid species introductions cannot be ignored. It is possible that the low species richness in the Western Cape populations is a result of stocking of these impoundments with stock from the Jonkershoek Hatchery, while the Eastern Cape impoundment received stock from both the Jonkershoek and Pirie Hatcheries (Hargrove *et al.*, 2017). These host populations could each have experienced independent enemy release upon

introduction and contributed to the combined parasite community of the EC impoundment as it is today. Similarities between the parasite communities of NW, KZN and EC should also not be ignored, but information on the introduction history of host into these regions are scant. With regard to host specificity, *C*. *bursatus*, *O*. *principalis* and *S. fusiformis* are extremely host specific, only parasitising *M*. *salmoides* even in the presence of other potential centrarchid hosts, but *O*. *dispar* and *O*. *furcatus* are less host specific, having been reported to also parasitise *L. cyanellus*, *L. macrochirus* and *Pomoxis nigromaculatus* (Lesueur, 1829) (see Collins and Janovy, 2003). As a result, the potential for spill-over to native fishes and the spill-back to other introduced centrarchids requires future investigation (see **Chapter 6**).

Individuals from the ciliate group *Trichodina* Ehrenberg, 1830 are found as symbionts to a broad range of aquatic invertebrate and vertebrate hosts e.g. newts, amphibians and fish. Although there are more than 150 trichodinid species described, little is still known on the diversity and their relationship with their hosts (Lom, 1985; van As and Basson, 1989; Basson and van As, 2006). From a pathological perspective, this symbiont can cause hyperplasia of mucus cells of the skin and gills of fish (Basson and van As, 2006). In the present study, these symptoms were not observed and infection was only with a few individuals from five fish specimens. Of the ten trichodinid species known to parasitise centrarchid fishes in their native range, only six, *Trichodina domerguei* (Wallengren, 1897); *Trichodina fultoni* Davis, 1974; *T. nigra*; *Trichodina heterodentata* Duncan, 1977; *Trichodina pediculus* Ehrenberg, 1831 and *Trichodina wellborni* Lom, 1970, were reported to parasitise *M. salmoides* (see Wellborn, 1967; Hoffman, 1999; Aguilar-Aguilar and Islas-Ortega, 2015). Only one of these species, *T. nigra*, was reported to be present in South African aquatic systems (Smit *et al.*, 2017). Other trichodinids present in South Africa have been described from freshwater fish and are currently categorised as indigenous. These are: *Trichodina compacta* van As & Basson, 1989 and *T. heterodentata*, and co-introduced *T. acuta*; *T. epizootica*; *Trichodina mutabilis* Kazubski & Migala, 1968; *Trichodina reticulata* Hirshcmann & Partsch, 1955 and *Trichodina uniforma* van As & Basson, 1989 (van As, 2015; Smit *et al.*, 2017). The specimens in the present study could only be identified to the genus level as

Trichodina sp. An additional survey is proposed to conduct for investigation of trichodinids of *M. salmoides* in South Africa in more detail.

The two nematode species found, *Spinitectus* sp. and *Contracaecum* sp. belong to parasitic groups comprising of a large number of species found in marine, freshwater fishes, amphibians and mammals (see Boomker, 1993). From the African continent, there are 16 *Spinitectus* species described of which two are from South Africa: *Spinitectus petterae* Boomker, 1993 from *C. gariepinus* and *Spinitectus zambezensis* Boomker, 1993 from *Synodontis zambezensis* Peters, 1852 collected in the Kruger National Park (see Boomker, 1993; 1994). Infections of *M. salmoides* with *Spinitectus* Fourment, 1883 have not been reported previously in South Africa, but four species are known to infect centrarchids in their native range: *Spinitectus carolini* Holl, 1928; *Spinitectus gracilis* Ward & Magath, 1917: *Spinitectus micranthus* Christian, 1972 and *Spinitectus macracanthus* (Bleeker, 1854) (see Hoffmann, 1999). Infection of fishes with *Contracaecum* Rialliet & Henry, 1912 in the body cavity is not uncommon and similarly as in the case of *Spinitectus,* the identification up to species level can be difficult. There are two species, *Contracaecum brachyurum* (Ward & Magath, 1917) and *Contracaecum spiculigerum* (Rudolphi, 1809) known to infect centrarchids in their native range (see Hoffmann, 1999). One report of infection in *M. salmoides* was published recently in South Africa (Tavakol *et al.*, 2015) and as in the present study, the identification of the species was only confirmed up to genus level. As the parasites of both nematode genera occur in the native range of *M. salmoides*, it's difficult to state whether the parasites found in South Africa represent native or introduced parasites. But there is a very high probability that *M. salmoides* can be a suitable host for the African parasites of both genera.

Table 3. 3. Morphometrics of *Onchocleidus dispar, O. furcatus and O. principalis,* from gills of *Micropterus salmoides* in South Africa.

	Onchocleidus dispar						*Onchocleidus furcatus*						*Onchocleidus principalis*					
	Mizelle and Cronan, 1943	Hanek and Fernando, 1972	Beverley-Burton and Suriano 1980		Present study		Mizelle, 1940		Wheeler and Beverley-Burton, 1989		Present study		Mizelle, 1940		Wheeler and Beverley-Burton, 1989		Present study	
	(n = 1)[a]	(n =10)[a]	(n = 20)		(n = 17)[c]		(n = 9)[a]		(n = 13)[a]		(n = 18)[c]		(n = 4)		(n = 10)		(n = 24)[d]	
Body length	650	540 – 732	410	(320 – 512)	654	(530 – 780)	352	(238 – 495)	424	(403 – 451)	800	(715 – 885)	237	(207 – 289)	411	(342 – 510)	430	(350 – 510)
Body width	86	84 – 108	100	(90 – 150)	120	(103 – 139)	107	(81 – 135)	128	(100 – 146)	146	(117 – 175)	97	(83 – 117)	88	(54 – 119)	108	(85 –131)
Haptor length	57	72 – 86	75	(50 – 100)	–		67	(58 – 82)	64	(46 – 77)	–		47	(37 – 58)	65	(60 – 75)	–	
Haptor width	71	116 – 132	125	(100 – 150)	–		82	(68 – 86)	69	(58 – 77)	–		71	(67 – 73)	85	(66 – 104)	–	
Pharynx	43	40 – 44	30	(28 – 40)	–		25	(22 – 30)	39		–		20	(19 – 22)	24	(18 – 33)	–	
Dorsal hamuli																		
Anchor length	69	51 – 54	71	(60 – 85)	74	(70 – 78)	74	(43 – 81)	79	(77 – 80)	67	(63 – 70)	38	(32 – 41)	34	(29 – 41)	37	(33 – 42)
Point length	–	–	–		27	(25 –30)	–		32	(28 – 34)	29	(27 – 31)	–		13	(8 – 16)	13	(12 – 15)
Shaft length	–	–	–		56	(53 – 60)	–		61	(59 – 63)	50	(46 – 55)	–		32	(29 – 36)	34	(30 – 38)
Inner root length	–	–	–		26	(24 – 28)	–		26	(23 – 29)	27	(25 – 30)	–		9	(8 – 10)	11	(9 – 12)
Outer root length	–	–	–		1	(1 – 2)	–		–		2	(1 – 3)	–		–		3	(2 – 4)
Aperture length	–	–	–		34	(32 – 36)	–		–		34	(31 –38)	–		–		23	(20 – 26)
Dorsal bar length	23	25 – 27	30	(28 – 35)	29	(27 – 32)	30	(20 – 37)	36	(33 – 39)	31	(29 – 34)	36	(32 – 38)	31	(26 – 36)	33	(31 – 35)
Dorsal bar median width	–	5 – 6	4	(3 – 5)	4	(3 – 4)	–		5	(4 – 6)	5	(4 – 7)	–		5	(4 – 6)	6	(4 – 7)
Ventral hamuli																		
Anchor length	36	32 – 34	40	(35 – 45)	41	(37 – 44)	35	(25 – 38)	36	(31 – 40)	33	(31 – 34)	42	(38 – 46)	37	(32 – 45)	38	(37 – 40)
Point length	–	–	–		19	(17 – 21)	–		17	(14 – 19)	17	(16 – 19)	–		14	(12 – 16)	13	(12 – 15)
Shaft length	–	–	–		32	(30 – 35)	–		31	(28 – 33)	27	(25 –30)			33	(28 – 41)	36	(34 – 38)
Inner root length	–	–	–		12	(10 – 14)	–		12	(9 – 16)	11	(9 – 14)	–		11	(8 – 14)	11	(10 – 13)
Outer root length	–	–	–		–		–		–		–		–		–	–	3	(2 – 4)
Aperture					18	(15 – 21)	–		–		16	(15 – 18)					24	(23 – 26)
Ventral bar length	14	16 – 18	21	(17 – 25)	20	(17 – 22)	28	(25 – 32)	–		22	(19 – 24)	33	(27 – 40)	31	(26 – 36)	34	(32 – 36)
Ventral bar median width	–	4 – 5	6	(5 – 7)	6	(4 – 7)	–		–		5	(4 – 7)	–		5	(4 – 6)	6	(5 – 7)
Marginal hooks								11 – 26						12 – 24				
Pair I	14 – 17	14 – 20	16	(15 – 20)	15	(14 – 16)	–		19	(18 – 21)	16	(13 – 18)	–		15	(12 – 19)	17	(15 – 19)
Pair II	14 – 17	14 – 15	18	(15 – 20)	15	(14 – 16)	–		17	(16 – 18)	16	(12 – 19)	–		13	(10 – 16)	14	(12 – 17)
Pair III – VII	"shorter than the rest"	14 – 20	16	(15 – 20)	18	(17 – 20)	–		20	(18 – 24)	20	(17 – 22)	–		17	(13 – 20)	20	(18 – 22)
Male copulatory complex																		
Penis length	26	26 – 29	31	(26 – 35)	29	(26 – 32)	64	(62 – 66)	71	(62 – 80)	53	(46 – 60)	41	(37 – 45)	44	(38 – 48)	47	(45 – 50)
Accessory piece		20 – 23	20	(17 – 25)	22	(20 – 24)	32	(20 – 38)	36	(30 – 40)	31	(22 – 39)	23	(19 – 26)	24	(19 – 31)	24	(21 – 26)
Spiral filament turns						4	–			9 – 10		7 – 9	–			7 – 9		7 – 9
Host(s)	*M. salmoides*	*M. dolomieu*	*L. gibbossus*		*M. salmoides*		*M. salmoides*			–	*M. salmoides*		*M. salmoides*			–	*M. salmoides*	
Geographic locality	Tennessee, USA	Ontario, Canada	Ontario, Canada		South Africa		Tennessee, USA			–	South Africa		Tennessee, USA			–	South Africa	

[a] Average, minima and maxima is given
[b] 6 specimens from the EC, 9 specimens from GV and 2 from VD
[c] 15 specimens from NW and 3 form KZN were examined
[d] 15 from EC, 4 from GV and 5 from VD were examined

Table 3. 4. Morphometrics of *Clavunculus bursatus* and *Syncleithrium fusiformis* from gills of *Micropterus salmoides* in South Africa.

	Clavunculus bursatus						*Syncleithrium fusiformis*					
	Mizelle, 1940 (n = 10)[a]		Beverley-Burton, 1986 (n = 3)[a]		Present study (n = 19)		Mizelle, 1940 (n = 4)[a]		Hanek and Fernando, 1972 (n = 15)	Beverley-Burton, 1986		Present study (n = 17)[b]	
Body length	820	(646 – 1006)	1086	(725 – 1431)	1668	(639 – 2835)[a]	483	(270 – 910)	1224 – 1692	437	(283 – 717)	1053	(756 – 1443)[a]
Body width	328	(172 – 405)	342	(205 – 450)	314	(203 – 425)	132	(68 – 180)	146 – 168	136	(83 – 183)	188	(147 – 229)
Haptor length	216	(144 – 270)	169	(98 – 257)	174	(85 – 282)[a]	89	(85 – 97)	168 – 180	78	(67 – 93)	136	(98 – 232)[a]
Haptor width	242	(134 – 294)	275	(197 – 325)	308	(85 – 457)[a]	–		216 – 240	98	73 110	178	(83 – 178)[a]
Pharynx length	111	(61 – 130)	–		137	(76 – 197)	35	(29 – 43)	96 – 108	–		76	(59 – 93)
Pharynx width	–		–		146	(91 – 202)	–		120 – 132	–		83	(64 – 103)
Dorsal hamuli			–										
Anchor length	27	(22 – 30)	25	(24 – 28)	26	(25 – 27)	51	(44 – 51)	49 – 52	40	(34 – 45)	54	(37 – 59)[a]
Point length	–		–		7	(6 – 9)	–		26 – 27	–		15	(12 – 18)[a]
Shaft length	–		–		24	(23 –28)	–		–	–		40	(31 – 44)[a]
Inner root length	–		–		6	(4 – 7)	–		17 – 19	–		20	(16 – 24)[a]
Outer root length	–		–		2	(1 – 3)	–		6 – 10	–		8	(4 – 12)[a]
Aperture length	–		–		14	(13 – 15)	–		–	–		26	(24 – 31)[a]
Dorsal bar length	19	(15 – 22)	10	(6 – 13)	16	(14 – 18)	46	(45 – 47)	38 – 42	33	(30 – 36)	41	(38 – 45)[a]
Dorsal bar median width	–		–		5	(4 – 6)	–		42 – 44	–		49	(45 – 55)[a]
Ventral hamuli	–		–				–						
Anchor length	27	(25 – 28)	26	(24 – 27)	26	(25 – 27)	42	(40 – 43)	43 – 45	39	(35 – 45)	47	(41 – 55)[a]
Point length	–		–		6	(6 – 7)	–		27 – 28	–		14	(12 – 18)[a]
Shaft length	–		–		23	(23 – 25)	–		–	–		39	(36 – 46)[a]
Inner root length	–		–		7	(6 – 8)	–		13 – 15	–		14	(9 – 20)[a]
Outer root length	–		–		2	(1 – 3)	–		8 – 9	–		6	(4 – 11)[a]
Aperture length	–		–		14	(11 – 16)	–			–		24	(21 – 30)[a]
Ventral bar length	31	(26 – 36)	30	(27 – 33)	18	(17 – 20)	64	(59 – 68)	69 – 72	46	(36 – 57)	65	(57 – 71)[a]
Ventral bar median width	–		–		4	(4 – 6)	–		9 – 10	–		7	(3 – 10)[a]
Marginal hooks													
Pair I					16	(15 – 18)			17 – 18			19	(17 – 22)[a]
Pair V		14 – 21	11 – 18		16	(15 – 17)	11 – 18		16 – 17	17 – 21		16	(12 – 20)[a]
Pair II - VII					17	(17 – 19)			18 – 21			19	(16 – 22)[a]
Male copulatory complex													
Vagina length	–		–		–		–		–	–		27	(21 – 36)[a]
Vagina width	–		–		–		–		–	–		15	(13 – 19)[a]
Penis length	41	(39 – 46)	55	(51 – 63)	69	(58 – 80)	49	(38 – 55)	37 – 41	61	(48 – 70)	69	(64 – 73)
Accessory piece	27	(25 – 28)	23	(19 – 27)	31	(27 – 34)			24 – 26	41	(29 – 46)	65	(59 – 71)
Host (s)	*M. punctulatus*		*M. salmoides*		*M. salmoides*		*M. salmoides*		*M. salmoides*	–		*M. salmoides*	
Geographic locality	Tennessee, USA		–		South Africa		Tennessee, USA		Ontario, Canada	–		South Africa	

[a] Average, minima and maxima is given
[b] Only 12 haptors and complete specimens could be studied.

Chapter 4: Molecular characterisation of ancyrocephalid monogeneans parasitising *Micropterus salmoides* in South Africa

4.1. Introduction

To date there is still uncertainty about the exact position of the Ancyrocephalidae within the Dactylogyridea. Several attempts have been made using morphological and molecular approaches to clarify the taxonomic position of the Ancyrocephalidae and taxa within the family. Beverley-Burton (1986), Kritsky and Boeger (1989) and Wheeler and Beverley-Burton (1989) all attempted to use morphological characteristics to group genera together. The latter emphasised that the main three factors contributing to taxonomic confusion are a) original descriptions, and inadequate redescriptions thereof; b) generic revisions based on characters that are questionable in taxonomic importance and c) the ignorance of scientists in the field to literature on the generic revisions. To resolve the confusion Beverley-Burton (1984) used morphology and grouped various individuals within the genus *Actinocleidus* based on the transverse haptoral bars that articulate from a single point from the anchors. Later Beverley-Burton (1986) noted that more supported features include the distinct penis types and that higher degrees of host affinity can be seen as adaptive radiations concurring with the evolutionary trend of the host. Kritsky and Boeger (1989) attempted a phylogenetic analysis on the status of Ancyrocephalidae and Ancyrocephalinae to test for monophyly. The phylogeny proved Ancyrocephalidae to be an unnatural group within the Dactylogyridea as it contained poly- and paraphilic features, not sharing members of a sister group and not containing any descendants of ancestors. Šimková *et al.* (2003) and Mendoza-Palmero *et al.* (2015) agreed with Kritsky and Boeger (1989) on the paraphyly of the Ancyropcephalidae and suggested a morphological revision.

Few studies have attempted to provide molecular characterisation on the ancyrocephalids parasitising centrarchid fishes. Sequences for only three species are available in the nucleotide database i.e. 28S rDNA for *Actinocleidus recurvatus* Mizelle & Donahue, 1944 (AJ969951.1) (Šimková *et al.*, 2006), 18S-ITS-1 and 28 rDNA for *Onchocleidus similis* (Mueller, 1936) (AJ490167.1, AJ969938.1) (Šimková *et al.*, 2003, 2006) and 28S rDNA of *Onchocleidus* sp. (AY841873.1) (Ding and Liao, 2005).

This section aimed to obtain nucleotide sequences for the co-introduced species of *M. salmoides*, addressing **Hypothesis 2**.

4.2. Materials and Methods

4.2.1. DNA extraction and Polymerase Chain Reaction

Molecular analysis[a] in conjunction with morphological characteristics were used to confirm identification of specimens. Monogenean specimens preserved in 96% molecular ethanol were evaporated in a shaker-heating block at 56°C until the ethanol was evaporated. DNA extraction followed using extraction kits PCRBIO Rapid Extract PCR Kit®, Qiagen DNeasy Blood & Tissue Kit® and the Machery-Nagel NucleoSpinTissue and Blood Extraction Kit® and associated protocols. Nuclear markers used for determination of species identification were 18S rDNA, ITS-1 and 28S rDNA (Šimková *et al.*, 2003; Vanhove *et al.*, 2013). All PCR reactions were performed in duplicate. Partial 18S rDNA and entire ITS-1 regions were amplified in one PCR reaction using primers S1 (5'-ATTCCGATAACGAACGAGACT-3') that anneals to the terminal region of the 18S gene and IR8 (5'-GCAGCTGCGTTCTTCATCGA-3') that anneal in the 5.8 rDNA (Šimková *et al.*, 2003). Each reaction was performed with a final volume of 50 µl containing 10 µM of each primer, 25 µl DreamTaq® polymerase and 1-2 µl of DNA. The thermal cycle program for this PCR reaction was as follow: 4 min. at 95°C followed by 35 cycles of 1 min. at 95°C, 1 min. at 55°C, and 1 min. 30 sec. at 72°C and 10 min. of final elongation at 72°C (Šimková *et al.*, 2004; Kmentová *et al.*, 2016). Partial 28S rDNA was amplified using primers C1 (5'-ACCCGCTGAATTTAAGCAT-3') and D2 (5'-TGGTCCGTGTTTCAAGAC-3') (Hassouna *et al.*, 1984). Each reaction was performed with a final volume of 50 µl containing 10 µM of each primer and 25 µl DreamTaq polymerase, 1-2µl of DNA. Thermal cycle program for this PCR reaction was initial denaturation at 94°C for 2 min. followed by 39 cycles of 20 sec. at 94°C, 30 sec. at 58°C, and 1 min. 30 sec. at 72°C and 10 min. of final elongation at 72°C (Kmentová *et al.*, 2016). Gel electrophoresis was performed on a 1% agarose gel stained with ethidium bromide for DNA visualisation. Successful PCR reaction products where sent for sequencing to Inqaba Biotec™.

[a] Molecular characterisation was only performed for monogenean specimens collected from *M. salmoides*.

4.2.2. Sequence alignment and phylogenetic analysis

Obtained nucleic acid sequences were aligned, visually inspected and corrected using MEGA7 (Kumar *et al.*, 2016) under default distance measures and sequence weighing schemes. Trimming of the resulting alignments was done in trimAL v1.2 using the '*gappyout*' automated parameter selection under default settings (Capella-Gutiérrez *et al.*, 2009). Pairwise distances (*p*-distances) were determined for each nuclear marker and optimal evolutionary models were selected in MEGA7 based on the Bayesian Information Criterion. Models were implemented in MrBayes v3.2 (Ronquist *et al.*, 2012): for 18S rDNA and ITS-1 the Kimura 2-Parameter (K80) and for 28S rDNA the Kimura + Γ (Γ = 0.295). All data was partitioned and probabilities were calculated for 1 million generations, sampling the Markov Chain Monte Carlo chain every 100th generation with a burn-in of the first 25% of the sample generations. A maximum likelihood (ML) analyses was performed using RAxML v8.2 (Stamatakis, 2014) following a general time reversible (GTR) model + Γ (Rodríguez *et al.*, 1990) with a rapid bootstrap analysis search for best-scoring ML tree using an automated number of replicates. Phylogenetic trees were visualised in FigTree v1.4.3.

Sequences were also subjected to a Basic Local Alignment Search Tool search (BLAST search) to identify closely related species. Species and accession numbers used in the phylogenetic analyses are presented in **Table 4.1**.

Table 4. 1. Species details and accession numbers of sequences used in phylogenetic analyses (AUS – Austria, CZ – Czech Republic, RSA – South Africa, UK – United Kingdom).

Parasite species	Host	Host family	Locality	Accession Number		Reference
				18S-ITS	28S	
Order Dactylogyridea						
Family Dactylogyridae						
Ancylodiscoidinae						
Thaparocleidus siluri (Zandt 1924)	*Silurus glanis* (Linnaeus)	Siluridae	Morava basin, CZ	AJ490164.1	AJ969940.1	Šimková *et al.*, 2003, 2006
Thaparocleidus vistulensis (Sivak 1932)	*Silurus glanis* (Linnaeus)	Siluridae	Morava basin, CZ	AJ490165.1	AJ969941.1	Šimková *et al.*, 2003, 2006
Quadriacanthus bagrae (Paperna 1979)	*Bagrus docmak* (Forskkål 1775)	Bagridae	Nile River Basin, Sudan	KX713993.1	KX685951.1	Francová *et al.*, 2017
Ancyrocephalinae						
Ancyrocephalus paradoxus Creplin 1839	*Sander lucioperca* Linnaeus	Percidae	Lake Constance, Germany Finland	KF499079.1	AJ96995.1	Behrman-Godel *et al.*, 2014; Šimková *et al.*, 2006
Ancyrocephalus percae (Ergens 1966)	*Perca fluviatili* (Linnaeus)	Percidae	Finland Lake Constance, Germany	AJ490166.1	KF499080.1	Šimková *et al.*, 2003; Behrman-Godel *et al.*, 2014
Cichlidogyrus sclerosus Paperna & Thurston 1969	*Oreochromis niloticus* (Linnaeus)	Cichlidae	Panyu, China	DQ537359.1	DQ157660.1	Wu *et al.*, 2007; Wu *et al.*, 2006
Cichlidogyrus tilapiae Paperna 1960	*Sarotherodon galilaeus* (Linnaeus) *Hemichromis fasciatus* (Peters)	Cichlidae	Comoe River, Ivory Coasts Senegal, Africa	AJ920277.1	HQ010029.1	Pouyaud *et al.*, 2006; Mendlova *et al.*, 2010
Clavunculus bursatus	*Micropterus salmoides* (Lacépède)	Centrarchidae	Eastern Cape & KwaZulu-Natal, RSA	Present study		
Cleidodiscus pricei Mueller 1936				AJ490168.1	AJ969939.1	Šmková *et al.*, 2003, 2006
Ergenstrema mugilis Paperna 1964	*Liza ramada* (Risso)	Mugilidae	Ebro Delta	JN996835.1	JN996800.1	Blasco-Costa *et al.*, 2012
Haliotrema angelopterum Plaisance & Bouamer, Morand 2004	*Chaetodon kleinii* Bloch	Chaetdontidae	Palau	AY820609.1	AY820620.1	Plaisance *et al.*, 2005
Haliotrema cromileptis Young 1968	*Epinephelus coioides* (Hamilton)	Serranidae	Nha Trang and Cam Bay, Vietnam	EU541306.1	EU523146.1	Dang *et al.*, 2009, 2010
Onchocleidus dispar	*Micropterus salmoides* (Lacépède)	Centrarchidae	Eastern & Western Cape, RSA	Present study		
Onchocleidus furcatus	*Micropterus salmoides* (Lacépède)	Centrarchidae	KwaZulu-Natal & North-West, RSA	Present study		
Onchocleidus principalis	*Micropterus salmoides* (Lacépède)	Centrarchidae	Eastern & Western Cape, RSA	Present study		
Onchocleidus similis (Mueller 1936)	Lepomis gibbosus (Linnaeus	Centrarchidae	Neusiedler Lake, Austria River Dunaj, Slovak Republic	AJ490167.1	AJ969938.1	Šimková *et al.*, 2003, 2006
Pseudohaliotrema sphincteroporus Yamaguti 1953	*Siganus doliatus* Guérin-Méneville	Siganidae	Green Island, Australia	AJ287568.1	AF382058.1	Littlewood and Olson (2001); Olson and Littlewood (2002)
Syncleithrium fusiformis	*Micropterus salmoides* (Lacépède)	Centrarchidae	KwaZulu-Natal, RSA	Present study		

Table 4.1. Species details and Accession numbers of sequences used in phylogenetic analyses (AUS – Austria, CZ – Czech Republic, RSA – South Africa, UK – United Kingdom) continued.

Parasite species	Host	Host family	Locality	Accession Number		Reference
				18S-ITS;	28S	
Dactylogyrinae						
Dactylogyrus anchoratus (Dujardin 1845)	*Cyprinus carpio* (Lnnaeus)	Cyprinidae	Morava Basin, CZ Mashhad, Iran	AJ490161.1	JX524546.1	Šimková *et al.*, 2003; Borji *et al.*, 2012
Dactylogyrus cryptomeres Bychowsky 1934	*Gobio gobio* (Linnaeus)	Cyprinidae	Morava Basin, CZ	AJ564123.1	AJ969947.1	Šimková *et al.*, 2004, 2006
Dactylogyrus extensus Mueller & Van Cleave 1932	*Cyprinus carpio* (Linnaeus)	Cyprinidae	Morava Basin, CZ	AJ564129.1	AJ969944.1	Šimková *et al.*, 2004, 2006
Dactylogyrus hemiamphibothrium Ergens 1956	*Gymnocephalus cernuus* (Linnaeus)	Percidae	Morava Basin, CZ	AJ564137.1	AJ969946.1	Šimková *et al.*, 2004, 2006
Dactylogyrus inexpactatus Izjumova 1955	*Carassius auratus* (Linnaeus)	Cyprinidae	Morava Basin, CZ	AJ564138.1	AJ969945.1	Šimková *et al.*, 2004, 2006
Dactylogyrus lamellatus Achmerov 1952	*Ctenopharyngodon Idella* (Linnaeus)	Cyprinidae	Morava Basin, CZ	AJ564141.1	AJ969948.1	Šimková *et al.*, 2004, 2006
Dactylogyrus nanus Dogiel & Bychowsky 1934	*Rutilus rutilus* (Linnaeus)	Cyprinidae	Morava Basin, CZ	AJ564145.1	AJ969942.1	Šimková *et al.*, 2004, 2006
Dactylogyrus sphyrna Linstow 1878	*Rutilus rutilus* (Linnaeus)	Cyprinidae	Morava Basin, CZ	AJ564154.1	AJ969943.1	Šimková *et al.*, 2004, 2006
Pseudodactylogyrinae						
Pseudoactylogyrus anguillae (Yin & Sporiston 1948)	*Anguilla anguilla* (Linaeus)	Anguillidae	Neusiedler Lake, AUS River Dunaj, Slovak Republic	AJ490162.1	AJ969950.1	Šimková *et al.*, 2003, 2006
Pseudoactylogyrus bini (Kikuchi 1929)	*Anguilla anguilla* (Linnaeus)	Anguillidae	Neusiedler lake, AUS	AJ490163.1	AJ969949.1	Šimková *et al.*, 2003, 2006
Tetraochinae						
Tetraonchus monenteron (Wagener 1857) *	*Esox lucius* (Linnaeus)	Esocidae	Morava Basin, CZ	AJ490159.1	AJ969953.1	Šimková *et al.*, 2003, 2006
Order Gyrodactylidea						
Family Gyrodactylidae						
Gyrodactylus cichlidarum Paperna 1968 *	*Orechromis niloticus niloticus* (Linnaeus)	Cichlidae	UK	DQ124228.1		Garcia-Vasquez *et al.*, 2007
Gyrodactylus hildae Garcia-Vasquez *et al.*, 2011 *	*Oreochromis niloticus niloticus* (Linnaeus)	Cichlidae	Baro River, Ethiopia	FJ231869.1		Garcia-Vasquez *et al.*, 2010
Gyrodactylus salaris Malmberg 1957 *	*Oncorhynchus mykiss* (Walbaum)	Salmonidae	Denmark	DQ823390.1		Kania *et al.*, 2007
Gyrodactylus ulinganisus Garcia-Vasquez *et al.*, 2011 *	*Oreochromis mossambicus* (Peters)	Cichlidae	Stellenbosch, RSA	FJ231870.1		Garcia-Vasquez *et al.*, 2010
Gyrodactylus zimbae Vanhove & Snoeks, Volkaert, Huyse 2011 *	*Simochromis diagramma* (Günther 1894)	Cichlidae	Lake Tanganyika, Zambia	HQ214482.1		Vanhove *et al.*, 2011

Species sequenced in present study are in bold.
* Species selected as outgroup.

4.3. Results

The sequences of five species *Clavunculus bursatus*, *Onchocleidus dispar*, *O. furcatus*, *O. principalis* and *Syncleithrium fusiformis*, that were also morphologically determined (see **Chapter 3**), were generated in the present study. A summary of successfully obtained sequences is given in **Table 4.2**. The length of the obtained fragments for all species constituted of 1121 bp. The 18S-ITS-1 region was 697 bp long with 443 bp corresponding to the 18S rDNA region and 254 bp to the ITS-1 region. The entire 28S rDNA was 424 bp long. The pairwise genetic distances between species for each nuclear marker included in the analysis are presented in **Table 4.3** for 18S rDNA, **Table 4.4** for the ITS-1 region and **Table 4.5** for 28S rDNA.

Table 4. 2. Successful sequences obtained of the 18S-ITS-1 and 28S rDNA regions of ancyrocephalid parasites in South African *Micropterus salmoides* populations.

Species	Locality	Sequenced	n
Clavunculus bursatus	KZN	+	3
	EC	+	2
Onchocleidus dispar	KZN	–	–
	EC	–	–
	VD	+	1
Onchocleidus furcatus	KZN	–	–
	NW	+	1
Onchocleidus principalis	EC	+	1
	GV	–	–
	VD	+	1
Syncleithrium fusiformis	KZN	+	1

+ successful sequences; – no sequences obtained

Table 4. 3. Uncorrected *p*-distances (in %) between 18S rDNA fragments of 443 bp length of ancyrocephalid parasites included in the analysis.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*O. dispar* (VD30)																
2	*O. furcatus* (NW90)	0.00															
3	*O. principalis* (EC55)	0.00	0.00														
4	*O. principalis* (VD27)	0.00	0.00	0.00													
5	*C. bursatus* (EC49)	2.02	2.02	2.02	2.02												
6	*C. bursatus* (EC48)	2.02	2.02	2.02	2.02	0.00											
7	*C. bursatus* (KZN37)	2.02	2.02	2.02	2.02	0.00	0.00										
8	*C. bursatus* (KZN46)	2.02	2.02	2.02	2.02	0.00	0.00	0.00									
9	*C. bursatus* (KZN47)	2.02	2.02	2.02	2.02	0.00	0.00	0.00	0.00								
10	*S. fusiformis (*KZN85)	2.24	2.242	2.242	2.242	1.57	1.57	1.57	1.57	1.57							
11	*A. paradoxus*	0.00	0.00	0.00	0.00	0.73	0.73	0.73	0.73	0.73	1.45						
12	*A. percae*	2.24	2.24	2.24	2.24	3.36	3.36	3.36	3.36	3.36	3.14	0.000					
13	*C. pricei*	1.79	1.794	1.79	1.79	1.57	1.57	1.57	1.57	1.57	2.24	1.45	3.14				
14	*O. similis*	0.89	0.89	0.89	0.89	2.47	2.47	2.47	2.47	2.47	2.24	0.000	2.24	2.02			
15	*Q. bagrae*	10.7	10.76	10.76	10.76	9.64	9.64	9.64	9.64	9.64	9.42	13.77	10.54	9.42	10.76		
16	*T. siluri*	8.97	8.97	8.97	8.97	8.07	8.07	8.07	8.07	8.07	8.52	11.59	9.87	7.62	8.52	6.94	
17	*T. vistulensis*	8.35	8.35	8.35	8.35	7.90	7.90	7.90	7.90	7.90	7.45	11.59	9.26	6.77	8.13	6.76	1.13

Table 4. 4. Uncorrected *p*-distances (in %) between ITS-1 fragments of 254 bp length of ancyrocephalid parasites included in the analysis.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*O. dispar* (VD30)																
2	*O. furcatus* (NW90)	4.36															
3	*O. principalis* (EC55)	7.22	6.43														
4	*O. principalis* (VD27)	7.22	6.43	0.00													
5	*C. bursatus* (EC49)	30.76	0.32	0.31	0.31												
6	*C. bursatus* (EC48)	30.76	31.62	30.77	30.77	0.00											
7	*C. bursatus* (KZN37)	30.76	31.62	30.77	30.77	0.00	0.00										
8	*C. bursatus* (KZN46)	30.76	31.62	30.77	30.77	0.000	0.000	0.00									
9	*C. bursatus* (KZN47)	30.76	31.62	30.77	30.77	0.00	0.00	0.00	0.00								
10	*S. fusiformis* (KZN85)	30.39	30.39	28.19	28.19	26.29	26.29	26.29	26.29	26.29							
11	*A. paradoxus*	19.06	19.07	19.92	19.92	29.82	29.83	29.83	29.83	29.83	26.34						
12	*A. percae*	17.44	18.29	18.72	18.72	28.19	28.19	28.19	28.19	28.19	26.46	7.63					
13	*C. pricei*	30.39	31.28	30.39	30.39	29.83	29.83	29.83	29.83	29.83	26.67	25.78	27.23				
14	*O. similis*	9.13	9.589	12.27	12.27	28.97	28.97	28.97	28.97	28.97	28.37	16.67	17.13	29.52			
15	*Q. bagrae*	57.92	57.43	57.64	57.64	56.31	56.31	56.31	56.31	56.31	56.19	58.91	59.41	58.97	57.00		
16	*T. siluri*	45.92	47.45	44.16	44.16	45.31	45.31	45.31	45.31	45.31	46.24	46.35	43.75	49.48	48.68	56.09	
17	*T. vistulensis*	47.26	47.76	46.54	46.54	45.18	45.18	45.18	45.18	45.18	46.11	47.74	45.73	48.49	51.27	53.97	8.92

Table 4. 5. Uncorrected *p*-distances (in %) between 28S rDNA fragments of 424 bp length of ancyrocephalid parasites included in the analysis.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*O. dispar* (VD30)																
2	*O. furcatus* (NW90)	8.65															
3	*O. principalis* (EC55)	8.65	1.93														
4	*O. principalis* (VD27)	8.65	1.93	0.00													
5	*C. bursatus* (EC49)	10.65	9.43	9.25	9.25												
6	*C. bursatus* (EC48)	10.65	9.43	9.25	9.25	0.00											
7	*C. bursatus* (KZN37)	10.65	9.43	9.25	9.25	0.00	0.00										
8	*C. bursatus* (KZN46)	10.65	9.431	9.25	9.25	0.00	0.00	0.00									
9	*C. bursatus* (KZN47)	10.65	9.431	9.25	9.25	0.00	0.00	0.00	0.00								
10	*S. fusiformis* (KZN85)	10.89	9.39	9.93	9.93	8.021	8.02	8.02	8.02	8.02							
11	*A. paradoxus*	8.80	5.51	5.68	5.684	9.16	9.16	9.16	9.16	9.16	7.51						
12	*A. percae*	9.97	5.86	6.57	6.57	9.35	9.35	9.35	9.35	9.35	8.96	3.23					
13	*C. pricei*	10.00	7.82	8.35	8.35	7.34	7.34	7.34	7.34	7.34	7.31	7.16	7.54				
14	*O. similis*	8.33	4.60	4.60	4.60	9.86	9.86	9.86	9.86	9.86	10.54	5.89	7.07	7.87			
15	*Q. bagrae*	19.16	26.22	26.76	26.76	27.74	27.74	27.74	27.74	27.74	27.74	26.33	26.51	27.87	26.78		
16	*T. siluri*	18.81	30.67	30.49	30.49	30.15	30.15	30.15	30.15	30.15	28.89	29.07	30.77	30.64	30.97	26.54	
17	*T. vistulensis*	18.54	30.73	30.55	30.55	29.65	29.65	29.65	29.65	29.65	28.94	29.25	31.01	30.46	31.20	26.59	1.44

Phylogenetic trees were constructed with Bayesian Inference (BI) and Maximum Likelihood (ML) methods (with the combined sequences of the three nuclear regions: 18S-ITS-1 and 28S rDNA) to determine their position within the Dactylogyridae (**Fig. 4.1**.). The five species in the present study is positioned within the group of Ancyrocephalinae, mainly parasitising freshwater fishes of the Suliformes and Perciformes. The five species (all specialists of the Centrarchidae) divide into two clades (**Fig. 4.2**), with the *Onchocleidus* spp. grouping, as a sister taxon to *Ancyrocephalus* spp. (specialists of Percidae), in Clade 1, supporting monophyly of the genus. *Clavunculus bursatus* and *S. fusiformis* forms Clade 2, a sister taxon to *Cleidodiscus pricei* (specialists of the Ictaluridae Gill, 1861).

No genetic divergence (interspecific diversity) was observed in the 18S rDNA, ITS-1 and 28S rDNA regions of *C. bursatus* from KZN and EC (see yellow section in **Table 4.2**, **Table 4.3** and **Table 4.4**), and *O. principalis* from EC and VD (see purple section for species in **Table 4.2**, **Table 4.3** and **Table 4.4**).

Among individual species of *Onchocleidus* there were no genetic divergence between species in the 18S rDNA region itself (only from *O. similis* for all 0.89%), but definite divergence is evident from the ITS-1 and 28S rDNA regions (see purple section **Table 4.2** and **Table 4.3**). The observed distances between the 28S rDNA fragments of *Onchocleidus* spp. in the study was 8.65% between *O. dispar* and the other two species, *O. furcatus* and *O. principalis*. The closest related species were *O. dispar* and *O. furcatus*, with only 4.36% differences in the ITS-1 region. Divergence from *O. similis* ranged between 9.13 – 12.27%.

Divergence between *C. bursatus* and *S. fusiformis* was already evident in the 18S rDNA region (1.57%). It is also possible to distinguish between the three genera using all three nuclear markers, see the green sections in **Table 4.2**, **Table 4.3** and **Table 4.4**).

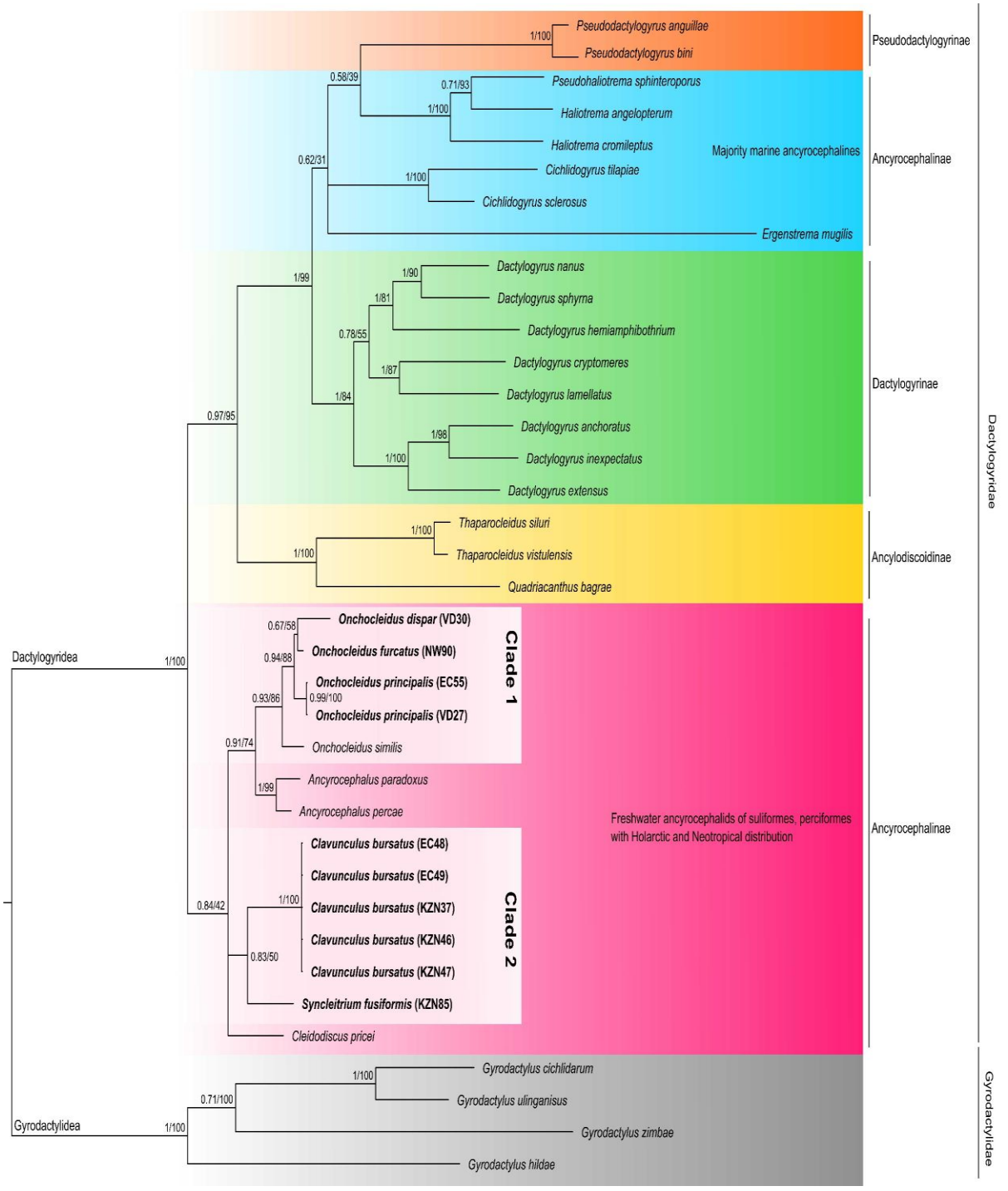

Figure 4. 1. Bayesian Inference tree from combined 18S-ITS-1 and 28S regions (1121 bp long) positioning ancyrocephalid monogeneans parasitising *Micropterus salmoides* in South Africa within the Dactylogyridae (Dactylogyridea). Numbers along branches indicate bootstrap values of BI on the left and ML on the right. The Gyrodactylidea was used as an outgroup.

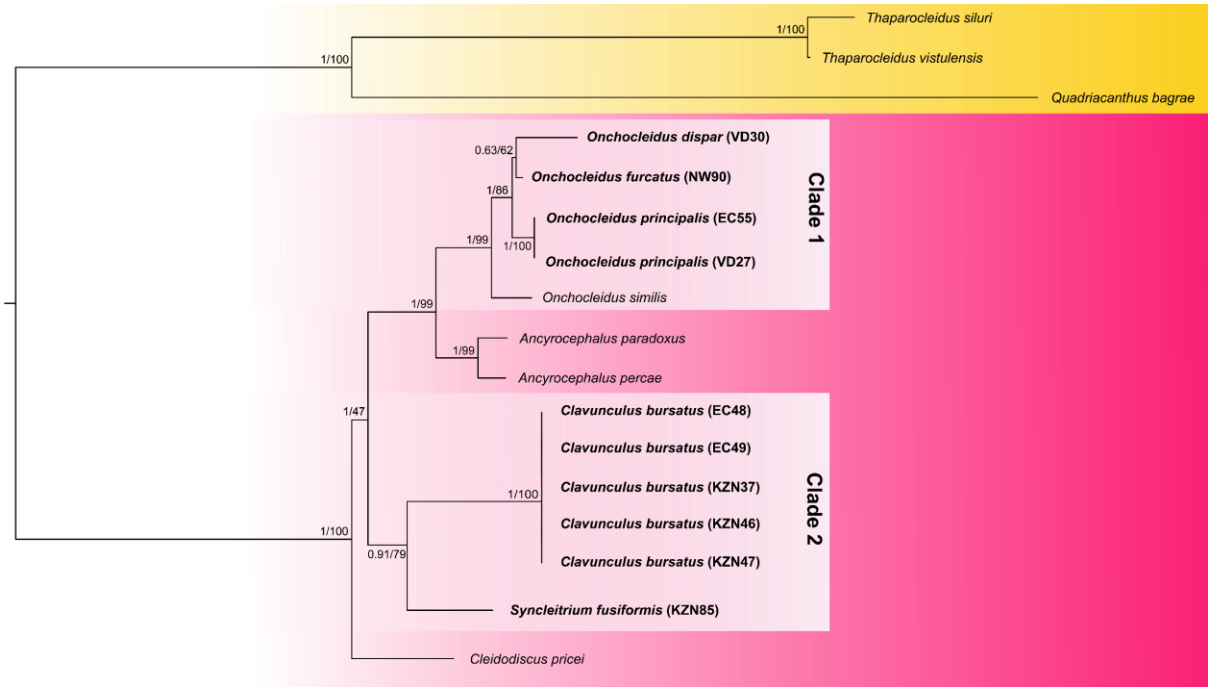

Figure 4. 2. Maximumlikelihood tree from combined 18S-ITS-1 and 28S regions (1121 bp long) positioning ancyrocephalid monogeneans parasitising *Micropterus salmoides* in South Africa within the Dactylogyridae. Numbers along branches indicate bootstrap values of BI on the left and ML on the right.

4.4. Discussion

Few studies have attempted to perform molecular characterisation of members within the Ancyrocephalidae to clarify their phylogenetic position within the Dactylogyridea (Kritsky and Boeger, 1989; Beverley-Burton and Klassen, 1990; Šimková *et al.*, 2003, 2006; Mendoza-Palmero *et al.*, 2015). The present study provides new molecular data for monogenean parasites found on largemouth bass in South Africa. The newly obtained data can potentially serve as a good base for taxonomic revision of these ancyrocephalids. In previous morphological revisions, the status of various ancyrocephalid genera, especially those specific to centrarchids, were reclassified, in differentiating between genera based on penis types (Beverley-Burton, 1986), as there were synonyms of *Cleidodiscus* and *Actinocleidus* with many of the ancyrocephalids. The present study supports the approach of Beverely-Burton (1986) in species differentiation primarily through penis types and then haptoral sclerite morphology. The evident monophyly within the genus *Onchocleidus* seen in Clade 1 and the position of *O. similis* (untill recently *U. similis*) confirm the synonymy of *Urocleidus* with *Onchocleidus* as proposed by Wheeler and Beverley-Burton (1989) and Beverley-Burton and Klassen (1990), despite the minor differences in anchor morphology within the genus. Here penis type is of relevance as all species within the genus have a Type 2 penis with variations in the size, spiral filament turns and morphology of the accessory piece.

Cleidodiscus pricei, Clavunculus bursatus and *S. fusiformis* all have articulating transverse haptoral bars, but definite species differentiation lies within penis types, as haptoral sclerites are primarily used to group specimens within a suitable genus. *Clavunculus bursatus* has a Type 1 penis (a sclerotised, tubular penis, with an inflated base, heavily sclerotised accessory piece with a distal finger-like projection and bifid base) and *S. fusiformis* a Type 8 penis (sclerotised penis with inflated, lightly sclerotised base, heavily sclerotised straight shaft with narrow, curved distal ejaculatory limb projection, accessory piece sclerotised, distal limb characterised by bifid tip acting as guide for distal extremity of penis, accessory piece attached to penis by strands of muscle) as defined in Beverley-Burton (1984, 1986). The sister relationship of *C. pricei* to Clade 2 (*C. bursatus* and *S. fusiformis*) in both trees can possibly be based on the morphology and composition of haptoral sclerites and more distinctively on penis Type 4, for this genus (see Beverley-Burton, 1984).

The evaluation of genetic differences between the studied species for each nuclear marker underline the relevance of each marker in the study of species phylogeny. The 18S rDNA, ITS-1 regions and 28S rDNA all evolve at different rates, making them suitable for assessment of genetic divergence at various levels (Hillis and Dixon, 1991). The 18S rDNA is a more conserved gene, while the ITS-1 is more variable and considered as one adequate to mirror morphological recognised species and are useful in identifying cryptic species. The 28S rDNA, together with the ITS-1 region, are also suitable for determination of inter- and intraspecific variation of species (Vanhove *et al.*, 2013). Efficacy of using the three nuclear markers in the present study is supported in differentiations between species, especially the ITS-1 rDNA indicating different species of the different genera, while this is not evident for all species with the 18S rDNA (see **Table 4.3** sections in brown).

Previous attempts to infer host and parasite relationships of the Ancyrocephlidae have been inconclusive (Beverley-Burton and Klassen, 1990). In the present study, no intraspecific variability was found in the studied nuclear markers of *O. principalis* from two different localities, suggesting that this parasite's origin concurs with the reconstruction of the host distribution proposed by Hargrove *et al.* (2017), who showed that *M. salmoides* for both EC and VD were sourced from a single host population. The same can be said for the lack of divergence between *C. bursatus* from the EC and KZN. On the other hand, the presence of these parasites in South African systems are less than a century, which is not a long enough period for divergence to have occurred in the conservative nuclear markers studied. Investigation on the genetic structures of the parasite populations using a mitochondrial marker with a more rapid evolutionary rate than the nuclear markers studied here, may reveal already existing or progressive genetic divergence of these parasite species.

This study presents molecular data that contributes to the advancement of molecular identification of the species found, supplementary to morphological identification (specifically penis types, and haptoral sclerites). To resolve the taxonomic status or other genera and species within the Ancyrocephalidae, and to eradicate synonymy, morphological re-examinations, in conjunction with molecular characterisation is recommended.

Chapter 5: Host health and parasitic infection

5.1. Introduction

The enemy release hypothesis (ERH) postulates that species that are introduced into a non-native region experience a decrease in, or in effect, escape their natural enemies that may give them an advantage to establishment success in novel environments (Van der Putten, 2000; Keane and Crawley, 2002; Mitchell and Power, 2003). Included among these natural enemies are the parasites that infect the introduced species in their native range (Lymbery *et al.*, 2014). In parasite/host relationships, host health can be affected by the presence of parasites and can also exert high energetic costs upon their hosts (Arnott *et al.*, 2000). Iwanowicz (2011) listed three categories of damage that are caused by parasites on their fish hosts: 1) mechanical damage, i.e. where fusion of gill lamellae or tissue replacement can occur; 2) physiological damage include cell proliferation, immunomodulation and behavioural responses and 3) reproductive damage that can affect growth, fecundity, survival and cause maladaptive alterations in the affected hosts. The aforementioned damage is often accompanied by symptoms such as hyperplasia of the gill epithelium, interference of respiratory function, tissue inflammation and fibrosis encapsulation (Lehtinen *et al.*, 1984; Iyaji and Eyo, 2008). Examples of such cases include infection with the monogenean *Ancyrocephalus percae* Ergens, 1966 that resulted in the detachment of the isthmus from the lower jaw in perch from a lake in Germany (Behrmann-Godel *et al.*, 2014); and excessive mucous production, eroded fins and behavioural changes (e.g., scratching of their skin against dam or pond walls) of salmon and rainbow trout infected with *Gyrodactylus salaris* Malmberg, 1957 in aquaculture conditions (see Buchmann and Bresciani, 1997; Olstad, 2013; Hansen *et al.*, 2016; Mo, 2017). Several other monogenean species are pathogenic or has the potential to become pathogenic to cultured and feral fish populations and include *Gyrodactylus luciopercae* Gussev, 1962 parasitising pike perch, *Gyrodactylus colemanensis* Mizelle & Kritsky, 1967 and *Gyrodactylus derjavini* Mikailov, 1975 on rainbow trout, *Pseudodactylogyrus anguillae* (Yin & Sproston, 1948) on farmed and feral eel populations, *Gyrodactylus cyprini* (Diarova, 1964)*, Dactylogyrus extensus, Dactylogyrus vastator, Dactylogyrus lamellatus* on cyprinids (see Buchmann, 1989;

Scholz, 1999; McHugh *et al.*, 2017) and *Diplectanum aequans* (Wagener, 1857) in cultured sea bass (Cognetti-Varialle *et al.*, 1993). Other symptoms of moderate infection associated with monogenean infections can include reduced feeding response, erratic swimming behaviour or discriminate mate selection against males by females (Houde and Torio, 1992; Houde, 1997; López, 1998; Bakke *et al.*, 2007). Most of the research, however, has been conducted under aquaculture conditions (see Reed *et al.*, 2012; Scholz, 1999; Turgut *et al.*, 2003) and few studies have investigated the association between fish health and parasitism in wild populations i.e. Rohlenová *et al.* (2011), Jerônimo *et al.* (2014), Sueiro *et al.* (2017) and McHugh *et al.* (2017).

Fish health assessments are relatively common in environmental assessments (Harris and Silveira, 1999; Crafford and Avenant-Oldewage, 2009; Watson *et al.*, 2012) using procedures such as the fish health assessment index (FHAI) developed by Adams *et al.* (1993). The FHAI procedure assesses four categories of health indicators: 1) three blood parameters (haematocrit, leukocrit, plasma protein); 2) length, weight and condition factor; 3) proportion of fish with abnormal eyes, gills, pseudobranchs, spleens, kidneys and livers and 4) index values of damage to skin, fins, thymus, hindgut inflammation, fat deposits and bile colour. In a recent paper, Sueiro *et al.* (2017) investigated the link between parasitism, immune function and general health state of feral rock fish *Sebastes oculates* Valenciennes, 1833 using ecological parasitological data (prevalence, abundance and intensity of infection) and components of the fish health assessment (i.e. white blood cell counts, haematocrit and condition factor). Jerônimo *et al.* (2014) included haematological parameters (haematocrit, leukocrit and plasma proteins) in their study on the monogenean infected pacu *Piaractus mesopatamicus* Holmberg, 1887 in Central Brazil and Rohlenová *et al.* (2011) included Fulton's condition factor, and organosomatic indices (i.e. HSI, SSI, GSI) to confirm if fish immune systems are affected by parasites.

In this Chapter the FHAI is utilised to assess for enemy release of *M. salmoides* in South Africa. The previous chapters demonstrated that *M. salmoides* in South Africa had not only escaped infection of parasites native to the novel environment but had also lost most of its native parasites. However, five species of monogeneans were co-introduced (see **Chapter 3**). To test whether these co-introduced parasites affect fitness

or condition of the introduced hosts and, by inference test the enemy release hypothesis, this chapter tests **Hypothesis 3** that “host health is negatively correlated with the degree of monogenean parasitic infection”.

5.2. Materials and Methods

5.2.1. General

The materials and methods used in this chapter are discussed in **Chapter 2**. For collection methods of host, see **Chapter 2**, **Section 2.2.1**. Necropsy procedure, blood parameters and biometric indices are discussed in **Chapter 2**, **Section 2.3**. Condition factor (CF) and FHAI was calculated for all individuals of *M. salmoides* collected from all sampling localities (see Adams *et al.*, 1993). Somatic indices (HSI, SSI and GSI) were only calculated for KZN, EC and GV. Blood parameters were only calculated for EC and KZN (see **Table 5.1** for values on ‘normal’, ‘below normal’ and ‘above normal’).

The following reduction can be made from blood parameters: the haematocrit is the percentage of the blood volume consisting of erythrocytes (red blood cells) and is responsible for transport of oxygen throughout an organism. Reduced haematocrit readings can be caused by stimulation with low oxygen concentrations and coagulation can occur causing excessive mucous production, resulting in the reduction of gas exchange and eventually tissue hypoxia (see Sniezko, 1974). Leukocrit and presence of leukocytes (white blood cells) are associated with the immune system and numbers or counts usually rise rapidly in response to infections or diseases (Barman *et al.*, 2013). The plasma protein consists of the fluid portion of vertebrate blood and consists of water and dissolved substances such as carbon dioxide, nitrogen, oxygen and nutrients with proteins that make up at least 8% of the total volume (Hopson and Wessels, 1990). The CF together with organ health can be indicators or gauges for the overall health of fish populations. Organs such as the liver that is responsible for maintaining the body amino acid pool, synthesis of proteins, bile production, maintenance in the uptake and conversion of substances and the drug metabolising system can also indicate exposure of fish to pollutants in water, and in association with lower plasma protein values it can be indicative of liver damage (see Heath, 1987). The spleen mainly functions as an erythrocyte reservoir but it also plays a role in destruction of aged cells, and recycling of

iron from haemoglobin cells. Its size is also used as a simple measurable parameter that potentially play a role in immune response to parasite infection. Gonadal development is indicative of sexual maturity and the GSI represents an assessment of reproductive maturity of individuals (Lefebvre *et al.*, 2004; Ottová *et al.*, 2005; Rohlenová *et al.*, 2011).

Table 5. 1. Description of blood parameter variables in the FHAI (from Adams *et al.*, 1993).

Health parameter	Description	FHAI score
Blood haematocrit value		
Within normal range	30-45%	0
Above normal range	>45%	10
Below normal range	19-29%	20
Below normal range	<18%	30
Blood plasma protein value		
Within normal range	30-69 mg/dL	0
Above normal range	70 mg/dL	10
Below normal range	< 39 mg/dL	30
Leukocrit value		
Within normal range	<4%	0
Below normal range	≥4%	30
White blood cell count		
Within normal range	<4%	0
Outside normal range	≥4%	30

5.2.2. Statistical analysis

Graphpad Prism 5 software was used to perform statistical analysis and comparisons of data. The D'Agostino & Pearson omnibus normality test was used to test for normality of fish size (SL) and mass. For parametric data sets (fish length, mass, intensity of infection and condition factor) a one-way analysis of variance (ANOVA) was performed with Turkey's multiple comparison test as post-test, and for non-parametric data sets (HSI, SSI, GSI and FHAI) the Kruskal-Wallis test was performed with Dunn's multiple comparison test as a post-hoc test. A $p < 0.05$ was considered as significant. Spearman's rank correlation analysis was used to determine if there were correlations between the intensity of infection, CF and the three organosomatic indices for each locality.

5.3. Results

5.3.1. Fish health and organosomatic indices

The biometric information from all localities, organosomatic indices and FHAI scores are presented in **Table 5.2**. Fish from the NW were significantly smaller in length (SL) ($p \leq 0.05$, $df = 4$) than those from all other localities, and had a significantly lower mass ($p \leq 0.05$, $df = 4$) than fish from localities KZN, GV and VD. Condition factor of fish from NW were also significantly lower than fish from KZN, GV and VD, the CF of EC were significantly lower than the CF of KZN and VD, and the CF of KZN were significantly higher than NW and EC ($p \leq 0.05$, $df = 4$) (**Fig. 5.1 A**). Ecto-parasitic infections with Monogenea from the gills were also significantly lower in NW, GV and VD relative to KZN and EC ($p \leq 0.05$). Highest IF was recorded from the EC. Endo-parasitic infections with nematodes were observed at all localities, 6 – 20% of hosts were infected in low numbers (see **Chapter 3**, **Section 3.3.1**). There was a weak relationship between the CF and monogenean (ecto-parasitic) intensity of infection (IF) for all localities (KZN $r = -0.07857$, EC $r = 0.08967$, GV $r = -0.1417$, VD $r = -0.09686$, for all localities $p \geq 0.05$), except in NW there was a moderate relationship ($r = 0.4788$) between the CF and IF, but not of statistical significance ($p \geq 0.05$).

From all localities, a selective macroscopic health assessment (attention given to liver, spleen, gonads, skin, fins, eyes, opercula, gills) yielded no evidence of external abnormalities, no frayed gills, fusion of gill lamellae or signs of excessive mucous production were observed. The only internal abnormality observed was the discolouration of livers in 46% of *M. salmoides* from GV. Statistical analysis indicated that there were significant differences between the FHAI scores of the different localities: NW, KZN, EC and GV had significantly higher scores than VD ($p \leq 0.05$, $df = 4$), KZN had a significant higher score than NW, EC and VD ($p \leq 0.05$, df = 4) (**Fig. 5.1 B**). The mean HSI of EC were significantly higher than that of KZN an GV (**Fig. 5.1 C**). The mean SSI of EC were significantly lower than KZN and GV ($p \leq 0.05$) (**Fig. 5.1 D**). The overall GSI (males + females) (**Fig. 5.1 E**) and the females (**Fig. 5.1 F**) did not differ statistically between the three localities, but the GSI for males from KZN and EC were significantly lower than GV ($p \leq 0.05$, $df = 2$) (**Fig. 5.1 G**), a moderate and statistical significant relationship was found between the condition factor and GSI from KZN ($r = 0.45$, $p \leq 0.05$).

Table 5. 2. Summary of fish biometrics and ecological paramaters of monogenean parasite load. n – number of fish studied; Mean SL– mean standard length; Mean IF – mean intensity of infection.

	n	Mean SL (mm)	Mean IF	Condition Factor	Organosomatic Indices				
					HSI	SSI	GSI		FHAI
							Male	Female	
NW	13	165.8 ± 79.24	32 (1 – 86)	0.5 ± 0.4	–	–	–	–	31.54 ± 3.76
KZN	15	248.3 ± 46.80	399 (60 –736)	0.9 ± 0.3	0.6 ± 0.2	0.05 ± 0.02	0.13 ± 0.12	0.46 ± 0.24	42 ± 4.14
EC	30	248.2 ± 131.5	448 (194 – 1668)	1.2 ± 0.4	0.7 ± 0.2	0.03 ± 0.06	0.11 ± 0.18	0.43 ± 0.12	31.33 ± 4.34
Western Cape									
GV	15	262.1 ± 17.27	35 (1 – 282)	1.1 ± 0.2	0.5 ± 0.2	0.04 ± 0.2	0.33 ± 0.20	0.67 ± 0.31	34 ± 15.95
VD	15	246.6 ± 24.12	25 (1 – 54)	1.2 ± 0.3	–	–	–	–	22.00 ± 10.82

5.3.2. Blood parameters

Mean haematocrit for KZN was 43.0 ± 12.7% and the mean haematocrit for EC was 31.7 ± 7. 2 (**Fig. 5.1 H**). KZN had 67% normal (30 – 45%), 13% below normal (19 – 29%) and 20% above normal (> 45%) haematocrit samples. EC had 67% normal, 27% below and 6% above normal haematocrit samples. There was a significant difference between the haematocrit value of KZN and EC ($p \leq 0.05$) (**Fig. 5.1 H** and **Fig. 5.1 I**). Mean protein plasma values for KZN were 87.0 ± 8.6 mg dl^{-1}, all were above normal range (70 mg dl^{-1}). The mean plasma values for EC were 63.5 ± 14.1 mg dl^{-1}, 87.5% were within normal range (30 – 69 mg dl^{-1}), 12.5% were above normal range.

The leukocrit and white blood cell counts for all individuals for KZN and EC were within normal range (< 4%), as proposed by Adams *et al.* (1993), although heavy infections with parasites were observed on the gills. No relationship between white blood cell counts (WBC's) and IF were found. There were significant differences between the WCB's of KZN and EC ($df = 14; p \leq 0.05$).

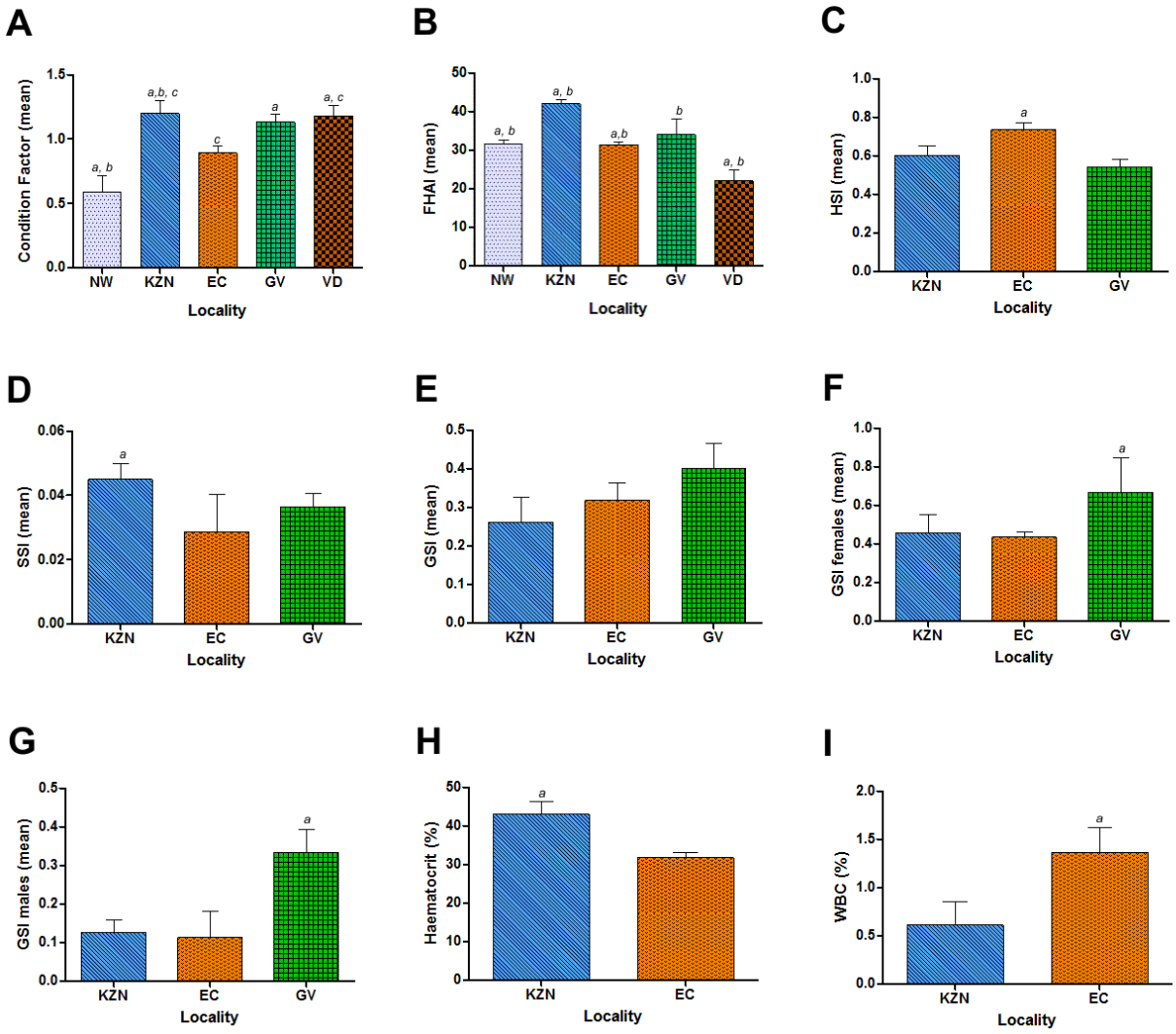

Figure 5. 1. Mean values for: A – Condition Factor and B – FHAI scores for all localities, and C – HSI, D – SSI, E – GSI, GSI of females (F) and males (G) from KZN, EC and GV. Mean percentages for H – haematocrit and I – white blood cell counts for KZN and EC.

5.4. Discussion

Overall no correlation was found between parasitic infection and any one of the parameters of the FHAI health indicators. Difference in CF of fish from NW relative to other localities can be attributed to smaller size of fish and the different feeding opportunities for fish at the different localities. The lower IF in NW, GV and VD can be a product of fluctuating environmental conditions such as water temperature or season of sampling at each locality (Blažek *et al.*, 2008; Öztürk and Özer, 2014). The size of fish can also play a role in IF i.e. smaller fish have not been exposed to the environment for a long enough period to accumulate a representative sample of the parasite population present in the system or even gill surface area, as in the case in NW where smaller fish were collected and low IF were recorded (Buchmann, 1989; Poulin, 2000). The weak correlation between CF and IF suggested that fish condition was not influenced by the presence of monogenetic parasites. Discoloration of the livers in GV may not be associated with ecto-parasitic infection, rather an indication of some other factor in the system such as pesticides (Fanta *et al.*, 2003; Velmurugan *et al.*, 2007; McHugh *et al.*, 2011) or infection with other parasite species e.g. a myxozoan (Feist and Longshaw, 2006).

The HSI value for KZN, EC and GV are within ranges reported for *M. salmoides* by McHugh (2015) and can be considered normal when considering the reproductive and temperature related metabolic demands for *M. salmoides*. Values of the HSI, SSI and GSI for all three localities are within ranges of previous studies for *M. salmoides* (Brown and Murphy, 2004; McHugh, 2015).

For both KZN and EC haematocrit and IF also showed no correlation of statistical significance, and although IF were the highest in EC there was a very weak correlation (and not of statistical significance) between WBC's and IF. The 'above normal' plasma protein for KZN is most probably inaccurate because of factors such as serum lipid interference with protein analysis (Adams *et al.*, 1993). Leukocrit and WBC values also indicate no unnatural immune responses, even when the fish had a high IF with monogeneans.

Compared to the study of McHugh (2015) on *M. salmoides*, the mean FHAI scores (of the present study) for all localities were higher, but within the same range (see **Table 5.2**), indicating good health condition of all fish. The FHAI scores of McHugh (2015) were, 21.8 ± 20.4, 17.4 ± 18.8 and 26.0 ± 19.9, and was attributed to the damaged tailfins and skin, frayed gills, granular spleens, discoloured and fatty livers. From the present study, the main factor contributing to the slightly higher FHAI scores are the presence of ecto-parasites (monogeneans) from each locality. The higher scores in KZN and EC are due to higher IF with gill parasites, while the significantly FHAI score in GV was still significantly lower despite discoloured livers of hosts.

In the few studies that investigate the link between immune function and the general health state of fish to parasitism, significant correlations between parasitism and health indicators are rarely reported. Although Jerônimo *et al.* (2014) found decreased levels of haematocrit and red blood cells in pacu that were heavily infected with monogeneans, Rohlenová *et al.* (2011) found that host immunity, physiological state and parasite infection were all highly dependent on seasonal variability, using the common carp as model. They rather found a relationship between the condition of fish and the selected organosomatic indices, and noted that spleen size (SSI) might not be a suitable representative measure for host immunocompetence and the HSI and GSI serves more effective in determination of the energy status of the host. Similarly, Sueiro *et al.* (2017) also found no correlation between the investigated immunological responses and health indicators in feral rock fish.

As in the aforementioned studies, no correlation between the parasitic infection, host health and immune response were found in the present study. Although the present study did not investigate immune responses to parasitism in such a broad sense, it is clear a health and parasitological assessment is not enough to conclude if parasites in high intensities can affect hosts, unless parasitic infection is unusually high or lead to secondary infection. Other factors to consider are the highly complex host-parasite interactions within ecosystems with many influencing factors such as seasonal changes in environmental factors, host age and abundance. In addition, and, as in the present study, infection of hosts with non-pathogenic parasites require a more in-depth investigation on a cellular level, including histopathological assessments (to determine if

low levels of infection show symptoms) and system health e.g. water quality and pollutant assessment from tissue for heavy metals. Several studies also link high ecto-parasitic parasitic infection with polluted systems (Crafford, 2000; Sueiro *et al.*, 2017). The correlation between immunity, condition of the host and parasitic infection is difficult to interpret, the evolved parasite tolerance strategies developed by hosts to their specific parasites can minimize the fitness cost of parasitised hosts (Baucom and de Roode, 2011), contributing to the lack in relationship between parasite loads and general health indices and condition of parasitised fish. The absence of a relationship between host health and parasitic infection found in the present study suggests the loss of parasite diversity are not related to fitness of the host in the novel environment, but rather to the co-evolution of the host and its parasites, therefor supporting the enemy release hypothesis.

Chapter 6: Parasite spill-over from largemouth bass *Micropterus salmoides* to native freshwater fish species: a biological invasion case study

6.1. Introduction

Mechanisms associated with the introduction of alien species and biological invasions are thoroughly discussed in **Chapter 1**, **Section 1** and briefly summarized in **Fig. 6.1**.

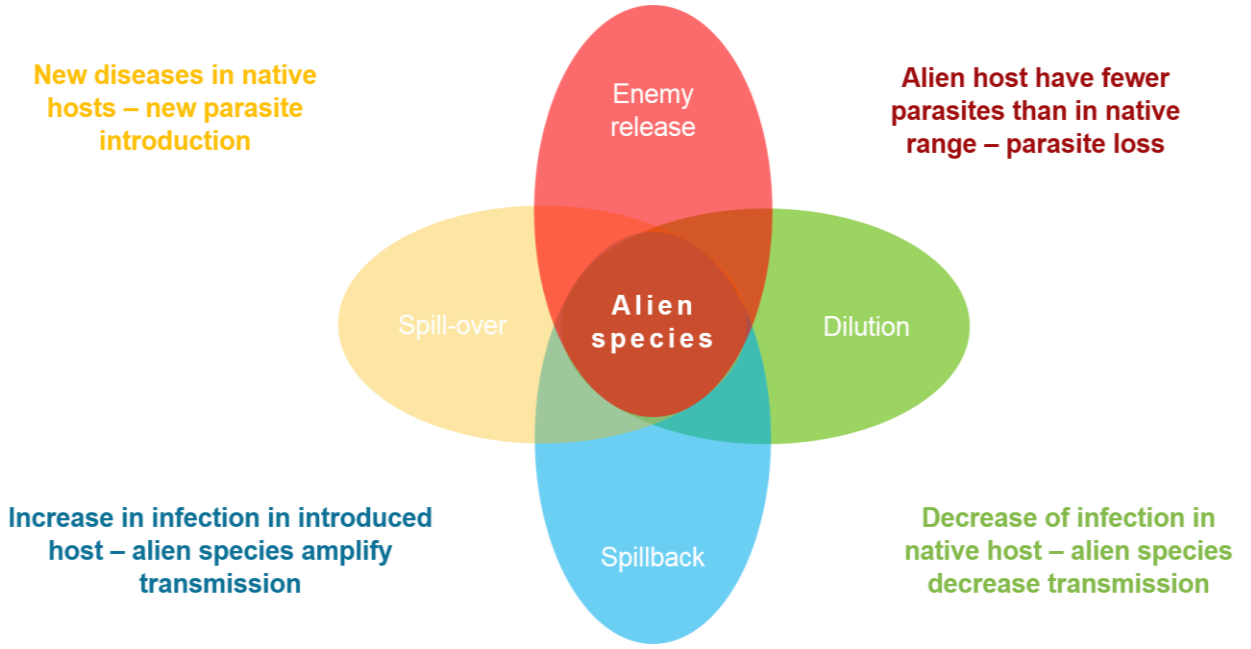

Figure 6. 1. Summary of mechanisms associated with the introduction of alien species into novel environments, referred to as biological invasion mechanisms.

As concluded in **Chapter 3** there are five co-introduced monogenean species identified to parasitise *Micropterus salmoides* in the various systems of South Africa surveyed in the present study. A scoping study conducted during October 2015, found that only one of these five monogenean species, *Onchocleidus furcatus* was present on *M. salmoides* in the Boskop Dam (pers. comm. Dr. I Přikrylová). In conjunction with this finding, the results of the present study (see **Chapter 3**) corroborate that *M. salmoides* from the Mooi River system are all infected with only *O. furcatus*. This monogenean is known to parasites centrarchid fishes (Beverley-Burton and Suriano, 1980) but is reported to be less host specific than other members of the Ancyrocephalidae that parasitise centrarchids (see Janovy and Collins, 2003; Cloutman, 1974). To date few studies have reported the spill-over of these ancyrocephalids to other non-centrarchid hosts. However, Havlatová *et al.* (2015) reported several co-introduced ancyrocephalid species from *Lepomis gibbosus* in

the River Durance, France including *Onchocleidus similis*, that is specific to *L. gibbosus* in its native range (Hoffman, 1998; Grupcheva and Neveda, 1999), to have spilled over to other introduced fishes.

There are several examples of co-introductions and co-invasion of alien species into Boskop Dam. These include the co-invasive Japanese fish louse *Argulus japonicus* recorded from *Labeobarbus aeneus*, *Labeo capensis* and *Labeo umbratus* (see van As and Basson, 1984) and the flatworm *Schyzocotyle acheilognathi* that spilled over from *Cyprinus carpio* to *Enteromius trimaculatus* (Peters, 1852) (van As *et al.*, 1981). Co-introduced parasitic species include *Trichodina acuta* recorded from *Pseudocrenilabrus philander* and *Tilapia sparrmanii* and *Trichodina heterodentata* from *P. philander* and *Trichodina mutabilis* from *E. trimaculatus* (see van As and Basson, 1984; Smit *et al.*, 2017).

Micropterus salmoides has been in the Boskop Dam for more than 60 years co-existing with native and other introduced species (see **Chapter 2**, **Section 2.1.1** and **Table 2.1**). To the authors knowledge, there have been no studies on their parasite community other than the compilation of a checklist of freshwater fish parasites in southern Africa by van As and Basson (1984) that included the Boskop Dam.

This section of the present study aimed to determine if parasite spill-over occurred from *M. salmoides* to other native or introduced species within the Boskop Dam, addressing **Hypothesis 4**.

6.2. Materials and Methods

6.2.1. General

The materials and methods used in this chapter are discussed in **Chapter 2**. For site selection, see **Chapter 2**, **Section 2.1**. For collection methods and identification of fish species, see **Chapter 2**, **Section 2.2.2** and for the necropsy and parasite screening, see **Chapter 2**, **Section 2.3**. Fixation and taxonomical important structures used for morphological identification are discussed in **Chapter 3**, **Section 3.2.2**. Literature used for identification of parasitic specimens in this section include Douëllou (1993), Oosthuizen and Siddall (2002) and Martens (2001) and Francová *et al.* (2017). Ecological parameters for parasitic infection with Monogenea was calculated and defined as in Bush *et al.* (1999).

6.3. Results

6.3.1. General and parasitic infection

A total of 38 fishes of five different species, *Clarias gariepinus*, *Labeobarbus aeneus*, *Labeo capensis*, *Labeo umbratus* and *Tilapia sparrmanii* were collected from the Boskop Dam and examined for ecto- and endoparasites. Fish biometrics and infection with monogenean parasites are summarized in **Table 6.1**. Presence and absence of other parasitic groups and Monogenea for each fish species is summarized in **Table 6.2**.

Table 6. 1. Fish biometrics and ecological parameters for infection with Monogenea from hosts collected from Boskop Dam.

	n	Mean M (g) ± SD	Mean SL ± SD	Monogenea	
				Prevalence (%)	IF
C. gariepinus	13	1072 ± 504.4	450 ± 65.3	53	3 (1 – 27)
L. aeneus	2	1270 ± 268.7	405 ± 21.2	50	2 (1 – 4)
L. capensis	4	1740 ± 177.4	413.8 ± 11.1	–	– –
L. umbratus	4	1950 ± 227.7	460.8 ± 15.1	–	– –
T. sparrmanii	15	59 ± 76.4	91.8 ± 31.93	100	38 (3 – 88)

Thirteen specimens of *C. gariepinus* were collected and were infected with parasitic species from the Monogenea, Cestoda, Nematoda, Hirudinea and Crustacea. A single specimen of the invasive branchiuran, *A. japonicus* was found from the body surface.

Two fish were infected with freshwater leeches from the Hirudinea Linnaeus, 1758 on the head region, that were identified as *Placobdelloides* sp. Infection with a single dactylogyrid species on the gills were also found in low intensities (see **Table 6.1**). The monogenean was identified as *Quadriacanthus* sp. 1 (see **Fig 6.2 A–C**). Larval stages of the nematode *Contracaecum* sp. (n = 11) were found in the muscle tissue, body cavity, kidneys and the liver. Altogether 25 nematode worms belonging to the Camallanidae Railliet & Henry, 1915 were also found in the intestine and stomach of five *C. gariepinus* individuals.

Only two specimens of *L. aenues* were collected. Both were infected on the fins and body surface, with three and four individuals of *A. japonicus*, respectively. An unknown species

of the Dactylogyridae was found to parasitise the gills in very low numbers (see **Table 6.1**) and is here referred to as *Dactylogyrus* sp. 1 (see **Fig. 6.2 D–G**).

Four specimens of *L. capensis* and *L. umbratus* each, were collected. The gills of neither species were infected with monogeneans. The former only had infection in the eye with two unidentified trematodes, and the fins and external body surface of the latter were infected with the alien branchiuran *A. japonicus* (n = 1), the copepod *Ergasilus* sp. (n = 10) and unidentified nematode worms from the intestine (n = 1) and liver (n = 3).

The fins of all 15 *T. sparrmanii* were infected with *Ergasilus* sp. and infection ranged from only a few specimens (n = 2) to hundreds per fish. The gills were also infected with individuals of two monogenean species from the Ancyrocephalidae i.e. *Cichlidogyrus* sp. 1 (**Fig. 6.3 A–C**) and *Cichlidogyrys* sp. 2 (**Fig. 6.3 D–E**). Two individuals of *Enterogyrys* sp. were also found in the intestine. Six of the 15 *T. sparrmanii* were also infected with metacercaria (Prevalence = 40%) from three unknown trematode species from the Diplostomidae Poirier, 1886, five fish had green trematode cyst in the muscle tissue and one were infected with a black trematode cysts on the fin.

Table 6. 2. Presence and absence of parasitic groups on hosts collected from Boskop Dam.

	Crustacea		**Nematoda**		**Trematoda**	**Monogenea**				**Hirudinea**
	Argulus japonicus	*Ergasilus* sp.	*Contracaecum* sp.	Camalanidae.	Diplostomidae	*Dactylogyrus* sp. 1	*Cichlidogyrus* sp. 1	*Cichilidogyrus* sp. 2	*Quadriacanthus* sp. 1	*Placobdelloides* sp.
C. gariepinus	+	–	+	+	–	–	–	–	+	+
L. aeneus	+	–	–	–	–	+	–	–	–	–
L. capensis	–	–	–	–	+	–	–	–	–	–
L. umbratus	–	–	–	–	–	–	–	–	–	–
T. sparrmanii	–	+	+	–	–	–	+	+	–	–

+ parasite species present; – parasite species absent

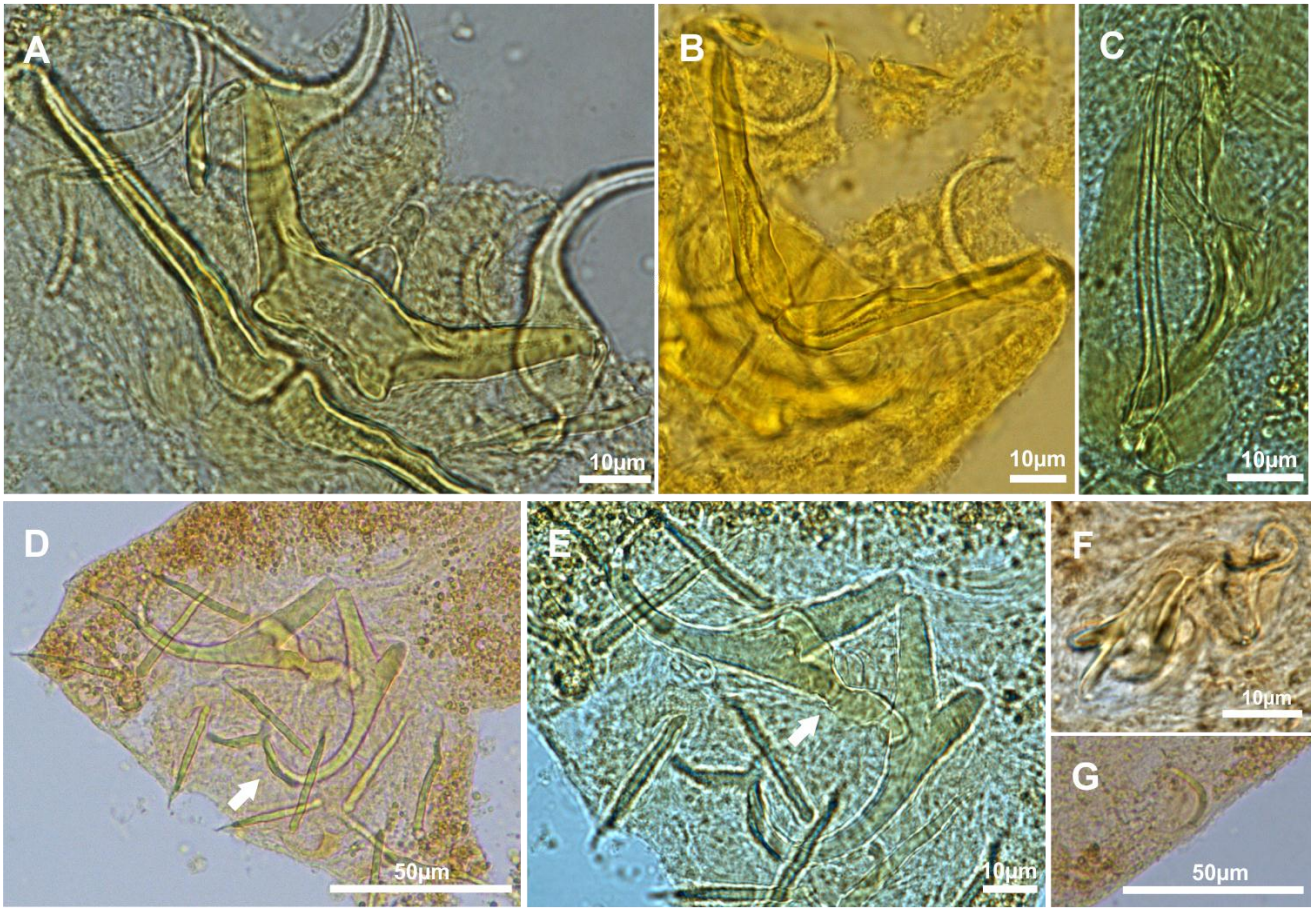

Figure 6. 2. Micrographs of *Quadriacanthus* sp. 1 from *Clarias gariepinus*, A – ventral bar, B – dorsal bar, C – male copulatory organ (MCO). Haptoral sclerites of *Dactylogyrus* sp. 1 from *Labeobarbus aeneus*, D – anchors and ventral sclerite (indicated with white arrow), E – dorsal bar (indicated with white arrow), F – MCO and G – vagina.

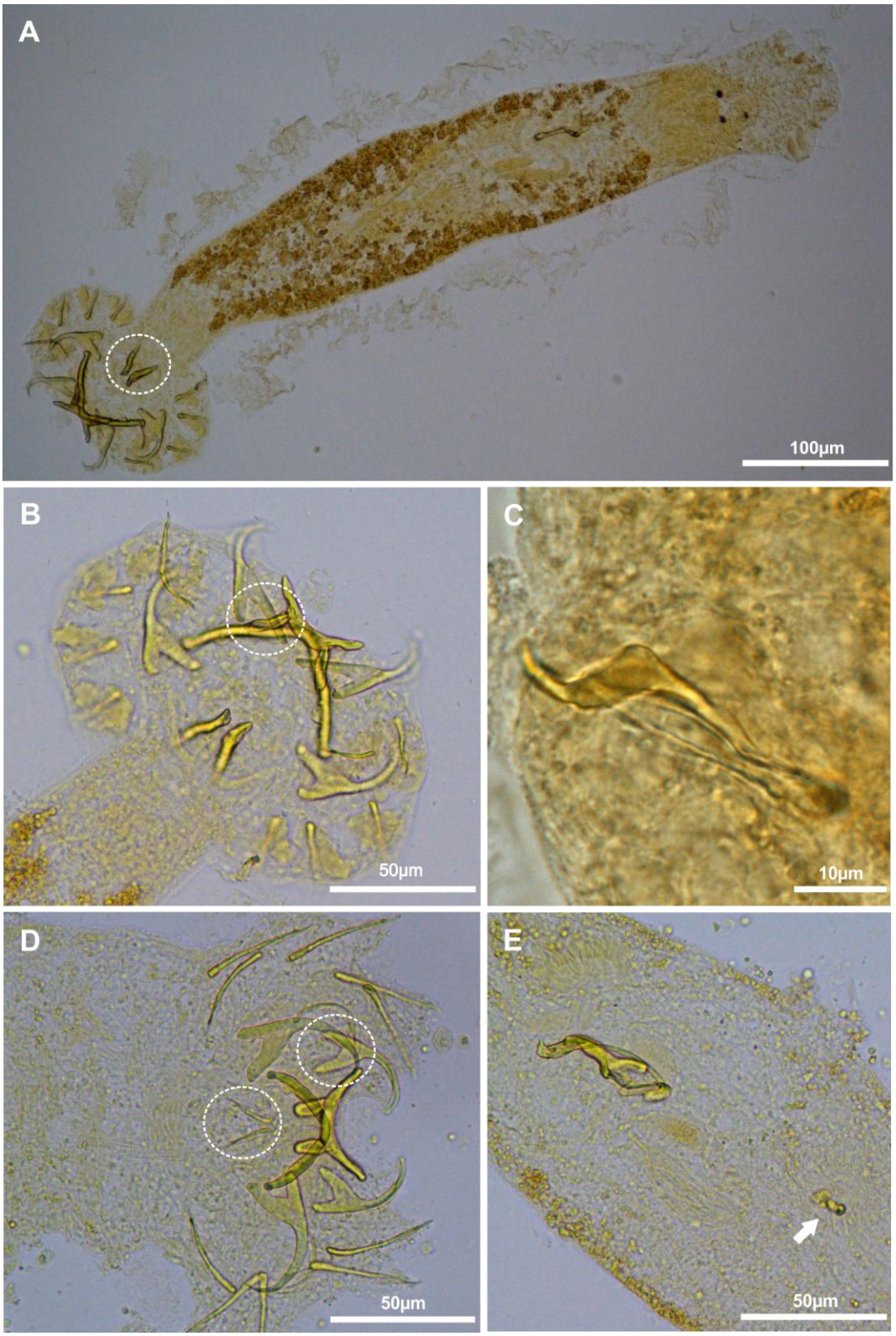

Figure 6. 3. Micrographs of *Cichlidogyrus* sp. 1, A – whole organism, B – anchors, dorsal and ventral bar complex, C – MCO and *Cichlidogyrus* sp. 2, D – anchors, dorsal and ventral sclerite complex, E – MCO and vagina (indicated with white arrow) from *Tilapia sparrmanii*. Differences in anchor, inner roots and hooklet morphology between species encircled with broken line.

6.4. Discussion

The co-invasive status of parasites is associated with the successful introduction and establishment of their introduced host. Generally, it is accepted that parasite spill-over is a common phenomenon observed with species that are less host specific, or have a cosmopolitan distribution (see **Chapter 1** and **Chapter 3**, **Section 3.1**). Within the Monogenea spill-over are rarely observed and the few that have been documented usually include reports of mass mortalities and health concerns from wild and cultured fishes. One of the best documented cases is that of *Gyrodactylus salaris* that spilled over from a Baltic salmon strain, from Sweden, to an Atlantic salmon strain of *Salmo salar* Linnaeus, 1758 in Norway (Peeler, *et al.*, 2010). Despite the fact that members of *Gyrodactylu*s von Nordmann, 1832 have broad host ranges, an extraordinary species diversity and has the ability to migrate to new hosts for survival if necessary, Boeger *et al.* (2005) found that continuous transmission to hosts enhances colonisation success, but ultimately successful colonisation of a new host means overcoming of 1) selective pressure of hosts to parasites; 2) the development of rapid resistance against parasites; 3) stress levels of hosts; 4) density and spatial distribution of hosts and 5) the ability of the parasite to track suitable hosts.

Other examples of spill-over in monogeneans include the capsalid gill monogenean *Nitzschia sturionis* (Abildgaard, 1794) and the pathogenic dactylogyrid eel parasite *Pseudodactylogyrus anguillae*. *Nitzschia sturionis* was co-introduced with the starry sturgeon *Acipenser stellatus* Pallas, 1771 from the Caspian Sea into the Aral Sea and spilled over to the native bastard sturgeon *Acipenser nudiventris* Lovetsky, 1828 causing mass mortalities of the native species (Bauer *et al.*, 2002; Strauss *et al.*, 2012), and the pathogenic dactylogyrid eel parasite *P. anguillae* is believed to have spilled over from the Japanese eel *Anguilla japonica* Temminck & Schlegel, 1846, through introduction of eels from Japan for aquaculture purposes, to European eel *Anguilla anguilla* (Linnaeus, 1758) and is now present in cultured and wild eels in Denmark, in Sweden, East Germany, south-eastern France and Southern Britain (Buchmann *et al.*, 1987; Køie, 1991). This monogenean is also listed as a co-introduced species in South Africa on the African longfin eel *Anguilla mossambica* (Peters, 1852) (Smit *et al.*, 2017) (although its source of introduction is still unclear) and McHugh *et al.* (2017) reported an expanded distribution range of *P. anguillae* in *A. mossambica* in South African freshwater systems, however, no spill-over event to non-anguillid fishes has been documented. So far all above spill-over

events mentioned were to other (native) host species within the same family as the introduced hosts.

Poulin and Keeney (2007) argued that host specificity is a fundamental property of parasitic organisms and determines the fate of the parasite i.e. the probability of its extinction and importantly, host specificity may reflect its ability to parasitise new hosts (i.e. spill-over or spillback) if the opportunity arises. They also agree with Boeger *et al.* (2005) that a parasite's ability or chance of infecting a native host is dependent on the resistance and tolerance of both the introduced parasite and the potential native host it can infect, in addition to the complexity of a parasites' life cycle. Species with indirect life cycles first need to succeed in finding a suitable native substitute or alternate between introduced and native hosts for completion of its complex life cycle (see Taraschewski *et al.*, 1987; Norton *et al.*, 2005; Britton, 2013; Goedknegt *et al.*, 2015). For parasites with direct life cycles, as in the case of the Monogenea in the examples given and for the parasites investigated for spill-over in the present study, impacts of spill-over or 'emerging disease' (i.e. mortality) on native species begins after the successful establishment of introduced host (after initial loss of parasites and its potential benefit in fitness and survival chance) (Britton, 2013; Goedknegt *et al.*, 2015). Šimková *et al.* (2001) investigated the role of host specificity and probability to spill-over in the event of co-introduction and found that three species of *Dactylogyrus* that are strict specialists to hosts from the Cyprnidae, did not spill over to native hosts in the introduced regions. The three species are *Dactylogyrus extensus* and *Dactylogurys minutus*, co-introduced with *C. carpio*, and *Dactylogyrus lamellatus* (co-introduced with *Ctenopharyngodon idella*) that have been co-introduced with their hosts globally (Gibson *et al.*, 1996; Dove and Ernst, 1998; Šimková *et al.*, 2001; Yang *et al.*, 2015). These three specialist species were found on larger hosts (reflection of predictable resources) and to co-occur with generalist parasite species, indicative of a rich parasite community. The specialisation of these three species also positively correlated with adaptation of attachment organs (Šimková *et al.*, 2001). Co-introduction of these species into South Africa was also documented by Crafford *et al.* (2014), and no spill-over events have been recorded. A single deviation of the findings of the above mentioned *Dactylogyrus* species is that of *Dactylogyrus extensus* in North America, where it was found to parasitise *Micropterus punctulatus* in the host's native range (Mizelle and McDougal, 1970), hereafter no record of this parasite on any centrarchid have been reported.

To date, there are records in South Africa of *C. gariepinus* being infected with *A. japonicus* (see van As and Basson, 1984), *Contracaecum* sp. and three species of *Quadriacanthus* Paperna, 1961 known to parasitise the gills i.e. *Quadriacanthus aegypticus* El-Naggar & Serag, 1986; *Quadriacanthus allobychowskiella* Paperna, 1979; *Quadriacanthus clariadis* Paperna, 1961 (Olivier *et al.*, 2009; Madanire-Moyo *et al.*, 2010). Infection of *C. gariepinus* with *Placobdelloides* sp. is seen as an accidental infection, as it may have washed downstream from upper reservoirs where hippopotami are present. Another possibility is that the leech may be the freshwater fish leech *Batracobdelloides tricarinata* (Blanchard, 1897).

The infection of *L. aeneus* with species of *Dactylogyrus* is not well studied in South Africa. Crafford *et al.* (2014) found an unidentified specimen, with similar features as *Dactylogyrus* sp. 1 from the present study, from *L. aeneus* in the Vaal Dam, Gauteng Province. Further investigation on more specimens and morphometric assessment are necessary for proper identification of this species.

Records of the infection of various cichlids with individuals of *Cichlidogyrus* Paperna, 1960 are also known from several South African systems and include seven species i.e. *Cichlidogyrus halli* (Price & Kirk, 1967) from *O. mossambicus* in the Middle Letaba Dam (Olivier *et al.*, 2009), *Cichlidogyrus papernastrema* Price, Peebles & Bamford, 1969 from *T. sparrmanii* in the Phongolo floodplains (Price *et al.*, 1969), *Cichlidogyrus philander* Douëllou, 1993 from *P. philander* in the Padda Dam (le Roux and Avenant-Oldewage, 2010), *Cichlidogyrus sclerosus* Paperna & Thruston, 1969 from *O. mossambicus*, *Cichlidogyrus tilapia* Paperna, 1960 from *O. mossambicus* and *P. philander*, and *Cichlidogyrus zambesensis* Douëllou, 1993 also from *O. mossambicus* in the Middle Letaba Dam (Olivier *et al.*, 2009).

All the parasites recorded from the five different fish species in the present study represents infection with parasite species known from the specific hosts. The absence of infection with any of the ancyrocephalids from *M. salmoides* confirms that no spill-over occurred. This then conforms to the statement of Poulin and Keeney, and as with the investigation of Šimková *et al.* (2001) and Boeger *et al.* (2005), emphasise just how important host specificity, or rather the lack thereof, is for the fate of introduced parasitic species. In the mentioned studies probability for survival and determinant factors for the success of a species spilling over to a native host is quite limited or reduced by factors such as to first surviving the initial introduction into a new environment, after which the type of parasitic life cycle, host and parasite tolerances and resistance, rapid adaptive

radiation of parasites, ability to find a suitable native host and already existing native parasite populations (lack of niche or enemy-free space) are challenges that need to be overcome by an co-introduced parasite. The possibility that no spill-over has occurred within the past 60 years suggest that it is unlikely that any of the Ancyrocephalidae will switch hosts. The possibility of host-switching or spill-over, however, should not be disregarded as little is known on the evolutionary relationship of these parasites with its centrarchid hosts.

Chapter 7: Concluding remarks and recommendations for future studies

7.1. Introduction

The ecological impacts such as predation on native invertebrates and fishes, habitat destruction, population alterations and competition with native species of largemouth bass are well documented in South Africa (e.g., Shelton *et al.*, 2008; Weyl *et al.*, 2010; Ellender *et al.*, 2011; Kimberg *et al.*, 2014). However, the parasite communities and possible invasion mechanisms (**Chapter 1**) that occur with the co-introduction of invasive hosts, have not received much attention (see Ellender and Weyl, 2014). In the native range, the parasite diversity and communities of largemouth bass has been documented extensively, and it is known to be parasitised by at least 150 parasite species across at least eight taxa (see Beverley-Burton, 1984; Hoffman, 1999). On the African continent, the few published records are mainly from Kenya, of Nematoda and Acantocephala parasitising *Micropterus salmoides* (see Schmidt and Canaris, 1967, 1968; Amin and Dezfuli, 1995; Khalil and Polling, 1997; Aloo and Dezfuli, 1997; Aloo, 1999). Knowledge on introduced populations in South Africa is limited to the few studies mentioned in **Chapter 1**, **Section 1.2**.

The present study focused on obtaining information on the parasite diversity of *M. salmoides* in a non-native range, also if any parasites were co-introduced from the native range, as well as to identify these parasites with the use of morphological and molecular approaches. The effect of these parasites on the health of the host in the novel environment and possible invasion mechanisms such as enemy release, parasite spill-over and spillback were also investigated.

7.2. Concluding remarks

This study firstly, presents the first comprehensive investigation of the parasitic communities and invasion mechanisms at play in introduced *M. salmoides* populations in South Africa. Previous parasitological studies in South Africa on *M. salmoides* investigated parasitic community of the host from a single locality (Matla, 2012), because of mortalities (Du Plessis, 1948), as part of larger studies where it was not the intended target species (Barson *et al.*, 2008; Tavakol *et al.*, 2015) and to compile checklists (van As and Basson, 1984; Khalil and Polling, 1999). No concrete conclusions on invasive status or potential of the parasites were made, some not even identified up to generic level or possible known species. Secondly, this study also presents the first molecular data on the five co-introduced species of *M. salmoides*. Thirdly, despite the ecological impact of this host in almost all systems where it is introduced, their parasite communities seem to have little to

no effect on the health of the host, as reports of severe infections with these specialist monogeneans from the native range of the host did not mention any pathogenicity to the host. Lastly, this study underlines that there is currently minimal threat of these co-introduced parasites to spill-over to native species (or reach co-invasive status), but this should be investigated in more populations.

The finding of only eight parasitic species in total, and only five of the known eight monogenean species in varying presence at the selected localities does not support the null hypothesis. Enemy release has occurred, therefor **Hypothesis 1** is not accepted.

The success in obtaining sequences from the selected three nuclear markers, and being able to distinguish between the five morphologically different species supports **Hypothesis 2**, this hypothesis is accepted.

The absence of a correlation between host health and parasitic infection found in the present study suggests the loss of parasite diversity are not related to fitness of the host in the novel environment, but rather to the co-evolution of the host and its parasites, supports the enemy release hypothesis, and also **Hypothesis 3**, the null hypotheis is accepted.

The absence of co-introduced ancrocephalids from *M. salmoides* on the native fishes, supports the null hypothesis of no spill-over, thus **Hypothesis 4** is accepted.

7.3. Future studies

Throughout the study the following possible research opportunities have been identified that will contribute to our understanding of introduced species, their parasitic communities, parasite-host relationship and potential for co-introduced parasites becoming co-invasive:

- There are other centrarchid species i.e. *Micropterus punctulatus*, *M. dolomieu* and *Lepomis macrochirus* present in the freshwater systems of South Africa. In the present study, two of the impoundments that were selected also harboured *L. macrochirus*. It would be beneficial to identify other impoundments where centrarchid species occur or co-exist with each other and investigate their parasite communities. A clear picture would be painted on how specialised the ancyrocephalid parasites of centrarchids are in a non-native range and possibly identify if cases of spill-over or spillback has already occurred in other systems.
- Molecular studies on the parasites of *M. salmoides* is almost non-existent. Apart from the present study, attempts should be made to identify these parasites through molecular characterisation that can assist is phylogenetic studies of the host-parasite relationships and their co-evolution.

- Apart from the attempt of Hargrove *et al.* (2017) to reconstruct the distribution history of *M. salmoides* throughout South Africa, additional molecular contributions of the host will be beneficial. Together with the phylogeny of both host and its parasites it is crucial to understand incidences of introductions of this invasive host, how it was distributed throughout the country and how its parasites co-evolved.
- Lastly, continuous monitoring of freshwater systems where this invasive host have been introduced, and other species within the Centrarchidae should be considered. The parasitic communities of this invasive host and other centrarchids are still new to science and the potential for spill-over to native species should not be ignored.

References

Adams, S.M., Brown, A., Goede, R.W. 1993. A quantitative health assessment index for rapid evaluation of fish condition in the field. *Transactions of the American Fisheries Society*, 122:63–73

Adams, S.M., McLean, R.B. 1985. Estimation of largemouth bass, *Micropterus salmoides* Lacepède, growth using the liver somatic index and physiological variables*. *Journal of Fish Biology,* 26:111–126

Aguilar-Aguilar, R., Islas-Ortega, A.G. 2015. A checklist of ciliate parasites (Ciliophora) of fishes from Mexico. *Zootaxa,* 4027:270–280

Aloo, P.A. 1999. Ecological studies of helminth parasites of the largemouth bass, *Micropterus salmoides*, from Lake Naivasha and the Oloidien Bay, Kenya. *Onderstepoort Journal of Veterinary Research,* 66:73–79

Aloo, P.A., Dezfuli, B.S. 1997. Occurrence of the cystacanths of *Polyacanthorhynchus kenyensis* larvae (Acanthocephala) in four teleostean fishes from a tropical lake, Lake Naivasha, Kenya. *Folia Parasitologica,* 44:233–238

Amin, O.M., Dezfuli, B.S. 1995. Taxonomic notes on *Polyacanthorhynchus kenyensis* (Acanthocephala: Polyacanthorhynchidae) from Lake Naivasha, Kenya. *Journal of Parasitology,* 81:76–79

Arnott, S.A., Barber, I., Huntingford, F.A. 2000. Parasite-associated growth enhancement in a fish-cestode system. *Proceedings of the Royal Society of London: Biological Sciences, Royal Society (Great Britain),* 267:657–663

Bakke, T.A., Cable, J., Harris, P.D. 2007. The biology of gyrodactylid monogeneans: The “Russian-Doll Killers”. *Advances in Parasitology,* 64:161–378

Barman, D., Nen, P., Mandal, S.C., Kumar, V. 2013. Immunostimulants for aquaculture health management. *Journal of Marine Science: Research and Development,* 3:1000134

Barnard, S., Venter, A., van Ginkel, C.E. 2013. Overview of the influences of mining-related pollution on the water quality of the Mooi River system’s reservoirs, using basic statistical analyses and self-organised mapping. *Water SA,* 39:655–662

Basson, L., van As, J.G. 2006. Trichodinidae and other ciliates (Phylum Ciliophora). In: *"Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections"*, 2nd ed. (Woo, P.T.K., ed). CABI. Cambrindge, MA p 339

Baucom, R.S., de Roode, J.C. 2011. Ecological immunology and tolerance in plants and animals. *Functional Ecology,* 25:18–28

Bauer, O.N., Pugachev, O.N., Voronin, V.N. 2002. Study of parasites and diseases of sturgeons in Russia: a review. *Journal for Applied Ichthyology,* 18:420–429

Behrmann-Godel, J., Roch, S., Brinker, A. 2014. Gill worm *Ancyrocephalus percae* (Ergens 1966) outbreak negatively impacts the Eurasian perch Perca fluviatilis L. stock of Lake Constance, Germany. *Journal of Fish Diseases,* 37:925–930

Beverley-Burton, M. 1984. Monogenea and Turbellaria. p. 5–209. *In* L. Margolis and Z. Kabata *eds*. Guide to the parasites of fishes of Canada. *Canadian Special Publication of Fisheries and Aquatic Sciences,* 74:209

Beverley-Burton, M. 1986. The taxonomic status of *Actinocleidus* Mueller, 1937; *Anchoradiscus* Mizelle, 1941; *Clavunculus* Mizelle *et al.*, 1956; *Anchoradiscoides* Rogers, 1067; Rogers, 1067; *Syncleithrium* Price, 1967 and *Crinicleidus* n. gen.: North American Ancyrocephalids (Monogenea) with articulating haptoral bars. *Journal of Parasitology* 72:22–44

Beverely-Burton, M., Klassen, G.J. 1990. New approaches to the systematics of the ancyrocephalid Monogenea from Nearctic Freshwater fishes. *Journal of Parasitology,* 76:1–21

Beverley-Burton, M., Suriano, D. M. 1980. *Haplocleidus dispar* (Mueller, 1936) and *Pterocleidus acer* (Mueller, 1936) (Monogenea: Ancyrocephalinae) from *Lepomis gibbosus* L. (Pisces: Centrarchidae) in Ontario, Canada: anatomy and systematic position. *Canadian Journal of Zoology*, 58:661–669. doi: 10.1139/z80-095

Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošik, V., Wilson, J.R.U., Richardson, D.M. 2011. A proposed unified framework for biological invasions. *Trends in Ecology and Evolution,* 26:333–339

Blasco-Costa, I., Miguez-Lozano, R., Sarabeev, V., Balbuena, J.A. 2012. Molecular phylogeny of species of *Ligophorus* (Monogenea: Dactylogyridae) and their affinities within the Dactylogyridae. *Parasitology International,* 61:619–627

Blažek, R., Jarkovský, J., Koubková, Gelnar, M. 2008. Seasonal variation in parasite occurrence and microhabitat distribution of monogenean parasites of gudgeon *Gobio gobio* (L.). *Helminthologia,* 45:185–191

Boeger, W.A., Kritsky, D.C., Pie, M.R., Engers, K.B. 2005. Mode of transmission, host switching, and escape from the red queen by viviparous gyrodactylids (Monogenoidea). *Journal of Parasitology,* 91:1000–1007

Boomker, J. 1993. Parasites of South African freshwater fish. V. Description of two new species of the genus *Spinitectus* Fourment, 1883 (Nematoda: Cystidicolidae), *Onderstepoort Journal of Veterinary Research*, 60:139–145

Boomker, J., Puylaert, F.A. 1994. Eight new Afrotropical *Spinitectus* spp. (Nematoda: Cystidicolidae) from freshwater fishes with a key to the members of the genus in the region, *Onderstepoort Journal of Veterinary Research*, 61:127–142

Borji, H., Naghibi, A., Nasiri, M.R., Ahmadi, A. 2012. Identification of *Dactylogyrus* spp. and other parasites of common carp in northeast of Iran. *Journal of Parasitic Diseases,* 36:234–238

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,*72:248–254

Britton, J.R. 2013. Introduced parasites in food webs: new species, shifting structures? *Trends in Ecology & Evolution,* 28:93–99

Brown, M.L., Murphy, B.R. 2004. Seasonal dynamics of direct and indirect condition indices in relation to energy allocation in largemouth bass *Micropterus salmoides* (Lacèpede). *Ecology of Freshwater Fish,* 13:23–36

Bruton, M.N., van As, J. 1986. Faunal invasions of aquatic ecosystems in southern Africa, with suggestions for their management. Pages 47–61 in I.A.W.

Buchmann, K. 1989. Relationship between host size of *Aguilla Anguilla* and the infection level of monogeneas *Pseudodactyloyrus* spp. *Journal of Fish Biology,* 35:599–601

Buchmann, K., Bresciani, J. 1997. Parasitic infecitons in pond-reared rainbow trout *Oncorhynchus mykiss* in Denmark. *Diseases of Quatic Organisms,* 28:15–138

Buchmann, K., Bresciani, J. 2006. Monogenea (Phylum Platyhelminthes), Chapter 9, In: *"Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections"*, 2nd ed. (Woo, P.T.K., ed). CABI. Cambridge, MA,339 pp

Buchmann, K., Mellergaard, S., Køie, M. 1987. *Pseudodactylogyrus* infections in eel: a review. *Diseases of Aquatic Organisms,* 3:51–57

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W. 1993. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology,* 84:575–583

Capella-Gutiérrez, S., Silla-Martínez, J.M., Gabaldón, T. 2009. trimAL: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, 25:1972–1973

Claussen, J.E. 2015. Black bass diversity: Multidisciplinary science for conservation. *American Fisheries Science Symposium,* 82:27–34

Cloutman, D.G. 1974. Monogenean and copepod parasites of fishes from the Smoky Hill River, Ellis County, Kansas. *Transactions of the Kansas Academy of Science,* 77:225–230

Cloutman, D.G., 1975. Parasite community structure of Largemouth bass, Warmouth, and Bluegill in Lake Fort Smith, Arkansas. *Transactions of the American Fisheries Society,* 104, 277–283

Cognetti-Varriale, A.M., Castelli, A., Cecchini, S. and Saroglia, M. 1993. Study of *Diplectanum aequans* (Monogenea), parasite of sea bass (*Dicentrarchus labrax*) in intensive farming. In:"*Production, Environment and Quality. Bordeaux Aquaculture '92*" (eds G. Barnabé and P. Kestemont) European Aquaculture Society, Special Publication 18, Ghent, Belgium, pp. 143–153

Colautti, R.I., MacIsaac, H.J. 2004. A neutral terminology to define 'invasive' species. *Diversity and Distributions,* 10:135–141

Collins, M.R., Janovy, J Jr. 2003. Host specificity among Ancyrocephalinae (Monogenoidea) of Nebraska Sunfish. *Journal of Parasitology,* 89:80–83

Crafford, D. 2000. Application of a fish health assessment index and associated parasite index on *Clarias gariepinus* (Sharptooth catfish) in the Vaal River system, with reference to heavy metals. MSc thesis Rand Afrikaans University, South Africa, 231 pp

Crafford, D., Avenant-Oldewage, A. 2009. Application of a fish health assessment index and associated parasite index to *Clarias gariepinus* (Teleostei: Clariidae) in the Vaal river system, South Africa. *African Journal of Aquatic Science,* 34:261–272

Crafford, D., Luus-Powell, W., Avenant-Oldewage, A. 2014. Monogenean parasites from fishes of the Vaal Dam, Gauteng Province, South Africa II. New locality records. *Acta Parasitologica,* 69:485–492

Cucherousset, J., Olden, J.D. 2011. Ecological impacts of non-native freshwater fishes. *Fisheries*, 36:215-230

Dang, B.T., Levsen, A., Schander, C., Bristow, G.A. 2010. Some iHaliotrema (Monogenea: Dactylogyridae) from cultured grouper (*Epinephelus* spp.) with emphasis on the phylogenetic position of *Haliotrema cromileptis*. *Journal of Parasitology,* 96:30–39

Daszak, P., Cunningham, A.A., Hyatt, A.D. 2000. Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science,* 287:443–449

De Moor, I.J. 1996. Case studies of the invasion by four alien fish species (*Cyprinus carpio*, *Micropterus salmoides*, *Oreochromis macrochis* and *O. mossambicus*) of freshwater ecosystems in Southern Africa. *Transactions of the Royal Society of South Africa*, 51:233-255

De Moor, I.J., Bruton, M.N. 1988. Atlas of alien and translocated indigenous aquatic animals in southern Africa. (p. 138). NMB, Port Elizabeth

Department of Water Affairs (DWAF). 2015. Water quality monitoring sites grouped by primary drainage region. http://www.dwaf.gov.za/iwqs/wms/data/WMS_pri_txt.asp Date of access: June 25 2017

Ding, X-J., Liao, X-H. 2005. Phylogenetic position of the monogeneans *Pseudodactylogyrus*, *Heteronchocleidus* and *Trianchoratus* inferred from the 5' terminal sequences of 28S rDNA. *Acta Zoot Sinica.* 30:244–51 (In Chinese)

Douëllou, L. 1993. Monogeneans of the genus *Cichlidogyrus* Paperna, 1960 (Dactylogyridae: Ancyrocephalidae) from cichlid fishes of Lake Kariba (Zimbabwe) with descriptions of five new species. *Systematic Parasitology,* 25:159–186

Dove, A.D.M., Ernst, I. 1998. Concurrent invaders—four exotic species of Monogenea now established on exotic freshwater fishes in Australia. *International Journal for Parasitology,* 28:1755–1764

Du Plessis, S.S. 1948. A gyrodactylid parasite from the ureters of Largemouth bass at the Jonkershoek Inland Fish Hatchery, South Africa. *Transactions of the American Fisheries Society,* 75:105–109

Ellender, B.R., Weyl, O.L.F. 2014. A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. *Aquatic Invasion*s, 9:117–132

Ellender, B.R., Weyl, O.L.F., Swartz, E.R. 2011. Invasion of a headwater stream by non-native fishes in the Swartkops River system, South Africa. *African Zoology,* 46:39–46

Ellender, B.R., Woodford, D.J., Weyl, O.L.F., Cowx, I.G. 2014. Managing conflicts arising from fisheries enhancements based on non-native fishes in southern Africa. *Journal of Fish Biology,* 85:1890–1906

Esch, G.W. 1971. Impact of ecological succesion on the parasitic fauna in centrarchids from oligotrophic and eutrophic ecosystems. *American Midland Naturalist,* 86:160–168

Fanta, E., Rios, F.S.A., Romão, S, Vianna, A.C.C., Freidberger, S. 2003. Histopatholgoy of the fish *Corydoras paleutus* contaminated with sublethal levels of organophosphorus in water and food. *Ecotoxicology and Environmental Safety,* 54:119–130

Feist, S.W., Longshaw, M. 2006. Phylum Myxozoa. In: "*Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections"*, 2nd ed. (Woo, P.T.K., ed). CABI. Cambridge, MA, p 230-296

Francová, K., Seifertová, M., Blažek, R., Gelnar, M., Mahmoud, Z. N., Řehulková, E. 2017. *Quadriacanthus* species (Monogenea: Dactylogyridae) from catfishes (Teleostei: Siluriformes) in eastern Africa: new species, new records and first insights into interspecific genetic relationships. *Parasites & Vectors,* 10:361 doi: 1 0.1186/s13071-017-2223-4

Fouché, P.S.O. 2016. Short Course in Aquatic Ectothermic Vertebrates Handling and Ethics. Faculty of Natural and Agricultural Sciences (AAAC 619), North-West University, 102pp

Galaviz-Silva, L., Iruegas-Buentello, F.J., Escobar-González, B., Molina-Garza, Z.J. 2016. Infection levels and seasonality of monogeneans in the largemouth bass *Micropterus salmoides* (Perciformes: Centrarchidae) from Nuevo León, Mexico. *Journal of Helminthology,* 90:685–692, doi: 10.1017/S0022149X15000954\

Garcia-Vasquez, A., Hansen, H. and Shinn, A.P. 2007. A revised description of *Gyrodactylus cichlidarum* Paperna, 1968 (Gyrodactylidae) from the Nile tilapia, *Oreochromis niloticus niloticus* (Cichlidae), and its synonymy with *G. niloticus* Cone, Arthur et Bondad-Reantaso, 1995. *Folia Parasitologica,* 54:129–140

Garcia-Vasquez, A., Hansen, H., Christison, K.W., Rubio-Godoy, M., Bron, J.E., Shinn, A.P. 2010. Gyrodactylids (Gyrodactylidae, Monogenea) infecting *Oreochromis niloticus niloticus* (L.) and *O. mossambicus* (Peters) (Cichlidae): A pan-global survey. *Acta Parasitologica,* 55:215–229

Gevers, E. 2016. Introduction of largemouth bass on the Fountain Hill Estate, Wartburg [correspondence]. Apr/Oct., South Africa

Gibson, D.I. 1996. Trematoda. In: Margolis, L., Kabata, Z. (eds). Guide to parasites of fishes of Canada. Part IV. *Canadian Special Publications of Fisheries and Aquatic Sciences,* 124:1–373

Grendron, A.D., Marcogliese, D.J., Thomas, M. 2012. Invasive species are less parasitized than native competitors, but for how long? The case of the round goby in the Great Lakes-St. Lawrence Basin. *Biological Invasions,* 14:367–384

Goedknegt, M.A., Feis, M.E., Wegner, K.M., Luttikhuizen, P.C., Buschbaum, C., Camphuysen, C.J., van der Meer, J., Thieltges, D.W. 2015. Parasites and marine invasions: Ecological and evolutionary perspectives. *Journal of Sea Reseach,* 113:11–27

Gozlan, R.E. 2008. Introduction of non-native freshwater fish: is it all bad? *Fish and Fisheries*, 9:106–115

Grupcheva, G. I., Nedeva, I. L. 1999. Parasite fauna of the crucian carp (*Carassius auratus gibelio* Bloch) in the Zrebchevo reservoir (Bulgaria). *Acta Zoologica Bulgarica*, 51:115–122

Hanek, G., Fernando, C.H. 1972. Monogenetic trematodes from the Bay of Quinte area, Ontaria. III. Genera *Actinocleidus, Cleidodiscus, Urocleidus* and *Tetraoncus*. *Canadian Journal of Zoology,* 50:1303–1312

Hansen, H., Cojocaru, C.D., Mo, T.A. 2016. Infections with *Gyrodactylus* spp. (Monogenea) in Romanian fish farms" *Gyrodactylus salaris* Malmberg, 1957 extends its range. *Parasites & Vectors,* 9:444–453

Hargis, W.J. Jr. 1953. Monogenetic trematodes of Westhampton Lake Fishes. III. Part I. Comparative morphology of the species encountered. *Journal of Parasitology,* 39:88–105

Hargrove, J.S., Weyl, O.L.F., Allen, M.S., Deacon, N.R. 2015. Using tournament angler data to rapidly assess the invasion status of alien sport fishes (*Micropterus* spp.) in southern Africa*. PLoS ONE* 10(6): e0130056

Hargrove, J.S., Weyl, O.L.F., Austin, J.D. 2017. Reconstructing the introduction history of an invasive fish predator in South Africa. *Biological Invasions,* 19:2261–2276 doi: 10.1007/s10530-017-1437-x

Harris, J.H, Silveira, R. 1999. Large-scale assessment of river health using Index of Biotic Integrity with low-diversity fish communities. *Freshwater Biology,* 41:235–252

Harrison, A.C. 1936. Black bass in the Cape Province: Second report on the progress of American largemouth black bass (*Micropterus salmoides*, Lacepede). South Africa Division of Fisheries Investigational Report. 119 pp

Hassouna, N., Michot, B., Bachellerie, J-P 1984. The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. Nucleic Acids Reseaerch,12:3563–83

Havlátová, L., Ondračková, M., Přikrylová, I. 2015. Monogenean parasites of *Lepomis gibbosus* Linnaeus introduced into the River durance, France. *Helminthologia,* 52:323–330

Heath, A.G. 1987. Water pollution and fish physiology. CRC Press Inc., Florida

Heath, R., du Preez, H., Genthe, B., Avenant-Oldewage, A. 2004. Freshwater fish and human health reference guide. WRC Report No TT213/04

Hillis, D., Dixon, M. 1991. Ribosomal DNA: Molecular Evolution and Phylogenetic Inference. *The Quarterly Review of Biology,* 66:411–453

Hockley, F.A., Williams, C.F., Reading, A.J., Taylor, N.G.H., Cable, J. 2011. Parasite fauna of introduced pumpkinseed fish *Lepomis gibbosus*: first British record of *Onchocleidus dispar* (Monogenea). *Diseases of Aquatic Organisms,* 97:65–73

Hoffman, G. L. 1998. *Parasites of North American freshwater fishes*. Ithaca, USA: Cornell University Press, pp. 97–121

Hoffman, G.L. 1999. Parasites of North American Freshwater Fishes. 2nd ed. Cornell University Press, Ithaca, NY, 539 pp

Hopson, J.L., Wessels, N.K. 1990. Essentials of biology. McGraw Hill Publishing Company, New York, 976 pp

Houde, A. E. 1997. Sex, color and mate choice in Guppies. Oxford: Princeton University Press,

Houde, A. E., Torio, A.J. 1992. Effect of parasitic infection on male color pattern and female choice in guppies. *Behavioural Ecology,* 3:346–351

Impson, D. 2017. Largemouth bass introductions in the Cape, Jonkershoek Hatchery, Stellenbosch [correspondence]. 7 Apr., South Africa

Iguchi, K., Matsuura, K., McNyset, K.M., Peterson, A.T., Scachetti-Pereira, R., Powers, K.A., Vieglais, D.A., Wiley, E.O. 2004. Predicting invasions of North American basses in Japan using native range data and a genetic algorithm. *Transactions of the American Fisheries Society,* 133:845–854

Iwanowicz, D.D. 2011. Overview on the effects of parasites on fish health. Proceedings of the Third Bilateral Conference between Russia and the United States, 12 – 20 July, 2009, Shepherdstown, West Virginia, pp 176 –184

Iyaji, F.O., Eyo, J.E. 2008. Parasites and their freshwater fish host. *Bio-Research,* 6:328–338

Jacobs, F.J. 2013. The behavioural ecology of the Orange-Vaal River yellowfish in lentic and lotic ecosystems, North-West Province, South Africa. MSc. Thesis 150pp

Jerônimo, G.T., Pádua, S.B., Bampi, D., Goncalves, E.L.T., Garcia, P, Ishikawa, M.M., Martins, M.L. 2014. Haematological and histopathological analysis in South American fish *Piaractus mesopotamicus* parasitized by monogenean (Dactylogyridae). *Brazilian Journal of Biology,* 74:1000–1006

Joy, J.E. 1984. Prevalence of the Ancyrocephalinae (Monogenea) on Largemouth and Spotted Basses in Beech Fork Lake, West Virginia. *Proceedings of the Helminthological Society of Washington,* 51:168–170

Kania, P.W., Jorgensen, T.R. and Buchmann, K. 2007. Differentiation between a pathogenic and a non-pathogenic form of *Gyrodactylus salaris* using PCR-RFLP. *Journal of Fish Diseases,* 30:123–126

Keane, R.M., Crawley, M.J. 2002. Exotic plant invaisons and the enemy release hypothesis. *Trends in Ecologyand Evolution,* 17:164–169

Keesing, F., Holt, R.D., Ostfeld, R.S. 2006. Effects of species diversity on disease risk. *Ecology Letters,* 9:485–498

Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R., Tompkins, D.M. 2009. Parasite spillback: A neglected concept in invasion ecology? *Ecology,* 90:2047–2056

Khalil, L.F., Polling, L. 1997. *Checklist of the helminth parasites of African freshwater fish*. Department Zoology/Biology. Sovenga: University of the North, South Africa, 185 pp

Kimberg, P.K., Woodford, D.J., Roux, H., Weyl, O.L.F. 2014. Species-specific impact of introduced largemouth bass *Micropterus salmoides* in the Groot Marico Freshwater Ecosystem Priority Area, South Africa. *African Journal of Aquatic Science,* 39:451–458

Kmentová, N., Gelnar, M., Mendlová, M., van Steenberge, M., Koblmüller, Vanhove, M.P.M. 2016. Reduced host-specificity in a parasite infecting non-littoral Lake Tanganyika cichlids evidence by intraspecific morphological and genetic diversity. *Scientific Reports,* 6:39605

Køie, M. 1991. Swimbladder nematodes (*Anguillicola* spp.) and gill monogeneans (*Pseudodactylogyrus* spp.) parasitic on the European eel (*Anguilla anguilla*). *International Council for the Exploration of the Sea,* 47:391–398

Kotze, D. 2017. Largemouth bass and other fish species in the Vergenoegd Farm Dam, Stellenbosch [email]. 8 Apr., South Africa

Kritsky, D.C., Boeger, W.A. 1989. The phylogenetic status of the Ancyrocephalidae Bychowsky, 1937 (Monogenea: Dactylogyridea). *Journal of Parasitology,* 75:207–211

Kumar, S., Stecher, G., Tamura, K. 2016. MEGA7: Molecular evolutionary genetics analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution,* 33:1870–1874

Lefebvre, F., Mounaix, B, Poizat, G., Crivelli, A.J. 2004. Impacts of the swimbladder neatode *Angiollicola crassus* on *Anguilla Anguilla*: variations in liver and spleen masses. *Journal of Fish Biology,* 64:435–447

Lehtinen, K.J., Notini, M., Landner, L.1984. Tissue damage and parasite frequenct in flounders, *Platichtys flesus* (L.) chronically exposed to bleached kraft pulp mill effluents. *Annales Zoologici Fennici,* 21:23–28

Lemly, A.D., Esch, G.W. 1984. Population biology of the trematode *Uvuifer ambloplitis* (Hughes, 1927) in juvenile bluegill sunfish, *Lepomis macrochirus*, and largemouth bass *Micropterus salmoides*. *Journal of Parasitology,* 70:466–474

Le Roux, L.E., Avenant-Oldewage, A. 2010. Checklist of the fish parasitic genus *Cichlidogyrus* (Monogenea), including its cosmopolitan distribution and host species. *African Journal of Aquatic Science,* 35:21–36

Littlewood, D.T.J. and Olson, P.D. 2001. SSU rDNA and the Platyhelminthes: signal, noise, conflict and compromise. In: "Littlewood, D.T.J., Bray, R.A. (Eds.); *INTERRELATIONSHIPS OF THE PLATYHELMINTHES*: 262–278; Taylor & Francis, London

Lom, J. 1958. A contribution to the systematics and morphology of endoparasitic trichodinids from Amphibians, with a proposal of uniform specific characteristic. *The Journal of Protozoology,* 5:251–263

López, S. 1998. Acquired resistance affects male sexual display and female choice in guppies. *Proceedings of the Royal Society of London: Biological Sciences, Royal Society (Great Britain),* 265:717–723

Lowe, S., Browne, M., Boudjelas, S., De Poorter, M. 2000. 100 of the World's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation (IUCN), 12pp. First published as special lift-out in Aliens 12, December 2000. Updated and reprinted version: November 2004

Lymbery, A.J., Morine, M., Kanani, H.G., Beatty, S.J., Morgan, D.L. 2014. Co-invaders: The effects of alien parasites on native hosts*. International Journal for Parasitology: Parasites and Wildlife,* 3:171–177

Madanire-Moyo, G.N., Luus-Powell, W.J., Olivier, P.A.S. 2010. Ecology of metazoan parasites of *Clarias gariepinus* (Osteichthyes: Clariidae) from the Nwanedi-Luphephe Dams of the Limpopo River System, South Africa. *African Zoology,* 45:233–246

Maitland, P.S., Price, C.E. 1969. *Urocleidus principalis* (Mizelle, 1936), a North American monogenetic trematode new to the British Isles, probably introduced with the Largemouth bass *Micropterus salmoides* (Lacépède, 1802). *Journal of Fish Biology,* 1:17–18

Malmberg, G.1970. The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Arkiv Zoologi*, 23:1–235

Martens, K. 2001. Ostracoda. In: Day, J. A., de Moor, I. J., Stewart, B. A. and Louw, A. E. eds. "*Guides to the Freshwater Invertebrates of Southern Africa, Crustacea II*", WRC Report No. TT 148/01. Pretoria: Water Research Commission. p. 9-77

Maseng, M.R. 2010. Risk analysis and potential transmission and implications of exotic *Gyrodactylus* specis on cultured and wild cyprinids in the Western Cape, South Africa. MSc thesis. University of the Western Cape, South Africa152 pp

Matla, M.M. 2012. Helminth ichthyo-parasitic fauna of South African sub-tropical lake. PhD thesis. University of Limpopo, South Africa, 160 pp

McCafferty, J.R., Ellender, B.R., Weyl, O.L.F., Britz, P.J. 2012. The use of water resources for inland fisheries in South Africa. *Water SA,* 38:327–344

McGee, M.N., Whitney, M.J., Heckmann, R.A. 2001. The monogenean *Haplocleidus furcatus* Mueller, 1937 (Phylum Platyhelminthes) on *Lepomis cyanellus* Rafinesque, 1819 from Utah: a range extension. *Western North American Naturalist,* 61:245–247

McHugh, K.J. 2015. Comprehensive fish health assessment and parasitological investigation of alien and indigenous fishes from the Amatola region, South Africa. PhD thesis. North-West University, South Africa, 218 pp

McHugh K.J., Smit, N.J., Van Vuren, J.H.J., Van Dyk, J.C., Bervoets, L., Covaci, A., Wepener, W. 2011. A histology-based fish health assessment of the tigerfish, *hydrocynus vittatus* from a DDT-affected area. *Physics and Chemistry of the Earth,* 36:895–904

McHugh, K.J., Weyl, O.L.F., Smit, N.J. 2017. Parasite diversity of African longfin eel *Anguilla mossambica* Peters with comments on hot response to the monogenean *Pseudodactylogyrus anguillae* (Yin and Sproston). *Journal of Fish Diseases,* 40:959–961 doi: 10.1111/jfd.12560

Mendlová, M., Pariselle, A., Vyskočilová, M. and Šimková, A. 2010. Molecular phylogeny of monogeneans parasitizing African freshwater Cichlidae inferred from LSU rDNA sequences. *Parasitology Research,* 107:1405–1413

Mendoza-Palmero, C.A., Blasco-Costa, I., Scholz, T. 2015. Molecualr phylogeny of Neotropical monogeneans (Platyhelminthes: Monogenea) from catfishes (Suliformes). *Parasites & Vectors,* 8:164 doi: 10.1186/s13071-015-0767-8

Mitchell, C.E., Power, A.G. 2003. Release of invasive plants from fungal and viral pathogens. *Nature,* 421:625–627

Mizelle, J.D. 1940. Studies on monogenetic Trematodes III: Redescriptions and variations in known species. *Journal of Parasitology,* 26:165–178

Mizelle, J.D., Crane, J.W. 1964. Studies on monogenetic trematodes, XXIII. Gill parasites of *Micropterus salmoides* (Lacepede) from a California Pond. *Transactions of the American Microscopical Society,* 83343–348

Mizelle, J.D., Cronan, J.P. 1943. Studies on Monogenetic Trematodes X. Gill parasites from Reelfoot lake fishes. *The American Midland Naturalist,* 30:196–222

Mo, T.A. 2017. Infection with *Gyrodactylus salaris* In: World Organisation for Animal Health - *"Manual of Diagnostics Tests for Aquatic Animals"*. [Web Access:] http://www.oie.int/index.php?id=2439&L=0&htmfile=chapitre_gyrodactylus_salaris.htm [Date of Access:] 30 September 2017

Molnar, K., Hanek, G., Fernando, C.H. 1974. Parasites of fishes from Laurel Creek, Ontario. *Journal of Fish Biology,* 6:717–728

Mueller, J.F. 1937. Further studies on North American Gyrodactyloidea. *The American Midland Naturalist,* 18:207–219

Norton, J., Rollinson, D., Lewis, J.W. 2005. Epidemiology of *Anguillicola crassus* in the European eel (*Anguilla anguilla*) from two rivers in southern England. *Parasitology,* 130:679–686

Olivier, P.A.S., Luus-Powell, W.J., Saayman, J.E. 2009. Report on some monogenean and clinostomid infestations of freshwater fish and waterbird hosts in Middle Letaba Dam, Limpopo Province, South Africa. *Onderstepoort Journal of Veterinary Research,* 76:187–199

Olson, P.D. and Littlewood, D.T. 2002. Phylogenetics of the Monogenea - evidence from a medley of molecules. *International Journal for Parasitology,* 32:233–244

Olstad, K. 2013. NOBANIS – Invasive alien species fact sheet – *Gyrodactylus salaris* – From: Online database of the European Network on Invasive Alien Species. [Web

access:] https://www.nobanis.org/globalassets/speciesinfo/g/gyrodactylus-salaris/gyrodactylus_salaris.pdf [Date of Access:] 30 August 2017

Ondračková, M., Dávidová, M., Přikrylová, I., Pečínkova, M. 2011. Monogenean parasites of introduced pumpkinseed *Lepomis gibbosus* (Centrarchidae) in the Danube River Basin. *Journal of Helminthology,* 85:435–441

Oosthuizen, J. H. and Siddall, M. E. 2002. Hirudinea. (In Day, J. A. and de Moor, I. J. eds. Guides to the Freshwater Invertebrates of Southern Africa, Non-Arthropods, WRC Report No. TT 167/02. Pretoria: Water Research Commission. p. 237-263

Öztürk, T., Özer, A. 2014. Monogenean fish parasites, their host preference and seasonal distributions in the Lower Kizilirmak Delta (Turkey). *Turkish Journal of Fisheries and Aquatic Sciences,* 14:367–378

Ottová, E.., Šimková, A., Jurajda, P., Dávidová, M., Ondračková, M., Pečínková, M., Gelnar, M. 2005. Sexual ornamentation and parasite infection in males of common bream (*Abramis brama*): a reflection of immunocompetence status or simple cost of reproduction? *Evolutionary Ecology Research,* 7:581–593

Parsons, R. 2009. Is Groenvlei really fed by groundwater discharged from the Table Mountain Group (TMG) Aquifer? *Water SA*, 35:657–662

Peeler, E.J., Oidtmann, B.C., Midtlyng, P.J., Miossec, L., Gozlan, R.E. 2011. Non-native aquatic animals introductions have driven disease emergence in Europe. *Biological Invasions,* 13:1291–1303

Petterson, R.A., Østbye, K., Holmen, J., Vøllestad, L.A., Atle Mo, T. 2016. *Gyrodactylus* spp. diversity in native and introduced minnow (*Phoxinus phoxinus)* populations: no support for “the enemy release hypothesis”. *Parasites & Vectors*, 9:51–63

Plaisance, L., Littlewood, D.T.J., Olson, P.D. and Morand, S. 2005. Molecular phylogeny of gill monogeneans (Platyhelminthes, Monogenea, Dactylogyridae) and colonization of Indo-West Pacific butterflyfish hosts (Perciformes, Chaetodontidae) *Zoologica Scripta,* 34:425–436

Poulin, R. 2000. Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes. *Journal of Fish Biology,* 56:123–137

Poulin, R., Keeney, D.B. Host specificity under molecular and experimental scrutiny. *Trends in Parasitology,* 24:24–28

Poulin, R., Paterson, R.A., Townsend, C.R., Tompkins, D.M., Kelly, D.W. 2011. Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. *Freshwater Biology,* 56:676–688

Pouyaud, L., Desmarais, E., Deveney, M., Pariselle, A. 2006. Phylogenetic relationships among monogenean gill parasites (Dactylogyridea, Ancyrocephalidae) infesting tilapiine hosts (Cichlidae): systematic and evolutionary implications. *Molecular Phylogenetics and Evolution,* 38:241–249

Price, C.E., Peebles, H.E., Bamford, T. 1969. The monogenean parasites of African fishes – IV: two new species from South African hosts. *Revue de Zoolgie et de Botanique Africaines,* 79:117–124

Rawson, M.V., Rogers, W.A. 1972. The seasonal abundance of the Ancyrocephalinae (Monogenea) on Largemouth bass in Walter F. George Reservoir. *Proceedings of the Helminthological Society of Washington,* 39:159–162

Reed, P., Francis-Floyd, R., Klinger, R. 2012. Monogenean parasites of fish[1]. Institure of Food and Agricultural Sciences, University of Florida, FA28: 10 pp. (Original publication date: June 1996, Reviewed May 2009, Revised June 2012)

Richardson, D.M., Pyšek, P., Rejmánek, M., Barbour, M.G., Panetta, F.D., West, C.J. 2000. Naturalizations and invasion of alien plants: concepts and definitions. *Diversity and Distribution,* 6:93–107

Roche, D.G., Leung, B., Franco, E.F.M., Torchin, M.E. 2010. Higher parasite richness, abundance and impact in native versus introduced cichlid fishes. *International Journal for Parasitology,* 40:1525–1530

Rodríguez, F., Olivier, J.L. Marín, A., Medina, J.R. 1990. The general stochastic model of nucleotide substitution. *Journal of Theoretical. Biology,*142:485–501

Rohlenová, K., Morand, S., Hyrši, Tolarová, S., Flajšhans, Šimková, A. 2011. Are fish immune systems really affected by parasites? An immunological study of common carp (*Cyprinus carpio*). *Parasites & Vectors,* 4:120

Ronquist, F., Teslenko, M., van der Mark, P., Ayers, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P. 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology,* 61:539–542

Rubtsova, N.Y. 2015. First record of *Onchocleidus dispar*, an alien monogenean from introduced pumpkinseed fish *Lepomis gibbosus* (Pisces, Centrarchidae) in Ukraine. *Scientific Parasitology,* 16:83–88

Savini, D., Occhipinti-Ambrogi, A., Marchini, E., Tricarico, F., Gherardi, S., Olenin, S., Gollasch, S. 2010. The top 27 animal alien species introduced into Europe for aquaculture and related activities. *Journal of Applied Ichthyology*, 26:1–7

Sayyadzahed, G., Esmaeili, H.R., Ghasemian, S., Mirghiyasi, S., Parsi, B., Zamanpoore, M., Akhalaghi, M. 2016. Co-invasion of anchor worms *Lernaea cyprinacea* (Copepoda: Lernaeidae) in some freshwater fishes of the Kor River Basin, Southwest of Iran with some remarks on the ecological aspects of lernaeosis in the country. *Iran Journal of Fish Science,*15:369–389

Schmidt, G. D., Canaris, A. G. 1967. Acanthocephala from Kenya with descriptions of two new species*. Journal of Parasitology,* 53:634–637

Schmidt, G.D., Canaris, A.G. 1968. Record of parasitic Nematodes in Kenya. *Journal of the East Africa Natural History Society and National Museum,* 27:155–156

Scholz, T. 1999. Parasits in cultured and feral fish. *Veterinary Parasitology,* 84:317–335

Sheath, D.J., Williams, C.F., Reading, A.J., Britton, J.R. 2015. Parasites of non-native freshwater fishes introduced into England and Wales suggest enemy release and parasites acquisition. *Biological Invasions,* 17):2235–2246. doi: 10.1007/s10530-015-0857-836:273–281

Shelton, J.M., Day, J.A., Griffiths, C.L. 2008. Influence of largemouth bass, *Micropterus salmoides*, on abundance and habitat selection of Cape galaxias, *Galaxias zebratus*, in a mountain stream in the Cape Floristic Region, South Africa. *African Journal of Aquatic Science*, 33:201–210

Šimková, A., Matějusová, I., Cunningham, C.O. 2006. A molecular phylogeny of the Dactylogyridae sensu Kritsky & Boeger (1989) (Monogenea) based on the D1-D3 domains of large subunit rDNA. *Parasitology,* 133(1):43–53

Šimková, A., Desdevises, Y., Gelnar, M., Morand, S. 2001. Morphometrics correlates of host specificity in *Dactylogyrus* species (Monogenea) parasites of European Cyprinid fish. *Parasitology,* 123:169–177

Šimková, A., Plaisance, L., Matějusová, I., Morand, S., Verneau. 2003. Phylogenetic relationships of Dactylogyridae Bychowsky, 1933 (Monogenea: Dactylogyridea): the

need for the systematic revision of the Ancyrocephalinae Bychowsky, 1937. *Systematic Parasitology,* 54:1–11

Šimková, A., Morand, S., Jobet, E., Gelnar, M. and Verneau, O. 2004. Molecular phylogeny of congeneric monogenean parasites (*Dactylogyrus*): a case of intra-host speciation. *Evolution,* 58:1001–1018

Skelton, P.H. 2001. A complete guide to the freshwater fishes of southern Africa. 2nd ed. Cape Town: Struik Publishers, 395 pp

Smit, N.J., Malherbe, W., Hadfield, K.A. 2017. Alien freshwater fish parasites from South Africa: Diversity, distribution, status and the way forward. *International Journal of Parasitology: Parasites and Wildlife,* doi: 10.1016/j.ijppaw.2017.06.001

Sniezko, S.F. 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. *Journal of Fish Biology,* 6:197–208

Spencer, K., Schutte-Vlok, A., Baker, N. 2016. Goukamma Nature Reserve Complex: Protected Area Management Plan. Unpublished CapeNature Report, Knysna

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30:1312–1313 doi:10.1093/bioinformatics/btu033

Sterud, E., Jørgensen, A. 2006. Pumkinseed *Lepomis gibbosus* (Linnaeus, 1758) (Centrarchidae) and associated parasites introduced to Norway. *Aquatic Invasions,* 1:278–280

Strauss, A., White, A., Boots, M. 2012. Invading with biological weapons: the importance of disease-mediated invasions. *Functional Ecology,* 26:1249–1261

Sueiro, M.C., Bagnato, E., Palacios, M.G. 2017. Parasite infection and immune and health-state in wild fish exposed to marine pollution. *Marine Pollution Bulletin,* doi: 10.1016/j.marpolbul.2017.04.011

Takamura, K. 2007. Performance as a fish predator of largemouth bass [*Micropterus salmoides* (Lacepède)] invading Japanese freshwaters: a review. *Ecology Research,* 22:940–946

Taraschewski, H. 2006. Hosts and parasites as aliens. *Journal of Helminthology,* 80:99–128

Tavakol, S., Smit, W.J., Sara, J.R., Halajian, A., Luus-Powell, W.J. 2015. Distribution of *Contracaecum* (Nematoda: Anisakidae) larvae in freshwater fish from the northern regions of South Africa. *African Zoology,* 50:133–139

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J., Kuris, A.M. 2003. Introduced species and their missing parasites. *Nature,* 421:628–630

Turgut, E., Akin, S. 2003. A review on Gyrodactylidae and Dactylogyridae (Monogeneans) and their importance in aquaculture. *Ziraat Fakültesi Dergisi,* 20:43–48

van As, J.G. 2015. A brief history of freshwater fish parasitology in southern Africa. *African Zoology,* 50:93–107

van As, J.G., Basson, L. 1984. Checklist of freshwater fish parasites from southern Africa. *Suid-Afrikaanse Tydskrif vir Natuurnavorsing,* 14:49–61

van As, J.G., Basson, L. 1989. A further contribution to the taxonomy of the Trichodinidae (Ciliophora: Pertrichia) and a review of the taxonomic status of some fish ectoparasitic trichodinids. *Systematic Parasitology,* 14:157–179

van As, J.G., Schoonbee, H.J., Brandt, F.D.W. 1981. Further records of the occurrence of *Bothriocephalus* (Cestoda: Pseudophyllidea) in the Transvaal. *South African Journal of Science,* 77:343

Van der Putten, W.H. 2002. How to be invasive. *Nature,* 417:32–33

Van Ginkel, C.E., Hols, B.C. 2001. Assessment of the Water Quality in the Wilderness Lakes, the Groenvlei Lake and the Swartvlei Lakes, 1998. Final Draft Internal Report No. N/0000/00/DEQ/0700. Institute for Water Quality Studies, Department of Water Affairs and Forestry. Pretoria,93 pp

Vanhove, M.P., Snoeks, J., Volckaert, F.A., Huyse, T. 2011. First description of monogenean parasites in Lake Tanganyika: the cichlid *Simochromis diagramma* (Teleostei, Cichlidae) harbours a high diversity of *Gyrodactylus* species (Platyhelminthes, Monogenea). *Parasitology,* 138:364–380

Vanhove, M.P.M., Tessens, B., Schoelinck, C., Jondelius, U., Littlewood, D.T.J., Artios, T., Huyse, T. 2013. Problematic barcoding in flatworms: A case-study on monogeneans and rhabdocoels (Platyhelminthes). *Zookeys,* 365:355–379

Velmurugan, B., Selvanayagam, M., Cengiz, E.I., Unlu, E. 2007. Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of *Cirrhinus mrigala*. *Environmental Toxicology and Pharmacology,* 24:286–291

Watson, R.M., Crafford, D., Avenant-Oldewage, A. 2012. Evaluation of the fish health assessment index in the Olifants River system, South Africa. *African Journal of Aquatic Science,* 37:235–251

Welcomme, R.L. 1992. A history of international introductions of inland aquatic species. *ICES Marine Science Symposium,* 194:3–14

Wellborn, T.L. 1967. *Trichodina* (Ciliata: Urceolariidae) of freshwater fishes of the southeastern United States. *Journal of Protozoology,* 14:399–412

Wessels, J. 2017. Information on the fish species of Boskop and Potchefstroom Dams, North West. Resort Manager, Potchefstroom Dam [jaapw@tlokwe.gov.za]. 13 Jul., South Africa

Weyl, P.S.R., de Moor, F.C., Hill, M.P., Weyl, O.L.F. 2010. The effect of largemouth bass *Micropterus salmoides* on aquatic macro-invertebrate communities in the Wit River, Eastern Cape, South Africa. *African Journal of Aquatic Science,* 35:273–281

Wheeler, T.A., Beverley-Burton, M. 1989. Systematics of *Onchocleidus* Mueller, 1936 (Monogenea: Ancyrocephalidae): generic revision. *Canadian Journal of Zoology,* 67:136–157

Whittington, I.D., Cribb, B.W., Hamwood, T.E., Halliday, J.A. 2000. Host-specificity of monogenean (platyhelminth) parasites: a role for anterior adhesive areas? *International Journal for Parasitology,* 30:305–320

Whitfield, A.K., Weerts, S.P., Weyl, O.L.F. 2017. A review of the influence of biogeography, riverine linkages, and marine connectivity on fish assemblages in evolving lagoons and lakes of coastal southern Africa. *Ecology and Evolution,* doi: 10.1002/ece3.3266

Wu, X.Y., Zhu, X.Q., Xie, M.Q. and Li, A.X. 2006. The radiation of Haliotrema (Monogenea: Dactylogyridae: Ancyrocephalinae): molecular evidence and explanation inferred from LSU rDNA sequences. *Parasitology,* 132:659–668

Wu, X.Y., Zhu, X.Q., Xie, M.Q., Li, A.X. 2006. The evaluation for generic-level monophyly of Ancyrocephalinae (Monogenea, Dactylogyridae) using ribosomal DNA sequence data. *Molecular Phylogenetics and Evolution,* 44:530–544

Yang, L., Sado, T., Hirt, MV., Pasco-Viel, E., Arunchalam, M., Li, J., Wang, X., Freyhof, J., Saitoh, K., Simons, A.M., Miya, M., He, S., Mayden, R.L. 2015. Phylogeny and

polyploidy: Resolving the classifiction of cyprinine fishes (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution,* 85:97–116