

**PARASITE DIVERSITY AND HEALTH OF *CHILOGLANIS PRETORIAE* AND
AMPHILIUS URANOSCOPIUS FROM THE LIMPOPO RIVER SYSTEM**

By

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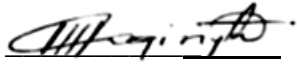
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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Zoology has not previously been submitted by me for a degree at this or any other university; that it is my work in design and execution, and that all material contained herein has been duly acknowledged.



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ABSTRACT

The African continent has rich freshwater fish fauna, but the parasite composition of only a few species is known. *Chiloglanis pretoriae* Van der Horst, 1931 and *Amphilius uranoscopus* (Pfeffer, 1889) are among African freshwater fish endemic to South Africa with limited information about their parasite composition and health status. A total of 171 fishes, *C. pretoriae* (n = 127) and *A. uranoscopus* (n = 44) from Nwanedi, Mutshundudi, Lutanandwa and Politsi rivers within the Limpopo River System, were collected to determine their parasite diversity and health. Both ecto- and endoparasites were recovered and preserved for identification. Scanning electron microscopy and morphological, morphometric and molecular analysis were used for parasite identification. Parasite infestation indices for all parasites were calculated and compared among the four rivers. The water quality of the rivers was investigated by measuring the selected physico-chemical parameters. Subsurface water samples were collected for analysis of selected metals and nutrients. The Health Assessment Index (HAI) in conjunction with the Inverted Parasite Index (IPI), condition factor (*K*) and regression analysis were calculated to determine the health of fish. Species richness and parasite diversity of *C. pretoriae* and *A. uranoscopus* from the four rivers were calculated using Margalef's richness index and Shannon-Weiner diversity index, respectively. Parasite diversity and species richness of *C. pretoriae* and *A. uranoscopus* were compared to the water quality of the four localities. Three parasitic groups (Monogenea, Digenea and Hirudinea) were found from *C. pretoriae* which comprised of two ectoparasite species [Dactylogyridae gen. sp. (n = 56) and a leech (n = 4)] and three endoparasite species [*Clinostomum* sp. (n = 4), Diplostomidae gen. sp. larva (n = 143) and Cephalogonimidae gen. sp. (n = 19)]. Ectoparasites were more abundant in the Lutanandwa River with a total of 54, while endoparasites were more abundant in the Mutshundudi River with a total of 106. Diplostomidae gen. sp. from *C. pretoriae* in the Mutshundudi River had the highest prevalence of 57.14%, and the leech from the Nwanedi River had the lowest prevalence of 0.25%. *Chiloglanis pretoriae* from Nwanedi River had the highest Shannon-Weiner and Margalef's index values of 0.53 and 0.54, respectively, indicating a healthy ecosystem. Two endoparasite groups, digeneans (*Clinostomum* sp., *Uvulifer* sp. and Cephalogonimidae gen. sp.) and nematodes (*Labeonema* sp., *Contracaecum* sp., *Rhabdochona* sp., *Gendria* sp. and *Gendria cf. paski*) were found in *A. uranoscopus*.

Uvulifer sp. was the most prevalent parasite with a 100% prevalence. *Amphilius uranoscopus* from Lutanandwa River had the highest Shannon-Weiner Index and Margalef's index values of 0.26 and 0.30, respectively, and a low evenness value of 0.37 due to uneven distribution of parasite species. The highest HAI value for *C. pretoriae* (36.19) was recorded from the Mutshundudi River and the lowest of 28.38 was recorded from the Lutanandwa River. The highest *K* score for *C. pretoriae* of 1.08 was recorded from Nwanedi River and the lowest of 1.01 from Lutanandwa River. The HAI of *A. uranoscopus* from the four selected localities indicated that the fish collected from the Lutanandwa River were in poorer health state than those from the other rivers due to the higher score. The highest HAI of 99.13 was recorded from the Lutanandwa River and the lowest HAI of 36.36 was recorded from the Nwanedi River. However, the highest mean *K* score of 0.84 was recorded from Nwanedi River while the lowest of 0.53 was recorded from Mutshundudi River. The results of both *C. pretoriae* and *A. uranoscopus* also indicated that the parasite burden did not affect the *K* of the fish. Generally, water quality results indicated that the rivers were in fair condition with most parameters being within the acceptable target water quality range (TWQR) of aquatic ecosystems. These rivers therefore have good water quality and indicate an oligotrophic state, however, Nwanedi and Politsi rivers are slightly impacted by anthropogenic factors which increased their nitrate concentration compared to the other rivers (Mutshundudi and Lutanandwa rivers). During this study, fish health, parasite diversity and species richness indices were used in combination to determine the water quality of the four localities. The results represent new geographical and host records for parasites from *C. pretoriae* and *A. uranoscopus* in the Nwanedi, Mutshundudi, Lutanandwa and Politsi rivers. Since it is the first record of Cephalogonimidae gen. sp. and Dactylogyridae gen. sp., more studies should be done to investigate other possible localities within the Limpopo River System and determine the life cycle of the digenean. Since both parasites represent new genera, papers on their description and identification are in preparation. Future studies on the parasites of the two fish species should employ the use of molecular analysis for larval forms as well as the leeches. It is proposed that the HAI using *A. uranoscopus* and *C. pretoriae* should not be employed for future studies because not all variables can be used due to the small size of these fish species. It is proposed that the study should be done seasonally at all localities to detect differences in the occurrence of parasites in relation to the seasonal water quality.

TABLE OF CONTENTS

DECLARATION	i
ACKNOWLEDGEMENTS	ii
RESEARCH OUTPUTS	iii
ABSTRACT	iv
CHAPTER 1	1
INTRODUCTION AND PURPOSE OF THE STUDY	1
1.1 Background information	1
1.2 Fish and parasites as biological monitoring tools	3
1.3 Previous studies on the two fish species	4
1.4 Motivation of study	4
1.5 Purpose of the study	5
1.5.1 Aim	5
1.5.2 Objectives	5
1.6 Research questions	6
1.7 Study layout	6
References	8
CHAPTER 2	13
MATERIALS AND METHODS	13
2.1 Study area	13
2.2 Sampling localities	14
2.3 Selected fish species	18
2.3.1 <i>Chiloglanis pretoriae</i>	18
2.3.2 <i>Amphilius uranoscopus</i>	19
2.4 Fish sampling and parasite collection	20
2.5 Parasite fixation	22
2.6 Morphological and molecular characterisation of parasites	22
2.7 Calculation of infestation indices, parasite diversity and species richness	26
2.8 Determination of the Health Assessment Index	26
2.9 Determination of the condition factor	30
2.10 Determination of the water quality	31
2.11 Data analyses	31
References	33
CHAPTER 3	40

PARASITE DIVERSITY OF <i>CHILOGLANIS PRETORIAE</i>	40
3.1 Introduction	40
3.2 Materials and Methods.....	42
3.3 Results	42
3.3.1 Parasites of <i>Chiloglanis pretoriae</i>	43
3.3.2 Seasonal variation of parasites infestation on <i>Chiloglanis pretoriae</i>	67
3.4 Discussion.....	67
3.5 Conclusion	72
References	73
CHAPTER 4	79
PARASITE DIVERSITY OF <i>AMPHILIUS URANOSCOPIUS</i>	79
4.1 Introduction	79
4.2 Materials and Methods.....	79
4.3 Results	79
4.3.1 Parasites found from <i>Amphilius uranoscopus</i>	80
4.4 Discussion.....	95
4.5 Conclusion	97
References	98
CHAPTER 5	101
FISH HEALTH AND WATER QUALITY FROM SELECTED RIVERS FROM THE LIMPOPO RIVER SYSTEM	101
5.1 Introduction	101
5.1.1 Water quality.....	101
5.1.2 Fish health for biological monitoring	102
5.2 Materials and method.....	103
5.3 Statistical analysis.....	104
5.4 Results	104
5.4.1 Water quality.....	104
5.4.2 Fish Health.....	106
5.4.3 Parasite diversity and species richness	111
5.5 Discussion.....	113
5.6 Conclusion	117
References	119
CHAPTER 6	123
SUMMARY AND RECOMMENDATIONS	123

6.1 General summary	123
6.2 Recommendations	125
References	127
Appendix A.....	128

CHAPTER 1

INTRODUCTION AND PURPOSE OF THE STUDY

1.1 Background information

Parasites

Parasites are organisms that live a proportion of their lives in (endoparasites) or on (ectoparasites) another organism, their host, are dependent on that host and benefit from the association at the host's expense without killing it (Poulin 2004). Parasites include representatives from many phyla mostly because free-living organisms possess parasites characteristics at one stage of their life cycle (Windsor 1998; Poulin & Morand 2000). Thus, parasites are more abundant than free-living organisms and they are found almost everywhere from subtropical regions to temperate regions (Marcogliese 2005; Faruk 2018).

Globally, several studies have revealed a rich parasitic fauna from freshwater fishes representing a wide variety of taxa (Iyaji & Eyo 2008), where freshwater fishes not only serve as their definitive hosts but also as intermediate hosts of many larval parasitic forms. These larvae that mature in a variety of vertebrates that can later cause diseases (Faruk 2018). They can also affect fish survival, health, growth and cause interference with spawning (Iyaji & Eyo 2008). Parasites cause this by weakening fish immune systems, increasing their susceptibility to secondary infestations (Ekanem et al. 2011). The presence of parasites can also alter the hosts morphology, physiology, reproduction, and behaviour. Behaviour alteration caused by the infestation of some parasites may aid the completion of their life cycles by making host to be easily captured (Lafferty 2008) because most freshwater fish are intermediate hosts for parasites (Marcogliese 2005).

Fish parasites are divided into microparasites and macroparasites depending on their size (Marcogliese 2005). Microparasites include fungi, viruses, protozoans, microsporidians, myxozoans and bacteria. Macroparasites are arthropods (copepods), annelids (leeches) and helminths which are comprised of monogeneans trematodes, cestodes, acanthocephalans and nematodes (Iyaji & Eyo 2008). These

taxa are further divided into endoparasites which are acquired through the ingestion of food or penetrate the host (e.g. cercariae) and ectoparasites which attach on the external organs of the fish such as skin, fins, and gills (Marcogliese 2002).

Africa has 12 large river systems that contain about 25% of the world's 13 000 freshwater fish species dominated by representatives of Cichlidae and Cyprinidae (Lévêque et al. 2008). Their parasites are still poorly known with a limited number of parasites identified compared to freshwater fish parasites from other continents. Many species of *Cichlidogyrus* Parpena, 1960 have been identified and a relative amount of attention has been given to parasitic crustaceans, specifically copepods (Scholz et al. 2018). Khalil & Polling (1997) created a checklist of parasites recorded from African freshwater fishes and they listed 568 adult helminth parasites comprising of 342 species of monogeneans, 62 trematodes, 61 cestodes, 80 nematodes and 21 acanthocephalans. The application of incorrect methods for collecting, processing, and identifying has led to the loss of valuable parasite material and the rate of discovery of new species has decreased in recent years (Scholz et al. 2018). For the parasites identified in Africa, studies on their life cycles are scarce (Khalil & Polling 1997). The relatively low number of parasites in African freshwater fishes is almost certainly not due to inherently low diversity, but rather to a lack of dedicated biodiversity research (Scholz et al. 2016). Because of the rich freshwater fish fauna of the African continent, Africa has a great potential to serve as an important model continent for ecological and evolutionary studies of fish parasites and their interactions (Scholz et al. 2018).

Water quality of the Limpopo River System

South Africa being a freshwater-scarce country, with a high and rapidly increasing human population, is faced with challenges of water quality and ecosystem deterioration because of urbanisation (Musingafi & Tom 2014). Freshwater in South Africa is also scarce due to less and unreliable rainfall patterns (Adewumi et al. 2010) and this is because it is in a region with a semi-arid climate (Kahinda et al. 2016). The country is subjected to droughts which are followed by floods leading to the destruction of freshwater bodies which are already threatened by anthropogenic effects (Ashton 2007). This is causing the country to experience an increase demand for water in agriculture, industrial and municipal sectors (Addo-Bediako 2020).

The scarcity of water is leading communities to exploit surface water. Since the Limpopo River System is shared among four neighbouring countries, the country is unable to manage its sustainability (Ashton 2007). The Limpopo River System is contaminated by heavy metals coming from its largest tributary, the Olifants River (Addo-Bediako 2020). The Olifants river is the most polluted river in the country (Addo-Bediako 2020), with contaminations due to past mining activities (Jooste et al. 2015). The overexploitation of the water from the Limpopo River System in the upper reaches causes a reduction of water and distribution of polluted water in the lower reaches (Kahinda et al. 2016). This affects the water quality by changing the physico-chemical composition of this system and the toxic metals get accumulated by aquatic organisms which in later trophic levels degrades the ecosystem (Sures et al. 2017).

1.2 Fish and parasites as biological monitoring tools

Fish and parasites are excellent biological indicators (Lafferty 2008) and it is due to their ability to respond to large amount of chemical, physical and biological changes compared to other aquatic organisms (Levin et al. 2019). The use of parasites and the interaction between parasites and fish serve as an effective way of monitoring freshwater ecosystem health. This is because alterations in the hosts may influence alterations in the parasites either directly or indirectly (Biswal & Chatterjee 2020). It is also because fish can serve as intermediate or final hosts for parasites and these hosts densities can affect parasite densities by altering the parasites' life cycle (Lafferty 1997).

Parasites are good biological indicators because water quality may affect their diversity. Different parasite species can survive in different water quality ranges (Sures 2006). Ectoparasites exposed directly to the environment are more affected and sensitive to water quality changes while endoparasites are less affected by the changes in the environment (Biswal & Chatterjee 2020). Reports indicate a trend towards the increase of ectoparasite infestations and a decrease of endoparasite infestations when pollution increases (Sures 2005). Stressful conditions caused by poor water quality may reduce the abundance of parasites (Marcogliese 2004; Morales-Serna et al. 2019). Disturbed water bodies thus need to be monitored to protect aquatic biodiversity (López-López & Sedeño-Díaz 2015).

Biological monitoring is a type of monitoring that is used where an organism is selected to be evaluated for its biological responses to identify and monitor changes in the environment (López-López & Sedeno-Díaz 2015). The Health Assessment Index (HAI) is one such method where fish health and parasites are used to evaluate the health of aquatic systems (Watson et al. 2012). The HAI can be used in conjunction with the Inverted Parasite Index (IPI), which is based on the premise that ectoparasites are more directly exposed to the effects of water quality than endoparasites, thus more ectoparasites are expected to be found where water quality is good (Crafford & Avenant-Oldewage 2009). This tool has successfully been used in South Africa as a biological monitoring tool (Crafford & Avenant-Oldewage 2009; Watson et al. 2012; Sara et al. 2014).

1.3 Previous studies on the two fish species

The Shortspine Suckermouth *Chiloglanis pretoriae* Van der Horst, 1931 and Stargazer Mountain catfish *Amphilius uranoscopus* (Pfeffer, 1889), are African freshwater Siluriformes with limited information about their parasites (Ngugi et al. 2009). Previous work on *Chiloglanis* focused on the genetic relationships between the species of this genus (Matlala et al. 2010). Currently, only two parasites have been recorded from *C. pretoriae* and they include the digenean *Clinostomum complanatum* (Rudolphi, 1814) which has been found on the peritoneum of the ventral surface of the swim bladder in the Limpopo River Drainage Systems (Olivier et al. 2009) and a ciliate *Trichodina compacta* Van As and Basson, 1989 (Scholz et al. 2018). A single parasite record, being *Clinostomum* larvae, has been documented for *A. uranoscopus* (Scholz et al. 2018). The current study will contribute meaningfully to the knowledge regarding the parasite fauna of these two fish species and provide baseline information in terms of fish health and parasite diversity.

1.4 Motivation of study

The poor water quality of South African freshwater ecosystems is being exacerbated by anthropogenic effects (Du Plessis 2021). Besides an increase in domestic demand, freshwater is essential for the abundance of biodiversity, and water quality needs to be within the acceptable range to sustain aquatic biodiversity and the health of the organisms therein (Department of Water Affairs and Forestry (DWAF) 1996; Hassan

et al. 2020). Previous research showed that the Limpopo River System was contaminated with various pollutants including heavy metals (Jooste et al. 2015) affecting the water quality by changing the physico-chemical composition of this system (Ashton et al. 2001). Besides water quality analysis, the diversity of fish parasites and the health of fish can be used as a tool to monitor the system since fish and parasites are sensitive to changes in water quality (Sures 2006; Roy 2019). Different types of pollution affect parasites in different ways, but in general, the diversity and abundance of parasites decrease when parasites are directly exposed to pollution (Lafferty 2008; Sures et al. 2017). There is inadequate data on the parasite diversity and health status of fish from the Limpopo River System. This is even more applicable to less abundant and smaller fish species such as *C. pretoriae* and *A. uranoscopus* of which information on the health status and parasite composition from most rivers is insufficient. Comprehensive information regarding the current status (water parameters and biotic data) of the Limpopo River System is thus essential and will be valuable for future ecosystem health studies.

1.5 Purpose of the study

1.5.1 Aim

The aim of the study was to determine the parasite diversity and health of *C. pretoriae* and *A. uranoscopus* from selected localities within the Limpopo River System.

1.5.2 Objectives

The objectives of the study were to:

- i. identify parasites to the lowest taxonomic level based on a combination of molecular analysis and morphometric features.
- ii. determine the prevalence, mean intensity, and mean abundance of parasites of *C. pretoriae* and *A. uranoscopus* from four different localities within the Limpopo River System.
- iii. determine the health of fish by assessing the HAI and calculate the condition factor of fish and diversity indices of parasites from each locality.
- iv. determine the water quality of the selected localities by analysing different constituents (ammonium, ammonia, nitrate, nitrite, fluoride, orthophosphate, sulphate and selected metals).

1.6 Research questions

- i. What is the parasite composition of each fish species at the different localities?
- ii. What is the parasite infestation levels of each parasite from the four selected localities?
- iii. What is the health status of the sampled fish and parasite diversity indices from the four localities?
- iv. What is the water quality of the different localities?

1.7 Study layout

Chapter 1: Introduction of the study giving information about the parasites, and impact of pollution on South African freshwater ecosystems mainly focusing on the Limpopo River System and how to use fish health and parasite composition to monitor ecosystem health. The motivation of the study, aims, objectives and research questions are also outlined.

Chapter 2: This chapter includes background information on the Limpopo River System, information on the four rivers within the Limpopo River System as well as the two studied fish species. The methods of fish sampling, parasite collection, parasite fixation and preservation, parasite identification, determination of the Health Assessment Index and condition factor, water quality analysis and data analysis are highlighted here.

Chapter 3: Investigating the parasite diversity of *C. pretoriae* from Nwanedi, Politsi, Lutanandwa and Mutshundudi rivers. Comparing parasite infestations and parasite diversity in the four different rivers using both infestation indices and diversity indices.

Chapter 4: Investigating the parasite diversity of *A. uranoscopus* from Nwanedi, Politsi, Lutanandwa and Mutshundudi rivers. Comparing parasite infestations in the four different rivers using the infestation indices.

Chapter 5: Investigating the water quality of the four localities, the health of *C. pretoriae* and *A. uranoscopus* from the four localities using the HAI, IPI and condition factor then parasite diversity and species richness of *C. pretoriae* and *A. uranoscopus*

is compared to the water quality of the four localities. Regression analysis was used to investigate the relationship between condition factor and parasite burden.

Chapter 6: Includes a summary of the overall results of the study and recommendations for future studies.

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CHAPTER 2

MATERIALS AND METHODS

2.1 Study area

The study was conducted in the Limpopo River System (Figure 2.1), which is located in the eastern part of southern Africa. It is the second largest river system in South Africa (Chilundo et al. 2008; Mosase et al. 2019). This system extends across four countries which include north-eastern South Africa, southern Zimbabwe, south-eastern Botswana and southern Mozambique (Rapolaki et al. 2019). It also forms a border between these four countries (van der Waal & Bills 2000) before it flows into the south-west Indian Ocean from Mozambique near the town of Xai-Xai (Rapolaki et al. 2019). It is about 1 770 km in length (Mosase et al. 2019) covering an area of approximately 413 300 km² (Kahinda et al. 2016). The area of the system covers 45% of South Africa, 19% of Botswana, 21% of Mozambique and 15% of Zimbabwe (Zhu & Ringler 2012). The rainfall in the different regions of the river system varies (Chilundo et al. 2008) with the average precipitation and annual runoff of 160 – 1100 mm and 5500 million m³, respectively (Sawunyama et al. 2006). Endemic freshwater fish species from the system are classified under 11 families and in total 25 species (Mheen 1997). It is home to over 14 million people divided between rural and urban areas (Zhu & Ringler 2012).

The upper reaches of the Limpopo River System form a border between Botswana and South Africa, the middle reaches form a border between Zimbabwe and South Africa and the lower reaches are entirely in Mozambique (Kahinda et al. 2016). The Shashe and Crocodile rivers are the two main tributaries of the upper reaches of the system and the Shashe River acts as a boundary between Zimbabwe and Botswana (Kahinda et al. 2016). The water in the upper reaches is mostly used for agriculture and domestic use with less quantities used for mining activities and power generation (Busari 2008). The lower Limpopo River System is comprised of the Limpopo River and its main tributary, the Olifants River. It is in an area that varies from both semi-arid to arid climate with the rainfall highly seasonal. The water from the lower reaches is mostly used for agricultural irrigation (Nhassengo et al. 2021). According to

Department of Water Affairs and Forestry DWAF (1996), most of the water from the overall system is used for agricultural irrigation.

The Nwanedi, Mutshundudi and Lutanandwa rivers form part of the Limpopo River while the Politsi River forms part of the Olifants River which then join the Limpopo River (Figure 2.1).

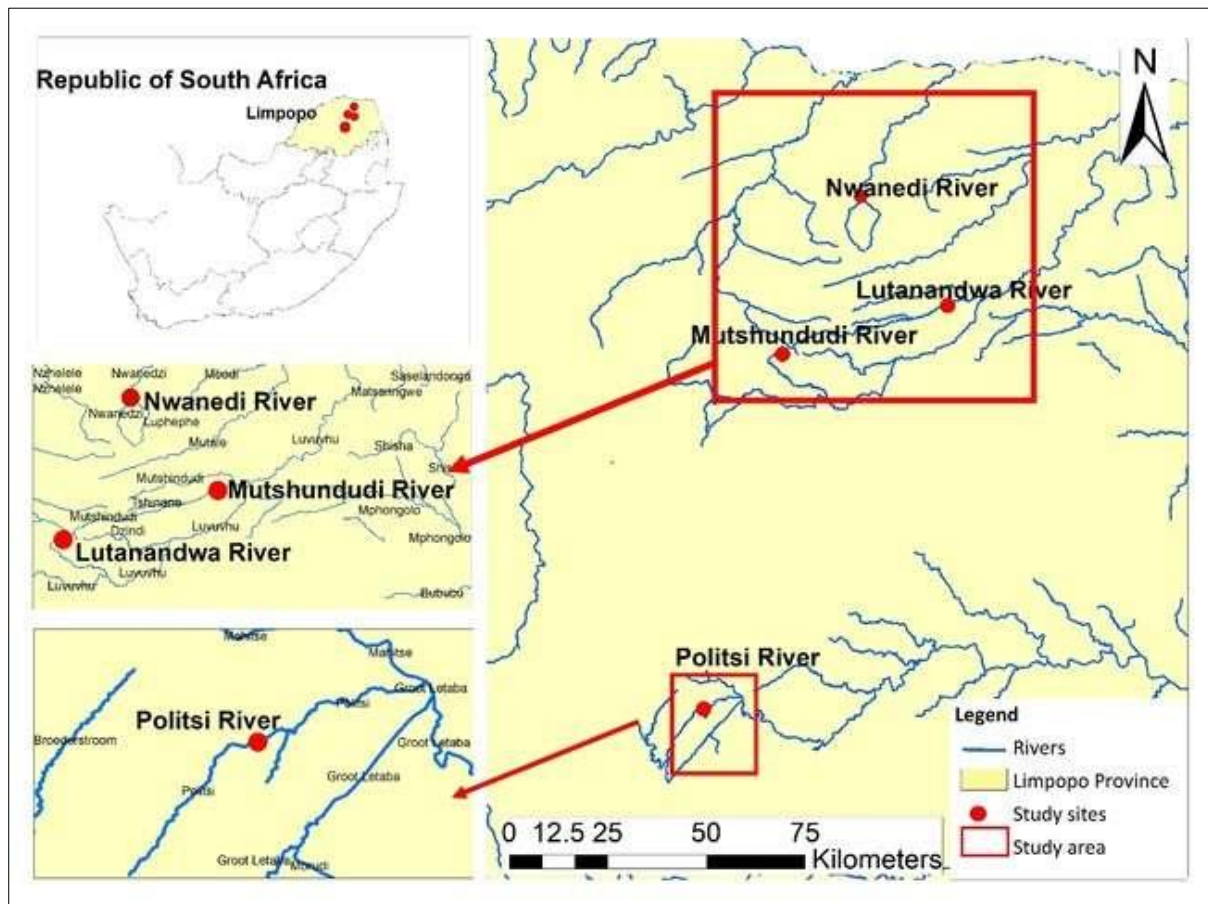


Figure 2.1: Location of the Limpopo River System in South Africa with the four sampling localities (Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers).

2.2 Sampling localities

Fish species (*C. pretoriae* and *A. uranoscopus*) were collected from the Nwanedi, Politsi, Mutshundudi and Lutanandwa rivers both within the Limpopo River System (Figure 2.1).

Nwanedi River (S 22°37'49'', E 30°23'59'')

The locality in the Nwanedi River is situated in the Nwanedi Reserve downstream of the confluence of the Luphephe and the Nwanedi rivers (Figures 2.1, 2.2). The Nwanedi River rises from the Southpansberg Mountains at an altitude of approximately 1100 meters above sea level and drops through a steep gorge before forming the Nwanedi-Luphephe Dams. The Nwanedi River then merges with the Luphephe River inside the Nwanedi Nature Reserve. From here the river continues to flow in the northern direction until it joins the Limpopo River at the Zimbabwe border. The river has a gross area of 1 136 km² with an annual runoff of 245 million m³ (Angliss 2007). Vegetation formed a canopy covering the locality, dominated by substrates and a lot of organic material.



Figure 2.2: The Nwanedi River in the Nwanedi Reserve.

Politsi River (S 23°49'0.9"; E 30°04'1.5")

The Politsi River is situated 1 km below Magoebaskloof Dam (Figures 2.1, 2.3) and the locality is dominated by cobbles with patches of sand and marginal vegetation. The flow of the locality is moderate to fast due to the prevailing cobbles and shallow riffles. This river is one of the Letaba River tributaries and it rises from the Northern Drakensberg Mountains between 1 100 to 1 800 meters above sea level and flows down the steep slopes in a north-easterly direction with very steep valleys. The land use close to the river is dominated by agriculture and forestry plantations (Fouche & Foord 2001).



Figure 2.3: The Politsi River.

Mutshundudi River (S 22°51'11", E 30°37'33.6")

Mutshundudi River is a small tributary of the Luvuvhu River which rises from the Soutpansberg Mountains (Figures 2.1, 2.4). Subsistence farming through plantations dominates the land use around the upper reaches of the river (Ramulifho et al. 2019). This tributary is dominated by rocks and grasses with no vegetation canopy.



Figure 2.4: The Mutshundudi River.

Lutanandwa River (S 22°59'56.4", E 30°14.6'30")

The Lutanandwa River is in the sub-catchment of the Luvuvhu River Basin (Figures 2.1, 2.5 A, B) occupying an area of 132.4 km² with an average annual rainfall of 1287 mm. The Lutanandwa River upper stream locality is situated close to a bridge and dominated by vegetation and plantation forming a canopy (Figure 2.5 A). The downstream locality situated close to the downstream Lutanandwa weir, and it is

dominated by vegetation forming a canopy (Figure 2.5 B). Forestry and agriculture are the main activities surrounding the catchment and the river (Obiero et al. 2019).



Figure 2.5: Lutanandwa River. A = upper stream, Lutanandwa River, B = downstream Lutanandwa River.

2.3 Selected fish species

2.3.1 *Chiloglanis pretoriae*

Order: Siluriformes Cuvier, 1817

Family: Mochokidae Jordan, 1923

Genus: *Chiloglanis* Peters, 1868

Species: *Chiloglanis pretoriae* van der Horst, 1931

Chiloglanis pretoriae (Figure 2.6) is a small-sized catfish with a maximum standard length of 6.5 cm. It is characterised by a sucker formed by jaws and lips which are used for holding on to objects in fast-flowing waters. It is generally small and has a forked caudal fin and a dorsal fin with one spine in the front. It has a dark colour with lighter patches on the sides (Skelton 2001).



Figure 2.6: Shortspine Suckermouth *Chiloglanis pretoriae* van der Horst, 1931.

Chiloglanis is a genus comprising of 35 species of which only eight species including *C. pretoriae* are found in southern Africa (Engelbrecht & Mulder 2000). *Chiloglanis pretoriae* is distributed throughout the Limpopo, Zambezi, Inkomati, Pungwe and Buzi river systems of southern Africa (Chakona et al. 2018). It is adapted to fast-flowing water, rocky riffles and rapids. It is very specific when it comes to habitat preferences and is vulnerable towards habitat destruction (Matlala et al. 2010).

2.3.2 *Amphilius uranoscopus*

Order: Siluriformes Cuvier, 1817

Family: Amphiliidae Regan, 1911

Genus: *Amphilius* Günther, 1864

Species: *Amphilius uranoscopus* (Pfeffer, 1889)

Amphilius uranoscopus (Figure 2.7) has a depressed body with a diffuse black stripe alongside its body. It has a forked caudal fin with a maximum total length of 19.5 cm. It has three pairs of barbels (Seegers et al. 2003; Thomson & Page 2010).



Figure 2.7: Stargazer mountain catfish *Amphilius uranoscopus* (Pfeffer, 1889).

The Stargazer mountain catfish is classified under Amphiliidae which has nine genera and more than 60 species. This species is native to South Africa and widespread around east and central Africa (Ngugi et al. 2009). It is nocturnal and it is found in clear upper streams in rocky habitats where there is rapid flowing water (Skelton 2001). It feeds on macroinvertebrates mainly on the order Trichoptera (Oosterhout et al. 2009). Although this fish species is not exploited commercially, its population is not abundant. Previous studies show that the species is being heavily predated by introduced exotic Rainbow trout *Oncorhynchus mykiss* Walbaum, 1792 (Ngugi et al. 2009; Oosterhout et al. 2009; Kadye et al. 2013). Levin et al. (2019) indicated that *A. uranoscopus* has the potential to be effective as a biological indicator for urban impacts on water quality and instream habitats.

2.4 Fish sampling and parasite collection

Sampling of fish was done between September 2021 and May 2022 (Permit number ZA/LP/109375; Animal Ethics approval AREC/06/2023: PG). Fish were collected by electrofishing (Figure 2.8 A) and identified in the field using a field guide (Skelton 2001) and were kept in well aerated containers (Figure 2.8 B). A field lab was set up on-site and fish were subjected to parasitological examinations (Figure 2.8 E). Skin/mucus smears were made from the body surface and fins of each host using microscope slides and they were examined for the possible presence of ectoparasites. Fish were then sacrificed by severing the spinal cord. Measurements of total, fork and standard

lengths were recorded (Figure 2.8 C), and the weight was recorded using an electronic balance (Figure 2.8 D). The fish were dissected and various organs (intestine, gall bladder, kidney, stomach) were separately examined for endoparasites. A stereomicroscope (LEICA EZ4) (Figure 2.8 F) was used to examine the mucus smears, fins and gills of the fish for ectoparasites and endoparasites (Figure 2.8 E).

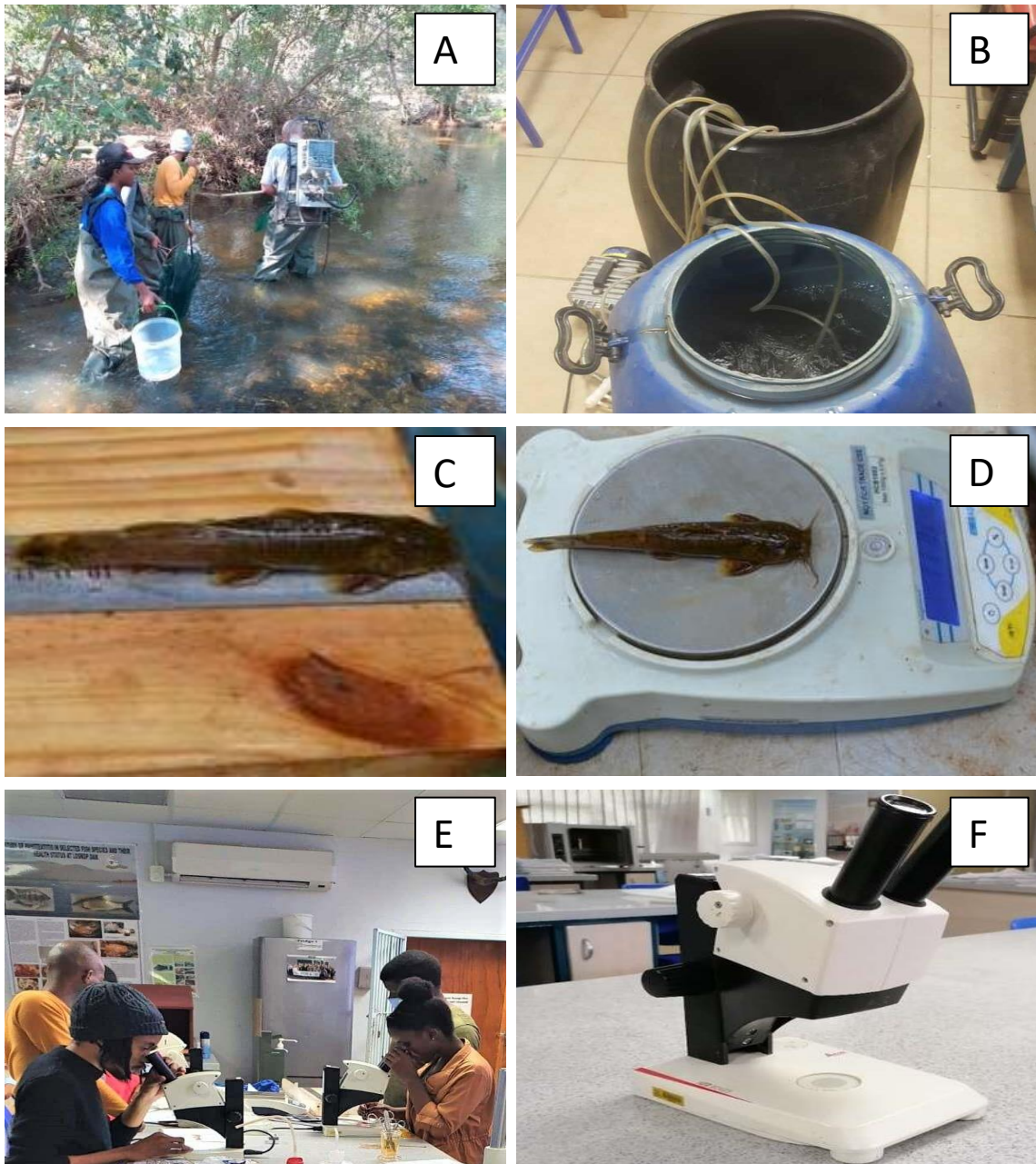


Figure 2.8: A = Electrofishing in Nwanedi River, B = Aerated fish tanks containing fish, C = Measuring fish length using a ruler, D = Weighing fish using the highland portable precision Balance-HCB 1002, E = Fish dissections and parasite examination, F = Stereomicroscope LEICA EZ4.

2.5 Parasite fixation

Parasite fixation and preservation for monogeneans were done following the guidelines proposed by Madanire-Moyo and Barson (2010). Monogeneans were mounted in ammonium picrate-glycerine (GAP) (Malmberg 1957). The slide was then sealed by using nail polish around the edges of the coverslip. Some of the monogeneans were cut in half and the anterior part was preserved in 96% ethanol for molecular analysis and the posterior part mounted on a slide for morphological analysis.

Smaller digeneans were directly placed in 70% ethanol while the large digeneans were fixed by placing them on a slide and using a cover slip to slightly press the specimen to straighten them. They were then placed in 70% ethanol as proposed by Cribb and Bray (2010). Nematodes were placed in a petri dish where after hot water was poured on them to uncoil and straighten the worms. Then they were placed in 70% ethanol. Leeches were preserved in 70% ethanol.

For staining, digeneans were fixed in hot Alcohol-Formol-Acid (AFA) and transferred to 70% ethanol. Digeneans were then placed in Acetocarmine, and the mixture transferred to 70% ethanol with 0.5% HCL for differentiation. They were then placed in 30%, 50%, 70%, 90%, 96% and 100% ethanol for 15 minutes during each dehydration step. The stained digeneans were cleared with creosote and mounted on a slide using Canada balsam as proposed by Thatcher (2006).

2.6 Morphological and molecular characterisation of parasites

Parasites were identified to the lowest taxonomic level using morphological features, their morphometrics and appropriate literature. Morphological features were studied using an Olympus BX50 compound microscope (Figure 2.9 A) and identified following the protocol for each parasitic group; monogeneans (Šimková et al. 2003), trematodes (Scholz et al. 2018, Vermaak et al. 2021) and nematodes (Moravec 2019, Nel et al. 2021). All measurements are expressed in micrometres (μm) unless stated differently. Drawings were made with the aid of a camera Lucida (Olympus BX50) image analysis software (Stream Essentials) (Figure 2.9 B) digitised with Adobe Illustrator® software (Adobe Inc., San Jose, California, USA) and a drawing tablet Wacom Intuos Pro (Wacom, Saitama, Japan) following the methods of Truter et al. (2017).

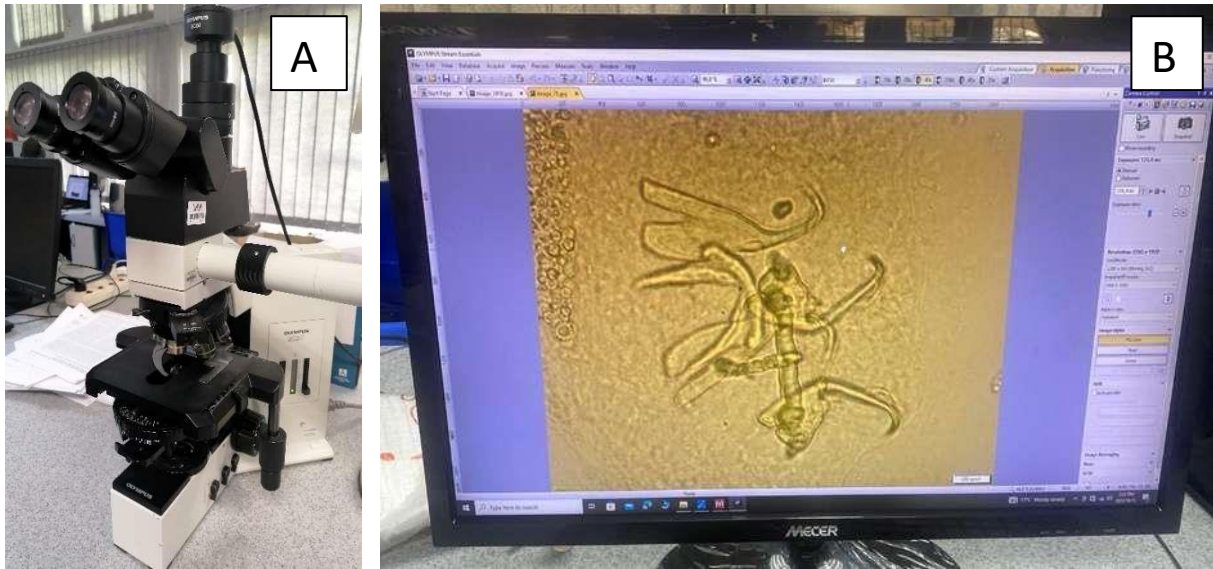


Figure 2.9: A = Compound microscope Olympus BX50, B = Stream Essentials image analysis software.

For scanning electron microscopy (SEM), the specimens which were preserved in 70% ethanol were prepared by dehydrating through graded ascending ethanol concentrations as per Rindoria et al. (2023). The samples were then dried using graded ascending series of Bis(trimethylsilyl)amine as outlined by Rindoria et al. (2023). The specimens were then transferred into a glass desiccator for 12 hours at room temperature and gold coated using a Quorum TM Q150T Emscope sputter coater (Quorum Technologies Ltd., Newhaven, U.K.) (Figure 2.10 B). The digeneans were then examined using a Zeiss Sigma 500VP scanning electron microscope (Jena, Germany) (Figure 2.10 A) at 4 kV acceleration voltages at the University of Limpopo.



Figure 2.10: A = Zeiss Sigma 500VP scanning electron microscope, B = Quorum TM Q150T Emscope sputter coater.

Molecular analysis was done at the Molecular Laboratory of the Water Research Group, North-West University, Potchefstroom Campus. The genomic DNA was extracted from the specimens which were preserved in 96% ethanol using PCRBio Rapid Extract Lysis Kit DNA (Figure 2.11) following manufacturer's instructions.

The partial 18S region of Dactylogyridae gen. sp. rDNA gene was amplified using the forward primer S1 (5'-ATTCCGATAACGAACGAGACT-3') and reverse primer IR8 (5'-GCTAGCTGCGTTCTTCATCGA-3'). Amplification reactions followed protocols optimised in Benovics et al. (2020). The partial 28S region was amplified using the forward primer C1 (5'-ACCCGCTGAATTTAAGCA-3') and reverse primer D2 (5'-TGGTCCGTGTTTCAAGAC-3') (Hassouna et al. 1984), following the PCR protocol optimised by Šimková et al. (2006).

Partial fragments of 28S rDNA of Cephalogonimidae gen. sp. and Diplostomidae gen. sp. were amplified using the forward primer combination DigI2 (5'-AAGCATATCACTAAGCGG-3') as per Snyder & Tkach (2001), and reverse primer 1500R (5'-GCTATCCTGAGGGAACTTCG-3') following Tkach et al. (2003).

PCR reactions were performed in a total volume of 25 μ L containing 1.25 μ L of each primer (10 μ M), 7 μ L of molecular-grade water, 12.5 μ L of DreamTaq™ Hot Start Green PCR Master Mix (2X) (ThermoFisher Scientific, Waltham, Massachusetts,

USA), and 3 μL of the DNA template, following the thermocycler conditions described by Tkach et al. (2003) and Van Steenkiste et al. (2015) for 28S rDNA for digeneans. Successful PCR products were verified using a 1% agarose gel electrophoresis and sent for purification and sequencing to Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa).



Figure 2.11: PCR Bio Rapid Extract Lysis Kit DNA. Adapted from: <https://pcrbio.com/app/uploads/PB15.11-08-1640x1231.jpg>.

Phylogenetic analysis

The sequences obtained from this study were inspected and aligned under default parameters of MUSCLE using MEGA X (Kumar et al. 2016). The resulting aligned sequences were subjected to a Basic Local Alignment Search Tool (BLAST, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Altschul et al. 1990) to identify the closest congeners. Alignments for each gene region were constructed in MEGA 7 (Kumar et al. 2016) and MrBayes using the CIPRES (Miller et al. 2010) computational resource. Phylogenetic trees [Maximum Likelihood (ML) and Bayesian Inference (BI)] generated were visualised in FigTree v1.4.4. (Rambaut 2018). The nodes with less than 60% bootstrap support were denoted with dashes. Uncorrected pairwise distances (p -distances) were estimated in MEGA7 according to Kumar et al. (2016).

2.7 Calculation of infestation indices, parasite diversity and species richness

The prevalence (percentage of hosts infested), mean abundance (mean number of parasites for all hosts examined) and mean intensity (mean number of parasites per infested host) of each parasite species found were calculated according to the protocol of Bush et al. (1997).

Shannon Wiener's index (H') was calculated according to Spellerberg and Fedor (2003) to determine the diversity of parasites for each locality. The Formula $H' = -\sum P_i \ln P_i$ was used to calculate, where: P_i = proportion of each species in the sample.

The evenness of the species which describes how equally the different species are distributed was calculated using the equation: $E' = H / \ln S$, where H = Shannon-Wiener diversity index; S = total number of species in a sample.

Margalef's richness index $(S-1) / \ln N$ was used as a simple measure of species richness where S is the number of species, and N is the number of individuals. Species richness refers to the number of different species represented in a community which can be calculated by simply counting the species available (Aslam 2009).

2.8 Determination of the Health Assessment Index

The Health Assessment Index (HAI) was determined for each fish following the protocol of Adams et al. (1993) and Heath et al. (2004). During dissection, the external and internal organs (stomach, fins, eyes, opercula, gills, mesenteric fat, spleen, hindgut, kidney, liver and gall bladder) were placed in different Petri dishes with distilled water to keep them hydrated. Internal organs were assessed using a colour chart developed by Watson (2001) (Figure 2.12) and a score was allocated to organs according to the condition of each following Adams et al. (1993) and Jooste et al. (2005) as shown in Table 2.1. The HAI value for each fish was calculated by adding the numerical values of the variables. The endo- and ectoparasites score were calculated using the inverted parasite index (IPI) [adapted from Jooste et al. (2005) and Crafford & Avenant-Oldewage (2009)] Table 2.2.



Figure 2.12: Colour chart used to compare the colour of liver, bile and spleen [Adapted from Watson (2001)].

Table 2.1: Fish health variables with assigned characters and values based on the norm and deviation from the norm [adapted from Adams et al. (1993) and Jooste et al. (2005)].

Variables	Condition of external variable	Original field designation	Substituted value for the HAI
Length	Total length in millimeters	Mm	-
Weight	Weight in grams	G	-
Eyes	Normal	N	0
	Exophthalmia	E1/E2	30
	Haemorrhagic	H1/H2	30
	Blind	B1/B2	30
	Missing	M1/M2	30
	Other	OT	30
Fins	No active erosion or previous erosion healed over	0	0
	Mild active erosion with no bleeding. >10 parasite cysts	1	10
	Severe active erosion with haemorrhage/secondary infection. >50 parasite cysts	2	20
Skin	Normal, no aberrations	0	0
	Mild skin aberrations. >10 parasite cysts	1	10
	Moderate skin aberrations. >50 parasite cysts	2	20
	Severe skin aberrations	3	30
Opercula	Normal/no shortening	0	0
	Mild/slight shortening	1	10
	Severe shortening	2	20
Gills	Normal	N	0
	Frayed	F	30
	Clubbed	C	30
	Marginate	M	30
	Pale	P	30
	Other	OT	30
Thymus	No haemorrhage	0	0
	Mild haemorrhage	1	10
	Moderate haemorrhage	2	20
	Severe haemorrhage	3	30

Mesenteric fat	(Internal body fat expressed regarding the amount present)		
	None	0	-
	Little where less than 50% of each cecum is covered	1	-
	50% of each cecum is covered	2	-
	More than 50% of each cecum is covered	3	-
	Cecae are completely covered by large amount of fat	4	-
Spleen	Black	B	0
	Red	R	0
	Granular	G	0
	Nodular	NO	30
	Enlarge	E	30
	Other	OT	30
Hindgut	Normal, no inflammation or reddening	0	0
	Slight inflammation or reddening	1	10
	Moderate inflammation or reddening	2	20
	Severe inflammation or reddening	3	30
Kidney	Normal	N	0
	Swollen	S	30
	Mottled	M	30
	Granular	G	30
	Urolithic	U	30
	Other	OT	30
Liver	Red	A	0
	Light red	B	30
	“Fatty” liver, “coffee with cream” colour	C	30
	Nodules in liver	D	30
	Focal discolouration	E	30
	General discolouration	F	30
	Other	OT	30
Bile	Yellow or straw colour, bladder empty or partially full	0	-
	Yellow or straw colour, bladder full, distended	1	-
	Light green to “grass” green	2	-
	Dark green to dark blue green	3	-
Blood (Haematocrit)	Normal range	30-45%	0
	Above normal range	>45%	10
	Below normal range	19-29%	20
	Below normal range	<18%	30

Endo- and ectoparasites were incorporated as a separate variable in the HAI tested in South Africa (Marx 1996; Robinson 1996; Luus-Powell 1997; Watson 2001), since contaminants affect endo- and ectoparasites differently. Endoparasites are usually much higher in number as compared to ectoparasites, where more than 1000 endoparasites can be observed in a single host, e.g. encysted digenean larvae of Diplostomidae gen. sp. Hence, the ranging values for endoparasites used in the current study were from zero to greater than 1000. Ectoparasites ranging values were from zero to greater than 20 (Table 2.2). Since ectoparasites are constantly exposed to the surrounding environment, they are expected to be found in water bodies with better water quality while larger numbers of endoparasites are expected in poorer water quality (depending on the specific group of parasites; see Gilbert & Avenant-Oldewage (2017) for a review on water quality affecting different types of parasites). The PI values of ectoparasites were inverted (the IPI) because zero is indicative of good water quality while 30 is representing poor water quality; thus if no ectoparasites were recorded it is indicative that the water quality is poor.

Table 2.2: Numerical scoring system for endo- and ectoparasites using the inverted parasite index (IPI) for ectoparasites [adapted from Jooste et al. (2005) and Crafford & Avenant-Oldewage (2009)].

Endoparasites		Ectoparasites	
Number	PI	Number	IPI
No parasites observed	0	No parasites observed	30
≤ 100	10	1 – 10	20
101 – 1000	20	11 – 20	10
> 1000	30	> 20	0

2.9 Determination of the condition factor

The condition factor (K) of the two fish species was calculated according to Heath et al. (2004). The formula $K = W \times 100 / L^3$ was used, where: W = weight in g; L = standard length in cm.

2.10 Determination of the water quality

A handheld multiparameter instrument (YSI Professional Plus) (Figure 2.13 A) was used to measure surface water quality parameters (Figure 2.13 B) [temperature (T), total dissolved solids (TDS), electrical conductivity (EC), dissolved oxygen (DO), pH and salinity] from each locality. Subsurface water samples were collected in acid-prepared sampling bottles during each survey. The samples were refrigerated immediately at -20°C and sent to an accredited laboratory (Capricorn Veterinary Laboratory, Polokwane) for analyses of selected water quality parameters which include ammonium, ammonia, nitrate, nitrite, fluoride, orthophosphate, sulphate and selected metals (aluminium, arsenic, cadmium, copper, manganese, mercury, lead, iron and zinc).

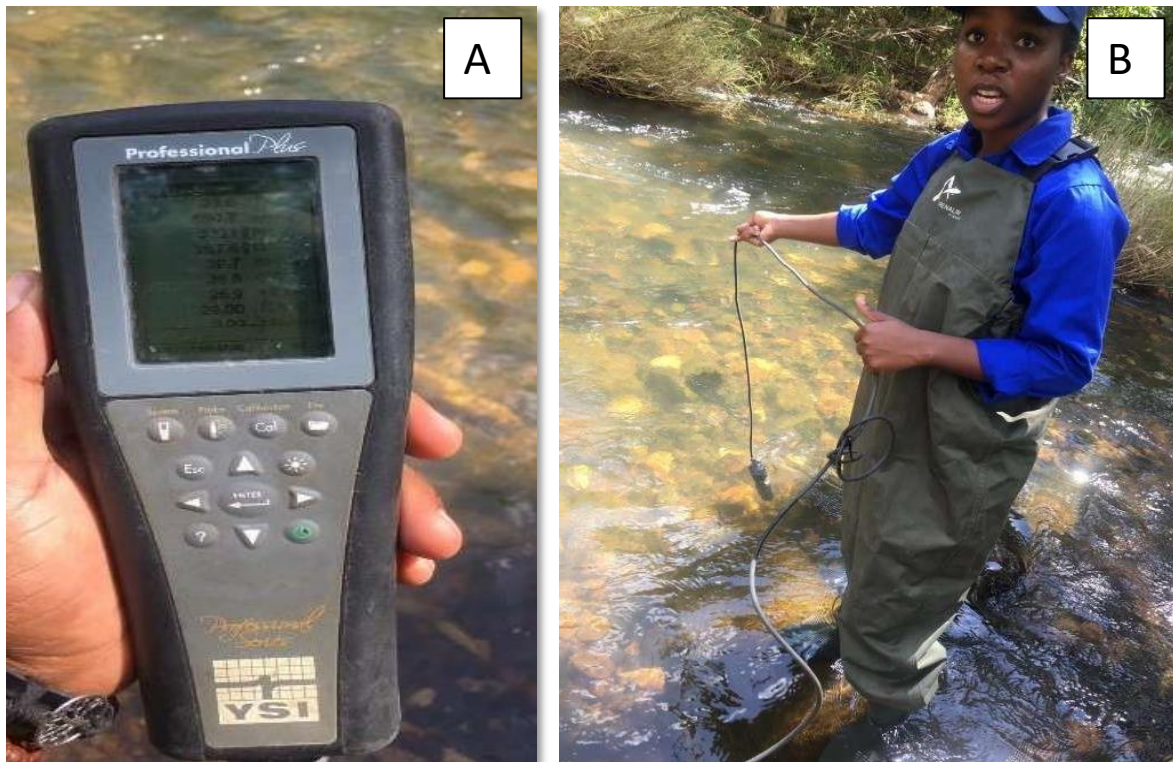


Figure 2.13: A = YSI Handheld multiparameter instrument (Professional plus), B = Measuring the surface water quality parameters.

2.11 Data analyses

One-way ANOVA was used to test the variance of *K* of *C. pretoriae* and *A. uranoscopus* from the four rivers. Regression analyses were used to test the

correlation between parasite burden and K . Statistical analyses were carried out using Microsoft Excel 2016.

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CHAPTER 3

PARASITE DIVERSITY OF *CHILOGLANIS PRETORIAE*

3.1 Introduction

African freshwater bodies contain almost 25% of the 13 000 freshwater fish species of which 670 species have known parasites (Moravec & Scholz 2017; Scholz et al. 2018). *Clarias gariepinus* Burchell, 1822 is one of the most studied freshwater fishes and has the highest number of more than 146 parasite species recorded followed by Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) infested by 54 parasite species (Scholz et al. 2018). *Oreochromis niloticus* is the African cichlid species with the highest number of formally reported helminth species (Scholz et al. 2018). Monogeneans, trematodes, nematodes and leeches are groups recovered from this study.

Monogeneans are majorly ectoparasitic, but few are endoparasitic flatworms, for example *Enterogyrus* Paperna, 1963 (Neves et al. 2020). They are mostly host specific and they have a direct life cycle which involves an infective free-swimming larva known as an oncomiracidium (Upadhyay & Rani 2019). They are divided into two groups according to their organ of attachment, the monopisthocotyleans with hook-like organs on the haptors and polyopisthocotyleans with clamp-like structures. They are found in a wide range of water temperatures (Öztürk & Özer 2014; Upadhyay & Rani 2019) and are commonly infesting freshwater and marine fishes, as well as invertebrates (Neves et al. 2020). Some of the adults become permanently attached to one host on a single site (Reed et al. 1996). More than 95% of monogeneans infest the fish's gills and skin and feed on the mucus (Euzet & Combes 1998). Their identification is mostly based on the difference in the shape and size of male copulatory organs and attachment structures which comprised of anchors, bars and marginal hooks (Iyaji & Eyo 2008). Monogeneans of the Dactylogyridae are among three families of monogeneans that have mostly been recorded from wild and captive fishes (Öztürk & Özer 2014). Due to their direct exposure to the water, the quality of water affects their infestation levels and their abundance on the hosts (Öztürk & Özer 2014).

Trematodes are parasitic flatworms with complex life cycles. The trematodes with developmental stages require intermediate hosts and to ensure completion of life their cycle, most exhibit reduced host specificity (Jousson et al. 2000). They have different larval stages, which are both parasitic and free-swimming, that need specific hosts to survive (Scholz et al. 2018). The first intermediate host is molluscs, especially gastropods and the second intermediate host can be organisms from several phyla, which are either invertebrate or vertebrate for example insects, molluscs, oligochaetes and fishes (Olsona et al. 2003; Iyaji & Eyo 2008). Their definitive host is almost always vertebrates (Bartoli & Boudouresque 2007).

Nematodes are unsegmented roundworms, pseudocoelomates with a hydrostatic skeleton (Abebe et al. 2008). They have complex life cycles; their eggs get deposited into the water through faeces of the final host, e.g., birds and humans. The eggs and larvae are then ingested by the first intermediate host which can be arthropods. Fish are the second intermediate host which harbour the third stage larvae (Vuic et al. 2022). They are distributed all over the world, occurring in soil habitats, marine habitats, and a few in freshwater ecosystems compared to the other habitats (Poinar 2020). Aquatic nematodes have a complex life cycle where fishes act as intermediate hosts as well as definitive hosts. Only some species of nematode parasites are host-specific in definitive hosts (Iyaji & Eyo 2008). Fishes have a relatively smaller number of nematode parasites compared to other vertebrate organisms (Anderson 1996). Their presence in fishes in small numbers cause no harm, but in large numbers, they can cause fatalities (Yanong 2002).

Leeches are hermaphroditic contractable segmented worms with anterior and posterior suckers. They are freshwater, marine and terrestrial ectoparasites of invertebrate and vertebrate organisms with a direct life cycle (Sket & Trontelj 2008). They often attach temporarily to the host although some attach to a host for most of their lives (Scholz et al. 2018). Damage to the site of infestation is observed on the host when there is heavy infestation of leeches which may kill the host (Iyaji & Eyo 2008). *Batracobdelloides tricarinata* (Blanchard, 1897) is the only leech identified as a parasite of African freshwater fish (Scholz et al. 2018).

Parasite diversity is affected by several factors including biotic (host and parasite sensitivity, host fitness, parasite life cycle) and abiotic factors (pollution, climate, and

eutrophication). Parasite distribution depends on the presence of intermediate and definite hosts. Although these parasites are directly affected by these factors, different groups are affected differently by the environment around them. The presence of many factors affects parasite diversity, which is why it's necessary to study the whole ecosystem (Wepener et al. 2022).

3.2 Materials and Methods

A total of 127 specimens of Shortspine Suckermouth, *C. pretoriae* (Nwanedi River = 52, Lutanandwa River = 40, Mutshundudi River = 21, Politsi River = 14) were collected for this study. Parasite collection, parasite fixation, preservation, identification and calculations of infestation levels, parasite diversity and parasite species richness indices methods are described in Chapter 2 sections 2.4; 2.5; 2.6 and 2.7.

3.3 Results

The fish collected had a total length ranging from 2.3 to 8.7 cm and a body mass ranging from 0.23 to 6.39 g. A total of 220 parasites representing three parasite groups (Monogenea, Digenea and Hirudinea) were collected from 47 fish. Parasites were most abundant in fish collected from the Mutshundudi River and less abundant in the Politsi River (Table 3.1). Digeneans were more abundant compared to the other groups.

Table 3.1: Total number of *Chiloglanis pretoriae* collected from Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers and the total number of parasites collected.

	Nwanedi	Lutanandwa	Mutshundudi	Politsi	Total
No. <i>C. pretoriae</i> collected	52	40	21	14	127
No. parasites collected	36	58	109	17	220

Parasites collected from *C. pretoriae* comprised of two ectoparasite species (monogenean Dactylogyridae gen. sp. and a leech) and three endoparasites species (*Clinostomum* sp., Diplostomidae gen. sp. larva and Cephalogonimidae gen. sp.). The number of endoparasites and ectoparasites collected from the four rivers are represented in Figure 3.1. Digeneans were overall the most abundant parasite group collected from *C. pretoriae* compared to monogeneans and hirudineans which were

recovered in lower numbers. The prevalence, mean intensity and mean abundance of the parasites are presented in Figures 3.4, 3.7, 3.13 and 3.16.

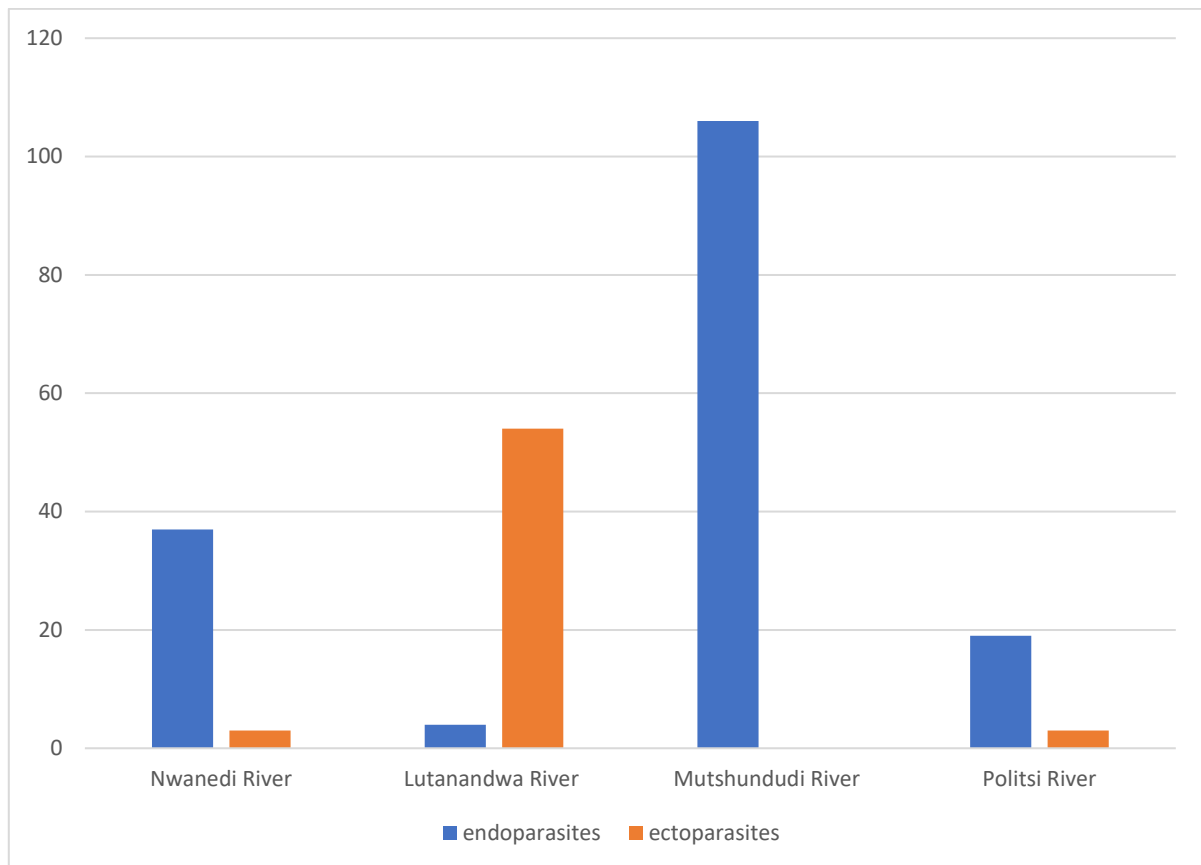


Figure 3.1: Total number of ectoparasites and endoparasites collected from *Chiloglanis pretoriae* from Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers.

3.3.1 Parasites of *Chiloglanis pretoriae*

Monogenea

ORDER: Polyonchoinea Bychowsky, 1937

FAMILY: Dactylogyridae (Bychowsky, 1933)

The monogenean Dactylogyridae gen. sp. was found on the gills of *C. pretoriae* collected from the Lutanandwa and Politsi rivers. The prevalence, mean intensity and mean abundance recorded at Lutanandwa River were higher than those recorded in Politsi River and presented in Figure 3.4. Morphological and molecular analysis is discussed below.

Morphology

The shape of the haptoral sclerites and the male copulatory organ do not look similar to any members of the genera within the Dactylogyridae. The morphology of the monogenean is shown in Figures 3.2 and 3.3.

Description: Based on 15 whole-mount specimens. Measurements in μm : mean \pm SD (minimum–maximum).

Body elongated 421.71 – 652.59 in length and 128.87 – 146.70 width (Figure 3.3 A). Four eye spots situated on extreme anterior of monogenean (Figure 3.3 A). A pair of ventral and dorsal anchors and two bars present (Figures 3.2; 3.3 B). Dorsal anchor with wide base, interior root 51.5 ± 3.1 (43.2 – 55.5), outer root 34.6 ± 2.7 (29.6 – 39.2) and short point 12.8 ± 1.2 (10.4 – 15.2) length (Figure 3.2). Ventral anchor with wide base 44.0 ± 3.9 (33.0 – 48.7) and elongated point 11.62 ± 1.8 (7.4 – 15.8) (Figure 3.2). Dorsal bar total length 35.9 ± 2.1 (32.24 – 39.40) and total width 5.5 ± 0.6 (4.0 – 6.6). Dorsal bar with two anterior projections (Figure 3.2). Ventral bar total length of 44.9 ± 2.2 (41 – 66-48.50). Anterior projection longer than posterior projection 4.6 ± 2.2 (3.3 – 6.7) vs 16.6 ± 2.2 (11.8 – 19.2) (Figure 3.2). Marginal hooks short and slender with handles 12.6 (11.0 – 14.1) (Figure 3.2). Male copulatory organ with a “C” shaped penis and a boat shaped accessory plate (Figures 3.2; 3.3 A; 3.3 C)

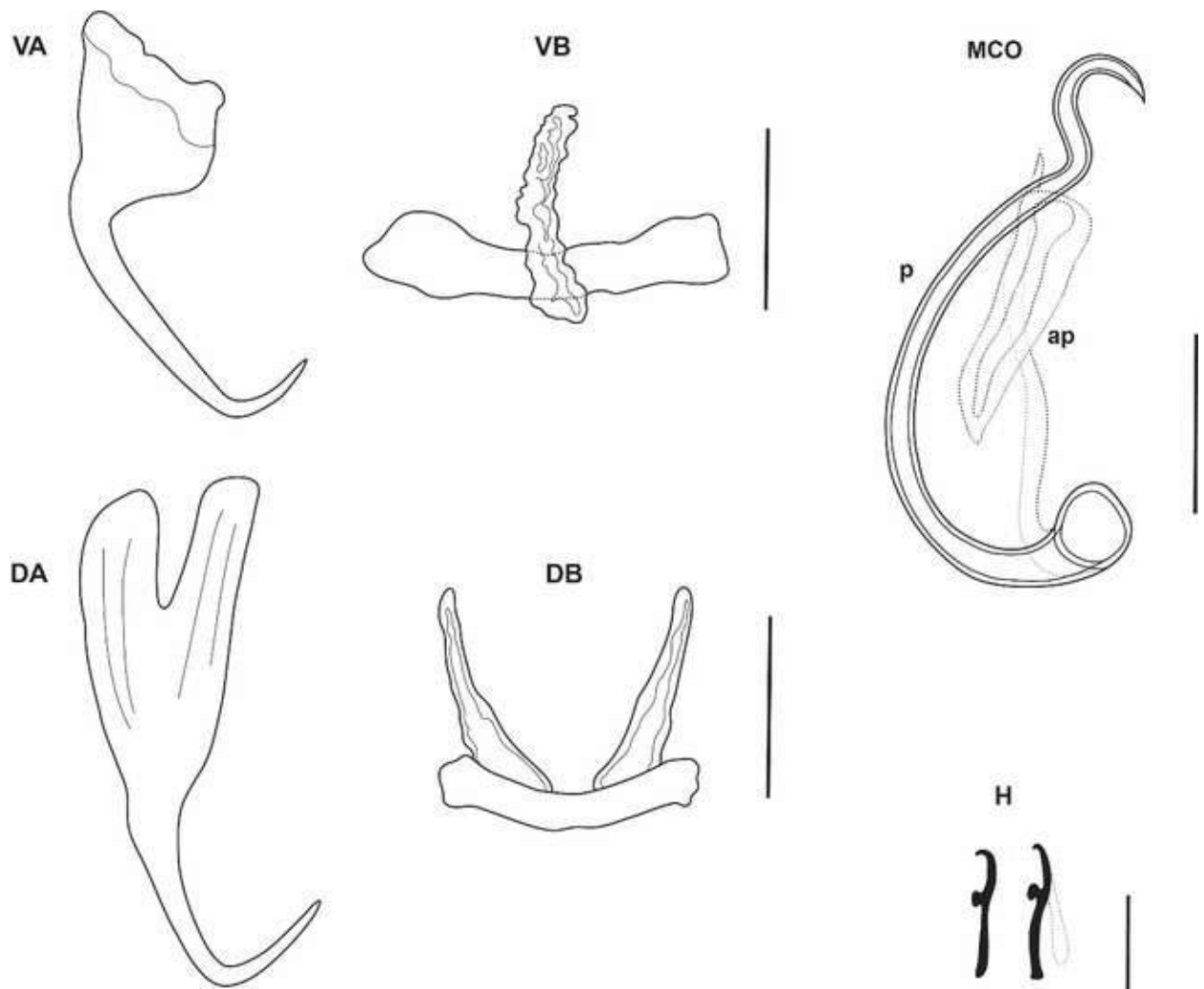


Figure 3.2: Line drawing of Dactylogyridae gen. sp. from *Chiloglanis pretoriae*. ap – Accessory plate, DA – Dorsal anchor, DB – Dorsal bar, H – Marginal hooks, MCO – Male copulatory organ, p – Penis, VA – Ventral anchor, VB – Ventral bar.

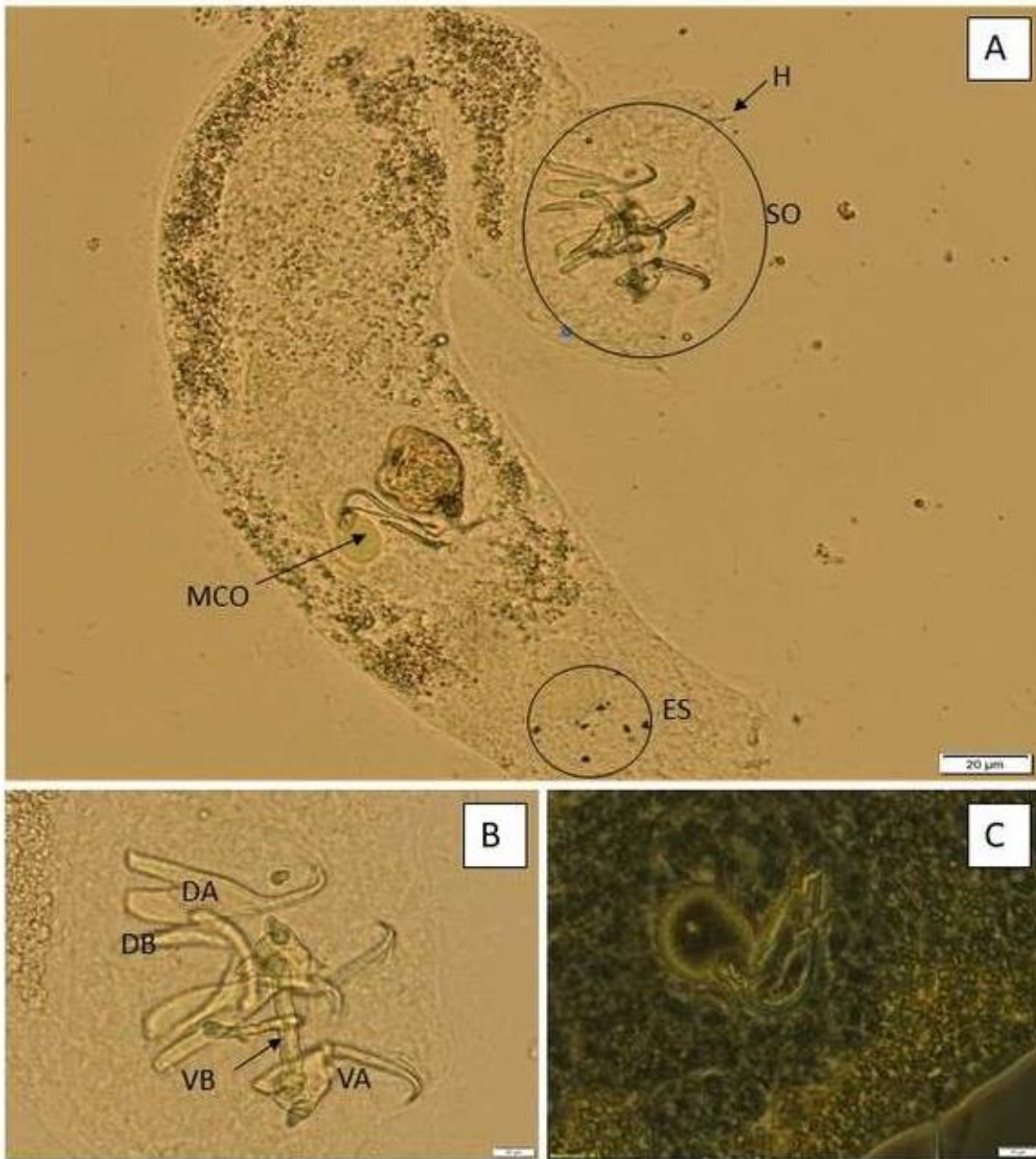


Figure 3.3: Micrograph of Dactylogyridae gen. sp. collected from the gills of *Chiloglanis pretoriae* from the Lutanandwa and Politsi rivers. A = Whole mount; B = Haptor sclerites of haptor; C = Male copulatory organ; DA = Dorsal anchor, DB = Dorsal bar, ES = eye spots, H = hook, MCO = Male copulatory organ, SO = haptor sclerites, VB = Ventral bar and VA = Ventral anchor.

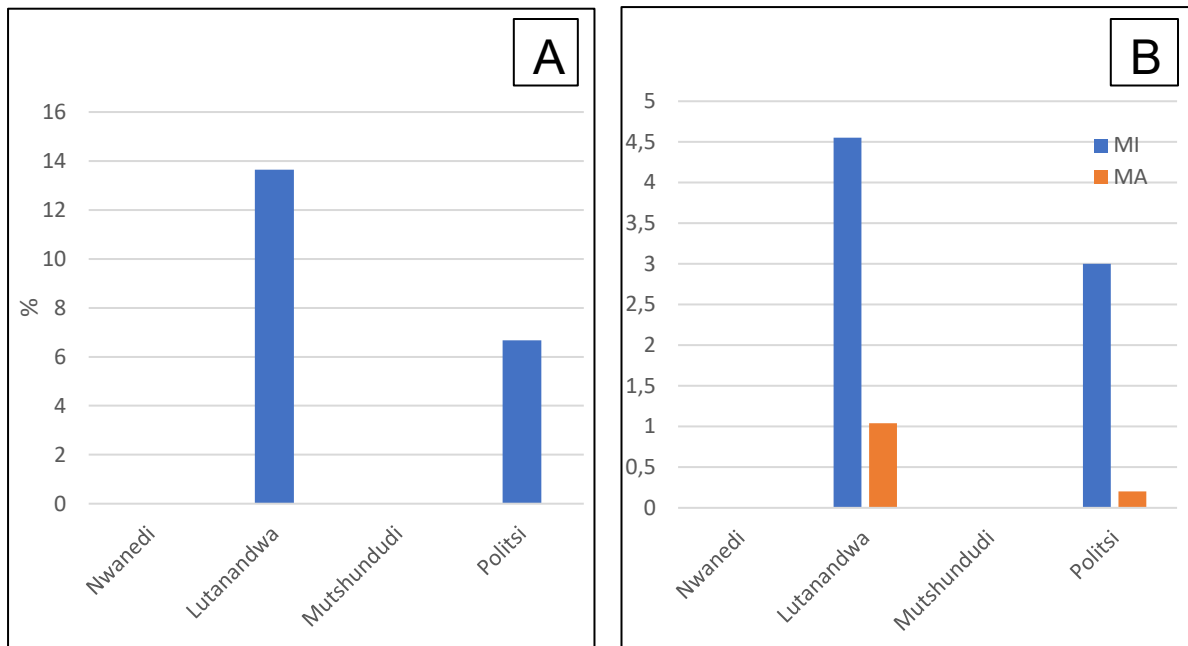


Figure 3.4: The prevalence (A), mean intensity and mean abundance (B) of the monogenean Dactylogyridae gen. sp. collected from the gills of *Chiloglanis pretoriae* from Lutanandwa and Politsi rivers. MA - mean abundance. MI - mean intensity.

Molecular results

Sequences of partial 18S and 28S rDNA 826 bp and 756 bp, respectively, were generated for Dactylogyridae gen. sp., and consensus trees were constructed (see Figure 3.5). Tree based on 18S sequences indicated that *Quadriacanthus* Paperna, 1961 and *Bykhowskyella* Achmerow, 1952 cluster did not lie on the same clade with other African genera (*Schilbetrema* Paperna and Thurston, 1968 with *Synodontella zambezensis* Douellou et Chishawa, 1995) and in the absence of *Pseudoancylodiscoides* Yamaguti, 1963 sequences (Figure 3.5 A). The generated tree based on 28S sequences consists of two main lineages (Figure 3.5 B). The first lineage includes species of genera *Thaparocleidus* Jain, 1952 and *Pseudoancylodiscoides*. In the second lineage, a very strong bootstrap support with two main clusters was observed: (1) African representative, *Quadriacanthus*, together with *Bykhowskyella* and (2) other two African genera (*Schilbetrema* sp. with *S. zambezensis*) and *Chauhunellus* Bychowsky & Nagibina, 1969. The newly identified genus appeared to be closest related to *Schilbetrema* sp. from *S. zambezensis* (Figures 3.5A, 3.5B).

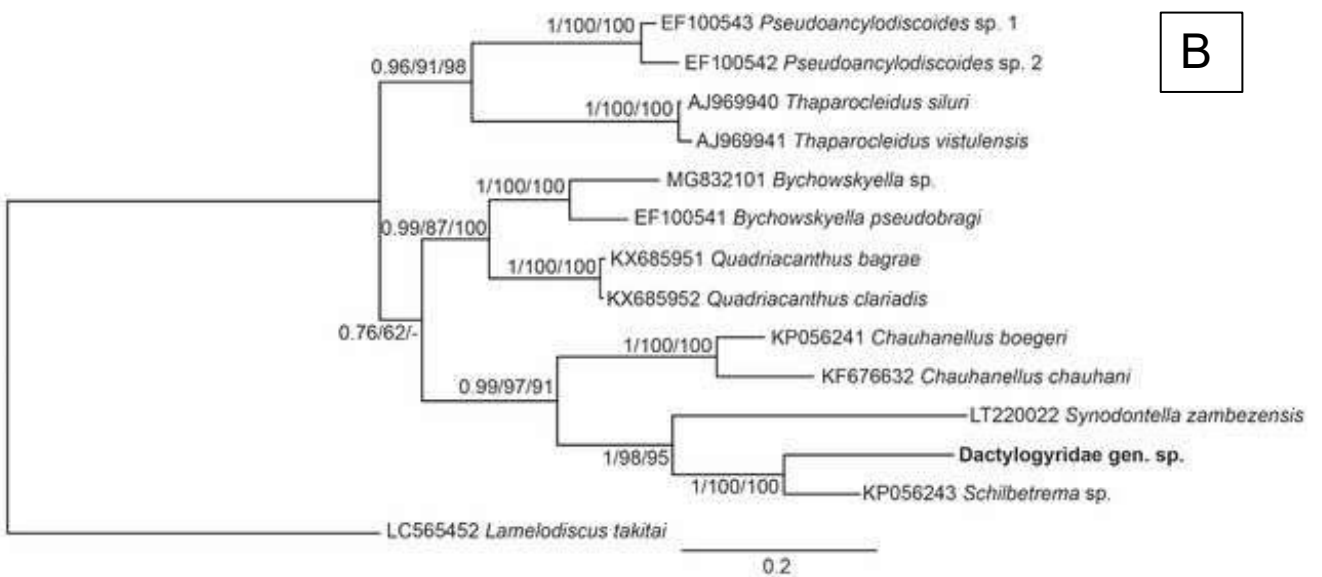
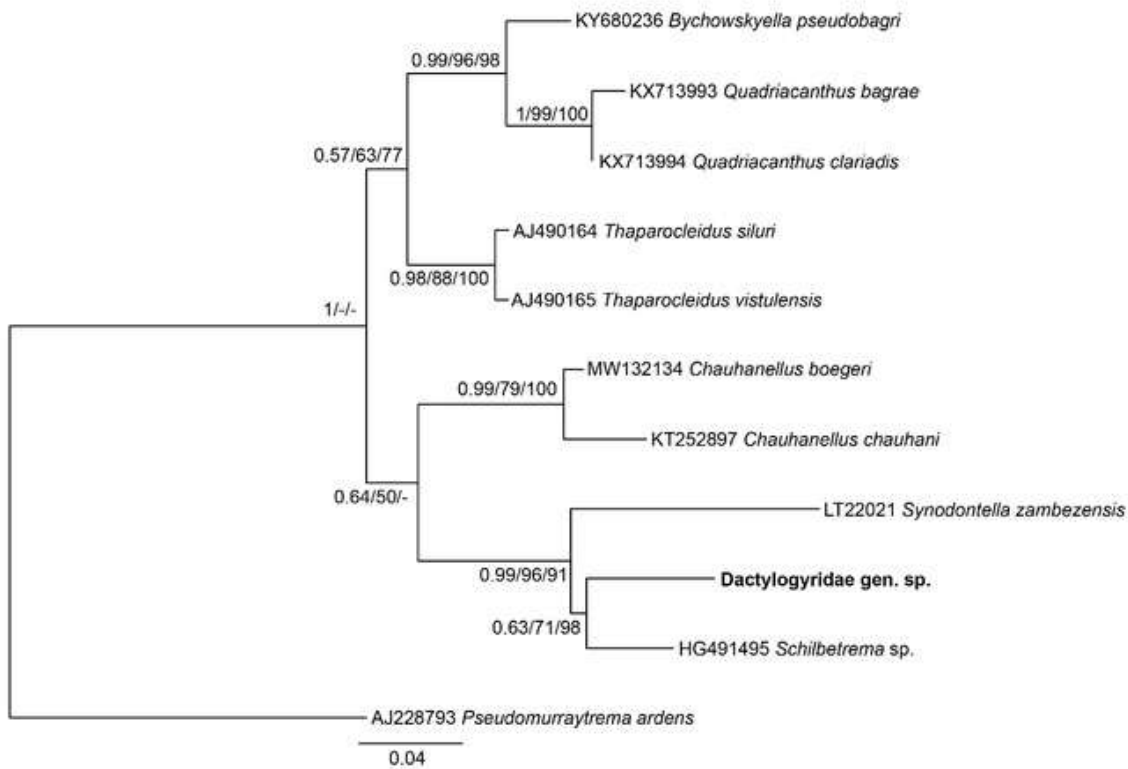


Figure 3.5: Phylogenetic tree based on maximum likelihood inferred from analysis of 18S (A) and 28S (B) rDNA data for *Dactylogyridae* gen. sp. with *Pseudomurraytrema ardens* Littlewood, Rohde & Clough, 1998 and *Lamelodiscus takitai* Ogawa & Eugusa, 1978 the designated outgroups, respectively. Bootstrap support values for both Bayesian inference and maximum likelihood, and neighbour-joining (BI/ML/NJ) are indicated at the respective nodes. Nodes with bootstrap values lower than 60% are indicated with dashes.

Based on 18S rDNA, the p -distances between the new genus and the representatives of the clusters were 6.52% – 18.20%. The base pair differences between the clusters and the new genus ranged from 27 to 75. *Schilbetrema* had the smallest base pair difference and a few base pair differences as compared to the other clusters as shown in Table 3.2. Based on 28S rDNA the p -distances were between 17% – 38% and the base pair differences between the clusters and the new genus ranged from 103 to 231 base pairs as shown in Table 3.3. *Schilbetrema* had the smallest p -difference and a few base pair differences as compared to the other clusters in both 18S and 28S rDNA.

Table 3.2: Pairwise genetic distances (%) (lower diagonal) and base pair difference (upper diagonal) between taxa included in the phylogenetic analysis of *Dactylogyridae* gen. sp. and other *Dactylogyridae* species based on 18S rDNA.

	1	2	3	4	5	6	7	8	9	10	11
1. <i>Dactylogyridae</i> gen. sp.		53	49	55	75	52	52	27	45	52	48
2. <i>Bychowskyella pseudobagri</i>	12.80		40	41	67	22	20	47	51	33	33
3. <i>Chauhanellus boegeri</i>	11.81	9.66		15	70	47	47	44	58	38	37
4. <i>Chauhanellus chauhani</i>	13.25	9.90	3.61		68	50	52	49	57	44	43
5. <i>Pseudomurraytrema ardens</i>	18.20	16.26	16.99	16.50		70	68	71	72	66	65
6. <i>Quadriacanthus bagrae</i>	12.53	5.31	11.33	12.05	16.99		5	52	59	36	34
7. <i>Quadriacanthus clariadis</i>	12.53	4.83	11.33	12.53	16.50	1.20		50	57	32	32
8. <i>Schilbetrema</i> sp.	6.52	11.38	10.63	11.84	17.27	12.56	12.08		40	43	45
9. <i>Synodontella zambezensis</i>	10.84	12.32	13.98	13.73	17.48	14.22	13.73	9.66		51	53
10. <i>Thaparocleidus siluri</i>	12.53	7.97	9.16	10.60	16.02	8.67	7.71	10.39	12.29		4
11. <i>Thaparocleidus vistulensis</i>	11.57	7.97	8.92	10.36	15.78	8.19	7.71	10.87	12.77	0.96	

Table 3.3: Pairwise genetic distances (%) (lower diagonal) and base pair difference (upper diagonal) between taxa included in the phylogenetic analysis of Dactylogyridae gen. sp. and other Dactylogyridae species based on 28S rDNA.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Dactylogyridae gen. sp.		190	190	182	180	185	192	188	184	103	175	201	196	231
2. <i>Bychowskyella</i> sp.	31.5		74	166	183	116	161	163	118	184	193	161	165	229
3. <i>Bychowskyella pseudobagri</i>	31.4	12.0		168	181	107	161	170	106	188	195	161	163	226
4. <i>Chauhanellus boegeri</i>	30.0	27.0	27.4		71	163	187	184	164	161	182	190	192	226
5. <i>Chaunhanellus chauhani</i>	29.7	29.7	29.4	11.5		177	190	193	173	161	181	197	200	237
6. <i>Quadriacanthus bagrae</i>	30.6	18.9	17.4	26.5	28.7		160	165	5	170	185	162	163	214
7. <i>pseudoancylodiscoides</i> sp. 1	31.8	26.3	26.3	30.5	30.8	26.1		30	161	186	198	145	146	232
8. <i>Pseudoancylodicoisdes</i> sp. 2	31.2	26.6	27.8	30.0	31.4	26.9	4.9		166	180	203	152	151	231
9. <i>Quadriacanthus clariadis</i>	30.4	19.2	17.2	26.7	28.0	0.8	26.2	27.1		171	186	161	162	215
10. <i>Schilbetrema</i> sp.	17.0	30.5	31.1	26.6	26.6	28.1	30.8	29.9	28.3		159	190	188	228
11. <i>Synodontella</i> sp.	28.9	31.9	32.2	30.0	29.8	30.5	32.7	33.6	30.7	26.3		204	209	240
12. <i>Thaparocleidus siluri</i>	33.4	26.4	26.4	31.1	32.1	26.5	23.7	24.9	26.4	31.7	33.9		9	225
13. <i>Thaparocleidus vistulensis</i>	32.8	27.2	26.9	31.6	32.8	26.8	24.0	24.8	26.6	31.5	34.9	1.5		227
14. <i>Lamelodiscus</i> sp.	38.2	37.3	36.8	36.7	38.4	34.8	37.8	37.7	35.0	37.7	39.6	36.8	37.3	

Trematoda

ORDER: Strigeatida (Larue, 1926) Sudarikou, 1959

FAMILY: Clinostomidae Lühe, 1901

GENUS: *Clinostomum* Leidy, 1856

Clinostomum sp. (Figure 3.6) was found in the branchial cavity of *C. pretoriae* from Nwanedi (n = 3) and Mutshundudi (n = 1). The highest prevalence and mean abundance of 5.77% and 0.06, respectively, were recorded from fish collected from the Nwanedi River. The mean intensity was 1 in both rivers (Figure 3.7).

Morphology

The body elongated with a small pharynx. Ventral sucker larger than oral sucker, both surrounded by oral collar. Long intestine and body widest below ventral sucker (Figure 3.6).

Remarks

The parasite found is similar to *Clinostomum ukoli* described by Caffara et al. (2020) which was found under skin tissue of *Synodontis bantensoda* (Siluriformes: Mochokidae). The present parasite has great similarities with *C. ukoli* in terms of the body shape and position of the oral and ventral sucker. Metacercaria stages of *Clinostomum* spp. are found in fish and adults found in birds that feed on fishes and molluscs (Rosser et al. 2017). Identification of this parasite is mostly possible through morphological analysis of adults since metacercaria have similar structures, thus the specimen was not identified to species level (Shamsi et al. 2021).

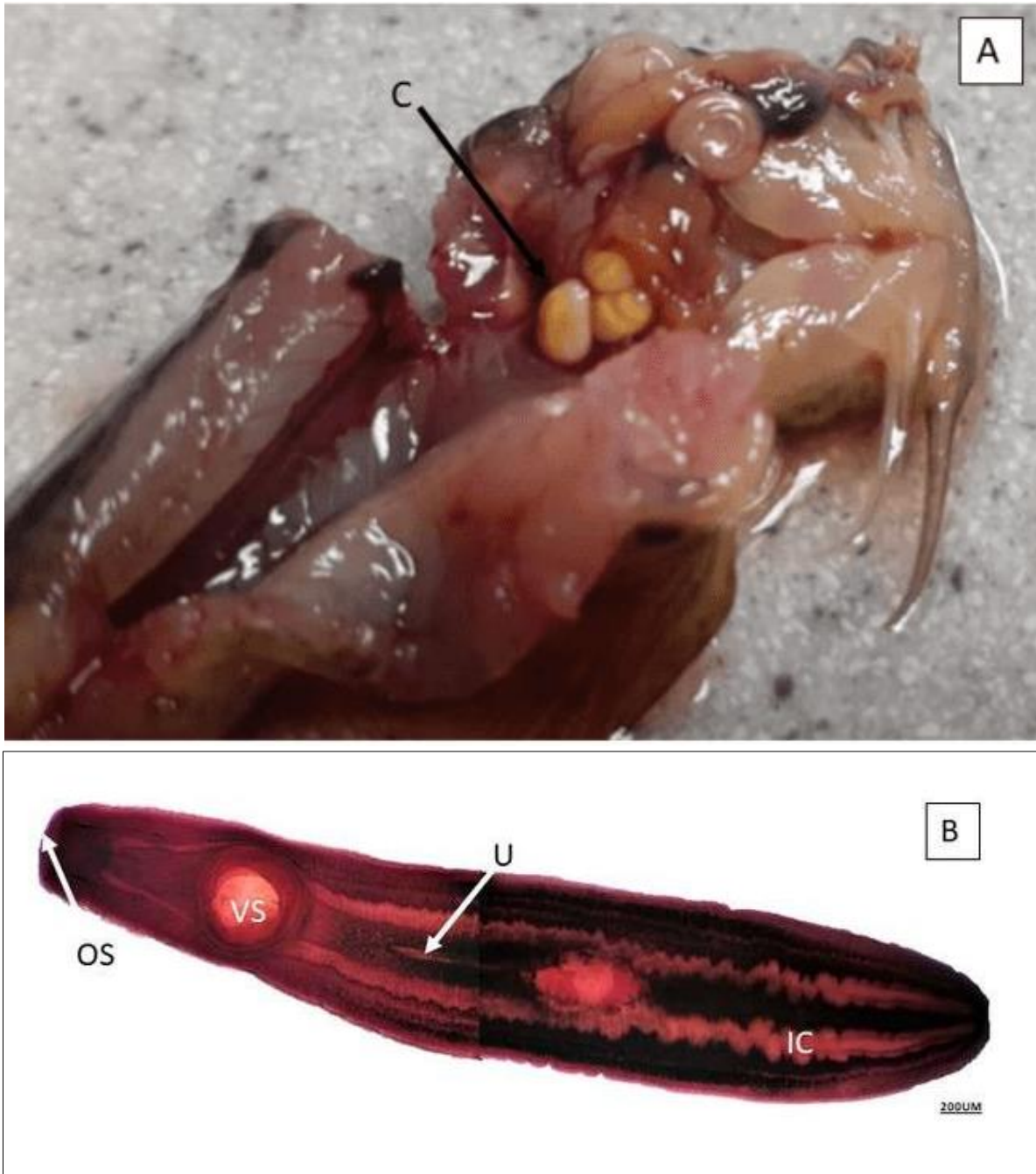


Figure 3.6: Micrographs of *Clinostomum* sp. metacercaria larva collected from the branchial cavity of *Chiloglanis pretoriae*; A = *Clinostomum* sp. cyst on the branchial cavity; B = whole mount; C = *Clinostomum* sp. cyst, IC = intestinal cecum, OS = oral sucker; VS= Ventral sucker; U = uterus.

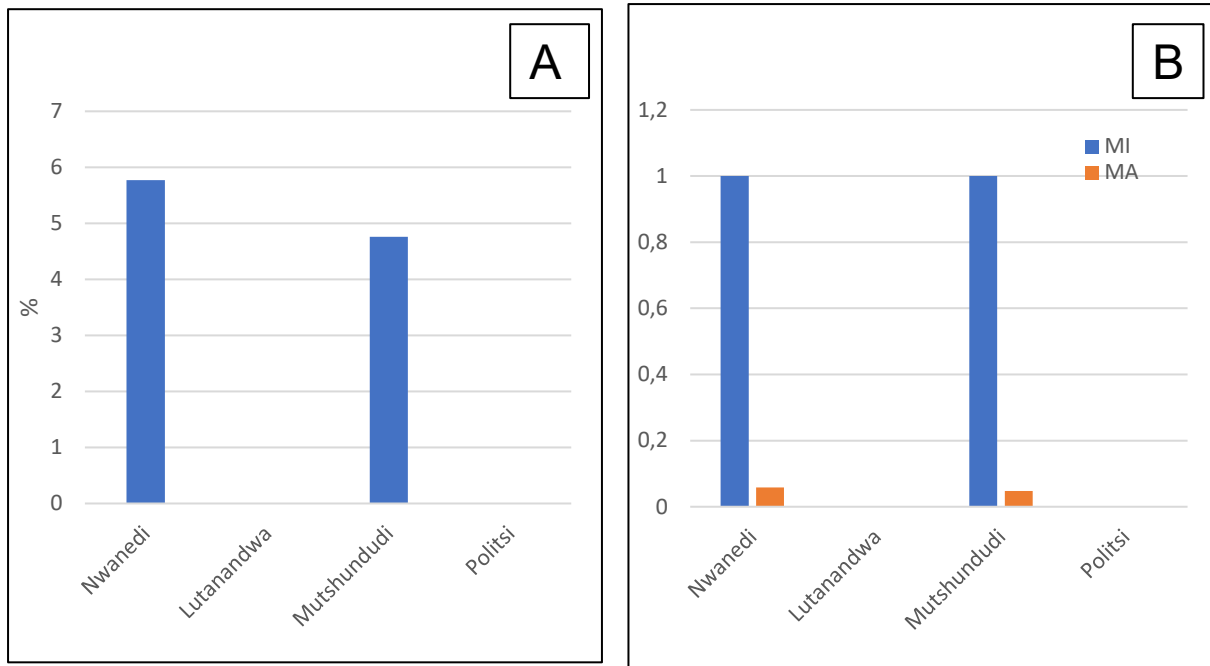


Figure 3.7: Prevalence (A), mean intensity and mean abundance (B) of *Clinostomum* sp. larva collected from the branchial cavity of *Chiloglanis pretoriae* from Nwanedi and Mutshundudi rivers. MA - mean abundance. MI - mean intensity.

ORDER: Plagiorchiida La Rue, 1957

FAMILY: Cephalogonimidae Looss, 1899

Morphology

Description: based on 10 whole mounts of Cephalogonimidae gen. sp. (Figure 3.9) and two specimens observed using SEM (Figure 3.10). Mean (minimum–maximum) measurements are in μm .

Body short, oval, and dorsoventrally flattened with 1099.0 (778 – 1587.5) long and 459.5 (371.8 – 607.5) wide (Figures 3.8, 3.9 A, 3.10 A). Body covered with spines, larger and many on the anterior end (Figure 3.10 B, C, D, F) compared to the posterior end (Figure 3.10 F). Oral sucker with length of 143.0 (106.4 – 156.7), cone shaped internally, longer than wide (Figure 3.9 C) and covered with two rows of circumoral spines (Figures 3.9 E, 3.10 B). Oral sucker and ventral sucker slightly similar in diameter [126.3 (110.9–169.4) vs 133.4 (114.8 – 128.4)]. Ventral sucker spherical with oral collar (Figures 3.8, 3.9 B). Long distance between the oral and ventral sucker

231.2 (170.2 – 336.0). Short pre-pharynx of 33.9 (21.5 – 42.5), long and small pharynx with the length of 48.1 (36.8 – 57.7) (Figure 3.8. 3.9 C). The male reproductive system consists of two testes situated at the posterior end with a diameter of 159.7 (93.6 – 237.3) (Figures 3.8, 3.9 D). The anterior testes oval and the posterior testes spherical in an oblique position. Elongated cirrus sac that covers space between the ventral sucker and the pharynx with length measuring 491.5 (317.9 – 851.9) long and 71.1 (60.9 – 110) wide (Figure 3.8). Female reproductive system with an oval ovary of 138.7 (129.4 – 152.7) long, close to the ventral sucker (Figure 3.8). Eggs oval measuring 22.7 (20.9 – 25.4) long. Excretory pore aligned with the oral sucker with the area surrounding it lacking spines (Figure 3.10 E).

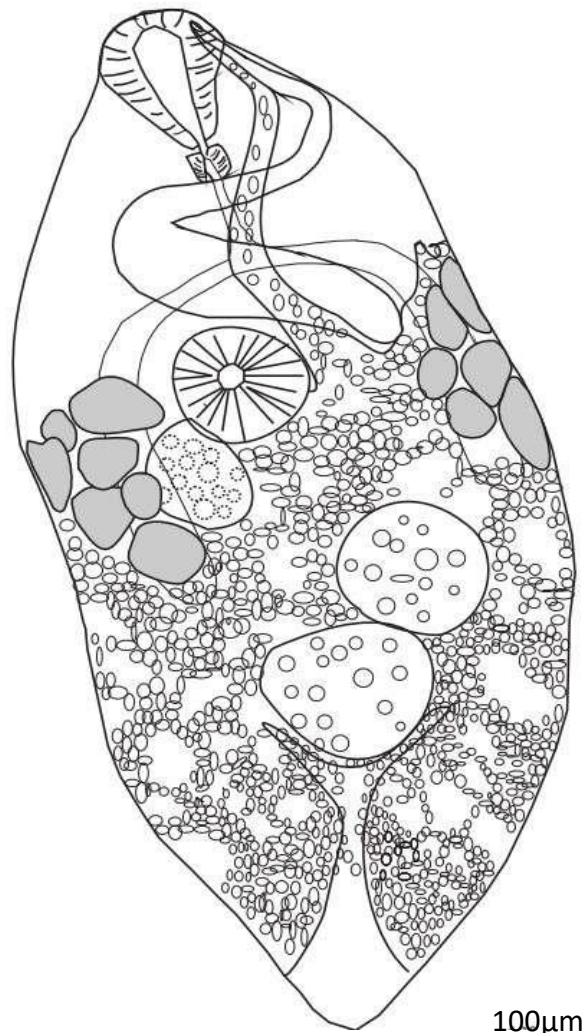


Figure 3.8: Line drawing of Cephalogonimidae gen. sp. from the intestine of *Chiloglanis pretoriae* from the Politsi River.

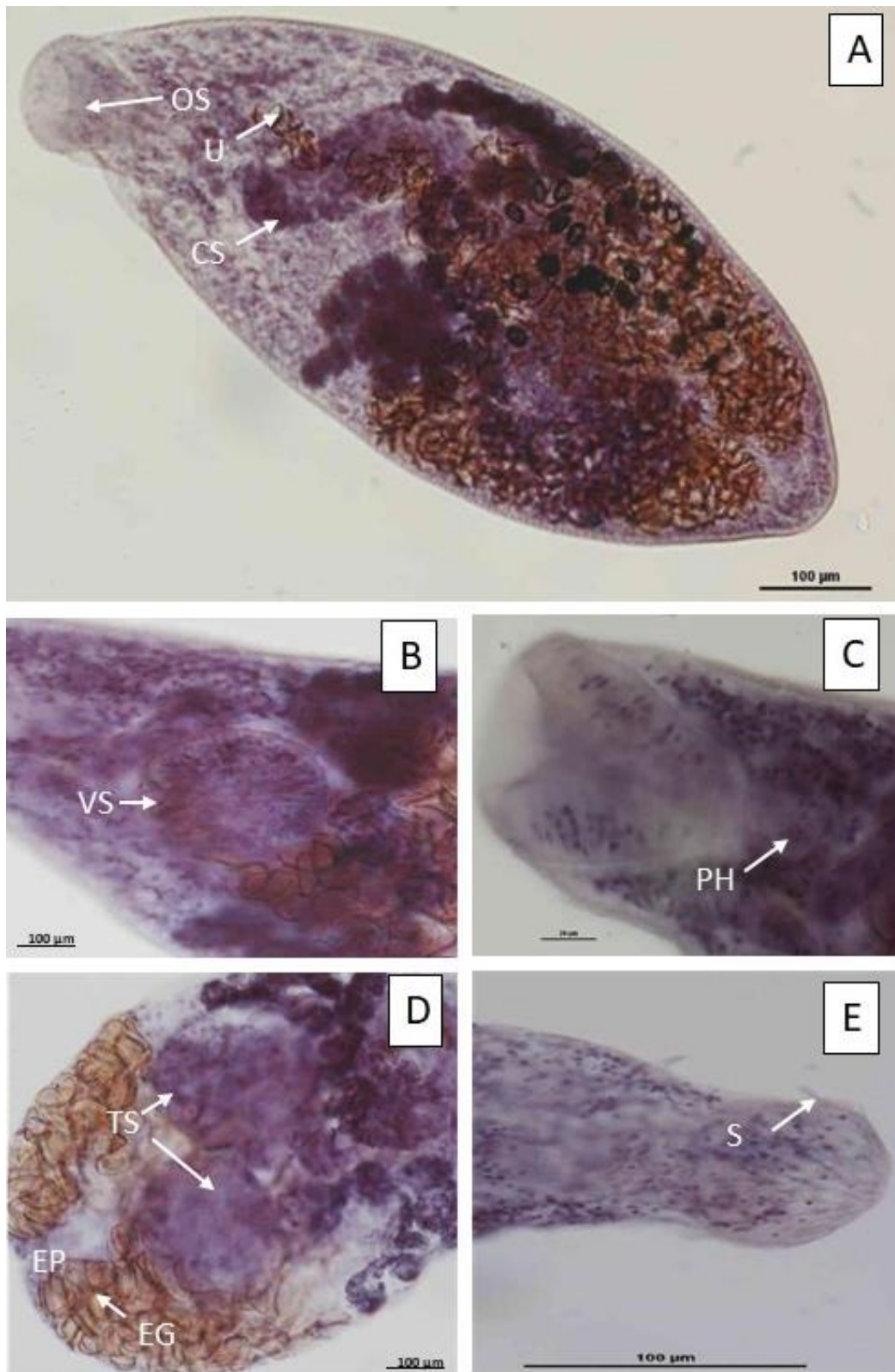


Figure 3.9: Micrographs of Cephalogonimidae gen. sp. collected from intestine of *Chiloglanis pretoriae* from Politsi River. A = whole mount; B = middle anterior part; C = Oral sucker; D = posterior part; E = Anterior part; CS = cirrus sac, EG = eggs, EP = excretory pore, OS = Oral sucker, PH = pharynx, S = circumoral spines, TS = testis, U = uterus with eggs, VS = ventral sucker.

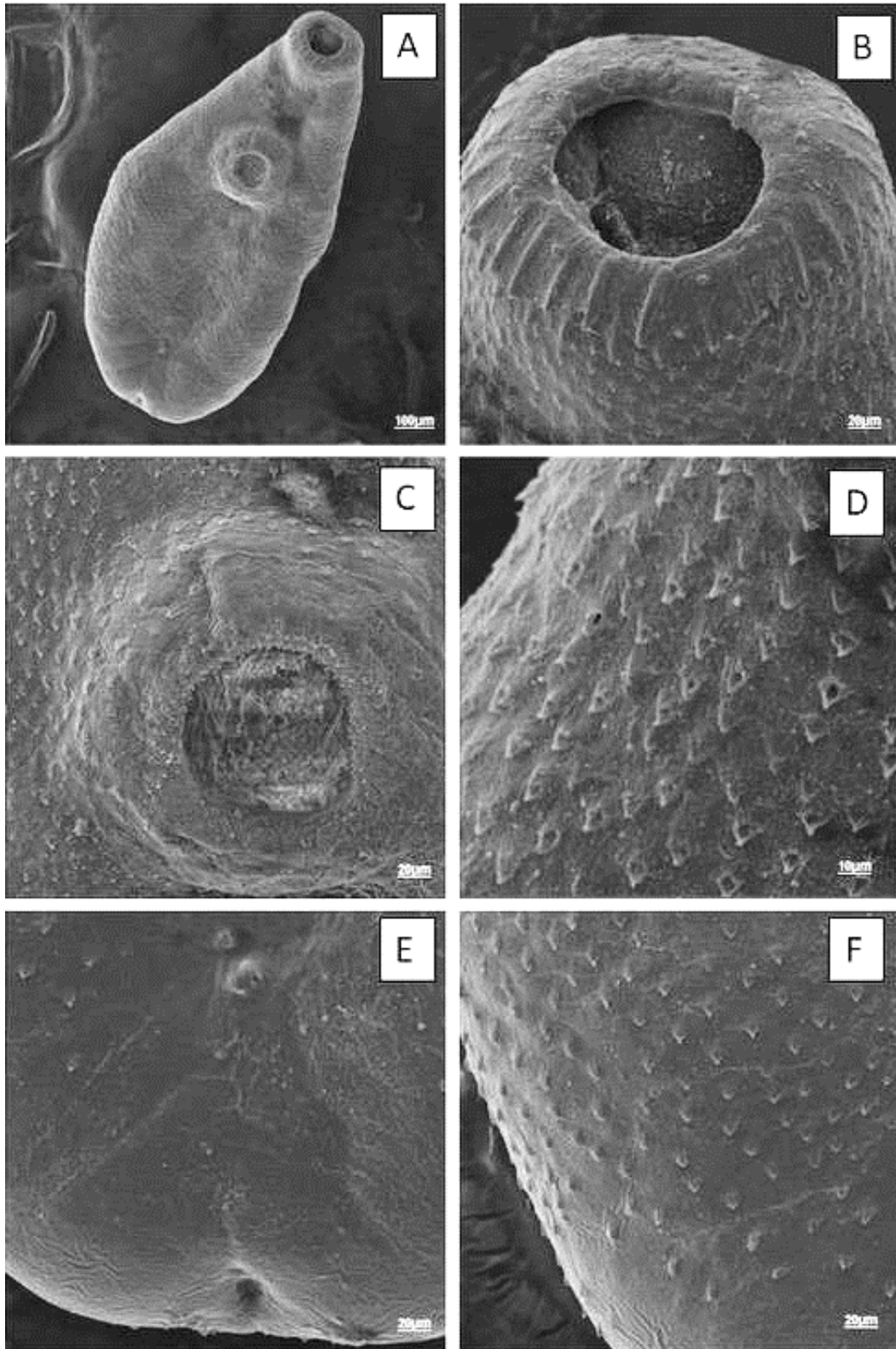


Figure 3.10: Scanning electron micrographs of Cephalogonimidae gen sp. from the intestine of *Chiloglanis pretoriae* and *Amphilius uranoscopus* from Politsi River. A = whole mount, B = oral sucker, C = ventral sucker, D = tegumental features between oral sucker and ventral sucker, E = anal opening, F = tegumental features of the posterior end.

Cephalogonimidae gen. sp. was found in both *C. pretoriae* and *A. uranoscopus*. The average width and length of the digenean found in *C. pretoriae* were higher than the ones found in *A. uranoscopus*. The average size of pre-pharynx and pharynx of the digenean found in *A. uranoscopus* is also smaller than that of the digenean found in *C. pretoriae*. The rest of the features are larger than those of *C. pretoriae*. The measurements are presented in Table 3.4.

Table 3.4: Measurements of Cephalogonimidae gen. sp. found in the intestine of *Chiloglanis pretoriae* and *Amphilius uranoscopus* from Politsi River. Mean (minimum-maximum) based on 10 specimens.

Measurement	<i>C. pretoriae</i>	<i>A. uranoscopus</i>
Width	470.4 (382.3 – 527.0)	451.8 (371.86 – 607.5)
Length	1120.5 (1092.7 – 1233.2)	1089.7 (778.3– 1587.7)
Pre-pharynx	35.4 (28.4 – 42.5)	33.2 (21.5 – 39.0)
Pharynx diameter	49.1 (45.5 – 52.7)	47.5 (36.8 – 57.8)
Oral sucker diameter	124.6 (110.9 – 169.4)	127. 0 (118.8 – 129.1)
Oral sucker length	141.3 (126.2 – 151.2)	143.8 (106.4 – 156.7)
Ventral sucker diameter	128.4 (118.2 – 142.1)	135.4 (111.9 – 178.5)
Distance between OS- VS	241. 4 (180.2 – 284.1)	226.9 (170.2 – 336.0)
Cirrus sac length	423.9 (391.7 – 473.6)	520.5 (317.9 – 851.9)
Cirrus sac width	68.5 (60.9 – 73.1)	72.2 (65-6 – 110.0)
Testes	137.7 (93.6 – 172.1)	172.9 (109.5 – 237.3)

Molecular analysis

Sequence for 28S rDNA was generated for Cephalogonimidae gen. sp. where the consensus tree formed a cluster of *Cephalogonimus* spp. and the lineage including *Masenia baroensis* Curran, Dutton, Warren, Du Preez & Bullard, 2021 and *Emoleptalea mozambiquensis* Curran, Dutton, Warren, Du Preez & Bullard, 2021. The phylogenetic tree is presented in Figure 3.11.

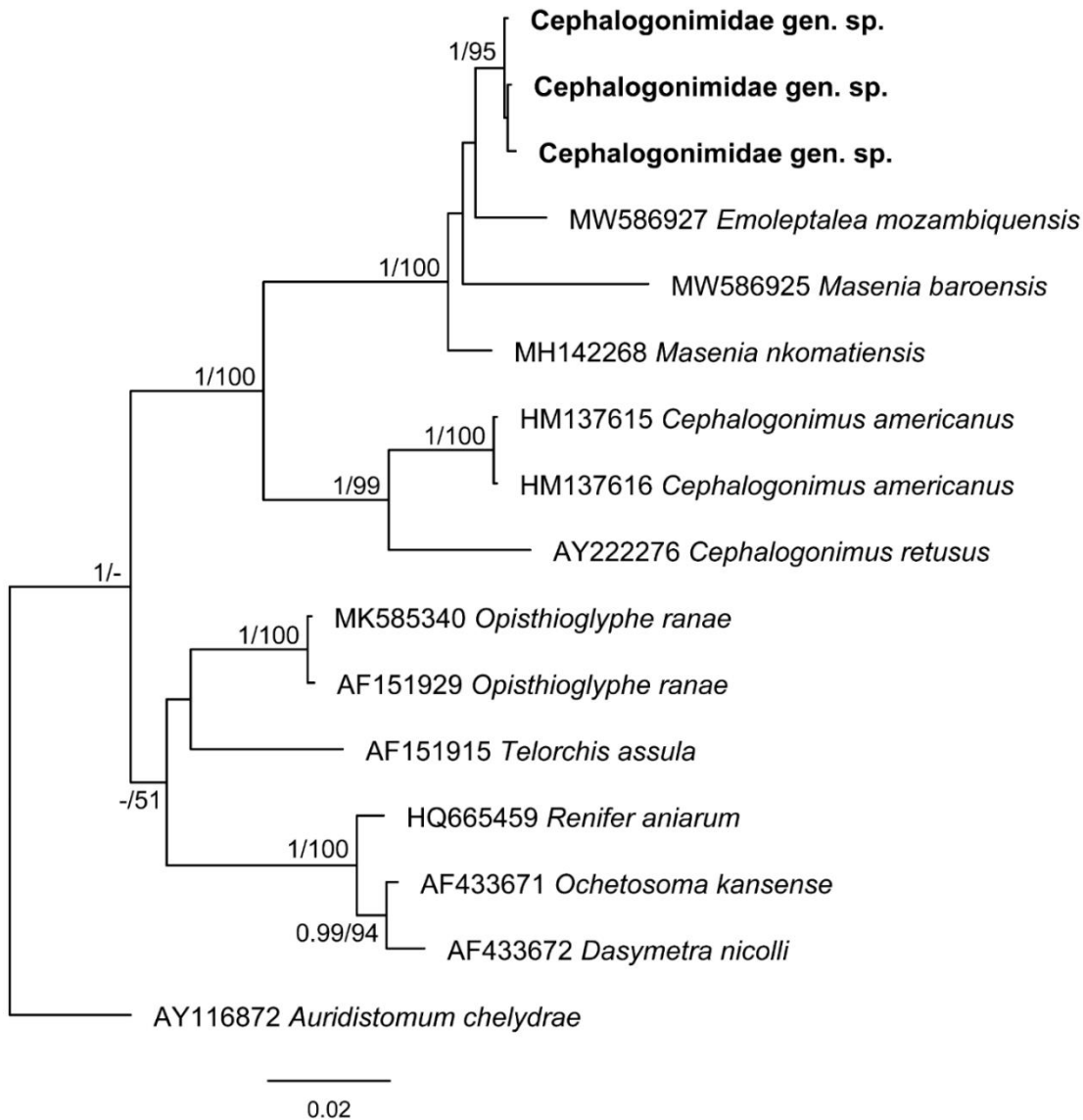


Figure 3.11: Phylogenetic tree based on maximum likelihood inferred from analysis of 28S rDNA data for Cephalogonimidae gen. sp. with *Auridistomum chelydrae* (Stafford, 1900) as the designated outgroup. Bootstrap support values for both Bayesian inference and maximum likelihood (BI/ML) are indicated at the respective nodes. Nodes with bootstrap values lower than 50% were indicated in dashes.

Based on 28S rDNA, the p -distances between the new genus and the representatives of the clusters ranged from 1.39% to 8.01% and the base pair differences between the clusters and the new genus ranged from 17 to 98 base pairs as shown in Table 3.5. The shortest p -distance to the new genus was with *Masenia nkomatesis* Dumbo, Dos Santos & Avenant-Oldewage, 2019 and the smallest number of base pair differences were observed in *M. nkomatesis*.

The Cephalogonimidae gen. sp. was collected only from Politsi River with the mean intensity, mean abundance and prevalence recorded to be 2.38, 1.27 and 53%, respectively.

Table 3.5: Pairwise genetic distances (%) (lower diagonal) and base pair difference (upper diagonal) between taxa included in the phylogenetic analysis of Cephalogonimidae gen. sp. and Cephalogonimidae species based on 28S rDNA.

	1	2	3	4	5	6	7	8	9	10	1	12	13	14
1. Cephalogonimidae gen. sp.		17	20	41	71	71	81	77	78	82	88	92	98	89
2. <i>Masenia nkomatiensis</i>	1.39		23	44	73	73	80	76	77	82	88	92	98	89
3. <i>Emoleptalea mozambiquensis</i>	1.63	1.87		49	76	76	86	77	78	82	92	94	100	87
4. <i>Masenia baroensis</i>	3.35	3.59	4.00		96	96	104	100	101	110	111	113	119	108
5. <i>Cephalogonimus americanus</i>	6.13	6.29	6.55	8.30		0	46	77	78	86	87	94	95	93
6. <i>Cephalogonimus americanus 2</i>	6.13	6.29	6.55	8.30	0.00		46	77	78	86	87	94	95	93
7. <i>Cephalogonimus retusus</i>	6.62	6.54	7.03	8.52	3.97	3.97		68	69	85	90	95	97	92
8 <i>Opisthioglyphe ranae</i>	6.38	6.29	6.37	8.31	6.64	6.64	5.64		1	49	62	60	62	72
9. <i>Opisthioglyphe ranae 2</i>	6.36	6.28	6.36	8.26	6.72	6.72	5.64	0.08		50	61	60	62	71
10 <i>Telorchis assula</i>	6.69	6.68	6.68	8.99	7.41	7.41	6.94	4.00	4.02		67	70	76	74
11. <i>Renifer aniarum</i>	7.59	7.59	7.93	9.60	7.76	7.76	7.77	5.33	5.18	5.69		12	17	78
12. <i>Ochetosoma kansense</i>	7.50	7.50	7.66	9.24	8.10	8.10	7.76	4.90	4.82	5.62	1.02		9	78
13. <i>Dasymetra nicolli</i>	8.01	8.00	8.16	9.75	8.20	8.20	7.94	5.07	4.99	6.11	1.44	0.72		84
14. <i>Auridistomum chelydrae</i>	7.26	7.25	7.09	8.83	8.02	8.02	7.52	5.88	5.71	5.94	6.62	6.27	6.76	

ORDER: Diplostomida Olson, Cribb, Tkach, Bray & Littlewood, 2003

FAMILY: Diplostomidae Poirier, 1886

The Diplostomidae gen. sp. specimens were found in cysts (Figure 3.12) from the fish collected from Nwanedi, Lutanandwa and Mutshundudi rivers. The cysts were found in the body cavity, muscle, under the skin, gills and in between the intestine. The highest prevalence, mean abundance and mean intensity of 57.14%, 8.41 and 4.86, respectively, were recorded from *C. pretoriae* collected from Mutshundudi River and the lowest of 10%, 1 and 0.07 were recorded in Lutanandwa River as represented in Figure 3.13. Molecular work was done to identify the larva and the results are indicated in the phylogenetic tree (Figure 3.14).

Remarks

Morphologically, the only visible feature of the larva is the oral sucker limiting identification of the digenean to lower levels by using morphology. Molecular work was used to identify the larva. The Diplostomidae gen. sp. larva possessed some lineage of diplostomid digeneans for which unfortunately there was no molecular data to relate to and this larva was thus not identified to genus level.

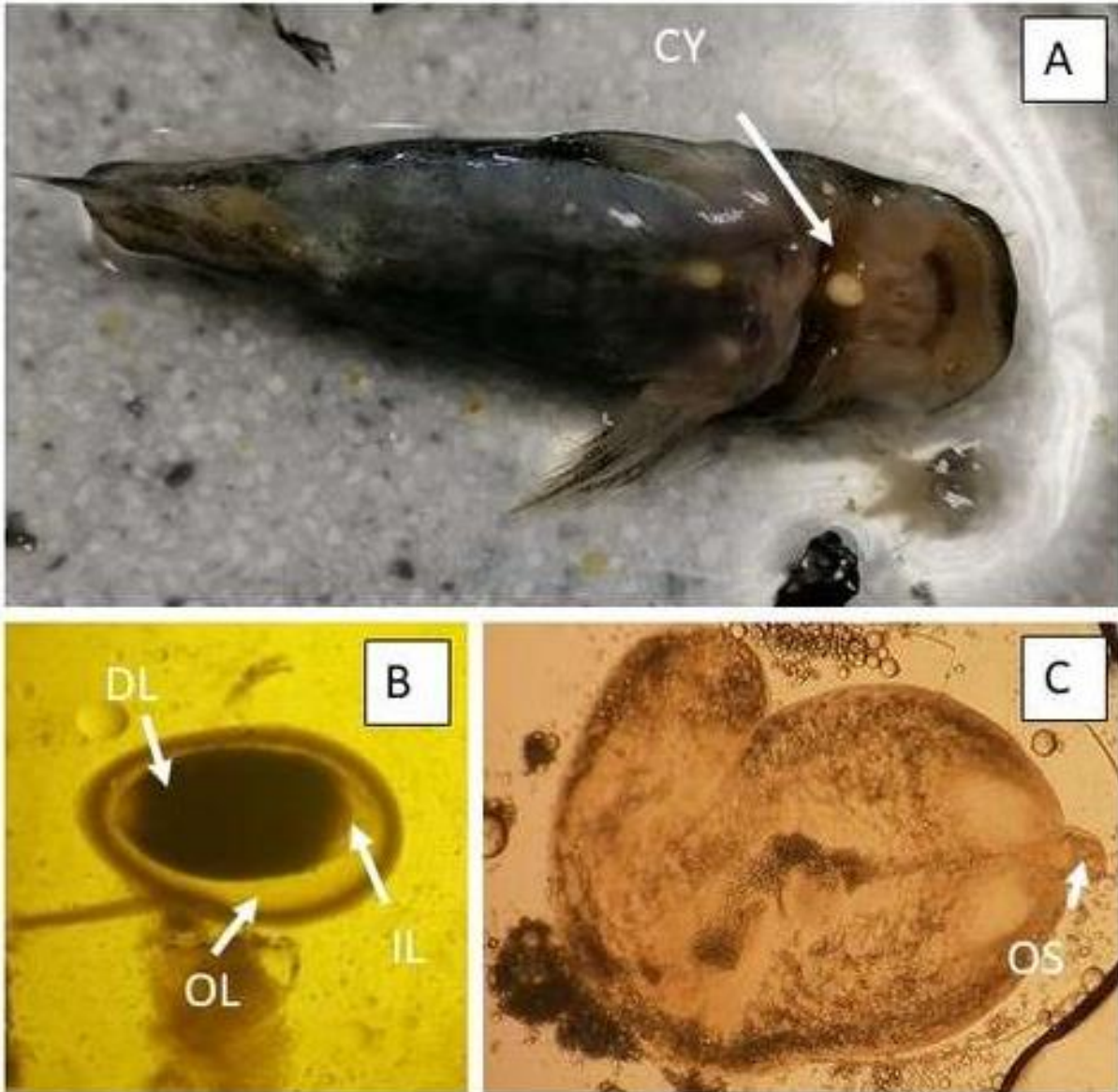


Figure 3.12: Diplostomidae gen. sp. larva found in *Chiloglanis pretoriae* from Nwanedi, Lutanandwa, Politsi and Mutshundudi rivers. A = Cyst on the body cavity, B = cyst; C = larva removed from cyst; DL = digenean larva; IL= inner layer; OL = outer layer, OS = oral sucker.

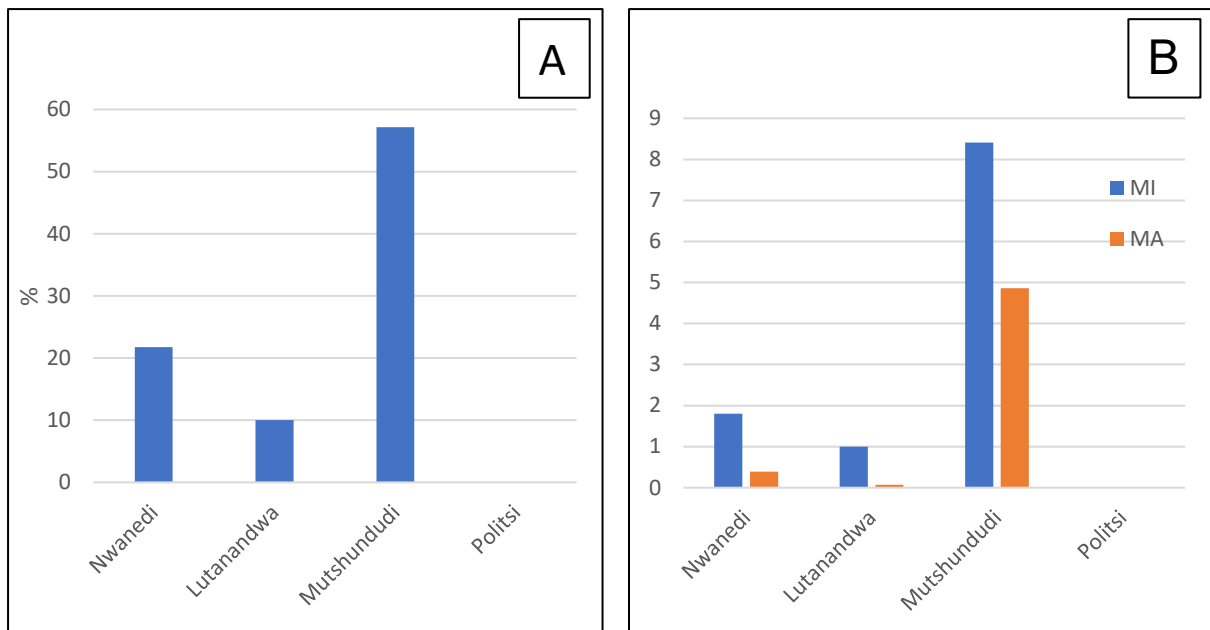


Figure 3.13: Prevalence (A), Mean intensity and mean abundance (B) of Diplostomidae gen. sp. larva collected from the muscle, gill cavity and skin of *Chiloglanis pretoriae* from Nwanedi, Lutanandwa and Mutshundudi rivers. MA - mean abundance. MI - mean intensity.

Sequences of partial 28S were generated for Diplostomidae gen. sp., and the consensus tree was constructed (see Figure 3.14). Tree based on 28S sequences formed two clusters which consisted of the Diplostomidae gen. sp. and ten genera (*Posthodiplostomum* Dubois, 1935; *Ornithodiplostomum* Dubois, 1935; *Bolbophorus* Dubois, 1935; *Nediodiplostomum* Railliet, 1919; *Tylodelphys* Diesing, 1850; *Austrodiplostomum* Szidat & Nani, 1935; *Alaria* Schran, 1788; *Hysteromorpha* Lutz, 1931; *Diplostomum* Nordmann, 1832; *Uvulifer* Yamaguti, 1934) under Diplostomidae. The larvae showed lineages of Diplostomidae digeneans but was not closely related to any of the genera and was thus unfortunately only identified to family level of Diplostomidae.

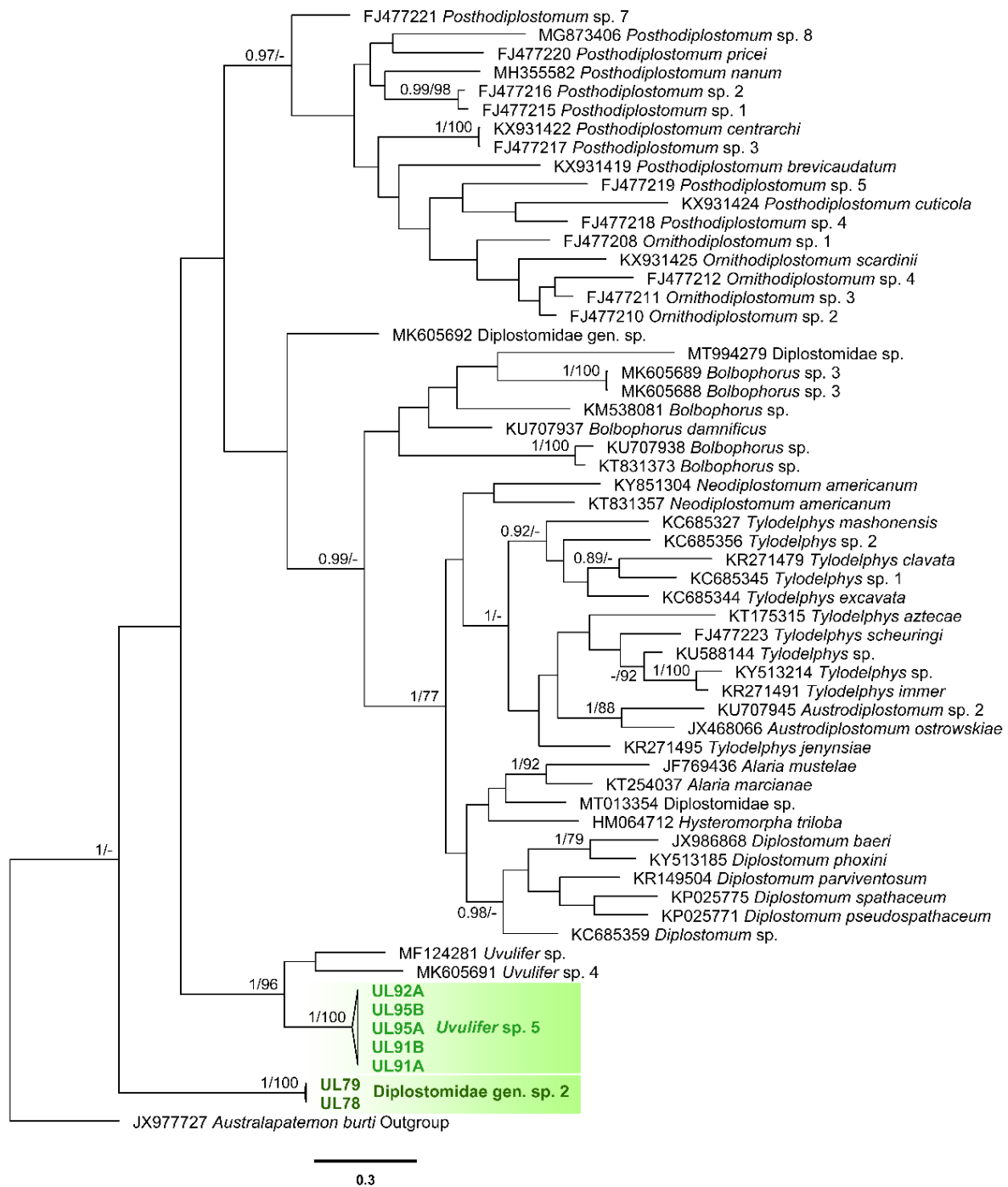


Figure 3.14: Phylogenetic tree based on maximum likelihood inferred from analysis of 28S rDNA data for *Diplostomidae* gen. sp. (UL78 and UL79) with *Australapatemon burti* (Miller, 1923) as the designated outgroup. Bootstrap support values for both Bayesian inference and maximum likelihood (BI/ML) are indicated at the respective nodes. Nodes with bootstrap values lower than 50% indicated in dashes.

Hirudinea

CLASS: Hirudinea Lamarck, 1818

The leech (Figure 3.15) was collected from the fins of *C. pretoriae* from Nwanedi and Lutanandwa rivers. Higher prevalence and mean abundance of 5.77% and 0.12 respectively in were recorded at the Nwanedi River and lower 0.25% and 0.83. A higher mean intensity of 1 was recorded Lutanandwa River and a lower mean intensity of 0.24 was recorded in Nwanedi River (Figure 3.16).

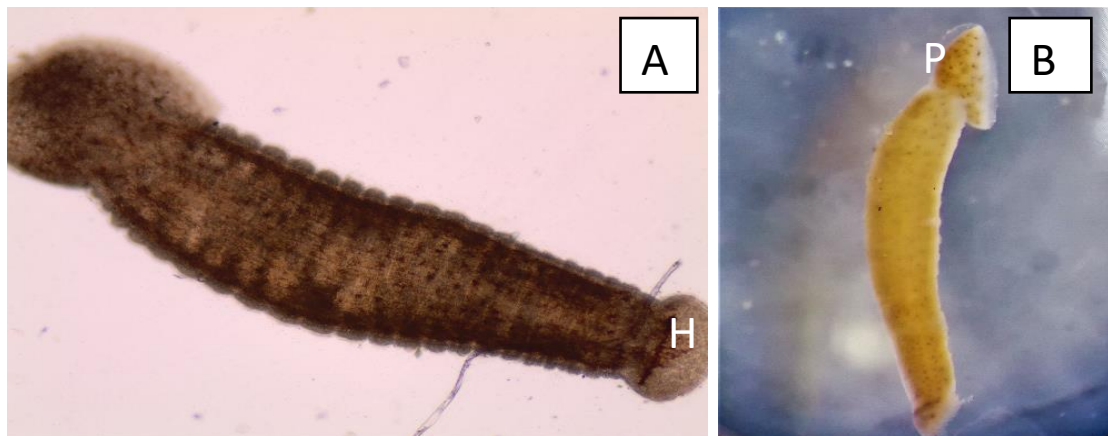


Figure 3.15: Micrograph of the leech collected from fins of *Chiloglanis pretoriae* from Nwanedi and Lutanandwa rivers. A = dorsal view; B = lateral view; H = Head region, P = posterior sucker.

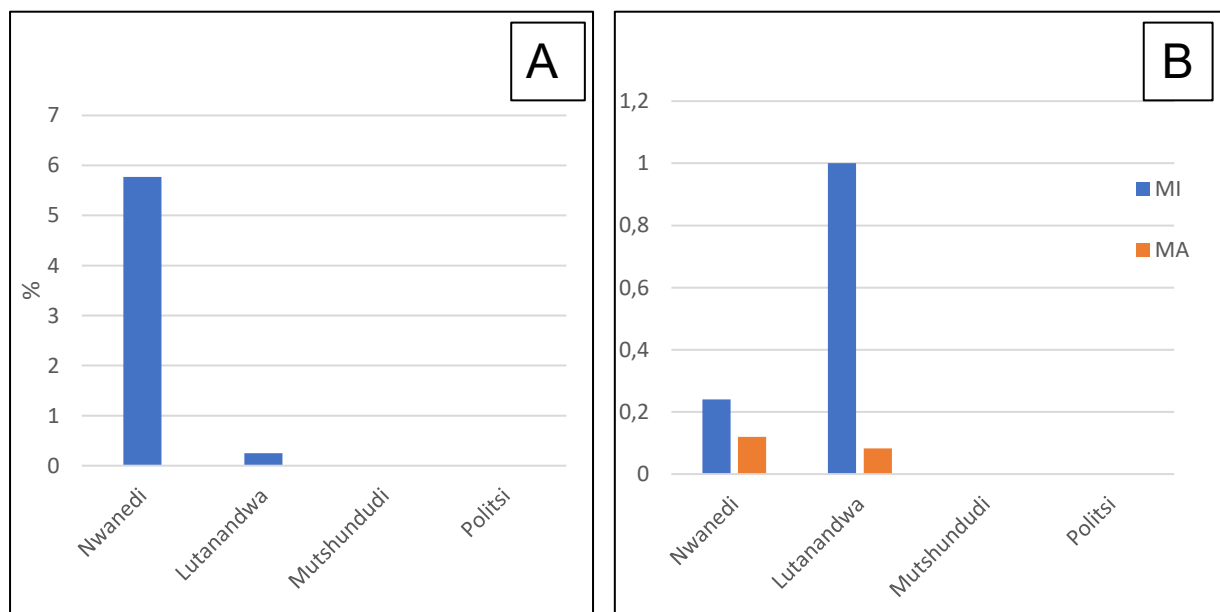


Figure 3.16: Prevalence (A), mean intensity and mean abundance (B) of the leech collected from the fins of *Chiloglanis pretoriae* from Nwanedi and Lutanandwa rivers.

3.3.2 Seasonal variation of parasite infestation from *Chiloglanis pretoriae*

Fish were collected seasonally (summer and winter) from only Nwanedi and Lutanandwa rivers. A total of 52 fish were collected from Nwanedi (summer n = 29 and winter n = 23) and 40 fish from Lutanandwa River (summer n = 22 and winter n = 18). Dactylogyridae gen. sp. collected from Lutanandwa River had higher prevalence of 44.44% in winter and lower 13.64% in summer. The leech collected from Nwanedi River had had higher prevalence of 13.04% and lower 0% in summer. The same was observed in Lutanandwa with higher prevalence of 5.56% in winter and lower 0% in summer. Diplostomidae gen. sp. from both rivers had a higher prevalence in winter than in summer. *Clinostomum* sp. from Nwanedi River had a higher prevalence of 6.90% in summer and lower prevalence of 4.35% in winter as reported in Table 3.6.

Table 3.6: Prevalence (P) (%), Mean abundance (MA) and Mean intensity (MI) of parasites of *Chiloglanis pretoriae* collected from Nwanedi and Lutanandwa rivers during winter and summer.

Parasite species	Nwanedi River						Lutanandwa River					
	Winter			Summer			Winter			Summer		
	P (%)	MA	MI	P (%)	MA	MI	P (%)	MA	MI	P (%)	MA	MI
Dactylogyridae gen. sp.	0	0	0	0	0	0	44.44	2.6	6	13.64	0.23	1.6
<i>Clinostomum</i> sp.	4.35	0.04	1	6.90	0.07	1	0	0	0	0	0	0
Diplostomidae gen. sp.	21.74	0.35	1.6	17.24	0.79	4.6	5.56	0.06	1	13.64	0.14	1
Leech	13.04	0.17	1.3	0	0	0	5.56	0.06	1	0	0	0

3.4 Discussion

Dactylogyridae is divided into nine subfamilies of which only two; Ancylo-discoidinae Gussev, 1961 and Ancyrocephalinae Bychowsky, 1937, consist of monogeneans that infest catfishes. A total number of 379 monogenean species from 31 genera have previously been reported from Siluriformes Cuvier, 1817 worldwide (Mendoza-Palmero et al. 2015). They prefer to feed on gills, and they are egg layers (Upadhyay & Rani 2019). These monogeneans have been widely used to study anthropogenic introductions, biogeographical history, phylogenetic relationships, and population

structure of hosts (Kmentová et al. 2022). According to Scholz et al. (2018) and Lim et al. (2001), seven genera (*Schilbetrema*, *Synodontella*, *Quadriacanthus* Paperna, 1961, *Schilbetrematoides* Kritsky & Kulo, 1992, *Bagrobdella* Paperna, 1969, *Paraquadriacanthus* Ergens, 1988) of Dactylogyridae monogeneans have been reported on several African Siluriformes. From these genera, *Synodontella* is the only genus that has been reported from fishes of Mochokidae (Dossou & Euzet 1993). Low phylogenetic coverage has caused the phylogenetic positions of African monogeneans to be poorly understood (Vanhove et al. 2018). Although that is the case, the positions of some of the Dactylogyridae monogeneans on African Siluriformes are known because they are well-studied due to their aquaculture potential (Mbondo et al. 2019). Morphological features such as the anchors and two connecting bars of these parasites and a majority of them having a single seminal vesicle, indicate how closely related they are (Lim et al. 2001) but only three genera (*Synodontella*, *Schilbetrema* and *Quadriacanthus*) have molecular data (Raphahlelo et al. 2016). Dactylogyridae gen. sp. possess unique dorsal and ventral anchors, ventral and dorsal bars and the MCO that are different from that of any other genera of the Dactylogyridae which is supported by molecular analysis. The molecular analysis included species representatives from twelve genera and confirmed the placement of the Dactylogyridae gen. sp. as a sister taxon to *Schilbetrema* sp. The phylogenetic tree showed a clade that consists of Dactylogyridae monogeneans found on African catfishes to be the ones closely related to the new species which is strongly supported by the BI, ML and MP values. The trees also indicate that *Schilbetrema* sp. is the closest related to Dactylogyridae n. gen. by forming a clade and the bootstrap values that have 100% support. The *p*-distance between Dactylogyridae gen. sp. and *Schilbetrema* sp. is 6.52% in 18S and 17% in 28S and base pair differences are 27 and 103, respectively. Morphologically five of six of the monogeneans parasitising on African Siluriformes possess a dactylogyrid type seminal vesicle excluding *Synodontella* (Lim 1996) and they are classified under Ancyrocephalinae Bychowsky, 1937 (Pugachev et al. 2009). Ancyrocephalinae is host specific (Upadhyay & Rani 2019), this was also observed for other Dactylogyridae monogeneans from African Siluriformes as they are limited to a maximum of two families while some are found on members of certain genera and others on certain species (Scholz et al. 2018). Although there is limited data on parasites found from *Chiloglanis* spp., it would be of no surprise for this new monogenean to be host specific to only *C. pretoriae*. Although

molecular analyses indicated that *Schilbetrema* forms a sister taxon with the new monogenean, both haptor sclerites and the Male Copulatory Organ (MCO) differ. The dorsal anchors of the members of *Schilbetrema* have elongated inner roots that are significantly longer than that of the outer roots. Dorsal and ventral bars have three anterior projections while dorsal anchors of Dactylogyridae gen. sp. have inner and outer roots that are elongated and slightly equal in length. Only the dorsal bar of the new monogenean has two projections, anterior and posterior. Given the great diversity of African Siluriformes with limited information about parasite fauna, monogeneans found are possibly underestimated and a great possibility of new species not yet discovered (Scholz et al. 2018).

Diplostomidae flatworms have complex life cycles with adult forms parasitic in piscivorous birds and mammals and larval forms found in a variety of freshwater fishes and snails as their intermediate hosts (Moema et al. 2013). These parasites are well-studied because their infestation has serious impacts on the health of their hosts (blindness), both wild and cultured, and may cause mortality (Chibwana et al. 2013; Locke et al. 2015). Diplostomids are usually found parasitising the eyes and the central nervous system of fishes. The identification of its metacercariae is usually morphologically impossible due to underdeveloped morphological features. Molecular analysis is usually used to identify them (Locke et al. 2015; Pelegrini et al. 2019).

Clinostomum sp. belongs to Clinostomidae, and the genus consists of over 50 species. They are cosmopolitan parasites and records exist all over the world (Shamsi et al. 2021). It has an indirect life cycle with three stages that require three hosts. The first stage is the cercariae, which is found in freshwater snails, metacercaria in the branchial cavity of fish and adults infest in the pharynx, oesophagus, and oral cavity of piscivorous birds (Rosser et al. 2017; Choudhary et al. 2022). The Infestation of *Clinostomum* in the branchial cavity of freshwater fish can cause a parasitic disease called clinostomiasis. The disease in fish can cause laryngo-pharyngitis disease in humans due to the consumption of raw or undercooked fish which can also lead to death due to asphyxia (Salem et al. 2021). Identification of the metacercariae of *Clinostomum* was difficult due to morphological similarities between larval forms. Recently the use of a combination of molecular and morphological analysis has assisted in the classification of this genus (Shamsi et al. 2021), although in this study,

obtaining genetic data was not successful and it could thus not be identified to species level.

Cephalogonimidae is a family of parasitic spinous digeneans that have their adult stage in the gastro-intestinal tract of African and Asian freshwater fishes, reptiles and amphibians (King et al. 2018; Curran et al. 2021). They are identified by adult forms that are characterised by a long cirrus-sac and genital pore near the anterior extremity. It is comprised of five genera: *Cephalogonimoides* Brooks & Buckner, 1976, *Emoleptalea* Looss, 1900, *Paracephhalogonimus* Skrjabin, 1950, *Masenia* Chatterji, 1933 and *Cephalogonimus* Poirier, 1886 (Dumbo et al. 2019). The genera in this family are differentiated by the number of rows of circumoral spines. Three of them (*Emoleptalea*, *Paracephhalogonimus*, *Cephalogonimus*) have no circumoral spines while members of *Cephalogonimoides* have three to six and *Masenia* with two rows (Dumbo & Avenant-Oldewage 2019). Molecular work of the Cephalogonimidae gen. sp. was done by Bayesian inference based on 28S rDNA. The results showed that the digenean specimens from the present study are closely related to *Emoleptalea* sp., strongly supported by a high BI value of 1. The clade of the digenean and *Emoleptalea mozambiquensis* forms a well-supported cluster with *Masenia baroensis* which is then followed by a close relationship with *Masenia nkomatiensis*. Both relationships have strong BI support of 1. The p -distances between the new digenean and the closest related species were short and approximately equal. The shortest p -distance was 1.39 followed by 1.63 between the new digenean and *M. nkomatiensis* and *E. mozambiquensis*, respectively. Divergence was confirmed by the number of pairwise distances of 17 and 20 of *M. nkomatiensis* and *E. mozambiquensis*, respectively. A few morphological features, such as the presence of two rows of circumoral spines, length and width were common in both Cephalogonimidae gen. sp. and *Masenia*. Cephalogonimidae gen. sp. is confirmed to represent a new genus morphologically due to the distinct characteristics of the reproductive system i.e., the position and shape of the ovary and the position of the two testes.

Not much work has been done on African freshwater leeches because infestation of leeches on fish is not common. Leeches can act as hosts for invertebrates and act as vectors for trypanosomes (Hayes et al. 2014).

Infestation statistics

Ectoparasites were more prevalent in the Lutanandwa River and the endoparasitic trematodes (*Clinostomum* sp. and Diplostomidae gen. sp.) were more prevalent in the Mutshundudi and Nwanedi rivers. The Cephalogonimidae gen. sp. was only collected from the Politsi River, and it had the second-highest overall prevalence of 53%. The presence of parasites can be influenced by the presence of intermediate and definitive hosts, environmental conditions, and host fitness (Lafferty 2008), thus the presence of the Cephalogonimidae gen. sp. in Politsi River may have been caused by those factors. Higher prevalence, mean intensity and mean abundance of monogeneans (Dactylogyridae gen. sp.) were recorded in the Lutanandwa River and lower in the Nwanedi River. Given that Dactylogyridae gen. sp. was only collected from *C. pretoriae* in all localities and has never been recorded on any siluriform, may suggest the possibility of it being host specific. Their lower prevalence on *C. pretoriae* and their absence in other localities may have been due to their sensitivity to environmental (water) stressors which affect them directly (Erasmus et al. 2022). *Clinostomum* sp. was more prevalent at Nwanedi River with equal mean intensity and mean abundance values from the Nwanedi and Mutshundudi rivers. The highest prevalence was lower than 10%. Olivier et al. (2009) found similar results regarding the prevalence of *Clinostomum* sp. in *C. pretoriae*.

The highest overall prevalence of 57.14% recorded was for Diplostomidae gen. sp. which was found in the Mutshundudi River and the lowest of 10% was recorded in the Lutanandwa River. Diplostomidae gen. sp. larvae were found in several organs (body cavity, gills, skin, between the intestines) in most fish collected from the Mutshundudi, Nwanedi and Lutanandwa rivers with gills and muscle as dominating sites of infestation. Despite their usual site of infestation (the eye and central nervous system) (Locke et al. 2015; Pelegrini et al. 2019), the sufficient data indicates the site of infestation was not accidental. The leech collected from the fins were more prevalent at Nwanedi River and a lower prevalence was recorded from Lutanandwa River. The leech was only recovered from these two rivers in low numbers. The overall highest prevalence was recorded from the Mutshundudi River for the Diplostomidae gen. sp., and the lowest prevalence was from the Nwanedi River for the leech.

Seasonal variations of parasites infestation on Chiloglanis pretoriae

According to Marcogliese (2016), water temperature and water quality influence the abundance of ectoparasites because they are exposed to the water and thermal tolerance may also differ according to ectoparasite species. This was observed in the ectoparasite species (Dactylogyridae gen. sp. and the leech) which were more prevalent in winter. The same was observed for Diplostomidae gen. sp. where it was more prevalent in winter than in summer. Diplostomidae gen. sp. which was found in Nwanedi and Lutanandwa rivers, its overall mean abundance and mean intensity recorded were higher in summer and lower in winter. The study was done during the middle of winter and the lower water temperatures might have weakened the immune response of the fish making them more susceptible to high infestations (Gilbert & Avenant-Oldewage 2017). The opposite was observed for *Clinostomum* sp. in Nwanedi River. These results are supported by Dallarés et al. (2016) who suggested that digeneans increase in numbers in high temperatures because it stimulates transmission from one host to the other.

3.5 Conclusion

Five species (Dactylogyridae gen. sp., Cephalogonimidae gen. sp. *Clinostomum* sp. Diplostomidae gen. sp. and a leech) from five genera were found from *C. pretoriae* from the four rivers. Fish from Nwanedi and Lutanandwa rivers had three genera while Mutshundudi and Politsi rivers had two. Cephalogonimidae gen. sp. was recorded only from the Politsi River. *Clinostomum* sp. and the leech were more prevalent in Nwanedi River while Dactylogyridae gen. sp. and Diplostomidae gen. sp. were more prevalent in Lutanandwa and Mutshundudi rivers, respectively. Two new genera (a monogenean and digenean) are described and all the parasites represent new host and geographic records.

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CHAPTER 4

PARASITE DIVERSITY OF *AMPHILIUS URANOSCOPIUS*

4.1 Introduction

There is limited information on parasites found in fish species from Amphiliidae. Previous records indicate that only four groups of parasites have been found in the family as a whole and they include monogeneans, digeneans, branchiurans and myxosporean cnidarians (Scholz et al. 2018). According to Ngugi et al. (2009), the same applies to *A. uranoscopus*, its information on several aspects is limited, including its biology, habitat and parasites found. The main food of *A. uranoscopus* is macroinvertebrates which represent a possible form of first intermediate hosts for digeneans (Oosterhout et al. 2009). In previous studies, *Clinostomum* sp. was the only recorded parasite found in the branchial cavity of *A. uranoscopus* (Scholz et al. 2018).

4.2 Materials and Methods

A total of 44 specimens of Stargazer Mountain Catfish, *A. uranoscopus* (Nwanedi River = 11, Lutanandwa River = 23, Mutshundudi River = 1 and Politsi River = 9) were collected from the four localities. Fish collection, fixing and preservation of parasites and identification methods are described in Chapter 2 sections 2.4; 2.5; 2.6 and 2.7.

4.3 Results

The fish collected had a total length ranging from 3.1 to 14.9 cm and a mass ranging from 0.19 to 24.16 g. A total of 3 169 parasites representing two parasite groups (Digenea and Nematoda) were collected from 31 *A. uranoscopus* (Table 4.1). Overall, parasites were more abundant in Lutanandwa River with digeneans being the most abundant. The present study represents new records of parasites and more especially nematodes which have never been recorded from *A. uranoscopus*.

Table 4.1: Summary of the collected number of *Amphilius uranoscopus* from Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers and an overview of parasites collected.

	Nwanedi River	Lutanandwa River	Mutshundudi River	Politsi River	Total
No. <i>A. uranoscopus</i> collected	11	13	1	9	44
No. parasites collected	28	3042	3	96	3169

4.3.1 Parasites found from *Amphilius uranoscopus*

Parasites identified from *A. uranoscopus* in the study were represented by eight species represented by two endoparasite groups, digeneans (*Clinostomum* sp., *Uvulifer* sp. and Cephalogonimidae gen. sp.) and nematodes (*Labeonema* sp., *Contraecum* sp., *Rhabdochona* sp., *Gendria* sp. and *Gendria* cf. *thysi*).

ORDER: Strigeatida (Larue, 1926) Sudarikou, 1959

FAMILY: Clinostomidae Lühe, 1901

GENUS: *Clinostomum* Leidy, 1856

Clinostomum sp. was collected from the branchial cavity of *A. uranoscopus* that was collected from the upper Lutanandwa River during winter. Only two were found from one fish. It had a low prevalence of 8.69% and low mean intensity and mean abundance of 0.66 and 0.13, respectively (Figure 4.1).

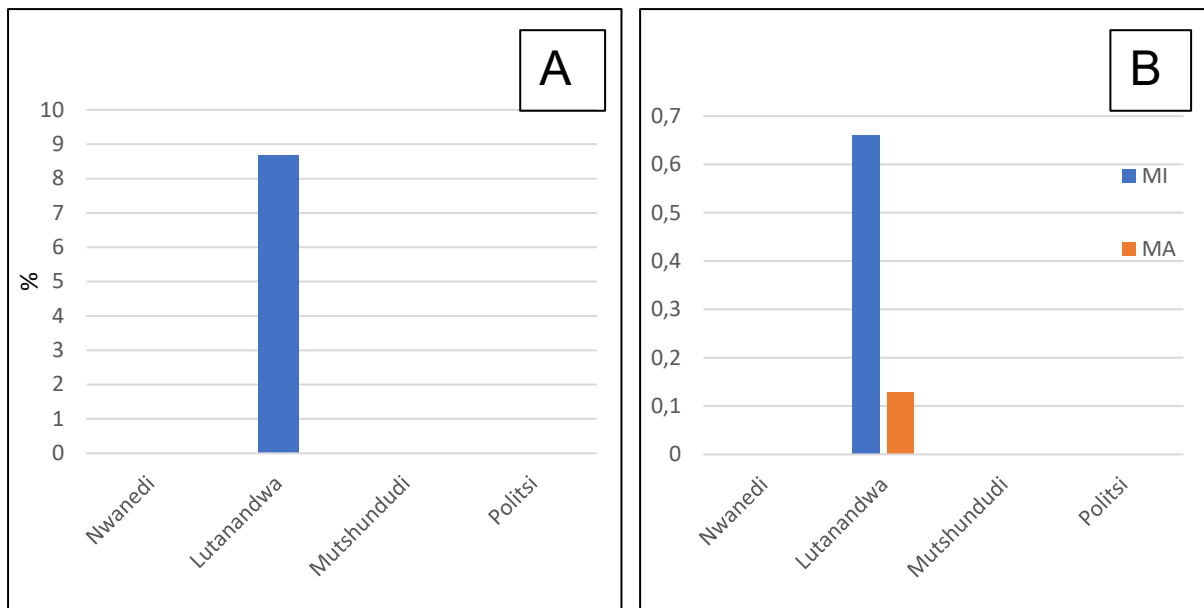


Figure 4.1: The prevalence (A), mean intensity and mean abundance (B) of *Clinostomum* sp. larva found in the branchial cavity of *Amphilius uranoscopus* collected from Lutanandwa River. MA - mean abundance. MI - mean intensity.

ORDER: Plagiorchiida

FAMILY: Cephalogonimidae Looss, 1899

Cephalogonimidae gen. sp. was found in the intestine of *A. uranoscopus* collected from Politsi River. A total of 62 specimens were collected from *A. uranoscopus* with a prevalence, mean intensity and mean abundance of 88.88%, 11.63 and 10.33, respectively (Figure 4.2).

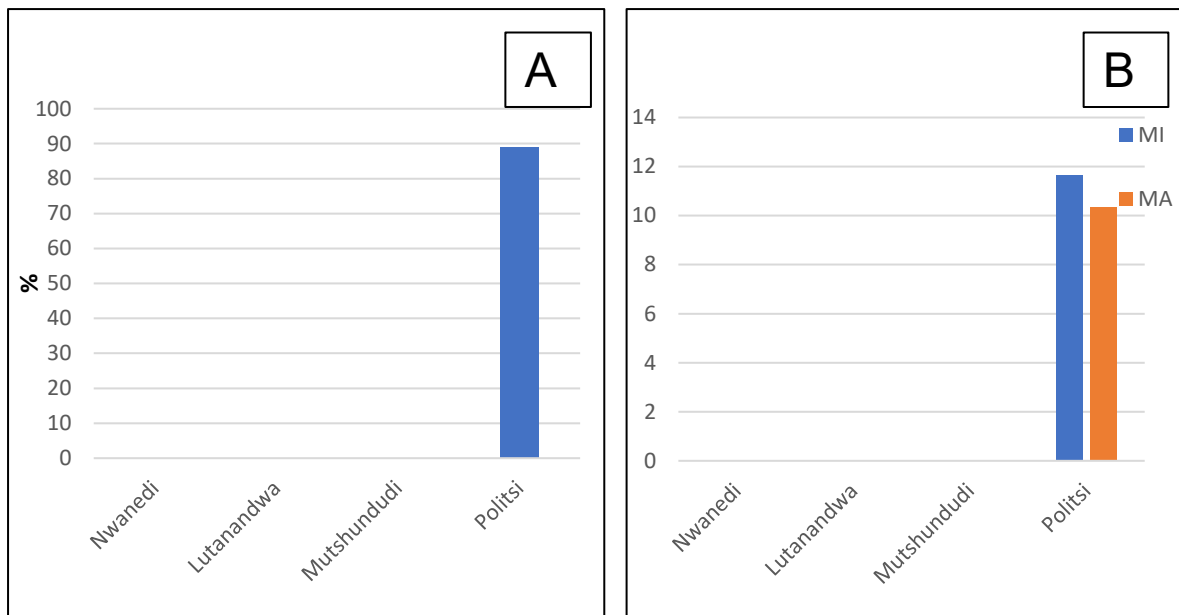


Figure 4.2: The prevalence (A), Mean intensity and mean abundance (B) of Cephalogonimidae gen. sp. collected from intestine of *Amphilius uranoscopus* from Politsi River. MA - mean abundance. MI - mean intensity.

ORDER: Strigeatida (Larue, 1926) Sudarikov, 1959

FAMILY: Diplostomidae Poirier, 1886

GENUS: *Uvulifer* (Yamaguti, 1934)

Uvulifer sp. collected from gill cavity (Figure 4.3 A), under skin (Figure 4.3 B), muscle, fins and body cavity of fish from Nwanedi, Lutanandwa and Politsi rivers. The digenean larvae were covered by cysts consisting of two layers; an outer layer (Figure 4.3 C) and inner layer (Figure 4.3 D). The highest number ($n = 3031$) of this parasite was recorded in Lutanandwa River with a 100% prevalence recorded (Figure 4.4).

Remarks

The cysts contained larval forms of the digenean with little to no morphological features to be used for identification. Molecular work was used to identify the digenean larva to genus level.

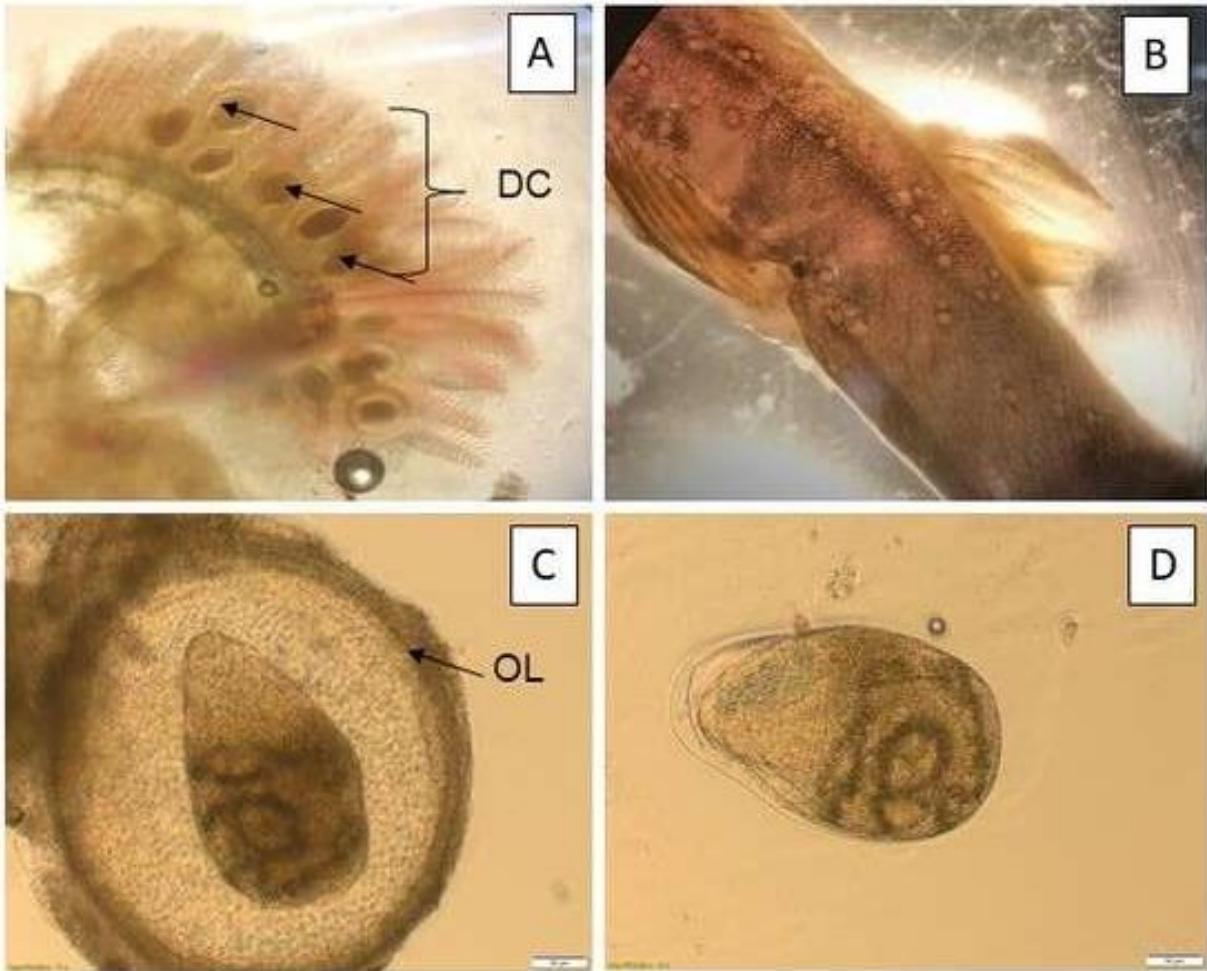


Figure 4.3: Micrographs of *Uvulifer* sp. larva collected from *Amphilius uranoscopus* from the Politsi and Lutanandwa rivers. A = several cysts on the gill; B = cysts under the skin of the fish; C = digenean larva in the cyst; D = digenean larva in the inner layer of the cyst; DC = digenean larvae in cysts, OL = outer layer.

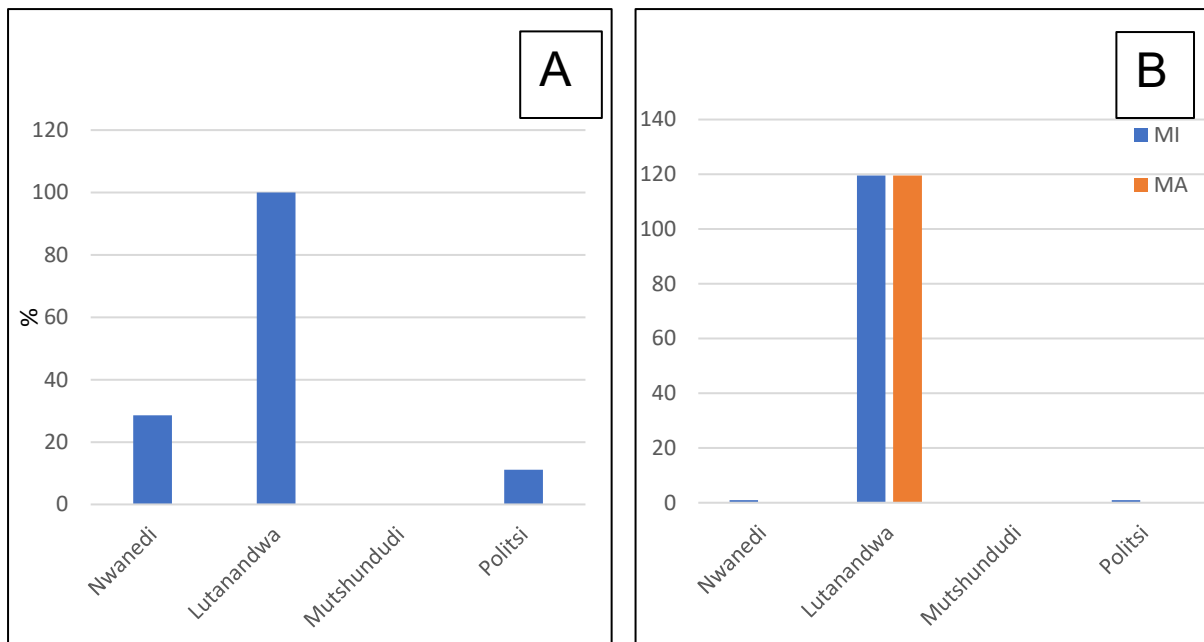


Figure 4.4: The prevalence (A), mean intensity and mean abundance (B) of *Uvulifer* sp. collected from the body cavity, fins, gills and muscle of *Amphilius uranoscopus* from Nwanedi and Lutanandwa rivers. MA - mean abundance. MI - mean intensity.

The phylogenetic tree generated indicated two clusters, (1) consisted of 10 genera of Diplostomidae (*Posthodiplostomum*, *Ornithodiplostomum*, *Bolbophorus*, *Nedioidiplostomum*, *Tylodelphys*, *Austrodiplostomum*, *Alaria*, *Hysteromorpha*, *Diplostomum* and *Uvulifer*) and (2) consisted of species of *Uvulifer* sp. 4 and *Uvulifer* sp. 5 digeneans with very high support. The digenean from the present study is represented by UL 91A to UL 95B and called *Uvulifer* sp. 5. *Uvulifer* sp. 5 is closely related to *Uvulifer* sp. 4 which has been previously recorded in *Tilapia sarrmanii* Smith, 1840 in Northwest South Africa (Hoogendoorn et al. 2020). The closest identified species level that is related to *Uvulifer* sp. 5 is *Uvulifer spinatus* Goodsir, 1845 (Figure 4.5).

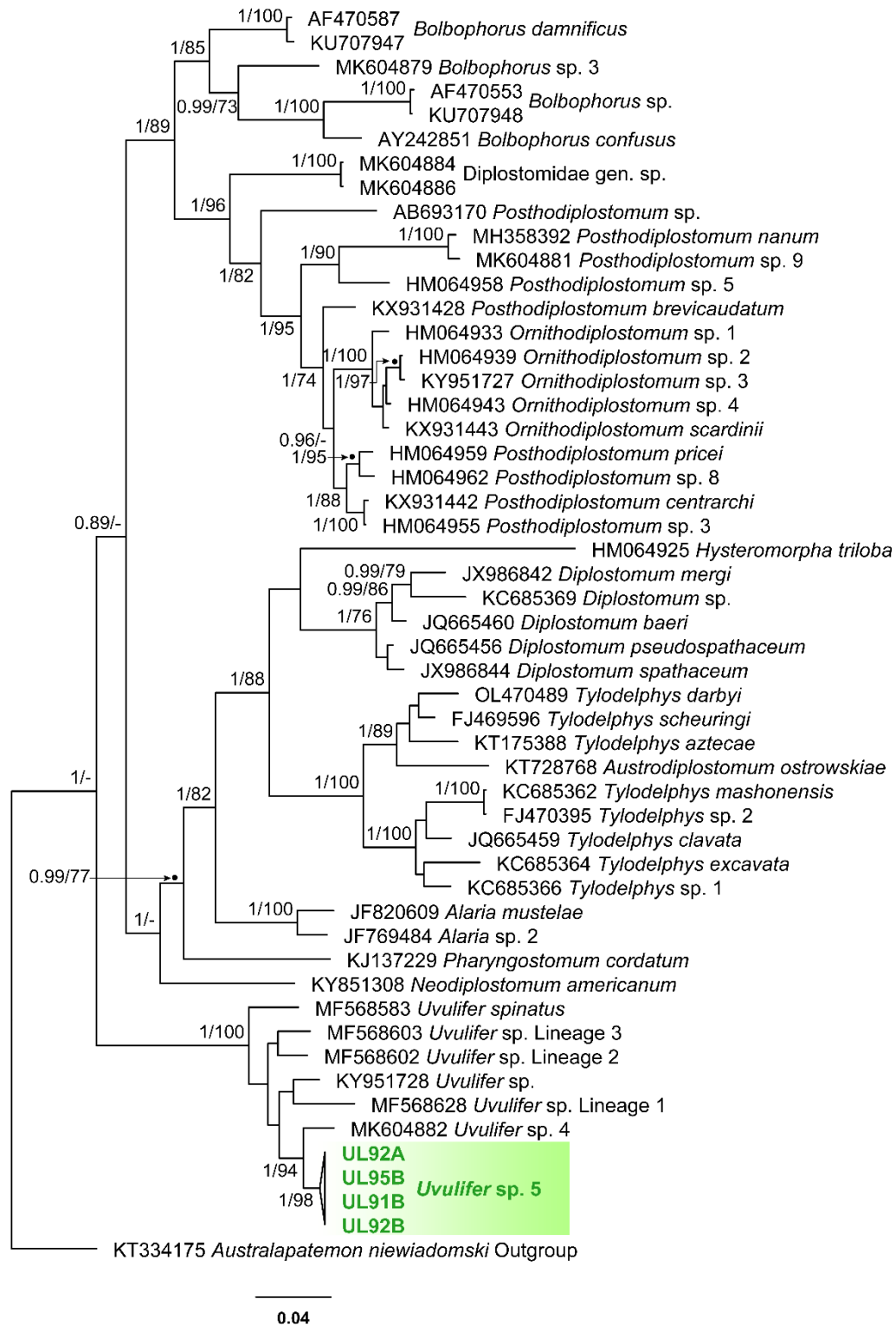


Figure 4.5: Phylogenetic tree based on maximum likelihood approaches inferred from analysis of 28S rDNA data for *Uvulifer* sp. with *Australapalemon niewiadomski* Blasco-Costa, Poulin & Presswell, 2016 as the designated outgroup. Bootstrap support values for both Bayesian inference and maximum likelihood (BI/ML) are indicated at the respective nodes. Nodes with bootstrap values lower than 50% were indicated in dashes.

ORDER: Cosmocercoidea Railliet, 1916

FAMILY: Kathlaniidae Lane, 1915

GENUS: *Gendria* Baylis, 1930

Gendria sp. (Figure 4.6) was collected from the intestine of *A. uranoscopus* collected from the upper and lower Lutanandwa River. Only two specimens were collected. The prevalence, mean intensity and mean abundance were 8.7%, 1 and 0.086, respectively, as shown in Figure 4.7.

Morphology

Whitish nematode with smooth cuticle with 14 mm total length, 144 μm total width. Anterior end roundish in shape, triangular buccal cavity with three well-developed lips surrounded by cuticle. Muscular oesophagus oval in anterior extremity with 395 μm length and glandular oesophagus absent, intestine long (Figure 4.6 A). Eggs diameter 34.7 μm . Tail long with a pointy end (Figure 4.6 B)

Remarks

Only two specimens were found. The shape of anterior part (lips and buccal cavity) was used for identification. Further identification to species level can be done with the presence of the male specimen using the number of papillae and the length of the left and right spicules.

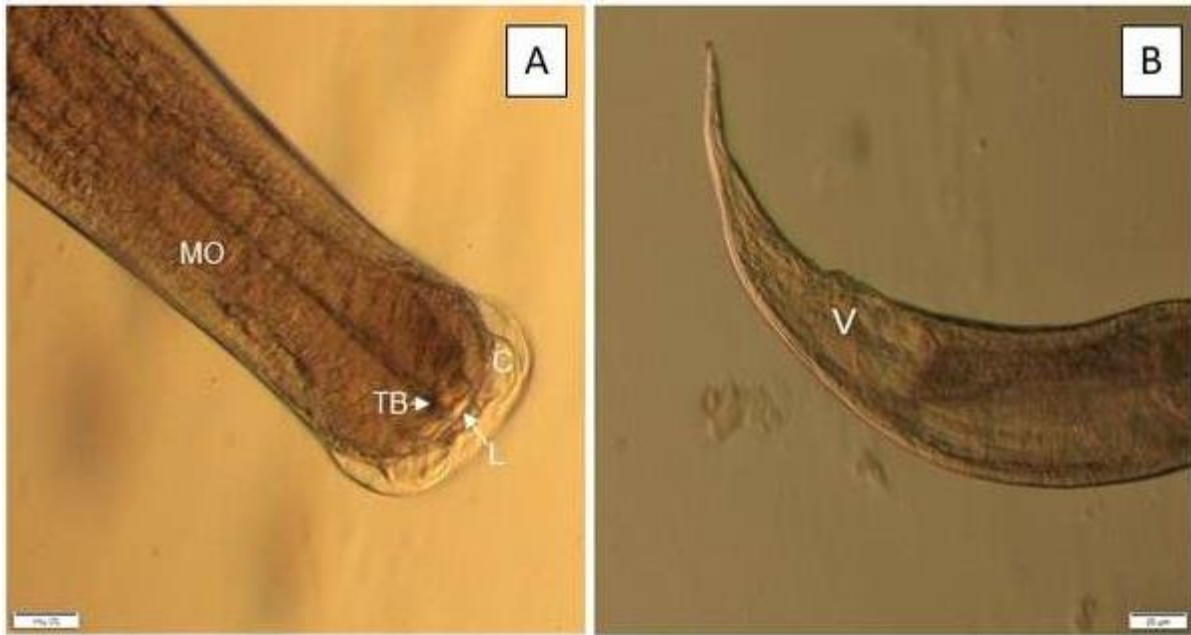


Figure 4.6: Micrograph of *Gendria* sp. collected from the intestine of *Amphilius uranoscopus* found in Lutanandwa River. A = Aterior end; B = posterior end; C = cuticle, L = inner lips, MO = muscular oesophagus, TB = Triangular buccal cavity, V = vagina.

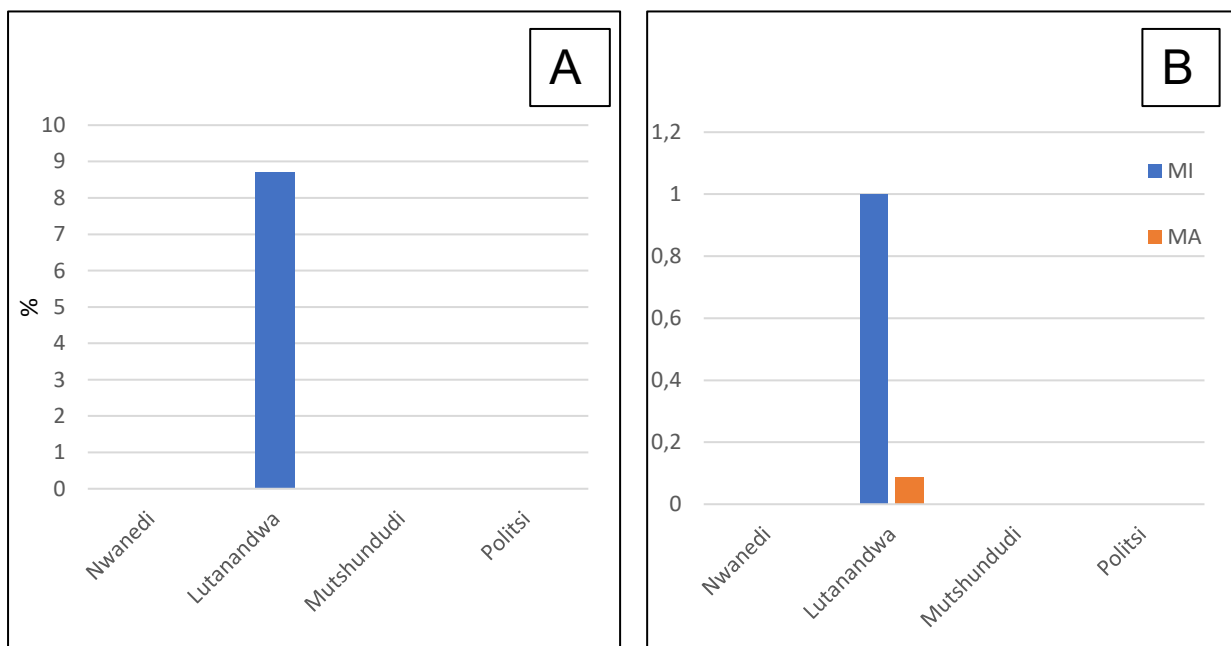


Figure 4.7: The prevalence (A), mean intensity and mean abundance (B) of *Gendria* sp. collected from the intestine of *Amphilius uranoscopus* from Lutanandwa MA - mean abundance. MI - mean intensity.

ORDER: Cosmocercoidea Railliet, 1916

FAMILY: Kathlaniidae Lane, 1915

GENUS: *Gendria* (Bailis 1930)

SPECIES: *Gendria cf thysi*

Gendria cf thysi (Figure 4.8) was collected from the intestine of *A. uranoscopus* from the Lutanandwa River. A single specimen was collected, and its infestation indices were calculated and represented in bar graphs (Figure 4.9).

Morphology

Elongated body total length of 11 mm and width of 153 μm with transversely striated cuticle. Lateral alae start a short distance from the cephalic vesicle and extend to the mid-anterior part of the muscular oesophagus (Figure 4.8 A). Mouth surrounded by lips with nerve endings. The entire oesophagus is muscular and clubbed. Short vagina and a long pointy tail (Figure 4.8 B)

Remarks

Only a single female specimen was collected. The specimen collected looked similar to *Gendria thysi* with similarities in shape, size and length.

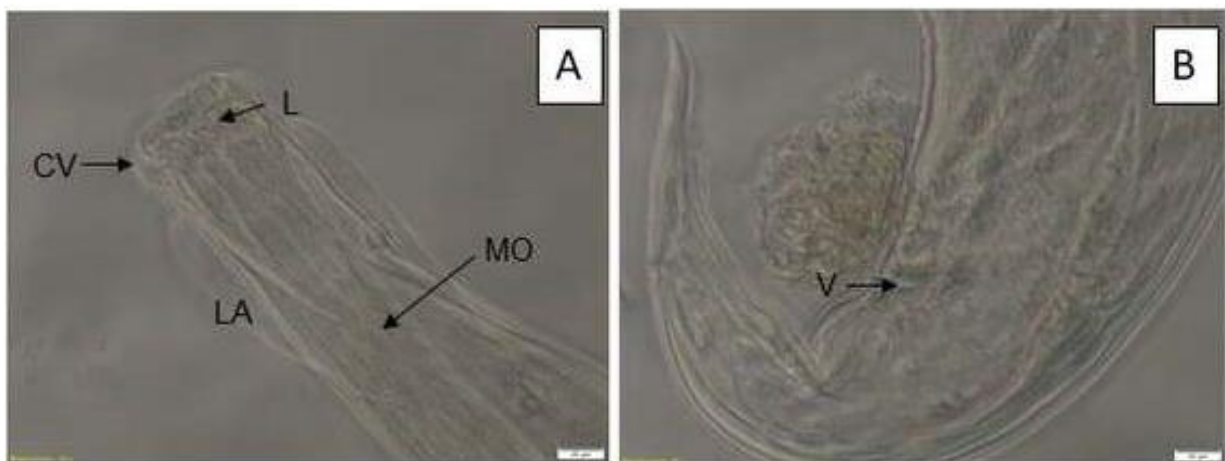


Figure 4.8: Micrograph of *Gendria cf thysi*, collected from the intestine of *Amphilius uranoscopus* from the Lutanandwa River. A = anterior end; B = posterior end; CV = cephalic vesicle, LA = lateral alae, L = lobes, MO = muscular oesophagus, V = vagina.

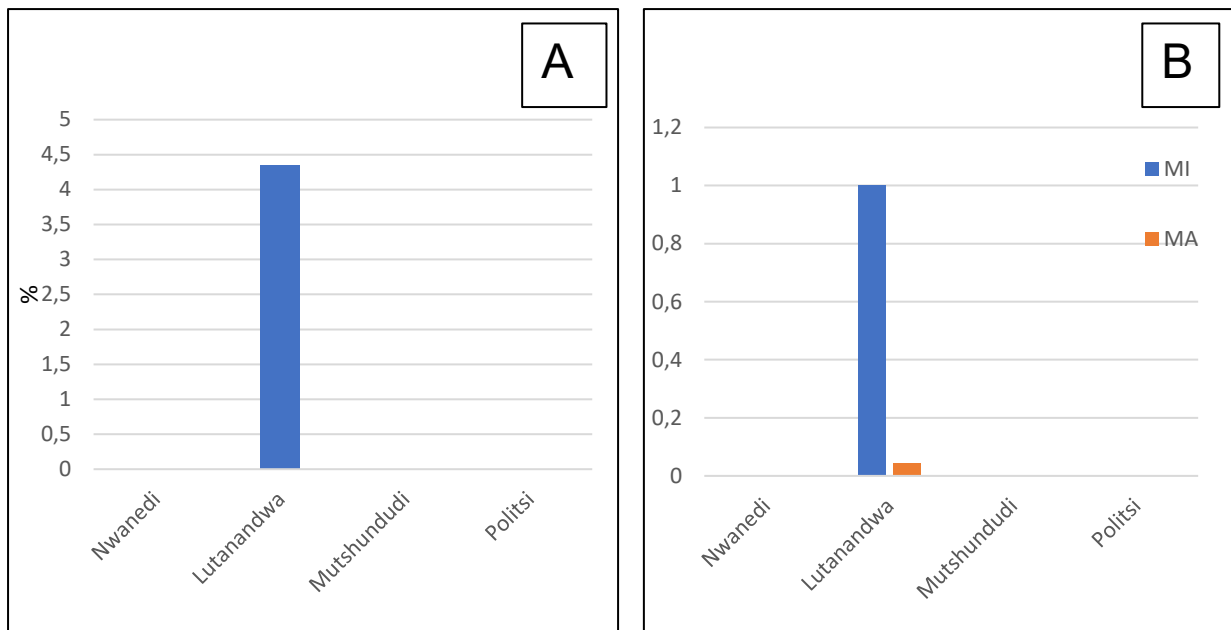


Figure 4.9: The prevalence (A), Mean intensity and mean abundance (B) of *Gendria cf. thysi*. collected from the intestine of *Amphilius uranoscopus* from the Lutanandwa River. MA - mean abundance. MI - mean intensity.

ORDER: Ascaridida Skrjabin & Schulz, 1940

FAMILY: Atractidae Railliet, 1917

GENUS: *Labeonema* Puyllaert, 1970

Labeonema sp. (Figure 4.10) was collected from the intestine of *A. uranoscopus* collected from the Mutshundudi River. Only a single female specimen was collected, and the infestation indices were calculated and represented in the bar graphs in Figure 4.11.

Morphology

Body whitish in colour with transversely striated cuticle. Small mouth, triangular with three lips surrounding it (Figure 4.10 A). Narrow lateral alae arise from a short distance posterior to anterior extremity and extend posteriorly to about the level of the anus. Intestine is straight and excretory pore is situated near posterior end of corpus. Conical tail with a sharp pointy end (Figure 4.10 B). Long uterus filled with large eggs (Figure 4.10 C).

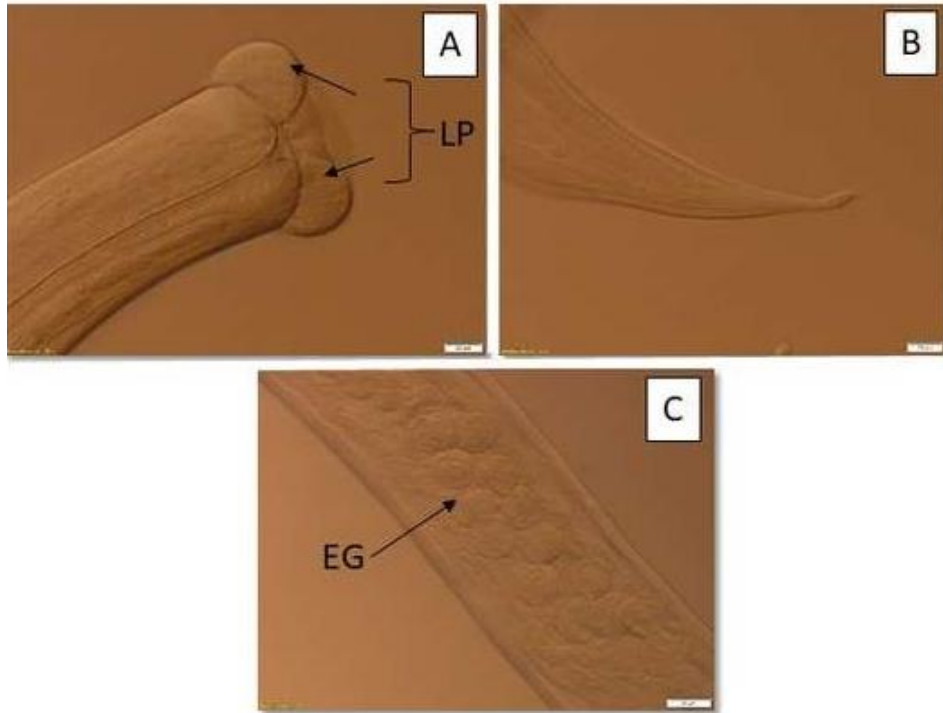


Figure 4.10: Micrograph of *Labeonema* sp. collected from the intestine of *Amphilius uranoscopus* from Mutshundudi River. Anterior end; B = posterior end; C = middle part of worm; EG = eggs in the uterus, LP = lips.

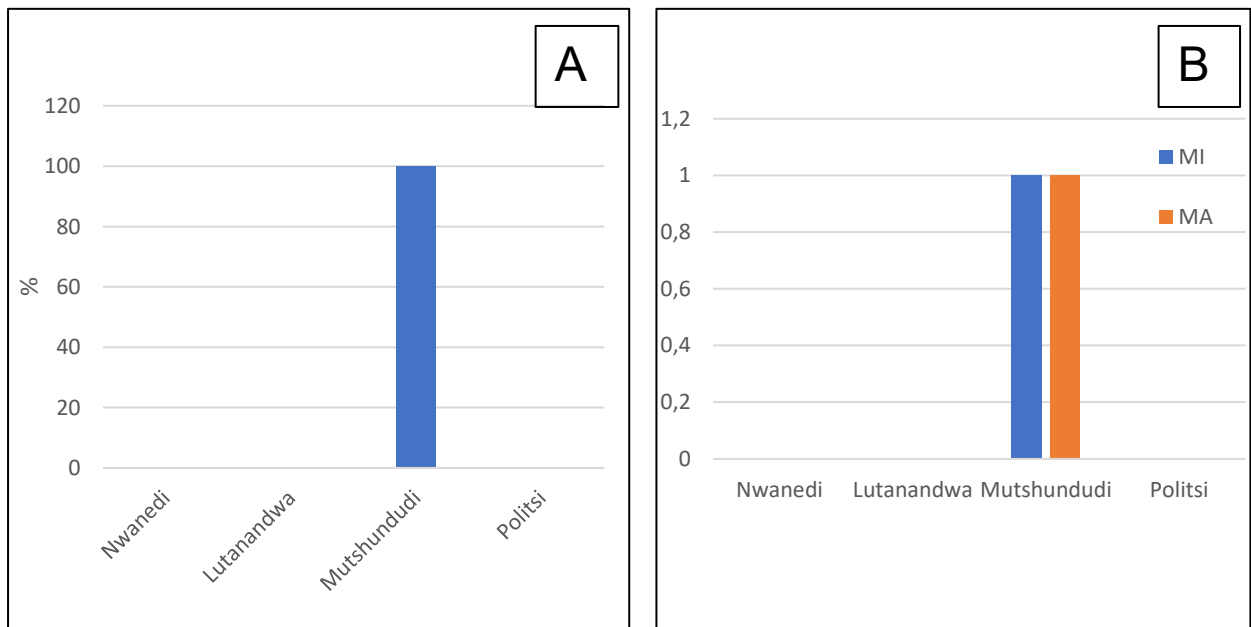


Figure 4.11: The prevalence (A), mean intensity and mean abundance (B) of the *Labeonema* sp. collected from the intestine of *Amphilius uranoscopus* from Nwanedi and Lutanandwa rivers. MA - mean abundance. MI - mean intensity.

ORDER: Ascaridida Baird, 1853

FAMILY: Rhabdochonidae Skrjabin, 1946

GENUS: *Rhabdochona* Railliet, 1916

SPECIES: *Rhabdochona cf paski*

Rhabdochona sp. (Figure 4.12) was collected from the intestine of *A. uranoscopus* collected from the Mutshundudi River. Only a single male specimen was collected, and the infestation indices were calculated and represented on the bar graphs in Figure 4.13.

Morphology

Medium-sized nematode with body elongated with slightly transversely striated cuticle. Anterior teeth in variable numbers. Prostom funnel shaped (Figure 4.12 A). Long buccal cavity with vestibule straight, relatively long. Muscular oesophagus (Figure 4.12 B) longer than glandular oesophagus (Figure 4.12 C). Tail conical with sharply pointed tip. Preanal papillae 5 number and 4 post anal papillae (Figures 4.12 D and E). Right spicule boat shaped and shorter than left spicule.

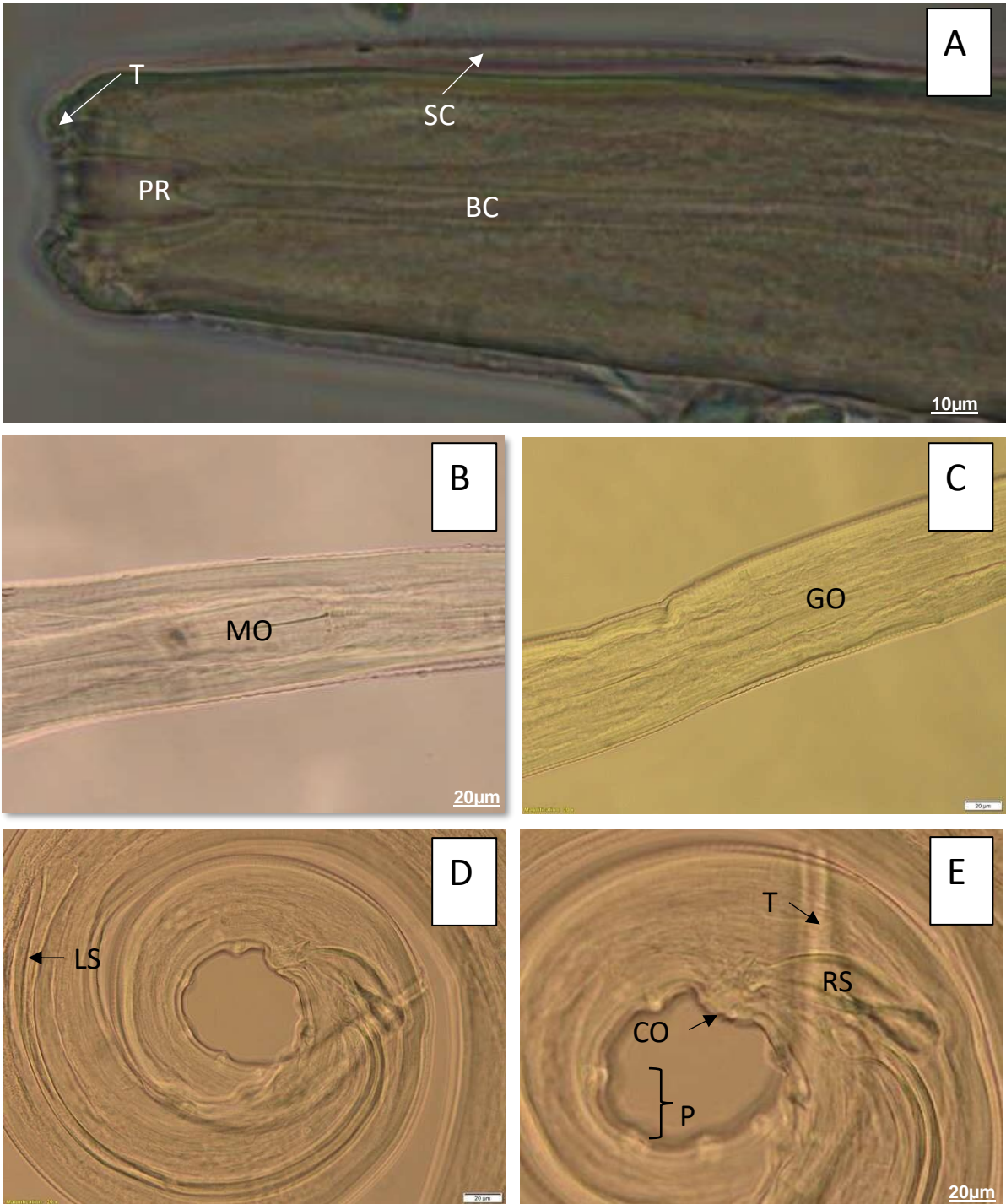


Figure 4.12: Micrographs of *Rhabdochona* sp. collected from the intestine of *Amphilius uranoscopus*. A = anterior end; B = anterior part; C = mid anterior part; D and E = posterior end; BC = buccal cavity, CO = cloacal opening, GO = glandular oesophagus, LS = left spicule, MO = muscular oesophagus, P = papillae, PR = Prostom, RS = right spicule, SC = striated cuticle, T = tail, T = teeth.

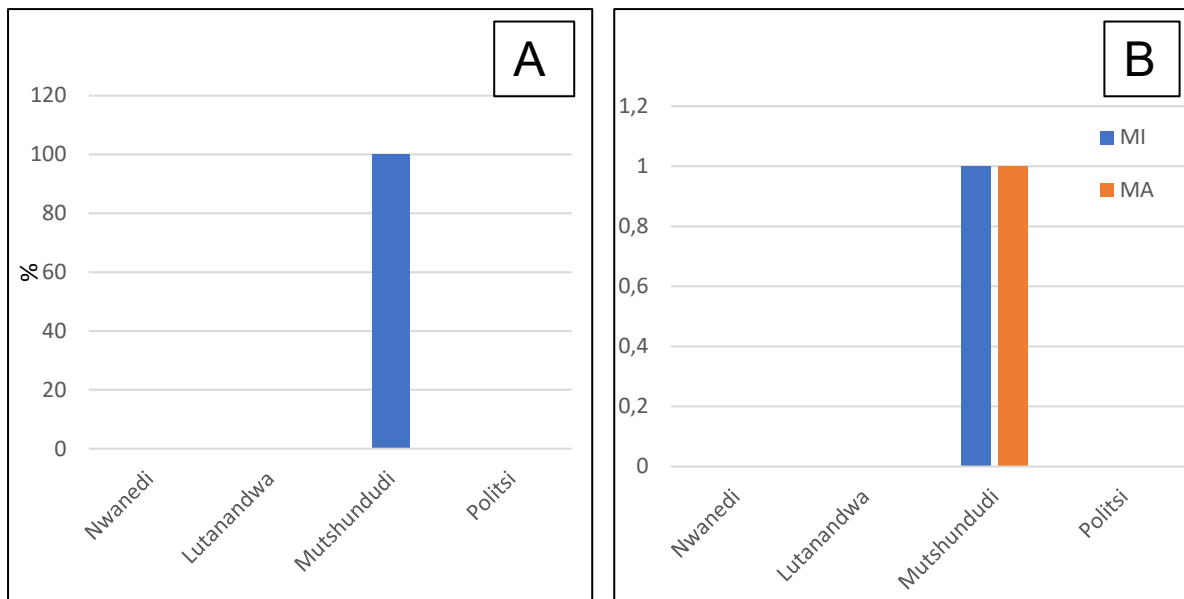


Figure 4.13: The prevalence (A), mean intensity and mean abundance (B) of *Rhabdochona* cf. *paski* collected from the intestine of *Amphilius uranoscopus* from Mutshundudi River. MA - mean abundance. MI - mean intensity.

ORDER: Rhabditida Chitwood, 1933

FAMILY: Anisakidae Skrjabin & Karokhin, 1945

GENUS: *Contracaecum* Railliet & Henry, 1912

Contracaecum sp. was collected from two rivers: Nwanedi (n = 27) and Lutanandwa (n = 2). The highest prevalence of 71.43% was recorded in Nwanedi River and 8.69% recorded in Lutanandwa River. The prevalence, mean intensity and mean abundance were calculated and presented in Figure 4.15.

Morphology

Body with transversely striated with a whitish colour. Head end rounded and a mouth with three lips. The dorsal lip has two lateral papillae. The two ventro-lateral lips have a small papilla in each. A cephalic tooth is found between these lips (Figure 4.14 A). The oesophagus is narrow, and the excretory pore opens at the head. Short conical tail (Figure 4.14 B).

Remarks

Contracaecum sp. adults are found in piscivorous birds, and the larvae in fish. The parasites collected were not identified to species level because they were larvae with inadequate features to aid in identifying the specimens using morphological analysis.

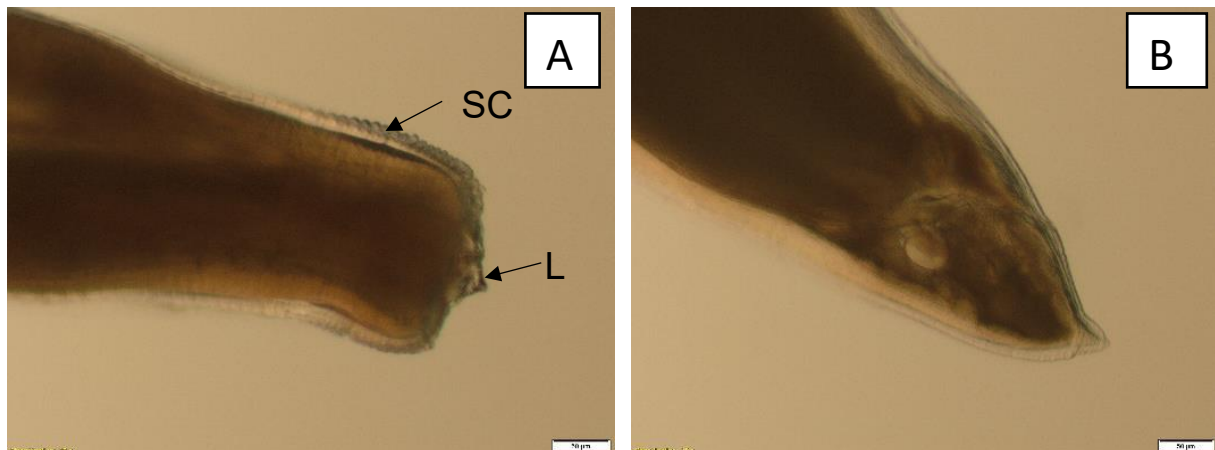


Figure 4.14: Micrographs of *Contracaecum* sp. collected from the body cavity of *Amphilius uranoscopus* from Nwanedi and Lutanandwa Rivers. A = anterior end; B = posterior end, short conical tail; SC = striated cuticle, L = lips.

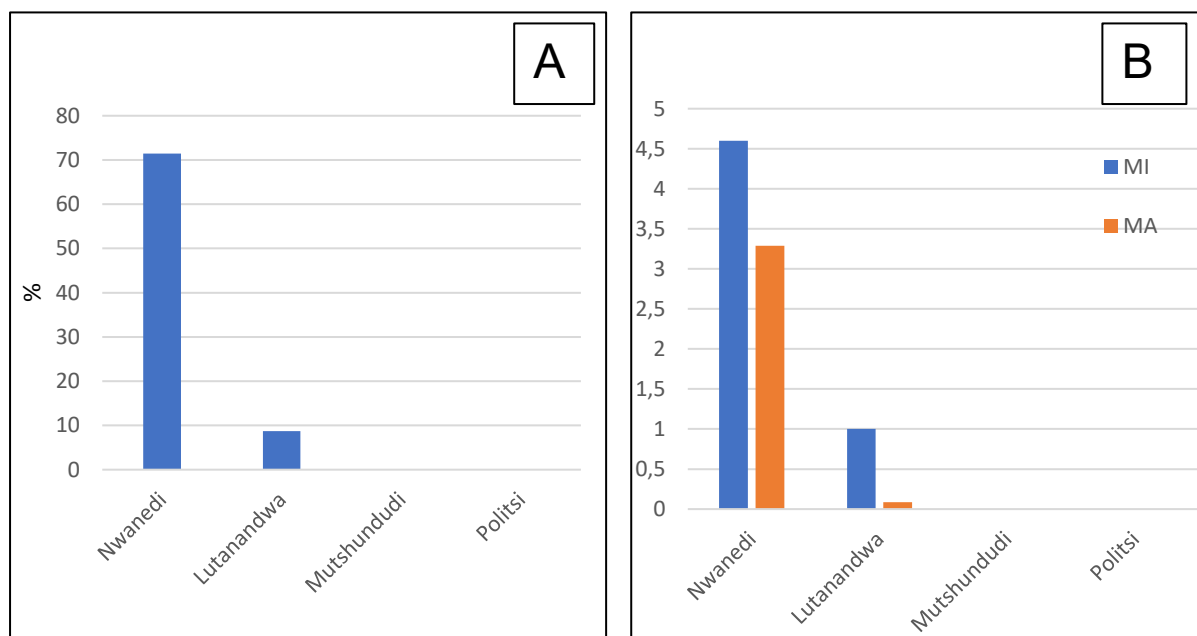


Figure 4.15: The prevalence (A), mean intensity and mean abundance (B) of *Contracaecum* sp. collected from the body cavity of *Amphilius uranoscopus* from Nwanedi and Lutanandwa rivers. MA - mean abundance. MI - mean intensity.

4.4 Discussion

Uvulifer is a genus of digeneans under Diplostomidae containing 19 species which is found all around the world (Subair et al. 2013). Its larval stages, cercariae and metacercariae encyst in or on snails and fishes, respectively, while the final hosts are piscivorous birds. The majority of these parasites are found in Kingfishers. The infestation of fish with these parasites often causes Black spot disease (Achatz et al. 2019).

The genus *Labeonema* consists of species that parasitise the intestine of fishes belonging to Cyprinidae, Schilbeidae, Citharinidae and Mochokidae (Koubková et al. 2008). The parasitic species from this genus are only from African freshwater fishes with four identified species (*Labeonema intermedium* Puylaert, 1970, *Labeonema bainaie* Baker, 1982, *Labeonema bakeri* Van Waerebeke, Chabaud, Bain & Georges, 1988 and *Labeonema africanum* Railliet, 1917). The morphology of all these species is very similar and the spicules are used to distinguish the different species (Moravec & Van As 2004; Koubková et al. 2008). In the present study, only a single female was found with no male to aid in identification by using the length of the spicules.

The genus *Gendria* is one of the three genera of Quimperidae with species that are reported to parasitise both freshwater amphibians and freshwater fishes from Africa and South Asia. Species of the genus *Gendria* possess three teeth, and a circular oral aperture. This parasite was previously found in Central Africa in the host *Pantodon buchholzi* (Moravec & Jirku 2017).

The genus *Rhabdochona* consists of many species that are parasitic in freshwater fishes from all zoogeographical regions (Moravec & Jirků 2014). These species are exclusively freshwater parasites (Moravec 2010). Mashego (1990) recorded *Rhabdochona esseniae* Mashego, 1990 in *Barbus* species (Cyprinidae) in South Africa while some records of *Rhabdochona paski* (Baylis, 1928) have been found in Central Africa from catfishes of the Mochokidae (Moravec & Van As 2015). Nine species are known from this genus, but it is suspected that some have not been discovered yet since little is known about the nematode fauna of the freshwater fishes in Africa (Moravec & Jirků 2014).

Contracaecum sp. is distributed in several fish species from both marine and freshwater environments and aquatic invertebrates serve as intermediate hosts. The larvae have mostly been reported from catfishes and cichlids from South Africa and Egypt (Martinsa et al. 2005; Younis et al. 2017). Its third-stage larva is commonly found in the body cavity, mesenteries and branchial chambers (Younis et al. 2017). The adults are commonly found in the gut of fish-eating birds. They are among the nematode species that are most prevalent in Africa (Mattiucci et al. 2008; Tavakol et al. 2015). *Contracaecum* can cause human anisakiasis, a disease caused by the consumption of raw fish with the parasite's larvae infesting humans as accidental hosts. Although these nematodes stop development in the alimentary canal of humans, their presence may have severe pathological consequences. This disease has recently become a major health and also of economic importance (Younis et al. 2017).

Infestation data

The ability of trematodes to parasitise the intestine and almost all body parts of fish allow them to exploit a wide range of hosts within aquatic ecosystems (Muller et al. 2015). This was observed for *Uvulifer* sp. as it was the most abundant parasite and found in all rivers except the Mutshundudi River. The absence in the Mutshundudi River was probably due to the collection of only one fish during the current study. Fish from the Lutanandwa River had a prevalence of 100% for *Uvulifer* sp. with a high mean intensity and mean abundance of 119.56. The presence of trematodes is mainly determined by the presence of the final host in the area (Gordy et al. 2020). The final hosts of *Clinostomum* spp. are piscivorous birds and the absence of their specific hosts in the area may have caused the low prevalence of *Clinostomum* sp. in the Lutanandwa River and its absence in the other rivers. *Contracaecum* sp. larva was the most prevalent nematode species. This may be due to high survival rate due to their hardened striated cuticle and being encysted that prevents them from being affected by the external factors such as pollution. High prevalence was expected because compared to the other collected nematode species, *Contracaecum* sp. is known to be among the most prevalent nematodes species in Africa and shows evidence of limited host specificity (Tavakol et al. 2015). *Gendria* cf. *thysi* had the lowest prevalence compared to other nematodes species, *Gendria* sp., *Labeonema* sp. and

Rhabdochona sp. had a low mean intensity of 1, due to collection of one specimen of each species. Adult digeneans of Cephalogonimidae gen. sp. were found only infish from the Politsi River with a high prevalence of 88.88%.

4.5 Conclusion

Eight species (Cephalogonimidae gen. sp., *Clinostomum* sp., *Uvulifer* sp., *Rhabdochona* cf. *Paski*, *Labeonema* sp., *Contracaecum* sp., *Gendria* sp., *Gendria thysi*) from seven genera were found from *A. uranoscopus* from the four rivers with a single species (*Clinostomum* sp.) previously recorded from the host.

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CHAPTER 5

FISH HEALTH AND WATER QUALITY FROM SELECTED RIVERS FROM THE LIMPOPO RIVER SYSTEM

5.1 Introduction

5.1.1 Water quality

Water quality refers to the physical, chemical, biological, organoleptic and appearance properties of the water (Department of Water affairs and Forestry (DWAF) 1996a). These properties affect a variety of factors such as the fitness for use, ecosystem health and diversity of aquatic organisms. Various water quality ranges are suited for various uses of water (Bartram & Ballance 1996). Water quality can be determined by measuring physical, chemical and biological constituents. Physical and chemical constituents are temperature, TDS, pH, EC, DO and metal concentration. Conductivity and TDS are physical parameters used to measure salinity and their values are correlated (Rusydi 2018). The physical and chemical properties of water in temporal regions vary in seasons (Forio & Goethals 2020). Analysing the water quality constituents assist in determining the state of the water quality (DWAF 1996a).

The chemical constituents can be affected by metals and nutrients present (Stevenson & Esselman 2013). Metals are divided into two groups: essential and non-essential. The essential metals are required by aquatic organisms in low amounts for nourishment and they naturally occur in aquatic ecosystems, for example calcium, zinc, iron, manganese, copper, and sodium. Their presence in water in high concentrations can have harmful effects on the organisms. Non-essential metals such as mercury, lead, nickel and cadmium have toxic effects on organisms at low concentrations, and they are not required by aquatic organisms for survival (Rai 2008; Gheorghe et al. 2017). They do not occur naturally in high concentrations in aquatic ecosystems but are deposited in water through mining and industrial discharges. Once these metals are introduced to aquatic ecosystems, they cannot degrade; instead they are distributed to different parts of the water including the sediments while some accumulate in the organisms found in the system. Aquatic ecosystems that are heavily contaminated by non-essential metals have reduced biodiversity (Rai 2008).

Nutrients are inorganic chemicals such as nitrogen, phosphorus and ammonium needed by aquatic organisms to survive. They are used by autotrophs to produce food (Stevenson & Esselman 2013). They are naturally found in the water from bacteria, decayed organisms, and the dissolution of rocks. A short supply of these nutrients can limit plant and algae growth while an excess supply causes nutrient pollution (eutrophication). Eutrophication causes reduced dissolved oxygen and increased pH levels which leads to imbalances in the ecosystem and alters biodiversity through the reduction of microorganisms and fishes that are oxygen-sensitive (De Villiersa & Thiar 2007; Stevenson & Esselman 2013).

Different ranges of water quality are required for different uses of water. The government of South Africa has identified a range of water quality suited for various water uses and this includes the targeted water quality range suitable to sustain healthy aquatic ecosystems (DWAF 1996a). The values are included in Tables 5.1, 5.2 and 5.3 for physical parameters, nutrients, and metals.

5.1.2 Fish health for biological monitoring

Various factors, both anthropogenic and natural, can alter the water quality through contamination of an aquatic ecosystem causing disturbances that lead to stress on aquatic organisms (Das 2005; Traoré et al. 2016). Other anthropogenic factors such as industrialisation cause the discharge of heavy metals into water bodies, and these changes have detrimental effects on the aquatic organisms in the latter stage (Behmel et al. 2016). The degraded water quality in the tributaries leads to altered species composition (Ouyang 2005). Poor water quality in South Africa has increased the need to monitor water bodies including the Limpopo River System. Monitoring is acquiring information on the chemical, biological and physical characteristics of the water body to indicate changes induced by anthropogenic activities and geological activities (Holt 2000; Strobl & Robillard 2008).

Monitoring ecosystems is essential for sustaining good water quality for the survival of aquatic organisms. Although many factors should be considered when assessing the health of fish in a certain river system (Watson et al. 2012), the water quality of a river can be reflected by the health of the aquatic organisms, in addition to the number and type of parasites present in/on fish (Adams et al. 1993). The Health Assessment Index

(HAI) is a biological monitoring tool which uses fish's health since the quality of water can have an impact on their health. These methods equate low HAI with good water quality. This method has successfully been used in South Africa to evaluate the health of river systems (Heath et al. 2004; Crafford and Avenant-Oldewage 2009; Madanire-Moyo et al. 2010; Sara et al. 2014).

Adams et al. (1993) indicated the use of health in conjunction with parasites as good indicators of water quality. The parasite index (PI) divides parasites into endo- and ectoparasites. This division is made because the two groups are affected differently by pollution. Ectoparasites are susceptible to pollution as they are exposed to the water and the opposite is observed for endoparasites as they are on the inside, and some are enclosed in cysts (Watson et al. 2012), and thus protected from direct exposure to water quality. Endoparasites are considered excellent accumulators of both organic pollutants and toxic metals (Sures et al. 2017). Crafford and Avenant-Oldewage (2009) used the inverted parasite index (IPI) indicating that larger numbers of ectoparasites will be present where there is good water quality while high numbers of endoparasites indicate poor water quality. This is based on a premise that ectoparasites are directly exposed to the effects of water quality such as pollution thus more ectoparasites will be found in good water quality.

Condition factor (K) expressed by $\text{weight}/\text{length}^3$, is another useful parameter for determining the health of an aquatic ecosystem (Adams et al. 1993; Nash et al. 2006). This is done by assessing the weight and the length of the fish. The value of K is a good indicator of fish health, with a value of 1 indicating a good health status. A value of less than 1 indicates a reduction in energy reserves and thus poorer health. The condition factor also assists in providing more information about the growth of a population. The depletion of energy reserves is caused by many factors including stress which can be caused by changes in the habitat brought by changes in water quality (Watson et al. 2012).

5.2 Materials and method

Nwanedi and Lutanandwa river's' water quality analyses were done once in winter and summer while Mutshundudi and Politsi rivers were done once in summer. Physical parameters were measured using a YSI combo meter. Water samples were collected

and transported to Capricorn Veterinary Laboratory for analysis of selected metals and nutrients. Materials and methods are discussed in detail in Chapter 2.

The HAI, K and parasite diversity index were calculated according to methods described in Chapter 2, sections: 2.8; 2.9; and 2.10.

5.3 Statistical analysis

ANOVA was used to analyse variance to test for the significant differences in the K of each fish species for the four localities. The statistical significance level was set at $p < 0.05$. Regression analysis was used to determine the correlation between parasite burden and K .

5.4 Results

5.4.1 Water quality

Physical parameters

The water temperature was the highest in the Politsi River (23°C) and lowest in the Mutshundudi River (12.5°C) (Table 5.1). The pH in the Nwanedi and Lutanandwa rivers was slightly alkaline while acidic in the Mutshundudi and Politsi rivers. The pH in all rivers was within the TWQR (Table 5.1). The DO of Lutanandwa River was the highest of those recorded (10.9 mg/l) and exceeded the TWQR. At the other rivers, the DO was within the TWQR with Nwanedi having the lowest DO of 7.66 mg/l. There is no TWQR for conductivity, but it was relatively low for all rivers with the value recorded from the Politsi River the lowest (4.8 mS/m). The turbidity of the Nwanedi and Mutshundudi rivers was relatively low and the salinity of the Nwanedi and Politsi rivers were within the range for freshwater ecosystems. The total alkalinity of Mutshundudi and Lutanandwa rivers were within the SA TWQR for aquaculture of 20-100 mg/l and the total alkalinity of Nwanedi and Politsi rivers was lower than 20 mg/l (Table 5.1).

Nutrients

The selected rivers were all oligotrophic due to low nutrient concentrations which were lower than the TWQR. The ammonia was lower than 0.2 mg/l in all sites and nitrite

was less than 0.01 mg/l. Nitrate was low in the Lutanandwa River (0.06 mg/l) and relatively high in the other three rivers. The orthophosphate of all rivers was less than 0.05 mg/l which is lower than the TWQR (Table 5.2).

Metals

Metal (aluminium, arsenic, copper, iron, mercury, manganese, lead, selenium and zinc) levels in all rivers were low. They were all lower than the TWQR and much lower than the Chronic Effect Value (CEV) (Table 5.3).

Table 5.1: Physical parameters measured in Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers in summer 2021 with results compared to the TWQR of aquatic ecosystems from DWAF (1996b). Values exceeding the TWQR are indicated with an asterisk (*).

Parameters	TWQR	Nwanedi River	Mutshundudi River	Lutanandwa River	Politsi River
Temperature (°C)	–	17.8	12.5	19.4	23
pH	6.5-9.0	6.9	7.28	6.6	7.4
Dissolved Oxygen (mg/l)	6.0-9.0	7.66	8.2	*10.9	8.06
TDS (mg/l)	–	59	54.12	86.53	31
Conductivity (mS/m)	–	9.1	6.32	13.11	4.8
Turbidity (NTU)	–	7.99	3.39	–	–
Salinity (ppt)	–	0.05	–	–	0.02
Total alkalinity(mg/l)	–	19.2	32.9	35.4	16.7

Table 5.2: Selected nutrients measured (mg/l) in Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers in summer 2021 with results compared to the TWQR from DWAF (1996b). Values exceeding the TWQR are indicated with an asterisk (*).

Nutrient	TWQR	Nwanedi summer	Mutshundudi	Lutanandwa River	Politsi
Ammonium NH4+	0.25	<0.20	–	–	<0.20
Nitrite NO2	0.06	<0.01	<0.01	<0.01	<0.01
Nitrate NO3	0.5	0.37	0.11	<0.06	0.22
Orthophosphate (PO4)	0.13	<0.05	<0.05	<0.05	<0.05
Chloride	0.2	13.9*	9.6*	4.7*	4.1*
Fluoride		<0.10	<0.10	<0.10	<0.10

Table 5.3: Selected metals recorded in mg/l from Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers. Results were compared to the target water quality range (TWQR) and Chronic Effect Value (CEV) for aquatic ecosystems (DWAF, 1996b). Dashes denote the unavailability of data.

Metals	TWQR	CEV	Nwanedi River	Lutanandwa River	Mutshundudi River	Politsi River
Aluminium	10.0	20.0	0.05	–	–	0.01
Arsenic	10.0	20.0	<0.03	–	–	<0.03
Copper	0.8	1.5	<0.01	<0.01	<0.01	<0.01
Iron	500.0	–	0.26	0.26	0.06	<0.01
Mercury	0.04	0.08	<0.01	<0.01	<0.01	<0.01
Manganese	180.0	370.0	<0.01	–	–	<0.05
Lead	0.5	1.0	<0.09	<0.09	<0.09	<0.01
Selenium	2	5	<0.02	–	–	–
Zinc	2.0	3.6	<0.01	<0.01	<0.01	<0.01

5.4.2 Fish Health

HAI and condition factor of *Chiloglanis pretoriae*

The value of HAI from this study indicated that *C. pretoriae* from the four localities had less abnormalities. The highest HAI value of 36.19 was recorded from Mutshundudi River and the lowest of 28.38 was recorded from Lutanandwa River. The HAI values were low as some parameters such as the haematocrit, were omitted as blood was not collected due to the fish being too small to draw blood. Liver, spleen and bile appeared normal for all fish and therefore did not contribute to the score (Table 5.4).

Table 5.4: The Health Assessment Index of *Chiloglanis pretoriae* from Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers.

	Inverted Parasite Index (IPI)	Total HAI
Nwanedi River	29.42	31.73
Mutshundudi River	30	36.19
Lutanandwa River	27	28.38
Politsi River	29.29	34.29

The average K score of *C. pretoriae* from all localities were similar and were slightly above 1 with the highest K score of 1.08 from Nwanedi River and lowest of 1.01 from Lutanandwa River (Figure 5.1).

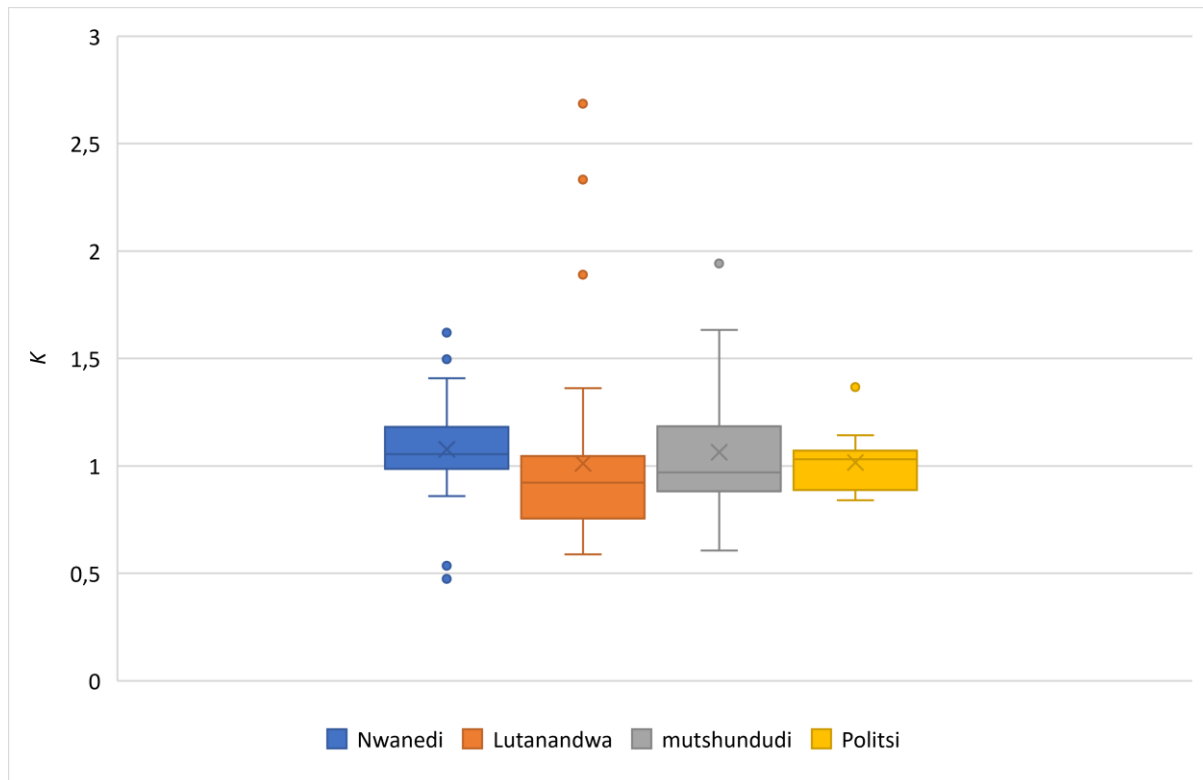


Figure 5.1: Box plot showing K values calculated for *Chiloglanis pretoriae* from Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers.

Parasite burden of *Chiloglanis pretoriae*

Parasite burden was examined to observe the influence of parasite burden on the K of both *C. pretoriae* and *A. uranoscopus* collected from the four rivers. Regression analysis showed that there was a weak negative correlation between K and parasite burden of *C. pretoriae* collected from all rivers, e.g., Nwanedi ($y = -0.0009x + 1.1034$), Lutanandwa ($y = -0.0152x + 1.0321$), Mutshundudi ($y = -0.0078x + 1.103$) and Politsi ($y = -0.0017x + 1.0176$) rivers (Figure 5.2).

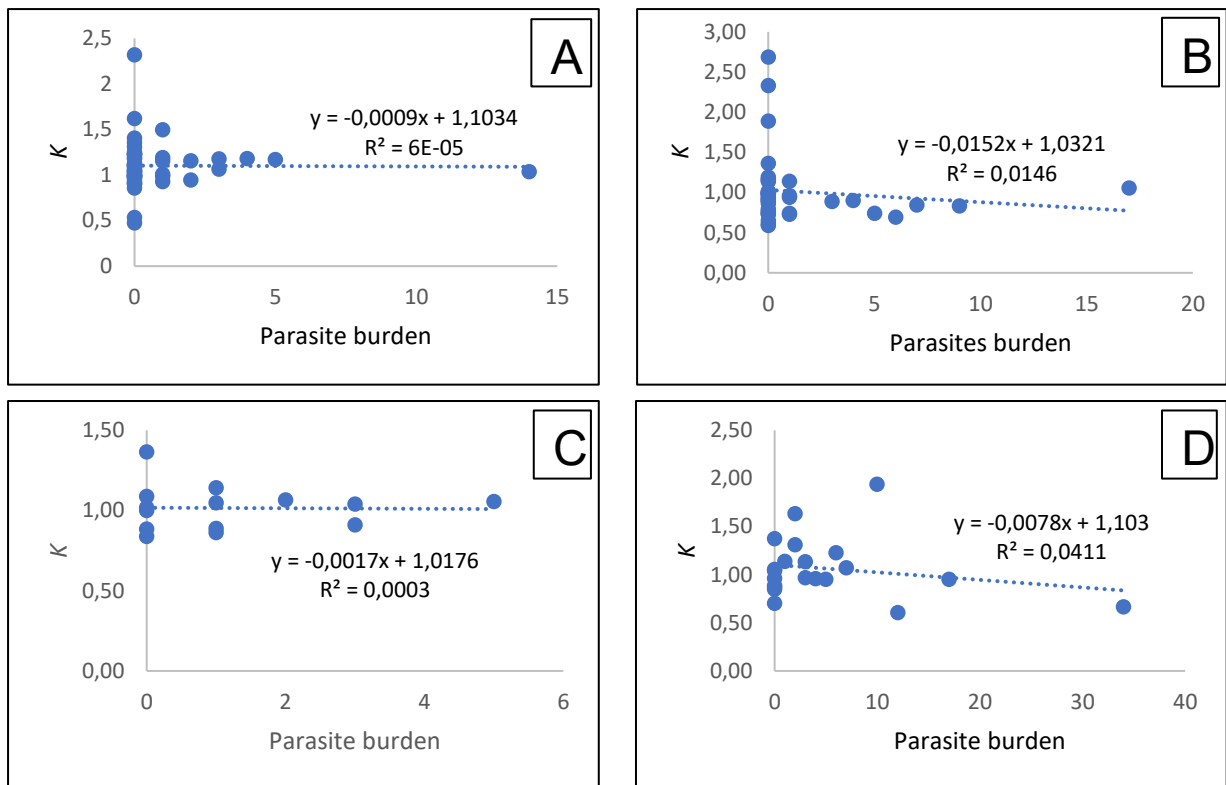


Figure 5.2: Regression analysis showing the effect of parasite burden on condition factor of *Chiloglanis pretoriae* collected from Nwanedi, Lutanandwa, Politsi and Mutshundudi rivers during winter and summer. A = Nwanedi; B = Lutanandwa; C = Politsi and D = Mutshundudi River.

HAI and condition factor of *Amphilius uranoscopus*

The HAI of *A. uranoscopus* from the four selected localities indicated that the fish collected from Lutanandwa River were not in good health. The highest HAI of 99.13 was recorded from Lutanandwa River and lowest HAI of 36.36 was recorded from Nwanedi River. The IPI for ectoparasites of 30 was observed in fish from all rivers because no ectoparasites were found on *A. uranoscopus* (Table 5.5).

Table 5.5: The Health Assessment Index of *Amphilius uranoscopus* from Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers.

	Inverted Parasite Index (IPI)	Total HAI
Nwanedi River	30	36.36
Mutshundudi River	30	40
Lutanandwa River	30	99.13
Politsi River	30	38.89

Large numbers of digenean cysts on or in fish organs caused damage to the organs. For example, digenean larvae encysted on the base of the gill filaments (Figure 5.3) can disrupt gill function and affect respiration. The abnormalities were on the skin (Figure 5.3 A), 33.33% on the gills (Figure 5.3 B) and 5.80% on the fins. All *A. uranoscopus* collected from the Lutanandwa River had impaired gills, 18 fish with impaired skin and four with damaged fins.

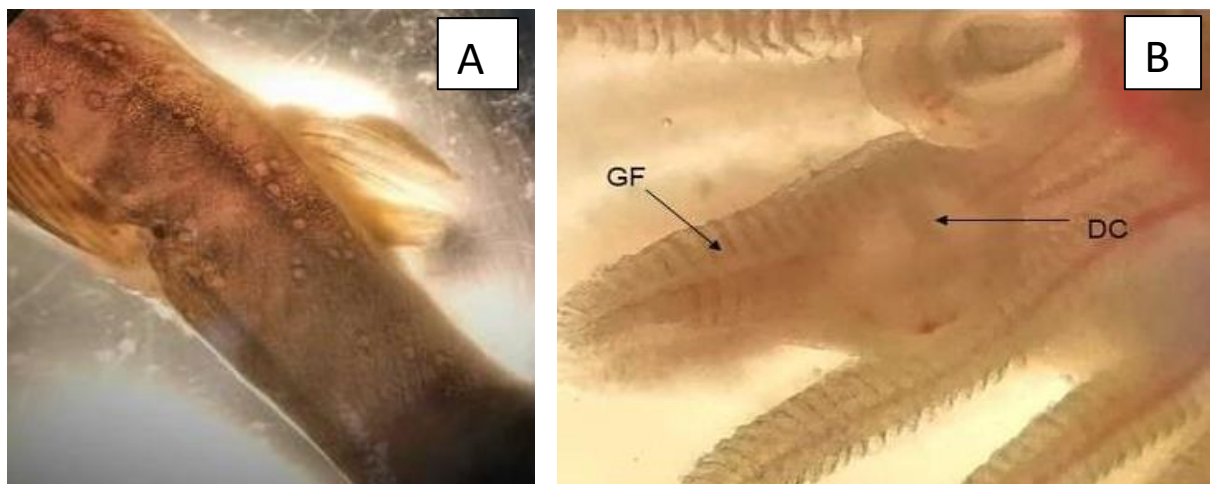


Figure 5.3: Micrographs of digenean infestation from *Amphilius uranoscopus* from Lutanandwa River. A = impaired skin by digenean infestation; B = impaired gill filament by infestation of digenean cysts; DC = digenean cyst GF = gill filament.

The average *K* of *A. uranoscopus* from all the rivers were lower than one. The highest mean *K* of 0.84 was recorded from Nwanedi River while the lowest of 0.53 was recorded from Mutshundudi River (Figure 5.4).

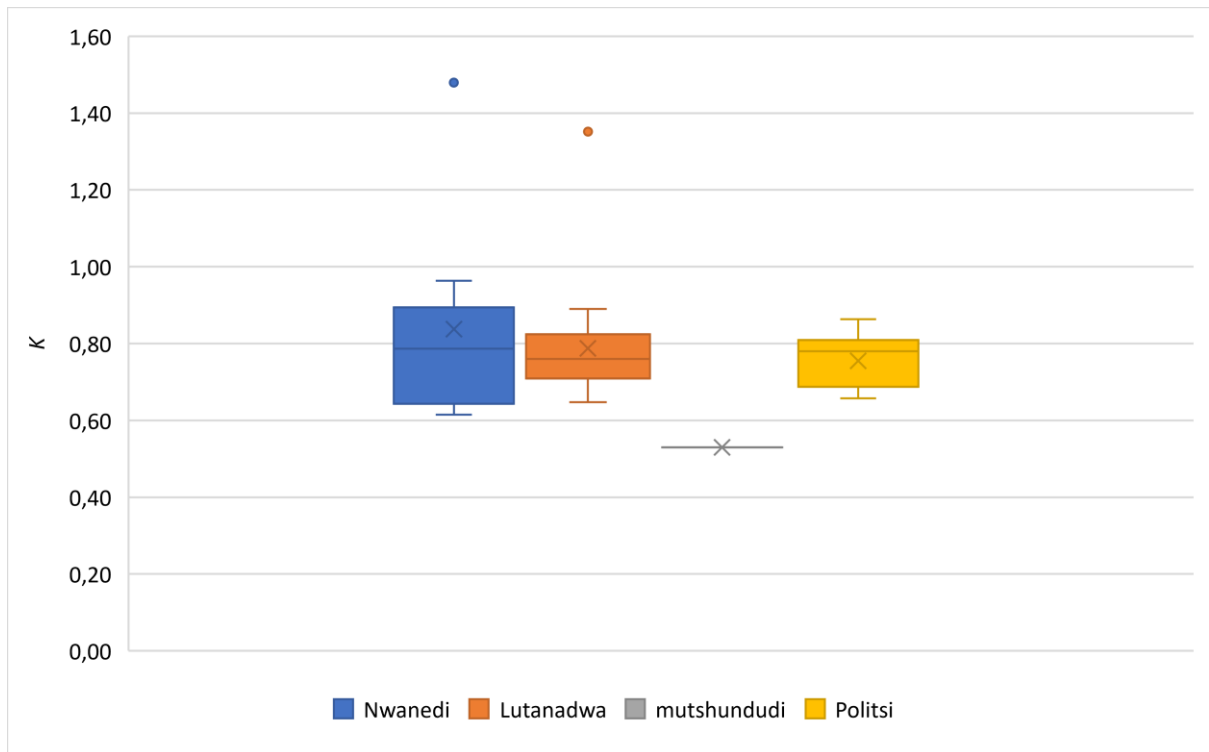


Figure 5.4: Box plot showing K values calculated for *Amphilius uranoscopus* from Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers.

Parasite burden of *Amphilius uranoscopus*

The regression analysis showed a weak negative correlation between parasite burden and K of *A. uranoscopus* collected from Nwanedi ($y = -0.0209x + 0.8903$) and Lutanandwa ($y = -8E-05x + 0.7976$) rivers but a weak positive correlation of *A. uranoscopus* collected from Politsi River ($y = 0.0044x + 0.7088$) (Figure: 5.5).

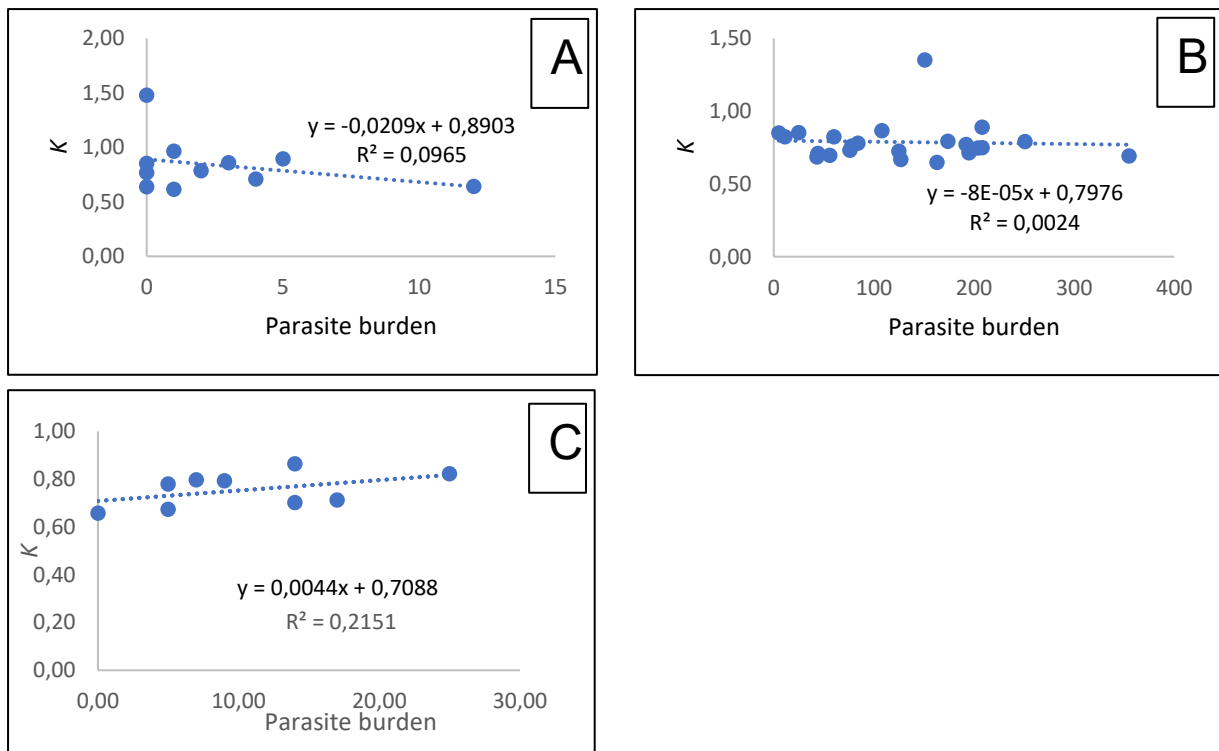


Figure 5.5: Regression analysis showing the effect of parasite burden on condition factor of *Amphilius uranoscopus* collected from Nwanedi, Lutanandwa and Politsi rivers. A = Nwanedi; B = Lutanandwa and C = Politsi.

5.4.3 Parasite diversity and species richness

A total of 3 389 parasites (*C. pretoriae*, n = 220 and *A. uranoscopus*, n = 3169) were collected from 171 fishes, *C. pretoriae* (n = 127) and *A. uranoscopus* (n = 44). Three parasite groups (monogeneans, digeneans and hirudineans) were collected from *C. pretoriae* and two groups (digeneans and nematodes) from *A. uranoscopus*.

The Shannon-Weiner and Margalef indices were used to calculate species diversity and species richness, respectively, and highest values of 0.53 and 0.54 were calculated for the Nwanedi River and lowest of 0.05 and 0.21 recorded for the Mutshundudi River. The results indicate that the Nwanedi River has better water quality as compared to the Mutshundudi River, since higher parasite species richness and parasite diversity are found in healthier aquatic ecosystems (Marcogliese 2005; Krause et al. 2010). According to the Intermediate Disturbance Hypothesis (Connell 1978), increased species diversity is influenced by less ecological disturbances (while pollution causes poor water quality). The acidic pH of the Mutshundudi water may have been caused by anthropogenic impacts such as deposition of agricultural fertilizers as

there are plantations around the upper reaches of the river. The Shannon-Weiner evenness of *C. pretoriae* indicated that the parasites were not evenly distributed, especially in Mutshundudi River. Diplostomidae gen. sp. was the most abundant parasite which was also the most dominant parasite in Nwanedi and Mutshundudi rivers. The Lutanandwa River was dominated Dactylogyridae gen. sp. and the Politsi River by Cephalogonimidae gen. sp. (Table 5.6).

The Shannon-Weiner and Margalef indices of *A. uranoscopus* were highest in the Mutshundudi River (0.69 and 1.44) and the lowest Shannon-Weiner in the Lutanandwa River. indicating greater parasite diversity and parasite richness. The results indicated the Mutshundudi River has better water quality as compared to the other rivers. Lutanandwa River (Marcogliese 2005; Krause et al. 2010). The Shannon-Weiner evenness indicated that the parasites were evenly distributed in the Mutshundudi River only. *Uvulifer* sp. was the most abundant parasite which was also the most dominant in the Lutanandwa River. The Nwanedi River was dominated by *Contracaecum* sp. *Labeonema* sp. and *Rhabdochona* sp. dominated in the Mutshundudi River, while the Politsi River was dominated by Cephalogonimidae gen. sp. (Table 5.7).

Table 5.6: The overall parasite diversity indices of metazoan parasites of *Chiloglanis pretoriae* from Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers.

	Nwanedi River	Lutanandwa River	Mutshundudi River	Politsi River
Species Richness	3	3	2	2
Total no. of parasites	36	58	109	17
Shannon-Weiner Index (H')	0.53	0.34	0.05	0.47
Evenness (H'E)	0.48	0.31	0.08	0.67
Margalef's richness index	0.54	0.49	0.21	0.32
Dominant Species	Diplostomidae gen. sp.	Dactylogyridae gen. sp.	Diplostomidae gen. sp.	Cephalogonimid ae gen. sp.

Table 5.7: The overall parasite diversity indices of metazoan parasites of *Amphilius uranoscopus* from Nwanedi, Lutanandwa, Mutshundudi and Politsi rivers.

	Nwanedi River	Lutanandwa River	Mutshundudi River	Politsi River
Total no. of species	2	5	2	3
Total no. of parasites	28	3042	3	96
Shannon-Weiner Index (H')	0.26	0.02	0.69	0.12
Evenness (H'E)	0.37	0.01	1	0.11
Margalef's richness index	0.30	0.50	1.44	0.22
Dominant Species	<i>Contracaecum</i> sp.	<i>Uvulifer</i> sp.	<i>Labeonema</i> sp. <i>Rhabdochona</i> sp.	Cephalogonimidae gen. sp.

5.5 Discussion

Water quality

The temperature of water in aquatic ecosystems can be affected by various aspects including vegetation cover, returning irrigation water, water volume and turbidity. Aquatic ecosystems' water can have daily fluctuations with regard to daily background temperatures (Dallas & Day 2004). As a result, temperature changes then affect aquatic ecosystem aspects such as DO, photosynthetic activity, chemical toxicity, and metabolism rate. The highest DO of 8.06 mg/l recorded in the Politsi River was within the TWQR which may have been influenced by the average (23°C) water temperature since temperature affects DO concentration (Dallas 2008; Febiyanto 2020). The highest DO (10.9 mg/l) recorded in Lutanandwa River exceeded the TWQR and this may have been caused by increased aeration of water as the river flows on uneven surfaces and during churning actions such as wind and waves (Kale 2016). An increase in turbidity can be due to soil erosion and organic organisms which can cause increase of water temperature (Dallas 2008). This was observed in the Nwanedi River where the temperature of 17.8°C was higher due to higher turbidity of 7.99 NTU as compared to the Mutshundudi River where the temperature of 12.5°C was lower with a lower turbidity of 3.39 NTU. Turbidity may have partially influenced water temperature as time of day of water quality samples collection differed which may have also contributed to the results since both samples were collected in summer. The total

alkalinity of the Lutanandwa River was the highest which caused the pH to be slightly alkaline. The opposite was observed at the Mutshundudi River where high total alkalinity did not affect the slightly acidic pH of the water. The acidic pH of the Mutshundudi water may have been caused by anthropogenic impacts such as deposition of agricultural fertilizers as there are plantations around the upper reaches of the river.

The nutrients are responsible for plant production in aquatic ecosystems and their presence in large amounts can cause eutrophication which can lead to imbalances in the aquatic plant communities that provide food and breeding habitats (Villiers 2007). Nutrient enrichment, particularly nitrogen pollution can also decrease pH and reduce dissolved oxygen (Stevenson & Esselman 2013; Mudaly & Van der Laan 2020). This was observed in the Nwanedi River where there was high nitrate concentration, and the pH was slightly acidic. Conversely, in the Politsi River, nitrate concentrations were also slightly high but that did not affect the pH of the water since it was slightly basic. Although nitrate occurs naturally in fresh water, its concentration and the concentration of orthophosphate can increase due to sewage effluents, agricultural runoffs and fertilisers. The phosphate gets absorbed by the sediments (Dallas & Day 2004). The concentration of ammonium in water can be altered by the deposition of nitrogen compounds of both nitrates and nitrites in the river body (DWAF 1996a). Ammonium concentrations in the Nwanedi and Politsi rivers were moderate. The Politsi River is situated close to an area of extreme agricultural activities causing large amounts of agricultural runoff to the river increasing the amount of ammonium. However, concentrations were moderate and less than 0.2 mg/l which is below the TWQR. Nitrite concentration was low in all rivers which may have caused the moderate concentration of ammonium.

According to DWAF (1996a), aluminium is described as a non-critical element, with the potential to cause harm in aquatic ecosystems depending on the species available. It is insoluble in neutral pH, soluble and toxic in acidic conditions and soluble in alkaline conditions. Its toxicity in acidic water can occur at a pH range of 4.4 to 5.2. It can be found in acidic mine drainages (Dallas & Day 2004). Aluminium was only detected in the Nwanedi and Politsi rivers where it was in very low concentrations with 0.05 mg/l as the highest recorded from the Nwanedi River. Its concentration in both rivers was

less than the TWQR and given the pH of both rivers being greater than 5.2, these rivers did not have aluminium toxicity levels during the time of sampling.

High levels of essential metals such as copper, iron and zinc can have hazardous effects on the ecosystem (Rai 2008). Although they occur naturally in water, they can also be deposited in water through sewage treatment, industrial effluents and groundwater contamination (DWAF 1996a). Generally, the concentration of elements in the four rivers was low. Copper is one of the most widely used metals globally and it is more soluble in acidic water and precipitates in alkaline water. It is toxic in low concentrations and the presence of zinc, sulphate and molybdenum can reduce its toxicity (Dallas & Day 2004). Copper was detected in all rivers in extremely low concentrations of less than 0.01 mg/l which were lower than the TWQR. Zinc was also detected in water which would have decreased the toxicity of copper if there were any.

Iron is one of the most abundant elements on Earth. It can be easily absorbed into fish's gastrointestinal tract (Dallas & Day 2004). According to DWAF (1996b), for aquaculture, the concentration of iron in unpolluted water ranges from 0.001 mg/l to 0.5 mg/l. Iron concentrations in all rivers was lower than 0.5 mg/l, although it was higher in the Nwanedi and Lutanandwa rivers, compared to the other localities. The iron concentrations recorded in these rivers during the current study can not cause any harm to the aquatic organisms.

Mercury and lead are heavy metals that get absorbed by sediments and their presence in water may cause a lot of harm to the environment. These non-essential metals were detected in all rivers in very low concentrations that were below the TWQR and none of them reached the Chronis Effect Values.

Fish health

There was no significant difference between the *K* of both *C. pretoriae* and *A. uranoscopus* collected from all rivers. Watson et al. (2012) indicated that a *K* close to 1 indicates good health while below 1 indicates poor health. The *K* of *C. pretoriae* indicated that the fish in all rivers were in good health as all their average *K* were greater than 1. The *K* of *A. uranoscopus* indicated different results which indicate slightly poorer fish health as *K* was below 1. The highest mean *K* of *A. uranoscopus*

was recorded for fish collected from the Nwanedi River (0.84). The mean *K* results were as follows: Nwanedi > Lutanandwa > Politsi > Mutshundudi River. This indicates that *A. uranoscopus* collected from Nwanedi River were in better condition although not in the best condition because the average condition was less than 1. The low sample size of *A. uranoscopus* from Mutshundudi River influenced the low average *K*. The same might be the case in the other three rivers since the sample size was not the same. The regression analysis indicated that there was no relationship between *K* and parasite burden since there is no significant difference ($p > 0.05$).

The use of HAI in conjunction with IPI [as used by Jooste et al. (2005)] indicated that *C. pretoriae* and *A. uranoscopus* scores ranged from 28.38 – 36.19 and 36.36 – 99.13, respectively. *Chiloglanis pretoriae* values indicated that generally all the rivers are in good health since low HAI scores indicate good water quality (see also Adams et al. 1993; Crafford & Avenant-Oldewage 2009). The low HAI value of *C. pretoriae* was a result of less to no morphological anomalies and the presence of more endoparasites compared to ectoparasites. The lowest HAI value of *C. pretoriae* was found in less impacted Mutshundudi River which correspond with water quality results. The low HAI values of *A. uranoscopus* indicated all rivers were in good condition with Lutanandwa slightly lower water quality. This was due highest total HAI values for *A. uranoscopus* recorded in Lutanandwa River. The HAI values from *A. uranoscopus* of the Lutanandwa River contradicted with water quality results which was caused by large numbers of endoparasites while presence of more ectoparasites indicate better water quality (Sara et al. 2014). Throughout the use the HAI as a tool for biological monitoring in South Africa, only large Siluriforme *C. gariepinus* has been used (Crafford & Avenant-Oldewage 2009; Madanire-Moyo et al. 2012; Watson et al. 2012; Sara et al. 2014) thus the results of this study through the use of small Siluriformes species were different and previous results cannot be used to compare the HAI recorded during the present study.

Parasite diversity and species richness

It has been proposed that higher parasite species richness and parasite diversity are found in healthy aquatic ecosystems (Marcogliese 2005; Krause et al. 2010). The use of parasites for biological monitoring was done by comparing the parasite diversity and species richness for each river using Shannon-Weiner and Margalef indices with

higher values representing higher parasite diversity and higher species richness, respectively. The Shannon-Weiner's evenness was used to compare the distribution of parasite species in different localities with the value of 1 indicating equal distribution and 0 indicating uneven distribution. The values of Shannon-Weiner and Margalef's indices for parasites collected from *C. pretoriae* were higher in the Nwanedi River (0.53 and 0.54) while those of parasites collected from *A. uranoscopus* were in higher in the Mutshundudi River (0.69 and 1.44). Although species richness and diversity results for *A. uranoscopus* were expected because the Mutshundudi River is less impacted, the value of the indices was affected by the small sample size of fish and parasites. Higher values of indices were not expected in the Nwanedi River as the water quality results indicated that the river is mildly impacted thus the results for *C. pretoriae* contradicted with the results expected. A high evenness value of 0.67 for *C. pretoriae* was observed in the Politsi River because the species were evenly distributed. The highest evenness of 1 for *A. uranoscopus* was observed in the Mutshundudi River which was caused by the presence of only two parasite species and a single specimen collected for each. An extremely low evenness of 0.01 for *A. uranoscopus* was found in the Lutanandwa River which was caused by a very high number of *Uvuliver* sp.

5.6 Conclusion

The water quality results indicated that physical parameters were all within the acceptable ranges of the TWQR at the four localities. The nutrient concentrations were low indicating the state of the rivers as oligotrophic, although the nitrate concentration of the Nwanedi and Politsi rivers were moderately high compared to the other two rivers. This can indicate their increased potential to be mesotrophic. From all the metals detected, both essential and non-essential, low concentrations were detected, and they were below the TWQR and none of them reached the CEV. The results indicated that the rivers have good water quality, but Nwanedi and Politsi are slightly impacted by anthropogenic factors compared to the other two rivers. The results of both *C. pretoriae* and *A. uranoscopus* also indicated that parasite burden did not affect *K* of the fish. Parasite diversity and species richness of *A. uranoscopus* indicated Mutshundudi and Lutanandwa to have good water quality due to high species richness and parasite diversity. The HAI values of both *C. pretoriae* and *A. uranoscopus* indicated that the rivers had good water quality, with Lutanandwa river slightly

impacted. Species richness and parasite diversity of *C. pretoriae* indicated that the Nwanedi, Politsi and Lutanandwa rivers had good water quality. The same was observed for *A. uranoscopus* given its low abundance and the small sample size as compared to *C. pretoriae*. Although some of the results were expected, they should be interpreted with caution as parasites are influenced by many factors like the presence of intermediate and final hosts, water quality, etc.

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CHAPTER 6

SUMMARY AND RECOMMENDATIONS

6.1 General summary

The study aimed to determine and compare the parasite composition and health of *C. pretoriae* and *A. uranoscopus* from the four rivers (Nwanedi, Mutshundudi, Lutanandwa and Politsi) within the Limpopo River System using the HAI, IPI and K. Four research questions were addressed. The research questions of this study were posed in response to limited information of parasite composition of *C. pretoriae* and *A. uranoscopus* and the health of the two fish species given the poor water quality of some localities within the Limpopo River System.

The results from the study indicated that *C. pretoriae* and *A. uranoscopus* have a diversity of parasite species with a couple of new species described during the current study which were hypothesised since the two fishes were understudied in terms of parasites infesting them. *Amphilius uranoscopus* possessed a higher species richness as compared to *C. pretoriae*. The higher number of parasite species recorded from *A. uranoscopus* may have been caused by factors such as its diet (macroinvertebrates) as parasites are accumulated along the food chain; their larger body size (compared to that of *C. pretoriae*) as larger body size allows diverse parasite colonisation for available niches (see for example Sasal et al. 1997). Parasites collected from *C. pretoriae* from the four localities were Cephalogonimidae gen. sp., Diplostomidae gen. sp., *Clinostomum* sp., Dactylogyridae gen. sp. and an unidentified leech. Parasites collected from *A. uranoscopus* from the four localities were Cephalogonimidae gen. sp., *Uvuliver* sp., *Clinostomum* sp., *Contracecum* sp., *Rhabdochona* cf. *paski*, *Labeonema* sp., *Gendria* sp. and *Gendria thysi*. Dactylogyridae gen. sp. and Cephalogonimidae gen. sp. are newly found genera firstly recorded from this study. A few parasites collected were not identified to genus and species level because molecular work was unsuccessful for some digeneans and limited specimens for nematodes since single specimens were recovered for most of them.

The overall highest prevalence of 57.14% for Diplostomidae gen. sp. from *C. pretoriae* was recorded from the Mutshundudi River, and the lowest prevalence of 0.25% was from the Nwanedi River for the leech. Higher prevalence, mean intensity, and mean abundance (13.64%, 4.55, 1.04) of Dactylogyridae gen. sp. were recorded in the Lutanandwa River. *Clinostomum* sp. was mostly prevalent at Nwanedi with equal mean intensity and mean abundance of 1 and 0.06, respectively, from the Nwanedi and Mutshundudi rivers. *Uvulifer* sp. was the most abundant parasite found in *A. uranoscopus* from Lutanandwa River with mean intensity and mean abundance levels of 119.56. *Uvulifer* sp., *Labeonema* sp. and *Rhabdochona* sp. had a prevalence of 100%, although the two nematodes and *Gendria* sp. had a lower mean intensity and mean abundance of 1. *Clinostomum* sp. was only recorded in the Lutanandwa River with a prevalence of 8.69%. *Contracaecum* sp. had higher prevalence, mean intensity and mean abundance of 71.43%, 4.6 and 3.29, respectively, from the Nwanedi River. *Gendria* cf. *thysi* had the lowest prevalence of 4.35% in the Lutanandwa River as compared to other nematodes. The Cephalogonimidae gen. sp. was only collected in the Politsi River in both fishes, and it was more prevalent in *A. uranoscopus* with 88.88%.

The condition factor indicated that the condition of *C. pretoriae* from all four rivers was generally good and condition factor of *A. uranoscopus* was slightly poor. It also indicated that the parasite burden of both endo- and ectoparasites did not have any significant negative effects on the *K* of both fish species. The results do not imply that parasites do not affect the health of fish because their presence may still disrupt the functionality of systems (Sures & Nachev 2022). The total HAI with IPI value of 99.13 for *A. uranoscopus* was the highest in the Lutanandwa River due to the presence of large numbers of endoparasites which did not correspond with water quality results as a high number of endoparasites has been linked to poor water quality in other studies (such as Madanire-Moyo et al. 2012).

The study indicated that the rivers have overall good water quality but two of the rivers, Nwanedi and Politsi rivers, were slightly impacted by anthropogenic factors when compared to Lutanandwa and Mutshundudi rivers. The results support the finding of the river's eco-status report of Christa & Nolusindiso (2019), who indicated that the Nwanedi and Politsi rivers are within the category of moderately to largely modified.

Forestry and agricultural activities near the Politsi River were probably the cause of the slightly elevated nutrient concentration in the river (Fouche & Foord 2001). The water quality results were also confirmed by similar results of HAI with IPI of *C. pretoriae* which were slightly higher in the Nwanedi River as compared to the other rivers where collected *C. pretoriae* had no abnormalities. Although two rivers were slightly impacted by anthropogenic factors, the quality of water did not have a significant effect on the health of the fish during the present study.

Species diversity and species richness indices distinguished between the four rivers in terms of the parasites collected from *C. pretoriae* and *A. uranoscopus* with the Nwanedi River (more impacted) having higher species richness and parasite diversity as compared to the other rivers. Although the results were not expected as high parasite diversity was expected in less impacted rivers as found by Sures (2004) and Marcogliese (2005), it was not a surprise since fish parasite communities are influenced by several factors including seasonality, host diet, parasite and host size and environmental factors (Galli et al. 2001; Iyaji et al. 2009).

6.2 Recommendations

Since it is the first record of Cephalogonimidae gen. sp. from the two fish species, it would be of value to also search for the first and second intermediate hosts in the life cycle of this parasite. The parasite was only found from the Politsi River, its presence in other rivers within the Limpopo River System should be further investigated. The trematode is a new genus in the Cephalogonimidae, therefore a paper on its description and identification is being prepared for publication. Dactylogyridae gen. sp. was another new species recorded in the present study in Lutanandwa and Politsi rivers. More studies should be done in different rivers within the Limpopo River System to determine its presence in other Limpopo River System tributaries. The monogenean is a new genus in the Dactylogyridae, therefore a paper on its description and identification is being prepared. The leech recorded from *C. pretoriae* was identified to order level because molecular work has not been done and there are not many records of leeches infesting African freshwater fishes to identify them based on morphology. Molecular work will be done to identify the leech and since there are no records of it infesting *C. pretoriae*, it may be a new species thus it will be described. Nematodes (*Labeonema* sp., *Rhabdochona* sp. and *Gendria thysi*) collected from *A. uranoscopus*

were not identified to species level using morphology since only one specimen (female specimen) of each genus was collected. More studies should be done on *A. uranoscopus* to further identify the nematodes, including using male specimens and using both molecular analyses and the SEM.

The HAI results obtained from this study indicated that the rivers were generally healthy although not all variables were used fish were too small to draw blood for haematocrit scoring. Thus, it is proposed that HAI using *A. uranoscopus* and *C. pretoriae* should not be employed rather use their parasite diversity and species richness indices.

Fish were collected seasonally in only two of the rivers. Thus, it is proposed that the study should be done seasonally for all four localities to detect differences in the occurrence of parasites in relation to the seasonal water quality.

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Appendix A

Research permit (LEDET)



LIMPOPO

PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF ECONOMIC DEVELOPMENT, ENVIRONMENT & TOURISM

DO SCIENTIFIC RESEARCH ON AQUATIC BIOTA

(Issued in terms of the provisions of the Limpopo Environmental Management Act 2003, Act no.7 of 2003).

In terms of and subject to the provisions of the abovementioned legislation and the regulation framed thereunder, the holder of this permit is hereby authorized to catch and/or collect the species and number of aquatic biota specified on the table below for scientific purpose in the property mentioned on this permit.

Permit Holder			
Name	PROF W J LUUS-POWELL		
Trade Name	N/A		
ID/Passport Number	7105290132083		
Address [Physical Postal]	UNIVERSITY OF LIMPOPO SOVENGA, MANKWENG 0727	PRIVATE BAG X 1106 UNIVERSITY OF LIMPOPO SOVENGA 0727	

Permit Details			
Permit No :	ZA/LP/109375		
Reference No :	CPM/40117/2021		
Date Issued :	2021-07-29	Stamp:	
Valid until :	2022-07-28		
Paid (ZAR):	R 60.00		
Receipt No :	1143525		

Farm Name / Organization	District	Province	Country
	N/A	Limpopo	South Africa

See Special Condition

Species Name	Scientific Name	Quantity	Note
	2021-07-30	2021-07-29	

Printed by: Seakamela TJ

Printed Date:

Effective Date:

W Powell

Signature of Permit Holder

I acknowledge, accept and understand fully the permit conditions as described.

WILDLIFE TRADE & REGULATION

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