HELMINTH ICHTHYO-PARASITIC FAUNA OF A SOUTH AFRICAN SUB-TROPICAL LAKE

PhD in Zoology

MM MATLA

2012

HELMINTH ICHTHYO-PARASITIC FAUNA OF A SOUTH AFRICAN SUB-TROPICAL LAKE

By

MATSOELE MOSES MATLA

Thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
(ZOOLOGY)

in the
Faculty of Science and Agriculture
(School of Molecular and Life Sciences)

at the
UNIVERSITY OF LIMPOPO

Promotor **Prof SN Mashego**
Co-promotor **Prof NM Mokgalong**

2012

DECLARATION

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

Matla, MM (Mr) 8 May 2012

[redacted]

Signature: 

ACKNOWLEDGEMENTS

I heartily acknowledge the significant contributions by the following people and institutions:

I am indebted in the completion of this thesis to my promotor Prof SN Mashego for a series of dialogues, guidance, support and interest throughout this project.

My heartfelt gratitude is expressed to my co-promotor Prof NM Mokgalong for his input during the birth of this project, financial and logistical arrangements, and his unfailing interest in my research.

I thank Prof L. Khalil (retired vice-president, International Institute of Parasitology, England) for enhancing my skills in parasitology, helpful comments and interest in my success.

To my colleagues in the Department of Biodiversity, University of Limpopo, I thank you for support and encouragement. Hendrik Hattingh, Wilmien Powell, Antoinette Jooste, Nathalie Viljoen (nee Nicolaai), Rick Viljoen and Bert Polling, I enjoyed your team work and company during the fieldwork.

Behind my success are my wife (Makgaile) and my children (Kgaile, Ntshediseng and Tshepang). My heartfelt thanks for your love, interest in my work and for allowing me to “steal” your precious time.

I express my gratitude to my sisters and brothers (Thakane, Poelo, Tshepo, Ntshepiseng, Molete and MaQueen) and their families for encouragement and having faith in me.

I am greatful to my dearest parents (Pheello and Mathakane) for their teachings, sacrifices, encouragement and their greatest love at all times.

This study was possible through the financial assistance of the University of Limpopo and the National Research Foundation (NRF).

DEDICATION

In Loving Memory of my late Dad and Mom

ABSTRACT

The diversity of the helminth parasites of fishes in a clear-water, subtropical Lake Tzaneen, in South Africa was investigated. Of the 527 fish specimens sampled approximately 9000 parasites were collected. There are 38 different parasite species discussed comprising 27 Monogenea, 3 Digenea, 4 Cestoda, 3 Nematoda and 1 Acanthocephala. Four new monogenean species are described and these are *Dactylogyrus* spp. 1 to 4. Three monogenean species are introduced as first records in Africa and these are *Actinocleidus fusiformis* (Mueller, 1934), *Haplocleidus furcatus* Mueller, 1937 and *Acolpenteron ureteroecetes* Fischthal & Allison, 1940. Fourteen monogenean and one acanthocephalan species are discussed as first geographical records for South Africa and these are *Gyrodactylus rysavyi* Ergens, 1973, *Dactylogyrus brevicirrus* Paperna, 1973, *Dactylogyrus cyclocirrus* Paperna, 1973, *Dogielius dublicornis* Paperna, 1973, *Dogielius* sp., *Schilbetrema quadricornis* Paperna & Thurston, 1968, *Quadriacanthus aegypticus* El Naggar & Serag, 1986, *Quadriacanthus clariadis* Paperna, 1961, *Scutogyrus gravivaginus* (Paperna & Thurston, 1969), *Cichlidogyrus quaestio* Douëllou, 1993, *Cichlidogyrus halli* Price & Kirk, 1967, *Cichlidogyrus sclerosus* Paperna & Thurston, 1969, *Cichlidogyrus dossoui* Douëllou, 1993, *Cichlidogyrus tilapiae* Paperna, 1960 and *Acanthosentis tilapiae* Baylis, 1948. Seven species are discussed as first records for their hosts and these are *Cichlidogyrus dossoui*, *Cichlidogyrus halli* and *Acanthosentis tilapiae* on *Oreochromis mossambicus; Dactylogyrus* sp. 1 on *Barbus radiatus* and *Barbus trimaculatus; Dactylogyrus* sp. 2 on *Barbus unitaeniatus; Dactylogyrus* sp. 3 and *Dactylogyrus* sp. 4 on *Labeo molybdinus*. *Gyrodactylus rysavyi* is the only species with a first site (gills) record. The other monogenean species discussed are *Macrogyrodactylus clarii* Gussev, 1961, *Macrogyrodactylus karibae* (Douellou and Chishawa, 1995), *Dactylogyrus afrolongicornis afrolongicornis* Paperna, 1973, *Dactylogyrus allolongionchus* Paperna, 1973, *Dactylogyrus spinicirrus* (Paperna & Thurston, 1968) and *Cichlidogyrus philander* (Douëllou, 1993). The digeneans discussed are *Glossidium pedatum* Looss, 1899 and the larvae of *Diplostomum* van Nordmann, 1832 and *Clinostomum* Leidy, 1856. The Cestodes discussed are *Proteocephalus glanduligerus* (Janicki, 1928) Fuhrmann, 1933, *Polyonchobothrium clarias* Woodland, 1925 and the larvae of *Ligula intestinalis* Goeze, 1782 and family

Gryporhynchidae. The nematodes discussed are *Procamallanus laevionchus* (Wedl, 1861), *Paracamallanus cyathopharynx* Baylis, 1923 and larvae of *Contracaecum* Railliet and Henry, 1912. Monogenea were commonly found on the gills but less on the skin and in the urinary bladder. Digenea were found mainly in the eyes, brain and visceral cavity, with only one species (*Glossidium pedatum*) present in the intestines of *Clarias gariepinus*. Cestoda and Nematoda were found in the intestine and body cavity. Only one species of Acanthocephala (*Acanthosentis tilapiae*) was found in the intestines of *Oreochromis mossambicus*. No definite seasonal variations of infection and parasite affinities towards the sexes and the sizes of the hosts could be determined. The lake is oligotrophic with the water quality having no influence on the parasite diversity and species richness.

CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

LIST OF ADDENDA

CHAPTER 1
INTRODUCTION

Research boat in Lake Tzaneen

CONTENTS

1.1 HELMINTHOLOGY OF AFRICAN FRESHWATER FISH FAUNA

The wealth of literature and information on fish parasites and diseases in many parts of the world can neither be overemphasised nor underestimated. The same is not said about Africa that is home to about 3 000 species of fish in its inland water-bodies. About 568 adult helminths and several larval forms have been recovered from only 359 species of its freshwater fish (Khalil & Polling 1997). This lack of comparable studies in Africa has shown tremendous improvement since the second half of the twentieth century. This period was characterised not only by the broad based investigations of the parasite fauna, but also of the commercial exploitation of its freshwater fishes (Gibson *et al.* 1996). Information was intermittent throughout the continent, non-existent in some countries whilst the Southern African region had a very low output.

Comprehensive literature on African freshwater fish helminths are mainly compilations and consolidations of existing information such as Paperna (1979) on the monogenea of inland water fish in Africa and Paperna (1980, 1996) on the parasites, infections and diseases of fishes in Africa. The check lists of the helminth parasites of African freshwater fishes (Khalil 1971a; Khalil & Polling 1997) provide sources of reference to the original records of the parasites, to their hosts and to their geographical distribution. Khalil (1971b, 1979) discusses the helminth parasites of African freshwater fishes and their zoogeographical affinities.

Consolidated efforts on the freshwater fish helminths are rare and incomplete but are serious attempts as they include most helminth groups. Looss (1896) reported on the parasitic fauna of Egypt and mentioned the few helminth parasites then known. Beauchamp (1914) and Baylis (1940) reported on the parasites of Congo. During the second half of the last century Dollfus (1951) on Morocco, Baer (1959) on Congo, Khalil (1969) on Sudan, Khalil & Thurston (1973) on Uganda and Van As & Basson (1984) on South Africa. Khalil & Polling (1997) lists the helminth fauna. A few other works are compiled on the regions/provinces within the countries.

Three approaches are present; the fish, the parasite and the water-body have been used as focus. For convenience these studies will be reported upon in this thesis in

their relevant categories even though many address more than one approach. Those that target a fish/fish group (chapter 3, section A) are characterised by chaos due to the complexity in the systematics of certain African fishes. Studies that target the parasite groups (chapter 3, sections B – F) are mostly taxonomic and are concerned mainly with the descriptions of species, subspecies or genera.

The studies adding information to the water-body category include those on the largest African lakes, Victoria, Tanganyika and Malawi as well as the lakes Albert, Edward and George. These include the studies of Fryer (1961) and Paperna (1973, 1979) on Lake Victoria; Beauchamp (1914), Fuhrmann & Baer (1925), Baylis (1928) and Prudhoe (1951) on Lake Tanganyika; Baylis (1948) and Prudhoe (1957) on lake Malawi; Golvan (1957), Campana-Rouget (1961) and Paperna (1973, 1979) on Lake Albert; Campana-Rouget (1961) and Paperna (1973, 1979) on Lake Edward; and Paperna (1973, 1979) on Lake George. Similarly, studies on parasites of the fishes of the Nile, the Congo, the Niger and other rivers have been conducted.

Many man-made dams in Africa were also studied and the approach was extended to Southern Africa by Brandt *et al.* (1981) on Vaal Dam; Boomker (1982) on Hartbeespoort Dam; Batra (1984) on an unnamed dam in Zambia; Earlwanger (1991), Douëllou (1991, 1992, 1993), Douëllou & Earlwanger (1993), and Douëllou & Chishawa (1995) on Lake Kariba; and Mashego *et al.* (1991) on Middle Letaba Dam. The present project belongs to this category in that it is confined to Lake Tzaneen in addressing the helminth parasites of its fishes.

The importance of fish parasites is usually viewed from their influence on the health of fish and any resultant economic loss they may cause. They also have adverse consequences for other animals that serve as hosts in the life cycles including man (Mokgalong 1996; Moema *et al.* 2006) and that some infections are zoonotic. Over and above, the usefulness of parasites has been demonstrated by contribution to studies on zoogeography and continental drift, use as biological tags and for host identification, as indicators to environmental degradation or pollution and improvement of genetic stock (Prudhoe & Hussey 1977; Paperna 1979; Mashego 1982a; Avenant-Oldewage 2006; Ramollo *et al.* 2006).

1.2 HELMINTHOLOGY OF SOUTH AFRICAN FRESHWATER FISH FAUNA

Literature on the helminth parasites of freshwater fish in South Africa are relatively limited (Monnig 1926; Ortlepp 1935; Du Plessis 1948, 1952; Lombard 1960, 1968; Junor & Price 1969; Price *et al.* 1969a, b & c; Price & McClellan 1969; Prudhoe & Hussey 1977; Mashego 1977, 1982a & b, 1983, 1988, 1989, 1990, 2000, 2001; Jackson 1978; Boomker *et al.* 1980; Hamilton-Atwell *et al*. 1980; Brandt *et al*. 1981; Mashego & Saayman 1981; Van As *et al*. 1981; Boomker 1982, 1984, 1993a & b, 1994; Britz 1983; Britz *et al*. 1984a & b, 1985; Hanert 1984; Van As & Basson 1984; Mashego & Saayman 1989; Mashego *et al.* 1991, 2006; Boomker & Fetter 1993; Boomker & Puylaert 1994; Khalil & Mashego 1998; Grobler *et al*. 1999; Barson 2003; Luus-Powell *et al*. 2003; Luus-Powell 2004; Barson & Avenant-Oldewage 2006a & b; Luus-Powell *et al*. 2006; Moema *et al.* 2006; Ramollo *et al*. 2006; Le Roux & Avenant-Oldewage 2009, 2010; Olivier *et al*. 2009; Madanire-Moyo *et al.* 2011).

Nevertheless, it has made some significant advance with more scientists with sporadic studies that are mainly taxonomic. The Committee for Inland Fisheries of Africa (CIFA) maintains that parasitism is an important factor in the management of lakes and fisheries (Okorie 1973). Paperna (1980) called for more comprehensive parasitological surveys in freshwater bodies in addition to the taxonomic approach. Sinderman (1987) requests the understanding of the quantitative data from field observations and experiments in their full ecological context. These pleas for more eco-parasitological research have been supported by Mashego *et al*. (1991) and should cover the in-depth studies on the dynamics of the population biology of freshwater fish parasites.

1.3 PROBLEM STATEMENT

Parasites infecting fish in natural waters may not be detrimental to fish, but do affect its quality as food for human consumption. Furthermore, some fish parasites are zoonotic. These problems are exacerbated when fish are produced under farming or aquacultural practices where mass mortalities frequently occur. Parasites therefore cause great economic loss. The basis of any parasitological studies is the identification of parasites (systematics) and followed by their biology and ecology.

This basic approach forms the aim of this study. With this knowledge economic gain is possible through practicing suitable prevention or treatment methods for parasites that infect the hosts in large quantities.

1.4 THE PRESENT INVESTIGATION

This study responds to the calls on population dynamics of parasites from fishes in a clear-water, subtropical lake. Lake Tzaneen, like many other large man-made impoundments in South Africa has under-exploited resources and requires proper conservation and management (Tomasson *et al*. 1985). Mashego (1982a) claims that parasite infections are heavier in lakes than in rivers and Kinne (1984) states that helminth-caused diseases are more frequent in captive than in the free-living fish and that parasites are of less importance to fishes in their natural habitats but may be fatal to the hosts in polluted environments, the fisheries or aquaculture practices. This study is necessary to advise accordingly for conservation, management and planning for fish farming purposes.

The study investigated the diversity of the helminth parasites found in and on the various fishes in the lake. A parasite-host list with location in/on the host is given. The prevalence, mean intensities and abundances of the parasites are calculated. Seasonal variations of infection and parasite affinities towards the sexes and the sizes of the hosts were also determined. The materials and methods are mentioned and discussed with regard to their influence on the results. The trophic status of the lake is discussed in relation to water quality of the lake with its parasite loads. Finally, the various helminth parasite species present in the lake are discussed. They are compared morphologically and also with regard to their host specificity and their zoogeographical affinities to those found elsewhere within the continent.

This investigation contributed to the database on Lake Tzaneen in that the diversity of the helminth parasites of its fishes and some of their population aspects are now known. Again, there are new additions to our knowledge on the helminth fauna of indigenous and exotic fishes of our region. Four new species are described. The study provided 15 first geographical records for South Africa as well as three for the continent. There are also seven first host records for parasites and one first site

(organ) record. The study also paves the way for follow-up investigations that may be undertaken either in the lake itself or in comparing with the parasite information from lakes in other regions.

CHAPTER 2

MATERIALS AND METHODS

Retrieving fish from the gill net

CONTENTS

2.1 STUDY AREA

Historical background

Lake Tzaneen (previously known as the Fanie Botha Dam) is a man-made impoundment on the outskirts of the town of Tzaneen in the Limpopo Province of South Africa. Its construction began in 1969 and was completed in 1976. It was built on the farm Doornhoek, northeast of the town in the valley of the Great Letaba River, a tributary of the Olifants River (figure 2.1). The lake was constructed such that it lies below the confluence of the rivers Great Letaba, Ramadiepa, Politsi and Selokwe (figure 2.2; Nicolaai 2008).

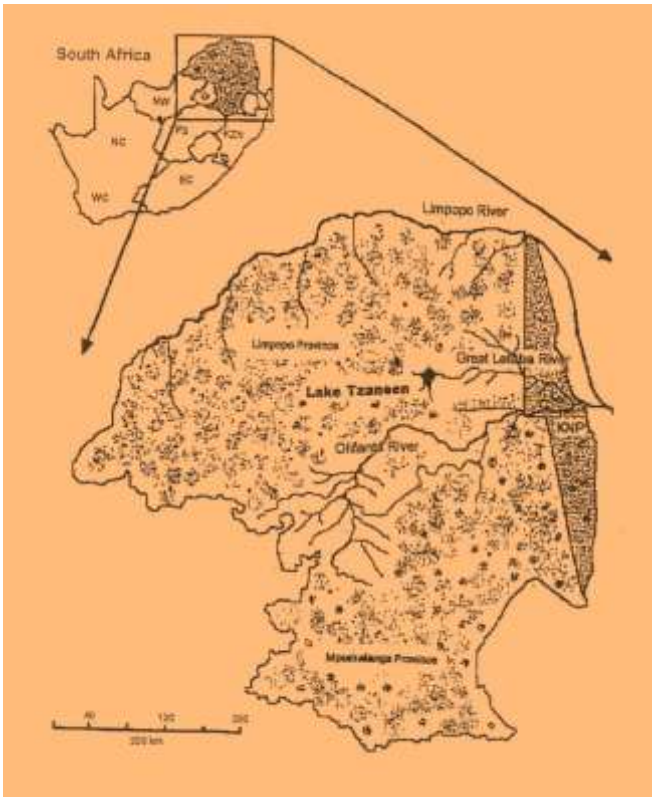


Figure 2.1 Lake Tzaneen on the Great Letaba River, a tributary of the Olifants River

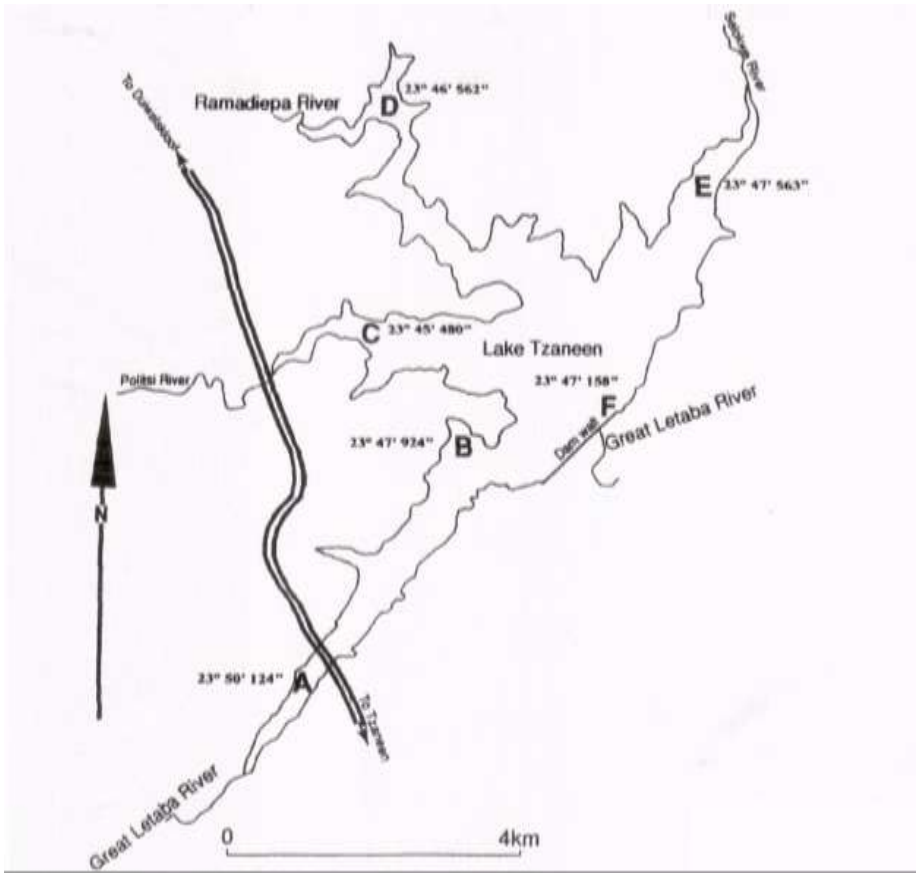


Figure 2.2 A map of Lake Tzaneen showing the sampling sites A – F

The construction of the dam is important in that much water is retained as there was little surface water available and the rivers ran dry during the long dry seasons. The water levels of the lake (figure 2.3) are shown in percentages from 1977 to the beginning of this project in 1999. The lake started at 5% of full capacity in 1976 and filled up for the first time in April 1977. The lake has experienced a dry period of less than 26% capacity in 1983 to 1987, and that of less than 8% capacity in 1993 to 1995 (Nicolaai 2008).

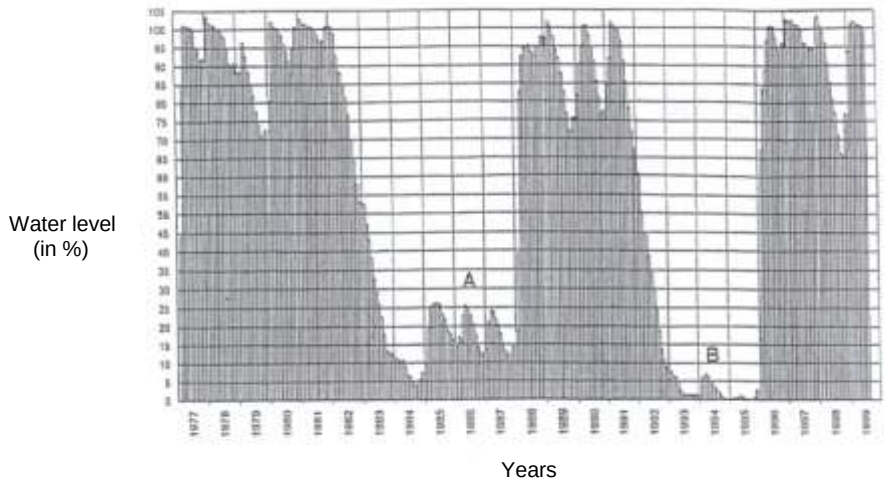


Figure 2.3 Water level (in %) of Lake Tzaneen from 1977 to 1999. A & B are the dry periods

The building of the dam changed the environment from the running water of the riverine system (lotic environment) to the standing water of the lacustrine system (lentic environment). This was accompanied by changes in the sediments and the vegetation in the lake and along the shore as well as the water quality and the biota. Likewise, the original populations of fishes in the four rivers contributed in the formation of the new lacustrine fish populations (Davies *et al.* 1975).

Geographical data and climate

The lake is situated below Tropic of Capricorn at 23° 47' 40" S and 30° 09' 40" E. It lies approximately 600 m above sea level and has a catchment area of 666 km² whilst that of the largest of the four rivers, the Great Letaba is 13 350 km². Its surface area covers 11.6 km² with a maximum depth of 35 m and has a full supply capacity of 160 million m³ (Nicolaai 2008). The sub-tropical lowveld of Limpopo has two periods that can be distinguished annually, namely a very hot, wet summer and a dry, frost-free mild winter. The rain falls mainly in the form of scattered thundershowers from September to March.

Features of the lake

The features of the lake are based on the six sites (A-F) in the dam (figure 2.2) that were identified for both fish and water quality samples by Nicolaai (2008). The selection of the sampling sites form an important part of the research and should represent as much as possible the habitat types, depth, bottom substrate, vegetation types as well as shoreline characteristics (Matla 1994). Four of the sampling sites (A, C, D and E) are situated at the inflows of each of the four rivers and measured 2-3 m deep. Site B is located near the areas of high human activities like camping and motorboat launching. It also measured 2-3 m in depth. One site (F) is near the dam wall and is 30 m deep. The substrate varied from sandy bottoms in the regions around A and B to predominantly soft mud or silt around C, E and F. Around site D were some patches of rare red hard mud (Nicolaai 2008).

The aquatic, fringing and riparian vegetation consist mainly of the thorny shrub weed *Mimosa pigra* that invaded the lake since 1992 and spread rapidly along the shores as well as inside the lake (Nicolaai 2008). The emergent aquatic macrophytes such as the reed *Phragmites australis* and the bulrush *Typha capensis* are present along the shores of riverine sections and mostly around sites C, D and E. Beyond the riparian vegetation are the plantations of banana (*Musa acuminata*), avocado (*Persea americana*), mango (*Mangifera indica*) and litchi (*Litchi chinensis*). There are also forests of blue-gum trees (*Eucalyptus grandis*) as well as pine trees (*Pinus silvestris*). Stumps of blue-gum trees are found inside the lake usually submerged during higher water levels.

Water usage and activities

The water of the lake is used by the municipality for business and household purposes as well as the sawmill industries near the banks of the lake. The farmers in the vicinity of the lake use the water for irrigation. Traditional fishing by the indigenous people and sport angling by the Bass Masters and other clubs also take place. Other water sports like boating and skiing are hobbies to many local residents. There are also municipal camping sites next to the lake. The beauty of the lake in a scenic environment with indigenous and planted forests adds to the aesthetic value.

Water quality of the lake

The water quality was determined from the stations A – F in the lake by Nicolaai (2008). This occurred from August 1997 to September 1999, a period just prior to, but also including the beginning of this parasite project. These physico-chemical parameters of the water regime are compared with the ecological Target Water Quality Range (DWAF 1996) in table 2.1.

Table 2.1 The water quality (minimum and maximum values) of Lake Tzaneen as compared to the Target Water Quality Range (TWQR).

Parameter	TWQR (DWAF 1996) Min - Max	Lake Tzaneen
pH (pH units)	6.5–10.0	5.0–8.5
Oxygen saturation (%)	80–120	61–105
Electrical conductivity (mS/m)	≤ 2.75	0.46–0.69
Total dissolved salts (mg/l)	≤ 17.88	3.06–4.49
Turbidity (NTU units)	---	1–8
Total hardness CaCO3 (mg/l)	≤ 60 (soft) ≥ 180 (hard)	11–29
Total nitrogen (mg/l)	≤ 0.5 (oligotrophic) ≥ 10 (hypertrophic)	1.0–4.2
Chlorine (µg/l)	≤ 0.20	0.000–0.006
Aluminium (µg/l)	≤ 5 (for pH < 6.5) ≤ 10 (for pH > 6.5)	0.0–0.18
Copper (µg/l)	≤ 0.3 (for soft water) ≤ 1.4 (for hard water)	0.0–0.02
Iron (µg/l)	≤ 0.1 (for soft water) ≤ 0.5 (for hard water)	0.0–0.03
Lead (µg/l)	≤ 0.2 (for soft water) ≤ 1.2 (for hard water)	0.0–0.07
Manganese (µg/l)	≤ 180	0–90
Zinc (µg/l)	≤ 2	0.0–0.2

More than 90% of the minimum and maximum values for all the water quality constituents are within their suitable ranges as required and are thus favourable for the survival of the fishes and their parasites (DWAF 1996). However, the occasional low pH levels may affect other water constituents that are dependent on it like ammonia and aluminium. The lower concentrations of dissolved oxygen in some instances have reached the sub-lethal levels of between 60% and 80%. Again, the nitrogen levels suggest that the lake may have already passed through the oligotrophic phase and is presently mesotrophic with some slight eutrophic conditions in a few areas of the lake.

The data on water quality suggests a clear, soft water impoundment with a good water quality for multi-purpose water usage. For the health of this aquatic ecosystem that carries a diversity of living organisms, it should be noted with concern the potential effects of the anthropogenic actions on the water quality of the lake. These include the boats with diesel-powered engines, the sawdust heaps that spill from the industries directly into the lake, the pesticides used in agriculture and the tin, plastic and other forms of pollution from the camping sites around the lake. They may in the long run alter the pH and oxygen levels as well as increase the toxic substances and heavy metals in the water. These will then affect the biota and may lead to a compounded stress response especially in fish, thus increasing their parasite loads.

Fish population of the lake

There are nineteen fish species on the records of the Bass Masters Club taken from their angling competitions in the lake. These fish species are the same as those recorded by Nicolaai (2008). *Barbus radiatus* Peters, 1853, the twentieth species was caught in one of the present investigations. Eighteen of these species are indigenous to the region (Skelton 2001). The two alien species, *Cyprinus carpio* and *Micropterus salmoides* were either introduced through active stocking programs or are a result of invasion (Nicolaai 2008). The following twenty species present in the lake are classified according to Jubb (1963, 1967), Jackson (1974, 1975) and Skelton (2001).

Phylum	:	Chordata	
Subphylum	:	Gnathostomata	
Superclass	:	Pisces	
Class	:	Osteichthyes	
Subclass	:	Actinopterygii	
(Division 1)	Superorder:	Ostariophysi	
Order	:	Cypriniformes	
Suborder	:	Cyprinoidei	
	Family:	**Cyprinidae**	
	Genus:	*Barbus* Cuvier, 1817	
	Species:	*B. paludinosus* Peters, 1852	Straightfin barb
		B. trimaculatus Peters, 1852	Threespot barb
		B. unitaeniatus Gunther, 1866	Longbeard barb
		B. radiatus Peters, 1853	Beira barb
	Genus:	*Labeobarbus* Rüppell, 1835	
		L. marequensis (Smith, 1841)	Largescale yellowfish
	Genus:	*Cyprinus* Linnaeus, 1758	
	Species:	*C. carpio* Linnaeus, 1758	Common carp
	Genus:	*Labeo* Cuvier, 1817	
	Species:	*L. cylindricus* Peters, 1852	Redeye labeo
		L. molybdinus Du Plessis, 1963	Leaden labeo
	Genus:	*Mesobola* Howes, 1984	
	Species:	*M. brevianalis* (Boulenger, 1908)	River sardine
Order	:	Characiformes	
Suborder	:	Characinoidei	
	Family:	**Characidae**	
	Genus:	*Micralestes* Boulenger, 1899	
	Species:	*M. acutidens* (Peters, 1852)	Silver robber

Order	:	Siluriformes	
Suborder	:	Siluroidei	
	Family:	**Clariidae**	
	Genus:	*Clarias* Gronow, 1763	
	Species:	*C. gariepinus* (Burchell, 1822)	Sharptooth catfish
	Family:	**Schilbeidae**	
	Genus:	*Schilbe* (Linnaeus, 1762)	
	Species:	*S. intermedius* Ruppel, 1832	Silver catfish

(Division 11) Superorder: Acanthopterygii

Order	:	Perciformes	
Suborder	:	Percoidei	
	Family:	**Cichlidae**	
	Genus:	*Oreochromis* Gunther, 1889	
	Species:	*O. mossambicus* (Peters, 1852)	Mozambique tilapia
	Genus:	*Tilapia* A. Smith, 1840	
	Species:	*T. rendalli* (Boulenger, 1896)	Redbreast tilapia
		T. sparrmanii Smith, 1840	Banded-tilapia
	Genus:	*Pseudocrenilabrus* Fowler, 1934	
	Species:	*P. philander* (Weber, 1897)	Southern mouthbrooder
	Genus:	*Chetia* Trewavas, 1961	
	Species:	*C. flaviventris* Trewavas, 1961	Canary kurper
	Family:	**Centrarchidae**	
	Genus:	*Micropterus* Lacepede, 1802	
	Species:	*M. salmoides* (Lacepede, 1802)	Largemouth-bass
Order	:	Mormyriformes	
	Family:	**Mormyridae**	
	Genus:	*Marcusenius* Gill, 1862	
	Species:	*M. macrolepidotus* (Peters, 1852)	Bulldog
	Genus:	*Petrocephalus* Marcusen, 1854	
	Species:	*P. catostoma* (Gunther, 1866)	Churchill

Originally all the fish species were to be investigated so as to have a complete list of the parasites in the lake. However, two species of the family mormyridae were already under investigation (Luus-Powell 2004). Of the remaining 18 species, Nicolaai (2008) obtained only 12 as six others never appeared in the catches. In this project 15 species (Plates 1 to 5; Skelton 2001) were investigated for parasites as three species, *M. acutidens, T. sparrmanii and B. paludinosus* were never caught throughout the project.

2.2 PERIOD OF INVESTIGATION

The collection of data commenced during the summer of 1999 and continued seasonally ending during the spring of 2003. The sub-tropical South African lowveld seasonal changes are difficult to distinguish and are usually not as clearly defined as in temperate regions. Seasons were selected according to the calendar months in which collections were made: Winter being in June, July and August; Spring in September, October and November; Summer in December, January and February; and Autumn in March, April and May.

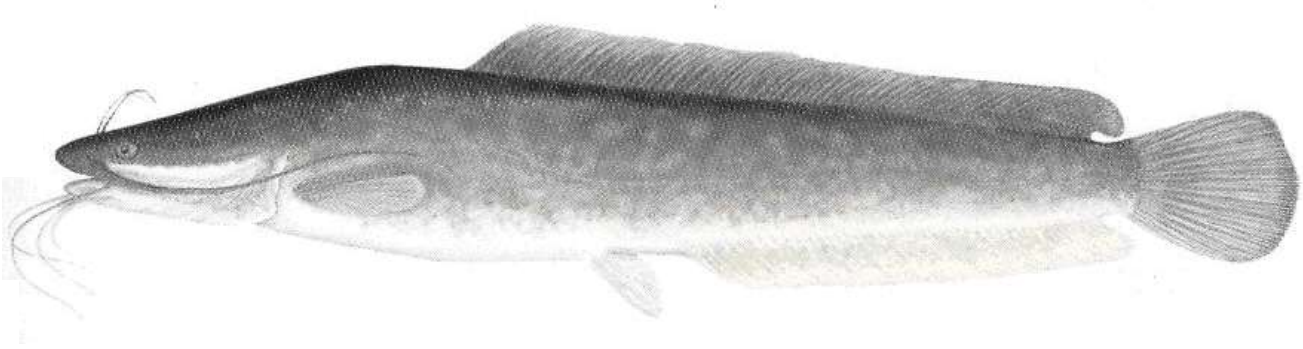
2.3 MATERIALS AND METHODS FOR HOSTS

Sampling of hosts

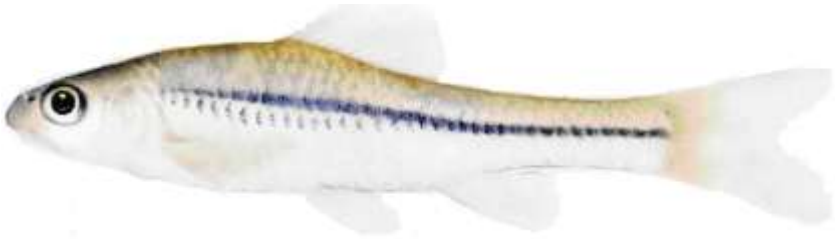
The sampling of host fish species was not an easy task as it was largely influenced by the following:

- a large number of fish species that were investigated
- the hosts that consisted of both larger and smaller fish species
- the sampling localities that could not strictly be adhered to.

Schilbe intermedius Ruppell, 1832

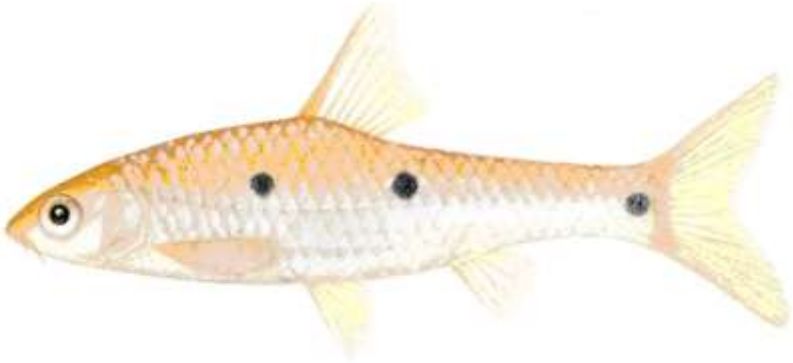


Clarias gariepinus (Burchell, 1822)

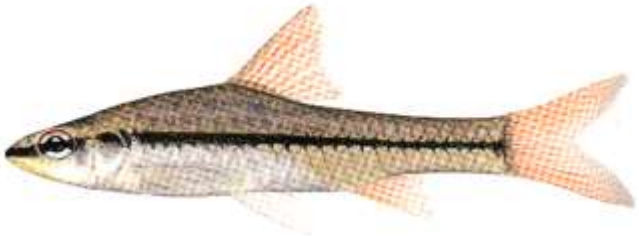


Barbus unitaeniatus Gunther, 1866

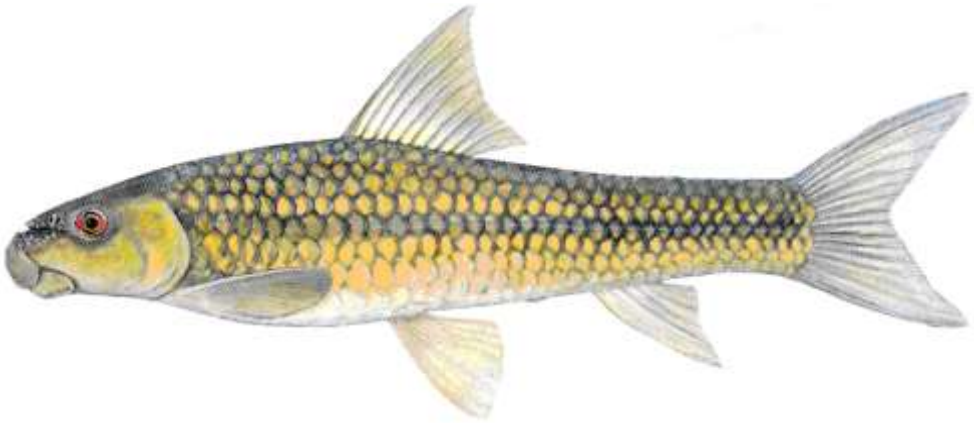
Plate 1 Some fish from Lake Tzaneen (photographs from Skelton 2001)



Barbus trimaculatus Peters, 1852



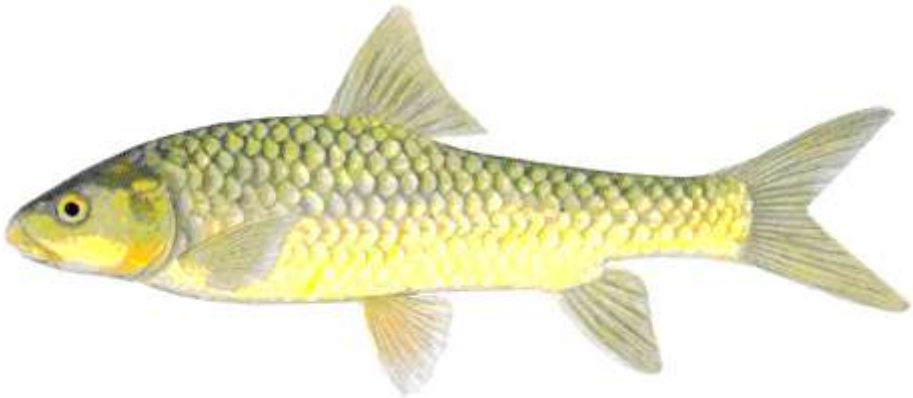
Barbus radiatus Peters, 1853



Labeo cylindricus Peters, 1852

Plate 2 Some fish from Lake Tzaneen (photographs from Skelton 2001)

Labeo molybdinus Du Plessis, 1963

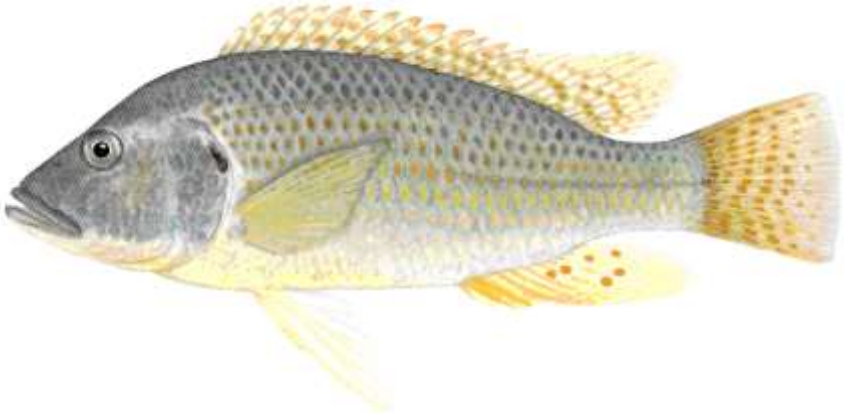


Labeobarbus marequensis (Smith, 1841)

Cyprinus carpio Linnaeus, 1758

Plate 3 Some fish from Lake Tzaneen (photographs from Skelton 2001)

Mesobola brevianalis (Boulenger, 1908)



Chetia flaviventris Trewavas, 1961

Pseudocrenilabrus philander (Weber, 1897)

Plate 4 Some fish from Lake Tzaneen (photographs from Skelton 2001)

Tilapia rendalli (Boulenger, 1896)

Oreochromis mossambicus (Peters, 1852)

Micropterus salmoides (Lacepede, 1802)

Plate 5 Some fish from Lake Tzaneen (photographs from Skelton 2001)

The six fish sampling stations (A – F) were surveyed in the beginning as they yielded catches of different fish species (Nicolaai 2008). However, the catches often did not provide the number of fish species and individuals required. Additional surveys per season were added to cover for this. Again, some sampling localities were abandoned and new ones were added due to heavy rains at the turn of the century that created newly submerged littoral areas as the dam filled to full capacity. The fishes caught could not always all be examined for parasites due to unbalanced catches in the numbers of species as well as individuals of the same species. Therefore sub-sampling was done to select the fish species and the number of individuals required for each survey.

Collection of host specimens

The gill nets with the following mesh sizes: 30, 50, 70, 90, 110, 130, 150, 180 mm, one with a length of 80 m and a depth of 2 m, the other with a length of 40 m and a depth of 2 m were laid overnight at two stations in the lake. The employment of this set-up was to collect different sizes. Leaving nets overnight for a fish parasite survey provides problems in that some fish die sooner and they lose some parasites. Another method that was employed even though scarcely, is the use of beach seine nets to collect smaller fish species as well as fingerlings of larger species. The nets were emptied and the hosts were sub-sampled and kept alive in separate containers and then transported ashore to the field laboratory.

Length, mass and sex of hosts

The length measurements of each intact host specimen were taken and recorded for total length (TL), standard length (SL) and, where applicable, fork (FL) to the nearest mm using a fish board with a measuring ruler. The mass of each specimen was determined to the nearest 0.1 g with the use of a Mettler SB 8001 electronic balance. Where possible the sex of each individual was recorded after dissection. These are important in correlating data with the parasites found and then determining the infestation trends.

Killing of hosts

At the field laboratory the fish were killed individually. The best methods chosen were to kill them manually either by asphyxiation or by severing the spinal cord immediately posterior to the cranium. The examination of hosts for parasites immediately after killing is advantageous in that it allows for easy spotting of the parasites by their movements and also by their colours. Again, when the parasites are collected alive, they can be allowed to relax before they are fixed especially the internal parasites that may degenerate quicker once the host is dead. The killing of hosts individually in this way also permits that specified fixation for a particular parasite group be carried out. Such collection, relaxation and fixation allow parts that are essential for identification to remain intact and subsequently make better microscopy preparations. However, few fish could be examined immediately after they were killed.

Preservation of hosts

As permission was not granted to transport fish alive from the field laboratory, this necessitated that many be preserved before examining for parasites. The employment of preservation of hosts prior to examination for parasites is disadvantageous in that it fixes all the parasite groups simultaneously and by similar method. However, the technical procedure of procuring the dead parasites from the hosts is simpler as they are no longer attached. Various methods of preservation were applied depending on the one that best suited the conditions at the time, and these were as follows:

- smaller fish specimens were frozen
- smaller fish specimens were kept in 10% formaldehyde
- organs of larger fish specimens were frozen separately
- organs of larger fish specimens were kept separately in 10% formaldehyde.

Examination of hosts for parasites

Due to the variety of both the host species investigated as well as the parasites found, the materials and methods described are general. Those employed for specific groups of parasites are discussed in the relevant sections (Chapter 3). In procuring the parasites from the hosts the standard procedures for parasitological examination suitable for both the qualitative and quantitative analyses were employed. These involved the use of a Wild M5A stereomicroscope and/or a compound light microscope. Firstly wet mucous smears of the skin were done, followed by scrutinizing of the mouth cavity and branchial chamber as well as the removal of the gills and their examination. Then the visceral cavity was examined. The alimentary canal was removed and studied followed by the removal and study of the brain, eyes and other visceral organs. Lastly, the muscles were checked through.

2.4 MATERIALS AND METHODS FOR PARASITES

Fixation and preservation of parasites

Two methods for fixing and preserving the parasites were applied. Firstly, in instances where the examination for parasites was done immediately after killing the host, the specific fixation and preservation discriminate methods were followed for Monogenea, Digenea, Cestoda, Nematoda and Acanthocephala. Secondly, where the preservation of hosts occurred before analyses for parasites, all types of parasites were fixed and preserved simultaneously with their hosts or the organs they infested. Through the application of these two different methods as many specimens of parasites as possible were collected. The collection of plenty parasite material was to ensure excellent preparations for later identification could be sourced. Furthermore, this prevented missing the mixed infections of macroscopically similar species that would later appear to be microscopically different, especially the gillworms (monogeneans).

Storage of parasites

The parasites were stored in 70% ethanol. Glass bottles with tops sealed with plastic shrives to reduce evaporation were used. Some specimens were stored inside glass vials with 70% ethanol and these were kept in larger bottles. Each storage bottle was labelled in pencil either on good quality paper put inside the bottle or with labels pasted externally on the bottles. Only the selected essential information appeared on the labels whilst further details on the hosts and parasites were kept in records.

Preparation of parasites for microscopy

The general procedure for preparing whole-mounts comprised of hydration (through a series of decreasing grades of ethanol), staining (using aceto alum carmine), dehydration (through a series of increasing grades of ethanol) and clearing (using clove oil). The parasite specimens were mounted on the microscopic slides using a preferred mounting medium for each group. The choices and quantities of stains, the clearing and mounting media as well as the length of time the parasites are subjected to these are influenced by the types and the sizes of the parasites.

Identification of parasites

The first level of identification occurred during the examination of hosts for parasites. This was macroscopic at first for larger specimens and then microscopic for smaller specimens. The macroscopic identification is used only for higher categories down to the class or order levels, whereas a light microscope can be used to identify to the genus or even the species level. However, for morphological details and species identification, an Olympus BX50 clinical microscope was used. The relevant literature was also consulted in the identification process.

Drawings, photographs and morphological measurements

The photographs, drawings and morphological measurements are used for presentation of results. To perform these functions two systems were available. They were both checked to verify the reliability of data so that only the user-friendly portion

of each system could be used to complement each other. The first system comprised of an Olympus BX50 clinical microscope with a fitted drawing tube, a calibrated eyepiece for measuring as well as a digital camera. The advantage of this system is the use of the drawing tube to make drawings. However, the taking of morphological measurements is a tedious task that involves too much of the re-orientation of the slides and the eyepiece as well as the numerical conversions when using different magnification objectives.

The second system comprised of a Zeiss HBO 50 microscope fitted with a Colorview 12 CCD digital camera, used in conjunction with the computer software package (Soft Imaging System GMBH 1986) for digital solutions in imaging and microscopy. In this system the taking of measurements is computer based and a lot can be covered within a short time. However, the drawings were made through tracing from the photographs.

Compilation, analysis and presentation of statistical data

The sampling units are the individual fish that were caught, and the data was collected and compiled on the individual fish. Each survey reflected for each host specimen its species name, sex and size (length and mass) and season. The parasites were recorded according to the genus or species and the numbers of individuals per genus/species were also recorded. The Microsoft Excel 2000 computer software program was used to manage the information in spread-sheets, and to perform calculations. The diversity, prevalence, mean intensity and abundance of various parasitic infections were calculated according to seasons, host sex and host size ranges.

CHAPTER 3

RESULTS AND DISCUSSIONS

Taking water quality readings

CONTENTS

CHAPTER 3

SECTION A

HOST - PARASITE DATA

Examining hosts for parasites in the field laboratory

CONTENTS

3A.1 INTRODUCTION

There are many cases where ecological terms are used without giving their exact meanings. In this case they are used according to Margolis *et al*. (1982).

Prevalence — The number of individuals of a host infected with a particular parasite divided by the number of hosts examined (usually expressed as a percentage).

Mean intensity — The number of individuals of a particular parasite divided by the number of infected individuals of the host (mean number of parasites per infected host).

Abundance — The number of individuals of a particular parasite divided by the number of host individuals examined (mean number of parasites per host examined).

The prevalence, mean intensity and abundance of each helminth group (Monogenea, Digenea, Cestoda, Nematoda and Acanthocephala) per host species were correlated with seasons, host sex and host size.

When determining the size groups for each host species the standard length (SL) was preferred because the caudal fins either dried out rapidly or got damaged. Nicolaai (2008) determined the standard length frequency (SLf) for each fish species in Lake Tzaneen and the data was used in this study. Hecht (1980) successfully correlated the standard length with fish age using the Von Bertalanffy growth curve.

A total of 527 fish hosts were sampled and studied for parasites. It was impossible to sample equal numbers of individuals per host species for each season, sex and size group. The total fish species sampled had a parasite load of 38 species comprising of 27 Monogenea, 3 Digenea, 4 Cestoda, 3 Nematoda and 1 Acanthocephala. There were also cysts of digeneans and cestodes (table 3A.1).

Table 3A.1 The parasite-host list for Lake Tzaneen with location of parasites in/on the host

Parasite species	Host species	Location in/on host
Monogenea		
Gyrodactylus rysavyi	*C. gariepinus*	skin, gills
Macrogyrodactylus clarii	*C. gariepinus*	gills
Macrogyrodactylus karibae	*C. gariepinus*	gills
Dactylogyrus afrolongicornis afrolongicornis	*B. trimaculatus*	gills
Dactylogyrus allolongionchus	*B. trimaculatus*	gills
Dactylogyrus sp. 1 (new species)	*B. trimaculatus,*	gills
Dactylogyrus sp. 1 (new species)	*B. radiatus*	gills
Dactylogyrus spinicirrus	*L. marequensis*	gills
Dactylogyrus sp. 2 (new species)	*B. unitaeniatus*	gills
Dactylogyrus brevicirrus	*L. cylindricus*	gills
Dactylogyrus cyclocirrus	*L. cylindricus*	gills
Dogielius dublicornis	*L. cylindricus*	gills
Dactylogyrus sp. 3 (new species)	*L. molybdinus*	gills
Dactylogyrus sp. 4 (new species)	*L. molybdinus*	gills
Dogielius sp.	*L. molybdinus*	gills
Schilbetrema quadricornis	*S. intermedius*	gills
Quadriacanthus aegypticus	*C. gariepinus*	gills
Quadriacanthus clariadis	*C. gariepinus*	gills
Cichlidogyrus dossoui	*T. rendalli*	gills
	O. mossambicus	gills
Cichlidogyrus halli	*O. mossambicus*	gills
	T. rendalli	gills
Cichlidogyrus philander	*P. philander*	gills
Cichlidogyrus quaestio	*T. rendalli*	gills
Cichlidogyrus sclerosus	*O. mossambicus*	gills
Cichlidogyrus tilapiae	*O. mossambicus*	gills
Scutogyrus gravivaginus	*O. mossambicus*	gills
Actinocleidus fusiformis	*M. salmoides*	gills
Haplocleidus furcatus	*M. salmoides*	gills
Acolpenteron ureterocoetes	*M. salmoides*	urinary bladder

Digenea

Glossidium pedatum	*C. gariepinus*	intestine
Diplostomum sp. metacercaria	*C. gariepinus,*	brain cavity, eyes
	L. marequensis	brain cavity, eyes
	C. flaviventris	eyes
	O. mossambicus	eyes
Clinostomum sp. metacercaria	*O. mossambicus*	brain, visceral, heart cavities
	S. intermedius	visceral cavity
cysts	*C. gariepinus*	visceral cavity
	O. mossambicus	skin, gills
	C. flaviventris	visceral cavity

Cestoda

Polyonchobothrium clarias	*C. gariepinus*	intestine
Proteocephalus glanduliger	*C. gariepinus*	intestine
Ligula intestinalis	*L. marequensis*	visceral cavity
	B. radiatus	visceral cavity
	B. unitaeniatus	visceral cavity
	M. brevianalis	visceral cavity
	M. salmoides	intestine
Gryporhynchidae (previously Dilepididae larva)	*O. mossambicus*	intestine
	T. rendalli	intestine
cysts	*C. gariepinus*	visceral cavity

Nematoda

Procamallanus laevionchus	*C. gariepinus*	stomach, intestine
Paracamallanus cyathopharynx	*C. gariepinus*	intestine
	S. intermedius	intestine
Contracaecum sp. larva	*C. gariepinus*	visceral cavity
	S. intermedius	visceral cavity
	O. mossambicus	visceral, pericardial cavities
	T. rendalli	visceral cavity
	B. trimaculatus	visceral cavity
	L. marequensis	visceral cavity
	M. salmoides	intestine

Acanthocephala

Acanthosentis (*Acanthogyrus*) *tilapiae*	*O. mossambicus*	intestine

The two mormyrid fishes of this lake harboured five species of Monogenea namely *Gyrodactylus* sp.*, Mormyrogyrodactylus gemini*, *Bouixella* sp. 1, *Bouixella* sp. 2 and *Archidiplectanum* sp.; the metacercariae of Digenea found belonged to the genera *Clinostomum* and *Diplostomum*; and only one Nematoda species (*Procamallanus laevionchus*) was found (Luus-Powell 2004).

3A.2 HOST PARASITE DATA

1 ***Clarias gariepinus*** (Burchell, 1822)

Clarias mossambicus and *C. lazera* have been synonymised with *C. gariepinus* (Skelton 2001). It is geographically the most widely distributed fish in Africa and has been prioritised for research mainly for its nutrition and aquaculture purposes. This species attains 1.4 m SL and may live for eight or more years (Skelton, 2001). Their growth depends on local conditions, they vary much from area to area, and they reach sexual maturity in one, two or more years (van Senus 1989; Skelton 2001). The fishes were arranged into three size groups (> 60 cm SL, 40-60 cm SL and < 40 cm SL) based on their SL*ƒ* (table 3A.2).

The southern African literature on the helminths of this fish host includes Prudhoe & Hussey (1977), Mashego (1977), Mashego & Saayman (1981, 1989), Boomker (1982, 1993, 1994), Van As & Basson (1984), Mashego *et al.* (1991), Douellou & Earlwanger (1993), Douellou & Chishawa (1995), Khalil & Mashego (1998), Mashego *et al.* (2006), Barson (2008), Barson *et al*. (2008a) and Madanire-Moyo & Barson (2010). According to Khalil & Polling (1997), more than 18 species of Monogenea, 16 Digenea, 6 Cestoda and 10 Nematoda (in all more than 50 species) are hosted by this fish.

Of the 53 specimens examined Monogenea consisted of *Gyrodactylus rysavyi*, *Macrogyrodactylus clarii*, *Macrogyrodactylus karibae*, *Quadriacanthus aegypticus* and *Quadriacanthus clariadis*; Digenea consisted of *Glossidium pedatum* and *Diplostomum* sp. metacercariae; Cestoda consisted of *Polyonchobothrium clarias* and *Proteocephalus glanduliger*; Nematoda consisted of *Contracaecum* sp. larvae, *Paracamallanus cyathopharynx* and *Procamallanus laevionchus* (table 3A.2).

Table 3A.2 Host-parasite data for *Clarias gariepinus* in Lake Tzaneen

Host	Variable		*Gyrodactylus rysavyi* (n=5) P (%)	MI	A	*Macrogyrodactylus clarii* (n=9) P (%)	MI	A	*Macrogyrodactylus karibae* (n=5) P (%)	MI	A	*Quadriacanthus clariadis* (n=12 P (%)	MI	A	*Quadriacanthus aegypticus* n=416) P (%)	MI	A	*Glossidium pedatum* (n=11) P (%)	MI	A
Cg n=53	Season	Summer (n=3)	0	0	0	0	0	0	33	3	1	100	4	4	67	4	3	0	0	0
		Autumn (n=9)	0	0	0	0	0	0	0	0	0	0	0	0	56	44	24	11	3	0.33
		Winter (n=15)	13	2	0.20	0	0	0	0	0	0	0	0	0	60	18	11	7	1	0.07
		Spring (n=26)	8	1	0.08	4	9	0.35	8	1	0.08	0	0	0	35	3	1	12	2	0.27
	Size	>60 cm (n=4)	25	2	0.50	0	0	0	0	0	0	0	0	0	50	40	20	25	1	0.25
		40-60 cm (n=45)	7	1	0.07	2	9	0.20	7	2	0.11	7	4	0.27	42	9	4	7	2	0.11
		<40 cm (n=4)	0	0	0	0	0	0	0	0	0	0	0	0	100	40	40	25	5	1
	Sex	Male (n=22)	10	2	0.15	0	0	0	9	2	0.18	14	4	0.55	64	17	11	14	3	0.41
		Female (n=31)	6	1	0.06	3	9	0.30	3	1	0.03	0	0	0	35	17	6	6	1	0.06
	Variable		*Diplostomum* sp (n=22) P (%)	MI	A	*Polyonchobothrium clarias* (n=172) P (%)	MI	A	*Proteocephalus glanduliger* (n=29) P (%)	MI	A	*Contracaecum* sp (n=500) P (%)	MI	A	*Paracamallanus cya - thopharynx* (n =107) P (%)	MI	A	*Procamallanus laevionchus* (n=5) P (%)	MI	A
	Season	Summer (n=3)	0	0	0	0	0	0	0	0	0	0	0	0	33	4	1	0	0	0
		Autumn (n=9)	0	0	0	11	2	0.22	0	0	0	33	11	4	0	0	0	0	0	0
		Winter (n=15)	0	0	0	20	6	1	13	5	0.6	47	25	12	27	6	2	0	0	0
		Spring (n=26)	8	11	0.85	27	22	6	23	3	0.77	62	18	11	31	10	3	4	5	0.19
	Size	>60 cm (n=4)	0	0	0	75	6	4	0	0	0	75	34	26	25	2	0.5	0	0	0
		40-60 cm (n=45)	4	11	0.49	16	22	3	16	4	0.62	49	18	9	27	9	2	0	0	0
		<40 cm (n=4)	0	0	0	25	1	0.25	25	1	0.25	25	1	0.25	0	0	0	100	5	5
	Sex	Male (n=22)	5	20	1	18	28	5	5	1	0.05	27	7	2	23	3	0.68	10	5	0.5
		Female (n=31)	3	2	0.06	23	9	2	23	4	1	65	23	15	26	12	3	0	0	0

Cg, Clarias gariepinus; P, prevalence; MI, mean intensity; A, abundance

The 12 species present do not include protozoan, annelid and arthropod parasites but still compare favourably with the 17, 11 and 4 parasite species obtained from *Clarias gariepinus* in Luphephe-Nwanedi, Flag Boshielo and Return Water Dams respectively (Madanire-Moyo 2011). In Asa Dam, Nigeria, Ayanda (2008) found 7 species. Mwita & Nkwengulila (2008) found 21 species in Lake Victoria, Tanzania. Barson *et al.* (2008) obtained 10 species in Save-Runde floodplains, Zimbabwe. Only 9% of the fishes were free from helminth parasites. This number may be lower if considered that hosts may lose some parasites if left as gills quickly become difficult to examine due to accumulation of mucus that may render small Monogenea not visible.

2 *Schilbe intermedius* Ruppell, 1832

This species was previously known as *Schilbe mystus* and *Eutropius depressirostris* (Skelton 2001). Khalil & Polling (1997) list this fish as a host to 12 species of Monogenea, all of the genus *Schilbetrema* except one genus *Schilbetrematoides*; two species of Digenea, unidentified cestode larvae and six nematode species. The southern African literature on the helminth parasites of this fish host includes Kritzky & Kulo (1992), Boomker (1994) and Douellou & Chishawa (1995).

A total of 92 fish specimens were examined. They were divided into three size groups (>20 cm; 10-20 cm and <10 cm). These hosted *Schilbetrema quadricornis*, *Clinostomum* sp. metacercariae, *Contracaecum* sp. larvae and *Paracamallanus cyathopharynx* (table 3A.3). Only 9% of the total sample was free from helminth parasites.

3 *Labeobarbus marequensis* (Smith, 1841)

The South African literature on the helminth parasites of this fish host includes Mashego (1982, 1983, 1989, 1990), Mashego *et al.* (1991) and Boomker (1994). Khalil & Polling (1997) have listed one Monogenea, two Digenea, one Cestoda, three Nematoda species and an unidentified nematode larva as hosted by this fish species.

Table 3A.3 Host-parasite data for *Schilbe intermedius*, *Labeobarbus marequensis* and *Barbus trimaculatus* in Lake Tzaneen

Host	Variable		*Schilbetrema quadricornis* (n=4230)			*Clinostomum* sp. (n=4)			*Contracaecum* sp. (n=353)			*Paracamallanus cyathopharynx* (n=4)		
			P (%)	MI	A	P (%)	MI	A	P (%)	MI	A	P (%)	MI	A
Si n=92	Season	Summer (n=32)	22	55	12	9	1	0.09	31	10	3	9	1	0.09
		Autumn (n=25)	40	69	28	0	0	0	36	6	2	0	0	0
		Winter (n=14)	100	90	90	0	0	0	57	11	6	0	0	0
		Spring (n=21)	52	172	90	5	1	0.05	43	13	5	5	1	0.05
	Size	>20 cm (n=38)	71	79	56	3	1	0.03	66	12	8	0	0	0
		10-20 cm (n=52)	29	140	40	6	1	0.06	25	5	1	6	1	0.06
		<10 cm (n=2)	0	0	0	0	0	0	0	0	0	0	0	0
	Sex	Male (n=32)	50	121	60	3	1	0.03	28	6	2	0	0	0
		Female (n=60)	43	89	38	5	1	0.05	48	10	5	5	1	0.05
Host	Variable		*Dactylogyrus spinicirrus* (n=247)			*Diplostomum* sp. (n=16)			*Ligula intestinalis* (n=5)			*Contracaecum* sp. (n=1)		
			P (%)	MI	A	P (%)	MI	A	P (%)	MI	A	P (%)	MI	A
Lbm n=38	Season	Summer (n=17)	53	23	12	0	0	0	0	0	0	0	0	0
		Autumn (n=1)	100	1	1	0	0	0	0	0	0	0	0	0
		Winter (n=9)	33	5	2	22	8	2	0	0	0	11	1	0.11
		Spring (n=11)	36	6	2	0	0	0	9	5	0.45	0	0	0
	Size	>30 cm (n=14)	14	3	0.36	7	1	0.07	0	0	0	0	0	0
		15-30 cm (n=18)	78	16	13	6	15	1	0	0	0	6	1	0.06
		<15 cm (n=6)	17	12	2	0	0	0	17	5	1	0	0	0
	Sex	Male (n=13)	85	20	17	0	0	0	8	5	0.38	0	0	0
		Female (n=25)	24	5	1	8	8	0.64	0	0	0	4	1	0.04
Host	Variable		*Dactylogyrus a. afrolongicornis* (n=16)			*Dactylogyrus allolongionchus* (n=5)			*Dactylogyrus* sp.1 (new sp.) (n=26)			*Contracaecum* sp. (n=5)		
			P (%)	MI	A	P (%)	MI	A	P (%)	MI	A	P (%)	MI	A
Bt n=21	Season	Summer (n=15)	33	3	1	14	1	0.08	27	7	2	13	3	0.33
		Autumn (n=3)	0	0	0	0	0	0	0	0	0	0	0	0
		Winter (n=1)	0	0	0	0	0	0	0	0	0	0	0	0
		Spring (n=2)	50	1	0.50	50	1	0.50	50	1	0.50	0	0	0
	Sex	Male (n=1)	0	0	0	100	1	1	0	0	0	0	0	0
		Female (n=12)	42	2	1	17	2	0.17	33	2	0.58	0	0	0
		Not determined (n=8)	13	7	1	13	2	0.25	13	19	2	25	3	0.63

Si, *Schilbe intermedius*; *Lbm*, *Labeobarbus marequensis*; *Bt*, *Barbus trimaculatus*; P, prevalence; MI, mean intensity; A, abundance

The following helminth parasites were found in this study: *Dactylogyrus spinicirrus*, *Diplostomum* sp. metacercariae, *Ligula intestinalis* plerocercoid larva and *Contracaecum* larvae (table 3A.3).

Previously this fish was classified under the genus *Barbus*. The females mature later, usually grow larger and live much longer than the males (Skelton 2001). A total of 38 fish specimens were examined. They were divided into three size groups (>30 cm, 15-30 cm and <15 cm) according to their SL*f* (table 3A.3).

4 ***Barbus trimaculatus*** Peters, 1852

The South African literature on the helminth parasites of this fish host includes Price *et al.* (1969), Van As *et al.* (1981), Mashego (1982, 1983, 1989, 1990) and Mashego *et al.* (1991). Khalil & Polling (1997) have listed four Monogenea, three Digenea, two Cestoda and three Nematoda species as hosted by this fish species. In this study the helminth parasites found were *Dactylogyrus afrolongicornis afrolongicornis*, *Dactylogyrus allolongionchus*, *Dactylogyrus* sp. 1 (new species) and *Contracaecum* sp. larvae (table 3A.3). This fish species is small and reach 15 cm SL (Skelton 2001). All the 21 fish specimens examined had a narrow SL range of 9-11 cm, hence no grouping into different sizes.

5 ***Barbus radiatus*** Peters, 1853

Previous records of parasites of this host species indicate only *Clinostomum* sp. metacercaria and *Ligula intestinalis* larva, both from the body cavity (Mashego 1982; Khalil & Polling 1997). This fish species was very scarce in the lake as only one specimen of 11 cm SL was caught during a spring survey. Nicolaai (2008) did not catch any during the 1997 – 1999 fish population survey in the lake. The parasites found are *Dactylogyrus* sp. 1 (new species) and *Ligula intestinalis* plerocercoid larva (table 3A.4).

Table 3A.4 Host-parasite data for *Barbus radiatus*, *Barbus unitaeniatus*, *Labeo cylindricus* and *Labeo molybdinus* in Lake Tzaneen

Host	Variable		*Dactylogyrus* sp. 1 (new sp.) (n=12)			*Ligula intestinalis* (n=1)		
			P (%)	MI	A	P (%)	MI	A
Br n=1	Season	Spring (n=1)	100	12	12	100	1	1
	Sex	Female (n=1)	100	12	12	100	1	1
Host	Variable		*Dactylogyrus* sp. 2 (new sp.) (n=2)			*Ligula intestinalis* (n=1)		
			P (%)	MI	A	P (%)	MI	A
Bu n=1	Season	Summer (n=1)	100	2	2	100	1	1
	Sex	Male (n=1)	100	2	2	100	1	1

Host	Variable		*Dactylogyrus brevicirrus* (n=29)			*Dactylogyrus cyclocirrus* (n=2)			*Dogielius dublicornis* (n=15)		
			P (%)	MI	A	P (%)	MI	A	P (%)	MI	A
Lc n=8	Season	Summer (n=0)	0	0	0	0	0	0	0	0	0
		Autumn (n=2)	0	0	0	0	0	0	0	0	0
		Winter (n=1)	100	24	24	100	2	2	100	10	10
		Spring (n=5)	60	2	1	0	0	0	40	3	1
	Size	>20 cm (n=3)	0	0	0	0	0	0	0	0	0
		≤20 cm (n=5)	80	7	6	20	2	0.4	60	5	3
	Sex	Male (n=4)	75	9	7	25	2	0.5	75	5	4
		Female (n=4)	50	1	0.5	0	0	0	0	0	0

Host	Variable		*Dactylogyrus* sp. 4 (new sp.) (n=150)			*Dactylogyrus* sp. 3 (new sp.) (n=20)			*Dogielius* sp. (n=6)		
			P (%)	MI	A	P (%)	MI	A	P (%)	MI	A
Lm n=20	Season	Summer (n=4)	75	33	25	50	10	5	25	2	0.5
		Autumn (n=2)	0	0	0	0	0	0	0	0	0
		Winter (n=6)	33	5	2	0	0	0	0	0	0
		Spring (n=8)	75	7	5	0	0	0	25	2	0.5
	Size	>20 cm (n=13)	62	16	10	15	10	2	23	2	0.5
		≤20 cm (n=7)	43	8	4	0	0	0	0	0	0
	Sex	Male (n=6)	50	35	18	17	18	3	34	2	0.67
		Female (n=12)	58	5	3	8	2	0.18	8	2	0.18
		Not determined (n=2)	50	8	4	0	0	0	0	0	0

Br, Barbus radiatus; *Bu, Barbus unitaeniatus*; *Lc, Labeo cylindricus*; *Lm, Labeo molybdinus*; P, prevalence; MI, mean intensity; A, abundance

6 ***Barbus unitaeniatus*** Gunther, 1866

This fish species was very scarce in the lake as only one male specimen of 7 cm SL was caught during a summer survey. Nicolaai (2008) did not catch any during the fish population survey in the lake. The parasites found are *Dactylogyrus* sp. 2 (new species) and *Ligula intestinalis* plerocercoid larva (table 3A.4). Previous records of parasites of this host species indicate *Diplostomum* sp. metacercaria, *Clinostomum* sp. metacercaria, *Ligula intestinalis* plerocercoid larva and *Contracaecum* sp. larva (Mashego 1982, 1989; Mashego *et al.* 1991; Khalil & Polling 1997).

7 ***Labeo cylindricus*** Peters, 1852

The South African literature on the helminth parasites of this fish host includes Paperna (1973, 1979) and Mashego *et al.* (1991). Khalil & Polling (1997) list only four monogenean species of the genera *Dactylogyrus* and *Dogielius* as hosted by this fish species. In this project, eight specimens were examined and three species of Monogenea (*Dactylogyrus brevicirrus, Dactylogyrus cyclocirrus* and *Dogielius dublicornis*) were collected (table 3A.4). This fish species reaches 23 cm SL (Skelton 2001) and are relatively scarce in the lake and Nicolaai (2008) obtained seven specimens. The fish were divided into two groups (>20 cm and ≤20 cm) based on their SL*f*.

8 ***Labeo molybdinus*** Du Plessis, 1963

The only South African record on the helminth parasites of this fish host is that of Boomker (1994). Khalil & Polling (1997) list only *Camallanus* sp. and an unidentified nematode larva as hosted by this fish species. In this project, three monogenean species (*Dactylogyrus* sp. 3 (new species), *Dactylogyrus* sp. 4 (new species) and *Dogielius* sp.) were present (table 3A.4). This fish species attains 38 cm SL (Skelton, 2001), are relatively scarce in the lake and Nicolaai (2008) obtained 11 specimens while 20 were examined in this project.

9 ***Cyprinus carpio*** Linnaeus, 1758

This alien species is a popular angling fish in Lake Tzaneen. However, only three specimens were caught during the fish survey of 1997 – 1999 (Nicolaai 2008) while in this study only one, large specimen of 8.1 kg was caught. These low catches may be due to the fish's ability to avoid capture by net (Beukema & de Vos 1974; Koch & Schoonbee 1980) and shallow water habitat preference (Allanson & Jackson 1983).

The South African literature on the helminth parasites of this fish host includes Boomker *et al*. (1980), Hamilton-Atwell *et al*. (1980), Brandt *et al.* (1981) and Van As *et al.* (1981). Khalil & Polling (1997) have listed only two cestode species from this fish. The many records of parasites of this fish are from elsewhere outside Africa. No parasites were found in/on the fish caught in this study.

10 ***Mesobola brevianalis*** (Boulenger, 1908)

No records of parasites of this fish species could be traced. This fish attains 7.5 cm SL (Skelton, 2001) but those caught were all within the range 3.7 – 4.8 cm SL. They were all caught using a seine net in autumn and could neither be differentiated into sexes nor sizes. Of the 104 fish specimens examined only *Ligula intestinalis* plerocercoid larvae were found in the visceral cavity of some (table 3A.5).

11 ***Pseudocrenilabrus philander*** (Weber, 1897)

The southern African literature on the helminths of this fish host includes Mashego *et al.* (1991), Douellou (1993), Christison *et al.* (2005) and Le Roux & Avenant-Oldewage (2009). Khalil & Polling (1997) list only *Cichlidogyrus philander* and this was the only parasite species found on this fish in Lake Tzaneen (table 3A.5). Christison *et al*. (2005) described G*yrodactylus thlapi* from this fish host. The 20 fish specimens examined were all within the size range of 3-6 cm SL.

Table 3A.5 Host-parasite data for *Mesobola brevianalis*, *Pseudocrenilabrus philander*, and *Chetia flaviventris* in Lake Tzaneen

Host	Variable		*Ligula intestinalis* (n=7) P (%)	MI	A
Mb n=104	Season	Summer (n=0)	0	0	0
		Autumn (n=104)	7	1	0.07
		Winter (n=0)	0	0	0
		Spring (n=0)	0	0	0
	Size	<5 cm (n=104)	7	1	0.07
	Sex	Not determined (n=104)	7	1	0.07
Host	Variable		*Cichlidogyrus philander* (n=73) P (%)	MI	A
Pp n=20	Season	Summer (n=0)	0	0	0
		Autumn (n=6)	100	4	4
		Winter (n=0)	0	0	0
		Spring (n=14)	57	6	3
	Size	>6 cm (n=0)	0	0	0
		<6 cm (n=20)	70	5	4
	Sex	Male (n=2)	100	5	5
		Female (n=18)	67	5	4
Host	Variable		*Diplostomum* (n=2) P (%)	MI	A
Cf n=3	Season	Summer (n=0)	0	0	0
		Autumn (n=3)	33	2	0.67
		Winter (n=0)	0	0	0
		Spring (n=0)	0	0	0
	Size	>10 cm (n=0)	0	0	0
		<10 cm (n=3)	33	2	0.67
	Sex	Not determined (n=3)	33	2	0.67

Mb, Mesobola brevianalis; *Pp, Pseudocrenilabrus philander*; *Cf, Chetia flaviventris*; P, prevalence; MI, mean intensity; A, abundance

12 ***Chetia flaviventris*** Trewavas, 1961

There are no records of parasites from this fish species that could be traced from literature sources. This is a very scarce species in the lake and Nicolaai (2008) obtained only one specimen. In this project only three sexually immature specimens (SL range of 6 - 10 cm) were caught during one autumn survey. Two of these were free of parasites. Two *Diplostomum* sp. metacercariae and three digenean cysts were retrieved from the eyes and visceral cavity respectively of the remaining specimen (table 3A.5).

13 ***Micropterus salmoides*** (Lacepede, 1802)

This is an alien fish species that was introduced in South Africa around the 1930's. (Skelton 2001). The African literature on the helminth parasites of this fish host includes Du Plessis (1948), Schmidt & Canaris (1967, 1968), Amin & Desfuli (1995) and Aloo (1999). Khalil & Polling (1997) list one species of Nematoda and one Acanthocephala as hosted by this fish.

Of the 32 fish specimens examined in this project, the parasites found are *Actinocleidus fusiformis* and *Haplocleidus furcatus* on the gills, *Acolpenteron ureteroecetes* in the urinary bladder, *Ligula intestinalis* plerocercoid larva in the intestines and *Contracaecum* sp. larva from the intestine (table 3A.6). The hosts were divided into three size groups (>30 cm SL, 15-30 cm SL and <15 cm SL).

14 ***Tilapia rendalli*** (Boulenger, 1896)

The southern African literature on the helminths of this fish includes Lombard (1968), Batra (1984), Douellou (1993) and Boomker (1994). Khalil & Polling (1997) listed five species of Monogeneans, two Digenea, one Nematoda and one Acanthocephala as hosted by this fish. Of the 20 fish specimens examined in this study the helminths

found are *Cichlidogyrus dossoui*, *C. halli*, *C. quaestio*, Gryporhynchidae larvae as well as *Contracaecum* sp. larvae (table 3A.6). The hosts were divided into two size groups (>15 cm and <15 cm) according to their SL*f*.

15 ***Oreochromis mossambicus*** (Peters, 1852)

This fish was previously known as *Tilapia mossambica*, later changed to *Sarotherodon mossambicus*, and is presently in the genus *Oreochromis* (Skelton 2001). The South African literature on helminth parasites of this fish host includes Lombard (1968), Prudhoe & Hussey (1977), Britz *et al*. (1984), Van As & Basson (1984), Mashego *et al.* (1991), Boomker (1994) and Madanire-Moyo *et al.* (2011). Khalil & Polling (1997) have listed three Monogenea, three Digenea, and an unidentified nematode as hosted by this fish.

Of the 92 fish specimens examined for Monogenea and 114 examined for other remaining groups, the parasites found are *Cichlidogyrus halli*, *C. dossoui*, *C. sclerosus*, *C. tilapiae*, *Scutogyrus gravivaginus*, *Clinostomum* sp. metacercariae, *Diplostomum* sp. metacercariae, Gryporhynchidae (previously Dilepididae) larvae, *Contracaecum* sp. larvae and *Acanthosentis* (*Acanthogyrus*) *tilapiae* (table 3A.7). This species reaches about 40 cm SL (Skelton, 2001). The hosts were divided into three size groups (>20 cm, 10-20 cm and <10 cm) according to their SL*f*.

Table 3A.6 Host-parasite data for *Micropterus salmoides* and *Tilapia rendalli* in Lake Tzaneen

Host	Variable		*Haplocleidus furcatus* (n=432)			*Actinocleidus fusiformis* (n=12)			*Acolpenteron ureteroecetes* (n=3)			*Ligula intestinalis* (n=4)			*Contracaecum* sp. (n=4)		
			P (%)	MI	A	P (%)	MI	A	P (%)	MI	A	P (%)	MI	A	P (%)	MI	A
Ms n=32	Season	Summer (n=6)	67	6	4	17	1	0.17	17	1	0.17	0	0	0	0	0	0
		Autumn (n=2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Winter (n=13)	62	10	6	8	2	0.15	8	1	0.08	8	2	0.15	8	1	0.08
		Spring (n=11)	64	47	30	9	9	1	9	1	0.09	9	2	0.18	18	2	0.27
	Size	>30 cm (n=7)	57	12	7	14	2	0.28	14	1	0.14	29	2	0.57	43	1	0.6
		15-30 cm (n=20)	65	29	19	10	5	0.50	10	1	0.10	0	0	0	0	0	0
		<15 cm (n=5)	40	3	1	0	0	0	0	0	0	0	0	0	0	0	0
	Sex	Male (n=10)	50	5	2	0	0	0	10	1	0.10	0	0	0	0	0	0
		Female (n=22)	64	29	19	14	4	0.55	9	1	0.09	9	2	0.14	14	1	0.18
Host	Variable		*Cichlidogyrus halli* (n=33)			*Cichlidogyrus dossoui* (n=14)			*Cichlidogyrus quaestio* (n=21)			Gryporhynchidae (n=38)			*Contracaecum* sp (n=3)		
			P (%)	MI	A	P (%)	MI	A	P (%)	MI	A	P (%)	MI	A	P (%)	MI	A
Tr n=20	Season	Summer (n=2)	50	4	2	0	0	0	0	0	0	0	0	0	0	0	0
		Autumn (n=1)	100	1	1	0	0	0	100	2	2	0	0	0	0	0	0
		Winter (n=3)	33	4	1	33	1	0.33	33	4	1	33	37	12	67	2	1
		Spring (n=14)	14	12	2	36	3	1	36	3	1	7	1	0.07	0	0	0
	Size	>15 cm (n=9)	44	8	4	22	3	0.55	33	3	1	23	19	4	23	2	0.33
		<15 cm (n=11)	9	1	0.09	36	2	1	36	3	1	0	0	0	0	0	0
	Sex	Male (n=1)	100	11	11	0	0	0	100	4	4	0	0	0	0	0	0
		Female (n=8)	38	7	3	13	4	0.5	13	5	0.63	25	19	5	25	2	0.38
		Not determined (n=11)	9	1	0.09	45	2	1	45	2	1	0	0	0	0	0	0

Ms, Micropterus salmoides; *Tr, Tilapia rendalli*; P, prevalence; MI, mean intensity; A, abundance

Table 3A.7 Host-parasite data for *Oreochromis mossambicus* in Lake Tzaneen

Host	Variable		*Cichlidogyrus halli* (n=419)			*Cichlidogyrus sclerosus* (n=89)			*Cichlidogyrus tilapiae* (n=37)			*Cichlidogyrus dossoui* (n=6)			*Scutogyrus gravivaginus* (n=9)		
			P (%)	MI	A	P (%)	MI	A	P (%)	MI	A	P (%)	MI	A	P (%)	MI	A
Om n=92	Season	Summer (n=29)	72	5	4	7	4	0.24	10	2	0.24	0	0	0	0	0	0
		Autumn (n=18)	100	5	5	33	5	2	22	5	1	11	3	0.33	6	5	0.28
		Winter (n=28)	79	8	6	32	5	2	7	6	0.43	0	0	0	4	4	0.14
		Spring (n=17)	100	3	3	18	4	0.65	0	0	0	0	0	0	0	0	0
	Size	>20 cm (n=60)	85	6	5	23	4	1	12	4	0.46	3	3	0.1	2	5	0.08
		10-20 cm (n=22)	77	6	5	18	5	1	0	0	0	0	0	0	5	4	0.18
		<10 cm (n=10)	100	3	3	20	7	1	20	5	1	0	0	0	0	0	0
	Sex	Male (n=32)	72	5	4	13	5	0.65	9	4	0.41	0	0	0	0	0	0
		Female (n=45)	89	7	6	33	4	1	13	4	0.53	4	3	0.13	4	5	0.20
		Not determined (n=15)	100	3	3	7	8	0.53	0	0	0	0	0	0	0	0	0
	Variable		*Diplostomum* sp. (n=55)			*Clinostomum* sp. (n=192)			Gryporhynchidae (n=1038)			*Contracaecum* sp (n=84)			*Acanthosentis tilapiae* (n=19)		
			P (%)	MI	A	P (%)	MI	A	P (%)	MI	A	P (%)	MI	A	P (%)	MI	A
Om n=114	Season	Summer (n=29)	10	5	0.55	41	3	1	10	33	3	21	1	0.28	0	0	0
		Autumn (n=32)	6	18	1	19	6	1	31	62	19	16	8	1	3	4	0.13
		Winter (n=28)	4	2	0.07	43	5	2	36	16	6	11	6	0.64	7	2	0.14
		Spring (n=25)	4	1	0.04	32	7	2	48	13	6	32	2	0.64	20	2	0.44
	Size	>20 cm (n=65)	11	8	0.85	42	4	2	28	19	5	15	2	0.29	3	3	0.08
		10-20 cm (n=39)	0	0	0	28	8	2	44	41	18	31	5	2	15	2	0.36
		<10 cm (n=10)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sex	Male (n=38)	18	8	1	39	4	2	34	24	8	21	5	1	0	0	0
		Female (n=61)	0	0	0	34	6	2	36	33	12	20	3	0.62	10	2	0.23
		Not determined (n=15)	0	0	0	13	8	1	0	0	0	13	2	0.2	13	3	0.33

Om, *Oreochromis mossambicus*; P, prevalence; MI, mean intensity; A, abundance

3A.3 DISCUSSION

The aquatic environment is rich in nutrients and various potentially opportunistic pathogens are present. In natural habitats, a diversity of parasites exists, but only in small numbers. They are considered normal findings and rarely cause disease problems as the relationship between the parasite and its host is stable. Fish with low numbers of parasites may not show signs of illness, but have reduced reproductive capacity while juveniles with low numbers may show signs of illness and have reduced growth rates (Snieszko 1974).

This study investigated a diversity of fish hosts and resulted in record of helminth diversity of both ectoparasites and endoparasites. Some fishes were very scarce to find in the lake. Furthermore, the literature revealed that some fishes were seldom investigated for parasites. First records for Africa, South Africa, new host records and new parasite species were found and will be discussed in Sections B – F.

Monogenea were commonly found on the gills but less on the skin and in the urinary bladder (table 3A.1). Digenea are commonly found in the eyes, brain and muscles, and fewer in the liver and intestines (Barnard 1990). In this study they were found mainly in the eyes and brain, with only one species (*Glossidium pedatum*) present in the intestines of *Clarias gariepinus*. Cestoda and Nematoda were found in the intestine and body cavity. Only one species of Acanthocephala (*Acanthosentis tilapiae*) was found in the intestines of *Oreochromis mossambicus*.

Host specificity of parasites varies within different parasite groups and different species (Marcogliese 2002). There is strict specificity where parasite species live on a single host species as in some Monogenea. In such cases the presence of a parasite can be used as diagnostic criterion for host species and is sufficient to identify the host with precision. This type of specificity is frequently used with dactylogyrid monogeneans of teleost fishes and can be considered as useful species indicators of their hosts (Lambert & El Gharbi 1995). Examples from this study are *Cichlidogyrus philander* from *Pseudocrenilabrus philander*, *Schilbetrema quadricornis* from *Schilbe intermedius*, *Macrogyrodactylus clarii* and *Macrogyrodactylus karibae* from *Clarias gariepinus*, *Dactylogyrus spinicirrus* from

Labeobarbus marequensis, and *Dactylogyrus allolongionchus* and *Dactylogyrus afrolongicornis afrolongicornis* from *Barbus trimaculatus*.

In narrow specificity the parasite species or even species of the same genus infect a few closely related hosts (species of the same genus/family). In this investigation such cases may be *Cichlidogyrus halli* infecting *O. mossambicus* and *T. rendalli* or *Cichlidogyrus* spp. infecting three cichlid fishes. In Trematoda, Cestoda and Nematoda there may be wide specificity where the parasite species/genus is found on several distantly related hosts (Rhode 1993). This may be due to ecological similarities or sharing the same biotope as in the larval stages of *Diplostomum, Clinostomum, Ligula intestinalis* and *Contracaecum* that were found in *Clarias, Oreochromis, Labeobarbus, Schilbe* and other species.

The introduction of exotic fish species into South African freshwater systems is a big threat to indigenous fish. They may bring along parasites that are host generalists with devastating effects on resident fish during co-adaptation. The Asian carp tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1961 is an example (Boomker *et al.* 1980; Van As *et al.* 1981; Brandt *et al.* 1981; Mashego 1982; Retief *et al.* 2007). In this study the only carp investigated was insufficient sample size to determine this threat. *Micropterus salmoides* has brought along three species of Monogenea to the lake, country or continent as they were never found in these before. These monogeneans are, however, host specialists and do not affect other resident fish species of Lake Tzaneen. Furthermore, the introduced fish species may also acquire new parasites. The introduced *Micropterus salmoides* is already acting as transport host for *Ligula intestinalis* in Lake Tzaneen.

A fish can serve as definitive, intermediate or paratenic host in the life cycles of many parasites (Hoffman & Bauer 1971). It serves as final host to Monogenea (direct life cycles with swimming onchomiracidia), also to *Glossidium*, *Polyonchobothrium, Proteocephalus*, *Paracamallanus, Procamallanus* and *Acanthogyrus* where adults settle in the intestine. It serves as transport host in cases where larvae are found in the intestine, and not the visceral cavity (e.g. *Ligula intestinalis* plerocercoid in *Micropterus salmoides*).

In parasites with indirect life cycles (Digenea, Cestoda, Nematoda) a fish may also serve as a second intermediate host. The metacercariae of digeneans (*Diplostomum* and *Clinostomum*), plerocercoids in cestodes (*Ligula intestinalis*) and larvae in nematodes (*Contracaecum*) usually migrate or settle in body cavities and tissues of fish. The free-living and other juvenile larval stages are found in water, snails or free-living copepods while the adults occur in piscivorous birds (Mashego *et al*. 1991).

Sometimes the cysts of Digenea, Cestoda and Nematoda are so undeveloped and they cannot be properly identified. The same can be said about the larvae that could be identified only to the genus level. If the larvae are encysted in the muscles they make fish unattractive for consumers and may develop in humans (zoonosis) if not killed during the cooking process. The life cycles of some parasites are thus so complex, involving more than one intermediate host, including fish, that the study of all stages enables one to understand the dynamics of aquatic ecosystems as a whole (Hoffman & Bauer 1971).

The distribution and infection levels of parasites (tables 3A.2 to 3A.7) are shown in terms of prevalence (%), mean intensity and abundance of each parasite species per fish host. These were calculated according to the predictor variables; season, host size and sex. From this data it was possible to determine diversity, dominant parasites and parasite associations per site on each fish host. However, to calculate the actual species richness, infra and component community structures, possible levels of associations in the component communities and the effects of the predictor variables need statistical software packages and mathematical models. To do these per fish host species (15 in all) is itself a topic on its own and is beyond the scope of this thesis. However, the data is sufficient to follow these up in journal publications.

The infection levels in *Gyrodactylus rysavyi, Macrogyrodactylus clarii* and *Macrogyrodactylus karibae* were very low but Khalil & Mashego (1998) indicated that these may increase rapidly in summer, from mean intensity of 1 to 700 in *M. clarii* and 150 in *M. karibae. Quadriacanthus clariadis* also had a low infection rate whilst *Quadriacanthus aegypticus* had prevalence of up to 67% in summer (table 3A.2). *Schilbetrema quadricornis* was very dominant reaching an abundance of 90 in both winter and spring (table 3A.3).

Previous studies on *Dactylogyrus* did not emphasize infection levels and thus made it difficult for comparative analysis. The inter-specific associations have been found among *D. a. afrolongicornis, D. allolongionchus and Dactylogyrus* sp. 1 on *Barbus trimaculatus*. Again, *Dactylogyrus* sp. 3 and *Dactylogyrus* sp. 4 were found sharing the gills on *Labeo molybdinus*. *Dogielius dublicornis* and *Dogielius* sp. also occurred in low Infection levels (tables 3A.3 & 3A.4).

Cichlidogyrus halli had higher prevalence in *Oreochromis mossambicus* (more than 70% in each season, size and sex) than in *Tilapia rendalli*. In Luphephe-Nwanedi Dams *C. halli* had prevalence of 73% (Madanire-Moyo *et al.* 2011). *Cichlidogyrus dossoui* was more prevalent in *T. rendalli* than in *O. mossambicus* but in low numbers, whilst in Luphephe-Nwanedi Dams it had a prevalence of 18% in *T.* rendalli. *Cichlidogyrus sclerosus* follows *C. halli* in prevalence as in Luphephe-Nwanedi Dams where prevalence was 45%. The infection levels of the other *Cichlidogyrus* spp. were very low (tables 3A.6 & 3A.7), a condition similar to that in Luphephe-Nwanedi Dams (Madanire-Moyo *et al.* 2011). Le Roux & Avenant-Oldewage (2009) found a high prevalence (100% in ten surveys) of *Cichlidogyrus philander* while in this study the lowest was 57% in spring (table 3A.5).

Glossidium pedatum was found from *Clarias gariepinus* in low infection levels (table 3A.2). Mashego (1977) (n=337) recorded this parasite from four waterbodies of the Olifants River System with prevalence of 33% and mean intensity of 62. Mashego *et al*. (1991) (n=27) found these worms with prevalence of 78% and mean intensity of 100. *Diplostomum* infections were low in Lake Tzaneen but Mashego (1977) (n=337) obtained these larvae with prevalence of 93% and mean intensity of 2 391. Mashego *et al*. (1991) (n=24) found these worms with prevalence of 83% and mean intensity of 68. *Clinostomum* occurred in low infection levels from *Oreochromis mossambicus* (n=157) in all the three dams surveyed by Madanire-Moyo (2011) with the highest prevalence of 22% and mean intensity of 4.

In South Africa all specimens of *Proteocephalus glanduligerus* were found from *Clarias gariepinus*, and in all instances (this included) the infection rates were low.

Mashego (1977) (n=337) recorded the first specimens from four waterbodies of the Olifants River System with prevalence of 3% and mean intensity of 7. Mashego *et al*. (1991) (n=28) found these worms with prevalence of 18% and mean intensity of 5.6. Mashego (2001) (n=115) recorded prevalence of 10% and mean intensity of 7 and Barson & Avenant-Oldewage (2006a) recorded prevalence of 14% and mean intensity of 2.

The prevalence of *Polyonchobothrium clarias* is lower and the mean intensity is higher as compared to the study of Mashego (1977) with a prevalence of 49% with mean intensity of 7. In other studies Mashego *et al*. (1991) found a prevalence of 14% and mean intensity of 6 while Barson & Avenant-Oldewage 2006a found a prevalence of 71.4% with mean intensity of 5. Mashego (1977) and Barson & Avenant-Oldewage (2006a) recorded intensities of up to 200 and 100+ respectively while this study had intensities of up to 105.

The infection statistics for *Ligula intestinalis* show both *Barbus radiatus* (n = 1) and *Barbus unitaeniatus* (n = 1) with prevalence of 100%, *Mesobola brevianalis* (n = 104) with 7%*, Micropterus salmoides* (n = 32) with 6% and *Labeobarbus marequensis* (n = 38) with 3%. All the five host species have each a mean intensity of 1. Both *B. radiatus* and *B. unitaeniatus* have abundance of 1 each, *M. brevianalis* with 0.07, *M. salmoides* with 0.06 and *L. marequensis* with 0.03. Epizootiological reports indicate that prevalence may be as high as 85% (Mashego 1982) with the mean intensity of one specimen per host in most fishes but several (two to three) specimens have been found in some (Paperna 1996).

The Gryporhynchid larvae had prevalence of 31% in *Oreochromis mossambicus* (n=114), a mean intensity of 29 and abundance of 9. In *Tilapia rendalli* (n=20) the metacestodes had prevalence of 5%, a mean intensity of 1 and abundance of 0.05. Mashego *et al*. (1991) found from *O. mossambicus* (n=128) the metacestodes with prevalence of 31% and mean intensity of 11. In the same study, the highest prevalence of 73% was from another cichlid, *Pseudocrenilabrus philander.* Several *Barbus* spp. were also found to host gryporhynchid metacestodes, but with very low infection rates (Mashego 1982; Mashego *et al*. 1991).

The prevalence of *Procamallanus laevionchus* in this study is 2% with the highest intensity of 5 parasites per fish. In other studies the prevalence and highest intensities were 32% and 15 parasites (Mashego *et al*. 1991), 30% (Boomker 1982), 14% and 13 parasites (Barson & Avenant-Oldewage 2006b) and 9% and 23 parasites (Mashego & Saayman 1981). The present results are lower than others and indicate that *P. laevionchus* is not well established in Lake Tzaneen. However, this result is higher than those from *C. gariepinus* in Nigeria where the prevalence were 0.8% (Oniye *et al*. 2004) and 0.6% (Ayanda 2009a). However, in the same country but from *Synodontis membranceous* the prevalence was higher at 16% (Owolabi 2008).

In this study, *Paracamallanus cyathopharynx* was procured from *C. gariepinus* with a prevalence of 25% and the mean intensity of 8 and from *Schilbe intermedius* with a prevalence of 4% and the mean intensity of 1. In other Southern African studies *C. gariepinus* was the host and the ecological statistics revealed a prevalence of 71% with the highest intensity of 241 parasites per host (Mashego & Saayman 1981), 62% with the highest intensity of 181 parasites per host (Mashego *et al*. 1991), 54% (Boomker 1982), and 80% with the highest intensity of 69 parasites per host (Moyo *et al*. 2009). Comparatively, *P. cyathopharynx* is not present in high numbers in Lake Tzaneen as is the case in these other Southern African studies.

In this study *Contracaecum* larvae were procured from seven fish hosts with the following statistics: prevalence of 49% (*C. gariepinus*), 38% (S. *intermedius*), 19% (*O. mossambicus*), 10% (*T. rendalli* & *B. trimaculatus*) and 3% (*M. salmoides* and *L. marequensis*); mean intensity and abundance respectively of 20 and 10 (*C. gariepinus*), 10 and 4 (S. *intermedius*), 4 and 0.8 (*O. mossambicus*), 3 and 0.2 (*B. trimaculatus*), 1.5 and 0.2 (*T. rendalli*), 2 and 0.1 (*M. salmoides*) and 1 and 0.03 (*L. marequensis*). In other Southern African studies (Mashego 1977, 1982, 1989; Mashego & Saayman 1981; Boomker 1982, 1994; Mashego *et al.* 1991; Barson & Avenant-Oldewage 2006b) there is also a wide variety of fish hosts with highest prevalence of 100% and intensity of 2860 parasites reached in *C. gariepinus*. It has been noted, however, that the prevalence is usually higher in *C. gariepinus* and is commonly below 50% in all other fish hosts.

Many African studies (Mbahinzireki 1980; Mashego & Saayman 1981; Paperna 1996) have reported higher prevalence and intensity of *Contracaecum* larvae with increasing age of fish. Although there may be excessive worm burdens in some fish hosts there are no major pathological disorders (Mashego *et al.* 1991). Whilst this may be so in wild fish populations, Mashego *et al.* (1991) warns of the harmful or lethal consequences that may be due to these larvae or an accumulative effect with other parasite species in fish farming conditions. *Contracaecum* carries the highest percentage of nematodes in some fishes, especially those like *Clarias gariepinus* where they may occur in excessive numbers. Cases of high intensity were found to have no direct lethal effects, but may be a way of ensuring that the parasites secure their chances of reaching the final host (Mashego *et al.* 1991).

Acanthogyrus (*Acanthosentis*) *tilapiae* specimens were retrieved from the intestines and had prevalence of 7%, mean intensity of 2.4 (range 1-4) and abundance of 0.2. Amin *et al.* (2008) found from 9 species of cichlids (n=219) in Lake Malawi *A. (A.) tilapiae* with prevalence of 98% and mean intensity of 9.1 (range 1-54). Douëllou (1992) found prevalence of 63% from *Tilapia rendalli.* When compared, it is clear that the infection statistics are very low in Lake Tzaneen and that *A. (A.) tilapiae* is not well established.

The lack of thorough experimentation and analysis of complex ecological relationships have resulted in several but somewhat conflicting postulations about the exact causes of changes in infection levels. Unless these factors are studied independently of one another, the confusion will still reign. Ecological science however, is not restricted to studying special or isolated cases but it aims to make generalizations (Rohde *et al.* 1995). To date, neither the experiments nor the mathematical models can provide definitive proof and how common abiotic, biotic and anthropogenic conditions lead to, or affect interrelationships (Holmes 1986).

There are several factors that may influence the distribution and infection levels. Climate, especially temperature is very important, but may not be the direct causative agent (Pearson & Dawson 2003) even though studies still prefer season as a predictor variable. Water level, quality and sediment type feature in studies that determine parasite community compositions (Khan & Thulin 1991). Habitat selection

and feeding behaviour by the fish host do influence diversity and levels of infection (Thomas 2002) and such a case in Lake Tzaneen is *Clarias gariepinus* as explained below. The genome, age, size and sex of fish host are also common as predictor variables in studies involving ecology of parasites. The history of host-parasite co-evolution is also important (Thomas 2002). The factors influencing distribution and infection levels of parasites are discussed below, with reference to Lake Tzaneen whenever possible.

The levels of infection of each fish species in this study were determined to compare parasite species richness in function of season, sex and size. These ecological parameters are important in fish farming or aquaculture planning to avoid serious losses of stock due to parasite loading. According to Barson *et al.* (2008b), little has been done on the ecology of freshwater fish parasites in tropical countries. In this study what was achieved in this regard is a contribution to the database and may be compared with studies from other similar or different lakes.

The data regarding distribution of parasites on the host are limited but in general agree with what was found for other fishes in many lakes. Species richness of Monogenea in Lake Tzaneen show an average of two species per host fish species and this has been a general trend in many lakes (Pariselle 2009, *pers. com*). Top of the list are *Clarias gariepinus* and *Oreochromis mossambicus* harboring five Monogenea species each. In total, *C. gariepinus* and *O. mossambicus* harboured 12 and 10 species of parasites respectively. The high numbers of species in these hosts are usually attributed to their old evolutionary host-parasite systems, persistent African habitation (their geographical area of origin) and omnivorous characteristics (Guégan & Kennedy 1993).

There are low concentrations of pollution in some areas in the lake due to anthropogenic actions. As this increases, the ecto-parasites directly exposed to polluted water will have their survival and reproductive rates reduced. The infections with endo-parasitic helminths with complex indirect life cycles also tend to decrease in number, while infections with endo-parasites with direct single-host life cycle tend to increase with increasing levels of pollution (Khan & Thulin 1991). In this way there

will be a decrease in the number of species of parasites found in various hosts due to pollution (Madanire-Moyo & Barson 2010).

Clarias gariepinus and *Oreochromis mossambicus* were among the numerically dominant species in the lake (Nicolaai 2008) and are top candidates for aquaculture purposes (Safriel & Bruton 1984). The diversity of Monogenea on their gills and skin pose a serious risk of parasitic epizootics for fisheries and aquaculture practices. There are many examples of epizootics in fish farming including *Macrogyrodactylus polypteri* on *Polypterus senegalus* in Sudan (Khalil 1964) and *Gyrodactylus* sp. on *Oreochromis mossambicus* in South Africa (Luus-Powell *et al*. 2006). Due to their complex life cycles, Digenea, Cestoda and Nematoda are not usually a problem in intensive aquaculture systems even though they are many in wild fish.

It is believed that seasonal variations in infection (prevalence, mean intensity and abundance) are caused by temperature (Chubb 1977) and alternating seasonal climatic conditions. Fluctuations in temperature have seasonal effects on the water of the lake. This will change the distribution of the host species, and all relevant stages in the life cycles of the parasites (Begon *et al.* 1996). Most groups of animals and plants have more species in warmer than in colder environments (Rohde 1993). During summer the increase in temperature is accompanied by higher rainfalls, more food availability, schooling behaviour and optimal breeding of fish, as well as increase in parasite reproduction and transfer of stages from host to host. In Lake Tzaneen casual observation shows high infection levels in summer even though the sample sizes were skewed.

The high infection levels of parasites (*Enterogyrus cichlidarum, Lamproglena clariae,* etc.) in winter were also reported previously (Khidr 1990; Banu *et al.* 1993; Tsotetsi *et al.* 2004) and may be ascribed to two causes. According to Hoffman & Bauer (1971), most fish aggregate in deeper water during winter, or the water level decreases during the dry periods (Marx & Avenant-Oldewage 1996) greatly increasing the opportunity for infection of hosts. Again, winter temperatures may be below the level at which antibodies are produced by the host, but above the minimum temperature required for parasite reproduction (Rawson & Rogers 1972; Cloutman 1978).

Host body size is a factor correlated with parasite community richness and infection levels and there are conflicting reports on size related differences. In larger hosts increased community richness and infection levels are obtained by consuming greater quantities and a variety of food contaminated with life cycle stages, or that they may offer more space and greater variety of niches for occupation by parasites (Poulin 1995). Again large hosts tolerate the stress of infestation better than smaller ones (Khan *et al.* 1993). On the contrary, smaller hosts may have higher infection levels due to their immune system that is still weaker and developing (Bakke *et al.* 2002).

The causes of parasites infecting the two sexes differently may be due to various circumstances. The male and female often have different feeding habits (Rohde 1993). As in *Labeobarbus marequensis* the females mature later, usually grow larger and live much longer than the males (Skelton 2001). The sample sizes (sex ratios) obtained (tables 2-7) also skew the results.

Constant challenge by pathogens requires the presence of an effective immune system for the survival of the fish. Weak points in the defense system of fish may be the large surface of the gills, their delicate structure and the direct contact with surrounding water. The feeding habits of fish also facilitate the invasion of pathogens (Rawson & Rogers 1972). Therefore, a basic understanding of the biology of parasites is essential for studying aquatic systems health and forms a basis for instituting mechanisms of control in fish farming.

CHAPTER 3

SECTION B

MONOGENEA

Gills habour monogeneans

CONTENTS

3B.1 INTRODUCTION

Southern African studies on monogenea include those of du Plessis (1948), Price & Kirk (1967), Junor & Price (1969), Price & McClellan (1969), Price & Pike (1969), Price *et al.* (1969a, b & c), Mashego (1982, 1983), Batra (1984), Mashego *et al.* (1991), Douellou (1993), Douellou & Chishawa (1995), Luus-Powell *et al.* (2003, 2006), Luus-Powell (2004), Modise *et al.* (2006, 2009), Ramollo *et al.* (2006), Le Roux & Avenant-Oldewage (2009, 2010) and Olivier *et al*. (2009).

According to Paperna (1996), the Monogenea are flatworms (Platyhelminthes), ectoparasitic and attached by special posteriorly positioned attachment organs to their host's skin or gills. Their anterior end contains apical sensory structures, a mouth with or without accessory suckers and special glands or clamps for attachment, and all are hermaphroditic. The testis is single or follicular; sperm are evacuated into a specialized, often sclerotized copulatory organ. Female organs include ovary and follicular vitelline glands. The uterus usually contains no more than one, or only a few eggs. One group is viviparous; the uterus contains prenatal offspring and vitelline follicles are lacking.

The monogeneans that are mostly found in freshwater fish are the Dactylogyroidea and all those collected in this study belong to this group. They are mostly gill parasites; some forms inhabit the skin and endoparasites. They are 0.3–2 mm long and usually have one or two anterior-dorsal pairs of eyes and a posterior-ventral attachment organ (the opisthaptor). This organ contains centrally positioned sclerotinoid anchors, connected to support bars and marginally located hooklets (Paperna 1996).

Further subdivision of the Dactylogyroidea results into many families of which the Dactylogyridae has been a subject of much confusion. The first scenario places the subfamily Ancyrocephalinae under the Dactylogyridae and Paperna (1979, 1996) continued using this system. The second scenario in which Bychowsky & Nagibina (1978) elevated this to the family Ancyrocephalidae has been used amongst others by Bunkley-Williams & Williams (1994), Pariselle & Euzet (1994, 1995a, 1996) and Khalil & Polling (1997). The third scenario is characterised by the confusion of

placing some of the dactylogyroids genera in either Dactylogyridae or Ancyrocephalidae. To avoid confusion in this study the monogenean genera represented in the lake are classified to only two families.

Family Gyrodactylidae Cobbold, 1864 are viviparous, eyes are absent, and the worm contains a distinct well differentiated embryo. Vitellaria are not distinct, with one pair of anchors firmly attached by two bars (Paperna 1996). This family has only four genera and are found in freshwater fish in Africa. Of the three genera present in the lake, the genus *Mormyrogyrodactylus* is hosted by *Marcusenius macrolepidotus* (Luus-Powell *et al.* 2003). The other two are *Gyrodactylus* which is the type genus whilst the larger *Macrogyrodactylus* has additional sclerites in the anchor-bar complex (Paperna 1996).

Family Dactylogyridae Bychowsky, 1933 are oviparous with prominent vitelline follicles, usually with one to two pairs of pigmented eyes, the anchor only loosely connected (through ligaments) with the bars and have no concentric platelets or squamodiscs (Paperna 1996). The three groups mentioned below are represented in the lake by the corresponding genera:

- have one pair of anchors - *Dactylogyrus* and *Dogielius*
- have two pairs of anchors - *Schilbetrema, Quadriacanthus, Cichlidogyrus, Scutogyrus, Actinocleidus* and *Haplocleidus*
- have only marginal hooklets - *Acolpenteron*

3B.2 MATERIALS AND METHODS

Monogeneans are usually small and when they occur in low numbers they may often be difficult to detect. Not noticing them in samples does not assure that fishes are free from these parasites (Bunkley-Williams & Williams 1994). Most species occur on the gills (hence gillworms), a few on the skin, in the ureter or urinary bladder and in the stomach. The skin examination is performed on either live or freshly killed fish by studying the wet mounts of skin scrapings and mucus with a compound microscope.

The gills should be removed from freshly killed fish without excessive hemorrhaging and before they become covered with copious mucus. They are dipped or immersed in saline or another appropriate medium to facilitate removal immediately under a stereo-microscope with both incident and transmitted light sources, or else the gills are preserved in 10% formalin for later examination. In some cases the fishes were frozen with their gills intact. This is not a preferred method as Paperna (1979) mentions that many monogeneans are distorted and destroyed during defrosting. The collected monogeneans were stored in 70% ethanol.

The worms were directly embedded under pressure in glycerine jelly which resulted in whole-mount preparations. Some specimens were subjected to glycerin jelly plus Berlese‘s fluid so as to dissolve softer tissues and leave the sclerotized organs intact. Identifications were done using drawings and photographs and the morphological measurements are in μm.

3B.3 RESULTS AND DISCUSSION

1 Genus ***Gyrodactylus*** von Nordmann, 1832

There have been taxonomic difficulties within the genus *Gyrodactylus* (Luus-Powell 2004). This was created, amongst others, by the fact that no standardized criteria for species differentiation exist (Malmberg 1970). Furthermore, the descriptions are based on one or two specimens, and usage of different terms for one structure or one term for different structures (Ergens 1973). They have conserved morphology and small size, as well as the fact that the earlier descriptions are difficult to relate to modern groupings and the confusion brought by the parasites' ability to transfer (accidental) between hosts as adults (Harris *et al.* 2004).

The literature on the genus has grown tremendously since 1980, with more than 100 papers between 1999 and 2003 (Harris *et al*. 2004). A list with 246 species was published (Malmberg 1970) whilst Bakke *et al*. (2002) indicate more than 400 nominal species with some still awaiting definition. Harris *et al*. (2004) attempted to evaluate the total diversity of the genus and succeeded to record 409 potentially valid species from 400 host species. Khalil & Polling (1997) listed only 17 species from African freshwater fishes but more species have been described thereafter (Luus-Powell 2004; Christison *et al.* 2005; Nack *et al*. 2005).

So far five species are hosted by *Clarias gariepinus* namely *G. alberti* and *G. clarii* (Paperna 1973), *G. groschafti* and *G. rysavyi* (Ergens 1973) and *G. transvaalensis* (Prudhoe & Hussey 1977). Of these *G. rysavyi* and *G. transvaalensis* are from the skin and the rest are from the gills. Only two studies on the genus are reported from South Africa. Prudhoe & Hussey (1977) described *G. transvaalensis* from the skin of *C. gariepinus*. Luus-Powell (2004) found *Gyrodactylus* sp. from the skin of *Marcusenius macrolepidotus* in Lake Tzaneen. In this study only five specimens of *G. rysavyi* were found from the skin (n=2) and the gills (n=3).

1.1 ***Gyrodactylus rysavyi*** Ergens, 1973

This parasite was originally described from the skin and fins of *Clarias gariepinus* in Egypt (Ergens 1973). No other records of this species could be traced from the literature. In this study the parasites were retrieved from the skin and gills of *Clarias gariepinus*. This is the first site (gills) and geographical record for southern Africa. In comparing the present material (figure 3B.1) with those used for the original description (Ergens 1973) they are morphologically identical but Tzaneen specimens are slightly larger (table 3B.1).

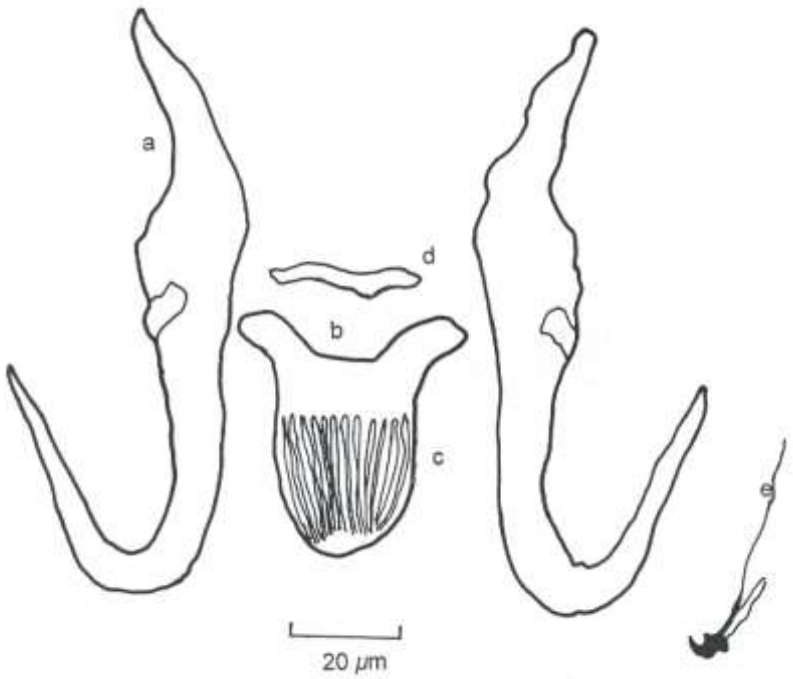


Figure 3B.1 *Gyrodactylus rysavyi* Ergens, 1973 – Haptoral parts a. anchor b. principal bar c. membranous extension d. fine auxillary bar e. marginal hook

Table 3B.1 Measurements (in μm) of *Gyrodactylus rysavyi*

G. rysavyi	Original (Ergens 1973)	Present
Number of specimens	10	5
Anchors		
length	81 - 91	100 - 102
basal part	56 - 61	52 - 54
point	41 - 48	42 - 45
root	36 - 43	41 - 42
Principal bar		
length	6 - 8	8 - 10
width	28 - 32	30 - 34
Membranous extension	18 - 19	21 - 23
Fine auxillary bar		
length	2 - 3	3 - 4
width	16 - 19	21 - 24
Marginal hooks		
overall length	28 - 30	30 - 33
hook itself	5 - 6	5 - 8

The most conspicuous feature differentiating this from the two other forms found in South Africa (*G. transvaalensis* and *G. tzangeni*) is the presence of a membrane to the transverse ventral bar of the opisthaptoral armature.

2 Genus ***Macrogyrodactylus*** Malmberg, 1957

This genus is endemic to Africa but appears to have received little attention even though they are larger than the other gyrodactylids. They are hosted mainly by the catfishes, but also found on two other genera (*Lates* and *Ctenopoma*). They are parasites of the skin and gills, are very mobile and may easily disappear once the host is caught or dies. At birth these worms already have developing embryos inside (hyper-viviparity) and they increase rapidly in aquaria and kill fish (Khalil 1964, 1970), a reason enough to have solicited attention in catfish farming.

Of the seven species described then, Khalil & Mashego (1998) regard only four namely *M. polypteri*, *M. congolensis*, *M. clarii* and *M. karibae* as valid species. The three others, namely *M. anabantii*, *M.ctenopomii* and *M. latesi* were described briefly from one specimen each by Paperna (1969, 1973) and are regarded as species inquirenda. Later, N'Douba & Lambert (1999) described *M. heterobranchii* from the gills of *Heterobranchus longifilis* and Přikrylová & Gelnar (2008) described *M. simentiensis* from *Polypterus senegalus* in Senegal. So far nine species have been described.

Khalil & Mashego (1998) emphasized the value of some taxonomic characters (sclerotized parts of the haptor) and the standard measurements used in differentiating the species of the genus. *Macrogyrodactylus heterobranchii* have in common with *M. clarii* the shape of the sclerites of the haptor, size of some haptoral features, and they are both from the gills. Only the minute spines of the cirrus are 8 - 10 in *M. heterobranchii* and 12 - 13 in *M. clarii*. It is suggested that more specimens of both species be collected and studied to verify the designation of *M. heterobranchii* or if need be, to revise the key to the species of the genus as given by Khalil & Mashego (1998).

Thus far only *M. polypteri* has been given more attention in studies (Malmberg 1957; Khalil 1964, 1969, 1970; Amirthalingham 1965; Saoud & Mageed 1969). The Southern African studies on the genus (Douellou & Chishawa 1995; Khalil & Mashego 1998; Barson *et al.* 2008) revealed only three species, namely *M. congolensis* from the skin, *M. clarii* and *M. karibae* from the gills of *C. gariepinus*. In this study *M. clarii* and *M. karibae* were found on the gills of *C. gariepinus*.

2.1 ***Macrogyrodactylus clarii*** Gussev, 1961

This parasite was originally described from *Clarias* sp. in Ethiopia (Gussev 1961) using only the shapes and sizes of the corpulatory organ and the haptoral sclerites. It was redescribed from *Clarias lazera* in Egypt (El-Naggar & Serag 1987) using detailed observations that included internal anatomy. The other records of this species are those of Paperna (1969), Shotter (1980), Faisal (1988), Khalil & Mashego (1998) and Barson *et al.* (2008). Most of these records are from *Clarias*

lazera with two from *Clarias gariepinus* and one each from *Clarias anguillaris* and aquaculture grown *Heterobranchus longifilis*. In this study the parasites were found on the gills of *Clarias gariepinus*.

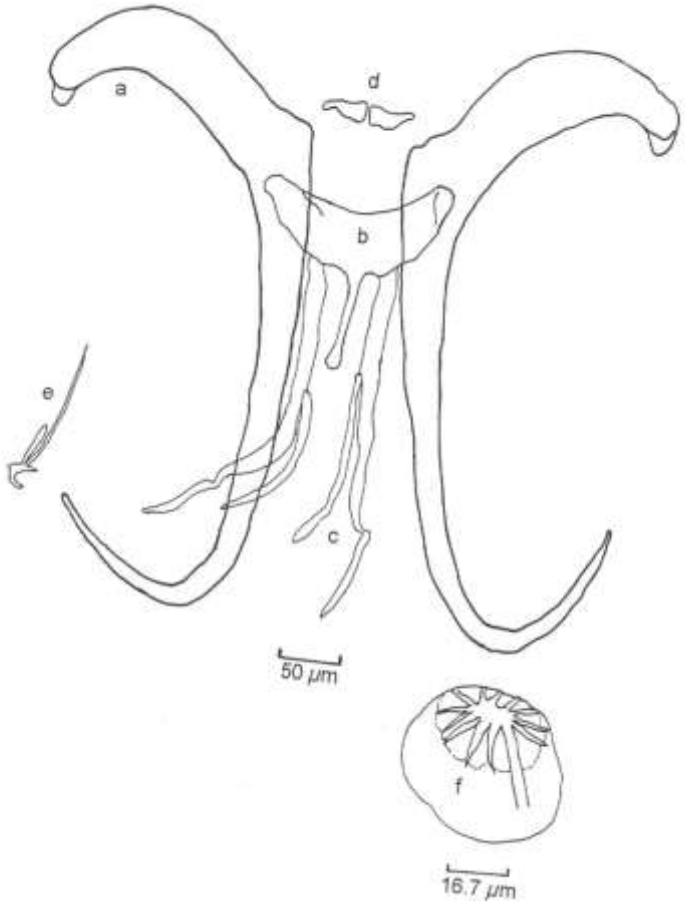


Figure 3B.2 *Macrogyrodactylus clarii* Gussev, 1961 – Haptoral sclerites a. anchor b. ventral bar c. accessory sclerites d. dorsal bar divided e. marginal hook f. cirrus

The anatomical features (figure 3B.2) and the dimensions (table 3B.2) of the present material resemble those of *M. clarii* (Gussev 1961; El-Naggar & Serag 1987) and the proposed taxonomic features and their measurements (Khalil & Mashego 1998).

Table 3B.2 Measurements (in *μ*m) of *Macrogyrodactylus clarii*

M.clarii	Original Gussev 1961	Rediscription El-Nagar & Serag 1987	Proposed scheme Khalil & Mashego 1998	Present
Host	*Clarias* sp.	*C. lazera*	*C. gariepinus*	*C. gariepinus*
Location	gills	gills	gills	gills
No. of specimens	–	10	–	9
Body				
length	–	2000 – 2580	1800 – 2600	1850 – 2000
width	–	360 – 440	320 – 440	330 – 400
Anchors/hamulus				
length	430	376 – 392	437 – 453	465 – 490
shaft	–	–	381 – 406	390 – 410
root	–	176 – 184	193 – 203	202 – 225
point	–	112 – 128	131 – 140	120 – 130
Ventral bar				
length	–	104 – 116	140 – 147	128 – 156
width	–	96 – 112	109 – 118	112 – 150
arm (ant-lat)	–	–	21 – 31	30 – 32
arm (pos-cen)	–	–	46 – 55	50 – 75
Dorsal bar divided				
length	40	64 – 72	68 – 75	70 – 75
width	–	–	15 – 18	15 – 20
Marginal hooks	110	91 – 101	109 – 125	115 – 125
Cirrus - spines	16	12	12 – 13	12

It should be noted however, that all the anchors (hamuli) of the present material are longer than those of the described material (table 3B.2). The measurements of Barson *et al.* (2008) for the anchors (274-465 *μ*m) were found to extend beyond the minimum-maximum of the proposed scheme. The shape and size of the anchor is a very reliable feature and this supports that the proposed taxonomic scheme (Khalil & Mashego 1998) be revised once sufficient and geographically representative materials have been collected.

2.2 ***Macrogyrodactylus karibae*** (Douellou and Chishawa, 1995)

The species was described from *Clarias gariepinus* in Zimbabwe (Douellou & Chishawa 1995) as a subspecies of *Macrogyrodactylus congolensis*. It was later elevated to the species level (Khalil & Mashego 1998) using specimens from South Africa.

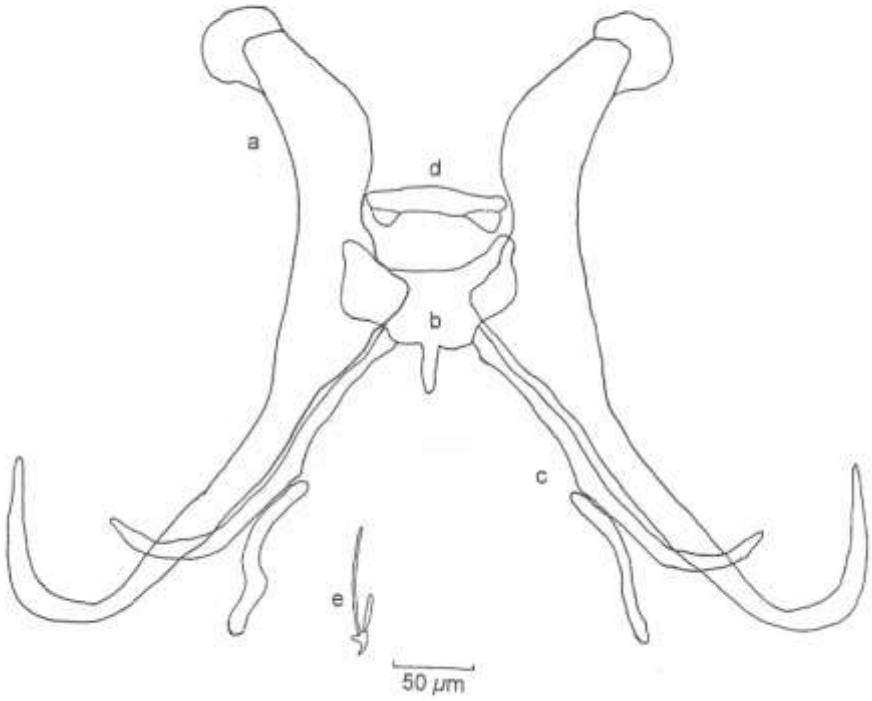
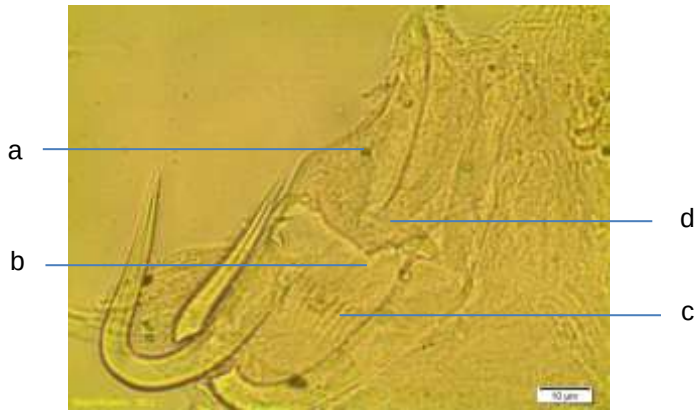


Figure 3B.3 *Macrogyrodactylus karibae* Douellou & Chishawa, 1995 – Haptoral sclerites a. anchor b. ventral bar c. accessory sclerites d. dorsal bar undivided e. marginal hook

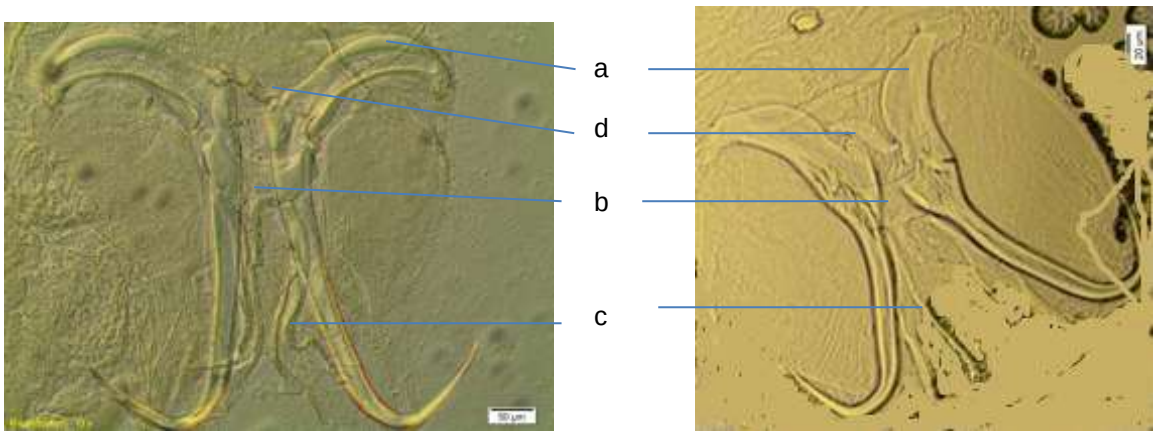
There are no other records about this parasite from the literature. In this study the parasites were procured from the gills of *Clarias gariepinus*. The morphology of the present specimens (figure 3B.3) and their measurements fall within the description and the proposed scheme. The anchors of material from Douellou & Chishawa (1995) (table 3B.3) and Barson *et al.* (2008) (n=1 of 286 *µ*m) overlap outside the proposed scheme. Plate 6 shows the micrographs of *G. rysavyi, M. clarii* and *M. karibae.*

Table 3B.3 Measurements (in μm) of *Macrogyrodactylus karibae*

M. karibae	Original Douellou & Chishawa 1995	Proposed scheme Khalil & Mashego 1998	Present
Host	*C. gariepinus*	*C. gariepinus*	*C. gariepinus*
Location	gills	gills	gills
No. of specimens	14	–	5
Body			
length	1300 – 3660	1200 – 3950	1650 – 2520
width	390 – 720	390 – 680	210 – 700
Anchors/hamulus			
length	252 – 314	296 – 375	310 – 375
shaft	106 – 128	251 – 296	255 – 290
root	69 – 101	125 – 156	125 – 160
point	73 – 114	68 – 93	80 – 100
Ventral bar			
length	93 – 110	84 – 111	90 – 100
width	_	78 – 111	85 – 110
arm (ant-lat)	16 – 27	21 – 31	20 – 22
arm (pos-cen)	3 – 49	24 – 40	30 – 35
Dorsal bar undivided			
length	78 – 93	68 – 78	85 – 92
width	14 – 18	15 – 21	20 – 22
Marginal hooks	71 – 88	68 – 78	68 – 80
Cirrus - spines	14	14 – 15	_



Gyrodactylus rysavyi haptor - a. anchor b. principal bar c. membranous extension d. fine auxillary bar



Macrogyrodactylus clarii *Macrogyrodactylus karibae*

a. anchor b. ventral bar c. accessory scleritesd. dorsal bar

Plate 6 *Gyrodactylus rysavyi, Macrogyrodactylus clarii & Macrogyrodactylus karibae*

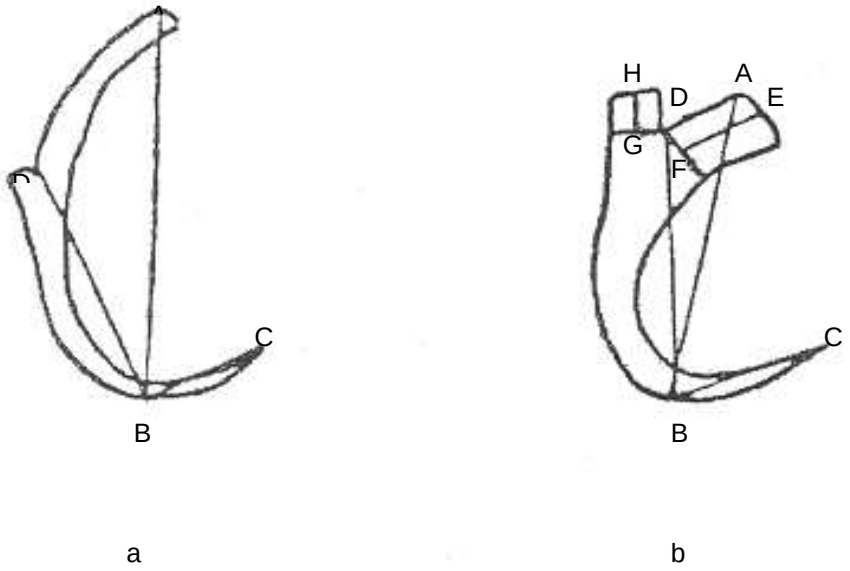
3 Genus ***Dactylogyrus*** Diesing, 1850

This largest helminth genus is in a state of considerable confusion (Gibson *et al.* 1996) due to a large extent to the high numbers of species that are described, many publications that are local and often obscure, as well as the confusing taxonomy of the large family of their fish hosts (Cyprinidae). The biogeography of the genus is closely linked to the evolutionary development of the cyprinid fishes (Gussev 1976, 1978). They are primarily (95%) parasitic on the gills of these fishes and the species are very host specific even within this fish family. Gibson *et al.* (1996) consolidated the existing information into 970 nominal species of which 11.5% have been described from Africa.

The studies on the genus *Dactylogyrus* in South Africa are those of Price *et al*. (1969a & b) and Mashego (1982, 1983). There are twelve species found in South Africa of which six were described locally, and all these are from only seven fish species. They are *Dactylogyrus myersi* described from the gills of *Barbus trimaculatus* by Price *et al*. (1969b); *Dactylogyrus afrolongicornis alberti*, *Dactylogyrus afrolongicornis afrolongicornis* and *Dactylogyrus allolongionchus* also from *Barbus trimaculatus* gills; *Dactylogyrus spinicirrus* from the gills of *Labeobarbus marequensis*; *Dactylogyrus dominici* and *Dactylogyrus teresae* described from the gills of *Barbus paludinosus* by Mashego (1983); *Dactylogyrus afrosclerovaginus* from *Barbus paludinosus* gills; *Dactylogyrus enidae* by Mashego (1983) from *Barbus neefi*; *Dactylogyrus varicorhini* from *Barbus kimberleyensis*; *Dactylogyrus pienaari* and *Dactylogyrus jubbstrema* by Price *et al.* (1969a) from the gills of *Labeo rosae* and *Glossogobius giuris* respectively.

This study recorded nine species of *Dactylogyrus.* One of them, namely *Dactylogyrus spinicirrus* belongs to the *Dactylogyrus varicorhini* species-group that is associated with the larger or large-scale African and Asian cyprinids. The remaining eight species belong to the *Dactylogyrus afrobarbae* species-group that, together with the *Dactylogyrus pseudoanchoratus* species-group, is associated with the smaller African cyprinids. These are *Dactylogyrus afrolongicornis afrolongicornis, D. allolongionchus, D. brevicirrus, D. cyclocirrus, Dactylogyrus* sp. 1 (new sp.), *Dactylogyrus* sp. 2 (new sp.), *Dactylogyrus* sp. 3 (new sp.) and *Dactylogyrus* sp. 4

(new sp.). The scheme below (figure 3B.4) used for measurements of morphological features is that of Paperna (1959).



a b

Figure 3B.4 Method of measuring the anchors (Paperna 1959) using two types, a & b

AB – total length, BD – shaft, BC – tip, EF – inner root, HG – outer root, AD (in a only) – root

3.1 ***Dactylogyrus afrolongicornis afrolongicornis*** Paperna, 1973

This parasite was described from *Barbus* cf. *kerstenii* in Uganda (Paperna 1973). Together with other *Dactylogyrus* spp. that were described by Paperna (1973), their illustrations only appeared for the first time in Paperna (1979). The other record of this species is that of Mashego (1982, 1983) from *Barbus trimaculatus* in South Africa. In the present study the parasites were also retrieved from the gills of *B. trimaculatus*. In comparing the present material (figure 3B.5) with previous finds (Paperna 1979; Mashego 1982) they are morphologically identical and fall within similar size ranges (table 3B.4).

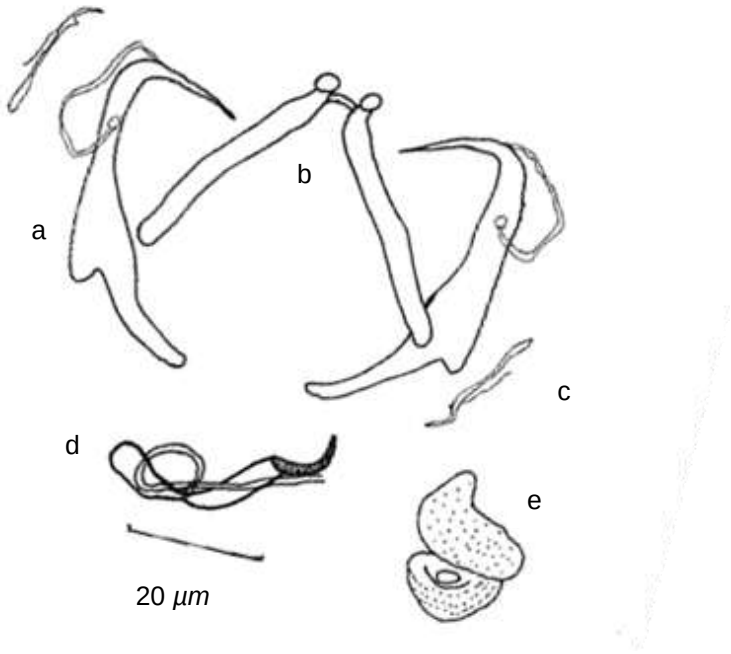


Figure 3B.5 *Dactylogyrus afrolongicornis afrolongicornis* Paperna, 1973 – Haptor and reproductive organs. a. anchor b. bar c. marginal hook d. copulatory organ e. vagina

The results confirm that only *D. afrolongicornis afrolongicornis* is present in the Olifants River system whilst together with *D. afrolongicornis alberti,* both were found in the Limpopo River system (Mashego 1982).

Table 3B.4 Measurements (in μm) of *Dactylogyrus afrolongicornis afrolongicornis*

D. afrolongicornis afrolongicornis	Original Paperna 1973	Mashego 1982	Present
Host	*B. kersteni, B. perince*	*B. trimaculatus*	*B. trimaculatus*
Location	gills	gills	gills
No. of specimens	10	7	16
Body			
length	180 – 440	213 – 413	325 – 480
width	60 – 100	25 – 63	75 – 120
Anchors/hamulus			
length	43 – 49	54 – 68	38 – 50
inner root	20 – 23 (25)	16 – 23	17 – 20
outer root	4 – 6	4 – 4	3 – 5
shaft	27 – 30	35 – 44	25 – 33
tip	13 – 16	13 – 19	15 – 18
Bar			
length	60 – 82	50 – 75	62 – 80
width	3 – 5	4 – 5	3 – 5
Marginal hooklets	16 – 25	13 – 21	16 – 21
Copulatory organ			
cirrus axis	26 – 29 (34)	29 – 38	32 – 40
accessory piece	17 – 22 (25)	19 – 28	20 – 27
Vagina			
length	24 – 28	13 – 21	15 – 30
width	11 – 13	9 – 13	10 – 17

3.2 ***Dactylogyrus allolongionchus*** Paperna, 1973

This parasite was described from *B.perince* in Uganda. The other record of this species is that of Mashego (1983) from *B. trimaculatus* in South Africa. In this study the parasites were retrieved from the gills of *B. trimaculatus*. The shapes and dimensions of the copulatory organ and opisthohaptoral features (figure 3B.6; table 3B.5) are comparable to those in previous studies, but the anchor is much longer in the specimens of Mashego (1983).

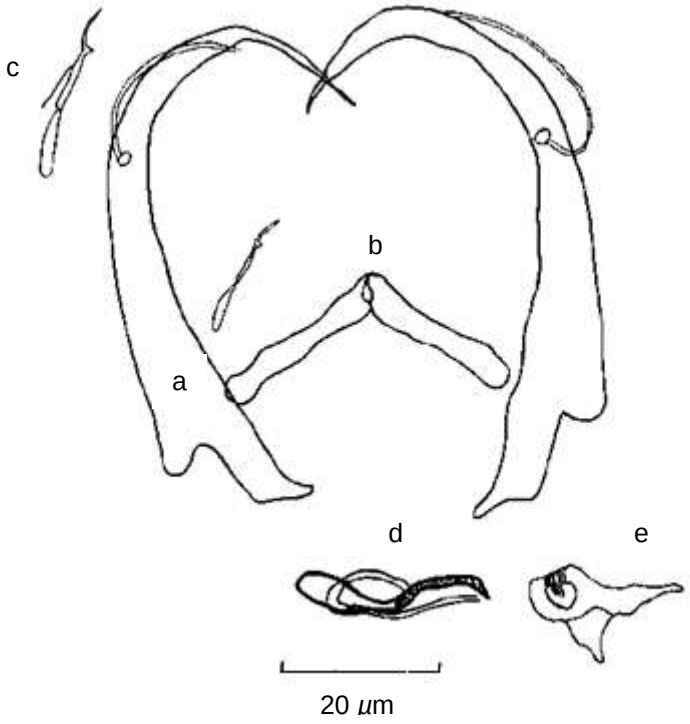


Figure 3B.6 *Dactylogyrus allolongionchus* Paperna, 1973 - Haptor and reproductive organs
a. anchor b. bar c. marginal hook d. copulatory organ e. vagina

Table 3B.5 Measurements (in μm) of *Dactylogyrus allolongionchus*

D. allolongionchus	Original Paperna 1973	Mashego 1982	Present
Host	*B. perince*	*B. trimaculatus*	*B. trimaculatus*
Location	gills	gills	gills
No. of specimens	5	5	3
Body			
length	200 – 310	188 – 331	225 – 365
width	80 – 160	44 – 69	60 – 88
Anchors/hamulus			
length	57 – 62	88 – 96	62 – 65
inner root	11 – 19	13 – 16	12 – 15
outer root	3 – 6	3 – 5	3 – 5
shaft	42 – 54	59 – 63	57 – 60
tip	16 – 20	19 – 19	18 – 20
Bar			
length	36 – 51	38 – 50	35 – 45
width	3 – 7	4 – 5	7 – 8
Marginal hooklets	15 – 21	16 – 25	17 – 20
Copulatory organ			
cirrus axis	22 – 25	21 – 31	28 – 32
accessory piece	17 – 22	13 – 19	18 – 20

3.3 ***Dactylogyrus* sp. 1** (new species) - figure 3B.7

TYPE HOST	:	*Barbus radiatus*
TYPE LOCALITY	:	Lake Tzaneen, South Africa
SITE OF INFECTION	:	Gills
MATERIAL STUDIED	:	30 specimens
DEPOSITION OF TYPES	:	Onderstepoort Veterinary Institute. Holotype accession number T.2196.1; paratypes accession number T.2196.2
OTHER HOST	:	*Barbus trimaculatus*
ETYMOLOGY	:	The name suggested is *Dactylogyrus radiatus* after the fish host Barbus radiatus

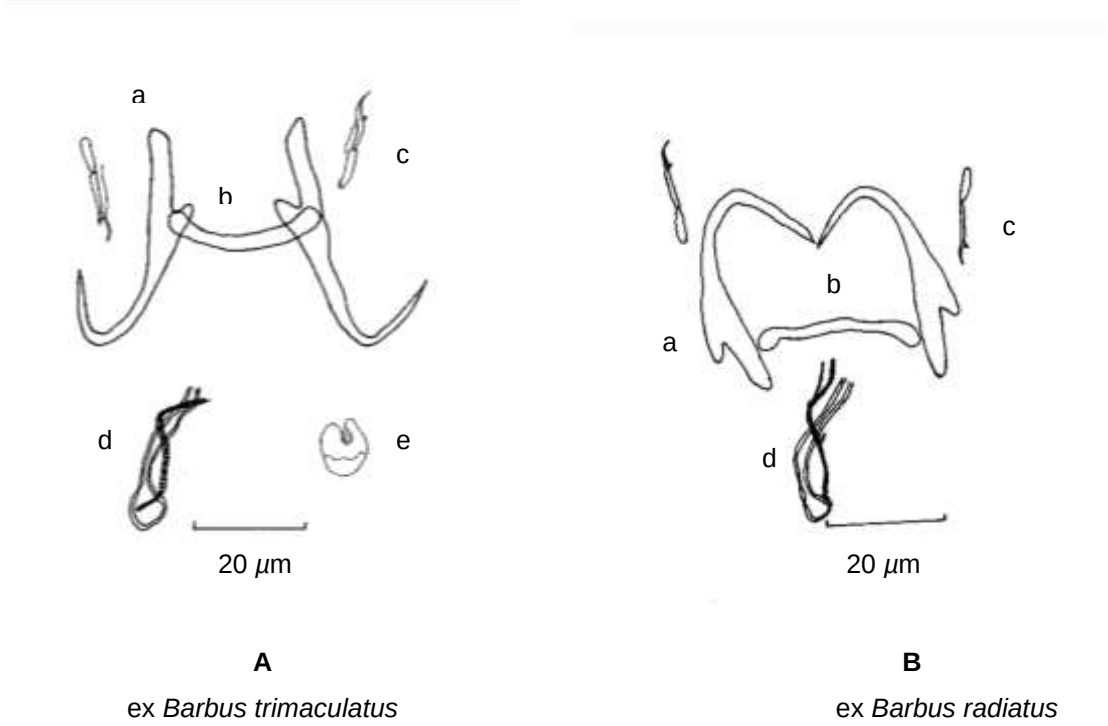


Figure 3B.7 *Dactylogyrus* sp. 1 - Haptor and reproductive organs.
a. anchor b. bar c. marginal hook d. copulatory organ e. vagina

DESCRIPTION (measurements in μm)

Body length 230-400, width 70-110. Anchors length 35-38, inner root 12-15, outer root 2-5, shaft 24-26, and tip 14-15. Bar length 24-28 and width 2-3. Marginal hooklets 17-19. Copulatory organ with cirrus axis 19-25 long, and accessory piece of 19-23 long. Vagina not visible in the type specimens but visible in specimens from B. trimaculatus.

DIAGNOSIS

Anchors have long inner root and vestigial outer root. Copulatory organ has basal ampulla (funnel) and slightly curved tubular cirrus. Accessory piece joined to the ampulla and is S-shaped. This species is closely related to *Dactylogyrus brevicirrus* but differs from it in that the rims of the funnel of the copulatory organ are not ornamented, the accessory piece is not forked, the cirrus axis is shorter and the bar is longer (Addenda 1a & 1b).

REMARKS

There are no previous records of Monogenea for *Barbus radiatus.* Khalil & Polling (1997) have listed four Monogenea from *Barbus trimaculatus* namely *Dactylogyrus afrolongicornis afrolongicornis*, *Dactylogyrus afrolongicornis alberti, Dactylogyrus allolongionchus* and *Dactylogyrus afrosclerovaginus*. In this study, this was the only species found on *B. radiatus* whilst the species co-occurred with both *D. afrolongicornis afrolongicornis* & *D. allolongionchus* on the gills of *B. trimaculatus*. As a new species, it is also the first host record for *B. trimaculatus*. The measurements of specimens from the two hosts are compared in table 3B.6 to show the conspecificity of these specimens.

Table 3B.6 Measurements (in μm) of *Dactylogyrus* sp. 1

Dactylogyrus sp. 1	Present	Present
Host	*B.trimaculatus*	*B. radiatus*
Location	gills	gills
No. of specimens	20	12
Body		
length	225 – 360	230 – 400
width	70 – 115	70 – 110
Anchors/hamulus		
length	35 – 40	35 – 38
inner root	12 – 15	12 – 15
outer root	2 – 4	2 – 5
shaft	25 – 30	24 – 26
tip	13 – 16	14 – 15
Bar		
length	26 – 30	24 – 28
width	2 – 3	2 – 3
Marginal hooklets	17 – 20	17 – 19
Copulatory organ		
cirrus axis	17 – 25	19 – 25
accessory piece	15 – 19	19 – 23
Vagina		
length	15 – 20	–
width	7 – 10	–

3.4 ***Dactylogyrus* sp. 2** (new species) - figure 3B.8

TYPE HOST	:	*Barbus unitaeniatus*
TYPE LOCALITY	:	Lake Tzaneen, South Africa
SITE OF INFECTION	:	Gills
MATERIAL STUDIED	:	2 specimens
DEPOSITION OF TYPES	:	Onderstepoort Veterinary Institute. Holotype accession number T.2197.1; paratypes accession number T.2197.2
ETYMOLOGY	:	The name suggested is *Dactylogyrus unitaeniatus* after the host *Barbus unitaeniatus*

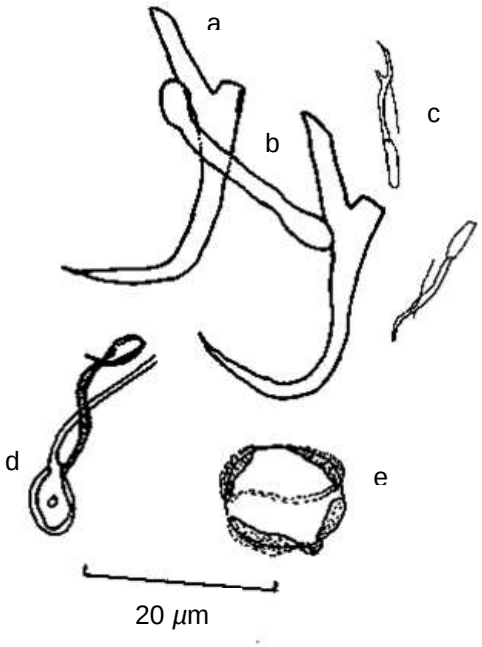


Figure 3B.8 *Dactylogyrus* sp. 2 - Haptor and reproductive organs
a. anchor b. bar c. marginal hook d. copulatory organ e. vagina

DESCRIPTION (measurements in *μ*m)

Body length 225-415, width 35-60. Anchors length 32-33, inner root 12-13, outer root 2, shaft 22-23, and tip 15. Bar length 25 and width 2. Marginal hooklets 16-17. Copulatory organ with cirrus axis 22 long, and accessory piece 18-20 long. Vagina length 14 and width 10-14.

DIAGNOSIS

Anchors have long inner root and short rectangular outer root. Copulatory organ has slightly curved tube-like cirrus. Accessory piece elongated, bifurcated distally and ending in movable hooks. Rims of the basal funnel not ornamented. Vagina sclerotised. This species is closely related to *Dactylogyrus longiphallus* and *Dactylogyrus* sp. 1. It differs from the former by its rectangular outer root of the anchor, movable hooks of the accessory piece and copulatory organ is smaller than that of *D. longiphallus* (Addenda 2a & 2b). The body size, copulatory organ and haptoral features of *Dactylogyrus* sp. 2 relative to those of *Dactylogyrus* sp. 1 correspond but differ in that the accessory piece of the latter is not bifurcated and does not have movable hooks.

REMARKS

This parasite species represents the first record of a monogenean on *Barbus unitaeniatus*.

3.5 ***Dactylogyrus* sp.3** (new species) - figure 3B.9

TYPE HOST	:	*Labeo molybdinus* Du Plessis, 1963
TYPE LOCALITY	:	Lake Tzaneen, South Africa
SITE OF INFECTION	:	Gills
MATERIAL STUDIED	:	8 specimens
DEPOSITION OF TYPES	:	Onderstepoort Veterinary Institute. Holotype accession number T.2198.1; paratypes accession number T.2198.2
ETYMOLOGY	:	The name suggested is *Dactylogyrus sevidi*, in honour of Prof. Sevid Mashego for his contribution to genus *Dactylogyrus* in South Africa

DESCRIPTION (measurements in μm)

Body length 260-400, width 60-130. Pharynx 18-20 in diameter. Anchors length 32-37, inner root 13-16, outer root very short 2-3, shaft 22-23, and tip 10-12. Bar length 17-29 and width 2-3. Marginal hooklets 14-15. Copulatory organ with cirrus axis of 16-25 long, and accessory piece of 14-20 long. Opisthohaptor length 44-60 and width 50-75. Vagina not distinct.

DIAGNOSIS

Anchors have long inner root and vestigial outer root. Copulatory organ has a basal ampulla followed by curvate tubular cirrus. Accessory piece joined to ampulla and bifurcates distally. The cirrus slides through the gutter-shaped distal branch of bifid accessory piece. This species is closely related to *Dactylogyrus pseudanchoratus* Price & Géry, 1968 and *Dactylogyrus falcilocus* Guégan, Lambert & Euzet, 1988, but differs from the former which has a trifid (not bifid) accessory piece, smaller body but longer anchors, and the latter which has a knob-like end of the proximal branch of bifid accessory piece and a sclerotised vagina (Addenda 3a & 3b).

REMARKS

This parasite species co-occurred with *Dactylogyrus* sp. 4 and *Dogielius* sp. on the gills of *Labeo molybdinus* and they together represent the first record of Monogenea on this fish species.

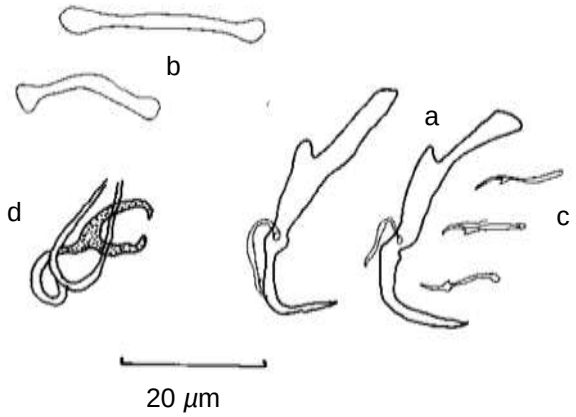


Figure 3B.9 *Dactylogyrus* sp. 3 – Haptoral features and copulatory organ
a. anchor b. bar c. marginal hook d. copulatory organ

3.6 ***Dactylogyrus* sp. 4** (new species) - figure 3B.10

TYPE HOST	:	*Labeo molybdinus* Du Plessis, 1963
TYPE LOCALITY	:	Lake Tzaneen, South Africa
SITE OF INFECTION	:	Gills
MATERIAL STUDIED	:	30 specimens
DEPOSITION OF TYPES	:	Onderstepoort Veterinary Institute. Holotype accession number T.2199.1; paratypes accession number T.2199.2
ETYMOLOGY	:	The name suggested is *Dactylogyrus molybdinus*, after the fish host *Labeo molybdinus*

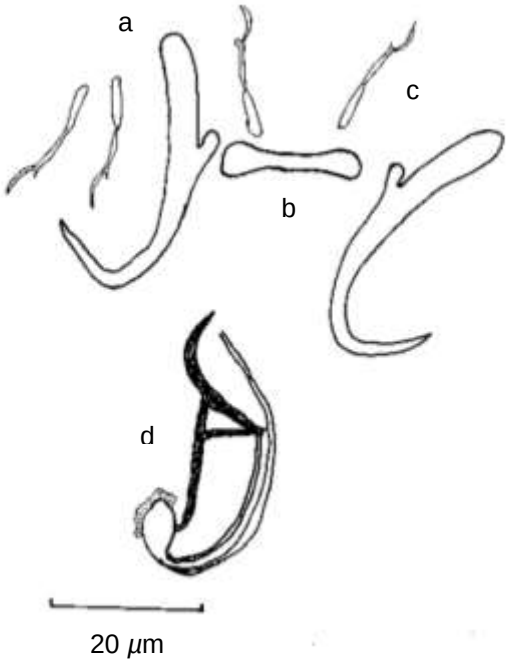


Figure 3B.10 *Dactylogyrus* sp. 4 - Haptoral features and copulatory organ
a. anchor b. bar c. marginal hook d. copulatory organ

DESCRIPTION (measurements in µm)

Body length 240-325, width 20-90. Anchors length 34-38, inner root long 14-16, outer root very short 2-3, shaft 20-22, and tip 8-11. Bar length 18-20 and width 2-3. Marginal hooklets 17-20. Copulatory organ very large with cirrus axis 25-36 long, and accessory piece 17-30 long. Opisthohaptor length 35-50 and width 30-50. Vagina not distinct.

DIAGNOSIS

Anchors with long inner root and the outer root is vestigial. Very large copulatory organ with a basal ampulla (funnel). Cirrus tubiform and curved. Accessory piece fixed to ampulla, elongated and bifurcated distally. Vagina non-sclerotised. The specimens are closely related to *Dactylogyrus longiphallus* Paperna, 1973 but differ in that the copulatory organ is larger in *D. longiphallus.* Furthermore, Guegan *et al*. (1988) redescribed *D. longiphallus* with a vestigial ventral bar, a feature absent in Lake Tzaneen specimens. The specimens also differ from *Dactylogyrus labeous* Paperna, 1969 which has a very short distal fork of the accessory piece (Addenda 4a & 4b).

REMARKS

This parasite species co-occurred with *Dactylogyrus* sp. 3 and *Dogielius* sp. on the gills of *Labeo molybdinus* and they together represent the first record of Monogenea on this fish species.

3.7 ***Dactylogyrus brevicirrus*** Paperna, 1973

This parasite was described from *Labeo victorianus* by Paperna (1973) but its first illustrations are found in Paperna (1979). The other records of this species are those of Guegan *et al.* (1988) and Guegan & Lambert (1991). In this study the parasites were retrieved from the gills of *Labeo cylindricus* and this is the first geographical record for South Africa. The shapes and dimensions of the copulatory organ and opisthohaptoral features (figure 3B.11; table 3B.7) are comparable to those in previous studies. The parasite is further characterized by a non-sclerotized vagina. The hooklets pairs1-4 is shorter than those of pairs 5-7. This species was found to co-occur with *Dactylogyrus cyclocirrus* and *Dogielius dublicornis* on the gills of *L. cylindricus*.

Table 3B.7 Measurements (in μm) of *Dactylogyrus brevicirrus*

D. brevicirrus	Paperna 1973	Guegan & Lambert 1988	Present
Host	*Labeo victorianus*	*Labeo parvus*	*Labeo cylindricus*
Location	gills	gills	gills
No. of specimens	-	32	5
Body			
length	230 - 380	350 - 500	240 - 430
width	40 - 100	40 - 80	70 - 100
Anchors/hamulus			
length	35 - 40	32 - 43	32 - 38
inner root	16 - 20	15 - 22	14 - 18
outer root	1 - 4	1 - 4	2 - 4
shaft	20 - 24	23 - 28	20 - 24
tip	10 - 14	10 - 15	12 - 13
Bar			
length	19 - 21	16 - 20	16 - 20
width	-	3 - 4	2 - 2
Marginal hooklets			
pairs 1 - 4	14 - 18	12 - 14	13 - 14
pairs 5 - 7	14 - 18	14 - 16	15 - 18
Copulatory organ			
cirrus axis	25 - 30	29 - 37	25 - 30
accessory piece	15 - 21	18 - 21	17 - 23
funnel length	7 - 10	4 - 5	8 - 8
width	3 - 6	-	3 - 3

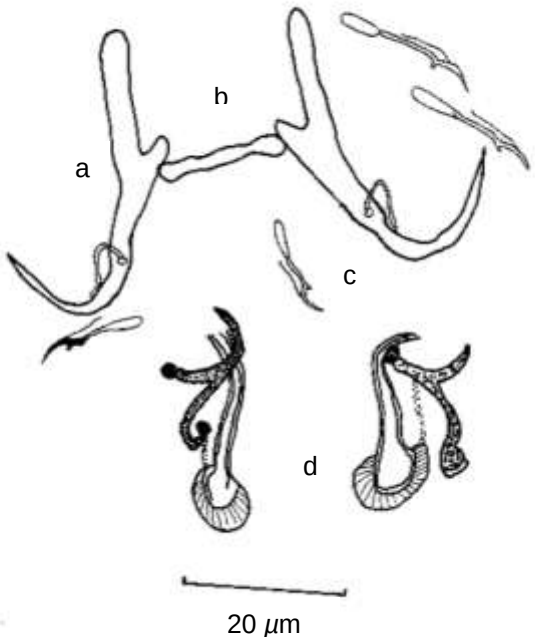


Figure 3B.11 *Dactylogyrus brevicirrus* Paperna, 1973 – Haptoral features and copulatory organ
a. anchor b. bar c. marginal hook d. copulatory organ

3.8 ***Dactylogyrus cyclocirrus*** Paperna, 1973

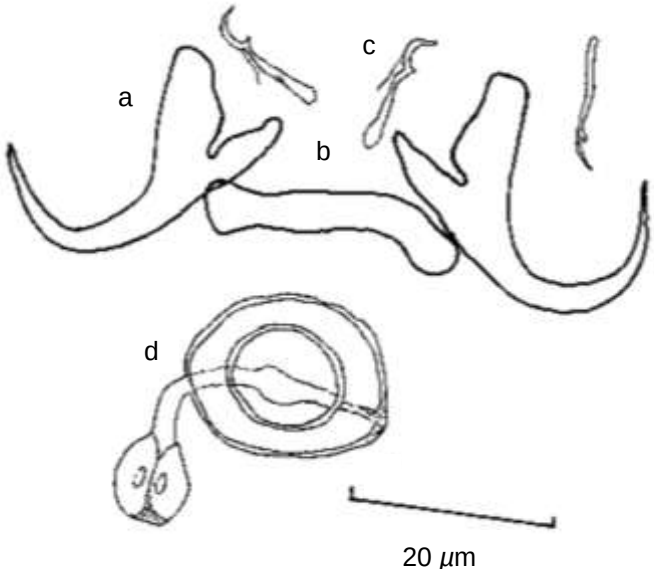


Figure 3B.12 *Dactylogyrus cyclocirrus* Paperna, 1973- Haptoral features and copulatory organ
a. anchor b. bar c. marginal hook d. copulatory organ

Table 3B.8 Measurements (in μm) of *Dactylogyrus cyclocirrus*

D.cyclocirrus	Paperna 1973	Guegan *et al.* 1988	Present
Host	*Labeo victorianus, L. senegalensis, L. cylindricus, L. cubie*	*Labeo senegalensis*	*Labeo cylindricus*
Location	gills	gills	gills
No. of specimens	5	30	2
Body			
length	420 - 460	130 - 700	330 - 400
width	110 - 150	110 - 140	130 - 150
Anchors/hamulus			
length	27 - 33	36 - 42	25 - 25
inner root	9 - 14	11 - 16	12 - 13
outer root	3 - 14	4 - 10	8 - 8
shaft	15 - 18	34 - 42	20 - 23
tip	7 - 9	12 - 16	12 - 14
Bar			
length	24 - 30	27 - 35	25 - 30
width	-	5 - 8	5 - 5
Marginal hooklets			
pairs 1 - 2	15 - 22	20 - 30	14 - 20
pairs 3 - 7	8 - 10	11 - 16	14 - 14
Copulatory organ			
cirrus axis	58 - 60	55 - 60	57 - 62
spiral	33 - 46	33 - 46	30 - 38

The parasite was described from *Labeo victorianus* by Paperna (1973), but was also found from several other *Labeo* spp. as well (table 3B.8). Its first illustrations are found in Paperna (1979). The other record of this species is that of Guégan *et al.* (1988). Musilová *et al.* (2009) studied the type and voucher specimens and redescribed *D. cyclocirrus,* also considered the specimens of Guégan *et al.* (1988) as senior subjective synonym of *Dactylogyrus yassensis* Musilová, Rehulkova & Gelnar, 2009.

In this study the parasites were retrieved from the gills of *Labeo cylindricus* and this is the first geographical record for South Africa. The shapes and dimensions of the copulatory organ and opisthohaptoral features (figure 3B.12; table 3B.8) are comparable to the type specimens. The parasite is further characterized by a non-sclerotized vagina. The hooklets pairs1-2 is longer than pairs 3-7. This species was found to co-occur with *Dactylogyrus brevicirrus* and *Dogielius dublicornis* on the gills of *L. cylindricus*.

3.9 ***Dactylogyrus spinicirrus*** (Paperna & Thurston, 1968)

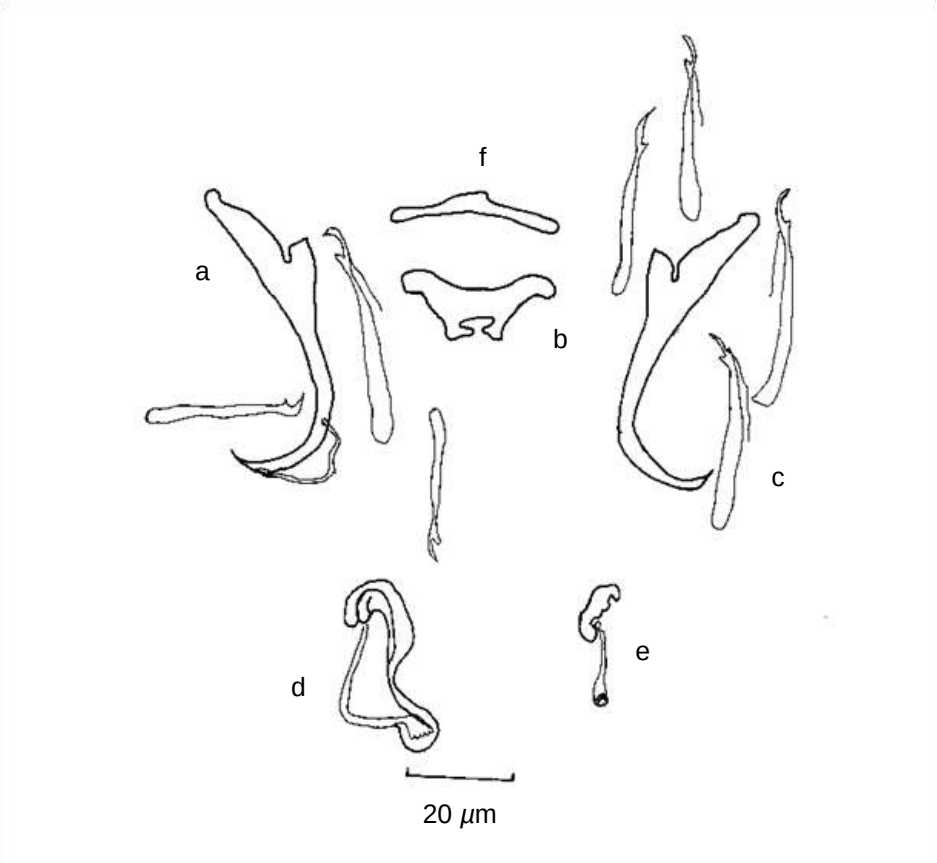


Figure 3B.13 *Dactylogyrus spinicirrus* (Paperna & Thurston, 1968) - Haptor and reproductive organs a. anchor b. main bar c. marginal hook d. copulatory organ e. vagina f. fine bar

This parasite was described from *Barbus altianalis* in Kenya. The other records of this species are those of Paperna (1973) from *B. nyanzae* and *B. somerini* in Uganda and Mashego (1983) from *Labeobarbus marequensis* in South Africa. In this study the parasites were retrieved from the gills of *Labeobarbus marequensis*. The shapes and dimensions of the copulatory organ and opisthohaptoral features (figure 3B.13; table 3B.9) are comparable to those in previous studies, but the anchors are shorter in the present study. The parasite is further characterized by two bars and the hooklets are very long, but of different lengths to one another.

Table 3B.9 Measurements (in μm) of *Dactylogyrus spinicirrus*

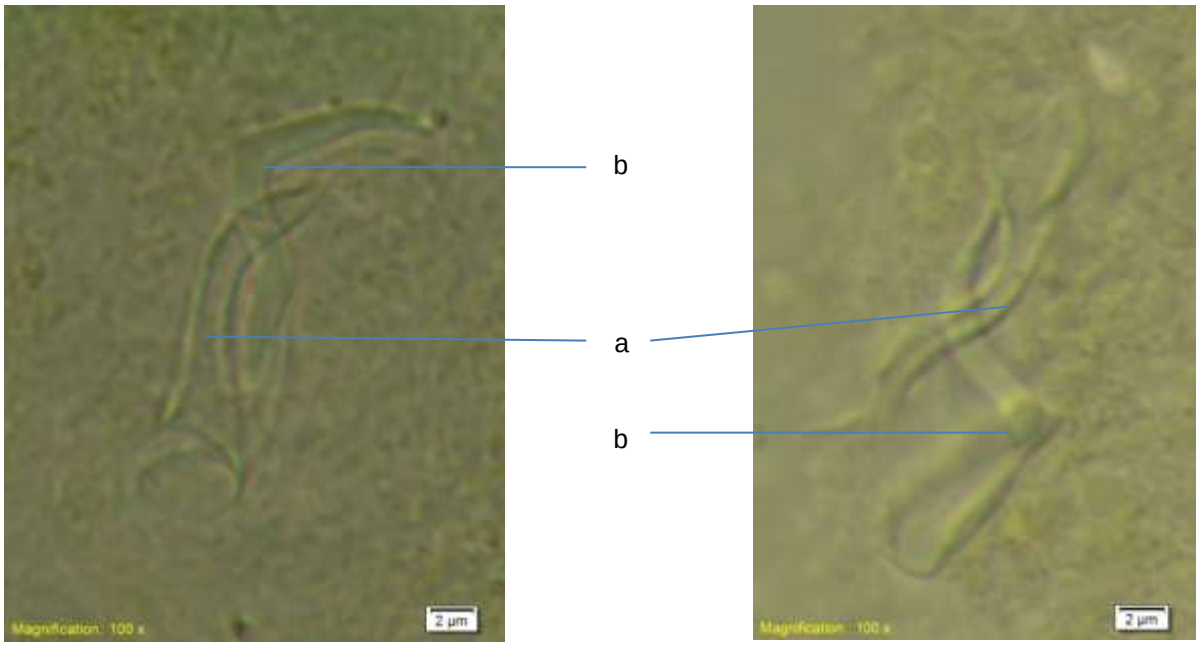
D. spinicirrus	Paperna & Thurston 1968	Mashego1982	Present
Host	*Barbus altianalis*	*Labeobarbus marequensis*	*Labeobarbus marequensis*
Location	gills	gills	gills
No. of specimens	2	3	10
Body			
length	400 - 450	338 - 669	400 - 670
width	-	44 - 75	60 - 70
Anchors/hamulus			
length	70 - 80	63 - 76	48 - 57
inner root	16 - 18	20 - 25	20 - 25
outer root	5 - 8	8 - 9	6 - 10
shaft	-	46 - 53	32 - 36
tip	-	14 - 14	13 - 14
Large bar	31 - 35	26 - 28	26 - 30
Small bar	25 - 28	25 - 28	22 - 29
Marginal hooklets (different lengths)	30 - 40	31 - 46	28 - 43
Copulatory organ			
cirrus axis	-	26 - 37	32 - 40
accessory piece	-	16 - 29	28 - 35
Vagina	-	-	22 - 25

Table 3B.10 compares the measurements of all nine species of the genus *Dactylogyrus* and indicates that *D. spinicirrus* is the largest among species of the genus. This larger size is an adaptation to allow the opisthohaptor to attach to the larger gill filaments of *Labeobarbus marequensis.* The four new species of *Dactylogyrus* that are being described are compared for copulatory organs as these are important diagnostic features (figure 3B.14).

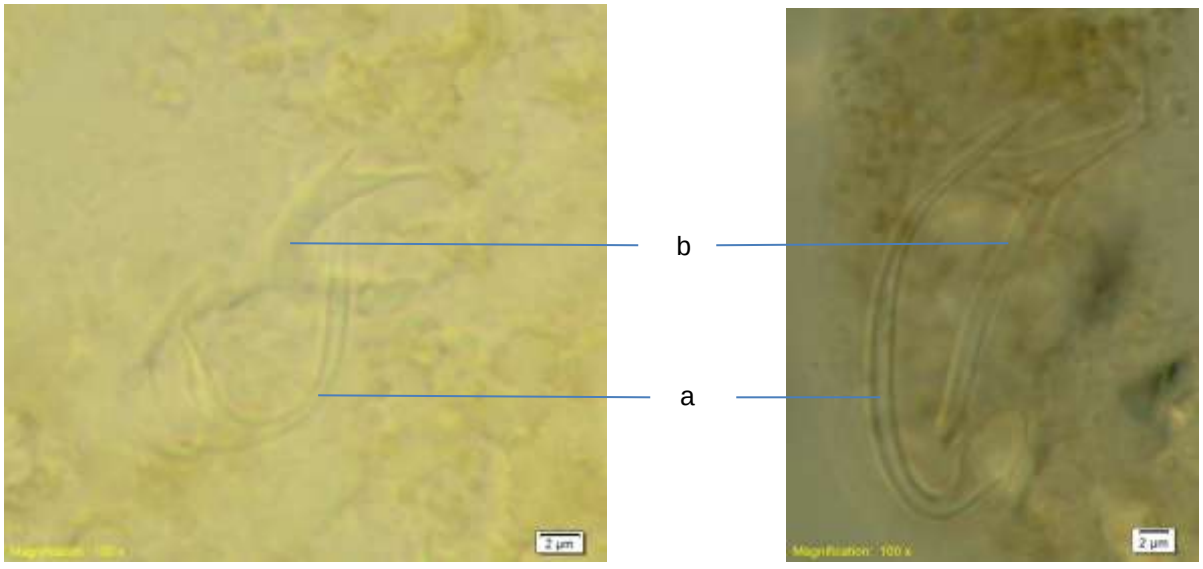
Plates 7 to 11 show the micrographs of all the nine species of the genus *Dactylogyrus.* The sclerotised parts that were used in identification (haptoral features, copulatory organ and vagina) are micrographed separately.

Table 3B.10 Measurements (in μm) of nine *Dactylogyrus* spp. found in Lake Tzaneen

Dactylogyrus sp.	*D. afrolongicornis*	*D. allolongionchus*	*D. radiatus*	*D. unitaeniatus*	*D. sevidi*	*D. molybdinus*	*D. brevicirrus*	*D. cyclocirrus*	*D. spinicirrus*
Host	*B. trimaculatus*	*B. trimaculatus*	*B. radiatus, B. trimaculatus*	*B. unitaeniatus*	*L. molybdinus*	*L. molybdinus*	*L. cylindricus*	*L. cylindricus*	*L. marequensis*
Location	gills	gills	gills	gills	gills	gills	gills	gills	gills
No. of specimens	16	3	12	2	8	30	5	2	10
Body length	325 – 480	225 – 365	230-400	225-415	260-400	240-325	240-430	330-400	400-670
width	75 – 120	60 – 68	70-110	35-60	60-130	20-90	70-100	130-150	60-70
Anchors/hamulus									
length	38 – 50	62 – 65	35-38	32-33	32-37	34-38	32-38	25-25	48-57
inner root	17 – 20	12 – 15	12-15	12-13	13-16	14-16	14-16	12-13	20-25
outer root	3 – 5	3 – 5	2-5	2	2-3	2-3	2-4	8-8	6-10
shaft	25 – 33	57 – 60	24-26	22-23	22-23	20-22	20-24	20-23	32-36
lip	15 – 18	18 – 20	14-15	15	10-12	8-11	12-13	12-14	13-14
Bar									
length	62 – 80	35 – 45	24-28	25	17-29	18-20	16-20	25-30	26-30 large
width	3 – 5	7 – 8	2-3	2	2-3	2-3	2-2	5-5	22-29 small
Marginal hooklets							13-14 p1-4	14-20 p1-4	
pairs 1 - 7	16 – 21	17 – 20	17-19	16-17	14-15	17-20	15-16 p5-7	14-14 p5-7	28-43
Copulatory organ									
cirrus axis	32 – 40	28 – 32	19-25	22	16-25	25-36	25-30	57-62	32-40
accessory piece	20 – 27	18 – 20	19-23	18-20	14-20	17-30	17-23	30-38	28-35
vagina length	15 – 30	-	15-20	14	-	-	-	-	22-25
width	10 – 17	-	7-10	10-14	-	-	-	-	-



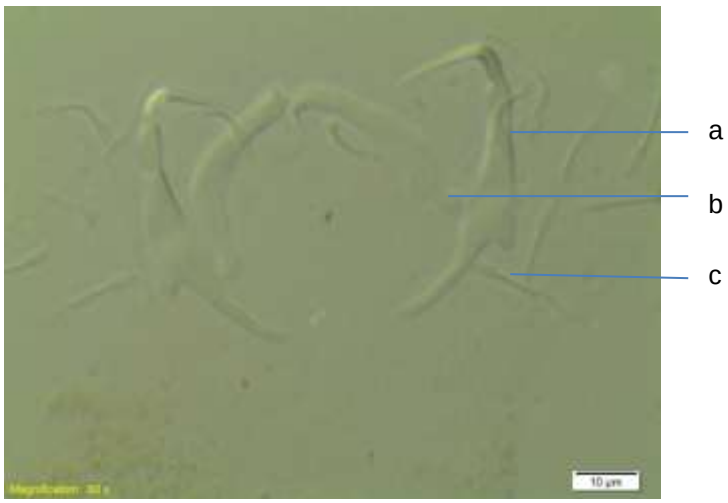
from *Dactylogyrus* sp. 1 from *Dactylogyrus* sp. 2



from *Dactylogyrus* sp. 3 from *Dactylogyrus* sp. 4

a. cirrus b. accessory piece

Figure 3B.14 The copulatory organs of the four new species of *Dactylogyrus* (spp. 1 to 4) found in Lake Tzaneen



haptor copulatory organ

Dactylogyrus afrolongicornis afrolongicornis - a. anchor b. bar c. marginal hook d. cirrus e. accessory piece

haptor copulatory organ

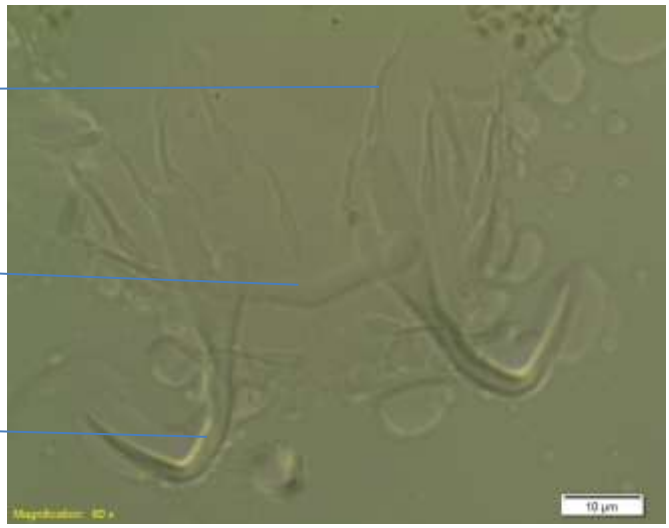
Dactylogyrus allolongionchus - a. anchor b. bar c. marginal hook d. cirrus e. accessory piece

Plate 7 *Dactylogyrus afrolongicornis afrolongicornis & Dactylogyrus allolongionchus*

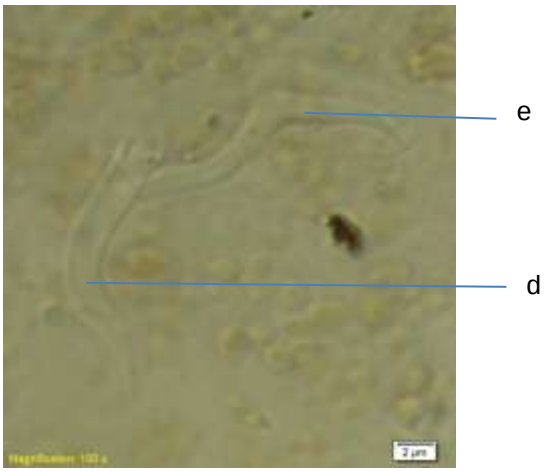
haptor

copulatory organ

Dactylogyrus sp. 1 from *Barbus radiatus* - a. anchor b. bar c. marginal hook d. cirrus e. accessory piece



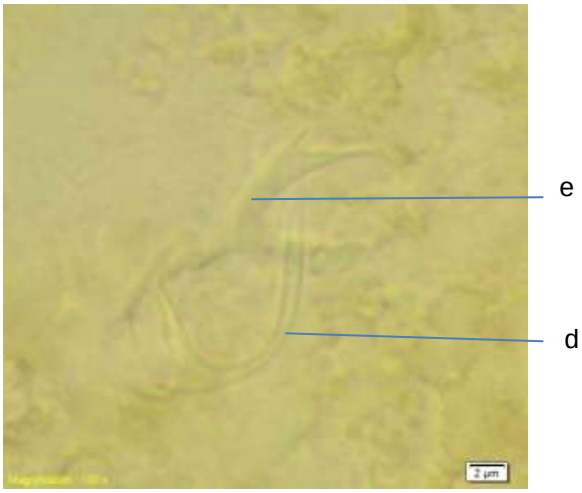
haptor



copulatory organ

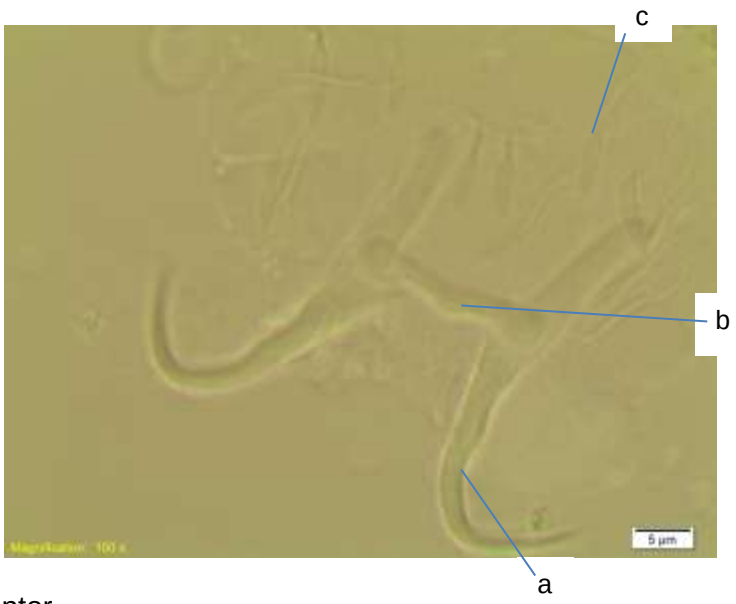
Dactylogyrus sp. 1 from *Barbus trimaculatus* - a. anchor b. bar c. marginal hook d. cirrus e. accessory piece

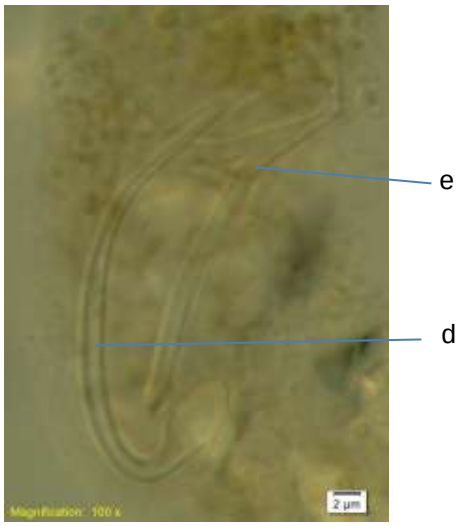
Plate 8 *Dactylogyrus* sp. 1



haptor copulatory organ

Dactylogyrus sp. 3 - a. anchor b. bar c. marginal hook d. cirrus e. accessory piece

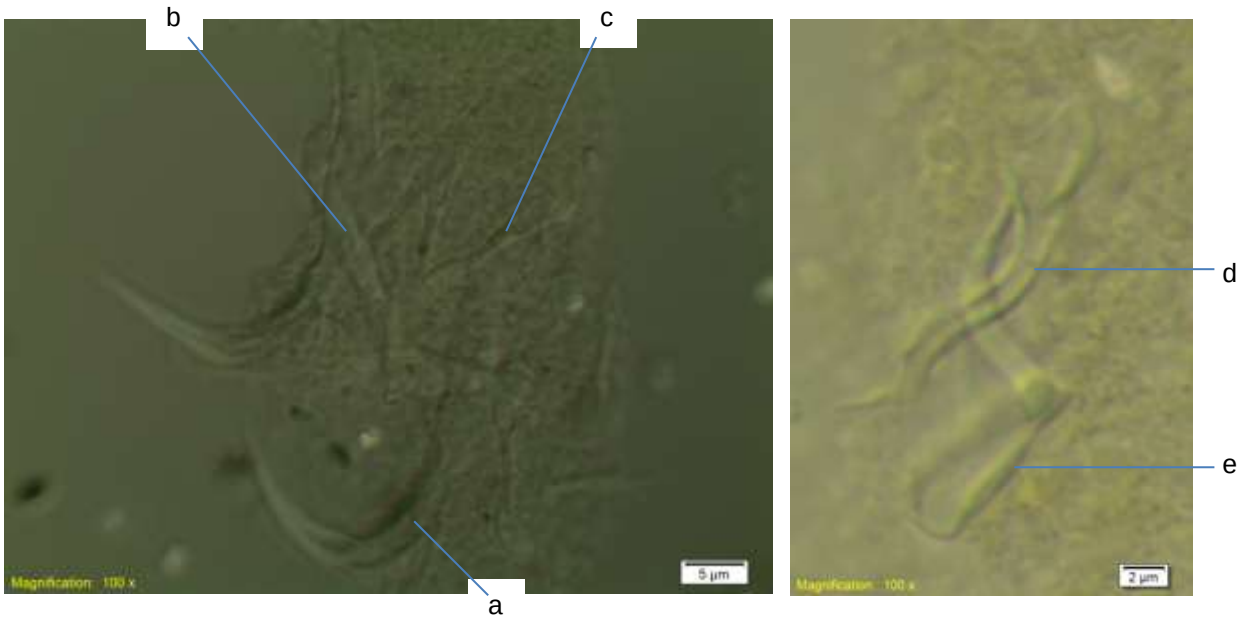




haptor copulatory organ

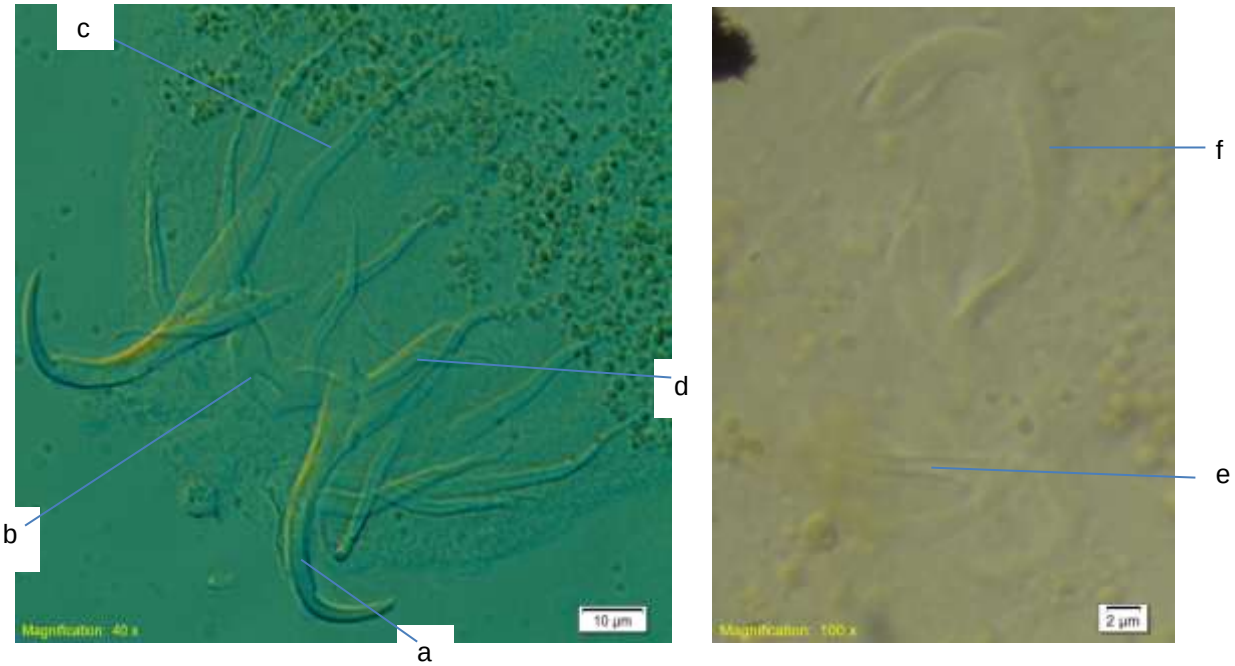
Dactylogyrus sp. 4 - a. anchor b. bar c. marginal hook d. cirrus e. accessory piece

Plate 9 *Dactylogyrus* sp. 3 & *Dactylogyrus* sp. 4



haptor copulatory organ

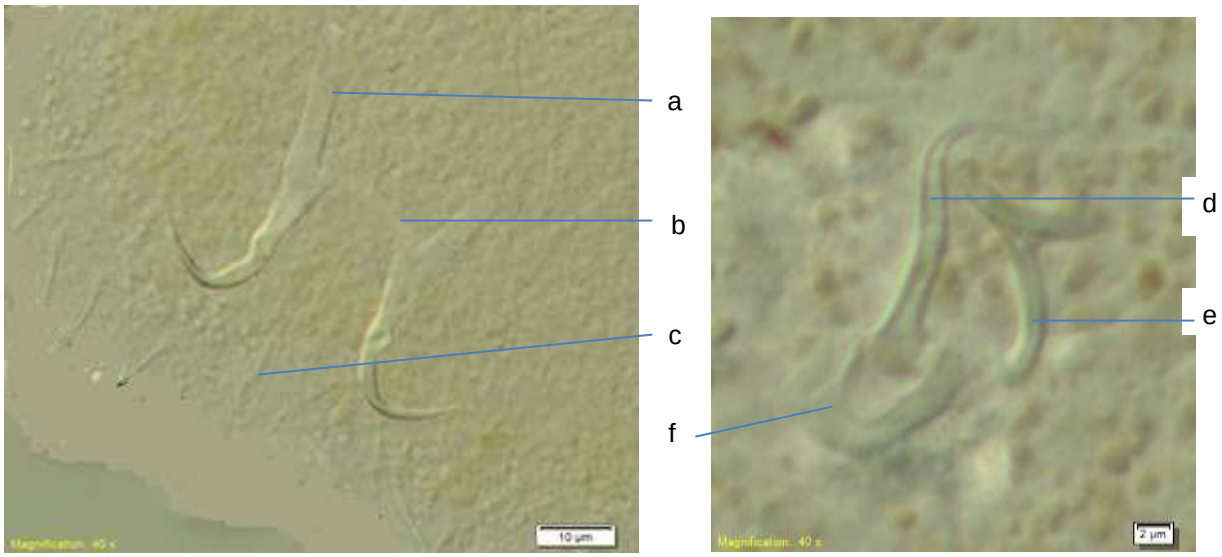
Dactylogyrus sp. 2 - a. anchor b. bar c. marginal hook d. cirrus e. accessory piece



haptor copulatory organ

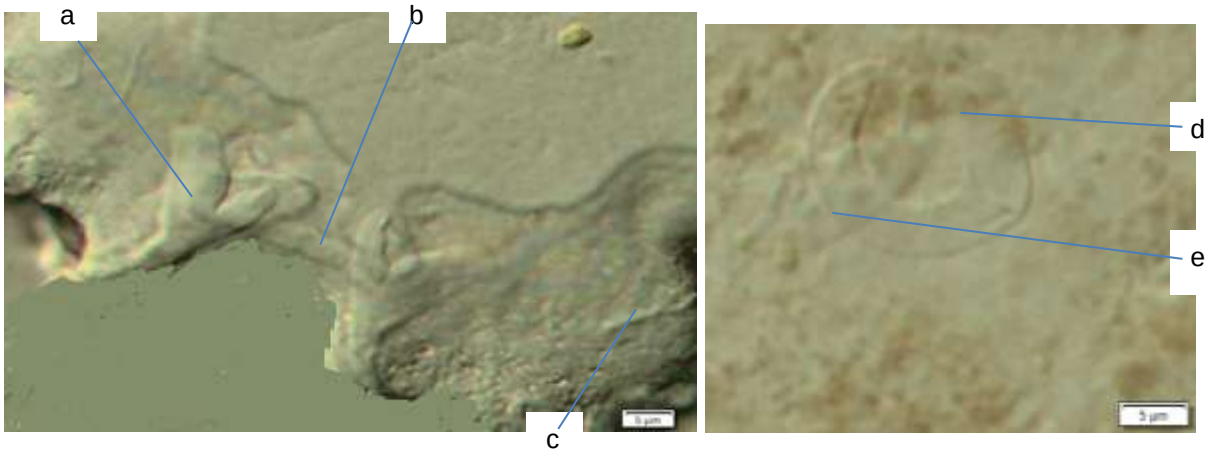
Dactylogyrus spinicirrus - a. anchor b. main bar c. marginal hook d. fine bar e. cirrus f. accessory piece

Plate 10 *Dactylogyrus* sp. 2 & *Dactylogyrus spinicirrus*



haptor copulatory organ

Dactylogyrus brevicirrus - a. anchor b. bar c. marginal hook d. cirrus e. accessory piece e. funnel



haptor copulatory organ

Dactylogyrus cyclocirrus - a. anchor b. bar c. marginal hook d. cirrus e. accessory piece

Plate 11 *Dactylogyrus brevicirrus* & *Dactylogyrus cyclocirrus*

4 Genus ***Dogielius*** Bychowsky, 1936

According to Paperna (1979), the genus *Dogielius* is closely related to *Dactylogyrus* and was apparently diverged from it. The two genera, both parasitic in cyprinids, have one pair of anchors. This genus is represented on cyprinid fish hosts of both Africa and Asia. Timofeeva *et al.* (1997) have listed 37 nominal species of *Dogielius.* The 22 species found in Africa are from host genera *Labeo, Barbus* and *Varicorhinus* and are listed in Khalil & Polling (1997).

African studies that include the genus Dogielius (Price & Yurkiewicz 1968; Paperna 1973; Birgi & Lambert 1987; Guégan *et al.* 1989, 1992; Paugy *et al.* 1990; Guégan & Lambert 1990, 1991; Guégan & Hugueny 1994; Lambert & El Gharbi 1995;Tombi & Bilong-Bilong 2004; Musilová *et al.* 2009; Jeannette *et al.* 2010) account for most species, whilst only few are from China, India and Iran. The type species, *Dogielius forceps* Bychowsky, 1936 was retrieved from *Schizothorax* in Kazakhstan. In this study, one species each, was found from the two *Labeo* fish species in the dam.

4.1 ***Dogielius dublicornis*** Paperna, 1973

This parasite was described by Paperna (1973) from *Labeo cylindricus* in Tanzania. In this study, the parasites were retrieved from the gills of *L. cylindricus.* This is a new geographical record for South Africa. When compared to the two specimens of Paperna (1973) the present specimens are found to be larger and with longer hooklets, but the anchors and a transverse bar are shorter (table 3B.11). The haptoral features and the copulatory organ (figure 3B.15) are similar. This species was found to co-occur with *Dactylogyrus brevicirrus* and *D. cyclocirrus* on the gills of *L. cylindricus*.

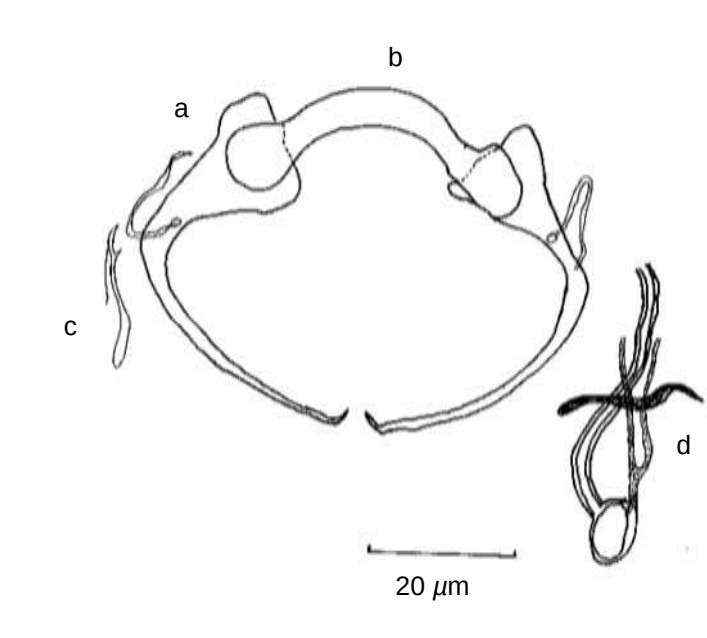


Figure 3B.15 *Dogielius dublicornis* Paperna, 1973 - Haptoral features and copulatory organ
a. anchor b. bar c. marginal hook d. copulatory organ

Table 3B.11 Measurements (in µm) of *Dogielius dublicornis*

Dogielius dublicornis	Paperna, 1973	Present
Host	*Labeo cylindricus*	*Labeo cylindricus*
Location	gills	gills
No. of specimens	2	10
Body		
length	150 - 200	190 - 280
width	80 - 80	80 - 110
Anchors/hamulus		
length	68 - 73	45 - 53
shaft	38 - 40	32 - 35
tip	4 - 5	2 - 3
Bar	50 - 54	42 - 47
Marginal hooklets	15 - 15	20 - 20
Copulatory organ		
cirrus axis	39 - 39	35 - 37
accessory piece	33 - 33	30 - 33

4.2 *Dogielius* sp.

There were only two specimens of this parasite that were retrieved from the gills of *Labeo molybdinus*.

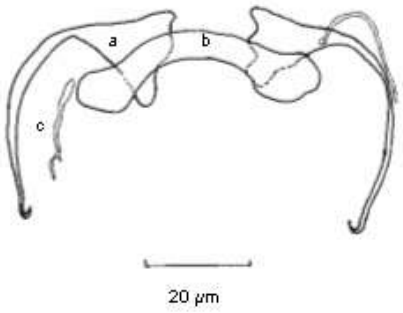


Figure 3B.16 *Dogielius* sp. – Haptoral features
a. anchor b. bar c. marginal hook

Table 3B.12 Measurements (in µm) of *Dogielius* sp.

Dogielius sp.	Present
Host	*Labeo molybdinus*
Location	gills
No. of specimens	2
Body	
length	210 - 210
width	110 - 110
Anchors/hamulus	
length	46 - 48
shaft	33 - 34
tip	2 - 2
Bar	47 - 47
Marginal hooklets	20 - 20
Copulatory organ	
cirrus axis	-
accessory piece	-

The opisthohaptoral features (figure 3B.16) are those of *Dogielius* and the copulatory organ was not detected in both specimens, rendering it difficult to be identified to the species level. Again, this is the first record of *Dogielius* from the gills of *Labeo molybdinus.* The parasite co-occurs with *Dactylogyrus sevidi* and *Dactylogyrus molybdinus*. The measurements of the two specimens are shown in table 3B.12.

5 Genus ***Schilbetrema*** Paperna & Thurston, 1968

Khalil & Polling (1997) recorded 12 species of the genus from African freshwater siluriform fishes. According to Lim *et al*. (2001), there are 16 nominal species in this genus and the hosts are freshwater schilbeid fishes from Africa. The main distinguishing features of the genus are based on the structure of the ventral anchors, which have a prominent knob in the inner surface of the base, and the projections (two terminal and one usually anteromedial) on the ventral bar. Like many other monogenean genera, their familial status has been in dispute and is still not resolved (Lim *et al*. 2001).

5.1 ***Schilbetrema quadricornis*** Paperna & Thurston, 1968

This is the type species of the genus and was found from *Schilbe intermedius* in Lake Victoria. This monogenean species was also found on the same fish host in Uganda (Paperna & Thurston 1968), Ghana (Paperna 1969, 1979), Tanzania (Paperna 1979), Togo (Kritzky & Kulo 1992) and in Zimbabwe (Douellou & Chishawa 1995). No record of this species could be found in South Africa. In this study, the parasites were found from *Schilbe intermedius*. The haptoral armature and the copulatory organ are shown in figure 3B.17.

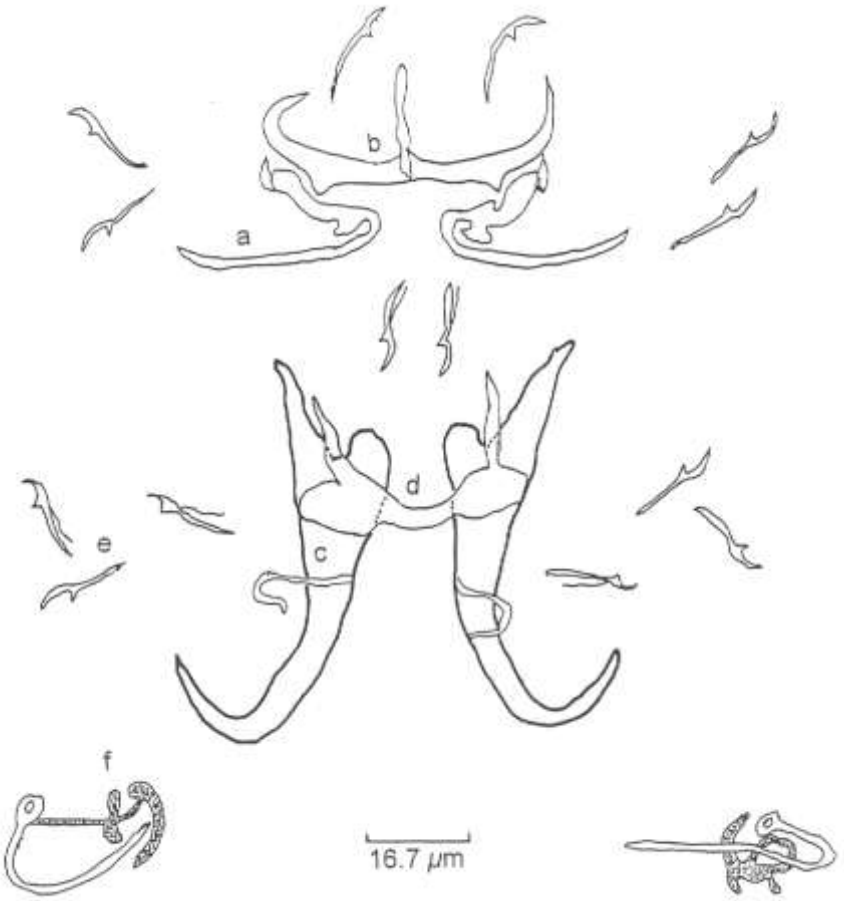


Figure 3B.17 *Schilbetrema quadricornis* Paperna & Thurston, 1968 – a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. copulatory organ

The diagnostic features are the following: dorsal anchors are simple, large, with straight superficial root and short deep root. The dorsal bar has two subterminal pointed projections and enlarged ends. The ventral anchors are much smaller than the dorsal, with sharp curved inner root. The ventral bar is W-shaped with two bilateral horns and delicate submedial projection. The hooks are similar in size. The corpulatory organ has a bent tube and an accessory piece with a branched distal sheath. The measurements of the present material and those of Paperna & Thurston (1968), Kritzky & Kulo (1992) and Douellou & Chishawa (1995) in table 3B.13 support the conspecificity of these specimens.

Table 3B.13 Measurements (in μm) of *Schilbetrema quadricornis*

S. quadricornis material	Original Paperna & Thurston 1968	Kritzky & Kulo 1992	Douellou & Chishawa 1995	Present
Host	*S. mystus*	*S. intermedius*	*S. intermedius*	*S. intermedius*
Location	gills	gills	gills	gills
No. of specimens	2 or 4	Variable up to 50	11	30
Body				
length	364-514	309-513	340-590	300-450
width	138-153	56-89	40-88	90-150
Haptor				
length	78-93	71-102	-	70-90
width	81-83	61-85	-	70-80
Dorsal anchors				
length	52-55	52-65	55-62	60-70
base width	20-26	17-26	-	20-25
Dorsal bar				
length	38-40	36-45	35-41	36-45
width	-	-	22-31	-
distal projection	-	-	13-19	18-20
Ventral anchors				
length	22-25	21-28	20-23	20-25
base width	8-9	7-11	-	-
Ventral bar				
length	40	36-47	36-46	45-55
width	-	-	17-23	-
medial projection	-	-	12-17	15-22
distal projection	-	-	12-17	12-14
Marginal hooks	15-16	16-18	13-17	15-17
Corpulatory organ				
length		38-56	23-31	26-32

6 Genus ***Quadriacanthus*** Paperna, 1961

The genus was established by Paperna (1961) for *Quadriacanthus clariadis* from the gills of *Clarias lazera* (= *C. gariepinus*) collected in the lake of Galilee, Israel. The genus was characterised, in part, by two unequal bars, each with a solid base, to which are attached narrower appendages. El-Naggar & Serag (1986), Kritsky & Kulo (1988) and Tripathi *et al*. (2007) emended the generic diagnosis, amongst others, by recognising the medially articulating ventral bar, unequal and dissimilar pairs of marginal hooklets, and the basally articulated, straight copulatory tube and accessory piece. There are 25 nominal species mainly from Clariidae (*Clarias* and *Heterobranchus*) and few from Bagridae (*Bagrus*) (Lim *et al.* 2001; Tripathi *et al*. 2007). Only one species was found from a cichlid and could have been, by admission, an accidental infection (Lim *et al.* 2001).

The few African studies (Paperna 1965, 1973, 1979; El-Naggar & Serag 1985, 1986; Birgi 1988; Kritsky & Kulo 1988; Doulloe & Chishawa 1995; N'douba *et al.* 1999; N'douba & Lambert 1999, 2001) indicate records from Egypt, Uganda, Ghana, Kenya, Tanzania, Ivory Coast, Cameroon and Zimbabwe only. In this study two species were found, both from the gills of *Clarias gariepinus*.

6.1 ***Quadriacanthus aegypticus*** El Naggar & Serag, 1986

This parasite was described by El-Naggar & Serag (1986) from the gills of *Clarias gariepinus* in Egypt. The other record of this species is that of Doulloe & Chishawa (1995) on *C. gariepinus* in Zimbabwe. In this study the monogenean species was found from the gills of *C. gariepinus* and is a first record for South Africa. This species have been found in this study to share the host species with other species of the genera *Gyrodactylus, Macrogyrodactylus* and *Quadriacanthus.*

The following characters for the genus can be seen: Dorsal anchors are larger than ventral anchors; dorsal bar with two arms and a process projecting posteriorly;

marginal hooklets unequal (figure 3B.18; table 3B.14). Generic diagnosis based mainly on accessory apparatus of copulatory organ that terminates in 2 hooks and possesses 2 distinctive lateral outgrowths (figure 3B.18). The morphological measurements of the Tzaneen specimens concur with those of El-Naggar & Serag (1986) (table 3B.14).

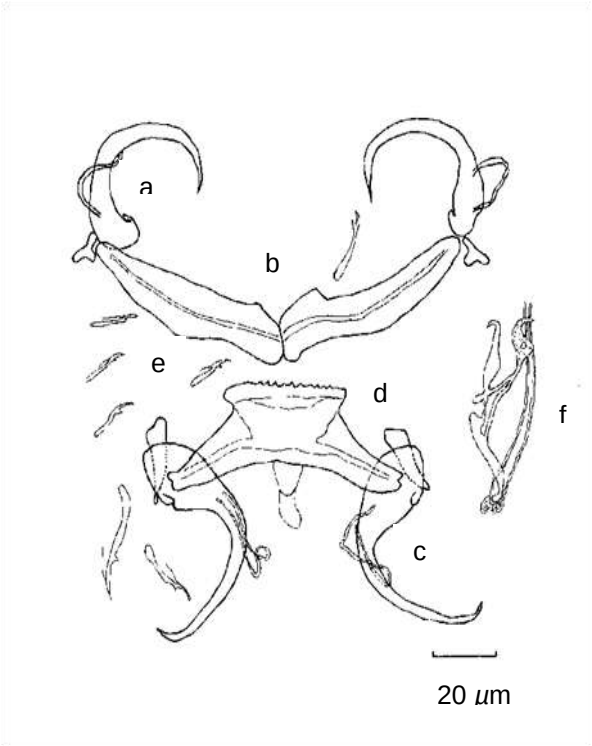


Figure 3B.18 *Quadriacanthus aegypticus* El Naggar & Serag, 1986. Haptoral features and copulatory organ.
a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook
f. copulatory organ

Table 3B.14 Measurements (in μm) of *Quadriacanthus aegypticus*

Q. aegypticus	El Naggar & Serag 1986	Present
Host	*C. gariepinus*	*C. gariepinus*
Location	gills	gills
No. of specimens	10	10
Body		
length	378 - 630	415 - 490
width	120 - 157	120 - 160
Opisthohaptor		
length	-	60 - 130
width	-	100 - 150
Ventral anchor		
length	35 - 43	40 - 46
base	9 - 13	10 - 14
process	8 - 11	6 - 10
Ventral bar	40 - 47	45 - 60
Dorsal anchors		
length	42 - 49	46 - 55
base	3 - 4	3 - 5
process	14 - 19	17 - 18
Dorsal bar		
a	35 - 47	43 - 54
b	24 - 30	32 - 43
c **(**process)	12 - 18	15 - 22
Hooklets		
1	16 - 22	20 - 21
3	16 - 22	20 - 21
4	27 - 36	33 - 36
2, 5, 6, 7	12 - 14	14 - 16
Copulatory tube	42 - 56	44 - 62
Vaginal duct	18 - 38	-

6.2 ***Quadriacanthus clariadis*** Paperna, 1961

This parasite was described as the type species of the genus by Paperna (1961) from the gills of *Clarias gariepinus* in Israel. The African records of this species (Paperna 1979; Kritsky & Kulo 1988; Doulloe & Chishawa 1995) are on fishes from Uganda, Ghana, Egypt and Zimbabwe. Tripathi *et al*. (2007) found this species in India. In the present study, this monogenean species was found from the gills of *C. gariepinus* and is a first record for South Africa.

The haptoral features (figure 3B.19) clearly indicate the genus. The copulatory organ is much smaller and more compact than in *Q. aegypticus.* The measurements of the present material are comparable to those of previous studies (table 3B.15).

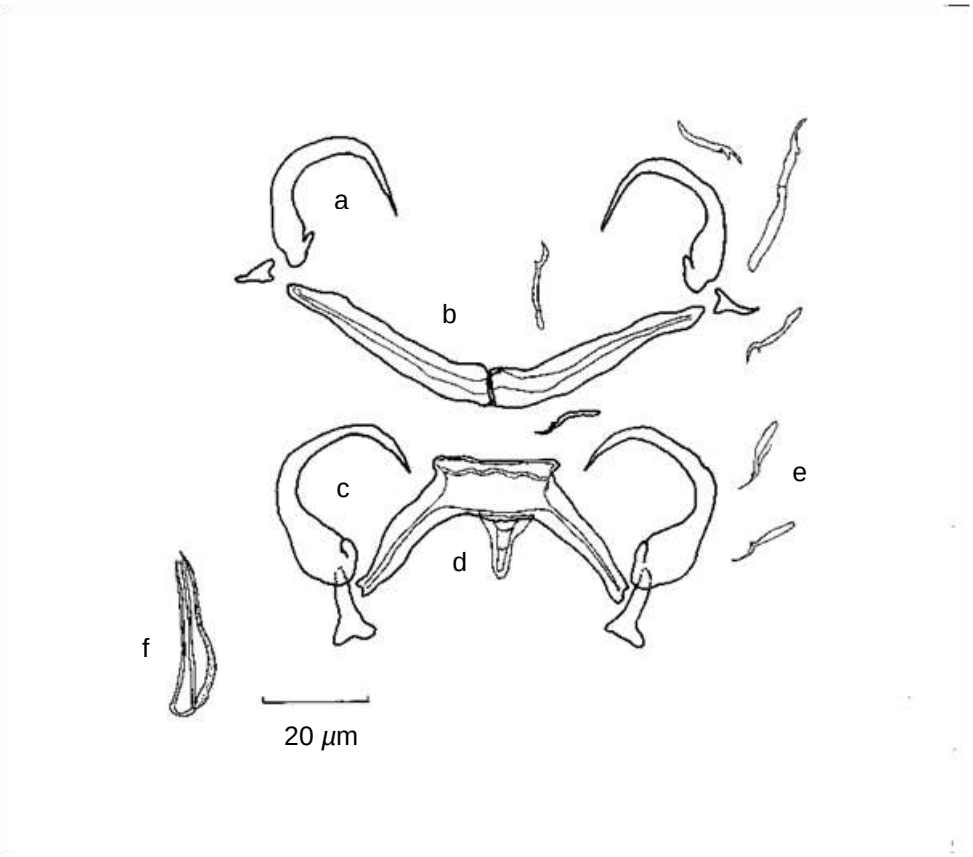
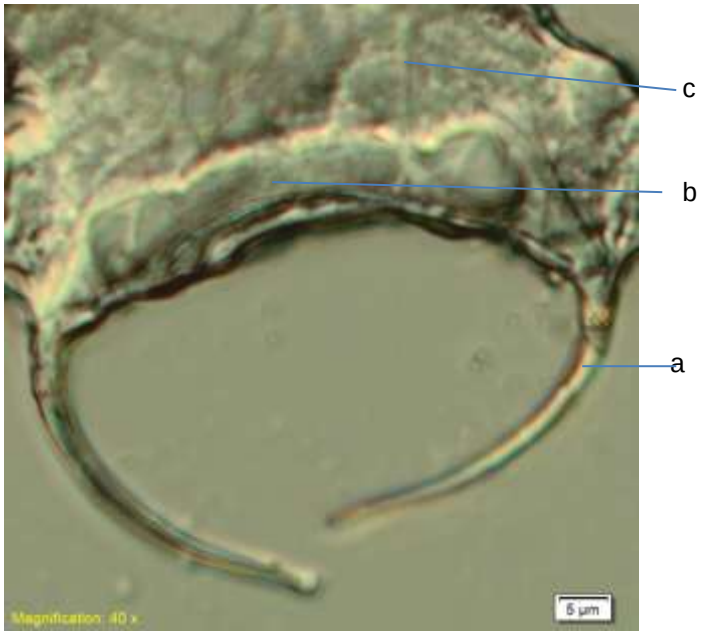


Figure 3B.19 *Quadriacanthus clariadis* Paperna, 1961. Haptoral features and copulatory organ. a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. copulatory organ

Table 3B.15 Measurements (in μm) of *Quadriacanthus clariadis*

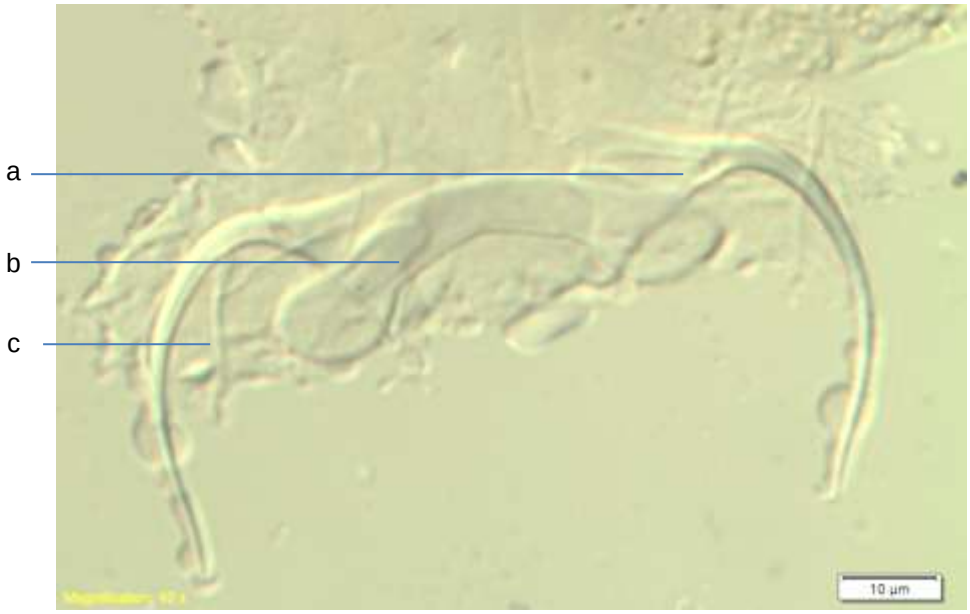
Q. clariadis	Paperna 1961	Kritzky & Kulo 1988	Tripathi *et al.* 2007	Present
Host	*C. gariepinus*	*C. gariepinus*	*C. gariepinus*	*C. gariepinus*
Location	gills	gills	gills	gills
No. of specimens	-	-	15	10
Body				
length	160 - 350	343 - 444	160-230	260-440
width	40 - 110	71 - 134	70-95	75-150
Haptor				
length	78 - 78	74 - 91	36-50	55-90
width	103 - 103	98 - 120	70-100	90-110
Pharynx diameter	20 - 40	20 - 31	16-22	20-21
Ventral anchors				
length	27 - 38	29 - 34	22-26	27-35
base	-	-	9-11	9-13
Ventral bar length	35 - 35	42 - 65	40-46	35-55
Dorsal anchors				
length	38 - 55	47-51	36-42	34-50
base	-	-	9-12	7-13
Dorsal bar				
length	-	52-72	42-54	52-70
process	-	-	9-14	11-14
Marginal hooks				
pair 1	15 - 24	18-20	15-18	19-23
pairs 2, 3, 4, & 5	8 - 12	13-15	12-13	12-15
pair 6	15 - 24	32-39	27-32	31-33
pair 7	8 - 12	16-17	13-16	15-18
Corpulatory tube	22 - 35	22-31	18-22	22-34
Accessory piece	22 - 35	19-22	21-24	22-34

The micrographs of the sclerotised parts of species of *Dogielius, Quadriacanthus and Schilbetrema* are shown in Plates 12, 13 & 14 respectively.



haptor copulatory organ

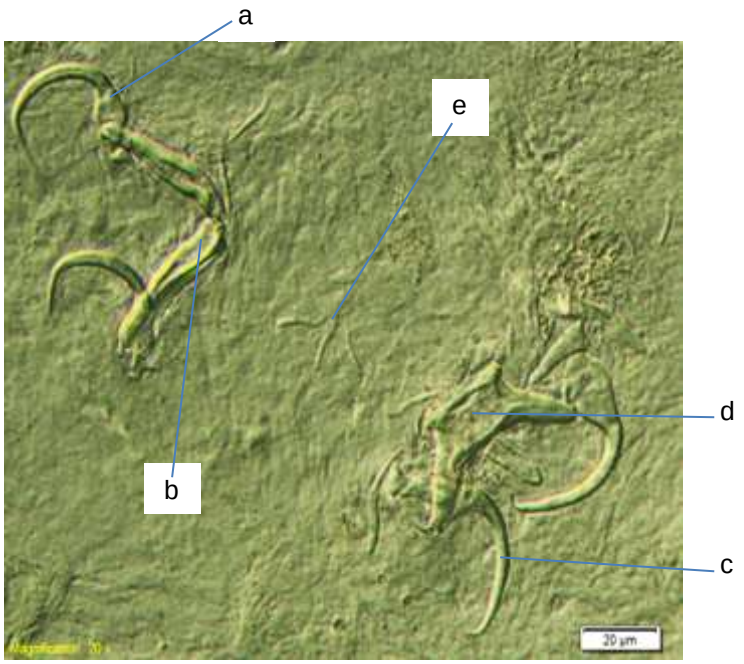
Dogielius dublicornis - a. anchor b. bar c. marginal hook d. cirrus e. accessory piece

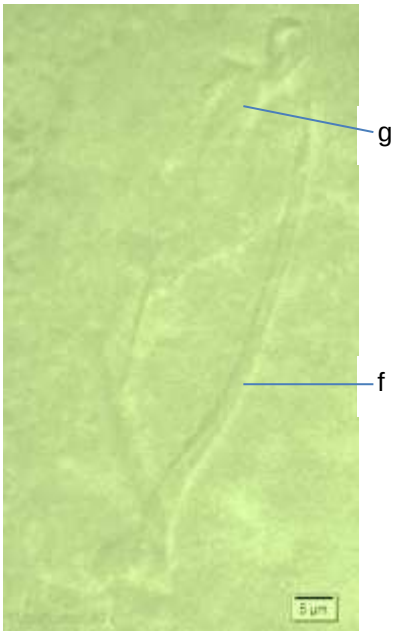


haptor

Dogielius sp. - a. anchor b. bar c. marginal hook

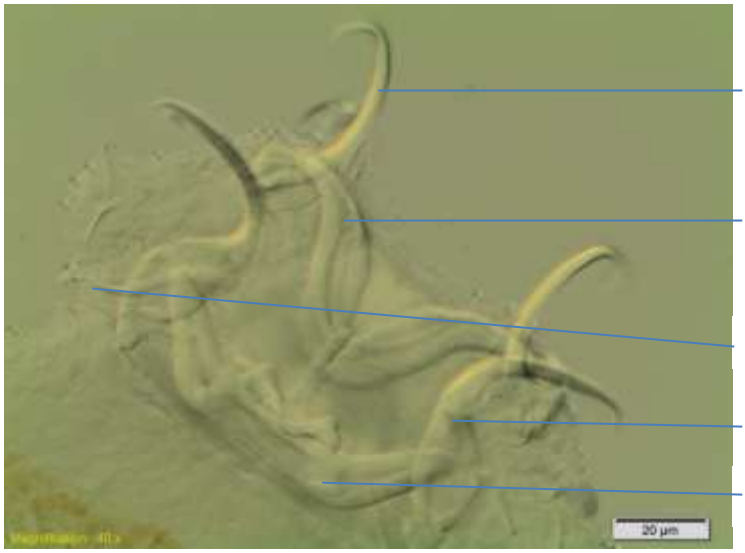
Plate 12 *Dogielius dublicornis* & *Dogielius* sp.

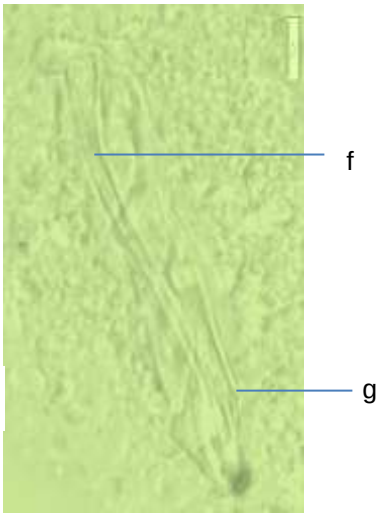




haptor copulatory organ

Quadriacanthus aegypticus - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. cirrus g. accessory piece

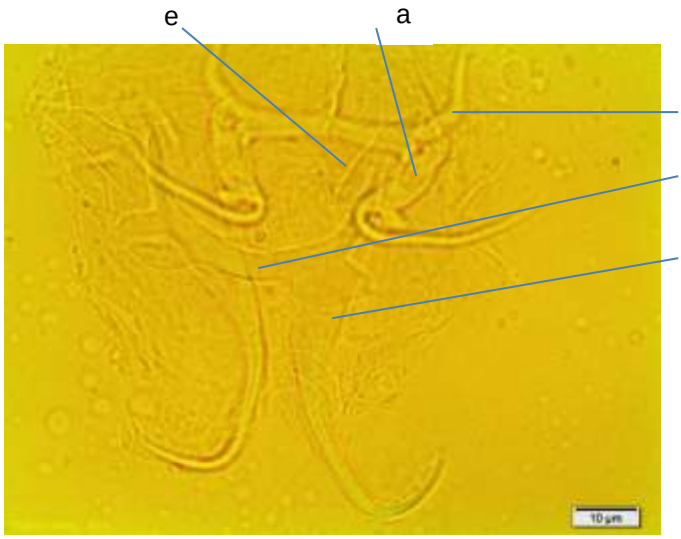




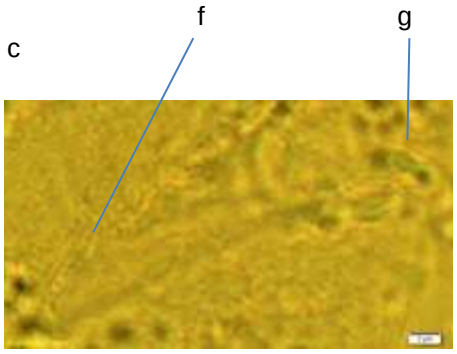
haptor copulatory organ

Quadriacanthus clariadis - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. cirrus g. accessory piece

Plate 13 *Quadriacanthus aegypticus* & *Quadriacanthus clariadis*

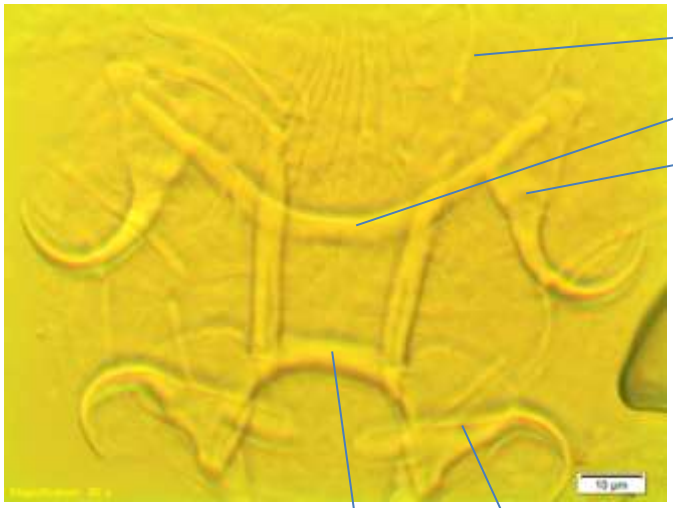


haptor

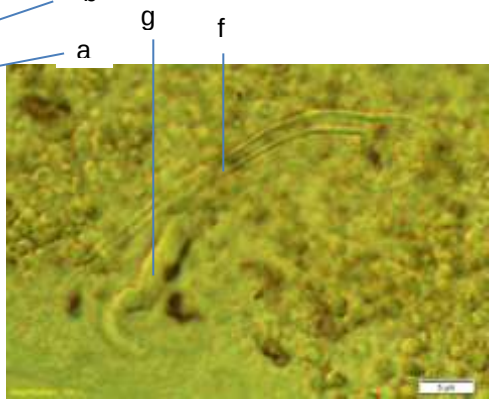


copulatory organ

Schilbetrema quadricornis - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. cirrus g. accessory piece



a haptor



b copulatory organ

Scutogyrus gravivaginus - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. cirrus g. accessory piece

Plate 14 *Schilbetrema quadricornis & Scutogyrus gravivaginus*

7 Genus ***Cichlidogyrus*** Paperna, 1960

This genus is restricted to cichlid fishes and was first described in Israel (Paperna 1960) with *Cichlidogyrus arthracanthus* as the type species. The taxonomy of these parasites is far from an easy task and will remain, together with the identification of cichlid hosts, a subject of frequent revision (Douëllou 1993). Khalil & Polling (1997) listed 51 species from a wide range (not less than 14 spp.) of African freshwater cichlid fishes.

The generic diagnosis is based mainly on the structure of the two bars that are characteristic for the genus. The ventral bar is V-shaped with some tooth-like projections on part of the inner margin. The dorsal bar has three articulating pieces, the central or basal piece to which are attached the two appendages on its sides, thus dividing it into three almost equal parts. There are 14 marginal hooklets on the opisthohaptor. In this genus the hooklets are remarkably polymorphic. The species diagnosis is therefore based on the copulatory organs, but hooklet and anchor polymorphism are also important (Paperna 1960).

The large diversity of species is due to numerous studies that were carried out in the African continent (Paperna 1965, 1968, 1969, 1979; Price & Kirk 1967; Paperna & Thurston 1969; Thurston 1970; Ergens 1981; Dossou 1982; Birgi & Euzet 1983; Batra 1984; Dossou & Birgi 1984; Birgi & Lambert 1986; Douëllou 1993; Pariselle & Euzet 1994, 1995a, 1996, 1997, 1998, 2003, 2004; Modise *et al.* 2006, 2009; Boungou *et al*. 2008; Le Roux & Avenant-Oldewage 2009, 2010). Southern African studies on the genus are only few (Price & Kirk 1967; Price *et al.* 1969c; Douëllou 1993; Modise *et al.* 2006, 2009; Le Roux & Avenant-Oldewage 2009). Pariselle & Euzet (2009) and Le Roux & Avenant-Oldewage (2010) produced a systematic review and a checklist respectively on species of the genus *Cichlidogyrus*.

During the identification of the different species of *Cichlidogyrus* the measurements (in *μ*m) of the sclerotised parts were done according to the scheme of Douëllou (1993) as shown below (figure 3B.20). They were compared with those in previous studies to ascertain the con-specificity of the materials under consideration.

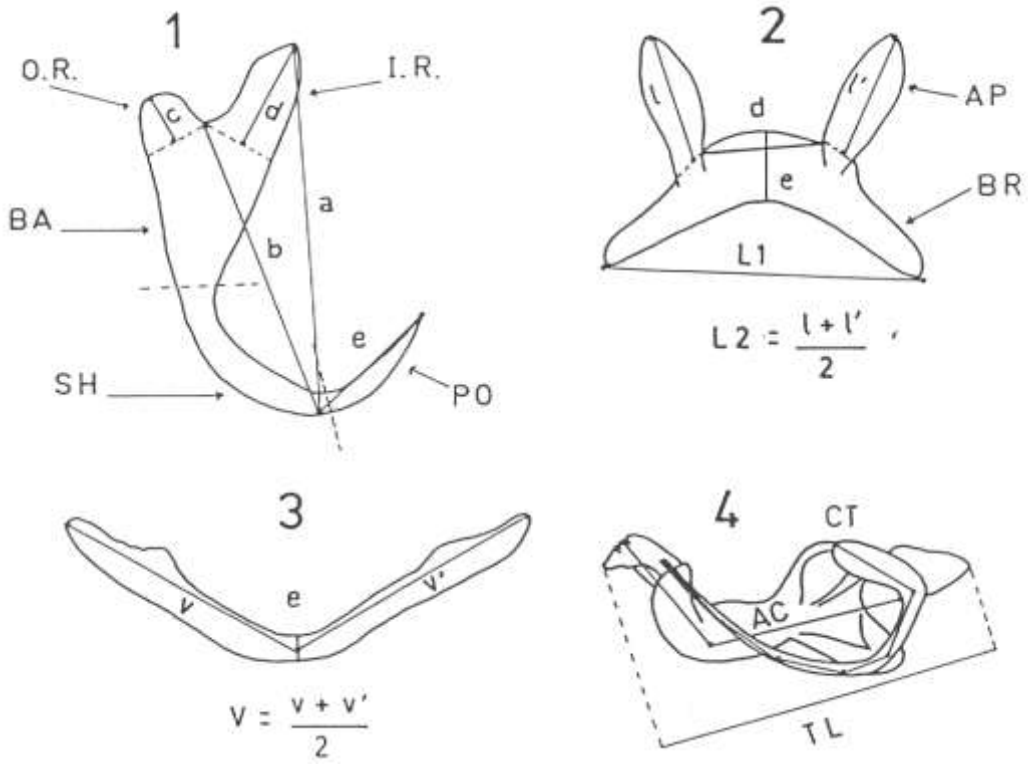


Figure 3B.20 Measurements of the sclerotized parts of *Cichlidogyrus* (from Douëllou 1993)
1. Anchor/Hamulus 2. Dorsal bar 3. Ventral bar 4. Copulatory organ
Abbreviations: O.R. - outer root; I.R. - inner root; BA – base; SH – shaft; PO – point; AP - appendage; BR – branch; CT – copulatory tube; AC – accessory piece

The present study provides more information on the geographical distribution of the species already found in various parts of Africa. In this study six species of *Cichlidogyrus* were found hosted by three cichlid fish species. *Oreochromis mossambicus* hosted *Cichlidogyrus halli, Cichlidogyrus sclerosus, Cichlidogyrus tilapiae* and *Cichlidogyrus dossoui*. *Tilapia rendalli* was a host to *Cichlidogyrus halli, Cichlidogyrus dossoui* and *Cichlidogyrus quaestio*. *Pseudocrenilabrus philander* hosted *Cichlidogyrus philander*. The four species found on *Oreochromis mossambicus* were in some cases found to co-occur on the gills of a single host individual. The same is also true of species co-occuring on *Tilapia rendalli*.

7.1 ***Cichlidogyrus dossoui*** Douëllou, 1993

This species was first described from the gills of *Tilapia rendalli* in Lake Kariba, Zimbabwe (Douëllou 1993), but it occurred also occasionally on *Oreochromis mortimeri* and *Serranochromis macrocephalus*. It was also found in the Okavango Delta, Botswana from the gills of *T. rendalli* (Modise *et al.* 2006). There are no other records of this species outside the southern African region.

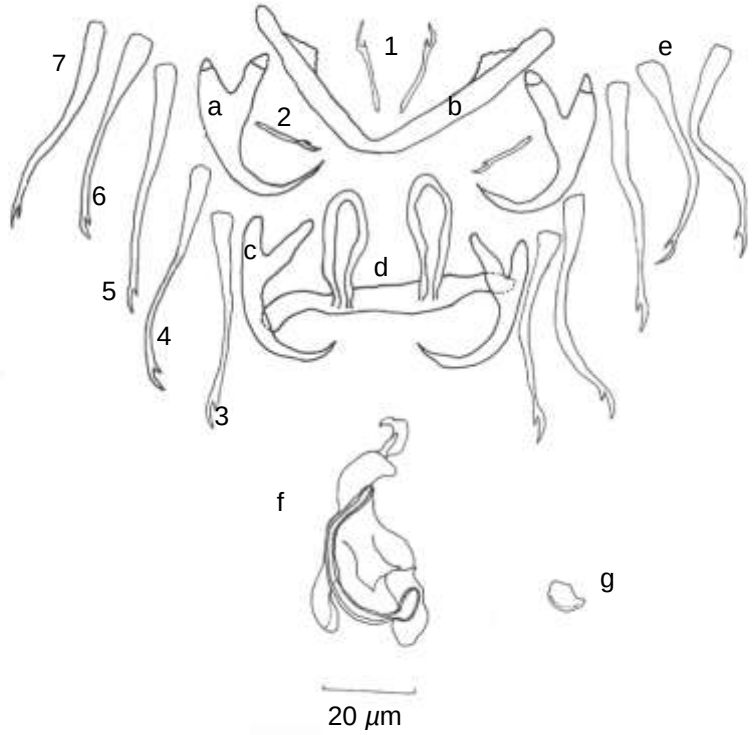


Figure 3B.21 *Cichlidogyrus dossoui* Douëllou, 1993 – Haptoral & reproductive features
a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hooks 1-7 f. copulatory organ g. vagina

Cichlidogyrus dossoui is a long parasite with two or four eyes and the haptor is broader than the body and not separated by a constriction. The hooklets are extremely long except pairs 1 and 2 which are shorter in size. They are longer in this species than in others found in the lake. The copulatory organ is large and has a tube that is arched with a curved basal enlargement and a tapering end.

Table 3B.16 Measurements (in μm) of *Cichlidogyrus dossoui*

C. dossoui material	Original, Douëllou 1993	Douëllou 1993	Present
Host	*T. rendalli*	*O. mortimeri*	*T. rendalli & O. mossambicus*
Location	gills	gills	gills
No. of specimens	15	15	10
L	430-680	800-1000	420-590
W	80-120	105-240	110-190
Ventral bar			
V	32-40	31-35	34-45
e	2-5	2-4	2-5
Dorsal bar			
L1	30-46	27-35	38-58
L2	12-18	14-16	15-22
d	10-15	8-13	9-15
e	4-8	4-6	4-8
Ventral anchors			
a	34-37	32-39	31-42
b	29-32	27-32	27-37
c	6-9	7-13	6-10
d	12-15	12-18	10-15
e	10-14	9-13	11-15
Dorsal anchors			
a	27-32	24-28	25-32
b	21-25	19-24	20-28
c	5-9	7-11	5-8
d	9-13	10-14	10-14
e	8-12	7-13	9-13
Hooklets			
1	17-20	12-15	15-22
2	12-15	12-13	13-17
3	36-45	38-45	35-49
4	38-46	43-50	35-50
5	41-49	40-47	40-56
6	40-48	42-46	42-51
7	36-42	38-44	37-46
Copulatory organ			
TL	46-56	46-51	50-60

The accessory piece is massive, S-shaped and has thick, fingerlike extensions. The end of the piece is slightly forked bearing denticles on convex part, reaching the end of the copulatory tube. The vagina is well sclerotized (figure 3B.21). This parasite concurs with the features and measurements (table 3B.16) of Douëllou (1993).

There are several *Cichlidogyrus* spp. with long hooklets and a copulatory organ looking the same namely *Cichlidogyrus tiberianus, Cichlidogyrus aegypticus, Cichlidogyrus thurstonae, Cichlidogyrus ergensi, Cichlidogyrus anthemocolpos* and *Cichlidogyrus testificatus* (Paperna 1960; Ergens 1981; Dossou 1982). They mostly differ in the details of the accessory piece and vaginas.

In this study the parasites were found from the gills of *T. rendalli* and *O. mossambicus*. The study presents *Oreochromis mossambicus* as the first host record for *C. dossoui.*

7.2 ***Cichlidogyrus halli*** Price & Kirk, 1967

Cichlidogyrus halli was described from the gills of *Oreochromis shiranus shiranus* in Malawi as *Cleidodiscus halli* by Price & Kirk (1967). It was later found and redescribed several times from a wide range of cichlid hosts and in several countries in Africa (Paperna 1968, 1969, 1979; Paperna & Thurston 1969; Thurston 1970; Ergens 1981; Dossou 1982; Douëllou 1993).

The species is relatively large as compared to other *Cichlidogyrus* spp. found in the lake. It has two eyes. The copulatory organ is simple and long with an S-shaped copulatory tube having an irregular basal portion. The accessory piece ends with a triangular extremity. Pairs 1 and 2 of hooklets are smaller than the other five pairs (figure 3B.22). The sclerotised features and their measurements (table 3B.17) were compared to those of Price & Kirk (1967) and Douëllou (1993) to confirm the species' identification.

In this study, the parasites were retrieved from the gills of *Oreochromis mossambicus* and *Tilapia rendalli.*

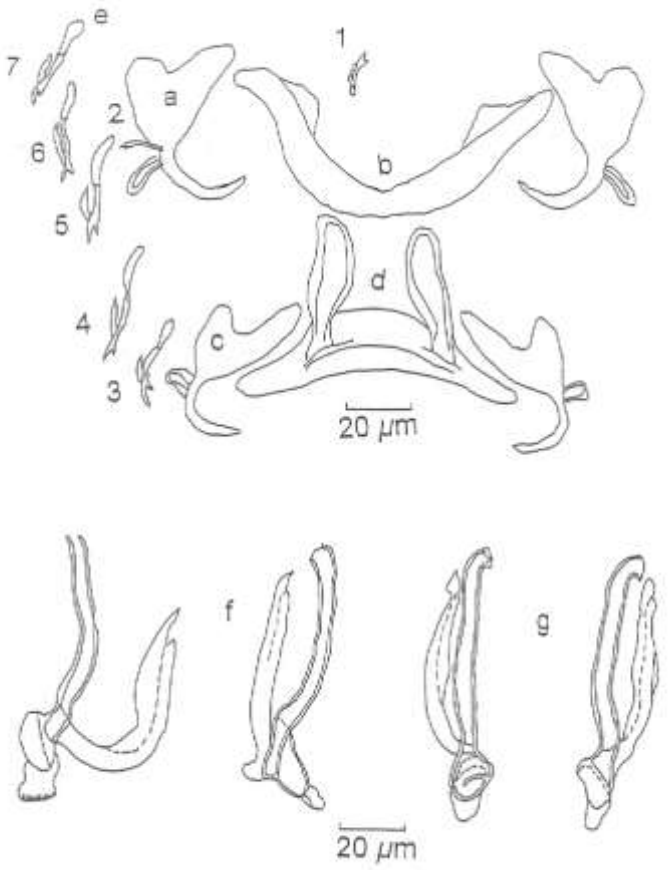


Figure 3B.22 *Cichlidogyrus halli* Price & Kirk, 1967 – Haptoral features & copulatory organ.
a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hooks 1-7
f .copulatory organs from *O. mossambicus* g. copulatory organs from *T. rendalli*

Table 3B.17 Measurements (in µm) of *Cichlidogyrus halli*

C. halli material	Price & Kirk 1967	Douëllou 1993	Present
Host	*O. shiranus*	*O. mortimeri*	*O. mossambicus & T. rendalli*
Location	gills	gills	gills
No. of specimens	8	15	10
L	525-721	700-1400	570-770
W	160-205	220-340	260-380
Ventral bar	104-122	104-144	124-170
V	51-61	51-72	62-85
e	-	-	8-10
Dorsal bar			
L1	68-79	51-73	55-80
L2	14	20-25	20-30
d	-	-	16-24
e	-	-	10-18
Ventral anchors			
a	54-62	49-60	50-58
b	-	-	38-45
c	-	-	6-10
d	-	-	15-20
e	-	-	10-16
Dorsal anchors			
a	53-60	42-56	48-55
b	-	-	30-38
c	-	-	6-10
d	-	-	20-27
e	-	-	10-13
Hooklets			
1	20-22	17-20	15-19
2	20-22	16-18	13-16
3	35-44	29-43	28-31
4	(pairs 3 to 7)	(pairs 3 to 7)	28-37
5			29-38
6			29-34
7			27-33
Copulatory organ			
TL	-	-	83-96
cop. tube	82-86	66-96	68-82
access. piece	61-67	54-66	62-72

7.3 ***Cichlidogyrus philander*** Douellou, 1993

The species was first described by Douëllou (1993) from the gills of *Pseudocrenilabrus philander* in Lake Kariba, Zimbabwe. The only other record of this species (Le Roux & Avenant-Oldewage 2009) was the first for South Africa even though the specimens were collected years after this study. It is the only *Cichlidogyrus* species ever found on the gills of *P. philander*. Christison *et al.* (2005) found and described *Gyrodactylus thlapi* from the gills of this fish in Botswana.

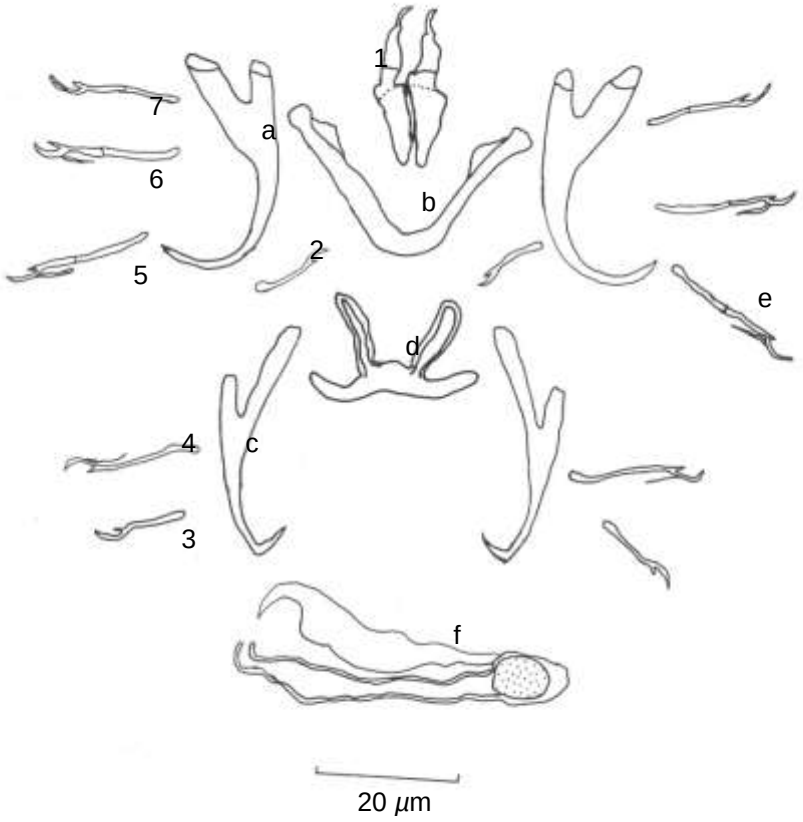


Figure 3B.23 *Cichlidogyrus philander* Douëllou, 1993 – Haptoral features & copulatory organ. a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hooks 1-7 f. copulatory organ

Cichlidogyrus philander is a relatively small parasite as compared to other *Cichlidogyrus* spp. and has either two or four eyes. It also has small-sized sclerotized parts of the haptor but the first pair of hooklets is large and stout. The species diagnosis is also based on the characteristic shape of its copulatory organ (figure 3B.23)

Table 3B.18 Measurements (in µm) of *Cichlidogyrus philander*

C. philander material	Douëllou 1993	Present
Host	*P. philander*	*P. philander*
Location	gills	gills
No. of specimens	15	12
L	260-400	260-390
W	55-80	70-90
Ventral bar		
V	25-29	23-30
e	3-5	2-4
Dorsal bar		
L1	24-31	25-32
L2	10-12	10-12
d	7-12	7-12
e	4-6	3-5
Ventral anchors		
a	28-32	28-34
b	23-27	25-28
c	4-7	4-6
d	8-12	7-12
e	8-11	10-12
Dorsal anchors		
a	29-36	31-35
b	18-23	21-23
c	3-7	4-5
d	12-16	11-13
e	6-9	7-9
Hooklets		
1	22-24	21-23
2	10-11	9-10
3	15-17	14-16
4	19-20	17-19
5	22-25	21-22
6	20-22	19-21
7	18-20	17-20
Copulatory organ		
TL	-	48-55
cop. tube	44-50	34-45
access. piece	27-35	32-35

It is identical to *Cichlidogyrus quaestio* in that both are the only ones with large, stout first pair of hooklets and nearly similar other structures of the haptor, but the two are easily separated by their copulatory organs. The measurements of the present material when compared with those of the original description (table 3B.18) confirm the identification of these parasites, moreover, it has previously been found only on *P. philander.*

7.4 ***Cichlidogyrus quaestio*** Douëllou, 1993

This parasite was first described from the gills of *Tilapia rendalli* in Lake Kariba, Zimbabwe (Douëllou 1993).

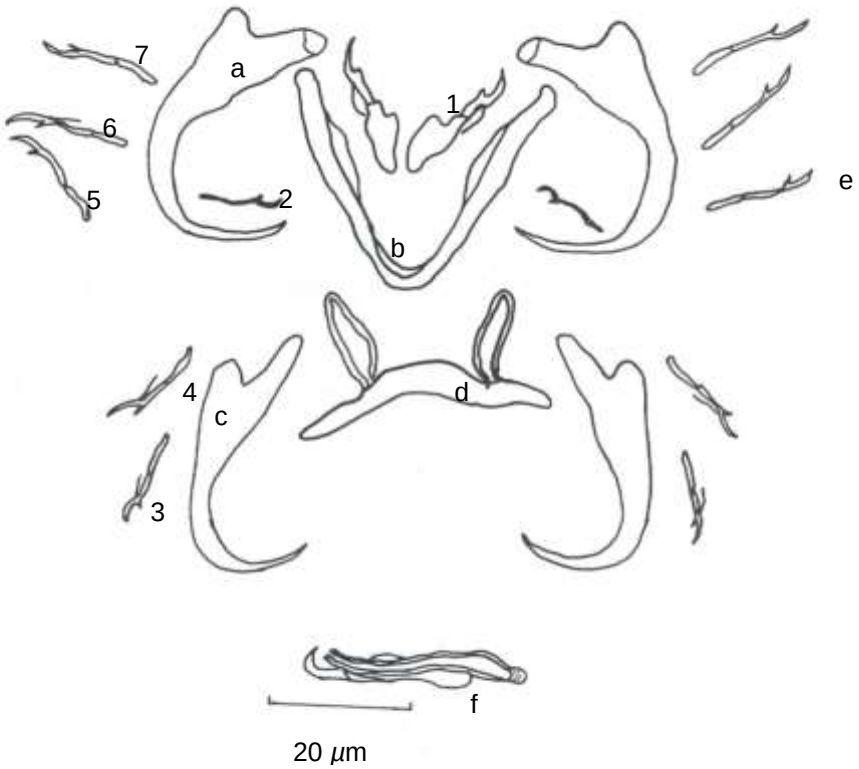


Figure 3B.24 *Cichlidogyrus quaestio* Douëllou, 1993 – Haptoral features & copulatory organ.
a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hooks 1-7
f. copulatory organ

The species occasionally occurred on *Sarotherodon codringtoni* and *S. macrocephalus* in Lake Kariba. The other record (Modise *et al.* 2009) is from the gills of *T. rendalli* in the Okavango Delta, Botswana

Table 3B.19 Measurements (in *µ*m) of *Cichlidogyrus quaestio*

C. quaestio material	Douëllou 1993	Present
Host	*T. rendalli*	*T. rendalli*
Location	gills	gills
No. of specimens	15	10
L	335-690	350-450
W	65-160	80-110
Ventral bar		
V	31-39	30-40
e	2-5	2-5
Dorsal bar		
L1	28-35	35-40
L2	9-17	11-17
d	9-14	12-15
e	5-7	4-7
Ventral anchors		
a	28-39	33-38
b	24-38	30-35
c	3-7	3-5
d	8-12	8-10
e	10-17	13-18
Dorsal anchors		
a	33-44	38-42
b	21-32	28-32
c	4-10	4-5
d	13-21	13-20
e	7-14	9-14
Hooklets		
1	23-31	20-25
2	10-12	9-11
3	16-20	16-20
4	18-22	18-22
5	21-25	19-22
6	21-26	20-22
7	18-22	17-21
Copulatory organ		
TL	-	30-37
cop. tube	27-32	27-33
access. piece	22-27	23-28

According to Douëllou (1993), there is a few other *Cichlidogyrus* spp. with a large first pair of hooklets. However, there has been some confusion with the descriptions of these specimens. *C. quaestio* agrees more closely with *C. brevicirrus* (Paperna & Thurston 1969) and *C. erectus* (Dossou 1982), but they all differ in the copulatory organs and their details.

It is small and the haptor is well separated from the body and is almost round in shape. The haptoral features and the copulatory organs (figure 3B.24) as well as the measurements of the present material (table 3B.19) fit well with those of Douëllou (1993) and agree with the identification of the species.

The parasites were found on the gills of *T. rendalli* and this forms the first record for South Africa.

7.5 ***Cichlidogyrus sclerosus*** Paperna & Thurston, 1969

Paperna & Thurston (1969) described this species for the first time from the gills of *Oreochromis mossambicus* in Uganda, but also found the species from other cichlid fishes. The species was also found in Zimbabwe (Douëllou 1993) and Botswana (Modise *et al.* 2009). There are some records of this species on cichlids outside Africa (Kritsky 1974; Jiménez-García *et al*. 2001; Mendoza-Franco *et al*. 2006).

This parasite is as large as *C. halli* and has two eyes. The ventral and dorsal anchors are robust and identical in size. The copulatory organ is large, long and thin, with arched copulatory tube attached to a large plate. Accessory piece is massive with protruding finger-like extension (figure 3B.25).

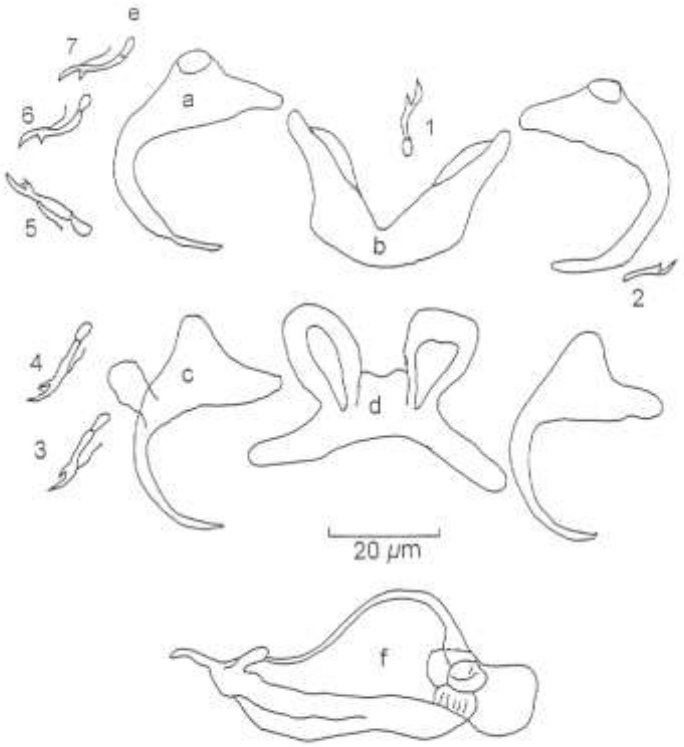


Figure 3B.25 *Cichlidogyrus sclerosus* Paperna & Thurston, 1969 – Haptoral features & copulatory organ. a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hooks 1 - 7 f. copulatory organ

This species is not confused with other *Cichlidogyrus* spp. because of the massive anchors with almost no roots, the solid short bars, the pyriform appendages of the dorsal bar and the copulatory organ, which are all characteristic (Douëllou 1993).

The measurements (table 3B.20) agree in many respects with those of Douëllou (1993) even though these specimens appear to be smaller in body length than those from Lake Kariba. The parasite species was retrieved from the gills of *O. mossambicus* and is the first record for South Africa.

Table 3B.20 Measurements (in μm) of *Cichlidogyrus sclerosus*

C. sclerosus material	Paperna & Thurston 1969	Douëllou 1993	Present
Host	*O. mossambicus*	*O. mortimeri*	*O. mossambicus*
Location	gills	gills	gills
No. of specimens	13	15	10
L	650-700	800-1400	500-740
W	100-200	180-300	250-300
Ventral bar			
V	42-53	31-35	30-38
e	-	3-8	4-8
Dorsal bar			
L1	37-40	31-44	42-50
L2	10-13	13-17	11-20
d	-	10-13	-
e	-	7-10	7-13
Ventral anchors			
a	29-37	33-36	30-35
b	-	32-36	30-35
c	-	3-8	1-5
d	-	9-14	5-10
e	-	12-15	8-13
Dorsal anchors			
a	26-27	32-35	28-35
b	-	31-34	26-32
c	-	4-9	1-5
d	-	9-13	3-8
e	-	9-13	8-12
Hooklets			
1	6-7	13-17	10-14
2	6-7	12-14	11-13
3	10-14 (pairs 3-7)	15-19	17-18
4		17-20	17-18
5		16-20	17-18
6		14-18	12-16
7		14-18	12-16
Copulatory organ			
TL	-	66-83	63-70
cop. tube	50-60	61-75	58-65
access. piece	39-50	49-62	43-56

7.6 ***Cichlidogyrus tilapiae*** Paperna, 1960

The original description of this species (Paperna 1960) was from the gills of *Oreochromis niloticus* in Israel. African records on the species are from various cichlid fishes in Ghana, Uganda, Tanzania, Egypt and Zimbabwe (Paperna 1965, 1968, 1969, 1979; Paperna & Thurston 1969; Thurston 1970; Ergens 1981; Douëllou 1993). There are other records outside Africa (Mendoza-Franco *et al*. 2006).

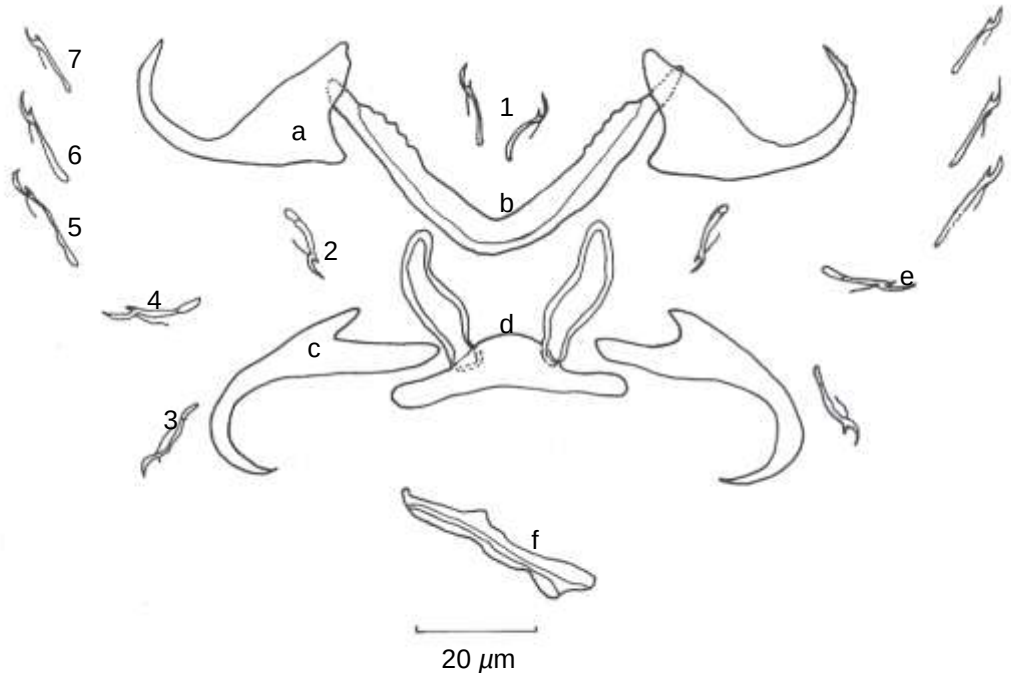


Figure 3B.26 *Cichlidogyrus tilapiae* Paperna, 1960 – Haptoral features & copulatory organ. a. ventral anchor b. ventral bar **C**. dorsal anchor d. dorsal bar e. marginal hooks 1-7 f. copulatory organ

They are very small with only two eyes and a haptor that is hardly separated from the body. The copulatory organ is used to identify the species. It is short and simple with a straight copulatory tube that is wider at the base. The accessory piece is straight with a swelling at about ⅔ its length and ends with a sharp hook (figure 3B.26). The measurements of specimens found (table 3B.21) agree mostly with those of Douëllou (1993).

Table 3B.21 Measurements (in *µ*m) of *Cichlidogyrus tilapiae*

C. tilapiae material	Paperna 1960	Douëllou 1993	Present
Host	*T. nilotica* etc.	*O. mortimeri*	*O. mossambicus*
Location	gills	gills	gills
No. of specimens	-	15	10
L	160-509	400-500	340-590
W	30-142	90-120	120-170
Ventral bar			
V	34-98	31-33	25-40
e	-	3-5	3-6
Dorsal bar			
L1	18-38	28-30	25-43
L2	9-19	13-17	18-22
d	-	12-16	10-15
e	-	5-6	4-8
Ventral anchors			
a	26-33	32-36	35-42
b	18-26	29-31	28-32
c	4-7	3-5	3-5
d	18	10-14	8-15
e	7	9-12	10-13
Dorsal anchors			
a	26-40	41-44	35-45
b	18-26	27-30	28-33
c	4-7	3-5	3-6
d	11-15	16-19	15-20
e	7-10	8-11	10-13
Hooklets			
1	7-17	13-14	12-14
2	13-17	9-12	9-11
3	13-20	13-17	15-17
4	13-20	16-17	16-18
5	11-15	16-19	16-19
6	15	17-18	15-17
7	21	14-16	14-16
Copulatory organ			
TL	26-48	-	32-42
cop. tube	-	30-36	30-37
access. piece	-	31-33	28-35

In this investigation the species was found on the gills of *O. mossambicus* and is the first record for South Africa.

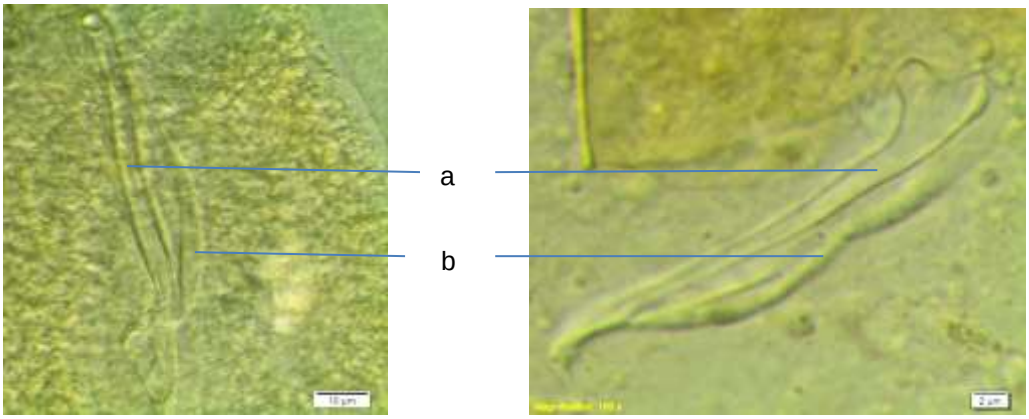
In considering the measurements of the six species of the genus (table 3B.22), *Cichlidogyrus halli* and *Cichlidogyrus sclerosus* are far larger in size than the rest and the same has been observed by Douëllou (1993). Again, the hooklet pair 2 is the shortest followed by pair 1 in the species present in Lake Tzaneen, except in *Cichlidogyrus quaestio* and *Cichlidogyrus philander* where pair 1 is stout and large. The copulatory organs of the six species are also compared (figure 3B.27) as these are important diagnostic features within the genus.

In terms of inter-specific associations *C. dossoui* was found to co-occur with *C. quaestio* on the gills of *T. rendalli* in some instances. However, no occurrences of these two species with *C. halli* could be found. On the other hand, on *O. mossambicus C. halli* co-occurred with *C. sclerosus* and *C. tilapiae*. No co-occurrence *of C. halli* with *C.dossoui* was found as only one specimen of *C dossoui* was retrieved on *O. mossambicus*. It can however be confirmed that the latter two species (*C. halli* and *C. dossoui*) do co-occur on *O. mossambicus* as was found in Flag Boshielo Dam, Limpopo (Madanire-Moyo *et.al.* 2011).

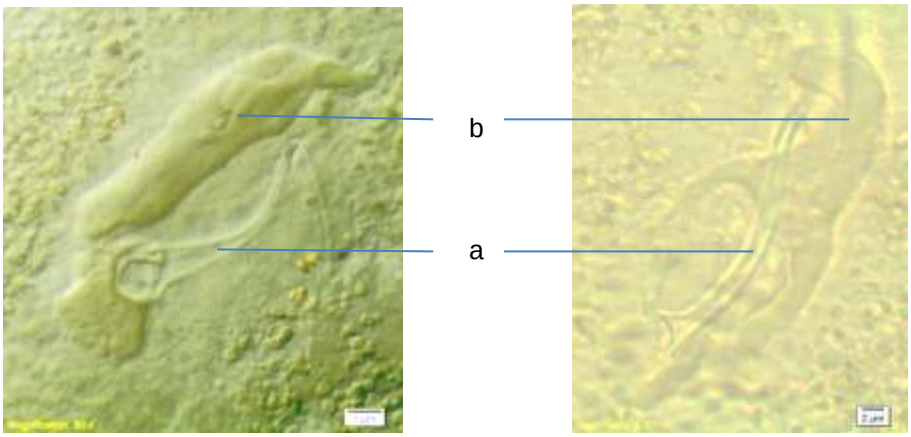
The micrographs of the sclerotised parts belonging to species of the genus *Cichlidogyrus* are shown in Plates 15, 16 & 17.

Table 3B.22 Measurements (in μm) of six *Cichlidogyrus* spp. found in Lake Tzaneen

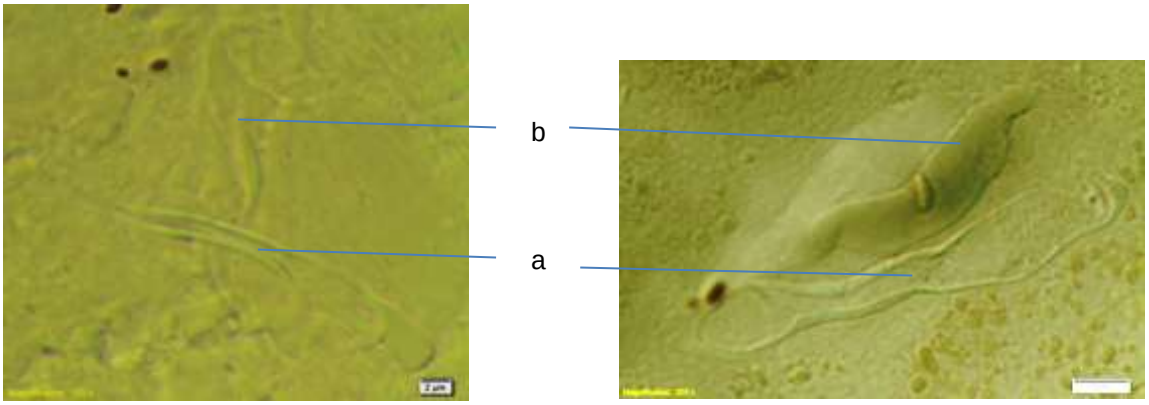
Parasite		*C. halli*	*C. sclero*	*C. dosso*	*C. tilap*	*C. quaes*	*C. phila*
Host		*O. moss & T. rend*	*O. moss*	*O. moss & T. rend*	*O. moss*	*T. rend*	*P. phila*
Location		gills	gills	gills	gills	gills	gills
No of specimens		10	10	10	10	10	12
L		570-770	500-740	420-590	340-590	350-450	260-390
W		260-380	250-300	110-190	120-170	80-110	70-90
Ventral bar	V	62-85	30-38	34-45	25-40	30-40	23-30
	e	8-10	4-8	2-5	3-6	2-5	2-4
Dorsal bar	L1	55-80	42-50	38-58	25-43	35-40	25-32
	L2	20-30	11-20	15-22	18-22	11-17	10-12
	d	16-24	-	9-15	10-15	12-15	7-12
	e	10-18	7-13	4-8	4-8	4-7	3-5
V. anchors	a	50-58	30-35	31-42	35-42	33-38	28-34
	b	38-45	30-35	27-37	28-32	30-35	25-28
	c	6-10	1-5	6-10	3-5	3-5	4-6
	d	15-20	5-10	10-15	8-15	8-10	7-12
	e	10-16	8-13	11-15	10-13	13-18	10-12
D. anchors	a	48-55	28-35	25-32	35-45	38-42	31-35
	b	30-38	26-32	20-28	28-33	28-32	21-23
	c	6-10	1-5	5-8	3-6	4-5	4-5
	d	20-27	3-8	10-14	15-20	13-20	11-13
	e	10-13	8-12	9-13	10-13	9-14	7-9
Hooklets	1	15-19	10-14	15-22	12-14	20-25	21-23
	2	13-16	11-13	13-17	9-11	9-11	9-10
	3	28-31	17-18	35-49	15-17	16-20	14-16
	4	28-37	17-18	35-50	16-18	18-22	17-19
	5	29-38	17-18	40-56	16-19	19-22	21-22
	6	29-34	12-16	42-51	15-17	20-22	19-21
	7	27-33	12-16	37-46	14-16	17-21	17-20
Cop. organ	TL	83-96	63-70	50-60	32-42	30-37	48-55
	ct	68-82	58-65	62-68	30-37	27-33	34-45
	ap	62-72	43-56	45-52	28-35	23-28	32-35



Cichlidogyrus halli *Cichlidogyrus tilapiae*



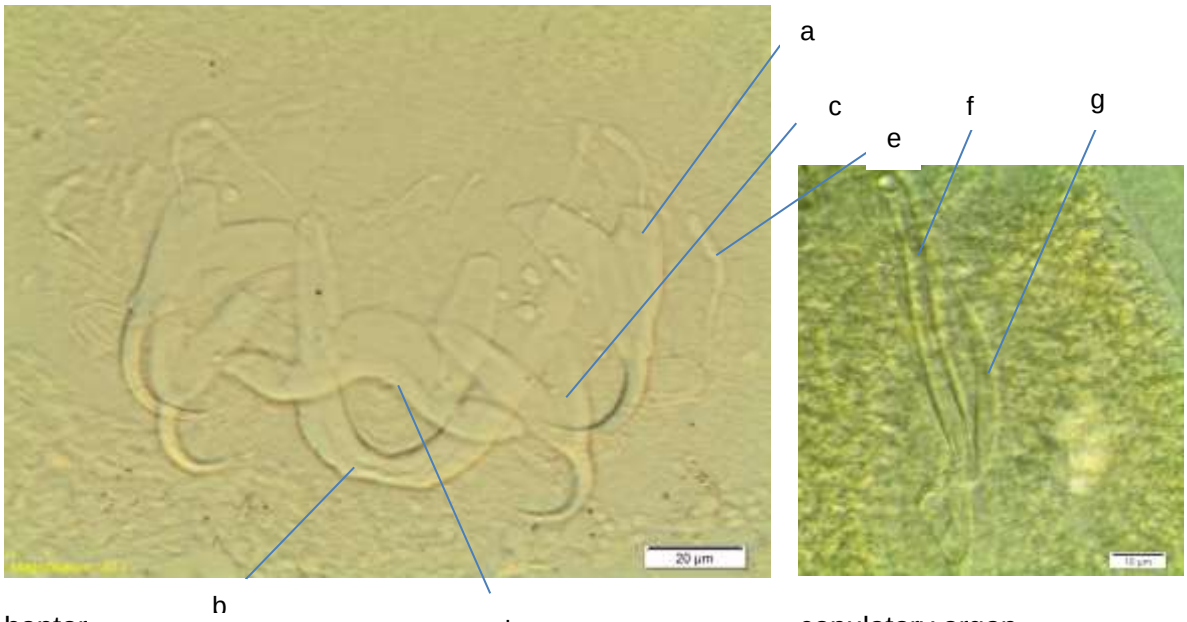
Cichlidogyrus sclerosus *Cichlidogyrus dossoui*



Cichlidogyrus quaestio *Cichlidogyrus philander*

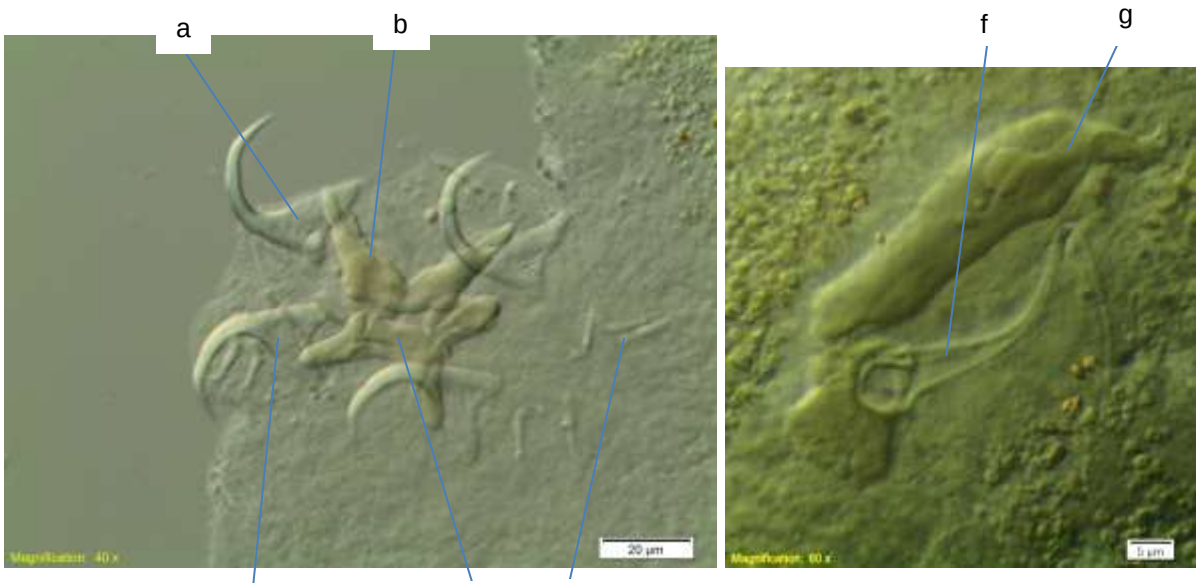
a. cirrus b. accessory piece

Figure 3B.27 The copulatory organs of the six species of *Cichlidogyrus* found in Lake Tzaneen



haptor copulatory organ

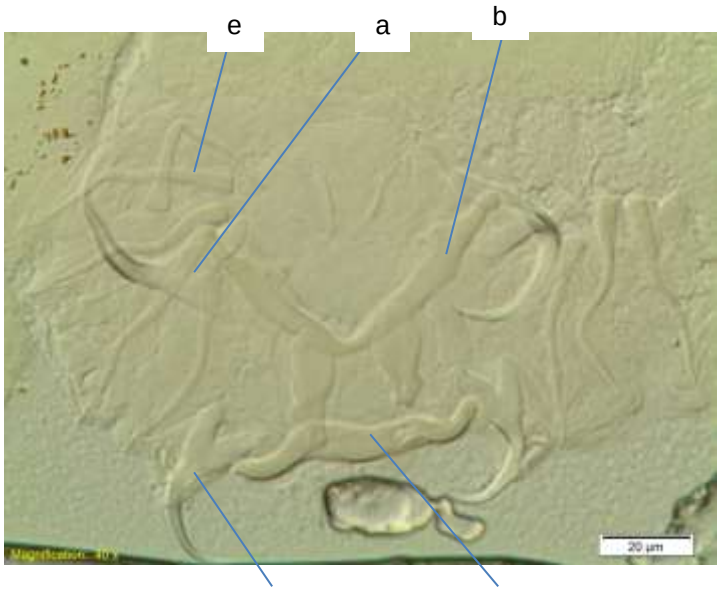
Cichlidogyrus halli - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. cirrus g. accessory piece



haptor copulatory organ

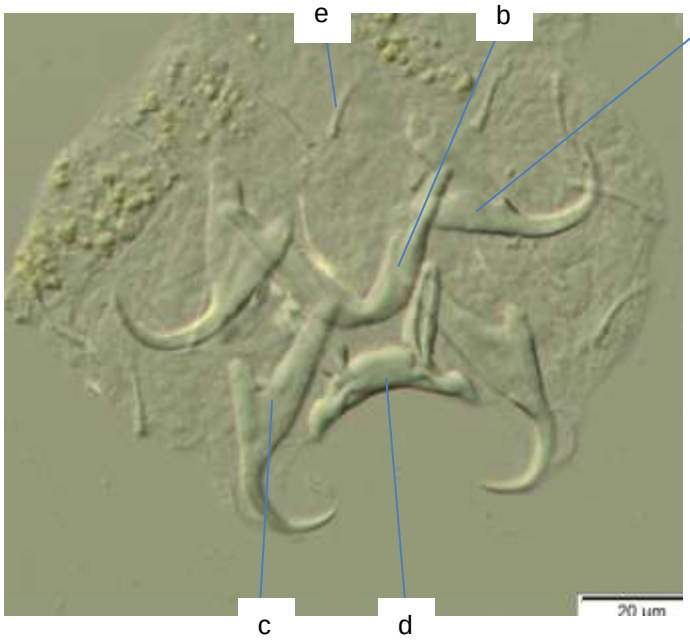
Cichlidogyrus sclerosus - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. cirrus g. accessory piece

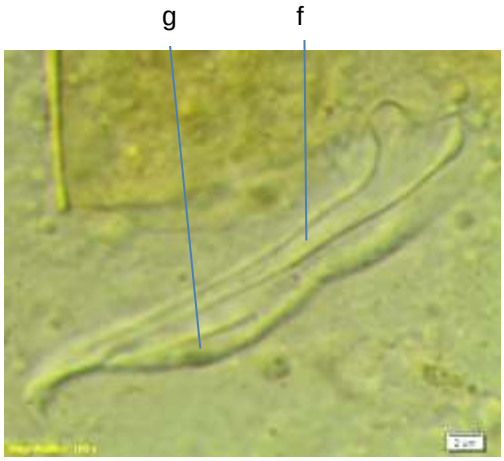
Plate 15 *Cichlidogyrus halli & Cichlidogyrus sclerosus*



haptor copulatory organ

Cichlidogyrus dossoui - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. cirrus g. accessory piece

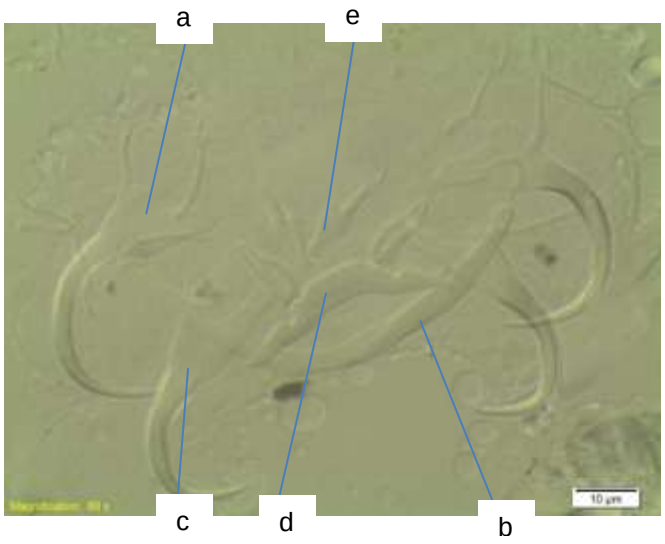


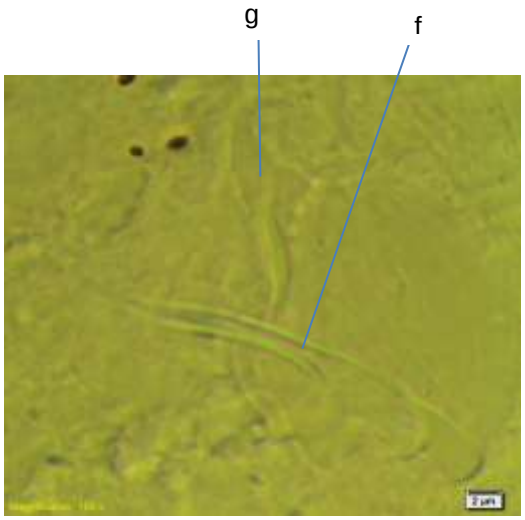


haptor copulatory organ

Cichlidogyrus tilapiae - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. cirrus g. accessory piece

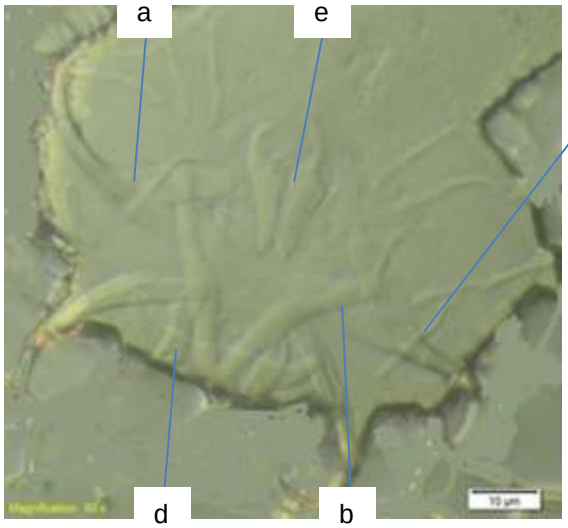
Plate 16 *Cichlidogyrus dossoui* & *Cichlidogyrus tilapiae*

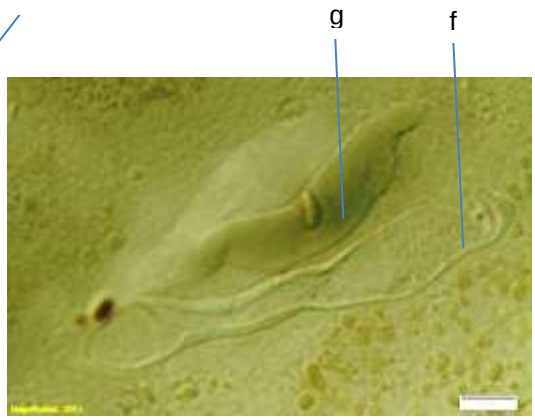




haptor copulatory organ

Cichlidogyrus quaestio - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. cirrus g. accessory piece





haptor copulatory organ

Cichlidogyrus philander - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hook f. cirrus g. accessory piece

Plate 17 *Cichlidogyrus quaestio & Cichlidogyrus philander*

8 Genus ***Scutogyrus*** Pariselle & Euzet, 1995

The genus *Scutogyrus* is relatively new and is distinguishable by a dorsal transverse bar enlarged laterally with, in its median portion, 2 very long auricles hollow at their base and by the ventral transverse bar arched, rigid, and supporting 1 large, thin, oval plate. There are two pairs of anchors (1 dorsal and 1 ventral) and 14 hooklets (Pariselle & Euzet 1995b). This genus is restricted to cichlid fishes.

Few members of the genus *Cichlidogyrus* (*C. longicornis minus* Dossou, 1982; *C. l. longicornis* and *C. l. gravivaginus* Paperna & Thurston, 1969) were removed and designated to the genus *Scutogyrus* with more species added to it (Pariselle & Euzet, 1995b). Using morphology of haptoral sclerites it was more suitable for Pouyaud *et al*. (2006) to infer phylogenetic relationships than using the genitalia that seemed more useful to resolve species-level identifications. Together with the usage of genetic data, Pouyaud *et al*. (2006) confirmed the validity of the genus *Scutogyrus* whilst Wu *et al*. (2007) questioned this status and suggested it be treated as *Cichlidogyrus*.

There are only six known species of the genus *Scutogyrus* and these are found restricted to fish hosts from *Oreochromis* and *Sarotherodon* (Pariselle & Euzet 1995b; Khalil & Polling 1997). Pariselle & Euzet (1995b) provided a key to their diagnoses. In this study, only one species of the genus (*S. gravivaginus*) was found.

8.1 ***Scutogyrus gravivaginus*** (Paperna & Thurston, 1969)

This parasite was first described as *Cichlidogyrus longicornis gravivaginus* by Paperna & Thurston (1969) from the gills of *Oreochromis leucosticus* in Uganda. It was described together with the other subspecies *Cichlidogyrus longicornis longicornis* from the gills of *Oreochromis niloticus niloticus.* The elevation to the species status as *C. gravivaginus* and *C. longicornis* was proposed by Douëllou (1993) in his redescription using specimens from the gills of *Oreochromis mortimeri* in Lake Kariba, Zimbabwe.

The other African records found are those of S*. longicornis* (Paperna 1968, 1969; Ergens 1981; Dossou 1982; Boungou *et al*. 2008). Douëllou (1993) and the present writer doubt all these, except the last, in their species identification either due to unclear descriptions of some parts or even their measurements.

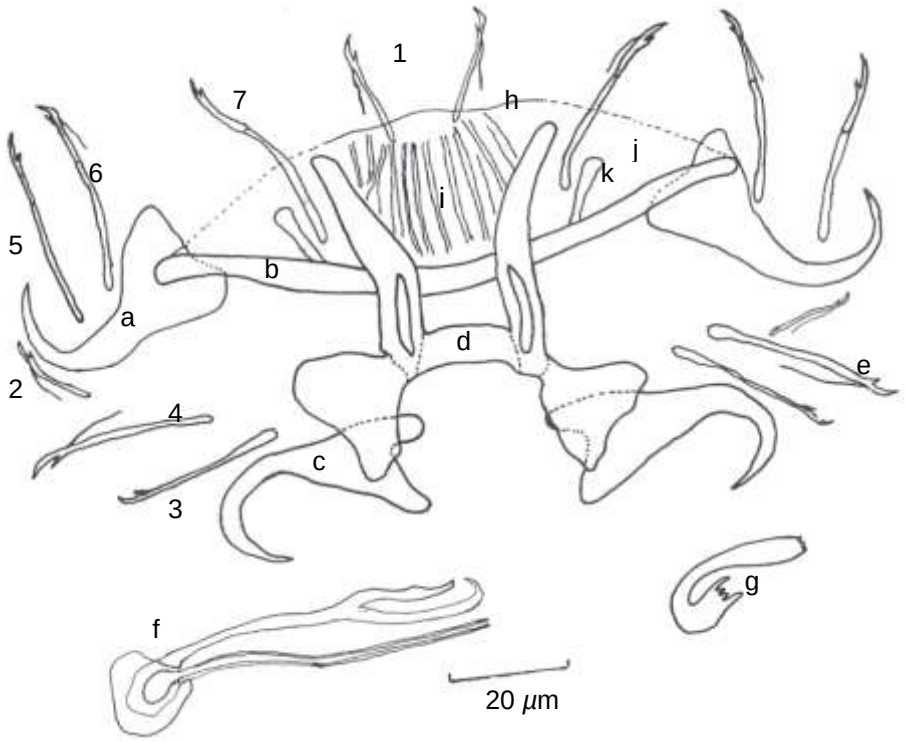


Figure 3B.28 *Scutogyrus gravivaginus* (Paperna & Thurston, 1969) – Haptoral features & copulatory organ. a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hooks 1 – 7 f. copulatory organ g. vagina h. thin bar i. ribbed portion j. membraneous portion k. process

Paperna & Thurston (1969) divided *S. longicornis* and *S. gravivaginus* based on the size of the parasite, the number of eyes, the sclerotization of the structure associated with the ventral bar and different morphology of the copulatory organs and the vaginas. This, together with other rediscriptions created problems as some drawings of reproductive organs were given with no descriptions or with incorrect measurements (Douëllou 1993).

Table 3B.23 Measurements (in μm) of *Scutogyrus gravivaginus*

S. gravivaginus	Paperna & Thurston 1969	Douëllou 1993	Present
Host	*Oreochromis leucosticus*	*O. mortimeri*	*O. mossambicus*
Location	gills	gills	gills
No. of specimens	10	15	1
L	600-700	530-856	720
W	100-120	90-150	180
Ventral bar			
V	66-79	36-45	50
e	-	3-5	4
Dorsal bar			
L1	66-79	60-67	63
L2	39-47	39-45	38
d	-	13-16	18
e	-	4-8	6
Ventral anchors			
a	33-34	31-36	35
b	-	29-34	33
c	-	3-7	5
d	-	7-12	10
e	-	10-15	12
Dorsal anchors			
a	33-37	31-36	32
b	-	25-30	27
c	-	7-11	9
d	-	8-13	12
e	-	7-12	10
Hooklets			
1	-	15-18	16
2	11-20	12-14	14
3	29-30	24-28	28
4	(pairs 3 to 7)	25-29	30
5		28-34	30
6		29-33	35
7		27-31	35
Copulatory organ			
TL	53-57	73-83	74
Vagina			
L	-	36-50	36
W	-	18-23	9

The confusion to separate the two species has been simplified by the attempts of Douëllou (1993) and is based on the structure of the reproductive organs. *Scutogyrus gravivaginus* has a larger copulatory organ with a basal portion and a heavily sclerotized vagina with a rounded part and an elongated part ending with

three finger-like extensions (figure 3B.28). *S. longicornis* has a shorter copulatory organ with no basal portion and the vagina is tube-like and flared distally. The morphology (figure 3B.28) and measurements (table 3B.23) of the specimens compared more favourably with those of *S. gravivaginus* than *S. longicornis.* The micrograph of *S. gravivaginus* is shown in plate 14.

9 Genus ***Actinocleidus*** Mueller, 1937

Sproston (1946) and Hoffman (1967) placed this genus under the subfamily Tetraonchinae whilst Bychowsky (1957) preferred the Ancyrocephalinae. There are two pairs of anchors, 14 marginal hooklets, bifurcate gut, two pairs of eyes with the second pair larger and the testis and ovary are unlobed. The generic diagnosis (Sproston 1946; Hoffman 1967) is based on two pairs of anchors that are ventral, approximately uniform in size and shape, two mid-articulated dissimilar bars, and the cirrus has a movable accessory piece articulated to its base. The ventrally positioned anchors were referred to as anterior and posterior pairs (Sproston 1946; Hoffman 1967) but are now, as usual practice, and due to their positions relative to each other, referred to as ventral and dorsal pairs respectively (Price 1967; Beverly-Burton 1986). There are no African records on this genus.

9.1 ***Actinocleidus fusiformis*** (Mueller, 1934)

In its first description, this parasite was found on the gills of *Micropterus dolomieu* and was named *Cleidodiscus fusiformis* by Mueller (1934). It was re-described by Mueller (1937) from *Micropterus salmoides* under its present genus. Some authors have in the past placed it under the genus *Syncleithrium* (Price 1967; Beverly-Burton 1986) because the dorsal bar is a fused mass (figure 3B.29). These descriptions and many other records of this species were from the USA (Meade & Bedinger 1972). With the introduction of the species of the fish genus *Micropterus* in other countries, many reports on their parasites also became available (Bunkley-Williams & Williams 1994; Aloo 1999).

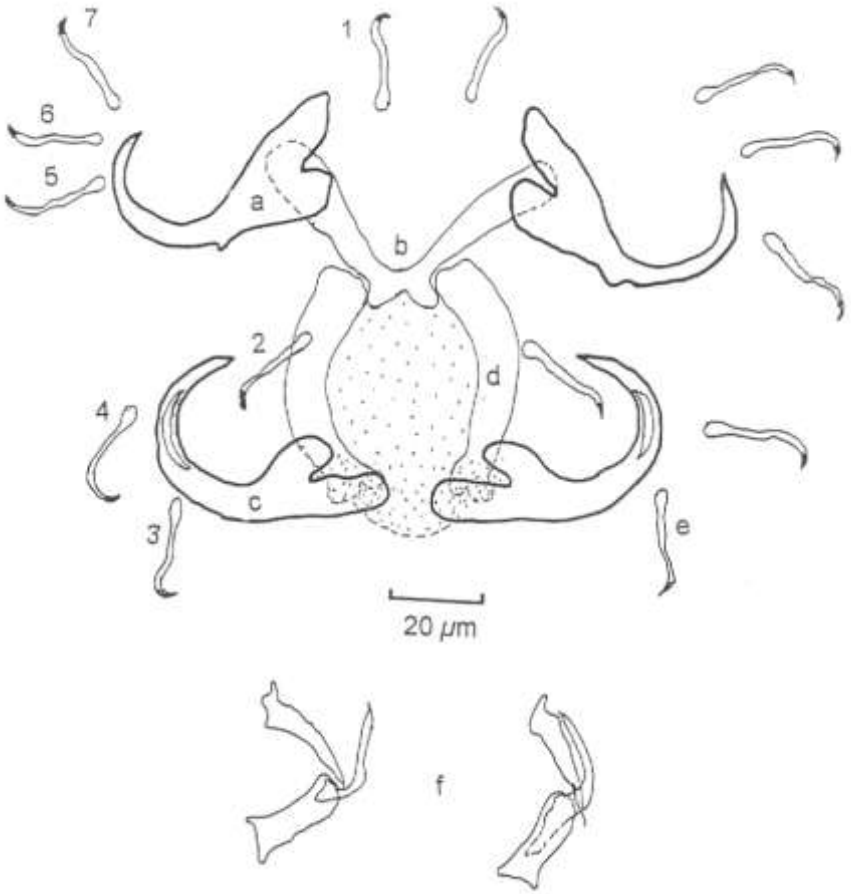


Figure 3B.29 *Actinocleidus fusiformis* (Mueller, 1934) – Haptoral features & copulatory organ. a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. marginal hooks 1-7 f. copulatory organ

This species is only host specific to *Micropterus* spp. in the USA, and was found only in *M. salmoides* in Puerto Rico (Bunkley-Williams & Williams 1994). Most species of this genus are hosted mainly by fish species of the genus *Lepomis*, with few other fish genera as well. The species differs from others of the genus in that the upper ("dorsal") bar is broad and fan-shaped (*A. gracilis* has both bars narrow and notched at ends), and the anterior anchors are hollow whilst the posterior ones are solid.

The morphology (figure 3B.29) and measurements (table 3B.24) are compared with those obtained by Beverly-Burton (1986) and Bunkley-Williams & Williams (1994) to support its identification as *A. fusiformis*.

Table 3B.24 Measurements (in μm) of *Actinocleidus fusiformis*

A. fusiformis	Original (Mueller 1934)	Beverly-Burton 1986	Present
Host	*Micropterus* spp.	-	*Micropterus salmoides*
Location	gills	gills	gills
No. of specimens	-	13	30
Body			
length	550-850	283-717	570-630
width	x-170	83-183	180-280
Haptor			
length	-	67-93	65-90
width	96	73-110	120-155
Dorsal anchors			
length	44	34-45	52-60
base width	13	-	-
Dorsal bar length	-	30-36	35-47
Ventral anchors			
length	-	35-45	50-63
Ventral bar length	-	36-57	38-50
Marginal hooks	-	17-21	15-22
Corpulatory organ			
length	-	48-70	38-53
acc. piece length	-	29-46	25-37
Pharynx	65	-	55-65

In this study, the parasites are from the gills of *Micropterus salmoides*. This is a new geographical record for Africa. The parasites (though very few in number) were found to share the gills with *Haplocleidus furcatus* in this study as in many others (Mueller 1937; Bunkley-Williams & Williams 1994).

10 *Genus **Haplocleidus** Mueller, 1937*

Confusion existed in the taxonomy of the sub-family Tetraonchinae to which this genus belongs. Several species were originally described in different genera hence some controversy over the synonymy involved (Sproston 1946; Hoffman 1967). The key to the genera that includes all such genera is useful as an aid to identification (Hoffman 1967) and avoids this controversy over generic synonymy. The generic diagnosis is based on the haptor with 2 separate non-articulated transverse bars, the accessory piece never basally articulated with the cirrus, vagina if present on right margin, and the anchors are similar in shape but markedly dissimilar in size.

10.1 ***Haplocleidus furcatus*** Mueller, 1937

This parasite was first described from *Micropterus salmoides* in Florida, USA (Mueller 1937). Confusion arose with some of its later re-descriptions (Mizelle & Hughes 1938) as *Urocleidus furcatus*. It has since been found hosted by several species of *Micropterus* and *Lepomis* in the USA and translocated elsewhere with the introduction of these fishes (Bunkley-Williams & Williams 1994).

The differential diagnosis is based on the accessory piece of the corpulatory organ that is Y-shaped and the associated cirrus is straight (Bunkley-Williams & Williams 1994). The morphology (figure 3B.30) and measurements (table 3B.25) are compared with those of Mueller (1937) and Mizelle (1940) to support its identification as *H. furcatus.*

In this study, the parasites were procured from the gills of *M. salmoides*. This is a new geographical record for Africa. The parasites were found to share the gills with *Actinocleidus fusiformis*.

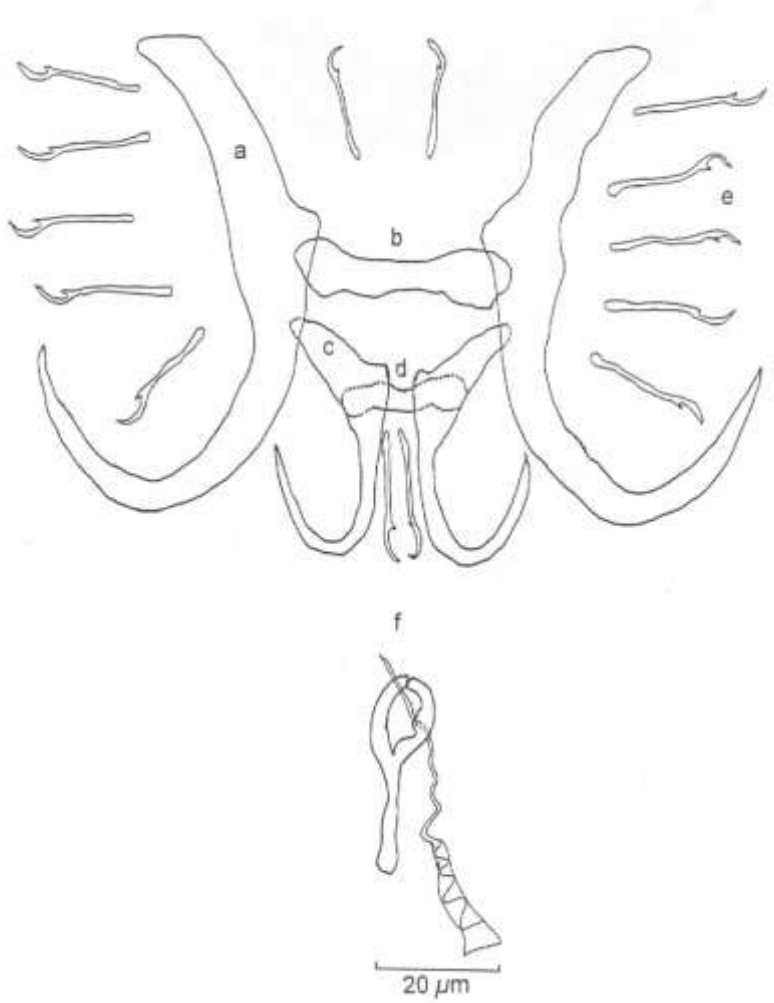


Figure 3B.30 *Haplocleidus furcatus* Mueller, 1937 – Haptoral features & copulatory organ.
a. dorsal anchor b. dorsal bar c. ventral anchor d. ventral bar e. marginal hooks f. copulatory organ

Table 3B.25 Measurements (in μm) of *Haplocleidus furcatus*

H. furcatus material	Original Mueller 1937	Mizelle 1940	Present
Host	*M. salmoides*	*M. salmoides*	*M. salmoides*
Location	gills	gills	gills
No. of specimens	-	10	30
Body			
length	-	238-495	360-600
width	-	81-135	140-170
Haptor			
length	-	58-82	40-90
width	-	68-86	75-140
Ventral anchors			
length	35	25-38	35-40
width	-	11-18	-
Ventral bar			
length	-	25-32	23-28
Dorsal anchors			
length	69	43-81	75
width	-	14-16	-
Dorsal bar			
length	-	20-37	28-35
Marginal hooks	-	-	18-22
Corpulatory organ			
cirrus length	49	62-66	53-55
accessory piece	23	20-38	30-35

11 Genus ***Acolpenteron*** Fischthal & Allison, 1940

The preliminary description of the genus and species first appeared in an abstract (Fischthal & Allison 1940) and a complete description followed a year later (Fischthal & Allison 1941). This was the first record of a monogenean from the ureters and urinary bladders of fishes. The generic diagnosis is based on the absence of anchors, eyes and head lappets; haptor cup-like, with 14 marginal hooklets; sensory hairs present; testis single, ovary median, vagina ventral and near median of the body; intestinal crura joined posteriorly, without diverticular.

There are seven species of the genus that have been described thus far from a wide variety of hosts that include centrarchids, catostomids and balitorids (all three from the northern hemisphere), with only *A. australe* from the percichthyid fishes in the southern hemisphere (Viozzi & Brugni 2003).

11.1 ***Acolpenteron ureteroecetes*** Fischthal & Allison, 1940

This parasite was described from largemouth bass (*Micropterus salmoides*) in USA (Fischthal & Allison 1940). The species occurs in other *Micropterus* spp. as well. With the introduction of centrarchid fishes, *A. ureteroecetes* has been found in other countries (Bunkley-Williams & Williams 1994). Petrie-Hanson (2001) discussed the mortality of aquaculture stocks due to extreme pathology.

The differential diagnosis is based on the presence of a forked accessory piece (absent in *A. australe*); overlapping gonads (absent in *A. nephriticum*) and have an unspined male corpulatory organ base (spined in *A. catostomi*) (Viozzi & Brugni 2003). The morphological features (figure 3B.31) and measurements (table 3B.26) of specimens collected were compared with those of Fischthal & Allison (1941).

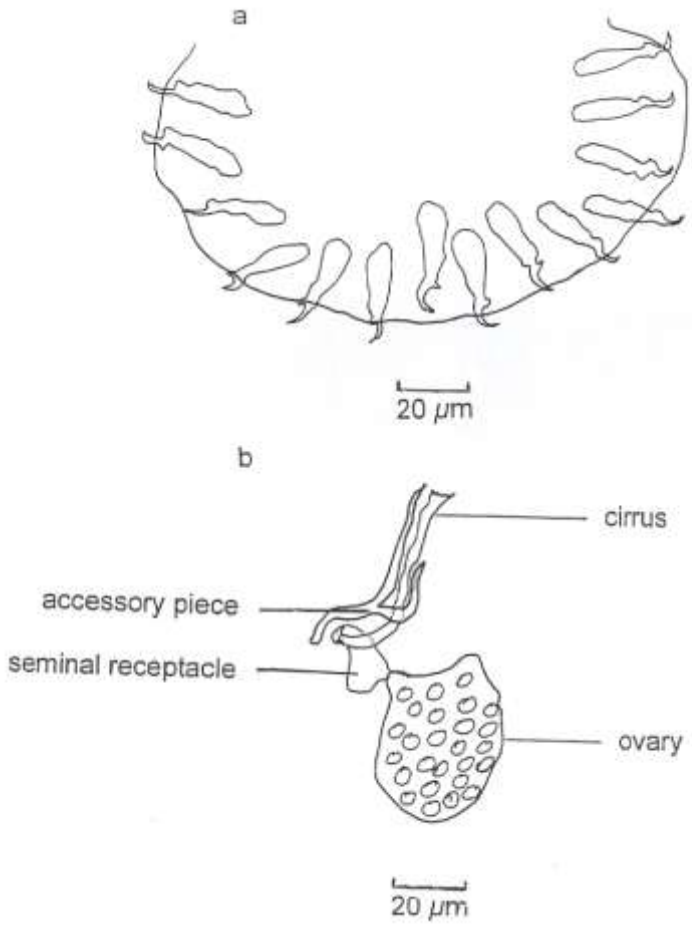


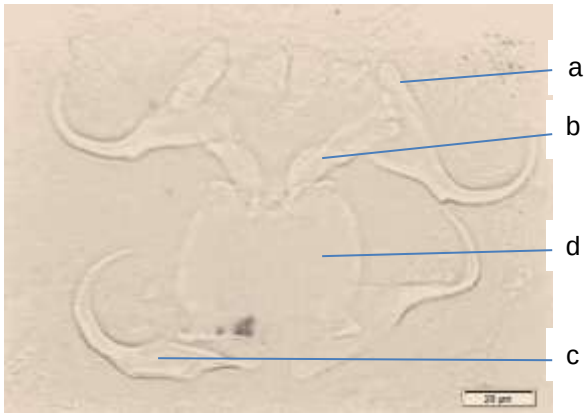
Figure 3B.31 *Acolpenteron ureteroecetes* Fischthal & Allison, 1940 – Haptoral features & reproductive system. a. haptor showing 14 marginal hooklets b. copulatory organ

The South African record of this species is the result of a direct translocation of infected *M. salmoides* host specimens from USA into a local hatchery (Du Plessis 1948). In this study, the parasites were collected from the ureter-urinary bladder complex in *M. salmoides*. This is the first record of *A. ureteroecetes* in African inland waters.

Table 3B.26 Measurements (in *μ*m) of *Acolpenteron ureteroecetes*

A. ureteroecetes material	Fischthal & Allison, 1941	Present
Number of specimens	-	3
Body		
length	931	750 - 1070
width	105	140 - 230
Haptor		
length	57	70
width	96	110 - 120
Marginal hooks	23	25 - 30
Testis		
length	53	-
width	16	-
cirrus	45	50
ovary		
length	62	44
width	38	35
Pharynx diameter	47	-

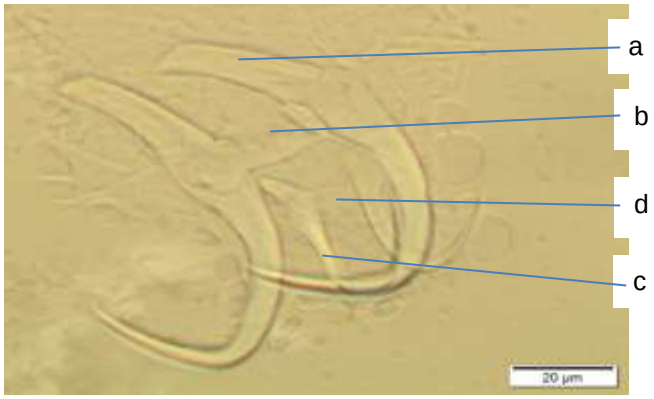
The micrographs of the sclerotised parts belonging to *Actinocleidus fusiformis, Haplocleidus furcatus* and *Acolpenteron ureteroecetes* are shown in Plate 18.



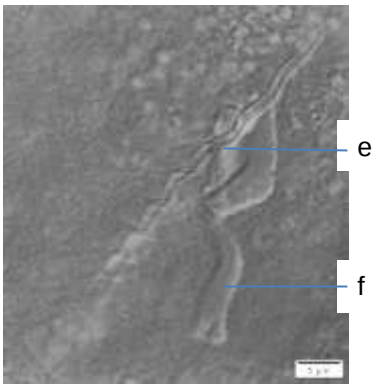
haptor

copulatory organ

Actinocleidus fusiformis - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. cirrus f. accessory piece

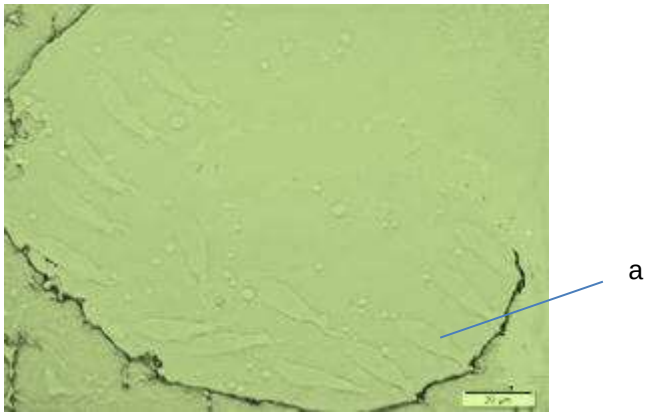


haptor

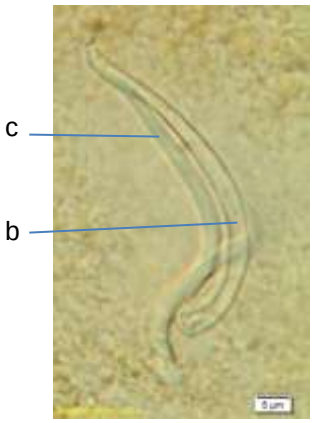


copulatory organ

Haplocleidus furcatus - a. ventral anchor b. ventral bar c. dorsal anchor d. dorsal bar e. cirrus f. accessory piece



haptor



copulatory organ

Acolpenteron ureteroecetes – a. marginal hooklets b. cirrus c. accessory piece

Plate 18 *Actinocleidus fusiformis, Haplocleidus furcatus & Acolpenteron ureteroecetes*

3B.4 GENERAL DISCUSSION

In previous parts of this section, a detailed literature review on each Monogenea species present in Lake Tzaneen was provided and the following observations were made: Little research was done in Southern Africa on monogenean parasites of freshwater fish. The only notable studies in this area are those of Mashego (1982, 1983) on *Dactylogyrus*, Douellou (1993) on *Cichlidogyrus*, and Khalil & Mashego (1998) on *Macrogyrodactylus*. Other Southern African reports were on taxonomy of single to two monogenean species. Most African studies concentrated on West African countries.

Because of many controversies due to doubtful species descriptions, the checklists, catalogues and updates (Paperna 1979; Kritsky & Kulo 1988; Gibson *et al.* 1996; Khalil & Polling 1997; Lim *et al*. 2001; Harris *et al.* 2004; Pariselle & Euzet 2009) came in handy as very useful sources, not only to clarify on the status of numerous species, but also to indicate studies done previously. More such works are encouraged, especially with large genera. As more species are being described it is recommended that genetic studies be carried out to confirm cases of dubious nature. In the long run, the genetic information on the parasites and their hosts will help solve problems pertaining to identification, evolution and ecology of parasites and their fish hosts.

There are new geographical records for Africa in this study and these are *Actinocleidus fusiformis, Haplocleidus furcatus* and *Acolpenteron ureterocoetes.* Many species were also recorded for the first time in Southern Africa and these are *Gyrodactylus rysavyi*, *Dactylogyrus brevicirrus*, *Dactylogyrus cyclocirrus*, *Dogielius dublicornis*, *Dogielius* sp., and *Schilbetrema quadricornis.* First finds for South Africa (already found in Zimbabwe and Botswana) are *Quadriacanthus aegypticus*, *Quadriacanthus clariadis*, *Cichlidogyrus dossoui*, *Cichlidogyrus halli*, *Cichlidogyrus quaestio*, *Cichlidogyrus sclerosus*, *Cichlidogyrus tilapiae* and *Scutogyrus gravivaginus*. *Cichlidogyrus dossoui* was found for the first time in *Oreochromis mossambicus* whilst *Gyrodactylus rysavyi* has the gills as its new site record.

The taxonomical account of individual species of Monogenea of fishes in Lake Tzaneen is also provided. The drawings and micrographs were used to aid in identification. The measurements were also taken and these were compared with those of their counterparts to confirm their status. This study has contributed four new species of monogenean parasites belonging to the genus *Dactylogyrus* that were retrieved from fish hosts never investigated prior to this study. These are *Dactylogyrus* sp. 1 from *Barbus radiatus*, *Dactylogyrus* sp. 2 from *Barbus unitaeniatus*, *Dactylogyrus* sp. 3 and *Dactylogyrus* sp. 4 from *Labeo molybdinus*.

Taking into consideration that there are 27 Monogenea species found at Lake Tzaneen, this figure indicates the significance of Monogenea parasites on fishes of this lake. Again, many hosts stayed longer or had to be preserved before examination and thus many monogeneans could have been missed due to accumulation of mucus on the gills or degeneration of specimens after thawing. Some specimens were lost during mounting and could thus not be identified to the species level.

CHAPTER 3

SECTION C

DIGENEA

“Sight for Site” - Eyes for some digenean larvae

CONTENTS

3C.1 INTRODUCTION

Southern African studies on digenea include those of Monnig (1926), Du Bois (1930, 1931, 1970), Ortlepp (1935), Lombard (1968), Beverly-Burton (1962, 1963), Prudhoe & Hussey (1977), Van As & Basson (1984), Mashego (1977, 1982), Britz (1983), Batra (1984), Britz *et al*. (1984a & b, 1985), Boomker (1984), Kabunda & Sommerville (1984), Mashego & Saayman (1989), Earlwanger (1991), Mashego *et al.* (1991), Douellou & Earlwanger (1993), Grobler *et al*. (1999), Luus-Powell (2004) and Ramollo *et al.* (2006). Paperna (1996) and Khalil & Polling (1997) are consolidated works that include Digenea.

According to Paperna (1996), Trematodes or Digenea are flatworms (Platyhelminthes), heteroxenous (with a multiple host life cycle) and require a mollusc as their first intermediate host. Adult-stage digeneans usually have a dorso-ventrally flattened, oval body with a smooth, spiny or corrugated surface, a sucker around the antero-ventral mouth, and an additional ventral sucker or acetabulum. Both suckers are used for attachment and locomotion. The digestive system consists of a pharynx connected to the mouth opening, a short oesophagus and two blind intestinal caeca. Most trematodes are hermaphrodite, containing both male organs (testes, ducts and copulatory system) and female organs (ovary, vitelline glands, ducts and uterus).

Eggs are evacuated through the genital opening, and are usually oval and operculated. Epizootiological studies indicate that all trematodes are host specific and transmission may, at most, involve species of the same or very close genera. The presence of suitable vector snails in the habitat is essential for transmission. Most freshwater and estuarine fish are potential hosts, but juvenile fish, bottom dwellers and shallow water inhabitants are most vulnerable. According to Paperna (1996), metacercarial infections were found in fish in all inland water bodies he studied in Africa. Piscivorous birds are the definitive hosts for many of the metacercariae found in fish.

Differential diagnosis is difficult and requires experience with trematode taxonomy (Paperna 1996). In fish hosts the adult digeneans may be identified to the species level, but this task is almost impossible with metacercarial stages. While some families or genera are recognizable by their structural and locality affinities, in other instances even family affinities cannot be determined. Again, experimental infection of known or suspected definitive hosts with metacercariae yield mature trematodes to a limited extent (Paperna 1996).

The present investigation yielded few adult members of one species of the family Macroderoididae (*Glossidium pedatum,* previously under Plagiorchidae) and mainly metacercariae belonging to the families Diplostomidae (*Diplostomum*) and Clinostomidae (*Clinostomum*). There were also small unidentified cysts that were lodged in the skin (black spots), the gills and the visceral cavity.

3C.2 MATERIALS AND METHODS

During the routine examination of hosts for parasites, adult digeneans were collected from the intestines whilst the metacercariae were procured from the brain, branchial, visceral and heart cavities as well as the eyes. The muscles and other body organs were checked but yielded none. Paintbrushes and pipettes were used to handle specimens, thus avoiding damage to the worms. The specimens from preserved hosts were directly transferred into 70% ethanol for storage. In freshly killed hosts the digeneans were killed and fixed in hot (±70 °C) alcohol-formal-acetate and preserved in 70% ethanol. The standard procedure for staining was followed and this comprised rehydration, staining with aceto alum carmine solution (Gurr 1956), dehydration and clearing with clove oil. Mounting of specimens was done with Canada balsam. The whole mounts were identified, drawn, measured and photographed.

3C.3 RESULTS AND DISCUSSION

1 Genus ***Glossidium*** Looss, 1899

The type species for the genus is *Glossidium pedatum* Looss, 1899 and is so far the only species of the genus. Yamaguti (1958) formulated the generic diagnosis for *Glossidium* as “Body elongate, tapered towards two extremities, spinulate. Oral sucker subterminal, prepharynx distinct, pharynx large, oesophagus practically absent. Ceca wide, reaching to posterior extremity. Acetabulum rather small, in anterior half of body. Testes placed obliquely tandem, a little behind acetabulum, separated from each other by uterus. Cirrus pouch claviform, enclosing bipartite seminal vesicle, prostatic complex and cirrus. Genital pore submedian, just in front of acetabulum. Ovary immediately postacetabular, a little out of the median line. Receptaculum seminis present. Uterus passing between testes and reaching to posterior extremity. Vitellaria extending in lateral fields in ovario-testicular zone. Excretory vesicle probably Y-shaped. Intestinal parasites of freshwater fishes”.

1.1 ***Glossidium pedatum*** Looss, 1899

Looss (1899) first described this parasite from *Bagras bayad* and *B. docmac* in the Nile River, Egypt. Further records (Fischthal 1973; Mashego 1977; Mashego & Saayman 1989; Mashego *et al*. 1991; Imam *et al.* 1991; Barson 2003; Bray & Hendrix 2007; Ibraheem 2007) indicate that this species was found, so far, only in Egypt, Ethiopia, Malawi and South Africa from catfishes of the families Bagridae and Clariidae. Mashego & Saayman (1989) considers *Afromacroderoides lazerae* Khalil, 1972 as a synonym of *Glossidium pedatum* thereby including Sudan in the country list. Bray *et al.* (2006) also consider *Astiotrema lazeri* as a synonym of *Glossidium pedatum.*

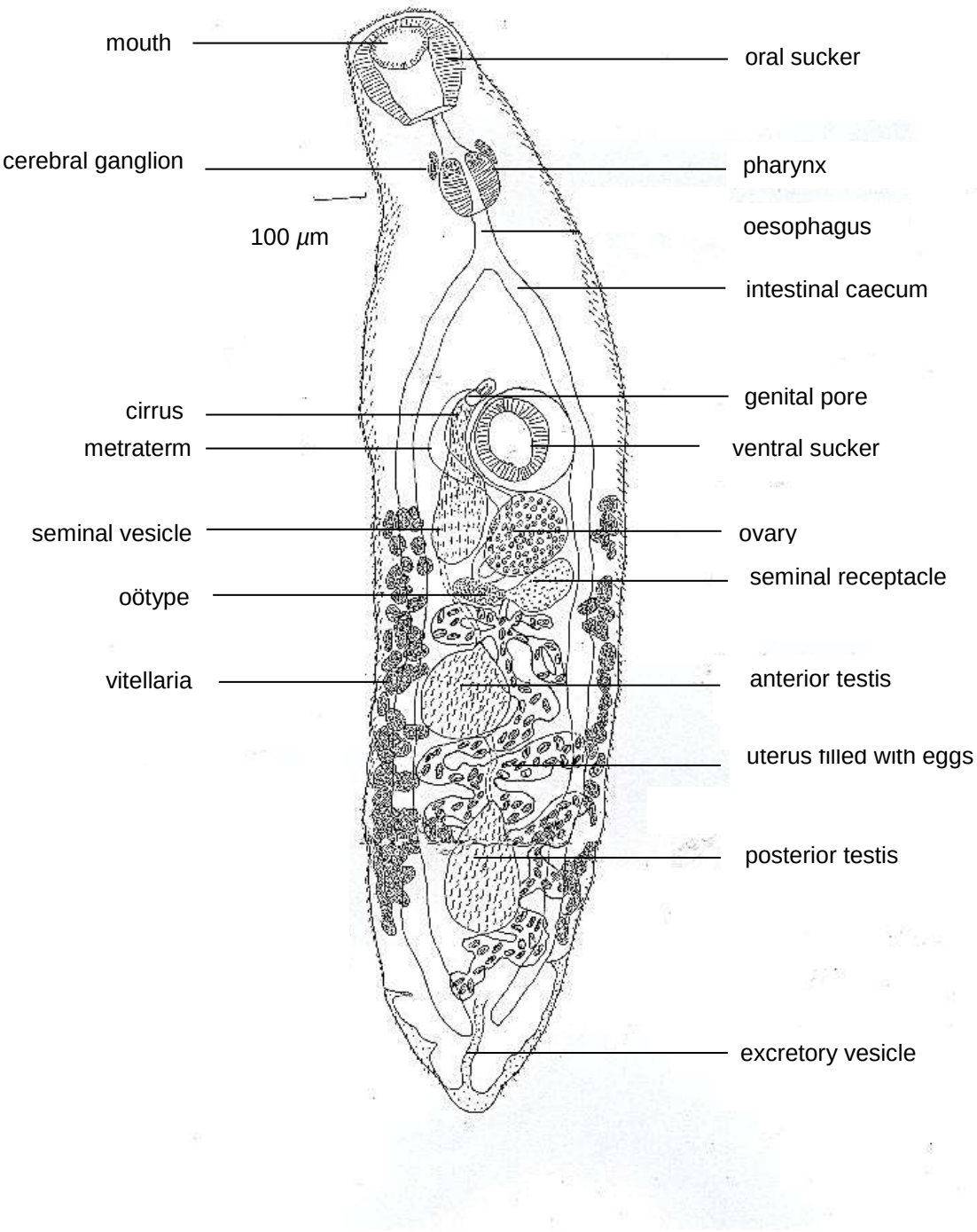


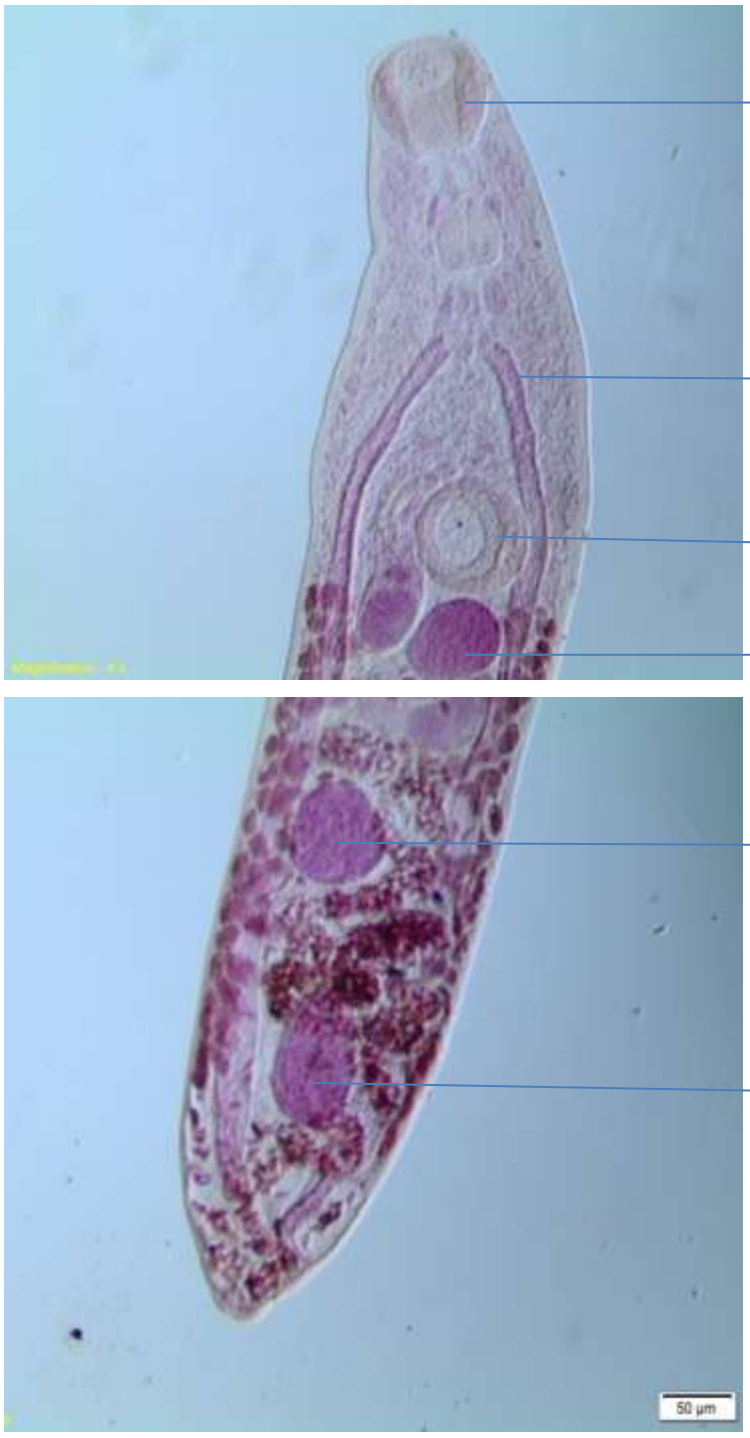
Figure 3C.1 *Glossidium pedatum* Looss, 1899

Table 3C.1 Measurements (in μm) of *Glossidium pedatum*

G. pedatum	Mashego 1977	Present
Number of specimens	-	6
Body length	1932	2250
width	312	525
Oral sucker length	210	212
width	160	140
Acetabulum diameter	170	160
from anterior tip	710	780
from intestine branch	220	237
Ant. testis length	150	175
width	110	175
from acetabulum	120	312
Post. testis length	200	250
width	110	150
from acetabulum	350	625
Ovary length	120	150
width	110	143

Fischthal (1973) redescribed this parasite to include more structures, Mashego (1977) included a graphic reconstruction of the parasite and Ibraheem (2007) studied the ultrastructure of its surface. Other studies only recorded its presence with other parasites from the hosts investigated. This parasite species seems to be host specific to *Clarias gariepinus* in the southern parts of Africa as, despite investigations of numerous other species of fishes, it was never found in any other species (Mashego *et al.* 1991). No record of its life cycle was found but Mashego *et al.* (1991) suggests that it includes a freshwater snail as its intermediate host.

In this investigation, the parasites were found from the intestines of *Clarias gariepinus*. The morphology (figure 3C.1) was compared to that of Mashego (1977) and together with the measurements (table 3C.1) they were convincing enough to be declared as *G. pedatum.* The micrographs of *G. pedatum* are shown in Plates 19 - 21. The life histories of the trematodes which (at the adult stage) infect African fish have so far not been studied and their first molluscan host and other intermediate hosts remain unknown (Paperna 1996).

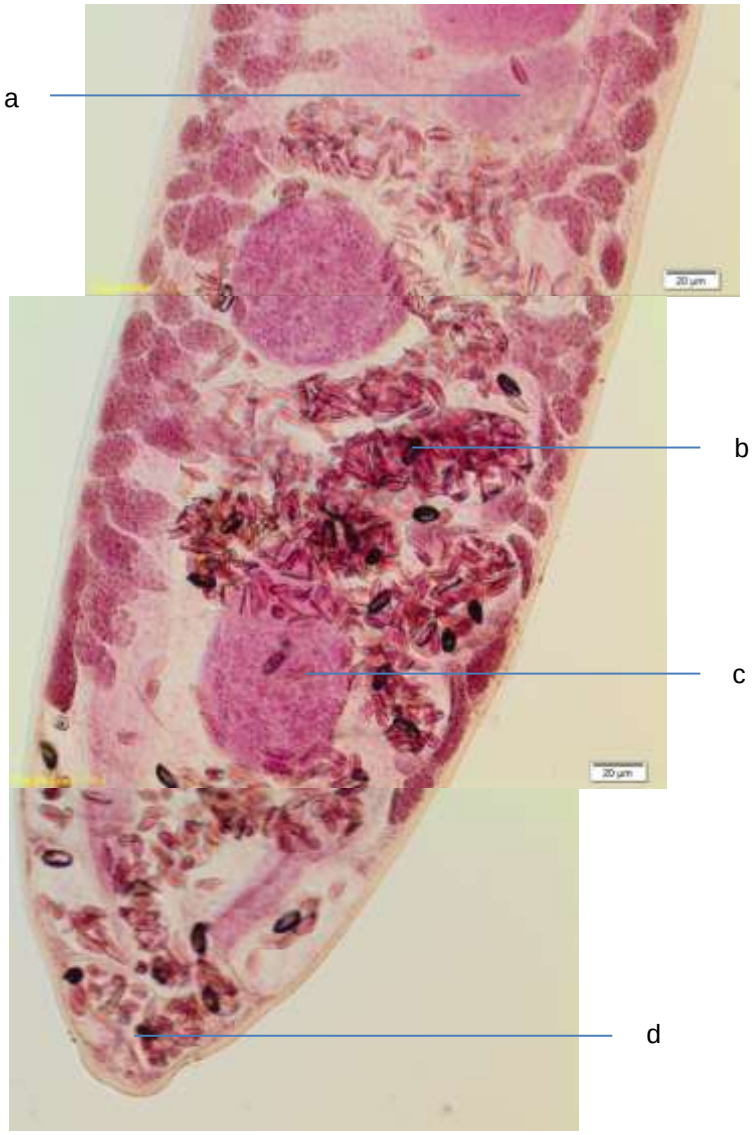


a. oral sucker b. intestinal caecum c. ventral sucker d. ovary
e. anterior testis f. posterior testis

Plate 19 *Glossidium pedatum* – two halves (anterior and posterior) together

a. mouth b. pharynx c. seminal vesicle

Plate 20 *Glossidium pedatum* – anterior half



a. seminal receptacle b. uterus c. posterior testis d. excretory vesicle

Plate 21 *Glossidium pedatum* – posterior half

2 Genus ***Diplostomum*** van Nordmann, 1832

Members of the genus *Diplostomum* are economically important in both natural and aquaculture systems worldwide due to their metacercariae which parasitize the eyes of fish (Chibwana & Nkwengulila 2010). The biology and life history of *Diplostomum* spp. have been studied extensively in the northern hemisphere (Niewiadomska 1996) with the south lagging behind. African species of the genus include *D. heterobranchi*, *D. magnicaudum*, *D. mashonense*, *D. tregenna* and *D. ghanense* (Mashego *et al*. 1991; Khalil & Polling 1997). Yamaguti (1971) gave a long generic diagnosis based mainly on morphological features, but included *Lymnaea* as the only snail first intermediate host, freshwater fish and various birds as second intermediate and definitive hosts respectively. Dubois (1970) came up with the compound genus *Diplostomum* to include three subgenera *Diplostomum*, *Tylodelphys* and *Dolichorchis*, often elevated to genus level.

2.1 ***Diplostomulum*** Brandes, 1892

The name *Diplostomulum* was coined for the larval metacercariae of *Diplostomum*. The metacercariae were found from many fish host species in all localities studied in Africa (Wedl 1861; Beverly-Burton 1963; Khalil 1963, 1969; Lombard 1968; Mashego 1977, 1982; Moravec 1977; Prudhoe & Hussey 1977; El Naffar 1979; Mashego & Saayman 1989; Mashego *et al*. 1991; Nkwengulila 1995; Musiba 2004; Chibwana & Nkwengulila 2010; Grobbelaar *et al*. 2010). Other than metacercariae of *Diplostomum,* Khalil & Polling (1997) list metacercariae of 20 genera found in African freshwater fishes, with still more unidentified.

According to Paperna (1996), it is not feasible to diagnose the diplostomid metacercariae to the species level. There are several factors that contribute to this difficulty; several developmental stages of a particular species may infect a single host organ at any particular time (Khalil 1963), some species may co-occur in the same host organ (Mashego *et al*. 1991), striking morphological similarity and phenotypic plasticity induced by age, host and fixation procedures, overlap in

morphological measurements within and among species, the lack of a key devoted to the metacercaria stage in the life cycle and that the identification of diplostomid metacercariae from Africa is dependent on scattered descriptions in the literature (Chibwana & Nkwengulila 2010).

Khalil (1963) attempted species identification of metacercariae by experimentally obtaining the adults from larvae but no certainty could be reached from his results in that the hosts were not natural and thus the adults were not normal. Mashego *et al*. (1991) made an identification attempt but could only provisionally designate the metacercariae to five species by associating them morphologically with the adult forms from the piscivorous birds within the same locality. Recently, multivariate analyses were used to separate morphologically similar *Diplostomum* species with increased success, but when extended to different populations, extensive morphometric variations introduced doubt about the reliability of measurements and it may lead to misidentifications. Genetic analysis, though problematic in cases of spatial separation of populations may shed some light in this regard (Chibwana & Nkwengulila 2010).

In this study the metacercariae were found in the brain cavity and eyes of *Clarias gariepinus* and *Labeobarbus marequensis*, and from the eyes of *Oreochromis mossambicus* and *Chetia flaviventris*. In Lake Tzaneen and the surrounding catchment no studies were conducted involving the adult stages as found in piscivorous birds and thus no morphological correlations would be possible. Again, it was not worthy, under the prevailing circumstances to attempt experimental infections of “hosts” with metacercariae, a tedious task, but usually with unsuccessful results.

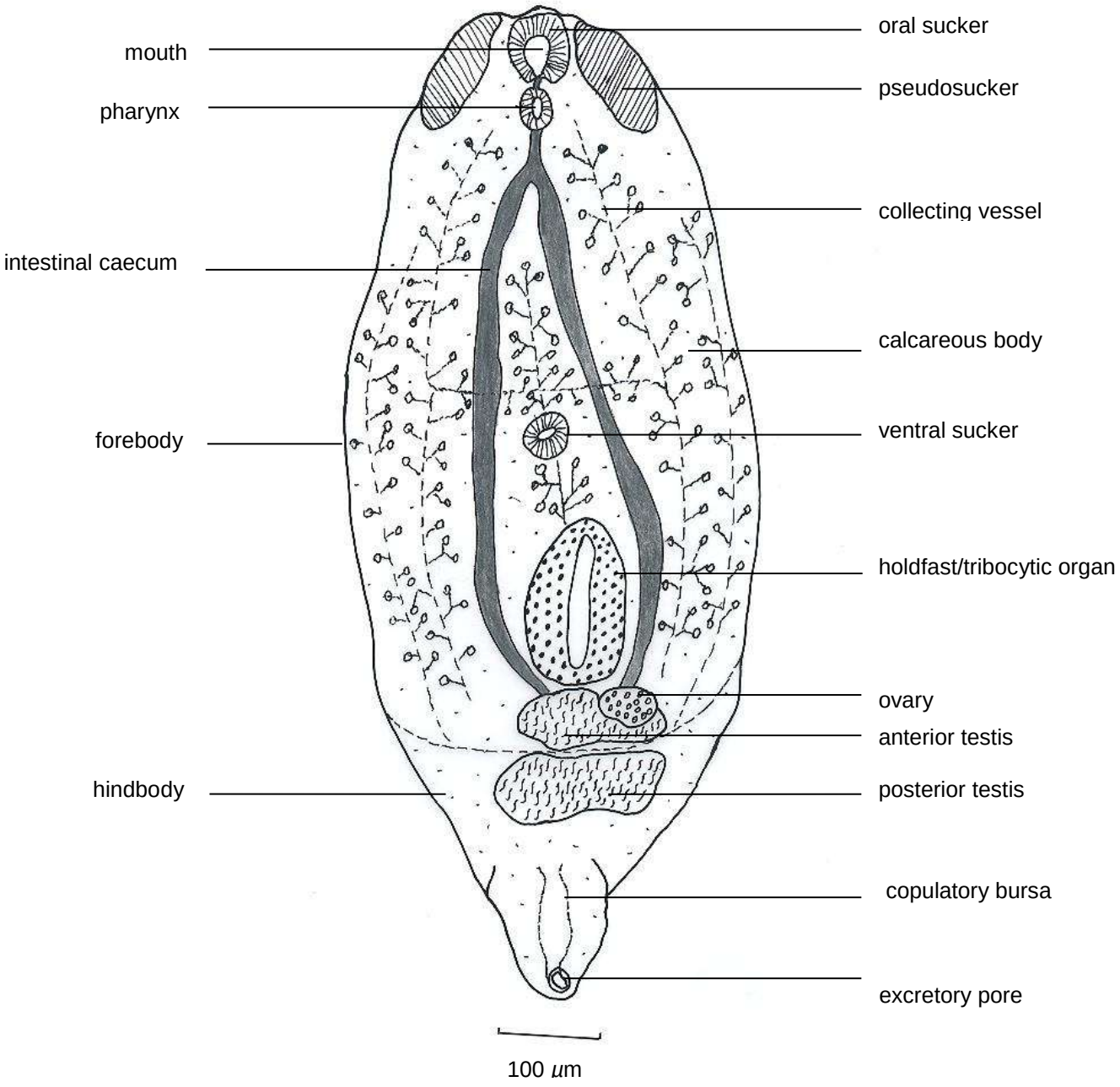


Figure 3C.2 *Diplostomulum* Brandes, 1892

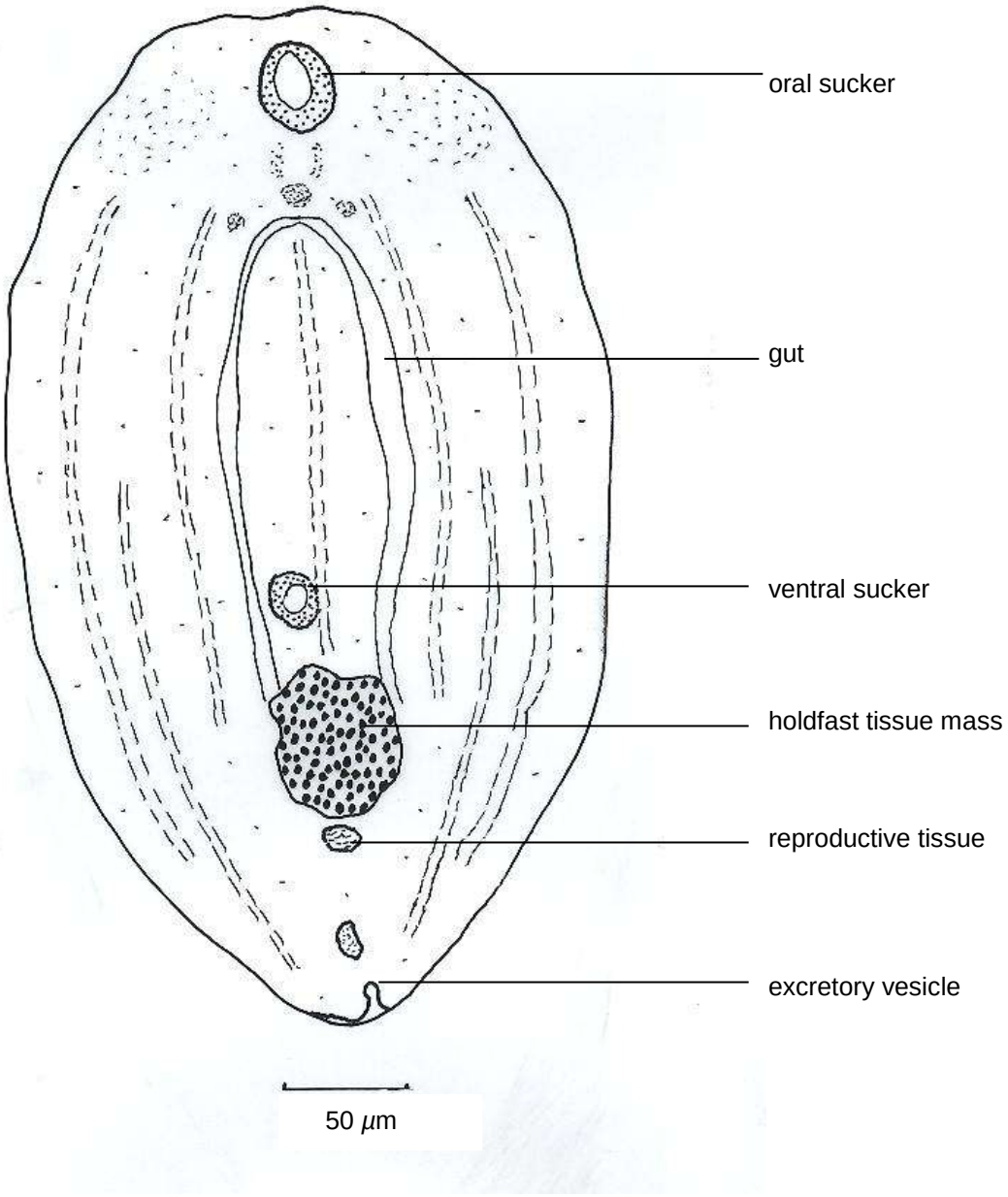


Figure 3C.3 *Diplostomulum* Brandes, 1892 immature

A study of the stained wholemount specimens of the metacercariae revealed only one type but this occurred at different developmental stages. The larger of these (figure 3C.2) closely resembles metacercaria of *D. tregenna* found by Khalil (1963), *D. mashonense* found by Mashego (1977) and metacercaria type 1 of Mashego

(1982) and were thus compared (table 3C.2). Dubois (1970) regarded *D. tregenna* and *D. mashonense* as synonyms due to similarities between their metacercariae. Mashego (1982) found that the metacercaria type 1 had no ventral sucker. In this study, the ventral sucker is very small and may not be easily visible under an ordinary light microscope. None of the specimens found could be compared to metacercaria type 2 and type 3 found by Mashego (1982).

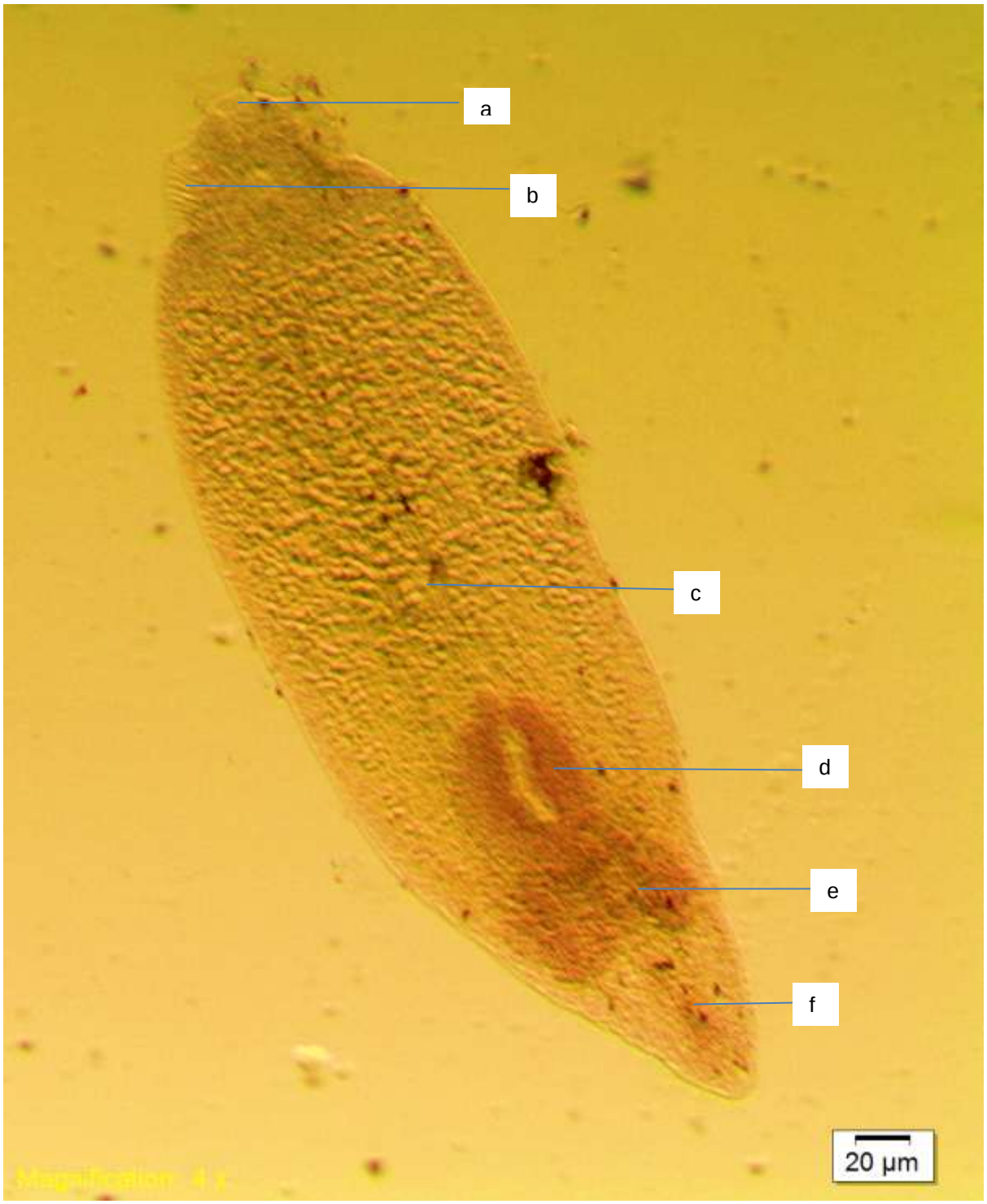
Morphological description (figure 3C.2, table 3C.2)

Body elongate, with dorso-ventrally flattened forebody and round hindbody; cuticle smooth and transparent; oral sucker terminal, round to triangular; ventral sucker much smaller than oral sucker; two pseudo-suckers adjacent but lateral to oral sucker; tribocytic organ posterior but very much larger than ventral sucker; anterior and posterior testes lie adjacent but posterior to tribocytic organ. Copulatory bursa on the hindbody and opens posteriorly; mouth opens into very short prepharynx (not visible in some specimens); pharynx muscular; short oesophagus; intestinal caeca extend to posterior end of forebody; excretory system with collecting vessels and calcareous bodies on the forebody, with a larger excretory vesicle on the hindbody.

The smaller metacercariae (figure 3C.3) may well be a developing stage of the former since both were found in all the hosts mentioned above, except in *C. flaviventris* where only the smaller was found. Both the oral and ventral suckers are present. Pseudosuckers are not yet that visible. Tribocytic (holdfast) organ is still a mass of tissue without a middle longitudinal slit. The intestinal caeca, reproductive and excretory systems are primordial. The micrographs of *Diplostomulum* are on Plates 22 to 24.

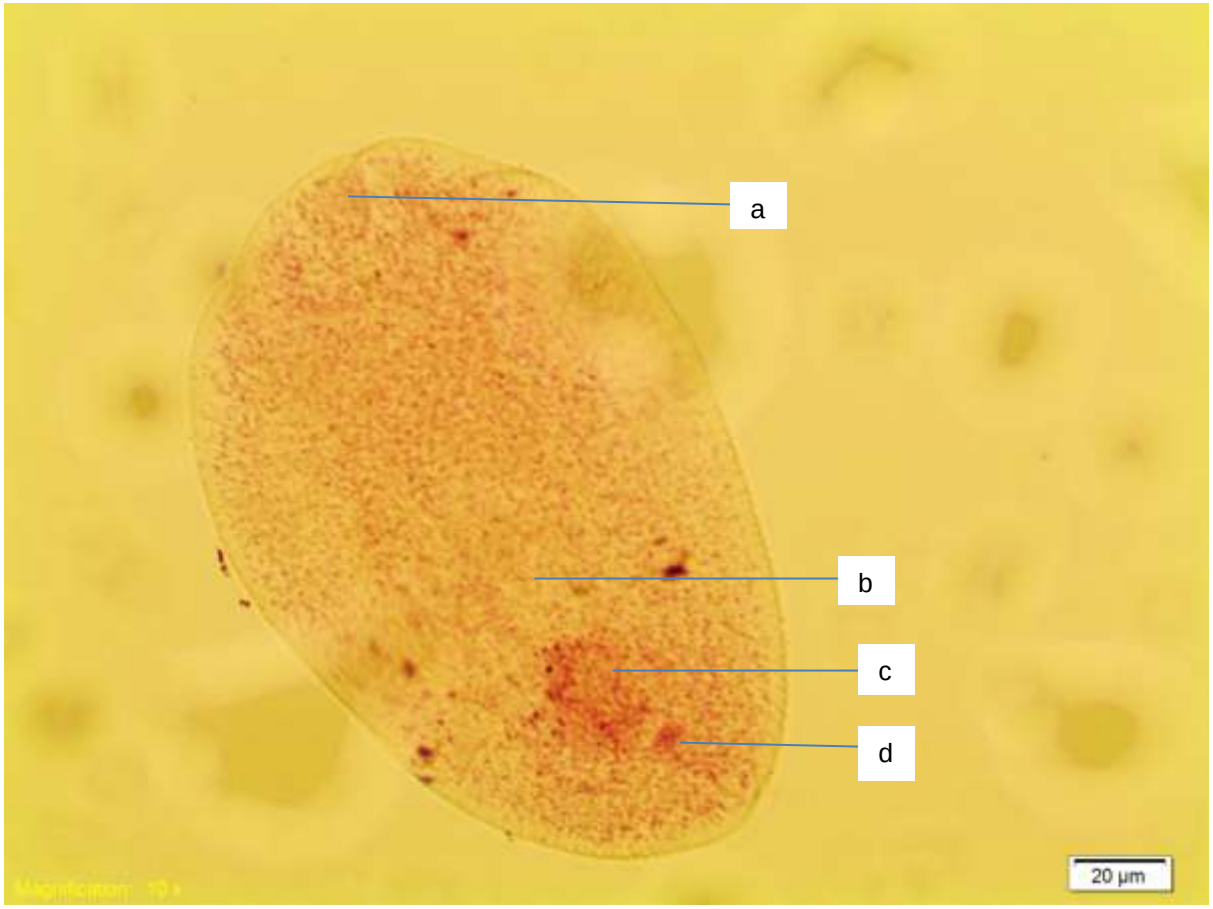
Table 3C. 2 Measurements (in *μ*m) of *Diplostomulum* Brandes, 1892

Diplostomulum	Khalil 1963 *D. tregenna*	Mashego 1977 *D. mashonense*	Mashego 1982 Type 1	Present
Host	*C. lazera* (= *C. gariepinus*)	*C. gariepinus*	*Barbus* spp.	*C. gariepinus* *O. mossambicus* *L. marequensis*
No. of specimens	-	1	10	10
Body length	960 - 1200	740	702 - 921	725 - 1246
width	-	268	301 - 340	284 - 420
Forebody length	720 - 860	-	-	502 - 845
width	220 - 300	-	-	284 - 420
Hindbody length	240 - 350	-	-	226 - 394
width	150 - 250	-	97 - 126	110 - 270
Oral sucker length	54 - 61	44	55 - 61	42 - 72
width	-	47	49 - 55	46 - 60
Ventral sucker length	43 - 49	47	absent	41 - 48
width	46 - 57	55		42 - 46
Pseudo-sucker length	-	-	61 - 92	90 - 140
width	-	-	40 - 43	40 - 48
Pharynx length	9 - 46	39	43 - 43	34 - 44
width	25 - 30	-	31 - 37	27 - 32
Holdfast organ length	100 - 120	97	110 - 140	94 - 164
width	90 - 110	45	61 -120	51 - 114
Distance of holdfast organ from anterior	-	-	427 - 610	398 - 544
Ovary length	34 - 39	-	-	31 - 43
width	25 - 29			42 - 59
Anterior testis length	46 - 71	-	-	52 - 64
width	89 - 114	-	-	94 - 148
Posterior testis length	56 - 71	-	-	54 - 69
width	107 - 121	-	-	118 - 166



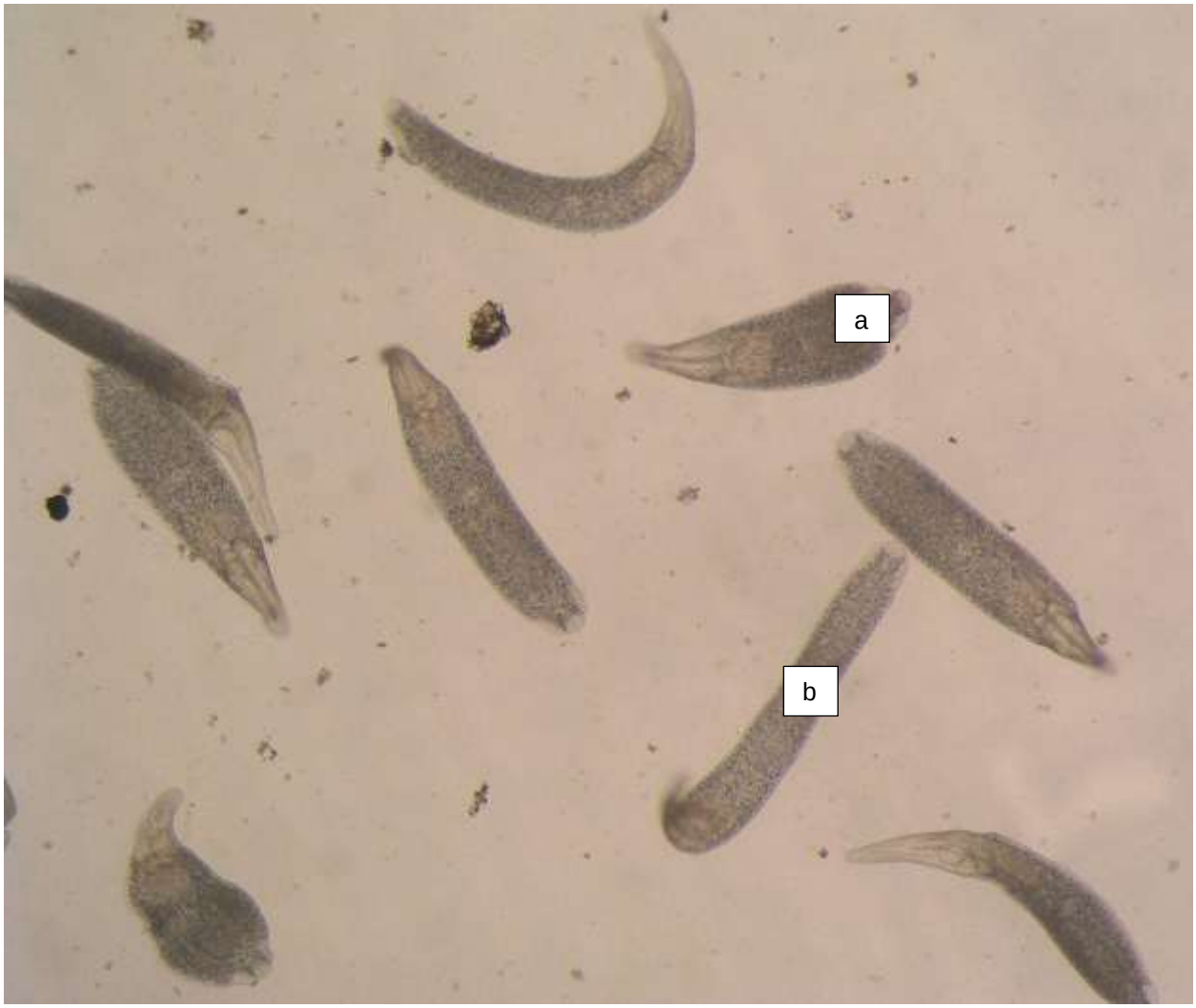
a. oral sucker b. pseudosucker c. ventral sucker d. tribocytic organ e. testis f. copulatory bursa

Plate 22 *Diplostomulum*



a. oral sucker b. ventral sucker c. holdfast tissue d. reproductive tissue

Plate 23 *Diplostomulum* – immature



a. contracted b. elongated

Plate 24 *Diplostomulum* from the brain of *Clarias gariepinus* - in motion in a petri dish

3 Genus ***Clinostomum*** Leidy, 1856

The clinostomes are of economic importance in that fish usually carry heavy infections of metacercariae and is, in many countries, condemned for human consumption (Prudhoe & Hussey 1977). The clinostome cysts and worms are the largest in diameter (5 mm) and size (10 x 3 mm) and the intestine is loaded with a yellow to orange substance (Paperna 1996). The genus is very prevalent and widespread in Africa with metacercariae in fish and adults in fish-eating birds (Manter & Pritchard 1969). Data on *Clinostomum* suggests transcontinental distribution, with more studies in the northern hemisphere (Paperna 1996).

African studies including metacercariae of the genus *Clinostomum* (Dubois 1930; Ortlepp 1935; Dollfus 1950; Ukoli 1966; Williams & Chaytor 1966; Lombard 1968; Khalil 1969; Manter & Pritchard 1969; Fischthal & Thomas 1970; Khalil & Thurston 1973; Imam *et al*. 1979; Mashego 1982; Britz 1983; Batra 1984; Van As & Basson 1984; Britz *et al*. 1984b; Saayman 1986; Mashego *et al*. 1991; Douellou & Earlwanger 1993; Paperna 1996) represent several fish host species in various parts of the continent. African species of the genus include *C. complanatum*, *C. vanderhosti*, *C. macrosomum*, *C. tilapiae* and *C. chrysichthys*. There are more metacercaria specimens that could not be designated to species level, and in some cases only referred to as type 1 and type 2 (Khalil 1969; Mashego 1982).

There has been controversy with the species identification of this group of parasites. According to Mashego (1982), this genus is morphologically uniform with minor differences to separate species. The identification of many species of this genus has been based on morphology of metacercariae alone since they are almost mature (Ukoli 1966). However, species identification is in most cases possible only with the reproductively mature adults (Mashego 1982). Price (1938), Agarwal (1959) and Ukoli (1966) provided keys to distinguish *Clinostomum* species using morphological characteristics as well as species lists whilst Yamaguti (1971) provided the generic diagnosis.

The life cycle of *Clinostomum* requires three hosts; adults occur in the alimentary canal of piscivorous birds, metacercariae encyst in several locations in fish whilst earlier larval stages use freshwater snails as first intermediate hosts. According to Mashego *et al*. (1991), in Africa fish hosts are from many families, bird hosts are mainly herons, darters, cormorants and pelicans while the snail hosts have not been identified with any certainty. Circumstantial evidence suggests that members of genera *Bulinus* and *Lymnaea* may be snail hosts for clinostomes (Britz 1983; Paperna 1996).

In this study, metacercariae were procured encysted from the branchial region musculature and visceral cavities of *Oreochromis mossambicus* and encysted in the visceral cavity (adjacent to swimbladder) of *Schilbe intermedius*. On very few occasions one specimen each was retrieved encysted from the heart cavity of *O. mossambicus*. The metacercariae from *O. mossambicus* were larger and yellowish in colour and those from *S. intermedius* were smaller and redish.

A study of the stained wholemount specimens of the metacercariae revealed only two types. The first type (figure 3C.4) is from *O. mossambicus*, the second type (figure 3C.5) is from *S. intermedius*, and they were compared with type 1 and type 2 found by Mashego (1982) respectively (table 3C.3). This was done because of the resemblances morphologically even though the first type in this study is far larger than type 1 found by Mashego (1982). In that study type 1 was compared to *C. tilapiae* and type 2 to *C. complanatum*. In South Africa, however, *C. tilapiae* was found from *O. mossambicus* (Britz 1983; Saayman 1986; Mashego *et al*. 1991) and *C. vanderhosti* was found from *Schilbe intermedius* (Saayman 1986; Mashego *et al*. 1991; Paperna 1996).

Among the five morphological differences pointed out by Mashego (1982) between the two types (reconstructed graphically), three of them stand out in the study of the present material. The posterior testis is fan-shaped with six lobes in the first, and heart shaped in the second; testes located in the middle third of the body in the first type and the anterior testis is partly in the middle and the posterior thirds of the body in the second type; the lumen of the uterine sac is diverticulated in the first type and without diverticular in the second type. The fourth difference was based on the

position of the cirrus pouch which could not be compared in this study because it was not present. However, the position of the cirrus itself, lateral to anterior testis in the first type and anterior to anterior testis in the second type is valid for this purpose (figures 3C.4 & 3C.5).

It was not possible in this study to identify these metacercariae to species level given the nature and scope of the study and to avoid further controversy. The identification of the metacercariae was outside the scope of this study as it would have involved ecological studies. The micrographs of stained *Clinostomum* metacercariae are on Plates 25 to 26.

4 Cysts

There were many cysts found externally in the skin and gills of *Oreochromis mossambicus* and internally in the visceral cavity of both *Clarias gariepinus* and *Chetia flaviventris*. Many of these cysts appear immature and are thus difficult to identify. The skin and the gills are used as the point of entry into the fish by these parasites (Paperna 1996).

Cysts consolidating around certain skin metacercariae may incorporate dermal melanophores and exceptionally, other chromophores. Such metacercariae, termed “black spot”, are formed in infections by the larvae of the genus *Neascus* and many others (Paperna 1996; Khalil & Polling 1997). In the gills the cysts are usually found attached to the filaments and when opened an immature, unidentifiable larva can be seen (Plate 27). In the visceral cavity small whitish cysts are usually seen attached to the mesentery.

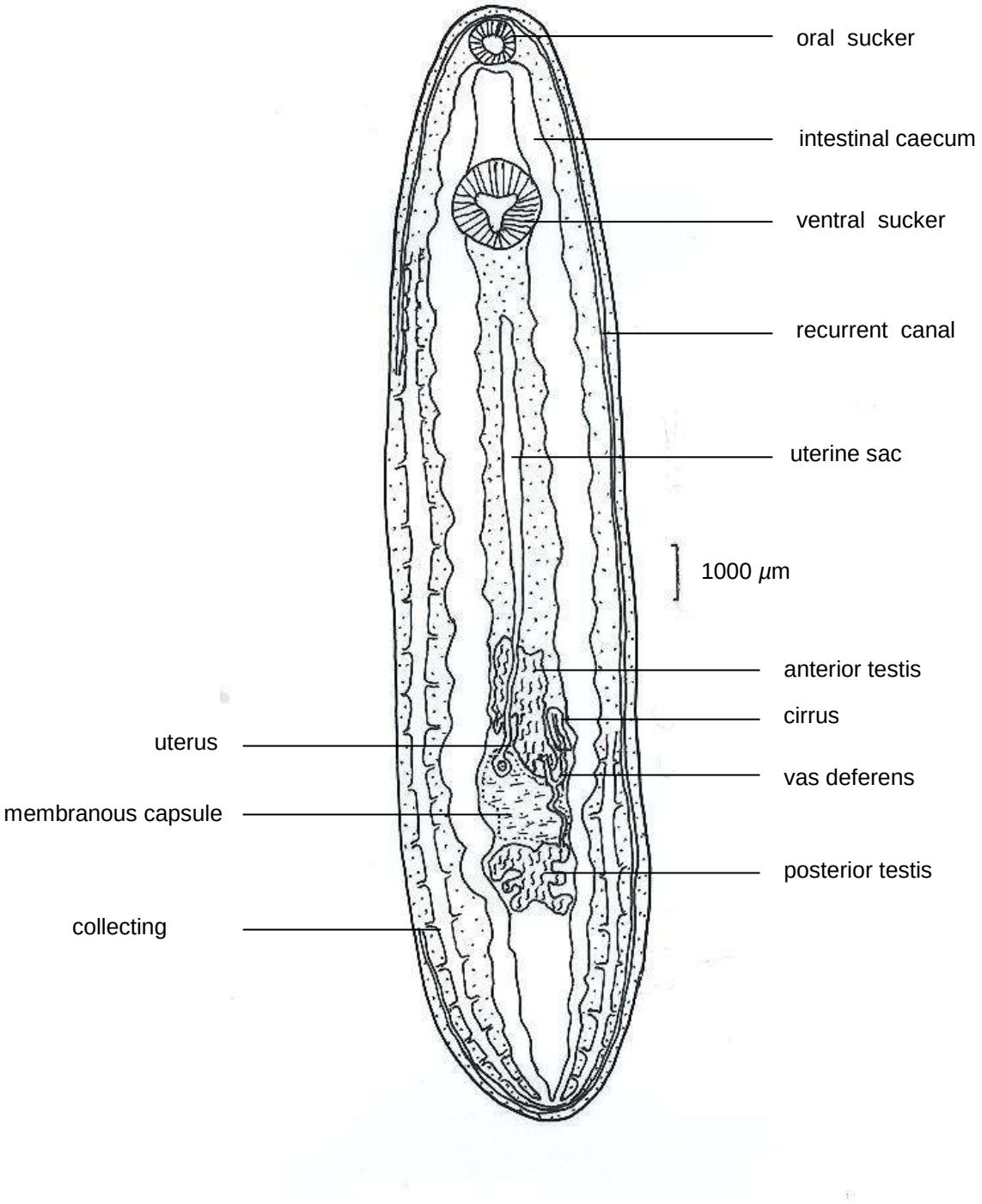


Figure 3C.4 *Clinostomum* Leidy, 1856 first type

Table 3C.3 Measurements (in *μ*m) of *Clinostomum* Leidy, 1856

Diplostomulum	Mashego 1982 Type 1	Present First type	Mashego 1982 Type 2	Present Second type
Host	*Barbus* spp.	*Oreochromis mossambicus*	*Barbus* spp.	*Schilbe intermedius*
No. of specimens	10	10	8	3
Body length	3802 - 6712	9614 - 21257	5490 - 7062	6204 - 7826
width	1264 - 1911	2835 - 6516	1358 - 1882	1920 - 2326
Oral sucker length	186 - 407	506 - 1223	310 - 416	362 - 485
width	262 - 485	611 - 1247	349 - 445	366 - 480
o.s. from anterior tip	78 - 116	114 - 405	97 - 136	187 - 275
Ventral sucker length	631 - 931	1238 - 2241	737 - 1057	844 - 1008
width	649 - 922	1274 - 2348	776 - 1048	838 - 986
v.s. from anterior tip	437 - 1222	2371 - 3888	941 - 1242	990 - 1114
v.s. from posterior tip	2871 - 4772	6002 - 15045	3686 - 5093	4823 - 5920
Ovary length	116 - 194	346 - 689	165 - 294	162 - 276
width	116 - 194	438 - 680	97 - 233	124 - 198
Anterior testis length	243 - 504	1554 - 2990	291 - 582	321 - 439
width	301 - 466	506 - 1210	291 - 446	446 - 555
Posterior testis length	243 - 504	750 - 1612	252 - 436	483 - 594
width	340 - 485	902 - 1532	349 - 631	566 - 687

o.s. – oral sucker

v.s. – ventral sucker

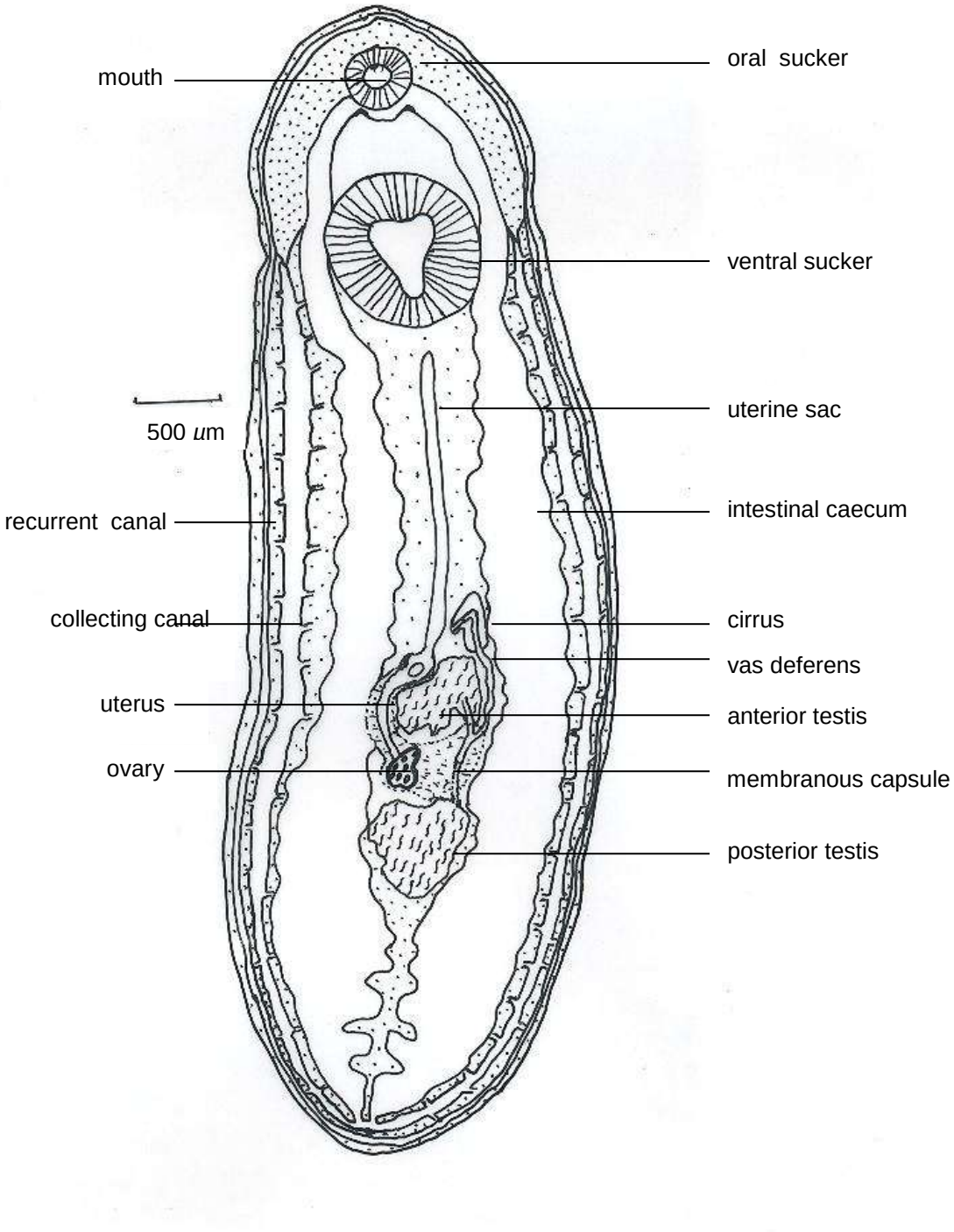
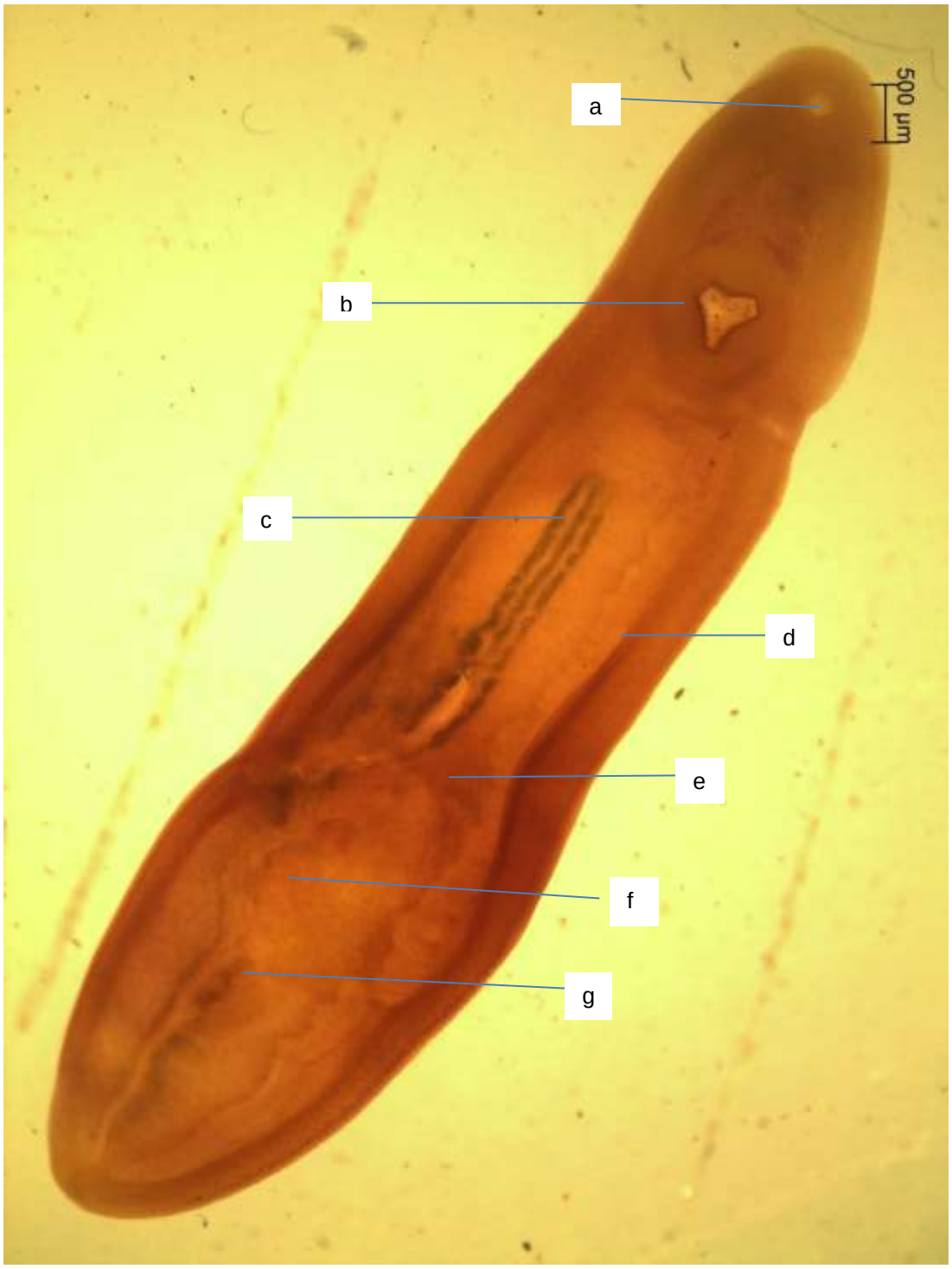
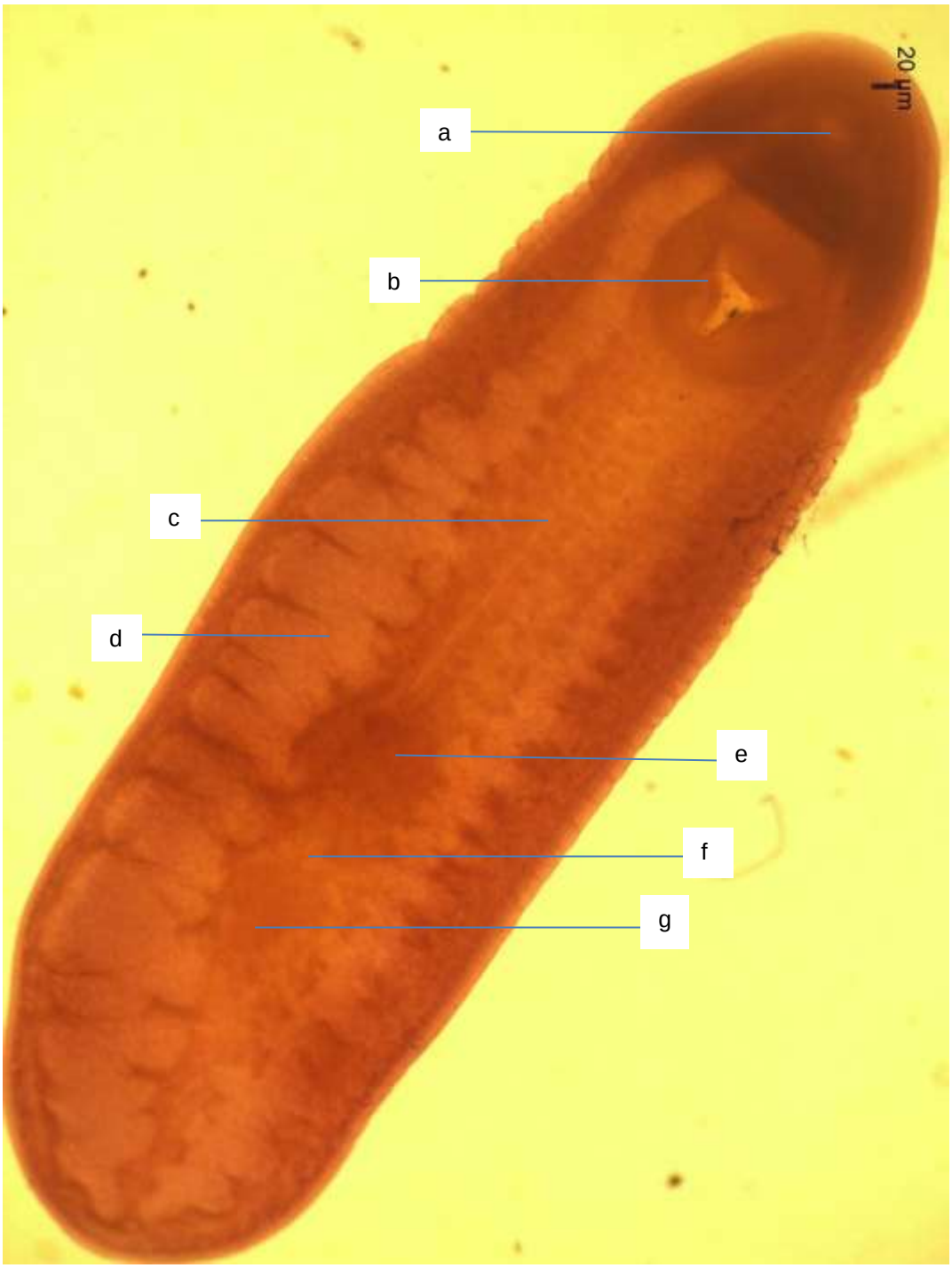


Figure 3C.5 *Clinostomum* Leidy, 1856 second type



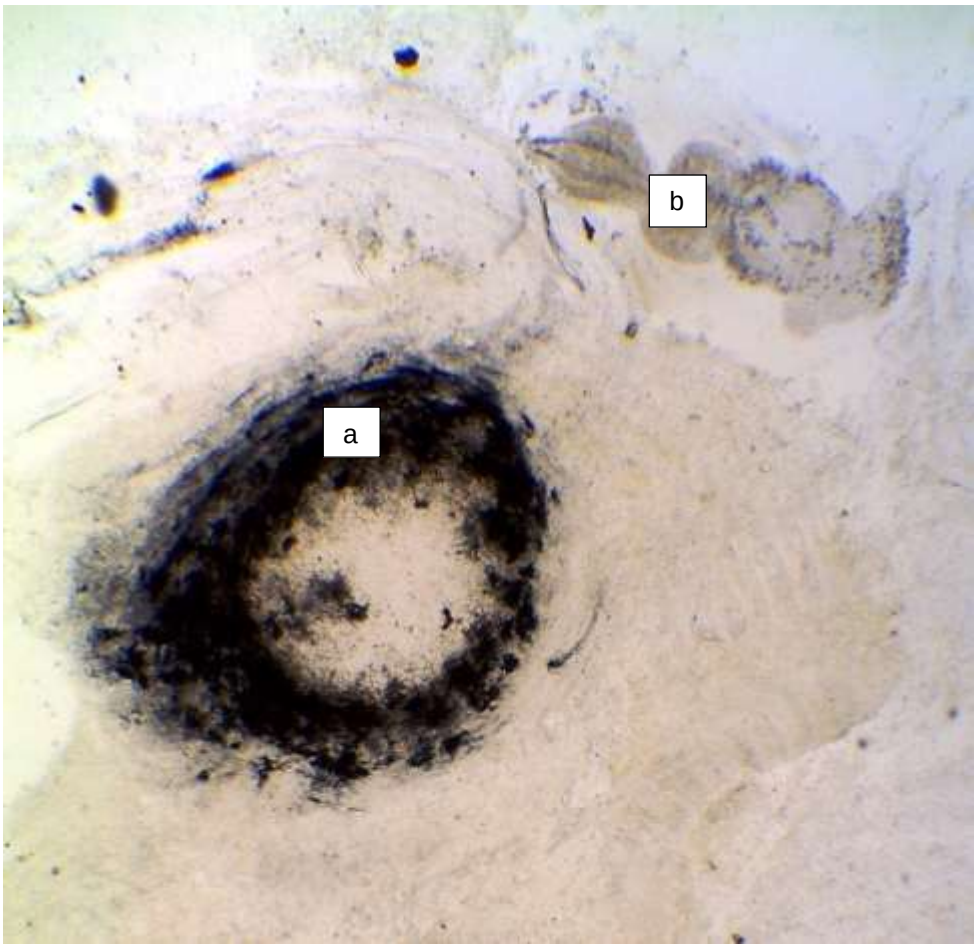
a. mouth b. ventral sucker c. uterine sac d. intestinal caecum e. anterior testis f. membranous capsule g. posterior testis

Plate 25 *Clinostomum* first type metacercaria



a. mouth b. ventral sucker c. uterine sac d. intestinal caecum e. anterior testis f. membranous capsule g. posterior testis

Plate 26 *Clinostomum* second type metacercaria



a. cyst wall b. metacercarial larva

Plate 27 Cyst opened to release metacercaria; ex gills of *Oreochromis mossambicus*

3C.4 GENERAL DISCUSSION

There have been more studies from continents other than Africa on the taxonomy, life cycles and diseases of trematodes and this is evident from the literature. Studies on trematodes of fish show that the metacercariae are more frequently studied than adult stages. This picture may be due to adult stages that appear easier to identify while the metacercariae, usually more in terms of individuals and sites in the hosts, are more difficult to identify and thus attract more attention.

In South Africa, it has been demonstrated that fish infections by diplostomid and clinostomid metacercariae is common (Ortlepp 1935; Lombard 1968; Mashego 1982; Britz 1983; Britz *et al*. 1985; Saayman 1986; Mashego *et al*. 1991) and that the prevalence is usually high. In the case of Lake Tzaneen this could also be true for more fish species had the sampling strategy not been compromised due to the large variety of hosts examined for all helminth parasites in/on all organs.

The greatest contributor to the dispersal of metacercarial infection is migrating birds along the eastern and western migration routes. This is due to the piscivorous birds being the main definitive hosts for many metacercariae found in fish (Paperna 1996). Another important factor is the presence of suitable mollusc intermediate hosts. The metacercarial infections are predominant in shallow waters where vector snails live. There have been suggestions that aquatic birds play a role in the dispersal of aquatic snails (Paperna 1996).

Trematodes demonstrate a high degree of specificity to their molluscan hosts. Eggs of gut dwelling digeneans are released via defaecation and in trematodes that reach the adult stage in fish the bivalve and gastropod molluscs serve as intermediate hosts. All flukes which attain maturity in piscine hosts reach their definitive host as waiting stage metacercariae. Adult trematodes, infecting the digestive tract of fish, are considered harmless, even when their numbers are high. Extra-intestinal trematode infections, on the other hand, are potentially pathogenic (Paperna 1996).

Cercariae penetrate fish via the skin and gills. According to Paperna (1996), exposure to massive numbers of cercariae may kill fry within a few hours but such

exposures are not usual in naturally occurring infections. Clinical effects of infection are often not obvious. Naturally, the presence of metacercariae in the brain, cranial nerves or spinal cord is not debilitating on the fish, even at relatively high infection loads (Paperna 1996).

Some metacercariae have a predilection or site-specificity for the eyes, or sometimes corneal infection is a side-effect of integument-encysting metacercariae which impair eye vision. This condition is aggravated when metacercariae are accompanied by melanophores (black spot). Several infections by diplostomid metacercariae have been reported invading the anterior or vitreous humor rather than the lens (Paperna 1996).

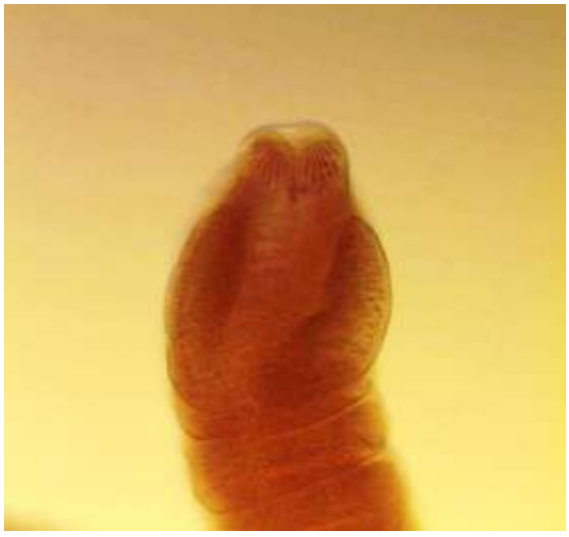
Metacercariae of fish may be recognized by the type and location of encystment in addition to their characteristic structural features, but this may be limited to the genus or even the family level. Again, at the genus level the metacercariae were found in various fish species not exhibiting host specificity. Cysts containing both juvenile metacercariae as well as some enveloped, more advanced metacercariae of the same or different species were also reported by Mashego (1982). In previous studies experimental infections of suspected avian hosts with metacercariae render mature worms only to a limited extent. These factors, together with morphological uniformity within genera have caused problems in the taxonomy of digeneans (Paperna 1996).

In future studies based on suprapopulations of *Diplostomum* and/or *Clinostomum* in an ecosystem should be done. These should be done also at the life cycle level, and the molluscan hosts, fish and birds should be investigated. Morphological data should be supported by genetical identifications in order to solve their taxonomic problems. Modern studies that involve taxonomy or even systematics are not complete without genetics especially where a thin line has to be drawn in species identification.

CHAPTER 3

SECTION D

CESTODA



Armed with hooks

or

Armed with eggs

CONTENTS

3D.1 INTRODUCTION

Cestoda are flatworms (Platyhelminthes) and their dominating morphological features are an elongated tape-like body and the absence of an alimentary canal. They are almost unique among parasites in that adult worms occupy only one particular habitat, the alimentary canal, in one particular group of animals, the vertebrates. Exceptions occur in the bile duct, gall bladder and pancreatic duct, sites that are, however, still related to the alimentary canal. In contrast larval cestodes occur in almost any location in the intermediate host, although many species show a predilection for a particular organ (Smyth & McManus 1989).

The life cycles of tapeworms involve more than one intermediate host, usually planktonic copepods and fish. The birds are definitive hosts that spread the infection and make it difficult to control worm infections across the waterbodies (Barson & Avenant-Oldewage 2006). Tapeworms are widespread throughout all major water systems of Africa and demonstrate a high degree of host specificity (Paperna 1996). Fish serve as definitive host for some tapeworms while others only occur as larvae (metacestodes) in their fish intermediate hosts.

The few Southern African studies on Cestoda included herein (Mettrick 1960; Mackiewicz & Beverly-Burton 1967; Mashego 1977, 1982; Boomker *et al.* 1980; Hamilton-Atwell *et al.* 1980; Brandt *et al.* 1980, 1981; Van As *et al.* 1981; Hanert 1984; Mashego & Saayman 1989; Mashego *et al*. 1991, 2006; Luus-Powell 2004; Bertasso & Avenant-Oldewage 2005; Barson & Avenant-Oldewage 2006a; Ramollo *et al*. 2006; Retief *et al.* 2006, 2007, 2009) show that this group of parasites have not as yet received much attention in this country.

In this study two species of adult and larval cestodes were found. The adult cestodes (*Proteocephalus glanduligerus* and *Polyonchobothrium clarias*) were both found in the alimentary tract of *Clarias gariepinus*. The plerocercoid larvae of *Ligula intestinalis* and of the family Gryporhynchidae were found in the visceral cavity and intestinal wall respectively in various fishes while their adults occur in fish-eating birds.

3D.2 MATERIALS AND METHODS

The examination for cestodes was done in the first instance from preserved host specimens. This method was poor in that the bodies of the worms disintegrated quickly. The scolices or the hooks that are essential for identification were easily lost. The second and useful method was to retrieve the parasites immediately after killing the hosts. Care was taken to collect the worms intact but in some cases the attachment of the worms to the intestinal mucosa was so firm and resulted in breakages. The worms were individually placed in bottles of saline or distilled water and shaken vigorously to dislodge some debris on the surface. They were then placed in petri dishes filled with saline or distilled water, put in a refrigerator and left to relax. The worms were then straightened and flattened on the microscopic slides using brushes and then fixed with hot (±70 °C) AFA. They were then stored in 70% ethanol. The standard procedure for staining was followed and this comprised rehydration, staining with aceto-alum carmine solution, dehydration and clearing with clove oil. Mounting of specimens was done with Canada balsam. The whole mounts were identified, drawn and photographed.

3D.3 RESULTS AND DISCUSSION

1 Genus ***Proteocephalus*** Weinland, 1858

The distribution of *Proteocephalus* is extensive around the world (Mashego *et al*. 1991). Yamaguti (1959) lists 71 species found in fish while Khalil & Polling (1997) lists 11 species from African freshwater fish. African studies on the genus *Proteocephalus* (Klaptocz 1906; Beauchamp 1914; Fuhrmann & Baer 1925; Woodland 1925, 1937; Baylis 1928; Janicki 1928; Prudhoe 1951; Mahon 1954; Khalil 1963, 1969, 1973; Mashego 1977; Shotter & Medaiyedu 1977; Troncy 1978; Jones 1980; El Naffar *et al*. 1984; Mashego & Saayman 1989; Mashego *et al*. 1991; Barson & Avenant-Oldewage 2006a) indicate that the genus occurs mainly in the clariid catfishes.

1.1 ***Proteocephalus glanduligerus*** (Janicki, 1928) Fuhrmann, 1933

Janicki (1928) first described this species as *Ichthyotaenia glanduligera* from *Clarias anguillaris* in Egypt and this was later transferred to *Proteocephalus* as *P. glanduliger.* The original description was brief and provided very limited data on the strobilar morphology. Mashego (2001) redescribed the species (still as *P. glanduliger*) from *Clarias gariepinus* in South Africa, but according to Scholz *et al*. (2009) it lacked important details of the strobilar morphology, including cross sections and description of eggs. In the latest redescription (Scholz *et al.* 2009) the species was named *P. glanduligerus* and included previously unreported morphological characteristics.

The most typical characteristic of *P. glanduligerus* is the presence of an extremely large glandular, spherical to widely oval apical organ, the size of which is 1.6–3.5 times larger than that of the suckers. It is the presence of this structure which gave the parasite its specific name (Janicki 1928). Other features, not reported in the original description or in its redescription by Mashego (2001) are a very low number of mature proglottids and the presence of testes in gravid proglottids. In addition the position of osmo-regulatory canals (situated close to each other, with the dorsal canal latero-ventral to testes) is unusual. There are also the presence of a vaginal sphincter, several (usually 3–5) uterine pores, and eggs with paired lateral auricular swellings (extensions) of the outer envelope (Scholz *et al.* 2009).

The material under investigation in this study showed a large glandular organ and four suckers on the scolex while the reproductive system in the proglottids showed alternating genital pores (figure 3D.1). The reproductive system corresponds with those in previous studies (Mashego 2001; Barson & Avenant-Oldewage 2006a; Scholz *et al*. 2009). Some morphological details that Scholz *et al*. (2009) pointed, could not be seen from the wholemount specimens investigated. The photographs of *P. glanduligerus* are on Plate 28.

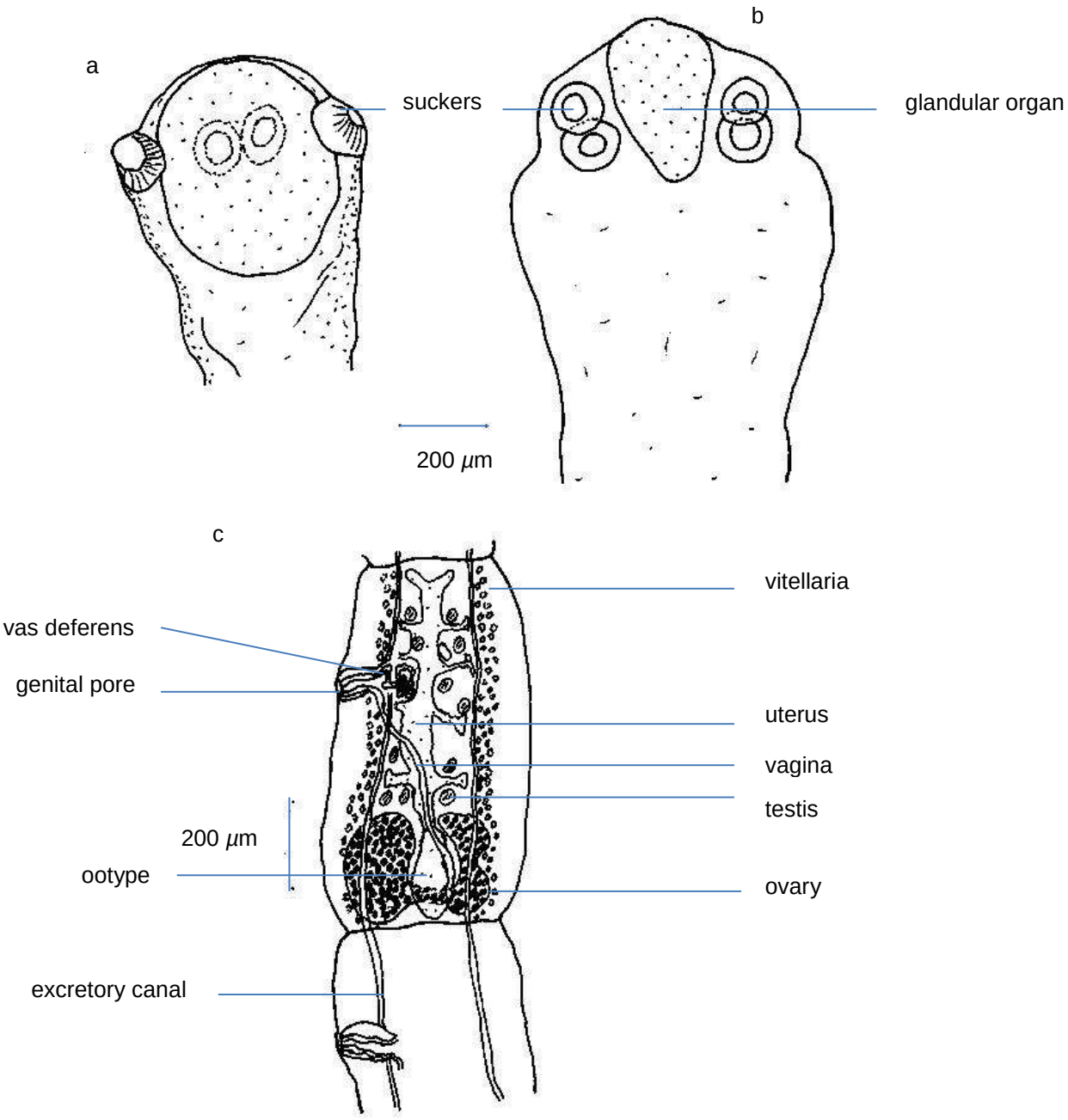
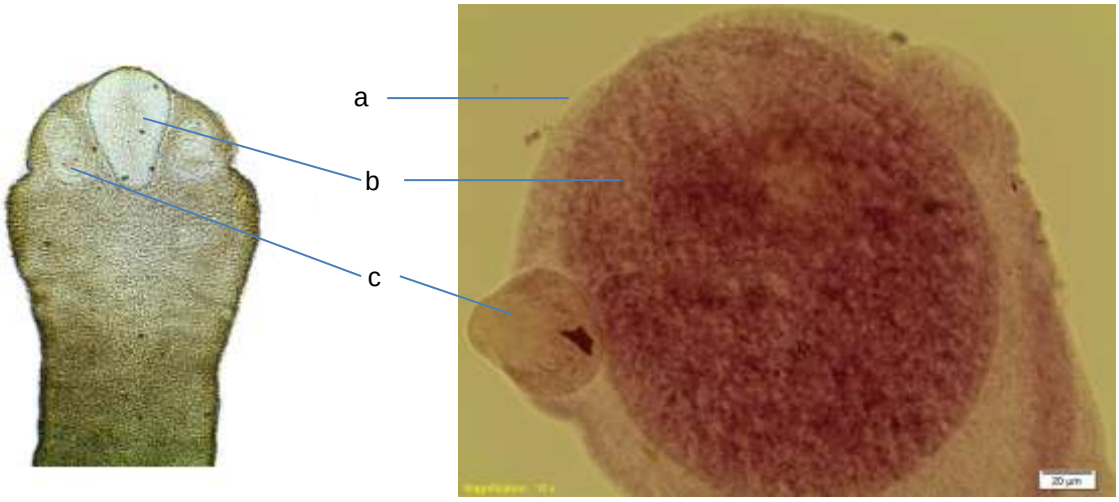
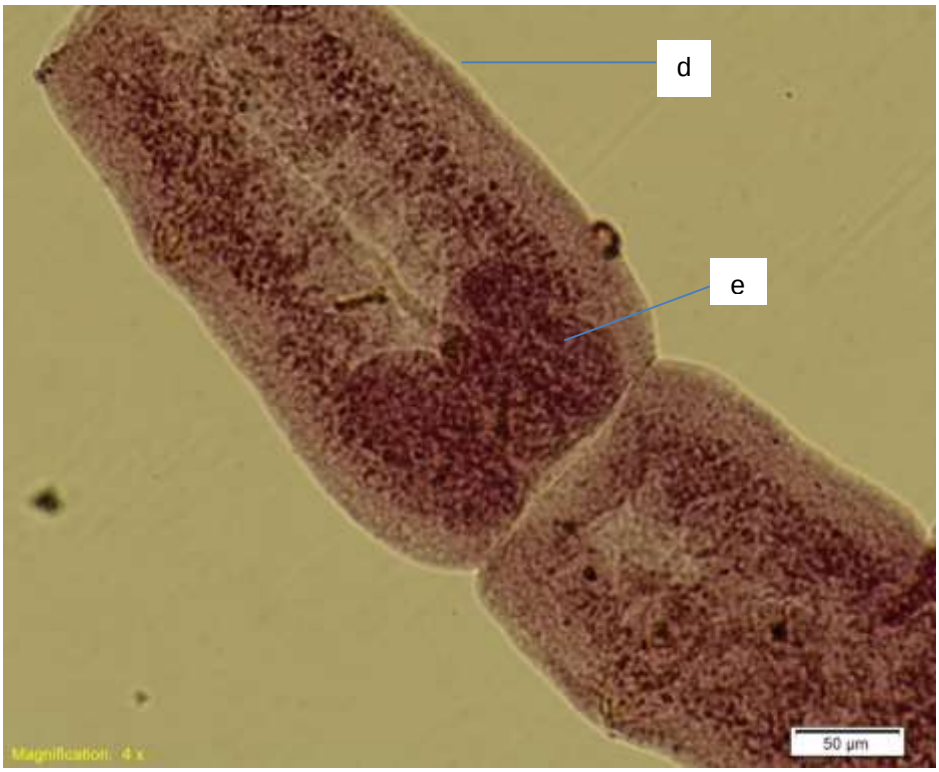


Figure 3D.1 *Proteocephalus glanduligerus* (Janicki, 1928) Fuhrmann, 1933 - a & b scolex c mature proglottid





a. scolex b. glandular organ c. suckers d. proglottids e. ovary

Plate 28 *Proteocephalus glanduligerus* (Janicki, 1928) Fuhrmann, 1933

Proteocephalid cestodes were found from *C. gariepinus* in Zimbabwe but were not further identified (Chishawa 1991; Douellou 1992; Barson 2004). Many proteocephalid cestodes were also found from different other fishes (including *C. gariepinus*) in other countries in Africa (Khalil & Polling 1997).

2 Genus ***Polyonchobothrium*** Diesing, 1854

Yamaguti (1959) provided the generic diagnosis: “Scolex nearly rectangular, with apex elevated, with shallow longitudinal groove on each flat surface, armed with a circle of numerous hooks arranged in four quadrants. Segmentation complete or rather incomplete. Inner longitudinal musculature well-developed. Testes medullary, lateral to ovary. Cirro-vaginal aperture mid-dorsal, posterior to uterine pore. Ovary transversely elongated or compact, in median posterior medulla. Vitellaria all round proglottid, divided into four (two dorsal and two ventral) cortical fields in genotype according to Klaptocz (1906). Uterus winding anterior to ovary, opening midventrally near anterior border of proglottid. Eggs thin-shelled, not operculate”. Khalil & Polling (1997) listed seven species of the genus from African freshwater fish. Notable in the African studies on the genus are the uncertainties and need for revisions on their taxonomy (Meggit 1930; Tadros 1968).

2.1 ***Polyonchobothrium clarias*** Woodland, 1925

Polyonchobothrium clarias was first described by Woodland (1925). It was later redescribed by Tadros (1968). Mashego (1977) reviewed the taxonomic history as well as the host and geographical distribution of *P. clarias* in Africa. African studies on the species (Meggit 1930; Tadros 1968; Khalil 1969, 1973; Imam 1971; Aderounmu & Adeniyi 1972; Khalil & Thurston 1973; Mashego 1977; Amin 1978; Wabuke-Bunoti 1980; Mashego & Saayman 1989; Barson & Avenant-Oldewage 2006a) indicate that it is widely distributed in various countries.

In South Africa this species was found from *C. gariepinus* (Mashego 1977; Mashego *et al*. 1991; Barson & Avenant-Oldewage 2006a). This species occurs in the intestine but specimens were also procured in the gall bladder attaching to the main bile duct (Mashego 1977; Wabuke-Bunoti 1980). This necessitates the study of its life cycle of which little is known.

Mashego (1977) and Mashego *et al*. (1991) described the morphology of *P. clarias* as follows: “Cestode parasites of the proximal intestine and main bile duct of *Clarias gariepinus*; Scolex triangular, bearing a marginal crown of hooks of different sizes. Main crown subdivided into two circles by dorsal and ventral indentations of the disc margin. The hooks adjacent to the indentations are smaller than those in the middle of the circles. The ovary is large, compact and bilobed. Testes in lateral fields of proglottid. Genital atrium in mid-dorsal line, uterine pore on ventral surface. Uterus anterior to ovary, occupies the greater portion of the gravid proglottid. Strobilum acrespedote”.

In this study the parasites were also found from the intestines of *C. gariepinus* (n=53). It was found that the morphology (figure 3D.2) resembles the above description in all respects. These adult worms were however, of remarkably different sizes with respect to their scolices and proglottids. In many cases the worms were not complete with anterior ends separated from their posterior ends due to preparation. Some gravid proglottids were more than twice their counterparts in size. Their attachment resisted their removal from the intestinal mucosa, thus breaking the worm into pieces. The damage caused by attachment is rarely evident but if they occur in high numbers they cause tissue inflammation around the point of attachment (Paperna 1996). The photographs of *P. clarias* are on Plate 29.

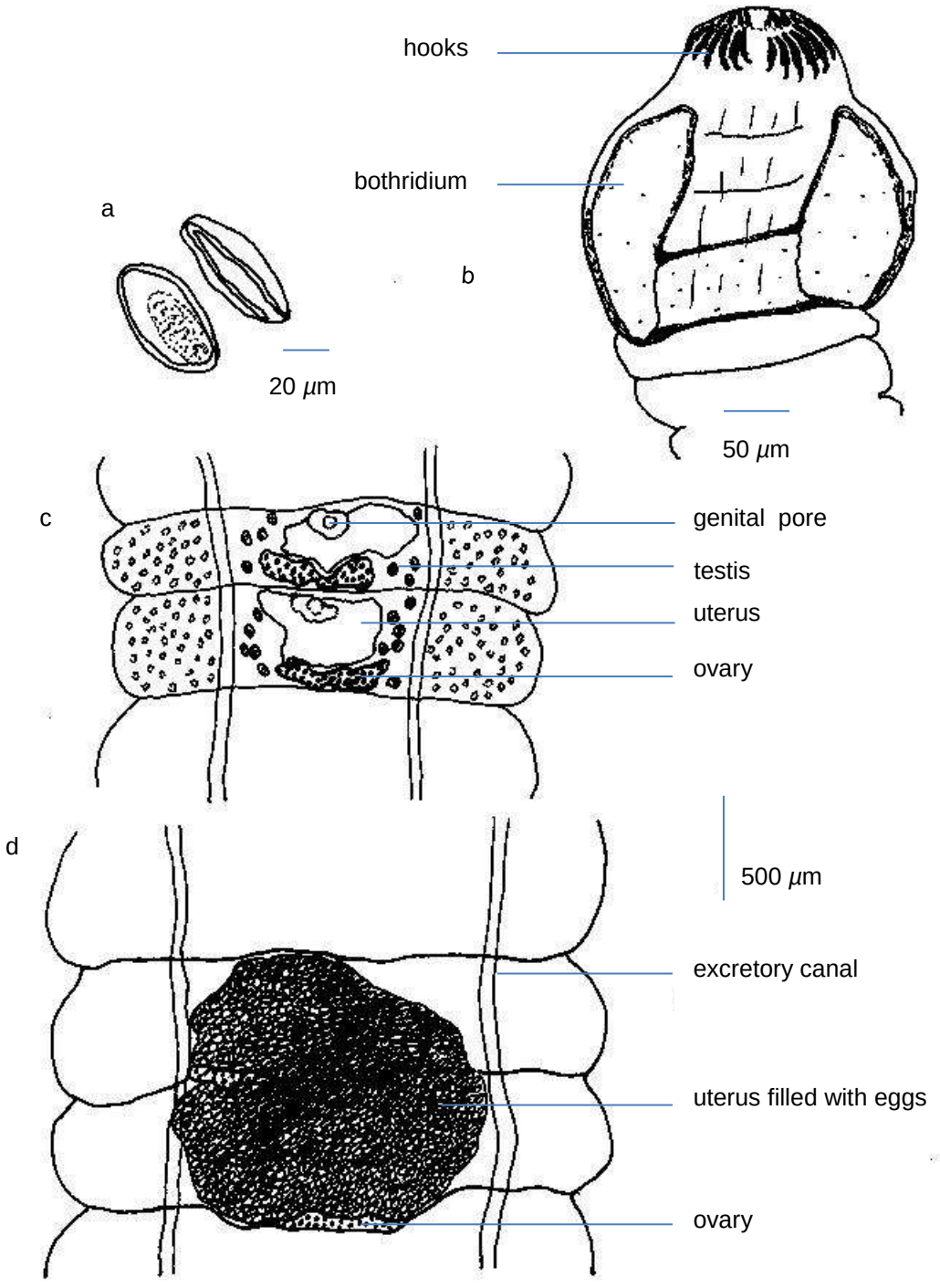
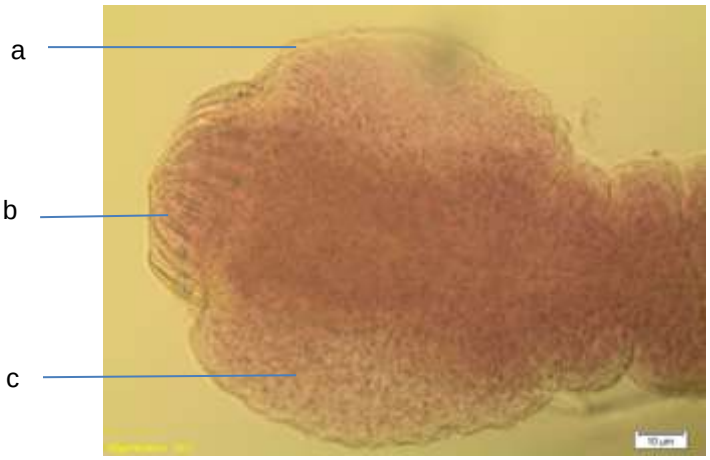
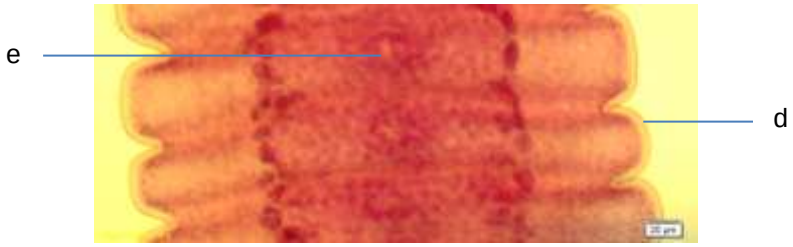
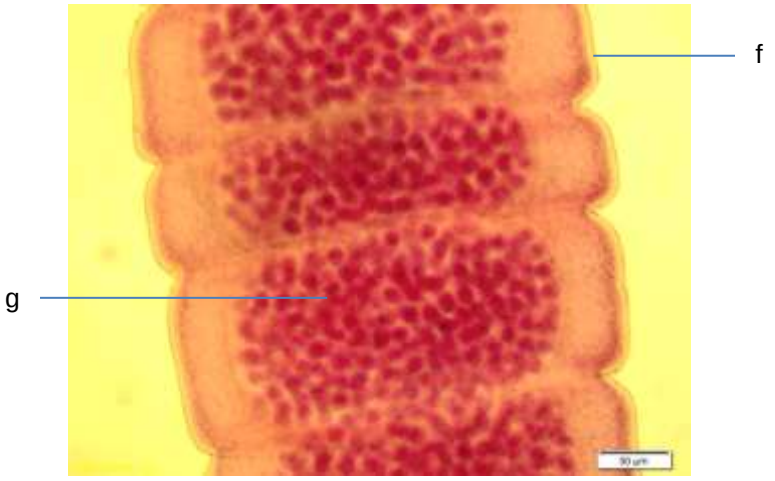


Figure 3D.2 *Polyonchobothrium clarias* Woodland, 1925 – a eggs b scolex c mature proglottid d gravid proglottid







a. scolex b. hooks c. bothridium d. mature proglottids e. genital pore
f. gravid proglottids g. uterine sac with eggs

Plate 29 *Polyonchobothrium clarias* Woodland, 1925

3 Genus ***Ligula*** Bloch, 1782

The *Ligula* larva is accommodated as a plerocercoid (an alacunate form with an everted scolex) (Freeman 1973). *Ligula* plerocercoid larvae are flat, unsegmented and have a tapering anterior end with two bothridia. Those from different host fish vary in size, which ranges from 6.7–24.5 cm in length, and 0.3–1.0 cm in width. The plerocercoids show very limited structural differentiation (Paperna 1996). Prudhoe & Hussey (1977) questions the conspecificity of *Ligula* from African fish with those from European fish, and if plerocercoids found in different fish families are of the same species. In Europe the plerocercoids from cyprinid fish develop to the adult stages mainly in gulls while in Africa the adults have been reported from cormorants and the darters (Prudhoe & Hussey, 1977; Mokgalong 1996).

3.1 ***Ligula intestinalis*** Goeze, 1782, plerocercoid

The plerocercoid of this cestode is cosmopolitan and present in the visceral cavities of a great variety of fishes. This may be made possible by the involvement of birds as final hosts that play a role in the distribution of the parasites (Dogiel *et al*. 1970; Khalil & Thurston 1973).

African studies on the plerocercoid larva of this species (Mahon 1954; Mettrick 1960; Williams & Chaytor 1966; Khalil 1973; Khalil & Thurston 1973; Brandt *et al.* 1980; Mashego 1982) indicate that various regions of the continent are affected. The South African records are from *Barbus* spp. (Brandt *et al.* 1980; Mashego 1982).

The life cycle of *L. intestinalis* needs at least three hosts to complete. According to Prudhoe & Hussey (1977), the first intermediate host is a copepod and it harbours a procercoid larva. If swallowed by a second intermediate host (a fish), the copepod releases the larvae into the intestine of a fish. These larvae burrow through the intestinal wall into the visceral cavity where they develop into the plerocercoid larvae. The plerocercoids develop rudimentary reproductive organs and grow large. In the alimentary canal of a piscivorous bird (final host) the larvae mature into adults within few days (Yamaguti 1959; Prudhoe & Hussey 1977).

In this study the plerocercoids were retrieved from the visceral cavities *of Barbus radiatus*, *Barbus unitaeniatus*, *Labeobarbus marequensis* and *Mesobola brevianalis* as well as the intestines of *Micropterus salmoides*. These fishes are the second intermediate hosts for this parasite. In the case of *Micropterus salmoides* the plerocercoids were procured from the intestines. The latter fish is carnivorous and acts as paratenic or transport host for the parasite species. The scolex bears no hooks and no suckers. The plerocercoid appears externally to have proglottids but these are not complete internally. There is a series of reproductive primordia bounded laterally by vitellaria and excretory canals on both sides (figure 3D.3). The photographs of *L. intestinalis* are on Plate 30.

The size of the plerocercoid larvae in the visceral cavity becomes so large that the infected, smaller fishes can be outwardly recognized by their bulged abdomens. When uncoiled the larva can be several times the length of the fish host and about 10% of their host weight (Paperna 1996). Mashego (1982) recorded the lengths 6.7-24.5 cm. In this study the plerocercoids measured 2.8-4.3 cm (n = 7) in *M. brevianalis*, 16.3 cm (n = 1) in *B. radiatus,* 17.8 cm (n = 1) in *L. marequensis,* 19.1 cm (n = 1) in *B. unitaeniatus* and 27.2 cm (n = 1) in *M. salmoides*.

Ligula intestinalis seems to be scarce in the larger *Labeobarbus marequensis* and more prevalent in the smaller *Barbus* spp. (Kennedy & Burrough 1981; Mashego 1982; Mashego *et al.* 1991).

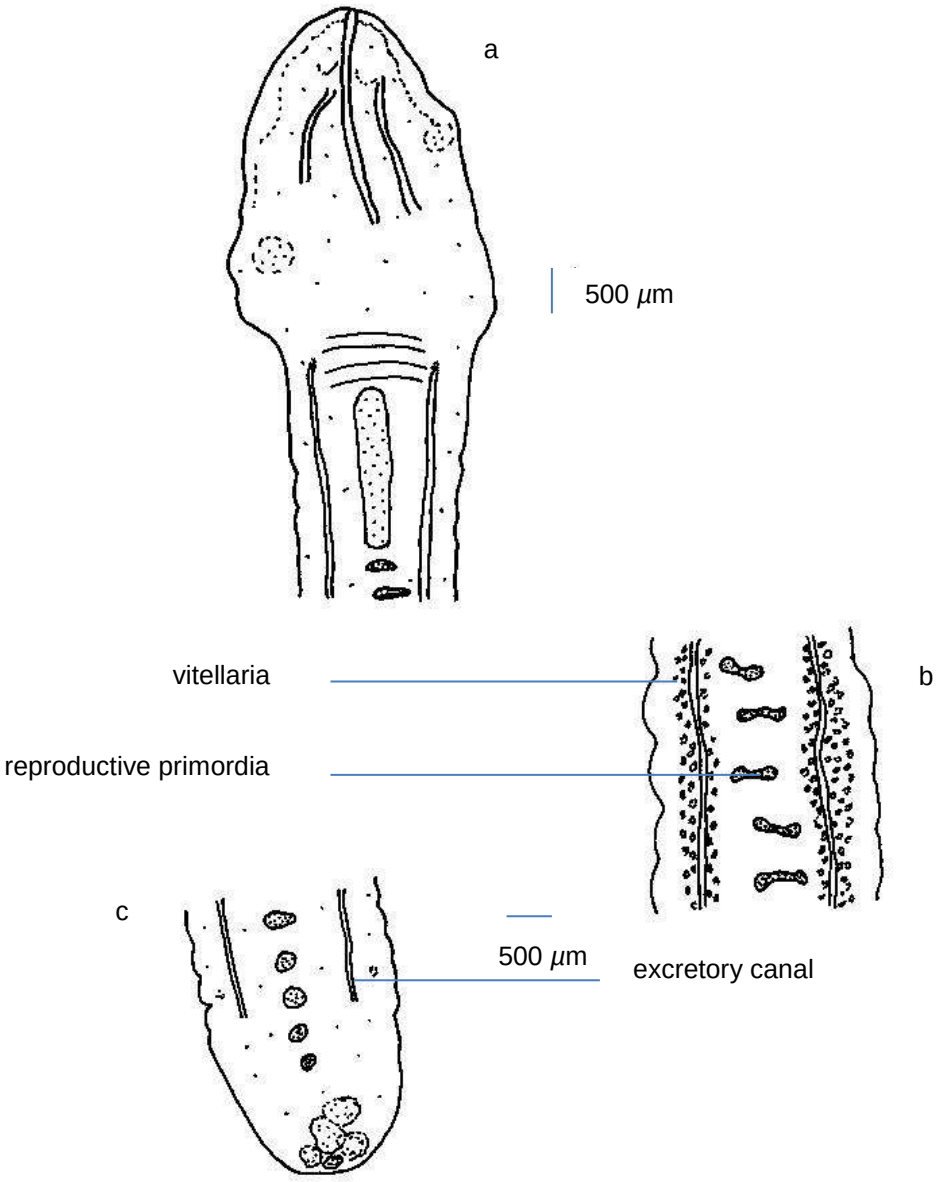
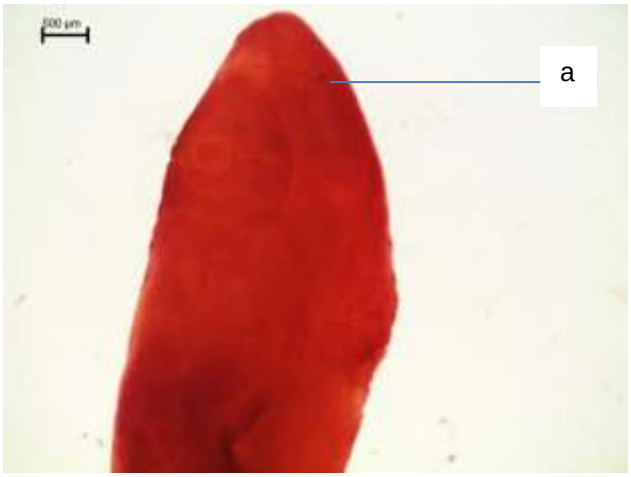
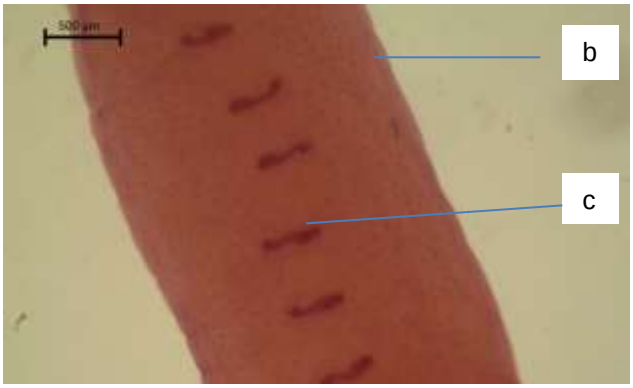
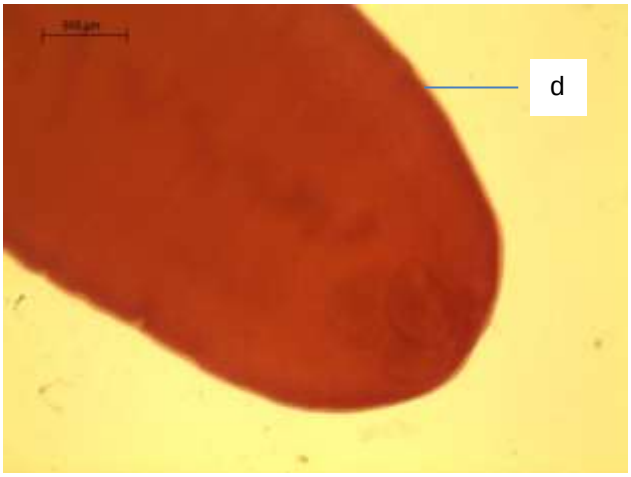


Figure 3D.3 *Ligula intestinalis* Goeze, 1782, plerocercoid – a anterior end with scolex b middle portion with proglottids c posterior end with proglottids







a. anterior end with scolex b. middle portion with proglottids c. reproductive primordial d. posterior end with proglottids

Plate 30 *Ligula intestinalis* Goeze, 1782, plerocercoid

Pathological effects caused by ligulosis have been reported in the northern hemisphere and include fibrosis, inflammation and atrophy of the viscera due to compression and displacement of organs by the parasites, accumulation of blood stained ascetic fluid and interruptions of reproductive functions (Paperna 1996).

4 Family **Gryporhynchidae**

Members of this family were previously placed in the Dilepididae (Cyclophyllidea). Spassky & Spasskaya (1973) proposed the Gryporhynchinae, later elevated to family level (Spassky 1995). The study on the phylogenetic analysis among the families of the order Cyclophyllidea based on comparative morphology (Hoberg *et al.* 1999) and molecular data (Mariaux 1998) equivocally confirmed the systematic position of the Gryporhynchidae as a separate family and as distinct from the Dilepididae. The family was erected to accommodate those species of dilepidids that mature in fish-eating birds and have larvae which occur in fish (Scholz *et al.* 2004).

The reviews of terminology associated with the nomenclature of larval cestodes or metacestodes (Freeman 1973; Jarecka 1975; Chervy 2002) caused further complications. Some terms are consistent, thus six basic types were identified (procercoid, plerocercus, plerocercoid, merocercoid, cysticercoid and cysticercus). The gryporhynchid larva is accommodated as plerocercoid (an alacunate form with an everted scolex) (Freeman 1973) and as merocercoids (an alacunate form with an invaginated scolex) (Jarecka 1975). As seen from the diagram of the gryporhynchid metacestode (figure 3D.4) it appears the latter is correct. Many other authors referred to them as cysticercus, plerocercus, and other names without really explaining or reviewing their origin.

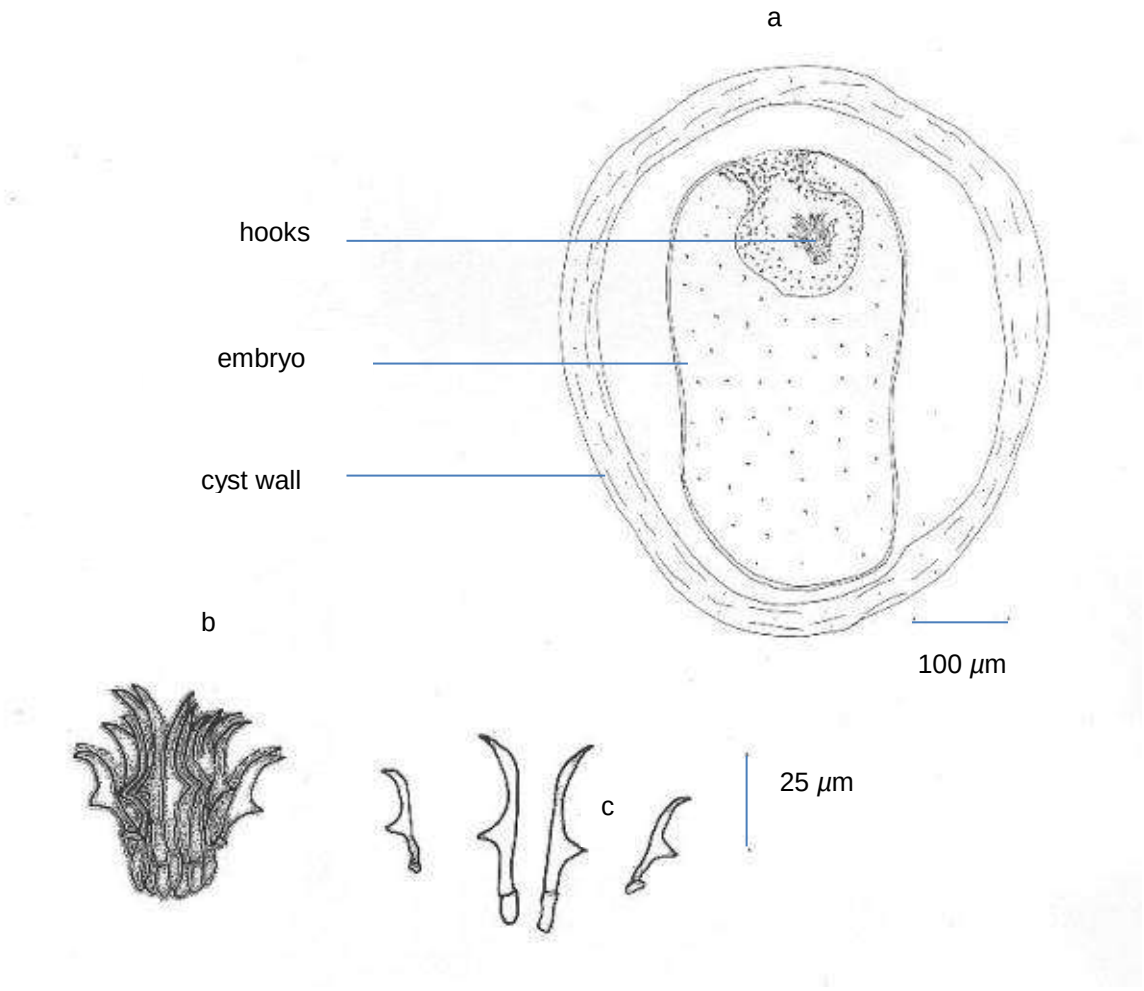


Figure 3D.4 Gryporhynchid metacestode - a cyst b hooks c small & large hooks

Generic diagnoses are mainly from adults that occur in birds and are based on rostellar hooks and reproductive organs (Mashego *et al*. 1991). The majority of papers report only the occurrence of larvae and there is scarcity of information on the taxonomy, life cycles, ecology and pathology. The site of infection of these metacestodes (mesenteries, liver, intestine wall etc.) partly relate to this state of affairs. Again, it is impossible to identify the metacestodes to the species level using morphological features (Mashego *et al*. 1991) and this is supported by numerous misidentifications and nomenclatural problems (Scholz *et al*. 2004).

The gryporhynchid metacestodes occur in many families of fishes. According to Scholz *et al*. (2004), cichlids harbor the highest number of gryporhynchid species while cyprinids are the most frequent fish intermediate hosts. African studies (Joyeaux & Baer 1935; Aderoumu & Adeniyi 1972; Khalil & Thurston 1973; Bray 1974; Mashego 1982; Mashego *et al*. 1991) indicate cichlids as principal intermediate hosts and cyprinids as mere reservoir hosts (Mashego *et al*. 1991).

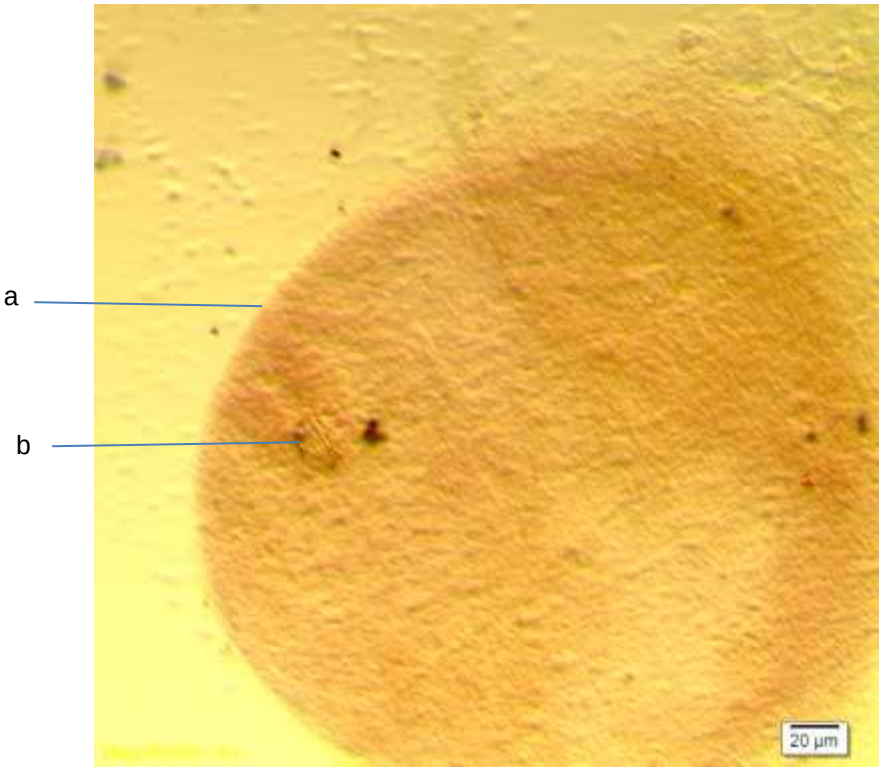
Reports on the geographical distribution of this larval group lack in that little has been done so far. Most data is from Europe and the former USSR (but with only three species). Canada and the USA have lots of misidentifications and unpublished work. Mexico alone has 13 species of larval gryporhynchids while in Africa there are doubtful records and most not identified to the species level (Scholz *et al.* 2004). It is suspected that the distribution of these metacestodes is far wider than indicated in the literature as they use birds as their final hosts.

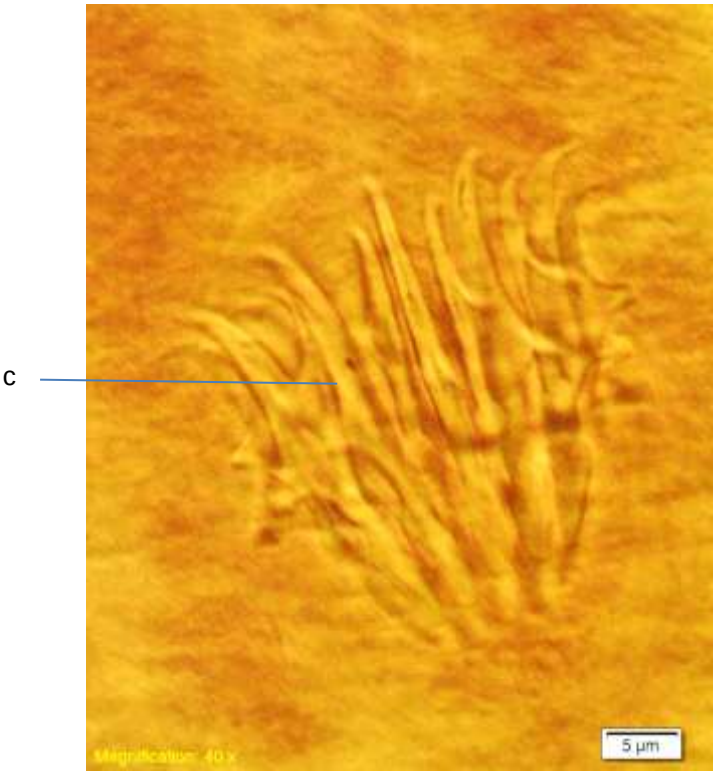
Data on the life cycle of gryporhynchids is limited to few experiments and studies. The copepods serve as primary intermediate hosts, fish as second intermediate hosts and fish-eating birds as definitive hosts. Many freshwater fish serve as hosts but few genera and species were found in brackish water fish and may be restricted to that water habitat.

In South Africa, studies on adult gryporhynchids (Prudhoe & Hussey 1977; Mokgalong 1996) are as few as on metacestodes (Mashego 1982; Mashego *et al*. 1991). From the birds three species have been found, namely *Paradilepis delachauxi, P. scolecina* and *Amirthalingamia macracantha*. The data from larvae were not precise in species diagnoses and the hosts are various fish species (Mashego *et al*. 1991). In this study the metacestodes of Gryporhynchidae were found encysted in the anterior third of the intestinal wall of *Oreochromis mossambicus* and *Tilapia rendalli*.

Table 3D.1 Measurements (in *μ*m) of rostellar hooks of gryporhynchid metacestodes

Species	Number	Large hook length	Small hook length	Source
Paradilepis delachauxi?	10 + 10	44-53	25-34	Present data
Paradilepis delachauxi	10 + 10	45-48	20-28	Khalil & Thurston 1973
Paradilepis scolecina	10 + 10	101-115	74-81	Scholz *et al.* 2004
Paradilepis cf. *urceus*	10 + 10	125-138	91-96	Scholz *et al.* 2004
Paradilepis caballeroi	12 + 12	110-121	83-88	Scholz *et al.* 2004
Paradilepis simoni	14 + 14	99-104	70-75	Scholz *et al.* 2004
Paradilepis rugovaginosus	16 + 16	93-108	67-76	Scholz *et al.* 2004
Amirthalingamia macracantha	10 + 10	390-480	240-290	Scholz *et al.* 2004
Ascodilepis transfuga	10 + 10	57-60	42-46	Scholz *et al.* 2004
Cyclustera cf. *ralli*	10 + 10	125-141	111-123	Scholz *et al.* 2004
Cyclustera magna	10 + 10	154-198	138-147	Scholz *et al.* 2004
Cyclustera ibisae	10 + 10	221-240	173-194	Scholz *et al.* 2004
Cyclustera capito	14 + 14	221-234	173-182	Scholz *et al.* 2004
Dendrouterina pilherodiae	10 + 10	48-49	40-45	Scholz *et al.* 2004
Neogryporhynchus cheilancristrotus	10 + 10	49-57	34-40	Scholz *et al.* 2004
Parvitaenia cochlearii	10 + 10	49-57	32-37	Scholz *et al.* 2004
Parvitaenia macropeos	10 + 10	43-46	26-30	Scholz *et al.* 2004
Glossocercus auritus	10 + 10	242-267	189-202	Scholz *et al.* 2004
Glossocercus caribaensis	10 + 10	189-211	124-146	Scholz *et al.* 2004
Glossocercus cyprinodontis	10 + 10	180-195	129-141	Scholz *et al.* 2004
Valipora campylancristrota	10 + 10	23-31	10-17	Scholz *et al.* 2004
Valipora minuta	10 + 10	36-40	18-22	Scholz *et al.* 2004
Valipora mutabilis	10 + 10	28-30	13-16	Scholz *et al.* 2004





a. cyst wall b. hooks in embryo c. hooks enlarged

Plate 31 Gryporhynchid metacestode

The encysted metacestode (figure 3D.4) shows the embryo while still in the cyst on the wall of the intestine with hooks. Khalil & Thurston (1973) found the metacestodes of *Paradilepis delachauxi* in the intestinal body wall in several stages of development. The biometrical data of the hooks from several species (Scholz *et al.* 2004) were compared with the present material (table 3D.1). By elimination, also using country/continent, host and site records, *Paradilepis delachauxi* is a probable candidate, but this cannot be confirmed without traces of some doubt. The photographs of gryporhynchid metacestodes are on Plate 31.

The pathological concern has been reported where there is a burden of larvae in which the growth of fish was retarded in fish rearing ponds (Scholz *et al.* 2004).

3D.4 GENERAL DISCUSSION

The existing knowledge about all the cestode parasites found in this study is fragmentary. For all these parasites one or more of their vital information (larval identification, life cycle, geographic distribution etc.) is missing due to fewer studies done on these cestodes, especially in Africa. The data also indicates that with the larval cestodes found in this study, there is no uniform pattern in the degree of specificity at the level of fish intermediate host, more so, also no site preference in gryporhynchid larvae.

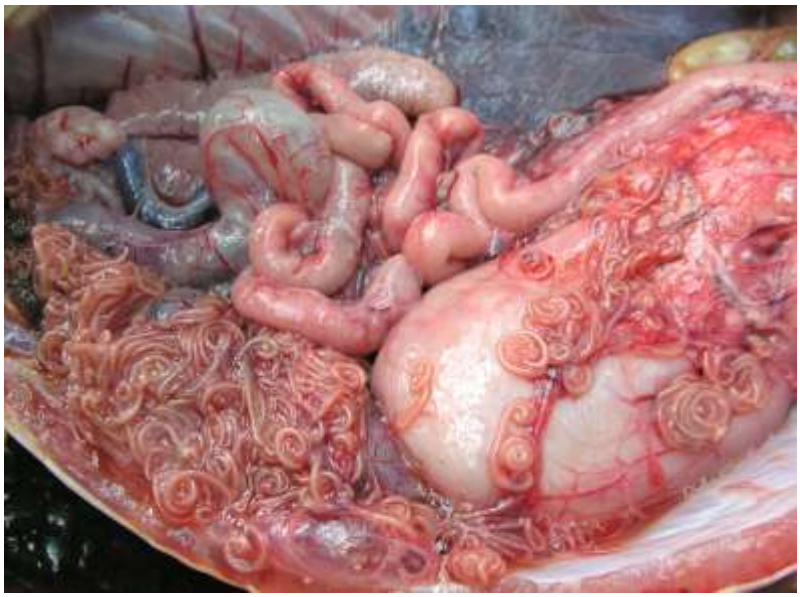
The supra-population of *Ligula intestinalis* may be well established in Lake Tzaneen with four intermediate and one paratenic fish hosts already. More hosts may be infected in the future as some fishes (*Barbus trimaculatus* and *Labeo* spp.) were found harbouring this parasite in some dams (Khalil 1973; Mashego 1982; Mashego *et al.* 1991).

Concurring with Scholz *et al.* (2004), future studies should focus wider on the missing data on the diversity, host specificity and distribution of these cestodes. Searching for the larvae previously over-looked, appropriate methods for fixing and processing, and the molecular characterization of these worms are necessary to provide new, reliable information about these cestode parasites.

CHAPTER 3

SECTION E

NEMATODA



They occur in large numbers. Can you count?

CONTENTS

3E.1 INTRODUCTION

The Nematoda are characterized by a cylindrical, filiform, or fusiform body with a cuticle that is smooth or have bristles and other types of ornamentations. The digestive system opens anteriorly by oral aperture usually (but not always) encircled by lips bearing sensory organs and with anus (or cloaca) on ventral surface terminally or subterminally. They are dioecious with rare exceptions. Genital tracts in male open by cloaca, in female by separate gonopore. Excretory system is of lateral canals and single excretory cells (no protonephridia or nephridia). Vascular system is absent (Skrjabin 1949).

The parasitic forms display all degrees of parasitism and attack virtually all groups of plants and animals (vertebrates and invertebrates) except that there are no ecto-parasitic forms on animals (Barnes 1974). The fish nematodes occur as endoparasites either as larval forms or adults. In the case where fish is the second intermediate host, aquatic invertebrates (copepods, amphipods, oligochaetes, etc.) serve as first intermediate hosts while birds or mammals are the final hosts. The adult fish nematodes use aquatic invertebrates as intermediate hosts (Mashego *et al*. 1991).

The records on fish nematodes worldwide are too numerous to mention. The same may also hold for Africa with many nematode families represented. In Southern Africa the few studies on fish nematodes (Yeh 1957; Moravec & Puylaert 1970; Khalil 1974; Prudhoe & Hussey 1977; Mashego 1977, 1982, 1989, 1990; Mashego & Saayman 1981; Boomker 1982, 1993a & b, 1994; Mashego *et al.* 1991; Chishawa 1991; Douëllou 1992; Boomker & Petter 1993; Boomker & Puylaert 1994; Barson 2003; Luus-Powell 2004; Barson & Avenant-Oldewage 2006b; Moyo *et al*. 2009; Madanire-Moyo & Barson 2010) indicate that not more than 15 nematode genera are represented. Few other studies (Ortlepp 1938; Whitefield & Heeg 1977; Mokgalong 1996) recorded the adults of these fish nematodes as found in piscivorous bird hosts.

In this study one larval and two adult nematode species are recorded from Lake Tzaneen. *Procamallanus laevionchus* was procured from the stomach and intestine

of *Clarias gariepinus* and *Paracamallanus cyathopharynx* was retrieved from the intestine of *C. gariepinus* and *Schilbe intermedius*. The larval forms of *Contracaecum* sp. were present within the peritoneum of the visceral cavities of *C. gariepinus, S. intermedius*, *Oreochromis mossambicus*, *Tilapia rendalli*, *Labeobarbus marequensis, Barbus trimaculatus* as well as in the pericardial cavity of *O. mossambicus* and the intestine of *Micropterus salmoides.*

3E.2 MATERIALS AND METHODS

Collecting the nematodes alive was preferable as they were in a good condition for fixing. The specimens were washed in distilled water to remove mucus and other debris and then placed in petri dishes where they were killed and fixed in glacial acetic acid. Ethanol (70%) was used for preservation. Temporary mounts were done on microscopic slides and the worms were drawn, photographed and identified.

3E.3 RESULTS AND DISCUSSION

1 Genus ***Procamallanus*** Baylis, 1923

According to Yamaguti (1961), the generic diagnosis is as follows: “Camallanidae; Buccal capsule continuous and not separated into paired lateral valves, the walls of the capsule may be smooth or provided with spiral thickenings; tridents absent; oesophagus divided into an anterior muscular, and a longer posterior glandular part. Male posterior extremity curved ventral; tail conical. Caudal alae present, uniting in front with 3 to 9 pairs of post-anal papillae; smaller additional papillae may be present out of the series. Spicules unequal. Female posterior extremity conical, ending in 3 very short blunt processes; vulva in front of middle of the body; posterior limb of uterus ending blindly. Viviparous. Parasites of silurid fishes, occasionally of amphibians”.

Yamaguti (1961) listed 34 species of the genus. There are several species worldwide with hosts representing various freshwater fish families, but other fishes

and frogs are also hosts. The genus is found with many species mainly in freshwater fishes of Tropical Asia. In Africa only one species is present in freshwater fish and two in frogs. It is absent in Europe, North and South America and Australia where only related genera (*Camallanus* & *Spirocamallanus*) are present (Stromberg & Crites 1974).

1.1 ***Procamallanus laevionchus*** (Wedl, 1861)

Several studies have been conducted in Africa (Wedl 1861; Baylis 1923; Campana-Rouget 1961; Khalil 1969, 1970; Vassiliades 1972, 1973; Khalil & Thurston 1973; Moravec 1974a, 1975; Mashego 1977; Mashego & Saayman 1981; Boomker 1982, 1994; Imam *et al*. 1991; Mashego *et al.* 1991; Chishawa 1991; Douëllou 1992; Barson 2003; Oniye *et al.* 2004; Barson & Avenant-Oldewage 2006b; Akinsanya & Otubanjo 2006; Ayanda 2008, 2009a & b; Mwita & Nkwengulila 2008; Owolabi 2008; Madanire-Moyo & Barson 2010) and they all indicate that within the genus *Procamallanus,* only one species, *P. laevionchus* is found from different freshwater fishes in various countries of the continent.

Myers *et al.* (1962) reported on *Procamallanus* sp. in Egypt from three different fish species. The other species were reclassified as *Spirocamallanus mazabukae* and *S. spiralis* (Khalil & Polling 1997). *Procamallanus laevionchus* is one of the most frequent and widespread nematode parasites of African freshwater fishes (Moravec 1975; Mashego *et al*. 1991) with hosts including members of the families Siluridae, Mormyridae, Characidae, Tetraodontidae and Cichlidae. In South Africa this nematode species has been found only in *Clarias gariepinus* stomach and intestine.

The diagnostic characteristics of *P. laevionchus*, according to Baylis (1923), are the following: “Cuticle striated; buccal capsule continuous and not separated into paired lateral valves; wall of buccal capsule smooth and not provided with spiral ribs; oesophagus differentiated into anterior muscular and posterior glandular portions; vulva in front of middle of the body; viviparous”. The buccal capsule, oesophagus, female reproductive system (ovary, oviduct, uterus filled with eggs and larvae, vagina and vulva) and the posterior of female worm are shown in figure 3E.1.

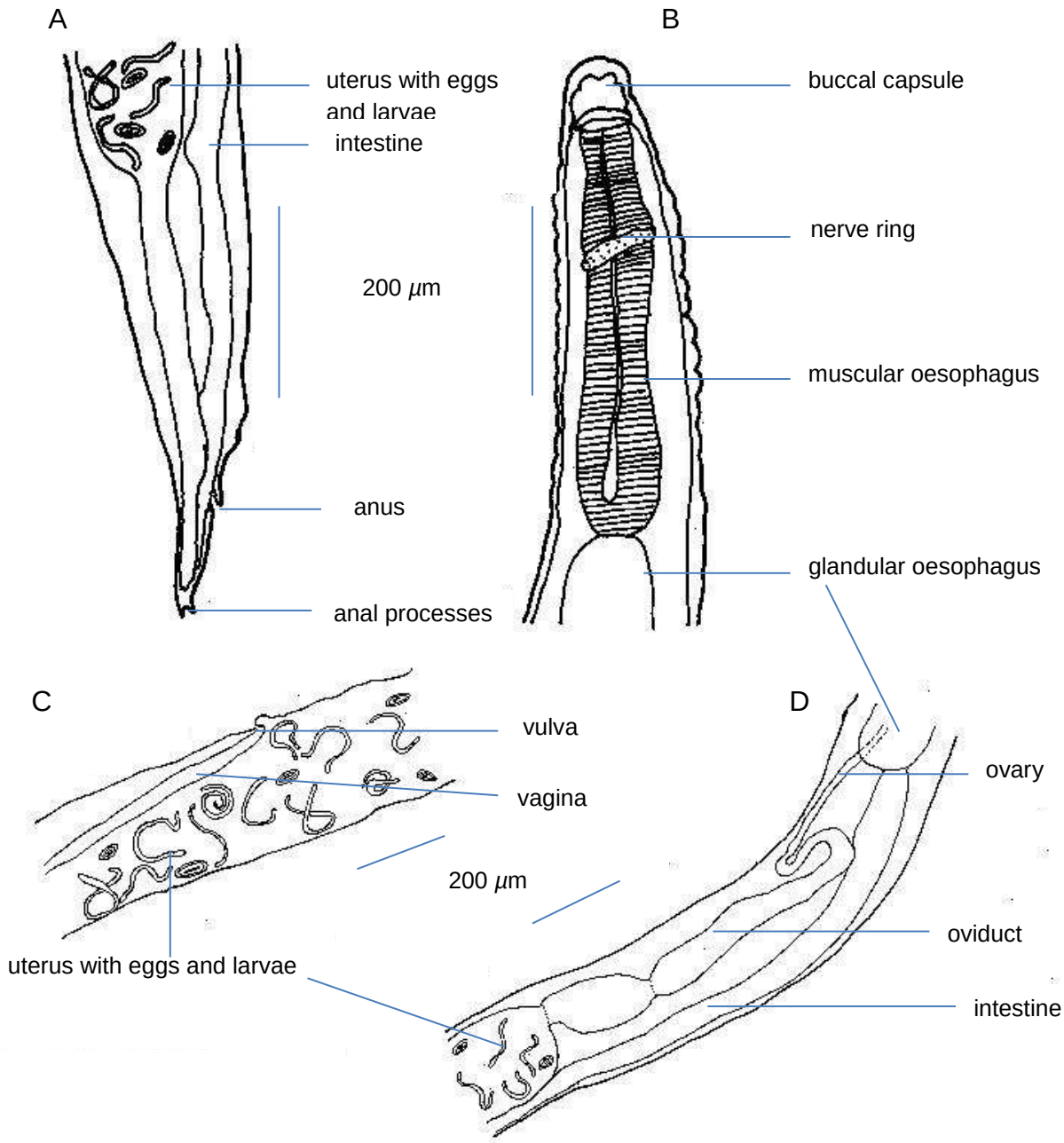


Figure 3E.1 *Procamallanus laevionchus* (Wedl, 1861) - A posterior end B anterior end C & D female reproductive system

The photographs of the above are shown in Plate 32. The female worms in this study (n=5) have a length range (6.5-8.7 mm) comparable with the range (7.0-8.9 mm) found by Boomker (1982) and the range (6.2-8.9 mm) found by Barson & Avenant-Oldewage (2006b).

According to Paperna (1996), even though infections by *P. laevionchus* are abundant and heavy (up to 20 or more) none were reported as pathogenic in respect of their buccal capsule attachment to the stomach or intestinal lining.

2 Genus ***Paracamallanus*** York and Maplestone, 1926

According to Yamaguti (1961), the generic diagnosis is as follows: “Camallanidae: Closely resembling *Camallanus* but differing in the presence of a large chitinous buccal cavity or pharynx behind buccal valves. Parasites of fishes”. Only two species occur in freshwater fishes of Tropical Asia whilst one species occurs in African freshwater fish. Like *Procamallanus*, it is absent in Europe, North and South America and Australia where only related genera (*Camallanus* & *Spirocamallanus*) are present (Stromberg & Crites 1974).

2.1 ***Paracamallanus cyathopharynx*** Baylis, 1923

African studies on the species (Baylis 1923; Campana-Rouget 1961; Myers *et al.* 1962; Khalil 1969; Vassiliades 1970, 1972; Moravec 1974a & b; Mashego 1977; Fahmy *et al.* 1978; Shotter, R. A. 1980; Mashego & Saayman 1981; Boomker 1982, 1994; Imam & El-askalany 1990; Mashego *et al.* 1991; Chishawa 1991; Douëllou 1992; Akinsanya & Otubanjo 2006; Akinsanya *et al*. 2007; Barson *et al*. 2008; Mwita & Nkwengulila 2008; Moyo *et al.* 2009; Madanire-Moyo & Barson 2010; Madanire-Moyo *et al.* 2010) have found only *Paracamallanus cyathopharynx* as the only species of African freshwater fishes. Ayanda (2008, 2009a & b) have found *Paracamallanus* sp. in Nigeria but this might have been *P. cyathopharynx*.

Baylis (1923) gave the species diagnosis as follows: “Large, chitinous buccal cavity or pharynx behind the paired buccal valves. Buccal valves provided with 10 – 12 longitudinal ribs of irregular lengths. Maximum length of males 5.9 mm, females 9.2 mm. Oesophagus (= pharynx) consisting of both muscular and glandular portions. General characteristics of female organs are those typical of *Camallanus*. Vulva situated slightly behind middle of body and without prominent lips. Viviparous”.

Figure 3E.2 shows the buccal cavity with longitudinal ribs, the buccal valves, the muscular and glandular portions of the oesophagus, the vulva, the uterus filled with eggs and larvae and the posterior end of female. These characteristics, typical of *P. cyathopharynx* can also be seen from Plate 33. The female worms in this study (n=10) have a length range (8.3-12.1 mm) compatible with the range (11.4-12.5 mm) found by Boomker (1982) and the range (5.2-9.1 mm) found by Barson *et al*. (2008).

P. cyathopharynx is a common parasite of catfishes of the family Clariidae in Africa. It is an ovoviviparous camallanid nematode whose larvae are liberated into the gut of the host and pass out with the faeces (Moravec 1974b). Most studies in Africa have found *C. gariepinus* as the host except those of Boomker (1994) with three host species (*Schilbe intermedius*, *Hydrocynus vittatus* & *Synodontis zambezensis*) and Campana-Rouget (1961) with *Heterobranchus longifilis* as another host.

3 Genus ***Contracaecum*** Railliet and Henry, 1912

According to Yamaguti (1961), the diagnosis of the genus is as follows: “Filocapsulariinae: Lips without dentigerous ridges; interlabia present, usually well developed. Ventriculus reduced,with solid posterior appendix. Intestinal cecum present. Male: without definite caudal alae. Postanal papillae up to seven pairs, partly subventral and partly lateral. Preanal papillae numerous. Spicules long, alate, equal or subequal; gybernaculum absent. Female: Vulva in anterior region of body. Oviparous. Parasites of fishes, birds and piscivorous mammals”.

There are many species of the genus identified from fishes (77), birds (61) and mammals (10) as listed by Yamaguti (1961).

3.1 *Contracaecum* sp. larvae

African studies that included this fish larval nematode (Baylis 1930, 1940; Myers *et al*. 1960, 1962; Campana-Rouget 1961; Khalil 1974; Paperna 1974; Mashego 1977, 1982, 1989; Prudhoe & Hussey 1977; Malvestuto & Ogambo-Ongoma 1978; Imam *et al*. 1979; Mashego & Saayman 1981; Mashego *et al.* 1991; Boomker 1982, 1994; Aloo 1999; Barson & Avenant-Oldewage 2006b; Kaddumukasa *et al*. 2006; Barson *et al*. 2008; Mwita & Nkwengulila 2008; Kassaye & Tadesse 2009; Moyo *et al.* 2009; Madanire-Moyo & Barson 2010; Madanire-Moyo *et al*. 2010) have confirmed beyond doubt the difficulty in identifying the larvae to the species level. Khalil & Polling (1997) have listed the *Contracaecum* sp. larvae without any species differentiation.

The life cycle involves the first stage larva (coracidium) in a microcrustacean, the second (procercoid) and the third stage (plerocercoid) in a fish, and the last moult in the gut of a suitable bird where the worm matures. The adult worms may be transferred from the gut of the parent bird to its offspring (Prudhoe & Hussey 1977). It is the plerocercoid larvae that were procured from the fish for study.

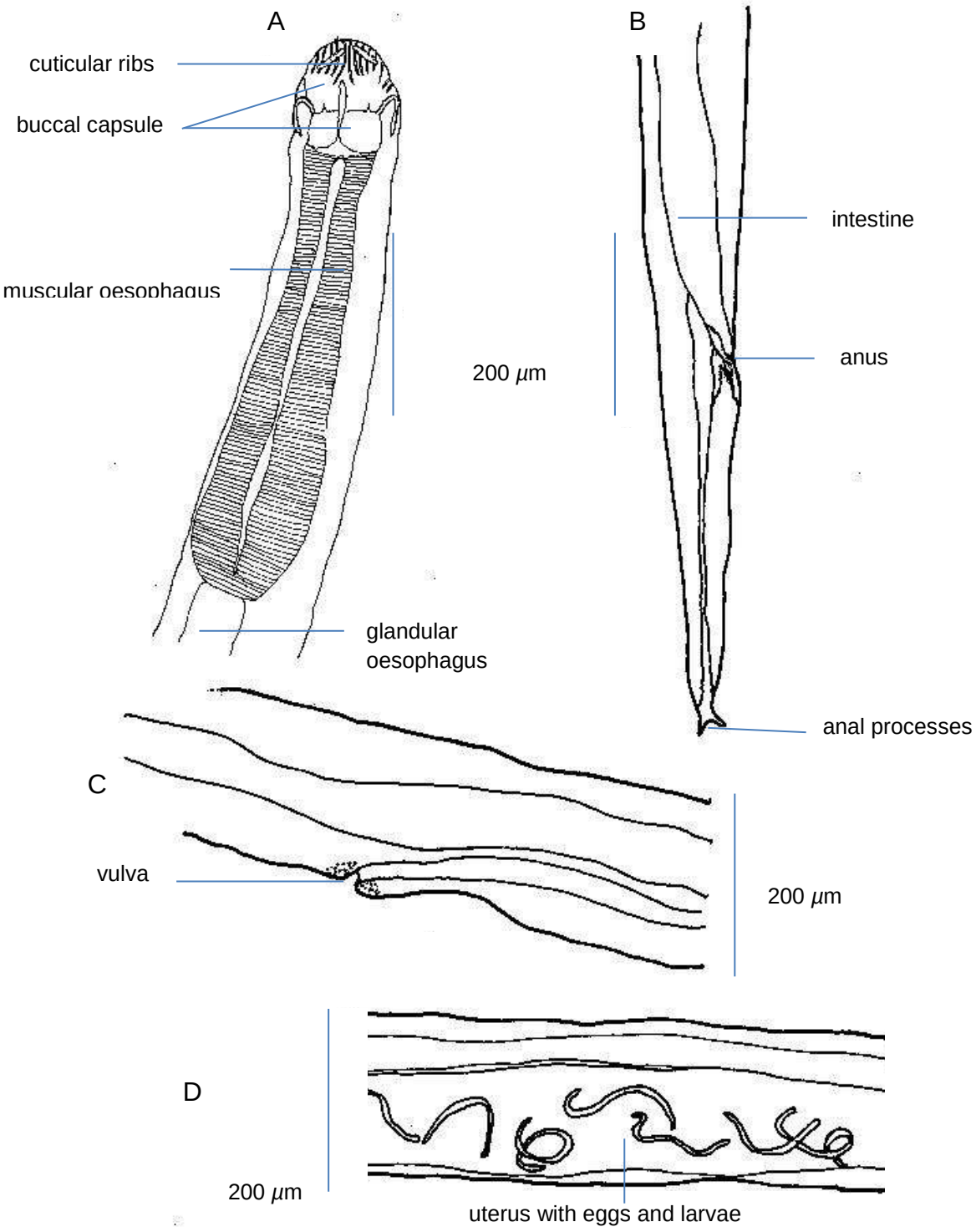


Figure 3E.2 *Paracamallanus cyathopharynx* Baylis, 1923 - A anterior end B posterior end C & D female reproductive system

The appearance of the generic features of the alimentary system, namely the intestinal caecum and the posterior appendix to the ventriculus in the early stages of larval development in *Contracaecum* have made it, at least, identifiable to the genus level, a condition not possible with larvae of other anisakid nematodes. However, it is impossible to diagnose *Contracaecum* larvae specifically as none of the diagnostic features of the adults develop until the last moult of the worm in the final hosts (Prudhoe & Hussey 1977). The intestinal caeca, ventricular appendix and the curved tail with a spine are visible features (figure 3E.3). These morphological characteristics are also shown in Plate 34. The larvae in this study (n=30) had a length range (6-38 mm; mean 28mm) compatible with the range (22-35 mm; mean 27.6) found by Barson & Avenant-Oldewage (2006b).

There are not less than nine species of *Contracaecum* identified from African birds (Canaries & Gardner 1967), with at least seven species present in South Africa (Mashego 1989). It has not been possible yet to differentiate the species from larvae in fishes (Mashego 1982). The adult *Contracaecum* species from African piscivorous birds were studied by Ortlepp (1938, cited by Mokgalong 1996), Canaries & Gardner (1967), Mokgalong (1996) and Barson & Marshall (2004). Likewise, it was not possible to determine the number of *Contracaecum* species in this study.

While the larvae usually infect the mesentery of the body wall or visceral cavity, infection of the pericardia is common in the cichlid fishes, and in this case *O. mossambicus.* The larvae in the pericardia were free and not encapsulated as in the visceral cavity. Aloo (1999) reported on *Contracaecum* in the caecum of the black bass (*Micropterus salmoides*) as a case of paratenic host. In this study, the larvae were also found in the intestine and this indicates the role of the bass as carnivorous on other fish species that harbour *Contracaecum* larvae.

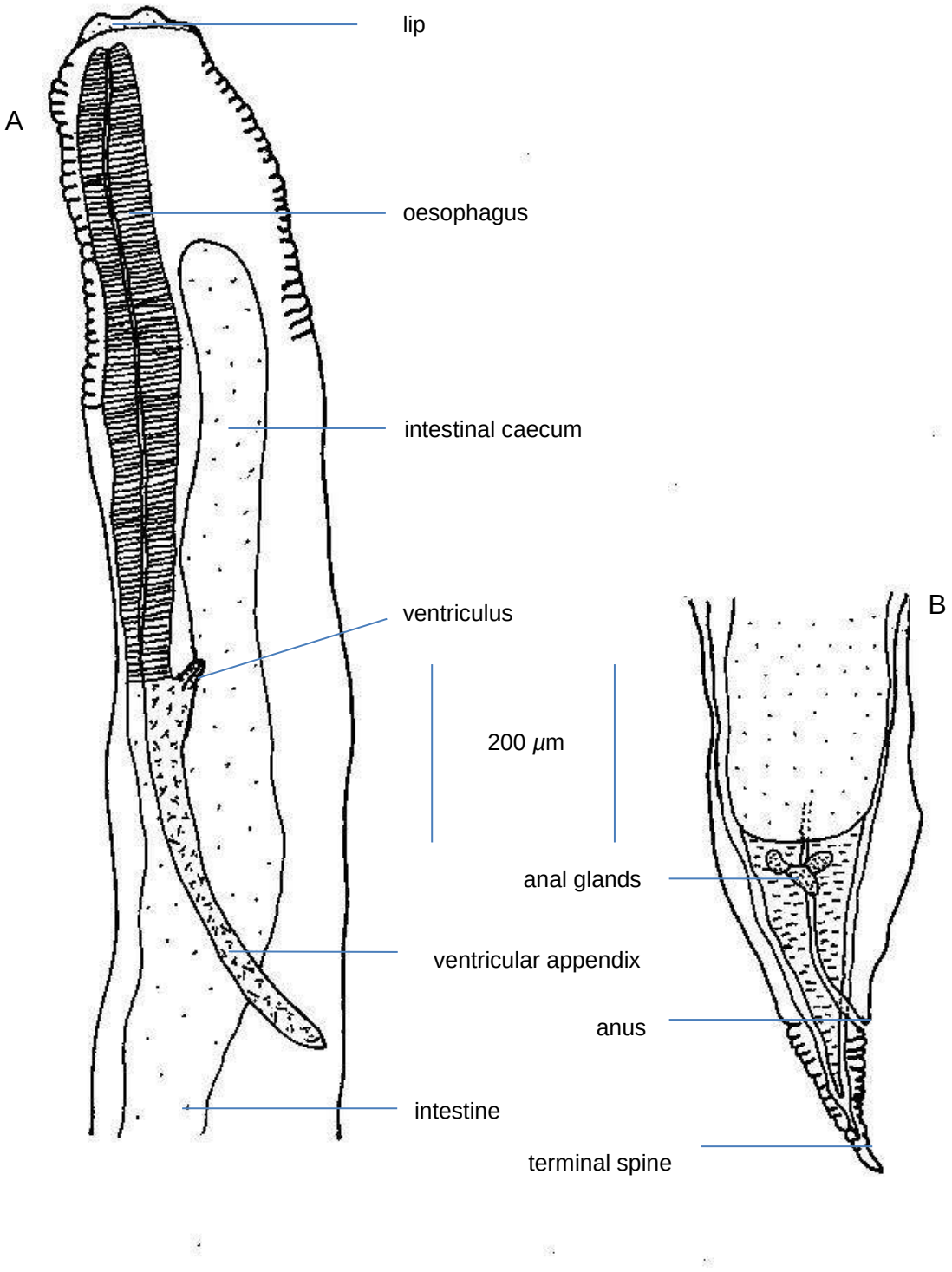


Figure 3E.3 *Contracaecum* sp. larva - A anterior end B posterior end

3E.4 GENERAL DISCUSSION

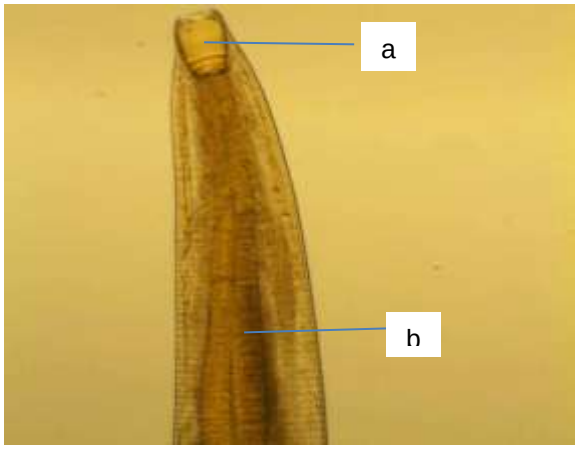
The nematodes are easily recognizable as most are large enough to be visible to the naked eye. Their shape is also distinctive and because of a solid, resistant cuticle they last longer after death (Paperna 1996). More experience is needed in nematological studies, however, in identifying the specimens to the genus or species level (Paperna 1996).

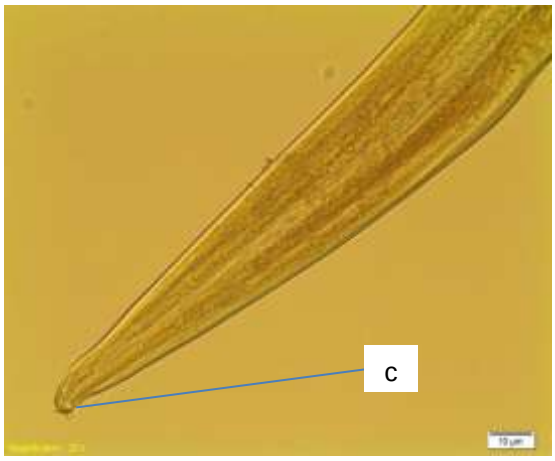
The adult forms of the two species *Procamallanus laevionchus* and *Paracamallanus cyathopharynx* use fish as their final hosts. They use a wide variety of fish hosts in Africa with *P. laevionchus* so far hosted by 10 genera within 6 families and *P. cyathopharynx* uses 5 genera of fishes (Paperna 1996; Khalil & Polling 1997). Their life cycles include copepods as intermediate hosts even though experimentally they rejected some common ones (Paperna 1996). Some species in these genera or their closely related genera in the family Camallanidae also use frogs as their final hosts (Stromberg & Crites 1974). While *P. laevionchus* seems to be more widely distributed than *P. cyathopharynx* in terms of host variety, it is the latter that is usually found in higher prevalence and intensity when present. Despite their occurrences in higher numbers, they do not seem to cause worrying pathological disorders (Paperna 1996).

The larval form of fish nematodes present in Lake Tzaneen is *Contracaecum.* As far as the intermediate fish host is concerned they seem not to be host specific. One reason is that they are only known to genus and not species level at the larval stage. Host specificity has been reported in as far as the final bird host (Mashego *et al.* 1991). The common bird hosts for *Contracaecum* are pelicans, cormorants and herons (Prudhoe & Hussey 1977; Boomker 1982).

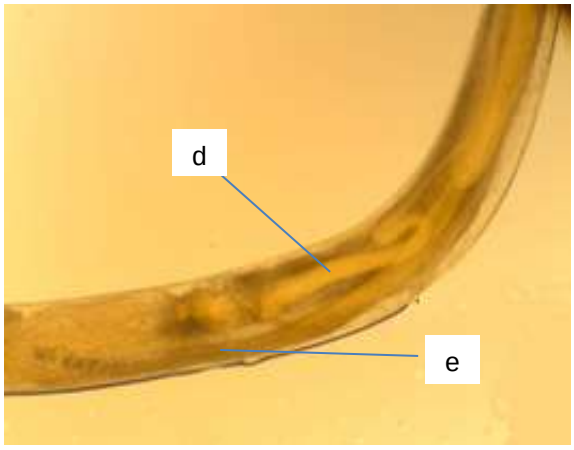
As there is an increase in studies involving larval nematodes of fish so must the studies on birds involving adult nematodes increase. Such studies are few in Africa but necessary to correlate with studies on larval forms from fish in a particular area

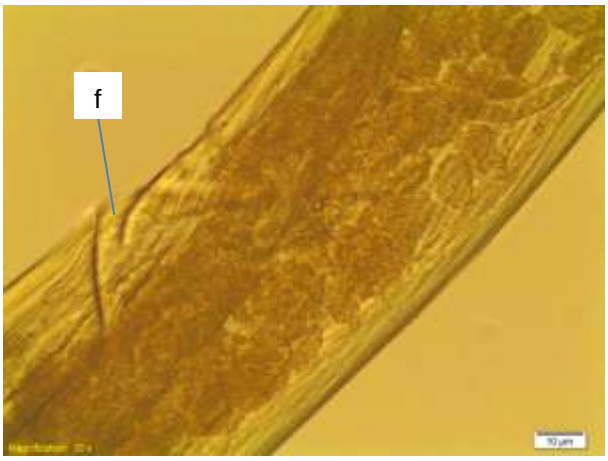
to match a larva to an adult. In cases where this is difficult a biochemical method using electrophoresis (Paperna 1996) to identify the larvae of Anisakidae or genetic studies should be included.





anterior end posterior end

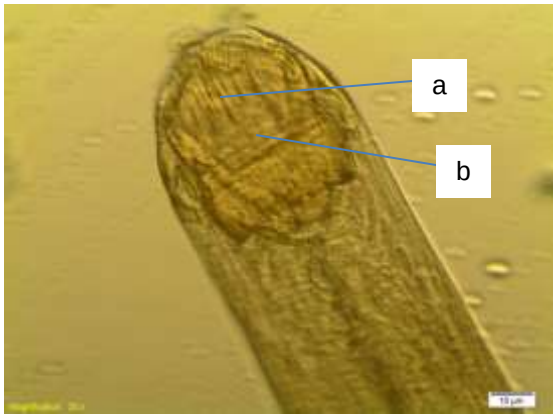




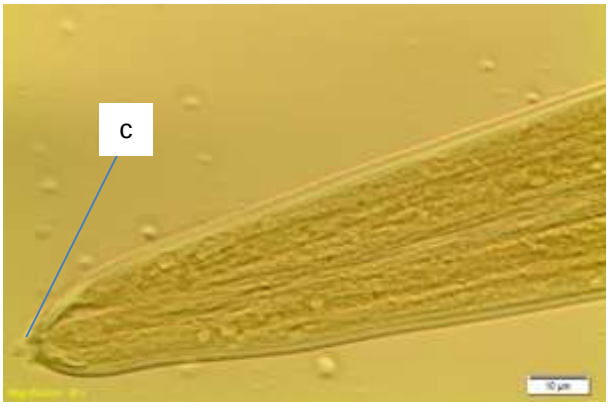
anterior female reproductive system posterior female reproductive system

a. buccal capsule b. oesophagus c. terminal spine d. oviduct e. intestine

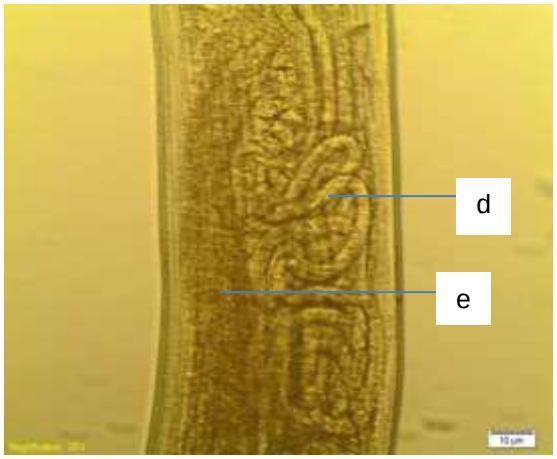
Plate 32 *Procamallanus laevionchus*



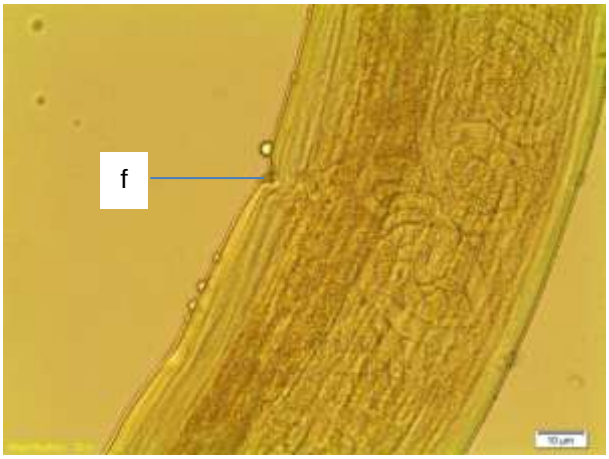
anterior end



posterior end



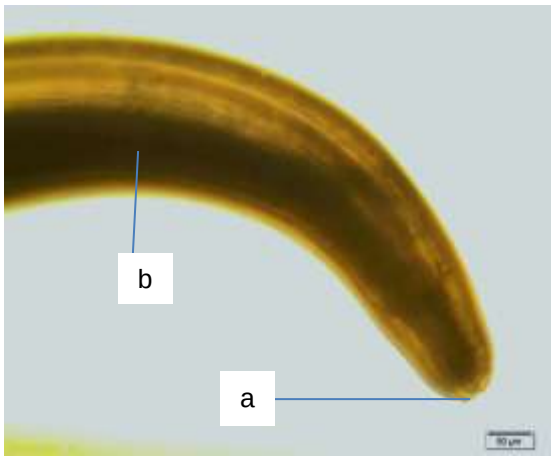
larvae in uterus



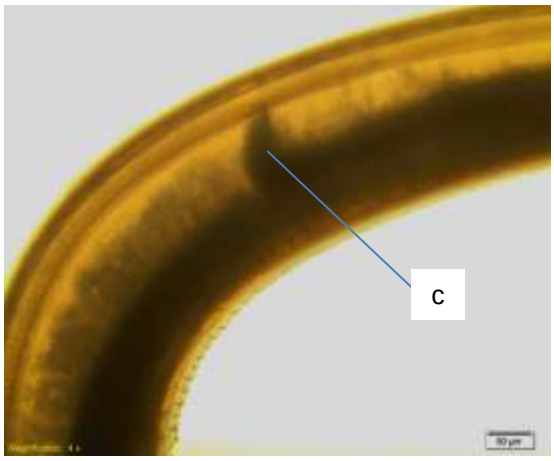
region of vulva

a. cuticular ribs b. buccal capsule c. terminal spine d. uterus e. intestine f. vulva

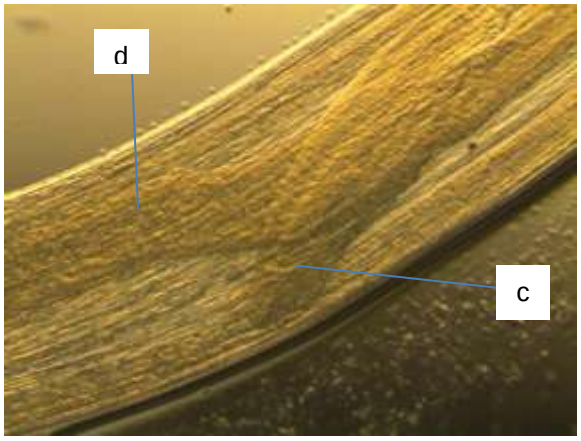
Plate 33 *Paracamallanus cyathopharynx*



anterior end



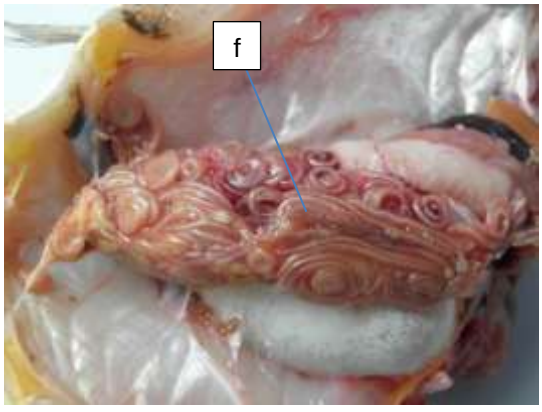
anterior end enlarged



anterior end enlarged

posterior end

complete larva



larvae in mesentery of body cavity

a. lip b. intestinal caecum c. ventricular appendix d. intestine e. terminal spine f. larvae in situ

Plate 34 *Contracaecum* sp. larva

CHAPTER 3

SECTION F

ACANTHOCEPHALA

Contests for a home in the intestines

CONTENTS

3F.1 INTRODUCTION

The Acanthocephala are parasites of peculiar structure whose distinctive feature, the anterior cylindrical proboscis bearing rows of spines, resulted in their name which means “spiny headed”. They live as larvae in arthropods and as adults in the intestines of vertebrates (fishes to mammals) where they attach with a proboscis to the host’s gut. Insects serve as intermediate hosts in species with terrestrial final hosts and crustaceans in aquatic environments (Storer *et al*. 1972).

The males are usually smaller than females with gonads in ligaments between the proboscis sheath and posterior end. Males have two testes and the ovaries of females are non-persistent. The lemnisci are present anteriorly to aid with proboscis retraction. Epidermis bears large nuclei and covers the pseudocoel. There are no digestive, circulatory and respiratory organs (Storer *et al*. 1972).

There are several studies that were conducted on fish acanthocephalans in Africa and they reveal 9 genera in 6 families from various fishes in not less than 12 countries of the continent (Khalil & Polling 1997). In South Africa there are 3 genera of acanthocephalans found from freshwater and marine fish (Mashego 1988). One of these is from a freshwater fish and is the only genus (this study included) found thus far. From South Africa only two reports (Mashego 1982, 1988) feature in the African literature on acanthocephalans of freshwater fish and both reports were actually from the same study.

In this study, only one species of Acanthocephala, namely *Acanthogyrus* (*Acanthosentis*) *tilapiae* (Baylis, 1948) was procured from the intestines of *Oreochromis mossambicus* in Lake Tzaneen. Batra (1984) and Douëllou (1992) found *A. (A.) tilapiae* from *Tilapia rendalli* in Zambia and Zimbabwe respectively, but this fish was found to host no acanthocephalans in this lake.

3F.2 MATERIALS AND METHODS

In analyzing the gut contents, the worms that were found were placed in distilled water. The petri dishes were then placed in a refrigerator until the worms were relaxed with the proboscis extended. They were killed in hot AFA and preserved in 70% ethanol. The standard procedure for staining was followed and this comprised rehydration, staining (using aceto alum carmine solution), dehydration and clearing with clove oil. Mounting of specimens was done with Canada balsam. The whole mounts were identified, drawn, measured and photographed.

3F.3 RESULTS AND DISCUSSION

1 Genus ***Acanthogyrus*** Thapar, 1927 = ***Acanthosentis*** Verma & Datta, 1929

Thapar (1927) gave the chief characters on which the generic diagnosis is based as follows: “(1) three rows of eight recurved hooks on the proboscis; (2) peculiar arrangement of the body spines; (3) presentation of pseudo-segmentation; (4) presence of only two prostate glands; (5) Y-shaped ductus ejaculatorius”.

Acanthogyrus and *Acanthosentis* were synonymized by Golvan (1959) who reduced *Acanthosentis* to a subgenus based on the number of hooks on the proboscis. *Acanthosentis* was later returned to full generic status by Golvan (1994) without any reasons given, but the subgenus is retained by some systematists (Amin 1985, 2005; Amin & Hendrix 1999).

There are 44 valid species of *Acanthogyrus* (*Acanthosentis*) worldwide with 6 of them found in Africa (Amin 2005). The only record within the genus in South Africa is that of *Acanthosentis phillipi* Mashego, 1988 from *Barbus neefi* (Mashego 1988).

1.1 ***Acanthogyrus* (*Acanthosentis*) *tilapiae*** (Baylis, 1948)
= ***Acanthosentis tilapiae*** Baylis, 1948

African studies (Baylis 1948; Prudhoe 1951; Golvan 1957, 1965; Khalil & Thurston 1973; Shotter 1974; Troncy 1974; Amin 1978; El-Naffar *et al*. 1983; Batra 1984; Hyslop 1988; Douëllou 1992; Amin *et al*. 2008) show *Acanthogyrus* (*Acanthosentis*) *tilapiae* (Baylis, 1948) to be the most common and widely distributed species of the genus within the continent. It was reported from 30 species of cichlids (with 28 of genus *Tilapia*) and 3 non-cichlid species from many countries of the continent (Amin & Hendrix 1999). *Acanthogyrus* (*Acanthosentis*) *tilapiae* found in the present study is the first genus and species record in *Oreochromis mossambicus* and first species record for South Africa (Khalil & Polling 1997).

The males are smaller than females and the length range obtained for males (n=5) is 1.1-2.1 mm and that for females (n=7) is 1.6-4.3 mm. The male shows an extended proboscis and that in the female is withdrawn (figure 3F.1). Male has two testes and the rest of the male system is posterior to the testes with the male opening terminal (figure 3F.1A). The female shows non-permanent ovarian balls in the pseudocoel and the female gonopore is also terminal (figure 3F.1B). The photographs of *Acanthogyrus* (*Acanthosentis*) *tilapiae* are on Plate 35.

There are several differences between the two species found in South Africa (Amin 2005). Some include the observations and measurements made in the present study as compared to Mashego (1988). *Acanthogyrus* (*Acanthosentis*) *phillipi* is larger (males 2.4-3.6 mm long; females 2.9-5.4 mm long) as compared to *Acanthogyrus* (*Acanthosentis*) *tilapiae* (males 1.1-2.1 mm long; females 1.6-4.3 mm long). Testes lie in posterior region of body in *A.* (*A.*) *phillipi* whilst anterior testis lies just posterior to proboscis receptacle in *A. (A.) tilapiae*. Lemnisci reach to the anterior testis in *A.* (*A.*) *phillipi* and to the posterior testis in *A. (A.) tilapiae*. In *A.* (*A.*) *phillipi* proboscis hooks in anterior and middle circles are equal and longer than those of posterior circle, but in *A. (A.) tilapiae* proboscis hooks gradually decrease in length posteriorly. Giant nuclei 6 or 7 with 5 dorsal and 1 or 2 ventral in *A.* (*A.*) *phillipi*, but usually 8 in *A. (A.) tilapiae* with 2-4 dorsal and 4-6 ventral.

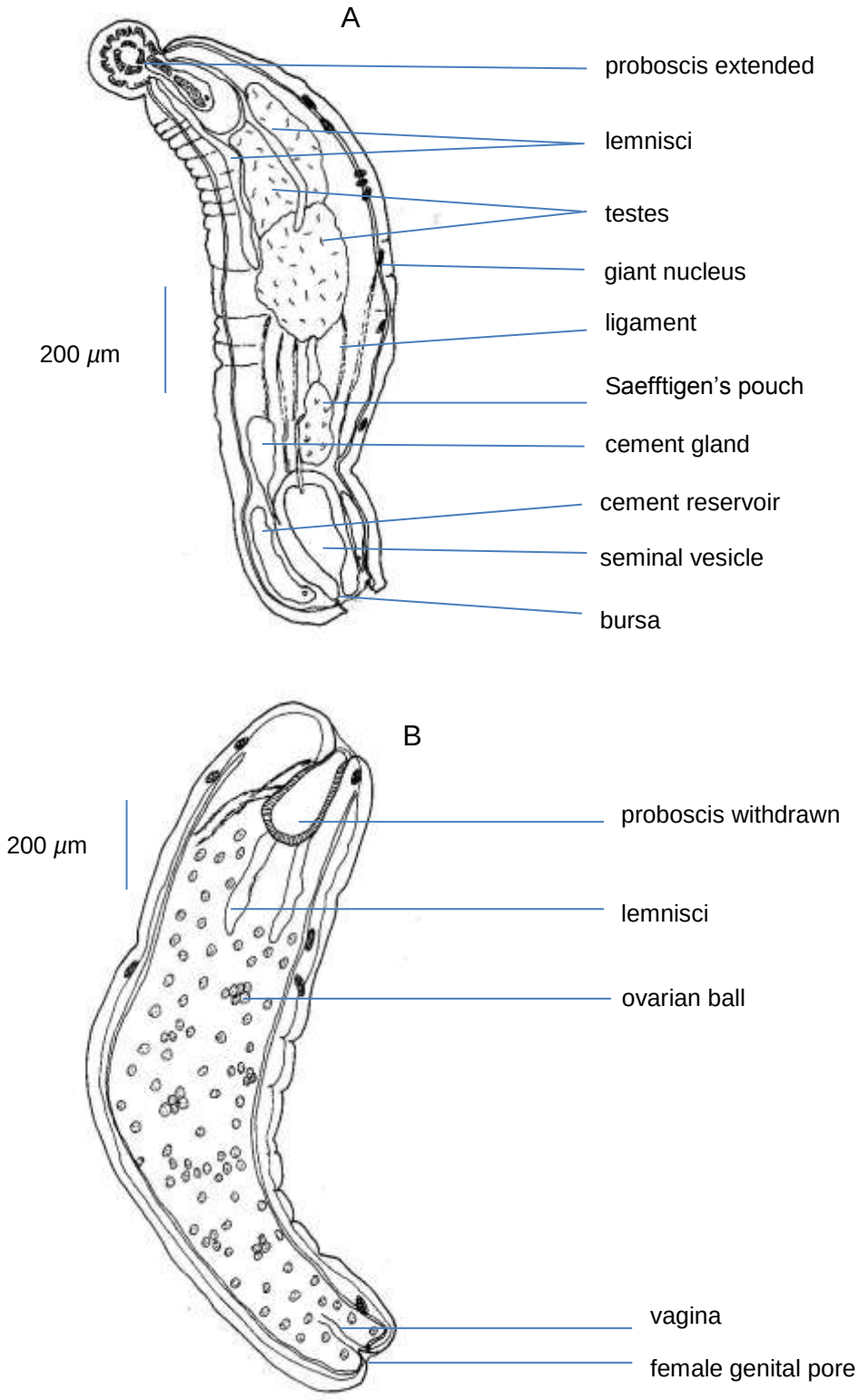
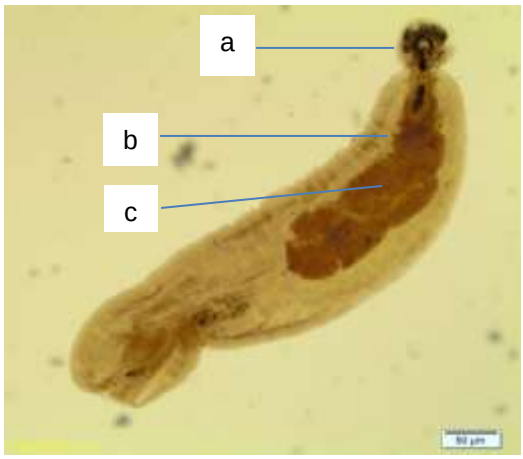
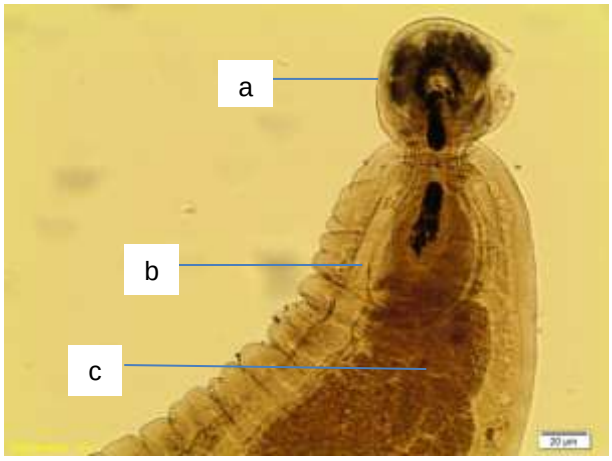


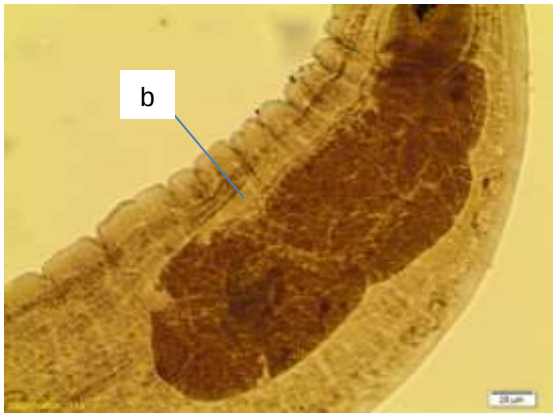
Figure 3F.1 *Acanthosentis tilapiae* - A male B female



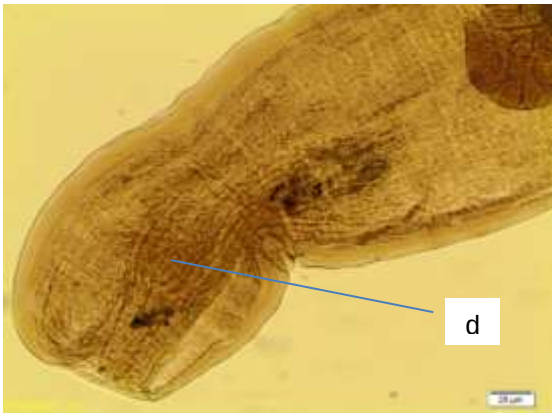
male



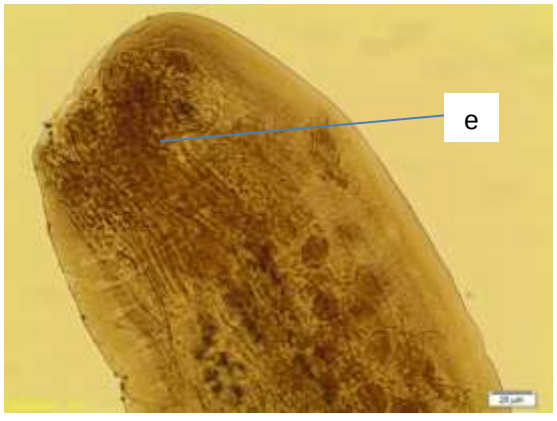
male anterior



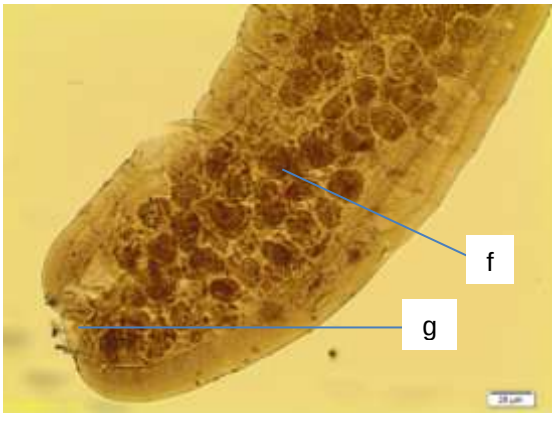
male middle



male posterior



female anterior



female posterior

a. proboscis b. lemnisci c. testis d. seminal vesicle e. proboscis withdrawn f. ovarian ball g. female genital pore

Plate 35 *Acanthogyrus* (*Acanthosentis*) *tilapiae*

There are no pathological effects reported on *A. (A.) tilapiae* more especially that the worms only attach to the mucosa and not the deeper muscles (Paperna 1996). Douëllou (1992) reported that the parasites usually occupy the whole lumen of the alimentary canal in small fish and may cause mechanical obstruction.

3F.4 GENERAL DISCUSSION

The Acanthocephala have received less attention as compared to other groups of helminths. This might have risen from the belief that infection by these worms is very rare (Thapar 1927). There have been disputes over the status of the group in the animal kingdom. More so, there were more debates on the classification of the group. All these were mainly because little is known about the group (Thapar 1927). Even today, though still a smaller phylum, the classification system and identification keys are debatable (Amin 2005).

The distribution of the 6 species of *Acanthosentis* in Africa is amazing in that only *Acanthogyrus* (*Acanthosentis*) *tilapiae* can be accounted for by the distribution of its hosts. The other five species are regional in distant countries of the continent (Amin & Hendrix 1999) *Acanthogyrus* (*Acanthosentis*) *maroccanus* in Morocco; *Acanthogyrus* (*Acanthosentis*) *malawiensis* in Malawi; *Acanthogyrus* (*Acanthosentis*) *nigeriensis* in Niger; *Acanthogyrus* (*Acanthosentis*) *papilio* in Senegal and *Acanthogyrus* (*Acanthosentis*) *phillipi* in South Africa). *Acanthogyrus* (*Acanthosentis*) *tilapiae* has been reported in Tanzania, Congo, Uganda, Chad, Nigeria, Egypt, Malawi (Khalil & Polling 1997; Amin & Hendrix 1999), Zimbabwe (Douëllou 1992) and now South Africa. It is believed that with more studies done in various countries this picture may change with the other five species represented in more countries.

CHAPTER 4

GENERAL DISCUSSION

Sunrise or Sunset? Life goes on for fish and parasites

CONTENTS

4.1 FISH PARASITOLOGY GLOBALLY

The study of fish parasites forms an exact science that contributes to the academic, research, social and economic markets worldwide. There are numerous Universities, Societies of Parasitologists, Journals of Parasitology and Conferences (scientific meetings) that ensure the advancement of Fish Parasitology through research, study and communication networks. The output from the above form a vast supply of knowledge important in many respects and to this study project in particular. Because of the immeasurable body of information, also due to geographic distribution of fish host species present in Lake Tzaneen, it was strategic to limit the literature search and references to Africa. Only at compelling circumstances were non-African but strongly relevant information used, especially compilations in which all species of a particular genus are evaluated.

In the present study four new species are introduced to the world data bank on fish parasites. These are *Dactylogyrus* sp. 1, *Dactylogyrus* sp. 2, *Dactylogyrus* sp. 3 and *Dactylogyrus* sp. 4.

4.2 AFRICAN FISH PARASITOLOGY

There are many literature sources on African freshwater fish parasites even though many of the fish species have not been subjected to studies (Khalil & Polling 1997). The Check List of the Helminth Parasites of African Freshwater fishes (Khalil & Polling 1997) helped direct the literature searches in this study. Volumes of articles were accumulated even though in some cases only the titles were glanced at or abstracts perused, especially those that relate to levels higher than the species or even genus. Others were only mentioned as they were related in terms of geographical area within the continent.

Whilst many fishes have been neglected in Africa the results obtained in this study indicate that only three species are introduced as first records to the data bank on African fish parasites. These are *Actinocleidus fusiformis* (Mueller, 1934), *Haplocleidus furcatus* Mueller, 1937 and *Acolpenteron ureteroecetes* Fischthal & Allison, 1940.

4.3 SOUTH AFRICAN SCENE

Studies on the helminth ichthyo-parasitological fauna in South Africa started increasing in the 1960's but can be regarded as still in infancy mainly due to little achievements thus far. They are scarce as compared to other aspects of aquatic parasitology and fish biology. The scene is more scaring when other aspects of parasitology (agricultural, wildlife and human) are considered. It is clear, however, that freshwater fish parasitology is at present more advanced than marine fish parasite studies. One reason why fish parasitology lags behind may be that aquaculture is not as strongly practiced as other forms of agriculture in South Africa.

Basic parasitological studies are not that innovative but do contribute to science as they are rarely done. The state of fish parasitology in South Africa is still at a taxonomic level and dictates that identifications should be done as a first priority. Such studies should be seen as being proactive, rather than reactive, and will be of greater contribution only when the state of aquaculture improves to the level of other farming practices. Epizootiology, pathology, and the more innovative prevention and control of diseases can only be maximised once the cause is known.

The country's contribution in basic fish parasitology is however, more visible than other partners in the southern part of the continent, with Zimbabwe improving in the recent past. The many first records of parasites in the present investigation bear testimony to the little achieved thus far. The following parasite records were presented for South Africa in this investigation: 15 first South African records, 7 first host records, 1 site record as well as 4 new species previously unknown to science.

Monogenean first geographical records are *Dactylogyrus brevicirrus* Paperna, 1973, *Dactylogyrus cyclocirrus* Paperna, 1973, *Dogielius dublicornis* Paperna, 1973, *Dogielius* sp., *Schilbetrema quadricornis* Paperna & Thurston, 1968, *Scutogyrus gravivaginus* (Paperna & Thurston, 1969) and *Cichlidogyrus quaestio* Douëllou, 1993. Three others (*Gyrodactylus rysavyi* Ergens, 1973; *Quadriacanthus aegypticus* El Naggar & Serag, 1986; *Quadriacanthus clariadis* Paperna, 1961) as well as four more (*Cichlidogyrus halli* Price & Kirk, 1967; *Cichlidogyrus sclerosus* Paperna & Thurston, 1969; *Cichlidogyrus dossoui* Douëllou, 1993; *Cichlidogyrus tilapiae*

Paperna, 1960) were also later identified by this writer (Madanire-Moyo *et al*. 2010, 2011) but published before completion of this thesis. The only non-monogenean first geographical record is an acanthocephalan, *Acanthosentis tilapiae* Baylis, 1948. The 7 first host records are the following: *Oreochromis mossambicus* for *C. dossoui*, *C. halli* and *A. tilapiae*; *Barbus radiatus* and *Barbus trimaculatus* for *Dactylogyrus* sp. 1; *Barbus unitaeniatus* for *Dactylogyrus* sp. 2; *Labeo molybdinus* for *Dactylogyrus* sp. 3 and *Dactylogyrus* sp. 4. The only site record is the gills for *Gyrodactylus rysavyi*.

4.4 FISH PARASITES OF LAKE TZANEEN

Divisions

The fish parasites of Lake Tzaneen include both the Protozoa and Metazoa. No study has been done on the protozoa whilst Luus-Powell (2004) studied the metazoan parasites of only two mormyrid fishes in the lake. This study involved only the Helminth parasites including the Platyhelminthes (Monogenea, Digenea and Cestoda), Nematoda and Acanthocephala. Monogeneans are ectoparasites on the gills with few species on the skin. The other groups are endoparasites mainly in the body cavities and intestines, but in other organs as well.

Although parasites make fish unattractive as food, few occupy the skin and muscles and are not generally seen as troublesome. Fish parasites are not problematic to hosts in lakes and rivers, but may only be so when the natural environment is altered, for instance by pollution (Bush *et al.* 2001).

Species richness and diversity

The fish species investigated in Lake Tzaneen had 38 different parasites that were discussed in this report. Monogeneans formed the bulk of species (27) with few from other groups comprised of 3 Digenea, 4 Cestoda, 3 Nematoda and 1 Acanthocephala. Of the 527 fish specimens sampled approximately 9000 parasites were collected in this study.

There are several survival strategies by the helminths and they include penetration, attachment, absorption and evasion of host defences. Their diverse major structural adaptations are the opisthohaptor with anchors and hooks in monogeneans, acetabula in digeneans, suckers and rostellar hooks in cestodes, sclerotized labia in nematodes and spines in acanthocephalans (Barson 2009).

Species richness and diversity, infection indices and epizootiology, the degree of interaction or association among parasites, their life cycles, pathology and any zoonotic threats they may cause confirm the importance of parasite community studies in lakes as a necessary preamble to any economic, ecologic or conservation planning.

Host specificity

The helminths present in Lake Tzaneen were compared within the continent and they demonstrate varying degrees of host specificity. Few may occur on one species only in South Africa but in many in other African countries. The review that follows is continental. In lakes with abundant intermediate and fish hosts, host specificity is lowered (Campana-Rouget 1961). Notable are the larval forms (*Diplostomulum*, *Clinostomum*, *Ligula intestinalis* and *Contracaecum*) that are generalists with regard to fish intermediate hosts, using hosts in many fish families.

Other species that use more than two fish families as hosts include *Actinocleidus fusiformis*, *Haplocleidus furcatus*, *Paracamallanus cyathopharynx*, *Procamallanus laevionchus* and *Acanthosentis tilapiae*. The following species are specific to a family: Each member of the genus *Cichlidogyrus* (except *C. philander*) and *Scutogyrus gravivaginus* are specific to Cichlidae and *Quadriacanthus clariadis* to Clariidae. *Glossidium pedatum* is host specific to families Clariidae and Bagridae.

Seven monogeneans and one cestode are genus specific. These are *Macrogyrodactylus clarii* and *Proteocephalus glanduligerus* on *Clarias*, *Dactylogyrus afrolongicornis afrolongicornis* and *Dactylogyrus allolongionchus* on *Barbus*, *Dactylogyrus brevicirrus* and *Dactylogyrus cyclocirrus* on *Labeo*, *Dactylogyrus spinicirrus* on *Labeobarbus* and *Acolpenteron ureteroecetes* on *Micropterus*. Six

monogeneans and one cestode are species specific. These are *Gyrodactylus rysavyi*, *Macrogyrodactylus karibae*, *Quadriacanthus aegypticus* and *Polyonchobothrium clarias* on *Clarias gariepinus*; *Schilbetrema quadricornis* on *Schilbe intermedius*; *Dogielius dublicornis* on *Labeo cylindricus* and *Cichlidogyrus philander* on *Pseudocrenilabrus philander*.

Host specificity assessments have gained prominence as a focus for practical research and as guideline for biological control of parasites in developed countries (Ehler 1991).

Morphological features and identification

The identification of parasites was based on morphological features and dimensions. To achieve this use of microscope with its accessories and literature were essential. Morphological observations through microscopy, measurements, photographs, drawings and comparisons with related hosts, site and parasite species were done in confirming earlier macroscopic identifications.

The process of identification was more tedious with numerous small species of Monogenea and relatively easier with other groups. In many cases monogeneans could be identified to genus level from host and site alone, but later confirmed microscopically by the haptoral features to the genus level and the copulatory organ alone or with the vagina to the species level.

In the Digenea, *Glossidium pedatum* was identified by host and site first and then confirmed microscopically by comparisons with previous finds in the literature. The larval digenea (*Diplostomum* and *Clinostomum*) were also identified to genus first by site and outward appearance as they are larger, and then to first and second types by means of processing and subjecting to microscopical observation.

The two cestodes (*Proteocephalus glanduligerus* and *Polyonchobothrium clarias*) were identified by the host (*Clarias gariepinus*) even though the two could not be separated into different species at this stage. Later through processing and subjecting to microscopy they could be identified to species level using morphological

characters of their scolices (glandular organ, hooks) and proglottids (position of genital opening and other reproductive organs). *Ligula intestialis* was identified to species level by sight alone and only confirmed using detailed features from microscopical observation. Gryporhynchid larvae were identified first by hosts and site and later confirmed through processing and microscopy.

The two nematodes (*Procamallanus laevionchus* and *Paracamallanus cyathopharynx*) could be identified from the host and site first, but also microscopically using the presence and arrangement of cuticular ribs in the buccal capsule (*Paracamallanus*) and the absence thereof (*Procamallanus*). *Contracaecum* sp. larvae usually occurred in aggregates in the body cavity but again microscopy was necessary for confirming features like the intestinal caecum and ventricular appendix.

The only acanthocephalan in this study was from the intestine of *Oreochromis mossambicus* and it could be designated to this group from external features, but microscopy and literature helped to recognise the species and males and females through the testes and the ovarian balls.

Life cycles

The study of life cycles is an important part of parasite taxonomy in that all stages and hosts are known. Furthermore, their transmission is better understood and their control, when planned becomes effective (Bush *et al.* 2001). The monogeneans have a direct life cycle and mature in fish hosts (are autogenic). Because of being more specific, they have co-evolved with their hosts and can be used as indicators of their host distribution (Pouyad *et al.* 2006). Few autogenic species among the digeneans, cestodes and nematodes that were procured in this study are each restricted to a little variety of hosts. The allogenic parasites in this study (all larvae of either digeneans, cestodes or nematodes) mature in birds and were found to have a wide geographical distribution that often is trans-continental (Paperna 1996).

4.5 MAJOR CHALLENGES AND RECOMMENDATIONS

- Africa, in particular the southern part lacks and need to strengthen scientific manpower towards freshwater fish parasitological studies

- Study strategies must be multidisciplinary to have more meaningful impact

- Southern African fish parasitologists must form partnerships and prioritise projects

- Strong candidates for fish culture must get full parasitological attention to pre-empt their full blown aquaculture capacity in the nearby future

- Consolidating and/or Updating of information for Africa is pivotal in simplifying future studies

- Molecular identification or phylogenetic studies should be encouraged

4.6 OUTPUTS

There are already outputs based on the the data from this project and they are listed below with the abstracts included as addenda 5 to 9.

International Conferences

1. The Sixth International Symposium on Monogenea (ISM6) held in August 2009 at Marine and Coastal Management Building, Cape Town, South Africa.

Oral presentation
MM Matla, WJ Luus-Powell, NM Mokgalong & SN Mashego: Lake Tzaneen – a sanctuary for monogenean parasites **Addendum 5**

2. The Eighth International Symposium on Fish Parasites (8thISFP) held in September 2011 at the Gala Hotel, Viña del Mar, Chile.

Poster presentation
MM Matla, SN Mashego and NM Mokgalong: Monogenea of the genus *Dactylogyrus* from South Africa **Addendum 6**

National Conferences

3. The 37th annual congress of the Parasitological Society of Southern Africa (PARSA) held in September 2008 at Onderstepoort (University of Pretoria).
Abstracts in *Journal of South African Veterinary Association* (2009) **80**(2):126-140.

Oral presentation
MM Matla, NM Mokgalong and SN Mashego: Monogenean parasites of largemouth bass *Micropterus salmoides* (Lacepede, 1802) in Tzaneen Dam **Addendum 7**

4. The 38th annual congress of the Parasitological Society of Southern Africa (PARSA) held in September 2009 at Magaliesberg Conference Centre.
Abstracts in *Journal of South African Veterinary Association* (2010) **81**(3):178-187.

Oral presentation
MM Matla, NM Mokgalong & SN Mashego: Monogenea of the genus *Dactylogyrus* from cyprinids of the genera *Barbus*, *Labeobarbus* and *Labeo* in Lake Tzaneen, South Africa **Addendum 8**

5. The 40th annual congress of the Parasitological Society of Southern Africa (PARSA) held in July 2011 at Stellenbosch University.
Abstracts to be published in *Journal of South African Veterinary Association* (2012).

Oral presentation
MM Matla, SN Mashego and NM Mokgalong: Helminth larval forms from freshwater fishes in a South African impoundment **Addendum 9**

4.7 CONCLUSION

It is believed that the achievements of this study project were elucidated first by the vast results, new species descriptions, first geographic and first host records obtained and discussed in this thesis. Secondly, outputs from the project were listed in the form of papers and posters presented in International and National Conferences and whose abstracts remain as records in worldwide websites and Journal of South African Veterinary Association. Lastly, this thesis will be published electronically and articles on the data from this project will culminate in a meaningful contribution to the relevant worldwide scientific community.

CHAPTER 5

REFERENCES

The true mystery of the world is the visible, not the invisible - **Oscar Wilde.** We still do not know one thousandth of one percent of what nature has revealed to us. **Albert Einstein.**

ADEROUNMU, E.A. & ADENIYI, F. 1972. Cestodes in fish from a pond at Ile-Ife, Nigeria. *The African Journal of Tropical Hydrobiology and Fisheries* **2**(2): 151-156.

AGARWAL, S.M. 1959. Studies on the morphology, systematics and life history of *Clinostomum giganticum* n. sp. (Trematoda: Clinostomatidae). *Indian Journal of Helminthology* **11**(2): 75-115.

AKINSANYA, B. & OTUBANJO, O.A. 2006. Helminth Parasites of *Clarias gariepinus* (Clariidae) in Lekki Lagoon, Lagos, Nigeria. *Revista de Biología Tropical* **54**(1): 93-99.

AKINSANYA, B., OTUBANJO, O.A. & IBIDAPO, C.A. 2007. Helminth bioload of *Chrysichthys nigrodigitatus* (Lacepede 1802) from Lekki Lagoon, Lagos, Nigeria. *Turkish Journal of Fisheries and Aquatic Sciences* **7**: 83-87.

ALLANSON, B.R. & JACKSON, P.B.N. 1983. The limnology and fisheries potential of Lake Le Roux. *South African National Scientific Programmes,* Report no. 77. CSIR, Pretoria.

ALOO, P.A. 1999. Ecological studies of helminth parasites of the largemouth bass, *Micropterus salmoides,* from Lake Naivasha and the Oloidien Bay, Kenya. *Onderstepoort Journal of Veterinary Research* **66**(2): 73-90.

AMIN, O.M. 1978. Intestinal helminthes of some Nile fishes near Cairo, Egypt, with redescriptions of *Camallanus kirandensis* Baylis, 1928 (Nematoda) and *Bothriocephalus aegyptiacus* Rysãvý and Moravec, 1975 (Cestoda). *Journal of Parasitology* **64**: 93-101.

AMIN, O.M. 1985. Classification. In: Crompton, D.W.T. & Nickol, B.B. (Eds). *Biology of the Acanthocephala.* Cambridge University Press, Cambridge.

AMIN, O.M. 2005. Occurrence of the subgenus *Acanthosentis* Verma & Datta, 1929 (Acanthocephala: Quadrigyridae) in Japan, with the description of *Acanthogyrus* (*Acanthosentis*) *alternatspinus* n. sp. and *A*. (*A*.) *parareceptaclis* n. sp. from Lake

Biwa drainage fishes and a key to the species of the subgenus. *Systematic Parasitology* **60**: 125–137.

AMIN, O.M. & DESFULI, B.S. 1995. Taxonomic notes on *Polyacanthorhynchus kenyensis* (Acanthocephala: Polycanthorhynchidae) from Lake Naivasha, Kenya. *Journal of Parasitology* **81**(1): 76-79.

AMIN, O.M. & HENDRIX, S.S. 1999. Acanthocephala of cichlids (Pisces) in Lake Malawi, Africa with a description of *Acanthogyrus (Acanthosentis) malawiensis* sp.n. (Quadrigyridae) from *Labeo cylindricus* Peters, 1852 (Cyprinidae). *Journal of the Helminthological Society of Washington*, **66**: 47–55.

AMIN, O.M., VAN OOSTERHOUT, C., BLAIS, J., ROBINSON, R.L. & CABLE, J. 2008. On the ecology and host relationships of *Acanthogyrus (Acanthosentis) tilapiae* (Acanthocephala: Quadrigyridae) from cichlids in Lake Malawi. *Comparative Parasitology* **75**(2): 278-282.

AMIRTHALINGAM, C. 1965. *Macrogyrodactylus polypteri* Malmberg on Polypterus senegalus in the Sudan. *Bulletin of the Fisheries Research Station*, Ceylon **17**(2): 237-239.

AVENANT-OLDEWAGE, A. 1998. Parasites as indicators for water pollution analysis. *South African Journal of Science* **94**(2): 34.

AVENANT-OLDEWAGE, A. 2006. The usefulness of parasites. *Journal of South African Veterinary Association* **77**(2): 92.

AYANDA, O.I. 2008. Comparison of parasitic helminth infection between the different age groups of *Clarias gariepinus* from Asa dam Ilorin, north-central Nigeria. *African Journal of Environmental Science and Technology* **2**(11): 404-406.

AYANDA, O.I. 2009a. Comparative parasitic helminth infection between cultured and wild species of *Clarias gariepinus* in Ilorin, North–Central Nigeria. *Scientific Research and Essay* **4**(1): 18-21.

AYANDA, O.I. 2009b. Comparison of parasitic helminthes infection between the sexes of *Clarias gariepinus* from Asa dam Ilorin, north-central Nigeria. *Scientific Research and Essay* **4**(4): 357-360.

BAER, J.G. 1959. Helminthes parasites. *Explorations du Parcs Nationales du Congo Belge, Mission J.G. Baer & W. Gerber* **1**: 1-163.

BAKKE, T.A., HARRIS, P.D. & CABLE, J. 2002. Host specificity dynamics: observations on gyrodactylid monogeneans. *International Journal for Parasitology* **32**: 281-308.

BANU, A.N.H., HOSSAIN, M.A. & KHAN, M.H. 1993. Investigation into the occurrence of parasites in carps, catfish and tilapia. *Progress Agriculture* **4**: 11–16.

BARNARD, C.J. 1990. Parasitic relationships. In: Barnard C.J & Behnke M.J. (eds), *Parasitism and Host Behavior*. Taylor and Francis, London, UK.

BARNES, R.D. 1974. *Invertebrate zoology*. W.B. Saunders Company, Philadelphia.

BARSON, M. 2003. Endoparasites of the sharptooth catfish, *Clarias gariepinus* (Burchell), from the Rietvlei Dam, Sesmyl Spruit system, South Africa. MSc dissertation, Rand Afrikaans University, South Africa.

BARSON, M. 2004. A study of the helminth parasites of fish and fish-eating birds in the Manyame catchment, Zimbabwe. M.Phil. thesis, University of Zimbabwe.

BARSON, M. 2009. Diversity and ecology of fishes and their helminth parasites in a subtropical river system (Save-Runde River, Zimbabwe). PhD thesis, K.U. Leuven, Belgium.

BARSON, M. & AVENANT-OLDEWAGE, A. 2006a. On cestode and digenean parasites of *Clarias gariepinus* (Burchell, 1822) from the Rietvlei Dam, South Africa. *Onderstepoort Journal of Veterinary Research* **73**: 101-110.

BARSON, M. & AVENANT-OLDEWAGE, A. 2006b. Nematode parasites of *Clarias gariepinus* (Burchell, 1822) from the Rietvlei Dam, South Africa. *Onderstepoort Journal of Veterinary Research* **73**: 87-94.

BARSON, M. & MARSHALL, B.E. 2004. First record of *Contracaecum* spp. (Nematoda: Anisakidae) in fish-eating birds from Zimbabwe. *Journal of the South African Veterinary Association* **75**: 74-78.

BARSON, M., BRAY, R., OLLEVIER, F. & HUYSE, T. 2008a. Taxonomy and Faunistics of the Helminth Parasites of *Clarias gariepinus* (Burchell, 1822), and *Oreochromis mossambicus* (Peters, 1852) from Temporary Pans and Pools in the Save-Runde River Floodplain, Zimbabwe. *Comparative Parasitology* **75**(2): 228-240.

BARSON, M., MULONGA, A. & NHIWATIWA, T. 2008b. Investigation of a parasitic outbreak of Lernaea cyprinicea Linnaeus, 1758 (Crustacea: Copepoda) in fish from Zimbabwe. *African Zoology* **43**: 175–183.

BATRA, V. 1984. Prevalence of helminth parasites in three species of cichlids from a man-made lake in Zambia. *Zoological Journal of the Linnean Society* **82**: 319-333.

BAYLIS, H.A. 1923. Report on a collection of parasitic nematodes mainly from Egypt. Part III. Camallanidae, etc., with a note on Probstmayria and an appendix on Acanthocephala. *Parasitology* **15**(1): 24-38.

BAYLIS, H.A. 1928. Some parasitic worms mainly from fishes from Lake Tanganyika. *Annals and Magazine of Natural History,* Series 10 **1**(4): 552-562.

BAYLIS, H.A. 1930. Mission Saharienne Augieras-Draper, 1927-1928. Parasitic nematodes. *Bulletin du Museum National d'Histoire Naturelle*, Paris 2e **2** (1): 117-130.

BAYLIS, H.A. 1940. On a further collection of parasitic worms from the Belgian Congo. *Annals and Magazine of Natural History* Series 11 **29**(36): 401-417.

BAYLIS, H.A. 1948. A new acanthocephalan from an East African freshwater fish. *Annals and Magazine of Natural History* **14**(11): 861–868.

BEAUCHAMP, P.M. 1914. Sur quelques parasites provenant du Congo Belge. *Revue de Zoologie et de Botanique Africaines* **4**(1): 109-116.

BEGON, M., HARPER, J.L & TOWNSEND, C.R. 1996. *Ecology individuals, Populations and Communities*. Blackwell Science Ltd.

BERTASSO, A & AVENANT-OLDEWAGE, A. 2005. Aspects of the ecology of the Asian tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 in the yellowfish in the Vaal Dam, South Africa. *Onderstepoort Journal of Veterinary Research*, **72**: 207–217.

BEUKEMA, J.J. & DE VOS, G.J. 1974. Experimental tests of a basic assumption of the capture-recapture method in pond populations of carp *Cyprinus carpio* L. *Journal of fish biology* **6**(3): 317.

BEVERLEY-BURTON, M. 1962. Some trematodes from *Clarias* spp. in the Rhodesias, including *Allocreadium mazoensis* n.sp. and *Eumasenia bangweulensis* n.sp., and comments on the species of the genus *Orientocreadium* Tubangui, 1931. *Proceedings of the Helminthological Society of Washington* **29**(2): 103-115.

BEVERLEY-BURTON, M. 1963. A new strigeid, *Diplostomum (Tylodelphys) mashonense* n.sp. (Trematoda: Diplostomatidae) from the Grey Heron, *Ardea cinerea* in Southern Rhodesia with an experimental demonstration of part of the life cycle. *Revue de Zoologie et de Botanique Africaines* **68**(3/4): 291-308.

BEVERLEY-BURTON, M. 1986. The taxonomic status of *Actinocleidus* Mueller, 37; *Anchoradiscus* Mizelle, 1941; *Clavunculus* Mizelle et al., 1956; *Anchoradiscoides* Rogers, 1967; *Syncleithrium* Price, 1967 and *Crinicleidus* n. gen.: North American Ancyrocephalids (Monogenea) with articulating haptoral bars. *The Journal of Parasitology* **72**(1): 22-44.

BIRGI, E. & EUZET, L. 1983. Monogènes parasites des poissons des eaux douces du Cameroun. Présence des genre *Cichlidogyrus* et *Dactylogyrus* chez *Aphyosemion* (Cyprinodontidae). *Bulletin de la Société zoologique de France* **108**: 101-106.

BIRGI, E. & LAMBERT, A. 1986. Presence chez un Nandidae (Teleosteen), *Polycentropsis abbreviate* Boulenger, 1901, du genre *Cichlidogyrus* (Monogenea, Monopisthocotylea, Ancyrocephalidae). Description de *Cichlidogyrus nandidae* n. sp. et de *Cichlidogyrus euzeti* n.sp. *Ann. Parasitol. Hum. Comp.* **61**(5): 521-528.

BIRGI, E. & LAMBERT, A. 1987. Les Dactylogyridae (Plathelminthes, Monogenea) des Cyprinidae du genre *Barbus* du Sud Cameroun. *Revue d'Hydrobiologie Tropicale* **20**: 37-47.

BIRGI, E. 1988. Monogeneans of genus *Quadriacanthus* Paperna, 1961, two gill parasites of Siluridae (Teleostei) *Clarias pachynema* Boulanger, 1903 and *Clarias jaensis* Boulanger, 1909 in south Cameroon. (Description of four new species). *Ann. Fac. Biol. Sci. Biochem*. **3**: 113-129.

BOOMKER, J. & PETTER, A.J. 1993. Parasites of South African freshwater fishes III. *Rhabdochona (Rhabdochona) versterae* n. sp. (Nematoda: Rhabdochonidae) from the spottailed robber *Alestes imberi* Peters, 1852. *Onderstepoort Journal of Veterinary Research* **60**: 23-27.

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

BOOMKER, J. 1982. Parasites of South African freshwater fish I. Some nematodes of catfish (*Clarias gariepinus* Burchell, 1822) from the Hartbeespoort Dam. *Onderstepoort Journal of Veterinary Research* **49**(1): 41-51.

BOOMKER, J. 1984. Parasites of South African freshwater fish II. Redescription of African species of the genus *Phyllodistomum* Braun, 1899 (Trematoda: Gorgoderinae) and the description of a new species. *Onderstepoort Journal of Veterinary Research* **51**(2): 129-139.

BOOMKER, J. 1993a. Parasites of South African freshwater fishes IV. Description of *Spirocamallanus daleneae* n. sp. ("Nematoda: Camallanidae) with comments on *Spirocamallanus spiralis* (Bayh's, 1923). *Onderstepoort Journal of Veterinary Research* **60**: 131-137.

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

BOOMKER, J. 1994. Parasites of South African freshwater fishes VI Nematode parasites of some fish species in the Kruger National Park. *Onderstepoort Journal of Veterinary Research* **61**: 35-43.

BOOMKER, J., HUCHZERMEYER, F.W. & NAUDE, T.W. 1980. Bothriocephalosis in the common carp in Eastern Transvaal. *Journal of the South African Veterinary Association* **51**: 263-264.

BOUNGOU, M., KABRE, G.B., MARQUES, A. & SAWADOGO, L. 2008. Dynamics of population of five parasitic monogeneans of *Oreochromis niloticus* Linné, 1757 in the Dam of Loumbila and possible interest in intensive pisciculture. *Pakistan Journal of Biological Sciences* **11**(10): 1317-1323.

BRANDT, F. DE W., SCHOONBEE, H.J. & VAN AS, J.G. 1980. Die voorkoms van ’n lintwurm parasite wat moontlik ’n bedreiging inhou vir visboere in Suid Afrika. *Fish Farmer* **28**: 7-10.

BRANDT, F. DE W., VAN AS, J.G., SCHOONBEE, H.J. & HAMILTON-ATWELL, V.L. 1981. The occurrence and treatment of bothriocephalpsis in the common carp *Cyprinus carpio* in fish ponds with notes on its presence in the large-mouth yellowfish *Barbus kimberleyensis* from the Vaal Dam, Transvaal. *Water, South Africa* **7**: 35-42.

BRAY, R.A. 1974. A new genus of dilepidid cestode in *Tilapia nilotica* (L., 1758) and *Phalacrocorax carbo* (L. 1758) in Sudan. *Journal of Natural History* **8**: 589-596.

BRAY, R.A. & HENDRIX, S.S. 2007. A new genus and species of macroderoididae, and other digeneans from fishes of Lake Malawi, Africa. *The Journal of Parasitology* **93**(4): 860-865.

BRAY, R.A., VAN OOSTERHOUT, C., BLAIS, J. & CABLE, J. 2006. *Astiotrema turneri* n. sp (Digenea: Plagiorchiidae) from cichlid fishes (Cichlidae: Perciformes) of Lake Malawi, south-eastern Africa. *Zootaxa* **1319**: 43-58.

BRITZ, J.B. 1983. Studies on Clinostomatid (Trematoda: Digenea) infections of fish in the Transvaal. M.Sc. dissertation, Rand Afrikaans University, Johannesburg, South Africa.

BRITZ, J., VAN AS, J.G. & SAAYMAN, J.E. 1984a. Anatomy of the metacercariae and adult of *Euclinostomum heterostomum* (Rudolphi, 1809)(Trematoda: Clinostomatidae). *South African Journal of Zoology* **19**: 91-96.

BRITZ, J., VAN AS, J.G. & SAAYMAN, J.E. 1984b. Notes on the morphology of an adult of *Clinostomum tilapiae* Ukoli, 1966 (Trematoda: Clinostomatidae). *South African Journal of Wildlife Research* **14**(3): 69-72.

BRITZ, J., VAN AS, J.G. & SAAYMAN, J.E. 1985. Occurrence and distribution of *Clinostomum tilapiae* Ukoli, 1966 and *Euclinostomum heterostomum* (Rudolphi, 1809) metacercarial infections of freshwater fish in Venda and Lebowa, Southern Africa. *Journal of Fish Biology* **26**(1): 21-28.

BUNKLEY-WILLIAMS, L. & WILLIAMS, E.H. 1994. *Parasites of Puerto Rican freshwater sport fishes.* Puerto Rico Department of Natural and Environmental Resources, San Juan, PR and Department of Marine Sciences, University of Puerto Rico, Mayaguez, PR.

BUSH, A.O., FERNANDEZ, J.C., ESCH, G.W. & SEED, R.J. 2001. *Parasitism: The diversity and ecology of animal parasites*. Cambridge University Press, Cambridge.

BYCHOWSKY, B.E. 1957. *Monogenetic trematodes, their systematics and phylogeny*. Leningrad Academy of Sciences, Moscow. (English translation edited by W.J. Hargis, Jr., 1961. American Institute of Biological Sciences, Washington)

BYCHOWSKY, B.E. & NAGIBINA, L.F. 1978. A revision of Ancyrocephalinae Bychowsky, 1937 (Monogenoidea). *Parazitologicheskii Sbornik* **28**: 5-15.

CAMPANA-ROUGET, Y. 1961. Nematodes de poissons. Institut Royal des Sciences Naturelles de Belgique. Exploration Hydrobiologique des Lacs Kivu, Edward et Albert (1952-54). *Resultats Scientifiques, Bruxelles* **3**(4): 1-61.

CANARIS, A.G & GARDNER, S.L. 1967. *A guide to helminth species described from African vertebrates.* Morgantown: West Virginia University Library.

CHERVY, L. 2002. The terminology of larval cestodes or metacestodes. *Systematic Parasitology* **52**: 1-33.

CHIBWANA, F.D. & NKWENGULILA, G. 2010. Variation in the morphometrics of diplostomid metacercariae (Digenea: Trematoda) infecting the catfish, *Clarias gariepinus* in Tanzania. *Journal of Helminthology* **84**: 61-70.

CHISHAWA, A.M.M. 1991. A survey of the parasites of three Siluriformes fish species in Lake Kariba. Kariba: University of Zimbabwe (University Lake Kariba Research Station Bulletin, 1/91): 8–25.

CHRISTISON, K.W, SHINN, A.P & VAN AS, J.G. 2005. *Gyrodactylus thlapi* n. sp. (Monogenea) from *Pseudocrenilabrus philander philander* (Weber) (Cichlidae) in the Okavango Delta, Botswana. *Systematic Parasitology* **60**(3): 165-173.

CHUBB, J.C. 1977. Seasonal occurrence of helminths in freshwater fishes. Part I. Monogenea. *Advances in Parasitology* **15**: 133-199.

CLOUTMAN, D.G. 1978. Abundance of *Cleidodiscus pricei* Mueller (Monogenea: Dactylogyridae) on the flat bullhead, *Ictalurus platycephalus* (Girard) in Lake Norman, North Carolina. *Journal of Parasitology* **64**: 170-172.

DAVIES, B.R., HALL, A. & JACKSON, P.B.N. 1975. Some ecological aspects of the Cabora Bassa Dam. *Biological Conservation* **8**: 189-201.

DOGIEL, V.A., PETRUSHEVSKI, G.K. & POLYANSKI, YU.I. 1970. *Parasitology of fishes.* T.F.H. Ltd., Surrey.

DOLLFUS, R.P. 1950. Trematodes recoltes au Congo Beige par le Professeur Paul Brien (Mai-Aout 1937). *Annales du Musee du Congo Beige Sine. 4, Zoologie* **1**(1): 1-136.

DOLLFUS, R.P. 1951. Miscellanea helminthologica Maroccana I. Quelques trematodes, cestodes et acanthocephales. *Archives de l' Institut Pasteur de Maroc* **4**(3): 104-229.

DOSSOU, C. 1982. Parasites de poissons d'eau douce du Bénin III. Espèces nouvelles du genre *Cichlidogyrus* (Monogenea) parasites de Cichlidae. *Bulletin de l'I.F.A.N.* **44**: 295-322.

DOSSOU, C. & BIRGI, E. 1984. Monogènes parasites d' *Hemichromis fasciatus* Peters, 1857 (Teleostei, Cichlidae). *Annales des Sciences naturelles, Zoologie* **6**: 101-109.

DOUËLLOU, L. 1991. A study of fish parasites in Lake Kariba. *University of Zimbabwe Lake Kariba Research Station Bulletin* **1/91**: 1-7.

DOUËLLOU, L. 1992. A survey of fish parasites in Lake Kariba. Kariba: University of Zimbabwe (University Lake Kariba Research Station Bulletin, 1/92): 1–71.

DOUËLLOU, L. 1993. Monogeneans of the genus *Cichlidogyrus* Paperna, I960 (Dactylogyridae: Ancyrocephalinae) from cichlid fishes of Lake Kariba (Zimbabwe) with descriptions of five new species. *Systematic Parasitology* **25**: 159-186.

DOUËLLOU, L. & CHISHAWA, A.M.M. 1995. Monogeneans of three Siluriform fish species in Lake Kariba, Zimbabwe. *Journal of African Zoology* **109**: 99-115.

DOUËLLOU, L. & EARLWANGER, K.H. 1993. Occurance and distribution of two clinostomatid metacercariae in fishes from Lake Kariba, Zimbabwe. *Transactions of the Zimbabwe Scientific Association* **66**: 35-40.

DU PLESSIS, S.S. 1948. A gyrodactyloid parasite from the ureters of large-mouth bass at the Jonkershoek inland fish hatchery, South Africa. *Transactions of the American Fish Society* **75**: 105-109.

DU PLESSIS, S.S. 1952. Fish diseases in Transvaal. *Symposium on Hydrobiology and Inland Fish, Entebbe* **(37)**: 128-130.

DUBOIS, G. 1930. Deux nouvelles especes de Clinostomidae. *Bulletin de la Societe neuchateloise des Sciences Naturelles* **54**: 61-72.

DUBOIS, G. 1931. Materiaux de la Mission scientifique suisse en Angola. Trematoda. *Bulletin de la Societe neuchateloise Sciences Naturelles* **55**: 73-883.

DUBOIS, G. 1970. Synopsis des Strigeidae et des Diplostomatidae (Trematoda). *Memoires de la Societe neuchatoise des Sciences naturelles* **10**: 1-727.

DWAF. 1996. South African water quality guidelines. Volume 7: Aquatic ecosystems. DEPARTMENT OF WATER AFFAIRS AND FORESTRY.

EARLWANGER, K. 1991. The occurance and distribution of *Dolops ranarum* Stuhlmann, 1891, *Clinostomum vanderhorsti* Ortlepp, 1935, *Clinostomum* sp., and *Centrocestus formosanus* Nishigori, 1924 in various fish species of Lake Kariba. *University of Zimbabwe Lake Kariba Research Station Bulletin* **1/91**: 26-36.

EHLER, L. 1991. Planned introductions in biological control. In Ginzburg L, ed. Assessing ecological risks of biotechnology. Butterworth-Heineman, Boston.

EL-NAFFAR, M.K. 1979. Parasites of the Egyptian Moorhens. I: *Diplostomum magnicaudatum* sp. nov. with part of its life cycle. *Journal of the Egyptian Society of Parasitology* **9**(2): 349-358.

EL-NAFFAR, M.K., SAOUD, M.F. & HASSAN, I.M. 1983. A general survey of the helminth parasites of some fishes of Lake Nasser at Aswan, A.R. Egypt. *Assiut Veterinary Medical Journal* **11**(21): 141-183.

EL-NAFFAR, M.K., SAOUD, M.F. & HASSAN, I.M. 1984. Some cestodes from Lake Nasser at Aswan including a new *Marsypocephalus*, *Marsypocephalus aegyptiacus* n. sp. *Assiut Veterinary Medical Journal* **12**(23): 69-76.

EL-NAGGAR, M.M & SERAG, H.M. 1985. The monogenean *Quadriacanthus kearni* n.sp. and a report of *Q. clariadis clariadis* Paperna, 1979 on the gills of *Clarias lazera* in Nile Delta. *Journal of the Egyptian Society of Parasitology* **15**: 479-492.

EL-NAGGAR, M.M & SERAG, H.M. 1986. *Quadriacanthus aegypticus* n. sp., a monogenean gill parasite from the Egyptian teleost *Clarias lazera*. *Systematic Parasitology* **8**: 129-140.

EL-NAGGAR, M.M. & SERAG, H.M. 1987. Redescription of *Macrogyrodactylus clarii* Gussev, 1961, a monogenean gill parasite of *Clarias lazera* in Egypt. *Arab Gulf Journal of Scientific Research,* B (Agricultural and Biological Sciences) **5**(2): 257-271.

ERGENS, R. 1973. Two new species of *Gyrodacylus* from *Clarias lazera. Revue de Zoologie et de Botanique Africaines* **87**(1): 77-80.

ERGENS, R. 1981. Nine species of the genus *Cichlidogyrus* Paperna, 1960 (Monogenea (Ancyrocephalinae) from Egyptian fishes. *Folia Parasitologica* **28**: 205-214.

FAHMY, M.A.M. MANDOUR, A.M. & EL-NAFFAR, M.K. 1978. On some nematode parasites of the freshwater fishes in Assuit Province, Egypt. *Veterinary Medical Journal*, Giza **24**: 263-276.

FAISAL, M. 1988. Prevalence of *Macrogyrodactylus clarii* among the African catfish *Clarias lazera* (CV) and possibilities of its treatment. *Veterinary Medical Review* **59**(1): 82-87.

FISCHTHAL, J.H. & ALLISON, L.N. 1940. *Acolpenteron ureteroecetes* n.g., n. sp., a monogenetic trematode from the ureters of Black Basses. *The Journal of Parasitology* **26**: 34-35.

FISCHTHAL, J.H. & ALLISON, L.N. 1941. *Acolpenteron ureteroecetes* Fischthal and Allison, 1940, a monogenetic trematode from the ureters of the Black Basses, with a revision of the family Calceostomatidae (Gyrodactyloidea). *The Journal of Parasitology* **27(**6): 517-524.

FISCHTHAL, J.H. & THOMAS, J.D. 1970. Some metacercariae of digenetic trematodes in fishes in Numgua Lake, Ghana. *Anales del Instituto de Biologia. Universidad de Mexico* **41**(1): 73-79.

FISCHTHAL, J.H. 1973. Three digenetic trematodes of *Clarias mossambicus* Peters (Clariidae) from Ethiopia. *Proceedings of the Helminthological Society of Washington* **40**(1): 166-167.

FREEMAN, R.S. 1973. Ontogeny of cestodes and its bearing on their phylogeny and systematics. *Advances in Parasitology* **11**: 481-557.

FRYER, G. 1961. Observations on the biology of the cichlid fish *Tilapia variabilis* Bouleinger in the northern waters of Lake Victoria(East Africa). *Revue de Zoologie et de Botanique Africaines* **64**(1/2): 1-33.

FUHRMANN, O. & BAER, J.G. 1925. Zoological results of the third Tanganyika Expedition conducted by Dr. W. A. Cunnington, 1904-1905. Report on the Cestoda. *Proceedings of the Zoological Society* **1**: 79-100.

GIBSON, D.I., TIMOFEEVA, T.A. & GERASEV, P.I. 1996. A catalogue of the nominal species of the monogenean genus *Dactylogyrus* Diesing, 1850 and their host genera. *Systematic Parasitology* **35**: 3-48.

GOLVAN, Y.J. 1957. Acanthocephala des poisons. *Exploration Hydrobiologique des Lacs Kivu, Edouard et Albert* (1952-54*), Brussels* **3**(2): 55-64.

GOLVAN, Y.J. 1959. Le Phylum des Acanthocephala. Deuxième note. La Classe de Eoacanthocephala (Van Cleave, 1936). *Annales de Parasitologie Humaine et Comparée* **34**: 5–52.

GOLVAN, Y.J. 1965. Acanthocephales de Madagascar recoltes par E. R. Brygoo. (Premiere note). *Annales de Parasitologie Humaine et Comparée* **40**(3): 303-316.

GOLVAN, Y.J. 1994. Nomenclature of the Acanthocephala. *Research and Reviews in Parasitology* **54**: 135–205.

GROBBELAAR, A., VAN AS, L.L. & BUTLER, H.J.B. 2010. Behavioural influence of eye flukes (Diplostomidae) on fish hosts. Paper delivered at a conference of Parasitological Association of Southern Africa, October 2010.

GROBLER, J.P., MOKGALONG, N.M. & SAAYMAN, J.E. 1999. Genetic divergence between two clinostomatid fish endoparasites, inferred from allozyme and RAPD data. *South African Journal of Zoology* **34**(3): 135-139.

GUÉGAN, J.F. & HUGUENY, B. 1994. A nested parasite species subset pattern in tropical fish: host as major determinant of parasite infracommunity structure. *Oecologia* **100**: 184-189.

GUÉGAN, J.F. & KENNEDY, C.R. 1993. Maximum helminth parasite community richness in British freshwater fish: A test of the colonisation time hypothesis. *Parasitology* **106**: 91-100.

GUÉGAN, J.F. & LAMBERT, A. 1990. Twelve new species of dactylogyrids (Platyhelminthes, Monogenea) from West African barbels (Teleostei, Cyprinidae) with some biogeographical implications. *Systematic Parasitology* **17**: 153-181.

GUÉGAN, J.F. & LAMBERT, A. 1991. Dactylogyrids (Platyhelminthes: Monogenea) of *Labeo* (Teleostei, Cyprinidae) from West African coastal rivers. *Journal for Helminthological Society of Washington* **58**: 85-99.

GUÉGAN, J.F., LAMBERT, A. & EUZET, L. 1988. Etudes des Monogenes des Cyprinidae du genre *Labeo* en Afrique del'Quest. I. Genre *Dactylogyrus* Diesing, 1850. *Revue d'Hydrobiologie Tropicale* **21**(2): 135-151.

GUÉGAN, J.F., LAMBERT, A. & EUZET, L. 1989. Monogeneans from cyprinid fishes of the genus *Labeo* in West African Rivers. II. Genus *Dogielius* Bychowsky, 1936. *Revue d'Hydrobiologie Tropicale* **22**(1): 35-48.

GUÉGAN, J.F., LAMBERT, A., LÉVÊQUE, C., COMBES, C. & EUZET, L. 1992. Can host body size explain the parasite species richness in tropical freshwater fishes? *Oecologia* **90**: 197-204.

GURR, E. 1956. *A practical manual of medical and biological staining techniques*. Leonard Hill, London.

GUSSEV, A.V. 1961. A viviparous monogenetic trematode from freshwater basins of Africa. *Doklady Akademu Nauk SSSR Seriya Biologiya* **136**(2): 490-493.

GUSSEV, A.V. 1976. Freshwater Indian Monogenoidea. Principles of systematics, analysis of the world faunas and their evolution. *Indian Journal of Helminthology* **25 &26**: 1-241.

GUSSEV, A.V. 1978. Freshwater Indian Monogenoidea. Principles of systematics, analysis of world fauna and its evolution. *Parazitologicheskii Sbornik.* **28**: 96-198.

HAMILTON-ATTWELL, V.L., TIEDT, L.R. & VAN AS, J.G. 1980. A SEM and TEM study of the integument of *Bothriocephalus gowkongensis* Yeh, 1955. *Proceedings of the Electron Microscopy Society of southern Africa* **10**: 105-106.

HANERT, E.T. 1984. ‘n Morfologiese studie van die Vislintwurm *Bothriocephalus* (Cestoda: Pseudophyllidae). M.Sc. dissertation, Rand Afrikaans University, Johannesburg, South Africa.

HARRIS, P.D., SHINN, A.P., CABLE, J. & BAKKE, T.A. 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. *Systematic Parasitology* **59**: 1-27.

HECHT, T. 1980. A comparison of the otolith and scale methods of ageing and the growth of *Sarotherodon mossambicus* (Pisces: Cichlidae) in a Venda impoundment (Southern Africa). *South African Journal of Zoology* **15**: 222–228.

HOBERG, P.E., JONES, A. & BRAY, R.A. 1999. Phylogenetic analysis among the families of the Cyclophyllidea (Eucestoda) based on comparative morphology, with new hypotheses for co-evolution in vertebrates. *Systematic Parasitology* **42**: 51-73.

HOFFMAN, G.L. & BAUER, O.N. 1971. Fish parasitology in water reservoirs: A review. In: G.E. Hall, (eds) *Reservoir fisheries and limnology* **8**: 495-511.

HOFFMAN, G.L. 1967. *Parasites of North American Freshwater Fishes*. University of California Press, Bekerly, California.

HOLMES, J.C. 1986. The structure of helminth communities. In: Parasitology-Quo vadit? *Proccedings of the Sixth International Congress of Parasitology.* Edited by Howell M.J.). Australian Academy of Science, Canberra.

HYSLOP, E.J. 1988. First occurance of *Acanthogyrus tilapiae* (Baylis, 1948) in *Hemichromis* species. *Journal of Fish Biology* **33**: 491-492.

IBRAHEEM, M.H. 2007. Surface ultrastructure of the plagiorchid trematode *Glossidium pedatum* Looss 1899 from bagrid fish in Egypt. *Acta Zoologica* **88**(2): 173-178.

IMAM, E.A. 1971. Morphological and biological studies of the enteric Helminthes infesting some of the Egyptian Nile fishes particularly *Polyonchobothrium clarias* of the karmotes *Clarias lazera* and *Clarias anguillaris*. Thesis for M.D.Vet. Faculty of Veterinary and Medical Sciences, Cairo University, Egypt.

IMAM, E.A., EL-ASKALANY, M.A. & RASHAD, S.M. 1991. The helminth parasites of *Synodontis schall* and *Bagrus bayad* from Beni-Suef water resources. *Assiut Veterinary Medical Journal* **24**(48): 137-152.

IMAM, E.A., GABR, N.S. & RASHAD, S.M. 1991. Studies on the enteric helminths infecting *Clarias lazera* in El-Minia Governorate, Egypt. *Journal of the Egyptian Society of Parasitologists* **21**(3): 669-673.

IMAM, E.A., HASSAN, A.A., EL-SHABRAWY, M.N. & TAWFIK, M.A. 1979. On some parasites of Nile Fishes in Egypt. *Journal of the Egyptian Veterinary Medical Association* **39**: 119-126.

IMAM, E.A.E. & EL-ASKALANY, M.A. 1990. An approach to helminth parasites of catfish (*Clarias lazera*) in Beni-Suef Governorate. *Assiut Veterinary Medical Journal* **24(**47): 96-107.

JACKSON, P.B.N. 1974. Common and scientific names of freshwater fishes in Southern Africa. *Piscator* **90**: 36-50.

JACKSON, P.B.N. 1975. Common and scientific names of fishes of Southern Africa. Part II. Freshwater fishes. *J.L.B. Smith Institute of Ichthyology Special Publication* **14**: 179-213.

JACKSON, P.B.N. 1978. Health and diseases in intensive aquaculture. *Journal of the South African Veterinary Association* **49**: 57-59.

JANICKI, C. 1928. Cestodes s. str. aus Fischen und Amphibien. Results of the Swedish zoological expedition to Egypt and the White Nile 1901 under the direction of L. A. Jagerskiold, The Library of the Royal University of Uppsala, Uppsala, Sweden.

JARECKA, L. 1975. Ontogeny and evolution of cestodes. *Acta Parasitology* **23**: 93-114.

JEANNETTE, T., JACQUES, N. & BILONG-BILONG, C.F. 2010. Spatial distribution of Monogenean and Myxosporidian gill parasites of *Barbus martorelli* Roman, 1971 (Teleostei, Cyprinidae): The role of intrinsic factors. *African Journal of Agricultural Research* **5**(13): 1662-1669.

JIMÉNEZ-GARCÍA, M.I., VIDAL-MARTÍNEZ, V.M. & LÓPEZ-JIMÉNEZ, S. 2001. Monogeneans in introduced and native cichlids in México: Evidence for Transfer. *Journal of Parasitology*, **87**(4): 907-909.

JONES, A. 1980. *Proteocephalus pentastoma* (Klaptocz, 1906) and *Polyonchobothrium polypteri* (Leydig, 1853) from species of *Polypterus* Geoffrey, 1802 in the Sudan. *Journal of Helminthology* **54**: 25-38.

JOYEAUX, C.E. & BAER, J.G. 1935. Recherches sur les helminthes de 1'Afrique occidental francaise. Cestodes. Collection de la Societe de Pathologic exotique. *Monographie* **2**: 17-54.

JUBB, R.A. 1963. A revised list of freshwater fishes of southern Africa. *Annals Cape Province Museum* **111**: 5-39.

JUBB, R.A. 1967. *Freshwater fishes of Southern Africa*. A.A. Balkema, Cape Town.

JUNOR, F.J.R. & PRICE, C.E. 1969. Monogenetic parasites of African fishes. Preliminary report on species recovered from hosts native to southern Africa. *South African Journal of Science* **65**: 119-120.

KABUNDA, M.Y. & SOMMERVILLE, C. 1984. Parasitic worms causing the rejection of tilapia (*Oreochromis* species) in Zaire. *British Veterinary Journal* **140**(3): 263-268.

KADDUMUKASA, M., KADDU, J.B. & MAKANGA, B. 2006. Occurence of nematodes in the Nile Tilapia *Oreochromis niloticus* (Linne) in Lake Wamala, Uganda. *Uganda Journal of Agricultural Sciences* **12**(2): 1-6.

KASSAYE, E. & TADESSE, B. 2009. The prevalence of internal and external parasites of *Oreochromis niloticus* L. collected from lakes and ponds in Ethiopia. *Proceedings of the First Annual Conference of EFASA*: 142-148.

KENNEDY, C.R. & BURROUGH, R.J. 1981. The establishment and subsequent history of a population of *Ligula intestinalis* in roach *Rutilus rutilus* (L.). *Journal of Fish Biology* **19**(1): 105-126.

KHALIL, L.F. & MASHEGO, S.N. 1998. The African monogenean gyrodactylid genus *Macrogyrodactylus* Malmberg, 1957, and the reporting of three species of the genus on *Clarias gariepinus* in South Africa. *Onderstepoort Journal of Veterinary Research* **65**: 223-231.

KHALIL, L.F. & POLLING, L. 1997. Check List of the Helminth Parasites of African Freshwater Fishes. University of the North, South Africa.

KHALIL, L.F. & THURSTON, J.P. 1973. Studies on the helminth parasites of freshwater fishes of Uganda including the description of two new species of digeneans. *Revue de Zoologie et de Botanique Africaines* **87**(2): 209-248.

KHALIL, L.F. 1963a. On *Diplostomulum tregenna*, the *Diplostomulum* stage of *Diplostomum tregenna* Nazmi Gohar, 1932 with an experimental demonstration of part of the life cycle. *Journal of Helminthology* **37**(3): 199-206.

KHALIL, L.F. 1963b. On some Proteocephalid cestodes from freshwater fishes in the Sudan. *Journal of Helminthology* **37**(4): 307-318.

KHALIL, L.F. 1964. On the biology of *Macrogyrodactylus polypteri* Malmberg, 1956, a monogenetic trematode on *Polypterus senegalus* in the Sudan. *Journal of Helminthology* **38**(3/4): 210-222.

KHALIL, L.F. 1969. Studies on the helminth parasites of freshwater fishes of the Sudan. *Journal of Zoology*, London **158**: 143-170.

KHALIL, L.F. 1970a. Further studies on *Macrogyrodactylus polypteri*, a monogenean on the African freshwater fish *Polypterus senegalus*. *Journal of Helminthology* **44**(3/4): 329-348.

KHALIL, L.F. 1970b. On some nematodes from the freshwater fishes of Ghana with the description of a new species *Spironoura petrel* n. sp. *Journal of Helminthology* **44**(1): 63-68.

KHALIL, L.F. 1971a. Check List of the Helminth Parasites of African Freshwater Fishes. *Commonwealth Agricultural Bureaux, Farnham Royal, Slaugh, England*.

KHALIL, L.F. 1971b. The helminth parasites of African freshwater fishes. Part 1. Zoogeographical affinities. *Revue de Zoologie et de Botanique Africaine* **84**(3/4): 236-263.

KHALIL, L.F. 1973. Some helminth parasites from African freshwater fishes with the description of two new species. *Review de Zoologie et de Botanique Africaine* **87**(4): 795-807.

KHALIL, L.F. 1974. Some nematodes from the freshwater fishes of Rhodesia with the description of a new species *Chathariniella petterae* n. sp. *Annales de Parasitologie Humaine et Comparee* 48(6): 811-818.

KHALIL, L.F. 1979. The helminth parasites of African freshwater fishes. Papers presented to the Societe Internationale de Limnologie meeting in Nairobi, 1979.

KHAN, R. A., RYAN, K., BARKER, D.E. AND LEE, E.M. 1993. Effect of a single *Lernaeocera branchialis* (Copepoda) on growth of Atlantic cod. *Journal of Parasitology* **79**: 954–958.

KHAN, R.A. & THULIN, J. 1991. Influence of pollution on parasites of aquatic animals. *Advanced Parasitology* **30**: 201-238.

KHIDR, A. A. 1990. Population dynamics of *Enterogyrus cichlidarum* (Monogenea: Ancyrocephalinae) from the stomach of Tilapia species in Egypt. *International Journal of Parasitology* **20** (6): 741-745.

KINNE, O. (ed.) 1984. *Diseases of marine animals*. Vol. IV. Part 1. Introduction, Pisces. Biologische Anstalt Helgoland, Hamburg, Federal Republic of Germany.

KLAPTOCZ, B. 1906. Ergebnisse der mit Subvention aus der Erbschaft der Dr. Franz Werner's in den aegyptischen Sudan und nach Nord-Uganda. *Cestoden aus Fischen, aus Varanus undHydrax. Sber. Akad. Wiss. Wien* **115**: 121-144.

KOCH, R.S. & SCHOONBEE, H.J. 1980. A fish mark-recapture study in Boskop Dam, Western Transvaal. *Water SA* **6**: 149-155.

KRITSKY, D.C. & KULO, S.D. 1988. The African species of *Quadriacanthus* with proposal of *Quadriacanthoides* gen. n. (Monogenea: Dactylogyridae). *Proceedings of the Helminthological Society of Washington* **55**: 175-187.

KRITSKY, D.C. & KULO, S.D. 1992. A revision of *Schilbetrema* (Monogenoidea: Dactylogyridae) with descriptions of four new species from African Schilbeidae (Siluriformes). *Transactions of the American Microscopical Society* **111**(4): 278-301.

KRITSKY, D.C. 1974. Monogenetic trematodes (Monopisthocotylea: Dactylogyridae) from freshwater fishes of Colombia, South America. *Journal of Helminthology*, **48**: 59-66.

LAMBERT, A. & EL GHARBI, S. 1995. Monogenean host specificity as a biological and taxonomical indicator for fish. *Biological Conservation* **72**: 227-235.

LE ROUX, L.E. & AVENANT-OLDEWAGE, A. 2009. Aspects of the ecology of *Cichlidogyrus philander* collected in the Padda Dam from *Pseudocrenilabrus philander philander*. *Journal of South African veterinary Association* **80**(2): 130.

LE ROUX, L.E. & AVENANT-OLDEWAGE, A. 2010. Checklist of the fish parasitic genus *Cichlidogyrus* (Monogenea), including its cosmopolitan distribution and host species. *African Journal of Aquatic Sciences* **35**(1): 21-36.

LIM, L.H.S., TIMOFEEVA, T.A. & GIBSON, D.I. 2001. Dactylogyridean monogeneans of the siluriform fishes of the Old World. *Systematic Parasitology.* **50**: 159-197.

LOMBARD, G.L. 1960. A preliminary survey of the occurrence of trematodes in fish and aquatic birds. *Publications Conseil Scientifique pour l'Afrique au sud du Sahara* **63**: 170-174.

LOMBARD, G.L. 1968. A survey of fish disease and parasites encountered in Transvaal. *Newsletter of the Limnological Society of Southern Africa* **11**: 23-29.

LOOSS, A. 1896. Recherches sur la faune parasitaire de l'Egypte. Première partie. *Mén. Inst.Egypte* **3**: 1-252.

LOOSS, A. 1899. Weitere Beiträge zur Kenntnis der Trematoden-Fauna Aegyptens, zugleich Versuch einer naturlichen Gliedering des Genus *Distomum* Retzius. *Zoologische Jahrbucher* **12**: 521-784.

LUUS-POWELL, W.J. 2004. Metazoan parasites of mormyrid fishes from South Africa. PhD. thesis, University of the North, South Africa.

LUUS-POWELL, W.J., MASHEGO, S.N. & KHALIL, L.F. 2003. *Mormyrogyrodactylus gemini* gen. et. sp. n. (Monogenea: Gyrodactylidae), a new gyrodactylid from *Marcusenius macrolepidotus* (Mormyridae) from South Africa. *Folia Parasitologica* **50**: 49-55.

LUUS-POWELL, W.J., THERON, J. & HATTINGH H.E. 2006. A small worm causing great losses in aquaculture. *Journal of South African Veterinary Association* **77**(2): 102.

MACKIEWICZ, J.S. & BEVERLY-BURTON, M. 1967.*Monobothrioides woodland* sp. Nov. (Cestoidea: Caryophyllidea) from *Clarias mellandi* Boulenger (Cypriniformes: Clariidae) in Zambia, Africa. *Proceedings of the Helminthological Society of Washington* **34**(2): 125-128.

MADANIRE-MOYO, G. & BARSON, M. 2010. Diversity of metazoan parasites of the African catfish *Clarias gariepinus* (Burchell, 1822) as indicators of pollution in a subtropical African river system. *Journal of Helminthology* **84**(2): 216-227.

MADANIRE-MOYO, G.N., LUUS-POWELL, W.J. & OLIVIER, P.A.S. 2010. Ecology of metazoan parasites of *Clarias gariepinus* (Osteichthyes: Clariidae) from the Nwanedi-Luphephe Dams of the Limpopo River System, South Africa. *African Zoology* **45**(2): 233-243.

MADANIRE-MOYO, G.N., MATLA, M.M., OLIVIER, P.A.S. & LUUS-POWELL , W.J. 2011. Population dynamics and spatial distribution of monogeneans on the gills of *Oreochromis mossambicus* (Peters, 1852) from two lakes of the Limpopo River System, South Africa. *Journal of Helminthology* **85**: 146-152.

MADANIRE-MOYO, G.N. 2011. A comparative evaluation of ecosystem health of selected water bodies in the Olifants and Limpopo River Systems using the health assessment index and parasite diversity as indicators. PhD thesis, University of Limpopo, South Africa.

MAHON, J. 1954. Contributions to the helminth fauna of tropical Africa. Tapeworms from the Belgian Congo. *Annales du Musee du Congo Belge,* Série 5 **1**(2): 141-261.

MALMBERG, G. 1957. On a new species of viviparous monogenetic trematodes. *Arkiv för Zoologie* **10**(3): 317-330.

MALMBERG, G. 1970. The excretory systems and the marginal hooks as a basis for the systematic of *Gyrodactylus* (Trematoda, Monogenea). *Arkiv för Zoologie* **23**: 1-237.

MALVESTUTO, S.P. & OGAMBO-ONGOMA, A. 1978. Observations on the infection of *Tilapia leucosticte* (Pisces: Cichlidae) with *Contracaecum* (Nematoda: Heterocheilidae) in Lake Naivasha, Kenya. *Journal of Parasitology* **64**(2): 383-384.

MANTER, H.W. & PRITCHARD, M.H. 1969. Some digenetic trematodes of Central Africa, chiefly from fishes (1). *Revue de Zoologie et de Botanique Africaine* **80**(1/2): 51-61.

MARCOGLIESE, D.J. 2002. Food webs and the transmission of parasites to marine fish. *Parasitology* **124**: S83-S99.

MARGOLIS, L., ESCH, G.W., HOLMES, J.C., KURIS, A.M. & SCHAD, G.A. 1982. The use of ecological terms in parasitology. *Journal of Parasitology* **68**: 131-133.

MARIAUX, J. 1998. A molecular phylogeny of the Eucestoda. *Journal of Parasitology* **84**: 114-124.

MARX, M.H. & AVENANT-OLDEWAGE, A. 1996. Redescription of *Lamproglena clariae* Fryer, 1956 (Copepoda, Lernaeidae), with notes on its occurrence and distribution. *Crustaceana* **69**: 503-523.

MASHEGO, S.N. & SAAYMAN, J.E. 1981. Observations on the prevalence of nematode parasites of catfish *Clarias gariepinus* (Burchell, 1822) in Lebowa, South Africa. *South African Journal of Wildlife Research* **11**(2): 46-48.

MASHEGO, S.N. & SAAYMAN, J.E. 1989. Digenetic trematodes and cestodes of *Clarias gariepinus* (Burchell, 1822) in Lebowa, South Africa, with taxonomic notes. *South African Journal of Wildlife Research* **19**(1): 17-20.

MASHEGO, S.N. 1977. A seasonal investigation of the ecto- and endoparasites of the barbel, *Clarias gariepinus* (Burchell, 1822) in Lebowa, South Africa. MSc dissertation, University of the North, South Africa.

MASHEGO, S.N. 1982. A seasonal investigation of the helminth parasites of *Barbus* species in water bodies in Lebowa and Venda, South Africa. PhD thesis, University of the North, South Africa.

MASHEGO, S.N. 1983. South African monogenetic parasites of the genus *Dactylogyrus*: new species and records (Dactylogyridae: Monogenea). *Annals of the Transvaal Museum* **33**(22): 337-346.

MASHEGO, S.N. 1988. A new species of *Acanthosentis* Verma & Datta, 1929 (Acanthocephala: Quadrigyridae) from *Barbus neefi* in South Africa. *Annals of the Transvaal Museum* **34**(23): 545-549.

MASHEGO, S.N. 1989. Nematode parasites of *Barbus* spp. in Lebowa and Venda, South Africa. *South African Journal of Wildlife Research* **19**(1): 35-37.

MASHEGO, S.N. 1990. A new species of *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochonidae) from *Barbus* species in South Africa. *Annals of the Transvaal Museum* **35**(7): 147-149.

MASHEGO, S. N. 2000. Occurrence of *Neodiplozoon polycotyleus* Paperna, 1973 (Diplozoidae: Monogenea) in cyprinid fish in South Africa. *Onderstepoort Journal of Veterinary Research* **67**: 153-154.

MASHEGO, S. N. 2001. Redescription of *Proteocephalus glanduliger* (Janicki, 1928) Fuhrmann, 1933 (Cestoda: Proteocephalidae: Proteocephalinae). *Annals of the Transvaal Museum* **38**: 13–17.

MASHEGO, S.N., SAAYMAN, J.E & MOKGALONG, N.M. 1991. Parasites of the fish population with notes on the helminth parasites of the water birds of Middle Letaba Dam. In: A post-impoundment ecological study of the Middle Letaba Dam, Gazankulu, with special reference to its fish production potential, (eds) J.E. Saayman & H.J. Schoonbee, pp. 8.1-8.210. University of the North, South Africa.

MASHEGO, S.N., WATSON, R.M. & AVENANT-OLDEWAGE, A. 2006. Presence of a cestode larva in the uterus of another cestode species. *Journal of South African Veterinary Association* **77**(2): 96.

MATLA, M.M. 1994. Investigations into the benthic macro-invertebrate fauna of the Middle Letaba Impoundment, Gazankulu. MSc dissertation, University of the North, South Africa.

MBAHINZIREKI, G.B. 1980. Observations on some common parasites of Bagrus docmac Forskahl (pisces: siluroidea) of lake Victoria. *Hydrobiologia* **75**(3): 273-280.

MEADE, T.G. & BEDINGER, C.A. 1972. Helminth parasitism in some species of freshwater fishes of Eastern Texas. *The Southwestern Naturalist* **16**(3&4): 281-295.

MEGGITT, F.J. 1930. Report on a collection of cestodes mainly from Egypt. IV. Conclusion. *Parasitology* **22**(3): 338-354.

MENDOZA-FRANCO, E.F., VIDAL-MARTINEZ, V.M., CRUZ-QUINTANA, Y. & PRATS LEON F.L. 2006. Monogeneans on native and introduced freshwater fishes from Cuba with the description of a new species of *Salsuginus* Beverley-Burton, 1984 from *Limia vittata* (Poeciliidae). *Systematic Parasitology*, **64**(3): 181-190.

METTRICK, D.F. 1960. Contribution to the helminth fauna of Central Africa. II. Some cestodes of the order Pseudophyllidea recorded from Southern Rhodesia. *Proceedings & Transactions of the Rhodesia Scientific Association* **48**: 54-62.

MIZELLE, J.D. & HUGHES, R.D. 1938. The North American freshwater Tetraonchinae. *American Midland Naturalist* **20**(2): 341-353**.**

MIZELLE, J.D. 1940. Studies on monogenetic trematodes III: Redescriptions and variations in known Species. *The Journal of Parasitology* **26**(3): 165-178.

MODISE, E.M., KING, P.H. & BAKER, C. 2009. *Cichlidogyrus* spp. Paperna, 1960 on the gills of *Tilapia rendalli* from the Okavango Delta, Botswana. *Journal of South African Veterinary Association* **80**(2): 139.

MODISE, E.M., KING, P.H., BAKER, C. & VAN AS, J.G. 2006. Monogenean parasites on gills of freshwater fish from the Okavango Delta, Botswana. *Journal of South African Veterinary Association* **77**(2): 96.

MOEMA, E.B.E., KING, P.H. & BAKER, C. 2006. Description of digenean trematode larval stages belonging to the family Strigeidae in proximity of Pretoria, Gauteng. *Journal of South African Veterinary Association* **77**(2): 97.

MOKGALONG, N.M. 1996. A study of the gastrointestinal helminthes of the Phalacrocoracidae and the Anhingidae in the Northern Province, South Africa. PhD thesis, University of the North, South Africa.

MONNIG, H.O. 1926. Helminthological notes. *11th & 12th Report Director Veterinary Education & Research, Deptartment of Agriculture, South Africa* (1): 221-228.

MORAVEC, F. & PUYLAERT, F. 1970. On *Johnstonmawsonia africana* sp. nov. (Nematoda: Rhabdochonidae) from the freshwater fish *Haplochromis schwetzi* of Angola. *Revue de Zoologie et de Botanique Africaines* **82**(3/4): 306-314.

MORAVEC, F. 1974a. On some nematodes from Egyptian freshwater fishes. *Vestnik Ceskoslovenske Spolecnosti Zoologicke* **38**: 32-51.

MORAVEC, F. 1974b. The development of *Paracamallanus cyathopharynx* (Baylis, 1923) (Nematoda: Camallanidae). *Folia Parasitologica* **21**: 333-343.

MORAVEC, F. 1975. The development of *Procamallanus laeviconchus* (Wedl, 1862) (Nematoda: Camallanidae). *Vestnik Ceskoslovenske Spolecnosti Zoologicke* **39**(1): 23-38.

MORAVEC, F. 1977. Some digenetic trematodes from Egyptian freshwater fishes. *Vestnik Ceskoslovenske Spolecnosti Zoologicke* **41**: 52-67.

MOYO, D.Z., CHIMBIRA, C. & YALALA, P. 2009. Observations on the helminth parasites of fish in Insukamini Dam, Zimbabwe. *Research Journal of Agriculture and Biological Sciences* **5**(5): 782-785.

MUELLER, J.F. 1934. Parasites of Oneida Lake fishes. Part 1V. Additional notes on parasites of Oneida Lake fishes, including descriptions of new species. *Roosevelt Wild Life Bulletin* **3**: 335-373.

MUELLER, J.F. 1937. Further studies on North American Gyrodactyloidea. *American Midland Naturalist* 18(2): 207-219.

MUSIBA, M.J. 2004. Some aspects of the life cycle of *Diplostomum* species (Digenea: Diplostomatidae) infecting *Clarias* species (Clariidae) of Lake Victoria. MSc Thesis, University of Dar es Salaam. 102 pp.

MUSILOVÁ, N., ŘEHULKOVÁ, E. & GELNAR, M. 2009. Dactylogyrids (Platyhelminthes: Monogenea) from the gills of the African carp, *Labeo coubie* Rüppell (Cyprinidae), from Senegal, with descriptions of three new species of *Dactylogyru*s and the redescription of *Dactylogyrus cyclocirrus* Paperna, 1973. *Zootaxa* **2241**: 47-68.

MWITA, C. & NKWENGULILA, G. 2008. Determinants of the parasite community of clariid fishes from Lake Victoria, Tanzania**.** *Journal of Helminthology* 82: 7-16**.**

MYERS, B.J, KUNTZ, R.E. & WELLS, W.H. 1962. Helminth parasites of reptiles, birds and mammals in Egypt. VII. Check list of nematodes collected from 1948 to 1955. *Canadian Journal of Zoology* **40**(4): 531-538.

MYERS, B.J., WOLFGANG, R.W. & KUNTZ, R.E. 1960. Helminth parasites from vertebrates taken in the Sudan (East Africa). *Canadian Journal of Zoology* **38**(4): 833-836.

N'DOUBA, V. & LAMBERT, A. 1999. Un nouveau *Macrogyrodactylus* (Monogenea, Gyrodactylidae) parasite de *Heterobranchus longifilis* Valenciennes, 1840 (Téléostéen, Siluriforme) en Côte d'Ivoire. *Zoosystema* **21**(1): 7-11.

N'DOUBA, V. & LAMBERT, A. 2001. Deux Monogènes nouveaux parasites branchiaux de *Clarias ebriensis* Pellegrin, 1920 (Siluriformes, Clariidae) en Côte-d'Ivoire. *Zoosystema* (In press).

N'DOUBA, V., LAMBERT, A. & EUZET, L. 1999. Seven new species of *Quadriacanthus* Paperna, 1961 (Monogenea) from the gills of *Heterobrancus longifilis* and *H. isopterus* from the Ivory Coast, West Africa. *Systematic Parasitology* **44**: 105-118.

NACK, J, BILONG-BILONG, C & EUZET, L. 2005. Monogenean parasites from Clariidae (Teleostei: Siluriformes) in Cameroon: Description of two new species of *Gyrodactylus* from the Nyong basin. *Parasite* 12(3): 213-220.

NICOLAAI, N.N. 2008. A study of the dynamics of the fish populations of a clear-water subtropical lake in the Limpopo Province, South Africa. PhD thesis, University of Limpopo, South Africa.

NIEWIADOMSKA, K. 1996. The genus *Diplostomum* – taxonomy, morphology and biology. *Acta Parasitologica* **41**: 55-66.

NKWENGULILA, G. 1995. Epidemiology and taxonomy of *Diplostomum* species (Trematoda: Diplostomatidae) infecting fish of Llyn tegid, North Wales and Ruvu basin, Tanzania. PhD Thesis, University of Liverpool. 321 pp.

OKORIE, O.O. 1973. Some major unsolved aspects of the dynamics of African fisheries as related to questions of rational development and management. *African Journal of Tropical Hydrobiology and Fisheries, Special Issue* **1**: 27-33.

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

ONIYE, S.J., ADEBOTE, D.A. & AYANDA, O.I. 2004. Helminth,parasites of *Clarias gariepinus* (Teugels) in Zaria, Nigeria. *Journal of Aquatic Sciences* **19**(2): 71-76.

ORTLEPP, R.J. 1935. On the metacercaria and adult of *Clinostomum vanderhorsti sp.nov*., a trematode parasite of fishes and herons. *Onderstepoort Journal of Veterinary Science* **5**(1): 51-58.

ORTLEPP, R.J. 1938. South African Helminths. Part V. Some avian and mammalian helminths. *Onderstepoort Journal of Veterinary Science and Animal Industry* **11**(1): 63-104.

OWOLABI, O.D. 2008. Endoparasitic helminthes of the upside-down catfish *Synodontis membranaceus* (Geoffroy Saint Hilarie) in Jebba Lake, Nigeria. *International Journal of Zoological Research* **4**(3): 181-188.

PAPERNA, I. 1959. Studies on monogenetic trematodes in Israel. 1. Three species of monogenetic trematodes of reared carp. *Bamidgeh, Bulletin of Fish Culture in Israel***11**(3): 51-67.

PAPERNA, I. 1960. Studies on Monogenetic Trematodes in Israel. 2 Monogenetic Trematodes of Cichlids. *Bamidgeh, Bulletin of Fish Culture in Israel* **12**: 20-33.

PAPERNA, I. 1961. Studies on monogenetic trematodes in Israel. 3. Monogenetic trematodes of the Cyprinidae and Clariidae of the Lake of Galilee*. Bamidge, Bulletin of Fish Culture in Israel* **13**: 14-29.

PAPERNA, I. 1965. Monogenetic trematodes of the fish of the Volta basin and South Ghana. *Bamidgeh, Bulletin of Fish Culture in Israel* **17**: 107-111.

PAPERNA, I. 1968. Monogenetic trematodes collected from freshwater fish in Ghana. *Bamidgeh, Bulletin of Fish Culture in Israel* **20**: 80–100.

PAPERNA, I. 1969. Monogenetic trematodes of the fish of the Volta basin and South Ghana. *Bulletin du Institute Français d'Afrique Noir* **31**: 840-880.

PAPERNA, I. 1973. New species of Monogenea (Vermes) from African freshwater fish. A preliminary report. *Revue de Zoologie et de Botanique Africaine* **87**(3): 505-518.

PAPERNA, I. 1974. Larval *Contracaecum* (Nematoda: Anisakidae) in the pericardium of fishes from East African Lakes. *Proceedings of the Helminthological Society of Washington* **41**(2): 252.

PAPERNA, I. 1979. Monogenea of inland water fish in Africa. *Annales de Musee Royal de I'Afrique Centrale, Science, Zoologie* 8, **226**: 1 -131.

PAPERNA, I. 1980. Parasites, infections and diseases of freshwater fishes in Africa. CIFA Technical Paper, no. 7. Rome, Italy.

PAPERNA, I. 1996. Parasites, infections and diseases of fishes in Africa – An update CIFA Technical Paper,no. 31. Rome, Italy.

PAPERNA, I. & THURSTON, J.P. 1968. Monogenetic trematodes (Dactylogyridae) from fish in Uganda. *Revue de Zoologique et de Botanique Africaines* **78**: 284-294.

PAPERNA, I. & THURSTON, J. P. 1969. Monogenetic Trematodes collected from cichlid fish in Uganda; including the description of five new species of *Cichlidogyrus*. *Revue de Zoologie et de Botanique africaines* **79**: 15-33.

PARISELLE, A. & EUZET, L. 1994. Three new species of *Cichlidogyrus* Paperna, 1960 (Monogenea, Ancyrocephalidae) parasitic on *Tylochromis jentinki* (Steindaichner, 1895) (Pisces, Cichlidae) in West Africa. *Systematic Parasitology* **29**: 229-234.

PARISELLE, A. & EUZET, L. 1995a. Gill parasites of the genus *Cichlidogyrus* Paperna, 1960 (Monogenea, Ancyrocephalidae) from *Tilapia guineensis* (Bleeker, 1862), with description of six new species. *Systematic Parasitology* **30**: 187-198.

PARISELLE, A. & EUZET, L. 1995b. *Scutogyrus* n. g. (Monogenea, Ancyrocephalidae) for *Cichlidogyrus longicornis minus* Dossou, 1982, *C. longicornis* and C*. l. gravivaginus* Paperna and Thurston, 1969, with descriptions of three new species parasitic on African Cichlids. *Journal of the Helminthological Society of Washington* **62**: 157-173.

PARISELLE, A. & EUZET, L. 1996. *Cichlidogyrus* Paperna, 1960 (Monogenea, Ancyrocephalidae): gill parasites from West African Cichlidae of the subgenus *Coptodon* Regan, 1920 (Pisces), with description of six new species. *Systematic Parasitology* **34**: 109-124.

PARISELLE, A. & EUZET, L. 1997. New species of *Cichlidogyrus* Paperna, 1960 (Monogenea, Ancyrocephalidae) from the gills of *Sarotherodon occidentalis* (Daget) (Osteichthyes, Cichlidae) in Guinea and Sierra Leone (West Africa). *Systematic Parasitology* **38**: 221-230.

PARISELLE, A. & EUZET, L. 1998. Five new species of *Cichlidogyrus* Paperna, 1960 (Monogenea: Ancyrocephalidae) from *Tilapia brevimanus, T. buttikoferi* and *T. cessiana* from Guinea, Côte d'Ivoire and Sierra Leone (West Africa). *Folia Parasitologica* **45**: 275-282.

PARISELLE, A. & EUZET, L. 2003. Four new species of *Cichlidogyrus* (Monogenea: Ancyrocephalidae), gill parasites of *Tilapia cabrae* (Teleostei: Cichlidae) with discussion on relative length of haptoral sclerites. *Folia Parasitologica* **50**: 195-201.

PARISELLE, A. & EUZET, L. 2004. Two new species of *Cichlidogyrus* Paperna, 1960 (Monogenea, Ancyrocephalidae) gill parasites on *Hemichromis fasciatus* (Pisces, Cichlidae) in Africa, with remarks on geographical distribution. *Parasite* **11**: 359-364.

PARISELLE, A. & EUZET, L. 2009. Systematic revision of dactylogyridean parasites (Monogenea) from cichlid fishes in Africa, the Levant and Madagascar. *Zoosystema* **31**(4): 849-898.

PARISELLE, A. 2009. *Personal communication.*

PAUGY, D., GUÉGAN, J.F. & Agnese, J. F. 1990. Three simultaneous and independent approaches to the characterization of a new species of *Labeo* (Teleostei,Cyprinidae) from West Africa. *Canadian Journal of Zoology* **68**: 1124-1131.

PEARSON, R.G. & DAWSON, T.P. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology & Biogeography* **12**: 361–371.

PETRIE-HANSON, L. 2001. First reported mortality and associated pathology attributed to *Acolpenteron ureteroecetes* in Largemouth Bass. *Journal of Aquatic Animal Health* **13**: 364-367.

POULIN, R. 1995. 'Adaptive' changes in the behaviour of parasitised animals: a critical review. *International Journal for Parasitology* **25**: 1371-1383.

POUYAUD, L., DESMARAIS, E., DEVENEY, M. & PARISELLE, A. 2006. Phylogenetic relationships among monogenean gill parasites (Dactylogyridae, Ancyrocephalinae) infecting tilapiine hosts (Cichlidae), systematic and evolutionary implications. *Molecular Phylogenetics and Evolution* **38**: 241-249.

PRICE, C.E. 1967. Proposal of *Syncleithrium*, a new genus of the North American Monogenea. *Texas Journal of Science* **19**: 175-183.

PRICE, C.E. & KIRK, R. 1967. First description of a monogenetic trematode from Malawi. *Revue de Zoologie et de Botanique Africaine* **76**(1/2): 137-143.

PRICE, C.E. & McCLELLAN, E.S. 1969. The monogenean parasites of African fishes. IX. A new genus, *Gussevstrema*, recovered from the gills of *Terapon jarbua* (Forskal) from South Africa. *Proceedings of the Biological Society of Washington* **82**: 171-176.

PRICE, C.E. & PIKE, T. 1969. The monogenean parasites of African fishes. VII. Dissolution of the family Protogyrodactylidae, Johnston and Tiegs, 1922. *Proceedings of the Helminthological Society of Washington* **36**(2): 259-264.

PRICE, C.E. & YURKIEWICZ, N.J. 1968. The monogenean parasites of African fishes. VIII. A re-evaluation of the genus *Dogielius* Bychowsky, 1936 with the description of a new species. *Revista Iberica de Parasitologia* **28**: 467–472.

PRICE, C.E., KORACH, K.S. & McPOTT, R. 1969a. The monogenean parasites of African fishes. V. Two new *Dactylogyrus* species from Natal cyprinids. *Revue de Zoologie et de Botanique Africaine* **79**(3/4): 273-279.

PRICE, C.E., McCLELLAN, E.S., DRUCKENMILLER, A. & JACOBS, L.G. 1969b. The monogenean parasites of African fishes. X. Two additional *Dactylogyrus* species from South African *Barbus* hosts. *Proceedings of the Biological Society of Washington* **82**: 461-468.

PRICE, C.E., PEEBLES, H.E., & BAMFORD, T. 1969c. The monogenean parasites of African fishes. IV. Two new species from South African hosts. *Revue de Zoologie et de Botanique Africaine* **79**(1/2): 117-24.

PRICE, E.W. 1938. A rediscription of *Clinostomum intermedialis* Lamont (Trematoda: Clinostomidae), with a key to the species of the genus. *Proceedings of the Helminthological Society of Washington* **5**(1): 11-13.

PŘIKRYLOVÁ, I. & GELNAR, M. 2008. The first record of *Macrogyrodactylus* species (Monogenea, Gyrodactylidae) on freshwater fishes in Senegal with the description of *Macrogyrodactylus simentiensis* sp. nov., a parasite of *Polypterus senegalus* Cuvier. *Acta Parasitologica* **53**(1): 1-8.

PRUDHOE, S. 1951. Trematoda, Cestoda and Acanthocephala. *Result. scient. Explor. hydrobiol. Lac Tanganyika* (1946-1947) **3**(2): 1-10.

PRUDHOE, S. 1957. Trematoda. *Exploration du Parc National de l'Upemba Mission G. F. de Witte en collaboration avec W. Adam, A. Janssens, L. van Meel & R. Verheyen (1946-49) Bruxelles* **48**: 1-28.

PRUDHOE, S. & HUSSEY, C.G. 1977. Some parasitic worms in freshwater fishes and fish-predators from the Transvaal, South Africa. *Zoologica Africana* **12**(1): 113-147.

RAMOLLO, P.P., LUUS-POWELL, W.J. & JOOSTE, A. 2006. Impact of water quality on abundance of parasites from *Oreochromis mossambicus* at the Phalaborwa Industrial Complex (PIC) and Barrage: preliminary results. *Journal of South African Veterinary Association* **77**(2): 103.

RAWSON, M.V. & ROGERS W.A. 1972. Seasonal abundance of ancyrocephalinean (Monogenoidea) parasites of blue gill, *Lepomis macrochirus* (Raf). *Journal of Wildlife Diseases* **8**: 255-260.

RETIEF, N.-R. , AVENANT-OLDEWAGE, A & DU PREEZ, H. 2006. The use of cestode parasites from the largemouth yellowfish, *Labeobarbus kimberleyensis* (Gilchrist and Thompson, 1913) in the Vaal Dam, South Africa as indicators of heavy metal bioaccumulation. *Physics and Chemistry of the Earth*, **31**: 840-847.

RETIEF, N.-R. , AVENANT-OLDEWAGE, A & DU PREEZ, H. 2007. Ecological aspects of the occurrence of asian tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 infection in the largemouth yellowfish, *Labeobarbus kimberleyensis* (Gilchrist and Thompson, 1913) in the Vaal Dam, South Africa. *Physics and Chemistry of the Earth*, **32**: 1384-1390.

RETIEF, N.-R. , AVENANT-OLDEWAGE, A & DU PREEZ, H. 2009. A seasonal study on asian tapeworm as indicators of metal pollution in the largemouth yellowfish of the Vaal dam, South Africa. *Water SA*, **35**(3): 315-322.

ROHDE, K. 1993. *Ecology of marine parasites*. CAB International, Wallingford.

ROHDE, K., HAYWARD, C. & HEAP, M. 1995. Aspects of the ecology of metazoan ectoparasites of marine fishes. *International Journal for Parasitology* **25**(8): 945-970.

SAAYMAN, J.E. 1986. Die limnologie van die waterliggame van Nasionale State van Noord-oos Transvaal. Final Report to the Department of Development Aid, Pretoria. Limnological Research Unit, University of the North, Pietersburg, South Africa.

SAFRIEL, O. & BRUTON, M.N. 1984. Aquaculture in South Africa: A cooperative research programme. South African National Scientific Programmes Report No. 89.

SAOUD, M.F.A. & MAGEED, A. 1969. Host-parasite relationships of *Macrogyrodactylus polypteri* (Trematoda: Monogenea) in some fishes of the Sudan. *Current Science* **38**(9): 209-210.

SCHMIDT, G.D. & CANARIS, A.G. 1967. Acanthocephala from Kenya with descriptions of two new species. *Journal of Parasitology* **53**(3): 634-637.

SCHMIDT, G.D. & CANARIS, A.G. 1968. Records of parasitic nematodes in Kenya. *Journal of the East African Natural History Society and National Museum* **27**(2): 155-156.

SCHOLZ, T., BRAY, R.A., KUCHTA, R. & REPOVA, R. 2004. Larvae of gryporhynchid cestodes (Cyclophyllidea) from fish: a review. *Folia Parasitologica* **51**: 131-152.

SCHOLZ, T., DE CHAMBRIER, A. BELETEW, M. & MAHMOUD, Z.N. 2009. Redescription of *Proteocephalus glanduligerus* (Cestoda: Proteocephalidea), a parasite of clariid catfishes in Africa with a unique glandular apical organ. *Journal of Parasitology* **95**(2): 443-449.

SHOTTER, R.A. 1974. Seasonal variation in the occurrence of the acanthocephalan *Acanthogyrus* (*Acanthosentis*) *tilapiae* (Baylis, 1948) in the intestine of the cichlid fish *Tilapia zillii* from a river and lake at Zaria in northern Nigeria. *Proceeding of the Third International Congress of Parasitology* **1**: 399.

SHOTTER, R. A. 1980. Aspects of the parasitology of the catfish *Clarias anguillaris* (L.) from a river and a lake at Zaria, Kaduna State, Nigeria. *Bulletin de l'Institut Fondamental d'Afrique Noire* **42(**4): 836-859.

SHOTTER, R.A. & MEDAIYEDU, J.A. 1977. The parasites of *Polypterus endlicheri* Heckel (Pisces: Polypteridae) from the river Galma at Zaria, Nigeria, with a note on its food. *Bulletin de I'lnstitute Fondamental d'Afrique Noire*, Serie A **39**(1): 177-189.

SINDERMAN, C.J. 1987. Effects of parasites on fish populations: Practical considerations. *International Journal for Parasitology,* **17**(2): 371-382.

SKELTON, P.H. 2001. *A complete guide to the freshwater fishes of Southern Africa*. Southern Book Publishers, South Africa.

SKRJABIN, K.I. 1949.Key to parasitic nematodes. Vol. 1. Spirurata and Filariata (In Russian). Translated in 1969 by M. Raveh, Israel Program for Scientific Translations Ltd. IPST Press, Jerusalem.

SMYTH, J.D. & MCMANUS, D.P. 1989. *The physiology and biochemistry of cestodes*. Cambridge University Press, Cambridge.

SNIESZKO, S.F. 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. *Journal of Fish Biology* **6**(2): 197-208.

SOFT IMAGING SYSTEM GMBH, 1986. Digital solutions for imaging and microscopy. Münster, Germany.

SPASSKY, A.A. & SPASSKAYA, L.P. 1973. New subfamily Gryporhynchinae, subfam. n. (Cestoda: Dilepididae). *Izv Akad. Nauk Mold. SSR*. **9**: 56-59.

SPASSKY, A.A. 1995. Changes in the type of symmetry in higher cestodes ontogeny. *Doklady biologicheskii nauk* **343**: 571-573.

SPROSTON, N.G. 1946. A synopsis of the monogenetic trematodes. *Transactions of the Zoological Society of London* **25**: 185-600.

STORER, T.I., USINGER, R.L., STEBBINS, R.C. & NYBAKKEN, J.W. 1972. *General Zoology*. McGraw-Hill Book Company. New York.

STROMBERG, P.C. & CRITES, J.L. 1974. Specialization, body volume, and geographical distribution of Camallanidae (Nematoda). *Systematic Zoology* **23**(2): 189-201.

TADROS, G. 1968. A redescription of *Polyonchobothrium clarias* (Woodland, 1925) Meggitt, 1930 (Bothriocephalidae: Cestoda) with a brief review of the genus *Polyonchobothrium* Diesling, 1854 and the identity of the genera *Tetracampos* Wedl, 1861, *Senga* Dollfus, 1935, and *Onchobothriocephalus* Yamaguti, 1959. *Journal of Veterinary Science*, United Arabic Republic **5**(1): 53-84.

THAPAR, G.S. 1927. On *Acanthogyrus* n.g. from the intestine of the Indian fish *Labeo rohita*, with a note on the classification of the Acanthocephala. *Journal of Helminthology* **5**: 109-120.

THOMAS, J.D. 2002. The ecology of fish parasites with particular reference to helminth parasites and their salmonid fish hosts in Welsh rivers: a review of some of the central questions. *Advances in Parasitology* **52**: 1-154.

THURSTON, J. P. 1970. The incidence of Monogenea and parasitic Crustacea on the gills of fish in Uganda. *Revue de Zoologie et de Botanique Africaines* **82**: 111-129.

TIMOFEEVA, T.A., GERASEV, P.I. & GIBSON, D.I. 1997. A catalogue of the nominal species of the monogenean family Dactylogyridae Bychowsky, 1933 (excluding *Dactylogyrus* Diesing, 1850). *Systematic Parasitology* **38**: 153-158.

TOMASSON, T., BRUTON, M.N. & HAMMAN, K.C.D. 1985. The demography and management of large cyprinids in a reservoir on the Orange River, South Africa. *Fisheries Research* **3**: 279-308.

TOMBI, J. & BILONG-BILONG, C.F. 2004. Distribution of gill parasites of the freshwater fish *Barbus martorelli* Roman, 1971 (Teleostei: Cyprinidae) and tendency to inverse intensity evolution between Mixosporidia and Monogenea as a function of the host age. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux* **57**(1-2): 71-76.

TRIPATHI, A., AGRAWAL, N. & PANDEY, K.C. 2007. The status of *Quadriacanthus* Paperna, 1961 and *Anacornuatus* Dudey *et al*., 1991 (Monogenoidea: Dactylogyridae) with Rediscription of *Q. kobiensis* Ha Ky, 1968, new geographical records for *Q. bagrae* Paperna, 1979 and *Q. clariadis* Paperna, 1961 from India and a Note on Speciation in Monogenoidea. *Parasitology International* **56**(1): 23-30.

TRONCY, P.M. 1974. Acanthocephales parasites de poissons du Tchad. Note de synthese. *Proceedings of the 3rd International Congress of Parasitology*, Munich **G2**: 1622-1623.

TRONCY, P.M. 1978. Nouvelles observations sur les parasites des poissons du bassin Tchadien. *Bulletin de I'Institute Fondamental d'Afrique Noire* **40**(3): 528-552.

TSOTETSI, A.M., AVENANT-OLDEWAGE, A. & MASHEGO, S.N. 2004. Aspects of the ecology of *Lamproglena clariae* (Copepoda: Lernaeidae) from the Vaal River System, South Africa. *Journal of Crustacean Biology* **24**(4): 529–536.

UKOLI, F.M.A. 1966. On *Clinostomum tilapiae* n. sp. and *C. phalacrocoracis* Dubois, 1931, from Ghana, and a discussion of the systematics of the genus *Clinostomum* Leidy, 1856. *Journal of Helminthology* **40**(1/2): 187-214.

VAN AS, J.G. & BASSON, L. 1984. Checklist of freshwater fish parasites from southern Africa. *South African Journal of Wildlife Research* **14**(2): 49-61.

VAN AS, J.G., SCHOONBEE, H.J. & BRANDT, F. DE W. 1981. Further records of the occurrence of *Bothriocephalus* (Cestoda: Pseudophyllidae) in the Transvaal. *South African Journal of Science* **77**: 343.

VAN SENUS, P. 1989. Investigations into the ecology of the larger fish species, with special reference to the numerically dominant species *Oreochromis mossambicus, Clarias gariepinus and Labeo ruddi*, in the Middle Letaba Dam, Gazankulu. PhD thesis, University of the North, South Africa.

VASSILIADES, G. 1970. Un nouveaux *Paracamallanus* (Nematoda: Camallanidae) chez un poissons Clariidae de Sangalkam (Senegal). *Annales de Parasitologie Humaine et Comparee* **45**(4): 441-448.

VASSILIADES, G. 1972. Nematodes parasites des poissons de la region de Sangalkam Senegal). *Bulletin de l'Institute Fondamental de l'Afrique Noire* **34**(3): 529-533.

VASSILIADES, G. 1973. Nematodes parasites de *Distichodus rostratus* poissons Citharinidae du Lac de Guiers (Senegal). *Annales de Parasitologie Humaine et Comparee* **48**: 469-475.

VIOZZI, G.P. & BRUGNI, N.L. 2003. *Acolpenteron australe* sp. n. (Dactylogyridae: Dactylogyrinae), a new species from the ureters of *Percichthys trucha* (Perciformes: Percichthyidae) in Patagonia (Argentina). *Folia Parasitologica* **50**: 105-108.

WABUKE-BUNOTI, M. A. N. 1980. The prevalence and the pathology of the cestode *Polyonchobothrium clarias* (Woodland, 1925) in the teleost, *Clarias mossambicus* (Peters). *Journal of Fish Diseases* **3**: 223-230.

WEDL, K. 1861. Zur Helminthenfauna Aegyptens. *Zitzungsen 15. Akademic der Wessenschafter*, *Wien, Math-Natur* **44**(9): 463-482.

WHITFIELD, A.K. & HEEG, J. 1977. On the life cycles of the cestodes *Ptychobothrium belones* and nematodes of the genus *Contracaecum* from Lake St. Lucia, Zululand, South Africa. *South African Journal of Science* **73**: 121-122.

WILLIAMS, M.O. & CHAYTOR, D.E.B. 1966. Some helminth parasites of freshwater fishes of the Freetown Peninsula, Sierra Leone. *Bulletin de I'lnstitute Fondamental d'Afrique Noire Serie A* **28**(2): 563-575.

WOODLAND, W.N.F. 1925. On three new Proteocephalids (Cestoda) and a revision of the genera of the family. *Parasitology* **17**: 370-394.

WOODLAND, W.N.F. 1937. Some cestodes from Sierra Leone. I. On *Wenyonia longicauda* sp. nov. and *Proteocephalus bivitellatus* sp. nov. *Proceedings of the Zoological Society of London* **4**: 931-937.

WU, X.Y., ZHU, X.Q., XIE, M.Q. & LI, A.X. 2007. The evaluation for generic-level monophyly of Ancyrocephalina (Monogenea, Dactylogyridae) using ribosomal DNA sequence data. *Molecular Phylogenetics and Evolution* **44**(2): 530-544.

YAMAGUTI, S. 1958. *Systema Helminthum. Vol. 1. The digenetic Trematodes of Vertebrates – Part 1 and 11.* Interscience Publishers, New York.

YAMAGUTI, S. 1959. *Systema Helminthum. Vol. 11. The Cestodes of Vertebrates*. Interscience Publishers, New York.

YAMAGUTI, S. 1961. *Systema Helminthum. Vol. 111. The Nematodes of Vertebrates*. Part 1 and 11. Interscience Publishers, New York.

YAMAGUTI, S. 1971. Synopsis of digenetic trematodes of vertebrates. Vol. 11. Keigaku Publishing Company, Tokyo, Japan.

YEH, L.S. 1957. On a new nematode, *Spirocamallanus mazabukae* sp.nov., from freshwater fish in Southern Africa. *Journal of Helminthology* **31**(3): 126-130.

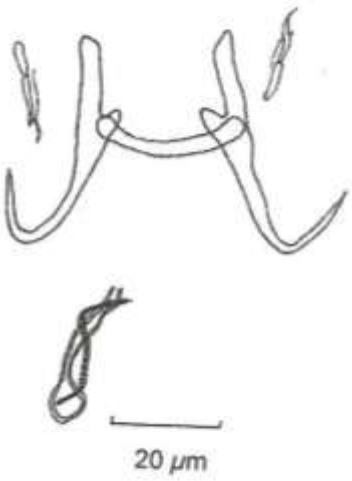
Addendum 1a

Measurements (in *µ*m) of *Dactylogyrus* sp. 1 compared to *Dactylogyrus brevicirrus* Paperna, 1973

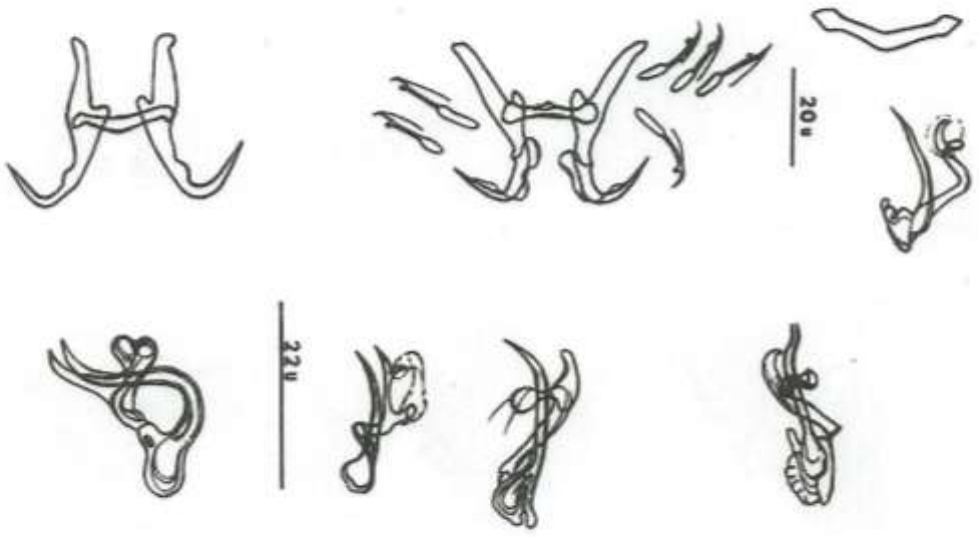
	Dactylogyrus sp. 1	*Dactylogyrus brevicirrus*
Type host	*Barbus radiatus*	*Labeo victorianus*
Location	gills	gills
No. of specimens	30	16
Body length	230 – 400	230 – 380
width	70 – 110	40 – 100
Anchors length	35 – 38	35 – 40
inner root	12 – 15	16 – 20
outer root	2 – 5	1 – 4
shaft	24 – 26	20 – 24
tip	14 – 15	10 – 14
Bar length	24 – 28	19 – 21
width	2 – 3	–
Marginal hooklets	17 – 19	15 – 18
Cirrus axis	19 – 25	25 – 30
Accessory piece	19 – 23	15 – 21

Addendum 1b

Dactylogyrus sp. 1 (this author's drawing) compared to *Dactylogyrus brevicirrus* Paperna, 1973 (Drawings from Paperna 1979)



Dactylogyrus sp. 1



Dactylogyrus brevicirrus

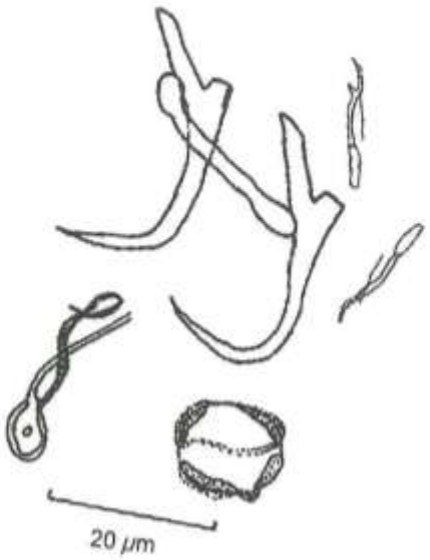
Addendum 2a

Measurements (in *µ*m) of *Dactylogyrus* sp. 2 compared to *Dactylogyrus longiphallus* Paperna, 1973

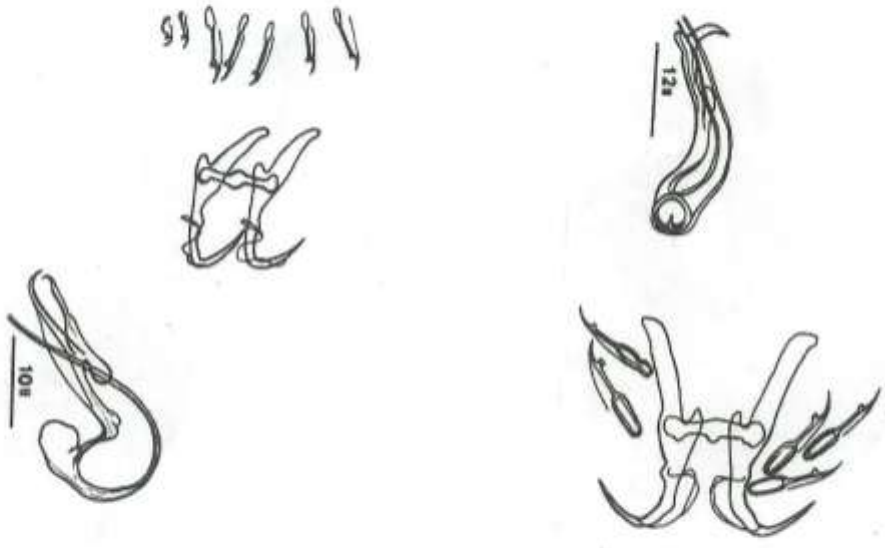
	Dactylogyrus sp. 2	*Dactylogyrus longiphallus*
Type host	*Barbus unitaeniatus*	*Labeo victorianus*
Location	gills	gills
No. of specimens	2	8
Body length	225 – 415	190 – 240
width	35 – 60	50 – 80
Anchors length	32 – 33	34 – 41
inner root	12 – 13	16 – 22
outer root	2	1 – 4
shaft	22 – 23	20 – 24
tip	15	11 – 17
Bar length	25	15 – 24
width	2	–
Marginal hooklets	16 – 17	10 – 15
Cirrus axis	22	45 – 63
Accessory piece	18 – 20	32 – 40

Addendum 2b

Dactylogyrus sp. 2 (this author's drawing) compared to *Dactylogyrus longiphallus* Paperna, 1973 (Drawings from Paperna 1979)



Dactylogyrus sp. 2



Dactylogyrus longiphallus

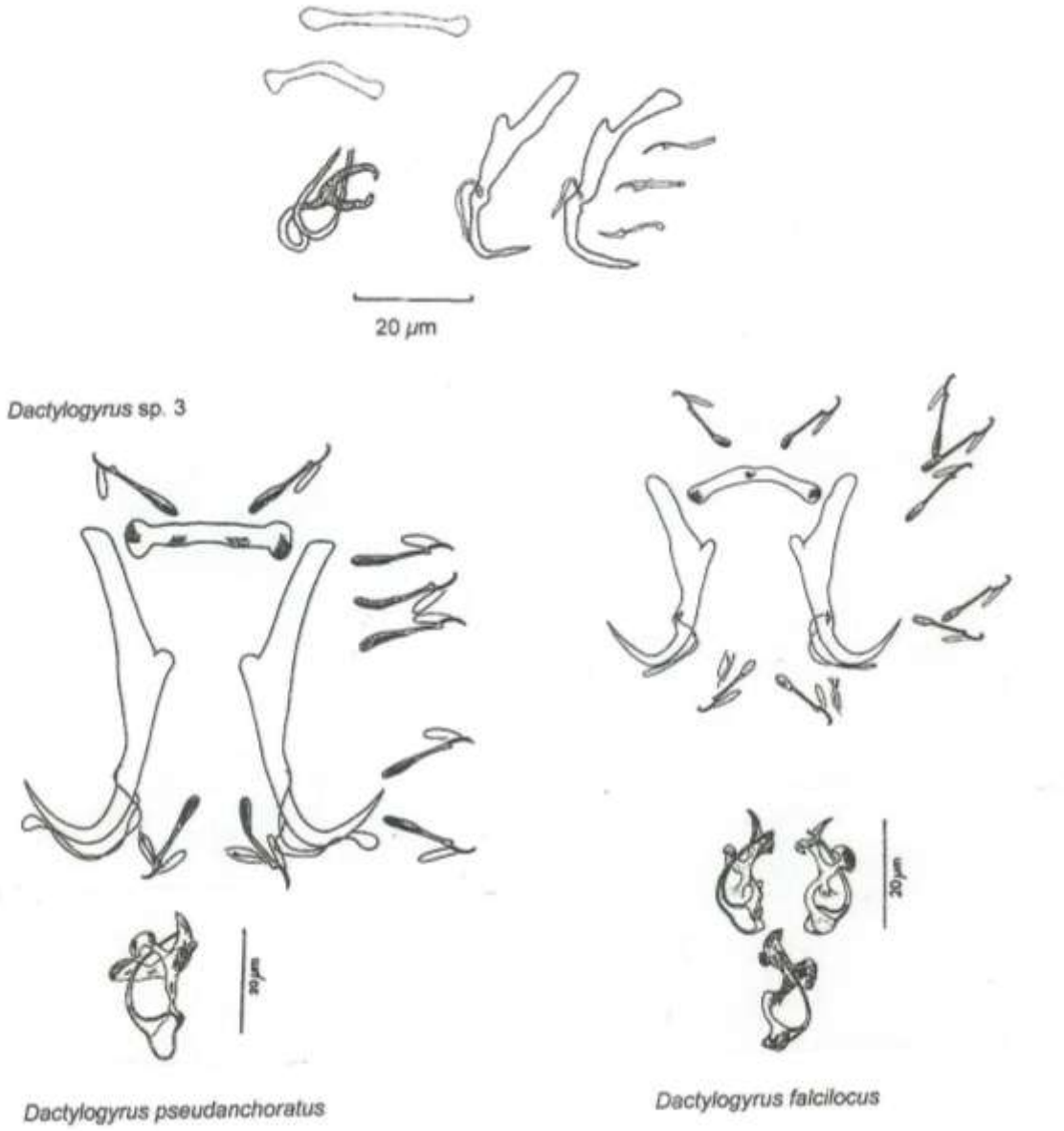
Addendum 3a

Measurements (in *µ*m) of *Dactylogyrus* sp. 3 compared to *Dactylogyrus pseudanchoratus* Price & Géry, 1968 and *Dactylogyrus falcilocus* Guégan, Lambert & Euzet, 1988

	Dactylogyrus sp. 3	*Dactylogyrus pseudanchoratus*	*Dactylogyrus falcilocus*
Type host	*Labeo molybdinus*	*Barbus* sp.	*Labeo coubie*
Location	gills	gills	gills
No. of specimens	8	23	20
Body length	260 – 400	210 – 240	320 – 520
width	60 – 130	56	60 – 100
Anchors length	32 – 37	52 – 61	34 – 39
inner root	13 – 16	–	14 – 18
outer root	2 – 3	–	1 – 3
shaft	22 – 23	–	22 – 24
tip	10 – 12	–	12 – 14
Bar length	17 – 29	23 – 28	23 – 27
width	2 – 3	–	2 – 4
Marginal hooklets	14 – 15	18 – 20	14 – 17
Cirrus axis	16 – 25	20 – 25	18 – 23
Accessory piece	14 – 20	15 – 20	15 – 20

Addendum 3b

Dactylogyrus sp. 3 (this author's drawing) compared to *Dactylogyrus pseudanchoratus* Price & Géry, 1968 and *Dactylogyrus falcilocus* Guégan, Lambert & Euzet, 1988 (Drawings from Guégan & Lambert 1990,1991)

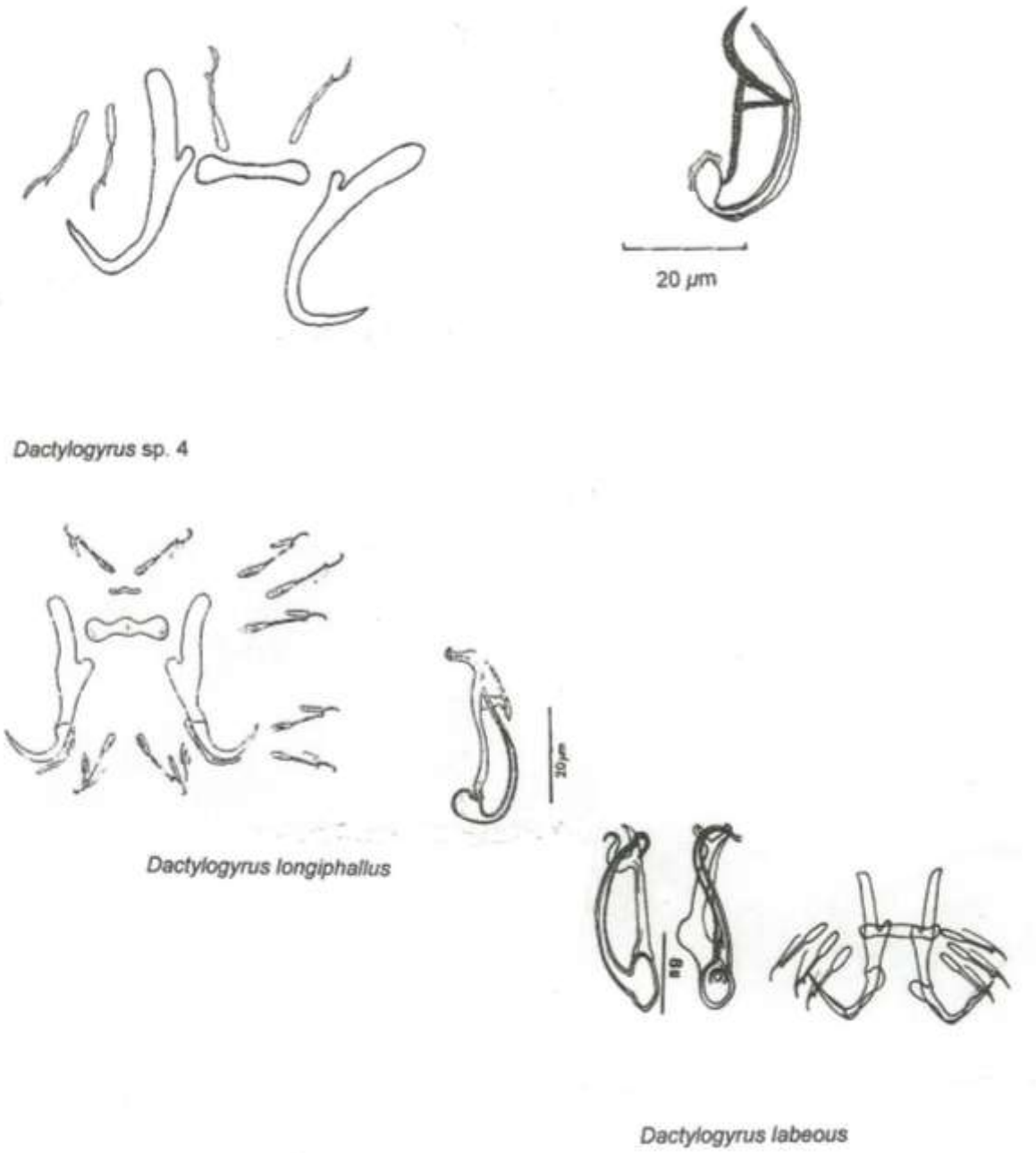


Addendum 4a

Measurements (in *µ*m) of *Dactylogyrus* sp. 4 compared to *Dactylogyrus longiphallus* Paperna, 1973 and *Dactylogyrus labeous* Paperna, 1969

	Dactylogyrus sp. 4	*Dactylogyrus longiphallus*	*Dactylogyrus labeous*
Type host	*Labeo molybdinus*	*Labeo victorianus*	*Labeo senegalensis*
Location	gills	gills	gills
No. of specimens	30	8	-
Body length	240 – 325	190 – 240	250 – 300
width	20 – 90	50 – 80	50 – 100
Anchors length	34 – 38	34 – 41	45
inner root	14 – 16	16 – 22	–
outer root	2 – 3	1 – 4	–
shaft	20 – 22	20 – 24	–
tip	8 – 11	11 – 17	–
Bar length	18 – 20	15 – 24	22 – 25
width	2 – 3	–	–
Marginal hooklets	17 – 20	10 – 15	10 – 35
Cirrus axis	25 – 36	45 – 63	30
Accessory piece	17 – 30	32 – 40	25

Addendum 4b



Dactylogyrus sp. 4 (this author's drawing) compared to *Dactylogyrus longiphallus* and *Dactylogyrus labeous* (Drawings from Paperna 1979 and Guégan & Lambert 1991)

Addendum 5

Lake Tzaneen – a sanctuary for monogenean parasites

MM Matla[1], WJ Luus-Powell[1], NM Mokgalong[1] & SN Mashego[2]

[1]*Department of Biodiversity, University of Limpopo, Prinate Bag X1106, Sovenga, 0727 South Africa.*
[2]*University of North-West, Private Bag X2046, Mmabatho, 2735 South Africa*
matlam@ul.ac.za

The research commitments on African freshwater fish monogeneans are uneven, non-existent in some countries and very low in the Southern African region. Lake Tzaneen (23°47'S & 30°09'E) is a sub-tropical impoundment in the Limpopo Province of South Africa. It has clear, soft water with good quality for its multi-purpose water usage. In a few areas it is already mesotrophic with some slight eutrophic conditions. Anthropogenic actions may in the long run alter the pH, oxygen, heavy metals and toxic substance levels in the water thus affecting the biota, fish and their monogenean compositions.

Freshwater fish monogeneans, being very small, may often be difficult to detect when they occur in low numbers. Most species occur on the gills, a few on the skin, in the stomach and in the urinary bladder. The skin examinations were performed on either live or freshly killed fish by studying the wet mounts, thereafter the gills were removed from fish and examined for the worms with a stereo-microscope with both incident and transmitted light sources. The collected specimens were stored in 70% ethanol, later mounted using glycerine jelly. Identifications were based on morphological analyses with the help of drawings, micrographs & dimensions.

The results comprise of 13 monogenean genera (with 33 species) belonging to 4 families (Gyrodactylidae, Dactylogyridae, Ancyrocephalidae & Diplectanidae) collected from various fish host species in the lake. These genera are *Gyrodactylus, Macrogyrodactylus, Mormyrogyrodactylus, Dactylogyrus, Dogielius, Schilbetrema, Quadriacanthus, Acolpenteron, Actinocleidus, Bouixella, Cichlidogyrus, Haplocleidus* & *Archidiplectanum.* This study resulted in newly described species, first records for Africa and first records for South Africa.

Addendum 6

Monogenea of the genus *Dactylogyrus* from South Africa

MM Matla[1], SN Mashego[2] and NM Mokgalong[1]

[1]*Department of Biodiversity, University of Limpopo, Private Bag X1106, Sovenga, 0727 South Africa.*
[2]*University of North-West, Private Bag X2046, Mmabatho, 2735 South Africa*
matlam@ul.ac.za

Monogenea of the genus *Dactylogyrus* are parasitic gillworms, predominantly on cyprinid fishes. They are a highly diverse group, with a distribution and zoogeography linked to the evolutionary history of their cyprinid hosts. In Africa, more than 92 species have been described from its fishes as compared to the more than 900 nominal species described worldwide, consequently causing confusion within this largest helminth genus. In South Africa only three studies serve as records for *Dactylogyrus* Monogenea with only 11 species present thus far. The present study adds 6 species to the list and 4 of them may be described as new.

In the present study undertaken in lake Tzaneen, South Africa, cyprinid fish hosts (n=88) were collected using gill nets. Nine *Dactylogyrus* spp. were collected from the various cyprinids sampled. Three of them previously found in South Africa are *D. afrolongicornis afrolongicornis* and *D. allolongionchus* collected from *Barbus trimaculatus* as well as *D. spinicirrus* from *Labeobarbus marequensis*. Two are new geographical records and these are *D. brevicirrus* and *D. cyclocirrus* on the gills of *Labeo cylindricus*. Four species to be described as new are *Dactylogyrus* sp. 1 from *Barbus radiatus, Dactylogyrus* sp. 2 from *Barbus unitaeniatus* and both *Dactylogyrus* sp. 3 and *Dactylogyrus* sp. 4 from *Labeo molybdinus*.

In South Africa, monogenean studies are still in the infancy stage of taxonomic identifications, probably due to very little aquaculture practices and fish farming. Price, Korach & McPott (1969) described 2 species. Price, McClellan, Druckenmiller & Jacobs (1969) described one species and found another species. Mashego (1983) described 3 new species, recorded new hosts for 5 species and reported one other species. The species are discussed with a focus on new species. It is envisaged that more species will be found in South Africa as more cyprinid hosts and freshwater bodies are subjected to investigations.

Addendum 7

Monogenean parasites of largemouth bass *Micropterus salmoides* (Lacepede, 1802) in Tzaneen Dam

M M Matla[1], N M Mokgalong[1] and S N Mashego[2]

[1]*Department of Biodiversity, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727 South Africa.*

[2]*North-West University, Private Bag X2046, Mmabatho, 2735 South Africa*

matlam@ul.ac.za

This forms part of a larger project on the helminth parasites of freshwater fish in Tzaneen Dam (23°47'40"S, 30°09'40"E). Micropterus salmoides is an alien fish species and was introduced into the dam through active stocking programmes. As a populargamefish species manysponsored bass fishing tournaments are organised at the dam. Monogenean parasites have been found on this fish species in the USA as well as in other countries where it was introduced, but no records of any monogeneans on this fish exist from Africa. The main focus was to examine the fish specimens for any monogenean parasites and identify them through morphological analyses. Host species (n = 32) were caught using gill nets as well as hook and line and were examined for monogeneans on the skin, gills and in the ureter-urinary bladder complex. Those procured were fixed in 70% alcohol and mounted on slides with the aid of glycerine jelly dissolved over a flame. Morphological analyses were done using a BX 50 Nomarski clinical microscope fitted with a drawing tube, a digital camera and an eyepiece with a µm calibrated measuring ruler. The results revealed a prevalence of 60 % for monogenean infections. Three species of monogeneans were found; Acolpenteron ureterocoetes (n = 3) from the ureter-urinary bladder; Actinocleidus fusiformis (n = 432) and Haplocleidus furcatus (n = 12) co-occur on the gills where they appear as macroscopically similar. Differential diagnoses of the gill monogeneans revealed A. fusiformis with 2 pairs of anchors approximately of uniform size and shape and H. furcatus with 2 pairs of anchors similar in shape but markedly dissimilar in size. A complete morphological analysis was done for each of the 3 monogeneans to confirm their status as new as well as the 1st records on M. salmoides in Africa.

Addendum 8

Monogenea of the genus *Dactylogyrus* from cyprinids of the genera *Barbus*, *Labeobarbus* and *Labeo* in Lake Tzaneen, South Africa

M M Matla[1], N M Mokgalong[1] and S N Mashego[2]

[1]*Department of Biodiversity, University of Limpopo, Prinate Bag X1106, Sovenga, 0727 South Africa.*
[2]*University of North-West, Private Bag X2046, Mmabatho, 2735 South Africa*
matlam@ul.ac.za

Monogenetic parasites are host specialists that naturally occur in intensities that are not very harmful to their fish hosts, but they usually cause epizootics under culture conditions. Their identification is vital for any subsequent ecological studies, their prevention or treatment so as to avoid the fatal effects on hosts when cultured. The hosts (*n*=88) were collected using gill nets and killed by cutting through the spinal cord. The gills were removed from fish and examined for the worms under a stereo-microscope with both incident and transmitted light sources. The collected specimens were stored in 70 % ethanol, later mounted using glycerine jelly. Identifications were based on morphological analyses with the help of drawings, micrographs and dimensions. Nine *Dactylogyrus* spp. were collected from the various cyprinids sampled. *Dactylogyrus afrolongicornis afrolongicornis, D. allolongionchus* and *Dactylogyrus* sp. 1 co-occurred on some specimens of *Barbus trimaculatus. Barbus radiatus* shared the same *Dactylogyrus* sp. with *B. trimaculatus*. *Barbus unitaeniatus* hosted *Dactylogyrus* sp. 2. *Labeobarbus marequensis* had only 1 species, *D. spinicirrus. Dactylogyrus brevicirrus* and *D. cyclocirrus* specimens co-occurred with *Dogielius dublicornis* on some *Labeo cylindricus* hosts. The 2 other *Dactylogyrus* spp. (species 2 & 3) co-occurred with *Dogielius* sp. on *Labeo molybdinus. Dactylogyrus brevicirrus* and *D. cyclocirrus* are the 1st records for South Africa. All 4 *Dactylogyrus* spp. are the 1st host records and 1st records for South Africa (Africa!) and may be described as new species. These data contribute to knowledge of the ichthyo-parasitic fauna of Lake Tzaneen.

Addendum 9

Helminth larval forms from freshwater fishes in a South African impoundment

MM Matla[1], SN Mashego[2] & NM Mokgalong[1]

[1]*Dept. of Biodiversity, University of Limpopo, P/Bag X1106, Sovenga, 0727, South Africa*
[2]*University of North-West, P/Bag X2046, Mmabatho, 2735, South Africa*
matlam@ul.ac.za

Parasites are of economic importance in both natural waters and more so in aquaculture. The starting point is usually their identification, then their ecology and lastly prevention and control. This study is part of a larger project on the helminth parasites of freshwater fishes and addresses the identification and ecological statistics of larval forms that use fish as intermediate hosts and birds as final hosts. Lake Tzaneen forms the major part of the Tzaneen Dam Nature Reserve with multitude activities that include fishing and angling, with fish farming and conservation strategies in the planning stages. During the routine examination of hosts for parasites, metacercariae were procured from the brain, branchial, visceral and heart cavities as well as the eyes. Metacestodes and plerocercoids were collected from the intestines and visceral cavity. Many other digenean cysts were retrieved externally on the skin and gills and internally in the visceral cavity. Metacercariae were killed and fixed in hot (±70 °C) alcohol-formal-acetate whilst nematodes were fixed using glacial acetic acid. The specimens were directly transferred into 70% ethanol for storage. The standard procedure for staining was followed and this comprised rehydration, staining (using aceto alum carmine solution), dehydration and clearing with clove oil. Temporary mounts were done on microscopic slides and the worms were identified using a BX51 clinical microscope through morphological analyses, photographs, drawings, dimensions and literature. *Diplostomulum* were obtained from *C. gariepinus*, *L. marequensis*, *O. mossambicus* and *C. flaviventris* with prevalence of 8% and mean intensity of 16. *Clinostomum* metacercariae had prevalence of 33% in *O. mossambicus* and a prevalence of 5% in *S. intermedius.* The prevalence for *Ligula intestinalis* in both *B. radiatus* and *B. unitaeniatus* was 100%, in *M. brevianalis* was 7%, in *M. salmoides* was 6% and in *L. marequensis* was 3%. All the five host species had mean intensity of 1 each. The gryporhynchid larvae had prevalence of 31% and mean intensity of 29 in *O. mossambicus*, and prevalence of 5% and mean intensity of 1 in *T. rendalli*. *Contracaecum* larvae had the following statistics: prevalence of 49% and mean intensity of 20 (*C. gariepinus*), 38% and 10 (S. *intermedius*), 19% and 4 (*O. mossambicus*), 10% and 3 (*B. trimaculatus*) 10% and 1.5 (*T. rendalli*) and 3% and 2 (*M. salmoides*). There were also numerous small unidentified cysts that were lodged in the skin (black spots), the gills and the visceral cavity. Though the life cycles of all these larvae are indirect and not troublesome in fish farming and aquaculture, the effects of their epizootiology and pathology cannot be underestimated.