Parasites of *Barbus* species (Cyprinidae) of southern Africa

By

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Chapter 1

Introduction

Although the fish parasite fauna of southern Africa has been studied extensively, information concerning parasites of freshwater fishes is by no means complete (Dejen *et al*. 2002). The present investigation was conducted to study the fish parasites from the fish genus *Barbus* Cuvier & Cloquet, 1816, in southern Africa to understand the general parasite infections, prevalences, seasonality, geographical distributions and the parasite fauna of this particular fish genus. This study will therefore contribute towards our knowledge of the parasite fauna of indigenous southern African freshwater fish.

What is a parasite? The word “parasite” is the composition of two Greek words, “para” and “sitos” which means “next to” and “grain or food”, respectively. This refers to organisms which live in or on a host, in this case a fish, and eats the food of the host or feeds on the host itself. Various different parasite groups can be found associated with fish, some are more pathogenic, while others do little or no harm (Buchmann *et al*. 2009). It is also important to know that parasites in natural conditions do not necessarily negatively impact fish populations, in fact the presence of indigenous parasites in natural conditions indicates a healthy system (Buchmann *et al*. 2009).

The genus *Barbus* was specifically selected for this investigation because of its wide distribution and the abundant occurrence of several species across most southern Africa freshwater bodies. Only a few studies on the parasites of *Barbus* have been conducted from southern Africa so far (Price *et al.* 1969; Mashego 1982, 1983, 1988, 2000; Basson *et al*. 1983; Viljoen & van As 1985; van As & Basson 1989; Reed *et al*. 2002; Barson & Marshall 2003; Schulz & Schoonbee 2006).

Most of the fish species from the genus *Barbus* in southern Africa are relatively unimportant in terms of aquaculture and food production, but some of the species are a major food source in Africa (Figure 1.1) and others are important as ornamental fish. Parasites can also be seen as problem animals in aquaculture and alien parasites can be harmful in natural systems. That is why it is very important to have a sound understanding of the diversity of indigenous as well as alien parasites, the life cycles of these parasites as well as the problems that these parasites may cause.

Figure 1.1: Basket fishing in the Okavango River System is a major food source for the people of Botswana living along the river. They use baskets to catch small fish and many of the fish that that are caught with these baskets are of the genus *Barbus* Cuvier & Cloquet, 1816.

This study is not only important because it expands our knowledge of the aquatic biodiversity, but could also provide valuable information about potential threats to humans. According to Mashego (1982), several different parasites from different parts in the world can infect humans through eating smoked or insufficiently cooked fish and a variety of fish diseases can be responsible for heavy mortalities in infested fish.

Fish parasite information can also be of great value to determine if the indigenous fish are infected by alien parasites. The introduced alien crustacean parasites *Argulus japonicus* Thiele, 1900*, Lernaea cyprinacea* Linnaeus, 1758 and the cestode *Bothriocephalus acheilognathi* Yamaguti, 1934, all associated with cyprinid hosts in their native distribution, were all introduced to Africa and are now widespread in the river systems across southern Africa where they are a threat to the indigenous fish fauna. According to Dejen *et al.* (2006), fish face a wide range of different enemies

in their natural conditions including competitors, predators and parasites, parasites can alter the condition, reproductive fitness and the mortality of their hosts.

Fish parasites have been the focus of numerous studies around the world, but these have mostly been of marine parasites, whilst studies of freshwater fish parasites in Africa are rare (Dejen *et al*. 2002; Tombi *et al.* 2011). Checklists of freshwater fish parasites of Africa have been provided by van As & Basson (1984), Paperna (1996) and Khalil & Polling (1997), and few parasites have been found associated with *Barbus* spp. in southern Arica. One of these parasites is *Ligula intestinalis* Linnaeus, 1758, a cestode parasite with a high prevalence for infecting small barbs. This parasite has a complex life cycle that involves three hosts. The first intermediate host involves cyclopoid copepods, the fish as the second intermediate host and a piscivorous bird as the final host. A plerocercoid larva infects and develops in the body cavity of the fish, which grows into a very large worm that causes swelling of the belly of its host. The worm can also change the behaviour of the fish, it increases their appetite, affects locomotion and finally the behaviour change facilitates predation by birds (Dejen *et al.* 2006). The plerocercoid larvae can occupy the body cavity of the fish for several years.

According to Mashego (2000), *Afrodiplozoon polycotyleus* Paperna, 1973 was found on *Barbus trimaculatus* Peters, 1852, larval forms were found on *Barbus neefi* Greenwood, 1962 and three new species of monogeneans were described from different *Barbus* hosts by Mashego (1983), i.e. *Dactylogyrus teresae* Mashego, 1983*, D. enidae* Mashego, 1983 and *D. dominici* Mashego, 1983, all from the Limpopo System. One monogenean species, *D. myersi* Price, McClellan, Druckenmiller, Jacobs, 1969, was described from the Pongola River System by Price *et al*. (1969).

A new species of Acanthocephala, *Acanthosentis phillipi* Mashego, 1988, was described from *Barbus neefi* by Mashego (1988), as well as the adult trematode *Allocreadium mazoensis* Beverley-Burton, 1962; larval forms of the genus *Diplostomum* Nordman, 1832 and *Clinostomum* Leidy, 1856, all found in the Limpopo system from barb species (Mashego 1982).

Three *Myxobolus* Bütschli, 1882 species, i.e., *Myxobolus nayongana* (Fomena, Bouix & Birgi, 1985) Fomena & Bouix 1997 from *Barbus poechii* Steindachner, 1911; *M. etsatsaensis* Reed, Basson & van As, 2002 from *Barbus thamalakanensis* Flower, 1935 and *M. paludinosus* Reed, Basson & van As, 2002 from *B. paludinosus* Peters, 1852 was found infesting the gill lamellae of the host in the Okavango Delta (Reed *et al*. 2002). Various ciliates of the genera *Trichodina* Ehrenberg, 1830, *Trichodinella* (Raabe, 1950) Sramek-Husek, 1953, *Tripartiella* Lom, 1959 and *Apiosoma* Blanchard, 1855 were also found on the skin and gills of *Barbus* species (Basson *et al*. 1983; Viljoen & van As 1985; Basson & van As 1987).

The aims of this study were:

1. To collect data on fish parasites of the genus *Barbus* in the Orange-Vaal, Pongola and Okavango river systems.
2. To identify new parasites and to provide taxonomical descriptions.
3. To identify previously described parasites and to add information on their taxonomy and distributions.
4. To identify infections of alien parasites.
5. To determine the geographical distribution, prevalence and abundance of parasites.
6. To expand our knowledge on the parasite fauna of southern Africa.

This study forms part of parasitic research by the Aquatic Parasitology group of the Department of Zoology & Entomology at the University of the Free State. This group of scientists has conducted a large variety of research topics in parasitology such as: phylogeny, taxonomy and life cycles of myxosporeans, trichodinid, peritrichs, trypanosomes, monogeneans, nematodes, trematodes, copepods and branchiurans. Research has also been conducted in biodiversity, phylogeny, parasite host interactions, as well as water quality and conservation studies (Grobbelaar 2011).

The dissertation comprises the Introduction (including information on the host) followed by a descriptive chapter of the three study sites, the Orange-Vaal, Pongola and Okavango river systems (Chapter 2), followed by four taxonomic chapters i.e.,

Ciliophora (Chapter 3), Myxozoa (Chapter 4), Monogenea (Chapter 5) and Cestoda together with the Trematoda (Chapter 6). The dissertation was written as if all the chapters were separate scientific journal articles according to the specifications proposed for each parasite group. Each chapter, except the study sites chapter contains their own introduction, materials and methods, results, discussion and references. The dissertation ends with a discussion (Chapter 7). The data of this dissertation is applicable to scientists, inland fisheries and aquaculture management.

Some results from this study have already been presented at an international conference. A presentation was given at the combined International Congress on Parasites of Wildlife and 43rd Annual Parasitological Society of Southern Africa (PARSA) in 2014 (Swanepoel & van As 2014).

CYPRINIDAE

Cyprinidae is the largest and most ecologically diverse freshwater fish family in the world with a cosmopolitan distribution and occurs abundantly in most water bodies in southern Africa (Ney & Helfrich 2009). The fish family consists of 275 genera and more than 2,000 species from Africa, Europe, Asia and North America (Tsigenopoulos *et al.* 2002). The earliest cyprinid fossils are of the Eocene Era and most of the cyprinids that live today in major land areas have no existence before the Miocene and Pliocene epochs. Since cyprinids dominate freshwaters today, it is difficult to believe that they have dominate for only the last 10-20 million years, in areas such as North-America, Europe, Africa and India (Winfield & Nelson 1991). The earliest cyprinid fossils have been found in Kazakhstan in Eurasia from the middle Eocene age, and it is believed that cyprinids evolved in Eurasia in this epoch, 40 million years ago. Cyprinids have a long history in Asia, probably dominated the freshwater ichthyofauna as early as the Oligocene in parts of Asia, but cyprinids were only abundant in Siberia, Europe and North-America until the Miocene. Africa was the last major land area to be invaded by cyprinids in the Miocene epoch 18 million years ago (Winfield & Nelson 1991) (Figure 1.3).

Cyprinids evolved in the Cenozoic Era where the continents moved into their current positions and the cyprinids distributed throughout the world through land bridges.

According to Winfield & Nelson (1991), cyprinid fish can be found on almost every continent, except in South America, Australia and Antarctica, and the reason for this is that during the Cenozoic Era these continents had no land bridges that connected them to other continents in order for the cyprinids to be able to invade these land masses (Figure 1.2).

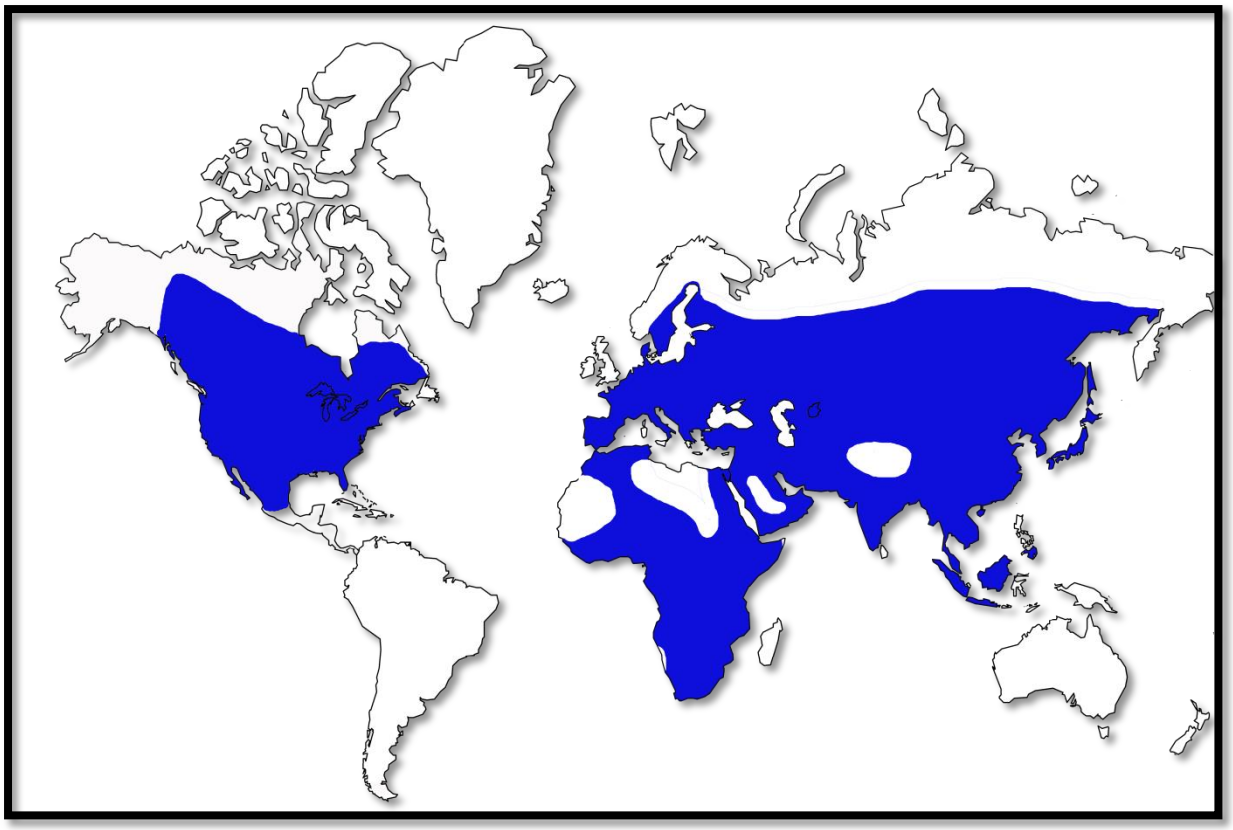

Figure 1.2: World distribution of Cyprinidae. Redrawn from Skelton (2001).

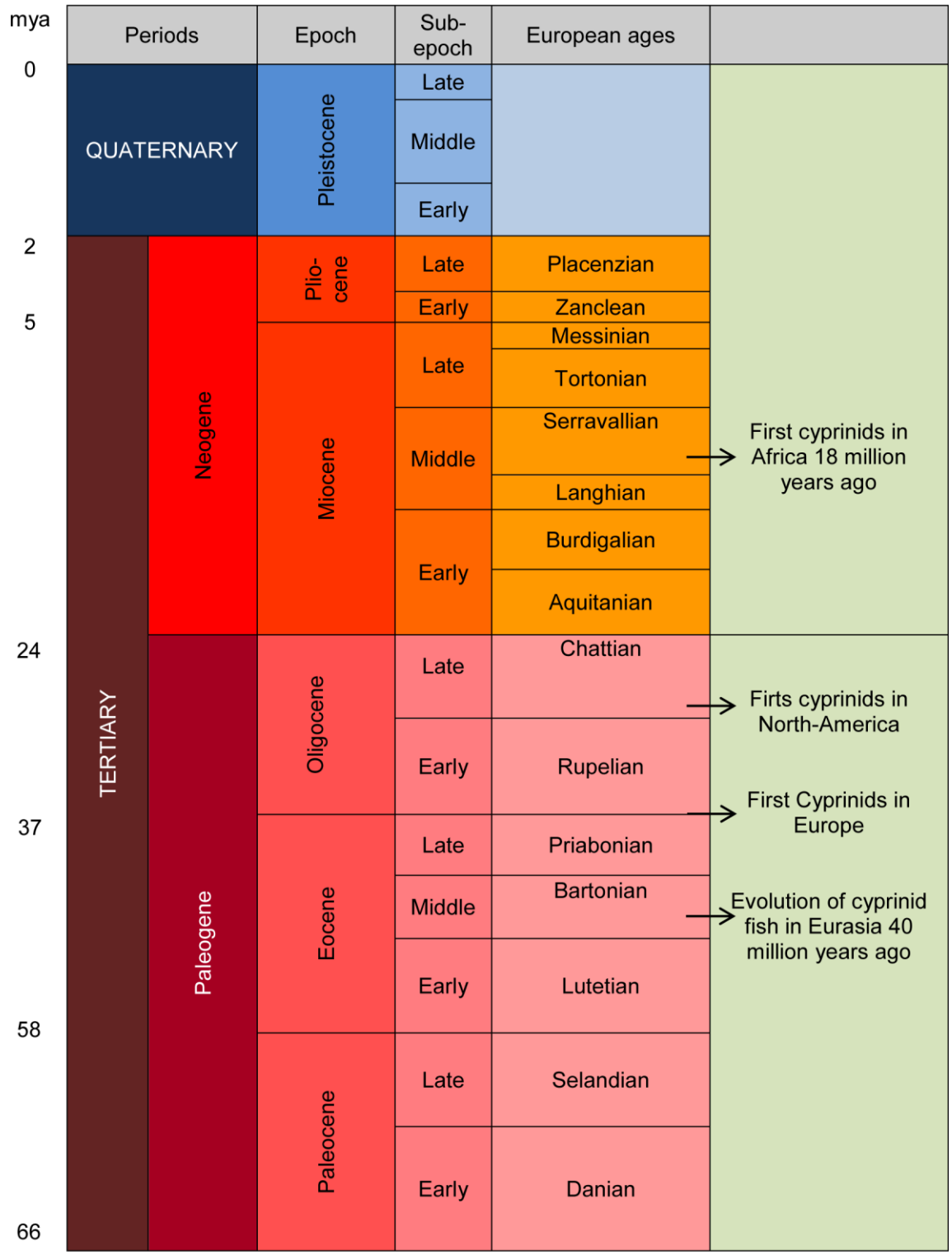

Figure 1.3: Geographic timescale of the Cenozoic era including subdivisions, European age names and the evolution and distribution of Cyprinidae. Adapted from Winfield & Nelson (1991).

Cyprinids are primarily freshwater fish with a wide range of sizes and shapes, and habitat preferences. The characteristics of the members of this family are that they have toothless jaws, but have strong pharyngeal (throat) bones with teeth. They lack a true stomach, as detritus and plant feeders have an extended and convoluted gut. Cyprinids are strong swimmers and some are distinctly modified to live in strong currents (Skelton 2001).

Certain species of cyprinids are economically important in fisheries around the world and some species have the potential to be aquaculture species. According to Sun & Liang (2004), the common carp, *Cyprinus carpio* Linnaeus, 1758, which is a cyprinid, is the most extensively cultured fish in the world. The grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) and the silver carp, *Hypophthalmichthys molitrix* (Valenciennes, 1844) are also important aquaculture species and the larger cyprinids of southern Africa, the yellow fish and labeo's are potential aquaculture species. Many of the smaller cyprinids are used in the aquarium trade and many more have the potential to be ornamental fish. The gold fish, *Carassius auratus* (Linnaeus, 1758) is extremely popular, if not the most popular ornamental fish in aquariums and garden ponds, and the koi fish industry has a large economic impact. Both gold fish and koi fish are cyprinids.

There are 24 genera and 475 cyprinid species in Africa, consisting of a few larger fish genera e.g., *Labeo* Cuvier, 1817 and the yellow fish species *Labeobarbus* Ruppëll, 1836 (Skelton 2001; Crafford *et al.* 2014). This is also the largest fish family in southern Africa with eight genera and 80 species described (Skelton 2001). The majority of cyprinids in southern Africa are small species, less than 200 mm in total length, of the genus *Barbus*; *Pseudobarbus* Smith, 1841; *Opsaridium* Peters, 1854 and *Mesobola* Howes, 1984.

It is generally accepted that the freshwater fish of southern Africa are derived from ancestors that occupied central tropical Africa (Mashego 1982). Unfortunately many of the Cyprinidae in southern Africa are under threat due to a variety of human related factors that affect the ecology of streams and lakes. According to Schulz & Schoonbee (2006), pollution from mines and industries have caused major and irreversible deterioration in the water quality and biology of freshwater systems. Habitat destruction and erection of weirs throughout southern Africa, threaten the

survival of many ecologically sensitive fish species due to the obstruction and siltation of their feeding and breeding grounds. Most of the indigenous cyprinid fish are ecologically sensitive and many are under threat, for example all the fish that belong to the endemic genus *Pseudobarbus*, are threatened through pollution, agriculture and the introduction of predatory fish and two species are critically endangered (Skelton 2001).

BARBUS

The genus *Barbus* in Africa, refers to the small cyprinids, but the genus is only valid for a certain tetraploid European species and a few species from the Maghreb region of north-west Africa (Skelton 2001). African small barbs are considered to belong to the subgenus *Enteromius* Cope, 1869 and differ from the European species by being diploid (2n = 50), they are characterised by an adult size of less than 20 cm standard length and by diverging striae on the exposed part of their scales (Agnese *et al*. 1990; Winfield & Nelson 1991; Dejen *et al*. 2002). African small barbs are also different from the larger cyprinids in Africa which are hexaploid (2n = 150), having parallel striae and having a larger dorsal spine, for example *Labeobarbus* (Dejen *et al.* 2002). According to Skelton (2001), the reclassification of African barbs is in progress based on genetics and morphology. The genus *Barbus* will still be used for small barbs in Africa in the current study until future reclassification.

According to Naran (1997), there are approximately 300 varied and widely different *Barbus* species in Africa. Many barbs have colour variations and a full range of characteristic pigmentation patterns, males may differ from the females by having longer fins and brighter breeding colours (Skelton 2001). These are common fish in streams and freshwater habitats in Africa; they usually occur in schools and are often well camouflaged from the surface. They are distinctly marked in aquariums with stripes, spots and other markings. They are opportunistic feeders, feasting on any small zooplankton, diatoms or detritus. They are also a valuable food source for larger fish and birds (Skelton 2001). They breed in pairs, small groups or large schools, the males usually develop bright colours, especially gold, yellow or red.

The genus *Barbus* in southern Africa is divided in three groups, sawfin, spinefin and soft-rayed barbs.

The genus *Barbus* is represented by 45 different species in southern Africa and that makes them the most represented genus of freshwater fish in southern Africa, but despite their abundance and ecological importance, they have hardly been studied (Dejen *et al*. 2002). Most fish studies have been done on commercially important species, but small barbs form the main link in the food chain between primary consumers and top-predators. Many commercially important fish are the top predators and in order to understand the food chain, the study of their prey is needed (Dejen *et al*. 2002).

Barbs have little commercial value and are therefore often overlooked by researchers. They are, however, of great importance in the ecosystems where they occur, because the different species occupy a variety of niches within the aquatic ecosystems. Many of them are also endemic species, often restricted to a single river. Some species are rare and some are endangered, and at least two species are critically endangered in southern Africa (Table 1.1). One of these species is the critically endangered Border barb, *Barbus trevelyani* Günther, 1877 and it is threatened due to anthropogenic factors such as habitat destruction by dam construction, water extraction, siltation and predation by introduced alien fish species and therefore there is very little hope for the survival of this species in the natural habitat (Cambray 1985).

Table 1.1: International Union of Conservation of Nature Red List of the genus *Barbus* Cuvier & Cloquet, 1816 from southern Africa. Compiled from Skelton (2001).

Species	**Common Name**	**Status**
Barbus erubescens Skelton, 1974	Twee River redfin	Critically Endangered
Barbus trevelyani Günther, 1877	Border barb	Critically Endangered
Barbus calidus Barnard, 1938	Clanwilliam redfin	Endangered
Barbus serra Peters, 1864	Clanwilliam sawfin	Endangered
Barbus motebensis Steindachner, 1894	Marico barb	Vulnerable
Barbus brevipinnis Jubb, 1966	Shortfin barb	Vulnerable
Barbus andrewi Barnard, 1937	Berg-Brede River whitefish	Vulnerable
Barbus treurensis Groenewald, 1958	Treur River barb	Near Threatened
Barbus hospes Barnard, 1938	Namaqua barb	Near Threatened

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Chapter 2

Study Sites

OKAVANGO RIVER SYSTEM

Importance

Due to the limited measureable anthropogenic impacts on the entire system, the Okavango is regarded as one of the most pristine rivers in the world. To date, there are no dams and channelling of the river that change the flow of the water in any way, and the natural vegetation is largely intact in the delta (Mendelsohn & El Obeid 2004). According to Varis *et al*. (2008), the delta is one of the most valuable global wetlands which was declared as one of the world's largest Ramsar sites in April 1997 and UNESCO also declared the Okavango Delta as the 1000th World Heritage Site in June 2014 (UNESCO 2014). The clarity, purity and quality of the water in the Okavango River System are astonishing. The water that comes from the catchment area has few nutrients and those that reach the delta are trapped in the aquatic plants and the Kalahari sand. The water that flows into the delta can be classified as ideal drinking water, and is about 40 times better in terms of purity and quality than the acceptable quality standards of Botswana's drinking water (Mendelsohn *et al.* 2010).

The Okavango Delta is not only special due to its functioning, but also the rich biological diversity with more than 400 species of birds, 122 mammal species, 64 reptile species, about 1,300 plant species, tens of thousands invertebrate species and more importantly in terms of this study, 71 fish species that occur naturally in the system, restricted to Botswana (Mendelsohn *et al*. 2010).

Hydrology

Rivers throughout the world flow down to the ocean and mix with saltwater, but the Okavango River never reaches the ocean. This river collects all its water in Angola from where it flows through hundreds of kilometres down a narrow waterway to the second country, Namibia and, finally the water reaches the inland delta in Botswana's Kalahari Desert (Mendelsohn & El Obeid 2004).

The Okavango River System originates in the pristine wilderness high up in the mountains of Angola with a catchment area of about 111,000 km^2. This catchment area in the central highlands of Angola receives an average annual rainfall of between 1,200 mm in the west and 1,800 mm in the east (Gutteridge & Reumerman 2011). The catchment area is divided into the western Cubango and eastern Cuito sub-catchment areas (Figure 2.1 A). These two rivers meet at the border of Namibia and then form the Okavango River before it crosses the Zambezi Region and finally forms an alluvial fan that covers up to 12,000 square kilometres, which is the largest wetland in southern Africa (Pallett *et al.* 1997; Mendelsohn & El Obeid 2004; Mendelsohn *et al.* 2010). The Okavango Delta (Figure 2.1 B) can be divided into three components, the panhandle, the permanent swamp and the seasonal swamp (Gutteridge & Reumerman 2011). The average flow over the longer term is about 10,000 m^2 (Varis *et al*. 2008). The annual rainfall at the delta is about 500 mm which peaks in January and February. The evaporation rate at the Okavango Delta is about 2,500 mm a year that is five times more than the average rainfall. This means that there would be no surface water for most of the year in the Kalahari Desert, which makes the Okavango Delta an oasis (Gutteridge & Reumerman 2011).

The first rain in the catchment area, the Angolan highlands, starts in October and the water will reach the top of the panhandle at Mohembo in Botswana, during January the following year; the water takes about eight to nine weeks from the source to Botswana. The floodwaters that enter Botswana peak around April, but the rainfall in Angola varies and the waters could arrive earlier or later. The waters then spread through the main alluvial fan during May, June and July with the Okavango Delta at its fullest in August (Gutteridge & Reumerman 2011)(Figure 2.4 A). The water will reach Maun in August and the Thamalakane will turn into a river four months after the period of the highest water volume that entered the Panhandle (Mendelsohn & El Obeid 2004). A graph (Figure 2.2) indicates high foods in the 1980's, low floods in the 1990's and a high flood in 2010 and 2011. It also indicates two peaks of floods, the first in March-February and the second in April.

According to van As *et al*. (2012), the world's largest inland delta is not really a delta in the true sense, as the name refers to the spread of a river before it flows into the sea. The Okavango River does not flow into the ocean, but the same mechanisms

are used, by which sediment is carried by the river and deposited as an alluvial fan across the Kalahari Desert. Three sets of fault lines, that are part of the East African Rift Valley, have a marked impact on the flow and distribution of the surface rivers and shape of the delta. The first fault line is the surface visible Gumare fault; dividing the panhandle from the alluvial fan. The second and third fault lines are the Kunyere and Thamalakane faults that block the water in the delta from flowing further south-eastwards, this forces the water to slow down and spread (Figure 2.1 B). These fault lines are the main cause of the delta's alluvial fan (Mendelsohn *et al*. 2010). In years of good rain in the catchment area (Angolan highlands), the water flows to the south-western areas to reach Lake Ngami and the Boteti River. The flow also seeps away through the Linyanti Swamps to the north-east and sometimes reaches Lake Liambezi (Mendelsohn *et al*. 2010; van As *et al*. 2012). According to Mendelsohn & El Obeid (2004), the Okavango Basin is the catchment area from which the water drains, which is the zone immediately around the flowing rivers and the delta, and also includes the Makgadikgadi Pans, an area into which the Okavango water flows during high rainfall sessions. Two million years ago, rivers larger than the Okavango, flowed into northern Botswana, leaving alluvial sediments of about 120,000 km^2 around the delta and the Makgadikgadi Pans. The Zambezi and Kwando rivers possibly ran here as recently as 50,000 years ago. Gutteridge & Reumerman (2011) state that many of the rivers from central Africa draining from Angola and Zambia today, originally flowed across the Kalahari as one single river into the Indian Ocean via the Limpopo River.

An estimated 96 % of the volume of water entering the delta through the river and rainfall are lost by evaporation; this includes evaporation directly into the atmosphere and through transpiration by plants. About 2.5 % of the delta's water ends up in groundwater aquifers and the remaining 1.5 % flows into the Boteti River (Mendelsohn *et al.* 2010).

Habitat and Vegetation

The total number of plant species currently known in the Okavango River System is 1,300 species, which belong to 530 genera and 134 families (Ramberg *et al.* 2006).

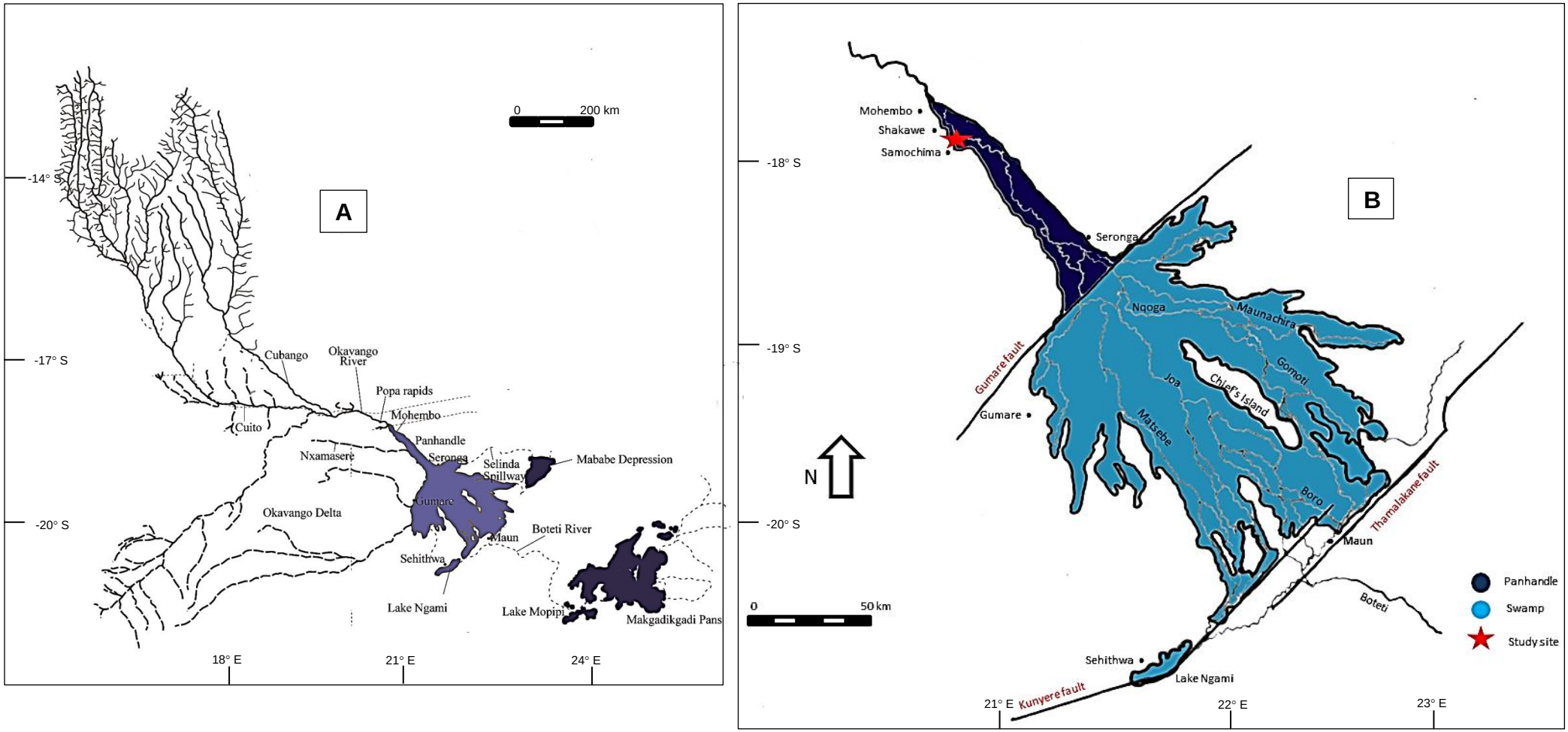

Figure 2.1: Map of the Okavango River Basin (**A**) (Courtesy of the Aquatic Parasitology Research Group, UFS) and Okavango System (**B**). Redrawn from Mendelsohn *et al*. (2010).

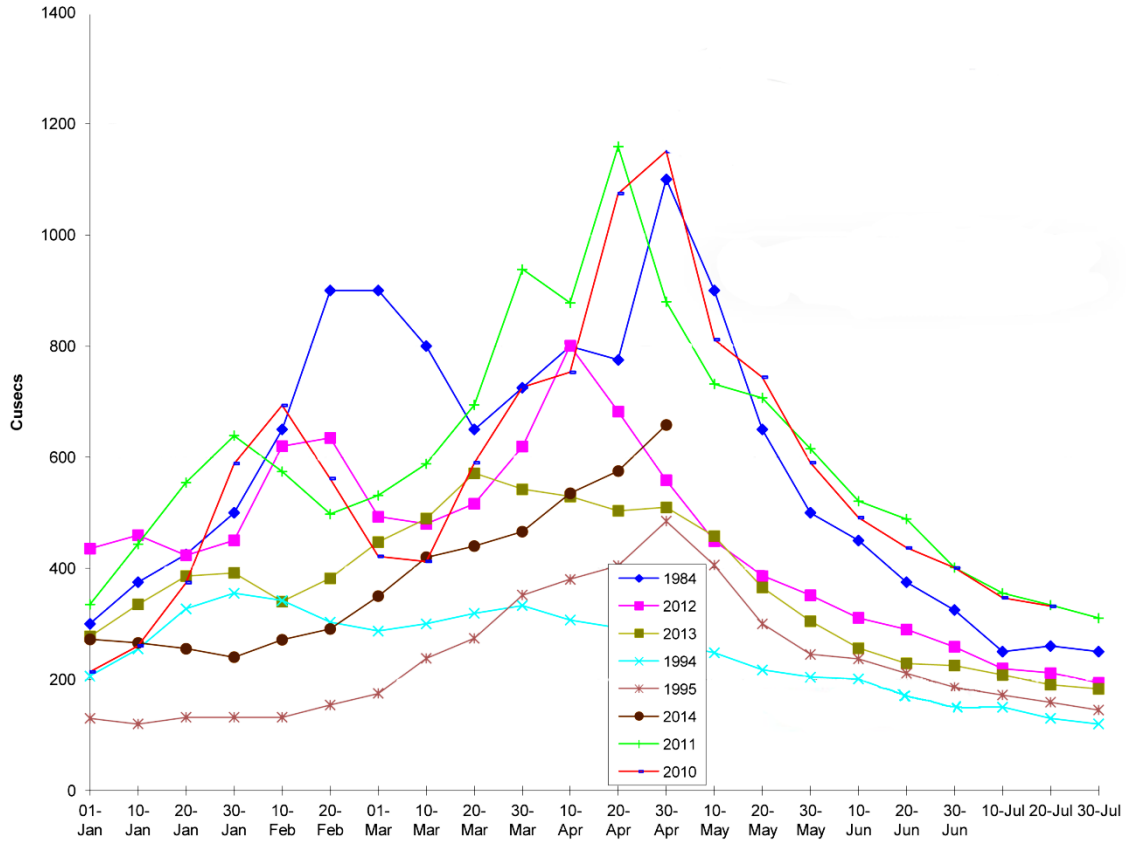

Figure 2.2: Flow graph of the flood levels of the Okavango Delta during different years that was measured at a measuring station at Mohembo. Graph obtained from Aliboats, Maun, Botswana.

The Okavango vegetation is characterised as a complex pattern: it varies from permanent to seasonal swamps, grasslands to riverine woodlands and, dry savannahs that are never under water (Figures 2.4 B-F). This pattern is mainly caused by the ever-changing river courses and the growth of new islands. Large variations of vegetation patterns over small distances can be found in the system, although the delta is very flat and is made up of homogeneous sand (Ramberg *et al*. 2006). Different habitats and vegetation can occur several times along a transect. A small difference in elevation makes a large difference in the duration of flooding, which causes large variations in vegetation in a flat environment (Ramberg *et al.* 2006).

The high diversity of plant species in the delta can be divided into wetland and dryland species. Surprisingly for a wetland, 60 % of all the species are dryland species, occurring on islands and river banks on dry Kalahari sand. However, most

of these species are absent in the dry Kalahari Desert because they need higher levels of air and soil moisture (Ramberg *et al*. 2006). Some of these species include the jackal-berry (*Diospyros mespiliformis* Hochst, 1844), knob-thorn (*Acacia nigrescens* Oliver, 1871) and lead-wood (*Combretum imberbe* Wawra, 1860). A large number of species occur in the swamps, but these are dominated by two plants: namely, papyrus (*Cyperus papyrus* Linnaeas, 1758) and phragmites reeds (*Phragmites australis* Steud, 1841 and *P. mauritianus* Kunth, 1829) (Mendelsohn *et al.* 2010).

The Okavango Delta has a high species richness of fish (Merron & Bruton 1995). The Okavango Basin hosts 86 different fish species and 71 of these species can be found in the Okavango River and Delta below the Popa Rapids (Ramberg *et al*. 2006). The Okavango Delta hosts highly diverse morphologies of fish species where different groups of species inhabit different delta habitats (Mosepele *et al*. 2009). Various habitat types were identified based on physical characteristics of these habitats. The various habitat types include the mainstream, river channels, floodplains, back swamps (backwaters), lagoons, perennial swamps (permanent swamps) and seasonal swamps (temporary swamps) (Christison 2002) (Figures 2.4 B-F). The diverse fish species of the Okavango River System belong to 15 families and 38 different genera (Ramberg *et al*. 2006). There are, however, no endemic fish species in the Okavango Delta. The fish fauna of the Okavango River System is part of the Zambezi System and, although these two rivers are predominantly isolated from one another, they are occasionally connected through the Magwegqana River. There are also similarities with the fish fauna from the Limpopo and Pongola Systems in South Africa (Mosepele *et al.* 2009). According to Ramberg *et al*. (2006), there have been no alien introductions or translocated fish species found in the Okavango River and Delta, to date.

Permanent swamps are characterised by a high abundance of tiger fish (*Hydrocynus vittatus* Castelnau, 1861), threespot tilapia (*Oreochromis andersonii* Castelnau, 1861) and sharptooth catfish (*Clarias gariepinus* (Burchell, 1822)), while the seasonal swamp are dominated by the silver catfish (*Schilbe intermedius* Rüppell, 1832) and African pike (*Hepsetus odoe* (Bloch, 1794)). The Okavango Delta is very important in terms of the current study since it hosts 17 different species of Cyprinidae and 13 of these species belong to the genus *Barbus* (Figure 2.3). The

only fish family with more species than Cyprinidae in the delta is the Cichlidae, with 18 different species (Merron & Bruton 1995; Ramberg *et al*. 2006).

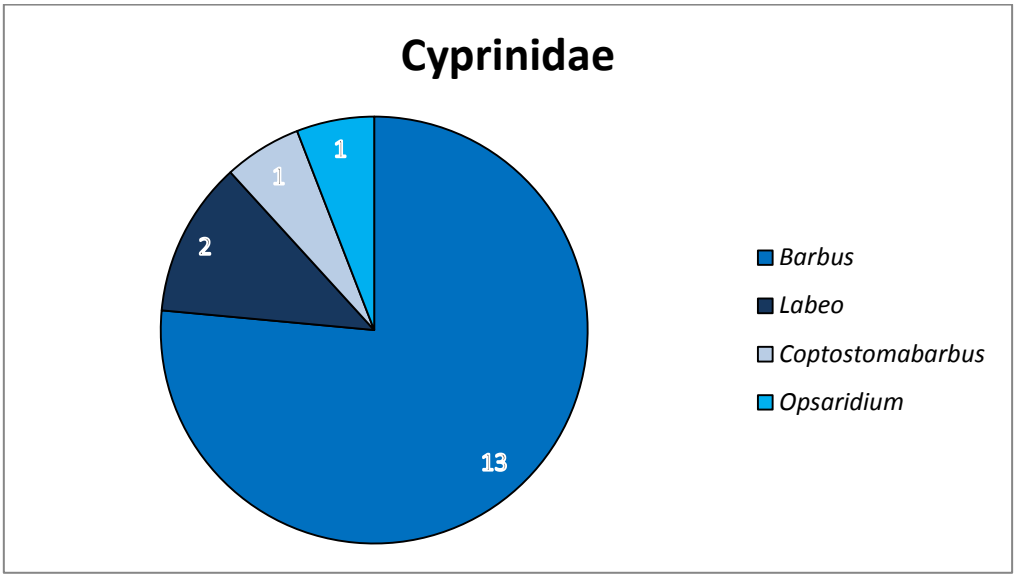

Figure 2.3: The number of fish per genera representing the fish family Cyprinidae of the Okavango Delta. Compiled from Merron & Bruton (1995) & Ramberg *et al.* (2006).

Leseding Research Camp

The field work for the current study at the Okavango Delta was conducted during July to August 2013 at the Leseding Research Camp. The camp is situated in the north-western part of Botswana's Panhandle (Figure 2.1 B). It is situated next to the Shamochima Lagoon on the Krokavango Crocodile Farm close to the village, 12.5 km from Shakawe. The research station was built by the University of the Free State's Aquatic Ecology group from the Department Zoology and Entomology. The main purpose of Leseding is to study Parasitology and the ecology of the Okavango System. The research station is equipped with two motorboats (*Synodontis* and *Labeo*), a permanent laboratory, aquariums, a fully equipped kitchen, six permanent canvas tents, and ablution facilities (Figures 2.4 G-H).

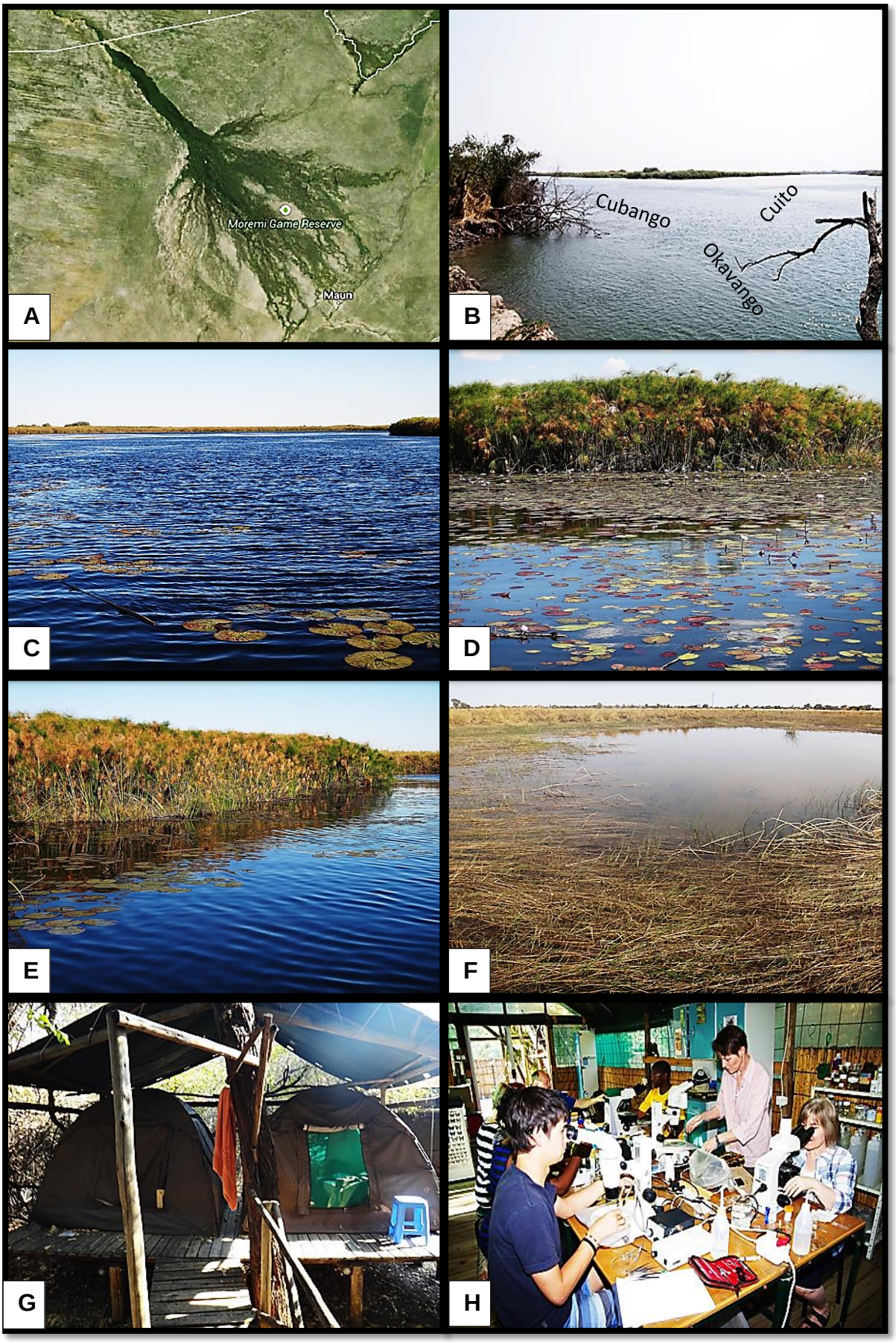

Figure 2.4: Photographs of the Okavango Delta. **A**- The panhandle and alluvial fan of the Okavango Delta (Google Maps 2014). **B**- The main stream in the Panhandle. **C**- Isolated lake in the Panhandle. **D**, **E**- Permanent and **F**- seasonal swamps. **G**- Canvas tents and **H**- laboratory at the Leseding Research camp.

PONGOLA RIVER SYSTEM

Importance

The Pongola River System is one of the most biologically diverse ecosystems in South Africa. The river rises 2,200 m above sea level in Mpumalanga near Wakkerstroom and flows through a variety of different ecosystems, ranging from mountain ranges to forests, oxbow lakes, lagoons, marshes and floodplain grasslands (Van Vuuren 2009). According to Rossouw (1985), the Pongola River down-stream is one of the few floodplains that exist in South Africa. This is also the biggest floodplain in South Africa which covers 10,000 ha (Heeg *et al.* 1980). The floodplain provides habitat for a wide variety of animals but, more importantly in terms of this study, a high diversity of fish species (Van Vuuren 2009).

The Pongola River is very important for various reasons: Firstly, it is the only major floodplain system with a series of pans within the borders of South Africa. It is also the most southern distribution of numerous tropical fish species and therefore important to scientific research. Lastly, the Pongola Floodplain is important for a large number of winter feeding water birds, notably the White Faced Duck and White Pelican (Heeg *et al.* 1980).

Unfortunately, the Pongolapoort Dam was constructed to impound the water of the river for irrigation, mainly for sugarcane, and the dam resulted in the absorption of floods (Figure 2.5). This may pose a threat to many fish species (tigerfish, mudsuckers, catfish) in the Pongola Floodplain that are totally flood dependant for their spawning (Heeg *et al*. 1980).

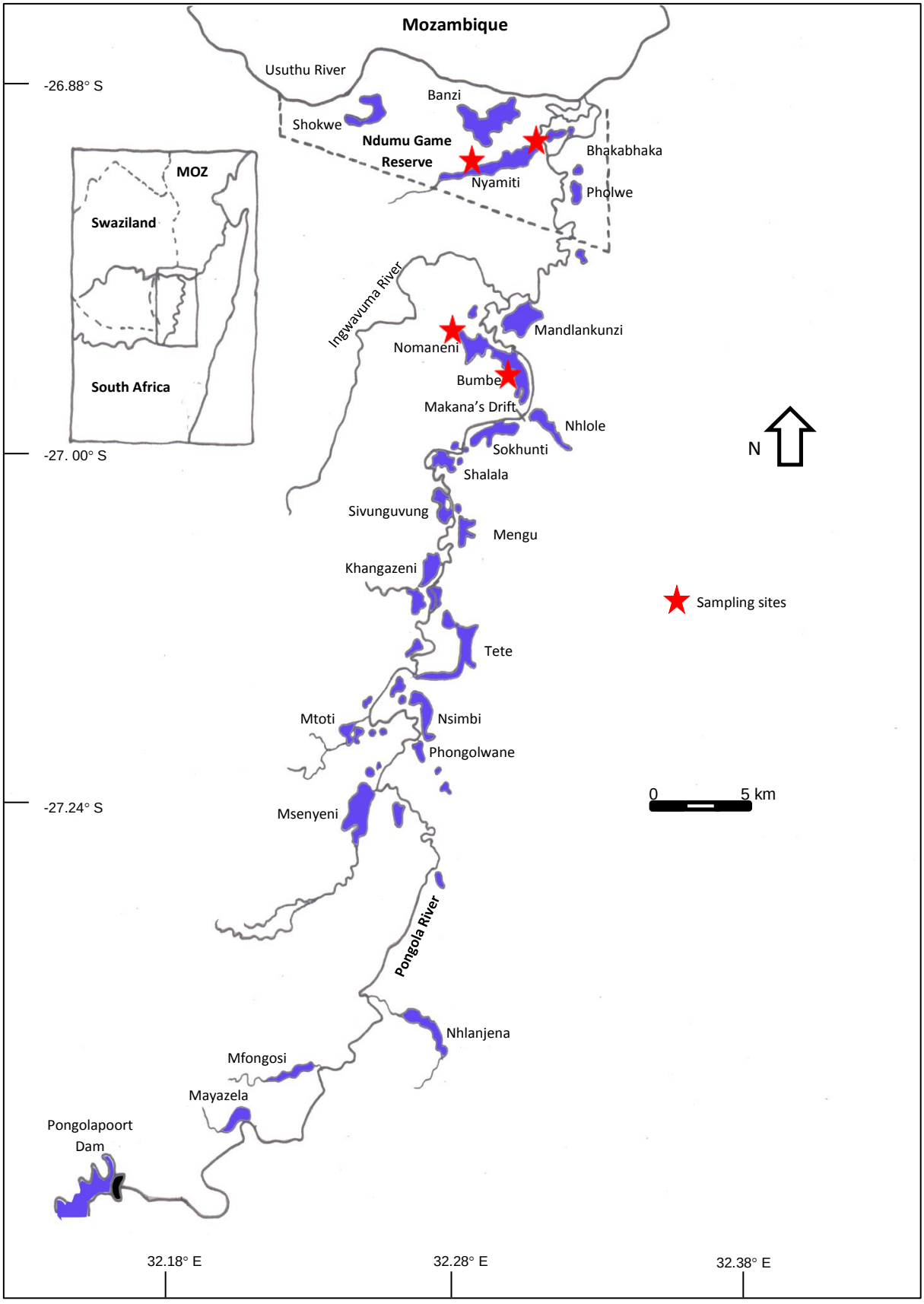

Figure 2.5: Map of the Pongola Floodplain and the sampling sites. Redrawn from Rossouw (1985).

Hydrology

The Pongola River rises in the Madlangampisi Mountains and passes between the Lebombo and Ubombo Mountains in KwaZulu-Natal, through a gorge known as the Pongolapoort before it reaches the lower Maputuland coastal plains. A change in gradient, changes the river into a slow flowing alluvial plain that results in the deposition of sediment which is called the Pongola Floodplain. The Pongola Floodplain (Figure 2.5) is the area downstream from the Pongolapoort up to where the Usutu River meets the Pongola River in the Ndumu Game Reserve (Rossouw 1985). This is a low-lying area with a series of shallow pans separated from the main river *(*Heeg *et al*. 1980). The Pongola Floodplain consists of 65 named and 25 unnamed pans of different sizes and retains floodwater for various lengths of time (Figures 2.6 A-C). According to Van Vuuren (2009), the Pongola Floodplains receive summer flooding that creates a diverse set of environmental conditions and when the floodwaters recede, rich soils are exposed to vegetation. Sadly, the floodwaters and sediments are being trapped behind the Pongolapoort Dam and are unable to reach the floodplains as it did before.

Habitat and Vegetation

The Pongola Floodplain vegetation is very similar to that of the Okavango River System, in the sense that both systems are dependent on the frequency and duration of floods and they are similar in the distribution of plant communities. The terrestrial plant communities are dependent on floodwaters for silt-borne nutrients and water. The terrestrial vegetation of the floodplain is comprised of high-lying vegetation such as *Ficus sycomorus* Linneaes, 1758*, Rauvolfia caffra* Linneaes, 1758*, Acacia xanthophloea* Benth, 1875 and *Dyschoriste depressa* Kuntze, 1832 that are not major contributors to the pans and are frequently flooded in short periods (Heeg *et al.* 1980). The marshy areas are occupied by *Cyperus fastigiatus* Enum, 1805 and *Echinochloa pyramidalis* Hitchc & Chase, 1917 which are known to be eaten by herbivorous fish for example redbreast tilapia (*Coptodon rendalli* (Boulenger, 1896)). One of the most important plants is the grass *Cynodon dactylon* Linnaeus, 1805 which forms extensive meadows around several pans. *Potamogeton crispus* Linnaeus, 1753 is the aquatic primary producer occurring in

permanent lakes in the winter and during summer floods germinates along seasonal swamps (Figures 2.6 D-E); this plant is eaten by herbivorous fish and White Faced Ducks (*Dendrocygnus viduata* Linnaeus, 1766). According to Heeg *et al.* (1980), a population of 8,000 of these ducks have been recorded in the Tete pan.

Fifty different fish species have been recorded from the floodplains; which represents the most diverse fish fauna in South Africa. There are several important fish species: for instance the Pongola River System is the most southern distribution of the economically important angling fish species, tigerfish (*Hydrocynus vittatus*). The Cyprinidae is represented by 22 different indigenous species, while 13 species belong to the genus *Barbus* Cuvier & Cloquet, 1816 (Skelton 2001) (Figure 2.7).

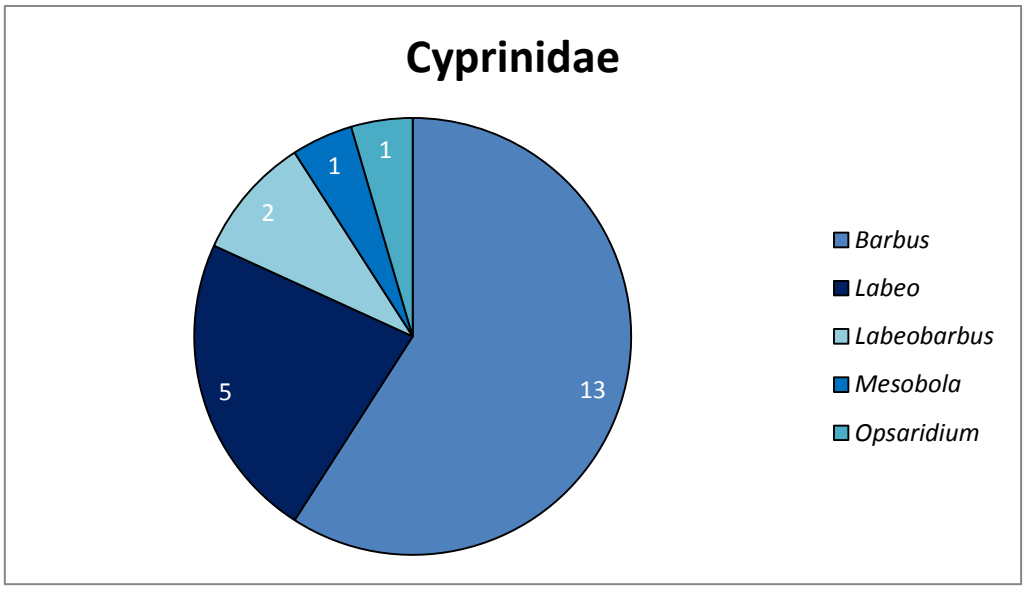

Figure 2.7: The number of fish per genera representing the fish family Cyprinidae of the Pongola River System. Compiled from Skelton (2001).

Ndumo Game Reserve

Field work for the current study in the Pongola Floodplain Pans was conducted in September 2013 at the Ndumo Game Reserve. The reserve is situated in the north-eastern part of South-Africa's KwaZulu-Natal Province, in the northern part of the Pongola Floodplains. The research station was set up in the campsite of Ndumo Rest Camp (Figure 2.6 F) with the collaboration of the North-West University, the University of Johannesburg, the University of Zululand and the University of the Free State's Aquatic Ecology group from the Department Zoology and Entomology. The

main purpose of this specific trip was to collect fish parasites of the Pongola Floodplain. This forms part of a longer Ecology project of the entire system. The research station was equipped with temporary laboratories and aquariums, a kitchen, canvas tents and ablutions facilities.

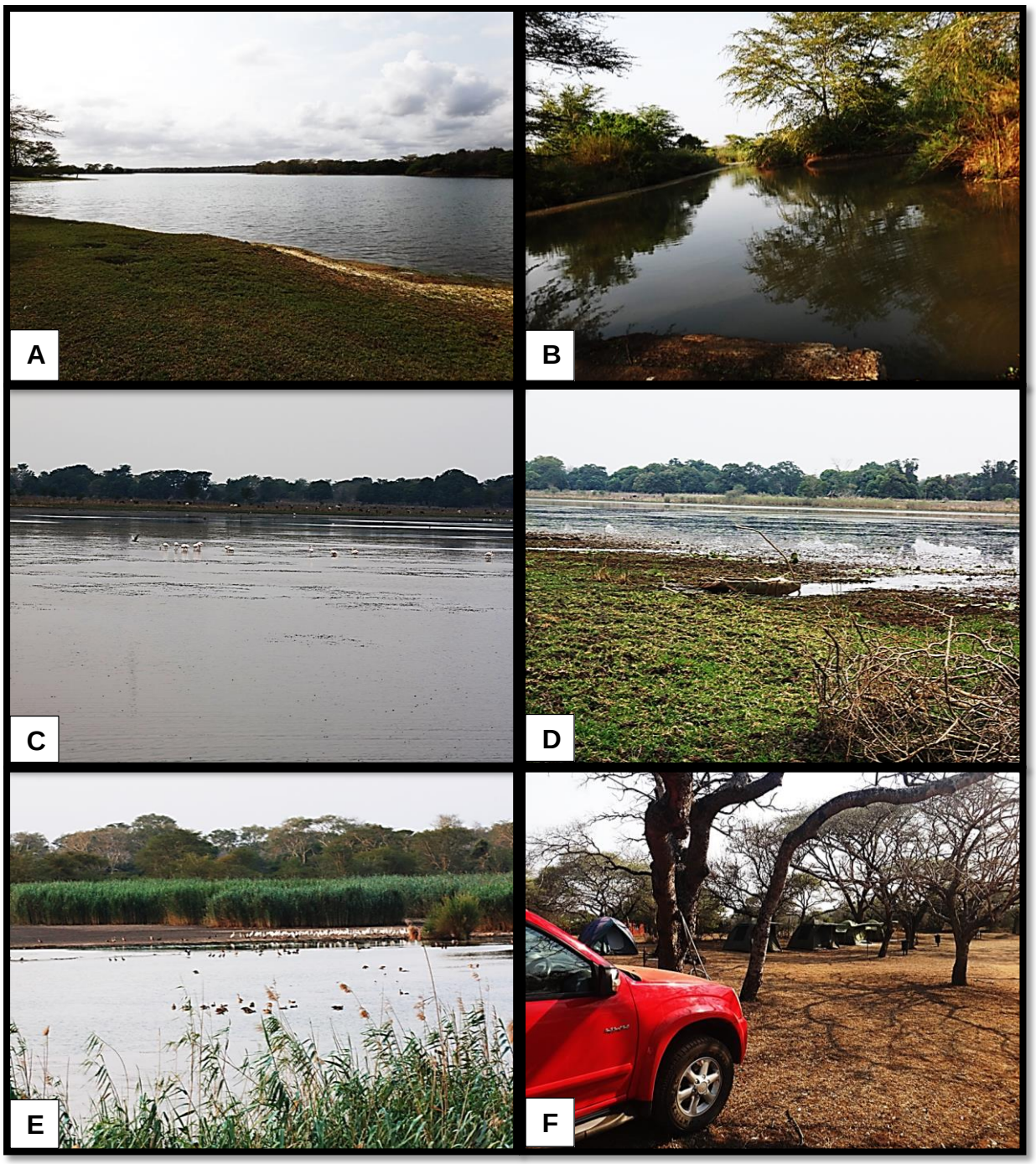

Figure 2.6: Photographs of the Pongola Floodplains and sampling sites. **A**- The permanent Nyamiti Pan and **B**- the Nyamiti Pan inlet mouth. **C**- The permanent Nomaneni Pan. **D**- The seasonal floodplain Bumbe. **E**- Seasonal floodplain. **F**- The Campsite at Ndumu Rest Camp.

ORANGE-VAAL RIVER SYSTEM

Importance

The river is called the Orange River not due to the reddish orange colour in appearance as some people may believe, but was named in 1779 by Colonel Robert Gordon, the commander of the garrison of the Dutch East Indian Company in honour of the Dutch House of Orange.

The Orange River basin consists of two Ramsar sites, namely Seekoeivlei and the Orange River Mouth Estuary. According to Earle *et al*. (2005), the Orange River Mouth Estuary is regarded to be the sixth most important estuary in southern Africa in terms of bird species, at times, as many as 57 bird species can be found with up to 26,000 individuals. To date, apart from diamond mining in the area, the environmental impacts caused locally at the estuary are low, but the presence of anthropogenic developments up-stream has a negative impact on the estuary. Two major dams upstream, Gariep (Figure 2.8 A) and Vanderkloof are considered to be the most significant threat to the Orange River Mouth wetland due to the obstruction of water and sediment. These dams trap sediment and water behind the dam walls in the middle reaches of the river, which restricts downstream flow and the sediment from reaching the Orange River Mouth wetland. This poses a serious threat to the integrity of the river mouth estuary. Agriculture and municipal water usage also have a negative impact on the wetland not only on the downstream flow, but also cause pollution due to the use of fertilisers. Earle *et al*. (2005) reported new economic developments close to the river mouth, such as the Kudu gas field station, which is likely to increase development and the demand for water from the river, furthering the potential of harmful effects on the river mouth.

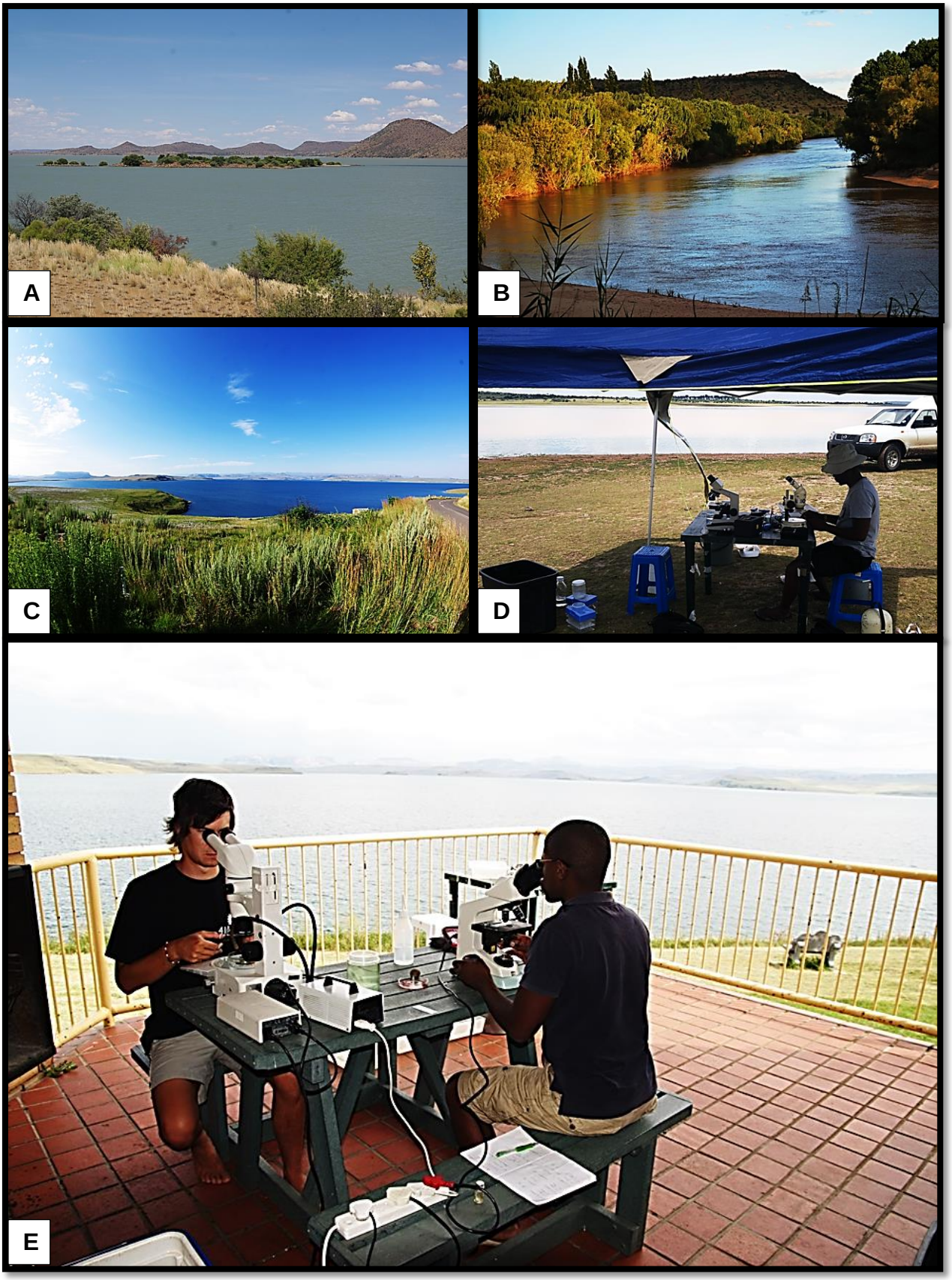

Figure 2.8: Photographs of some of the study sites of the Orange-Vaal River System. **A**- The Caledon River that flows into Welbedacht Dam. **B**- The Gariep Dam. **C**- Sterkfontein Dam. **D**- Temporary laboratory's at the Allemanskraal Dam and **E**- Sterkfontein Dam.

Seekoeivlei wetland is in the Klip River, which drains in the Vaal River and forms part of the Orange River Basin. This wetland is the largest wetland on the South African highveld and is valued for its ability to regulate stream flow and enhance water quality. This wetland supports a large number of resident and migrating bird species and is a breeding site for endangered bird species (Dini 1997).

Due to anthropogenic developments, the Orange River is the most developed river system in southern Africa, however, the developments are everything but positive for the environment (Swanevelder 1981). According to Ramollo (2011), human interferences in the river system have already resulted in a threat to the survival of certain fish species, such as the largemouth yellowfish (*Labeobarbus kimberleyensis* Gilchrist & Thompson, 1913). The Maluti minnow (*Pseudobarbus quathalambe* (Barnard, 1938)) is critically endangered due to the predation pressure by the introduced Rainbow trout (*Oncorhyncus mykiss* (Walbaum, 1792)), while the rock catfish (*Austroglanis sclateri* (Boulenger, 1901)) is endangered due to habitat destruction. Unfortunately there are also 9 introduced alien fish species in the Orange River Basin and, according to Ramollo (2011), the Mozambique tilapia (*Oreochromis mossambicus* (Peters, 1852)) has also been translocated to the Orange River Basin.

Hydrology

The Orange River Basin is the largest river south of the Zambezi (Earle *et al*. 2005; Ramollo 2011) and covers almost one million km^2 (Figure 2.9). According to Swanevelder (1981), the Orange River Basin is by far the most important river system in South Africa with a total basin area of 896,368 km^2. The Orange River Basin drains 48 % of the total area of South Africa and carries 22 % of the total downstream flow (Swanevelder 1981). It is also the most developed transboundary river basin in southern Africa, with a variety of water transfer schemes that supply water to municipalities, farms and industries (Earle *et al*. 2005). According to Ramollo (2011), the river originates in the Maluti mountains in Lesotho where the river is called the Senqu and the entire river is sometimes referred to as the Orange-Senqu River (Earle *et al*. 2005). The name Orange River will rather be used in this study as it is the internationally recognised name.

This river basin is shared by four countries, namely Botswana, Namibia, South Africa and Lesotho. From Lesotho the river flows westwards towards the semi-arid and arid regions in the Karoo, the Free State and Northern Cape Province. The Vaal River meets the Orange River in the Northern Cape Province at Mazelsfontein and then flows into the Atlantic Ocean at Alexander Bay (Swanevelder 1981; Ramollo 2011). According to Ramollo (2011), the river is highly regulated through dams and weirs such as the Gariep and Vanderkloof dams to provide water for human consumption, mining, electricity generation, flood control and agriculture.

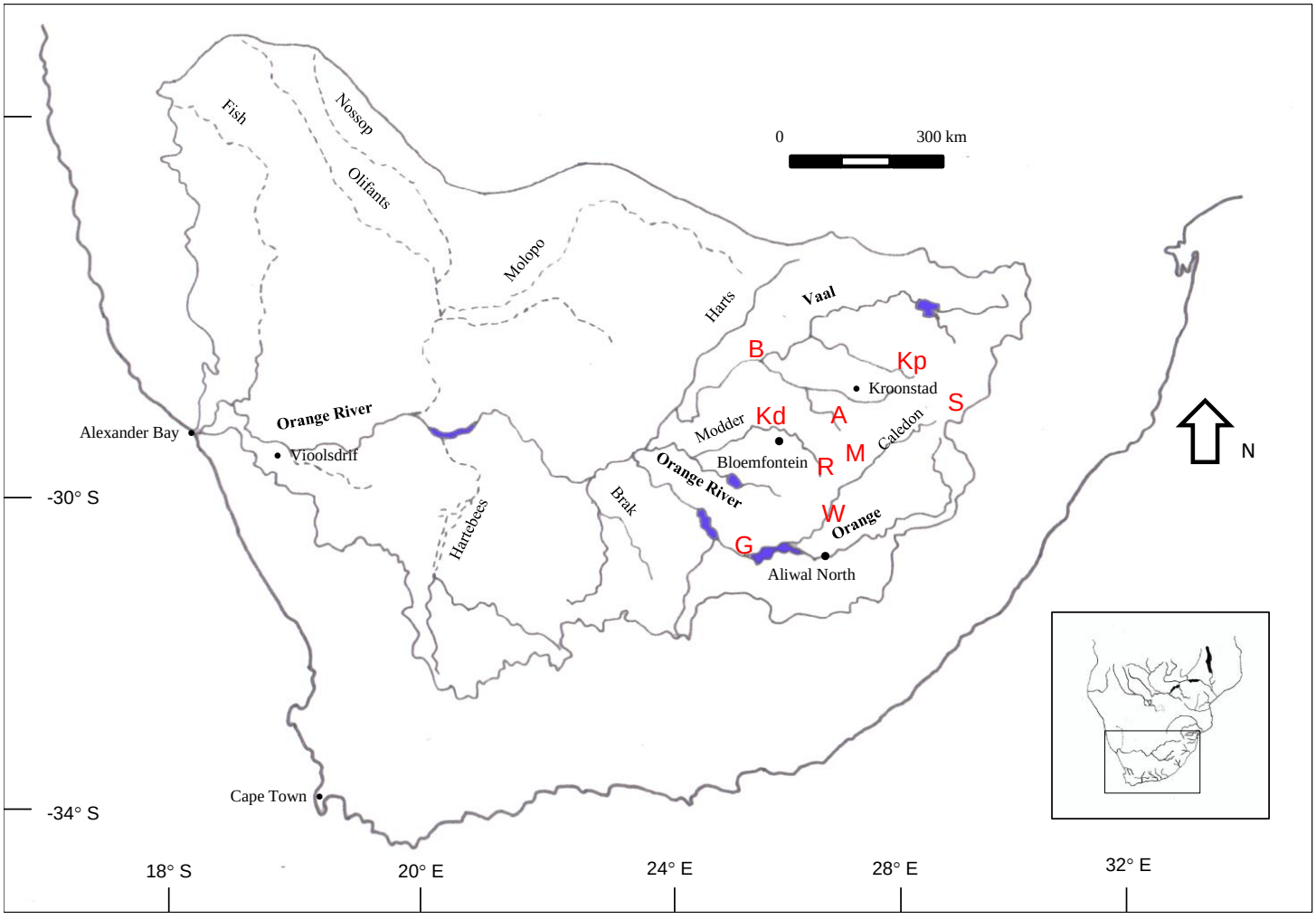

Figure 2.9: Map the Orange-Vaal River Basin and of some of the study sites. Redrawn from Tooth & McCarthy (2004). The Free State dams are indicated as **A**- Allemanskraal, **G**-Gariep, **Kd**- Krugersdrift, **Kp**- Koppies, **M**- Maria Moroka, **R**- Rustfontein, **S**-Sterkfontein and **W**-Welbedacht.

Habitat and vegetation

Ramollo (2011) asserts that the Orange-Vaal River System is a hostile environment due to climatic fluctuations, water obstructions, hydrological regimes, agricultural activities and environmental changes. Environmental factors such as water quality and depth, water current, food availability and substratum along the river are changing constantly. Due to the length of the Orange River, the altitude and climatic zones change dramatically and therefore the basin covers wide ranges of ecological systems. The Orange River Basin includes several biomes from grasslands, to the Karoo and arid savannah biomes. This also means that the basin contains a vast array of faunal and floral species with several endemic species (Earle *et al*. 2005).

Tooth & McCarthy (2004) and Earle *et al.* (2005) agree that the Orange River flows through hyper arid regions with an average of less than 200 mm of rain per annum in the summer and with an annual evaporation of 3,000 mm per year. Controversially the upper reaches in Lesotho, which is only 5 % present of the total basin area, contributes over 40 % of the total stream flow due to high rainfall (up to 2,000 mm per year) and runoff from snowmelt. The habitat of the river ranges from fast flowing rocky rivers in the mostly treeless and overgrazed landscape of Lesotho to slow flowing sandy rivers (Figure 2.8 B) in the arid parts of South Africa (Earle *et al.* 2005).

The Orange River System hosts a relatively low indigenous species diversity that is dominated by the fish family Cyprinidae. The other indigenous fishes in the Orange River System belong to the families Clariidae, Cichlidae and Austroglanidiaea (Skelton 2001). Only 11 indigenous fish species occur in the Orange River Basin and of importance for this study is the nine species belonging to the family Cyprinidae and the five species of the genus *Barbus*. They are the straightfin barb (*Barbus paludinosus* Peters, 1852), chubbyhead barb (*B. anoplus* Weber, 1897), goldie barb (*B. pallidus* Smith, 1841), threespot barb (*B. trimaculatus* Petes, 1952) and the near threatened Namaqua barb (*B. hospes* Barnard, 1938) (Figure 2.10).

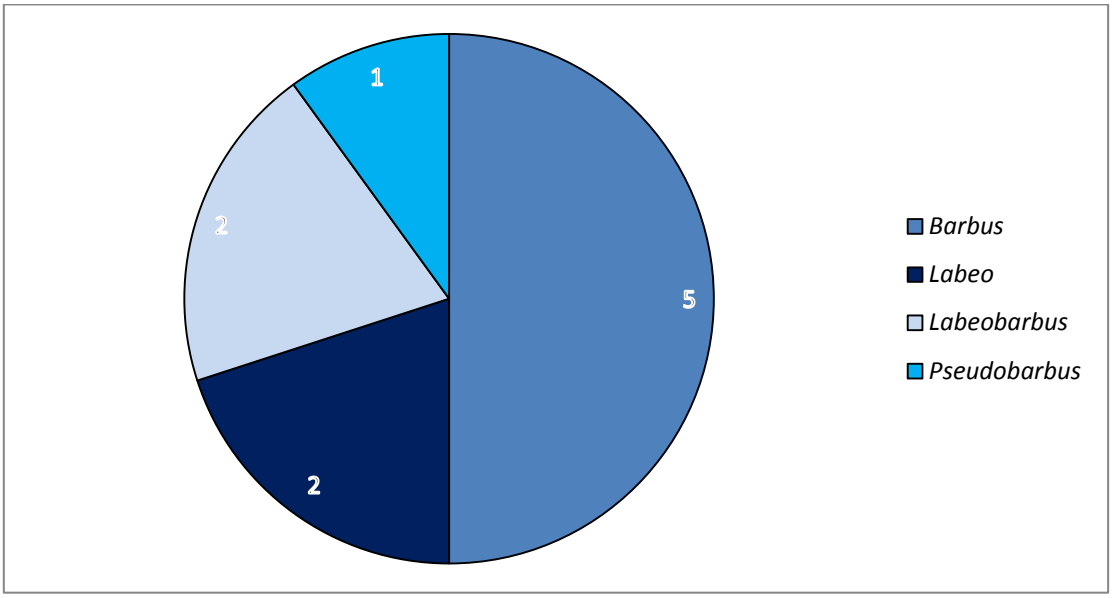

Figure 2.10: The number of indigenous fish per genera representing the fish family Cyprinidae of the Orange-Vaal River system. Compiled from Skelton (2001).

Free State Provincial Dams

The field work for the current study in the Orange-Vaal River Basin was conducted between November 2012 and February 2013 (Figures 2.8 B-C). The Free State provincial government have nature reserves across the province with dams where the field work for this study was carried out (Table 2.1). Temporary research stations were set up at the self-catering chalets or alongside the dams (Figures 2.8 D-E) in collaboration with Mr Leon Barkhuizen from The Department of Economic Development, Tourism and Environmental Affairs (DETEA) and The University of the Free State's Aquatic Ecology group from the Department Zoology and Entomology. The main purpose was to collect fish parasites of the Free State dams.

Table 2.1: Dams of the Free State where the field work for this study was conducted.

Free State dams	
Allemanskraal	Maria Moroka
Bloemhof	Rustfontein
Gariep	Sterkfontein
Koppies	Welbedacht
Krugersdrift	

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Chapter 3

Ciliophora

INTRODUCTION

According to Basson & van As (2006), ciliophorans are amongst the most common and widely distributed symbionts of fishes. They are one of the most commonly encountered symbiont groups in the aquatic environment; however, much of their diversity is virtually unknown. This chapter aims to contribute to the diversity, distribution and taxonomy of these ciliophorans from the fish genus, *Barbus* Cuvier & Cloquet, 1816 in southern Africa.

These symbionts belong to the Protozoans from the Phylum Ciliophora. During the current study, trichodinids that belong to the order Mobilina Kahl, 1933 and sessile ciliates that belong to the order Sessilina Kahl, 1935 were found associated with *Barbus* species.

Trichodinids have been the cause of fish mortalities of wild as well as cultured fish, but they are essentially commensals, and use fish as a substrate upon which they glide and to which they temporarily attach (Basson & van As 2006). Trichodinids also never occur in large numbers on healthy fish, but in aquaculture conditions, large numbers can occur. These large numbers of trichodinids can cause skin irritation and damage epithelial or epidermal cells that could lead to heavy losses of fish stocks (Basson & van As 2006).

More than 260 species of trichodinids from 10 genera have been described where seven of these genera are associated with fish. Roughly 259 species were described from fishes from marine, estuarine and freshwater habitats (Basson & van As 2006). The family Trichodinidae Ehrenberg, 1830 from freshwater fishes in Africa are represented by five genera, *Trichodina* Ehrenberg, 1830, *Trichodinella* (Raabe, 1950) Sramek-Husek, 1953, *Tripartiella* Lom, 1959, *Paratrichodina* Lom, 1963 and *Hemitrichodina* Basson & van As, 1989 (Basson & van As 1989).

Trichodinid ciliophorans from southern African fish have been extensively studied, with various species described from the genera *Trichodina*, *Trichodinella* and *Tripartiella*, and all three of these genera have been associated with the genus *Barbus* (van As & Basson 1984, 1989; Basson & van As 1987, 1989). Five species of *Trichodina*, two species of *Tripartiella* and one species of *Trichodinella* have been

found infecting *Barbus* species in southern Africa (Basson *et al*. 1983; van As & Basson 1984; Basson & van As 1987).

The sessiline peritrichs comprise 12 families that are associated with aquatic organisms, of which four are associated with fish. According to Basson & van As (2006), peritrichs associated with fish are permanently attached to the host with a scopula that is attached either directly to the substrate or it secretes a stalk that it attaches with. Sessiline peritrichs are therefore ectocommensals, using their host as a living substrate to feed on organic debris and waterborne bacteria. As with the trichodinids, sessiline peritrichs never occur in large numbers on healthy fish. Their attachment does not harm the host's epithelium. Sessilines are very often considered to be responsible for diseases, but they are actually very seldom involved in pathology (Basson & van As 2006).

Very little is known of sessile peritrichs of freshwater fish in southern Africa and only a few shed records exist (Viljoen & van As 1983, 1985; van As & Viljoen 1984). Only three species have been reported from southern Africa utilising *Barbus* hosts as a substrate, *Apiosoma phiala* Viljoen & van As, 1985, *A. piscicola* Blanchard, 1885 and *Scyphidia dermata* Viljoen & van As, 1983.

MATERIALS AND METHODS

Fish collections

The collection methods for the fish varied according to their habitat preferences. Fish were collected from the different river systems, using gill nets, comprised of six 20 m long panels of different stretched mesh sizes. The minimum mesh size was 28 mm and the maximum was 144 mm (28, 44, 50, 75, 100, and 144 mm). A 100 m x 3 m deep seine net with a mesh size of 75 mm was used for the collection of larger fish, and a 10 m x 2 m deep seine net with a mesh size of 10 mm was used for the smaller fish species. Line fishing, fyke, cast and scoop nets were also used for smaller fish species and fingerlings, mainly barbs. Motorboats were used to move around the dams and river systems, to catch the fish and to access remote locations,

especially in the Okavango Delta. The fish were collected in different habitats within the rivers and dams, including shallow or deep water, still standing or flowing water, or rocky or sand bottoms (Figures 3.1 A-D).

The fish were kept in aerated containers at the dams or rivers, and later transferred to aerated aquariums in the department or field laboratories for examination. The total length (from the tip of the mouth to the end of the caudal fin) of the fish were measured in millimetres and the fish species were identified using Skelton (2001). Alternatively, temporary laboratories were set up at the dams and the fish were examined immediately after collection (Figures 3.1 E-F). Smaller fish were killed by severing the spinal cord behind the head and bigger fish were killed by using Benzocaine.

Host examination, fixation and preservation of parasites

Microscopes were used to examine freshly killed fish directly after collection. Wet skin smears were done by scraping off the fish mucus with a slide or a scalpel onto another slide. The slides were examined under a compound light microscope (Nikon Eclipse 50i). If parasites were found, the slides were given a number and documented. Positive smears were air dried or fixed in Bouin's, depending on which parasites were found. The slides were air dried for trichodinids and Bouin's fixative was used for fixing sessiline peritrichs. The gills of the fish were dissected and examined for larger parasites using a compound or a dissection microscope (Nikon SMZ800) depending on the size of the gills. The gills were then used to make gill smears on slides; where after the smears were examined under a compound microscope. The slides were also air dried or fixed as previously explained for skin smears.

Figure 3.1: Photographs of fish collection and examination methods. Fish collections **A**- using a small seine net, **B**- using a large seine net, **C**- using a fishing rod and **D**- using a scoop net. **E**- A temporary laboratory at Sterkfonein dam and **F**- Allemanskraal dam.

Light microscopy preparations

Trichodinids:

Air dried skin or gill smears were impregnated with sliver-nitrate ($AgNO_3$) in order to study details of the adhesive disc of trichodinids using a modification of Klein's technique as described by Lom (1958). For studying the nuclear apparatus of trichodinids, Harris's Haematoxylin (Humason 1979) was used to stain air-dried smears; the haematoxylin was prepared according to the method from Humason (1979).

Silver-nitrate was prepared by mixing 4g $AgNO_3$ with 200 ml distilled water (H_2O). The slides were dipped back to back upright in slide trays in silver-nitrate solution for 10 min. The slides were rinsed by immerging it in distilled water for a few times (2-3 times). The slides were moved to white staining dishes facing upwards covered in distilled water. The dishes with the slides were exposed to UV-light from 45 min to 1 hour until the parasites were impregnated correctly. The slides were examined under a compound light microscope to determine if the parasites were impregnated correctly (light brown colour) (Lom 1958). The slides were dried and permanently mounted with Eukitt quick-hardening mounting medium with the correct sized cover slip; excess mounting medium was cleaned with xylene.

Harris's Haematoxylin was used by staining air dried smears in a tray staining dish 5-10 min in 200 ml haematoxylin. The slides were rinsed in running tap water (pH=6) for 2 min and then dipped twice in distilled water. The slides were dehydrated in 90 % ethanol for 1 min and in 100 % ethanol for 2 to 3 min. The slides were cleared in Xylene for 2 to 3 min and permanently mounted as described above with Eukitt quick-hardening mounting medium (Basson *et al.* 1983).

Sessiline Peritrichs:

The sessiline peritrichs were fixed in Bouin's fixative, stored in 70 % ethanol and later stained with Harris's Haematoxylin for the microscopic investigation of the macro- and micronuclei. The same method was used for sessiline peritrichs as previously explained for staining trichodinid dried smears with Harris's haematoxylin.

HARRIS'S HAEMATOXYLIN

Haematoxylin	10 g
Absolute Ethanol	100 ml
Potassium Alum	200 g
Distilled Water	2000 ml
Mercuric Oxide	5 g

Morphological measurements

All the measurements were done from micrographs taken with a Zeiss Axiophot compound microscope equipped with a Zeiss AxioCam ICc camera using Zeiss ZEN Imaging Software program with photo stacking. All measurements given in the results below are in micrometres unless otherwise stated. Minimum and maximum values are given, followed by the arithmetic mean, standard deviation and number of specimens measured. The mode is given for the number of denticles and number of radial pins instead of arithmetic mean.

Trichodinids:

The uniform specific characteristics proposed by Lom (1958), van As & Basson (1989) and Basson & van As (2002) were followed for species identifications and descriptions. The body diameter, border membrane, adhesive disc, the thickness of the macronucleus and the length and width of the micronucleus were measured (Basson *et al*. 1983). Measurements of the adhesive disc, denticles and nuclei were measured as indicated in Figure 3.2. The descriptions of the denticles were done using guidelines provided by van As & Basson (1989) for *Trichodina* and Basson & van As (2002) for *Tripartiella* and *Trichodinella* based on drawing y and x lines (Figures 3.3 and 3.6). Denticle descriptions were done from a single population from one fish host.

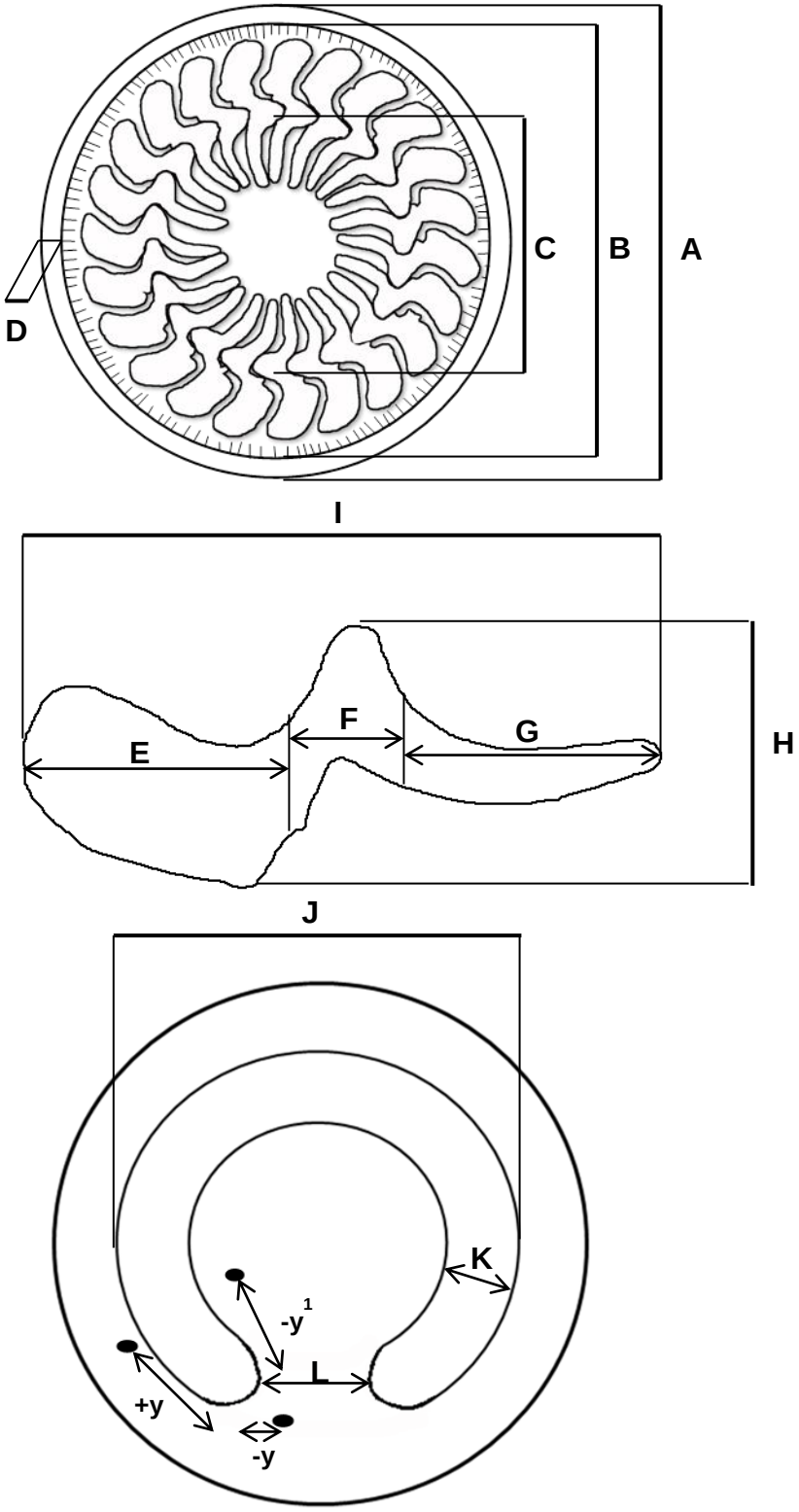

Figure 3.2: Measurements of the adhesive disc (top), the denticle (middle) and, the macronucleus and micronucleus (bottom) of trichodinids. **A**-Diameter of body, **B**-Diameter of adhesive disc, **C**-Diameter of denticle ring, **D**-Width of border membrane, **E**-Length of blade, **F**-Width of central part, **G**-Length of ray, **H**-Width of denticle, **I**-Span of denticle, **J**-External diameter of macronucleus, **K**-Thickness of macronucleus, **L**-Distance between terminations of macronucleus (x-value) and -y, +y, $-y^1$= different positions of micronucleus in relation to macronucleus (Lom 1958).

Sessiline peritrichs:

Systematic observations and measurements were done from haematoxylin-stained specimens. The body length was measured from the base of the scopula to the peristomial cap, body width was measured at the widest part of the body and the width of the scopula was measured (Viljoen & van As 1983). The length and width of the macro- and micronuclei were measured and also the width of the scopula. Species descriptions were compared to that of known species described by van As & Viljoen (1984).

RESULTS

This chapter consists of taxonomic diagnoses of species of the families Trichodinidae Raabe, 1959 and Epistylidae, Kahl, 1935 collected from different localities from *Barbus* hosts. Based on taxonomic diagnoses of the species morphology, seven different species from four different genera have been found. Keys for protozoan parasites of fishes provided by Lom & Dyková (1992) were used to identify the genera. Species were identified by descriptions provided by Viljoen & van As (1985); Basson & van As (1987) and van As & Basson (1989).

Phylum: Ciliophora Doflein, 1901

Class: Oligohymenophorea de Puytorac, Batisse, Bohatier, Corliss, Deroux, Didier, Dragesco &Tuffrau, 1974

Order: Mobilina Kahl, 1933

Family: Trichodinidae, Raabe, 1959

Genus: *Trichodina* Ehrenberg, 1838

***Trichodina kazubski* van As & Basson, 1989** (Figures 3.3 A, 3.4 A-B)

Host and localities: *Barbus afrovernayi* Nichols & Boulton, 1927 and *Barbus radiatus* Peters, 1853 from the Okavango River System, Shakawe (S18°42'93'85" E21°89'64'98"). *Barbus paludinosus* Peters, 1852 and *Barbus toppini* Boulenger, 1916 from Pongola River System, Bumbe (S26°99'57'72" E32°30'05'33").

Location on host: Skin and gills

Reference material: Slide 2013/08/08-09 for *B. afrovernayi* from the Okavango River System and slide 2013/09/14-19 for *B. paludinosus* from the Pongola River System in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements were made using light microscopy from two populations from the Okavango River System and two populations from the Pongola River System, stained with silver-nitrate and Harris's haematoxylin, mounted with Eukitt quick-hardening mounting medium (Table 3.1).

Comparative description:

Trichodina kazubski from the population of *Barbus radiatus*, Okavango River System. Biometrical data is presented in Table 3.1. Medium sized trichodinid with a disc shaped body, 42.3-50.3 (46.7±2.4, 12) in diameter. Adhesive disc concave, 34.6-42.8 (39.5±2.6, 12) in diameter; surrounded by finely striated border membrane 1.8-4.9 (3.9±1, 11) wide. Diameter of denticle ring 18.7-25 (22.7±1.7, 12). Number of denticles 22-26 (23,19). Span of denticle 10.3-14.7 (12.6±1.1, 12); width of denticle 4.5-6.4 (5.8±0.6, 12); length of ray 3.6-6 (5.2±0.7, 12); width of central part 2.1-2.9 (2.6±0.3, 12); length of blade 4.2-6.2 (5±0.6, 12). Blade broad, distal margin rounded, tangent point rounded and lower than distal surface. Curve of posterior margin L-shaped with deepest point where blade connects to the central part. Apex of anterior margin slightly pointed, extending beyond y + 1 line, but apophysis of blade is beyond y + 1. Apophysis of blade prominent. Posterior projection of blade absent. Point of central part rounded extending less than half past y-axis, central part above x-axis similar to part below x-axis. Medium sized rays of same thickness throughout, with rounded points parallel with y-axis that is slightly curved. Apophysis

of ray not prominent. Ratio of denticle above x axis to denticle below x axis one (Figure 3.3 A). Number of radial pins per denticle 8-12 (9,19). Macronucleus C-shaped with external diameter 12.1-20.8 (17.1±2.5, 22), thickness 2.1-3.9 (3.1±0.4, 22) and distance between terminations 2.3-7.8 (4.8±1.4, 22). Micronucleus oval, 0.9 (1) x 0.8 (1) in +y position with y-value of 4.5 (1).

Remarks:

The current trichodinid species was identified as *T. kazubski* that was described by van As & Basson (1989) from *B. paludinosus* from the Limpopo System. All four populations that were found in the current study have the same denticle shape and the size and important morphological characteristics that fall into the parameters that were given in the original description. All four populations were found on *Barbus* hosts, the same as in the original description. Species that are similar in denticle shape and dimensions are *T. uniforma* van As & Basson, 1989 and *T. mutabilis* Kazubski & Migala, 1968 that were described by van As & Basson (1989) and Kazubski & Migala (1968) respectively. *Trichodina kazubski* was previously believed to be *T. mutabilis* by Basson *et al.* (1983). Through closer examination of the denticles it was shown that these two species are in fact different species. *Trichodina kazubski* can easily be distinguished from the above mentioned species on the basis of its smaller body size and the blade which is much larger and broader in appearance with a rounded distal edge.

The two populations that were found in the Okavango River System from *B. afrovernayi* and *B. radiatus* are clearly larger in body diameter than the populations that were found from the Pongola River System from *B. paludinosus* and *B. toppini* (Table 3.1). The population from the Pongola River System is very similar to the type population description from the Limpopo System (van As & Basson 1989). The difference is size from the populations could be seasonal; the Okavango populations were collected in winter and the Pongola population in spring. Trichodinids live longer and therefore grow larger in winter before cell division.

There are slight differences in the denticle shape in the population from *B. radiatus* from the Okavango River System compared to the original description. The tangent point is more round-shaped in the Okavango population and the anterior aphophysis

is not prominent and is sometimes absent. *Trichodina kazubski* also contain a horseshoe-shaped macronucleus, but the nuclear data from the current study revealed that the macronuclei are more C-shaped.

This represents new host and locality records for *T. kazubski* that was found on the skin and gills of *B. afrovernayi* and *B. radiatus* from the Okavango River System and *B. paludinosus* and *B. toppini* from the Pongola River System.

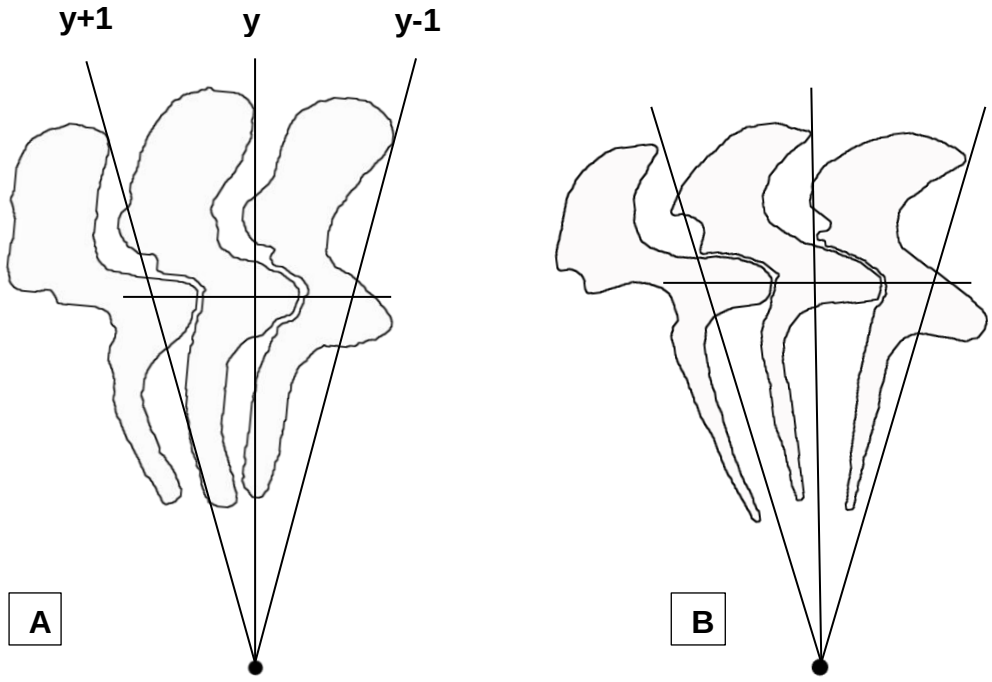

Figure 3.3: Diagrammatic drawings of the denticles of trichodinids. **A**- *Trichodina kazubski* van As & Basson, 1989 and **B**- *Trichodina heterodentata* Duncan, 1977 from *Barbus afrovernayi* Nichols & Boulton, 1927 from the Okavango River System, Botswana.

Table 3.1: Biometrical data of four populations of *Trichodina kazubski* van As & Basson, 1989 from the Okavango and Pongola river systems with comparative measurements of the same species original description from the Limpopo System (van As & Basson 1989).

Host	***Barbus afrovernayi***	***Barbus radiatus***	***Barbus paludinosus***	***Barbus toppini***	***Barbus paludinosus***
Locality	Okavango System	Okavango System	Pongola System	Pongola System	Limpopo System
Position on host	Skin and Gills	Skin and Gills	Skin and Gills	Skin and Gills	Skin, fins and gills
Reference	Current study	Current study	Current study	Current study	(van As & Basson 1989)
Body diameter	39.4-60.7 (50.3±5.2,41)	42.3-50.3 (46.7±2.4,12)	34.6-42.3 (37.5±1.9,16)	29.6-37.3 (32.8±2.9,8)	34.3-54.6 (41.2±4.5,23)
Adhesive disc diameter	30.9-51.9 (41.7±4.6,40)	34.6-42.8 (39.5±2.6,12)	28.6-35.8 (32.2±2.1,16)	22.8-29.9 (26.3±2.3,8)	26.7-39.5 (32.9±3.7,23)
Denticle ring diameter	17.8-31.4 (24.4±3.1,42)	18.7-25 (22.7±1.7,12)	15.4-21.6 (18.8±1.8,16)	13.1-17.8 (15.3±1.8,8)	16.4-26.3 (20.2±2.4,23)
Border membrane width	1.7-5.8 (4.6±0.9,40)	1.8-4.9 (3.9±1,11)	0.9-4.1 (2.7±1,16)	2-3.9 (3.3±0.7,8)	3.2-5.9 (4.3±0.6,23)
Blade length	4.5-6.8 (5.4±0.4,42)	4.2-6.2 (5±0.6,12)	3.4-4.8 (4.2±0.3,16)	3.3-4.6 (3.9±0.5,8)	3.5-5.7 (4.6±0.7,23)
Central part width	1.6-3.8 (2.8±0.6,42)	2.1-2.9 (2.6±0.3,12)	1.5-2.2 (1.9±0.2,16)	1.5-2.4 (1.7±0.3,8)	1.5-3.2 (2-0.4,23)
Ray length	4.2-6.9 (5.5±0.6,42)	3.6-6 (5.2±0.7,12)	3.5-5.8 (5.1±0.6,16)	3.5-4.5 (4.2±0.3,8)	3.6-6.4 (5.2±0.6,23)
Denticle width	4.4-7.2 (6.1±0.6,42)	4.5-6.4 (5.8±0.6,12)	3.8-5.7 (4.8±0.5,16)	3.8-5.2 (4.4±0.4,8)	3.6-5.7 (4.3±0.5,23)
Denticle span	10.8-15.6 (13.5±1.2,42)	10.3-14.7 (12.6±1.1,12)	8.1-12.4 (11.1±0.9,16)	8.6-11.6 (9.7±1, 8)	-
Denticle number	21-27 (24,46)	22-26 (23,19)	22-25 (24,15)	19-21 (20,8)	22-26 (23,23)
Radial pins/denticle	8-12 (9,43)	8-12 (9,19)	8-11 (9,12)	8 (8,8)	7-10 (8,23)
Macronucleus-shape	-	C-shaped	C-shaped	C-shaped	Horseshoe-shaped
Macronucleus-external diameter	-	12.1-20.8 (17.1±2.5,22)	21.6-30.8 (25.1±3.3,8)	20.5-32 (25.4±4.3,7)	-
Macronucleus-thickness	-	2.1-3.9 (3.1±0.4,22)	4.2-8.5 (5.6±1.5,8)	4.9-7.7 (6.1±1.2,7)	-
Macronucleus-x value	-	2.3-7.8 (4.8±1.4,22)	4.5-12.9 (7.3±2.8,8)	6.8-23.2 (12.8±5.4,7)	-
Micronucleus-shape	-	Oval	-	Oval	-
Micronucleus-length	-	0.9 (1)	-	2.3-3.1 (2.6±0.3,5)	-
Micronucleus-width	-	0.8 (1)	-	1.2-1.8 (1.5±0.2,5)	-
Micronucleus-y position	-	+y position	-	+y position	-
Micronucleus-y value	-	4.5 (1)	-	2.2-9.6 (5.1±3.1,5)	-
Adoral spiral	390°	-	370°-385°	-	400°

*- = Not observed

***Trichodina heterodentata* Duncan, 1977** (Figures 3.3B, 3.4 C-D)

Hosts and locality: *Barbus radiatus* Peters, 1853*, Barbus haasianus* David, 1936 and *Barbus afrovernayi* Nichols & Boulton, 1927 from the Okavango River System, Shakawe (S18°42'93'85" E21°89'64'98").

Location on hosts: Skin and gills

Reference material: Slide 2013/07/24-12 for *B. radiatus* from the Okavango River System in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements were made using light microscopy from three populations from the Okavango River System, stained with silver-nitrate and Harris's haematoxylin, mounted with Eukitt quick-hardening mounting medium (Table 3.2).

Comparative description:

Trichodina heterodentata from the population of *Barbus radiatus*, Okavango River System. Biometrical data is presented in Table 3.2. Large trichodinid with a disc-shaped body, adhesive disc concave, surrounded by finely striated border membrane. Blade curve sickle-shaped. Distal surface rounded with sharp tangent point, slightly below distal surface. Anterior surface rounded, slightly pointed extending to the y +1 axis. Blade apophysis prominent and sharp, forming an indentation in the blade at the same level as deepest point of curve. Posterior surface possesses a deep curve, deepest point opposing blade apex. Blade connection with central part thin. Posterior projection absent. Central part with rounded tip extending more than halfway past y-axis, fitting tightly in preceding denticle. Section above and below x-axis similar. Ray apophysis not clearly visible in most specimens. Ray straight that tapers to a sharp rounded point. Ratio of denticle above x-axis to denticle below, less than one (Figure 3.3 B).

Table 3.2: Biometrical data of *Trichodina heterodentata* Duncan, 1977 from *Barbus radiatus* Peters, 1853 from the Okavango River System with comparative measurements of the same species from Boskop Dam, South Africa (Basson *et al*. 1983).

Host	***Barbus radiatus***	***Pseudocrenilabrus philander***
Locality	Okavango System	Boskop dam
Position on host	Skin and gills	Skin, fins and gills
Reference	Current study	Basson *et al.* (1983)
Body diameter	44.5-70.3 (3)	47.5-69.1 (55.3±3.8,51)
Adhesive disc diameter	37.3-62.2 (3)	39.5-59.8 (46.9±4.1,51)
Denticle ring diameter	23.5-39.3 (3)	23.2-37.8 (29.3±2.9,51)
Border membrane width	1.7-5.8 (3)	3.2-6.2 (4.2±0.6,51)
Blade length	3.6-5.4 (3)	3.4-5.5 (4.3±0.4,51)
Central part width	2.2-3.3 (3)	1.6-3.3 (2.7±0.9,51)
Ray length	4.3-9.8 (3)	4.6-8.1 (6.3±0.9,51)
Denticle width	6.2-11.1 (3)	5.1-8.6 (6.6±0.8,51)
Denticle span	11.5-18 (3)	-
Denticle number	22-24 (3)	22-29 (25,51)
Radial pins/denticle	10-13 (3)	9-13 (10,51)
Macronucleus-shape	C-shape	U-shape
Macronucleus-external diameter	26.2-45.1 (5)	23.7-53.4 (40.5±5.6,57)
Macronucleus-thickness	3.6-10.3 (5)	3.2-11.2 (5.7±1.4,57)
Macronucleus-x value	6.9-24.4	7.8-33.8 (19.5±6.6,57)
Micronucleus-shape	Oval	-
Micronucleus-length	2.7-5.2 (2)	-
Micronucleus-width	2.2-3.3 (2)	-
Micronucleus-y position	+y position	-
Micronucleus-y value	11.6-15 (2)	-
Adoral spiral	-	400°

*- = Not observed

Remarks:

Trichodina heterodentata was originally described by Duncan (1977) from cichlids in the Philippines, but this parasite has been found widespread on various fish and amphibians throughout the world (Duncan 1977; Basson *et al.* 1983; van As & Basson 1984, 1989; Kruger *et al.* 1993). This parasite is also known to have considerable variation within populations (Kruger *et al*. 1993). According to Kruger *et al*. (1993), this parasite is most likely a cichlid parasite that originated from Africa and was distributed worldwide through translocation of cichlids for the purpose of aquaculture. This might explain the existence of *T. heterodentata* in the Okavango River System that has so far not been exposed to any introductions of translocations of fish species (Basson & van As 2002).

During the current parasitic investigation of *Barbus* species, very few individuals of *T. heterodentata* were found on the different fish species and they were only found from the Okavango River System. As stated above, *T. heterodentata* is most likely a cichlid parasite, but it is believed that due to their considerable size variation, the parasite can adapt and be found on other fish families. This is probably the reason why such low numbers were found. This parasite was previously found on *B. paludinosus* and *B. trimaculatus* from the Limpopo System, South Africa (Basson *et al.* 1983; van As & Basson 1989).

The current specimens from three populations of *B. radiatus*, *B. afrovernayi* and *B. haasianus* were identified as *T. heterodentata* by their unique denticle shape, the shape of the blade and central part. Very few specimens were found during the current study, but *T. heterodentata* is a parasite species that has been very well documented and studied throughout the world and could be positively identified, even when low numbers were found. With a small sample size the variation within the population, that is a characteristic of *T. heterodentata*, can be seen in Table 3.2.

The only considerable difference that can be distinguished between the population that was found on *P. philander* by van As & Basson (1989) and the current population from *B. radiatus*, is the thickness of the ray. The current population possesses a slender ray that tapers down to a sharp point, where the former population possesses a straight thick ray that tapers down to a blunt point.

Clear differences can be distinguished between *T. kazubski* and *T. heterodentata*, the most obvious is the overall size; *T. heterodentata* is much larger than *T. kazubski*. *Trichodina kazubski* possesses a broad blade with a rounded distal margin where *T. heterodentata* possesses a narrow blade with a sharp tangent point.

This represents new host and locality records for *T. heterodentata* that was found on the skin and gills of *B. afrovernayi*, *B. radiatus* and *B. haasianus* from the Okavango River System, Botswana.

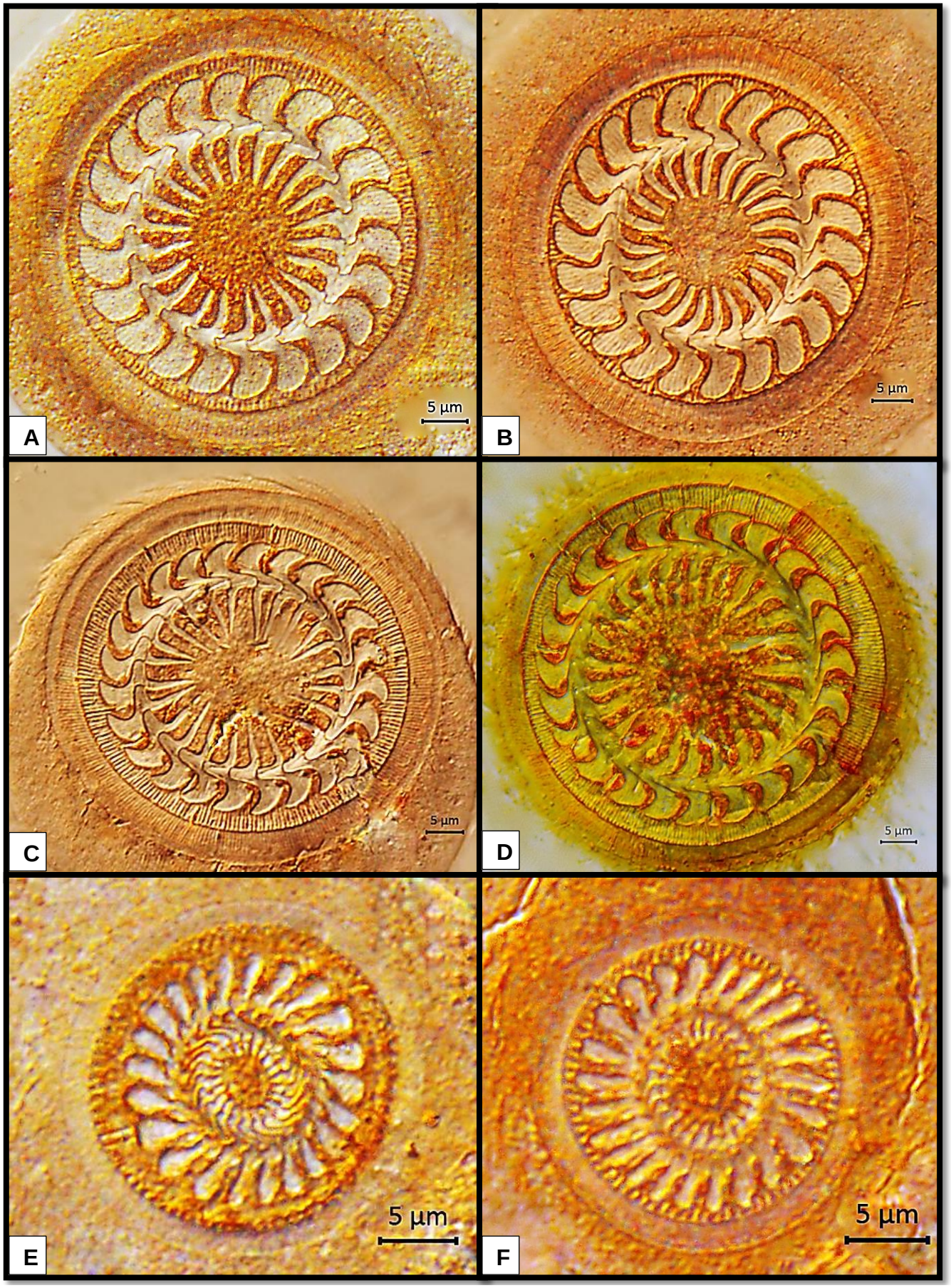

Figure 3.4: Micrographs of silver impregnated adhesive discs of trichodinids. **A** & **B**- *Trichodina kazubski* van As & Basson, 1989 from *Barbus radiatus* Peters, 1853 and *Barbus afrovernayi* Nichols & Boulton, 1927, respectively. **C** & **D**- *Trichodina heterodentata* Duncan, 1977 from *B. afrovernayi* and *B. radiatus*, respectively. **E**- *Tripartiella macrosoma* Basson & van As, 1987 from *Barbus paludinosus* Peters, 1852 and **F**- *Tripartiella lechridens* Basson & van As, 1987 from *Barbus afrohamiltoni* Crass, 1960.

Genus: *Tripartiella* Lom, 1963

***Tripartiella macrosoma* Basson & van As, 1987** (Figures 3.4 E, 3.5 A, 3.6 A)

Hosts and localities: *Barbus toppini* Boulenger, 1916 and *Barbus paludinosus* from Pongola River System, Bumbe (S26°99'57'72" E32°30'05'33") and *Barbus afrohamiltoni* Cass, 1960 and *Barbus gurneyi* Günther, 1868 from Pongola River System, Nyamiti Pan Outlet (S26°88'27'75" E32°31'13'93").

Location on hosts: Gills

Reference material: Slide 2013/09/14-29a for *B. toppini* from the Pongola River System in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements were made using light microscopy from four populations from the Pongola River System, stained with silver-nitrate and Harris's haematoxylin, mounted with Eukitt quick-hardening mounting medium (Table 3.3).

Comparative description:

Tripartiella macrosoma from the population of *B. toppini* from the Pongola River System. Biometrical data is presented in Table 3.3. Blades broad with rounded bulbous distal parts. Blade thickness varies from very thick to thin within same specimen. Distal margin rounded, with tangent point lower than distal margin. Anterior blade margin sloping downwards, extending past y+1 axis for half of its length. Second apophysis large and rounded corresponding to indentation in posterior margin of next bade. Blade apophysis distinct, extending tightly into indentation of next denticle. Posterior blade margin extending from y-axis sloping down towards y+1 axis. Posterior projection large and distinct, corresponding to indentation between apophysis and second apophysis of next blade. Blade connection medium sized and long, of equal thickness throughout. Blade connection sloping downward through y+1 and y-axis. Angle of posterior blade margin to blade connection sharp, more or less 90°. Section of blade from tangent point to blade apophysis extending over one y segment. Ratio of denticle above to below x-axis more than two (2.5) (Figure 3.6 A).

Remarks:

Tripartiella macrosoma was originally described by Basson & van As (1987) from *Barbus eutaenia* Boulenger, 1904 from the Olifants River, Mpumalanga. The current species was identified as *T. macrosoma* due to the unique bulbous blade shape, distinct apophysis and posterior projections. This species also has a long sloping blade connection which is clearly present in the current species. The biometrical data of the current study also falls within the parameters of the measurements from Basson & van As (1987) (Table 3.3). The species were also found on the same fish genus, *Barbus* and both study locations are part of the Limpopo System.

Table 3.3: Biometrical data of *Tripartiella macrosoma* Basson & van As, 1987 from *Barbus toppini* Boulenger, 1916 from the Pongola River System with comparative measurements of the same species from the Olifants River, South Africa (Basson & van As 1987).

Host	***Barbus toppini***	***Barbus eutaenia***
Locality	Pongola System	Olifants River System
Position in host	Gills	Gills
Reference	Current study	Basson & van As (1987)
Body diameter	16.2-23.3 (19.9±1.8, 27)	18-23.4 (21.2±1.5, 25)
Adhesive disc diameter	13.5-19.9 (16.2±1.7, 27)	13.6-19.9 (16.9±1.6, 25)
Denticle ring diameter	4-7.4 (5.2±0.8, 27)	6.4-9.3 (7.8±0.8, 25)
Border membrane width	1.4-2.6 (2±0.4, 27)	1.7-2.7 (2.2±0.3, 25)
Blade length	3.3-5 (4.1±0.5, 27)	2.4-4.3 (3.3±0.4, 25)
Central part width	0.6-1.1 (0.8±0.1, 27)	0.9-1.4 (1.2±0.1, 25)
Ray length	0.3-1 (0.6±0.2, 27)	1-2.3 (1.7±0.4, 25)
Denticle width	1.4-2.7 (2.1±0.3, 27	2.5-4 (3.3±0.4, 25)
Denticle span	4.6-6.9 (5.6±0.5, 27)	
Denticle number	20-26 (23, 26)	22-25 (23, 25)
Radial pins/denticle	5-6 (5, 24)	4 (4, 25)
Macronucleus-shape		C-shape
Macronucleus-external diameter	13.2-34 (21.3±4.7, 26)	15.5-32.3 (24.7±4.8, 18)
Macronucleus-thickness	2.6-6.5 (4±0.9, 26)	3.1-7.4 (4.6±1.0, 18)
Macronucleus-x value	3-23.1 (11.3±5.9, 26)	4.3-20.3 (13.2±4.6, 18)
Micronucleus-shape	Oval	-
Micronucleus-length	2.6-6.2 (4.5±0.9, 11)	-
Micronucleus-width	1.5-4.4 (3.4±1, 11)	-
Micronucleus-y position	Mostly +y	-
Micronucleus-y value	2.9-18.5 (10.3±5.5, 11)	-
Adoral spiral	180°-225°	170-230°

*- = Not observed

Tripartiella macrosoma shows resemblance to *T. lechridens*, but clear differences can be distinguished. The blade shape of *T. lechridens* is robust, broad and does not taper down as in the case of *T. macrosoma*. *Tripartiella lechridens* also differs by possessing a shorter and thinner blade connection than *T. macrosoma*. Both species were described originally, by possessing C-shaped macronuclei, but new information on the nuclear data from the current study suggests otherwise. *Tripartiella macrosoma* does possess a C-shaped macronucleus with one end thick and rounded and the other end tapered down to a sharp point. *Tripartiella lechridens* does not possess a C-shaped macronucleus, but the nucleus is in the shape of four oval structures that are connected or not, that occur around the trichodinid body (Figure 3.5 B). This phenomenon is also supported by the raw data from the original description (Basson & van As 1987).

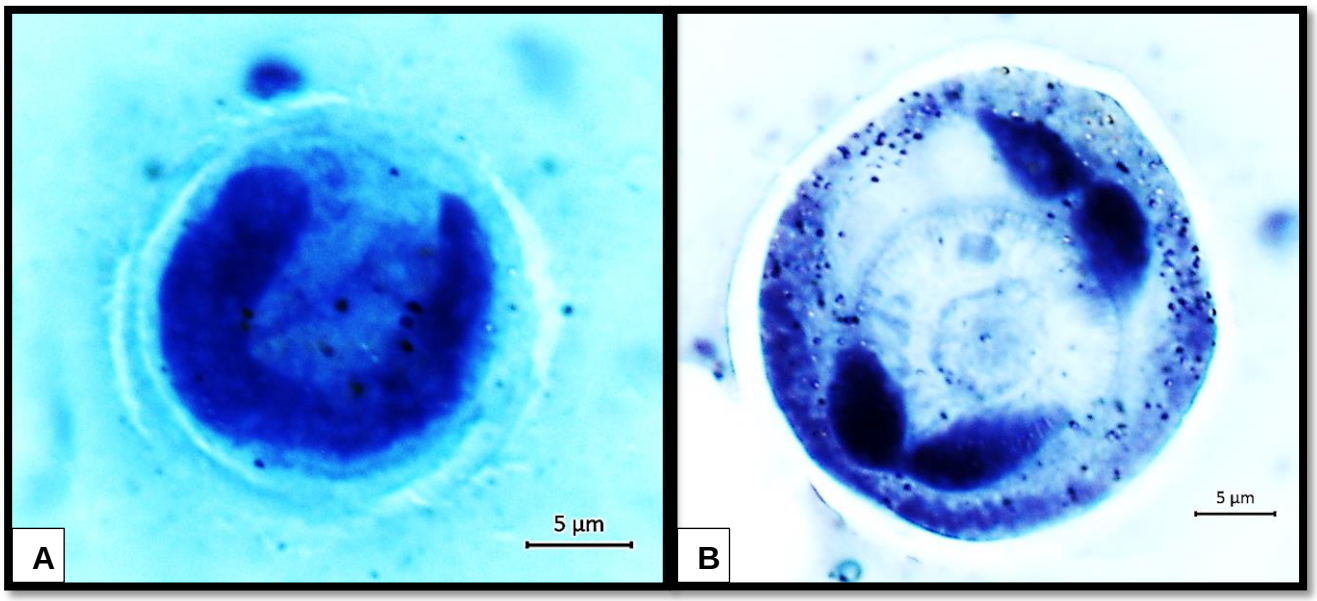

Figure 3.5: Micrographs of Harris's Haematoxylin stained specimens of **A**-*Tripartiella macrosoma* Basson & van As, 1987 and **B**-*Tripartiella lechridens* Basson & van As, 1987 from *Barbus afrohamiltoni* Crass, 1960 from the Pongola River System indicating the difference between the macronuclei of the two species.

The current study provides new information on *T. macrosoma* by providing a description of the denticles as proposed by van As & Basson (1989) and Basson & van As (2002) for the genus of *Tripartiella*. New information of the micronucleus is also provided in Table 3.3, the micronuclei were not observed in the original description.

This represents new hosts for *T. macrosoma* that was found on the gills of *B. toppini*, *B. paludinosus*, *B. afrohamiltoni* and *B. gurneyi* from the Pongola River System, South Africa.

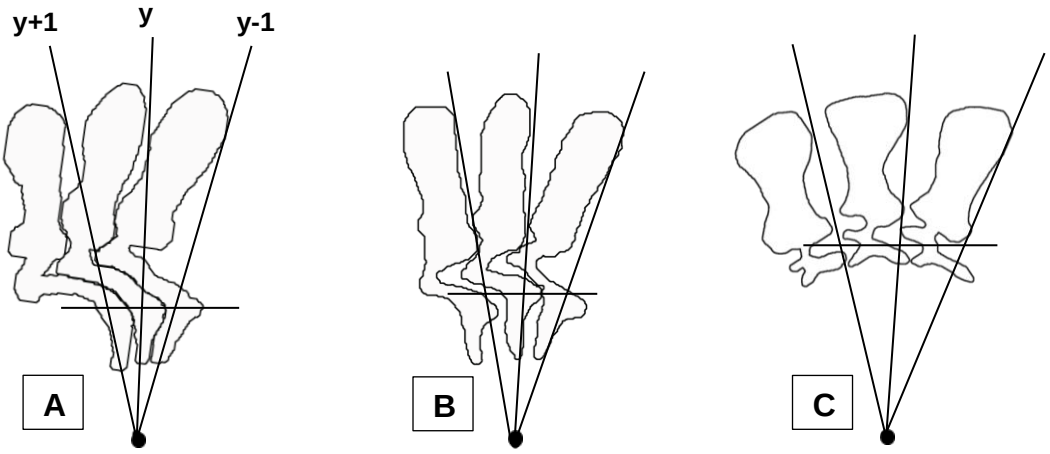

Figure 3.6: Diagrammatic drawings of denticles of **A**-*Tripartiella macrosoma* Basson & van As, 1987 from *Barbus toppini* Boulenger, 1916, **B**-*Tripartiella lechridens* Basson & van As, 1987 from *Barbus afrohamiltoni* Crass, 1960 and **C**-*Trichodinella epizootica* (Raabe, 1950) Šrámek-Hušek, 1953 from *Barbus radiatus* Peters, 1853.

***Tripartiella lechridens* Basson & van As, 1987** (Figures 3.4 F, 3.5 B, 3.6 B)

Hosts and Localities: *Barbus afrohamiltoni* Crass, 1960 and *Barbus gurneyi* Günther, 1868 from Pongola River System, Nyamiti Pan Outlet (S26°88'27'75" E32°31'13'93").

Location on hosts: Gills

Reference material: Slide 2013/09/11-02 for *B. afrohamiltoni* from the Pongola River System in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements were made using light microscopy from two populations from the Pongola River System, stained with silver-nitrate and Harris's haematoxylin, mounted with Eukitt quick-hardening mounting medium (Table 3.4).

Comparative description:

Tripartiella lechridens from the population of *B. afrohamiltoni* from the Pongola River System. Biometrical data is presented in Table 3.4. A small parasite with a bell-shaped body, adhesive disc cup-shaped, surrounded by finely striated border membrane. Blades broad with rounded distal parts, posterior and anterior margins of equal thickness throughout length. Distal margin rounded, with rounded tangent point lower than distal margin. Anterior blade margin extending downwards, parallel with y-axes, not extending past y+1 axis. Second blade apophysis present, but not distinct. Blade apophysis distinct extending past y+1 axis, into indentation of next denticle. Posterior projection present, corresponding to indentation between blade apophysis and second blade apophysis of proceeding denticle. Blade connection short and thin, extending down from y+1 axis towards y-axis. Central part small and delicate with ray extending directly from central part, parallel with y-axis. Angle of posterior blade margin to blade connection sharp and less than 90°. Rays are of unequal lengths in one species. Ratio of denticle above to below x-axis more than two (2.5) (Figure 3.6 B).

Table 3.4: Biometrical data of *Tripartiella lechridens* Basson & van As, 1987 from *Barbus afrohamiltoni* Crass, 1960 from the Pongola River System with comparative measurements of the same species from the Olifants River, South Africa (Basson & van As 1987).

Host	***Barbus afrohamiltoni***	***Labeo cylindricus***
Locality	Pongola System	Limpopo River System
Position on host	Gills	Gills
Reference	Current study	(Basson & van As 1987)
Body diameter	19.9-22 (5)	19.1-24.7 (21.7±3.4, 50)
Adhesive disc diameter	14.7-19.3 (5)	15.4-20.4 (17.9±1.3, 50)
Denticle ring diameter	6-7.8 (5)	7.1-11 (8.7±1.5, 50)
Border membrane width	1.7-2.3 (5)	1.6-2.8 (2.3±0.3, 50)
Blade length	4.1-4.6 (5)	3-4.7 (3.8±0.4, 50)
Central part width	0.7-0.9 (5)	0.8-1.6 (1.2±0.2, 50)
Ray length	0.6-0.7 (5)	0.6-1.7 (1.1±0.3, 50)
Denticle width	1.5-2.4 (5)	1.8-4.3 (3±0.4, 50)
Denticle span	5.5-6.1 (5)	
Denticle number	24-25 (5)	20-26 (24, 50)
Radial pins/denticle	5-6 (5)	3-5 (4, 50)
Macronucleus-shape		C-shape
Macronucleus-external diameter	17.7-27.3 (23.2±2.3, 19)	17.2-30.9 (23.9±2.9, 52)
Macronucleus-thickness	2.9-6 (4.4±0.8, 19)	3.6-8.7 (5.5±0.9, 52)
Macronucleus-x value	4.1-18.4 (12.7±3.8, 19)	5.5-22.1 (13.5±4.1, 52)
Adoral spiral	240°	120-205°

Remarks:

The current trichodinid species was identified as *Tripartiella lechridens* that was described by Basson & van As (1987) from *Labeo cylindricus* Peters, 1852 from the Limpopo River. The current species was identified as *T. lechridens* by possessing the same broad blade and short thin blade connections. Although small populations of this species were found, the biometrical data corresponds with the original description and therefore it could be identified as the same species. In both the studies, the current study and the original description, specimens were collected from the Limpopo System and were found on two congeneric fish species i.e. *B. paludinosus* and *B. trimaculatus*.

As previously stated, there are some similarities in the general shape of the denticles between the current species and *T. macrosoma*, but clear differences are present. A new denticle description was given for *T. lechridens* in this study as proposed by van As & Basson (1989) and Basson & van As (2002). New evidence of the shape of the macronucleus is also presented for *T. lechridens* in Figure 3.5 B.

This represents new host records for *T. lechridens* found on the gills of *B. afrohamiltoni* and *B. gurneyi* from the Pongola River System, South Africa.

Genus: *Trichodinella* Šrámek-Hušek, 1953

***Trichodinella epizootica* (Raabe, 1950) Šrámek-Hušek, 1953** (Figures 3.7 A-B)

Hosts and localities: *Barbus radiatus* Peters, 1853 from the Okavango River System, Shakawe (S18°42'93'85" E21°89'64'98")

Location on host: Gills

Reference material: Slide 2013/07/24-14 for *B. radiatus* from the Okavango River System in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements were made using light microscopy from one population from the Okavango River System, stained with silver-nitrate and Harris's haematoxylin, mounted with Eukitt quick-hardening mounting medium.

Comparative description:

A small parasite with a disc-shaped body, 17-23.6 (20.9±1.8,22) in diameter. Adhesive disc, 14-19.9 (17.5±1.6,22) in diameter; surrounded with finely striated border membrane 1.2-2.4 (1.9±0.3,22) wide. Diameter of denticle ring 7.1-10.7 (8.7±1,21). Span of denticle 3.7-5.1 (4.4±0.4,22) and width of denticle 2.1-3.1 (2.6±0.3, 22). Length of blade 2.7-3.7 (3.3±0.3,22); width of central part 0.7-1.4 (1±0.2, 22) and ray absent. Number of denticles 18-22 (20,22) with 5-6 (5,19) radial pins per denticle. Blade broad, not curved, distal blade margin flat bulbous shaped. Tangent point rounded, lower than distal blade margin or on same level. Anterior and posterior blade margins both slightly curved shaped and do not extend through y-segments. Second blade apophysis large and rounded. Blade apophysis prominent, extending to y+1 axis. Posterior projection present and distinct, extending to y-1 axis. Blade connection thin and short of equal thickness, extending down parallel with y-axis. Central part small, elongated and flat. No indentation

present in central part. In most specimens rays are not visible, sometimes very short and thin rays are present. No angle between posterior blade margin and blade connection present, both are linear with y-axis (Figure 3.6 C). Ratio of denticle above to below x-axis more than eight. Adoral spiral makes a turn of 190°-220°.

Remarks:

Trichodinella epizootica was originally described from different cyprinid fishes in Poland and Hungary (Basson & van As 1989), but this species is one of the most widely distributed freshwater trichodinids that has previously been reported from Africa, the Pacific region and North-America (Basson 2010). This species shows very little host specificity and has been reported from more than 90 fishes from various fish families (Basson 2010). According to Basson & van As (1987), there are also two distinct groups that can be associated with *T. epizootica*, the first group having a broad blade with lateral sides almost parallel and the second group with slender blades (Figure 3.7).

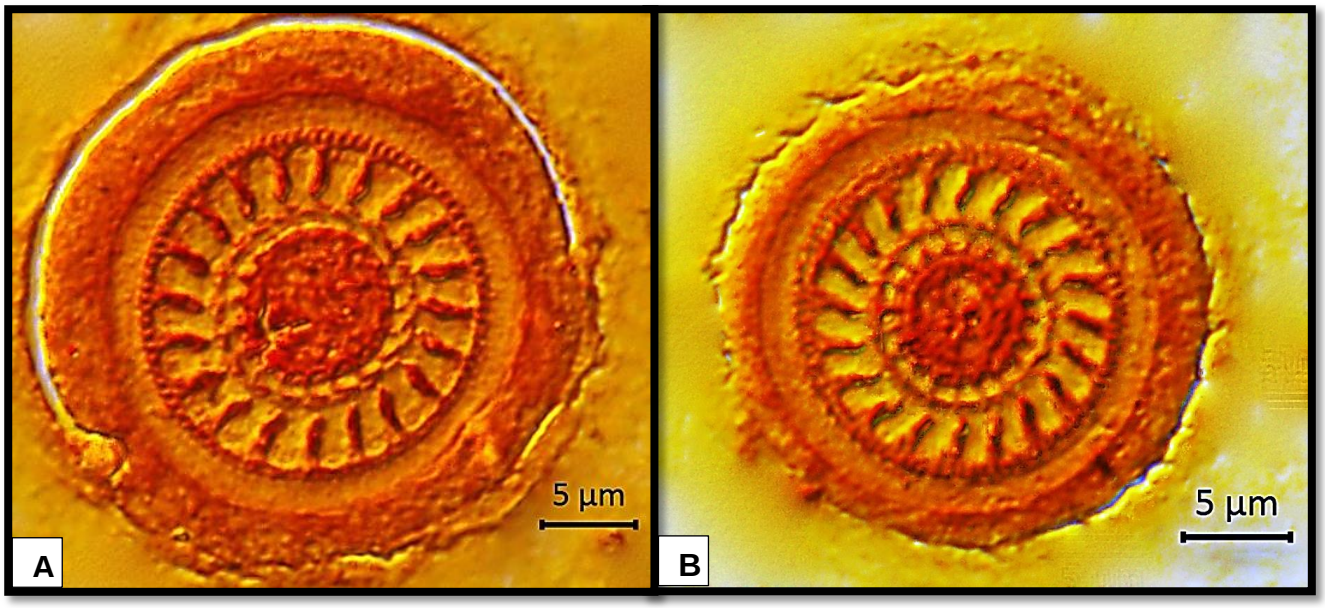

Figure 3.7: Micrographs of silver impregnated adhesive discs of *Trichodinella epizootica* (Raabe, 1950) Šrámek-Hušek, 1953 from *Barbus radiatus* Peters, 1853 from the Okavango River System, indicating the two distinctive groups, **A**- with broad blades and **B**- with slender blades.

Only two species of the genus *Trichodinella* have previously been identified in southern African freshwaters, *Trichodinella crennulata* Basson & van As, 1987 and *T. epizootica*. The current species was identified as *T. epizootica* by possessing the

same blade margins and the biometrical data corresponds to the parameters from the description given by Basson *et al*. (1983) from *Cyprinus carpio*. This parasite is also known to occur on cyprinid fishes and the same species was previously found on *B. trimaculatus* and *B. paludinosus* from the Limpopo System, South Africa (Basson & van As 1987).

According to Basson & van As (1987), *T. epizootica* could possibly be divided into two species due to the large variation that exists between the two groups. Findings indicate that the larger specimens with the broad blade were associated with the exotic *C. carpio* and the smaller slender specimens were more associated with indigenous fish species. The current specimens were found in the Okavango River System and both variations were found on the same species, but *C. carpio* does not occur in the system and no other introductions of alien fish species have been reported (Basson & van As 2002). This rejects the theory that we are dealing with separate species, but rather considerable variation within one species. *Trichodinella epizootica* therefore occurs naturally in the Okavango River System and southern Africa. There are, however, slight differences in denticle morphology from different populations around the world and molecular work on this species is needed to give clarity on whether they are different species or not[1]. A re-evaluation of the different populations from different localities is required for *T. epizootica*; van As & Basson (1992) and Basson & van As (1993) are convinced that the populations from southern Africa do not represent the same species as those occurring in Europe. For the purpose of this study, the species will be referred to as *T. epizootica* until further studies suggest otherwise.

This represents a new host and locality for *T. epizootica* found on the gills of *B. radiatus* from the Okavango River System, Botswana.

[1] Personal communication with Professor Linda Basson, Department of Zoology and Entomology, University of the Free State , Bloemfontein, South Africa

Phylum: Ciliophora Doflein, 1901

Class: Oligohymenophorea de Puytorac *et. al*., 1974

Order: Sessilina Kahl, 1935

Family: Epistylidae, Kahl, 1935

Genus: *Apiosoma* Blanchard, 1855

***Apiosoma caulata* Viljoen & van As, 1985** (Figures 3.8 A-B, 3.9 A)

Host and localities: *Barbus trimaculatus* Peters, 1952 from the Pongola River System, Bumbe (S26°99'57'72" E32°30'05'33")

Location on host: Skin

Reference material: Slide 2013/09/14-12 for *B. trimaculatus* from the Pongola River System in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements were made using light microscopy from one population from the Pongola River System stained with Harris's haematoxylin, mounted with Eukitt quick-hardening mounting medium (Table 3.5).

Comparative description:

Biometrical data is presented in Table 3.5. Body cylindrical when expanded, broad at peristome, tapers down towards scopula, pear-shaped body. Peristomial disc flat, with broad lip. Very prominent peristomial apex, open as wide as body when expanded. Widest region of body located towards the anterior region, close to peristome. Macronucleus round, situated at base of body, with micronucleus oval-shaped, situated below macronucleus. Zoochlorellae were present in cytoplasm in upper two thirds of body. Small scopula, attached to stalk present (Figure 3.8 B).

Table 3.5: Biometrical data of *Apiosoma caulata* Viljoen & van As, 1985 from *Barbus trimaculatus* Peters, 1952 from the Pongola River System with comparative measurements of the same species from the Limpopo System, South Africa (Viljoen & van As 1985).

Host	***Barbus trimaculatus***	***Mesobola brevianalis***
Locality	Pongola System	Limpopo System
Position in host	Skin	Skin & gills
Reference	Current study	(Viljoen & van As 1985)
Body length	28.2-96.8 (49.6±17.4,29)	46.4-73.9 (61.4±7.7,26)
Body width	20-66.2 (31.9±12.8,29)	31.6-60.6 (42±6.2,26)
Scopula	3.3-6.9 (4.6±0.8,28)	5.8-11.4 (8.4±1.7,20)
Macronucleus length	9.5-28.7 (15.8±4.8,29)	13.8-31.2 (19.5±3.9,20)
Macronucleus width	8.4-23.2 (13.4±3.8,29)	15-25.6 (20.4±3,20)
Micronucleus length	2.9-10.9 (4.9±2.1,29)	2.8-8.2 (5±1.3,25)
Micronucleus width	2.1-6.8 (3.9±1.3,29)	3.1-9.4 (5.9±1.2,25)

Remarks:

This species corresponds to the description of *A. caulata* that was described by Viljoen & van As (1985) from *Mesobola brevianalis* (Boulenger, 1908) from the Limpopo System. Although the current population is slightly smaller than the original description, the measurements still fall into the parameters for this species. Both populations were also found on small fish from the family Cyprinidae. According to Viljoen & van As (1985), an outstanding feature of *A. caulata* is the zoochorellae that are present in the cytoplasm. This is also the only known species of the genus *Apiosoma* that zoochorellae have been found in. The zoochorellae are clearly visible in the current specimens, see Figures 3.8 A-B.

Only two other species of *Apiosoma* have previously been recorded from *Barbus* in southern Africa, *A. piscicola* Blanchard, 1885 and *A. phiala* Viljoen & van As, 1885. *Apiosoma caulata* differs from *A. piscicola* by having a pear-shaped body and is not elongated as in the case of *A. piscicola*. *Apiosoma phiala* is smaller than *A. caulata* and possesses a triangular macronucleus with a micronucleus situated above the macronucleus.

This represents a new host and locality for *A. caulata* that was found on the gills of *B. trimaculatus* from the Pongola River System, South Africa.

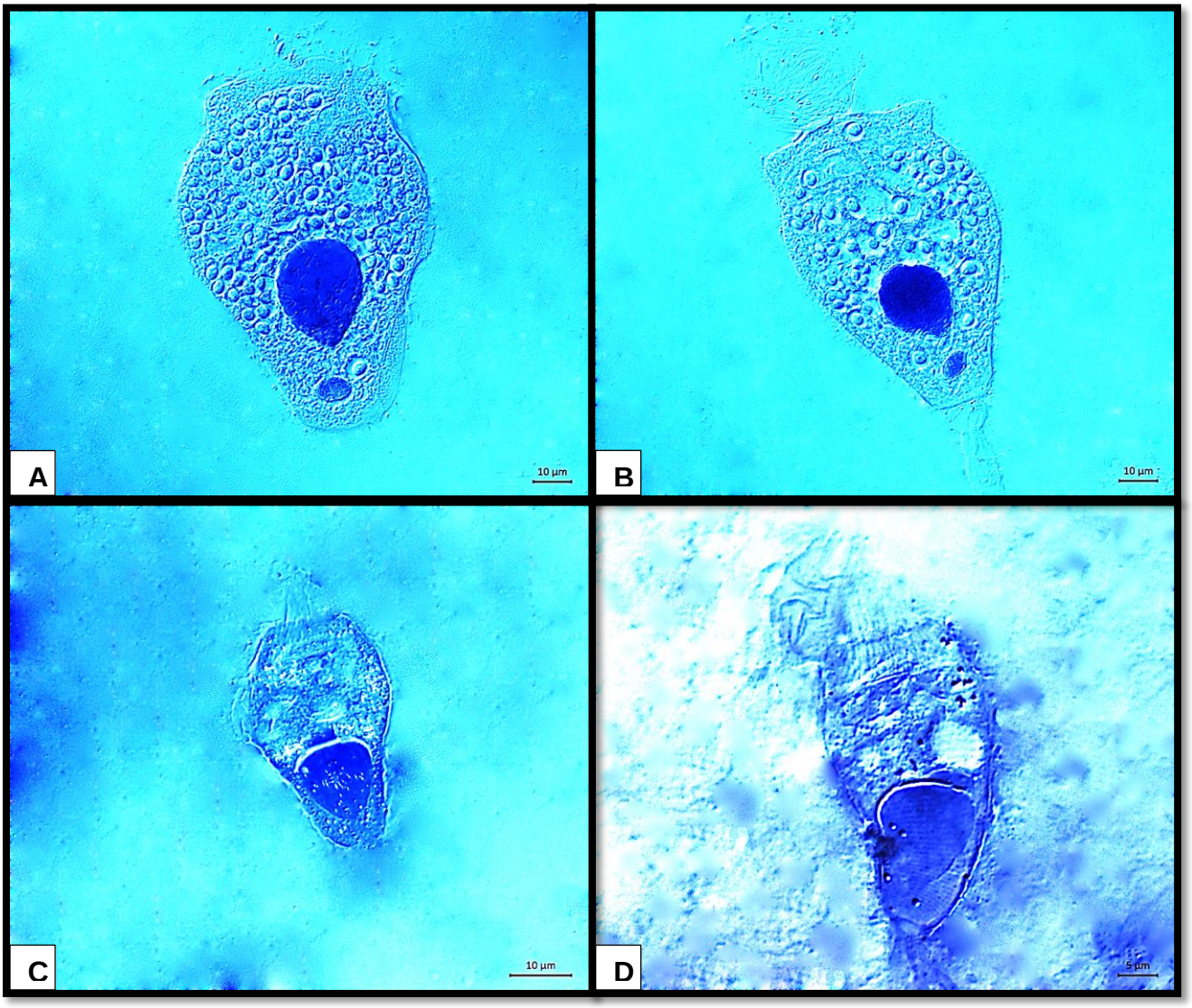

Figure 3.8: Micrographs of Harris's Haematoxylin stained specimens of **A** and **B**- *Apiosoma caulata* Viljoen & van As, 1985 from *Barbus trimaculatus* Peters, 1952 from the Pongola River System and **C** and **D**- *Apiosoma phiala* Vijloen & van As, 1985 from *Barbus radiatus* Peters, 1853 from the Okavango RIver System.

***Apiosoma phiala* Viljoen & van As, 1985** (Figures 3.8 C-D, 3.9 B)

Host and localities: *Barbus radiatus* Peters, 1853 from the Okavango River System, Nxamasere (S18°37'34'9" E22°06'24'4")

Location on host: Gills

Reference material: Slide 2013/07/25-03 for *B. radiatus* from the Okavango River System in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements were made using light microscopy from one population from the Okavango River System, stained with Harris's haematoxylin, mounted with Eukitt quick-hardening mounting medium (Table 3.6).

Comparative description:

Biometrical data is presented in Table 3.6. Body cylindrical above groove, tapers down sharply toward scopula. Small scopula present, stalk absent. Groove centrally placed. Peristomial disc flat. Peristomial lip broad, open as wide as body when expanded. Food vacuoles present in region above groove. Macronucleus triangular, situated below groove. Micronucleus oval-shaped, elongated, situated above macronucleus. Cytoplasm homogenous to granular.

Table 3.6: Biometrical data of *Apiosoma phiala* Viljoen & van As, 1985 from *Barbus radiatus* Peters, 1853 from the Okavango River System with comparative measurements of the same species from the Limpopo System, South Africa (Viljoen & van As 1985).

Host	***Barbus radiatus***	***Barbus trimaculatus***
Locality	Okavango System	Limpopo System
Position in host	Gills	Skin & gills
Reference	Current study	(Viljoen & van As 1985)
Body length	27.8-47 (39.2±5.4,25)	22.5-49.8 (36.6±5.3,28)
Body width	16.3-27.9 (22.7±3.3,25)	15.1-34.1 (21.5±3.7,28)
Scopula	2.6-6.5 (4.3±1.1,25)	3.4-9.6 (5.9±1.8,15)
Macronucleus length	9.4-19.4 (14.4±2.5,25)	9.7-20.5 (14.6±2.5,28)
Macronucleus width	8-16.7 (12.5±2.3,25)	8.8-19.6 (13.6±2.7,28)
Micronucleus length	2.4-7.2 (4.4±1.2,25)	3.6-6.7 (5±0.7,28)
Micronucleus width	0.6-4.7 (2.4±0.7,25)	1.7-4.6 (3.2±0.7,28)

Remarks:

The current specimens conform to the description of *A. phiala* that was previously described from *B. trimaculatus* in the Limpopo System by Viljoen & van As (1985). The same species was also recorded from *B. paludinosus* and *B. unitaeniatus* and several other species of cyprinid and cichlid fishes (Viljoen & van As 1985). The current population possesses the same biometrical data as the original description and the species is known to use *Barbus* species as a living substrate.

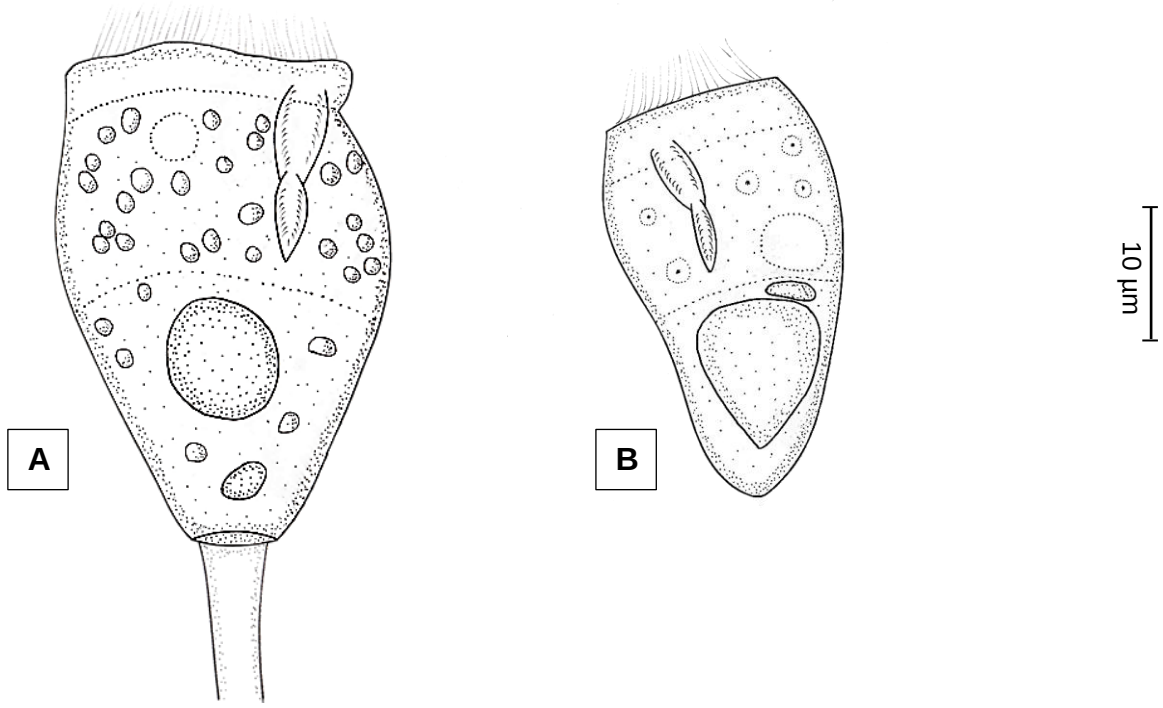

Figure 3.9: Microscope projection drawings of Harris's Haematoxylin stained specimens of *Apiosoma* Blanchard, 1855 species. **A**- *Apiosoma caulata* Viljoen & van As, 1985 from skin of *Barbus trimaculatus* Peters, 1952 collected from the Pongola River System and **B**- *Apiosoma phiala* Vijloen & van As, 1985 from the gills *Barbus radiatus* Peters, 1853 collected from the Okavango River System.

The species possesses a unique triangular macronucleus with the micronucleus situated above the macronucleus. This species can be distinguished from other species by this feature. *Apiosoma phiala* is also different from *A. piscicola* by being much smaller and less elongated. *Apiosoma mothapitsis* Viljoen & van As, 1985 that has been described from *Labeobarbus marequensis* (Smith, 1841) also possesses a triangular macronucleus with the micronucleus situated above, but differs from *A. phiala* in site of infection and by being much smaller (Viljoen & van As 1985).

This represents a new host and locality record for *A. phiala* present on the gills of *B. radiatus* from the Okavango River System, Botswana.

DISCUSSION

During the current study, no new species of trichodinids were found associated with the genus *Barbus* from southern Africa. The first taxonomic descriptions of trichodinids from freshwater fish in southern Africa were provided by Basson *et al*. (1983) and from then on trichodinid ciliophorans from southern Africa received considerable attention (Basson & van As 1987, 1989, 1993, 2002, 2006; van As & Basson 1989, 1992). These studies also include trichodinids from fish of the genus *Barbus*, and these authors reported the same species as those presented in the current study.

According to van As & Basson (1992), trichodinids vary from having no specificity to a high degree of host specificity among different species. *Trichodina heterodentata* utilise a broad spectrum of fishes from different fish families, but is mostly found on cichlids (van As & Basson 1992). This species was found on *B. radiatus* and *B. haasianus* and support the theory that this species does not show any host specificity. *Trichodina kazubski* on the other hand, has previously only been found on *Barbus* species (van As & Basson 1989) with specimens from the current study also found on the same genus. This indicates that *T. kazubski* shows a high degree of host specificity for fish of the genus *Barbus*.

According to Tang *et al*. (2007), *T. kazubski* was found on the gills of *Carassius auratus* (Linnaeus, 1758) and *Hypophthalmichthys nobilis* Richardson, 1848 in China. Although the biometrical data is comparable to the original description, it is very unlikely that *T. kazubski* occur on fishes in China. *Trichodina kazubski* was only previously found on indigenous *Barbus* fish from southern Africa and shows a high degree of specificity (van As & Basson 1989). The description from the work of Tang *et al*. (2007) is only based on one infected fish from each host and therefore more information is needed to confirm the occurrence of *T. kazubski* in China.

Van As & Basson (1992) determined the host specificity of trichodinids based on their site of infection. They concluded that trichodinids that occur predominantly on the skin show a low degree of specificity and trichodinids that occur on the gills show a higher degree of specificity. The two species of *Trichodina* that were collected during the current study were found on both skin and gills, but show different

degrees of specificity. This indicates that the site of infection does not always determine the host specificity in trichodinids and the current two species have no clear pattern.

Tripartiella lechridens and *T. macrosoma* were found on different *Barbus* species from the Pongola River System and they were originally described from the greater Limpopo System, of which the Pongola River System is a part of (Basson & van As 1987). They were also described from *Barbus* species and therefore expected to be recorded during the current study. *Tripartiella lechridens* has previously been found on different fish species from three different fish families, indicating that this trichodinid does not show any host specificity. On the other hand, *T. macrosoma* has previously only been found infecting the genus *Barbus*, but during the current study only *Barbus* fish were investigated and therefore its host specificity is unknown. More research is needed from different fish families to give clarity on the host specificity of *T. macrosoma*. Various parasitological studies were conducted throughout southern Africa and these two species were only found within the Limpopo System, so it is safe to say that there distribution is restricted to this system only.

The current study contributes by providing information on the geographical distribution of the species and by providing new host records. Denticle descriptions of *T. lechridens* and *T. macrosoma* are also provided that were previously not included in the original description.

As previously mentioned, very little work has been done on sessile ciliates of freshwater fishes of southern Africa. During the current study, only two species were found from two different *Barbus* species from different localities. This indicates that sessile ciliates do use *Barbus* fish as a living substrate, but they are not as abundant as other fish parasites or symbionts. *Apiosoma caulata* was found on a new host, but was found in the Pongola River System which forms part of its original distribution. *Apiosoma phiala* was found in the Okavango River System and this finding increases its distribution range from the Limpopo System to the Okavango River System.

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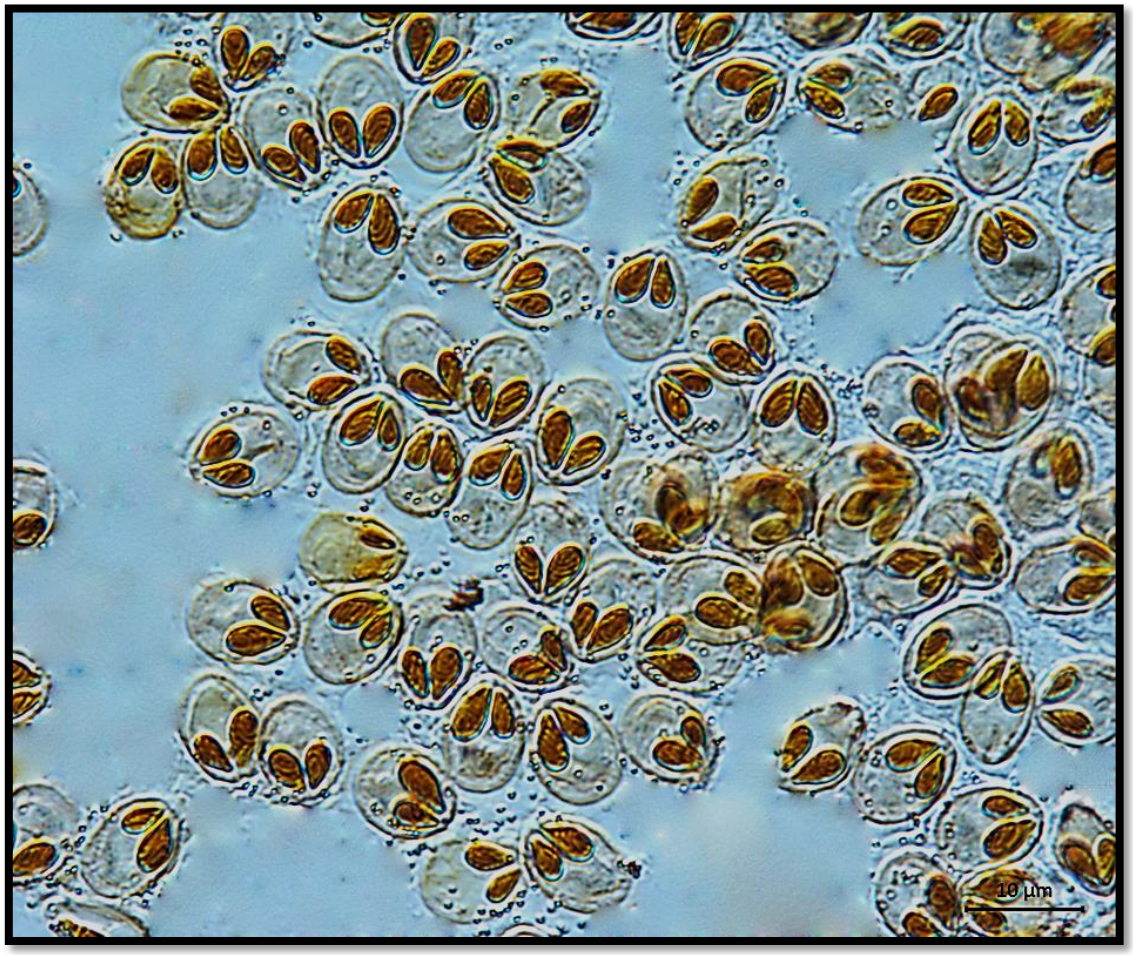
Chapter 4



Myxozoa

INTRODUCTION

Myxozoans are extremely simplified parasites of aquatic vertebrates, but their classification and position in the phylogenetic tree has long been uncertain. According to Foox & Siddall (2015), myxozoans were previously believed to belong to the protozoans, but studies revealed that these organism are in fact multicellular. They were transferred to the Metazoa, a subkingdom of Animalia (Lom & Dyková 2006), but suggestions were frequently made that they possessed characters similar to the phylum Cnidaria. It is now clear that a clade of cnidarians diverged to become endoparasites that are now the Myxozoa (Okamura *et al*. 2015). Myxozoa are now classified as a subphylum and a clade of highly derived cnidarians (Foox & Siddall 2015) (Figure 4.1).

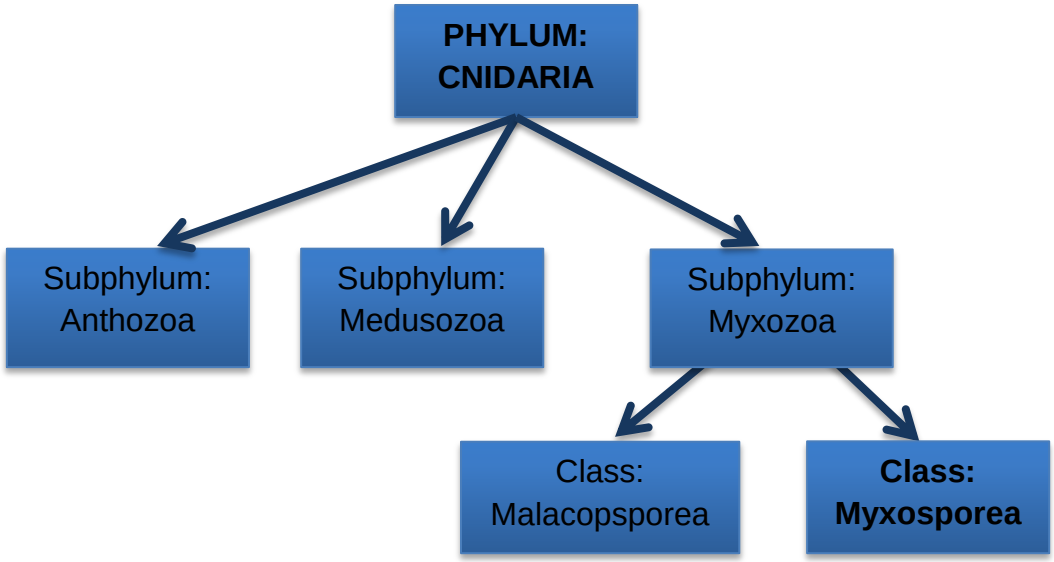


Figure 4.1: Phylogenetic tree of Phylum Cindaria indicating the evolutionary relationship with the Class Myxosporea (Collins 2009).

The class Myxosporea Büetschli, 1881 represents an abundant and diverse group of parasites, mostly parasitising teleost fish (Ali *et al*. 2002). Myxosporeans are abundant and more than 1,350 species have been described worldwide (Fomena & Bouix 1997; Fomena *et al.* 2007). More than 135 species have been described in Africa with just over a 100 species described from primarily freshwater fishes, most of these from northern Africa (Fomena *et al.* 2007).

Although many species have been recorded from Africa, relatively few have been identified as serious pathogens (Ali *et al.* 2002). However, myxosporeans are economically important fish parasites and should be regarded as a potential hazard to the fitness of their hosts directly or indirectly, particularly in captive fish populations. According to Fomena *et al*. (2007), myxosporeans have been the direct cause of fish mortalities in aquaculture in Africa before; unidentified myxosporeans were the cause of devastating mortalities in fish stocks in Ivory Coast. Gbankoto *et al*. (2001) reported the indirect negative impacts of myxosporeans that caused damage to the ovaries of their hosts negatively affecting reproduction in these fish.

Thirteen different genera of Myxosporea are currently known to infect freshwater, brackish and marine fish in Africa, i.e. *Myxidium* Buetschli,1882, *Myxobolus* Bütschli, 1882, *Sphaerospora* Thelohan, 1882, *Chloromyxum* Mingazzini, 1890, *Henneguya* Thelohan, 1892, *Thelohanellus* Kudo, 1933, *Myxobilatus* Davis, 1944, *Unicauda* Davis, 1944 and *Kudoa* Meglitsch, 1947 (Fomena & Bouix 1997), *Chloromyxum* Mingazzini, 1890, *Ortholinea* Shulman, 1962, *Ceratomyxa* Thelohan, 1892 and *Sphaeromyxa* Thelohan, 1892 (Ali 2000; Reed *et al.* 2007; Bartošová-Sojková *et al*. 2015). *Myxobolus* is the largest genus within Myxosporea in the world, with 857 valid species described, with more than 50 described in Africa (Fomena & Bouix 1997; Eiras *et al*. 2005; Abdel-Baki *et al*. in press).

Not much research has been done in southern Africa on myxosporean freshwater parasites and only a few species have been described. The latest publications on freshwater myxosporeans from southern Africa were by Reed *et al*. (2002, 2003). Two new species were described from *Barbus paludinosus* Peters, 1852 and *Barbus thamalakanensis* Flower, 1935 in the Okavango River System belonging to the genus *Myxobolus*, namely *Myxobolus paludinosus* Reed, Basson & van As, 2002 and *Myxobolus etsatsaensis* Reed, Basson & van As, 2002, respectively. One known species, *Myxobolus nyongana* (Fomena, Bouix & Birgi, 1985) was also found on *Barbus poechii* Steindachner, 1911 (Reed *et al*. 2002).

Table 4.1: Measurements of *Myxobolus* Bütschli, 1882 species previously described from *Barbus* Cuvier & Cloquet, 1816 hosts in Africa (Fomena & Bouix 1997; Ali *et al*. 2002; Reed *et al*. 2002; Eiras *et al*. 2005). All measurements are given in micrometres.

Species names	Spore Length	Spore Width	Polar Capsule Length	Polar Capsule Width	No. of Coils	Infected Organ	Type Host	Type Locality	Reference
***M. njinei* Fomena, Bouix & Birgi, 1985**	14-20	11.2-18.5	6.5-9	3.5-5.5	-	-	*Barbus* spp.	Cameroon	Fomena & Bouix (1997)
***M. oloi* Fomena & Bouix 1994**	6.5-11.5	5-9.5	4-7	2-5	4-5	Gill arches	*Barbus aspilus*	Cameroon	Fomena & Bouix (1997)
***M. nkolyaensis* Fomena & Bouix 1994**	8-11	7-11	3.5-5.5	2-3.5	-	Caudal muscles	*Barbus jae*	Cameroon	Fomena & Bouix (1997)
***M. nyongana* (Fomena, Bouix & Birgi, 1985)**	7.5-13	5-7	5-7	1.5-2.5	6-9	Gill arches	*Barbus aspilus*	Cameroon	Fomena & Bouix (1997)
***M. naffari* Abdel Ghaffar *et al*. 1998**	10.8-13.2	7.8-9.8	4.5-6.2	2.5-3	7-9	Mouth	*Barbus bynni*	Egypt	Eiras *et al.* (2005)
***M. caudatus* Ali *et al*. 2002**	16-19.2	11-13.6	6.4-9	3.2-4.5	8-9	Caudal fin	*Barbus bynni*	Egypt	Ali *et al.* (2002)
***M. intestinalis* Ali *et al*., 2002**	12-13.6	8-9.6	7.2-8	3.2-3.6	5-6	Intestine	*Barbus bynni*	Egypt	Ali *et al.* (2002)
***M. fahmii* Ali *et al*. 2002**	10.8-12	6.4-8	6.4-7.2	2.8-3.8	7-9	Gill filaments	*Barbus bynni*	Egypt	Ali *et al*. (2002)
***M. etsatsaensis* Reed, Basson & van As, 2002**	12.8-15	6.2-8	7-8	1.25-2.5	7-8	Secondary gill lamellae	*Barbus thamalaka-nensis*	Botswana	Reed *et al.* (2002)
***M. paludinosus* Reed, Basson & van As, 2002**	11.2-13.7	7.5-10	5-6.8	2-2.5	6-7	Secondary gill lamellae	*Barbus paludinosus*	Botswana	Reed *et al.* (2002)

During the present study, a survey of myxosporeans was carried out on selected species from *Barbus* in southern Africa. There are currently 10 myxosporean species known from *Barbus* in Africa, all belonging to *Myxobolus* (Table 4.1). Only three of these species have been found in southern Africa, *Myxobolus nyongana* (Fomena, Bouix & Birgi, 1985), *M. etsatsaensis* Reed, Basson & van As, 2002 and *M. paludinosus* Reed, Basson & van As, 2002 (Reed *et al*. 2002; Eiras *et al*. 2005).

This chapter reports on the occurrences of seven myxosporeans of the genus *Myxobolus* found infecting five different *Barbus* host fish species from the Okavango River System, Botswana and Pongola River System, South Africa.

MATERIALS AND METHODS

Fish collections

See chapter 3.

Host examination, fixation and preservation of parasites

Skin smears were made by scraping off the fish mucus with a slide or a scalpel onto another slide. The slides were examined under a compound microscope (Nikon Eclipse 50i) for parasites. If myxosporean parasites were found, the slides were given a number, documented and smears were air dried. The gills of the fish were dissected and examined for myxosporean parasites and myxosporean plasmodia using a compound or a dissection microscope (Nikon SMZ800) depending on the size of the gills. The gills were used to make gill smears on slides, using the same method as with skin smears; the smears were examined under a compound microscope. The slides were also air dried as previously explained for skin smears.

Light microscopy preparations

The method that was used for staining myxosporeans is the same as described in Chapter 3 for trichodinids, a modification of Klein's technique that was described by Lom (1958) that is typically used for trichodinid staining. The reason for using the latter preparation technique is, because both trichodinids and myxosporean parasites were found on the same skin and gill smears. This technique provides sufficient morphological information for identification of the myxosporeans to species level and can also be used for trichodinid identifications from the same slides.

Morphological measurements

All measurements given in the results are in micrometres unless otherwise stated.

All the spores that were measured were from fully matured plasmodia, stained with silver-nitrate and were measured according to the guidelines provided by Lom & Arthur (1989). All the species that were found in the study belong to the genus *Myxobolus* and according to Lom & Arthur (1989), there are four basic measurements for this genus (Figure 4.2). *Myxobolus* spp. possess two polar capsules at the anterior end or the apex, the length of the spore is the distance from the apex to the posterior or opposite end. The width of the spore is measured perpendicular to the length, in the plain of the suture. Polar capsule length and width were measured as indicated in Figure 4.2. The measurements are presented in the descriptions of the species collected as follows.

Minimum - maximum (average ± standard deviation)

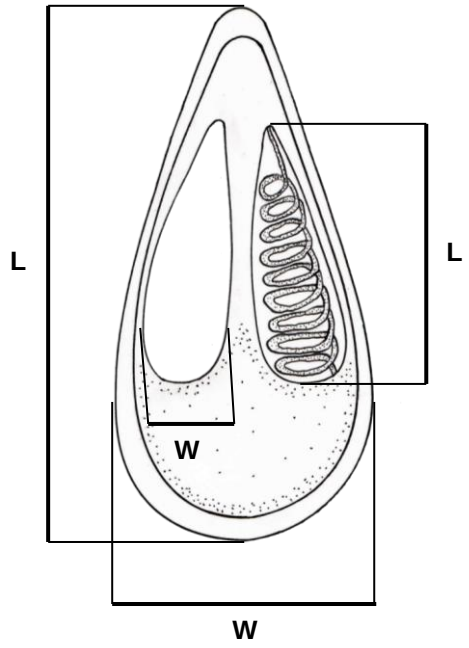


Figure 4.2: Methods of measuring myxosporeans spores of the genera *Myxobolus* Bütschli, 1882 using the guidelines as illustrated by Lom & Arthur (1989). **L**- Length of spore and polar capsule and **W**- Width of spore and polar capsule. Adapted from Lom & Arthur (1989).

RESULTS

This chapter report of the taxonomical diagnoses of *Myxobolus* species collected from different localities. Based on taxonomical diagnosis of the spore morphology, seven species of the genus *Myxobolus* have been found, five known and two unknown species. Keys of African Myxosporea provided by Fomena & Bouix (1997) were used to identify the genus and some of the species.

Phylum: Cnidaria Hatschek, 1888

Subphylum: Myxozoa Grassè, 1970

Class: Myxosporea Bütschli, 1881

Suborder: Platysporina Kudo, 1919

Order: Bivalvulida Shulman, 1959

Family: Myxobolidae Thélohan, 1892

Genus: *Myxobolus* Bütschli, 1882

***Myxobolus nyongana* (Fomena, Bouix & Birgi, 1985) Fomena & Bouix, 1997** (Figures 4.3 A-D and 4.5 C)

Description of the vegetative stage:

Sporogonic plasmodia found attached to secondary gill lamellae. Plasmodia small, rounded, greyish in colour (Figure 4.3 A). No histopathological observations were made.

Description of spores (based on 50 specimens, 25 from *B. radiatus* and 25 from *B. multilineatus*, from fully mature plasmodia) (Table 4.2):

Anterior or apex of spore pointed, posterior end rounded, spore body pyriform teardrop-shape with blunt point. Widest region of spore observed towards centre of sporoplasm. Two smooth shell valves visible. Two pyriform-shape polar capsules situated in anterior end of spore; polar capsules occasionally unequal in size, occupy half of spore body. Position of sporoplasm in posterior half of spore cavity. Polar filament coils five to seven times within polar capsules. Measurements of spores are presented in Table 4.2.

Host: *Barbus radiatus* Peters, 1853

Additional host: *Barbus multilineatus* Worthington, 1933

Locality: Okavango River System, Shakawe (S18°42’93’85” E21°89’64’98”)

Prevalence: 6/9 = 66.67% for *B. radiatus* and 4/17 = 23.53% for *B. multilineatus*

Site of infection: Gill lamellae and skin

Type material: 2013/07/24-17 for *B. radiatus* and 2013/07/29-04 for *B. multilineatus* in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Morphological measurements were made from micrographs using Zeiss Zen Imaging software program from 25 specimens from each host from

the Okavango River System, stained with silver-nitrate. Detailed morphologic drawings were made from micrographs (Figure 4.5 C).

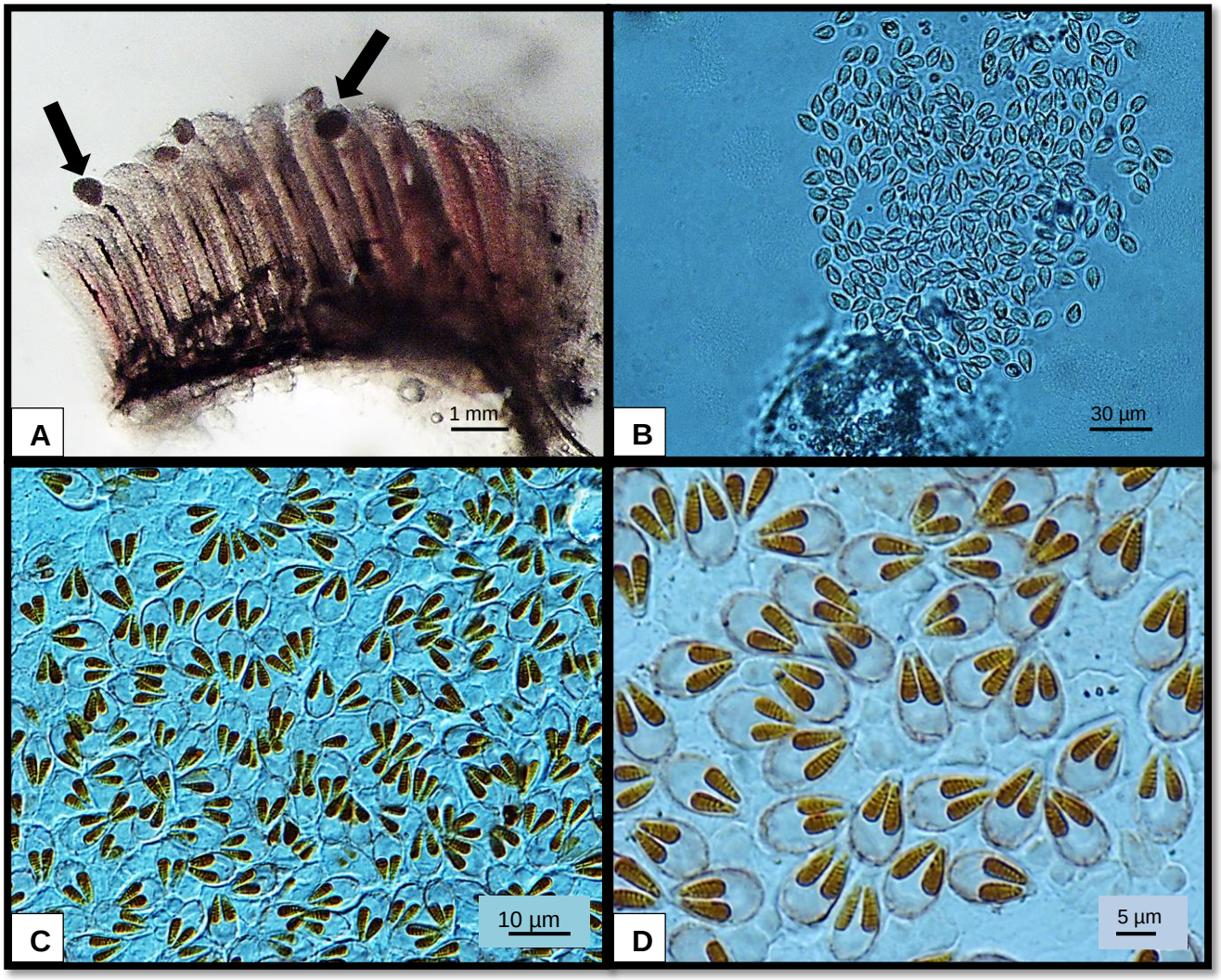


Figure 4.3: *Myxobolus nyongana* (Fomena, Bouix & Birgi, 1985) collected from the Okavango River System, Botswana. Light micrographs of live specimens and silver-nitrate stained spores. **A**- Polysporous plasmodia found within secondary gill lamellae in *Barbus radiatus Peters*, 1853 (indicated by arrows). **B**- *Myxobolus nyongana* spores emerging from the polysporous plasmodia. **C** and **D**- Micrographs of silver impregnated spores of *M. nyongana* from *Barbus radiatus*.

Remarks:

The morphology of these spore concur that of *M. nyongana* which was originally described by Fomena *et al.* (1985) from the gills of *Barbus jae* Boulenger, 1903 in Cameroon. The populations studied here were found on congeners of the type host and the same species were previously identified in the same locality, the Okavango River System from *Barbus poechii* Steindachner, 1911 (Reed *et al*. 2002). As previously mentioned, ten *Myxobolus* species have been described from fish species

from the genus *Barbus* in Africa, including *M. nyongana* (Table 4.1). *Myxobolus nyongana* differs from the other nine species.

Myxobolus njinei have much larger spores and the spore shape is spherical to ovoid with rounded anterior and posterior ends. *Myxobolus oloi* also differs in the shape and size of the spores; they have smaller oval spores, asymmetric polar capsules and four to five coils in the polar capsules. *Myxobolus nkolyaensis* has almost a spherical spore with subspherical polar capsules and the spore and polar capsules width does not fall within the range reported for the various populations of for *M. nyongana.*

Table 4.2: Measurements of *Myxobolus nyongana* (Fomena, Bouix & Birgi, 1985) from *Barbus radiatus* Peters, 1853 and *Barbus multilineatus* Worthington, 1933 from the Okavango River System and a comparison with published descriptions from Fomena *et al.* (1985) and Reed *et al*. (2002). All measurements are given in micrometres.

Myxosporean	*Myxobolus nyongana* (n=25)	*Myxobolus nyongana* (n=25)	*Myxobolus nyongana* (n=12)	*Myxobolus nyongana* (n=not available)
Host	*Barbus radiatus*	*Barbus multilineatus*	*Barbus poechii*	*Barbus* spp.
Locality	Okavango System Current study	Okavango System Current study	Okavango System (Reed *et al.* 2002)	Cameroon (Fomena *et al.* 1985)
Spore body				
Length	9.6-12.1 (10.7±0.70)	9.9-13 (11.1±0.63)	11-11.2 (11.1±0.26)	7.3-13 (10.8)
Width	5.3-7.3 (6.4±0.49)	5.7-7.3 (6.3±0.40)	6.1-7 (6.5±0.31)	5-7 (6.1)
Polar capsules				
Length	4.7-6.5 (5.5±0.49)	4.5-7.3 (5.3±0.59)	3-5.5 (4.4±0.79)	5-7 (5.9)
Width	1.7-2.5 (2.2±0.2)	1.8-3 (2.1±0.25)	1.25-2.5 (1.6±0.44)	1.4-2.5 (1.9)

Myxobolus naffari spore shape is subspherical to elliptical and the polar capsules are oval, they are also slightly larger in overall length compared to *M. nyongana*. *Myxobolus caudatus* are elliptical in shape with a blunt rounded anterior end and oval polar capsules. The spores are also much larger in size than *M. nyongana*. *Myxobolus intestinalis* are oval in frontal view with a blunt apex, the polar capsules occupy two thirds of the spore length and the site of infection is the intestines. *Myxobolus fahmii* are pear-shape and taper into an anterior tip, the polar capsules are larger than those of *M. nyongana* which are pyriform and that occupy more than half of the spore.

Myxobolus etsatsaensis and *M. paludinosus* were described from the same river system, the Okavango, where the current species were found, but clear differences are present. *Myxobolus etsatsaensis* are extremely elongated, pyriform, with the

anterior end tapering sharply to a blunt point. Two extremely elongated, pyriform polar capsules of unequal length are situated in the anterior part of spore. *Myxobolus nyongana* has a far less extended spore body than *M. etsatsaensis*. *Myxobolus paludinosus* differs from *M. nyongana* being ovoid with the anterior end tapering to a blunt point and a rounded posterior end.

This report represents new host records for *M. nyongana* found on the gills of *Barbus radiatus* and *B. multilineatus* in the Okavango River System, Botswana.

***Myxobolus* sp. 1** (Figures 4.4 and 4.5 G)

Description of the vegetative stage:

Sporogonic plasmodia not observed.

Description of spores (based on 25 spores):

Spores extremely large in valvular view; spores teardrop-shape with anterior end tapering to blunt rounded point, posterior end rounded, 19.3-23.7 (21±1.07) in length. Widest region of spore observed more or less in middle of spore body, 8.8-11.2 (9.7±0.64) in width. Two smooth shell valves visible. Two pyriform polar capsules of equal size situated in anterior side of spore, occupying half of spore body, 7.8-9.9 (9±0.48) long x 3.2-4.2 (3.7±0.24) wide. Polar filaments have seven to nine coils within polar capsules and sporoplasm situated in the posterior half of the spore.

Host: *Barbus radiatus*

Locality: Okavango River System, Shakawe (S18°42’93’85” E21°89’64’98”)

Prevalence: 1/9 = 11.11%

Site of infection: Skin

Type material: 2013/07/24-16 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Morphological measurements were made from micrographs using Zeiss Zen Imaging software program from 25 specimens from the Okavango

River System, stained with silver-nitrate. Detailed morphologic drawings were made from micrographs (Figure 4.5 G).

Figure 4.4: Light micrograph of silver-nitrate stained spores of *Myxobolus* sp.1 collected the skin from the host *Barbus radiatus* Peters, 1853 in the Okavango River System, Botswana.

Remarks:

Myxobolus sp. 1 is extremely large and none of the species of the genus *Myxobolus* currently described from Africa correspond to the length of the current species, therefore *Myxobolus* sp. 1 does not conform to the description of any other *Myxobolus* species in Africa. *Myxobolus* sp. 1 are almost twice as long as most of the *Myxobolus* species of Africa.

Myxobolus njinei differs from the current species by having a truncated anterior end and *M. oloi* differs by having oval spores and asymmetric polar capsules that coil four to five times. *Myxobolus nkolyaensis*, *M. naffari, M. caudatus* and *M. intestinalis* have subspherical to elliptical spores.

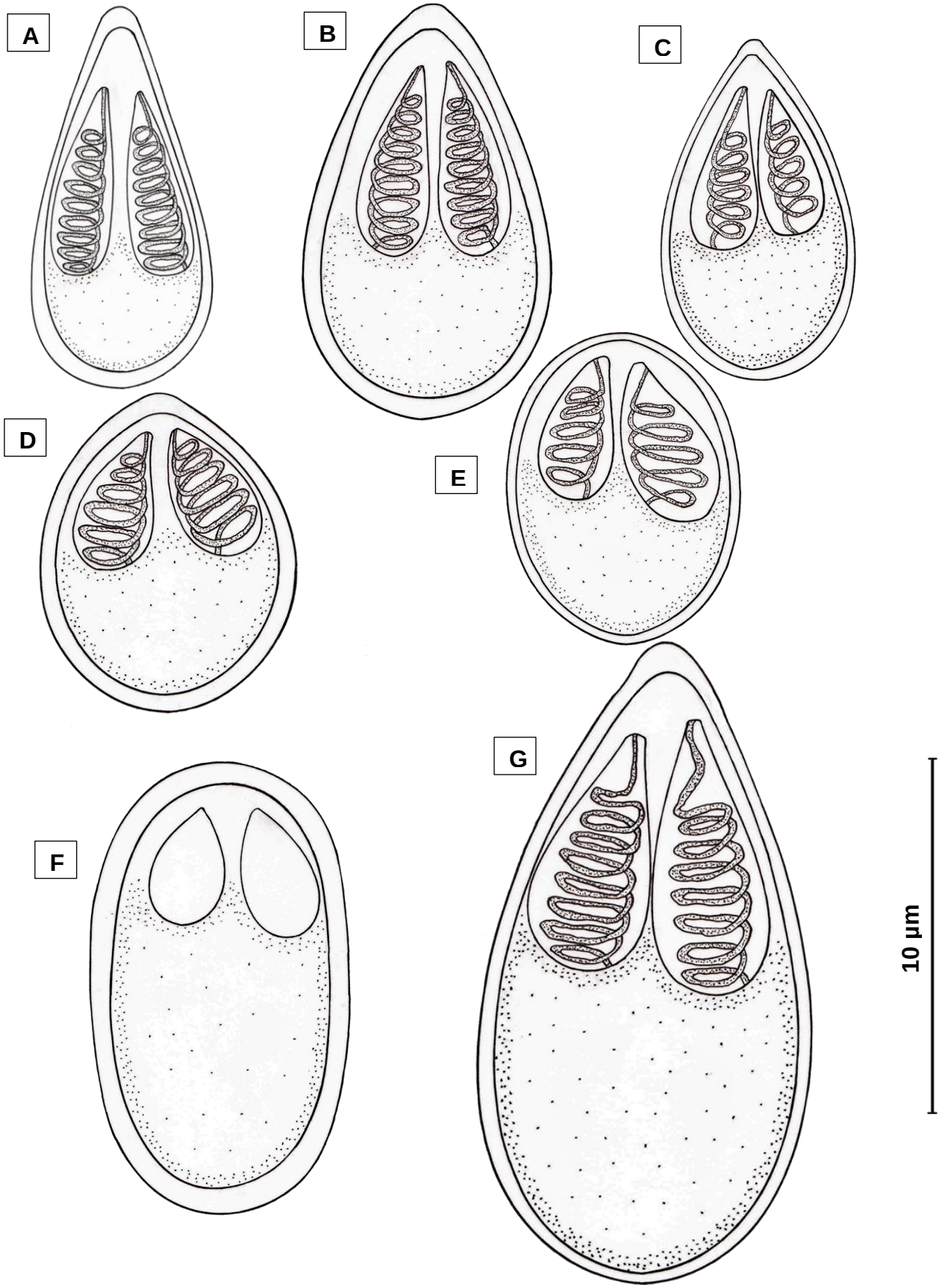


Figure 4.5: *Myxobolus* Bütschli, 1882 species collected from the Okavango and Pongola River Systems. Microscope projection drawings of silver impregnated spores. **A**- *Myxobolus etsatsaensis* Reed, Basson & van As, 2002 from the gills of *Barbus afrohamiltoni* Crass,1960 , **B**- *Myxobolus* sp. 2 from the gills of *Barbus paludinosus* Peters, 1852, **C**- *Myxobolus nayongana* (Fomena, Bouix & Birgi, 1985) from the gills of *Barbus radiatus*, Peters, 1853, **D**- *Myxobolus paludinosus* Reed, Basson & van As, 2002 from the gills of *Barbus paludinosus* Peters, 1852, **E**- *Myxobolus oloi* Fomena & Bouix, 1994 from the gills of *Barbus radiatus* Peters, 1853, **F**- *Myxobolus heterosporus* Baker, 1963 from the skin of *Barbus afrohamiltoni* Crass, 1960 and **G**- *Myxobolus* sp.1 from the skin of *Barbus radiatus* Peters, 1853.

The spore shape of *Myxobolus* sp. 1 show similarities with *M. nyongana* and *M. fahmii,* being teardrop-shape and pear-shape, respectively, but both differ from *Myxobolus* sp. 1 by being half the size of the current species (Table 4.1). *Myxobolus etsatsaensis* are also teardrop-shape, but differ from *Myxobolus* sp. 1 by being extremely elongated and taper to a sharp blunt anterior point. *Myxobolus paludinosus* has a pyriform ovoid spore body tapering to a blunt point. Therefore *Myxobolus* sp. 1 is a possible new species.

***Myxobolus oloi* Fomena & Bouix, 1994** (Figures 4.6 A-D and 4.5 E)

Description of the vegetative stage:

Sporogonic plasmodia not observed.

Description of spores: (based on 50 spores, 25 from *B. radiatus* and 25 from *B. haasianus*, from fully mature plasmodia) (Table 4.3)

In valvular view, spores oval, shell valves thick and smooth. Polar capsules thick teardrop-shape, of unequal size close together occupying anterior half of spore length. Polar filaments coiled four to five times oblique within polar capsules. Sporoplasm filled posterior half of spore. Measurements of spores are presented in Table 4.3.

Host: *Barbus radiatus*

Additional host: *Barbus haasianus* David, 1936

Locality: Okavango River System, Shakawe (S18°42’93’85” E21°89’64’98”)

Additional locality: Okavango River System, Nxamasere (S18°37’34’.9” E22°06’24.4”)

Prevalence: 3/9 = 33.3% for *B. radiatus* and 4/13 = 30.77% for *B. haasianus*

Site of infection: Gill lamellae and skin

Type material: 2013/07/24-14 for *B. radiatus* and 2013/07/17-01 for *B. haasianus* in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Morphological measurements were made from micrographs using Zeiss Zen Imaging software program from 25 specimens from each host from the Okavango River System, stained with Silver nitrate. Detailed morphological drawings were made from micrographs (Figure 4.5 E).

Table 4.3: Measurements of *Myxobolus oloi* Fomena & Bouix, 1994 from *Barbus radiatus* Peters, 1853 and *Barbus haasianus* David, 1936 from the Okavango River System and a comparison with published descriptions of the same species from Fomena & Bouix (1997). All measurements are given in micrometres.

Myxosporean	*Myxobolus oloi* (n=25)	*Myxobolus oloi* (n=25)	*Myxobolus oloi* (n=not available)
Host	*Barbus radiatus*	*Barbus haasianus*	*Barbus* spp.
Locality	Okavango System Current study	Okavango System Current study	Cameroon (Fomena & Bouix, 1994)
Spore body			
Length	9.4-12.5 (10.5±0.67)	11.4-14.6 (12.9±0.81)	6.5-11.5
Width	6.1-8.4 (7.5±0.55)	7.7-10.6 (9.4±0.74)	5-9.5
Polar capsules			
Length	4.8-5.7 (5.2±0.26)	5.6-7.3 (6.3±0.37)	4-7
Width	2.5-3.3 (2.9±0.18)	2.9-3.9 (3.3±0.28)	2-5

Remarks:

The morphology of the current spore concur to the description of *Myxobolus oloi* that was originally described by Fomena & Bouix (1994) from gills of an unknown *Barbus* species in Cameroon. The description of *M. oloi* is similar to the current specimens in having unequal lengths of the polar capsules with four to five oblique coils in the capsules, but differs by having a slightly anterior protrusion that the current specimens lack. The current specimens are also slightly larger than the original description (Table 4.3). The population of *M. oloi* found on *B. haasianus* are also slightly larger in overall length than the population that was found on *B. radiatus*.

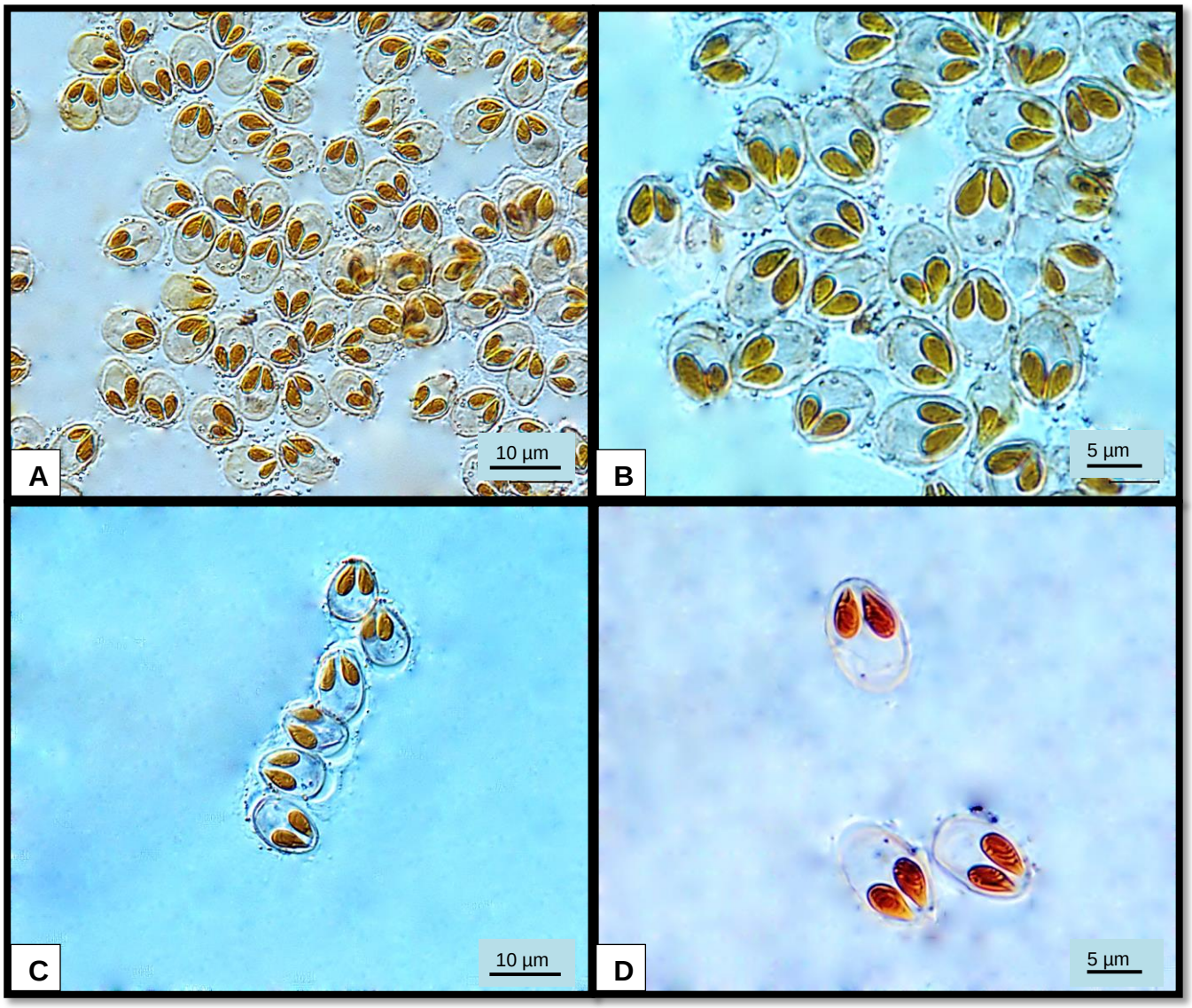


Figure 4.6: Light micrographs of silver-nitrate stained spores of *Myxobolus oloi* Fomena & Bouix, 1994 collected within secondary gill lamellae from *Barbus radiatus* Peters, 1853 (**A** & **C**) and *Barbus haasianus* David, 1936 (**B** & **D**) in the Okavango River System, Botswana.

Myxobolus oloi differs from the other species that were described from Africa from *Barbus* species in the following ways; *Myxobolus njinei*, *M. nkolyaensis*, *M. naffari*, *M. caudatus* and *M. intestinalis* are all spherical to oval in frontal view, but differ from *M. oloi* by having polar capsules of equal sizes and more polar filament coils. *Myxobolus nyongana* and *M. fahmii* differ from the current species by having pyriform to teardrop and pear-shape spores respectively. *Myxobolus etsatsaensis* spores have an elongated pyriform-shape that tapers sharply to a blunt point and *M.*

paludinosus spores are pyriform to ovoid with a blunt posterior point, both species have polar capsules with equal sizes (Fomena & Bouix 1994; Ali *et al*. 2002; Reed *et al*. 2002).

Myxobolus oloi differs from *Myxobolus* sp. 1 in spore length and width, the spores of *M. oloi* spore is half the size of that of *Myxbolus* sp. 1. They also have different spore and polar capsule morphology, *M. oloi* spores are oval shape and consists of unequal oblique polar capsule that coil four to five times where *Myxobolus* sp. 1 has a teardrop-shape spore with polar capsules that are equal in length with filaments with seven to nine coils.

This represents new hosts and a locality record for *M. oloi* that was found on the gills and skin of *B. radiatus* and *B. haasianus* in the Okavango River System, Botswana.

***Myxobolus* sp. 2** (Figures 4.7 A-B and 4.5B)

Description of vegetative stage:

Sporogonic plasmodia not observed.

Description of spores (based on 27 spores):

Spores pyriform to teardrop-shape in valvular view with anterior end tapering to blunt point, posterior end rounded. Spore elongated with length of 11.2-14 (12.3±0.86) and width 5.7-9 (7.1±0.59). Two smooth shell valves visible, two elongated pyriform polar capsules of equal length parallel with each other in anterior half of spore, 5.1-7.7 (6.5±0.65) in length and 1.9-3.2 (2.3±0.28) in width. Polar filaments coil nine to eleven times within polar capsules. Sporoplasm situated in posterior half of spore that occupies half of spore body.

Host: *Barbus paludinosus* Peters, 1852

Locality: Pongola Floodplains, Nyamiti Pan Outlet (S26°88’27’75” E32°31’13’93”)

Prevalence: 5/6 = 83.33%

Site of infection: Gill lamellae

Type material: 2013/09/10-03 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Morphological measurements were made from micrographs using Zeiss Zen Imaging software program from 27 specimens from the host from the Pongola River System, stained with silver-nitrate. Detailed morphologic drawings were made from micrographs.

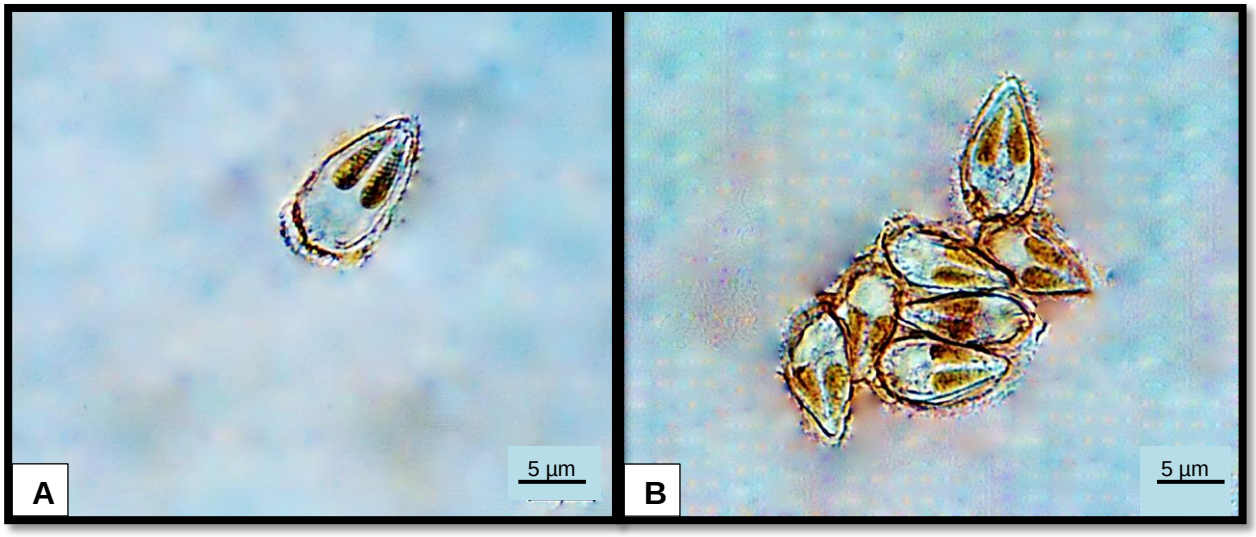


Figure 4.7: Light micrographs of silver-nitrate stained spores of *Myxobolus* sp. 2 (**A** & **B**) collected within secondary gill lamellae from the host *Barbus paludinosus* Peters, 1852 in the Pongola River System, South Africa.

Remarks:

Of the ten species of *Myxobolus* that have been described from *Barbus* hosts in Africa, *Myxobolus* sp. 2, is teardrop-shape and can easily be distinguished from *M. njinei*, *M. nkolyaensis*, *M. oloi*, *M. naffari*, *M. caudatus*, *M. intestinalis* and *M. paludinosus* where all of these species are either subspherical or oval.

Myxobolus sp. 2 closely resembles *M. nyongana*, *M. naffari* and *M. etsatsaensis* by also being teardrop or pear-shape, but differs from these species in the following way. *Myxobolus nyongana* are different from *Myxobolus* sp. 2 by being smaller in overall length and width, and by not having an elongated spore body. *Myxobolus naffari* spores are more pear-shape and the polar capsules occupy more than half of

the spore body (Ali *et al*. 2002), where *Myxobolus* sp. 2 are more teardrop-shape, with polar capsules occupy only half of the spore body.

Myxobolus etsatsaensis is very similar to *Myxobolus* sp. 2 in both overall size and polar capsule morphology, but differ in the spore body morphology. *Myxobolus etsatsaensis* are described by Reed *et al.* (2002) as having a slender spore body where the anterior end tapers sharply to a blunt point forming a narrow anterior end, where *Myxobolus* sp. 2 does not possess a narrow anterior end. They also differ as *Myxobolus* sp. 2 has parallel polar capsules of equal size, while *M. etsatsaensis* possesses unequal polar capsules.

Myxobolus sp. 2 is different from *Myxobolus* sp. 1 in the current study in overall size, despite both being teardrop-shape in spore morphology. The length and width parameters in the two species do not correspond; *Myxobolus* sp. 1 is much larger than *Myxobolus* sp. 2.

This possibly represents a new species of *Myxobolus* that was found on the gills of *B. paludinosus* in the Pongola River System, South Africa.

***Myxobolus paludinosus* Reed, Basson & van As, 2002** (Figures 4.8 A-B and 4.5 D)

Description of vegetative stage:

Sporogonic plasmodia not observed.

Description of spores (based on 24 spores):

Spores pyriform to ovoid with anterior end pointed and posterior end rounded. Shell valves smooth and sutural lines along edge of spore becoming broader posteriorly. Two polar capsules, thick, pyriform in shape, of equal sizes situated in anterior end of spore. Polar filaments have six to seven coils within polar capsules and sporoplasm situated in posterior half of spore that occupies half of spore body. Measurements of spores presented in Table 4.4.

Host: *Barbus paludinosus*

Locality: Pongola Floodplains, Nyamiti Pan Outlet (S26°88'27'75" E32°31'13'93")

Prevalence: 5/6 = 83.33%

Site of infection: Gill lamellae

Type material: 2013/09/10-03 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Morphological measurements were made from micrographs using Zeiss Zen Imaging software program from 24 specimens the host from the Pongola River System, stained with silver-nitrate. Detailed morphologic drawings were made from micrographs (Figure 4.5 D).

Table 4.4: Measurements of *Myxobolus paludinosus* Reed, Basson & van As, 2002 from *Barbus paludinosus* Peters, 1852 from the Pongola River System and a comparison with published descriptions of the same species from Reed *et al*. (2002). All measurements are given in micrometres.

Myxosporean	*Myxobolus paludinosus* (n=24)	*Myxobolus paludinosus* (n=10)
Host	*Barbus paludinosus*	*Barbus paludinosus*
Locality	Pongola System Current study	Okavango System (Reed *et al*. 2002)
Spore body		
Length	10-11.8 (10.9±0.54)	11.2-13.7 (12.0±0.87)
Width	7.9-9.5 (8.4±0.42)	7.5-10 (8.6±0.75)
Polar capsules		
Length	4.3-5.6 (5±0.3)	5-6.8 (5.7±0.88)
Width	2.2-3.2 (2.7±0.26)	2-2.5 (2.4±0.21)

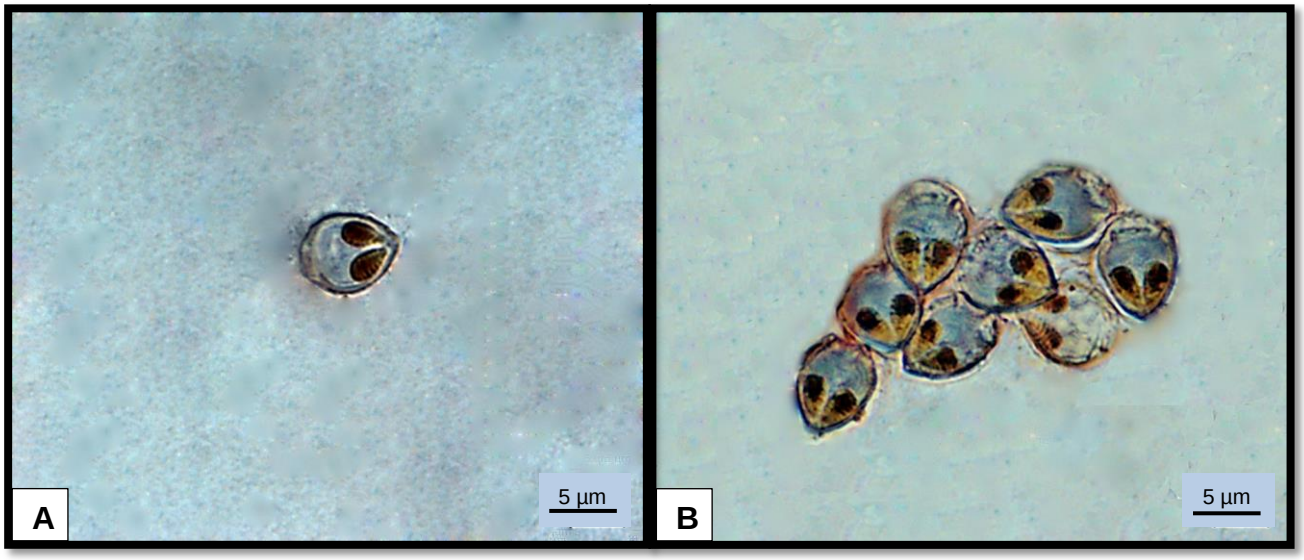


Figure 1.8: Light micrographs of silver-nitrate stained spores of *Myxobolus paludinosus* Reed, Basson & van As, 2002 (**A** & **B**) collected within secondary gill lamellae from the host *Barbus paludinosus* Peters, 1852 in the Pongola River System, South Africa.

Remarks:

The overall spore measurements and morphology of this species conforms to the description of *M. paludinosus* that was described by Reed *et al.* (2002) from the secondary gill filaments from *B. paludinosus* in the Okavango River System, Botswana. The material from the present study was also found on the same host as in the original description.

Myxobolus paludinosus are different from the other nine known species in Africa by having the following differences: *M. nyongana*, *M. naffari* and *M. etsatsaensis* are teardrop or pear-shape with elongated bodies, whilst *M. paludinosus* spores are more ovoid shape, with a blunt anterior end.

The current species, *M. paludinosus*, has similarities with *M. njinei*, *M. nkolyaensis*, *M. oloi*, *M. naffari*, *M. caudatus* and *M. intestinalis*, as all these species are either subspherical or oval, but differences are present. *Myxobolus njinei* is distinct from *M. paludinosus* by having a truncated anterior end and by being longer in spore length. *Myxobolus nkolyaenis* and *M. oloi* are almost spherical in shape with subspherical polar capsules and therefore differs from *M. paludinosus*. *Myxobolus caudatus* has larger polar capsules and a longer spore length than *M. paludinosus*.

Myxobolus intestinalis is different from *M. paludinosus* by having polar capsules that occupy two thirds of the spore body (Fomena & Bouix 1997; Ali *et al*. 2002).

Myxobolus naffari possesses characteristics that are very comparable to *M. paludinosus*, all their measurements fall into the same parameters and they have exactly the same amount of polar filament coils in the polar capsules, six to seven. The only difference that can be distinguished is that *M. naffari* does not taper down to the anterior end as much as *M. paludinosus* (Ali *et al*. 2002). There are, however, not enough morphological differences to distinguish these two species as separate species, therefore genetic differences need to be investigated. For the purpose of this study, the current species will be assigned to *M. paludinosus*, because the species were found on the same fish host as the original description by Reed *et al*. (2002).

Myxobolus paludinosus differ from the possible new species that was found during the current study by possessing a more oval spore body, where *Myxobolus* sp.1 and *Myxobolus* sp. 2 have teardrop-shape spore bodies. Both these species are also larger in overall spore lengths.

This represents a new locality record for *M. paludinosus* that was found on the gills of *B. paludinosus* in the Pongola River System, South Africa.

***Myxobolus etsatsaensis* Reed, Basson & van As, 2002** (Figures 4.9 A-B and 4.5 A)

Description of vegetative stage:

Sporogonic plasmodia not observed.

Description of spores (based on 27 spores):

In valvular view, spores pyriform teardrop-shape with posterior end rounded and anterior end tapers sharply down to narrow blunt point. Spore body length measured 10.1-12.5 (11.3±0.66) and width 4.6-6.5 (5.5±0.56) with widest region parallel to posterior end of polar capsules. Two smooth shell valves visible. Polar capsules pyriform, situated in middle of spore, occupying two thirds of spore body. Polar

capsules of equal sizes 4.9-7 (6.1±0.56) x 1.8-2.4 (2±0.18) are set apart from each other parallel to one another, but some specimens possessed polar capsules of unequal sizes within same population. Polar filaments in polar capsules coil seven to nine times. Sporoplasm occupies one third of the spore body.

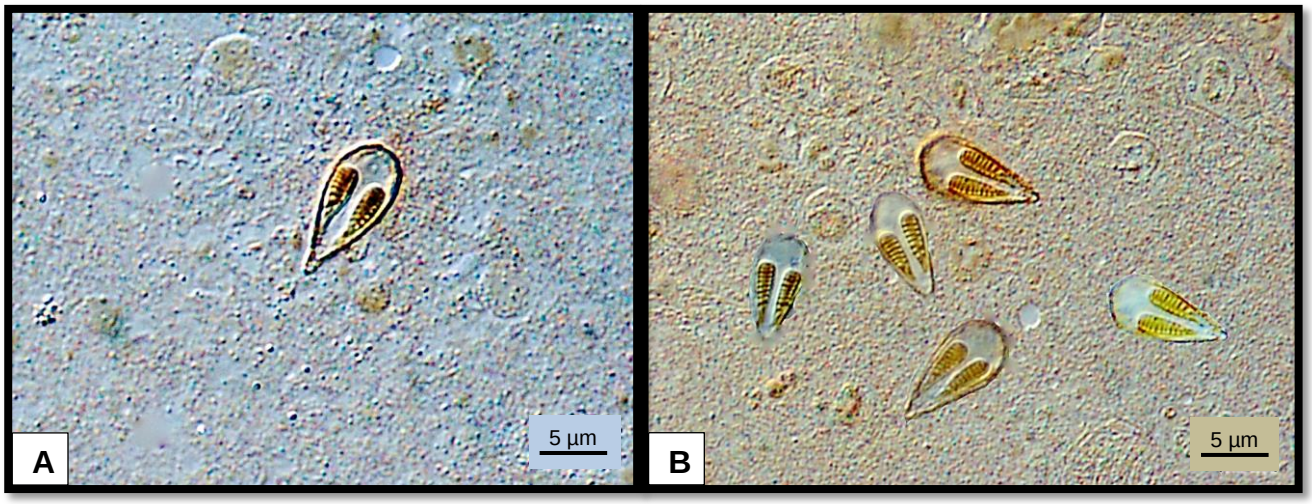


Figure 4.9: Light micrographs of silver-nitrate stained spores of *Myxobolus etsatsaensis* Reed, Basson & van As, 2002 (**A** & **B**) collected within secondary gill lamellae from the host *Barbus afrohamiltoni* Crass, 1960 in the Pongola River System, South Africa.

Host: *Barbus afrohamiltoni* Crass, 1960

Locality: Pongola Floodplains, Nyamiti Pan Outlet (S26°88'27'75" E32°31'13'93")

Additional localities: Pongola Floodplains, Pomphuis (S26°90'52'83" E32°32'35'00")

Prevalence: 7/19 = 36.84%

Site of infection: Gill lamellae

Type material: 2013/09/11-08 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Morphological measurements were made from micrographs using Zeiss Zen Imaging software program from 27 specimens from the host from the Pongola River System, stained with silver-nitrate. Detailed morphologic drawings were made from micrographs (Figure 4.5 A).

Remarks:

The morphology of these spores conform to the description of *M. etsatsaensis*, which was originally described by Reed *et al*. (2002) from the secondary gill filaments of *Barbus thamalakanensis* Flower, 1935 in the Okavango River System, Botswana. The spores of the *M. etsatsaensis* population in the present study are slightly smaller than the spores described for the original population, but are similar in spore morphology and both populations have a distinct narrow blunt anterior point.

Myxobolus njinei, *M. nkolyaensis*, *M. oloi*, *M. naffari*, *M. caudatus*, *M. intestinalis* and *M. paludinosus* that were also found on *Barbus* hosts are either subspherical or oval and therefore are different in spore morphology to the more elongated pyriform spore body of *M. etsatsaensis*. *Myxobolus nyongana* and *M. fahmii* closely resemble the current species by also being pyriform-shape. *Myxobolus nyongana* is, however, different in having a much less extended spore body. Compared to *M. fahmii*, *M. etsatsaensis* has a more slender spore body and differs also in the spore width.

This represents a new host and locality record for *M. etsatsaensis* that was found on the gills of *B. afrohamiltoni* in the Pongola River System, South Africa.

***Myxobolus heterosporus* Baker, 1963 type 2** (Figures 4.10 A-B and 4.5 F)

Description of vegetative stage:

Sporogonic plasmodia not observed.

Description of spores (based on 25 spores):

In valvular view, spore body oblong to oval with both anterior and posterior ends rounded, spore length 11.4-13.4 (12.5±0.63) and width 7.1-9.3 (8.1±0.53). Two smooth shell valves visible. Two oval, almost rounded, small pyriform polar capsules situated in anterior region of spore body. Polar capsules occupy one third of spore body parallel to each other and of equal length 3.3-4.5 (3.9±0.32) and width 1.9-3 (2.4±0.22). Number of polar filament coils within polar capsules difficult to observe. Sporoplasm occupies two thirds of spore body.

Host: *Barbus afrohamiltoni* Crass, 1960

Locality: Pongola Floodplains, Nyamiti Pan Outlet (S26°88’27’75” E32°31’13’93”)

Additional localities: Pongola Floodplains, Pomphuis (S26°90’52’83” E32°32’35’00”)

Prevalence: 5/19 = 26.32%

Site of infection: Skin

Type material: 2013/09/11-14 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Morphological measurements were made from micrographs using Zeiss Zen Imaging software program from 25 specimens from each host from the Okavango River System, stained with silver-nitrate. Detailed morphologic drawings were made from micrographs (Figure 4.5 F).

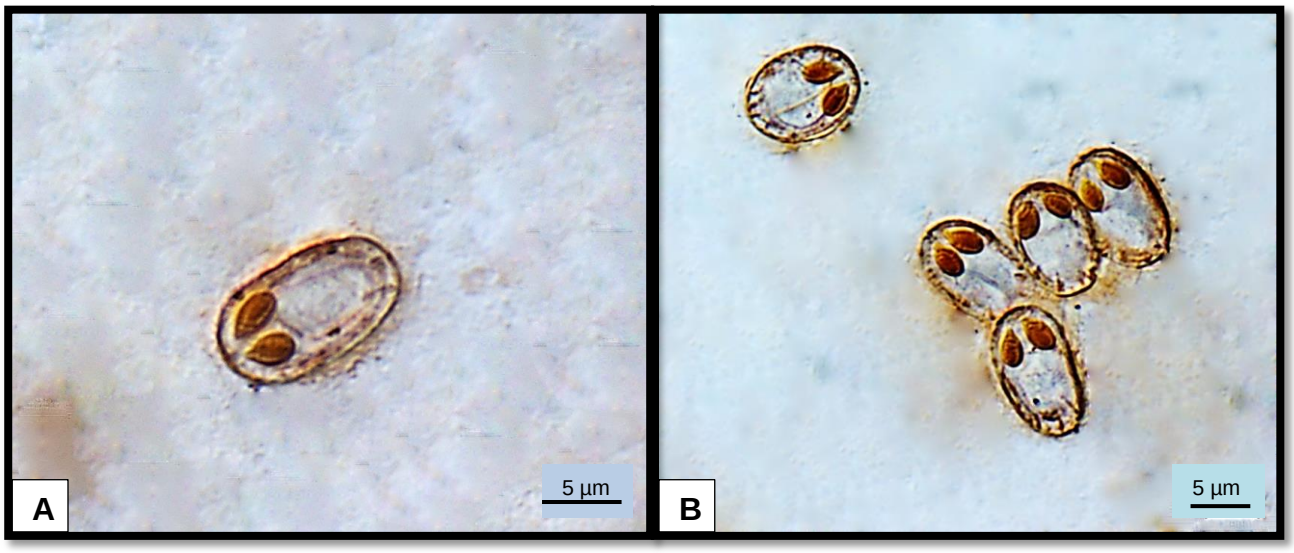


Figure 4.10: Light micrographs of silver-nitrate stained spores of *Myxobolus heterosporus* Baker, 1963 (**A** & **B**) collected the skin from the host *Barbus afrohamiltoni* Crass, 1960 in the Pongola River System, South Africa.

Remarks:

The current species was identified as *Myxobolus heterosporus* that was previously described from *Oreochromis esculentus* Graham, 1928, *Oreochromis variabilis* Boulenger, 1906, *Oreochromis niloticus*, Linnaeus 1758 and *Haplochromis* Hilgendorf, 1888 species in east Africa (El-Mansy 2005). According to El-Mansy (2005), three different spore types can be distinguished in *M. heterosporus*, with considerable differences between them. The current species was identified as *M. heterosporus* type 2 due to the similarity in spore shape and the current species corresponds to the measurements of the original description (Fomena & Bouix 1997). According to Lom & Arthur (1989), finding myxosporea in a different host species, site of infection or new geographical locality is no justification for creating a new species.

As previously mentioned, *M. heterosporus* has an oblong spore body with rounded ends at both anterior and posterior ends. No other *Myxobolus* species from *Barbus* hosts in Africa have been described with a similar morphology.

The ten known *Myxobolus* species of *Barbus* hosts differ in the following ways: *Myxobolus heterosporus* spores are different from those of *M. etsatsaensis*, *M. nyongana* and *M. fahmii*, because they are pyriform-shape and taper to a blunt anterior end, where the current species has a rounded anterior end. *Myxobolus njinei*, *M. oloi*, *M. naffari, M. paludinosus* and *M. intestinalis* are distinct from *M. heterosporus* in having anterior ends that are slightly narrower than the posterior end and by having blunt pointed anterior ends.

Myxobolus nkolyaensis and *M. caudatus* are subspherical and oval, respectively, and are distinct from *M. heterosporus* that possesses an oblong body shape that is longer than wide. *Myxobolus heterosporus* also possesses smaller polar capsules compared to its spore body and larger overall spore size to the previously mentioned species. *Myxobolus* sp. 1 and *Myxobolus* sp. 2 from the current study differs from *M. heterosporus* by being both pyriform-shape with anterior ends tapering to blunt points.

Another species that was found infecting African fish species that is morphologically similar to *M. heterosporus* is *Myxobolus tilapiae* Abolarin, 1974. They are similar by

having the same oblong spore shape, but differ in the shape of the polar capsules. *Myxobolus heterosporus* has more pyriform-shape polar capsules whereas *M. tilapiae* possesses almost spherical polar capsules (Reed *et al*. 2002).

This represents a new host and locality record for *M. heterosporus* that was found on the skin of *B. afrohamiltoni* in the Pongola River System, South Africa.

DISCUSSION

According to Lom & Arthur (1989), many species of Myxosporea do not display strict specificity with regards to host species or even higher host taxa or site of infection within the host. While certain species have very strict specificity and can colonise only one species, most of the species of Myxosporea share several closely related fish species as hosts (Molnár 1994). Many species occur naturally over a large geographical area and others have been introduced to new continents along with their fish hosts through human activities (Lom & Arthur 1989).

The current study clearly supports the statement that myxosporeans share several closely related fish species as hosts. Three of the seven species found, i.e. *M. nyongana*, *M. oloi* and *M. etsatsaensis*, were previously found infecting different fish species than in the current study, but all the fish hosts belong to the genus *Barbus*. Therefore these myxosporean species infect different fish species that are phylogenetically closely related.

On the other hand, *M. heterosporus* was found infecting *B. afrohamiltoni*, but was previously only found on different species from the family Cichlidae. *Myxobolus heterosporus* shows a variety of spore shapes that express remarkable heteromorphism (El-Mansy 2005) and therefore it is believed that this parasite can adapt to parasitise different fish species from different fish families. The results of the current study then give supportive evidence to the fact that *M. heterosporus* does not display specificity with regards to different fish families.

Myxobolus paludinosus was previously found infecting *B. paludinosus* in the Okavango River System (Reed *et al*. 2002) and the same species was found on the same fish host in the Pongola River System in the current study. The results of this

study could indicate that *M. paludinosus* is species specific to *B. paludinosus* with a wide geographical distribution.

Two species were found during the current study that do not conform to any species known in Africa, i.e. *Myxobolus* sp. 1 and *Myxobolus* sp. 2. These two species could be new species from Africa based on the morphology of the spores.

There are an extraordinarily large number of myxosporean species that have been described and most of the taxonomical classification is based on the morphology of their spores (Molnár 1994). There are however, only slight structural differences between myxosporean spores; therefor descriptions from only the spore morphology do not provide enough variance to describe species. To describe a new species of myxosporean, morphology should be used hand to hand with molecular studies to give the most accurate description of the species. Myxosporean classification is in the transition from spore-based morphology to a new arrangement where molecular characters should also be taken into account (Lom & Dyková 2006).

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Chapter 5

Monogenea

INTRODUCTION

Monogenean infections generally have negligible impacts on natural host populations (Cusack 1986), however the study of monogeneans is still important for various reasons. Firstly, knowledge on monogenean taxonomy and biology is essential in aquaculture and fisheries management for potential aquaculture species. Monogenean parasite infections may cause mortalities and retarded growth in aquaculture and aquarium conditions (Crafford *et al.* 2014). In terms of the genus *Barbus* Cuvier & Cloquet, 1816 the monogenean knowledge is widely recognised for the ornamental fish trade and for their effects on biological diversity (Crafford *et al.* 2012). The current study was an investigation into the parasite fauna of species of the cyprinid fish genus *Barbus* in southern Africa.

The monogenea fauna of southern Africa has been studied from as early as 1969 through the work of Price *et al.* (1969), but is still far from complete. A recent increase in interest in monogeneans of southern Africa can be shown through a number of recent publications (Christison & Baker 2007; Le Roux & Avenant-Oldewage 2010; Crafford *et al*. 2012, 2014; Dos Santos *et al*. 2013), including one publication on monogeneans of the genus *Barbus* by Mbokane *et al.* (2015). Very few monogeneans have been described from this fish genus in southern Africa, but more recent descriptions were done by Matla (2012).

The majority of monogeneans described from *Barbus* belong to the genus *Dactylogyrus* Diesing, 1850, which is the largest genus of monogeneans, with more than 900 species described. Ninety five precent of all the *Dactylogyrus* species were described from cyprinid fish, but only 11,5 % are known from African fish (Gibson *et al.* 1996). More than sixty species have been described from the genus *Barbus* in Africa with most deriving from western Africa through the work of Guégan & Lambert (1990) and east Africa by Paperna (1979), but only six species have been described from southern African freshwater systems (Gibson *et al.* 1996; Matla 2012).

While most of the barb monogeneans belong to the genus *Dactylogyrus*, it is also important to know that other monogeneans also parasitised these fish, for example, *Afrodiplozoon polycotyleus* Paperna, 1973 was found on *Barbus trimaculatus* Peters, 1852 (Mashego 2000), and *Gyrodactylus* von Nordmann, 1832 sp. and *Dogielius* sp.

Bychowski, 1957 on *Barbus radiatus* Peters, 1853 (Mbokane 2011) in Limpopo, South Africa.

The first species of *Dactylogyrus* that was described from barbs in southern Africa is *Dactylogyrus myersi* Price, McClellan, Druckenmiller & Jacobs 1969 from *B. trimaculatus* in Pongola (Price *et al.* 1969). Mashego (1983) further described three species from the Limpopo Province, i.e. *Dactylogyrus teresae* Mashego, 1983, *Dactylogyrus enidae* Mashego, 1983 and *Dactylogyrus dominici* Mashego, 1983 from different *Barbus* hosts. Two species have been recorded through the work of Christison (2002), i.e. *Dactylogyrus barrilus* and *Dactylogyrus viviersii* from *B. radiatus* and *B. multilineatus* Worthington, 1933, respectively, from the Okavango River System, they were found during his PhD study and will only be accepted as new species once published in an accredited systematic journal. Two species of the genus *Dactylogyrus* were also described by Malta (2012) from *B. trimaculatus* and *B. radiatus* in his PhD study.

Price *et al.* (1969) described *Dactylogyrus varicorini* Price, McClellan, Druckenmiller & Jacobs 1969 from *Labeobarbus kimberleyensis* (Gilchrist & Thompson, 1913) previously known as *Barbus kimberleyensis*, but the genus was changed and therefore this monogenean is no longer from a *Barbus* host, although it is still a cyprinid. Although only a few species have been described from southern African freshwaters, previously described species have been found associated with southern African barbs. Mashego (1982) found four species in the Limpopo System, South Africa, that were also previously described by Paperna (1979) from Uganda on different *Barbus* hosts. These species are: *D. allolongionchus* Paperna, 1979 plus two subspecies *D. afrolongicornis afrolongicornis* Paperna, 1979 and *D. afrolongicornis alberti* Paperna, 1979 all from *B. trimaculatus* and *D. afrosclerovaginus* Paperna, 1979 from *B. paludinosus* Peters, 1852. *Dactylogyrus spinicirrus* Paperna & Thurston, 1968 that was originally described in Kenya were also recorded from *B. trimaculatus* by Mbokane *et al.* (2015) in the Limpopo System. These five species also form part of the *Barbus* monogeneans of southern Africa.

The objective of the present study was to contribute towards the knowledge of monogenean parasite fauna of the genus *Barbus* that occur in southern Africa through describing, identifying and comparing monogeneans.

MATERIALS AND METHODS

Fish collections

See chapter 3.

Host examination, fixation and preservation of parasites

The surface of the fish was carefully examined for external parasites by making a skin smear. The slides were examined for parasites using a compound microscope (Nikon Eclipse 50i). If monogeneans were found, they were rinsed off with 70 % EtOH in McCartney Bottles and given a specimen number and documented. The gills of the fish were removed and live observations were made using a compound or a dissection microscope (Nikon SMZ800), depending on the size of the gills. The monogeneans were removed from the gills and fixed in 70 % EtOH or, alternatively, the gills with monogeneans attached were fixed in 10 % neutral buffered formalin and documented. The fixed specimens were re-examined in Bloemfontein where individual parasites were identified.

Light microscopy preparations

The fixed monogeneans were transferred to either glycerine ammonium picrate solution (GAP) or glycerine jelly and mounted to study the sclerotised organs. The glycerine ammonium picrate solution and glycerine jelly were prepared according to methods by Malmberg (1970).

The monogeneans that were transferred in GAP were removed from the fixed gills and placed in a drop of water in the middle of a microscope slide. Excess water was carefully absorbed using tissue paper to achieve the desired level of compression of the specimen. The specimens were mounted between the slide and a cover slip, by adding a drop of GAP to the edge of the cover slip. The mounting medium quickly mixed with the water under the cover slip, a day or two was allowed for the water to

evaporate so that the specimen was totally embedded in GAP, where after the cover slip was sealed with clear nail varnish. This method was adapted by Dr Kevin Christison[2] from Malmberg (1970).

The material that was mounted in glycerine jelly was fixed in 70 % EtOH. As much of the excess alcohol was pipetted off and glycerine alcohol was added. The alcohol was allowed to evaporate and a small amount of glycerine jelly was placed on a slide and then transferred to a slide dryer until the jelly melted. The monogeneans were mounted in the glycerine jelly and covered with a cover slip. The slides were allowed to dry for a few hours and sealed with clear nail varnish.

GLYCERINE AMMONIUM PICRATE

Neutral Buffered Formalin 10%	1 part
Glycerine	9 parts

Mix formalin and glycerine. Add 1 drop of picric acid for every 10 ml solution.

GLYCERINE JELLY

Gelatin	10 g
Distilled Water	60 ml
Glycerine	70 ml
Phenol (C_6H_5OH)	0.25 g

Morphological measurements

Measurements of the sclerotised hard parts were done according to the recommendations made by Gusev (1962). The sclerotised hard parts include the opisthaptoral anchors, dorsal bar, marginal hooklets, male copulatory organ (MCO) and the accessory piece. The opisthaptoral anchors were measured using six basic measurements, i.e. total length (A), anchor shaft length (B), length of outer root (C), length of inner root (D), anchor point length (E) and anchor aperture (F) (Figure 5.1 I). The length (G) and width (H) of the dorsal bar (Figure 5.1 II) and the total length (I) of all seven marginal hooklets (Figure 5.1 III) were measured. The MCO measurements include the total length of the copulatory organ (J) and the tube trace

[2] Dr Kevin Christison from the Department of Agriculture, Forestry and fisheries, Cape Town, South Africa

length of the male copulatory organ (K) (Figure 5.1 IV). The total body length and the body width at the level of the MCO were measured.

All the sclerotised parts were drawn with the aid of a drawing tube and a Leitz Wetzlar Laborlux D compound microscope. All the measurements were made directly from the drawings with a Super Inoxvdable Vernier calliper and the MCO tube trace length was measured by using a string. All the measurements were in micrometres (µm), tabulated and used for species descriptions and comparisons.

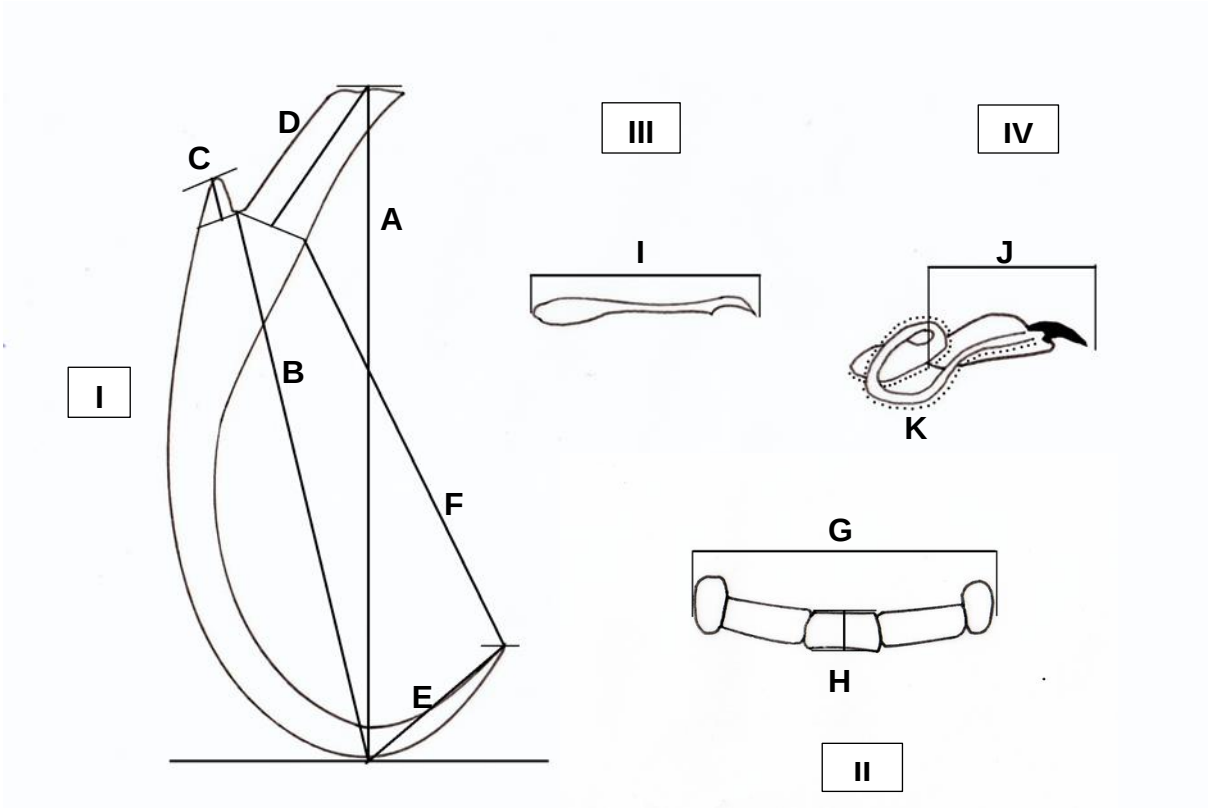

Figure 5.1: Illustration of the measurements of the sclerotised structures of *Dactylogyrus* Diesing, 1850 spp.: **I**, Anchor; **II**, Dorsal bar; **III**, Marginal hooklets; **IV**, Male copulatory organ. **A** - Anchor total length; **B** – anchor shaft length; **C** – length of outer root; **D** – length of inner root; **E** – length of tip/point; **F** – anchor aperture; **G** – length of dorsal bar; **H** – width of dorsal bar; **I** – marginal hooklet total length; **J** – accessory piece length; **K** – male copulatory organ trace length. Adapted from Crafford *et al.* (2012).

Data analysis

The raw data was analysed to determine the distribution and the prevalence of the monogeneans on the fish host at the various study sites. The mean, standard deviation and range (minimum and maximum values) were calculated for all measurement data from all species for descriptions.

RESULTS

This part of the chapter comprises the taxonomical diagnosis of *Dactylogyrus* species collected from fishes of the genus *Barbus* from the Okavango, Pongola and Orange-Vaal river systems.

Based on the sclerotised parts of the monogeneans found during the current study, four species of the *D. afrobarbae* group (Paperna 1979) have been found. Of these, three are potentially new species and one species is known.

According to Paperna (1979), the *D. afrobarbae* group are characterised by anchors with long inner roots and short vestigial outer roots; singular dorsal bar, often long and subdivided into two halves by a median constriction. The MCO is tubular and may be coiled once or twice. The distal portion of the penis is embedded by the accessory piece which terminates in a fixed or movable hook. The vagina is often sclerotised and the vaginal plug can occasionally be spiny or dentated, in some species it may be absent.

Class: Monogenea (Van Beneden, 1858)

Subclass: Polyonchoinea Bychowsky, 1937

Order: Dactylogyridea Bychowsky, 1937

Family: Dactylogyridae Bychowsky, 1933

Subfamily: Dactylogyrinae (Boeger & Kritsky, 1987)

Genus: *Dactylogyrus* Diesing, 1850

***Dactylogyrus dominici* Mashego, 1983** (Figures 5.2 A-D, 5.6 A-B)

Host: *Barbus paludinosus* Peters, 1852

Locality: Pongola Floodplains, Ingwavuma River (S27°03’35’95” E32°18’26’54”)

Additional localitys: Orange-Vaal River System, Fish River (S24°49’70’21” E17°86’98’68”), Pongola Floodplains, Nyamiti Pan Outlet (S26°88’27’75” E32°31’13’93”)

Site of infection: Gill lamellae

Reference material: 2013/09/10-02 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

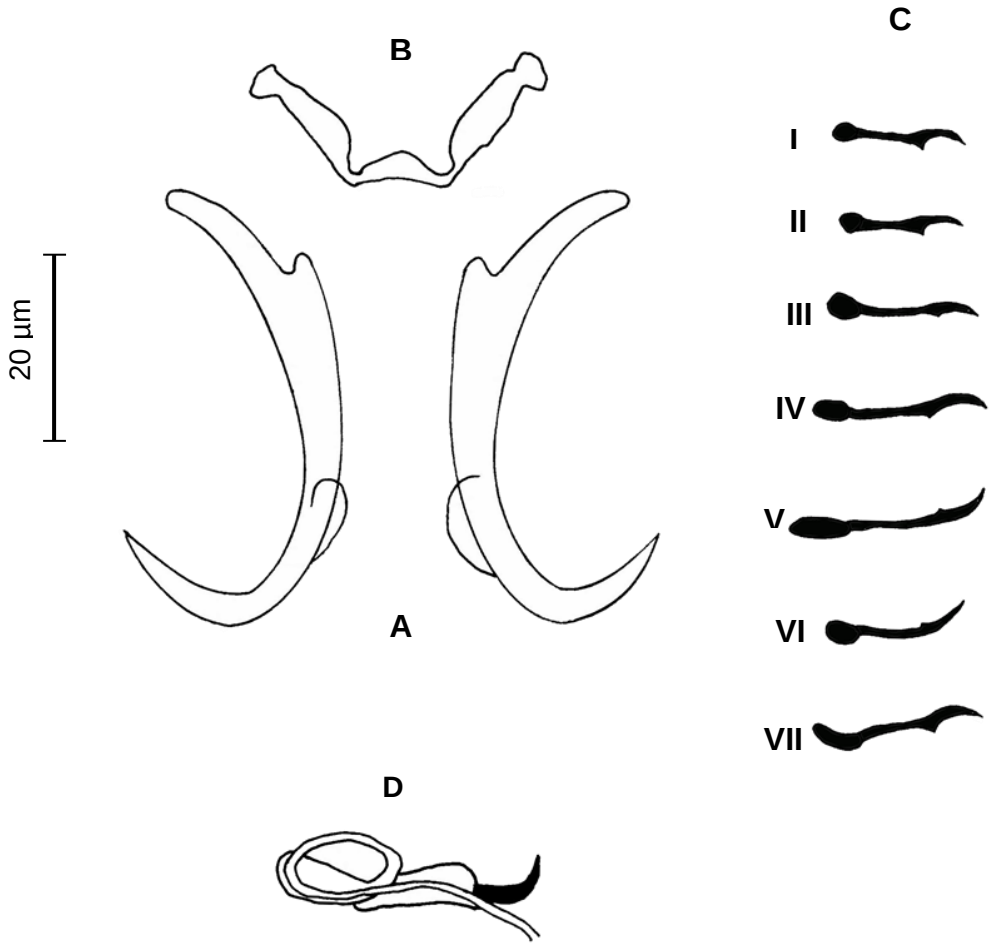

Figure 5.2: Microscope projection drawings of the sclerotinoid organs of *Dactylogyrus dominici* Mashego, 1983 from the gills of *Barbus paludinosus*, Peters, 1852 collected from the Pongola River System. **A** – anchors, **B** – Dorsal bar, **C** – Marginal hooklets (**I** to **VII**) and **D** – Male copulatory organ.

Material examined: Detailed morphometric measurements and drawings were made using light microscopy from 19 specimens from the Pongola and Orange-Vaal river systems, mounted in glycerine ammonium picrate.

Description and measurements:

Body length 249.3 ± 38.5 (181.2 – 317.4) width 61.3 ± 10.7 (42.2 – 83.9), perpendicular to male copulatory organ. Anchor total length 46 ± 5.1 (35.5 – 52.5), anchor shaft length 32.9 ± 6.3 (23.7- 41.4), anchor outer root length 3.6 ± 0.6 (2.3 - 4.7), anchor inner root length 18.7 ± 1.9 (15.5 - 21.3), anchor point length 15.3 ± 1.8 (11.4 - 17.6), anchor aperture 24.2 ± 5.8 (15.8 - 38.6). Dorsal bar length 36.6 ± 4.6 (29.3 - 46.8), dorsal bar width 2.9 ± 0.5 (2 - 3.8). Seven pairs of marginal hooklets; I = 13.5 ± 1.9 (10.3 - 16.9), II = 15.5 ± 2 (11.3 - 18), III = 16.1 ± 3 (10.8 - 22.7), IV = 16.8 ± 3.2 (10.9 – 22.1), V = 17.3 ±3 (10.8 – 23.2), VI = 17.6 ± 3.1 (9.9 - 22.9), VII = 18.9 ± 2.2 (14.4 - 22.8). Male copulatory organ 53.7 ± 5.7 (40 - 66.2), accessory piece 18.3 ± 2.9 (12.2 - 24.5). Ratio between handle and hook is 1/3.

Remarks:

Dactylogyrus dominici was originally described from *B. paludinosus* by Mashego (1983) from Limpopo Province, South Africa. *Dactylogyrus dominici* was found in the Orange-Vaal and Pongola river systems in the current study. The two populations were compared to material from the Limpopo system (Mashego 1983) and from the Okavango River System, Botswana (Christison 2002). These four populations are considered to be the same species based on the unique morphology of the dorsal bar, the morphology and size of the MCO and the size of the marginal hooklets (Table 5.1). All four populations were found on the same host, i.e. *B. paludinosus*.

The Limpopo River population appears to be larger than the Okavango, Orange-Vaal and Pongola populations in the size of total body lengths and anchors. The marginal hooklets also appear to be larger, although only the average length of the hooklets was given in Mashego's work (Mashego 1983). The dorsal bar length of the Okavango population is remarkably shorter in comparison to the other three populations.

Table 5.1: Measurements of *Dactylogyrus dominici* Mashego, 1983 from *Barbus paludinosus* Peters, 1852 from four river systems and a comparison with published descriptions of the

same species. *Dactylogyrus afropsilovaginus* Paperna, 1979 is also used for comparison. All measurements are given in micrometres.

Monogenean	*Dactylogyrus dominici* (n=9)	*Dactylogyrus dominici* (n=10)	*Dactylogyrus dominici* (n=20)	*Dactylogyrus dominici* (n=5)	*Dactylogyrus afropsilovaginus* (n=5)
Host	*Barbus paludinosus*	*Barbus paludinosus*	*Barbus paludinosus*	*Barbus paludinosus*	*Barbus paludinosus*
Locality	Orange-Vaal System Current study	Pongola System Current study	Limpopo System (Mashego 1983)	Okavango System (Christison 2002)	Uganda (Paperna 1979)
Body					
Total length	215.2-317.4	181.2-289.3	218-419	134.2-290.2	210-260
Width	51.8-83.9	42.2-77.6	31-75	43-122.4	70-100
Anchor					
Total length	48.6-52.5	35.5-49.4	58-80	37.1-44.3	31-40
Shaft length	36.1-38.8	23.7-41.4	40-54	21.1-35.3	17-24
Outer root length	2.9-4.7	2.3-4.3	3-5	2-5	2-4
Inner root length	15.5-21.3	15.8-19.9	16-23	15.6-22.3	16-18
Point length	11.8-17.6	11.4-16.4	15-19	10.4-16.6	9-12
Aperture	24.9-28.8	15.8-38.6	-	-	-
Dorsal bar					
Length	32.2-45.1	29.3-46.8	43-58	18.8-28.4	44-56
Width	2.7-3.8	2-3.5	4-5	3.7-4.7	-
Marginal hooklets					
I	11.6-16.9	10.3-14.8	19-25	9.2-15.3	19-25
II	11.3-18	11.7-17.5	-	10.5-17.5	
III	10.8-21.6	12-22.7	-	14.5-19.8	
IV	15.6-22.1	10.9-20.4	-	16.1-20	
V	14.2-23.2	10.8-21.9	-	17.7-21.5	
VI	15-22.5	9.9-22.9	-	14-18.6	
VII	18.6-22.8	14.4-22.5	-	12.2-18.1	
Male copulatory organ					
Male copulatory organ	40-58.8	47.4-66.2	25-45	47.9-50	20-24
Accessory piece	12.2-19.9	16-24.5	15-19	22.9-26.7	15

The present material resembles *D. afropsilovaginus* that was described and found on *B. paludinosus* in Uganda, with the only difference the presence of a sclerotised vagina (Paperna 1979). Morphometrics are presented in Table 5.1. The sclerotised vagina is not always present in *Dactylogyrus* monogeneans and Paperna (1979) described the species from only five specimens. The sclerotised vagina of the specimens from Paperna (1979) could not have developed yet, this means that *D. afropsilovaginus* and *D. dominici* could be the same species. Both these species were also described from the same host. The possible discrepancy in description illustrates the need for the redescription of the type material of *D. afropsilovaginus*.

***Dactylogyrus* sp. 1** (Figures 5.3 A-D, 5.6 C-D)

Type host: *Barbus fasciolatus* Günther, 1868

Additional host: *Barbus afrovernayi* Nichols & Boulton, 1927

Type locality: Okavango River System, Shakawe (S18°42'93'85" E21°89'64'98")

Site of infection: Gill lamellae

Type material: 2013/08/01-02 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements and drawings were made by using light microscopy from 12 specimens from the Okavango River System, mounted in GAP. Not all the sclerotised parts were visible in all of the Okavango specimens mounted; the total number per specimen measured per sclerotised part is indicated in the description.

Description and measurements:

Body length 257.8 ± 23.9 (217.4 – 290, n=12), width 66.2 ± 14.1 (51.3 - 97.3, n=12) perpendicular to male copulatory organ. Anchor total length 38.6 ± 1.2 (36.1 – 40, n=12), anchor shaft length 30.8 ± 1.7 (27.5 - 33.7, n=10), anchor outer root length 3.3 ± 0.7 (1.7 - 4.1, n=9), anchor inner root length 13 ± 1.9 (9.9 - 15.2, n=9), anchor point length 7.5 ± 0.9 (6.3 - 9.6, n=9), anchor aperture 24.5 ± 1.9 (22.4 - 28.3, n=9). Dorsal bar length 22.7 ± 2.5 (17.9 - 26.5, n=12), dorsal bar width 1.2 ± 0.2 (0.9 - 1.7, n=12). Seven pairs of marginal hooklets; I = 9.6 ± 2.2 (6.6 - 14.4, n=12), II = 10 ± 2.3 (6.9 - 15.5, n=12), III = 13.3 ± 2.7 (7.9 - 16.5, n=12), IV = 14.4 ± 2.1 (10.5 - 17.8, n=12), V = 15 ± 2.7 (10.3 - 19.3, n=11), VI = 16.8 ± 2.2 (13.9 - 20.8, n=11), VII = 18.5 ± 1.6 (15.5 - 21.2, n=12). Male copulatory organ 33.2 ± 5 (22.8 - 38.8, n=11), accessory piece 13.2 ± 3.9 (6.7 - 17.9, n=11). Ratio between handle and hook is 1/4.

Table 5.2: Measurements of *Dactylogyrus* Diesing, 1850 sp. 1 from *Barbus fasciolatus* Günther, 1868 and *B. afrovernayi* Nicholas & Boulton, 1927 from the Okavango River System and a comparison with the published descriptions of the similar *D. afrosclerovaginus* Paperna, 1973. All measurements are given in micrometres.

Monogenean	*Dactylogyrus* sp. 1 (n=12)		*Dactylogyrus* sp. 1 (n=5)		*Dactylogyrus afrosclerovaginus* (n=3)
Host	*Barbus fasciolatus*		*Barbus afrovernayi*		*Barbus neglectus*
Locality	Okavango System Current study		Okavango System Current study		Lake George, Uganda (Paperna 1979)
	Mean ± SD	Range	Mean	Range	Range
Body					
Length	257.8±23.9	217.4-290	215.4	201.5-229.4	130-400
Width	66.2±14.1	51.3-97.3	48.3	42.3-58.7	45-100
Anchor					
Length	38.6±1.2	36.1-40	29.7	26.6-33	36-41
Shaft	30.8±1.7	27.5-33.7	21.5	19.9-23.4	27-32
Tip	7.5±0.9	6.3-9.6	9.3	7.6-10.9	7-12
Dorsal bar					
Length	22.7±2.5	17.9-26.5	27.5	24-29.3	38-40
Width	1.2±0.2	0.9-1.7	1.2	1.1-2	2-4
Male copulatory organ					
Male copulatory organ	33.2±5	22.8-38.8	33.3	28-42.3	22-30
Accessory piece	13.2±3.9	6.7-17.9	11.6	7-17.3	16-23

Remarks:

These are small to medium size worms with anchors that have relatively short inner roots. The bar is subdivided into heavy plates by a median constriction. Hooklets are small, and small MCO are present (Figure 5.3).

Differential diagnosis:

The present material resembles *D. afrosclerovaginus*, described by Paperna (1979) from *Barbus neglectus* Boulenger, 1903 in Uganda from Lake George. Both species have the same dorsal bar pattern, but *Dactylogyrus* sp. 1 differs from *D. afrosclerovaginus* in the presence of heavy thick plates and in the length and width of the dorsal bar. The dorsal bar of *Dactylogyrus* sp. 1 is shorter and thinner than *D. afrosclerovaginus*. In addition, the accessory piece is smaller and the body length is shorter (Paperna 1979). The populations found on *B. fasciolatus* and *B. afrovernayi* appear to be very similar in size (Table 5.2). *Dactylogyrus* sp. 1 differs from *D. dominici* in the pattern and shape of the dorsal bar.

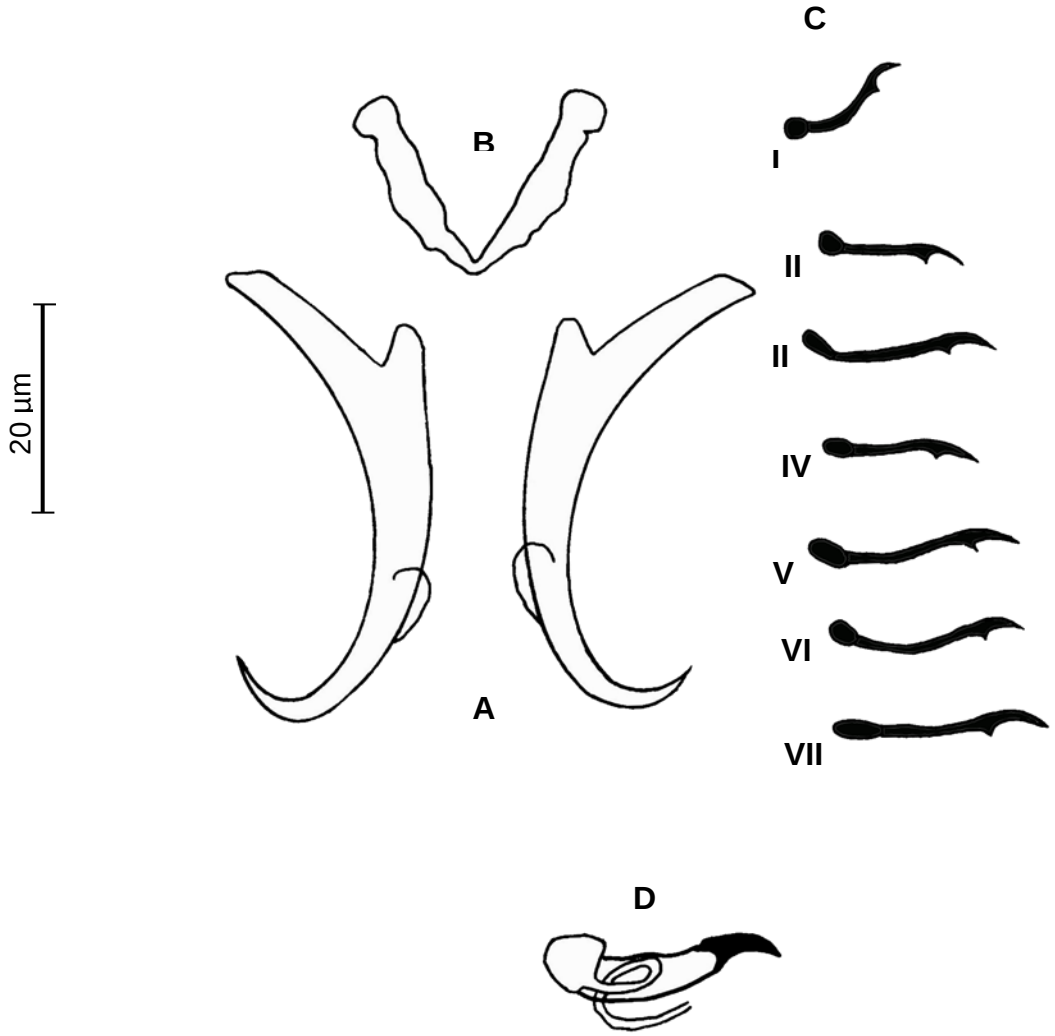

Figure 5.3: Microscope projection drawings of the sclerotinoid organs of *Dactylogyrus* Diesing, 1850 sp. 1 from the gills of *Barbus fasciolatus* Günther, 1868, collected from the Okavango River System. **A** – anchors, **B** – Dorsal bar, **C** – Marginal hooklets (**I** to **VII**) and **D** – Male copulatory organ.

***Dactylogyrus* sp. 2** (Figures 5.4 A-D, 5.6 E)

Type host: *Barbus paludinosus* Peters, 1852

Type locality: Pongola Floodplains, Ingwavuma River (S27°03'35'95" E32°18'26'54")

Additional locality: Pongola Floodplains, Nyamiti Pan Outlet (S26°88'27'75" E32°31'13'93")

Site of infection: Gill lamellae

Type material: 2013/09/10-02 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements and drawings were made by using light microscopy from nine specimens from the Pongola River System, mounted in glycerine ammonium picrate.

Description and measurements:

Body length 189.2 ± 43.2 (115.8 – 253.8), width 55.3 ± 7 (43.9 - 62.2) perpendicular to male copulatory organ. Anchor total length 73.8 ± 3.3 (68.6 - 78.6), anchor shaft length 62.3 ± 3.3 (57.3 - 66.8), anchor outer root length 5.6 ± 0.8 (3.9 - 6.5), anchor inner root length 20.7 ± 1.6 (17.9 - 22.3), anchor point length 19.5 ± 1.4 (18 - 21.3), anchor aperture 49.3 ± 4.1 (42.5 - 55.1). Dorsal bar length 37.2 ± 6.3 (29.2 - 48.7), dorsal bar width 4.5 ± 0.9 (3.2 - 5.8). Seven pairs of marginal hooklets; I = 14.1 ± 2 (9.2 - 16.3), II = 15.7 ± 3.6 (10.6 - 19.8), III = 15.7 ± 2.3 (12.5 - 20.1), IV = 21.2 ± 2.9 (14.4 - 24), V = 21.4 ± 2.4 (16.4 - 24.7), VI = 17 ± 3.7 (9.6 - 23.5), VII = 17.6 ± 2.6 (12.9 - 20.7). Male copulatory organ 43.6 ± 3.6 (38.8 - 48.5), accessory piece 16.8 ± 2.2 (13.5 - 19.8). Ratio between handle and hook is one.

Table 5.3: Measurements of *Dactylogyrus* Diesing, 1850 sp. 2 from *Barbus paludinosus* Peters, 1852 from the Pongola River System and comparison with published descriptions of similar *D. afrochelatus*, Paperna, 1973. All measurements are given in micrometres.

Monogenean	*Dactylogyrus* sp. 2 (n=9)		*Dactylogyrus afrochelatus* (n=4)
Host	*Barbus paludinosus*		*Barbus paludinosus*
Locality	Pongola System, South Africa		Nzoia River, Kenya
	Current study		(Paperna 1979)
	Mean ± SD	Range	Range
Body			
Length	189.2±43.2	115.8-253.8	280-360
Width	55.3±7	43.9-62.2	80-130
Anchor			
Length	73.8±3.3	68.6-78.6	58-68
Shaft	62.3±3.3	57.3-66.8	46-55
Tip	19.5±1.4	18-21.3	15-16
Outer Root	5.6±0.8	3.9-6.5	1-2
Inner Root	20.7±1.6	17.9-22.3	13-19
Dorsal bar			
Length	37.2±6.3	29.2-48.7	42-58
Width	4.5±0.9	3.2-5.8	-
Male copulatory organ			
Male copulatory organ	43.6±3.6	38.8-48.5	24-32
Accessory piece	16.8±2.2	13.5-19.8	18-24

Remarks:

These are small worms with a wide opisthaptor and large anchors, the MCO is small. The anchors are large, with short inner roots and long shafts. The bar is robust, subdivided by two submedian folds into one central and two lateral plates (Figure 5.4 B).

Differential diagnosis:

This species resembles *Dactylogyrus afrochelatus* Paperna, 1973, described by Paperna (1979) from *B. paludinosus* in Kenya from the Nzoia River. *Dactylogyrus* sp. 2 possesses the same pattern in the dorsal bar and large anchors compared to body size, but differs from *D. afrochelatus* in the size of the anchors. It possesses a larger total anchor length with a shorter length of the dorsal bar. In addition, the outer and inner roots are longer and body length and width is significantly smaller than *D. afrochelatus* (Table 5.3). *Dactylogyrus* sp. 2 differs from *D. dominici* and *Dactylogyrus* sp. 1 in the pattern of the dorsal bar and the sizes of the anchors; *Dactylogyrus* sp. 1 has larger anchors.

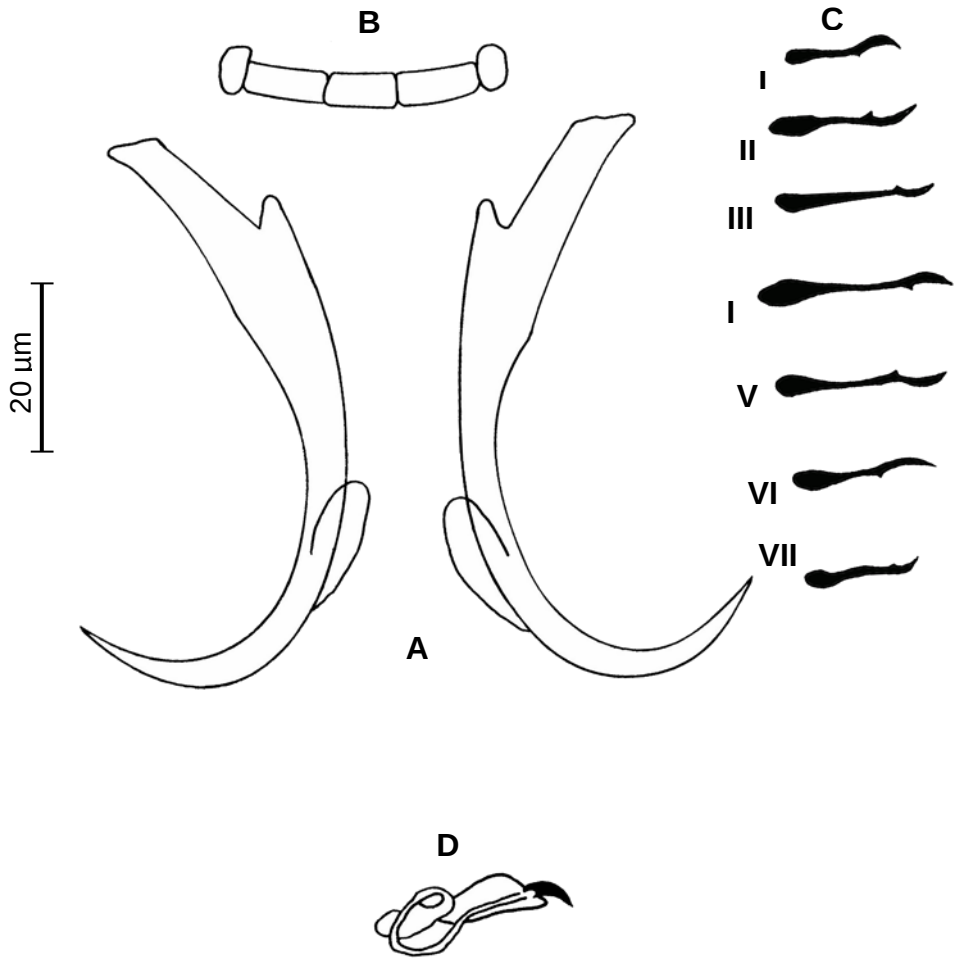

Figure 5.4: Microscope projection drawings of the sclerotinoid organs of *Dactylogyrus* Diesing, 1850 sp. 2 from the gills of *Barbus paludinosus*, Peters, 1852, collected in the Pongola River System. **A** – Anchors, **B** – Dorsal bar, **C** – Marginal hooklets (**I** to **VII**) and **D** – Male copulatory organ.

***Dactylogyrus* sp. 3** (Figures 5.5 A-D, 5.6 F)

Type host: *Barbus afrohamiltoni* Crass, 1960

Type locality: Pongola Floodplains, Nyamiti Pan Outlet (S26°88'27'75" E32°31'13'93")

Additional localities: Pongola Floodplains, Pomphuis (S26°90'52'83" E32°32'35'00")

Site of infection: Gill lamellae

Type material: 2013/09/11-18 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements and drawings were made by using light microscopy from 12 specimens from the Pongola River System, mounted in glycerine ammonium picrate.

Description and measurements:

Body length 316.8 ± 61.5 (251.2 - 477.1), width 52.7 ± 10.6 (36.7 - 70.5) perpendicular to male copulatory organ. Anchor total length 47.9 ± 1.4 (45.7 - 50.3), anchor shaft length 36.8 ± 1.1 (35.5 - 38.8), anchor outer root length 4.8 ± 0.8 (3.9 - 6.6), anchor inner root length 15.2 ± 2 (12.1 - 18.7), anchor point length 11.5 ± 0.5 (10.8 - 12.8), anchor aperture 28.2 ± 1.7 (25.3 - 30.8). Dorsal bar length 31.7 ± 3.9 (25.9 - 38.3), dorsal bar width 1.7 ± 0.5 (1 - 2.5). Seven pairs of marginal hooklets; I = 29.2 ± 4.9 (19.9 - 34.7), II = 30.3 ± 3.6 (23.1 - 34.5), III = 24.4 ± 2.7 (17.6 - 28), IV = 24.2 ± 5.5 (8.2 - 29.3), V = 26 ± 3.9 (18.1 - 30.8), VI = 26.6 ± 2.1 (21.9 - 28.8), VII = 26.6 ± 2.5 (22.4 - 29.5). Male copulatory organ 40.5 ± 5.9 (31.4 - 53.1), accessory piece 17.2 ± 1.9 (13.4 - 21.1). Ratio between handle and hook is four to five.

Table 5.4: Measurements of *Dactylogyrus* Diesing, 1850 sp. 3 from *Barbus afrohamiltoni* Crass, 1960 from the Pongola River System and comparison with the published description of similar *D. afroruahae*, Paperna, 1973. All measurements are given in micrometres.

Monogenean	*Dactylogyrus* sp. 3		*Dactylogyrus afroruahae*
Host	*Barbus afrohamiltoni*		*Barbus* sp.
Locality	Pongola System, South Africa Current study		Ruaha River, Tanzania (Paperna 1979)
	Mean ± SD	Range	Range
Body			
Length	316.8±61.5	251.2-477.1	350-400
Width	52.7±10.6	36.7-70.5	70-100
Anchor			
Length	47.9±1.4	45.7-50.3	36-41
Shaft	36.8±1.1	35.5-38.8	25-35
Tip	11.5±0.5	10.8-12.8	11-12
Outer Root	4.8±0.8	3.9-6.6	2
Inner Root	15.2±2	12.1-18.7	14-15
Dorsal bar			
Length	31.7±3.9	25.9-38.3	43-46
Width	1.7±0.5	1-2.5	-
Male copulatory organ			
Male copulatory organ	40.5±5.9	31.4-53.1	25-29
Accessory piece	17.2±1.9	13.4-21.1	15-19

Remarks:

These are medium to large worms, the worms have of a long slender body with relatively short inner roots and the dorsal bar is divided into heavy, wide plates by a median constriction. The hooklets are very large with thick handles (Table 5.4).

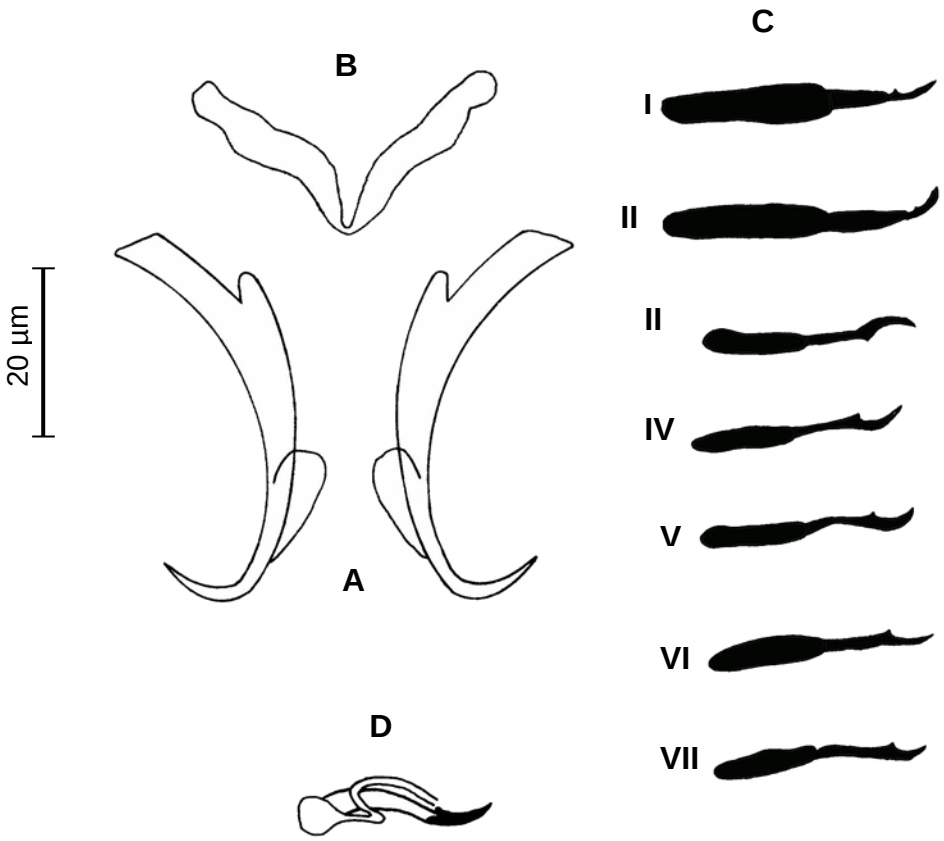

Figure 5.5: Microscope projection drawings of the sclerotinoid organs of *Dactylogyrus* sp. 3 from the gills of *Barbus afrohamiltoni Crass*, 1960, collected from the Pongola River System. Abbreviations: **A** – anchors, **B** – Dorsal bar, **C** – Marginal hooklets (**I** to **VII**) and **D** – Male copulatory organ.

Differential diagnosis:

The present material resembles *Dactylogyrus afroruahae* described from a unknown small *Barbus* sp. by Paperna (1979) in Tanzania from the Ruaha River. *Dactylogyrus* sp. 3 differs from *D. afroruahae* by a larger total anchor length and outer root length. The present material also consists of a shorter dorsal bar and a

longer MCO length. Furthermore, the body length and width is smaller. *Dactylogyrus* sp. 3 differs from *D. dominici*, *Dactylogyrus* sp. 1 and *Dactylogyrus* sp. 2 by possessing much larger marginal hooklets with thick long handles. The dorsal bar of *Dactylogyrus* sp. 3 and *Dactylogyrus* sp. 1 is very similar, but the marginal hooklets are different. The ratio between handle and hook for *Dactylogyrus* sp. 3 is 4/5, whereas the ratio for *Dactylogyrus* sp. 1 is 1/4.

DISCUSSION

All the monogeneans found during the present study belong to the genus *Dactylogyrus*. According to Paperna (1979), three species groups can be singled out among the genus *Dactylogyrus* from African fish. These three species groups are *D. pseudanchoratus* and *D. afrobarbae* (so far only known from African cyprinids) and *D. varicorhini* that are common in both Asia and Africa. The *D. afrobarbae* group is endemic to Africa and is associated with smaller *Barbus* species as well as with *Labeo* species (Mashego 1982).

Eighteen different species of *Barbus* were examined and only four fish species were found infested with enough material to compile taxonomic descriptions. Four different species of *Dactylogyrus,* based on the measurements and morphology of the sclerotised parts of the monogeneans were found. All the species were from the *D. afrobarbae* group and were distinguished from each other using the size of the MCO, size of the hooklets, the anchor inner roots as well as the size and pattern of the dorsal bar (Paperna 1979).

According to Guégan & Lambert (1990), *Dactylogyrus* monogeneans found on small *Barbus* species seems to be restricted to this genus, and species described from *Barbus* were therefore used for comparisons and identifications. Some species of *Dactylogyrus* are host specific and their distribution would be limited to that of their hosts, but some species are more restricted to their specific distribution than their respective hosts (Mashego 1982).

Dactylogyrus sp. 1 was found on two different hosts i.e. *B. fasciolatus* and *B. afrovernayi* (Figures 5.6 C-D), and therefore, the range of the distribution seems to be on more than one fish host. These host species have the same distribution and *Dactylogyrus* sp. 1 could have the same restricted distribution. The other three species were only found on one host and, therefore, their range of distribution seems to be restricted to one host.

Dactylogyrus sp. 3 (Figure 5.6 F) was found on *B. afrohamiltoni* that can be found in the lowveld reaches of the tropical east coast rivers and this could also be the distribution of the parasite. *Dactylogyrus dominici* (Figures 5.6 A-B) seems to be widely distributed through the Orange-Vaal, Pongola, Limpopo and the Okavango river systems and so are their hosts, i.e. *B. paludinosus*. If future studies reveal that *D. afropsilovaginus* and *D. dominici* are the same species, the distribution will be as far as Uganda. *Dactylogyrus* sp. 2 (Figure 5.6 E) was also found on *B. paludinosus* and the host's parasites are well studied throughout its wide distribution in Africa, but this monogenean was only found in the Pongola River System and the distribution could therefore be restricted to this system only. Due to small sample sizes in the current study, more data needs to be collected to support this theory.

Six species already have been described from *B. paludinosus* in Africa; *D. afrochelatus, D. afropsilovaginus, D. afrosclerovaginus*, *D. clavatovaginus, D. dominici* and *D. teresae* which seem to be restricted to their systems and not to the distribution of the host, except *D. dominici* that has a wider distribution. *Dactylogyrus* sp. 2 is possibly a new species found on the same widely distributed host, *B. paludinosus,* but is restricted to the Pongola River System.

Further studies on this subject could benefit by focussing on a larger sample size, more frequent sampling, seasonal studies and molecular work on the monogeneans. Morphologically based taxonomy and molecular work can also be used to describe the species more accurately and to acquire a better understanding of their evolutionary relationships (Crafford *et al.* 2012).

Figure 5.6: Light microscope photos of *Dactylogyrus dominici* Mashego, 1983 from **A**- Pongola and **B**- Orange-Vaal River System from the host *Barbus paludinosus* Peters, 1852. *Dactylogyrus* Diesing, 1850 sp. 1 from **C**- *B. fasciolatus* Günther, 1868 and **D**- *Barbus afrovernayi* Nichols & Boulton, 1927 from the Okavango River System. **E**- *Dactylogyrus* Diesing, 1850 sp. 2 from *B. paludinosus* Peters, 1852 and **F**- *Dactylogyrus* Diesing, 1850 sp. 3 from *Barbus afrohamiltoni* Crass, 1960 from the Pongola River System.

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Chapter 6

Cestoda and Trematoda

INTRODUCTION

Cestodes and trematodes are very poorly studied in southern Africa and only a few publications have been written on these parasites (Whitfield & Heeg 1977; Brandt *et al*. 1981; Britz *et al.* 1984; Mashego & Saayman 1989; Barson & Marshall 2003; Bertasso & Avenant-Oldewage 2005; Barson & Avenant-Oldewage 2006; Barson *et al.* 2008; Stadtlander *et al.* 2011; Kuchta *et al*. 2012; Jansen van Rensburg *et al*. 2013). Cestodes and trematodes associated with the genus *Barbus* in southern Africa is even less known and very little work has been done on these fish species. Mashego (1982) did some work for his PhD thesis and Barson & Marshall (2003) published one article on cestodes from this fish genus.

The known cestodes from Africa include 61 adult and larval forms from different fish species (Kuchta *et al.* 2012). The adult tapeworms that were identified to species level belong to the orders Amphilinidea (1), Caryophyllidea (20), Bothriocephalidea (6) and Proteocephalidea (19) (Kuchta *et al.* 2012).

Cestodes of freshwater fish are a relatively unknown field in southern Africa and only a few species have been found. One of these species is *Proteocephalus glanduligerus* (Janicki, 1928) that was described from the mudfish catfish *Clarias anguilaris* (Linneaus, 1758) from the Nile River. This tapeworm was also found by Mashego (2001) from the sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), in the Limpopo River of South Africa and the same species was found on the same host by Barson & Avenant-Oldewage (2006) from the Rietvlei Dam in Gauteng, South Africa.

Tetracampos ciliotheca Wedl, 1861, previously known as *Polyonchobothrium clarias* (Woodland, 1925) (Kuchta *et al*. 2008, 2012; Madanire-Moyo & Avenant-Oldewage 2013), is a widely distributed cestode that parasitises catfish from Africa. It has been found infesting *C. anguillaris* in Egypt and Senegal, *C. gariepinus* in Nigeria and *Chtysichthys thonneri* Steindachner, 1912 in Gabon. In southern Africa this tapeworm was found infesting *C. gariepinus* in the Limpopo and Gauteng Provinces of South Africa and was found in Lake Kariba in Zimbabwe from *Synodontis zambezensis* Peters, 1852 (Barson & Avenant-Oldewage 2006).

One species of alien tapeworm that is now widely distributed in southern Africa was first recorded by Brandt *et al.* (1981) from the alien fish, *Cyprinus carpio* Linnaeus, 1758. This tapeworm is *Bothriocephalus acheilognathi* Yamaguti, 1934 and according to Brandt *et al.* (1981), it was described from Japan and its original distribution area is East Asia. This tapeworm originally parasitised cyprinid fish from East Asia, but this parasite is now spread worldwide and it has been able to infect more than 100 different fish species from several unrelated fish families and even amphibians (Stadtlander *et al.* 2011). This parasite was introduced with cyprinid fish, most probably from their original hosts *Ctenopharyngodon idella* Valenciennes, 1844 and *Hypothalmichthys molitrix* Valenciennes, 1844 that were introduced in rivers across South Africa (Bertasso & Avenant-Oldewage 2005). The distribution of this tapeworm in Africa stretches from the Great Fish River (Stadtlander *et al.* 2011) and the Orange-Vaal River Basin (Bertasso & Avenant-Oldewage 2005) to the Maputo, Incomati and Limpopo Basins in South Africa, the Congo Basin in the Democratic Republic of Congo and the Nile Basin in Egypt (Kuchta *et al*. 2012).

Bothriocephalus acheilognathi has been synonymised with various tapeworms throughout the world and in Africa. *Bothriocephalus kivuensis* was described by Baer & Fain (1957) and *B. aegyptiacus* described by Rysavy & Moravec (1975) from Africa, but molecular studies that were done by Kuchta *et al.* (2012) revealed that they are both *B. acheilognathi* and not different species. Seven bothriocephalidean tapeworms have been found and described in Africa and only one species of the genus *Bothriocephalus* exists, the alien tapeworm *B. acheilognathi*, while the other six species of five genera are endemic to Africa (Kuchta *et al.* 2012).

There are only three cestodes records from the genus *Barbus* in southern Africa, one is the cosmopolitan tapeworm *Ligula intestinalis* (Linnaeus, 1758) of which the plerocercoid larva was found in *B. paludinosus* in Zimbabwe (Barson & Marshall 2003), and in the Limpopo System, South Africa from *Barbus paludinosus* Peters, 1852, *B. unitaeniatus* Günther, 1866 and *B. radiatus* Peters, 1853 (Mashego 1982; Matla 2012). This is a common tapeworm that can be found associated with the genus *Barbus* and it has also been found in *B. humilis* Boulenger, 1902 in Ethiopia (Dejen 2006).

According to Kuchta *et al.* (2012), the alien tapeworm *B. acheilognathi* has been recorded from seven different *Barbus* species in Africa and in southern Africa it was found in *B. trimaculatus* and *B. paludinosus* from the Limpopo System (Mashego 1982). It was found in the threatened *B. brevipinis* Jubb, 1966 in Mpumalanga (Schulz & Schoonbee 2006), as well as *B. argenteus* Gunther, 1868, and *B. mattozi* Guimaraes, 1884 (Stadtlander *et al.* 2011). The third cestode that was found in barbs from southern Africa is the dilepidid larva from the genus *Parvitaenia* Burt, 1940, five different fish species were infested with this parasitic larvae (Mashego 1982).

During the current study, five different species of cestodes were found infesting different barbs, i.e. three adult and two larval tapeworms. Two known species were found, the alien parasite *B. acheilognathi,* the plerocercoid larva *L. intestinalis* and also a dilepidid larval form of the genus *Parvitaenia.* Two possibly new species have been found belonging to the genera *Proteocephalus* Weinland, 1858 and *Ichthybothrium* Khalil, 1971.

Trematode parasites of southern Africa are mostly known from economically important fishes, for example the sharptooth catfish *C. gariepinus* and *Oreochromis mossambicus* (Peters, 1852) which have been extensively studied (Britz *et al*. 1984; Mashego & Saayman 1989; Barson & Avenant-Oldewage 2006; Barson *et al*. 2008; Madanire-Moyo *et al.* 2012; Jansen van Rensburg *et al*. 2013).

Only a few studies have been done on the trematodes of the genus *Barbus* in southern Africa. A few species of metacercariae from the genus *Clinostomum* Leidy, 1856 and *Diplostomum* von Nordmann, 1832 have been found through work of Mashego (1982); King & van As (1997); Jansen van Rensburg (2006) and Grobbelaar (2011). *Clinostomum* sp. and *Diplostomum* sp. are trematodes of barbs according to the checklist of helminth parasites of African fish (Khalil & Polling 1997). *Posthodiplostomum* sp. and *Ornithodiplostomum* sp. were also recorded from southern African barbs (Mbokane *et al*. 2015).

MATERIALS AND METHODS

Fish collections

See chapter 3

Host examination, fixation and preservation of parasites

The fish viscera, liver, spleen, intestine as well as the gall and swim bladders were examined for parasites in 1% saline solution. The intestines swim and gall bladders were carefully teased apart using dissection needles and fine tipped forceps in search for cestodes and trematodes. The parasites were removed and transferred to saline.

All the cestodes and trematodes collected were swirled in 1% saline or placed in a fridge to relax the worms. Various fixation methods were used for the parasites for various microscopic preparations. The parasites used for staining for permanent slides were fixed flat by quickly adding warm (70°C) alcohol-formaldehyde-acetic acid (AFA) or 5% or 10% buffered neutral formaldehyde (BNF). They were then preserved in 70% EtOH in McCarteny bottles. The worms that were fixed for scanning electron microscopy (SEM) were fixed in warm 70% EtOH and also preserved in 70% EtOH.

Light microscopy preparations

The cestodes were stained and mounted by using adapted preparation methods from de Chambrier (2001). The worms were stained by using two different stains, namely Mayer's Hydrochloric Carmine and Weigert's Haematoxylin solution and were prepared by using methods from de Chambrier (2001). The Mayer's Hydrochloric Carmine stain was used by placing the worms in the stain for 15 min; the worms were then rinsed in 70 % EtOH and destained in acid 75 % EtOH for 20-30 min until no carmine diffused from the worms. The worms were then dehydrated

in an ethanol series by placing them in 80, 96 and twice in 100 % EtOH for 30 min. The worms were cleared in increasing concentrations of Eugenol (clove oil) that was diluted with 100 % EtOH to 50, 75, 90 and 100 % for 15 min in each. The worms were permanently mounted on a frosted end slide with Eukitt quick-hardening mounting medium by using a cover slide.

The worms were placed in distilled water (H_2O) for 5 min before the Haematoxylin stain were used. Weigert's Haematoxylin solution was then used by placing the worms in the stain between 5 and 10 min (depending on the size of the worm). The worms were destained in acid 75 % ethanol for 20-30 min and then transferred to tap water until the worm turned a blue colour (approximately 10 min). The worms were dehydrated in an ethanol series, as described above for Carmine staining.

MAYER'S HYDROCHLORIDE CARMINE

Carmine	5 g
18% Hydrochloric acid	10 ml
95% Ethanol	200 ml
Iron	Piece

WEIGERT'S HAEMATOXYLIN SOLUTION

1% Haematoxylin in ethanol	5 m
1.2% Iron (III) Chloride in distilled water	5 ml
18% Hydrochloric acid	5 drops

Trematodes were stained and mounted by using adapted methods from Professor Robin Overstreet[3]. Delafield's, Ehrlich's and Van Cleave's Haematoxylin were prepared by using methods from Professor Robin Overstreet[3]. The specimens were cleaned in 70 % EtOH and hydrated with distilled water. The specimens were then stained with Van Cleave's Haematoxylin stain overnight. The stain was removed and replaced with 70 % EtOH and dehydrated with a series of 80, 90, 96 and 100 % EtOH respectively. The specimens were then cleared with either Xylene of Cederwood Oil and mounted with Eukitt quick-hardening mounting medium on a frosted end slide and a cover slide.

[3] Professor Robin Overstreet, Department of Coastal Sciences, University of Southern Mississippi, United States of America

DELAFIELD'S HAEMATOXYLIN

Haematoxylin	4 gm
95 % Ethyl alcohol	25 ml
Aluminium ammonium sulphate, sat. aq. Sol.	400 ml
Glycerine	100 ml
Methyl alcohol	100 ml

EHRLICH'S HAEMATOXYLIN

Haematoxylin	2 gm
95 % Ethyl alcohol	100 ml
Glycerine	100 ml
Distilled water	100 ml
Glacial acetic acid	10 ml
Potassium alum ($K_2SO_4AL_2(SO_2)_324H_2O$)	3 gm

VAN CLEAVE'S HAEMATOXYLIN

Delafield's haematoxylin	1 ml
Ehrlich's haematoxylin	1 ml
Aluminium ammonium sulphate	6.5 g
Distilled water	100 ml

The specimens that were used for Scanning Electron Microscopy were prepared with a Quorum K850 Critical Point Drier, an Emscope SC500 Sputter Coater and a Shimadzu SSX550 Scanning Electron Microscope, using standard preparation methods.

Morphological measurements

The parasites were identified based on morphological drawings, light microscopy and scanning electron microscope (SEM) micrographs. Morphological drawings were made with the aid of a drawing tube and a Leitz Weltzar Laborlux D compound microscope. Light micrographs were taken using a Zeiss Axiophot microscope with a Zeiss AxioCam ICc 5 camera. The measurements were made from the micrographs with ZEN image-processing and analysis software program.

The cestodes were identified to genus level using Khalil *et al*. (1994) and Kuchta *et al*. (2008), and trematodes were identified to genus level using Kanev *et al*. (2002).

RESULTS

This chapter comprises the taxonomical diagnoses of cestode and digenean species collected from fishes of the genus *Barbus* in the various localities.

The parasites that were found belong to different orders and families, and each of the parasites were identified on different criteria for the specific parasites (Khalil *et al*. 1994; Rego *et al*. 1998; Kanev *et al*. 2002; Kuchta *et al*. 2008, 2012).

Class: Cestoda

Order: Proteocephalidea Mola, 1928

Family: Proteocephalidae La Rue, 1911

Subfamily: Proteocephalinae Mola, 1929

Genus: *Proteocephalus* Weinland, 1858

***Proteocephalus* sp. 1** (Figures 6.1 A-B, 6.2 A-F, 6.3 A-E)

Type host: *Barbus miolepis* Boulenger, 1902

Additional host: *Barbus multilineatus* Worthington, 1933 and *Barbus afrovernayi* Nichols & Boulton, 1927

Type locality: Okavango River System, Shakawe (S18°42’93’85” E21°89’64’98”)

Prevalence: 1/2 = 50% for *B. miolepis*, 1/16 = 6.25% for *B. multilineatus* and 1/21 = 4.8% for *B. afrovernayi*

Intensity: 1 to 2 specimens per fish host

Site of infection: Small intestine

Type material: 2013/08/12-02 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

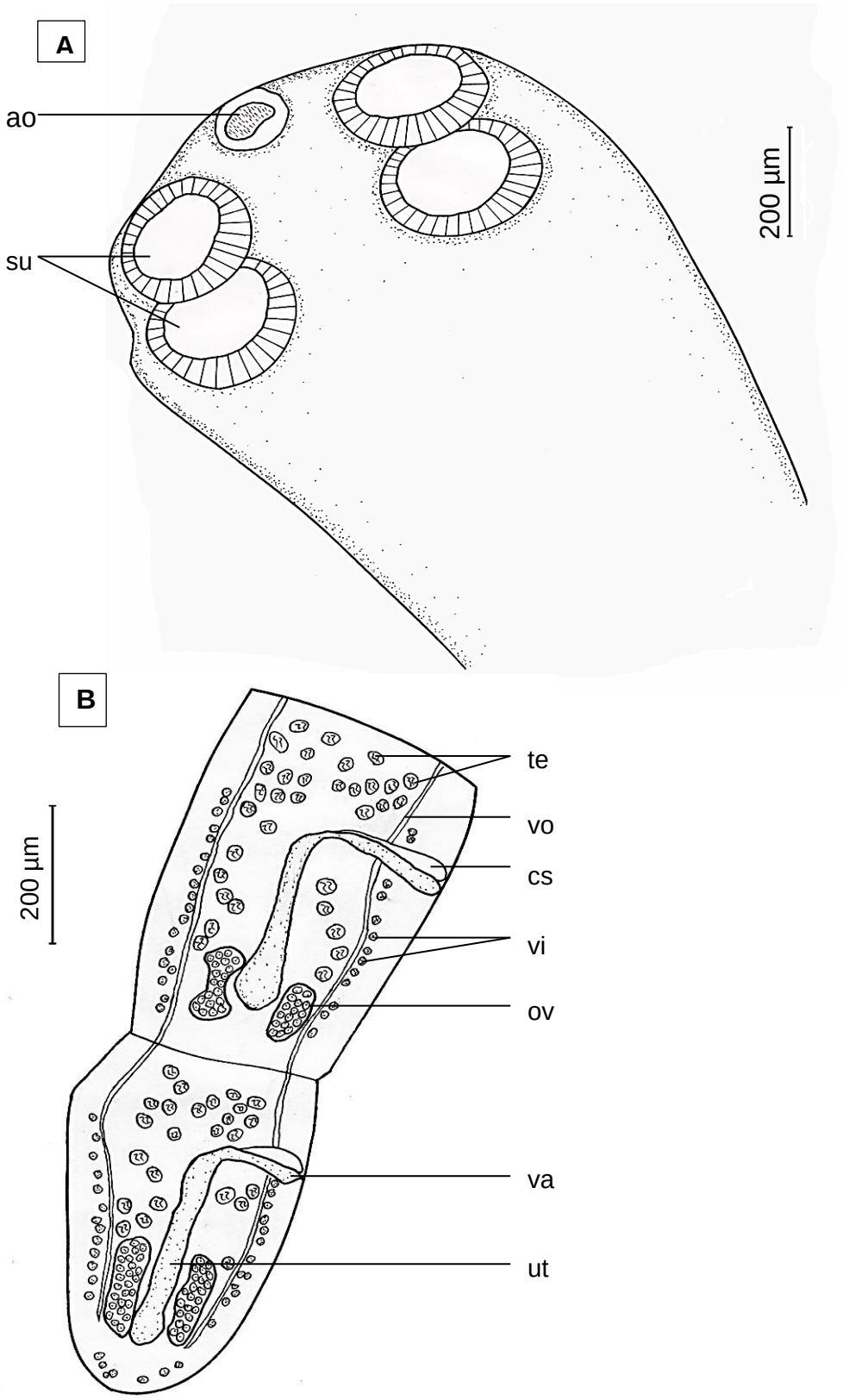

Figure 6.1: Microscope projection drawings of *Proteocephalus* Weinland sp. 1, 1858 from the Okavango River System from *B. miolepis* Boulenger, 1902. **A**- Scolex with ao- apical organ and su- suckers. **B**- Mature proglottids, cs- cirrus sac, ov- ovary, te-testes, ut- uterus, va- vagina, vi- vitelline follicles and vo- ventral osmomoregulatory canal.

Material examined: Detailed morphometric measurements and drawings were made using light microscopy from three specimens from the Okavango River System, stained in either Mayer's Hydrochloric Carmine or Weigert's Haematoxylin solution and mounted with Eukitt quick-hardening mounting medium. One specimen was used for scanning electron microscopy preparations and micrographs.

Description:

The character analysis based on morphology and taxonomy was used to examine the genus *Proteocephalus* through methods given by Rego *et al.* (1998).

This is a small tapeworm that is less than seven millimetres in total length (Figures 6.2 A-B). The type of proglottid is acraspedote, with 14-16 proglottids per worm. Immature proglottids are wider than long and mature proglottids are longer than wide (Figures 6.2 E-F). The scolex is well separated from the strobila and a metascolex is absent. An apical organ is present that does not contain a sucker (Figures 6.2 C-D). Four prominent spherical suckers that open slightly anteriorly are present (Figures 6.3 A-C, E). The scolex is unarmed and transverse tegmental wrinkles are absent (Figures 6.3 D).

The position of the vitelline follicles is cortical in two lateral fields. The spherical oval testes are medullary in two fields, connected anteriorly without overlapping the excretory canal. The position of the ovary is medullary posterior in the proglottid. The uterus is located medullary. The vaginal sphincter was not observed and the position of the vagina is posterior to the cirrus-sac (Figures 6.1 B). The eggs were not observed in the specimens and no gravid proglottids were present, this indicates immature worms.

Remarks:

Proteocephalus sp. 1 is a small worm characterised by a small apical organ that is neither glandular nor muscular with four suckers. The vitellaria are medullary and the uterus length is two thirds the length of mature proglottids. The ovaries are in two lateral fields, longitudinal and posterior in the proglottids.

Differential diagnosis:

In total 19 species of proteocephalidean tapeworms from different genera have been described from different fish in Africa (de Chambrier *et al.* 2009). Ten different species of the genus *Proteocephalus* have been described in Africa, but despite the abundance and diversity, only one species was found in southern Africa, *P. glanduligerus* (Barson & Avenant-Oldewage 2006).

Table 6.1: Measurements of *Proteocephalus* Weinland, 1858 sp. 1 from *Barbus miolepis* Boulenger, 1858 from the Okavango River System and a comparison with published descriptions of *P. glanduligerus* (Janicki, 1928). All measurements are given in micrometres unless otherwise stated.

Cestode	*Proteocephalus* Weinland, 1858 sp. 1	*P. glanduligerus* (Janicki, 1928)
Host	*Barbus miolepis* Boulenger, 1902	*Clarias anguillaris* (Linnaeus, 1758)
Locality	Okavango System Current study	Nile River (Scholz *et al*. 2014)
Total length (mm)	5.79-6.19	8-25
Maximum width	572.1-744.2	655
No. proglottids	14-16	30-38
Scolex width	572.1-744.2	420-560
Diameter of suckers	165.5-213.6	110-170
Apical organ		
Length	74.7-110.4	250-400
Width	71.4-76.4	245-280
Immature proglottids		
Length	164.1-243.5	115-310
Width	387.8-560.7	300-365
Mature proglottids		
Length	476.9-557	545-665
Width	306.6-470.8	425-595
Testes		
Diameter	25.4-28	40-60
Number	37-39	37-48
Cirrus-sac		
Length	97.6-108.4	135-170
Width	26.7-27.9	70-75
l:w ratio	3.7-3.9	1.9-2.2
Relative size	23-31.8%	29-35%
Ovary		
Length	139.1-206.5	140-210
Width	42.1-84.5	200-310

A comparison of *P. glanduligerus* and *Proteocephalus* sp. 1 was made in Table 6.1. The apical organ of *P. glanduligerus* is glandular and large, larger than the diameter of the suckers, compared to *Proteocephalus* sp. 1 which has a small apical organ, which is also smaller than the suckers. *Proteocephalus glanduligerus* is much longer

and contains more proglottids (Table 6.1). *Proteocephalus* sp. 1 was found in a different fish family (Cyprinidae) than *P. glanduligerus* (Clariidae).

Proteocephalus sp. 1 was identified to genus level by using the keys provided by Khalil *et al*. (1994) and the species was described by the character analysis that was provided for the genus *Proteocephalus* in the paper of Rego *et al*. (1998). The current species could be a possible new species from the genus *Proteocephalus* occurring in *B. miolepis* and *B. multilineatus* from the Okavango River System.

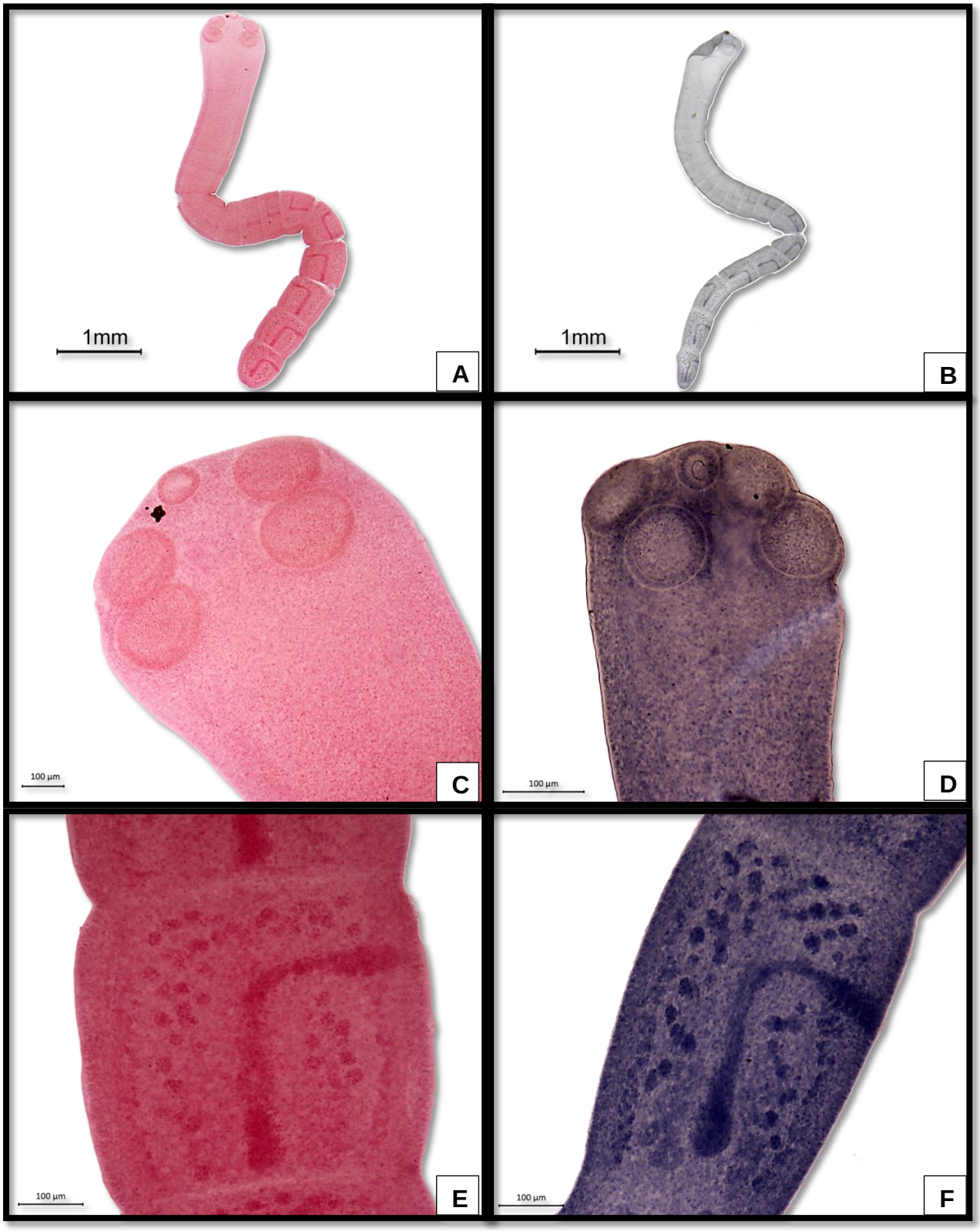

Figure 6.2: Light micrographs of *Proteocephalus* Weinland sp. 1, 1858 from the Okavango River System from *Barbus miolepis* Boulenger, 1902. A and B – Entire worm, **C** and **D** – the scolex and **E** and **F** – mature proglottids. **A**, **C** and **E** (pink) were stained with Mayer's Hydrochloric Carmine and **B**, **D** and **F** (blue) were stained with Weigert's Haematoxylin solution.

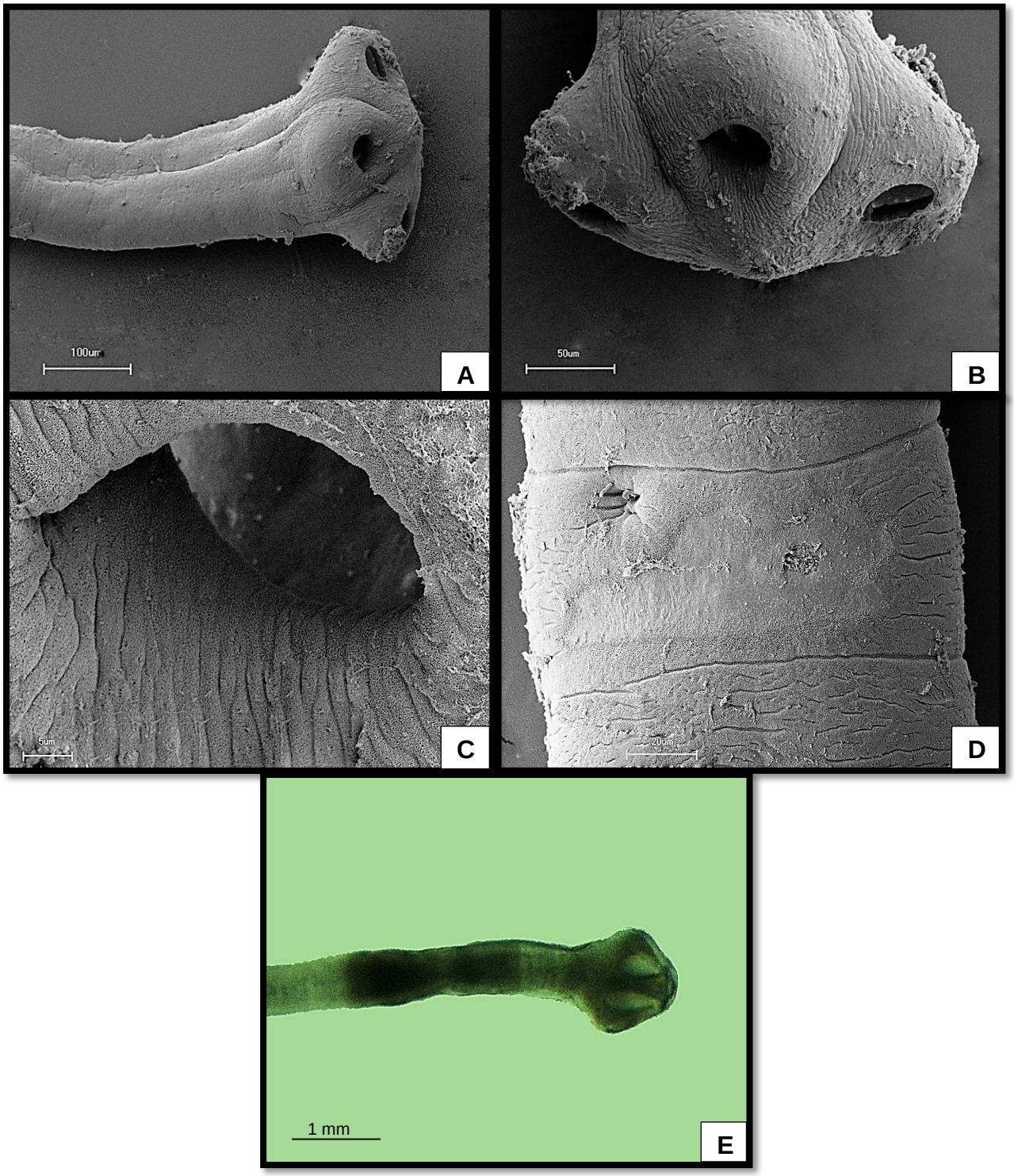

Figure 6.3: Scanning electron micrographs of *Proteocephalus* Weinland sp. 1, 1858 from the Okavango River System from *Barbus miolepis* Boulenger, 1902. **A**- Scolex and neck, **B**- Scolex, **C**- enlarge view of sucker and **D**- enlarge view of anterior margin of mature proglottids. **E**- Micrograph from a dissecting microscope of a live specimen.

Order: Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008

Family: Bothriocephalidae Blanchard, 1849

Genus: *Ichthybothrium* Khalil, 1971

***Ichthybothrium* sp. 1** (Figures 6.4 A-B, 6.5 A-C)

Type host: *Barbus afrovernayi* Nichols & Boulton, 1927

Type locality: Okavango River System, Shakawe (S18°42'93'85" E21°89'64'98")

Prevalence: 3/21 = 14.3% for *B. afrovernayi*

Intensity: 1 to 2 specimens per fish host

Site of infection: Small intestine

Type material: 2013/08/09-01 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements and drawings were made using light microscopy from one specimen from the Okavango River System, stained in Mayer's Hydrochloric Carmine and mounted with Eukitt quick-hardening mounting medium.

Description:

The character analysis based on morphology and taxonomy was used to examine the genus *Ichthybothrium* through the work of Khalil (1971).

The oval-shape scolex is medium sized, unarmed with two shallow bothria and no apical disc is present (Figure 6.5 A). The cestode is small to medium sized with a maximum length of almost 16 mm and a width of 678.4 µm. The segmentation of the strobila is difficult to distinguish in the first immature segmentations and the segmentations is wider than long. The segmentations gradually increase in length and the proglottids become more marked. Mature proglottids have one set of reproductive organs and can only be seen from the 20th proglottid (Figure 6.5 B). Ten mature proglottids are present and the last two are gravid proglottids (Figure 6.5 C). In total 32 acraspedote proglottids are present.

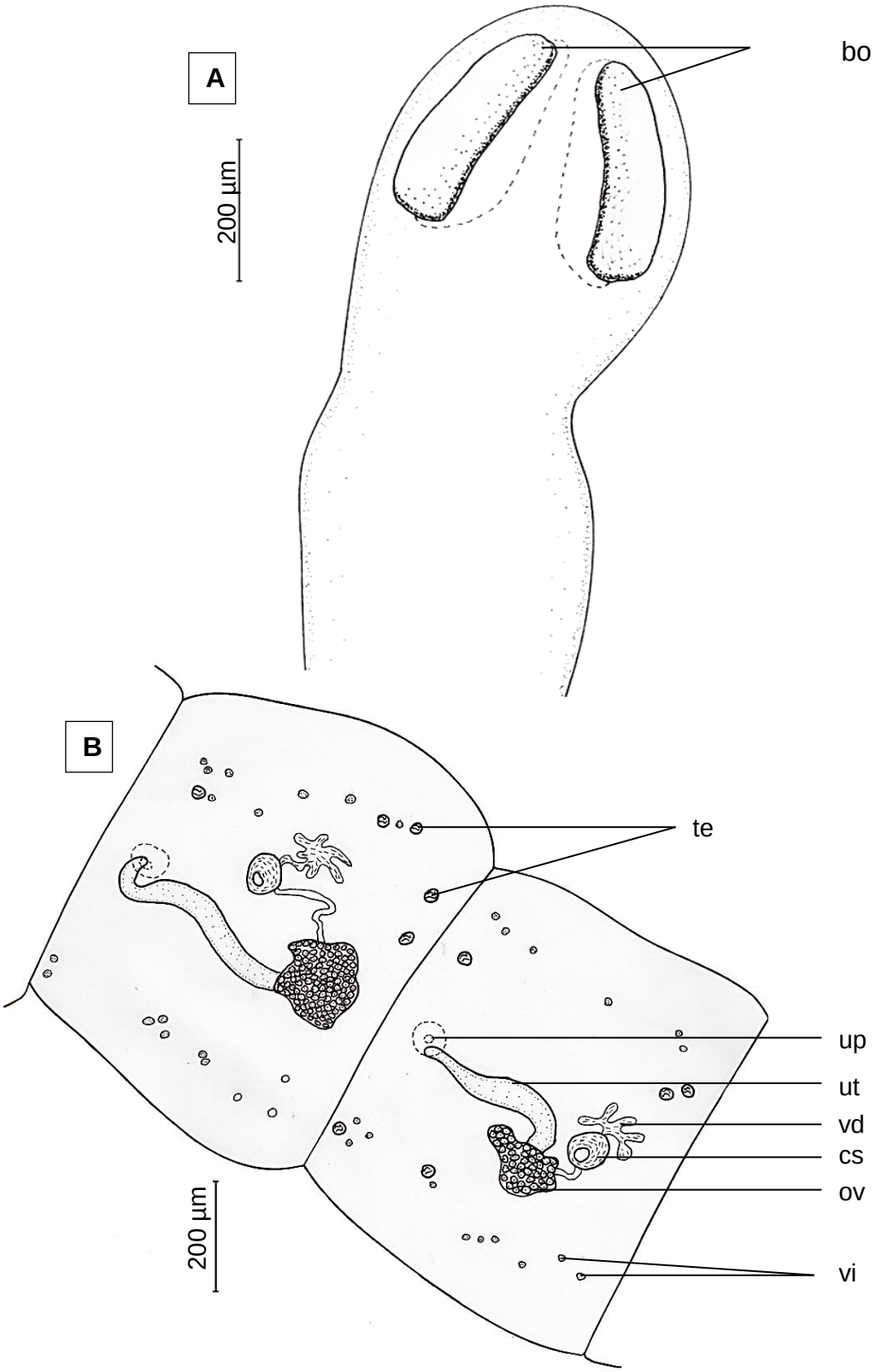

Figure 6.4: Microscope projection drawings of *Ichthybothrium* Khalil, 1971 sp. 1 from the Okavango River System from *Barbus afrovernayi* Nichols & Boulton, 1927. **A**- Scolex with bo- bothria. **B**- Mature proglottids, cs- cirrus sac, ov- ovary, te-testes, up- uterine pore, ut- uterus, vd- vas deferens and vi- vitelline follicles.

The position of the testes is medullary and cortical, scattered irregularly in the lateral margins of the strobila on each side (Figure 6.4 B). There are more or less eight testes per proglottid that is spherically shaped. The cirrus sac is spherical and perpendicular to the dorsal surface.

The ovary is oval and the position is medullary posterior in the proglottid. The uterus is located medullary and the uterine pore opens ventrally anterior of the proglottid. The eggs are non-operculated and the last proglottids were full of eggs. The position of the vitelline follicles is cortical in two lateral fields.

Table 6.2: Measurements of *Ichthybothrium* Khalil, 1974 sp. 1 from *Barbus afrovernayi* Nichols & Boulton, 1927 from the Okavango River System and a comparison with published descriptions of *Ichthybothrium ichthybori* Khalil, 1971. All measurements are given in micrometres unless otherwise stated.

Cestode	*Ichthybothrium* Khalil, 1971 sp. 1	*Ichthybothrium ichthybori* Khalil, 1971
Host	*Barbus afrovernayi* Nichols & Boulton, 1927	*Ichthyborus besse* (Joannis, 1835)
Locality	Okavango System Current study	White Nile (Khalil 1971)
Total length (mm)	15.3	-
Maximum width	678.4	800-1500
No. of proglottids		
Immature	20	8-13
Mature	10	6-17
Gravid	2	-
Length of proglottids		
Pregenital (mm)	9.4	22
Early genital (mm)	2.4	7
Mature (mm)	2.9	9
Scolex		
Length	560.3	490-610
Width	388.9	340-530
Bothria		
Length	308.3	240-360
Width	121.4	140-200
Strobila		
Length (mm)	14.7	35-41.8
Average	-	38
Immature proglottids		
Length	332.2	-
Width	510.1	-
Mature proglottids		
Length	659.3	-
Width	476.4	-
Testes		
Diameter	22.5	77-96
Number	8	53-58
Cirrus-sac		
Length	59.4	116-135

Remarks:

The current species was identified as belonging to genus *Ichthybothrium* based on its morphology, by using keys to cestode parasites provided by Khalil *et al*. (1994). Conversely, a more recent publication by Kuchta *et al*. (2008), a revision of the order Bothriocephalidae provided new keys to families and genera for this order and if one compares the current species with this article, it seems as if the current species shares a few characteristics with the genus *Bothriocephalus* and not just with *Ichthybothrium*. However, according to Kuchta *et al*. (2012) only one species of the genus *Bothriocephalus* can be found in Africa and it is the introduced alien parasite, *B. acheilognathi*. The current species is not *B. acheilognathi*, because there are in fact more morphological differences when compared to the alien tapeworm (See page 147). The cestode species of the current study was also found in the Okavango River System, which is still a pristine system where no introduced alien fish species present (Basson & van As 2002), thus *B. acheilognathi* does not occur in this system. The material from the Okavango can also not belong to the genus *Bothriocephalus*, because, according to Scholz (1997) this genus is not associated with African fish cestodes and occur naturally only in the Holarctic region and not in Africa. The species is preliminary assigned to the genus *Ichthybothrium*, as the genus is endemic to Africa and the species of the current study has more similarities with this genus.

Ichthybothrium sp. 1 is a small to medium sized worm, characterised by a small unarmed oval scolex with two shallow bothria. The genital pore is dorsal, median and the uterine pore ventral. The vitellaria are cortical and testes are both cortical and medullary in two lateral fields with relatively few testes per proglottid.

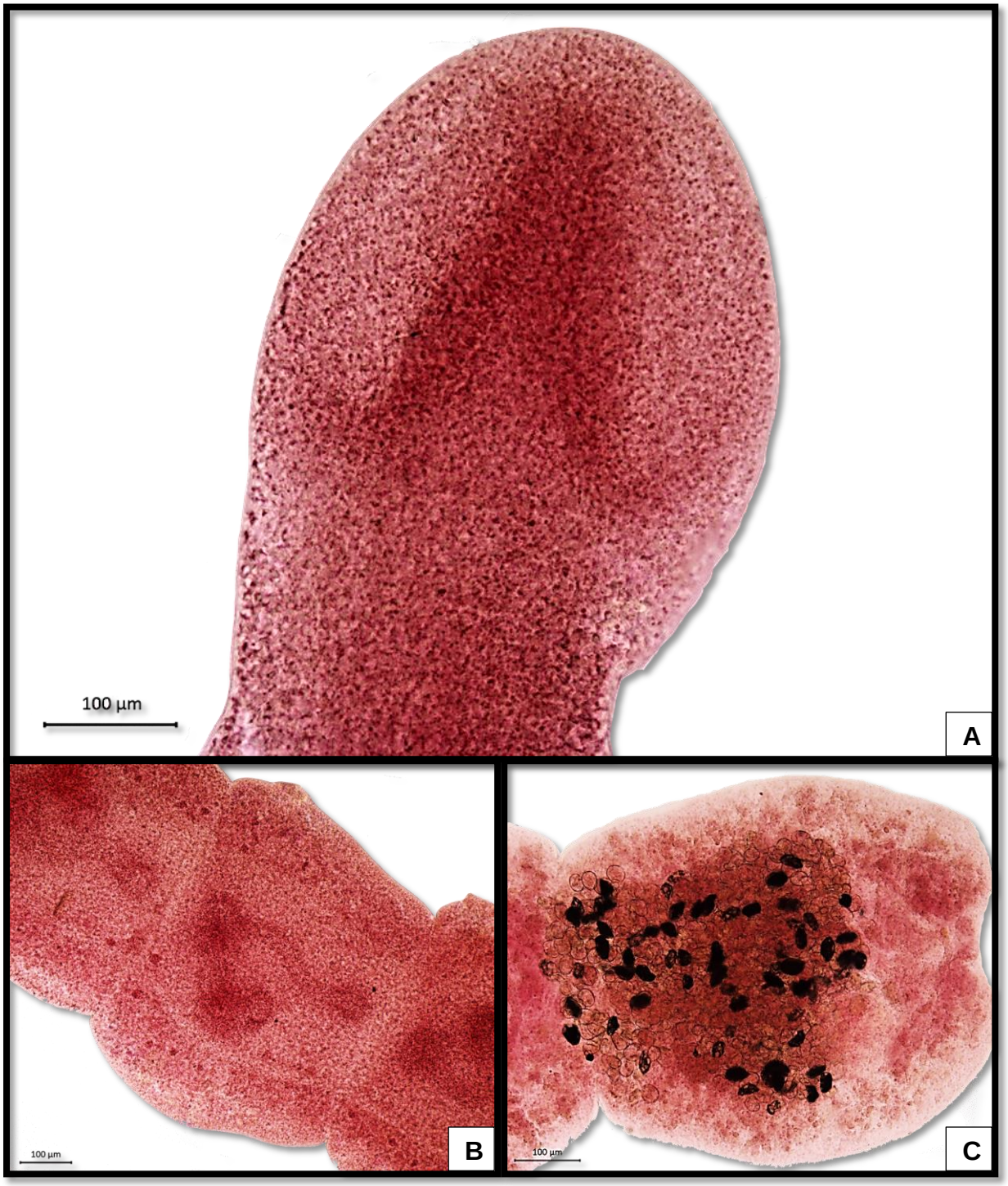

Figure 6.5: Light micrographs of *Ichthybothrium* Khalil, 1971 sp. 1 from the Okavango River System from *Barbus afrovernayi* Nichols & Boulton, 1927. **A**- The scolex, **B**- mature proglottids and **C**- gravid proglottids. Stained with Mayer's Hydrochloric Carmine.

Differential diagnosis:

Only one species of *Ichthybothrium* Khalil, 1971 was described and is restricted to African freshwaters, *I. ichthybori* Khalil, 1971.

Ichthybothrium ichthybori and *Ichthybothrium* sp. 1 was compared in Table 6.2. *Ichthybothrium ichthybori* has a very small thimble-shaped scolex that is narrower than the beginning of the strobila and *Ichthybothrium* sp.1 has a larger oval scolex that is larger than the beginning of the strobila, both have shallow bothria. *Ichthybothrium ichthybori* has a wider maximum width and has more testes than *Ichthybothrium* sp. 1 (Table 6.2). *Ichthybothrium* sp. 1 was found in a different fish family (Cyprinidae) than *I. ichthybori* (i.e. Distichodontidae).

The current species could possibly be a new species that belongs to the family Bothriocephalidae occurring in *B. afrovernayi* from the Okavango River System.

Family: Bothriocephalidae Blanchard, 1849

Genus: *Bothriocephalus* Rudolphi, 1808

Species: *Bothriocephalus acheilognathi* Yamaguti, 1934 (Figures 6.6 A-B, 6.7 A-D, 6.8 A-C)

Host: *Barbus bifrenatus* Flower, 1935

Additional host: *Barbus annectens* Gilchrist & Thompson, 1917

Locality: Pongola River System, Pomphuis (S26°90’52’83” E32°32’35’00”)

Additional localities: Pongola River System, Nyamiti Pan Outlet (S26°88’27’75” E32°31’13’93”), Ingwavuma River (S27°03’35’95” E32°18’26’54”)

Prevalence: 1/1 = 100% for *B. bifrenatus,* 3/13 = 23.1% for *B. annectens*

Intensity: 1 to 2 specimens per fish host

Site of infection: Small intestine

Material: 2013/09/11-22 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements and drawings were made using light microscopy from four specimens from the Pongola River System, stained

in Mayer's Hydrochloric Carmine or Weigert's Haematoxylin solution and mounted with Eukitt quick-hardening mounting medium. Two specimen were used for scanning electron microscope preparations and micrographs.

Description:

This is a rather large worm that is up to 34 mm long. This species was identified by its unique heart-shaped scolex that is unarmed with an apical disc; the scolex is also larger than other worms of the same genus (Figures 6.8 A-B). There are two long deep bothria in the scolex, one dorsal and one ventral in live specimens, however, they both appear to be lateral when mounted on a slide (Figures 6.7 A-B). External segmentation is present with proglottids wider than long (Hoffman 1980) (Figures 6.7 C-D). The genital pores are dorsomedian and the testes are medullary in two lateral fields. The cirrus is unarmed (Figure 6.8 C). The ovary is transversely elongated. A uterine sac is present and the uterine pore is ventral anterior to the genital pore. The eggs are operculated.

Differential Diagnosis:

The current species was identified to genus level by using keys on cestode parasites provided by Khalil *et al*. (1994). Only one species of the genus *Bothriocephalus* has been found in Africa, the alien *B. acheilognathi*. Various synonyms exist for this species, but molecular work and comparisons with type material by Kuchta *et al.* (2012) suggests that they are all the same species. The current species was compared to *B. acheilognathi*, previously known as *B. aegyptiacus*, from the Nile River that was found infesting *Barbus bynni* Forsskal, 1775 (Rysavy & Moravec 1975). The current species differs in total length of the specimens found by Rysavy & Moravec (1975), but *B. bynni* is a much larger fish (maximum length = 52 cm) than the small *Barbus* of the current study (Table 6.3). *Bothriocephalus acheilognathi* is a very versatile parasite and can adapt to the host size, therefore different lengths can be found of the same species (Kuchta *et al.* 2012). The description of the reproductive organs and scolex of *B. acheilognathi* is the same as the description of the current species.

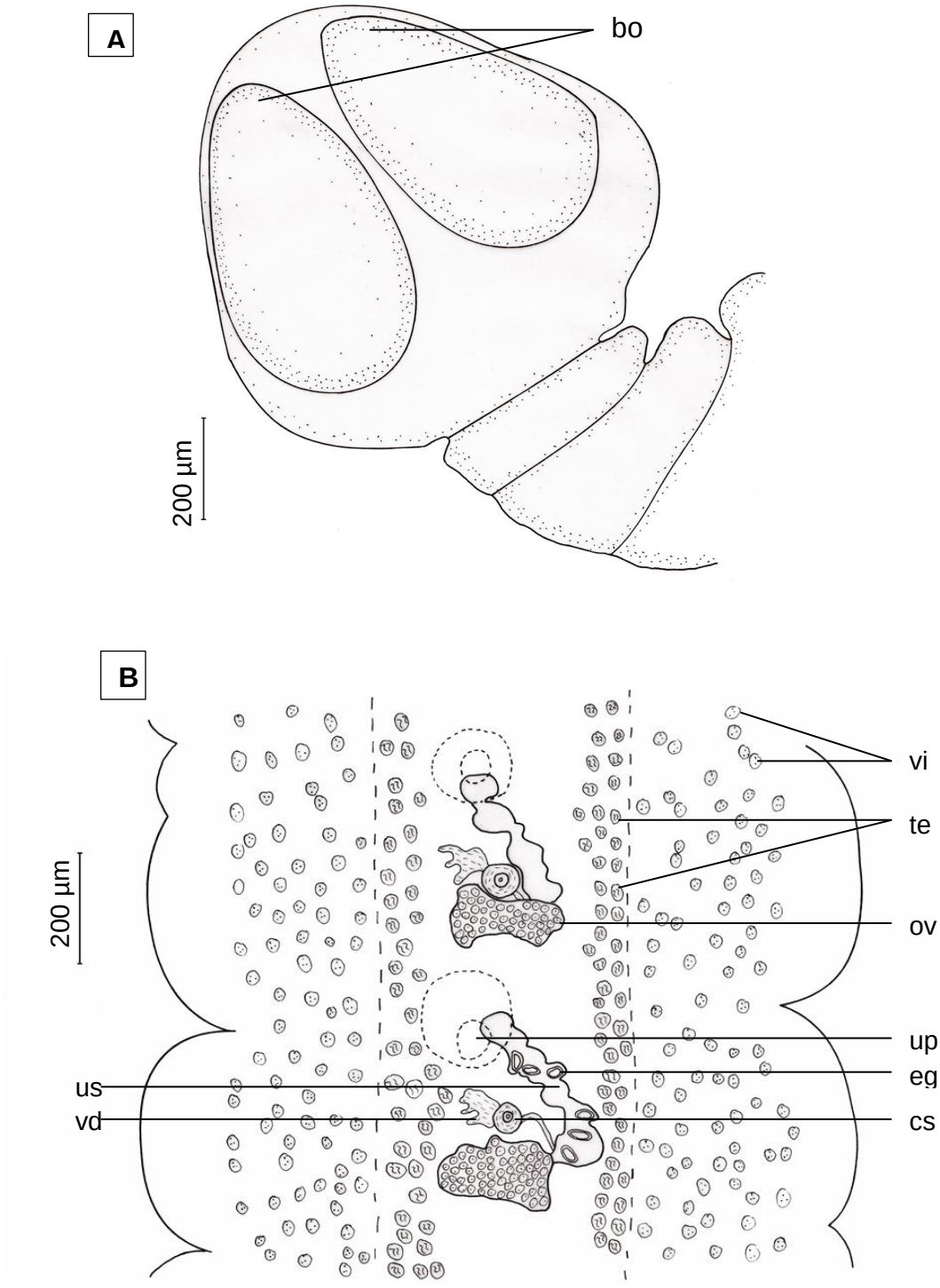

Figure 6.6: Microscope projection drawings of *Bothriocephalus acheilognathi* Yamaguti, 1934 from the Pongola River System from *Barbus bifrenatus* Flower, 1935. **A**- scolex with bo- bothria. **B**- Mature proglottids, cs- cirrus sac, eg- eggs, ov- ovary, te-testis, up- uterine pore, us- uterine sac, vd- vas deferens and vi- vitelline follicles.

The species of the current study possesses characters similar to *B. acheilognathi* and is assigned to this species. This is the first documented record of *B. acheilognathi* occurring in *B. bifrenatus* and *B. annectens* from the Pongola River System.

Table 6.3: Measurements of *Bothriocephalus acheilognathi* Yamaguti, 1934 from *Barbus bifrenatus* Flower, 1935 from the Pongola River System and a comparison with published descriptions of the same species from Rysavy & Moravec (1975). All measurements are given in micrometres unless otherwise stated.

Cestode	*Bothriocephalus acheilognathi* Yamaguti, 1934	*Bothriocephalus acheilognathi* Yamaguti, 1934
Host	*Barbus bifrineatus* Flower, 1935	*Barbus bynni*, Forsskal, 1775
Locality	Pongola System Current study	Nile River (Rysavy & Moravec 1975)
Total length (mm)	28.2-34.1	598
Maximum width (mm)	0.94-1.2	4
Scolex width	781.3-1000	1100
Mature proglottids		
Length	271.4-291.7	-
Width	687.5-820.7	-
Ratio*	2-3	7-8
Gravid proglottids		
Length	364.6-458.3	-
Width	708.3-1218.8	-
Ratio	2-3 wider	3-4
Testes		
Diameter	25.3-33.3	26-48
Number	102-112	140-200
Cirrus-sac		
Length	91.5-93.1	130-156
Ovary		
Length	175.4-203-3	480-540
Vitelline follicles		
Diameter	14.5-15.6	6-20
Eggs		
Length	54.4-54.6	34-46
Width	20.5-21.5	66

*Proglottids wider than long

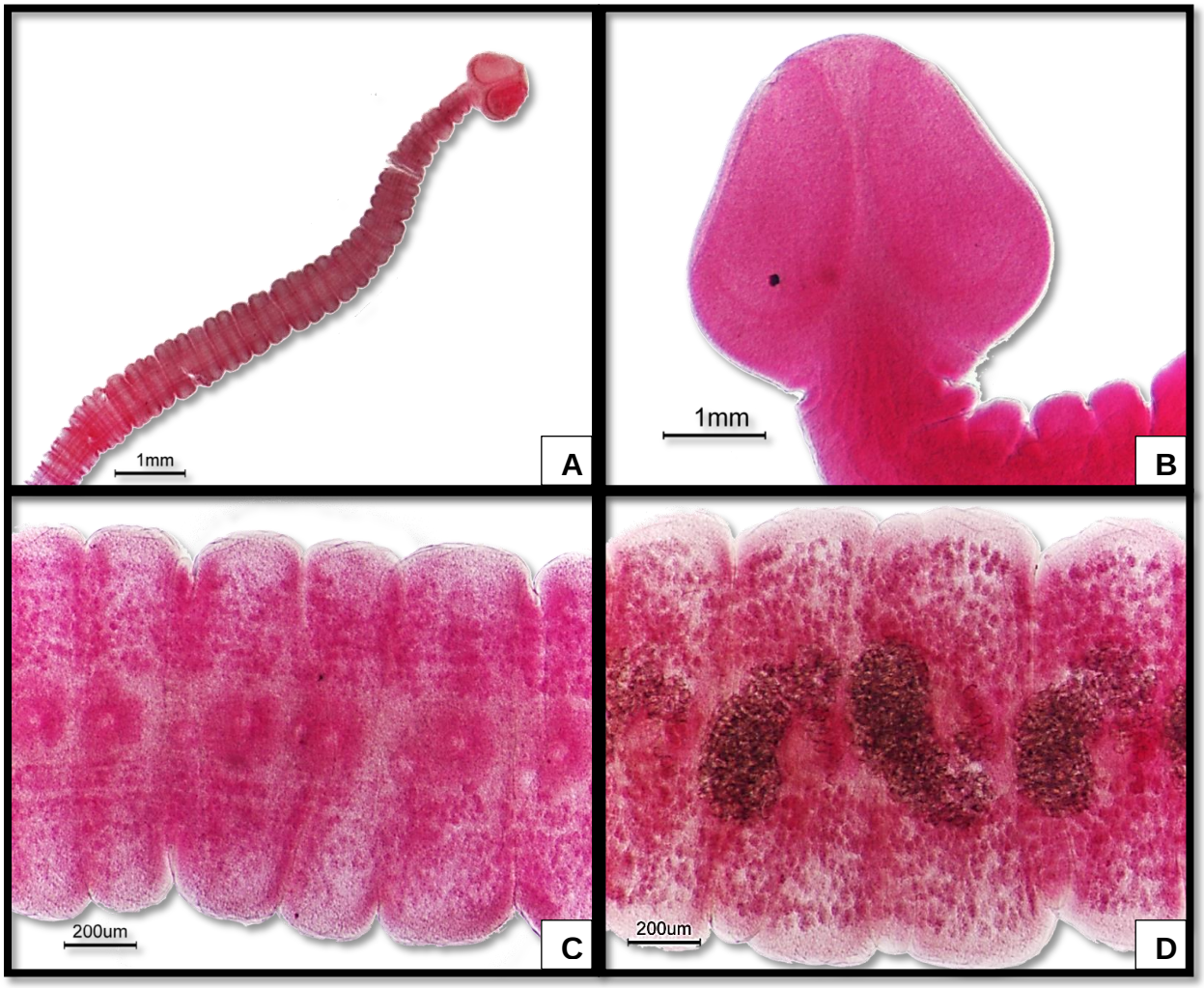

Figure 6.7: Light micrographs of *Bothriocephalus acheilognathi* Yamaguti, 1934 from the Pongola River System from *Barbus bifrenatus* Flower, 1935. **A**- Scolex and strobila, **B** - scolex, **C** - mature proglottids and **D** - gravid proglottids. Stained with Mayer's hydrochloric carmine.

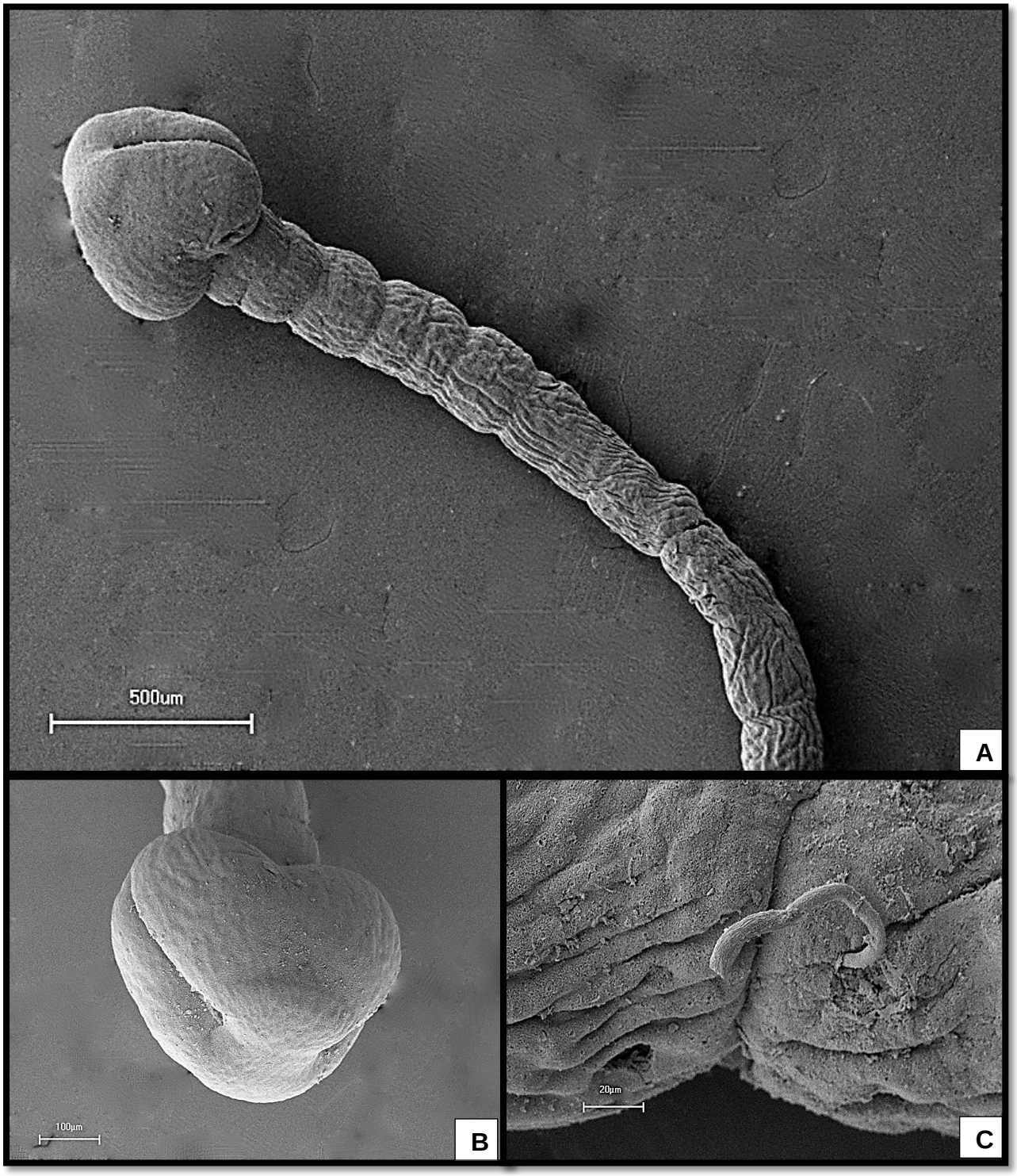

Figure 6.8: Scanning electron micrographs of *Bothriocephalus acheilognathi* Yamaguti, 1934 from the Pongola River System from *Barbus bifrenatus* Flower, 1935. **A**- Scolex and strobila, **B**- Scolex, **C**- enlarge view of the male cirrus.

Family: Diphyllobothriidae Lühe, 1910

Genus: *Ligula* Bloch, 1782

Species: *Ligula intestinalis* (Linnaeus, 1758) (Figures 6.9 A-B)

Host: *Barbus toppini* Boulenger, 1916

Additional host: *Barbus annectens* Gilchrist & Thompson, 1917 and *Barbus paludinosus* Peters, 1852

Locality: Pongola River System, Bumbe (S26°99'57'72" E32°30'05'33")

Additional localities: Pongola River System, Nyamiti Pan Outlet (S26°88'27'75" E32°31'13'93"), Ingwavuma River (S27°03'35'95" E32°18'26'54")

Prevalence and intensity: Presented in Table 6.4

Table 6.4: The prevalence and intensity of plerocercoids of *Ligula intestinalis* (L., 1758) in *Barbus* Cuvier & Cloquet, 1816 sp.

Host	Prevalence	%	Intensity range
B. toppini Boulenger, 1916	4/5	80%	1-2
B. paludinosus Peters, 1852	1/6	16.7%	4
B. annectens Gilchrist & Thompson, 1917	4/13	30.8%	1-2

Site of infection: Viscera

Material: 2013/09/14-14 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Micrographs were taken using light microscopy from one specimen from the Pongola River System, stained in Mayer's Hydrochloric Carmine and mounted with Eukitt quick-hardening mounting medium.

Description:

The present material of *L. intestinalis* is very large, measuring from 20 mm to 140 mm in length and a width of 2 mm to 5 mm (n=24). This is more or less the same sizes as the material collected by Mashego (1982) in Limpopo River, South Africa. The plerocercoid larvae are thin anteriorly and gradually become broader to reach

their maximum width near the posterior end. The bothria are absent and there are no signs of external segmentation in the larval stages(Figures 6.9 A-B), they only develop in the adult stage in the fish-eating birds (Mashego 1982).

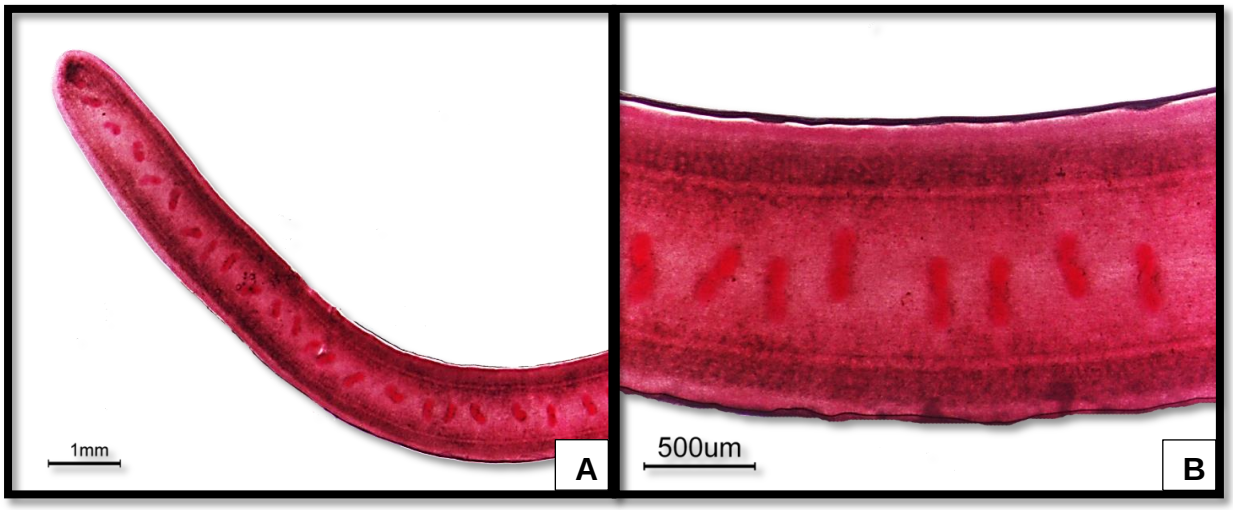

Figure 6.9: Light micrographs of a plerocercoid larva of *Ligula intestinalis* (Linnaeus, 1758) from the Pongola River System from *Barbus toppini* Boulenger, 1916. **A** – Strobili, **B** – immature proglottids. Stained with Mayer's hydrochloric carmine.

Differential Diagnosis:

Ligula intestinalis was identified to genus level by using keys of cestode parasites provided by Khalil *et al*. (1994). It was identified to species level with various publications that positively identified the plerocercoid larva infesting the genus *Barbus* in various localities in southern Africa (Mashego 1982; van As & Basson 1984 and Barson & Marshall 2003).

Ligula intestinalis are fleshy worms up to 400 mm long and 7-8 mm wide in the adult stage. The anterior end is bluntly pointed with a poorly developed scolex, bothria are represented by two tiny slits. This parasite has a complex life cycle that involves three hosts. The first intermediate host involves cyclopoid copepods, the fish as the second intermediate host and a piscivorous bird as the final host. The adults can be found in fish-eating birds and fleshy plerocercoids can be found in the body cavity of cyprinids (Khalil *et al.* 1994).

Ligula intestinalis has a high prevalence for infecting small barbs. Plerocercoid larvae infect and develop in the body cavity of the fish that grow into a very large

worm within the body cavity that causes swelling of the belly of its host (Figure 6.10). The worm can also change the behaviour of the fish, it increase the appetite, impedes locomotion and finally the behavioural change facilitates predation by birds (Dejen *et al.* 2006). The plerocercoid larvae can occupy the body cavity of the fish for several years. According to Dejen *et al.* (2006), the plerocercoid larvae can harm the gonadal development and cause sterilisation of infected fish. There have also been reports that the larvae have damaged the internal viscera and caused retarding maturity of the ovary.

Cestode parasites have been found in lakes and reservoirs throughout the world infecting various cyprinids, 26 different cyprinids have been reported as being the second intermediate host for *L. intestinalis* (Mashego 1982). In Africa, infections by *L. intestinalis* have been reported for the Nile River in Egypt, from Sudan and from the East African Lakes (Dejen *et al.* 2002). In southern Africa these parasites have been reported in various dams in South Africa (Mashego 1982; van As & Basson 1984) and Barson & Marshall (2003) reported the tapeworm from Zimbabwe; all the fish hosts were from the genus *Barbus.*

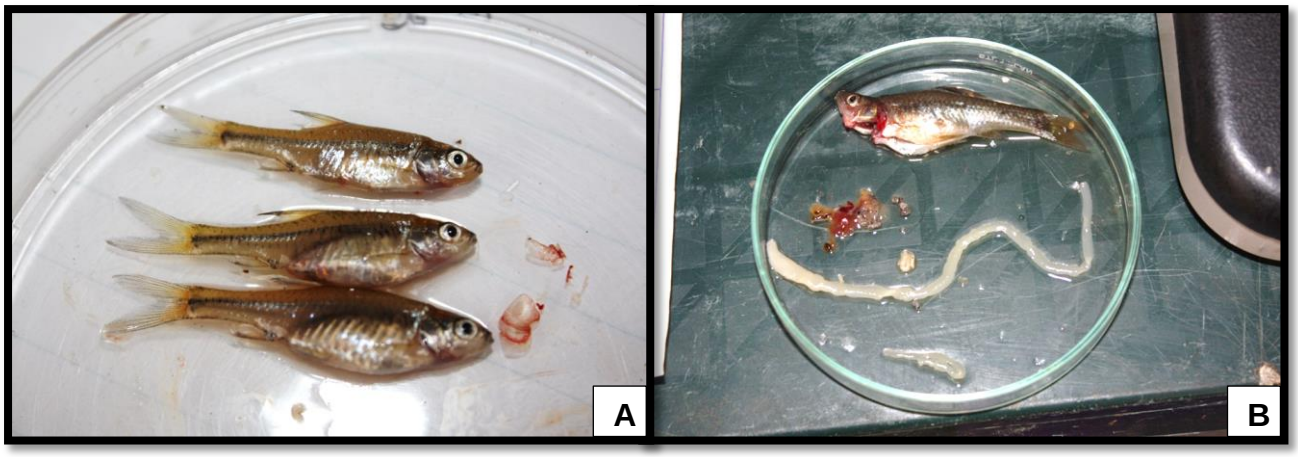

Figure 6.10: **A**- A Photo of *Barbus annectens* Gilchrist & Thompson, 1917 with plerocercoid larva of *Ligula intestinalis* (Linnaeus, 1758) in the viscera (the top fish was not infected and the bottom two were infected). **B**- A Photo of *Barbus paludinosus* Peters, 1852 that was dissected and was infected with the plerocercoid larva. Both these fish species were collected from the Pongola River System.

The heavy infection of the fish host is clearly visible due to the swollen bodies (Figure 6.10 A). Very large plerocercoid larvae, twice the length of the fish host were found in the fish viscera, with up to four worms per fish host (Figure 6.10 B).

The species of the current study possesses characters similar to *L. intestinalis* and is thus assigned to this species. This is the first documented record of *L. intestinalis* occurring in *B. toppini* and *B. annectens* from the Pongola River System.

Order: Cyclophyllidea van Beneden in Braun, 1900

Family: Dilepidae Raillie & Henry, 1909

Genus: *Parvitaenia* Burt, 1940 (Figures 6.11, 6.12 A-B, 6.13 A-E)

Host: *Barbus gurneyi* Günther, 1868

Additional host: *Barbus radiatus* Peters, 1853 and *Barbus unitaeniatus* Günther, 1866

Locality: Pongola River System, Nyamiti Pan Outlet (S26°88’27’75” E32°31’13’93”)

Additional localities: Pongola River System, Pomphuis (S26°90’52’83” E32°32’35’00”)

Prevalence: 2/10 = 20% for *B. gurneyi,* 1/1 = 100% for *B. radiatus*

Intensity: 1 to 2 specimens per fish host

Site of infection: Gall bladder

Material: 2013/09/13-05 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements and drawings were made using light microscopy from two specimens from the Pongola River System, stained in Mayer’s Hydrochloric Carmine and mounted with Eukitt quick-hardening mounting medium. Two specimens were used for scanning electron microscope preparations and micrographs.

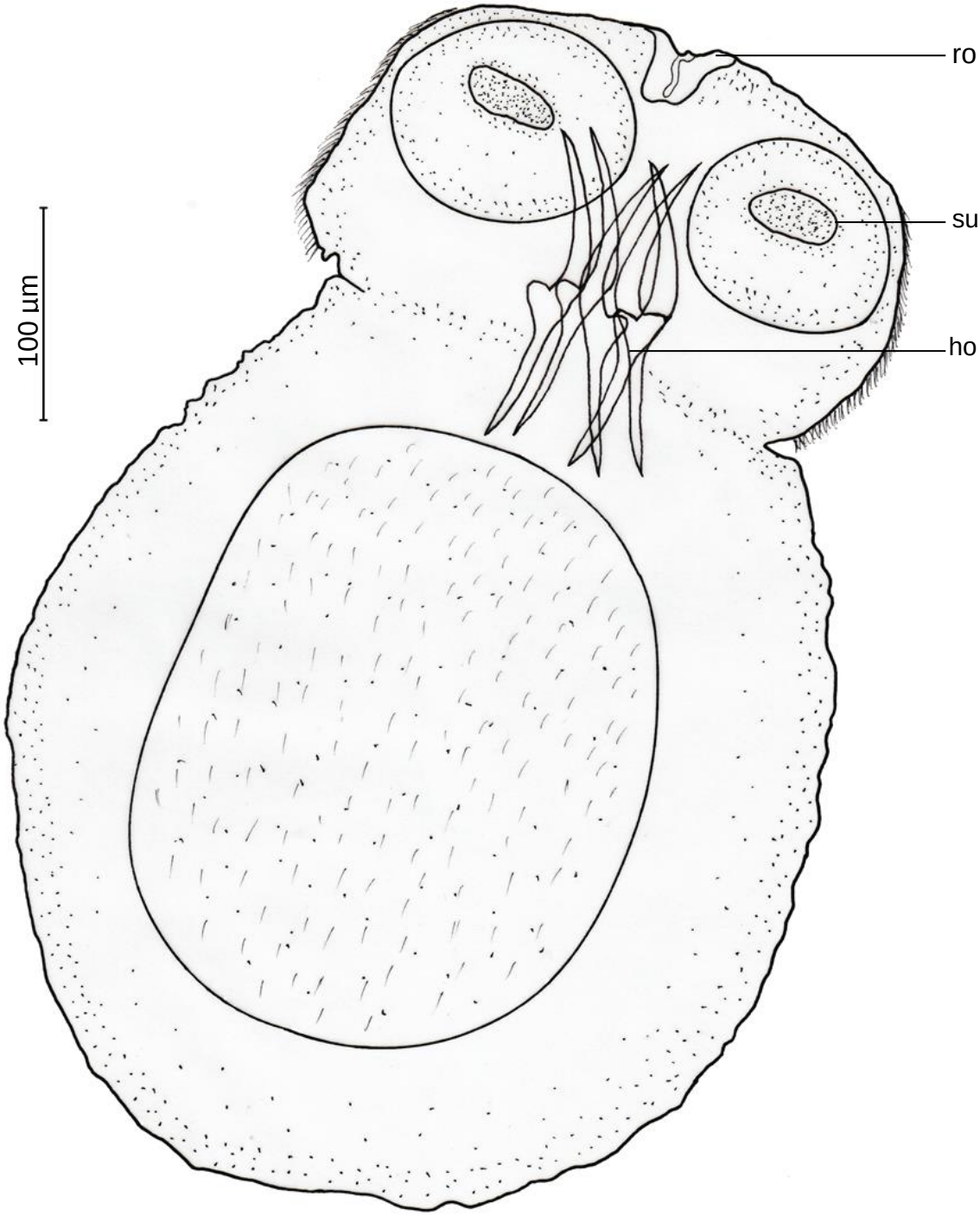

Figure 6.11: Microscope projection drawing of *Parvitaenia* Burt, 1940 from the Pongola River System from *Barbus gurneyi* Günther, 1868, ho – hooks, ro- rostellum and su- suckers.

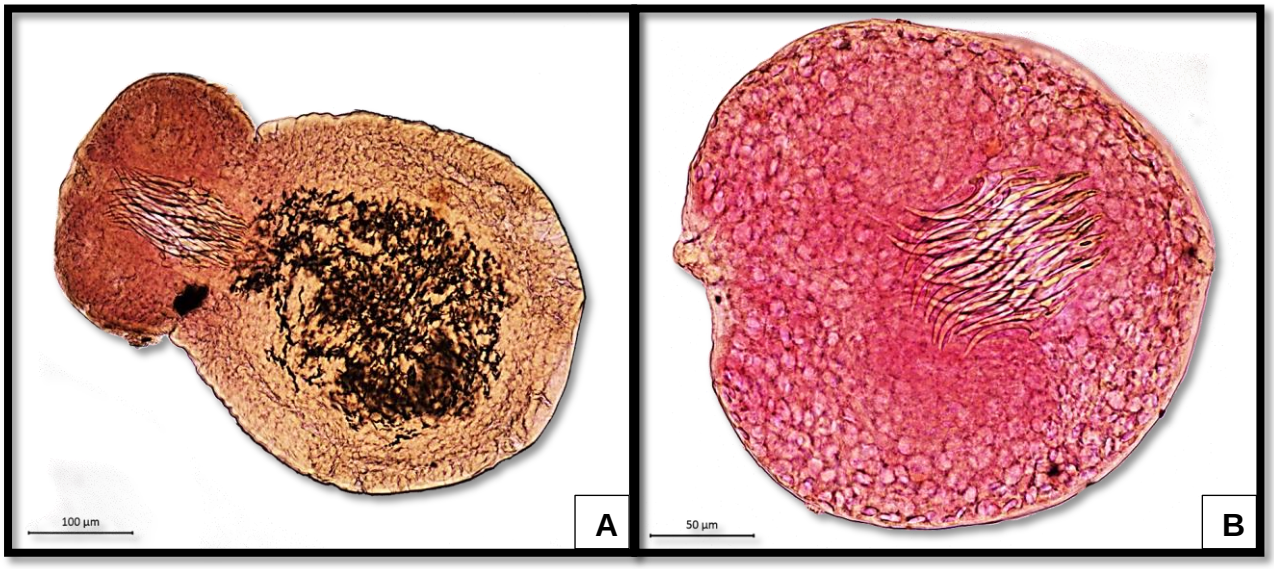

Figure 6.12: Light micrographs of dilepidid larva *Parvitaenia* Burt, 1940 from the Pongola River System from *Barbus gurneyi* Günther, 1868. **A** –Relaxed worm and **B** – contracted worm. Stained with Mayer's Hydrochloric Carmine.

Description:

This parasite was found in two different *Barbus* species in the Pongola River System in the gallbladder of the hosts. They were actively moving around within the gallbladder and were not enclosed in a cyst. The larvae can be divided into two parts, the scolex and the strobila. The scolex is armed with a rostellum with an uncountable number of hooks and a slit-like opening through which the rostellum is withdrawn or protruded (Figures 6.12 A-B). The scolex has four suckers slightly anteriorly and a hair-like tegument is present (Figures 6.13 A-E). The second part is the sac-like strobila with no segmentations visible and no reproductive organs visible.

Remarks:

It is difficult to assign a cestode parasite to a specific genus from just the larval form, but Mashego (1982) found similar cestode larvae from different *Barbus* hosts in the Limpopo System. According to Mashego (1982), the structure of the rostellum of the larva corresponds with the general characteristics of the family Dilepididae and that the hook pattern and form seem to be of the genus *Parvitaenia* (Figures 6.12 A-B).

The current species was compared with the species found by Mashego (1982) and the morphology is similar in both cases (Table 6.5).

Table 6.5: Measurements of *Parvitaenia* Burt, 1940 from *Barbus gurneyi* Günther, 1868 from the Pongola River System and a comparison with descriptions by Mashego (1982).

Cestode	*Parvitaenia* Burt, 1940	*Parvitaenia* Burt, 1940
Host	*Barbus gurneyi* Günther, 1868	*Barbus* sp. Cuvier & Cloquet, 1816
Locality	Pongola System Current study	Limpopo System (Mashego 1982)
Scolex		
Length	187.9	128-154
Width	244	153-200
Suckers		
Diameter	91.5-92.4	61-73
Rostellum		
Length	168.2	122-134
Width	91	73
Crown of hooks	53.6	61-68
Strobila		
Length	393.2	170-250
Width	344.4	232-242

Differential diagnosis:

Mashego (1982) believed that the parasite that was found in the Limpopo System is a dilepidid larva from the genus *Parvitaenia.* The main difference from the current study is that the site of infection is different. In Mashego's (1982) study, the parasites were found in an enclosed cyst, loosely attached in the body cavity and in the current study the parasites were found actively moving around in the gallbladder without being enclosed in a cyst. The dilepidid larva is also slightly larger in the current study (Table 6.5). The hook pattern, rostellum, suckers and strobila seem to be the same in both studies.

The species of the current study possesses similar characteristic hook shapes as the dilepidid larvae of the genus *Parvitaenia* and is thus assigned to this genus. This is the first documented record of *Parvitaenia* occurring in *B. gurneyi* from the Pongola River System.

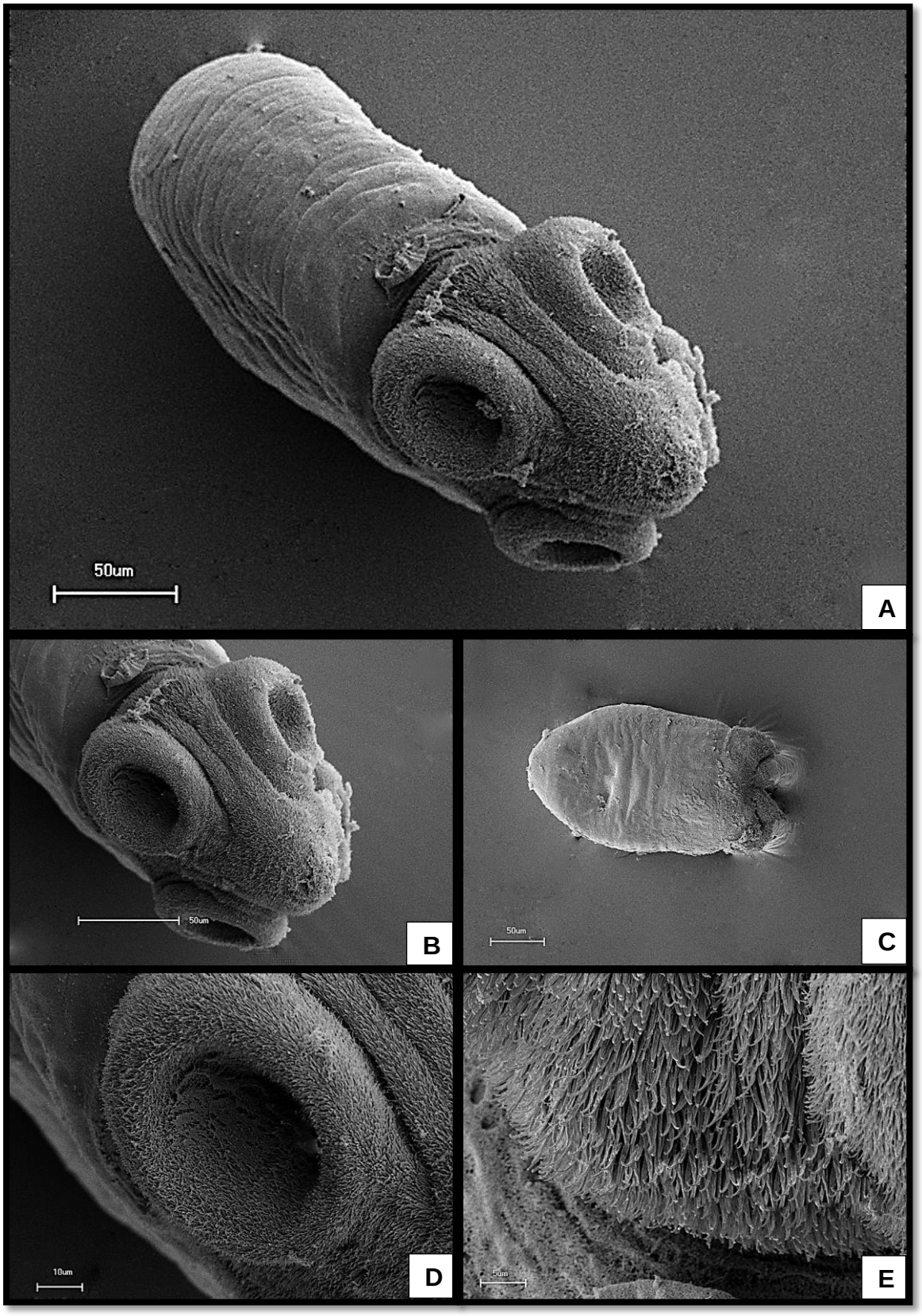

Figure 6.13: Scanning electron micrographs of *Parvitaenia* Burt, 1940 from the Pongola River System from *Barbus gurneyi* Günther, 1868. **A**- entire dilepidid larva, **B**- scolex, **C**- contracted dilepidid larva, **D**- sucker and **E**- hair-like tegument.

Class: Trematoda Rudolphi, 1808

Sub-class: Digenea Carus, 1863

Order: Strigeida La Rue, 1926

Superfamily: Clinostomoidea Lühe, 1901

Family: Clinostomidae Lühe, 1901

Subfamily: Clinostominae Lühe, 1901

Genus: *Clinostomum* Leidy, 1856

Species: *Clinostomum complanatum* (Rudolphi, 1819) (Figures 6.14, 6.15 A-E)

Host: *Barbus multilineatus* Worthington, 1933

Locality: Okavango River System, Shakawe (S18°42’93’85” E21°89’64’98”)

Prevalence: 3/15 = 20%

Intensity: 2 to 4 specimens per fish host

Site of infection: Viscera

Material: 2013/07/30-13 in the collection of the Aquatic Parasitology group at the Department of Zoology and Entomology at the University of the Free State.

Material examined: Detailed morphometric measurements and drawings were made using light microscopy from 2 specimens from the Okavango River System, stained with Van Cleave’s Haematoxylin stain and mounted with Eukitt quick-hardening mounting medium.

Description of larval form: (All measurements are in micrometres unless otherwise stated).

The character analysis based on morphology and taxonomy was used to examine the genus *Clinostomum* using Kanev *et al*. (2002).

Metacercariae move around freely when removed from the cysts. Body medium to large, elongate, measuring 3.51-4.26 mm in length and 1.11-1.17 mm wide at acetabulum level. Both ends are rounded (Figures 6.15 A, E). Oral sucker small (216.2-226.5 x 290.1-290.7), oval shaped (Figure 6.15 B). Acetabulum round, muscular, well developed and larger than oral sucker, measuring 734.5-789.5 x 700.3-734, situated 393.7-449.3 from oral sucker (Figure 6.15 C). Prepharynx measuring 66.3-71.3. Cirrus sac and genital pore lateral to anterior testis. Two testes are present, anterior testis round, measuring 94.5-125.2 x 144.1-147 and posterior testis Y-shaped, measuring 49.3-69.8 x 175.2 x 211.5. Both testes situated just posterior to middle body (Figure 6.15 D). Caeca long, simple, with more or less sinuous wall without long lateral branches or diverticula. Ovary intertesticular and smaller than cirrus, cirrus sac measuring 59.4-60.1 x 60.8-79.4. Uterus extending anteriorly intercaecal between acetabulum and cirrus sac. Vitelline follicles undeveloped and no eggs are present (Figure 6.14).

Remarks:

The current species resembles *Clinostomum tilapiae* described by Ukoli (1966) from different fish species from the genus *Tilapia* Smith, 1840. The species in the present study is similar to *C. tilapiae* in the position of the testes in relation to the body, the tubular uterine sac and the pouch which is larger than the ovary. The body size of the current species is also similar to the description of *C. tilapiae*.

The current species also resembles the morphology of the *C. tilapiae* given by Britz *et al.* (1984) and Jansen van Rensburg (2006) collected from *O. mossambicus* and *Oreochromis andersonni* (Castelnau, 1861), respectively. However, many problems were experienced in the past with diagnosis of species of the family Clinostomidae. According to Olivier *et al*. (2009), the problem was solved by synonimising 35 species of the genus *Clinostomum* Leidy, 1856 as one species, including *C. tilapiae* to *Clinostomum complanatum* (Rudolphi, 1819).

Mashego (1982) found two *Clinostomum* metacercariae species from six different *Barbus* spp. from the Limpopo System; the current species resembles his *Clinostomum* type 1. The current species is similar to *Clinostomum* type 1 in body length, the sizes of the oral sucker and acetabulum and the position of the

reproductive organs. *Clinostomum* type 1 also possesses characters of *Clinostomum complanatum* and can be assigned to this species.

The possible life cycle of the family Clinostomatidae involves a definitive host and two intermediate hosts. According to Britz *et al.* (1984) and Olivier *et al*. (2009), adults of *Clinostumum* sp. can be found in the oesophagus of piscivorous birds and the first intermediate stage in snails. The second intermediate stage can be found in the muscle, body cavity, gills and eye sockets of freshwater fish. It is believed that *C. complanatum* has the same life cycle.

The species of the current study possesses characters similar to *Clinostomum complanatum* and is thus assigned to this species. This is the first documented record of *C. complanatum* metacercariae occurring in *B. multilineatus* from the Okavango River System.

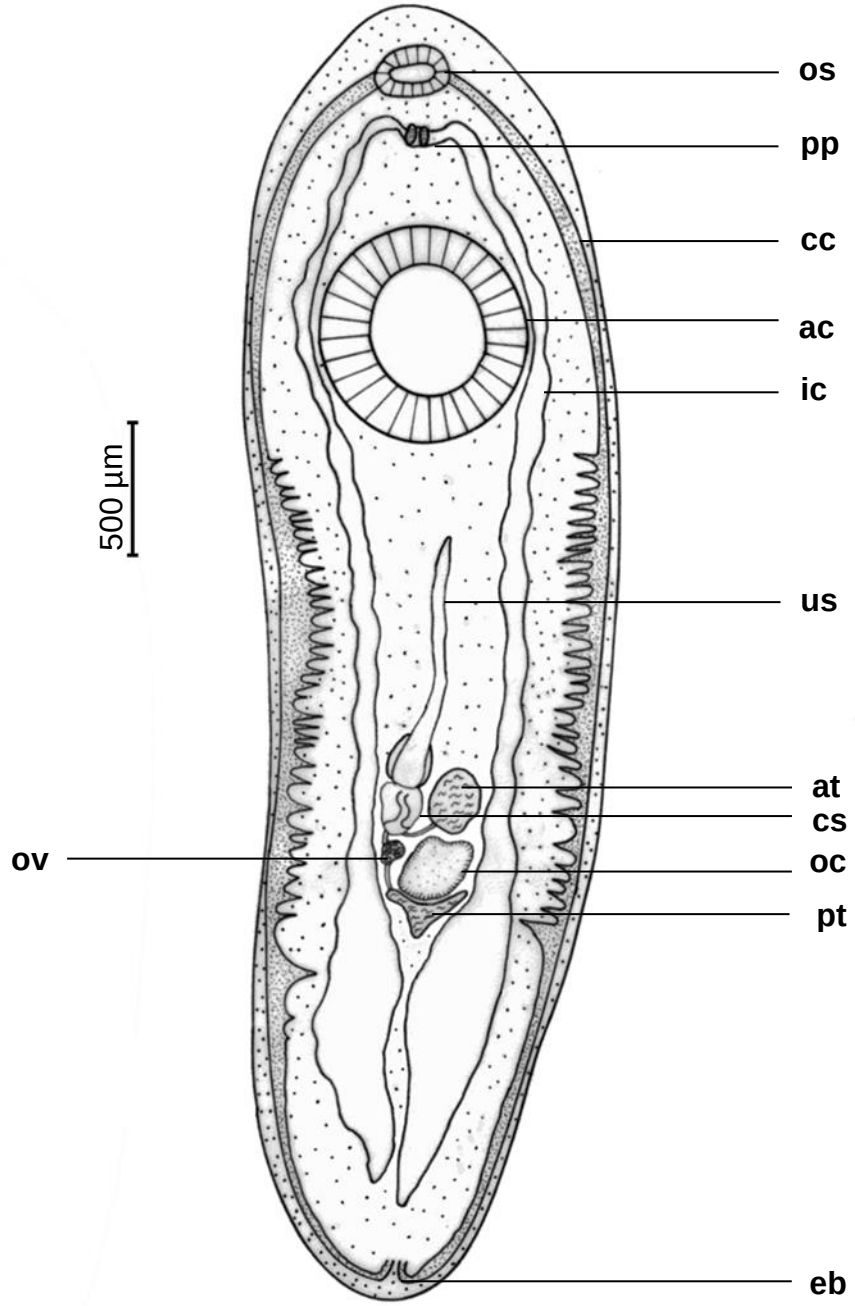

Figure 6.14: Microscope projection drawings of metacercaria *Clinostomum complanatum* (Rudolphi, 1819) from the Okavango River System from *Barbus multilineatus* Worthington, 1933, ac- acetabulum (ventral sucker), at- anterior testis, cc- collecting canal, cs- cirrus sac, eb- excretory blader, ic- intestinal caecum, oc- ootype complex, os- oral suckers, ov- ovary, pp- prepharynx, pt- posterior testis and us- uterine sac.

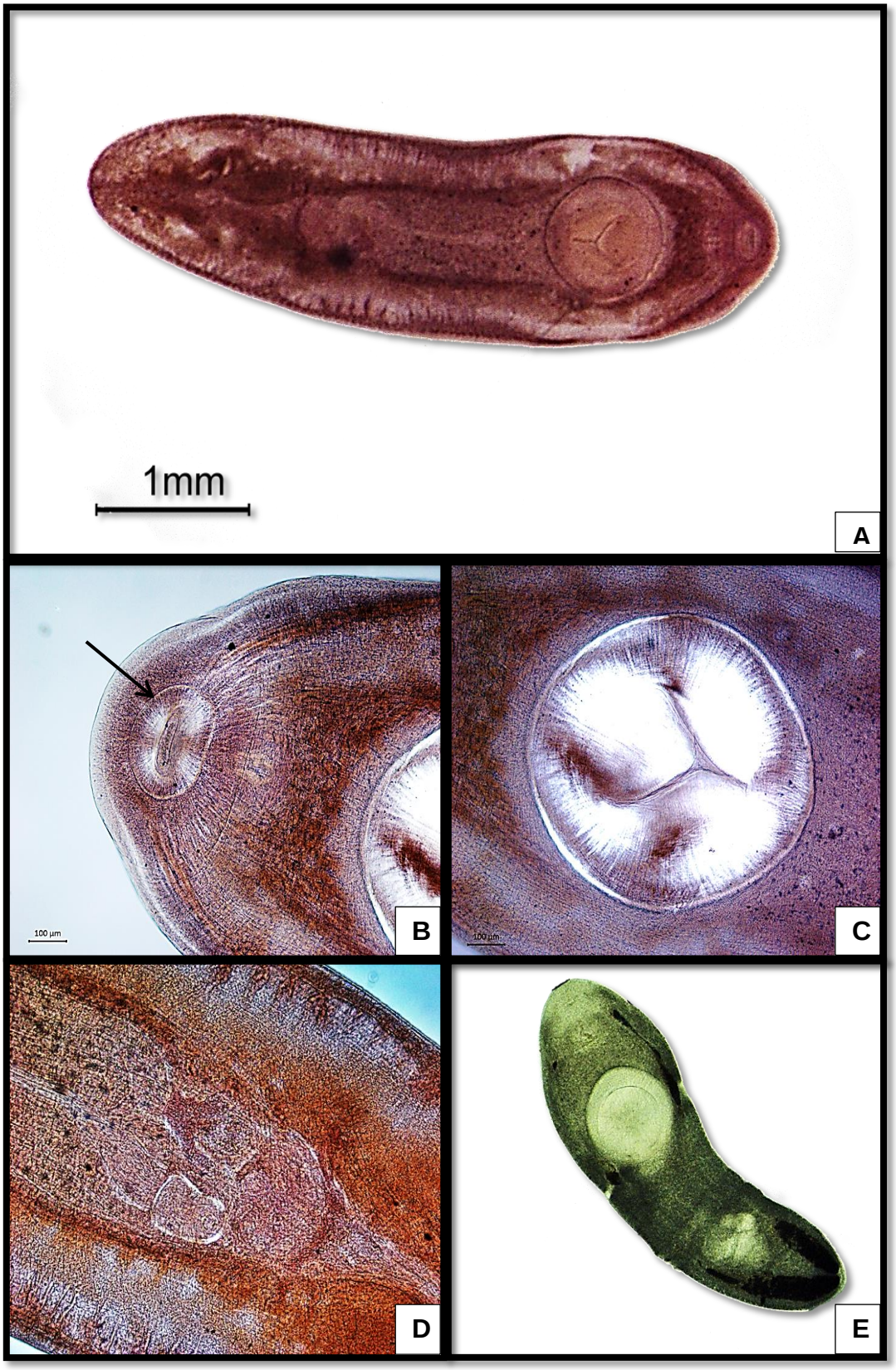

Figure 6.15: Light micrographs of metacercaria *Clinostomum complanatum* (Rudolphi, 1819) from the Okavango River System from *Barbus multilineatus* Worthington, 1933. **A** - Entire worm, **B** - oral sucker (arrow), **C** – acetabulum, **D** – reproductive organs and **E** – live specimen. **A** to **D** were stained with Van Cleave's Hematoxylin stain.

DISCUSSION

According to Jansen van Rensburg (2006) fish susceptibility to infection with helminth parasites differs and is dependent on the morphology, physiology and diet of the host. The endoparasite fauna therefore depends on the host's feeding. Fish belonging to the families Mochokidae and Clariidae show high parasite diversity, as they feed on almost anything, but on the other hand *Barbus* species have a restricted food source due to their morphology and physiology. As a result the endoparasite diversity of *Barbus* species is smaller.

Fish of the family Cyprinidae feed only on invertebrates and plant material (Skelton 2001) and therefore they host a low diversity of internal parasites. Cestode parasites use aquatic invertebrates as an intermediate host, which barbs feed on and therefore more cestodes can be found than trematodes. The barbs are the second intermediate host for some trematodes and therefore only larval trematodes were found, but it is also important to mention that barbs can also serve as final hosts.

Five different cestodes were found, three adult tapeworms and two larval stages. Two species of cestodes were found in low numbers in the Okavango River System. Zooplankton that acts as an intermediate stage for cestode parasites is only present in very low numbers in the Okavango River, due to the continuous flow and purity of the water. This might be the reason why low numbers of cestodes were found in the Okavango River System. No previous studies on the cestode parasites of the *Barbus* were conducted in Okavango River System and thus it is no surprise to have found two possible new species of tapeworms representing two different orders. The first species is from the genus *Proteocephalus* that belongs to the order Proteocephalidea and the second species is from the genus *Ichthybothrium* from the order Pseudophyllidea. This is also only the second species of the genus *Ichthybothrium* that has been recorded in Africa.

Three different species of cestodes were collected from the Pongola River System; one species was the alien Asian tapeworm. They parasitise cyprinid fish that were probably introduced to the river system via the common carp, *Cyprinus carpio*, that

were recently found in the Pongola Floodplain[4]. The other two cestode species were both larval forms, using the barbs as an intermediate host. *Ligula intestinalis* is a common plerocercoid tapeworm of small cyprinids that were found in the viscera of some barb species. They form large fleshy plerocercoid larvae that cause swelling of the belly, which makes them more susceptible for predation by a piscivorous bird that is the final host of the parasite. The fifth cestode that was found was the dilepidid larva of the genus *Parvitaenia* from the harsh environment of the barbs gallbladder.

Only one species of Trematoda was found from *Barbus* during the current study from the Okavango River System. This was the metacercaria larva *C. complanatum*; this species has 35 synonyms including *C. tilapae* that has been found infesting southern African fish. Only larval species of trematoda have been found infesting the genus *Barbus* in Africa (Khalil & Polling 1997), because they are the second intermediate host for species of trematodes.

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Chapter 7

Discussion

INTRODUCTION

This is one of only few studies in existence that provides taxonomic parasitic data of indigenous fish from the genus *Barbus* from southern Africa. This chapter is a discussion that combines the dissertation results and findings to understand the purpose of the study and answer questions that were proposed. During the current study, fish parasites from different fish species belonging to the genus *Barbus* were collected from three river systems, i.e. the Orange-Vaal, Okavango and Pongola river systems. All the parasites were collected, identified and provided with a taxonomic description.

The three study sites that were selected for the study are all part of the three largest river systems in southern Africa. The Okavango River System forms part of the larger Zambezi System, they are sometimes connected via the Magwegqana River. The Pongola River System forms part of the larger Limpopo River System. The Orange-Vaal River System is the longest river system in South Africa. These study sites therefore give an ideal representation of the genus *Barbus* and their parasites of southern Africa. The Zambezi and Limpopo river systems have previously been the main two systems for the study of fish parasites in southern Africa, with fewer studies conducted in Okavango and Pongola river systems. The current study adds new information to the existing parasitic data from southern Africa.

ALIEN PARASITES

The three river systems were used to study infections of alien parasites. The Okavango River System is a pristine river system that has so far not been exposed to introductions or translocated fish species and is affected by very few human impacts (Basson & van As 2002). On the other hand, the Orange-Vaal River System has various invasive species that were introduced, along with their parasites and the river has also been negatively affected by human impacts in the form of agriculture and pollution for many years. The Pongola River System was an almost untouched river system, but has been affected by the construction of the Pongolapoort Dam, and lately also the introduction of alien fish. The three river systems can be compared to a pristine river, (Okavango River System) to a river that has recently

been introduced with alien fish (Pongola River System) and to a river that has been affected by alien fish for a long time (Orange-Vaal River System).

The results of the study show that the Okavango River System can still be considered pristine in terms of the genus *Barbus*, all the parasites that were currently found are indigenous to the system. *Cyprinus carpio* has only been introduced to the Pongola Floodplains. Already alien fish parasites usually associated with *C. carpio*, were present in indigenous fish examined during this study. This therefore confirms the first positive identification of *Bothriocephalus acheilognathi* in the Pongola Floodplains infecting indigenous fish species, such as *Barbus bifrenatus* and *B. annectens.* Very little data was recorded from the Orange-Vaal River System from the barbs, as only a few specimens were collected. Ten different dams in the Free State Province of South Africa were investigated for *Barbus* species and very few specimens were found. There are only three species of the genus *Barbus* that occur in the Free State and only two species with very few individual parasites were found. This could be because these fish species do not occur in the dam habitat where the data was collected or these ecologically sensitive species population size has declined due to the effect of alien fish, alien parasites and human pollution in the river system. More information is needed to determine the cause of the absence of specimens in the Free State dams.

No other alien parasites, except *B. acheilognathi*, were found infecting the genus *Barbus* in southern Africa. However, other alien parasites have been recorded from the Orange-Vaal and Pongola River Systems parasitising cyprinid fishes during the current study, i.e. the crustacean parasites *Argulus japonicus* and *Lernaea cyprinacea*. The reason why they did not parasitise the genus *Barbus* is unknown, a possible reason could be that both these parasites are large, and possibly too large for the small *Barbus* species. *Trichodinella epizootica* was thought to be an alien parasite from Europe, but during the current study this species was found on the gills of *Barbus radiatus* from the Okavango River System. This system has no introductions or translocations and therefore *T. epizootica* cannot be an alien parasite and is indigenous to southern Africa or maybe represents a cosmopolitan species, but a controversy exists whether this parasite is the same as the European *T. epizootica*, or rather a different species. Although the Okavango River System is

still considered to be pristine, recently Huchzermeyer & van der Waal (2012) reported epizootic ulcerative syndrome (EUS) from the Okavango Delta and McHugh *et al.* (2014) reported this disease from *Barbus haasianus* and *B. unitaeniatus* from Lake Liambezi in the northern part of Namibia. According to McHugh *et al*. (2014), epizootic ulcerative syndrome is caused by an infection of the oomycete, *Aphanomyces invadans* David & Krik, 1997 and does not seem to be species specific, this disease has been reported in the Okavango River System and is known to infect *Barbus*, and that is a great concern for the system.

BARBUS **RECLASSIFICATION**

As previously explained in the introduction (Chapter 1), using the genus name *Barbus* is not valid for the southern African barbs, but only for certain fish genera from Europe. The fish taxonomy from Africa is therefore not accurate and the barbs are in transition, to be relocated to a new genus. The small barbs from southern Africa that are less than 200 mm long, all seems to belong to the same genus, but there are larger fish in the rest of Africa that are also currently placed in the genus *Barbus* and appear not to belong to the same genus as the small barbs from southern Africa, they may belong to the genus *Labeobarbus*. These larger fish are also not parasitised by the same parasites as the small barbs and therefore the parasites that were found during the study were only compared with known species from southern Africa from small barbs. African fish taxonomy requires attention and the fish species need to be re-allocated to their correct genera. When the fish taxonomy of Africa is sorted out, the fish parasites can then be compared more effectively to related fish species. In the case from *Barbus* monogeneans, which can be highly host specific fish parasites, it is irrelevant to compare all the known African monogeneans of the genus *Barbus* to the species that were found in the current study, unless the taxonomy of the fish species is understood. It is no easy task to relocate the small African barbs to a new genus, but the most important factor for planning this is that the morphology of the species should be used hand in hand with molecular data to give the most accurate descriptions of species and genera.

PARASITE DISTRIBUTION AND HOST SPECIFICITY

During the current study, 23 different parasite species from different parasite groups were identified from 18 *Barbus* species. Considering the constraints of small sample sizes and few sampling localities, the data may provide some support to a hypothesis of higher parasite diversity correlating to geographical range, in this case *Barbus radiatus* and *B. paludinosus*. *Barbus radiatus* was found in both the Okavango and Pongola river systems, and eight different parasites were found infesting this fish species. *Barbus paludinosus* was found in all three of the study sites and seven different parasites were found infesting this fish species (Table 7.2).

Dactylogyrus dominici was also found in both the Pongola and Orange-Vaal River Systems during the current study, and was previously found in the Okavango River System by Christison (2002) from the same fish, *B. paludinosus*. *Trichodina kazubski* was also found in the Okavango and Pongola river systems from four different barbs. These two parasites have a wide distribution in southern Africa, but are only known to parasitise *Barbus* hosts. *Tripartiella macrosoma* and *T. lechridens* was found in the Pongola and in the Limpopo river systems by Basson & van As (1987) and appears therefore to be restricted to these systems only (Table 7.1).

Myxobolus paludinosus and *D. dominici* were previously found on *B. paludinosus* by Reed *et al*. (2002) and Mashego (1983), respectively, and the same two species were found on the same host during the current study, and thus these species are most likely host specific for *B. paludinosus*. These parasite distributions are therefore determined by the distribution pattern of their hosts.

Ligula intestinalis and *Trichodina heterodentata* were previously known to parasitise different fish species from different fish families and they were also found in *Barbus* species in the current study, and for this reason they are not regarded as family specific and can be found on different fish families, and in the case of *T. heterodentata* even on amphibians (Kruger *et al*. 1993).

Table 7.1: Parasite / host checklist for the genus *Barbus* Cuvier & Cloquet, 1816 collected during the current study.

No.	Species	*Barbus* Hosts	Locality
	***Trichodina* Raabe, 1959**		
1	*T. kazubski* van As & Basson, 1989	*Barbus afrovernayi*	Okavango
		Barbus radiatus	Okavango
		Barbus paludinosus	Pongola
		Barbus toppini	Pongola
2	*T. heterodentata* Duncan, 1977	*Barbus radiatus*	Okavango
		Barbus haasianus	Okavango
	***Tripartiella* Lom, 1963**		
3	*T. macrosoma* Basson & van As, 1987	*Barbus toppini*	Pongola
		Barbus paludinosus	Pongola
		Barbus afrohamiltoni	Pongola
		Barbus gurneyi	Pongola
4	*T. lechridens* Basson & van As, 1987	*Barbus afrohamiltoni*	Pongola
		Barbus gurneyi	Pongola
	***Trichodinella* Šrámek-Hušek, 1953**		
5	*T. epizootica* (Raabe, 1950) Šrámek-Hušek, 1953	*Barbus radiatus*	Okavango
	***Apiosoma* Blanchard, 1855**		
6	*A. caulata* Viljoen & van As, 1985	*Barbus trimaculatus*	Pongola
7	*A. phiala* Viljoen & van As, 1985	*Barbus radiatus*	Okavango
	***Myxobolus* Bütschli, 1882**		
8	*M. nyongana* (Fomena, Bouix, & Birgi, 1985) Fomena & Bouix, 1997	*Barbus radiatus*	Okavango
		Barbus multilineatus	Okavango
9	*Myxobolus* sp.1	*Barbus radiatus*	Okavango
10	*M. oloi* Fomena, & Bouix, 1994	*Barbus radiatus*	Okavango
		Barbus haasianus	Okavango
11	*Myxobolus* sp. 2	*Barbus paludinosus*	Pongola
12	*M. paludinosus* Reed, Basson & van As, 2002	*Barbus paludinosus*	Pongola
13	*M. etsatsaensis* Reed, Basson & van As, 2002	*Barbus afrohamiltoni*	Pongola
14	*M. heterosporus* Baker, 1963 type 2	*Barbus afrohamiltoni*	Pongola
	***Dactylogyrus* Diesing, 1850**		
15	*D. dominici* Mashego, 1983	*Barbus paludinosus*	Pongola+Orange
16	*Dactylogyrus* sp.1	*Barbus fasciolatus*	Okavango
		Barbus afrovernayi	Okavango
17	*Dactylogyrus* sp.2	*Barbus paludinosus*	Pongola
18	*Dactylogyrus* sp.3	*Barbus afrohamiltoni*	Pongola
	***Proteocephalus* Weinland, 1858**		
19	*Proteocephalus* sp. 1	*Barbus miolepis*	Okavango
		Barbus multilineatus	Okavango
		Barbus afrovernayi	Okavango
	***Ichthybothrium* Khalil, 1971**		
20	*Ichthybothrium* sp.1	*Barbus afrovernayi*	Okavango
	***Bothriocephalus* Rudolphi, 1808**		
21	*B. acheilognathi* Yamaguti, 1934	*Barbus bifrenatus*	Pongola
		Barbus annectens	Pongola
	***Ligula* Bloch, 1782**		
22	*L. intestinalis* (Linnaeus, 1758)	*Barbus toppini*	Pongola
		Barbus annectens	Pongola
		Barbus paludinosus	Pongola
	***Parvitaenia* Burt, 1940**		
23	*Parvitaenia* sp.	*Barbus gurneyi*	Pongola
		Barbus radiatus	Pongola
		Barbus unitaeniatus	Pongola
	***Clinostomum* Leidy, 1856**		
24	*C. complanatum* (Rudolphi, 1819)	*Barbus multilineatus*	Okavango

Table 7.2: Host / parasite checklist for the genus *Barbus* Cuvier & Cloquet, 1816 collected during the current study.

No.	Fish Host	Parasites	Locality
1	***Barbus anoplus* Weber, 1897**	-	
2	***Barbus gurneyi* Günther, 1868**	*Tripartiella macrosoma* Basson & van As, 1987	Pongola
		Parvitaenia sp.	Pongola
		Tripartiella lechridens Basson & van As, 1987	Pongola
3	***Barbus annectens* Gilchrist & Thompson, 1917**	*Bothriocephalus acheilognathi* Yamaguti, 1934	Pongola
		Ligula intestinalis (L., 1758)	Pongola
4	***Barbus unitaeniatus* Günther, 1866**	*Parvitaenia* sp.	Pongola
5	***Barbus bifrenatus* Fowler, 1935**	*Bothriocephalus acheilognathi* Yamaguti, 1934	Pongola
6	***Barbus viviparous* Weber, 1897**	-	
7	***Barbus barnardi* Jubb, 1965**	-	
8	***Barbus toppini* Boulenger, 1916**	*Trichodina kazubski* van As & Basson, 1989	Pongola
		Tripartiella macrosoma Basson & van As, 1987	Pongola
		Ligula intestinalis (L., 1758)	Pongola
9	***Barbus fasciolatus* Günther, 1868**	*Dactylogyrus* sp. 1	Okavango
10	***Barbus radiatus* Peters, 1853**	*Trichodina kazubski* van As & Basson, 1989	Okavango
		Trichodina heterodentata Duncan, 1977	Okavango
		Trichodinella epizootica (Raabe, 1950) Šrámek-Hušek, 1953	Okavango
		Apiosoma phiala Viljoen & van As, 1985	Okavango
		Myxobolus nyongana (Fomena, Bouix, & Birgi, 1985) Fomena & Bouix, 1997	Okavango
		Myxobolus sp. 1	Okavango
		Myxobolus oloi Fomena, & Bouix, 1994	Okavango
		Parvitaenia sp.	
11	***Barbus haasianus* David, 1936**	*Trichodina heterodentata* Duncan, 1977	Okavango
		Myxobolus oloi Fomena, & Bouix, 1994	Okavango
12	***Barbus trimaculatus* Peters, 1952**	*Apiosoma caulata* Viljoen & van As, 1985	Pongola
13	***Barbus poechii* Steindachner, 1911**	-	
14	***Barbus miolepis* Boulenger, 1902**	*Proteocephalus* sp. 1	Okavango
15	***Barbus multilineatus* Worthington, 1933**	*Myxobolus nyongana* (Fomena, Bouix, & Birgi, 1985) Fomena & Bouix, 1997	Okavango
		Proteocephalus sp. 1	Okavango
		Clinostomum complanatum (Rudolphi, 1819)	Okavango
16	***Barbus afrovernayi* Nichols & Boulton, 1927**	*Trichodina kazubski* van As & Basson, 1989	Okavango
		Trichodina heterodentata Duncan, 1977	Okavango
		Dactylogyrus sp. 1	Okavango
		Proteocephalus sp. 1	Okavango
		Ichthybothrium sp. 1	Okavango
17	***Barbus paludinosus Peters, 1852***	*Trichodina kazubski* van As & Basson, 1989	Pongola
		Tripartiella macrosoma Basson & van As, 1987	Pongola
		Myxobolus sp. 2	Pongola
		Myxobolus paludinosus Reed, Basson & van As, 2002	Pongola
		Dactylogyrus dominici Mashego, 1983	Pongola+ Orange
		Dactylogyrus sp. 2	Pongola
		Ligula intestinalis (L., 1758)	Pongola
18	***Barbus afrohamiltoni* Crass, 1960**	*Tripartiella macrosoma* Basson & van As, 1987	Pongola
		Tripartiella lechridens Basson & van As, 1987	Pongola
		Myxobolus etsatsaensis Reed, Basson & van As, 2002	Pongola
		Myxobolus heterosporus Baker, 1963 type 2	Pongola
		Dactylogyrus sp. 3	Pongola

PARASITE INFECTIONS

During the parasitic investigation, most of the parasites that were found seemed to cause no harm to the fish species in any way and occurred in very low intensities. The only visible case of a parasite infection that affects the fish negatively was infections by *Ligula intestinalis*. This large cestode caused swollen bellies that impedes swimming efficacy and facilitates predation by birds and other fish; the fish were also easy to catch with scoop nets, possibly because of the infection. This parasite can have a negative impact on both aquaculture and aquarium fishes causing slow growth and even death. Although *Bothriocephalus acheilognathi* was found and is known to cause heavy infections in southern Africa indigenous fish in natural and in aquaculture conditions, only four fish specimens were found infected with a low number of worms per fish (one to two). To provide a conclusion about the parasite infections of *Barbus* fish in southern Africa under natural conditions, one could say that it is of least concern, but these parasites could cause problems in aquaculture and aquarium conditions.

HUMAN INFECTION

The fish from southern Africa that belong to the genus *Barbus* are not known to be a major food source for humans, but it is known that the people from Botswana and South Africa that live next to the Okavango and Pongola rivers, catch these small fish for food. Even though the consumption rates of *Barbus* fish appears negligible, it is still important to determine whether any parasites could be a potential threat for humans. Cestodes are known to infect humans, and *Ligula intestinalis* and *Parvitaenia* are both cestode larvae that are in their second intermediate stage in the fish host. *Ligula intestinalis* is known to infect piscivorous birds in their adult stage and it is unknown if this cestode will also infect humans, but in the case of *Parvitaenia* larvae, the adult stage of this species is unknown and further investigation is needed to determine if this parasite could infect humans. According to Grobbelaar (2011), a few reports exist of trematode larvae that have been found within the eyes of humans and it is also known that diplostomatid flukes can be found in warm-blooded animals that cause severe ocular damage and blindness.

Clinostomum complanatum that was found during the current study, has also previously been found in the pharyngeal wall of a human in Korea that used to eat raw freshwater fish, but no remarkable injury was found (Chung *et al*. 1995).

PARASITE DIVERSITY

Throughout the parasite investigation, 15 parasite species were found that had been previously described and eight species are new to science. Two species from the genus *Myxobolus*, three species from the genus *Dactylogyrus*, one species from the genus *Proteocephalus* and an unknown species from the genus *Parvitaenia* were found. One cestode species from *Barbus afrovernayi* was found in the Okavango River System that belongs to the family Bothriocephalidae Blanchard, 1849, the closest genus to this species is *Ichthybothrium* and it was presented as this genus in the description, but the material collected during this study possibly belongs to a new genus. More specimens need to be collected to give an accurate description of possibly a new genus. The 15 known parasites were found with new information on their descriptions, hosts and distributions. The study produced eight possible new parasite species and one possible new genus from the indigenous *Barbus* species from southern Africa. The information compiled in this dissertation expands our knowledge on the parasite fauna of the fish genus *Barbus* in southern Africa.

CONCLUDING REMARKS

Parasites do not have a negative impact on fish populations in natural conditions, on the contrary, indigenous parasites indicate a healthy ecosystem, and most of the fish parasites species that were found during the current study are indigenous to the hosts. The current study was a taxonomical investigation on fish parasites from the genus *Barbus* in southern Africa and this occurrence of fish parasites confirms a healthy distribution of natural fish parasites across the three study sites.

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ABSTRACT

The fish family Cyprinidae is represented by eight genera and 80 species in southern African rivers, consisting of a few large fish genera of the genus *Labeo* Ruppëll, 1836 and the yellow fish species *Labeobarbus* Cuvier, 1817. The majority of the cyprinids are, however, small e.g. *Barbus* Cuvier & Cloquet, 1816. Many species are endemic, often restricted to a single river, endangered and at least two species are critically endangered. This study focused on the parasite diversity of *Barbus* in southern Africa. Fish parasitological surveys were conducted within the Orange-Vaal, Pongola and Okavango River systems. During the study 23 different parasite species were identified from 18 *Barbus* fish species. These parasites belong to different parasite groups, i.e. Ciliophora Doflein, 1901, Myxozoa Grassé, 1970, Monogenea (Van Beneden, 1858), Cestoda and Trematoda Rudolphi, 1808. Two possible new myxozoans, three monogeneans and two cestodes, with one of the latter possibly new a genus, were found. The results showed low intensities of infections and no obvious pathology was observed in the infected fish. Parasites normally have little negative impact on their hosts under natural conditions, but the knowledge gathered is widely recognised for the ornamental fish trade and for the contribution to biological diversity. This study contributes to the biodiversity, taxonomy, distribution and abundance of the symbionts found associated with *Barbus* species.

OPSOMMING

Die vis verteenwoordigers van die familie Cyprinidae bestaan uit agt genera en 80 spesies in suidelike-Afrika se rivierstelsels wat uit ‘n paar groter visspesies van die genus *Labeo* Ruppëll, 1836 en die geelvis genus *Labeobarbus* Cuvier, 1817 bestaan. Die meerderheid van die verteenwoordigers van Cyprinidae is egter klein en behoort aan die genus *Barbus* Cuvier & Cloquet, 1816. Baie van hierdie kleiner spesies is endemies, soms beperk tot ‘n enkele rivier, bedreig en ten minste twee spesies is krities bedreig. Die studie fokus op die parasietdiversiteit van die genus *Barbus* in suidelike-Afrika. Visparasietopnames is in die Oranje-Vaal, Pongola en Okavangorivierstelsels uitgevoer. Tydens die studie is 23 verskillende parasiete van 18 *Barbus* visspesies geïdentifiseer. Die parasiete behoort aan verskillende parasietgroepe naamlik, Ciliophora Doflein, 1901, Myxozoa Grassé, 1970, Monogenea (Van Beneden, 1858), Cestoda & Trematoda Rudolphi, 1808. Twee moontlike nuwe verteenoordigers van die Myxozoa, drie Monogenea en twee Cestoda, waarvan laasgenoemde ‘n moontlike nuwe genus insluit, is geïdentifiseer. Die resultate het ‘n lae intensiteit van parasietbesmettings getoon, met geen duidelik sigbare patologiese effekte op geïnfekteerde gashere nie. Parasiete het gewoonlik ‘n baie klein negatiewe invloed op gashere onder natuurlike omstandighede en die inligting vanuit hierdie studie kan in die ornamentele visbedryf gebruik word. Die studie dra by tot die biologiese diversiteit, taksonomie, verspreiding en aantal simbionte wat geassosieer word met *Barbus* spesies in suidelike Afrika.

APPENDIX

The department of economic development, tourism and environmental affairs

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FREE STATE PROVINCE

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Cell:

This Permit is issued in terms of the Biodiversity Act 10 of 2004 (Threatened or Protected Species Regulations) and In Terms of Nature Conservation Ordinance no 8 of 1969, permission is hereby granted to the holder of this permit to;

General Permit

To catch,collect, capture, transport, convey and hold in possession freshwater fishes in stream, rivers, state dams and Nature Reserves in the Free State Province and keep indigenous and exotic fishes at the Biology building, University of the Free State for research purposes.

Permitee's Signature	**Approved** on behalf of the MEC department of economic development, tourism and environmental affairs .	
Expiry Date 2015-12-31 Return Permit After Expiry Date	Permit Number 01/16566 Cynthia Kgoboko	Date Issued 2013-01-28

metpppmnippr

DEPARTMENT OF TOURISM, ENVIRONMENTAL AND ECONOMIC AFFAIRS
2013 -01- 28
PRIVATE BAG X20601
BLOEMFONTEIN 9300
FREE STATE PROVINCE

Subject to the following conditions;

1. The permit is invalid unless all reqiurements of any other legislation in respect of the act mentioned are complied with.

2. This permit is invalid if it is not signed by the permitee and is not transferable.

3. This permit is only valid in its original form.

Special Conditions

1. A report must be submitted to the Free State Department of Economic Development, Tourism and Environmental Affairs highlighting the localities where the fish surveys and sampling were done, as well as which fish species and parasite species that were caught.