REVIEW OF AFRICAN DIPLOZOIDAE

By

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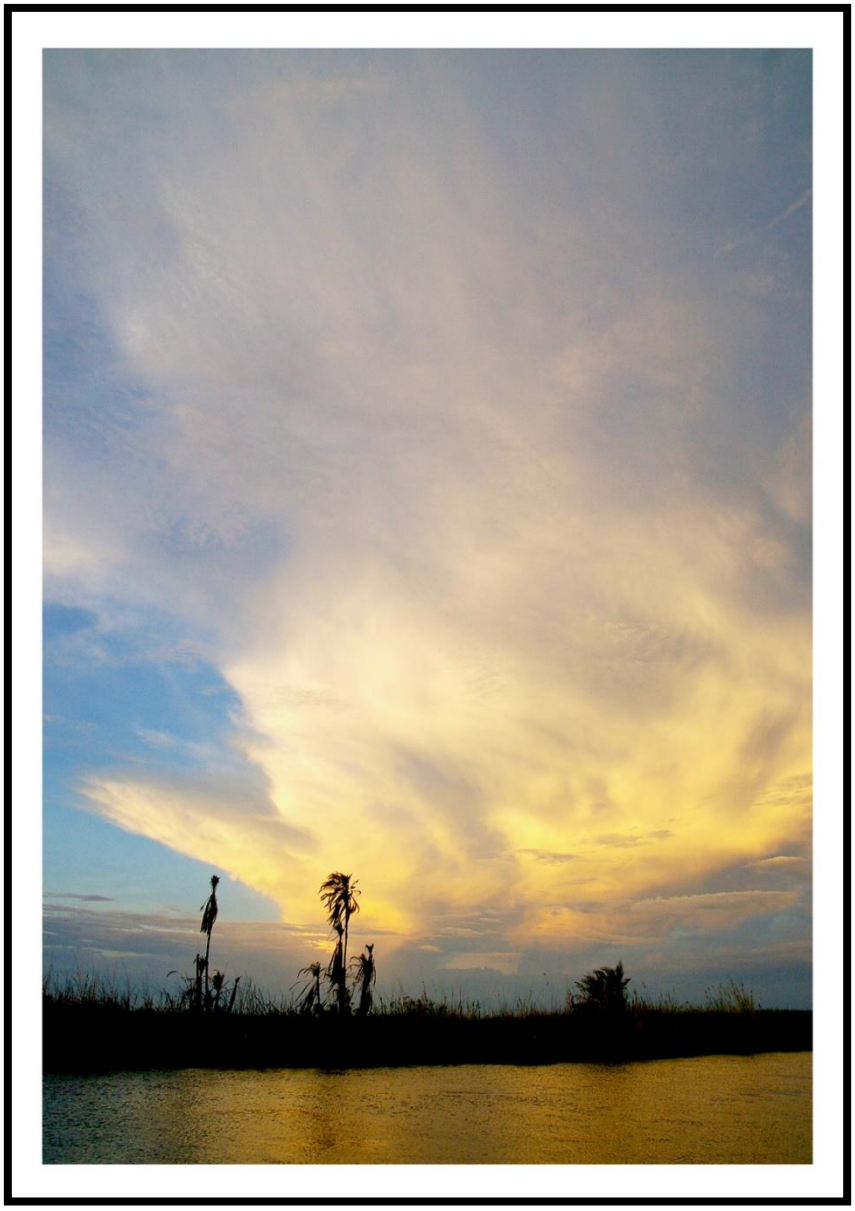


Introduction

The class Monogenea (Van Beneden, 1858) comprises diverse groups of parasitic flatworms. One of these groups of parasites belongs to the family Diplozoidae Palombi, 1949 that contains unique parasites, present on the gills of mostly cyprinid fish. Diplozoids are well known for their distinctive monogamous life style consisting of two hermaphroditic adult worms fused in permanent copula. The gradual decrease in morphological diversity of

diplozoids from east to west, suggests that these parasites have originated in Asia, after which they spread throughout Eurasia and Africa. In 1987, Le Brun and his co-workers expressed concern about the lack of valid morphometric criteria for the determination of diplozoid species. More than 20 years later, this concern is still valid and the identification of diplozoid species has lead to a great deal of confusion. Research done on the taxonomy of this group of parasites is presented in Chapter 2 and aims to clarify some of the confusion regarding this group of parasites.

Not much is known about the African diplozoid fauna, with only three species described from this continent, two from the genus *Paradiplozoon* Achmerov, 1974 and one from the African genus *Afrodiplozoon* Khotenovsky, 1980. These species exhibit high host specificity to their cyprinid fish hosts. In Chapter 3, the history of African cyprinids is discussed as well as the cyprinid hosts, found to be infested with diplozoids, from the Okavango and Orange-Vaal River Systems. The Aquatic Parasitology Team from the University of the Free State, collected a variety of fish parasites and various species of diplozoids during fish parasitological surveys over the past years. These surveys focussed on the Okavango River in Botswana, as well as various sites in the Orange-Vaal River System. The material and methods applied are presented in Chapter 4, briefly focusing on the study areas, fieldwork, microscopy and the morphological measurements used for species determination. The previously collected material together with material collected during the present study formed one of the major objectives of this study i.e. to identify and establish the taxonomic position of the collected diplozoids in order to broaden the knowledge on the biodiversity of African diplozoids. The results obtained during the study are presented in Chapter 5 with species descriptions on two newly described species and the proposal of a new identification key developed for the determination of African diplozoid families, genera and species. In Chapter 6, the problems concerning the taxonomic confusion of the family Diplozoidae are discussed as well as host specificity, and the various associated interactions. This is followed by a discussion of the Siamese life style of these unique parasites and lastly the interactions between parasites, their hosts and the environment. Chapter 7 contains a list of references used throughout the study and lastly the appendix with supplementary tables is provided in Chapter 8.

The aims of the present study were to:

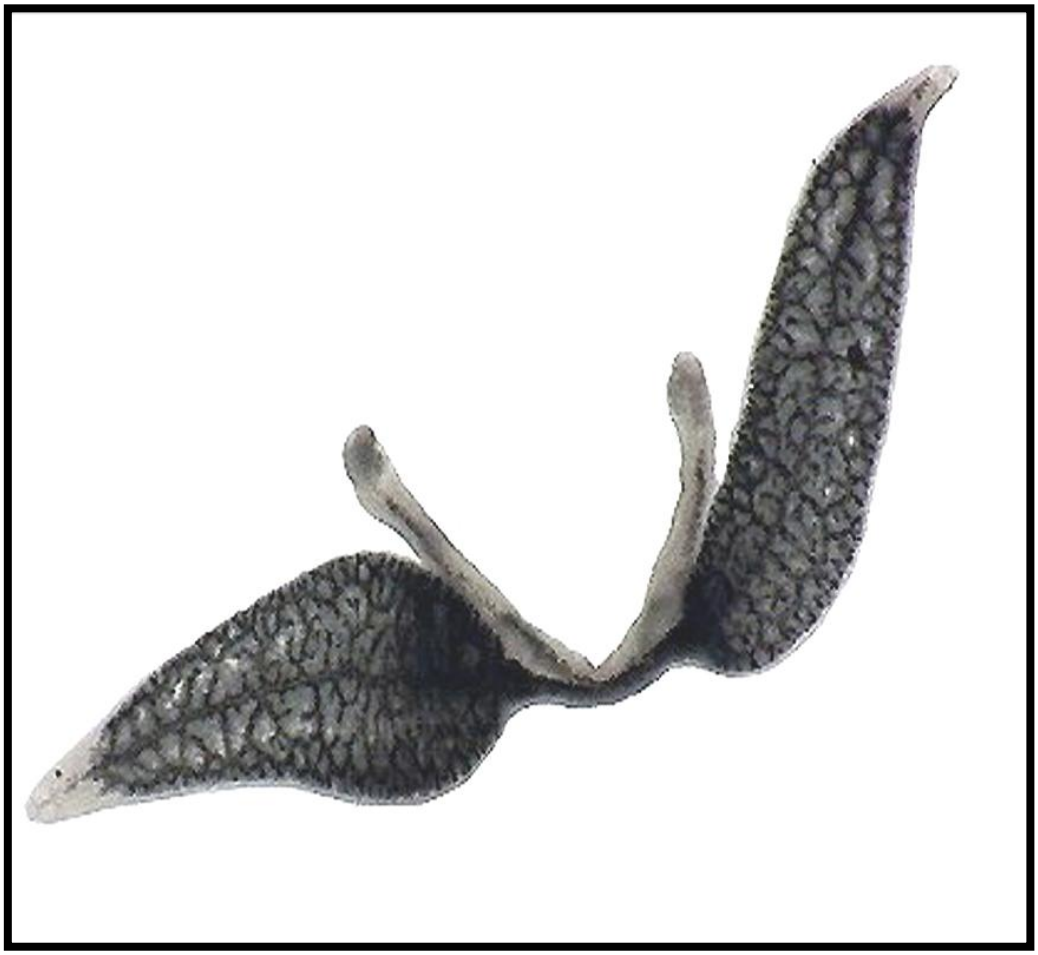
1. Clarify the taxonomic confusion present in the family Diplozoidae.
2. Compile an overview of the African diplozoid fauna and especially focussing on southern Africa.
3. Identify the diplozoid species collected by means of morphometric analysis.
4. Determine the host range and host/parasite interaction in both the Okavango and Orange-Vaal River Systems.

The overview of diplozoids from southern Africa comprises the identification and descriptions of two new species of diplozoids from the genera *Paradiplozoon* and *Diplozoon* von Nordmann, 1832 respectively, as well as an expansion of the host range for *Afrodiplozoon polycotyleus* (Paperna, 1973). The present study falls within the realm of a larger ongoing aquatic biodiversity project carried out by the team from Aquatic Parasitology at the University of the Free State.

"Among the solutions adopted by animals to satisfy their energy requirements, there are two main strategies: predation – where the larger eat the smaller – and parasitism, which is in some respects the revenge of the small."

(Lambert & Gharbi 1995)

Chapter 2



Literature Review

THE CLASS MONOGENEA

The class Monogenea have had a great deal of controversial phylogenetic relationships over time. Together with the Turbellaria Ehrenberg, 1831, Trematoda Rudolphi, 1808 and Cestoda Rudolphi, 1808, Monogenea belong to the parasitic Phylum Platyhelminthes (Jovelin & Justine 2001). Monogeneans are an extremely diverse group of species, not just due to their vast numbers but also with respect to their morphology and ecology. Poulin (2002) stated that monogeneans may be a product of an adaptive radiation and have

expanded from parasites on the skin of early vertebrates to a diversity of designs, colonising internal and external organs of various aquatic vertebrates. This is apparently due to the fact that monogeneans are known to be mainly fish gill parasites, infesting mostly actinopterygian and chondrichtyan fish and in some cases tetrapods like freshwater turtles and amphibians (Bentz *et al.* 2003). Monogeneans are, however, also found on the skin, open cavities and urinary bladder as well as other parts of the excretory system of fish. One species, *Oculotrema hippopotami* Stunkard, 1924, is even found in the conjuctival sac of the hippopotamus, *Hippopotamus amphibious* Linnaeus, 1758 (Lebedev 1988). Of all platyhelminth fish parasites monogenea are also the most dominant external parasites, while digeneans dominate the internal parasites (Cribb *et al.* 2002). Monogeneans are generally recognised by having a free-swimming, oncomiracidium stage with cilia responsible for finding and attaching to a host fish. There are, however, exceptions such as gyrodactylids, which transfer directly, from host to host.

According to Hickman *et al.* (2004), the body of adult monogenean flatworms are leaf-like to cylindrical and covered by a syncytial tegument with no cilia. A combination of attachment organs comprising hooks, suckers or clamps are usually located on the posterior part of the body. The Monogenea contains more than 53 recognised families, most of which displaying high host specificity (Olson & Littlewood 2002).

Monogenea comprises two primary clades, namely the subclass Polyonchoinea Bychowsky, 1937, also known as Monopisthocotylea Odhner, 1912 with 18 families and a second clade Heteronchoinea Boeger & Kritsky, 2001 containing the infraclasses Polystomatoinea Lebedev, 1986 with two families and the Oligonchoinea Bychowsky, 1937, otherwise known as Polyopisthocotylea Odhner, 1912, with 30 families (Boeger & Kritsky 1993; Justine 1998). These infraclasses were mainly distinguished from one another based on the larval attachment organ, Oligonchoinea with an oral sucker absent or weakly developed and Polystomatoinea with a mouth surrounded by a prohaptor consisting of one or two suckers.

Khotenovsky (1985) stated that the infra-subclass Oligonchoinea is divided into the order Mazocraeidea Bychowsky, 1937 and suborder Octomacrinea Khotenovsky, 1985 with two

families namely, Octomacridae Yamaguti, 1963 and Diplozoidae. In 1997, Boeger & Kritsky revised the hypothesis for the phylogeny of monogeneans and also placed Diplozoidae in the infra-subclass Oligonchoinea and order Mazocraeidea, with the family falling in the suborder Discocotylinea Bychowsky, 1957. Jovelin & Justine (2001) conversely reported that previous phylogenetic studies did not include a sequence of the genus *Diplozoon* and demonstrated that Diplozoidae is rather a sister-taxon of the Microcotylinea Lebedev, 1972, but added that their findings need to be confirmed. For this reason the higher classification of the class Monogenea and the placement of the family Diplozoidae, is followed as proposed by Boeger & Kristy (1997 & 2001), which is mostly accepted (Table 2.1).

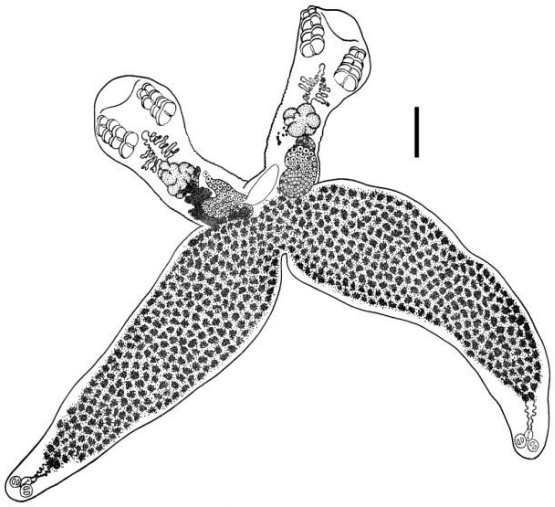
CLASSIFICATION OF DIPLOZOIDS

The most recent and extensive revision of diplozoids was given by Khotenovsky in his 1985 Russian manuscript. According to Khotenovsky (1981 & 1985) the family Diplozoidae is divided into two subfamilies, Diplozoinae Palombi, 1949 and Neodiplozoinae Khotenovsky, 1985. Diplozoinae includes the genera *Diplozoon*, *Paradiplozoon*, *Inustiatus* Khotenovsky, 1978 and *Sindiplozoon* Khotenovsky, 1981 (Khotenovsky 1979, 1981, 1982 & 1985). The genus *Eudiplozoon* Khotenovsky, 1985 was also added to this subfamily. The subfamily Neodiplozoinae contains the genera *Neodiplozoon* Tripathi, 1959 and *Afrodiplozoon*. According to Matejusova *et al.* (2001 & 2002), there are 60 described species of diplozoids. Gao *et al.* (2007) reported that 43 species are listed in the Diplozoinae with six species inquirenda from the former Soviet Union. The diplozoid fauna of Europe consists of about 18 species from the genera *Diplozoon* and *Paradiplozoon*, as well as *Eudiplozoon nipponicum* (Goto, 1891). China has 31 species of diplozoids with 22 species from the genus *Paradiplozoon*, *Sindiplozoon* with six species, *Inustiatus* with two species and lastly the genus *Eudiplozoon* with one species. A list of diplozoid species from around the world, as compiled from the literature, is given in Table 2.2.

Subfamily: Diplozoinae Palombi, 1949

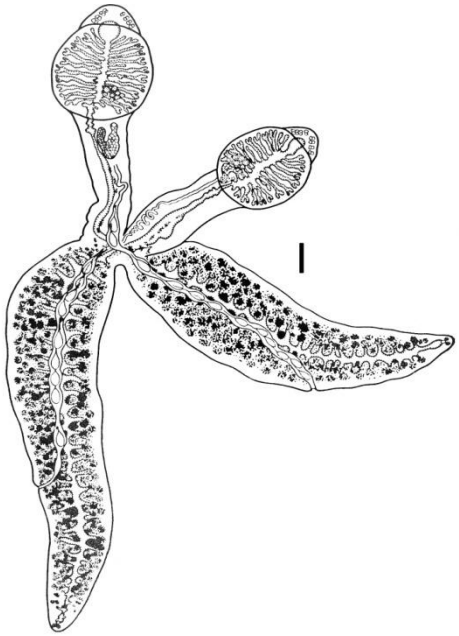
Characteristics of the genera as summarised and redrawn from Khotenovsky (1981 & 1985):

- ***Paradiplozoon*** Achmerov, 1974



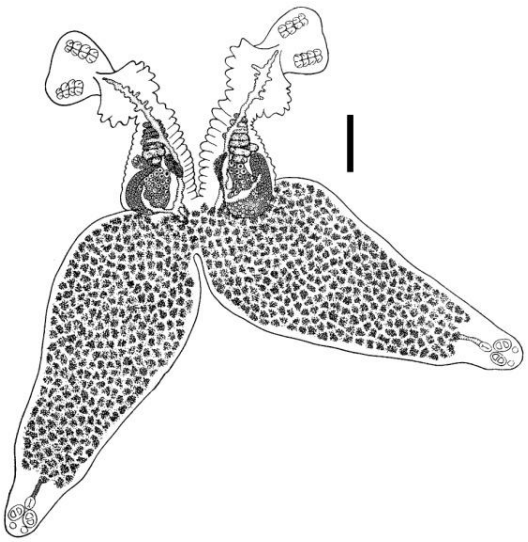
Of all the species of *Paradiplozoon, P. homoion* (Bychowsky and Nagibina, 1959) which was first described from *Rutilus* rutilus (Linnaeus, 1758) in the former Soviet Union, is probably the most studied. The main characteristic of this genus is the absence of enlargements in the middle section of the posterior body part. **Scale: 0.5 mm**

- ***Inustiatus*** Khotenovsky, 1978



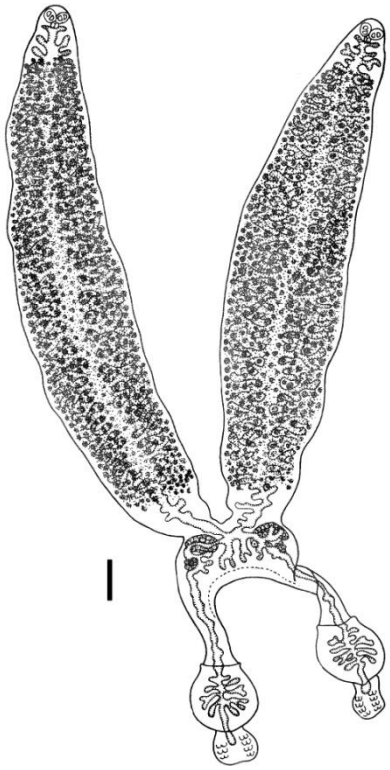
According to Khotenovsky (1985) *Inustiatus inustiatus* (Nagibina, 1965) is the only species belonging to this genus. *Inustiatus aritichthysi,* however, is also reported from Chinese fish by Gao *et al.* (2007). This genus is characterised by the most primitive type of enlargement in the middle section of the posterior body part, with a disk-like extension, resembling horse-like bolsters, pierced by a thick net of intestinal diverticula. **Scale: 0.5 mm**

- ***Eudiplozoon*** Khotenovsky, 1985



Eudiplozoon nipponicum is a monotypic genus and was collected in East Asia on *Cyprinus carpio* Linnaeus, 1758, where after it was introduced and spread throughout Europe (Hodova *et al.* 2010). The genus *Eudiplozoon* is characterised by enlargements in the middle section of the posterior body part in the form of tooth-like folds with very well developed musculature. **Scale: 0.5 mm**

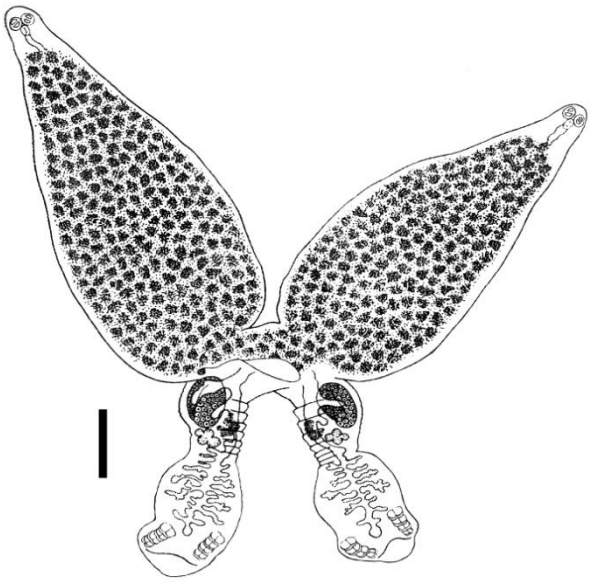
- ***Sindiplozoon*** Khotenovsky, 1981



The type species for this genus is *Sindiplozoon strelkowi* (Nagibina, 1965). According to Khotenovsky (1985), only two species belong to this genus, namely *S. strelkowi* and *S. diplodiscus* (Nagibina, 1965), both occurring in China. Gao *et al.* (2007), however, reported on six species from the genus *Sindiplozoon* occurring in China. Xiao-Qin *et al.* (2000) reported that diplozoids from this genus infest fish from the cyprinid subfamilies Culterinae, Hypophthalmichthyinae and Xenocyprinae from inland waters in China. These species are characterised by four small pairs of clamps and an enlargement in the mid-posterior part of the body with well-developed musculature. Khotenovsky (1985) also

stated that enlargements in this genus are in the form of glass-like cavities present on the ventral side of the posterior body and in addition that the intestinal diverticula do not form a thick compact net. **Scale: 1 mm**

- ***Diplozoon*** Von Nordmann, 1832



Diplozoon paradoxum von Nordmann, 1832 is the type specimen for the genus *Diplozoon,* collected from the gills of *Abramis brama* (Von Nordmann, 1832). The genus contains only two described and accepted *Diplozoon* species namely *D. paradoxum* and *Diplozoon scardinii* Komarova, 1966. Enlargements in the middle section of the posterior body are almost like a combination of that found in the genera *Sindiplozoon* and *Eudiplozoon.* This genus is characterised by more developed enlargements with a glass-like form and a small number of big folds on the ventral side of the posterior body part. The intestinal diverticula do not form a thick net. **Scale: 0.5 mm**

Diplozoon* vs. *Paradiplozoon

Over the years, many species have been incorrectly assigned to the genus *Diplozoon* and after more accurate examination re-assigned to the genus *Paradiplozoon.* An ample amount of speculation still exists on the validity of these two genera. When Von Nordmann

described the genus *Diplozoon* in 1832, he considered only one character, the worm coalescence in pairs. Later on in 1974, Achmerov divided the genus *Diplozoon* in two subgenera according to the presence or absence of enlargements in the middle section of the posterior body part. He, however, did not take into consideration morphological particulates as well as the geographical occurrence of diplozoids and their hosts. The subgenus *Diplozoon* was not recognised, but the subgenus *Paradiplozoon* was recognised as a genus, sometime later by Khotenovsky (1981). After doing extensive work on diplozoids, Khotenovsky (1981) reported the genus *Diplozoon* to be a miscellaneous genus with only two species, i.e. *D. paradoxum* and *D. scardinii.* It was later found that *D.* scardinii was identical to *P. homoion*, leaving only one species in the genus *Diplozoon* (Gao *et al.* 2007). About 21 species were moved to the genus *Paradiplozoon*. This caused a lot of confusion, seeing that Khotenovsky's work was mainly published in Russian and consequently inaccessible or not known of, by various scientists working on diplozoids. The outcome is that to date a lot of diplozoid species are still being incorrectly designated to the genus *Diplozoon* without adopting the published changes.

Khotenovsky (1985) stated that the genera *Diplozoon* and *Paradiplozoon* are distinguished in the original description only in the shape of soft parts of the body, with a typical cup-shaped extension of the distal part of the posterior part of the body of *Diplozoon*. According to Khotenovsky (1985), all diplozoids can be divided into two groups in terms of their external morphology. The worms without enlargements in the middle section of the posterior body part, can be placed in the genus *Paradiplozoon* while those with enlargements, belong to one of either the genera *Inustiatus, Eudiplozoon, Sindiplozoon* or *Diplozoon*. It is believed that diplozoids with larger clamps, compared to diplozoids from other genera, do not need enlargements, as is the case in the genus *Paradiplozoon.* Diplozoids from the genus *Inustiatus* on the other hand, have very small clamps, 28 to 50 times smaller than the posterior body part, but diplozoids from this genus have well expressed enlargements that aid the attachment function of the clamps.

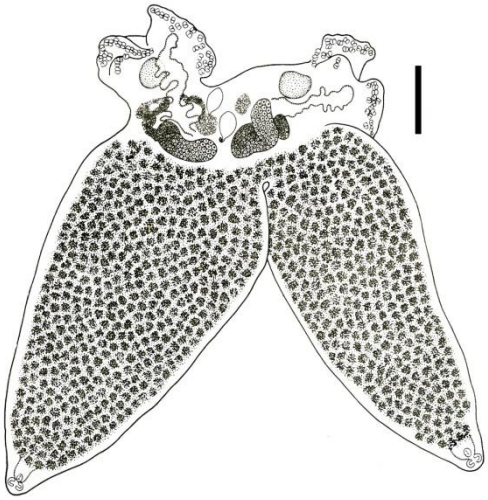
The folds present in the anterior section of the posterior body part appear after the joining of the juvenile diporpae. Contraction or relaxation of the posterior body part does not

influence the form of the folds, only the distances between the folds can change. Khotenovsky (1985) stated that diplozoids could be divided in two groups due to the presence or absence of these folds. The genera *Neodiplozoon, Afrodiplozoon*, *Inustiatus* and *Sindiplozoon* have no folds and this is the same with most of the species of *Paradiplozoon* from India and Africa.

Subfamily: Neodiplozoinae Khotenovsky, 1985

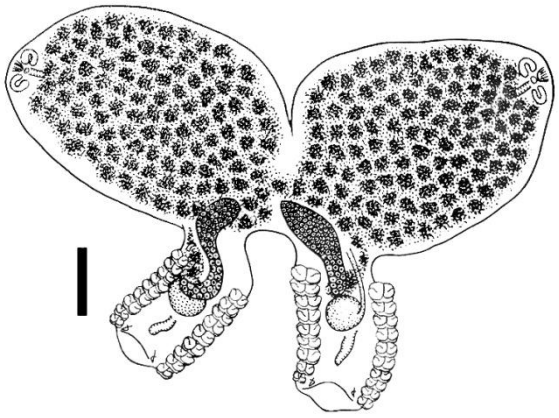
Adult worms from the subfamily Neodiplozoinae are characterised by the presence of eight or more pairs of clamps. Khotenovsky (1981) divided the subfamily Neodiplozoinae into two genera namely *Neodiplozoon*, from India and a new genus, *Afrodiplozoon* from Africa. Characteristics of the genera as summarised and redrawn from Khotenovsky (1981 & 1985) are as followed:

- ***Neodiplozoon*** Tripathi, 1959



Neodiplozoon barbi (Tripathi, 1959) is the only species in the genus *Neodiplozoon*. According to Khotenovsky (1985), this species is characterised by a number of clamps, mostly 16 pairs, divided into two horizontal lobes, situated underneath the back edge of the opisthaptor. **Scale: 0.5 mm**

- ***Afrodiplozoon*** Khotenovsky, 1980



Afrodiplozoon polycotyleus was originally described as *Neodiplozoon polycotyleus* by Paperna (1973), where after it was separated from the genus *Neodiplozoon* due to differences and modifications in terms of worm attachment to the gills of the fish host. This species is characterised by having up to 13 laterally situated clamps. As mentioned, the genus *Neodiplozoon* is characterised by clamp pairs divided into two horizontal lobes. These lobes are missing in species from the genus *Afrodiplozoon* and clamps are situated in two lines underneath the lateral edges of the opisthaptor. Khotenovsky (1981) used this as an important distinguishing indicator, which led to the creation of an independent genus *Afrodiplozoon* of which *A. polycotyleus* is the only species belonging to this African genus. **Scale: 0.2 mm**

Table 2.1 Classification of the class Monogenea (Van Beneden, 1858) adapted from Boeger and Kritsky (2001) and Khotenovsky (1985).

PHYLUM	CLASS	SUB CLASS	INFRA CLASS	ORDER	SUB ORDER	INFRA ORDER	SUPER FAMILY	FAMILY	SUB FAMILY	GENERA
Platyhelminthes	Turbellaria									
	Cestoidea									
	Trematoda									
	Monogenea	Polyoncholinea		Monocotylidea						
				Capsalidea						
				Lagarocotyllidea						
				Motchadskyellidea						
				Gyrodactylidea						
				Dactylogaridea						
		Heteroncholinea	Polystomatoinea	Polystomatidea				Polystomatidae		
								Sphyranuridae		
			Oligoncholinea	Chimaericolidea				Chimaericolidae		
				Diclybothriidea				Diclybothriidae		
								Hexabothriidae		
				Mazocraeidea	Mazocraeinea			Plectanocotylidae		
								Mazoplectidae		
								Mazocraeidae		
					Gastrocotylinea	Anthocotylinea		Anthocotylidae		
						Gastrocotylinea		Pseudodiclidophoridae		
							Protomicrocotyloidea	Allodiscocotylidae		
								Pseudomazocraeidae		
								Chauhaneidae		
							Gastrocotyloidea	Bychowskycotylidae		
								Gastrocotylidae		
								Neothoracocotylidae		
								Gotocotylidae		
					Discoctylinea			Discocotylidae		
								Diplozoidae	Neodiplozoinae	*Neodiplozoon*
										Afrodiplozoon
									Diplozoinae	*Paradiplozoon*
										Inustiatus
										Eudiplozoon
										Sindiplozoon
										Diplozoon
								Octomacridae		
					Hexostomatinea			Hexostomatidae		
					Microcotylinea	Microcotyloidea		Axinidae		
								Diplasiocotylidae		
								Heteraxinidae		
								Microcotylidae		
						Allopyragraphoroidea		Allopyragraphoridae		
						Diclidophoroidea		Diclidophoridae		
						Pyragraphoroidea		Pterinotrematidae		
								Rhinecotylidae		
								Pragraphoridae		
								Heteromicrocotylidae		

DIPLOZOIDS FROM AFRICA

The history of the African diplozoid fauna began with the first species described in 1957 from cyprinid fish and since then no extensive work has been done on the African diplozoids. To date diplozoid species have been described belonging to the genera *Paradiplozoon* and *Afrodiplozoon*. Species described as belonging to the genus *Diplozoon* have later been reassigned to the genus *Paradiplozoon*.

Paradiplozoon ghanense (Thomas, 1957):

In 1957, Thomas identified a new species of the genus *Diplozoon* from the Black Volta River in the Northern region of Ghana on the gills of *Brycinus macrolepidotus* (Valenciennes, 1850) and proposed the name *Diplozoon ghanense* (Thomas, 1957). This species was, however, moved to the genus *Paradiplozoon* by Khotenovsky (1981) and it was thereafter known as *Paradiplozoon ghanense* (Thomas, 1957). Thomas (1957) reported that *P. ghanense* was only found on the gills of the characin *B. macrolepidotus* and not on any of the other fish families, Cyprinidae, Mormyridae, Gymnarchidae, Citharinidae, Bagridae, Schilbeidae, Clariidae, Mochocidae or Cichlidae, from the same body of water. Since the description of the species, it has also been reported by Echi & Ezenwaji (2009) from *B. macrolepidotus* in the Anambra River, Nigeria. According to the identification key of Thomas (1957), this species can be distinguished from other known species by the placement of the compact testis in the region of fusion.

Paradiplozoon aegyptensis (Fischthal & Kuntz, 1963):

Fischthal & Kuntz (1963) described a new species of diplozoid from the Nile in Egypt, found on the gills of *Labeo forskalii* Rüppel, 1835. The species was named *Diplozoon aegyptensis* Fischthal & Kuntz, 1963, but was also moved to the genus *Paradiplozoon* by Khotenovsky (1981) and thereafter know as *Paradiplozoon aegyptensis* (Fischthal & Kuntz, 1963). In addition, *P. aegyptensis* has since been found on *L. coubie* Rüppel, 1832 from the Volta Lake in Ghana, *L. cylindricus* Peters, 1852 from the Ruaha River, Tanzania, *L. victorianus* Boulenger, 1901 from the Nzoia River, Kenya and lastly on *Brycinus macrolepidotus* from Lake Albert (Paperna 1979). Fischthal & Kuntz (1963) stated that this species could be

distinguished from other *Paradiplozoon* species by the placement of the testis in the posterior body part of the worm, while the ovaries are situated in the area of fusion. Another unique characteristic is the size of the eggs, which are quite large with a length of 254 to 313 µm and a width of 81 to 132 µm.

Hempel *et al.* (2001) found representatives of the genus *Paradiplozoon* on *Labeobarbus aeneus* (Burchell, 1822) in the Vaaldam. Milne & Avenant-Oldewage (2006) also collected adults and larvae of this *Paradiplozoon* sp. on both *L. aeneus* and *L. kimberleyensis* (Gilchrist & Thompson, 1913). Very little characteristics are given and no morphological measurements of the *Paradiplozoon* sp. are provided in these articles.

***Afrodiplozoon polycotyleus* (Paperna, 1973):**

Afrodiplozoon polycotyleus was described by Paperna (1973) from the host fish *Barbus paludinosus* Peters, 1852, *B. cercops* Whitehead, 1960 and *Labeo victorianus* Boulenger, 1901 from the Nzoia River in Kenya as well as from *B. macrolepis* Pfeffer, 1889 from the Ruaha River in Tanzania. When the new genus *Afrodiplozoon* was created by Khotenovsky (1980), this species was re-assigned to this genus, from the genus *Neodiplozoon*. In addition the number of hosts increased with the finding of *A. polycotyleus* on *B. kerstenii* Peters, 1868 by Paperna (1979) and Chapman *et al.* (2000) added *B. neumayeri* Fischer, 1884 from the Mpanga River System in Uganda. Mashego (1982 & 2000) reported this species from *B. neefi* Greenwood, 1962, *B. marequensis* Smith, 1842 and *B. trimaculatus* Peters, 1852 from the Lingwe River as well as the Luphephe and Nwanedzi Dams in South Africa. Recently Echi & Ezenwaji (2009) found *A. polycotyleus* on *Alestes baremoze* (Joannis, 1835) from the Anambra River in Nigeria. *Afrodiplozoon polycotyleus* is characterised by having 8 to 13 pairs of clamps situated on the opisthaptor.

See Chapter 5, Table 5.6 for comparisons between the African diplozoid species as well as species collected during the present study.

A SIAMESE LIFE CYCLE

Diplozoids have a simple life cycle, with a unique mating behaviour. The life cycle is direct as shown in Figure 2.2, including a free-swimming oncomiracidium and a post-oncomiracidial racidial stage known as the diporpa. The oncomiracidium is swept into the gill chambers of the fish host and attaches to the gill filaments. It is at this stage of the life cycle where the uniqueness becomes apparent (Khotenovsky 1977 & Pecinkova *et al.* 2007). Two individual diporpa larvae find each other on the gills of a fish host and become permanently fused into a diplozoid pair (Zurawski *et al.* 2001). Fusion initiates metamorphosis of the joined pair during which there is reciprocal fusion of the external openings of the male and female genital ducts, ensuring cross-fertilisation between the two hermaphroditic partners and ultimately leading to eventual sexual maturity (Zurawski *et al.* 2003). It is vital for the diporpa to find a mate and fuse because each of the individuals are unable to continue further development alone and therefore will die without being able to reproduce.

Paperna (1996) reported that diplozoid development is slower than found in dactylogyroids, taking longer periods to reach maturity, with a life span of several months to two years. Maturity is only reached after four months in *Diplozoon paradoxum*, whereas species from tropical fish exhibit a considerable shorter development period, taking only a few weeks to mature and with a life span of less than a year.

A diplozoid pair forms a permanent monogamous association, which is rather unusual in the animal kingdom and has become widely known and discussed, even in popular magazines and newspapers such as the New York Times. Such an article was written by Angier (2008) with the title "In most species, faithfulness is a

"Sexual promiscuity is rampant throughout nature, and true faithfulness a fond fantasy. Male and female diplozoid worms meet each other as adolescents, and their bodies literally fuse together, whereupon they remain faithful until death. ... That is the only known species in which there seems to be 100 percent monogamy and where the only hearts burned belong to the unlucky host fish."

Figure 2.1: Extract from an article by Angier (2008).

fantasy", in which diplozoid worms were said to be the only known species in which there seems to be 100 percent monogamy (Figure 2.1). Tchuente *et al.* (1996) reported on comparable phenomena found in two other groups of parasites: Didymozoidae Monticelli, 1888 from the class Trematoda and Syngamidae Leiper, 1912 from the phylum Nematoda Diesing, 1861. In both cases, however, only temporary relationships exist.

MORPHOLOGY OF THE DIFFERENT STAGES IN THE DIPLOZOID LIFE CYCLE

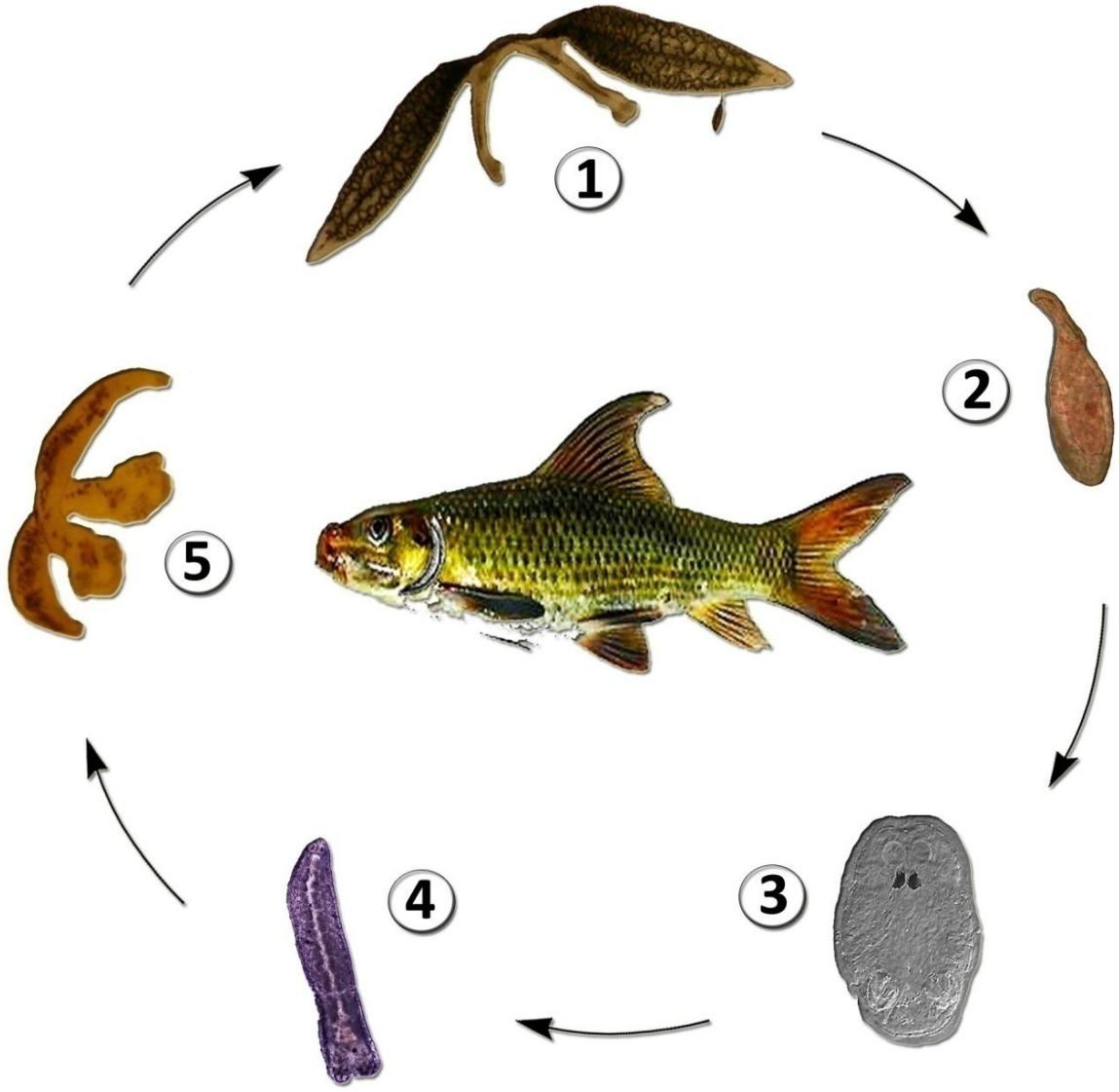


Figure 2.2: Diagrammatic representation of the diplozoid life cycle illustrating the various developmental stages: 1 – Adult diplozoid, 2 – Egg, 3 – Oncomiracidium, adapted from Valigurova *et al.* (2010), 4 – Diporpa, 5 – Juvenile stage.

1 - Adult worm

The body of each of the paired adult individuals can be separated into anterior and posterior parts. These two parts in turn, are divided by the fusion area, the area where union between the two juvenile worms takes place. According to Khotenovsky (1985), diplozoid worms attain a total body length of between 0.3 to 14.9 mm. The anterior body part has a lance-like shape that is quite mobile, resulting in the form being able to change easily in order to manoeuvre among the gill filaments of the fish host. At the end of the anterior part, the mouth is positioned between two oral suckers (Figure 2.3). The suckers are mostly horseshoe-shaped, consisting of radial muscle fibres with their main function assisting in the feeding process by attaching to gill filaments long enough for food to be absorbed by the mouth. The suckers also aid while moving about on the gills as they are used to hold on to gill filaments in order for the clamps to be repositioned.

The oval-shaped pharynx stretches from the subterminal mouth opening where after it is followed by the oesophagus, which in turn leads into the intestinal track up to numerous lateral, blind-ending branches. The intestinal tract is obscured from view by a compact network of vitellaria extending through the area of fusion into the posterior part of the body. Khotenovsky (1985) stated that the intestinal tubes of both individuals are connected by anastomosis in the area of fusion. The size of the body, clamps suckers and pharynx depend on the worm's body size and that in turn is related to the age and length, as well as other characteristics of the worm.

Both individual worms are hermaphroditic and possess both testes and ovaries (Justine *et al.* 1985). The gonads are usually situated in the area of fusion, but this may differ from species to species, with either the testes or ovaries, or even both, extending into the posterior part. The female reproductive organs consist of an ovary, oviduct, ootype and uterus. Placement of the gonads is also a distinguishing factor when discriminating between species. The vas deferens is situated in the area of fusion and passes in parallel with the uterus as a very thin tube, linking the testis of one individual with a sperm-receiving canal of the other individual, acting as a vagina (Gerasev 1994). The sperm-receiving canal opens near the place of connection between the yolk canal and the oviduct, this canal is always full

of sperm extending at the ovary level. The female vitelloduct with reservoir is connected to the anterior part of the oviduct (Figure 2.3). The posterior body part is usually shorter in length than the anterior part and according to Khotenovsky (1985), it can be divided into two or three sections. Firstly, the anterior section with a fold on the surface and secondly, the middle section, with various bolsters, folds or cavities present. This area is sometimes impossible to tell apart from the anterior area if these enlargements are absent. The last area, is the posterior section, which carries the rows of attachment clamps (Figure 2.3).

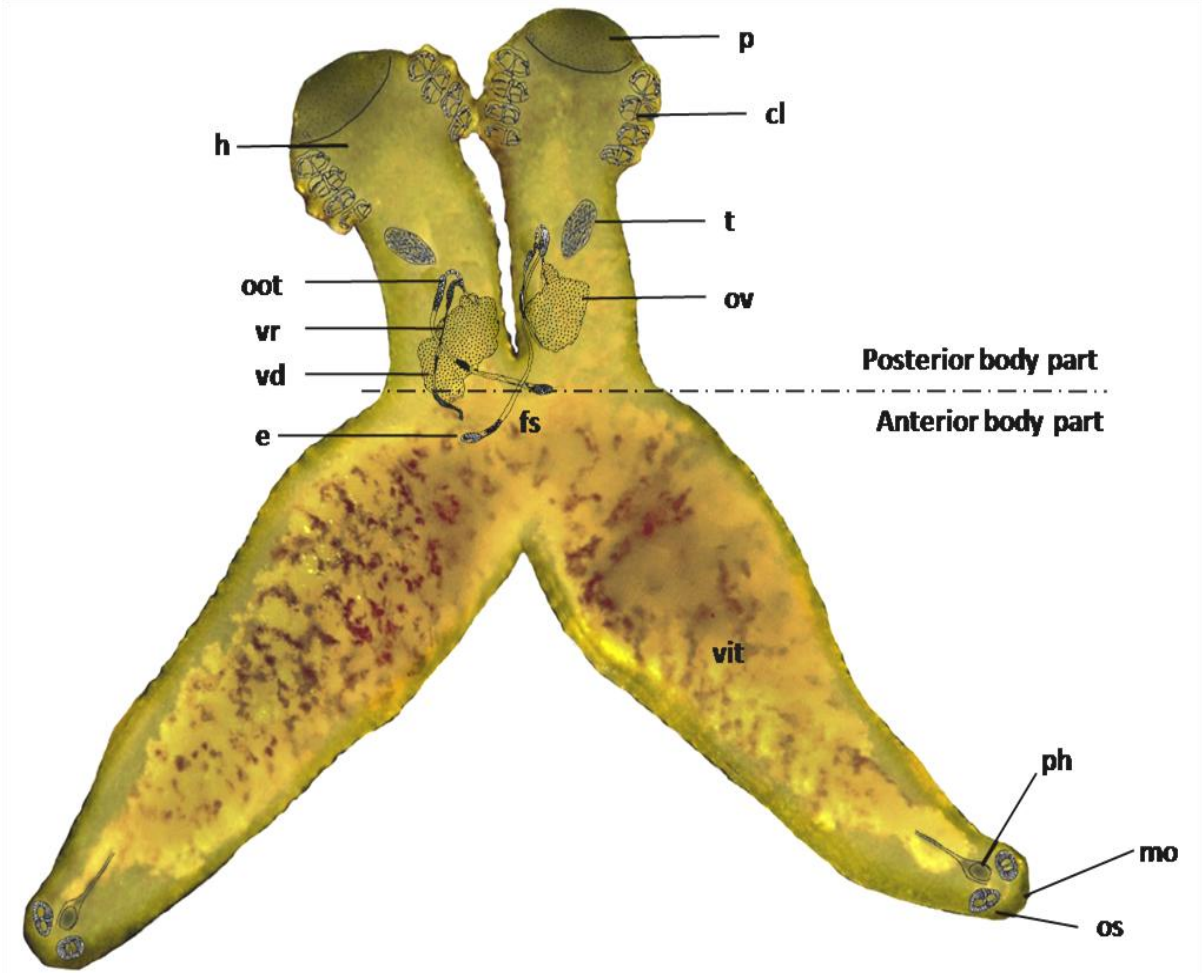


Figure 2.3: Diagrammatic representation of a paired, adult diplozoid worm illustrating morphological features. The reproductive system is partly redrawn from Valigurova *et al.* (2010): clamps (cl), egg (e), fusion area (fs), haptor (h), mouth opening (mo), ootype (oot), oral suckers (os), ovary (ov), opisthaptor protrusion (p), pharynx (ph), testis (t), vitelloduct (vd), vittelaria (vit) and vitelloduct reservoir (vr).

2 - Egg

The diplozoid egg consists of an almost oval-shaped shell with a point anopercular end leading to a long coiled filament. MacDonald (1978) stated that diplozoids from different hosts display behavioural characteristics specific to those hosts. It was found that the eggs of a *Diplozoon* sp. from the minnow (*Phoxinus laevis* Fitzinger, 1832) hatches in the early morning and light activated hatching has been reported from roach and bream hosts. It was also found that *Paradiplozoon homoion gracile* (Reichenbach-Klinke, 1961) from the Mediterranean barbel *Barbus meridionalis* Risso, 1827, exhibits both egg-laying and hatching rhythms. A significant greater number of eggs are laid at night than during daytime and it can be said that the egg laying rhythms displayed are synchronised to the behaviour of the host in order to better the chances of successful invasion by larvae. The Mediterranean barbel spends most of the day actively swimming and feeding while at night it rests under banks and ledges. The eggs of *P. homoion gracile* therefore accumulates during the day and hatch at night in the areas of the riverbed in which host fish are most commonly present during night-time.

3 - Oncomiracidium

The ciliated oncomiracidium already possesses oral suckers, a well developed pharynx and a blind ending intestinal caecum. The cilia of the oncomiracidium are arranged in anterolateral, medial and posterior zones. Hodova *et al.* (2010), reports that this arrangement facilitates movement during the free-living stage and might serve as mechano-receptors or proprio-receptors. One pair of clamps and the central hooks are arranged on the ventral side of the larva's body. Two pigmented eyes are located on the dorsal side of the body near the front (Khotenovsky 1985). The eyes contain retinal cells and are composed of glass-like parts with a light-reflecting lens. Whittington *et al.* (2000) reported that in the oncomiracidium of *Diplozoon paradoxum* the single pair of median laterally directed pigment shields each contain a single rhabdomere with no lens evident. It is believed that these eyes are responsible for monitoring day or night length in order to control hatching rhythms. The oncomiracidia exhibit two behavioural phases, i.e. an early photopositive period during which the larva is not able to attach and therefore acts as a dispersal period. This is followed by an infestation phase, lasting throughout the free-

swimming life in which photopositive behaviour is lost and sometimes replaced by photonegative behaviour (Kearn 1978). When the larva attaches to the gills of the fish, it undergoes various changes in morphology. It loses the surface cilia as well as the eyespots and develops a branched intestine (Valigurova *et al.* 2010). Another change is the development of a muscular sucker on the mid-ventral surface and a papilla on the dorsal surface. The larva now enters its post-oncomiracidium stage where after it is known as a diporpa (Zurawski *et al.* 2002).

4 - Diporpa

The diporpa is able to migrate over the gill surface by using the mouth, hooks and clamps. After meeting another diporpa on the same gill arch fusion takes place. During the process of fusion, which takes a few hours, the two diporpa align their bodies parallel to one another (Zurawski *et al.* 2002). This is a complicated process with each diporpa grasping the dorsal papillae of the other by means of its ventral sucker, during which shortening, elongation, and twisting of their bodies take place. As soon as both the ventral and dorsal papillae of one diporpa are attached to that of the other, fusion of the adjacent tissue takes place. Following the fusion process, the juvenile stage undergoes further development with successive pairs of clamps appearing on the opisthaptor and the progressive reduction in size of the ventral sucker (Zurawski *et al.* 2001). Unpaired diporpa are also able to develop up to four pairs of clamps, but without fusion, it will not fully mature (Valigurova *et al.* 2010). Zurawski *et al.* (2002) explain that it is at this point that reproductive development will commence and gonads will appear in order for the male and female genital ducts of one individual to become fused with that of the other.

5 - Juvenile stage

As soon as two diporpae fuse into a coalescent pair they are in the juvenile stage of the diplozoid life cycle. According to Valigurova *et al.* (2010), juvenile worms migrate from the marginal part of the gills to the medial part where they will remain as adults. The fourth clamp will go through the final development in order for the developing worm to exhibit a haptor with well developed clamps. The reproductive tract starts to develop after fusion of the diporpae, but sexual maturity will only be reached in the adult stage.

ATTACHMENT TO THE HOST

The attachment apparatus responsible for maintaining a hold fast on the host fish, consists of a pair of central hooks and in most cases, four pairs of clamps on each haptor of the pair. Clamps appear gradually as the larvae differentiate and the development is asymmetric, developing worms may therefore show unpaired numbers of clamps at different stages in their development (Paperna, 1996). Valigurova *et al.* (2010) believe that newly forming clamps already possess musculature, except that it is still less developed than that of the fully developed clamp. The attachment formations are divided in two groups, namely sclerotic formations comprising valves and hooks, and secondary, parenchim-muscular formations, consisting of enlargements and folds on the posterior body part. These folds and lobular extensions play an important role in fixing the worm in between the gill lamellae (Valigurova *et al.* 2010). Most diplozoids have four pairs of clamps situated in two lateral rows on the opisthaptor, but the arrangements and number of clamps may vary largely from species to species. Each clamp is made up of a pair of opposable, hinged jaws supported by a complex array of sclerites, acting as a skeleton.

Measurements of the clamps were previously not recommended for species determination because of their variability together with the fact that clamps are not stable, but growing gradually (Matejusova *et al.,* 2002). Clamps and central hooks, on the other hand are currently seen as the major morphological characters used for species determination (Matejusova *et al.*, 2004). Khotenovsky (1985) reported that the third clamp is best to use for genus determination, seeing that the first and second clamps have less distinguished structural elements, while the fourth continues to grow. The length of the posterior body part in correlation with the width of the third clamp, together with other characteristics, can be used to differentiate between genera.

Two central hooks are placed on the posterior edge of the body and are mainly for attachment. These hooks are rather primitive formations and arise when the oncomiracidium is formed. Khotenovsky (1985) reported that the central hooks already reach their final size during the embryo stage and might only differ slightly as the worm

reaches maturity. The central hook consists of three parts, e.g., the body, on which is carried the hook, with a handle. A strong muscle cluster is attached to the handle of the central hook and aid in drawing in the hook. During the diporpa stage up to four pairs of clamps will develop (Pecinkova *et al.* 2005).

NEUROMUSCULATURE

The nervous system of monogeneans can be divided in central and peripheral nervous systems (Halton & Jennings 1964; Halton *et al.* 1998). Worms from the genus *Eudiplozoon* display a nervous system typically orthogonal in arrangement (Zurawski *et al.* 2001). This, however, changes as two worms unite in a pair and the tracts of the paired longitudinal nerve cords of both worms cross over that of the other at the point of fusion. Zurawski *et al.* (2002), stated that not only the musculature of the two diporpae become fused during pairing, but also their nervous systems at the level of the central nervous system. Adults have highly developed body wall musculature composed of outer circular, intermediate longitudinal, inner diagonal and dorsoventral muscle fibers (Valigurova *et al.* 2010). The strongest nerve roots extend into the opisthaptor to support a complex network of neurons that innervate the muscles of the clamps. The presence of neural connectivity between the central nervous systems of both individuals in a diplozoid pair was established for all three known major groups of mediators (Zurawski *et al.* 2003). Zurawski *et al.* (2002) found that neural elements are pulled into the ventral sucker during fusion providing a base for lateral growth of the inter-specimen commissures, connecting the central nervous system of both worms.

How neural connectivity is established between the two worms during pairing cannot exactly be explained yet. It is likely that there is continuity between the peripheral nervous systems of the worm pair, seeing that there is a rich array of peripheral nerve plexuses around the ventral sucker and dorsal papilla. These nerves are thought to aid in coordinating the pairing of the two diporpae and therefore have a sensory function (Zurawski *et al.* 2002). The nervous system plays an important role in coordinating behavioural aspects such as motility, attachment, feeding and reproduction in the diplozoid

life cycle. During the present study the neuromusculature of adult and diporpa diplozoids were investigated and results presented in Chapter 5.

Table 2.2: A list of species from the family Diplozoidae Palombi, 1949 from around the world as compiled from literature.

Species	Author	Host	Country	Reference
Family: **Diplozoidae** Palombi, 1949				
Subfamily: **Diplozoinae** Palombi, 1949				
Genus: ***Paradiplozoon*** Achmerov, 1974				
P. aegyptensis	(Fischthal and Kuntz, 1963)	*Labeo forskalii* *L. coubie* *L. cylindricus* *L. victorianus* *Brycinus macrolepidotus*	Egypt Ghana Tanzania Kenya	Khotenovsky (1985) Fischthal & Kuntz (1963) Paperna (1979)
P. alburni	Khotenovsky, 1982	*Alburnus alburnus*	Ukraine	Khotenovsky (1985)
P. amurense	(Achmerov, 1974)	-	-	Khotenovsky (1985)
P. barbi	(Reichenbach-Klinke, 1951)	*Barbus semifasciolatus* *Puntius tetrazona*	Germany	Reichenbach-Klinke (1980) Khotenovsky (1985) Thomas (1957)
P. bliccae	(Reichenbach-Klinke, 1961)	*Blicca bjoerkna*	Ukraine	Khotenovsky (1985)
P. capoetobrama	(Gavrilova, 1964)	-	Russia	Khotenovsky (1985)
P. cauveryi	(Tripathi, 1959)	*Cirrhinia cirrhosa*	India	Khotenovsky (1985)
P. cyprini	Khotenovsky, 1982	*Cyprinus carpio haematopterus*	China Ukraine	Khotenovsky (1982 & 1985)
P. doi	(Ha Ky, 1971)	-	Vietnam	Khotenovsky (1985)
P. ergensi	(Pejcoch, 1968)	-	-	Khotenovsky (1985)
P. ghanense	(Thomas, 1957)	*Brycinus macrolepidotus*	Ghana Nigeria	Khotenovsky (1985) Thomas (1957) Echi & Ezenwaji (2009)
P. homoion	(Bychowsky and Nagibina, 1959)	*Hypophthalmichthys molitrix* *Rutilus rutilus* *Phoxinus phoxinus* *Cyprinidae* sp.	Ukraine Poland Russia	Lucky (1981) Khotenovsky (1985)
P. h. gracile	(Reichenbach-Klinke, 1961)	*Barbus meridionalis* *Gobio gobio*	France Russia Poland	Khotenovsky (1985) Koval & Pashkevichute (1973)
P. h. homoion	(Bychowsky and Nagibina, 1959)	*Rutilus rutilus*	Russia Finland	Khotenovsky (1985) Koskivaara & Valtonen (1991)
P. hemiculteri	(Ling, 1973)	*Hemiculter leucisculus*	China	Khotenovsky (1985)
P. indicum	(Dayal, 1941)	*Barbus sarana*	India	Khotenovsky (1985) Thomas (1957)

Table 2.2 (continue): A list of species from the family Diplozoidae Palombi, 1949 from around the world as compiled from literature.

P. jiangxiensis	(Jiang, Wu & Wang, 1985)	*Cultrichthys erythropterus*	China	Gao *et al.* (2007)
P. kashmirense	(Kaw, 1950)	*Schizothorax* sp.	India	Khotenovsky (1985) Thomas (1957)
P. leucisci	Khotenovsky, 1982	*Leuciscus cephalus* *Leuciscus leuciscus*	Czechoslovakia Ukraine	Khotenovsky (1982 & 1985)
P. magnum	Lim lee Hong and Khotenovsky, 1984	-	-	Khotenovsky (1985)
P. malayense	Lim lee Hong and Khotenovsky, 1984	-	-	Khotenovsky (1985)
P. marinae	(Achmerov, 1974)	-	-	Khotenovsky (1982 & 1985)
P. megalobramae	Khotenovsky, 1982	*Megalobrama terminalis*	Russia	Khotenovsky (1982 & 1985)
P. megan	(Bychowsky and Nagibina, 1959)	*Leuciscus idus*	Russia	Khotenovsky (1985)
P. microclampi	(Kulkarni, 1971)	*Barbus sarana*	India	Kulkarni (1970) Khotenovsky (1985)
P. minutum	(Paperna, 1964)	*Phoxinellus kervellei* *Tylognathus steinitziorum*	Israel	Khotenovsky (1985)
P. opsariichthydis	(Jiang, Wu & Wang, 1984)	*Opsariichthys uncirostris*	China	Gao *et al.* (2007)
P. nagibinae	(Glaser, 1965)	*Abramis ballerus*	Russia	Khotenovsky (1985)
P. parabramisi	(Ling, 1973)	-	-	Khotenovsky (1982 & 1985)
P. pavlovskii	(Bychowsky and Nagibina, 1959)	*Aspius aspius*	Russia	Khotenovsky (1982 & 1985)
P. rutili	(Glaser, 1967)	*Rutilus rutilus* *Cyprinidae* sp.	France Russia	Khotenovsky (1985)
P. sapae	(Reichenbach-Klinke, 1961)	*Abramis sapa bergi*	Russia	Khotenovsky (1985)
P. schizothorazi	(Iksanov, 1965)	-	Russia	Khotenovsky (1985) Koval & Pashkevichute (1973)
P. skrjabini	(Achmerov, 1974)	-	-	Khotenovsky (1985)
P. soni	(Tripathi, 1959)	*Oxygaster bacaila*	India	Khotenovsky (1985) Koval & Pashkevichute (1973)
P. tadzhikistanicum	(Gavrilova and Dzhalilov, 1965)	-	Russia	Khotenovsky (1985)
P. tetragonopterini	(Sterba, 1957)	*Ctenobrycen spilurus*	Russia	Khotenovsky (1985) Koval & Pashkevichute (1973)
P. tisae	Khotenovsky, 1982	*Barbus meridionalis petenyi*	Ukraine	Khotenovsky (1982 & 1985)

Table 2.2 (continue): A list of species from the family Diplozoidae Palombi, 1949 from around the world as compiled from literature.

P. vietnamicum	Khotenovsky, 1982	*Cirrhinus chinensis*	Vietnam	Khotenovsky (1982 & 1985)
P. vojteki	(Pejcoch, 1968)	-	-	Khotenovsky (1985)
P. zeller	(Gyntovt, 1967)	*Cyprinus carpio*	Bulgaria Russia	Khotenovsky (1982 & 1985)
Species inquirenda:				
P. agdamicum	(Mikailov, 1973)	*Leuciscus cephalus orientalis*	Azerbaijan	Khotenovsky (1985)
P. balleri	(Nagibina, Ergens & Pashkevichute, 1970)	*Abramis ballerus*	Russia	Koval & Pashkevichute (1973)
P. bergi	(Gavrilova, 1964)	*Abramis sapa*	Russia	Khotenovsky (1985)
P. chazaricum	(Mikailov, 1973)	-	-	Khotenovsky (1985)
P. erithroculteris	(Achmerov, 1974)	-	-	Khotenovsky (1985)
P. kasimii	(Rahemo, 1980)	-	-	Khotenovsky (1985)
P. kuthkaschenicum	(Mikailov, 1973)	-	-	Khotenovsky (1985)
P. schulmani	(Mikailov, 1973)	-	-	Khotenovsky (1985)
Genus: ***Inustiatus*** Khotenovsky, 1978				
I. aritichthysi	(Ling, 1973)	*Aristichthys nobilis*	China	Gao *et al.* (2007)
I. inustiatus	(Nagibina, 1965)	*Hypophthalmichthys molitrix*	China	Khotenovsky (1985)
Genus: ***Eudiplozoon*** *Khotenovsky, 1985*				
E. nipponicum	(Goto, 1891)	*Carrassius vulgaris* *Cyprinus carpio* *Cyprinid* sp.	China Russia Ukraine	Kamegai (1968) Khotenovsky (1985)
Genus: ***Sindiplozoon*** Khotenovsky, 1981				
S. diplodiscus	(Nagibina, 1965)	*Elopichthys bambusa*	Russia	Khotenovsky (1985)
S. trelkowi	(Nagibina, 1965)	*Hemibarbus labeo*	Russia	Khotenovsky (1985)
S. ctenopharyngodoni	(Ling, 1973)	*Ctenopharyngodon idella*	China	Gao *et al.* (2007)
Genus: ***Diplozoon*** von Nordmann, 1832				
D. paradoxum	Von Nordmann, 1832	*Abramis brama* *Cyprinid* sp. *Rutilus rutilus* *Gobio gobio* *Blicca bjoekna* *Squalius cephalus* Bream	Ukraine Russia Europe Ireland Poland Germany Asia	Reichenbach-Klinke (1980) Khotenovsky (1985) Koval & Pashkevichute (1973) Stranock (1979) Fotedar & Parveen (1987)

Table2.2 (continue): A list of species from the family Diplozoidae Palombi, 1949 from around the world as compiled from literature.

Subfamily: **Neodiplozoinae** Khotenovsky, 1985				
Genus: ***Neodiplozoon*** Tripathi, 1960				
N. barbi	(Tripathi, 1959)	*Barbus chagunio*	India	Khotenovsky (1985) Reichenback-Klinke (1980) Mashego (2000)
Genus: ***Afrodiplozoon*** Khotenovsky, 1981				
A. polycotyleus	(Paperna, 1973)	*Labeo victorianus*	Kenya	Khotenovsky (1985)
		Barbus cercops	Tanzania	Paperna (1973 & 1979)
		B. kerstenii		
		B. macrolepis	South Africa	Mashego (2000)
		B. paludinosus		
		B. neefi		
		B. marequensis		
		B. trimaculatus		
		Alestes baremoze	Nigeria	Echi & Ezenwaji (2009)

Comment:

According to Khotenovsky (1985), Koval & Pashkevichute (1973) & Gao *et al.* (2007):

D. scardinii (Komarova, 1966) is identical to *P. homoion*

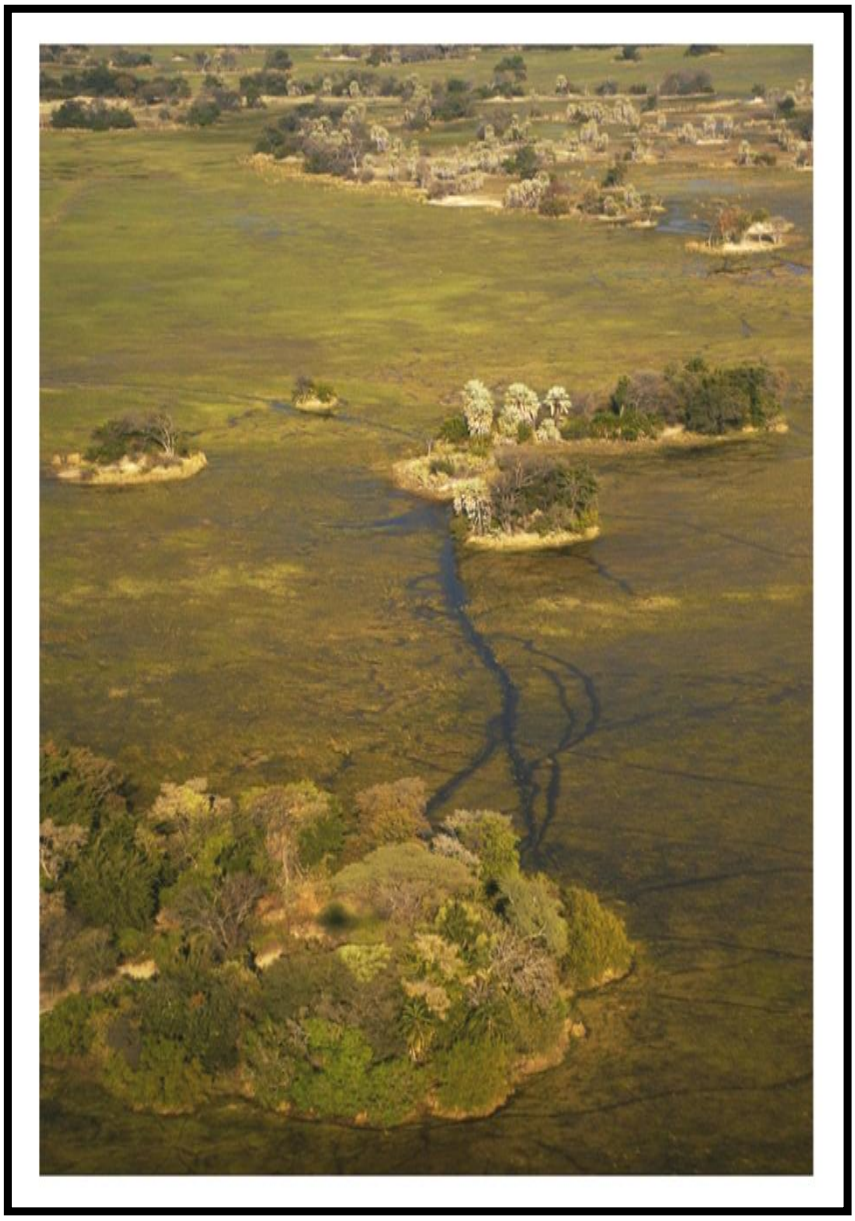
D. paradoxum sapae (Reichenbach-Klinke, 1961) is identical to *P. bergi*

P. bychowski (Nagibina, 1965) identical to *S. strelkowi*

List of species of which the current classification is unclear:

Species:	**Author:**	**Reference:**
D. paradoxum sapae	Reichenbach-Klinke, 1961	Koval & Pashkevichute (1973)
D. paradoxum ballerus	Komarova, 1964	Koval & Pashkevichute (1973)
D. paradoxum bliccae	Reichenbach-Klinke, 1961	Koval & Pashkevichute (1973)
D. cauveryi		Koval & Pashkevichute (1973)
D. balleri	Nagibina, Ergens & Pashkevichute, 1970	Koval & Pashkevichute (1973)
D. bergi	Gavrilova, 1964	Koval & Pashkevichute (1973)
D. gussevi	Glaser, 1964	Koval & Pashkevichute (1973)
D. markewitschi	Bychowsky, Gintovt & Koval, 1964	Koval & Pashkevichute (1973)
D. ctenpharynogodoni	Nagibina, -	Khotenovsky (1979)

Chapter 3



History of African Cyprinids

AFRICA'S FISH FAUNA

Africa contains an extremely diverse fish fauna with some 3 000 species inhabiting the inland waters. The families Denticipitidae, Distichodontidae, Pantodontidae, Phractolemidae, Kneriidae, Mormyridae and Gymnarchidae are endemic to Africa, dating

back to the Early Mesozoic (Leveque 1997). According to Skelton (1988) the Cyprinidae, Characidae, Bagridae, Schilbeidae, Amphiliidae, Clariidae, Mochokidae, Cyprinodontidae, Cichlidae and Gobiidae are present in most east and west Afro-tropical river systems. Leveque (1997) reported on Africa having over 2 000 non-cichlid species belonging to 340 genera and 75 families of which the majority belong to the families Cyprinidae and Characidae. The continental waters of southern Africa contains 280 species of fish in 105 genera and 39 families. Skelton (2001) states that the southern African fish fauna is rather poor especially when compared to certain regions in Africa such as the Congo River with more than 700 fish species, Lake Malawi with 845 species, Lake Tanganyika with an estimated 325 species and lastly Lake Victoria with 545 species.

The family Cyprinidae is an extremely abundant and widespread family with respect to the range of sizes, shapes and habitat preferences. Jubb (1967) stated that when considering the indigenous fish families included in the freshwater fish fauna south of the South Equatorial Divide and excluding the large endemic population of Cichlidae from Lake Nyasa, the family Cyprinidae has the highest number of fish species. According to Skelton (2001), the family comprises 275 genera, and more than 1 600 species, of which 24 genera and 475 species dominate the riverine faunas of southern, south-eastern and eastern Africa. It is also the largest fish family in southern Africa with eight genera and more or less 80 species of which some are threatened. Cyprinidae species are furthermore widely distributed throughout Europe, Asia and North America, but none native in South America and Australia (Tang *et al*. 2009). The majority of cyprinids in Africa belong to two large genera, namely *Barbus* Cuvier & Cloquet, 1816 (minnows) and *Labeo* Cuvier, 1817 (mudsuckers and yellowfishes) with *Barbus* probably being the only true pan-African genus. Even though both these genera are distributed throughout the Afro-tropical region, they are probably polyphyletic assemblages (Skelton 1988).

EVOLUTION OF CYPRINIDS IN AFRICA

Zoogeographers believe that the family Cyprinidae evolved in East Asia during the Tertiary Era where after they dispersed to Europe, North America and Africa. Ancestral forms of the

genera *Labeo* and *Barbus* spread to rivers in the southern tip of Africa where endemic species evolved (Skelton 2001). Various theories exist on the biogeographic history of cyprinid fish and whether these fish arose in Africa and then dispersed into Asia, or if they dispersed into Africa after arising in Asia. By using molecular phylogenetic analysis, Tang *et al.* (2009) found that the African *Labeo* and Asian *Cirrhinus* Oken (ex Cuvier), 1817 species shared a common ancestor with an Asian origin and therefore, proved the in-to-Africa dispersal route to be accurate. Almaca (1994) stated that the migration of Iberian *Barbus* populations to North Africa could have occurred during the late Miocene. This theory was also supported by Tang *et al.* (2009) who established that cyprinids probably entered the Nile area of northeast Africa, around nine million years ago. Cyprinids are not the only fish that undertook significant migrations, fish from the families Clariidae and Anabantidae also evolved in Asia and invaded Africa during the Upper Eocene. Dispersal of these stenohaline, true freshwater fishes had to take place via freshwater links associated with the slow progress of hydrographical and physiographical changes that occurred during the evolution of the continents (Jubb 1967).

Skelton (1993 & 2001) reported that fishes entered the southern parts of Africa in "waves" of invasion where each wave moved further south in times when different river basins were interconnected. The first wave most likely took place two to three million years ago during the mid-Pliocene with the Cape and Karoo fauna moving into the Orange and Cape coastal rivers. At some stage in the late Pliocene, about 1.8 to two million years ago, the second invasion took place. During this time, the Okavango-upper Zambezi and Limpopo Basin was thought to be connected. It is believed that less than 1.8 million years ago, in the Pleistocene, an invasion occurred linking the lower Zambezi and the Limpopo Basin (Skelton 2001). Jubb (1967) declared that the fish fauna of the present Orange and Vaal River Systems and the Olifants River of the south-western Cape display similarities, which is proof of recent connections between these river systems. This is also the case with fish faunas of the Kunene, Okavango, Mashi, Upper Zambezi and Kafue Rivers, which are quite similar but differ considerably from fish faunas in the Zambezi River System below the Victoria Falls. The suggested previous link between the Kunene and Okavango Rivers is supported by

Curtis *et al.* (1998) who states that fifty-nine of the Kunene species also occur in the Okavango River.

The temporary link between the Okavango-Ngami drainage and the Limpopo Basin is supported by the fact that the fish fauna of the Limpopo River shows similarities to fish fauna in rivers of both the east and west of southern Africa (Jubb 1967). According to Skelton (2001), the closest relatives of the *Labeo umbratus* (Smith, 1841) group of species are Asiatic *Labeo* species. This would propose that the fish fauna from southern Africa were linked with the fish from India before the separation of these two landmasses, about 120 million years ago. Most of the freshwater fish fauna of Africa have therefore speciated and evolved after dispersing into southern Africa from fish families that migrated southwards from Asia and Africa, north and south of the South Equatorial Divide.

In an article by Gabie (1965), it is mentioned that numbers and diversity of fishes in southern African rivers decrease from north to south. About 134 freshwater fish species make up the Zambezi River System's fish fauna, which is considerably more than when compared to the fish fauna of rivers situated to the south. The Cunene has 66 species, the Limpopo 50, the Phongolo 40, the Orange 16, the Tugela 12, the Olifants 10 and lastly the Berg River with four species. Tweddle *et al.* (2009) stated that this pattern of fish species numbers can be seen as a result of two factors, namely a general pattern of declining species numbers from tropical to temperate zones along with a pattern of fish distribution reflecting the drainage history.

THE RISE OF SOUTHERN AFRICAN RIVER SYSTEMS

About 60 million years ago, southern Africa's most important river systems arose from Africa. During this time, arches started forming in the interior of the African continent, which was followed by a series of events resulting in the formation of the Kalahari Basin, a depression in the interior of southern Africa. According to Tweddle *et al.* (2009), it is generally agreed upon, that an early large river system flowed south-west from the Lake Bangweulu region in Zambia, into the Kafue, Upper Zambezi, Okavango and Kunene Rivers.

These rivers in turn flowed into a large central lake in the Okavango Delta-Makgadikgadi region. Another theory is that these rivers flowed into the Atlantic Ocean. McCarthy & Rubidge (2005) stated that as the Kalahari-Zimbabwe Axis cut off the headwaters of the Limpopo, Lake Makgadikgadi started forming in the interior. It is during this region of time that the uplift of the Transvaal-Griqualand Axis resulted in the capture of the Karoo River by the Kalahari River, which brought forth the Orange River system.

According to McCarthy & Rubidge (2005), the asymmetrical appearance of South Africa's drainage is attributable to the Vaal and Orange Rivers rising close to the east coast and flowing westwards across the entire country. This phenomenon was a result of plume activity that caused a breakup in the east. In an article by Skelton & Cambray (1981), it was suggested that the Orange River was formerly two separate river systems. The south west via the Olifants to the sea was drained by the upper Orange; and the lower Orange had an enlarged northern drainage of which the Molopo River and its tributaries are remnant.

More or less 14 million years ago, arid conditions developed in the west of southern Africa due to the upwelling of cold water on the west coast. This occurrence together with the progressive capture of inflow by die Zambezi River led to the drying up of lakes in the Kalahari Basin. While the East African Rift system continued south-eastwards, it resulted in the lower Zambezi capturing rivers such as the Kafue and most recently the Kwando. The Kunene River was thought to be the first to break away from this central complex of rivers and therefore broke away before the Kafue (Tweddle *et al.* 2009). McCarthy & Rubidge (2005) suggested that the Okavango River was next in line to be diverted by the Zambezi, but the outcome has temporarily been blocked by the rift related faults resulting in northern Botswana's magnificent Okavango Delta. The Okavango and Upper Zambezi Rivers are today again connected via the Selinda spillway, after a 20 to 30 year drought.

FISH FROM THE OKAVANGO RIVER

According to Mendelsohn & El Obeid (2004) the Okavango River contains 83 species of fish from the families Anabantidae, Amphiliidae, Characidae, Cichlidae, Clariidae, Claroteidae, Cyprinidae, Cyprinodontidae, Distichodontidae, Hepsetidae, Kneriidae, Mastacembelidae,

Mochokidae, Mormyridae and Schilbeidae. It has been found that a stretch of river in the Delta could usually be occupied by 15 to 30 species at any one time. Tweddle *et al.* (2003) found the highest diversity of fish species in the Okavango River at Shakawe, which is situated in the Upper Panhandle and where more than 54 fish species were recorded. A list of all the fish collected during the 1997 to 2009 survey by the Aquatic Parasitology Research Group from the Okavango River are given in the Appendix, Table 8.1. Only the fish species found to be infested with diplozoids are discussed below. Photographs of *Labeo capensis* and *L. umbratus* are from the Aquatic Parasitology Group. The rest of the fish photographs were adapted from J.R. Tweddle (used with his permission).

Barbus afrovernayi Nichols & Boulton, 1927

Common name: Spottail barb

Size: 45 mm

Habitat and Ecology: Benthopelagic species, present in various habitats such as swamps, lagoons, pools as well as main river channels and under and along the edges of papyrus mats (Marshall *et al.* 2009[a]). It prefers quiet, well-vegetated waters and feeds from the surface and on small invertebrates living on plant surfaces (Skelton 2001). This species is known to tolerate low oxygen conditions (Tweddle *et al.* 2003).

Distribution: Widespread in the upper Zambezi River System as well as the Cunene, Okavango, Kafue and Congo Rivers Systems. In central Africa, this species is present in the Lualaba River, Lake Upemba and Luapula-Mweru System.

Barbus multilineatus Worthington, 1933

Common name: Copperstripe barb

Size: 45 mm

Habitat and Ecology: Benthopelagic species (Marshall *et al.* 2009[b]). It inhabits shallow lagoons and well-vegetated water in backwaters, floodplains as well as river margins (Skelton 2001).

Distribution: Present in various rivers of southern Africa, i.e. the Cunene, Okavango, Upper and Middle Zambezi and Kafue Rivers as well as Zambian Congo in the Lake Bangweule area (Skelton 2001).

Barbus paludinosus Peters, 1852

Common name: Straightfin barb

Size: 150 mm

Habitat and Ecology: Hardy benthopelagic species (Bills *et al.* 2009[a]). It occupies a wide range of habitats ranging from quiet, well-vegetated waters in lakes, swamps and marshes to large rivers and small streams. This species does not occur in densely vegetated swamps and prefers larger open pools with high plant diversity. *Barbus paludinosus* feeds on a range of small organisms, i.e. insects, small snails and crustaceans, algae, diatoms and detritus (Skelton 2001).

Distribution: Typical pioneer species widespread throughout Africa (Tweddle *et al.* 2003). According to Bills *et al.* (2009[a]) in Central Africa this species occurs in the headwaters of the Lualaba and Sankuru Rivers in the Congo. In eastern Africa, it can be found in the Lake Victoria basin, Athi and Tana River Systems. This species also inhabits the upper Pangani System, Amboseli swamps as well as Lake Naivasha and its effluents. It has also been reported from Lakes Tanganyika and Malawi with their various streams and rivers (Delaney *et al.* 2006). In northern Africa, it reaches the Awash Basin and rift lakes of Ethiopia. Lastly, *B. paludinosus* is widespread in southern Africa's east coastal rivers from East Africa down to Kwazulu-Natal and from the Quanza in Angola to the Orange River.

Barbus poechii Steindachner, 1911

Common name: Dashtail barb

Size: 110 mm

Habitat and Ecology: Benthopelagic species (Marshall *et al.* 2009[c]). According to Skelton (2001), it is regularly found in association with *Brycinus lateralis* (Boulenger, 1900), the striped robber. These two species resemble one another quite closely and this phenomenon can be explained as mimicry. *Barbus poechii* is usually present in riverine and floodplain habitats and also in open waters of main river channels and open lagoons where they feed on small insects and organisms (Marshall *et al.* 2009[c]).

Distribution: This species is distributed through the Upper Zambezi River System and Kafue River (Jubb 1967). According to Marshall *et al.* (2009[c])

possible records of *B. poechii* from the Kasai River System in the Central Congo River Basin has been reported. Other known localities are the Cunene and Okavango Rivers as well as a few records in the Middle Zambezi River.

Barbus radiatus Peters, 1853

Common name:	Beira barb
Size:	120 mm
Habitat and Ecology:	Benthopelagic species (Bills *et al.* 2009[b]). According to Skelton (2001) it is active in subdued light and at night, favouring marshes and marginal vegetation of streams, rivers and lakes. They are also found in rock pools in the Komati River, Swaziland and have even been observed on rocky shore in Lake Malawi (Bills *et al.* 2009[b]).
Distribution:	Widespread through central, eastern and southern Africa. In central Africa, records have been confirmed in the Lulua from the Kasai River System. In eastern Africa, the species is present in the Lake Victoria Basin, the Tana River System, Malagarasi River and Rukwa System (Bills *et al.* 2009[b]). In southern Africa, it ranges from Uganda to the Zambian Congo, Cunene, Okavango, Zambezi and east coast rivers south of the Phongolo System (Skelton 2001).

Labeo lunatus Jubb, 1963

Common name:	Upper Zambezi labeo
Size:	400 mm
Habitat and ecology:	Generally absent from rocky habitats and prefers the main river channel and large soft-bottomed floodplain lagoons (Marshall & Tweddle 2007). It grazes on algae, "aufwuchs" and detritus. According to Skelton (2001), it is a shoaling species and breeds in flooded marginal habitats.
Distribution	Present in the upper Zambezi and Okavango Rivers (Skelton 2001).

FISH FROM THE ORANGE-VAAL SYSTEM

According to Skelton & Cambray (1981), fishes of the Orange River System were of the first species of fish to be described in southern Africa. Fish belonging to the families Cyprinidae, Cichlidae, Austroglanididae and Clariidae are present in the various rivers of the Orange-Vaal System. Skelton (2001) puts the number of indigenous fish species in the Orange River at 16, of which four are endemic and two of these, *Barbus hospes* Barnard, 1938 and *Austroglanis sclateri* (Boulenger 1901) are listed as rare in the red data book. A list of fish species present in the Orange-Vaal River System is given in the Appendix, Table 8.2. The two species of fish found to be infested with diplozoids during the study are discussed below.

Labeo capensis Smith, 1841

Common name: Orange River mudfish

Size: 500 mm

Habitat and ecology: Successful lotic species. According to Skelton (2001), they prefer running water of large rivers but are also present in large impoundments. Their range of habitats also include quiet, weedy backwaters, standing open waters, flowing open waters, sandy rocky stretches as well as rocky rapids (Skelton & Cambray 1981). This species feeds on plants, grazes from firm rock surfaces and are known to dredge the bottom of sand for small animals (Jubb 1967).

Distribution: Most common large fish species in the Orange and Vaal Rivers, below and above the Augrabies Falls and is endemic to this system (Skelton & Cambray 1981).

Labeo umbratus Smith, 1841

Common name: Moggel or Mud mullet

Size: 500 mm

Habitat and ecology: *Labeo umbratus* favours a lentic environment and is abundant in shallow impoundments and farm dams and prefers standing or gently flowing water where they feed on soft sediments and

detritus (Skelton & Cambray 1981). This species is recognised for being able to tolerate a wide range of water conditions such as temperature and quality of the water (Jubb 1967). *Labeo umbratus* is able to hybridise with *L. capensis* especially when both species are present in impounded waters (Skelton 2001).

Distribution: *Labeo umbratus* is a familiar fish species in the Orange-Vaal River System, above the Augrabies Falls and with a broad distribution including the Gourits, Gamtoos, Sundays, Bushmans, Fish and Keiskamma Rivers in the Eastern Cape as well as the Olifants-Limpopo System (Skelton 2001). According to Swartz & Impson (2007), because of inter-basin transfer schemes, there may also be other areas where this species has become established. The species is also present in the Buffalo River and this is presumably due to it being translocated by anglers who used it for bait (Jubb 1967).

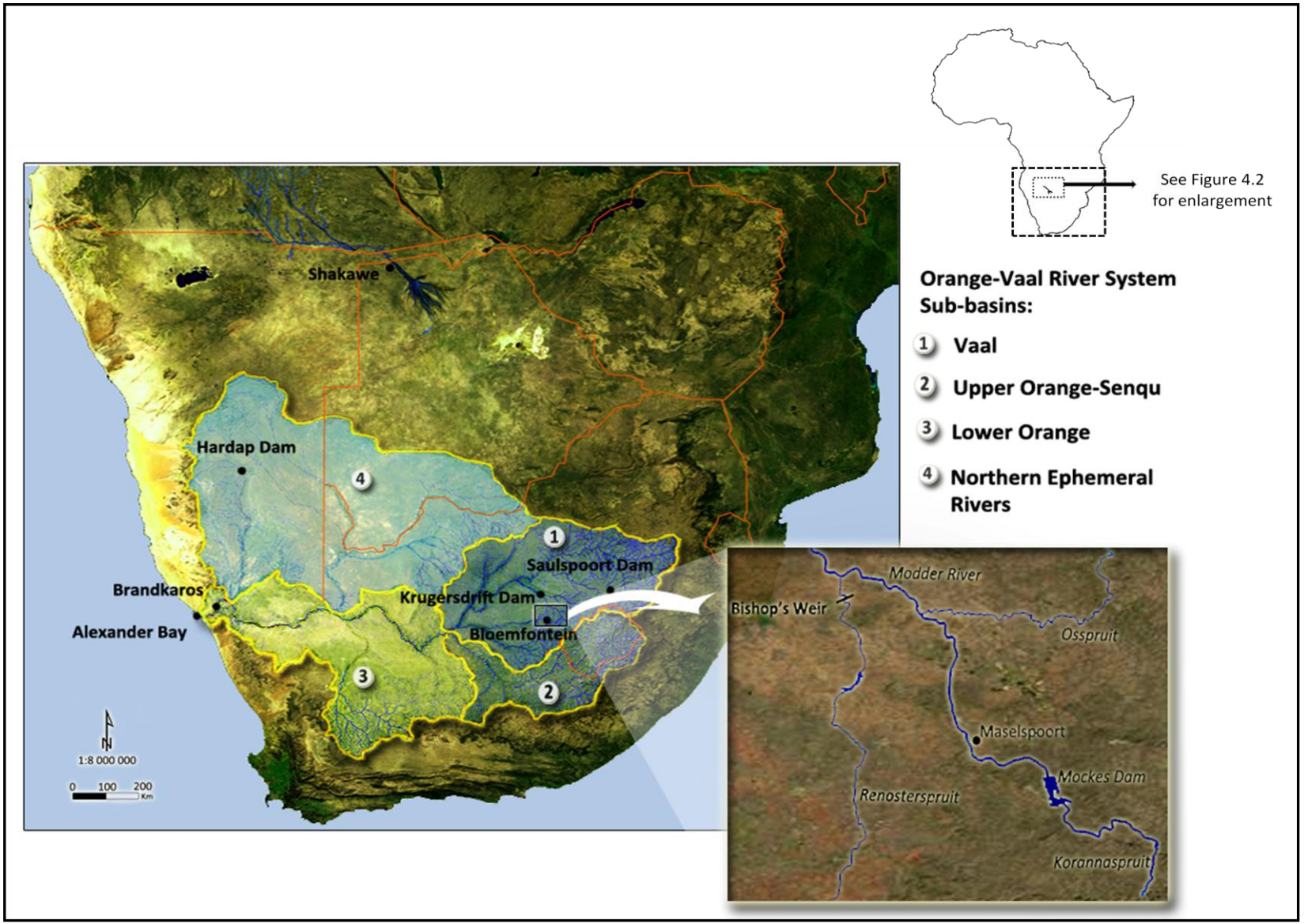


Figure 4.1: Map of southern Africa showing the specific locations of all the sampling sites in the Orange-Vaal River System.

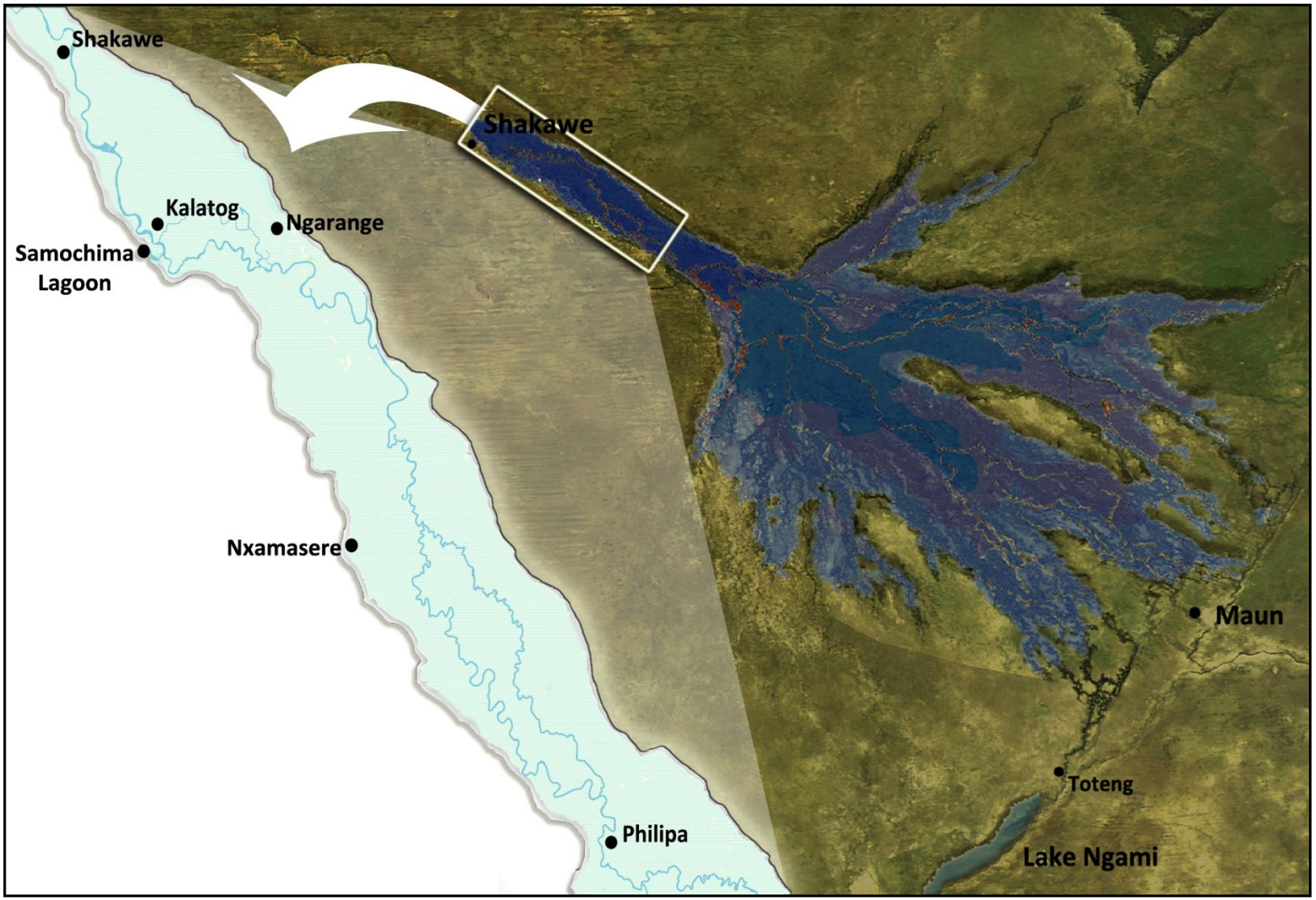


Figure 4.2: Map of the Okavango River and Delta showing the specific sampling locations in the Panhandle part of the river.

Chapter 4

Materials and Methods

The Okavango Delta is a pristine environment, known as the jewel of the Kalahari, appealing to birders, anglers, scientists, tourists and anyone with a love for nature. The Orange-Vaal River System on the other hand, differs greatly from the Okavango, being a much larger system flowing through a diversity of ever changing areas. In this chapter, the study areas in both these systems will be discussed only briefly, but plenty of books and magazine articles are available for further reading especially on the Okavango system, i.e. Okavango Delta:

Floods of life (Mendelsohn *et al.* 2010) and Okavango: Jewel of the Kalahari (Ross 2003), to name only a few.

STUDY AREA

Okavango River

Cuando Cubango, or better known as the Okavango River is one of Africa's great rivers. It has its origins in the Angolan highlands where a series of headwater streams form the boundary between Angola and Namibia, before crossing over to the Caprivi Strip and entering Botswana (Alonso & Nordin 2003). It does, however, not flow down to an ocean as expected with rivers, but disperses across one of the largest inland alluvial fans in the world, as the renowned Okavango Delta (Figures 4.1 & 4.2). The Okavango River forms part of the larger Okavango basin, being comprised of perennial and ephemeral sub-catchments and the basin is believed to drain about 725 000 km^2 in central southern Africa (Pinheiro *et al.* 2003). When the Okavango enters Botswana, it does so as the panhandle, with a river width of around 200 m and a depth of 2 to 8 m. The river is known to have a mean annual discharge of about 9.86 x 10^9 m^3 with wet periods in the panhandle, during February to March and reaching the distal end of the delta in July (Mosepele *et al*. 2009). Another localised wet period also occurs in December to March, caused by rains. Low flows are usually during October and November (Ramberg *et al.* 2006). The aquatic ecosystems are incredibly complex and completely dependent upon these annual floods and the flooding can be seen as the main driving force for fish breeding.

The fish communities in the Okavango River can be grouped into two dimensions according to their food preferences and secondly to the habitats they choose to occupy (Mendelsohn & El Obeid 2004). Wide varieties of fish are present in the river, each with different food preferences which range from detritivorous, e.g. various barbs and minnows, herbivorous for example breams and lastly predators like the notorious Tigerfish, *Hydrocynus vittatus* Castelnau, 1861. The river contains diverse habitats ranging from the Okavango mainstream and endless river channels to backwaters, floodplains, lagoons and stretching out into perennial and seasonal swamps. Of these, the floodplains and seasonal swamps

play an extremely important role in acting as the breeding and nursery spots for fish, shielding young from larger predators. The flooded areas contain affluent amounts of nutrients sufficient for sustaining vast plant growth, insects and other small animals (Mendelsohn & El Obeid 2004).

Orange-Vaal River System

The Orange-Senqu River Basin is one of the largest river basins in the world and the largest in Africa south of the Zambesi River Basin (Figure 4.1). An area of approximately 900 000 km^2 is covered by the basin of which 62% is situated in South Africa (Knoesen *et al.* 2009). Runoff in the basin is disproportionately distributed making it amongst the most water rich and most water scarce region in Africa. The basin encapsulates a diverse landscape ranging from the Senqu River in the Lesotho highlands, through the grasslands and savannah of central South Africa and southern Botswana. It also stretches through the Nama and Succulent Karoo of western South Africa and southern Namibia, before spilling into the Atlantic Ocean at Alexander Bay (Earle *et al.* 2005).

According to Knoesen *et al.* (2009), the Orange-Senqu River basin is the most developed transboundary river basin in southern Africa and supplies water to municipalities, industries and farms in and around the basin. The two main tributaries to the Orange River are the Senqu River, originating in the Maluti mountain range in Lesotho, and the Vaal River, which rises on the eastern highveld escarpment in the north-eastern parts of South Africa (Earle *et al.* 2005). The Vaal River joins the Orange River 13 km west of Douglas, in the Northern Cape. Other major tributaries include the Harts, Fish, Caledon, Molopo, Modder and Nossob, all supporting the livelihoods of millions of people dependant on these rivers.

The basin can be divided into four sub-basins (Figure 4.1) first of which is the Vaal River (1) followed by the Upper Orange-Senqu River (2), the Lower Orange River (3) and lastly the Northern Ephemeral Rivers (4). Collections for this study were made at localities in the Vaal River sub-basin, the Northern Ephemeral Rivers and the Lower Orange River.

Figure 4.1

Figure 4.2

The Vaal River sub-basin

The Vaal River has its origins on the plateau west of the Drakensberg escarpment and drains most of the north-eastern part of the basin. Tributaries to the Vaal River include the Klip, Wilge, Liebenbergsvlei, Mooi, Schoonspruit and Harts Rivers as well as the Riet River, of which the Modder River is a tributary (Avenant 2008). Collections were made at three sites in the Vaal River sub-basin, one of which was at the Saulspoort Dam and two in the Modder River, namely Bishop's Weir in the Renosterspruit and Krugersdrift Dam in the Soetdoring Nature Reserve.

Site 1: Saulspoort Dam

The Saulspoort Dam was constructed in 1969, in the Liebenbergsvlei River and is situated outside Bethlehem. The dam forms part of the Lesotho-highlands Water Project and water is transferred from Lesotho via the Ash River into the Saulspoort Dam. Water then flows along the Liebenbergsvlei River, which in turn flows to the Wilge River before reaching the Vaal Dam (Hall & Jennings 2007). The Liebenbergsvlei River together with the Wilge River contribute to 36% of the surface flow in the Upper Vaal River area.

Site 2: Bishop's Weir in the Renosterspruit

Renosterspruit is a tributary of the Modder River, which receives Bloemfontein's run-off and wastewater via the Bloemspruit (Avenant 2008). The Modder River is a non-perennial river associated with intermittent flow and has its origins in the hills of south-eastern Free State, from where it flows in a north-westerly direction before turning west. The catchment area covers an area of about 17 360 km^2 and is largely situated in the south central Free State Province with a smaller part in the Northern Cape Province (Seaman *et al.* 2002). The Modder River unites with the Riet River, where after it flows in a westerly direction and joins with the Vaal River west of Douglas. Close to where the Renosterspruit flows into the Modder River a concrete weir was erected known as Bishop's Weir, situated about 20 km outside Bloemfontein, in a north-easterly direction (Figures 4.1 & 4.4 H). According to Avenant (2008), fish species in this river and its tributaries are adapted to survive and tolerate periods of low-flow to no-flow as well as the coinciding changes in water quality and habitat conditions. In a study done by Avenant (2008) on fish biomonitoring of the

Modder River, the Lower Middle Modder River was sampled. This fish habitat segment stretches from below the Mazelspoort Weir to the Krugersdrift Dam, including the Renosterspruit. During this survey, seven indigenous species were observed, namely *Barbus anoplus* Weber, 1897, *Labeobarbus aeneus*, *Labeo umbratus*, *L. capensis* (Smith, 1841), *Clarias gariepinus* (Burchell, 1822), *Pseudocrenilabrus philander* (Weber, 1897) and *Tilapia sparrmanii* Smith, 1840. Of these species, *Tilapia sparrmanii* and *Labeo capensis* were the most abundant.

Site 3: Krugersdrift Dam

The Krugersdrift Dam was built in 1970 and provides irrigation water to farmers along the lower reaches of the Modder River. The dam is situated in the Soetdoring Nature Reserve about 45 km north-west of Bloemfontein, on the Modder River. According to Avenant (2008), seven indigenous species are present in the Soetdoring Reserve, near the inflow to the dam. These species are *Labeobarbus aeneus*, *Barbus anoplus*, *Labeo capensis*, *L. umbratus*, *Clarias gariepinus*, *Pseudocrenilabrus philander* and *Tilapia sparrmanii*. *Labeo capensis* was found to be the most abundant of these species. The two exotic species *Cyprinus carpio* Linnaeus, 1758 and *Gambusia affinis* (Baird & Girard, 1853) are also known to be present, although they were not collected during Avenant's (2008) survey. Collections made further into the Krugersdrift Dam produced the same indigenous species with the addition of *Barbus paludinosus.*

Northern Ephemeral Rivers

The Northern Ephemeral Rivers are distributed throughout southern Africa, with 27% of the catchment area in South Africa, 42% in Namibia and 31% in Botswana. The sub-basin is comprised of the Molopo, Luruman, Nossob, Auob and Fish Rivers. The Fish River flows a distance of 636 km from the south of Windhoek, before joining the Orange River about 100 km upstream from Alexander Bay (Earle *et al.* 2005).

Site 4: Hardap Dam

Hardap Dam is situated in the ephemeral Fish River. According to Curtis *et al.* (1998) seepage water from Hardap together with permanent springs, sustain the Fish River. The

dam maintains all of the annual floodwater from the upper catchment and only in exceptional floods water is released. The Lower Orange and Fish Rivers contains fourteen freshwater fish species, of which two are endemic (Curtis *et al.* 1998). According to Okeyo (2003) *Labeobarbus aeneus*, *L. kimberleyensis, Labeo capensis, L. umbratus, Barbus paludinosus, Clarias gariepinus, Oreochromis mossambicus* (Peters, 1852) and *Cyprinus carpio* are present in the Hardap Dam.

Lower Orange sub-basin

The Upper Orange sub-basin originates in the Lesotho Highlands and extends to where the Orange and Vaal Rivers meet. From this point of confluence, the Orange River flows west as part of the Lower Orange sub-basin. The major tributaries to the sub-basin are the Ongers River, that joins the Orange River about 80 km downstream from Upington and the Sak River from the northern Karoo. In addition, the Kuruman and Molopo Rivers from the Cape Province and the Fish River from the southern part of Namibia, which also form part of the main tributaries to the Orange River. From here the Orange River flows into the Orange River mouth at Alexander Bay and ultimately the Atlantic Ocean.

Site 5: Brandkaros

The small community of Brandkaros is situated 120 km downstream to where the Fish River meets the Orange River and about 27 km upstream from Alexander Bay. This is next to the last stretch of the Lower Orange River before it becomes an estuary at Alexander Bay and flows into the South Atlantic Ocean. The Lower Orange is over 1 000 km long and stretches from the confluence of the Orange and Vaal Rivers to its point at Alexander Bay (DWAF 2009).

FIELDWORK

Diplozoid material investigated during the present study was collected from a number of different localities using different methods. The material comprised of preserved specimens collected over the past fifteen years during fish parasitological surveys by the Aquatic Parasitology Research Group of the Department of Zoology and Entomology (University of the Free State), as well as material collected during the present study.

Fieldtrips to the Okavango River, Botswana, ranged from four weeks up to two months, and surveys were carried out during the periods November 2008 to January 2009 and August to September 2009. Throughout this time the Leseding Research Camp of the Aquatic Parasitology Research Group, situated on the periphery of Samochima village near Shakawe, acted as base camp for the most part of these trips (Figures 4.5 C – F & 4.6 A). The tented camp is located next to Samochima Lagoon and is equipped with a laboratory and aquarium; therefore, fish could be kept in holding tanks over periods to aid in the fish examination process. Fish were collected during various boat trips to lagoons and channels up and down stream of the base camp. As many different habitat types as possible were sampled, ranging from the mainstream to channels, lagoons, backwaters and floodplains (Figure 4.4 A – F). Collections sites included, the mainstream near Shakawe, Samochima Lagoon, Kalatog, Philipa and Ngarange Channel. Various fish collection trips were also undertaken with 4x4 vehicles to remote areas such as the Nxamasere Floodplains (Figure 4.6 E) and Lake Ngami where fish was collected at Toteng (Figure 4.5 A & B). The latter trips occasionally called for the setup of a temporary camp and field laboratory. In such cases fixation and preservation methods had to be kept as simple as possible.

Most of the preserved material was collected from various locations in the Orange-Vaal River System as described above and show shown in Figure 4.1. During the present study, collections mainly took place at Bishop's Weir (site 2) (Figure 4.4 H).

COLLECTION AND EXAMINATION OF FISH

Various fish collection methods were employed in order to sample a wide range of fish species with diverse habitat preferences (Figures 4.4 A – H & 4.6 A, C - E). Fish collection in the Okavango River posed a challenge due to the vast variety of habitats and resulted in a number of interesting collecting techniques. Cast nets turned out to be the most rewarding method especially on shallows banks of lagoons, in swamps and floodplains. Hoop nets were effectively used for the collection of smaller *Barbus* species, in narrow channels (Figure 4.4 E). Collection in deeper lagoons were done by means of gill nets, which were put out at

dusk, left overnight and removed early the next morning. This method was not frequently used and less successful, as the gill nets served as a convenient food source for crocodiles. In some of the isolated pools in the Nxamasere Floodplains, seine nets were employed in order to collect bottom dwelling fish species (Figure 4.4 B). Fish from the Orange-Vaal River System was mostly collected by means of cast nets and an electro-fishing apparatus. The cast nets proved to be more efficient in pools while the electro-fishing apparatus worked better in the faster flowing streams.

After collection, fish were kept alive and transported to the Leseding Research Camp where they were transferred to a well-equipped aquarium with various holding tanks (Figure 4.5 E & F). Collection of fish in the Okavango River was sometimes carried out in remote areas and in such conditions that a temporary field laboratory was set up (Figure 4.5 A & B). Upon examination, the fish were anaesthetised with MS222 and examined by the aquatic parasitology group for ectoparasites as well as endoparasites (Figure 4.5 C & D). Live diplozoid specimens were removed from the gills with a fine brush (5/0) and fixed in either 70% ethanol or 10% formalin. A few specimens were fixed in osmium for use in scanning electron microscopy. During field trips in the Bloemfontein area, fish were collected and transported in temporary tanks to the laboratory at the Department of Zoology and Entomology. Once there, they were also kept alive in holding tanks for examination.

MICROSCOPY PREPARATION

Light microscopy

In preparation for compound light microscopy, whole mounts were prepared with a range of staining methods and mounted in Eukitt. In order to study a variety of diplozoid characteristics, ranging from internal to opisthaptoral structures, a number of staining methods were employed including Mayer's Hematoxylin, Mayer's Paracarmine, Mayer's Carmalum, Harris's Alum Hematoxylin as well as Grenacher's Alum Carmine (see Table 8.5 in appendix). Not all stains were equally effective; Mayer's hematoxylin worked well for fixed specimens whereas other specimens produced dark stained internal organs and hooks.

Mayer's Hematoxylin

Diplozoid specimens were gradually hydrated from 70% ethanol and rinsed in tap water. The specimens were then stained in Mayer's Hematoxylin for 20 minutes and differentiated in acid alcohol. Hereafter the specimens were again rinsed in tap water and placed in Scott's solution until blue. After another rinse in tap water the specimens were gradually dehydrated to 100%, cleared in xylene and mounted in Eukitt.

Mayer's Paracarmine

The specimens were slowly passed through distilled water where after they were stained in the dye solution for 30 minutes and differentiated in acid alcohol. Afterwards the specimens were rinsed in 70% ethanol and soaked in 70% for 1 minute and this was repeated three times. The same was done with 96% ethanol. Finally, the specimens were dehydrated in absolute ethanol and mounted under a cover glass with Eukitt.

Mayer's Carmalum

This technique was carried out by slowly hydrating the specimens in water. The specimens were stained overnight in the Carmalum working solution. The following day the specimens were transferred to 30% ethanol for 30 minutes. The same was done for 50%, 70%, 80% and 90% ethanol concentrations. Specimens were transferred to 96% ethanol for 30 minutes, twice. The same process was followed when the ethanol was replaced with 100% ethanol. After completing the dehydration process, the specimens were transferred to a 1:1 solution of absolute ethanol and xylene for 30 minutes. This step was repeated with 1:2 and 1:4 solutions of ethanol and xylene consecutively until the specimens were cleared in pure xylene for 60 minutes. The final part of the process was completed by repeating the last three steps, but with 1:1, 1:2 and 1:4 solutions of xylene and Eukitt, each for 15 minutes. Lastly, the specimens were mounted in Eukitt under a cover slide.

Harris's Alum Hematoxylin

In this technique, specimens were hydrated from 70% ethanol to distilled water overnight. The specimens were stained with the Alum Hematoxylin solution for 2 to 3 hours and

transferred to 30% ethanol for 30 minutes. From this point on the same steps were followed as described in the Mayer's Carmalum staining technique.

Grenacher's Alum Carmine

This was the last staining technique that was used. The specimens were also hydrated from 70% ethanol to distilled water and stained in the Alum Carmine solution for 2 hours after which the same procedure followed as with the previous two techniques.

Scanning Electron Microscopy (SEM)

Some specimens were post fixed in osmium to establish if this technique would allow less 'charging' to take place during the SEM process. Specimens used for SEM studies were cleaned with a fine brush (5/0) to remove mucus and debris, whereupon they were dehydrated in graded ethanol concentrations, ranging from 70% to 100%, critical point dried, gold coated using an Emscope SC500 sputter coater and viewed with a Jeol Winsem JSM 6400 SEM at 10 kV.

Osmium

Osmium was mixed up to 2% and 5 ml double distilled water added. The specimens were placed into the osmium solution, covered and left to stand for 20 minute where after it was rinsed with phosphate buffer for 20 to 30 minutes. The dehydration process was then followed by transferring specimens to 30% ethanol for 20 minutes, this was repeated to 100% ethanol and critical point dried.

Confocal Laser Scanning Microscopy

Whole mount slides of specimens prepared for light microscopy and stained with hematoxylin, were used for examination with the confocal light microscope and produced very clear images. Specimens fixed in 70% ethanol were also stained with Orange G ($C_{16}H_{10}N_2NA_2O_7S_2$), an Azo dye, for 10 to 15 minutes and mounted on a temporary slide.

MORPHOLOGICAL MEASUREMENTS

Whole mounted specimens where measured in a similar fashion to that proposed by Khotenovsky (1985). Species descriptions of African diplozoids by Thomas (1957), Fischthal & Kuntz (1963) and Paperna (1973) were also examined to ensure no structures were overlooked during the measuring process. Measurements of whole mounts and preserved specimens were made with the aid of a Leitz light microscope and drawing attachment, as well as a Wild dissection microscope and drawing attachment. In total, the length and width of 12 characters were measured as shown in Figure 4.3.

Unless otherwise indicated, all measurements are in micrometers. Measurements are presented in the following manner: mean and standard deviation followed in parentheses by the minimum and maximum values. Where less than ten specimens where measured, no standard deviation is provided. A list of all the measurements taken during the study are given in the Appendix (Table 8.4).

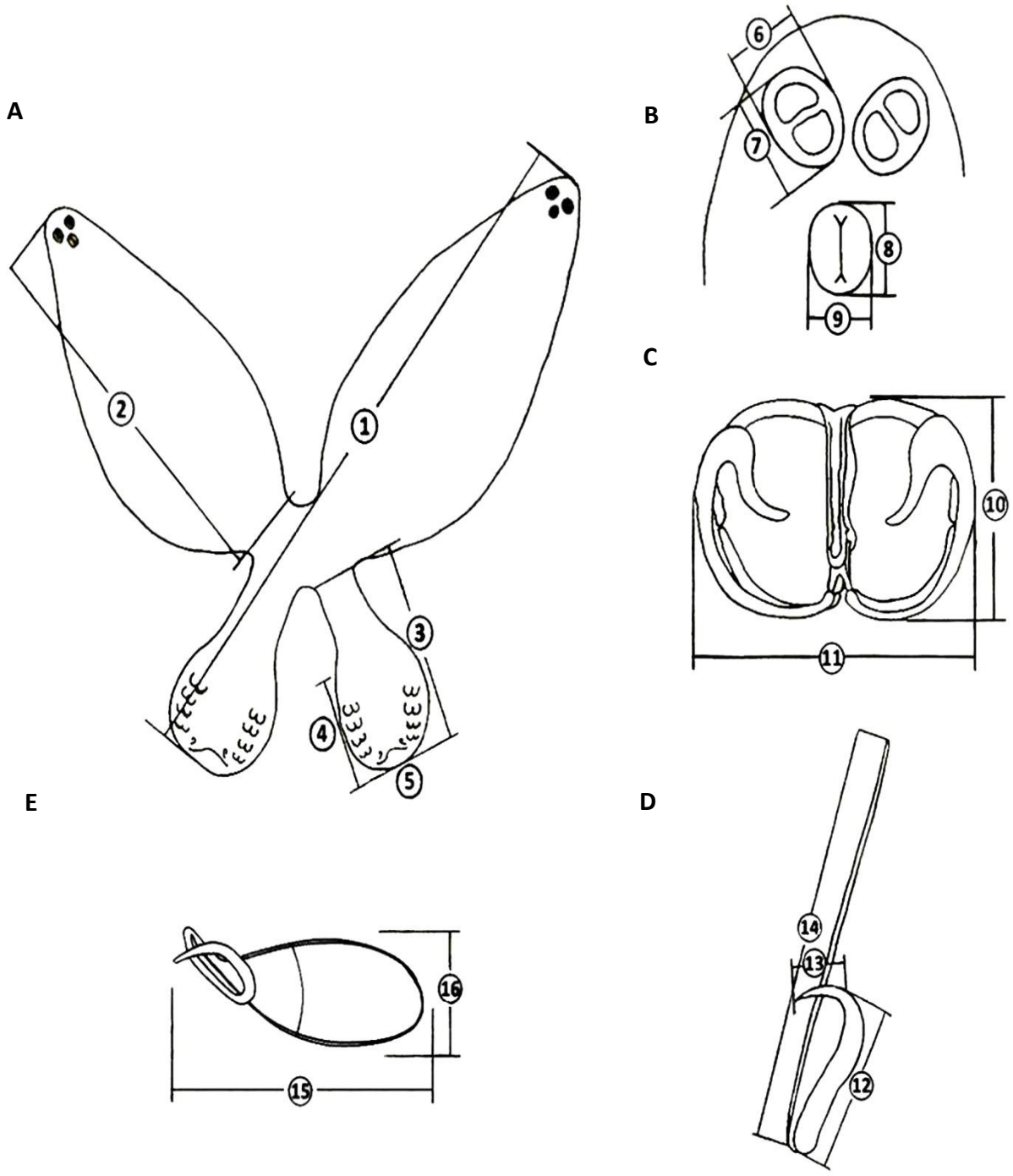


Figure 4.3: Illustrations adapted from Khotenovsky (1985), to indicate the measurements taken in order to obtain morphometric data for use in identification. **A:** 1 - total body length, 2 - anterior length, 3 - posterior length, 4 – opisthaptor length and 5 – opisthaptor width. **B:** 6 - oral sucker length, 7 – oral sucker width, 8 – pharynx length and 9 – pharynx width. **C:** 10 –clamp length and 11 – clamp width. **D:** 12 – central hook length, 13 – length of handle and 14 – length of hook body. **E:** 15 – egg length and 16 – egg width.

IMAGING

Digital images of the relevant structures were taken using a Zeiss Axiophot compound microscope and a Nikon DXM1200F digital camera. These images were analysed and additional measurements made using the ImageJ software package.

TYPE AND REFERENCE MATERIAL

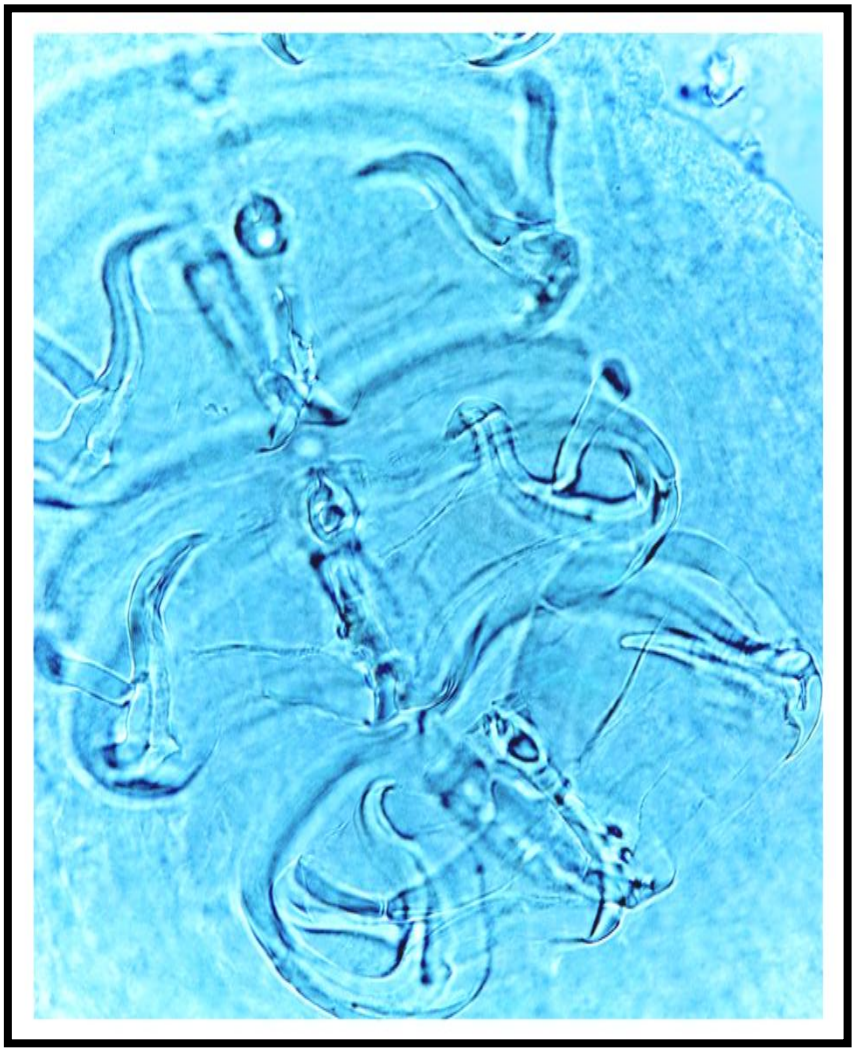
All type and reference material is deposited in the collection of the Aquatic Parasitology Research Group, Department Zoology and Entomology, University of the Free State. Descriptions of two new species are contained in this dissertation, i.e. *Diplozoon* sp. A and *Paradiplozoon* sp. A. These species have not yet been named, to avoid confusion and descriptions will only be regarded as valid once they have appeared in an accredited systematic journal.

Figure 4.4: **A** - Constructing a fish 'net'. **B – G** - Fish collection in the various habitats of the Okavango River, Botswana. **H** - Fish collection by means of a cast net at Bishop's Weir in the Renosterspruit.

Figure 4.5: **A & B** – Field trip to Lake Ngami. **C & D:** Fish dissection and lab work at Leseding Research Camp, Okavango River, Botswana. **E & F** – Outdoor aquarium with holding tanks at Leseding Research Camp. **G & H** - Relaxing after a hard day's work.

Figure 4.6: **A** – Samochima Lagoon with Leseding Research Camp, indicated by the arrow. **B** – Samochima Village. **C** – Swamps and islands in Okavango River. **D** – Typical lagoon in the Okavango River. **E** – The Nxamasere Floodplain.

Chapter 5



Results

SPECIES DESCRIPTIONS

***Afrodiplozoon polycotyleus* (Paperna, 1973)**

Hosts: *Barbus afrovernayi* Nichols & Boulton, 1927, *B. multilineatus* Worthington, 1933, *B. paludinosus* Peters, 1852, *B. poechii* Steindachner, 1911 *&* *B. radiatus* Peters, 1853 (Table 5.1 & 5.2).

Locality: Okavango River (Samochima Lagoon, Phillipa, Ngarange and Kalatog Channels), Botswana (Figure 4.2).

Material examined: Morphometric measurements and drawings made using light microscopy and confocal light microscopy (Figure 5.2). Description based on eight specimens.

Description

Measurements in µm except where indicated otherwise.

External features:

Adults permanently fused in copula in the shape of a cross (Figure 5.1 A). Anterior body part prior to point of fusion dorsoventrally flattened, length 0.91 ± 0.14 (0.72-1.24) mm. Length of posterior body part behind area of fusion 0.37 ± 0.07 (0.32-0.52) mm. Total body length 1.55 ± 0.33 (1.26-2.14) mm. Opisthaptor length 328 ± 19 (300-350) and width 268 ± 19 (240-290) carries cotylophore with clamps (Figure 5.2 B). Opisthaptor ends in folded lip-like protrusion on posterior edge (Figure 5.2 D).

Clamps:

Eight to 11 pairs of clamps situated along the postero-lateral margins of the cotylophore (Figure 5.2 A – D). Each clamp consists of pair of opposable hinged jaws supported by complex array of sclerites (Figure 5.1 B). First and second pair of clamps smaller in size, length 31.24 ± 9.53 (18.28-39.38) and width 23.74 ± 5.97 (14.81-27.13). Rest of clamp pairs more or less equal in size. Fifth pair 63.63 ± 1.54 (61.97-65.01) in length and 46.04 ± 18.31 (24.92-57.21) in width. Two small crooked anchors present near posterior end of opisthaptor parallel to rows of clamps. Central anchors (Figures 5.1 C & 5.2 B) with hook length 6.07 (5.1-7.1) and shaft length 13.22 (12.65-14.01).

Alimentary canal:

Mouth situated on anterior end of prohaptor between pair of horseshoe-shaped oral suckers 42.22 ± 4.84 (31-50) in length and 36.32 ± 5.71 (22-45) in width. Pharynx oval-

shaped, length 47.71 ± 12.01 (25-66) and width 32.57 ± 7.98 (18-42). Intestine not bifurcate but with numerous granular diverticula. Stretches from behind pharynx to as far as ovaries in area of fusion.

Reproductive system:

Ovary large, elongated structure extending into area of fusion (Figure 5.2 A & F) with vitelline duct clearly visible (Figure 5.2 A & E). Testis much smaller than ovary, non-lobed and located in posterior body part. No eggs present in any specimens.

Remarks:

Specimens collected during this study resemble *Afrodiplozoon polycotyleus* described by Paperna (1973). After comparing measurements obtained from the present study to those of Paperna (1973 & 1979) it became evident that there were immense differences in the sizes of the structures measured (Table 5.1). This led to the re-measuring of the original drawings of *A. polycotyleus* from Paperna (1979) and the discovery that inaccuracies possibly occurred during the measuring process by Paperna (1973 & 1979). These indiscretions were also carried over by Khotenovsky in his 1985 manual on the suborder Octomacrinae. It would therefore seem that *A. polycotyleus* was inaccurately described in terms of the measurements provided for characteristics such as the length and width of the first and middle pair of clamps, length of the middle hooks, as well as the length and width of the oral suckers. Dr. Sevid Mashego published an article on *A. polycotyleus* in 2000 from his PhD, which was completed in 1982. In this article, he referred to *A. polycotyleus* as *Neodiplozoon polycotyleus* even though Khotenovsky changed *Neodiplozoon polycotyleus* to *Afrodiplozoon polycotyleus* in 1985. The published article (Mashego 2000) also does not contain any measurements or drawings and comparisons could only be made by acquiring Mashego's unpublished Ph.D. The re-measured values, comparisons to Paperna's original values and the values from the present study, as well as measurements from Mashego (1982) are provided in Table 5.1 and illustrate that Paperna (1973 & 1979) probably made an error while converting values from mm to µm or vice versa.

When taking these changes into consideration, it is evident that the size of the clamps, central hooks and oral suckers from the present study, which were previously believed to be much smaller when compared to Paperna (1973 & 1979), were actually similar in size and the clamps somewhat larger. Mashego (1982) also found the size of the clamps and oral suckers to be smaller when compared to the description by Paperna (1973 & 1979) and it could be that the errors in the measurements were overlooked by Mashego (1982 & 2000). If this is the case the specimens examined and measured by Mashego (1982) are as noted more robust than the specimens from both the present study and that of Paperna (1973), with the anterior and posterior lengths being a great deal larger. The size of the smaller first clamps and larger middle clamps, central hooks as well as the oral suckers are actually to a great extent, larger than that of Paperna (1973) and to a lesser extent larger than the measurements from the present study. Paperna (1973) described *A. polycotyleus* as having eight pairs of clamps and up to 10 pairs in gravid specimens. The documented number of clamps harboured by *A. polycotyleus* increased with the discovery of *A. polycotyleus* with up to 13 pairs of clamps by Mashego (1982). All of the diplozoids removed from *Barbus paludinosus* from the Okavango River possessed eight pairs of clamps. *Afrodiplozoon polycotyleus* from *B. poechii* contained 10 pairs of clamps and *B. multilineatus* and *B. radiatus* both 11 pairs. No eggs were present in the specimens collected, however, according to Paperna (1973) and Khotenovsky (1985) the eggs have a length of 0.18-0.22 mm and width of 0.14-0.16 mm with a long filament. A list of documented fish hosts for *A. polycotyleus* as well as hosts from the present study in the Okavango River, are provided in Table 5.2.

Diagnostic features proposed for use in the description and identification of *Afrodiplozoon polycotyleus* (Paperna, 1973):

- Mean ratio of posterior length to anterior length, 1: 2.446
- Mean ratio of anterior length to total length, 1: 1.698
- Mean ratio of posterior length to total length, 1: 4.152
- Posterior body part ending in opisthaptor with 8 to 13 pairs of clamps
- Mean ratio of opisthaptor length to total posterior length, 1: 1.129
- Body of the central hook 5.1 - 7.1µm in length

- No folds on posterior body part
- 8 to 13 pairs of clamps
- 1st and 2nd pair of clamps much smaller
- 3rd to 11th pairs of clamps marginally equal in size
- Testis and ovary located in area of fusion extending into posterior body part
- Intestinal tract does not bifurcate but with numerous granular diverticula up to distal termination

This is the first record of *A. polycotyleus* on *Barbus afrovernayi* Nichols & Boulton, 1927*, B. multilineatus* Worthington, 1933*, B radiatus* Peters, 1853 and *B. poechii* Steindachner, 1911 from the Okavango River, Botswana.

The specimens from this study are therefore assigned to *Afrodiplozoon polycotyleus* (Paperna, 1973).

Table 5.1

Table 5.2: List of documented fish hosts as well as hosts from the present study in the Okavango River, found to be parasitised by *Afrodiplozoon polycotyleus* (Paperna, 1973) and their localities in Africa.

Host	Localities	Reference
Labeo victorianus Boulenger, 1901	Nzoia River, Kenya	Paperna (1973)
L. cylindricus Peters, 1852*	Swamps of Lake Kyoga, Tanzania	Paperna (1973)
Barbus paludinosus Peters, 1852	Ruaha River, Tanzania	Paperna (1973)
B. cercops Whitehead, 1960	Ruaha River, Tanzania	Paperna (1973)
B. macrolepis Pfeffer, 1889	Ruaha River, Tanzania	Paperna (1973)
B. kerstenii Peters, 1868*	Swamps of Lake Kyoga, Tanzania	Paperna (1973)
B. neumayeri Fischer 1884	Kibale National Park, Uganda	Chapman *et al.* (2000)
B. marequensis Smith, 1841	Luphephe & Nwanedzi Dams, South Africa	Mashego (2000)
B. trimaculatus Peters, 1852	Luphephe & Nwanedzi Dams, South Africa	Mashego (2000)
B. neefi Greenwood, 1962*	Lingwe River, South Africa	Mashego (2000)
Alestes baremoze (Joannis, 1835)	Anambra River, Nigeria	Echi & Ezenwaji (2009)
Okavango River, Botswana		**Reference Number**
B. afrovernayi Nichols & Boulton, 1927	Phillipa Channel	2002/12/27 – 46
B. multilineatus Worthington, 1933	Ngarange Channel	2003/12/15 – 29
B. paludinosus Peters, 1852	Phillipa Channel	2002/12/27 – 40
	Ngarange Channel	2003/12/10 – 74
	Samochima Lagoon	2003/12/16 – 01
B. poechii Steindachner, 1911	Kalatog Channel	2001/10/18 – 17
B. radiatus Peters, 1853	Samochima Lagoon	2008/11/29 – 04

*Diplozoid larval forms

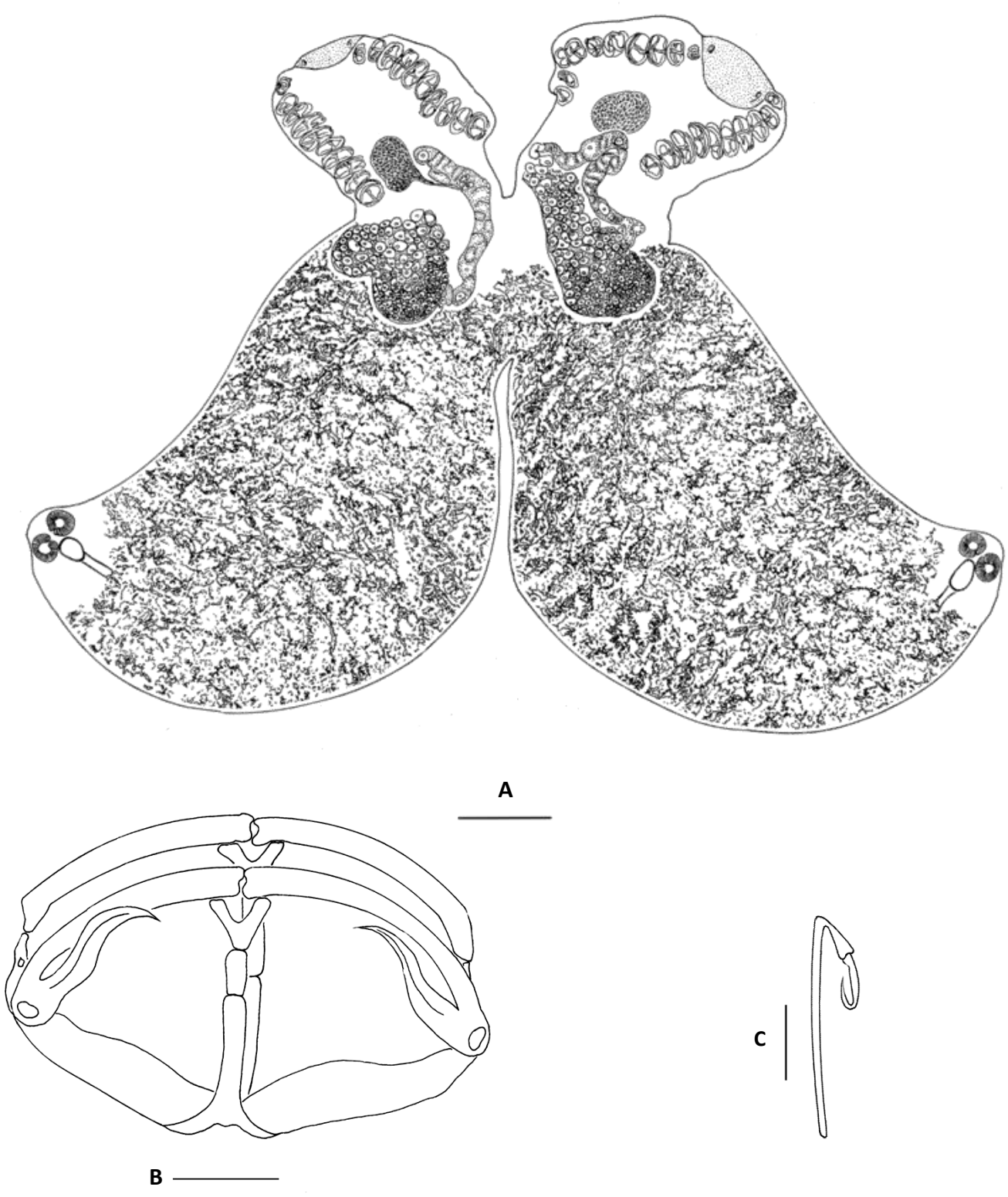


Figure 5.1: Microscope projection drawings of *Afrodiplozoon polycotyleus* (Paperna, 1973) from the gills of *Barbus paludinosus* Peters, 1852 collected from the Okavango River, Botswana. **A** - Whole mount. Scale bar: 0.1 mm. **B** – Middle clamp. **C** – Central hook. Scale Bar B & C: 1 µm

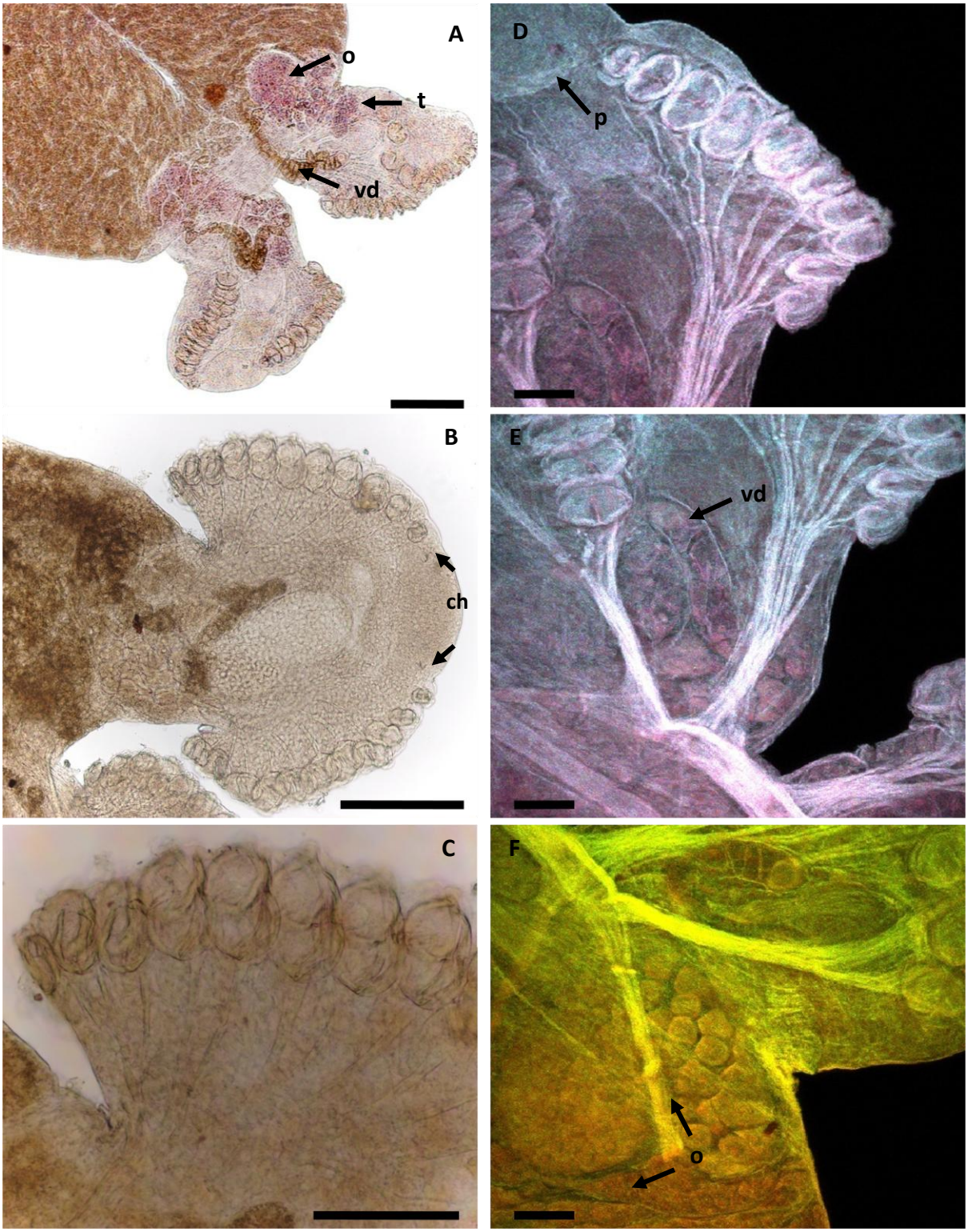


Figure 5.2: Micrographs of compound light (A-C) and confocal (D-F) microscopy of *Afrodiplozoon polycotyleus* (Paperna 1973). **A** – Whole mounted specimen with 10 pairs of clamps. **B & C** - Whole mounted specimen with 11 pairs of clamps. **D, E & F** – Micrographs with lip-like protrusion, ovaries & vitelline duct. **ch** – central hooks, **o**- ovaries, **p** – lip-like protrusion, , **t** – testis & **vd** - vitelline duct. Scale bar A & B: 0.1 mm, C - F: 50 µm

***Paradiplozoon* sp. A**

Host: *Labeo lunatus* Jubb, 1963.

Locality: Okavango River (Shakawe), Botswana (Figure 4.2).

Material examined: Morphometric measurements and drawings made using light microscopy and confocal light microscopy (Figure 5.3). Description based on 13 specimens.

Description

Measurements in µm except where indicated otherwise.

External features:

Adults permanently united in copulating pair (Figures 5.3 A & 5.4 A). Anterior body part prior to point of fusion broad, length 2.55 ± 0.78 (1.18-3.5) mm. Posterior body part behind area of fusion elongated and narrow without folds, length 1.15 ± 0.32 (0.52-1.7) mm. Total body length 4.38 ± 1.21 (1.92-5.8) mm. Opisthaptor slightly widened in area of clamps, length 343 ± 69 (280-440) and width 299 ± 76 (220-375), carries cotylophore with clamps (Figure 5.4 B). Folded lip-like protrusion on posterior edge of opisthaptor reduced and not clearly visible.

Clamps:

Four large clamps situated in two longitudinal rows along the postero-lateral margins of the cotylophore (Figure 5.4 B). Each clamp is made up of complex array of robust sclerites supporting opposable hinged jaws (Figure 5.3 C). Clamps even in size, length 102 ± 5.21 (92-110), width 66 ± 8 (52-79). Two central anchors very small, lunate hook, length 10 ± 1.69 (8-11) and long shaft, length 21 ± 1.52 (20-22) (Figures 5.3 D & 5.4 C).

Alimentary canal:

Mouth subterminal, situated on anterior end of prohaptor between pair of horseshoe-shaped oral suckers 89 ± 16 (60-125) in length and 103 ± 22 (69-145) in width. Pharynx oval-shaped, length 78 ± 22 (40-95) and width 51 ± 19 (20-71). Intestine divides into three branches short distance behind pharynx, one median and two longitudinal divisions with numerous diverticula and compact vitellaria.

Reproductive system:

Ovary situated in area of fusion and partly into posterior body part. Testis single, non-lobed situated in posterior body part near ovary. Egg oval-shaped with pointed anopercular end leading to long coiled filament. Egg length 175 ± 7.07 (170-180) and width 69 ± 0.71 (68-70) (Figures 5.3 B & 5.4 D).

Immature stage:

Two solitary diporpa larvae were collected from two fishes of which the average measurements are given in Table 5.3. The diporpa (Figure 5.3 E) had an elongate body form with a slight indentation indicating the division between the anterior and shorter posterior body parts (Figure 5.4 E). Four pairs of irregular sized clamps, in two longitudinal rows are carried on the opisthaptor at the end of the posterior body part. The oral opening leading to the pharynx was situated at the end of the anterior body part, between two oral suckers with the intestine branching into a prominent median division and two more unobtrusive longitudinal branches. The ventral sucker was placed medially in the centre of the division between the anterior and posterior body parts (Figure 5.4 F).

Remarks:

With the aid of the key by Khotenovsky (1985) it was determined that the diplozoids from the present study belong to the genus *Paradiplozoon*. The identification key from Fischthal & Kuntz (1963) as modified from Thomas (1957) and Tripathi (1959) were used as well as the key translated from Khotenovsky (1985), for the determination of species from south-eastern Asia and Africa. Morphometric measurements and various characteristics from the

present material were compared with the two described species of *Paradiplozoon* from Africa namely, *P. ghanense* and *P. aegyptensis*. According to Khotenovsky (1985) the determination of species within more diverse genera such as *Paradiplozoon* is difficult and the attachment clamps and central hook are the main morphological characters to use, together with host specificity. Matejusova *et al.* (2002), however, stated that these morphological characters are not stable, because the clamps grow gradually.

Diplozoon ghanense Thomas, 1957 was collected and described from *Brycinus macrolepidotus*, in Ghana. Fischthal & Kuntz (1963) described a new species of diplozoon from the gills of *Labeo forskalii* in Egypt, as *Diplozoon aegyptensis*. When the subgenus *Paradiplozoon* was raised to genus status by Khotenovsky (1981) and after Khotenovsky's extensive revision of diplozoids in 1985, both *Diplozoon* were subsequently moved to the genus *Paradiplozoon*.

The diplozoid from the current study shows very little resemblance to *P. ghanense* and differs in overall appearance of specifically the posterior body part. The opisthaptor of *P. ghanense*, carrying the cotylophore, is broad and the posterior part of the body is much more shortened. Another difference is seen in the position of the ovaries and testis, in *P. ghanense* both of these structures are situated in the area of fusion while in the diplozoid from the present study the testes are located in the posterior part of the body. The diplozoids from the present study therefore do not fit the characteristics of *P. ghanense*.

A prominent difference between material from the present study and *P. aegyptensis* is the shape of the intestine. According to the descriptions provided by Fischthal & Kuntz (1963), the intestine is a single cecum, not bifurcate with many branched diverticula. The material from the present study, however, illustrate three very prominent divisions occurring in the anterior body part, some distance behind the pharynx with the intestine splitting up in one median and two longitudinal branches. No indication could be found in the literature and drawings of this characteristic and it is difficult to believe that Fischthal & Kuntz (1963), when describing *P. aegyptensis* could have overlooked such a prominent feature. Differences were also encountered with the size of the central anchor as shown in Table 5.4

and the measurements from the present study being twice as small as that described by Fischthal & Kuntz (1963). This was also the case with the length and width of the eggs.

When comparing the material from the present study to the morphological measurements and characteristics of the African *Paradiplozoon* species, it can be said that a closer resemblance is shared with *P. aegyptensis* than *P. ghanense*, but the differences cannot be ruled out. The specimens collected from *Labeo lunatus* do not fit the key of Thomas (1957), seeing that the intestine is not without bifurcation and does not re-unite behind the testis. The key of Khotenovsky (1985) is a bit more detailed, but does not take into account the differences in egg and central anchor size, as well as the shape of the intestine. Khotenovsky's (1985) key demonstrates that the present material bears close resemblances to *P. aegyptensis*, although a solid identification cannot be made.

When contemplating the above meantioned differences it is clear that the material from the present study does not fit the desription for *P. aegyptensis*. Khotenovsky (1985) stated that the attachment clamps and central anchor are two of the main characters, but these characters have not been precisely described for African *Paradiplozoon* species, making it difficult to use for accurate species determination. Another main character used by Khotenovsky (1985) for determining species is host specificity. The only other host of *P. aegyptensis* occurring in the Okavango River together with *Labeo lunatus* Jubb, 1963 is *Labeo cylindricus* and might account for the diplozoid from the present study being *P. aegyptensis*. This reason of thinking can, however, be ruled out on the following principles. *Labeo cylindricus* prefers rocky, clear, fast flowing streams; and habitats like this only occurs in the Popa Falls region in the Caprivi north of the Pan-handle (Skelton, 2001). *Labeo lunatus* on the other hand is a fish favouring lagoons and floodplains with a muddy substrate and over the past years during fish parasitological surveys by the Aquatic Parasitology group, has only been found south of the Popa Falls region. It is therefore highly unlikely for oncomiracidium of *P. aegyptensis* from *L. cylindricus*, which is the only free-living stage in the diplozoid life cycle and not known to travel long distances, to locate and infest *L. lunatus*.

During the extensive work over the past years by the Aquatic Parasitology group, to date *L. cylindricus* from the Okavango River has not been found to be infested with any diplozoid species, a list of all the hosts from which other monogenean parasites were collected during the past years, are given in the Appendix (Tables 8.1 & 8.3). It is therefore proposed that this is the first record of a previously undescribed species of *Paradiplozoon* from *Labeo lunatus* from the Okavango River, Botswana.

This is unfortunately not where the confusion ends. Other inaccuracies were uncovered from various articles adding to the uncertainty in correclty identifying diplozoid species. For one, *P. aegyptensis* and *P. ghanense* are still widely described as *Diplozoon aegyptensis* and *D. ghanense* in various articles (Hussain & Ahmad, 2010; Echi & Ezenwaji, 2009 and Ahmad & Christi, 1999) . A point of confusion was also established on the spelling of *Paradiplozoon aegyptensis*, as described by Fischthal & Kuntz (1963). However, Khotenovsky (1985) refers to *P. aegyptense*. Both 'ese' and 'iensis' are Latin suffix indicating a place or a characteristic of a place. The differences in the spelling of this species name may be due to a difference in bending of the location, Egypt, where *P. aegyptensis* was originally described. *Paradiplozoon aegyptensis*, however, was described as such by Fischthal & Kuntz after which the suffix was adapted without indicating a reason. This change was also carried over by Matejusova *et al.* (2004) where fishes of the Alestidae in Africa are referred to being recorded hosts of *P. aegyptense*. Article 69.2.1 of the International Code of Zoological Nomenclature (1999) states that: "If an author subsequently designates a species by using an unjustified emendation or an incorrect spelling of the name of one of the originally included nominal species, he or she is deemed to have designated the type species under its correctly spelled name".

No description on the diporpa of *P. aegyptensis* is available and also no measurements to use for comparison.

Diagnostic features proposed for use in the description and identification of *Paradiplozoon* sp. A*:*

- Clamps in two series of four each with width 52 to 79 µm.
- The average diameter of the 3rd clamp is 10 to 15 times smaller than the length of the posterior body part
- The surface of the posterior part of the body has no folds/plicae
- The posterior body part is shorter than the anterior part
- The pharynx is smaller than the suckers or equal in size
- The length of the suckers is 69 to 145 µm.
- Intestine with three divisions, one median and two longitudinal
- Testis entire, occurring in opisthaptoral region
- Ovary located in area of fusion
- Egg large, 170 – 180 x 68 – 70 µm with filaments

The specimens from this study are therefore described as *Paradiplozoon* sp. A

Note:

For the purpose of this M.Sc. dissertation a name is not assigned to the newly described *Paradiplozoon* sp. A, to avoid adding confusion to an already puzzling group of diplozoids. Further work will be done together with molecular analysis of material from *L. forskalii* in Egypt compared to that of *Paradiplozoon* sp. A from *L. lunatus,* if possible, for publication in the near future.

Table 5.3: List of average measurements of two juvenile *Paradiplozoon* sp. A diporpae collected from the gills of *Labeo lunatus* Jubb, 1963 in the Okavango River, Botswana. Measurements of the total, anterior and posterior body lengths are in mm and the rest of the measurements in µm.

Characteristics	Measurements
Total body length	1.42
Anterior body length	0.95
Posterior body length	0.47
Oral suckers length	64
Oral suckers width	55
1st Clamp length	50
1st Clamp width	40
2nd Clamp length	55
2nd Clamp width	45
3rd Clamp length	54
3rd Clamp width	35
4th Clamp length	50
4th Clamp width	30

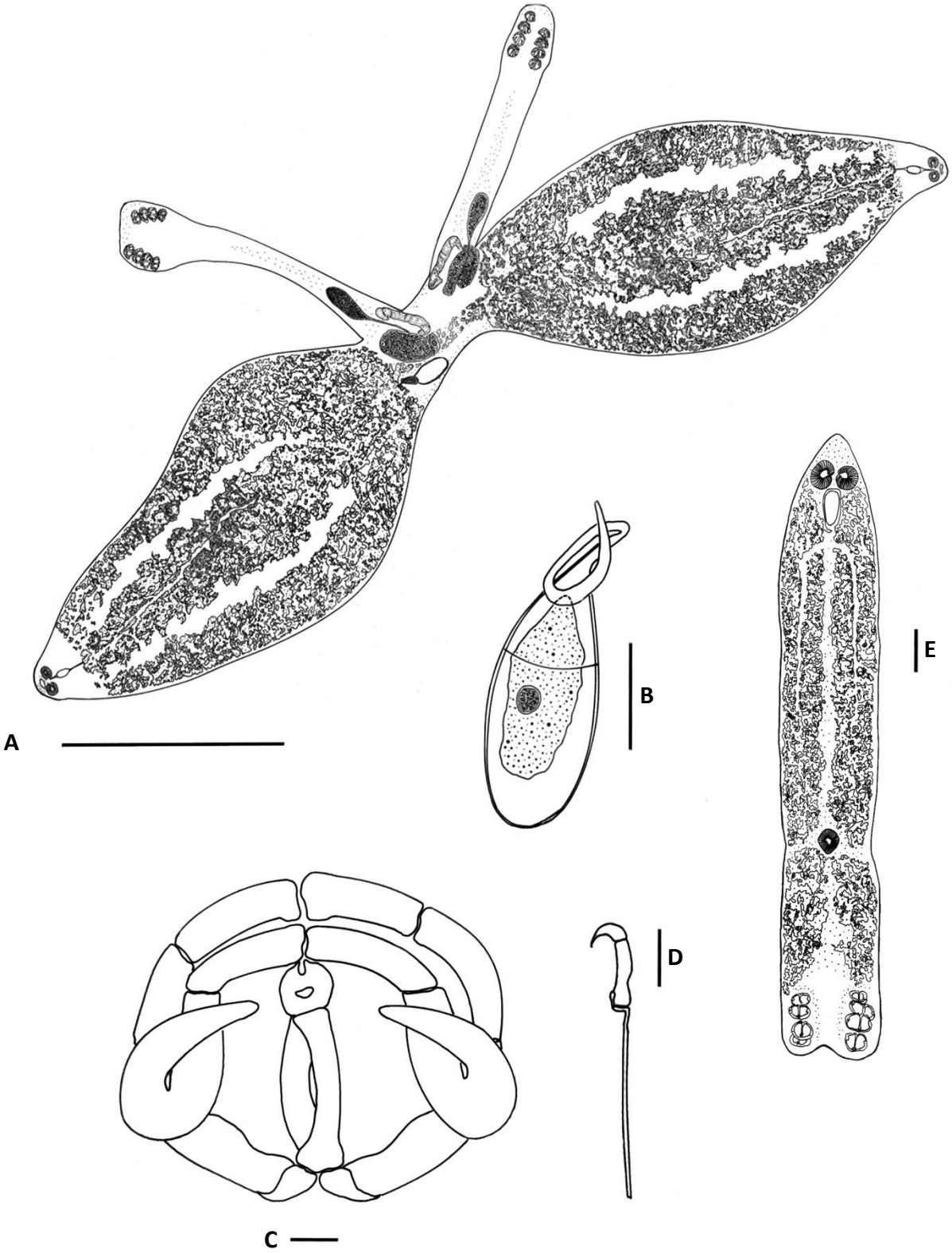


Figure 5.3: Microscope projection drawings of *Paradiplozoon* sp. A from the gills of *Labeo lunatus* Jubb, 1963 collected from the Okavango River, Botswana. **A** - Whole mount. **B** – Egg. **C** – Clamp. **D** – Central anchor. **E –** Diporpa**.** Scale bar A: 1 mm, B & E: 0.1 mm and C & D: 10 µm.

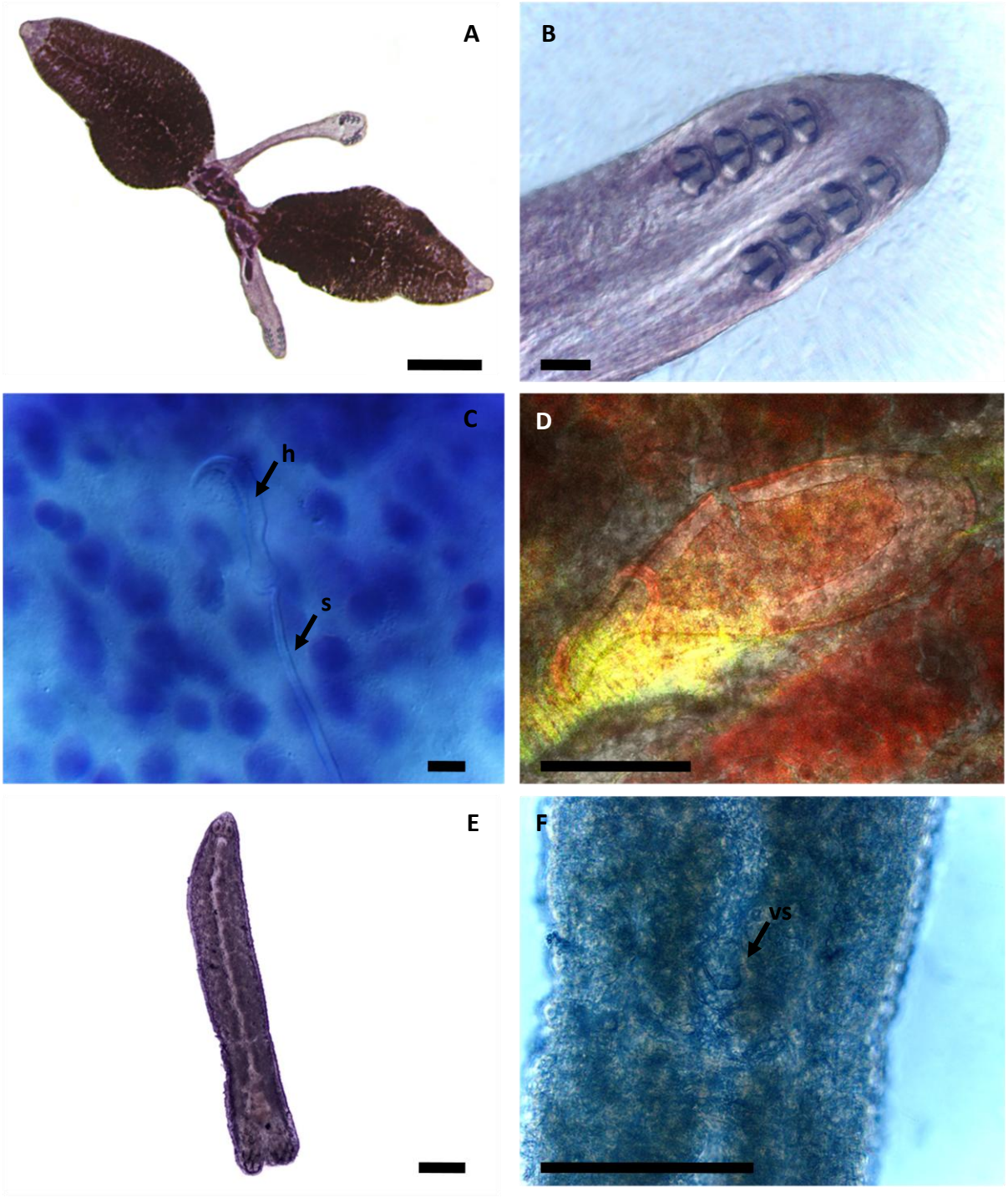


Figure 5.4: Micrographs of dissecting (A & E), compound light (B, C & F) and confocal (D) microscopy of *Paradiplozoon* sp. A. **A** – Whole mounted specimen of mature worm. **B** – Opisthaptor with four pairs of clamps. **C** – Central anchor with handle (**h**) and shaft (**s**). **D** – Micrograph showing intra-uterine egg. **E** – Whole mount of juvenile diporpa. **F** – Ventral sucker (**vs**) of diporpa.
Scale bar A, E & F: 0.1 mm, B: 50 µm, C & D: 1 µm.

***Diplozoon* sp. A**

Host: *Labeo capensis* (Smith, 1841) and *L. umbratus* (Smith, 1841)

Locality: Orange-Vaal River System (Soetdoring Nature Reserve, Modder Rivier, Renoster Spruit, Saulspoort Dam, Alexander Bay, Brandkaros) (Figure 4.1).

Material examined: Morphometric measurements and drawings made using light microscopy and confocal light microscopy (Figure 5.5). Description based on 53 specimens.

Description

Measurements in µm except where indicated otherwise.

External features:

Adults united in copulating pair (Figures 5.5 A, 5.6 A & B). Anterior body part prior to point of fusion, length 1.73 ± 0.61 (0.57-3.1) mm. Posterior body part behind area of fusion length 0.86 ± 0.31 (0.28-1.4) mm, with seven to 14 folds/plicae (Figures 5.6 C & D). Total body length 2.92 ± 1.01 (1.03-4.4) mm. Opisthaptor, length 410 ± 101 (162-660) and width 320 ± 96 (140-600), carries cotylophore with clamps (Figure 5.6 B). Folded lip-like protrusion on posterior edge of opisthaptor prominent (Figure 5.6 C).

Clamps:

Four large clamps situated in two longitudinal rows along the postero-lateral margins of the cotylophore (Figure 5.6 C). Each clamp is made up of complex array of sclerites supporting opposable hinged jaws (Figures 5.5 C & 5.6 E). Clamps almost even in size, length 65 ± 15 (42-118), width 34 ± 11 (18-71). Two central anchors very small, lunate hook, length 2.95 ± 0.29 (2.6-3.3) and long shaft, length 15 ± 2.19 (13.2-17.22) (Figures 5.5 D & 5.6 F).

Alimentary canal:

Mouth subterminal, situated on anterior end of prohaptor between pair of horseshoe-shaped oral suckers, 65.7 ± 15.39 (37-100) in length and 62.8 ± 13.85 (30-92) in width. Pharynx oval-shaped, length 62.68 ± 12.98 (34-100) and width 41.78 ± 8.11 (20-61).

Intestine stretching into posterior body part, does not bifurcate, but with numerous diverticula and compact vitellaria.

Reproductive system:

Ovary situated in posterior body part. Testis single, non-lobed situated in posterior body part behind ovary. Egg oval-shaped with pointed anopercular end leading to long coiled filament. Egg length 260 $\pm$ 61.88 (150-320) and width 116.25 $\pm$ 19.96 (90-150) (Figure 5.5 B). Measurements based on 17 eggs.

Remarks:

According to the literature, diplozoids from the genera *Afrodiplozoon*, *Diplozoon* and *Paradiplozoon* are present from host fish in Africa. The genus *Afrodiplozoon* contains only one species namely *Afrodiplozoon polycotyleus,* characterised by eight to 13 pairs of clamps. Material from the present study contains only four pairs of clamps, which along with other morphometric characteristics eliminates the possibility of the present material belonging to the genus *Afrodiplozoon*.

According to the identification key of Khotenovsky (1985), diplozoids from the present study may possibly belong to the genus *Paradiplozoon*, due to the lack of enlargements formed in the middle section of the posterior body part. It can, however, also be assumed that material from the present study belongs to the genus *Diplozoon*, because of the presence of plicae in the anterior section of the posterior body part.

Paradiplozoon kashmiriensis (Kaw, 1950), known from south-eastern Asia, is the only species characterised by plicae on the posterior part of the body (Khotenovsky 1985). This species was found on hosts such as *Carassius carassius* (Linnaeus, 1758), *Cyprinus carpio communis* Linnaeus, 1758, *Cyprinus carpio spicularis* Linnaeus, 1758, *Schizothorax niger* Heckel, 1838, *S. esocinus* Heckel, 1838, *S. curvifrons* Heckel, 1838, *Oreinus sinuatus* (Heckel, 1838), *O. plagiostomus* (Heckel, 1838) and a *Labeo* sp. (Hussain & Ahmad 2010). The present material from the Orange-Vaal River System, however, does not resemble other

characters for this species. Two species from the genus *Paradiplozoon* have been described from Africa, i.e. *P. aegyptensis* and *P. ghanense*, as well as the *Paradiplozoon* material collected from *Labeo lunatus* in the Okavango River in the present study (see p. 62). It can be concluded that the Orange-Vaal material do not belong to the genus *Paradiplozoon* due to various morphological differences (Table 5.5). These differences include the lack of folds in the posterior body parts of these African *Paradiplozoon* species, overall difference in the morphology of both the anterior and posterior body parts, as well as differences in the size of attachment clamps and central hooks.

To date no published species from the genus *Diplozoon* have been described from Africa and as a result, no identification key for African *Diplozoon* species exists and also no measurements or descriptions to accurately compare material with. There are only two described and accepted *Diplozoon* species from Europe, namely *Diplozoon paradoxum*, designated as the type species when von Nordmann erected the genus *Diplozoon* in 1832, and *Diplozoon scardinii*. According to Khotenovsky (1985), *D. paradoxum* is characterised by four to eight large folds on the posterior body part and central hooks with a length of 28 – 33 µm. *Diplozoon scardinii* on the other hand is characterised by eight to 13 shallow folds in the front section of the posterior body part and central hooks with a length of 22 – 26 µm.

Material from the present study shows more resemblance to the genus *Diplozoon* than *Paradiplozoon*, but differs immensely when compared to the two known *Diplozoon* species. It is therefore proposed that this is the first record of an undescribed new species of *Diplozoon* in southern Africa, from *Labeo capensis* and *L. umbratus* from the Orange-Vaal River System.

Diagnostic features proposed for use in the description and identification of *Diplozoon* sp. A:

- Clamps in two series of four each with width 42 to 118 µm
- The surface of the middle section of the posterior part of the body has seven to 14 folds/plicae

- The posterior body part is shorter than the anterior part
- The pharynx is smaller than the suckers or equal in size
- The length of the suckers is 37 to 100 µm.
- Intestine with various diverticula, does not bifurcate
- Intestine stretches into posterior body part
- Testis and ovaries occurring in opisthaptoral region
- Egg large, 150 – 320 x 90 – 150 µm with long filament

The specimens from this study are therefore described as *Diplozoon* sp. A

Note:

For the purpose of this M.Sc. dissertation a name is not assigned to the newly described *Diplozoon* sp. A, to avoid adding confusion to an already puzzling group of diplozoids. Further work will be done together with molecular analysis of material, if possible, for publication in the near future.

Table 5.4

Table 5.5: Comparison of the morphological measurements and characteristics of *Diplozoon paradoxum* Nordmann, 1832, *Paradiplozoon* sp. A from *Labeo lunatus*, Jubb, 1963 and *Diplozoon* sp. A occurring on *Labeo capensis* (Smith, 1841) and *L. umbratus* (Smith, 1841), from the Orange-Vaal River System. Measurements of the total, anterior and posterior body lengths are in mm and the rest of the measurements in µm.

	***D. paradoxum* Nordmann, 1832**	***Paradiplozoon* sp. A**	***Diplozoon* sp. A**
	Locations and Hosts		
	Europe and Asia *Abramis brama* (Linnaeus, 1758) Various species from families Cyprinidae, Gadidae and Esocidae	Okavango River, Botswana *Labeo lunatus* Jubb, 1963	Orange-Vaal River System *Labeo capensis* (Smith, 1841) *Labeo umbratus* (Smith, 1841)
	Measurements		
Total body length	2.2-10	1.92-5.8	1.03-4.4
Anterior body length	1.5-6.4	1.18-3.5	0.57-3.1
Posterior body length	0.3-3	0.52-1.7	0.28-1.4
Plicae/ Folds	4-8	No folds	8-10
Enlargement	Middle section of posterior body part	No enlargement	No enlargement
1st Clamp length	60-119	92-104	42-90
1st Clamp width	76-190	52-74	19-68
2nd Clamp length	54-119	98-104	49-112
2nd Clamp width	114-228	60-74	19-71
3rd Clamp length	54-130	104-110	48-118
3rd Clamp width	130-255	63-77	18-55
4th Clamp length	60-141	70-104	47-103
4th Clamp width	125-255	67-110	21-50
Anchor hook	28-33	8-11	13-17
Anchor shaft	10-13	20-22	3-4
Oral sucker length	65-195	69-145	37-100
Oral sucker width	60-190	60-125	30-92
Pharynx	54-146	40-95	34-100
Eggs length	317-434	170-180	150-320
Eggs width	84-117	68-70	90-150

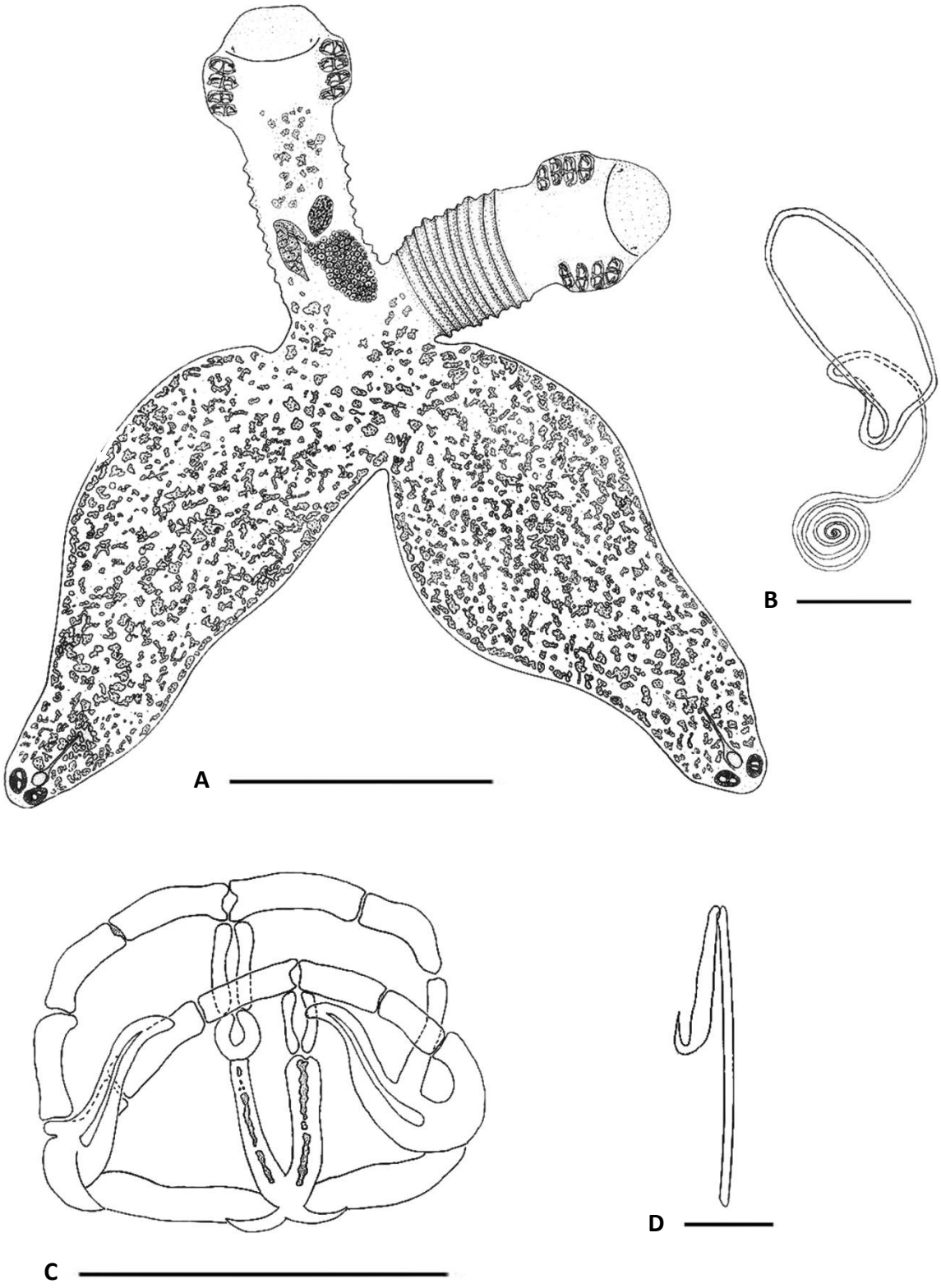


Figure 5.5: Microscope projection drawings of *Diplozoon* sp. A from the gills of *Labeo capensis* (Smith, 1841) collected from the Orange-Vaal River System. **A** - Whole mount. **B** – Egg. **C** – Clamp. **D** – Central anchor. Scale bar A: 1 mm, B & C: 0.1 mm and D: 1 µm.

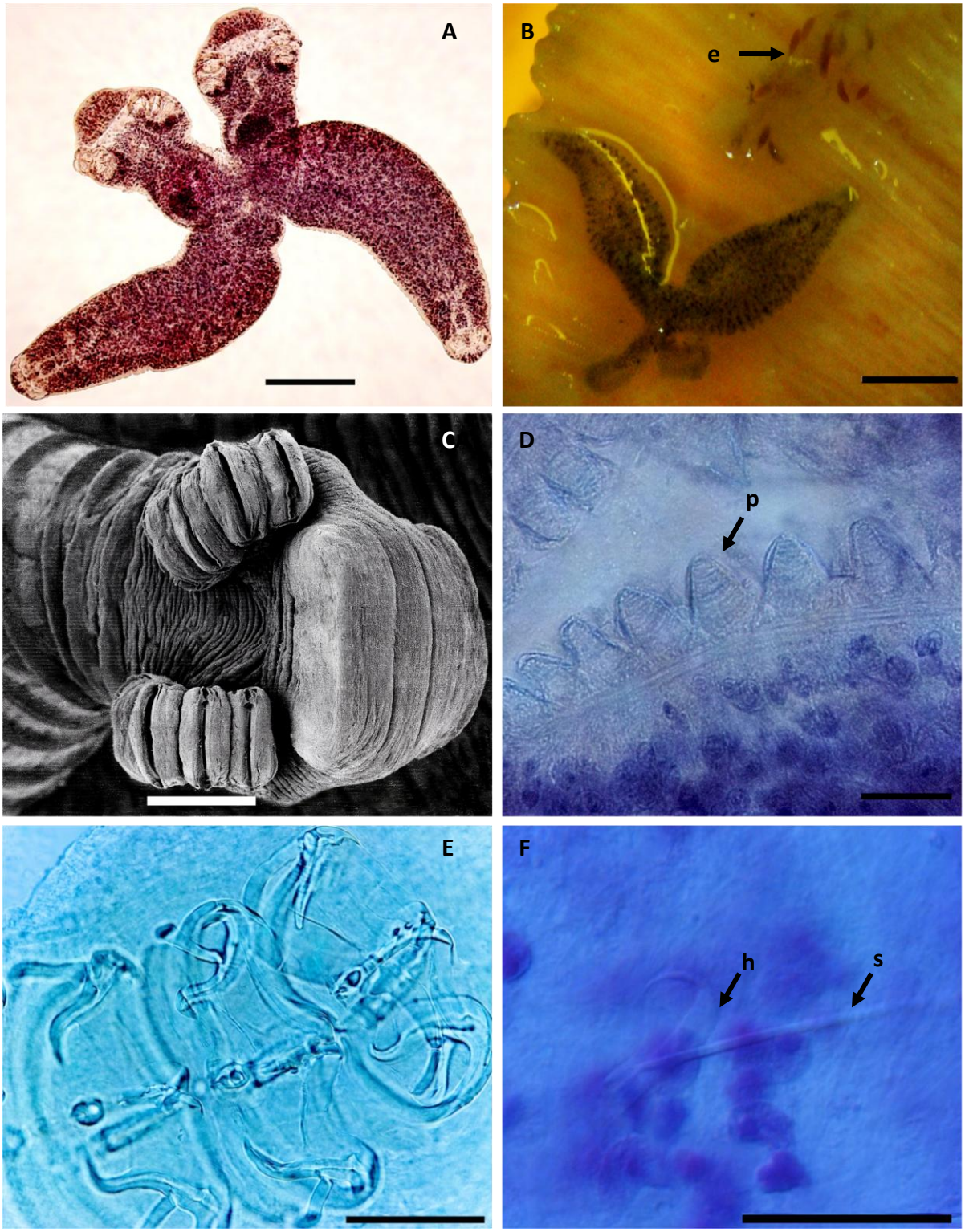


Figure 5.6: Micrographs of dissecting (B), compound light (A, D,E & F) and scanning electron (C) microscopy of *Diplozoon* sp. A. **A** – Whole mounted specimen of mature worms. **B** – Adult paired worm on gill of *Labeo capensis* (Smith, 1841) with string of eggs (**e**). **C** – Posterior part of body with four pairs of clamps **D** – Plicae (**p**) on posterior body part. **E** – Attachment clamps. **F** – Central anchor with handle (**h**) and shaft (**s**). Scale bar A & B: 1 mm, C & D: 0.1 mm, E: 5 µm and F: 2 µm

A list of characteristics used for species discrimination by Khotenovsky (1985), for the known African diplozoid species, as well as the two new species described during the present study, *Paradiplozoon* sp. A from *Labeo lunatus* Jubb, 1963, in the Okavango River, Botswana and *Diplozoon* sp. A from *Labeo capensis* and *L. umbratus*, both from the Orange-Vaal River System are presented in Table 5.6.

STAINING AND CONFOCAL MICROSCOPE IMAGING

The various hematoxylin stains and Orange G used as described in Chapter 4 worked well with confocal laser scanning microscopy and can be used for the measuring of both external and internal structures. The result can be clearly seen in Figures 5.7 A to D, where images from normal light microscopy (A & C) is compared to that of confocal microscopy (B & D). In most cases staining resulted in highlighting the neuromusculature system especially in the area of fusion (Figures 5.7 E & F) and the muscles leading to the clamps (Fig 5.7 B & Fig 5.8 C). Clamp structures can also be easily distinguished as shown in Figures 5.8 A to D, as well as the oral suckers, mouth opening and pharynx (Figures 5.8 E & F).

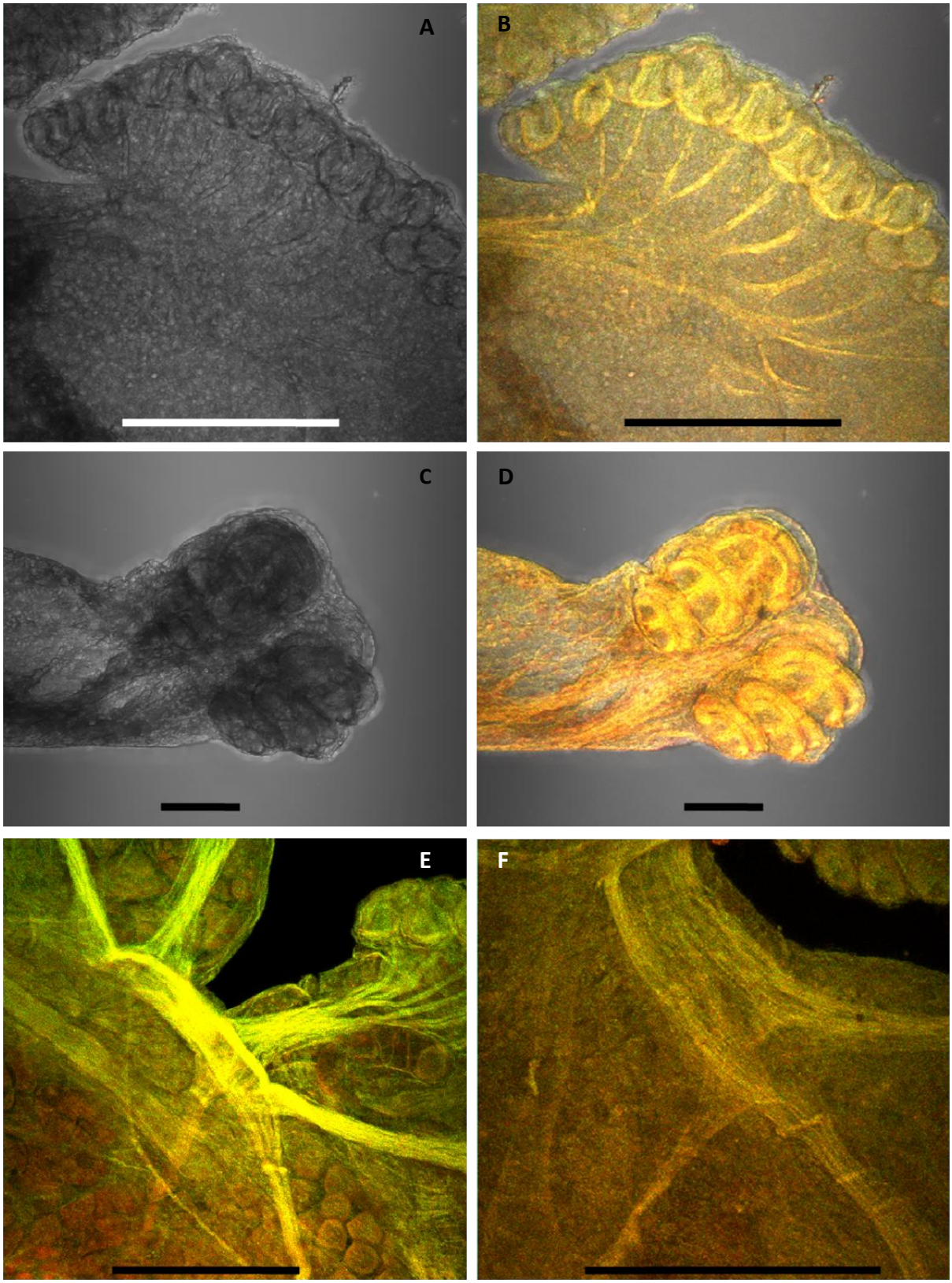


Figure 5.7: Micrographs of light (A & C) and confocal (B & D-F) microscopy of *Afrodiplozoon polycotyleus* (Paperna 1973) (A, B, E & F) and the diporpa of *Paradiplozoon* sp. A. **A & B –** Opisthaptor with neuromusculature leading to the clamps. **C & D –** Posterior end of diporpa carrying clamps. **E & F -** Fusion area of adult *A. polycotyleus* . Scale bar A, B, E & F: 0.1 mm and C & D: 50 µm.

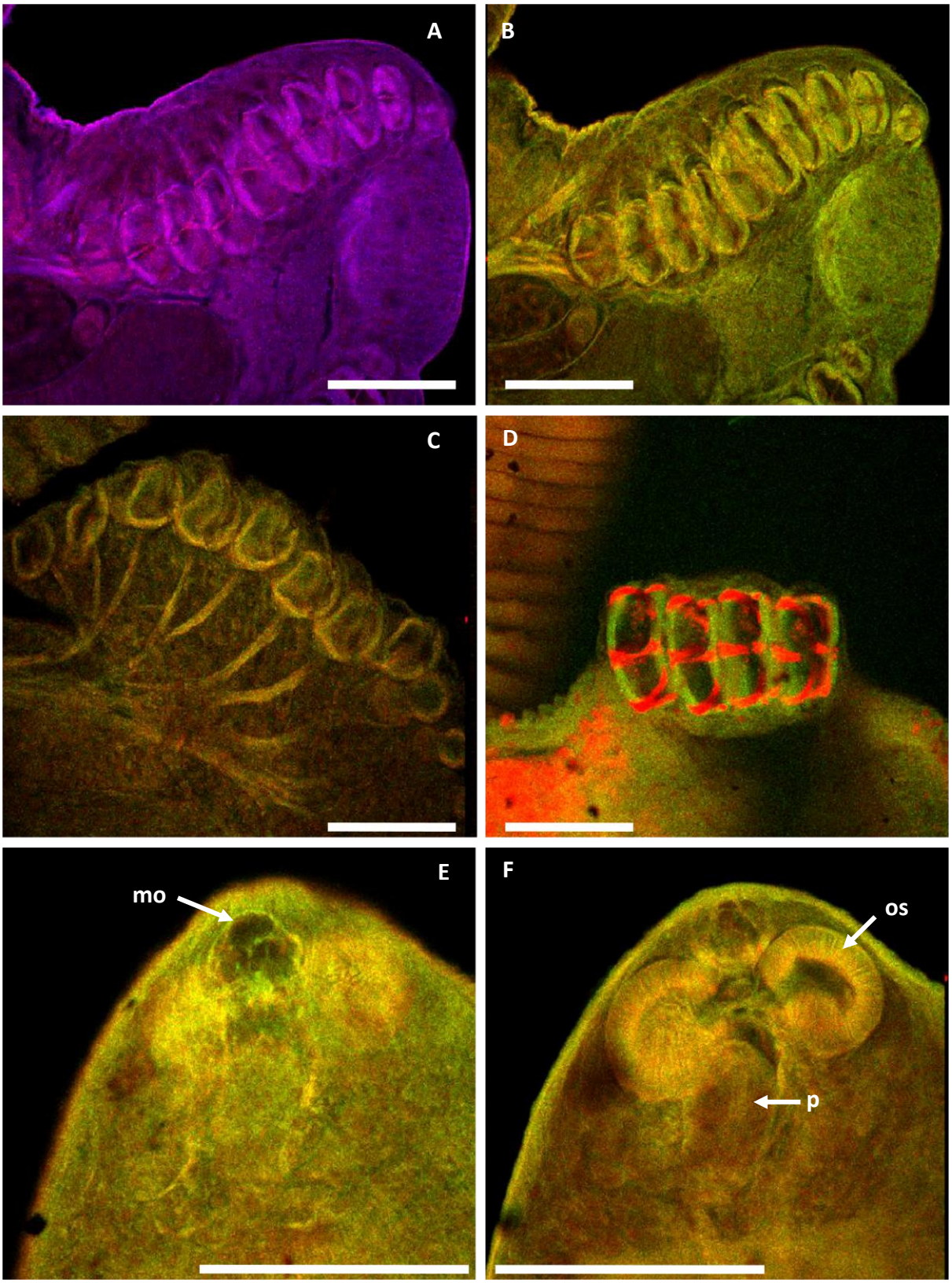


Figure 5.8: Micrographs of confocal microscopy of *Afrodiplozoon polycotyleus* (Paperna 1973) (A-C, E & F) and *Diplozoon* sp. A (D). **A, B & C –** Opisthaptor with clamps focused on different levels. **D –** Opisthaptor with four clamps carried on the cotylophore. **E & F -** Anterior end with mouth opening (**mo**), oral sucker (**os**) and pharynx (**p**). Scale bar A - D: 50 µm and E & F: 100 µm.

IDENTIFICATION KEYS

Various diplozoid identification keys have been developed by Thomas (1957), Tripathi (1959) Fischthal & Kuntz (1963) and Khotenovsky (1981 & 1985) (Table 5.7 & 5.8). These keys, however, mainly focus on European and Asian diplozoid species and are of little help with the identification of African species, seeing that not much is known about the African diplozoid fauna. The keys developed by Khotenovsky (1980 & 1985) for determining genera are, except for being in Russian, useful up to when accurate discrimination is needed between the genera *Diplozoon* and *Paradiplozoon*. Species from these two genera are sometimes very difficult to distinguish, especially when confronted with new or vaguely described species.

Fischthal & Kuntz (1963) modified identification keys from Thomas (1957) and Tripathi (1959) for determining African species, such as *Paradiplozoon ghanese* and *P. aegyptensis*. After Khotenovsky (1985) moved various species from the genus *Diplozoon* to *Paradiplozoon*, this key was adapted again (Table 5.8), but as of then a key has not been devised for exclusively African diplozoids. The reason for this is probably due to the fact that the African diplozoid fauna has not been accurately described or accepted, making it difficult to compile an identification key with insufficient or imprecise characteristics. A new key for African diplozoids, adapted from that of Khotenovsky (1985) together with characteristics from the newly described species mentioned in this chapter, is therefore proposed in Table 5.9.

Table 5.6

Table 5.7: Keys for determining subfamilies and genera as translated from Khotenovsky (1980 & 1985).

Subfamilies of Diplozoidae Palombi, 1949			
1	(2)	No more than 4 pairs of clamps.	Diplozoinae Palombi, 1949
2	(1)	More than 8 pairs of clamps.	Neodiplozoinae Khotenovsky, 1980
Genera of subfamily Neodiplozoinae Khotenovsky, 1980			
1	(2)	Posterior body part divided into 2 horizontal blades. Clamps, which are more than 15 pairs, placed on sides of blades.	*Neodiplozoon* Tripathi, 1960
2	(1)	Posterior body part not divided into 2 blades. Clamps, which are less than 15 pairs, placed as two vertical groups laterally on sides of the body	*Afrodiplozoon* Khotenovsky, 1980
Genera of subfamily Diplozoinae Palombi, 1949			
1	(2)	Middle section of posterior body part does not form enlargements.	*Paradiplozoon* Achmerov, 1974
2	(1)	Middle section of posterior body part forms different enlargements.	
3	(4)	Ahead of pharynx two big musculo-glandular organs present. Enlargements with big lateral plicae.	*Eudiplozoon* Khotenovsky, 1985
4	(3)	Ahead of oral suckers musculo-glandular organs missing. Enlargements with no plicae.	
5	(6)	Enlargement disc-shaped. Intestinal branches form compact net. Uterine opening lateral, placed in middle section of anterior part of body.	*Inustiatus* Khotenovsky, 1978
6	(5)	Enlargement glass/cup-shaped. Intestinal branches do not form thick net. Uterine opening is on border between anterior and posterior body parts.	
7	(8)	Anterior section of posterior body part with plicae.	*Diplozoon* Nordmann, 1832
8	(7)	Anterior section of posterior body part with no plicae.	*Sindiplozoon* Khotenovsky, 1981

Table 5.8: Key for the determination of species from south-eastern Asia and Africa, as translated from Khotenovsky (1985).

1	(2)	Surface of posterior body part plicated / folded. Posterior body part 1.5 times shorter than anterior part. Diameter of 3rd clamp approximately 10 to 20 times smaller than length of posterior body part.	*P. kashmirense* (Kaw)
2	(1)	Surface of posterior part with no folds / plicae.	
3	(6)	Anterior and posterior body parts almost equal in length.	
4	(5)	Testis single and extremely integral.	*P. barbi* (Reichenbach-Klinke)
5	(4)	Testis formed from numerous shallow bladder-like formations / lobes.	*P. tetragonopterini* (Sterba)
6	(3)	Posterior body part shorter than anterior part.	
7	(16)	Average diameter of 3rd clamp 3 to 8 times smaller than length of posterior body part.	
8	(11)	Middle hooks 20 µm long.	
9	(10)	Pharynx is smaller than suckers.	*P. doi* (Ha kyu)
10	(9)	Pharynx is bigger than suckers.	*P. malayense* Lim Lee Hong & Khotenovsky
11	(8)	Middle hooks over 25 µm long.	
12	(15)	3rd clamp over 200 µm wide.	
13	(14)	Intestinal diverticula / branches of anterior body part placed perpendicular to longitudinal axis of body.	*P. indicum* (Dayal)
14	(13)	Intestinal branches in anterior part of body directed to back. Testis spiral-like twisted.	*P. magnum* Lim Lee Hong & Khotenovsky
15	(12)	Attachment clamps 120 to 160 µm wide.	*P. ghanense* (Thomas)
16	(7)	Average diameter of 3rd clamp 10 to 38 times smaller than length of posterior body part.	
17	(24)	Average diameter of 3rd clamp is 10 to 20 times smaller than length of posterior body part.	
18	(19)	Pharynx larger than suckers.	*P. soni* (Tripathi)
19	(18)	Pharynx smaller than suckers, or equal to them.	
20	(21)	Clamps are 125 to 190µm wide. Eggs with no filament.	*P. cauveryi* (Tripathi)
21	(20)	Clamps are 80 to 117µm wide. Eggs with filaments.	
22	(23)	Length of suckers 67 to 83 µm.	*P. vietnamicum* Khotenovsky
23	(22)	Length of suckers 95 to 125 µm.	*P. aegyptense* (Fishthal & Kuntz)
24	(17)	Average diameter of 3rd clamp 25 to 38 times smaller than length of posterior body part.	*P. microclampi* (Kulkarni)

Table 5.9: Newly proposed key for determining of African diplozoid species, partly adapted from Khotenovsky (1985).

1	(2)	4 pairs of attachment clamps in lateral lines on opisthaptor.	Diplozoinae Palombi, 1949
1'	(3)	More than 8 pairs of attachment clamps in lateral lines on opisthaptor.	Neodiplozoinae Khotenovsky
2	(5)	Middle section of posterior body part without enlargements.	*Paradiplozoon* Achmerov, 1974
2'	(7)	Anterior section of posterior body part with plicae.	*Diplozoon* Nordmann, 1832
3	(4)	Posterior body part not divided into 2 blades. Less than 15 attachment clamp pairs, in 2 vertical groups laterally on sides of opisthaptor.	*Afrodiplozoon* Khotenovsky, 1981
4		8 to 13 pairs of clamps laterally on sides of opisthaptor.	*Afrodiplozoon polycotyleus* (Paperna, 1973)
5	(6)	Intestine single cecum, not bifurcate with many branched diverticula.	
5'		Intestine splits into 3 branches, 1 median, 2 lateral.	*Paradiplozoon* sp. A.
6		Testis and ovaries situated in area of fusion.	*P. ghanense* (Thomas, 1957)
6'		Ovaries situated in area of fusion, testis extends into posterior body part.	*P. aegyptensis* (Fishthal & Kuntz, 1963)
7		Intestine stretches into posterior body part. 3rd clamp length 104-110 and width 67-79.	*Diplozoon* sp. A

OCCURRENCE

The prevalence, abundance and mean intensity, as standardised by Margolis *et al.* (1982), was used for the diplozoids collected during the study. It was, however, found that the level of infestation as indicated by collections over the past few years, as well as during the present study, were too low for accurate statistical analysis. Hosts from the Okavango and Orange-Vaal Rivers Systems, were never found to harbour more than three or four diplozoids at once. On only one occasion, six *Diplozoon* sp. A specimens were collected from the gills of *Labeo capensis* from the Renosterspruit, while *Labeo lunatus* from the Okavango River, never harboured more than three *Paradiplozoon* sp. A individuals at a time. *Afrodiplozoon polycotyleus*, when present, were always reduced to two specimens on the gills of a *Barbus* host.

Chapman *et al.* (2000) found that 37.1% of *Barbus neumayeri* from the Kibale National Park in Uganda, were infested with one specimen of *A. polycotyleus* and 62.9% with two. Of the

377 *B. neumayeri* examined during their study, 47.2 % were infested. These infestation levels presented by Chapman *et al.* (2002) are much higher than that found in the Okavango River, from the various *Barbus* species collected during the present study. Echi & Ezenwaji (2009) reported a low prevalence of 1.9% for both *A. polycotyleus* and *Diplozoon ghanense* from *Brycinus macrolepidotus* and *Alestes baremoze* respectively, from the Anambra River in Nigeria. Reasons for the low levels of infestation found during the present study are discussed in Chapter 6.

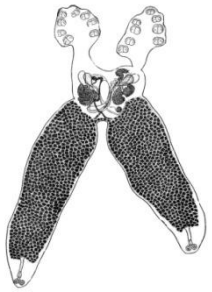
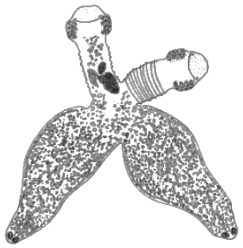
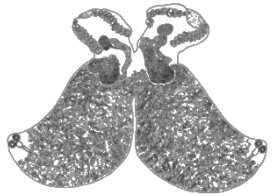
Table 5.1. Comparison of original measurements of *Afrodiplozoon polycotyleus* (Paperna, 1973) for various morphometric characteristics by Paperna (1973 & 1979) with re-measurements of Paperna's original drawings, measurements by Mashego (1982) and measurements from the Okavango River, Botswana material. All measurements are in µm, except anterior and posterior body lengths, which are in mm.

	Paperna (1973 & 1979)		**Mashego (1982)**	**Present Material**
Hosts	*Labeo victorianus* Boulenger, 1901 *Barbus cercops* Whitehead, 1960 *B. kerstenii* Peters, 1868 *B. macrolepis* Pfeffer, 1889 *B. paludinosus* Peters, 1852		*B. neefi* Greenwood, 1962 *B. marequensis* Smith, 1842 *B. trimaculatus* Peters, 1852	*B. afrovernayi* Nichols & Boulton, 1927 *B. multilineatus* Worthington, 1933 *B. paludinosus* Peters, 1852 *B. poechii* Steindachner, 1911 *B. radiatus* Peters, 1853
	Original measurements	Re-measurements	Measurements from unpublished PhD.	Measurements from present study
Anterior body length	0.68 – 1.05	0.7	0.97 – 2.66	0.72 – 1.24
Posterior body length	0.42 – 0.59	0.5	0.5 – 0.63	0.3 – 0.52
Clamps:				
1st pair of clamps length	80 – 150	10	30 – 45	18.28 – 37.38
1st pair of clamps width	100 – 180	13	30 – 40	14.8 – 27
Middle pair of clamps length	190 – 250	29	60 – 65	24.9 – 57
Middle pair of clamps width	300 – 340	38	85 – 90	61.9 - 65
Central hooks	7 – 11	5	28 – 35	5 – 7
Suckers:				
Oral suckers length	140 – 190	40	50 – 65	33 – 50
Oral suckers width	120 – 200	40	80 – 100	22 – 45
Pharynx	80	50	50 - 65	25 – 66
Eggs length	180 – 220	20	Not found	Not found
Eggs width	140 – 160	14	Not found	Not found

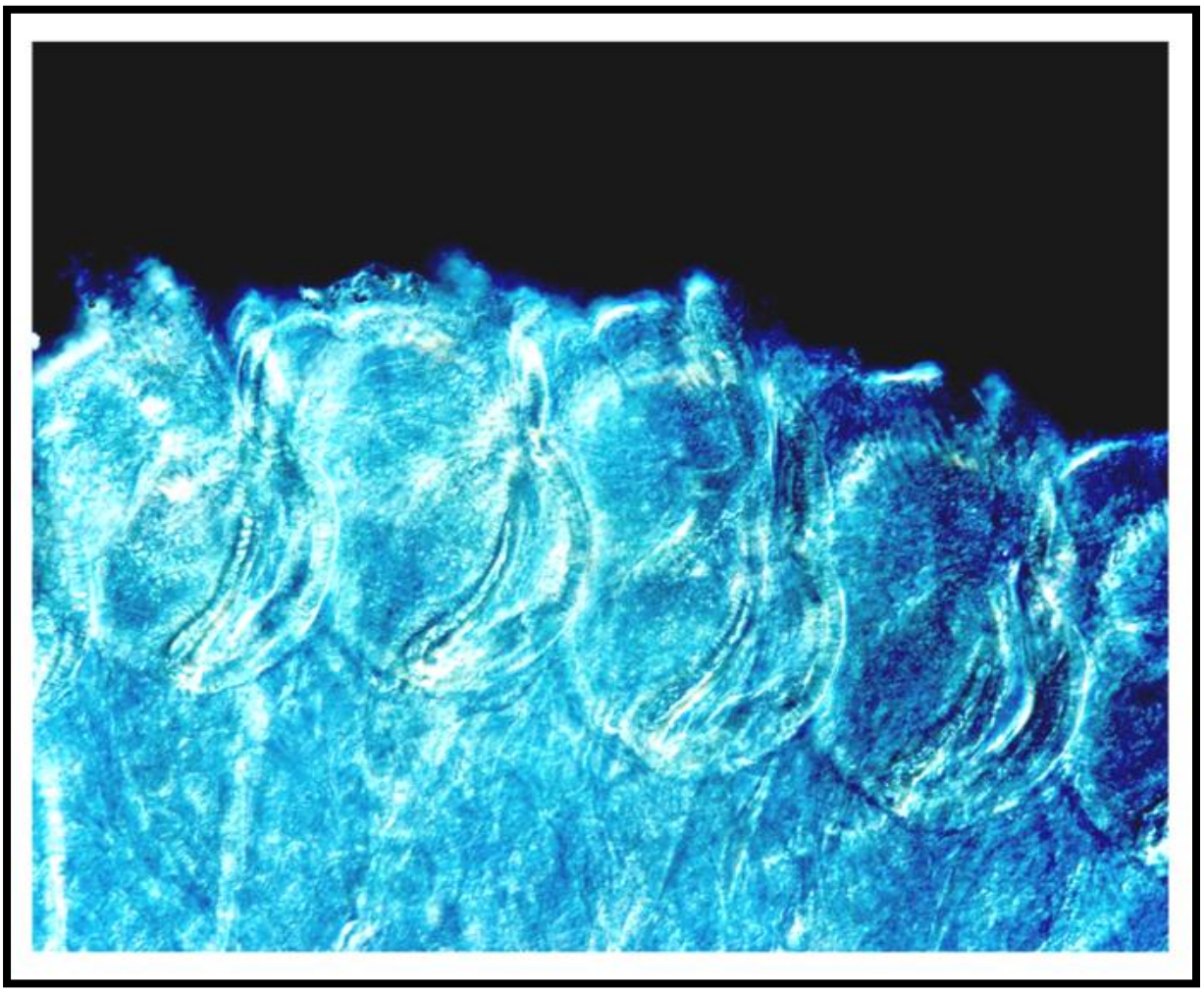
Table 5.4. Comparison of the original morphological measurements of *Paradiplozoon ghanense* (Thomas, 1957)*, P. aegyptensis* (Fischthal & Kuntz, 1963) and measurements from the present study, hosts and localities. Measurements of the total, anterior and posterior body lengths are in mm and the rest of the measurements in µm.

	***P. ghanense* (Thomas, 1957)**	***P. aegyptensis* (Fischthal & Kuntz, 1963)**	**Present Material**
	Hosts and Location		
	Brycinus macrolepidotus (Valenciennes, 1850) (Black Volta River, Ghana) (Anambra River, Nigeria)	*Labeo forskalii* Rüppel, 1835 (Egypt) *L. coubie* Rüppel, 1832 (Volta Lake, Ghana) *L. cylindricus* Peters, 1852 (Ruaha River, Tanzania) *L. victorianus* Boulenger, 1901 (Nzoia River, Kenya) *Brycinus macrolepidotus* (Valenciennes, 1850) (Lake Albert)	*Labeo lunatus* Jubb, 1963 (Okavango River, Botswana)
	Measurements		
Total body length	3.21 – 3.83	4.53 (3.62 – 5.77)	4.38 (1.92 – 5.8)
Anterior body length	1.86 – 2.73	2.67 (1.88 – 3.45)	2.55 (1.18 – 3.5)
Posterior body length	0.38 – 0.48	1.13 (0.87 – 1.87)	1.15 (0.52 – 1.7)
Clamp length	120 – 160	97 (92 – 102)	102 (92 – 110)
Clamp width	100 - 110	70 (65 – 79)	66 (52 – 79)
Anchor hook	None detected	16.5 (16 – 17)	10 (8 – 11)
Anchor shaft		49 (48 – 49)	21 (20 - 22)
Oral sucker length	50 – 75	110 (95 – 125)	103 (69-145)
Oral sucker width	50 - 70	95 (78 – 103)	89 (60-125)
Pharynx	45 - 70	62 (51 – 75)	78 (40 – 95)
Eggs length	115 - 260	292 (254 – 313)	175 (170 – 180)
Eggs width		107 (81 - 132)	69 (68 – 70)

Table 5.6: List of characteristics used for species discrimination by Khotenovsky (1985), for the known African diplozoid species as well as the two species described during the present study, *Paradiplozoon* sp. A from *Labeo lunatus* Jubb, 1963, in the Okavango River, Botswana and *Diplozoon* sp. A from *L. capensis* (Smith, 1841) and *L. umbratus* (Smith, 1841), both from the Orange-Vaal River System.

	***Paradiplozoon ghanense* (Thomas, 1957)**	***Paradiplozoon aegyptensis* (Fischthal & Kuntz, 1963)**	***Paradiplozoon* sp. A**	***Diplozoon* sp. A**	***Afrodiplozoon polycotyleus* (Paperna, 1973)**
					
Clamps	4 pairs	4 pairs	4 pairs	4 pairs	8 to 13 pairs
3rd Clamp mean length and width	105 x 140 µm	67 x 97 µm	107 x 67 µm	69 x 34 µm	60 x 51 µm
Folds	None present	None present	None present	Folds in the posterior body part	None present
Position of genitals	Testes and ovaries located in area of fusion	Testes located in posterior body part, ovaries located in area of fusion	Testes located in posterior body part, ovaries located in area of fusion	Testes and ovaries located in posterior body part	Testes located in posterior body part, ovaries located partly in posterior body part and area of fusion
Egg	With filament 115 - 260 µm	With filament 254-313 µm x 81-132 µm.	With filament 170-180 µm x 68-70 µm	With filament 150-320 µm x 68-70 µm	With filament 180-220 µm x 140- 160 µm
Intestine	Does not bifurcate	Does not bifurcate	Divides in three branches	Does not bifurcate	Does not bifurcate
Ratio of width of 3rd clamp to length of posterior body part	4 : 1	14 : 1	17 : 1	25 : 1	7 : 1

Chapter 6



Discussion

During this study, two new diplozoid species were described from cyprinid hosts in the Okavango River, Botswana and the Orange Vaal-River System. These species were not yet named in the present study and for the purpose of this study they were only referred to as *Paradiplozoon* sp. A and *Diplozoon* sp. A. In order to give a complete review of the family Diplozoidae in Africa, a comprehensive study was done on the taxonomy of these parasites and it was established, that literature on this topic contains a great deal of confusion.

Diplozoids were found to be an immensely interesting group of parasites, well adapted to their peculiar life style with high host specificity. The data obtained during this study will be discussed under the following headings:

- Taxonomic Turmoil
- Host Specificity
    - What is Host Specificity?
    - Parasite Strategies
    - Faithfull to the Family Cyprinidae?
    - Habitat Selection and Host Location
    - Diplozoids and Other Parasites
- Siamese Life Style
    - Finding a Mate
    - Reproductive Strategies
    - Seasonal Influence
- Parasites, Hosts and their Environment
    - Pathological Effects
    - Environmental Effects
    - Monogeneans as Species Indicators

TAXONOMIC TURMOIL

Literature on the taxonomy of the family Diplozoidae is littered with irregularities, errors, incomplete descriptions and overall confusion. Most articles on the phylogeny of this group were published in Russian, French or German and translations of these works probably produced some inaccurate or imprecise renditions in some places, leaving questions and uncertainties. Since Khotenovsky's (1985) Russian manual on the suborder Octomacrinae, in which the family Diplozoidae was reviewed and discussed, various species are still inquirenda, while others were not included in the manual. This, together with incomplete descriptions and doubts on the placement of various species in certain genera, only adds to the confusion. Plenty of contradictions on the classification of certain species are apparent

throughout the literature studied, as various authors place the same species in different genera.

The exact number of species for each of the different diplozoid genera is unclear at the current stage, seeing that the changes brought on by Khotenovsky (1985), have not been applied by many authors since then. This could be due to either inaccurate translations or the lack of knowledge of previously published works. Whatever the reason, various species have been incorrectly identified and described over the years, leading to some species having various synonyms. In the last few years, scientists and experts in the diplozoid field, have begun making sense of some of these uncertainties, with the help of molecular markers (Matejusova *et al.* 2001, 2002 & 2004; Gao *et al*. 2007). Goa *et al.* (2007) stated that molecular markers provide useful information for discrimination of diplozoids. This puts forward the question on the soundness of morphological characteristics and morphometric measurements as the only method for describing species.

With the description of the species in Chapter 5, it was found that morphological characteristics and morphometric measurements may not be interpreted similarly by all. This leaves ground for irregularities to occur seeing that some authors might choose to measure a distinctive characteristic, deemed important for identification, in a different way than what it was originally intended. Therefore, a standard set of characteristics is needed when it comes to measuring specific features for identification, especially when it comes to certain species of diplozoids that are difficult to distinguish from one another. The place of morphological characters and the description thereof cannot entirely be replaced by molecular studies, as it fulfils an important taxonomic duty. These two techniques should rather be used together in order to build a better understanding of the adaptations and phylogeny of diplozoids. It is evident that some morphological characteristics might vary within species, making these an unreliable for species identification, on their own, especially since different views exist on the identification of some species in the genera *Diplozoon* and *Paradiplozoon*. Gao *et al*. (2007) suggested the nucleic acid sequence of the second internal transcribed spacer of ribosomal DNA (ITS-2 rDNA) to be used as a tool for separating species of these genera.

As mentioned in Chapter 5, not much is known about the African diplozoid fauna and even less was known with the publishing of Khotenovsky's (1985) manual. The key for the determination of species from south-eastern Asia and Africa from Khotenovsky (1985) (see Table 5.8) is rather puzzling, with the numbers, as allocated to certain characteristics, not always clearly specifying the exact characteristics to follow. For this reason and to include the two newly described species, a new, more simplified key (Table 5.9) was developed for the determination of the currently known African diplozoid species.

HOST SPECIFICITY

During the present study diplozoids were found on only cyprinid fish from both the Okavango and Orange-Vaal River Systems. The Okavango River is home to 24 species of cyprinid fish, while 11 cyprinid species are present in the Orange-Vaal River System. *Paradiplozoon* sp. A was found to infest only *Labeo lunatus* in the Okavango River, one of two known *Labeo* species in this system. As mentioned in Chapter 5, no diplozoids have been collected from *Labeo cylindricus* (the other *Labeo* species occurring in this system) over the past years during parasitological surveys on the Okavango River. *Afrodiplozoon polycotyleus* was present on five of the 18 *Barbus* species from the Okavango River, *Barbus afrovernayi, B. multilineatus, B. paludinosus, B. poechii* and *B. radiatus* (see Table 8.1). *Diplozoon* sp. A was present on *Labeo capensis* and *Labeo umbratus*, the two only species from the genus *Labeo*, present in the Orange-Vaal System.

In a study by Raymond *et al.* (2006) *Afrodiplozoon polycotyleus* was found on four *Barbus* species from a single location in the Mpanga River in western Uganda. It was, however, found that *A. polycotyleus* only parasitised *B. neumayeri*, suggesting high host specificity that may relate to host physiology, ecology or behaviour. Host physiology was ruled out as it is unlikely to account for the observed host selection of *A. polycotyleus* as *Barbus cercops* and *B. kersteni* were also parasitised by *A. polycotyleus* in the Nzola River, Kenya, and in the swamps of south-eastern Kyoga, respectively, but not at the Mpanga River site. It was evident that ecological and ethological factors play an important role in host selection of cyprinids by diplozoids. During their study it was found that noticeable ecological

differentiation between *Barbus* species of the Mpanga River, did not exist, as *Barbus apleurogramma, B. kerstenii and B. neumayeri* are all bottom dwellers. Due to their similar feeding preferences, all of these species are possibly exposed to *A. polycotyleus* larvae at least part of their lives. It is thought that microhabitat differences or temporal differences in microhabitat used among these *Barbus* species may add to the narrow host specificity of *A. polycotyleus* encountered in the Mpanga River.

What is Host Specificity?

Whittington (1997) defines parasite host specificity as "the restriction or exclusivity of the occurrence of a given species of parasite in one or more species of hosts". Host specificity can be seen as a way of characterising the relationship between parasites and their hosts. Various physiological, ethological and ecological factors together with specific nutritional requirements of the parasite, determine whether the relationship will result in an equilibrium (Lambert & Gharbi 1995). Monogeneans are stenoxenic, referring to their high host specificity, being among the most host-specific of parasites in general and infesting mostly closely related host species (Guégan & Agnése 1991). This occurrence is probably a result of coevolution between monogenean parasites and their hosts. Jovelin & Justine (2001) stated that when looking at coevolution in monogeneans, it must be seen as a result of coaccommodation and cospeciation rather than reciprocal selection pressures acting on the host-parasite couple. Coaccommodation in this case is adaptation without speciation of neither the parasite nor the host and cospeciation refers to speciation of the parasite and host being parallel.

The question, however, arises, what is the advantages of coevolution for parasites? Whittington *et al.* (2000) stated that coevolution between parasites and hosts allow them to adapt to one another so closely that parasites are able to predict host behaviour, physiology and biochemistry, in order to better make use of the host. This continuous adaptation is also said to improve parasite "fitness". Coevolution is therefore closely linked with host specificity based on the assumption that if a parasite is better adapted to the behaviour of a specific host, it would be better adapted to locate that host. The result is enhanced

encounters with the host and a better chance to successfully attach and infest the host. This in turn would reduce encounters with other non-hosts in the environment. Another advantage of high host specificity is a simple, direct life cycle, without an intermediate host. There also exists a disadvantage to such narrow host specificity. If a parasite becomes so specific to survive on only one host, it is possible that when that host goes extinct, the parasite will probably suffer the same fate.

Parasite Strategies

Why have some parasite species become adapted to only certain host species, while others infect a variety of hosts, either from the same genus or family, to unrelated host species or in some cases infect at random if the opportunity presents itself? It is probably due to the fact that parasites have evolved different strategies to enhance their chances of host location. These strategies lead to coevolution and adaption to either a specific host species, or to a broader range of hosts, depending on the behaviour, physiology or biochemistry of the hosts. Opportunistic parasites on the other hand rely less on specific strategies for infecting specific hosts. Krasnov *et al.* (2006) found that opportunistic parasites exert more negative effects on hosts, whereas host specific parasites will have almost no negative effect on their specific hosts under natural conditions, emphasising the result of adaptation and the development of strategies specific to a certain host, over time.

These strategies may include a wide range of adaptations such as the attachment of parasite eggs to the host. According to Whittington (1997), various monogenean species make use of this strategy in order to promote reinfestation, seeing that if the eggs are attached to the host the larvae that hatch do not have to locate a new host, therefore completing the direct life cycle. In the case of most diplozoids, the eggs are attached to the gills by means of long coiled filaments. This filament probably enables hatching to take place in the protected gill chamber of the fish, where after it would be easy for the ciliated oncomiracidium to be swept from the gill chamber in order to locate a host during its infestive, free-living stage.

The influence of host chemicals on egg hatching is another strategy exploited by almost all platyhelminths. Chemical substances such as mucus from the surface of a fish host, may lead to a hatching response in monogenean eggs. The strategy of rhythmical emergence refers to the infestive stages of the parasite, timing its emergence in order to coincide with the host behaviour so that it can infest the host when it is more susceptible to infestation. This is a strategy exploited by *Paradiplozoon homoion gracile*, from the Mediterranean barbel *Barbus meridionalis*, as discussed in Chapter 2. *Paradiplozoon homoion gracile* therefore hatches during the night and infests its host *B. meridionalis* at which time the behaviour of the host leans itself to being more susceptible.

Another strategy utilised by monogenean parasites is the development of special behaviour by the infestive stages. This means that parasites have become adapted so that transmission can take place actively, either during a free-swimming stage or passively in which larvae are ingested by an intermediate or final host. The last strategy proposed by Whittington (1997) is host recognition. Research on host identification of monogeneans is unfortunately still filled with uncertainties. It was determined that oncomiracidia respond to a substance secreted by the epidermis of the fish host, making it possible to recognise a specific fish host. Buchmann & Lindenstrom (2002) stated that monogenean larvae are able to actively seek hosts over short distances after which chemo-, photo and mechanoreceptors possibly play a role in recognising a specific host.

When taking the bigger picture into consideration it is evident that it is not only the above mentioned factors that influence host specificity, but it is vital to also understand the role of the environment, and how the composition of the system affects the interactions taking place within it. Interactions within two diverse river systems such as the Okavango River System and Orange-Vaal River System would therefore be expected to vary to some extent. The Okavango River with its more than 83 fish species has a much higher diversity of fish than the Orange-Vaal River System, with 16 species. As a result, the Okavango River would probably also harbour a more diverse fish parasite fauna.

Faithfull to the Family Cyprinidae?

Diplozoids are generally considered to be host specific to only cyprinid species (Matejusova *et al.* 2001). In Eurasia, diplozoid occurrence is restricted to cyprinid fish. However, in Africa, diplozoids have also been collected from two hosts species of the family Characidae. *Paradiplozoon ghanense* has been reported from *Brycinus macrolepidotus* in Ghana as well as from the Anambra River, Nigeria (Thomas 1957; Echi & Ezenwaji 2009). Another diplozoid that has been found to infest a characid host is *Afrodiplozoon polycotyleus* collected from *Alestes baremoze*, also from the Anambra River, Nigeria (Echi & Ezenwaji 2009). It is therefore apparent that host specificity differs from river system to river system. If these fish were identified correctly, it would appear that host specificity differs in different river systems.

The evolution of African cyprinids was discussed in Chapter 2, together with the classification and world occurrence of diplozoids. It is evident that the relationship between diplozoids and their cyprinid hosts has undergone various adaptations and it can probably be said that diplozoids favour cyprinid hosts. The reason for this, as discussed previously is ecological, ethological and physiological and these factors might have contributed to the occurrence of a few non-cyprinid fish hosts exhibiting similar characters resembling that of the original diplozoid hosts. Seeing that diplozoids are host specific and fall in an overall host specific group of parasites, pure opportunistic infestation can probably be ruled out as a reason for this occurrence. The composition of the system and fish fauna present are also important influences and together with geographical origin can contribute to the interactions present in a system.

Habitat Selection and Host Location

The Mpanga River in Uganda and Okavango River, share some similarities since both consists of large, open water stretches and papyrus swamps. The study by Chapman *et al*. (2000) and Raymond *et al.* (2006) stated that *Barbus neumayeri* is the only *Barbus* species that penetrate into the papyrus swamps of the Mpanga River. It was found that *A.*

polycotyleus is more abundant in swamp habitats than in the well-oxygenated river and infestation levels were found to be much lower in fast flowing currents with the fast-flowing waters of the river possibly limiting successful infestation of the host. This would imply that *A. polycotyleus* does not actively seek a specific host in the river, but host selection would then be seen as an artefact of macrohabitat selection by the host species. Most *Barbus* species in the Okavango River favour swamps, marshes, floodplains and lagoons, areas with lower oxygen conditions. Most fish species in the Okavango River use these floodplains for breeding and as nurseries for the young fish. This is probably where infestation by *A. polycotyleus* takes place and would account for the higher number of *Barbus* species being parasitised in the Okavango River than in the Mpanga River. The reason why only five of the 18 *Barbus* species of the Okavango River is parasitised, however, is unclear. Not much is known about the ecology and behaviour of these fish, making it difficult to make accurate assumptions on possible reasons of infestation by *A. polycotyleus*.

Diplozoids and Other Parasites

During the collection of fish from the Okavango River *Afrodiplozoon polycotyleus* as well as *Paradiplozoon* sp A. were in most cases accompanied by other parasites on the gills of the hosts examined. Other parasites found to be present with both diplozoid species ranged from species from the monogenean genus *Dactylogyrus* Diesing, 1850 to mobile peritrichs such as *Trichodina* Ehrenberg, 1838*, Trichodinella* Sramek-Husek, 1953 and some from the family Myxosporidea (see Table 8.3). Contrary to the variety of parasites present on the gills of diplozoid hosts, fish hosts from the Orange-Vaal River System in most cases during sampling in the present study, did not harbour any other parasites on their gills together with the occurrence of *Diplozoon* sp. A.

The lower diversity of parasite species present on hosts from the Orange-Vaal River System can be explained by the lower diversity of fish species present in this system, as described in Chapter 3 and is not a surprise to find a poorer diversity of parasites than what is present in the Okavango River. Very few rivers in the Orange-Vaal System are in a pristine condition and most have suffered habitat deterioration due to pollution, agriculture, damming and

the introduction of alien fish species, to name only a few. These factors may lead to the increase of infestation by exotic parasites species, such as has been reported by Kruger & Van As (1983). It was found that *Argulus japonicus* Thiele, 1900, infested all endemic fish species from the Bloemhof Dam and Lake Barberspan. The Okavango River, on the other hand, is one of the last pristine freshwater systems in southern Africa, with no introduced fish species, although it is also facing threats due to human interactions.

SIAMESE LIFE STYLE

Diplozoids possess an extremely unique life style, being firstly hermaphrodites and secondly fusing into a monogamous pair, for life. Hermaphroditism, as a breeding system is common in monogeneans and is a topic that has been widely discussed with various hypotheses and models developed on the matter. According to the low density model of Tomlinson (1966) hermaphrodites have less difficulty finding a suitable mate, seeing that they are both male and female. Another hypothesis states that the evolution of an organism's breeding system is influenced by its efficiency to find a mate (Ghiselin 1969; Eppley & Jesson 2008). It is also stated that since hermaphrodites are able to self-fertilise, it allows for reproductive success even without a mate, even though cross-fertilisation is the rule whenever possible (Brown *et al.* 2001). Hermaphrodites are furthermore characterised by the fact that individuals are able to mate with any conspecific mate it comes across, whereas organisms with separate sexes must wait to encounter a mate from the opposite sex (Eppley & Jesson 2008). All of these add up to reproductive assurance for organisms not very effective in finding a mate.

Diplozoids, however, do not exactly fit the above mentioned description of most hermaphrodites, seeing that mate location is of the upmost importance and if juvenile diporpae do not find a mate, they cannot sexually mature and ultimately die without reproducing. One statement that does fit the diplozoid life style is the fact that hermaphrodites are able to mate with any mate they come across and when taking into consideration that juvenile diporpae are 'dead set' on finding a mate, this is a large advantage. Eppley & Jesson (2008) stated that mate-search efficiency shows a relationship

with the evolution of hermaphroditism. Why did diplozoids evolve their very specific, monogamous life style and which came first, hermaphroditism or the merging of pairs?

Finding a Mate

If a diporpa larva does not find a mate to pair with, it fails to reach sexual maturity and consequently dies. In such a unique system as the Okavango River, with diverse and vast habitats stretching as far as the eye can see, it would surely seem like a miracle if two diporpae do come across each other on the gills of a fish host. It would therefore make sense that mate location needs to be as efficient as possible, which correlates with the evolution of hermaphroditism in diplozoids. During the present study *Afrodiplozoon polycotyleus* had a very low prevalence, being present in almost less than one out of a hundred fish collected, highlighting the scarceness of these parasites. Chapman *et al.* (2000) stated that intrinsic factors play some role in determining niches in *A. polycotyleus* and suggested that narrow microhabitats may function to enhance the chance of locating a mate.

In order to optimise the chances of fusion with another diporpa there has to be one of two factors at work. Firstly, *A. polycotyleus* must have become highly host specific to these five fish species with their specific ecology, behaviour or physiology putting them apart from other *Barbus* species in the system and therefore illustrating a long line of coevolution, in order to successfully locate these hosts. The second possibility is that, as with *A. polycotyleus* in the Mpanga River, this diplozoid might not be actively seeking for specific hosts, but host selection could be seen as an artefact of macrohabitat selection by the host species. The *Barbus* fauna of the Okavango River mostly overlaps in terms of habitat and feeding preferences and it might be likely that other *Barbus* species may also occasionally become infested with *A. polycotyleus.* If the last statement proves to be correct, it would suggest that *A. polycotyleus* is not as highly host specific in the Okavango River, although up to now, *A. polycotyleus* has not been found on any other *Barbus* species.

In order to conserve the energy of going through the process of mate location time after time, it would seem that diplozoids came up with a solution, merge to a mate and form a monogamous pair. Monogamy would seem like a strange choice, seeing that it is an occurrence not at all abundant in nature, so there must have been additional selective pressures at work, owing to the evolution of such a permanent bond. It has been hypothesised that diporpae do not just unite in pairs for cross-insemination purposes, but that fusion of reproductive structures is a secondary development. It is rather thought that ecological factors was the primary driving force leading to the fusion of pairs and that paired worms are afforded better protection against dislodgement from the gills by ventilating water currents (Whittington 1997). According to Morrow (2004), the sperm of diplozoids are immotile. With the reciprocal exchange of sperm between the two fused individuals, the possibility of sperm competition is eliminated entirely. Could this be another adaptation developed due to the monogamous life style?

It can therefore be said that the evolution of the siamese life style has developed due to reproductive as well as ecological selection pressures. Hermaphroditism in a sense lightens the burden for diplozoid diporpae to find a mate in a diverse, vast system while also acting as a reproductive advantage. Fusion, while ensuring cross fertilisation, also has a very important ecological role, assisting in establishment of the juvenile diplozoid in its microhabitat. A question, however, comes to mind, why is this monogamous, siamese life style not more common in monogenean parasites, seeing that it seems to be an effective life style choice?

Reproductive strategies

Monogenean parasites comprise a wide assortment of interesting reproductive strategies, whether it be viviparous or ovoviviparous. These strategies range from parasites that seem to be more ordinarily adapted to life on its host, to siamese worms and even worms carrying up to four generations in the uterus of the parent. Parasites have therefore adapted to a variety of circumstances, undergoing coevolution with their hosts in order to best adjust to their specific niche. For instance, the monogenean flatworm *Gyrodactylus thlapi* Christison,

Shinn & Van As, 2005 was described from *Pseudocrenilabrus philander philander* (Weber, 1897) in the Okavango River (Christison *et al.* 2005). Gyrodactylids reproduce through polyembryony where the embryo in the uterus of the mother, produces another embryo, which develop inside the first. This second embryo produces a third, which in turn, produces a fourth embryo. The second embryo will only complete its development after the first embryo is released and this will result in a continuous process. It was stated that a single worm could produce as many as 2 453 descendants, within 30 days (Craig *et al.* 1997).

To answer the previous question, Paperna (1979) stated that specific monogenean parasites infest certain African fish and this is probably due to a long association between host and parasite. In certain parasitic groups, specific reproduction strategies evolved which best suit the relationship with their host and ultimately leading to a particular life style. The energy cost of being host specific, locating a mate in a vast habitat and forming a permanent bond through fusion, together with a variety of ecological selection pressures, probably does not fit the energy budget of most parasites. Parasites adapt diverse strategies in order to infest hosts in a wide array of habitats and under various different conditions. The fact is that not much is known about host-parasite interactions of specifically diplozoids. Le Brun *et al.* (1990) showed that various factors affect the infestation of European fish populations by *Diplozoon gracile*. These factors include schooling behaviour of fish, diet patterns of host activity and refuge zones where hosts and parasites are concentrated. The life style of any certain group of parasites is therefore dependant on an array of characters, pressures and adaptations.

The Seasonal Influence

During the present study, collections were made during the summer months of October to February as well as during the winter months, June to August. In the Okavango River, *Paradiplozoon* sp. A and *Afrodiplozoon polycotyleus* was mostly present during summer, with only two separate collections of *Paradiplozoon* sp. A, being present from *Labeo lunatus*, during June. This was also the case for *Diplozoon* sp. A, from the Orange-Vaal River System, which was present on collected hosts during the period from October to February

and to a much lesser extent in June. Both of these systems are situated in regions of summer rainfall.

According to Chapman *et al.* (2000) and Raymond *et al.* (2006) seasonal peaks of rainfall coincided with lower prevalence of *A. polycotyleus* in western Uganda. It was stated that a higher frequency of occurrence in the dry season might result from relatively higher host susceptibility, as water availability is much reduced and land barriers isolate sections of the river. This leads to concentrations of *Barbus neumayeri* in dry season refuges and higher prevalence of infestation by increasing host availability. According to Le Brun *et al.* (1990), diplozoid larvae spend about 60% of their lifetime on river bottoms, favouring infestation of benthic hosts. Chapman *et al.* (2000) found *Barbus neumayeri* to spend a great deal of time hidden under bottom materials, especially during the dry season.

As mentioned in Chapter 4, the panhandle region of the Okavango River is in flood during February to March and the delta in July, while the river is at its lowest during October and November. Diplozoids exhibited higher levels of infestation during the months when the Okavango River is at its lowest, exposing hosts to isolated pools and receded lagoons, channels and backwaters, coinciding with the findings of Chapman *et al.* (2000) and Raymond *et al.* (2006). This means that fish are more prone to infestation by *A. polycotyleus* or *Paradiplozoon* sp A. The Orange-Vaal River System, however, is most prone to floods during the wet summer months.

Collections in the Orange-Vaal River and especially at the Renosterspruit, mainly took place during the months before the onset of the floods, as most rivers become a brown surge carrying loads of sedimentation, conditions not favourable for fishing. At the Bishop's Weir, most specimens of *Diplozoon* sp. A were collected from fish in the impoundment near the weir and from other collection sites in the Saulspoort, Krugersdrift and Hardap Dams, most of which are susceptible to filling only after floods later in summer. Fish were therefore, as described by Chapman *et al.* (2000) and Raymond *et al.* (2006), present in areas of reduced water availability and ultimately more susceptible to infestation by *Diplozoon* sp. A.

PARASITES, HOSTS AND THEIR ENVIRONMENT

Parasites, their hosts and the environment in which they exist are three entities that cannot be considered in isolation from one another. The effects exerted by a parasite on its host will have an impact on the condition of the host, which in turn will influence the environment in which it lives, and the other way around. It is therefore important to take all the possible interactions between parasites, their hosts and the environment into consideration before drawing conclusions on any one of the three.

Pathological Effects

Lambert & Gharbi (1995) stated that it is essential for parasites to establish relationships with their hosts, which act in the best interest of both the parasite and the host; if not, by destroying their host, they ultimately destroy their habitat and their own biotope. Monogeneans exert little negative effect on their host especially when present in small numbers. As soon as host populations become overcrowded, as is mostly the case in unnatural conditions such as aquaculture, high levels of infestation become prominent with the result of pathogenic effects. Under such circumstances, damage caused by monogeneans may include anaemia, haemorrhages and ulceration of host epithelium, development of epithelial outgrowths and the production of excessive amounts of mucus. The latter may lead to the disturbance of the respiratory function of the gills as well as ionic exchange (Chapman *et al.* 2000).

Kawatsu (1978) reported that *Diplozoon nipponicum* causes hypochromic microcytic anemia in *Carassius carassius*. This is a condition characterised by smaller than normal blood cells, which are poorly filled with haemoglobin. It was found that the haemoglobin levels decreased approximately with an increase in the number of parasites. The infested goldfish were bathed in a 1.0 ppm solution of trichlorfon for 48 hours at a water temperature of 23 to 25˚C. Trichlorfon was recommended as an effective treatment. Lastly, it was established that only diplozoids with a total body length of larger than 2.5 mm, exhibit harmful effects under natural conditions.

Fish are known to co-exist with their specific monogeneans even in cases where infestation is intense (Paperna 1996). During the present study no visible harmful effects on the gills of any of the hosts were noted, due to the infestation of *Diplozoon* sp. A, *Paradiplozoon* sp. A or *Afrodiplozoon polycotyleus*. Chapman *et al.* (2000) also did not find any evidence of significant effects on the condition of *Barbus neumayeri*, because of infestation by *A. polycotyleus* and Barson & Marshall (2003) showed that African *Barbus* species are able to survive infestation of more than ten parasites per fish without this drastically affecting the host. The life span of most diplozoon species is unknown, but it has been shown that *Diplozoon paradoxum* can live for more than two years. It is therefore apparent that a longer life span of a parasite and host could cause more hosts to be parasitised with older hosts having a higher parasite load (Le Brun *et al.* 1990).

Environmental effects

Water quality due to pollution

Parasite communities are known to act as good indicators of environmental quality and stress. This is due to their association with trophic relations as parasites are situated at the top of the food web and integrate the adverse effects of contaminants moving through the food web (Galli *et al.* 2001; Lafferty 1997). In a study by Sebelova *et al.* (2002), it was stated that pollution and environmental stress affect fish and the monogeneans inhabiting the gills of the host fish. Non-optimal environmental conditions was found to trigger changes in the morphology of attachments clamps of diplozoids (Pecinkova *et al.* 2005). These changes may occur in various ways such as changes in the size of the clamps without accompanying changes in their structure. Other changes include morphological modifications of the standard clamp size. It was also found that some clamps consisted of abnormal sclerites while in others, rudimentary clamps were present and in some cases, clamps were absent or additional clamps were present.

Water quality due to seasonal changes

Chapman *et al.* (2000) and Echi & Ezenwaji (2009) found that fish that are under stress or in deprived conditions are generally less resistant to parasites. In river systems prone to seasonal flooding, dry season conditions lead to habitat contraction with decreased oxygen availability. This is followed by higher fish densities and in extreme cases, lower fish condition with eventually higher fish mortality. If higher host densities contribute to higher levels of stress, dry season conditions may lead to higher levels of parasitism (Chapman *et al.* 2000).

In a study by Echi & Ezenwaji (2009) on *Diplozoon ghanense* and *Afrodiplozoon polycotyleus* from the Anambra River in Nigeria, it was found that *A. polycotyleus* peaked in December and January when the water was somewhat acidic with a pH of 6.6 to 6.7. Dissolved oxygen was about 10.0 mg litre^{-1} during this time. The lower distribution of oxygen during the dry period was thought to be a result of waste inputs by the local populations, its attendant biological activities and other characteristics of the water system. It was concluded that the relative low pH, perhaps, affected the direct life cycle of these diplozoids. Chapman *et al.* (2000) found *A. polycotyleus* in western Uganda, to tolerate low levels of oxygen, averaging 2.5 mg litre^{-1}. Raymond *et al.* (2006) also confirmed this occurrence and stated that areas such as swamps, with slower currents and lower dissolved oxygen were characterised by higher levels of parasitism by *A. polycotyleus.* Le Brun *et al.* (1990) also confirmed this, with lower prevalence of *D. gracile* noted in large deep rivers with rapid currents. This pattern was ascribed to higher host-finding abilities for the larvae in slower flowing waters.

Monogeneans as Species Indicators

Monogeneans with their high host specificity can play an important role as species indicators. Various monogenean fish parasites have shown to be useful in describing new fish species. A few examples were given by Lambert & Gharbi (1995), one of which referred to the discovery of *Annulotrema pikoïdes* (Guega, 1988) (Monogenea: Ancyrocephalidae). This monogenean parasite is strictly specific to the tigerfish, *Hydrocynus vittatus* Castelnau,

1861 and confirmed *H. forskalli* (Cuvier, 1819) and *H. vittatus* from the Niger basin, which was previously believed to be synonyms for the same species, to be distinct species.

Monogeneans are also good indicators of host hybridisation and monogeneans with a very narrow specificity for one of the host parent species would be present on the hybrids, but not on the other parent species (Lambert & Gharbi 1995). A good example is the mudfish *Labeo umbratus* (Smith, 1841) and *L. capensis* (Smith, 1841), discussed in Chapter 3. These two species are closely related and are known to hybridise. Du Toit *et al.* (1973) reported that these two species could not be regarded as different species after using the haemoglobin electropherogram to try to differentiate between the two species. During the present study *Diplozoon* sp. A was present on both *L. umbratus* and *L. capensis*, but not on any other cyprinids from the Orange-Vaal River System. This would suggest that *Diplozoon* sp. A is host specific to the genus *Labeo* and also confirm the close relation of these two species. During this study no hybrids of these two *Labeo* species were collected, but it would be expected that the hybrids would also be infested with *Diplozoon* sp. A.

CONCLUSION

To conclude, in view of the past 2010 International Year of Biodiversity, in an article by Thompson *et al.* (2010) the conservation value of parasite biodiversity was briefly discussed. It was stated that the overall negative view of parasites, need to make way for a more comprehensive understanding of the complex relationships between parasites and their hosts. The extinction of free living species is accompanied by the coextinction of the parasites dependant on these species as hosts, especially in the case of host specific parasites. This means that in most cases, host species will go extinct without even recognising the numerous parasite species that will be lost as well. Parasites are important components of biological communities, playing key roles in community food webs and being responsible for influencing host behaviour, the regulation of host population sizes, mediating competitive interactions among hosts as well as acting as ecosystem engineers. It is therefore clear that a loss of parasite biodiversity will have significant impacts on ecosystem functioning.

The role parasites play in the environment cannot be overlooked and it is necessary to better understand the vital interactions they regulate. Diplozoids are not the only group of parasites in Africa lacking research on both their ecology as well as that of their hosts. By finding and describing new species, the knowledge on the evolution of parasites and the relationships with their hosts can be expanded. Further investigation into the relationship of diplozoids and their cyprinid hosts from the Okavango River, might also aid in better understanding the behaviour and ecology of its occupants. In the case of the Orange-Vaal River System which is threatened by pollution and the degradation of it endemic composition, parasites and their contribution as bio-indicators, might be able to play a supporting role in monitoring river condition in this diverse system.

Chapter 7

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Abstract & Opsomming

ABSTRACT

The family Diplozoidae Palombi, 1949 comprises host specific worms found on the gills of cyprinid fish. Diplozoids are the only members of the Monogenea characterised by a unique monogamous life style where two hermaphroditic adults fuse and live in permanent copula. Little is known of the African Diplozoidae fauna and even less of the Southern African fauna, especially when compared to the European and Asian fauna. The taxonomy of this group of parasites contains a great deal of irregularities and confusion on the placement of species in certain genera. To date two species of *Paradiplozoon* Achmerov, 1974 are known from Northern Africa, i.e. *P. aegyptensis* (Fischtal & Kuntz, 1963) collected from *Labeo* spp. and *P. ghanense* (Thomas, 1957) described from *Alestes* spp. *Afrodiplozoon polycotyleus* (Paperna, 1973), previously of the genus *Neodiplozoon* Tripathi 1959, was described from *Labeo victorianus* Boulenger, 1901 in Kenya and from *Barbus* spp. in Uganda and South Africa. During the present study, it was attempted to clarify the taxonomic disorder and give a review of the African diplozoid fauna. Members of the Diplozoidae were collected from eight cyprinid fish species during fish parasitological surveys over the last 15 years from the Okavango and Orange-Vaal River Systems. Identification of species was mainly done through morphometric analysis. Three different species have been identified belonging to the genera *Paradiplozoon, Diplozoon* Von Nordmann, 1832 and *Afrodiplozoon* Khotenovsky, 1980 of which two are newly described species. *Paradiplozoon* sp. A was collected from *Labeo lunatus* Jubb, 1963 and *Afrodiplozoon polycotyleus* from *Barbus afrovernayi* Nichols & Boulton, 1927, *B. multilineatus* Worthington, 1933, *B. paludinosus* Peters, 1852, *B. poechii* Steindachner, 1911 and *B. radiatus* Peters, 1853, all from the Okavango River, Botswana. This is also the first records of *A. polycotyleus* from these hosts. *Diplozoon* sp. A was collected from *Labeo capensis* Smith, 1841 and *Labeo umbratus* Smith, 1841 from the Orange-Vaal River System.

Keywords: *Monogenea; Diplozoidae; Cyprinids; Okavango River; Orange-Vaal River System; Afrodiplozoon; Paradiplozoon; Diplozoon; Morphometric analysis; Host specificity.*

OPSOMMING

Die familie Diplozoidae Palombi, 1949 bestaan uit gasheerspesifieke wurms wat op die kieue van verteenwoordigers van cyprinid visse voorkom. Diplozoidae is die enigste lede van die Monogenea wat deur 'n unieke monogame leefstyl gekenmerk word, bestaande uit twee volwasse hermafroditiese wurms wat aanmekaar vasgroei om sodoende ‘n permanente band te vorm. Min is bekend oor die Afrika diplozoid fauna en selfs minder oor die Suider-Afrikaanse fauna, veral in vergelyking met dit wat oor diplozoids van Europa en Asië bekend is. Groot-skaalse verwarring bestaan oor die taksonomie van hierdie groep parasiete, met onreëlmatighede oor die plasing van spesies in sekere genera. Tot op hede is twee *Paradiplozoon* Achmerov, 1974 spesies uit Noord-Afrika, naamlik *P. aegyptensis* (Fischtal & Kuntz, 1963), van *Labeo* spp. en *P. ghanense* (Thomas, 1957), vanaf *Alestes* spp. versamel *Afrodiplozoon polycotyleus* (Paperna, 1973), voorheen van die genus *Neodiplozoon* Tripathi 1959, is vanaf *Labeo victorianus* Boulenger, 1901 in Kenia asook vanaf *Barbus* spp. uit Uganda en Suid-Afrika beskryf. Gedurende die huidige studie is gepoog om duidelikheid oor die heersende taksonomiese verwarring te bied asook om 'n oorsig van die diplozoid fauna van Afrika te verskaf. Lede van die verteenwoordigers van die Diplozoidae is vanaf agt cyprinid visspesies gedurende parasitologiese opnames wat oor die afgelope 15 jaar uitgevoer is, vanaf visse uit die Okavango en Oranje-Vaal Rivier Stelsels, versamel. Deur middel van morfometriese analises is verskillende spesies, naamlik van die genera *Paradiplozoon*, *Diplozoon* Von Nordmann, 1832 en *Afrodiplozoon* Khotenovsky, 1981 geïdentifiseer waarvan twee as nuwe spesies beskryf word. *Paradiplozoon* sp. A vanaf *Labeo lunatus* Jubb, 1963 beskryf, terwyl *Afrodiplozoon polycotyleus* op *Barbus afrovernayi* Nichols & Boulton, 1927, *B. multilineatus* Worthington, 1933, *B. paludinosus* Peters, 1852, *B. poechii* Steindachner, 1911 en *B. radiatus* Peters, 1853, vanuit die Okavangorivier, Botswana versamel is. Dit is ook die eerste aangetekende rekord van *A. polycotyleus* van hierdie gashere. *Diplozoon* sp. A is vanaf *Labeo capensis* Smith, 1841 en *Labeo umbratus* Smith, 1841 onderskeidelik, uit die Oranje-Vaalrivierstelsel versamel.

Sleutelwoorde: Monogenea; Diplozoidae; Cyprinids; Okavango Rivier; Oranje-Vaal Rivier Stelsel; Afrodiplozoon; Paradiplozoon; Diplozoon; Morfometriese analiese; Gasheerspesifisiteit.

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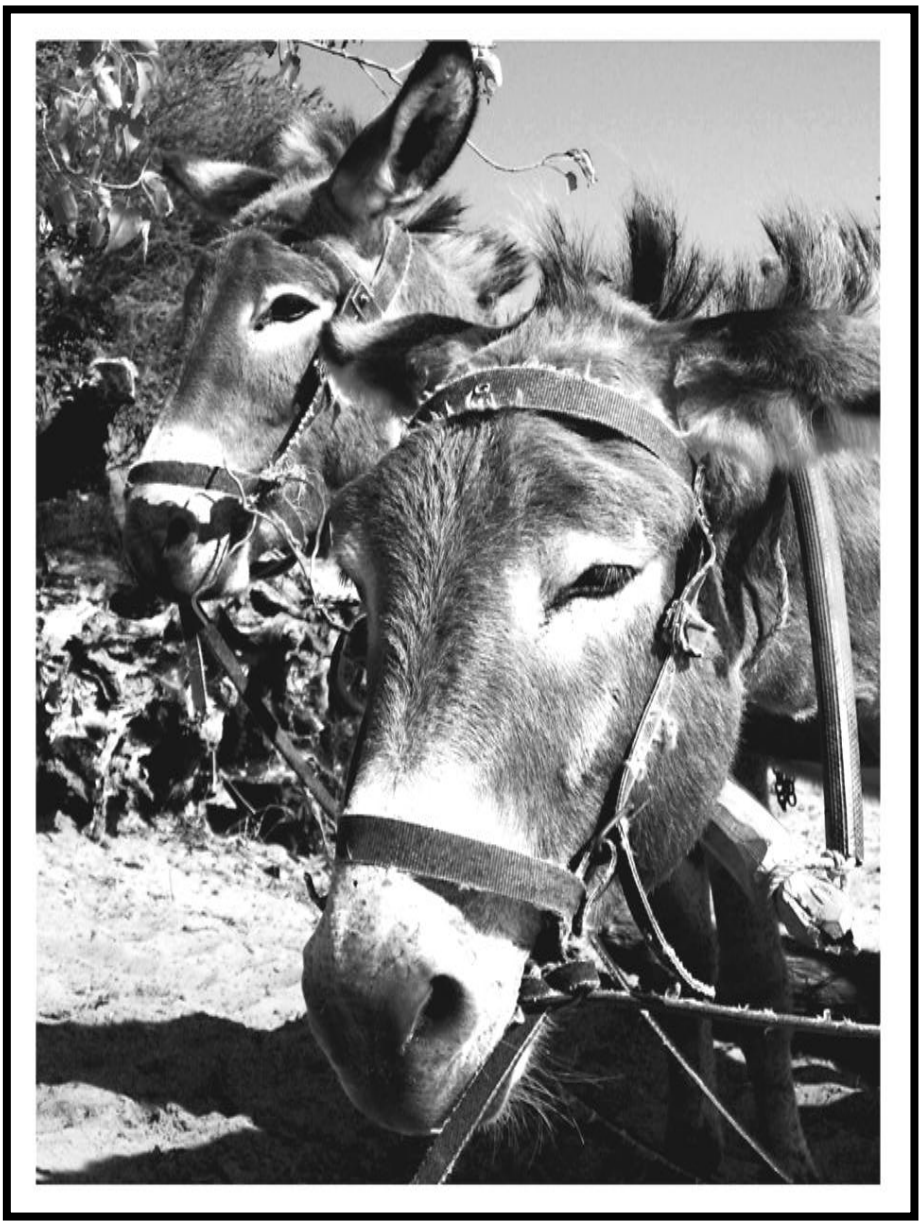
Chapter 8



Appendix

Table 8.1: List of all the fish species occurring in the Okavango River, Botswana, below Popa Falls according to Skelton (2001) and as found by the Aquatic Parasitology survey, 1997 to 2009.

	Author and Date	Common name
Mormyridae		
Hippopotamyrus ansorgii	(Boulenger, 1905)	Slender Stonebasher
Cyphomyrus discorhynchus	(Peters, 1852)	Zambezi Parrotfish
*Marcusenius macrolepidotus**	(Peters, 1852)	Bulldog
*Mormyrus lacerda**	Castelnau, 1861	Western Bottlenose
*Petrocephalus catostoma**	(Günter, 1866)	Northern Churchill
*Petrocephalus wesselsi**	(Kramer & van der Bank, 2000)	Southern Churchill
*Pollimyrus castelnaui**	(Boulenger, 1911)	Dwarf Stonebasher
Kneriidae		
Kneria polli	Trewavas, 1936	Northern Kneria
Parakneria fortuita	Penrith, 1973	Cubango Kneria
Cyprinidae		
Barbus afrovernayi*	Nichols & Boulton, 1927	Spottail Barb
Barbus barotseensis	Pellegrin, 1920	Barotse Barb
*Barbus barnardi**	Jubb, 1965	Blackback Barb
*Barbus bifrenatus**	Fowler, 1935	Hyphen Barb
Barbus brevidorsalis	Boulenger, 1915	Dwarf Barb
Barbus breviceps	Trewavas, 1936	Shorthead Barb
Barbus eutaenia	Boulenger, 1904	Orangefin Barb
*Barbus fasciolatus**	Günter, 1868	Red Barb
*Barbus haasianus**	David, 1936	Sickle-fin Barb
Barbus kerstenii	Peters, 1868	Redspot Barb
Barbus lineomaculatus	Boulenger, 1903	Line-spotted Barb
*Barbus miolepis**	Boulenger, 1902	Zigzag Barb
Barbus multilineatus*	Worthington, 1933	Copperstripe Barb
Barbus paludinosus*	Peters, 1852	Straightfin Barb
Barbus poechii*	Steindachner, 1911	Dashtail Barb
Barbus radiatus*	Peters, 1853	Beira Barb
*Barbus thamalakanensis**	Fowler, 1935	Thamalakane Barb
*Barbus unitaeniatus**	Günter, 1866	Longbeard Barb

Table 8.1 (continue): List of all the fish species occurring in the Okavango River, Botswana, below Popa Falls according to Skelton (2001) and as found by the Aquatic Parasitology survey, 1997 – 2009, continued.

*Coptostomabarbus wittei**	David & Poll, 1937	Upjaw Barb
Labeobarbus codringtoni	(Boulenger, 1908)	Upper Zambezi yellowfish
*Labeo cylindricus**	Peters, 1852	Redeye Labeo
Labeo lunatus*	Jubb, 1963	Upper Zambezi Labeo
Mesobola brevianalis	(Boulenger, 1908)	River Sardine
Opsaridium zambezense	Peters, 1852	Northern Barred Minnow
Distichodontidae		
*Hemigrammocharax machadoi**	Poll, 1967	Dwarth Citharine
*Hemigrammocharax multifasciatus**	Boulenger, 1923	Multibar Citharine
Nannocharax macropterus	Pellegrin, 1925	Broadbar Citharine
Characidae		
*Brycinus lateralis**	(Boulenger, 1900)	Stiped Robber
*Hydrocynus vittatus**	Castelnau, 1861	Tigerfish
*Micralestes acutidens**	(Peters, 1852)	Silver Robber
*Rhabdalestes maunensis**	(Fowler, 1935)	Slender Robber
Hepsetidae		
*Hepsetus odoe**	(Bloch, 1794)	African Pike
Claroteidae		
Parauchenoglanis ngamensis	(Boulenger, 1911)	Zambezi Grunter
Amphiliidae		
Zaireichtys rotundiceps	(Hilgendorf, 1905)	Spotted Sand Catlet
*Leptoglanis rotundiceps**	Boulenger, 1902	Chobe Sand Catlet
Amphilius uranoscopus	Pfeffer, 1889	Common or Stargazer Mountain Catfish
Schilbeidae		
*Schilbe intermedius**	Rüppell, 1832	Silver Catfish (Butter barbel)
Clariidae		
*Clarias gariepinus**	(Burchell, 1822)	Sharptooth Catfish
Clarias liocephalus	Boulenger, 1898	Smoothhead Catfish
*Clarias ngamensis**	Castelnau, 1861	Blunttooth Catfish

Table 8.1 (continue): List of all the fish species occurring in the Okavango River, Botswana, below Popa Falls according to Skelton (2001) and as found by the Aquatic Parasitology survey, 1997 – 2009, continued.

*Clarias stappersii**	Boulenger, 1915	Blotched Catfish
*Clarias theodorae**	Weber, 1897	Snake Catfish
Clariallabes platyprosopos	Jubb, 1964	Broadhead Catfish
Mochokidae		
Chiloglanis fasciatus	Pellegrin, 1936	Okavango Suckermouth (Okavango Rock Catlet)
*Synodontis leopardinus**	Pellegrin, 1914	Leopard Squeaker
Synodontis macrostigma	Boulenger, 1911	Largespot Squeaker
Synodontis macrostoma	Skelton & White, 1990	Largemouth Squeaker
*Synodontis nigromaculatus**	Boulenger, 1905	Spotted Squeaker
*Synodontis thamalakanensis**	Fowler, 1935	Bubblebarb Squeaker
*Synodontis vanderwaali**	Skelton & White, 1990	Finetooth Squeaker
Synodontis woosnami	Boulenger, 1911	Upper Zambezi Squeaker
Cyprinodontidae		
*Aplocheilichthys hutereaui**	(Boulenger, 1913)	Meshscaled Topminnow
*Aplocheilichthys johnstonii**	Günther, 1893	Johnston's Topminnow
Aplocheilichthys katangae	(Boulenger, 1912)	Striped Topminnow
Cichlidae		
Hemichromis elongatus	(Guichenot, 1859)	Banded Jewelfish
*Oreochromis andersonii**	(Castelnau, 1861)	Threespot Tilapia
*Oreochromis macrochir**	(Boulenger, 1912)	Greenhead Tilapia
*Pharyngochromis acuticeps**	(Steindachner, 1866)	Zambezi River Bream
*Pseudocrenilabrus philander**	(Weber, 1897)	Southern Mouthbrooder
*Sargochromis carlottae**	(Boulenger, 1905)	Rainbow Bream
*Sargochromis codringtoni**	(Boulenger, 1908)	Green Bream
*Sargochromis giardi**	(Pellegrin, 1903)	Pink Bream
*Sargochromis greenwoodi**	(Bell-Cross, 1975)	Deepcheek Bream
*Serranochromis altus**	Winemiller & Kelso-Winemiller, 1990	Humpback Largemouth
*Serranochromis angusticeps**	(Boulenger, 1907)	Thinface Largemouth
Serranochromis longimanus	(Boulenger, 1911)	Longfin Largemouth

Table 8.1 (continue): List of all the fish species occurring in the Okavango River, Botswana, below Popa Falls according to Skelton (2001) and as found by the Aquatic Parasitology survey, 1997 – 2009, continued.

*Serranochromis robustus**	(Günther, 1864)	Nembwe (Tsungwa)
*Serranochromis macrocephalus**	(Boulenger, 1899)	Purpleface Largemouth
Serranochromis thumbergi	(Castelnau, 1861)	Brownspot Largemouth
*Tilapia rendalli rendalli**	(Boulenger, 1896)	Redbreast Tilapia
*Tilapia ruweti**	Poll & Thys van den Audenaerde, 1965	Okavango Tilapia
*Tilapia sparrmanii**	A. Smith, 1840	Branded Tilapia
Anabantidae		
*Microctenopoma intermedium**	(Pellegrin, 1920)	Blackspot Climbing Perch
*Ctenopoma multispine**	Peters, 1844	Manyspined Climbing Perch
Mastacembelidae		
Aethiomastacembelus frenatus	(Boulenger, 1901)	Longtail Spiny Eel
Aethiomastacembelus vanderwaali	(Skelton, 1976)	Ocellated Spiny Eel

*Hosts of monogenean gill parasites. Host of diplozoids from this study are indicated in bold.

Table 8.2: List of all the fish species occurring in the Orange-Vaal River, according to Skelton (2001).

	Author and Date	Common name
Cyprinidae		
Mesobola brevianalis	(Boulenger, 1908)	River Sardine
Barbus anoplus	Weber, 1897	Chubbyhead Barb
Barbus pallidus	Smith, 1841	Goldie Barb
Barbus trimaculatus	Peters, 1852	Threespot Barb
Barbus hospes	Barnard, 1938	Namaqua Barb
Barbus paludinosus	Peters, 1852	Straightfin Barb
Labeobarbus kimberleyensis	(Gilchrist & Thompson, 1913)	Vaal-Orange Largemouth Yellowfish
Labeobarbus aeneus	(Burchell, 1822)	Vaal-Orange Smallmouth Yellowfish
*Labeo umbratus**	(Smith, 1841)	Moggel
*Labeo capensis**	(Smith, 1841)	Orange River Mudfish
Cyprinus carpio	Linnaeus, 1758	Carp
Austroglanididae		
Austroglanis sclateri	(Boulenger, 1901)	Rock Catfish
Clariidae		
Clarias gariepinus	(Burchell, 1822)	Sharptooth catfish
Centrarchidae		
Micropterus salmoides	(Lacepede, 1802)	Largemouth Bass
Cichlidae		
Oreochromis mossambicus	(Peters, 1852)	Mozambique Tilapia
Pseudocrenilabrus philander	(Weber, 1897)	Southern Mouthbrooder
Tilapia sparrmanii	Smith, 1840	Banded Tilapia

*Hosts of diplozoids from this study.

Table 8.3: List of the genera of parasites collected from fish in the Okavango River, Botswana, from 1997 - 2009.

Parasite genera	Number of species	Fish Species Affected
Protozoa		
Suctoria	2	22
Apiosoma	5	34
Scopulata	2	22
Epistylis	2	18
Trichodina	15	39
Hemitrichodina	1	1
Paratrichodina	1	1
Tripartiella	8	34
Trichodinella	1	4
Ichthyophthirius	1	7
Chilodonella	1	8
Ichthyobodo	1	4
Trypanosoma	1	1
Myxosporidea		
Henneguya	3	3
Myxobolus	2	1
Parasitic crustacea		
Copepoda:		
Lamproglena	3	12
Afrolernaea	1-2	1
Opistolernaea	1	3
Ergasilus	3-4	6
Branchiura:		
Dolops	1	3
Chonopeltis	2	2
Argulus	1	1
Worms		
Monogenea:		
Annulotrema	5	5
Characidotrema	1	1
Cichlidotrema	10	11
Schilbetrema	2	1
Gyrodactylis	20	13
Macrogyrodactylis	1	1
Dactylogyrus	6	20
Anchyrocephalus	2	10
Paradiplozoon	1	1
Afrodiplozoon	1	5
Larval Trematoda	4	11
Adult Trematoda	2	8
Larval Cestoda	2	2
Adult Cestoda	2	2
Larval Nematoda	3-4	9
Adult Nematoda	4	8
Larval Acantocephala	2	4
Adult Acantocephala	2	3
Hirudinea	1	1

Table 8.4: Comparisons of all the morphometric characteristics between the three diplozoid species, A*frodiplozoon polycotyleus* Paperna, 1973*, Paradiplozoon* sp. A and *Diplozoon* sp. A, collected during the present study. Measurements of the total, anterior and posterior body lengths are in mm and the rest of the measurements in µm.

	A. polycotyleus	***Paradiplozoon* sp. A**	***Diplozoon* sp. A**
	Okavango River, Botswana		Orange-Vaal River System
Total body length	1.54 ± 0.328 (1.26-2.14)	4.38 ± 1.21 (1.92-5.8)	2.92 ± 1.01 (1.03-4.4)
Anterior body length	0.91 ± 0.144 (0.72-1.24)	2.55 ± 0.78 (1.18-3.5)	1.73 ± 062 (0.57-3.1)
Posterior body length	0.37 ± 0.072 (0.3-0.52)	1.15 ± 0.32 (0.52-1.7)	0.86 ± 0.31 (0.28-1.4)
Oral sucker 1 length	42.82 ± 4.69 (34-49)	104 ± 22 (69-145)	65.38 ± 15.44 (38-100)
Oral sucker 1 width	35.36 ± 5.66 (22-42)	89 ± 18 (60-125)	61.74 ± 13.52 (32-90)
Sucker 2 length	41.64 ± 5.14 (31-50)	103 ± 23 (69-145)	66.02 ± 15.43 (37-100)
Sucker 2 width	37.27 ± 5.87 (22-45)	89 ± 15 (70-120)	63.8 ± 14.17 (30-92)
Opisthaptor length	328 ± 19 (300-350)	343 ± 69 (280-440)	410 ± 101 (162-660)
Opisthaptor width	268 ± 19 (240-270)	299 ± 76 (220-375)	320 ± 96 (140-600)
Pharynx length	47.71 ± 12.01 (25-66)	78 ± 22 (40-95)	62.68 ± 12.99 (34-100)
Pharynx width	32.57 ± 7.98 (18-42)	51 ± 19 (20-71)	41.79 ± 8.11 (20-61)
Anchor hook “handle”	6.07 (5-7)	10 ± 1.69 (8-11)	2.95 ± 0.29 (3-3)
Shaft	13.22 (13-14)	21 ± 1.52 (20-22)	15.13 ± 2.2 (13-17)
1st Clamp length	31.24 ± 9.53 (18-39)	98 ± 5.04 (92-104)	58.53 ± 12.1 (42-90)
Width	23.74 ± 5.97 (15-27)	59 ± 9.04 (52-74)	34.8 ± 14.3 (19-90)
2nd Clamp length	49.35 ± 5.13 (44-54)	101 ± 3.23 (98-104)	67 ± 16.4 (49-112)
Width	28.58 ± 7.02 (21-34)	65 ± 6.06 (60-74)	34.82 ± 13.29 (19-71)
3rd Clamp length	59.73 ± 13.72 (42-71)	107 ± 2.58 (104-110)	69.1 ± 16.4 (48-118)
Width	51.39 ± 19.25 (23-65)	67 ± 6.74 (63-77)	34.12 ± 11.1 (18-55)
4th Clamp length	66.16 ± 15.1 (45-79)	102 ± 5.66 (94-110)	64.9 ± 15.3 (47-103)
Width	52.38 ± 22.49 (25-80)	72 ± 4.45 (67-79)	34.1 ± 9.76 (21-50)
5th Clamp length	63.63 ± 1.54 (62-65)	–	–
Width	46.04 ± 18.31 (25-57)	–	–
6th Clamp length	62.56 ± 14.63 (49-78)	–	–
Width	43.42 ± 14.26 (27-53)	–	–
7th Clamp length	62.75 ± 5.48 (59-69)	–	–
Width	41.33 ± 15.46 (24-51)	–	–
8th Clamp length	64.15 ± 3.38 (61-68)	–	–
Width	35.47 ± 7.89 (26-41)	–	–
9th Clamp length	61.69 ± 14.82 (51-72)	–	–
Width	40.96 ± 16.97 (29-53)	–	–
10th Clamp length	55.56	–	–
Width	44.71	–	–
11th Clamp length	54.98	–	–
Width	44.2	–	–
Egg length	–	175 ± 7.07 (170-180)	260 ± 61.9 (150-320)
Width	–	69 ± 0.71 (68-70)	116 ± 19.9 (90-150)

Table 8.5: Recipes for stains used

Mayer's Carmalum	
Stock solution:	
Carminic acid (C.P. carmine) (C.I. 75470)	1g
Ammonium alum	10g
Distilled water	20g
When dissolved, filter and add:	
Formalin	1ml
To create the working solution, mix the following:	
Carmalum stock solution	5ml
Glacial acetic acid	0.4ml
Distilled water	100ml

Glacial acetic acid	
70% Ethanol	100ml
Concentrated HCL	1ml

Mayer's Paracarmine	
Dissolve:	
Aluminium chloride	0.5g
In heated, 70% ethanol	100ml
Add:	
Calcium chloride	4g
Carmine	1g
Filter the mixture after it has cooled down.	

Harris's Alum Hematoxylin	
Hematoxylin crystals	5g
Absolute ethanol	50ml
Ammonium or potassium alum	100g
Distilled water	1000ml
Mercuric oxide (red)	2.5g

Procedure:

Dissolve the hematoxylin in the alcohol and the alum in the water by heating the respective liquids. Remove from heat and mix the two solutions. Bring to boil as rapidly as possible. Remove from the heat and add the mercuric oxide slowly. Reheat until it becomes dark purple, remove from flame immediately and plunge the vessel into a basin of cold water until cool. The stain is ready for use as soon as it cools. Addition of 2 to 4ml of glacial acetic acid per 100ml of solution increases the precision of the nuclear stain. Filter before use.

Grenacher's Alum Carmine	
Carmine powder	1g
Distilled water	100ml
Ammonium Alum	5g

Procedure:

Dissolve alum in water and add the carmine. Bring to boil for 10 to 20 minutes. Cool the solution and filter. Let the solution rest for 1 to 2 days before use.

Phosphate buffer	
Solution A:	
Na_2HPO_4	35.81g/l
NaH_2PO_4	14.19g/l
Solution B:	
KH_2PO_4	13.61g/l

Procedure:

Mix 80 part of solution A with 20 parts of solution B.