

On cestode and digenean parasites of *Clarias gariepinus* (Burchell, 1822) from the Rietvlei Dam, South Africa

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ABSTRACT

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Sharptooth catfish, *Clarias gariepinus*, from the Rietvlei Dam near Pretoria, South Africa were examined for internal platyhelminth parasites. Two adult cestodes, *Polyonchobothrium clarias* (stomach) (prevalence 71 %, mean intensity = 5, $n = 7$) and *Proteocephalus glanduliger* (anterior intestine) (prevalence 14 %, mean intensity = 2, $n = 7$), were found in the gut while metacercariae of one larval digenean, *Ornithodiplostomum* sp. (prevalence 14 %, mean intensity = 140, $n = 7$), were found encysted in the muscles. The morphology of these species, based on light and scanning electron microscopy as well as histological analysis, and how they differ from previously described specimens, are discussed. *Ornithodiplostomum* is a new record in southern Africa. Infection levels of the host fish were mild compared to records from previous surveys.

Keywords: Cestode, *Clarias gariepinus*, digenean, platyhelminth, Rietvlei Dam, South Africa

INTRODUCTION

Fish helminthology in southern Africa is not as widely studied as other aspects of aquatic parasitology and fish biology. This is probably because helminths mainly infect the internal organs, predominantly the gastrointestinal tract which, for humans, does not comprise the edible portion of the fish. Although fishermen and anglers regularly encounter encysted “grubs” (metacercariae) in the skin and muscles of fish (B. Marshall, personal communication 2002), they regard them as just a nuisance, notwithstanding the biological and economic impact they may have on the fish species.

Only a few studies on cestode and trematode parasites of fish in South Africa have been documented (Whitfield & Heeg 1977; Boomker, Huchzermayer & Naude 1980; Mashego 1977, 1981, 1982, 2001; Mashego & Saayman 1989; Brandt, Van As, Schoonbee & Hamilton-Attwell 1981; Van As, Schoonbee & Brandt 1981; Britz, Van As & Saayman 1985; Saayman, Mashego & Mokgalong 1991, Luus-Powell & Mashego 2003). In 1984, Van As and Basson compiled a checklist of South African freshwater fish parasites, but many new species have been discovered since then (Khalil & Polling 1997). Paperna (1996) published a concise update of the parasitic diseases of fish in Africa, in which the occurrence and geographical distribution, life cycles, pathology, epizootiology and control of the parasites is described.

The tapeworms (Cestoda) and flukes (Digenea) infect internal organs of fish (Roberts & Janovy 2000) and their life cycles involve more than one intermediate host, including planktonic copepods, molluscs and fish. Piscivorous birds, in which some helminths

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develop into adult stages, are important in that they can disseminate parasite eggs over long distances, making it difficult to control the spread of infections between water bodies in different catchments (Saayman *et al.* 1991). In wetland systems such as the Rietvlei Dam locality, there is a high diversity of aquatic birds, both resident species (e.g. ducks) and migratory species (e.g. cormorants) (M. Barson, personal observation 2003). While there is much need to understand the interactions between fish and birds in the transmission of helminth infections, only a few parasitological studies on piscivorous birds in Africa are documented (Beverly-Burton 1963; Ukoli 1968; Saayman *et al.* 1991; Mokgalong 1996; Barson 2004; Barson & Marshall 2004).

The sharptooth catfish, *Clarias gariepinus*, (Burchell, 1822) investigated in this study, is a widely distributed food fish in Africa (Safriel & Bruton 1984; Skelton 2001) and is one of the best species being targeted for aquaculture and biological research.

This study was carried out as a survey of the internal parasites that are found in *C. gariepinus* from the Rietvlei Dam, which can be used in the fish health assessment index that has been developed for South African fish by Avenant-Oldewage (2001). The objectives of this paper were to specifically identify and classify the internal platyhelminth parasites collected from the host fish (*Clarias gariepinus*) based on their morphological features, and to note their prevalence and mean intensity in the Rietvlei Dam.

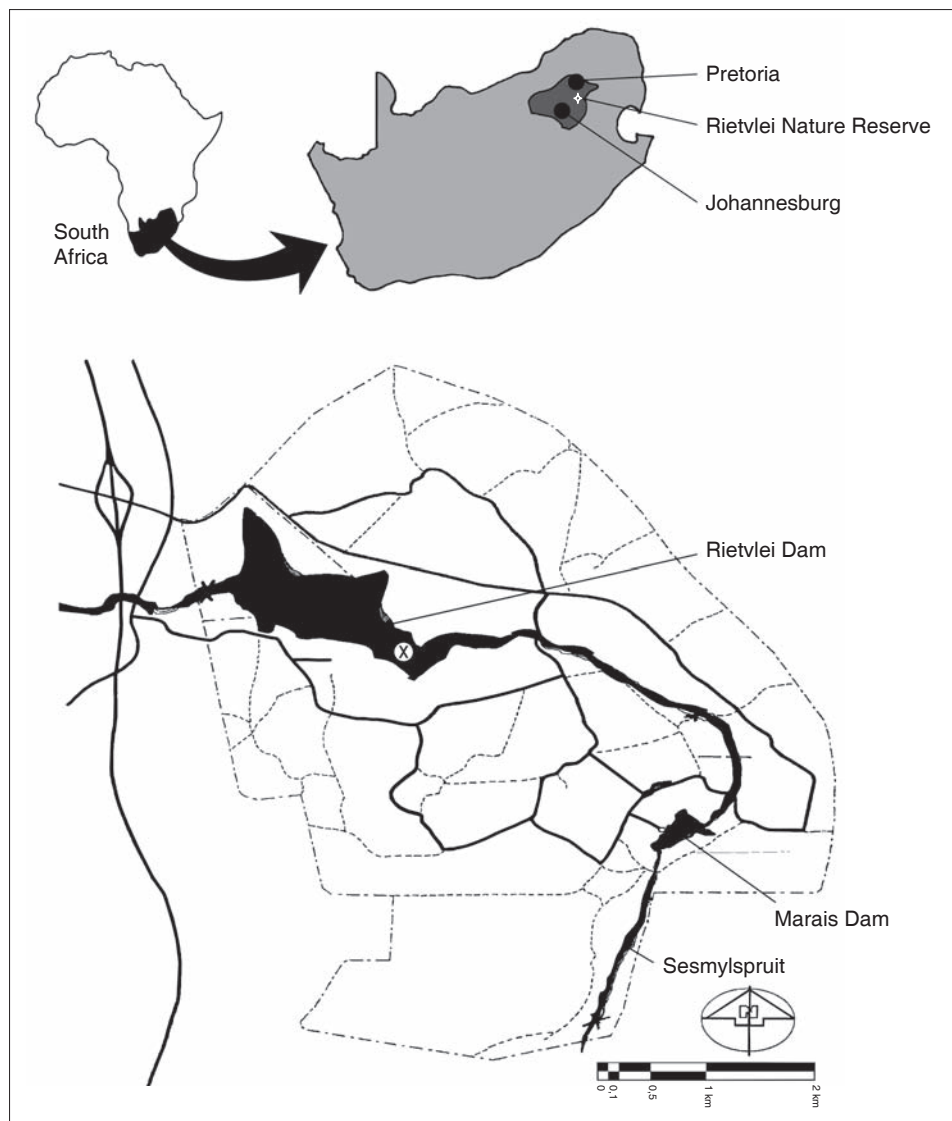


FIG. 1 The location of the Rietvlei Dam inside the Rietvlei Nature Reserve. X indicates point of sampling. Bar = 2 km

MATERIALS AND METHODS

Study area

The Rietvlei Nature Reserve (25°41'22" S, 26°37'48" E) lies between Pretoria and Johannesburg in the Gauteng Province, the economic hub of South Africa (Fig. 1). It is solely responsible for conservation of the Sesmylspruit catchment area, and the Rietvlei Dam (25°32'30" S, 28°16'46" E) currently supplies 27 % of Pretoria's water requirements (Wessels 1998). The dam is supported by the smaller Marais Dam, which lies approximately 4 km upstream, and the two are separated by a wetland (Fig. 1). Further upstream, just before the Sesmylspruit enters the reserve, effluent from a number of industries and a wastewater treatment plant is discharged into the stream, directly affecting the two dams.

Collection of fish and parasites

Fish were collected from the Rietvlei Dam using large mesh gill nets in May 2003. The gastrointestinal tract was dissected from the rectum to the oesophagus and parasites encountered were carefully detached from the stomach or intestinal mucosa. Portions of the skin between the lateral line and dorsal fin of each fish were peeled off with forceps and fillets of muscle tissue were cut and examined for encysted parasitic forms. The liver, spleen, gall bladder and kidneys of each fish were also examined for parasites or cysts.

Trematode cysts from the muscle were teased manually to release metacercariae, which were fixed in hot alcohol-formal-acetate (AFA) and preserved in 70 % ethyl alcohol. Cestodes from the intestinal tract were swirled in 0.1 % sodium chloride (saline) to relax them, fixed in hot AFA and preserved in 70 % ethyl alcohol.

Cestodes were stained with aqueous acetocarmine solution as described by Khalil (1991). Digenean trematode metacercariae were stained in Delafield's haematoxylin and counterstained in eosin. Standard microtechnique procedures were used to prepare transverse serial sections of cestodes, which were embedded in synthetic resin (Transmit LM) with a curing point of 70 °C. The specimens were sectioned with a rotary resin microtome (Anglio Scientific) at 5 µm thickness, and the sections were stained with AZAN, a trichrome stain.

The parasites were identified based on their morphology, and using drawings and light micrographs taken with a Zeiss Axioplan microscope. Scanning electron micrographs were taken with a JEOL 6100 scanning electron microscope (SEM). Drawings and

measurements were done with a Zeiss Standard 25 microscope equipped with a drawing tube. The following keys were consulted for identification: Yamaguti (1958) and Gibson, Jones & Bray (2002) for the trematodes, and Yamaguti (1959), Freze (1965), Schmidt (1986), Khalil, Jones & Bray (1994) and Rego (1994) for the cestodes. Parasite prevalence and mean intensities were calculated as defined by Margolis, Esch, Holmes, Kuris & Schad 1982.

Voucher specimens were deposited in the zoological collection of the University of Johannesburg (formerly Rand Afrikaans University), South Africa.

RESULTS AND DISCUSSION

The platyhelminth endoparasites found in *C. gariepinus* include two adult cestode species, *Polyonchobothrium clarias* and *Proteocephalus glanduliger*, and digenean metacercariae of the genus *Ornithodiplostomum* (Table 1).

Cestoda

Polyonchobothrium clarias (Woodland, 1925), (Fig. 2 and 5)

Scolex rectangular with a flat to slightly raised rostellum armed with a crown of 26–30 hooks (mean 28; $n = 6$). Rostellum divided into two semicircles each bearing 13–15 hooks. Hooks at the end of each semicircle smaller than the others. Two longitudinally elongated bothria in line with the gaps between the crowns of hooks. Immature proglottids of strobila not completely segmented. Some mature segments apparently fused as shown by SEM (Fig. 5E). Testes medullary; uterus anterior to ovary, highly folded and occupying the greater portion of gravid proglottids. Vitellaria cortical. Eggs unoperculate and embryonated. Measurements of structures are given in Table 2.

Polyonchobothrium clarias is widely distributed in siluroid fishes from African freshwater fishes, having been recorded from Nigeria in the North African catfish *Clarias lazera* (= *C. gariepinus*) Cuvier & Valenciennes, 1840 (Aderounmu & Adeniyi 1972). It was also reported in the Bagrid catfish *Chrysichthys thonneri* Steindachner, 1912 from Gabon, the mudfish *Clarias anguillaris* (Linnaeus, 1758) and *Heterobranchius bidorsalis* Geoffroy Saint-Hilaire, 1809 from Senegal (Khalil 1973), and in *C. anguillaris* from Egypt (Amin 1978). In southern Africa, *P. clarias* was first observed and recorded by Mashego (1977) from *C. gariepinus* in seven dams in the Lebowa region, Limpopo Province, South Africa. The only

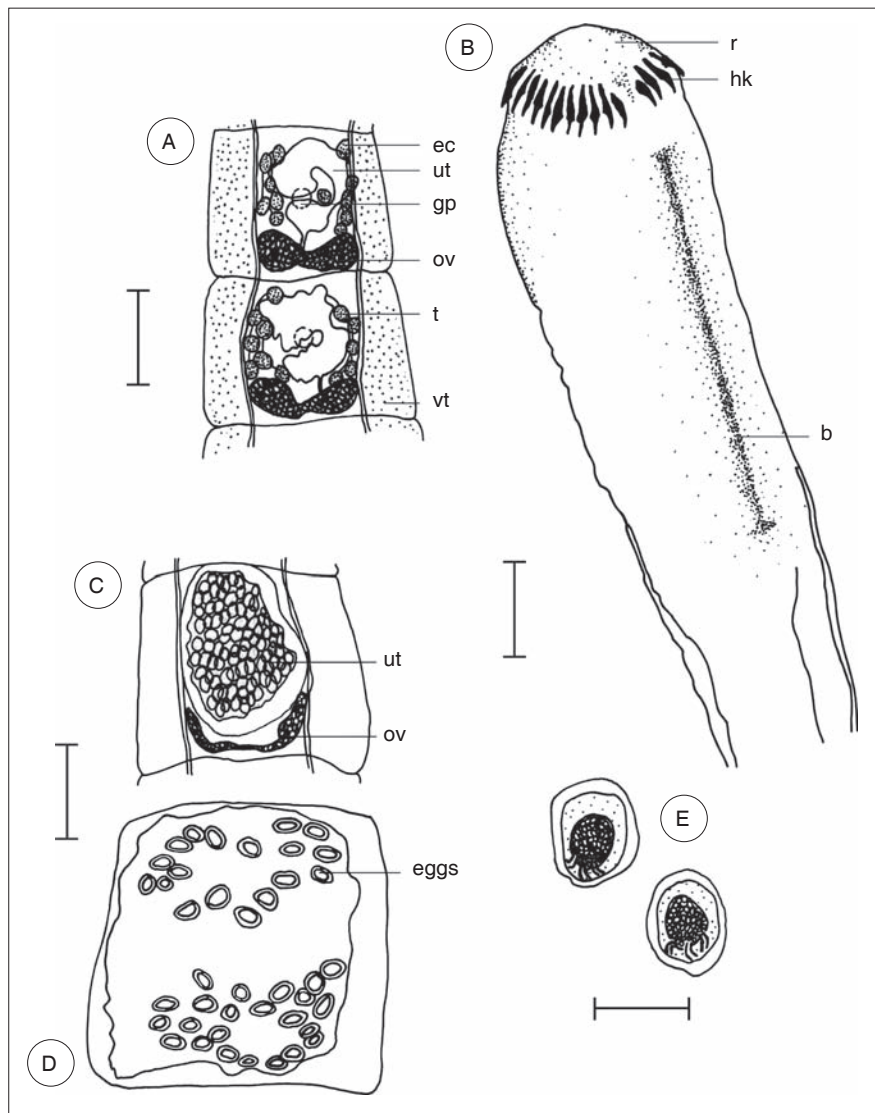


FIG. 2 *Polyonchobothrium clarias* (A) mature proglottid, (B) scolex, (C), (D) gravid proglottids (scale bar = 200 μ m), (E) eggs (scale bar = 50 μ m), b = bothrium, ec = excretory canal, gp = genital pore, hk = hook, ov = ovary, r = rostellum, t = testis, ut = uterus, vt = vitellaria

other record of the parasite was from the Middle Letaba Dam (Saayman *et al.* 1991), also in the Limpopo Province. Its high prevalence in the Rietvlei system, as well as in the Vaal Dam (M. Barson & A. Avenant-Oldewage, unpublished data 2003), seems to suggest that the cestode is widely distributed in *C. gariepinus* in the country.

In Zimbabwe, Chishawa (1992) and Douëllou (1992) recorded an intestinal cestode from the brown squeaker, *Synodontis zambezensis* Peters, 1852, and *C. gariepinus* from Lake Kariba. Although they mistook it for larval *P. clarias*, it was apparently an adult with 38 hooks on its apical crown. Larvae only occur in copepod intermediate hosts.

The low intensity of *P. clarias* in hosts from the Rietvlei Dam (up to 11 worms per fish) would be expected to inflict minimal damage on the host tissue (Paperna 1996), whereas high parasitic loads in the gall bladder have been shown to cause granulomatous nodules and fibrosis (Wabuke-Bunoti 1980). Mashego (1977) recorded intensities of up to 200 in *C. gariepinus* from Lebowa. In Nigeria, Aderounmu & Adeniyi (1972) reported a heavy infection of 123 worms per host in *C. gariepinus*, causing nodules at the point of attachment. Barson & Avenant-Oldewage (unpublished data 2003) recorded more than 100 individuals infecting one specimen of *C. gariepinus* in the Vaal Dam. The fact that the tapeworms physically

TABLE 1 Prevalence and intensity of endohelminths of *C. gariepinus* from Rietvlei Dam

Parasite species	Location in host	n*	Prevalence (%)	Intensity	Mean intensity
<i>Polyonchobothrium clarias</i>	Stomach mucosa, anterior intestine	7	71.4	1–11	5.0
<i>Proteocephalus glanduliger</i>	Anterior and mid- intestine	7	14.0	1–2	2.0
<i>Ornithodiplostomum</i> sp. metacercariae	Mid-dorsal muscles between dorsal fin and lateral line	7	14.0	0–140	140.0

*n = sample size

TABLE 2 *Polyonchobothrium clarias* measurements (in µm, unless otherwise stated)

n = 6	Range	Mean
Length (mm)	23–43	29.8
Maximum width	280–701	506
Number of proglottids	133–230	183.0
Scolex size (L*W) ¹	420–680*160–230	527*198
Number of hooks in apical rostellum	26–30	28
Mean size of hooks	35–57	44
Mean size of immature proglottids (L*W)	80–180*146–170	150*151
Mean size of mature proglottids (L*W)	122–179*129–222	151*186
Mean size of gravid proglottids (L*W)	240–378*280–710	330*506
Mean egg size	28–42.5*21–38	32.8*27.0

¹ L*W : L = length, W = widthTABLE 3 *Proteocephalus glanduliger* measurements (in mm, unless otherwise stated)

Measurement	1	2	Mean
Strobila length	7.04	13.0	10.02
Strobila maximum width	0.52	1.08	0.80
No. of proglottids	58	45	51.5
Scolex (L*W) ¹	1.08*0.82	1.28*0.92	1.20*0.88
Size of apical organ (L*W)	0.36*0.28	–	0.36*0.28
Size of suckers (mean L*W)	0.36*0.32	0.48*0.40	0.44*0.36
Size of mature proglottid (L*W)	–	–	–
Size of gravid proglottid (L*W)	0.13*0.45	–	–
Size of cirrus sac (L*W)	0.14*0.03	–	–
Size of testes	–	–	–

¹ L*W : L = length, W = widths

resisted detachment from the gut mucosa suggests that the suction created by the bothria and the clasp of the apical hooks could cause severe pathological effects in heavy infections.

Proteocephalus glanduliger (Janicki, 1928)
Fuhrmann, 1933 (Fig. 3 and 5F)

Scolex unarmed, with four cup-shaped suckers arranged symmetrically around a protrusible rostellum; neck region not differentiated into well-formed proglottids. Glandular organ present in one specimen but not apparent in the other, approximately similar in size to suckers. All proglottids broader

than long. Genital pores lateral and alternating. Testes medullary as shown in resin sections (Fig. 3E and 5F). Specimens much larger than those described by Freze (1965) and Mashego (2001) (Table 3).

Despite the abundance and diversity of proteocephalid cestodes in African freshwater fish (Khalil & Polling 1997), only one species, *Proteocephalus glanduliger* has been recorded in South Africa from *C. gariepinus* (Mashego 1977, 2001; Van As & Basson 1984; Saayman *et al.* 1991). Only two specimens of this cestode were recovered from one of the seven catfish from the Rietvlei Dam. Mashego

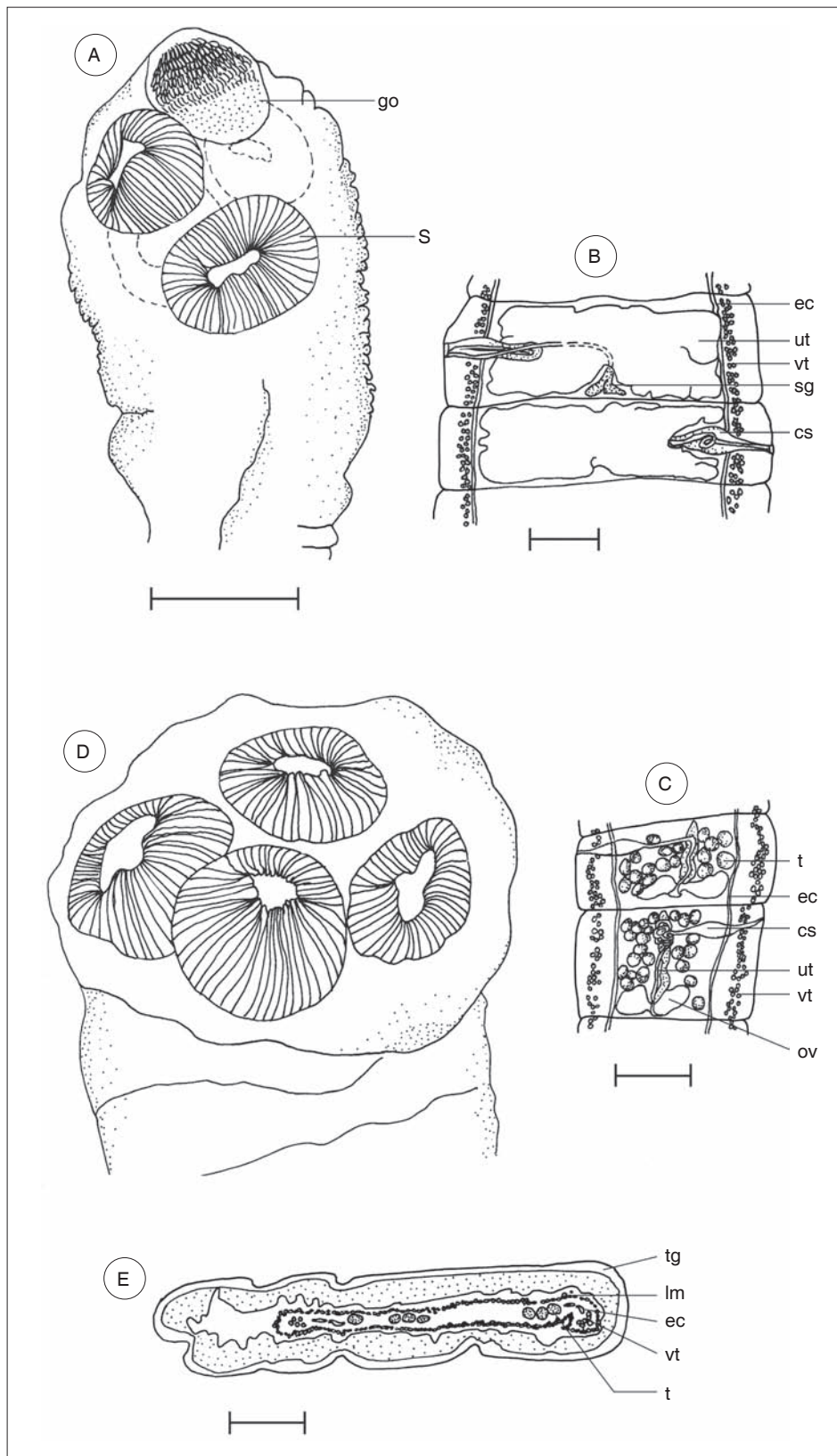


FIG. 3 *Proteocephalus glanduliger* (A, D) scolex (scale bar = 400 µm), (B) gravid proglottid, (C) mature proglottid, (E) transverse section (scale bars = 200 µm), ec = excretory canal, go = glandular organ, cs = cirrus sac, lm = longitudinal muscles, s = sucker, sg = shell gland, t = testis, tg = tegument, ut = uterus, vt = vitellaria

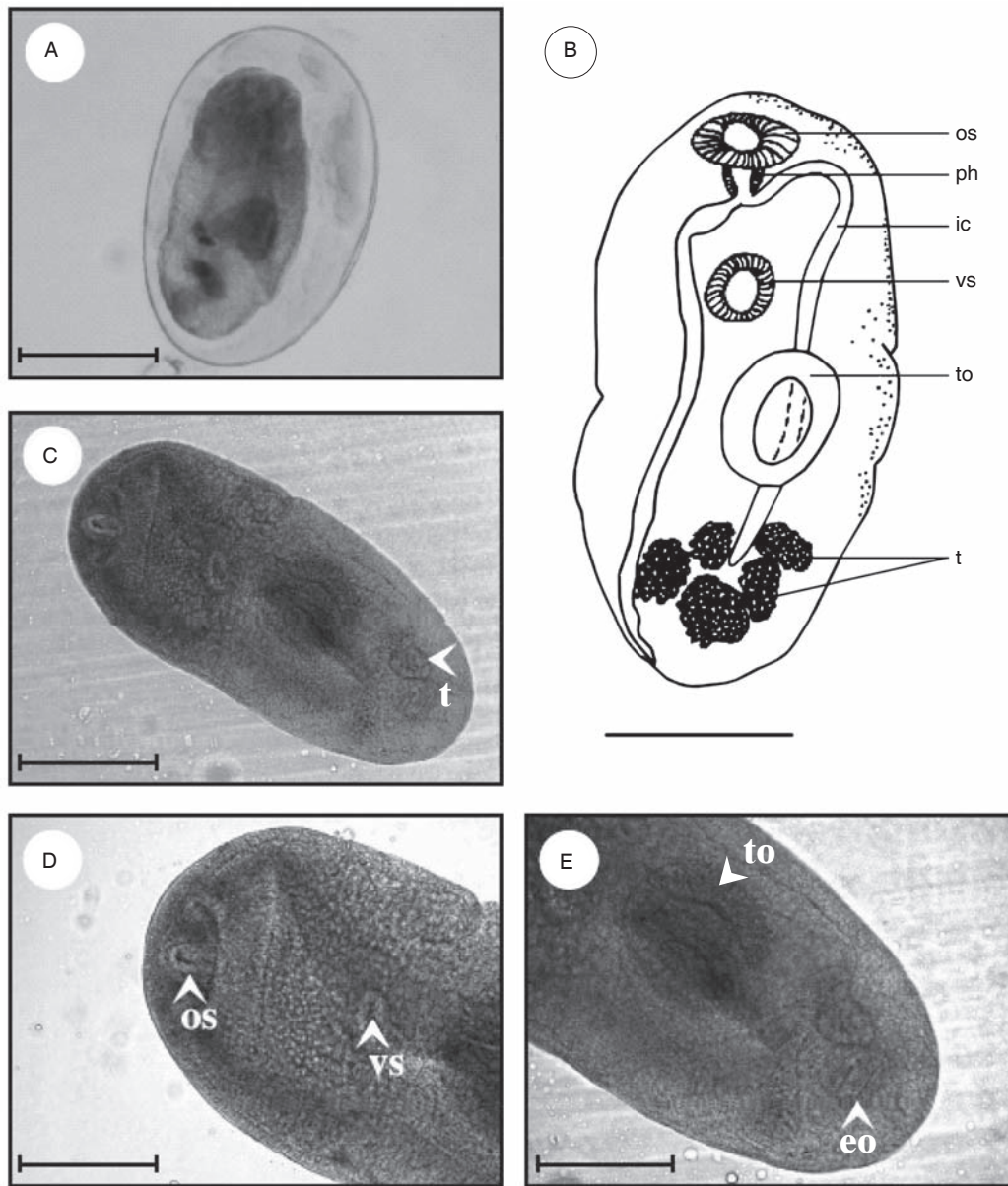


FIG. 4 *Ornithodiplostomum* sp. Metacercariae (A) encysted (scale bar = 400 µm), (B) drawing showing main features (scale bar = 350 µm), (C) whole worm (scale bar = 250 µm), (D) anterior end showing oral sucker and pharynx (left arrow) and ventral sucker (right arrow), (E) posterior end showing rudimentary genital/excretory opening (scale bars = 150 µm), eo = excretory opening, ic = intestinal caecum, os = oral sucker, ph = pharynx, t = testes, to = tribocytic organ, vs = ventral sucker

(1977) recorded it in 11 of 337 hosts and Mashego (2001) in 11 of 115 hosts, with a mean intensity of seven parasites per fish. While the present specimens were much larger (Table 3) than those described by Janicki (Freze 1965) and Mashego (2001) from *C. anguillaris* and *C. gariepinus*, respectively, the glandular organ at the apex of the scolex was much smaller and almost equal in size to the suckers (Fig 3A). Serial sectioning of the present material confirmed the medullary positioning of the testis and

vitellaria (Fig. 3E and 5F), a characteristic of the genus *Proteocephalus* (Freze 1965; Schmidt 1986; Rego 1994).

The histology of the worm as observed from the sections resembles the description of *P. glanduliger* by Mashego (2001) in *C. gariepinus* from four South African dams. Proteocephalid cestodes have been found in *C. gariepinus* from the neighbouring Zimbabwe (Barson 2004; Chishawa 1991; Douëllou 1992) but as they were not specifically identified, compari-

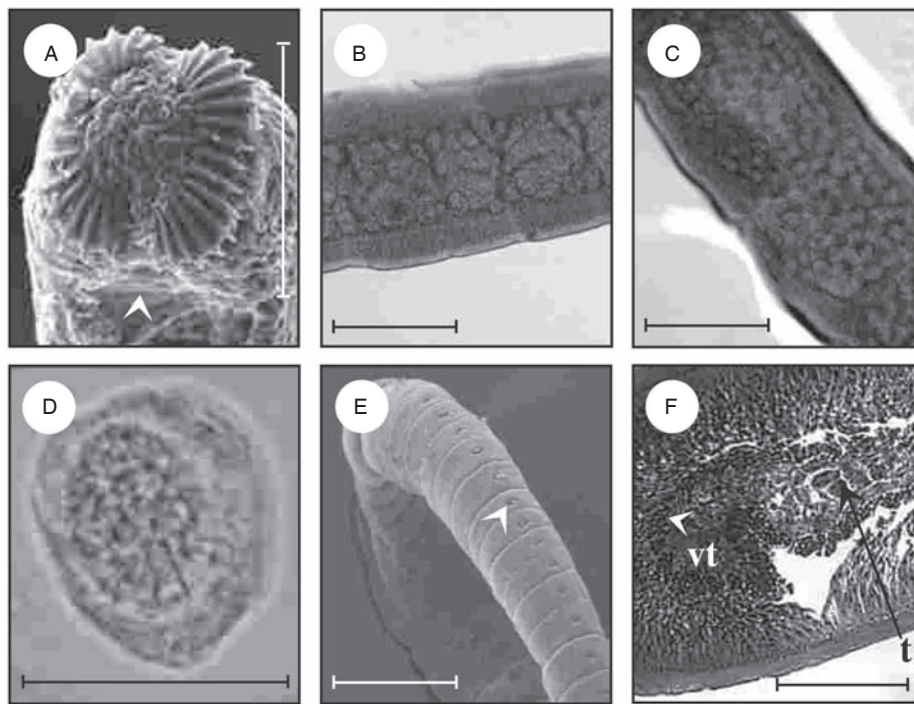


FIG. 5 *Polyonchobothrium clarias* (photographs) (A) scolex (SEM) showing the arrangement of hooks around the rostellum (scale bar = 200 µm); (B) mature proglottid (LM); (C) gravid proglottid (LM) (scale bars = 400 µm); (D) embryonated egg (LM) (scale bar = 50 µm); (E) strobila (SEM) showing genital openings (go) and fused segment (fs) on the ventral surface; (F) light micrographs of AZAN stained transverse section through early mature proglottid (scale bar = 60 µm), t = testes, vt = vitellaria

TABLE 4 *Ornithodiplostomum* sp. measurements (µm)

<i>n</i> = 3	Range	Mean
Length	544–656	602
Maximum width	320–356	336
Size of oral sucker (L*W) ¹	97–115*60–75	108*66.5
Size of pharynx (L*W)	48–55*45–52	50*49.5
Size of ventral sucker (L*W)	75–82*64–70	80–68
Size of tribocytic organ (L*W)	138–150*115–124	142.3*119.5
Size of anterior testes	44–60*31.5–42.5	53.3*40
Size of posterior testes	62–73.3*50–58	67*52.5

¹ L*W : L = length, W = width

son with their South African counterparts cannot be made. In African fish species other than the Clariidae, many proteocephalid species have been described from the Sudan (Khalil 1963, 1973; Jones 1980), the Democratic Republic of Congo, Egypt, Liberia and Senegal (Khalil 1973; Khalil & Polling 1997).

Trematoda

Ornithodiplostomum sp. metacercariae (Fig. 4)

Metacercariae coiled and encysted in tough white cysts formed by the host in the dorso-lateral muscles and a thin transparent wall, secreted by the para-

site (Fig. 4A), thus difficult to identify. The few successfully excysted metacercariae were identified as belonging to the family Diplostomidae and closely resemble *Ornithodiplostomum* sp. Body indistinctly bipartite with no pseudosuckers (as in other diplostomes), but with a large circular holdfast organ (tribocytic organ). Developing, immature testes apparent; Subterminal eversible genital apparatus also clear from photomicrographs (Fig. 4C–E).

The present specimens match the description of adult *Ornithodiplostomum* sp. that occurs in the Podicipedidae (grebes) and Anatidae (ducks) from the

Holarctic region and Africa (Niewiadomska 2002). This is the first record of this genus in South Africa, neither appearing in the latest checklist (Khalil & Polling 1997) nor in Canaris & Gardner's (1967) checklist of helminths of African vertebrates. However, related genera such as *Diplostomum*, *Neodiplostomum* and *Postodiplostomum* are quite common in southern Africa (Mashego 1977; Prudhoe & Hussey 1977; Khalil & Polling 1997). The prevalence of this parasite was low (14%) but the intensity of infestation was very high (Table 1).

Only three metacercariae were successfully recovered and measured (Table 4) from the cysts. Some were indistinct and difficult to identify (e.g. Fig 4A), thus it cannot be concluded that all the metacercariae found were *Ornithodiplostomum* sp., or even exclusively diplostomid. Experimental infection of suitable bird hosts with these metacercariae is the only way to obtain adult parasites which can then identified to species level.

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