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New records of digenean parasites of *Clarias gariepinus* (Pisces: Clariidae) from the Okavango Delta, Botswana, with description of *Thaparotrema botswanensis* sp. n. (Plathelminthes: Trematoda)

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ABSTRACT

Forty-two specimens of African sharptooth catfish *Clarias gariepinus* (Clariidae) were collected from a number of localities in the Okavango Delta in Botswana and examined for digenean parasites. The morphology of these parasites was studied using light and scanning electron microscopy. Six digenean species, four represented by adults and two by metacercariae, belonging to five different genera were collected from various organs within the fish. *Clinostomoides brieni* is described from the branchial chamber; a *Neodiplostomum* type metacercariae from the musculature; *Phyllodistomum bavuri* and *Phyllodistomum vanderwaali* from the urinary bladder; *Glossidium pedatum* from the intestine and one new species, *Thaparotrema botswanensis* sp. n. from the gall bladder. These are all new geographical records for southern Africa.

KEY WORDS: Digenea, flukes, Clariidae, *Clarias gariepinus*, African sharptooth catfish, new species, Afrotropical, Okavango.

INTRODUCTION

Digeneans were collected from a number of fish hosts, including the African sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), during fish parasitological studies carried out in the Okavango Delta, Botswana. According to Safriel and Bruton (1984) and Skelton (2001), *C. gariepinus* is a widely distributed food fish in Africa and is a popular species being targeted for aquaculture and biological research. When compared to other African fish, *C. gariepinus* is known to possess one of the richest parasite fauna, which can be attributed to its diet and the number of predator species feeding on them. According to Khalil (2003), Zhokhov (2010) and Zhokhov *et al.* (2010), 19 species of monogeneans, nine digeneans and one species of acanthocephalan, together with 16 various larval forms have been found infecting this catfish; although the number of named species will undoubtedly grow in the future (Chibwana *et al.* 2013).

Information concerning digenean parasites of freshwater fish in southern Africa and parts of Africa is scant and far from complete. The life history of trematodes, which (at the adult stage) infect African fish, has so far not been studied in detail and their first molluscan and other intermediate hosts remain unknown as yet. When consulting literature, most of the studies carried out in South Africa have been surveys on helminth parasites in general, not concentrating on a specific group as such.

Khalil and Polling (1997) provided a checklist of helminth parasites of African freshwater fish, while Canaris and Gardner (2003) compiled a guide to helminth species described from African vertebrates. In both publications no information is given on digenean parasites from the Okavango Delta.

This paper provides additional information on the morphology, distribution and host records for known and new digenean species from *C. gariepinus*.

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MATERIAL AND METHODS

Forty-two specimens of African sharptooth catfish *Clarias gariepinus* were collected from a number of localities in the Okavango Delta by gill nets, rod and line. Fish were taken to the field laboratory and kept in aerated aquariums until examination. Fish species were identified using Skelton' (2001) guide. The fish were anesthetized using MS222 and killed, the internal organs were removed, dissected and examined under a Nikon SMZ 800 dissection microscope for the presence of digenetic trematodes.

All digeneans were removed and fixed in warm 70% ethanol. Whole-mounts were stained using Van Cleave's haematoxylin with additional drops of Ehrlich's haematoxylin. The stain was then made alkaline at 70% ethanol with lithium carbonate and butylamine. Specimens were then dehydrated in increasing percentages of ethanol and cleared using cedarwood oil and mounted in Canada Balsam. These were then drawn with the aid of a microscope drawing tube.

Some specimens were prepared for scanning electron microscopy (SEM) using standard techniques for digenetic trematodes, then examined at different accelerating voltages (5–10 kV) and working distances, using a Leica Stereoscan 420 SEM. Reference material is kept in the Department of Zoology and Entomology Parasite Collection, University of the Free State, Bloemfontein. Type material is stored in the parasite collection of the National Museum, Bloemfontein (NMBP), South Africa and a voucher specimen is deposited in the Natural History Museum (BMNH), London, United Kingdom. Measurements are reported in millimetres as the range, followed by the arithmetic mean and standard deviation in parentheses.

TAXONOMY

Family Clinostomidae Lühe, 1901

Genus *Clinostomoides* Dollfus, 1950

Clinostomoides brieni Dollfus, 1950

Figs 1A, 2A–E; Table 1

Clinostomoides brieni: Dollfus 1950: 77, fig. 54; Manter & Pritchard 1969: 54–56, figs 1–4; Fischthal & Thomas 1970: 74–76, fig. 1.

Clinostomoides ophicephali (Tubangui & Masilungan, 1944): Agarwal 1959: 17; Manter & Pritchard 1969: 56.

Clinostomoides dollfusi Agarwal, 1959: 13–16, fig. 1; Manter & Pritchard 1969: 56.

Redescription:

Metacercaria.

Excysted metacercariae from gill chamber or branchial region move around freely (Fig. 2A). Body elongate, narrow, parallel lateral margins, extremities round with maximum width at acetabulum level or just pre-acetabular (Figs 1A, 2B). Cuticular spines fingerlike projections, pointed, occurring from level of posterior part of oral sucker or prepharynx to posterior extremity, unspined anteriorly (Fig. 2C). Oral sucker small. Acetabulum larger than oral sucker. Prepharynx short; pharynx muscular, 0.18 in diameter; no apparent oesophagus. Caecal bifurcation pre-acetabular closer to oral sucker than to acetabulum, forming caecal shoulders before beginning descent from acetabular level; posteriorly caeca thick-walled, with short diverticula on median and lateral sides terminating just above excretory bladder (Fig. 1A). Testes transversely elongated, in posterior ⅙–⅐ body length; anterior

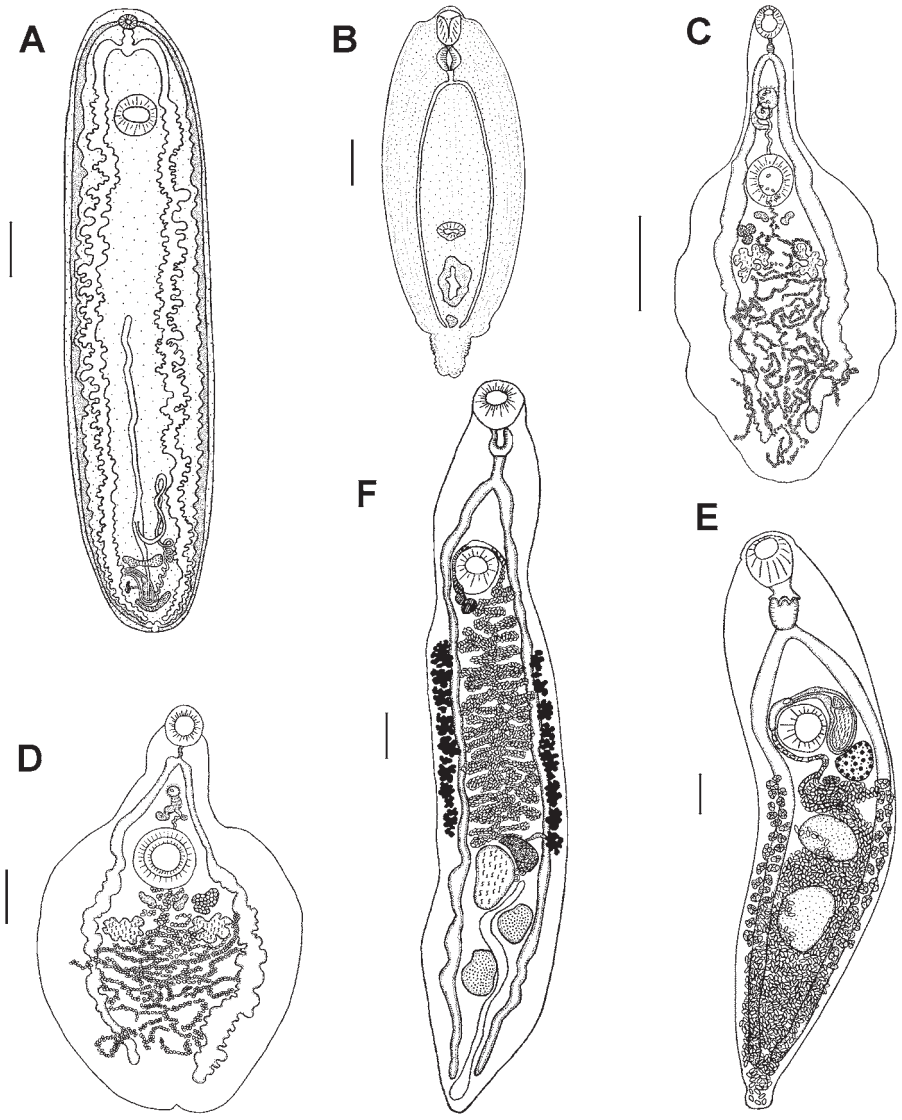

Fig. 1. Light microscope projection drawings of digeneans from *Clarias gariepinus* in the Okavango Delta: (A) *Clinostomoides brieni* Dollfus, 1950; (B) *Neodiplostomum* Railliet, 1919 type 1 metacercaria; (C) *Phyllodistomum bavuri* Boomker, 1984; (D) *Phyllodistomum vanderwaali* Prudhoe & Hussey, 1977; (E) *Glossidium pedatum* Looss, 1899; (F) *Thaparotrema botswanensis* sp. n. Scale bars: A, C – 1 mm; D – 0.3 mm; B, E – 0.1 mm; F – 0.2 mm.

testis crescent shaped extending across intercaecal space; posterior testis crescent to Y-shaped sometimes less wide, mostly dextral (Fig. 2D). Cirrus sac thick-walled, comma-shaped, inter-testicular, dextral (Fig. 1A). Genital pore just submedian to right, immediately anterior to posterior testis. Ovary small, intercaecal, longitudinally elongate. Uterus extending anteriorly on left side; uterine sac median, without lateral sacculations in posterior half of body, well separated from acetabulum, receiving uterus near middle;

vitellarium undeveloped; no eggs (Fig. 2D). Excretory pore dorso-terminal (Fig. 2E); vesicle small, Y to V-shaped, entirely postcaecal, thick-walled, arms extending anteriorly on each side of body in extra-caecal position, uniting at anterior margin of oral sucker (Fig. 1A).

Material examined: BOTSWANA: 24 metacercariae, Okavango Delta, 5 Shakawe mainstream (18°26'05.0"S 21°54'23.0"E); 9 Kalatog channel (18°25'08.2"S 21°54'05.0"E); 5 Nxamaseri backwaters (18°37'34.9"S 22°06'24.4"E); 3 Mokoro lagoon (18°29'10.1"S 21°55'10.1"E); 2 Samochima lagoon (18°25'44.0"S 21°54'01.0"E).

Site of infection: Gill chamber/branchial region.

Prevalence of infection: 26.1%.

Remarks: *Clinostomoides brieni* was described by Dollfus (1950) from the Goliath heron, *Ardea goliath* Cretzschmar, 1827 from the Congo. Agarwal (1959) described *C. dollfusi* occurring in siluroid fishes from India; however, this species was later synonymised with *C. brieni* by Manter and Pritchard (1969). They briefly redescribed the metacercaria from *Clarias* sp. from Rwanda and synonymised *C. ophiocephali* with *C. dollfusi*. They also suggested that *C. brieni* is the only species in the genus with its synonyms being *C. dollfusi* and *C. ophicephali*.

The material from the current survey is similar to *C. brieni* in having the testes, ovary and uteroduct in the same position, the uterine sac being removed far from the acetabulum and the latter being larger than the oral sucker. Specimens from this study differ from *C. brieni* described by Fischthal and Thomas (1970) in that the metacercariae are larger, oral and ventral suckers are slightly larger and the testes differ in shape. Considering the above mentioned morphological differences and similarities, specimens of the present material are assigned to *C. brieni*. This is the first record of *C. brieni* in the Okavango Delta, and the first documented record from Botswana.

Family Diplostomidae Poirier, 1886
Genus *Neodiplostomum* Railliet, 1919
Neodiplostomum type 1 metacercaria

Figs 1B, 2F–H; Table 1

Neodiplostomum type metacercaria: Prudhoe & Hussey 1977: 136–139, fig. 12.

Description:

Metacercaria.

Cysts white. Body divided into a relatively large, dorsoventrally flattened forebody, much smaller hindbody with maximum width occurring pre-acetabular (Fig. 1B). Oral sucker subterminal, longitudinally oval (Fig. 2G). Acetabulum round to transversely oval. Prepharynx absent. Pharynx longitudinally elongate, opens into oesophagus (Fig. 2G). Caecal bifurcation pre-acetabular, extending to level of gonads. Caeca only visible in live specimens. Holdfast organ elliptical, longitudinally elongate, situated post-acetabular (Fig. 2H). Single reproductive anlagen round to longitudinally elongate, lying posterior to holdfast organ in forebody (Fig. 1B).

Material examined: BOTSWANA: 36 metacercariae, Okavango Delta, 18 Shakawe mainstream (18°26'05.0"S 21°54'23.0"E); 18 Xaro mainstream (18°25'19.7"S 21°56'19.9"E).

Site of infection: Cysts distributed throughout musculature (Fig. 2F).

Prevalence of infection: 4.7%.

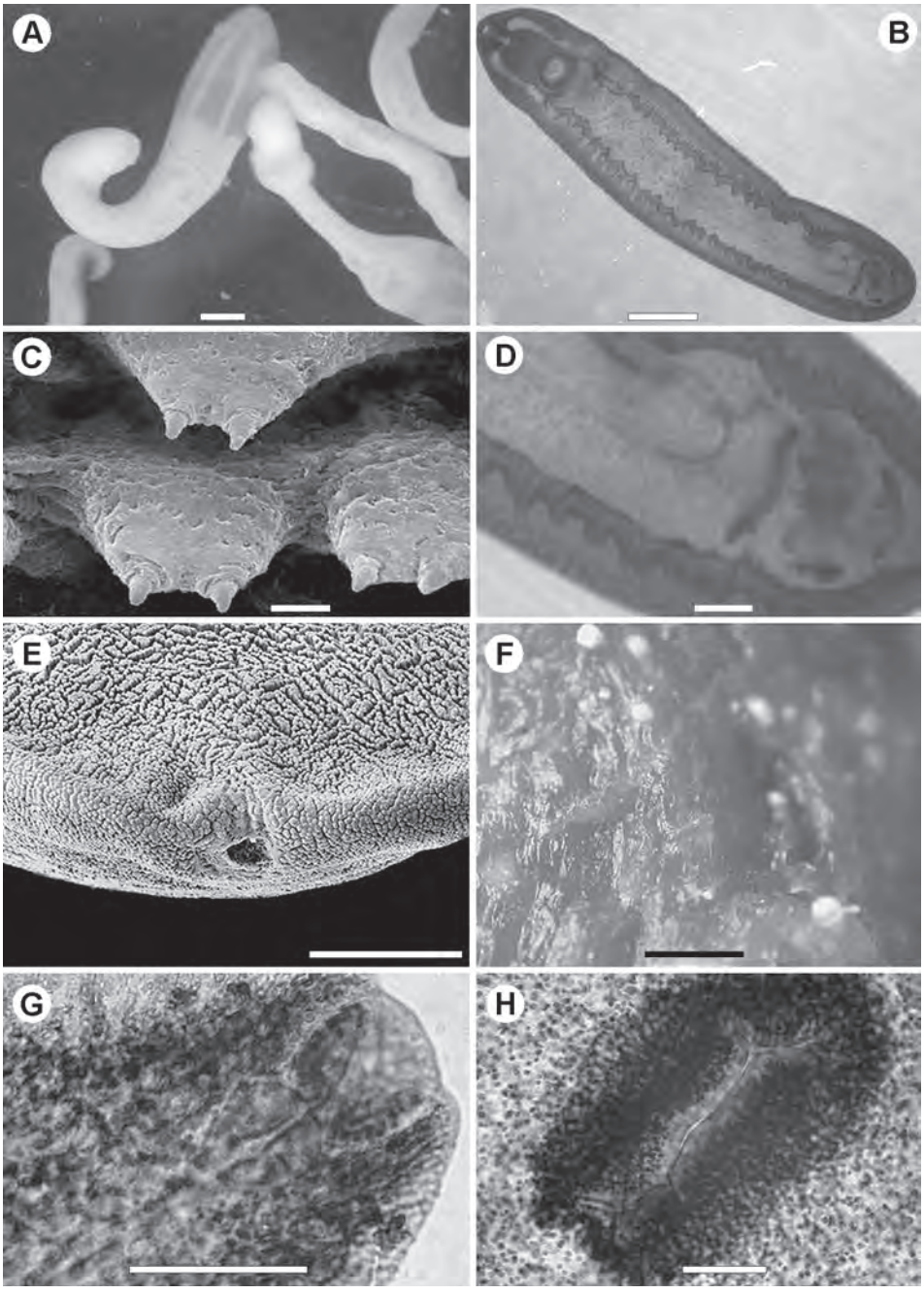

Fig. 2. Light and scanning electron micrographs of *Clinostomoides brieni* Dollfus, 1950 (A–E) and *Neodiplostomum* type 1 metacercaria (F–H) from *Clarias gariepinus* in the Okavango Delta: (A) excysted metacercariae; (B) whole mount; (C) spines on body surface; (D) reproductive system; (E) excretory pore; (F) metacercariae encysted in muscle; (G) oral sucker and pharynx; (H) holdfast organ. Scale bars: A, B – 1 mm; C – 2 μm, D, F – 0.1 mm; E – 20 μm; G – 0.05 mm; H – 0.01 mm.

Remarks: Prudhoe and Hussey (1977) described three diplostomid metacercariae from *Clarias gariepinus* in South Africa. The first *Diplostomum* type I was found in the eyes and does not conform to the species in the present study. The second was *Diplostomum* type II found in the mesenteries. The species from the current survey is similar to it in having the body divided into a fore- and hindbody and possessing a pharynx just posterior to the oral sucker. It differs from *Diplostomum* type II in that the fore and hind bodies are not as well developed, the gonads are a small mass and not horseshoe-shaped, and the pseudosuckers are not prominent.

The third type was identified as a *Neodiplostomum* type. The species on hand is similar to it in possessing a fore- and hindbody and has a transversely oval acetabulum. It differs, however, in that the gonads are not situated in the hindbody and the holdfast organ is not situated immediately posterior to the end of the forebody. Bisseru (1956) described three *Neodiplostomum* species from birds of prey from Africa as well as from the Nile crocodile (Bisseru 1957), but these were all adult specimens and could not be compared to the larval forms. According to Niewiadomska (2002), *Neodiplostomum* type metacercaria cannot be assigned to adult genera with certainty without knowing their complete life cycle; metacercariae are then referred to as types. This metacercaria is thus assigned to *Neodiplostomum* type 1. This is the first record of a *Neodiplostomum* metacercaria in the Okavango Delta, and the first documented record from Botswana.

Family Gorgoderidae Looss, 1901
Genus *Phyllodistomum* Braun, 1899

Eight species of *Phyllodistomum* have been recorded from African fishes (Khalil & Polling 1997; Zhokhov 2010): *Ph. linguale* Odhner, 1902 from *Gymnarchus niloticus* Cuvier, 1829, *Ph. spatula* (Odhner, 1902) from *Bagrus bajad* (Forskål, 1775) and *Bagrus docmak* (Forskål, 1775), and *Ph. spatulaeforme* (Odhner, 1902) from *Malapterurus electricus* (Gmelin, 1789) from the Sudan; *Ph. tana* Zhokhov, 2010 from *C. gariepinus* from Ethiopia; *Ph. ghanense* Thomas, 1958 from *Ctenopoma kingsleyae* Günther, 1896 and *Caecomastacembelus nigromarginatus* (Boulenger, 1898), and *Ph. symmetrorchis* Thomas, 1958 from *Auchenoglanis occidentalis* (Valenciennes, 1840) from Ghana; and *Ph. vanderwaali* Prudhoe & Hussey, 1977 and *Ph. bavuri* Boomker, 1984 from *C. gariepinus* from South Africa. The genus has also been recorded from several fish species in the Nile River in Egypt (Mansour *et al*. 2003).

Phyllodistomum bavuri Boomker, 1984
Figs 1C, 3A–C; Table 1

Phyllodistomum bavuri: Boomker 1984: 129–130, fig. 1.

Redescription:

Body aspinose, ampullate in shape (Fig. 1C). Anterior part subcylindrical, amounts to ⅓ total body length. Posterior part of body thin, flattened dorsoventally, various internal structures microscopically visible without staining (Fig. 3A). Oral sucker round, situated subventrally. Acetabulum circular, larger than oral sucker, situated just posterior to junction of anterior end of hind body (Fig. 1C). Pharynx absent. Oesophagus short. Caecal bifurcation nearer to oral sucker than to acetabulum. Intestinal caeca terminate almost at end of posterior body margin. Excretory vesicle, excretory pore indistinct.

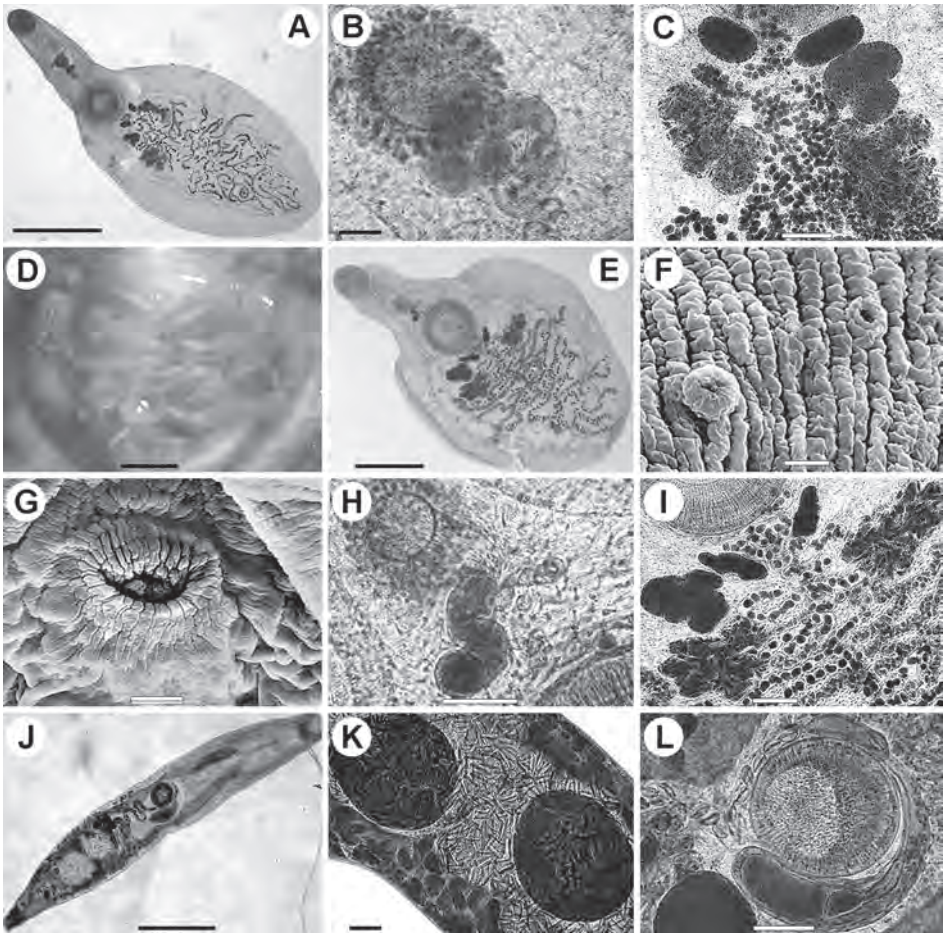

Fig. 3. Light and scanning electron micrographs of *Phyllodistomum bavuri* Boomker, 1984 (A–C), *Phyllodistomum vanderwaali* Prudhoe & Hussey, 1977 (D–I) and *Glossidium pedatum* Looss, 1899 (J–L) collected from *Clarias gariepinus* in the Okavango Delta: (A) whole mount; (B) genital pore; (C) vitellaria, ovary and testes; (D) adults attached to urinary bladder; (E) whole mount; (F) body surface with papillae; (G) genital pore; (H) genital pore and pars prostatica; (I) vitellaria, ovary, uterus and testes; (J) whole mount; (K) anterior and posterior testes; (L) acetabulum and cirrus sac. Scale bars: A – 1 mm; B–D, H – 0.1 mm; E, I, K, L – 0.05 mm; J – 0.5 mm; F – 2 μm; G – 20 μm.

Genital pore lies in midline of body halfway between oral and ventral suckers beneath gut bifurcation, in some specimens slightly more towards oral sucker (Fig. 3B). Cirrus sac absent. Two fairly large testes, deeply and irregularly lobed lying in middle of body on either side of midline between intestinal caeca. In some specimens testes slightly displaced one in front of other but mostly opposed (Fig. 3C). Ovary trilobed, sometimes round; situated to right of body midline in three specimens, to left in five specimens, between vitellarium and testes. Vitellarium compact, roughly round to lobed, opposed, lies just posterior to rim of acetabulum, anterior to ovary. Uterus consists of numerous loops, runs between testes and vitellarium to reach genital pore, loops rarely extend laterally beyond caeca (Fig. 3C). Uterine loops full of eggs. Eggs rounded to oval.

Material examined: BOTSWANA: 8 adult specimens, Okavango Delta, Shakawe mainstream (18°26'05.0"S 21°54'23.0"E).

Site of infection: Urinary bladder.

Prevalence of infection: 2.3 %.

Remarks: According to Boomker (1984) and Brooks and MacDonald (1986), species in the genus can mainly be distinguished from each other by the position of the ovary relative to the vitellarium, i.e. lying anterior, opposite or posterior to the vitellarium.

The species from the present study resembles *Ph. linguale* occurring in the urinary bladder of *G. niloticus* from Sudan, in having similar body size, the ovary being irregularly lobed, uterine coils that seldom cross the intestinal caeca laterally and the ovary lies posterior to the vitellarium. It differs in that the caeca does not terminate a distance from the posterior margin and the intestinal bifurcation being closer to the oral sucker in this species. The genital pore in *Ph. linguale* lies between the gut bifurcation and rim of the ventral sucker, while in our specimens it is halfway between the bifurcation and the ventral sucker.

The studied material also resembles *Ph. bavuri* described by Boomker (1984) from the urinary bladder of *C. gariepinus* from the Bangu River, Transvaal (presently Gauteng), in having the same body size and shape, the ovary being round to weakly trilobed and the vitellarium also being weakly lobed. It differs in that the gut bifurcation occurs near to the oral sucker and not halfway between the oral and ventral suckers. It also differs in that the intestinal caeca terminate close to the posterior margin of the body. The general morphology and measurements of specimens from the present material correspond to a certain degree with those in *Ph. bavuri* as described by Boomker (1984). However, in contrast to data by Boomker (1984), the present specimens show more variation in ovary shape, and vitellarium being slightly more lobed and irregularly shaped.

The above mentioned morphological differences are, however, considered to be within the intraspecific variability of *Ph. bavuri* and therefore the specimens of the present material are assigned to this species. This is the first record of *Ph. bavuri* in the Okavango Delta, and the first documented record from Botswana.

Phyllodistomum vanderwaali Prudhoe & Hussey, 1977

Figs 1D, 3D–I; Table 1

Phyllodistomum vanderwaali: Prudhoe & Hussey 1977: 116–119, fig. 2; Boomker 1984: 131, fig. 7.

Redescription:

Body ampullaceous with subcylindrical forebody and leaf-like dorsoventrally flattened hindbody (Figs 1D, 3E). Forebody constitutes ¼ of total length, hindbody almost circular. Body surface unspined, papillae present on the lateral sides of body (Fig. 3F). Oral sucker terminal, almost round. Acetabulum circular, larger than oral sucker, situated at anterior level of hindbody (Figs 1D, 3F). Pharynx absent. Oesophagus short. Caecal bifurcation immediately posterior to oral sucker, terminating near posterior body margin. Excretory pore opens dorsally near posterior extremity of body. Genital pore opens halfway between gut bifurcation and acetabulum (Fig. 3G, H). No cirrus sac, terminal organs of male and female complexes lie free in parenchyma. Ejaculatory duct short, runs from genital pore to open into small pars prostatica, which is endowed with a large number of deeply staining gland-cells (Fig. 3H). Two indented, irregularly lobed

TABLE 1

Morphological measurements of the digenean species collected from *Clarias gariepinus* (Burchell, 1822) in the Okavango River and Delta, Botswana. Abbreviations: L – length, W – width.

	Clinostomoides brieni	***Neodiplostomum* type 1**	***Phyllodistomum bavuri***	***Phyllodistomum vanderwaali***	***Glossidium pedatum***	***Thaparotrema botswanensis* sp. n.**
Body (L×W)	6.60–14.30 (10.00±2.21)× 1.20–1.98 (1.57±0.19)	0.45–1.92 (0.10±0.46)× 0.12–0.45 (0.24±0.11)	3.20–5.65 (4.33±0.89)× 1.35–2.45 (1.86±0.37)	1.07–4.25 (2.45±1.16)× 0.49–2.52 (1.15±0.68)	1.25–2.98 (1.81±0.58)× 0.27–0.55 (0.40±0.08)	1.74–4.80 (2.96±0.96)× 0.30–0.75 (0.52±0.12)
Oral sucker (L×W)	0.12–0.30 (0.24±0.05)× 0.20–0.45 (0.30±0.07)	0.02–0.06 (0.04±0.01)× 0.02–0.05 (0.04±0.01)	0.30–0.42 (0.37±0.04)× 0.25–0.35 (0.32±0.04)	0.12–0.33 (0.22±0.07)× 0.15–0.35 (0.24±0.08)	0.10–0.30 (0.16±0.05)× 0.12–0.20 (0.16±0.03)	0.17–0.44 (0.25±0.08)× 0.16–0.45 (0.26±0.09)
Acetabulum (L×W)	0.60–0.95 (0.74±0.09)× 0.60–0.95 (0.76±0.08)	0.02–0.10 (0.05±0.03)× 0.01–0.12 (0.06±0.03)	0.38–0.61 (0.52±0.08)× 0.40–0.58 (0.51±0.07)	0.20–0.53 (0.33±0.12)× 0.21–0.60 (0.35±0.13)	0.12–0.24 (0.17±0.03)× 0.13–0.23 (0.17±0.03)	0.14–0.35 (0.21±0.06)× 0.15–0.34 (0.22±0.06)
Distance of acetabulum from oral sucker	0.60–1.50 (0.99±0.27)	0.24–0.91 (0.39±0.21)	0.46–1.39 (0.81±0.31)	0.40–1.30 (0.62±0.28)	0.22–0.68 (0.37±0.14)	0.10–1.20 (0.51±0.27)
Prepharynx length	0.05–0.20 (0.12±0.08)	–	–	–	0.02–0.05 (0.04±0.01)	–
Pharynx length	–	0.01–0.05 (0.03±0.01)	–	–	0.05–0.10 (0.08±0.02)	0.05–0.12 (0.09±0.02)
Oesophagus	–	0.01–0.10 (0.04±0.02)	0.05–0.20 (0.11±0.06)	0.06–0.20 (0.12±0.05)	–	0.03–0.18 (0.10±0.05)
Holdfast organ (L×W)	–	0.02–0.28 (0.12±0.07)× 0.03–0.16 (0.07±0.04)	–	–	–	–
Reproductive anlagen	–	0.01–0.08 (0.04±0.02)× 0.01–0.22 (0.04±0.04)	–	–	–	–
Anterior testis (L×W)	0.05–1.16 (0.21±0.23)× 0.10–0.75 (0.54±0.15)	–	0.20–0.47 (0.33±0.10)× 0.19–0.45 (0.29±0.10)	0.05–0.28 (0.15±0.09)× 0.06–0.37 (0.17±0.11)	0.12–0.24 (0.17±0.04)× 0.16–0.24 (0.19±0.02)	0.11–0.40 (0.20±0.07)× 0.13–0.34 (0.19±0.06)
Posterior testis (L×W)	0.09–2.00 (0.28±0.39)× 0.05–0.51 (0.29±0.13)	–	0.24–0.43 (0.35±0.06)× 0.20–0.45 (0.30±0.08)	0.04–0.30 (0.15±0.09)× 0.05–0.33 (0.15±0.10)	0.12–0.24 (0.18±0.03)× 0.13–0.24 (0.18±0.03)	0.12–0.40 (0.22±0.07)× 0.11–0.36 (0.20±0.07)
Seminal vesicle length	–	–	–	–	–	0.24–2.33 (0.08±0.67)
Ovary (L×W)	0.12–0.27 (0.20±0.04)× 0.06–0.20 (0.11±0.04)	–	0.17–0.25 (0.20±0.03)× 0.18–0.25 (0.21±0.02)	0.04–0.22 (0.12±0.07)× 0.06–0.25 (0.13±0.08)	0.10–0.19 (0.14±0.02)× 0.10–0.20 (0.16±0.01)	0.06–0.33 (0.19±0.08)× 0.06–0.34 (0.17±0.07)
Left vitellarium (L×W)	–	–	0.15–0.17 (0.16±0.01)× 0.08–0.19 (0.12±0.04)	0.04–0.20 (0.10±0.06)× 0.02–0.13 (0.06±0.04)	–	–
Right vitellarium (L×W)	–	–	0.13–0.22 (0.17±0.03)× 0.08–0.13 (0.10±0.02)	0.04–0.20 (0.1±0.06)× 0.02–0.19 (0.6±0.05)	–	–
Eggs (L×W)	–	–	0.02–0.04 (0.03±0.01)× 0.02–0.03 (0.03±0.004)	0.03–0.04 (0.04±0.00)× 0.01–0.04 (0.03±0.01)	0.02–0.09 (0.03±0.02)× 0.01–0.04 (0.02±0.01)	0.01–0.03 (0.02±0.01)× 0.004–0.01 (0.01±0.02)

testes situated on either side of median line of mid region of body in between intestinal caeca, may lie opposite each other or one slightly in front of other (Fig. 3I). Ovary lies to right of median line in six specimens; to left in other six specimens, but always in front of testes and posterior to vitellarium. Ovary irregularly round or lobed mass (Fig. 3I). Vitellarium compact diagonally opposed bodies, lie posterior to rim of acetabulum, anterior to ovary between intestinal caeca (Fig. 3I). Uterus consists of numerous loops that pass anteriorly along the median line between testes and vitelline bodies to reach genital pore, may extend beyond ends of caeca occasionally crossing caeca laterally (Fig. 1D). Uterus contains thin-shelled oval eggs. Eggs larger towards the distal end.

Material examined: BOTSWANA: 12 adult specimens, Okavango Delta, 7 Xaro mainstream (18°25'19.7"S 21°56'19.9"E); 5 Xaro lagoon (18°25'19.7"S 21°56'19.9"E).

Site of infection: Numerous worms observed in urinary bladder (Fig. 3D).

Prevalence of infection: 4.7%.

Remarks: This species differs from *Ph. spatula*, *Ph. symmetrorchis* and *Ph. ghanense* in having the ovary not anterior or slightly posterior to the vitellarium. It also differs from *Ph. ghanense* in having the uterine coils not filling the entire posterior two-thirds of the body. In *Ph. spatula* the ovary is completely round, while in *Ph. spatulaeforme* the ovary is kidney-shaped and the testes are small in relation to the body size.

Ph. vanderwaali differs from *Ph. linguale* and *Ph. bavuri* in being much smaller and in having the vitellarium immediately posterior of the acetabulum and the intestine bifurcating almost immediately posterior of the oral sucker. It also differs from *Ph. bavuri*, as redescribed in this paper, in that the testes, ovary and vitellarium are different in shape and size, the ovary lies more adjacent to the vitellarium and the body size is far smaller. The general morphology and measurements of specimens of the present material also correspond to a certain degree with *Ph. vanderwaali* as described by Prudhoe and Hussey (1977) and redescribed by Boomker (1984). However, in contrast to data by Prudhoe and Hussey (1977) and Boomker (1984), the present specimens show more variation in the shape, size and position of the testes, which in different specimens may be directly diagonal or nearly at the same level and dissimilar in size. Generally, the testes are larger than the ovary but in some specimens they are smaller.

All the above mentioned morphological differences are considered to be within the intraspecific variability of *Ph. vanderwaali* and therefore the specimens of the present material are assigned to this species. This is the first record of *Ph. vanderwaali* in the Okavango Delta, and the first documented record from Botswana.

Family Macroderoididae McMullen, 1937
Genus *Glossidium* Looss, 1899
Glossidium pedatum Looss, 1899

Figs 1E, 3J–L; Table 1

Glossidium pedatum: Looss 1899: 705–706, fig. 27; Fischthal 1973: 166–167.
Afromacroderoides lazerae Khalil, 1972: 341–344, fig. 1; Mashego & Saayman 1989: 19.

Redescription:

Elongate trematodes with truncated posterior end (Figs 1E, 3J). Body with maximum width at acetabulum level. Cuticle covered with tiny spines which are dense on anterior

end of body, gradually diminishing posteriorly. Both suckers well developed, almost equal in size. Oral sucker slightly oval to spherical in shape (Fig. 1E). Acetabulum spherical. Prepharynx short. Pharynx fairly large, opens into oesophagus which may sometimes be indistinct. Intestinal caeca extend posteriorly, ending near posterior extremity of body. Testes tandem, oval-shaped, smooth (Figs. 1E, 3K). Testes separated from each other by number of eggs. Cirrus sac large, elongate, curves to right of acetabulum, contains bipartite seminal vesicle with posterior half being larger than anterior part (Fig. 3L). Common genital pore median or submedian, immediately anterior to acetabulum. Ovary round to oval situated posterior to, or next to cirrus sac. Uterus forming upward and downward loops reaching to posterior end of body, where it fills in almost whole post testicular space (Fig. 1E). Numerous yellow to brown operculate eggs present. Vitellarium irregularly shaped, extends laterally on either side of body from ovarian level to posterior margin of posterior testis.

Material examined: BOTSWANA: 21 adult specimens, Okavango Delta, Shakawe mainstream (18°26'05.0"S 21°54'23.0"E).

Site of infection: Posterior third of intestine.

Prevalence of infection: 11.9%.

Remarks: *Glossidium pedatum* was first reported by Looss (1899) from *Bagrus bajad* (Forskål, 1775) and *Bagrus docmak* (Forskål, 1775) from the lower reaches of the Nile River. Fischthal (1973) collected and redescribed specimens of *Glossidium pedatum* from the intestine of *C. gariepinus* in Ethiopia, and noted that there were similarities between his material and *Afromacroderoides lazerae* Khalil, 1972 (also from *C. gariepinus*). It was found that Khalil's (1972) description corresponded to those of *G. pedatum*, and Mashego and Saayman (1989) thus synonymised *A. lazerae* with *G. pedatum*. Although Tkach (2008) treated *Afromacroderoides* as a synonym of *Glossidium*; however, he did not consider *G. pedatum* and *G. lazerae* to be synonyms and thus made the new combination *Glossidium lazerae* on the basis of the length of the oesophagus.

The specimens from the present study are similar to *G. pedatum* described by Fischthal (1973) in that it possesses a four-lobed pharynx, the ovary and testes are in the same position; the vitellaria extend from the ovarian level to the posterior margin of the posterior testis; the prepharynx has a sphincter-like structure situated just anterior to the pharynx. On the basis of the above mentioned characteristics, specimens of the present material are thus assigned to *G. pedatum*. This is the first record of *G. pedatum* in the Okavango Delta, and the first documented record from Botswana.

Family Opisthorchiidae Braun, 1901
Genus *Thaparotrema* Gupta, 1955

The genus *Thaparotrema* was erected for *Thaparotrema vittalani* Gupta, 1955 that was described from the intestine of the bagrid catfish *Rita rita* (Hamilton, 1822) in India (Gupta 1955). Scholz (2008) transferred species with a small, elongate or fusiform body covered with tegumental spines, having no prepharynx and with sinuous excretory system, which had been previously placed in the genus *Opisthorchis* Blanchard, 1895, to *Thaparotrema*. *Thaparotrema piscicola* (Odhner, 1902) was described by Odhner (1902) from the gall bladder of *Gymnarchus niloticus* from Sudan.

Thaparotrema botswanensis sp. n.

Figs 1F, 4A–H; Table 1

Etymology: This species is named after Botswana, where the material was collected.

Description:

Body elongate, narrow with round extremities, maximum width at level of acetabulum (Figs 1F, 4A). Tegument spined except for extreme part of post-testicular end of body. Suckers muscular. Oral sucker terminal round to oval in shape. Acetabulum slightly smaller than oral sucker, situated in first quarter of anterior part of body (Fig. 1F). Prepharynx absent. Pharynx round, some instances overlaps oral sucker dorsally (Fig. 4B). Oesophagus long; caecal bifurcation pre-acetabular closer to oral sucker; caeca narrow, extend to almost end of posterior extremity (Fig. 1F). Two testes, slightly oval, tandem to diagonal within inter-caecal space near to posterior extremity of body, separated from each other by excretory bladder (Fig. 4C). Posterior testis slightly bigger than anterior. Cirrus sac absent. Seminal vesicle tubular commencing post-acetabular (Fig. 4D). Genital pore median, just pre-acetabular (Fig. 4E). Ovary smooth, intercaecal (Figs 1F, 4F). Mehlis' gland present. Seminal receptacle situated obliquely anterior to ovary (Fig. 4F). Laurer's canal not observed. Vitelline follicles extending post-acetabular to level of ovary, slightly overlapping caeca (Figs 1F, 4G). Uterus extensively coiled, intercaecal, with ascending limb only, sinistral to seminal vesicle (Fig. 4G). Eggs yellow-brown, operculate. Excretory bladder saccular, passing between testes ending at posterior level of ovary; pore subterminal (Figs 1F, 4H).

Comparison: The new species is similar to *Th. piscicola* with the body being elongate and spined, having suckers that are almost of equal size and position, the absence of the cirrus sac, as well as the vitellarium beginning post-acetabular and extending to the level of the ovary, but differs in having a prominent seminal receptacle, greater space between the testes, and the testes being also not situated as close to the posterior extremity. The vitelline follicles are also much larger than those in *Th. piscicola*.

The new species is also similar to *Th. pedicellatum* (Verma, 1927) from the intestine of *Rita rita* from India in having suckers of almost equal size and a more prominent seminal receptacle. It differs, however, in that the body is not as wide nearer to the posterior extremity, the distance between the oral sucker and acetabulum is shorter and the vitelline follicles begin a distance post-acetabular and extend to the level of the ovary. *Thaparotrema botswanensis* sp. n. also differs from *Th. vitallani* in that the vitelline follicles do not begin anterior to the level of the acetabulum, the vitelline follicles are also more compact and the testes are situated close together. Based on the above remarks and the differences between *Th. botswanensis* sp. n. and the known species, it can be regarded as a new species. This is the first record of *Thaparotrema* from *Clarias gariepinus*, as well as the first report of the genus from the Okavango Delta.

Holotype: BOTSWANA: Okavango Delta, Shakawe mainstream (18°26'05.0"S 21°54'23.0"E), 01/10/25-9c (NMBP 355).

Paratype: BOTSWANA: Okavango Delta, Kalatog channel (18°25'08.2"S 21°54'05.0"E), 01/10/25-9b (NMBP 356).

Other material examined: same as paratype, 01/10/25-9e (BMNH 2013.9.27.1).

Site of infection: Gall bladder.

Prevalence of infection: 4.7%.

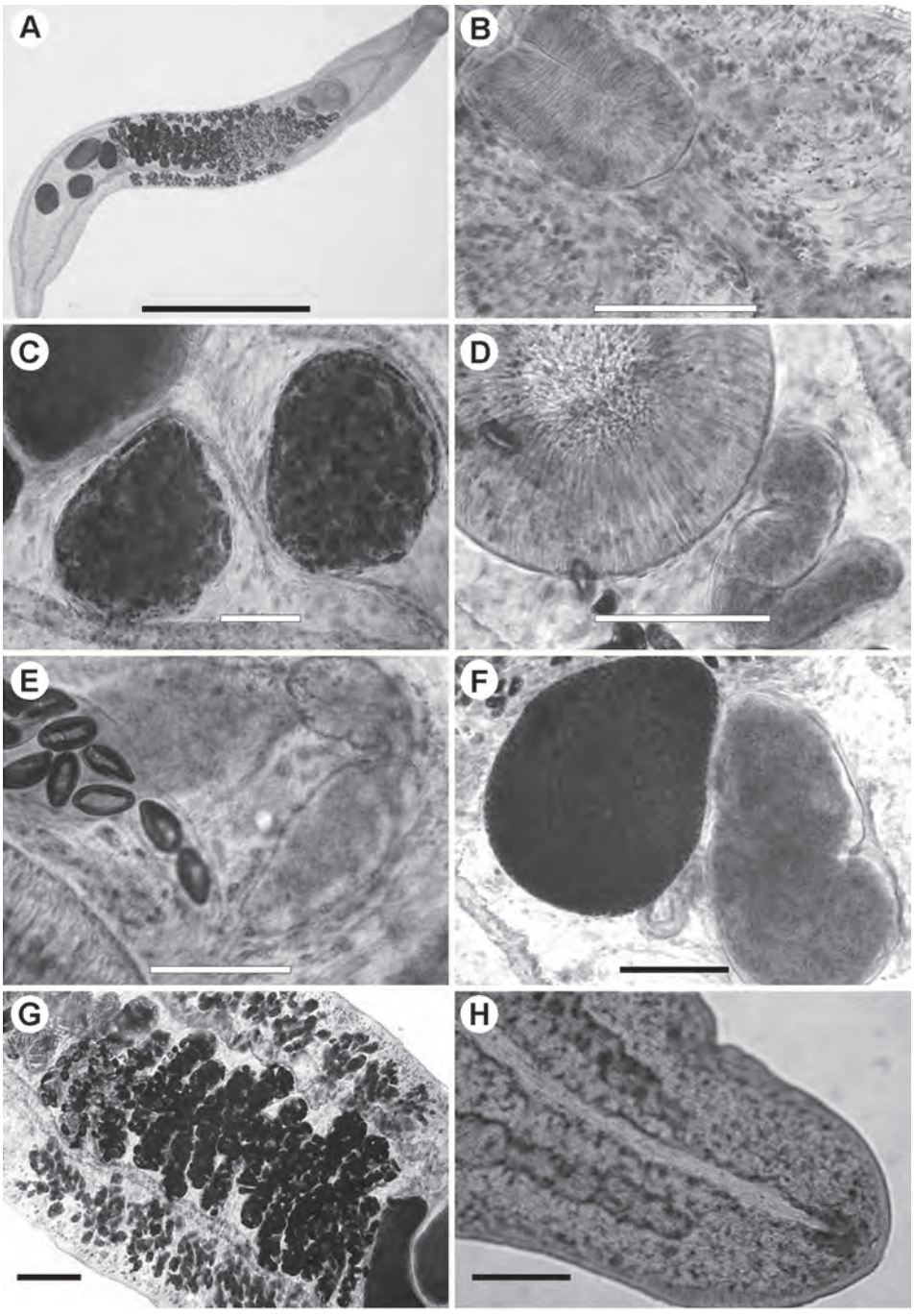

Fig. 4. Light micrographs of *Thaparotrema botswanensis* sp. n. collected from *Clarias gariepinus* in the Okavango Delta: (A) whole mount; (B) pharynx; (C) anterior and posterior testes; (D) seminal vesicle; (E) genital opening; (F) ovary and seminal receptacle; (G) vitellaria and uterus filled with eggs; (H) excretory bladder. Scale bars: A – 1 mm; B, C, E, F, H – 0.1 mm; D, G – 0.05 mm.

CONCLUDING REMARKS

Among the digeneans collected during the present investigation only *Clinostomoides brieni*, *Neodiplostomum* sp. (both metacercariae), *Glossidium pedatum*, *Phyllodistomum vanderwaali*, *Phyllodistomum bavuri* and *Thaparotrema botswanensis* sp. n. were found infecting *C. gariepinus*. A certain degree of site specificity was also observed, with *T. botswanensis* sp. n. being found only in the gall bladder of *C. gariepinus*, while both *Phyllodistomum* species were found only in the urinary bladder and thus seem to occupy a certain niche within their host. At any one time a single *C. gariepinus* may host four species of digeneans, each occupying its own niche within the host. Not only are these fish infected with adult and larval digeneans, but they also host other ecto- and endoparasites, sometimes having a heavy parasite burden (Jansen van Rensburg *et al.* 2001).

Clarias gariepinus plays a notably important role in the life cycles of digenean parasites in the Okavango Delta, as it acts as a definitive host for adults of four species, and as a second intermediate host to two metacercarial trematodes.

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