

## Annotated record of digenean parasites collected from fishes of the River Nile in Qena and Aswan governorates, Egypt

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### Abstract

A general investigation of digenean parasites was carried out from four fish species; Fahka puffer fish (*Tetraodon lineatus*), Nile tilapia (*Oreochromis niloticus*), Sharp tooth catfish (*Clarias gariepinus*) and Bayad (*Bagrus bajad*) from different localities of the River Nile in Qena and Aswan governorates, Egypt during October 2015 to August 2016. Four adult digeneans were found, belonging to four genera; *Astiotrema*, *Orientocreadium*, *Glossidium* and *Acanthostomum*. These were *Astiotrema impletum* 90% (27/30), *Orientocreadium batrachoides* 83.33% (25/30), *Glossidium pedatum* 60% (12/20) and *Acanthostomum spiniceps* 86.66% (26/30). Additionally, two larval trematodes (metacercariae) were detected, belonging to two genera; *Clinostomum* and *Euclinostomum*. These were *Clinostomum complantum* 85.57% (60/70) and *Euclinostomum heterostomum* 70% (35/50). Morphological and morphometric characters were discussed for each species in details and compared with the previously published data. This study considered *Glossidium pedatum*, *Acanthostomum spiniceps* and *Euclinostomum heterostomum* as new records from the River Nile in Qena Governorate.

**Keywords:** Digenea, Fish, River Nile, Egypt.

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## Introduction

Fresh water includes 41.24% of all known fish species. Freshwater fishes are one of the important sources of parasitic infection to human and fish-eating mammals (Mohamed, 1996). Adult trematodes are normally endoparasitic in the gastrointestinal tract, blood vessels or other natural body cavities of vertebrates (Hassan, 2006). Adult trematodes in fishes are usually host specific and rarely cause significant harm to their hosts, although there are some notable exceptions (Paperna and Dzikowski, 2006). The main pathogenic effects from trematode infections in fish hosts occur from larval metacercariae. Metacercariae tend to be less specific host than adult fluke and may affect fish growth and survival, as well as being a source of infection for final hosts and other fish-eating vertebrates, including people (Paperna and Dzikowski, 2006).

In the River Nile, Egypt, digeneans have been studied principally by many authors (Looss, 1896; Looss, 1899; Fischthal and Kuntz, 1963 a,b,c; El-Naffar, 1970; Moravec, 1976; El-Naffar, 1984; El-Shahawi and Al-Bassel, 1992; Soliman and El-Damarany, 1995; Hamada, 2002; Saad, 2007; El-Siefy, 2008; El-Mansy et al., 2011; Mansour, 2012; Morsy et al., 2013; Abdel-Gaber, 2015; El-Shahawy et al., 2017). The present study considered *Glossidium pedatum*, *Acanthostomum spiniceps* and *Euclinostomum heterostomum* as first records from the River Nile in Qena Governorate, Egypt.

This study aimed to conduct a general examination of some larval and adult digeneans infecting four species of important freshwater fishes from Qena and Aswan governorates in Egypt. The present study also aimed to improve the existing data on digeneans of freshwater fishes and to provide a preliminary estimation of digenean biodiversity in different localities along the River Nile, Egypt.

## Material and Methods

Fish were caught from commercial fishermen from different localities of the River Nile in Qena and Aswan governorates, Egypt during the period from October 2015 to August 2016. The collected fish were immediately transported to Parasitology Laboratory, Zoology Department, Faculty of Science, South Valley University, Qena, Egypt using special tanks supplied with ice.

Fish samples were dissected and examined for digenean worms. Macroscopic examination of the different organs carried out for the detection of any visible metacercariae. The alimentary canal was opened by a fine pair of scissors and left in an isotonic saline solution (0.9% NaCl) for few minutes in a petri-dish with occasional shaking (for the parasites to set free from the tissue of the alimentary canal). Repeated changes of the saline solution were done to keep it clear, then the alimentary canal was examined using a binocular dissecting microscope.

The recovered parasites were transferred to a clean saline solution using a brush or small pipettes and washed several times by saline solution to remove any mucous or debris which is usually adhere to their body surface. Worms were placed in between two glass slides or between a glass slide and cover slip in 5% formalin, subjected to a pressure until relaxed; some worms were preserved in 5% formalin and others in 70% ethanol. Worms were stained with acetic acid alum carmine for 5-10 minutes (Carleton, 1967), dehydrated in an ascending series of ethyl alcohol, cleared in xylene, mounted in DPX, covered with cover slip and finally they were examined under light microscope.

## Results

**Superfamily: Plagiorchioidea Lühe, 1901**

**Family: Opisthorchiidae Braun, 1901**  
*Astiotrema impletum* (Looss, 1899)

**Looss, 1900**

**Synonym:** *Astia impleta* Looss, 1899

**Host:** Puffer fish, *Tetraodon lineatus* Linnaeus, 1758 (Tetraodontidae)

**Infection site:** Intestine

**Locality:** The River Nile in Qena Governorate (Locality of Dandara), Egypt.

**Prevalence:** 90% (27/ 30).

**Specimens deposited** in Parasitology Laboratory at Zoology Department, Faculty of Science, South Valley University, Qena, Egypt

**Description: (Table 1, Fig. 1A):**

Body was oval in shape and measured 1.63-2.54 (1.75±0.02) mm in length and 0.63-0.98 (0.75±0.02) mm in width. The tegument provided with spines in the anterior third of the body. Oral sucker measured 0.12-0.3 (0.19±0.02) mm in length by 0.13-0.34 (0.25±0.02) mm in width. Ventral sucker measured 0.11-0.18 (0.16±0.02) mm in length and 0.15-0.21 (0.18±0.02) in width. Mouth was subterminal and led to a well-developed spherical to oval pharynx which measured

0.09-0.95 (0.52±0.02) mm long by 0.06-0.12 (0.09±0.002) mm wide. Pharynx was followed by a long esophagus which bifurcated into two simple intestinal caeca which extended posteriorly to the level of the posterior testis.

Testes were smooth, more or less rounded and more diagonal in position. Anterior testis was 0.22-0.26 (0.25±0.02) mm in length, 0.24-0.41 (0.32±0.02) mm in width and posterior testis measured 0.24-0.32 (0.29±0.02) mm long by 0.26-0.5 (0.42±0.02) mm wide. Ovary was spherical, measured 0.13-0.16 (0.15±0.02) mm in length and 0.12-0.17 (0.14±0.02) in width. Vitelline follicles occupied lateral fields extended from the level of intestinal bifurcation to the ends of the intestinal caeca.

Uterus was convoluted, descended from zone and pass between the two testes to the posterior end of the parasite, before ascending to open in the genital atrium. Eggs were numerous and elongated.

**Table 1. Comparative measurements (mm) of *A. impletum* in the present study with those described previously.**

Reference	Fischthal and Kuntz, 1963a	El-Naffar, 1970	El-Naffar et al., 1984	Present study
<b>Host</b>	<i>Tetraodon fahaka</i>		<i>Tetraodon lineatus</i>	
<b>Locality</b>	Cairo	Assiut	Assiut	Qena
<b>Body length</b>	1.105-2.530	1.8-2.8	1.33-3.33	1.63-2.54 (1.75±0.02)
<b>Body width</b>	0.405-0.750	0.79-1.14	0.617-1.045	0.63-0.98 (0.75±0.02)
<b>Oral sucker length</b>	0.185-0.290	0.26-0.32	0.199-0.380	0.12-0.3 (0.19±0.02)
<b>Oral sucker width</b>	0.225-0.360	0.30-0.36	0.228-0.427	0.13-0.34 (0.25±0.02)
<b>Ventral sucker length</b>	0.125-0.195	0.16-0.19	0.095-0.218	0.11-0.18 (0.16±0.02)
<b>Ventral sucker width</b>	0.130-0.215	-----	0.123-0.237	0.15-0.21 (0.18±0.02)
<b>Pharynx length</b>	0.65-0.105	0.10-0.12	0.085-0.142	0.09-0.95 (0.52±0.02)
<b>Pharynx width</b>	0.102-0.150	0.088-0.092	0.076-0.152	0.06-0.12 (0.09±0.02)
<b>Ant. testis length</b>	0.195-0.340	0.25-0.29	0.209-0.418	0.22-0.26 (0.25±0.02)
<b>Ant. testis width</b>	0.135-0.335	0.26-0.31	0.247-0.332	0.24-0.41 (0.32±0.02)
<b>Post. testis length</b>	0.215-0.345	0.25-0.29	0.237-0.437	0.24-0.32 (0.29±0.02)
<b>Post. testis width</b>	0.160-0.340	0.26-0.31	0.237-0.313	0.26-0.50 (0.42±0.02)
<b>Ovary length</b>	0.120-0.188	0.132-0.188	0.085-0.19	0.13-0.16 (0.15±0.02)
<b>Ovary width</b>	0.130-0.185	-----	0.085-0.196	0.12-0.17 (0.14±0.02)

**Superfamily: Plagiorchioidea Lühe, 1901**  
**Family: Orientocreadiidae Yamaguti, 1958**

***Orientocreadium batrachoides* Tubangu, 1931**

**Synonym: *Ganada barabankiae* (Dayal, 1938) Fischthal & Kuntz, 1963**

**Host:** Sharptooth catfish, *Clarias gariepinus* Burchell, 1822 (Clariidae).

**Infection site:** Intestine

**Locality:** The River Nile in Qena Governorate (Locality of Dandara), Egypt.

**Prevalence:** 83.33% (25/ 30).

**Specimens deposited** in Parasitology Laboratory at Zoology Department, Faculty of Science, South Valley University, Qena, Egypt

**Description: (Table 2, Fig. 1B):**

Body was elongated and measured 1.63-2.82 (0.20±0.2) mm long by 0.21-0.53 (0.42±0.02) mm wide. Cuticle was armed with minute spines. Oral sucker was subterminal and measured 0.14-0.28 (0.21±0.02) mm long by 0.12-0.23 (0.17±0.02) mm wide; pharynx measured 0.06-0.17 (0.12±0.02) mm long by 0.12-0.19 (0.17±0.02) mm wide; intestinal caeca extended behind uterine coils, almost into posterior body margin. Ventral sucker was 0.17-0.31 (0.25±0.02) mm in length and 0.15-0.28 (0.19±0.02) mm in width, it occupied about one third of body length.

Testes were rounded, tandem with anterior testis situated in mid region of body. Anterior testis measured 0.18-0.27 (0.25±0.02) mm in length and posterior testis was 0.24-0.39 (0.35±0.02) mm in length. Cirrus sac was also variable depending on the state of contraction, it may curve round to right or left of ventral sucker or may be anterior to it. Common genital pore was median, situated between ventral sucker and bifurcation of intestine. Ovary was rounded, its length was 0.17-0.28 (0.23±0.02) mm and it often separated from the ventral sucker by cirrus sac and/or seminal vesicle. Receptaculum seminis was not observed.

Uterus with descending and ascending limbs formed a series of irregular transverse

coils. Uterine coils separated from the posterior margin of the body by vitelline follicles. Vitellaria lie in two extracaecal bands extended from the level of ventral sucker to posterior extremity. Eggs were numerous and operculated.

**Superfamily: Plagiorchioidea Lühe, 1901**  
***Glossidium pedatum* Looss, 1899**

**Host:** Sharptooth catfish, *Clarias gariepinus* Burchell, 1822 (Clariidae).

**Infection site:** Intestine

**Locality:** The River Nile in Qena Governorate (Locality of Al- Ashraf Al-Qibliyyah), Egypt.

**Prevalence:** 60% (12/ 20).

**Specimens deposited** in Parasitology Laboratory at Zoology Department, Faculty of Science, South Valley University, Qena, Egypt

**Description: (Table 3, Fig. 1C):**

Body was entirely spined, slightly rounded anteriorly, bifid and expanded laterally at posterior extremity, measured 1.11-2.36 (1.52±0.2) mm long by 0.21-0.45 (0.32±0.02) mm wide. Oral sucker was slightly rounded to truncate or slightly concave anteriorly, wider than long, measured 0.08-0.28 (0.15±0.02) mm long by 0.18-0.26 (0.19±0.02) mm wide; acetabulum was rounded to slightly longitudinally or transversely elongated, measured 0.31-0.42 (0.38±0.02) mm long by 0.17-0.32 (0.26±0.02) wide. Pharynx was 0.04-0.15 (0.14±0.02) mm in length, 0.08-0.12 (0.1±0.02) mm in width, four-lobed anteriorly with dorsal lobe longer and ventral lobe shorter than two lateral ones; esophagus was distinct, thick-walled and muscular.

Testes were longitudinally elongated, smooth, diagonal or sometimes nearly tandem; anterior testis was dextral, measured 0.13-0.35 (0.25±0.02) mm long by 0.14-0.26 (0.46±0.02) mm wide, posterior testis was sinistral, measured 0.11-0.3 (0.15±0.02) mm long by 0.13-0.26 (0.19±0.02) mm wide. Cirrus sac was thick-walled and muscular. Ovary was sinistral, smooth, longitudinally elongated but

sometimes nearly rounded, measured 0.11-0.18 (0.16±0.02) mm long by 0.12-0.25 (0.23±0.02) mm wide. Vitelline follicles were overlapping ceca in lateral fields; uterus in mature adults was filling most of hindbody and posttesticular space, lying

ventral to gonads and proximal part of cirrus sac, occasionally overlapping ceca and vitellaria laterally. Eggs were yellow to yellow-brown and operculated.

**Table 2. Comparative measurements (mm) of *O. batrachoides* in the present study with those described previously.**

References	Beverley-Burton, 1962	Abdel-Gaber et al., 2015	Dumbo et al., 2019	Present study
Host	<i>Clarias mossambicus</i>	<i>Clarias gariepinus</i>	<i>Clarias gariepinus</i>	<i>Clarias gariepinus</i>
Locality	Rhodesia	Egypt	Mozambique	Egypt
Body length	1.11-2.58	1.83-2.72	1.47-2.67	1.63-2.82 (0.20±0.2)
Body width	0.22-0.77	0.31-0.46	314-663	0.21-0.53 (0.42±0.02)
Oral sucker length	0.11-0.23	0.16-0.24	0.1-0.2	0.14-0.28 (0.21±0.02)
Oral sucker width	0.11-0.33	0.15-0.20	0.11-0.22	0.12-0.23 (0.17±0.02)
Ventral sucker length	0.12-0.24	0.18-0.23	0.14-0.21	0.17-0.31 (0.25±0.02)
Ventral sucker width	0.12-0.25	0.19-0.27	0.11-0.22	0.15-0.28 (0.19±0.02)
Pharynx length	0.08-0.16	0.07-0.12	0.11-0.16	0.06-0.17 (0.12±0.02)
Pharynx width	0.09-0.16	0.10-0.18	0.08-0.13	0.12-0.19 (0.17±0.02)
Anterior testis length	0.11-0.25	0.19-0.24	0.11-0.22	0.18-0.27 (0.25±0.02)
Posterior testis length	0.11-0.30	0.21-0.31	0.16-0.3	0.24-0.39 (0.35±0.02)
Ovary length	0.13-0.27	0.16-0.21	0.12-0.23	0.17-0.28 (0.23±0.02)

**Table 3. Comparative measurements (mm) of *G. pedatum* in the present study with those described previously.**

References	Fischthal, 1973	Mashego, 1977	van Rensburg, 2013	Present study
Host	<i>Clarias mossambicus</i>		<i>Clarias gariepinus</i>	
Location	Ethiopia	South Africa	Botswana	Egypt
Body length	1.17-1.92	1.93	1.25-2.98	1.11-2.36 (1.52±0.2)
Body Width	0.2-0.31	0.31	0.27-0.55	0.21-0.45 (0.32±0.02)
Oral sucker length	0.11-0.15	0.21	0.10-0.30	0.08-0.28 (0.15±0.02)
Oral sucker Width	0.12-0.17	0.16	0.12-0.20	0.18-0.26 (0.19±0.02)
Acetabulum length	.....	.....	.....	0.31-0.42 (0.38±0.02)
Acetabulum width	0.11-0.15	0.17	0.13-0.23	0.17-0.32 (0.26±0.02)
Pharynx length	0.06-0.09	.....	0.05-0.10	0.04-0.15 (0.14±0.02)
Pharynx width	0.06-0.1	.....	0.11-0.15	0.08-0.12 (0.1±0.02)
Ant. testis length	0.1-0.13	0.15	0.12-0.24	0.13-0.35 (0.25±0.02)
Ant. testis width	0.08-0.1	0.11	0.16-0.24	0.14-0.26 (0.46±0.02)
Post. testis length	0.12-0.17	0.2	0.12-0.24	0.11-0.3 (0.15±0.02)
Post. testis width	0.08-0.11	0.11	0.13-0.24	0.13-0.26 (0.19±0.02)
Ovary length	0.07-0.11	0.12	0.10-0.19	0.11-0.18 (0.16±0.02)
Ovary width	0.06-0.09	0.11	0.10-0.20	0.12-0.25 (0.23±0.02)

**Superfamily: Opisthorchioidea Looss, 1899**

**Family: Cryptogonimidae Ward, 1917**

*Acanthostomum spiniceps* (Looss, 1896)  
**Looss, 1899**

**Synonym: *Distomum spiniceps* Looss, 1896**

**Host:** Bayad, *Bagrus bajad* Forskål, 1775 (Bgridae).

**Infection site:** Intestine

**Locality:** The River Nile in Qena Governorate (Locality of Dandara), Egypt.

**Prevalence:** 86.66% (26/ 30).

**Specimens deposited** in Parasitology Laboratory at Zoology Department, Faculty of Science, South Valley University, Qena, Egypt

**Description: (Table 4, Fig. 1D):**

Body length was 1.21-1.63 ( $1.45 \pm 0.2$ ) mm in length and 0.3-0.52 ( $0.41 \pm 0.02$ ) mm in width. Body surface was covered with fine spines, which were most numerous on the anterior portion of body and became more scarce toward the posterior end. Oral sucker was funnel-shaped, measured 0.15-0.4 ( $0.35 \pm 0.02$ ) mm in length and 0.23-0.47 ( $0.30 \pm 0.02$ ) mm in width. Outer surface of this sucker was armed with a row of large simple spines. Ventral sucker was almost rounded, measured 0.12-0.28 ( $0.25 \pm 0.02$ ) mm x 0.1-0.29 ( $0.24 \pm 0.02$ ) mm. Pharynx was strongly muscular and oval, measured 0.04-0.15 ( $0.07 \pm 0.02$ ) mm x 0.05-0.11 ( $0.08 \pm 0.02$ ) mm. Esophagus was relatively short. Intestinal caeca were relatively narrow and run along the body approximately up to the level of the posterior margin of the second testis, where they open to the body surface by distinct anal openings.

Testes were tandem and located near the posterior end of the body. Anterior testis measured 0.12-0.33 ( $0.23 \pm 0.02$ ) mm long x 0.23-0.41 ( $0.34 \pm 0.02$ ) mm wide and posterior testis measured 0.11-0.34 ( $0.22 \pm 0.02$ ) mm long x 0.2-0.26 ( $0.23 \pm 0.02$ ) mm wide. Ovary was almost spherical and subterminal, measured 0.15-0.2 ( $0.17 \pm 0.02$ ) long and 0.12-0.19 ( $0.15 \pm 0.02$ ) wide. Vitellaria were follicular

and situated on sides of body in its posterior half.

**Family: Clinostomidae Lühe, 1901**

**Subfamily: Clinostominae Lühe, 1901**

*Clinostomum complantum* (Rudolphi, 1814) Braum, 1899

**Synonym: *Clinostomum marginatum* (Rudolphi, 1819) Braun, 1899**

**Host:** Nile tilapia, *Oreochromis niloticus* Linnaeus, 1758 (Cichlidae)

**Infection site:** Buccal cavity and heart

**Locality:** The River Nile in Aswan Governorate (Locality of Shniarha), Egypt.

**Prevalence:** 85.57% (60/ 70).

**Specimens deposited** in Parasitology Laboratory at Zoology Department, Faculty of Science, South Valley University, Qena, Egypt

**Description: (Table 5, Fig. 1E):**

Body was linguiform and aspinose with blunt ends, measured 2.12-5.17 ( $3.89 \pm 0.2$ ) mm in length and 1.74-2.36 ( $2.15 \pm 0.2$ ) mm in width. Oral sucker was sub-terminal and measured 0.13-0.21 ( $0.17 \pm 0.02$ ) mm long by 0.14-0.35 ( $0.25 \pm 0.02$ ) mm wide. Ventral sucker situated at the anterior third of the body, measured 0.42-0.86 ( $0.65 \pm 0.02$ ) mm long and 0.4-0.88 ( $0.66 \pm 0.02$ ) mm wide. Mouth occupied the center of oral sucker and opened into a small prepharynx and the pharynx which measured 0.1-0.21 ( $0.15 \pm 0.02$ ) mm x 0.03-0.09 ( $0.05 \pm 0.02$ ) mm.

Caeca occupied a greater part of the body on either side and supported with small lateral diverticula. Testes were large digitate; anterior testis was 0.15-0.28 ( $0.22 \pm 0.02$ ) mm x 0.14-0.49 ( $0.3 \pm 0.02$ ) mm and posterior testis was 0.14-0.36 ( $0.26 \pm 0.02$ ) mm x 0.11-0.52 ( $0.33 \pm 0.02$ ) mm. Ovary was small, rounded to oval and intertesticular, measured 0.08-0.27 ( $0.17 \pm 0.02$ ) mm x 0.06-0.24 ( $0.16 \pm 0.02$ ) mm. Excretory bladder was small, V-shaped and opened out by a sub-terminal pore.

**Table 4. Comparative measurements (mm) of *A. spiniceps* in the present study with those described previously.**

References	Fernandes et al., 2002	Morsy et al., 2013	Abdel-Gaber et al., 2018	Present study
<b>Host</b>	<i>Astroscopus sexspinosus</i>	<i>Lates niloticus</i>	<i>Lates niloticus</i>	<i>Bagrus bajad</i>
<b>Locality</b>	Brazil	Egypt	Egypt	Egypt
<b>Body length</b>	3.37	1.203–2.074	1.291–2.871 (2.210 ± 0.1)	1.2-1.63 (1.45±0. 2)
<b>Body width</b>	0.57	0.311–0.534	0.298–0.476 (0.392 ± 0.01)	0.30-0.52 (0.41±0.0 2)
<b>Oral sucker length</b>	0.318	0.201–0.353	0.232–0.397 (0.321 ± 0.01)	0.15-0.40 (0.35±0. 02)
<b>Oral sucker width</b>	0.270	0.245–0.310	0.257–0.344 (0.322 ± 0.01)	0.23-0.47 (0.30±0. 02)
<b>Ventral sucker length</b>	0.150	0.110–0.140	0.123–0.151 (0.143 ± 0.01)	0.12-0.28 (0.25±0. 02)
<b>Ventral sucker width</b>	0.138	0.120–0.204	0.132–0.198 (0.157 ± 0.01)	0.10-0.29 (0.24±0. 02)
<b>Pharynx</b>	0.138 × 0.135	0.163–0.177 × 0.150–0.190	0.154–0.173 (0.165 ± 0.01) × 0.162–0.189 (0.175 ± 0.01)	0.04-0.15 × 0.05-0.11 (0.07±0.02x0.08±0.02)
<b>anterior testis</b>	0.285	0.130–0.220×0.314–0.358	0.128–0.286 (0.212 ± 0.01) × 0.343–0.430 (0.358 ± 0.01)	0.12-0.33×0.23-0.41 (0.23±0.02x0.34±0.02)
<b>posterior testis</b>	0.315×0.281	0.180–0.274×0.390–0.327	0.194–0.287 (0.254 ± 0.01)×0.354–0.420 (0.386 ± 0.01)	0.11-0.34×0.2-0.26 (0.22±0.02x0.23±0.02)
<b>Ovary length</b>	0.243	0.102–0.166	0.143–0.178 (0.156 ± 0.01)	0.15-0.2 (0.17±0.02)
<b>Ovary width</b>	0.187	0.100–0.164	0.098–0.143 (0.121 ± 0.01)	0.12-0.19 (0.15±0.02)
<b>No. spines</b>	27	23	23	23

**Table 5. Comparative measurements (mm) of *C. complantum* in the present study with those described previously.**

Reference	Malik et al., 2016	Simsek et al., 2018	Present study
<b>Host</b>	-----	-----	<i>Oreochromis niloticus</i>
<b>Location</b>	-----	-----	Egypt
<b>Body length</b>	1.917-2.700 (0.231±0.2)	3.998–6.718 (5.1 ± 0.34)	2.12- 5.17 (3.89±0. 2)
<b>Body width</b>	0.64-1.05 (0.08±0.1)	1.197–2.131 (1.69 ± 0.16)	1.74-2.36 (2.15±0. 2)
<b>Oral sucker length</b>	0.14-0.23 (0.17±0.02)	0.24–0.31 (0.27 ± 0.01)	0.13-0.21 (0.17±0. 02)
<b>Oral sucker width</b>	0.14-0.24 (0.19±0.02)	0.26–0.48 (0.33 ± 0.03)	0.14- 0.35 (0.25±0. 02)
<b>Ventral sucker length</b>	0.33-0.44 (0.39±0.03)	0.58–0.82 (0.68 ± 0.03)	0.42- 0.86 (0.65±0. 02)
<b>Ventral sucker width</b>	0.32-0.42 (0.37±0.02)	0.67–0.89 (0.76 ± 0.03)	0.4-0.88 (0.66±0. 02)
<b>Pharynx</b>	-----	-----	0.10-0.21 x 0.03-0.09 (0.15±0.02 x 0.05±0.02)
<b>Anterior testis</b>	0.11-0.20 (0.15±0.02) x 0.08-0.26 (0.14±0.02)	0.29–0.71 (0.47 ± 0.06) x 0.2–0.49 (0.4 ± 0.04)	0.15-0.28 x 0.14-0.49 (0.22±0.02x0.3±0.02)
<b>Posterior testis</b>	0.1-0.19 (0.14±0.028) x 0.1-0.32 (0.21±0.05)	0.21–0.39 (0.32 ± 0.02) x 0.38–0.56 (0.45 ± 0.02)	0.14-0.36 x 0.11-0.52 (0.26±0.02x0.33±0.02)
<b>Ovary</b>	0.03-0.1 (0.06±0.02) x 0.04-0.08 (0.05±0.02)	0.12–0.15 (0.13 ± 0.005) x 0.08–0.14 (0.11 ± 0.009)	0.08-0.27x0.06-0.24 (0.17±0.02x0.16±0.02)

**Family: Clinostomidae Lühe, 1901**

**Subfamily: Euclinostominae Yamaguti, 1958**

***Euclinostomum heterostomum* (Rudolphi, 1809) Travassos, 1928**

**Host:** Nile tilapia, *Oreochromis niloticus* Linnaeus, 1758 (Cichlidae).

**Infection site:** Buccal cavity and kidney

**Locality:** The River Nile in Qena

Governorate (Locality of Al- Ashraf Al-Qibliyyah), Egypt.

**Prevalence:** 70% (35/ 50).

**Specimens deposited** in Parasitology Laboratory at Zoology Department, Faculty of Science, South Valley University.

**Description: (Table 6, Fig. 1F):**

Body was stout, fleshy, linguiform, attenuated anteriorly and slightly rounded posteriorly, measured 4.11-5.52 (4.55±0.2) mm long and 1.53-2.67 (1.72±0.02) mm wide. Cuticle was thin and smooth. Oral sucker was small and subterminal, measured 0.16-0.34 (0.26±0.02) mm long and 0.2-0.76 (0.52±0.02) mm wide. Ventral sucker was well developed, strongly muscular, almost rounded marginally and

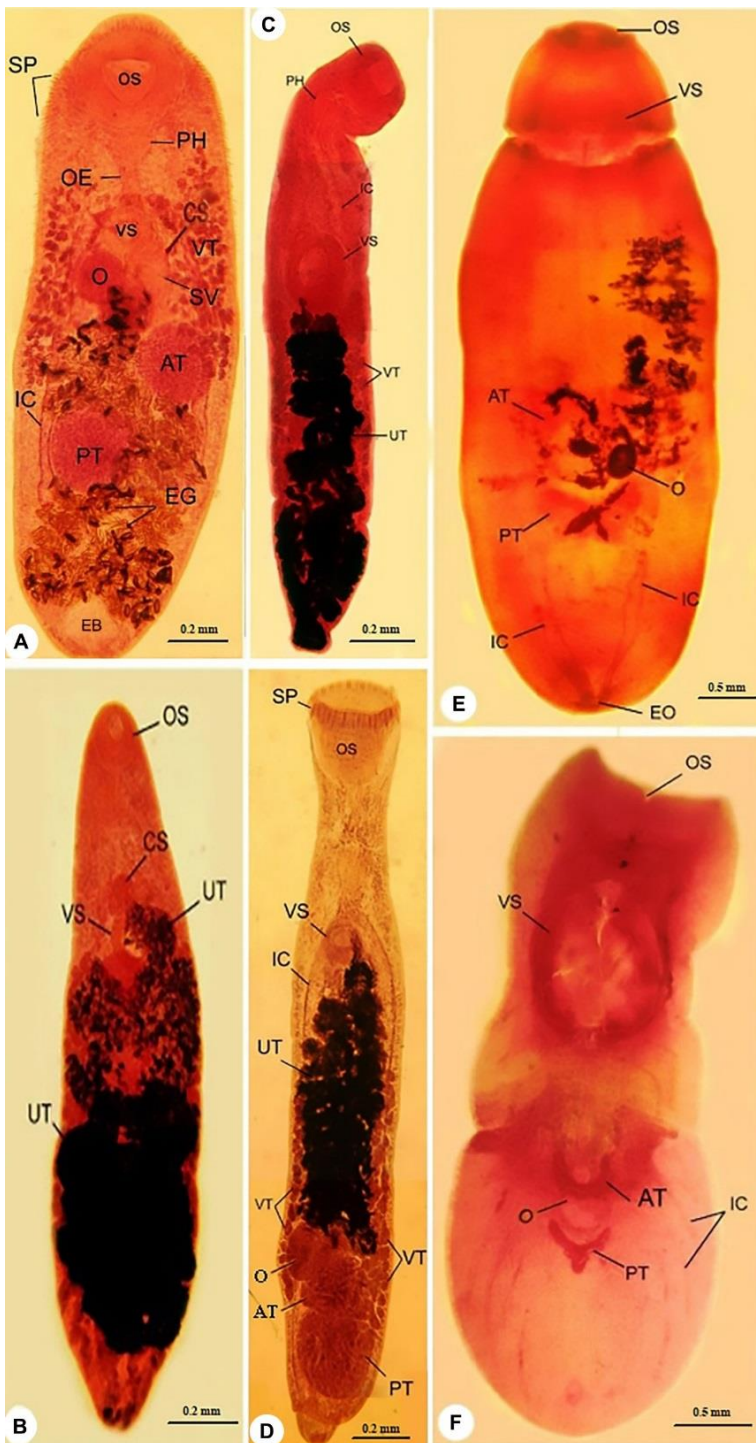
heart shaped internally, situated at the end of the first quarter of the body, measured 0.52-1.04 (0.83±0.02) mm long and 0.72-0.96 (0.85±0.02) mm wide. Pharynx was short and measured 0.05-0.08 (0.06±0.002) mm x 0.04-0.07 (0.07±002) mm. Esophagus was very short and bifurcated into long diverticulated ceca extended up to posterior extremity. Left cecum was supported with seven simple branches while right cecum with 12 simple branches.

Caecal branches were elongated, lobed and appeared finger like processes with somewhat swollen extremities. Testes were asymmetrical, tandem and situated in third quarter of body. Anterior testis was wider than long and measured 0.01-0.02 (0.01±0.002) mm long x 0.51-0.61 (0.06±002) mm wide. Posterior testis was also asymmetrical, slightly lobed and measured 0.16-0.22 (0.19±0.02) mm long x 0.59-0.63 (0.61±002) mm wide. Ovary was almost rounded, small, lie laterally on upper corner of anterior testis and measured 0.13-0.14 (0.13±0.02) mm x 0.12-0.15 (0.14±002) mm.

**Table 6. Comparative measurements (mm) of *E. heterostomum* in the present study with those described previously.**

Reference	Rai, 1970	Jhansilakshmbai and Madhavi, 1997	Vankara, 2011	Present study
<b>Host</b>		<i>Channa punctatus</i>		<i>Oreochromis niloticus</i>
<b>Location</b>	India	India	India	Egypt
<b>Body length</b>	5.41-5.62	0.183-4.534	3.45-8.41	4.11-5.52 (4.55±0. 2)
<b>Body width</b>	1.46-2.32	0.089-1.627	1.28-2.36	1.53-2.67 (1.72±0.02)
<b>Oral sucker length</b>	0.18-0.23	0.042-0.168	0.14-0.34	0.16-0.34 (0.26±0.02)
<b>Oral sucker width</b>	0.21-0.25	0.05 - 0.217	0.20-0.52	0.2-0.76 (0.52±0.02)
<b>Ventral sucker length</b>	0.75-0.86	0.07-0.929	0.60-1.16	0.52-1.04 (0.83±0.02)
<b>Ventral sucker width</b>	0.82-0.87	0.07-0.914	0.55-1.34	0.72-0.96 (0.85±0.02)
<b>Pharynx</b>	0.06-0.09 x 0.05-0.08	-----	0.137-0.17 x 0.16-0.44	0.053-0.089x 0.041-0.076 (0.063±0.002x0.072±002)
<b>Anterior testis</b>	0.013-0.014 x 0.61-0.72	0.126-0.314 x 0.150- 0.491	0.22- 0.63 x 0.31-0.78	0.010-0.023 x 0.51-0.61 (0.013±0.002x0.060±002)
<b>Posterior testis</b>	0.18-0.21x 0.51-0.52	0.111-0.370 x 0.145- 0.420	0.07-0.39 x 0.10-0.22	0.16-0.22x 0.59-0.42 (0.19±0.02x0.61±002)
<b>Ovary</b>	0.12-0.15 x 0.12-0.14	0.052-0.136 x 0.050- 0.146	-----	0.13-0.14 x 0.12-0.15 (0.13±0.02x0.14±0.02)





**Fig. 1. Light photomicrographs of the recorded digenean parasites among the examined fish species from Qena and Aswan governorates, Egypt. (1A):** Whole mount preparation of the adult worm *Astiotrema impletum* with an oval body, consists of: a tegument provided with spines (SP) in the anterior third of the body, an oral sucker (OS), followed by pharynx (PH), oesophagus (OE), small median ventral sucker (VS), two intestinal caeca (IC), spherical ovary (O), convoluted uterus filled with numerous and elongated eggs (EG), two testes, anterior testis (AT) and posterior testis (PT), seminal vesicle (SV), cirrus sac (CS), laterally distributed vitellaria (VT) and an excretory bladder (EB), scale bar 0.2 mm. **(1B):** Whole mount preparation of the adult worm *Orientocreadium batrachoides* with elongated body consists of: an oral sucker (OS), ventral sucker (VS), uterus (UT) filled with numerous and small eggs and cirrus sac (CS), scale bar 0.2 mm. **(1C):** Whole mount preparation of the adult worm *Glossidium pedatum* with an elongated oral sucker (OS) leading to pharynx (PH), intestinal caeca (IC), ventral sucker (VS), uterus (UT) and lateral vitellaria (VT), scale bar 0.2 mm. **(1D):** Whole mount preparation of the adult worm *Acanthostomum spiniceps* with elongated body consists of: a funnel-shaped oral sucker (OS) armed with a row of large simple spines (SP), ventral sucker (VS), intestinal caeca (IC), small oval ovary (O), uterus (UT) filled with numerous and mature eggs, anterior testis (AT), posterior testis (PT) and laterally scattered vitellaria (VT), scale bar 0.2 mm. **(1E):** Whole mount preparation of digenean metacercaria *Clinostomum complantum* showing a subterminal oral sucker (OS), ventral sucker (VS), intestinal caeca (IC), small rounded to oval ovary (O), anterior testis (AT), posterior testis (PT) and an excretory opening (EO), scale bar 0.5 mm. **(1F):** Whole mount preparation of digenean metacercaria *Euclinostomum heterostomum* consisting of a small oral sucker (OS), well developed ventral sucker (VS), diverticulated intestinal caeca (IC), a small ovary (O), tandem testis; anterior testis (AT) and posterior testis (PT), scale bar 0.5 mm.

## Discussion

### ***Astiotrema impletum* (Looss, 1899) Looss, 1900:**

*Astiotrema impletum* was originally described by Looss (1899) from *Tetraodon lineatus* (as *Tetraodon fahaka*) (Tetraodontidae) in the Nile at Cairo, Egypt. Looss (1900) proposed the name *Astiotrema* and both species *Distoma reniferum* and *Astia impleta* were renamed to *A. renferuim* (Looss, 1898) Looss, 1900 and *A. impletum* (Looss, 1898) Looss, 1900 respectively. This species is known only from North Africa from the freshwater fish, *Tetraodon lineatus* (as *Tetraodon fahaka*). Further studies reported *Astiotrema impletum* (Looss, 1899) Looss, 1900 from different localities of Egypt among *Tetraodon lineatus* (as *Tetraodon fahaka*) (Fischthal and Kuntz, 1963a; El-Naffar, 1970; El-Naffar, 1984; El-Siefy, 2008), *Tetraodon lineatus* (Karar et al., 2021) and *Oreochromis niloticus* (Hamada, 2002) and from Sudan among *Tetraodon lineatus* (as *Tetraodon fahaka*) (Odhner, 1911; Yeh and Fotedar, 1958; Khalil, 1969).

This species is distinguished from the rest members of *Astiotrema* by the following characters; the oral sucker is large and twice that of the ventral, the caeca is short in which it terminates at the posterior border of the second testis or the junction of the third- and fourth-quarters of the body and vitellaria are restricted to anterior half of the worm. The eggs in our specimens were elongated, thin shell operculated whereas no mention was made of this by Looss (1899), Odhner (1911) and Yeh and Fotedar (1958).

Odhner (1911) noted that the testes occasionally were slightly lobed and almost symmetrical; whereas, all the previous authors and the present study noted smooth testes at all times and a more diagonal position. Testes in the present study were separated by a distinct intertesticular space while Odhner (1911) and Fischthal & Kuntz (1963a) noted that the testes were in contact or closer together. El-Naffar (1984)

stated that vitellaria in his specimens extending from the level of intestinal bifurcation to the end of the intestinal caeca but his illustration shows that the distribution of vitellaria reaches only to the beginning of posterior testis. Morphometry of *A. impletum* specimens collected by the previous authors were comparatively similar to our specimens as shown in Table 1.

### ***Orientocreadium batrachoides* Tubangui, 1931:**

*Orientocreadium batrachoides* was first described by Tubangui (1931) from *Clarias batrachus* in Philippines. More studies were carried out on this species (Beverley-Burton, 1962; Yamaguti, 1971; Tang and Lin, 1973; Sirikantayakul, 1976; Moravec, 1977; Moravec and Sey, 1989; Ruangpan, 1989; Tepe et al., 2013; Bhure and Nanware, 2011; Bhure et al., 2013; Bhure and Nanware, 2016; Zhokhov et al., 2017; Dumbo et al., 2019). This species was previously recorded from Egypt in *Clarias gariepinus* (Abdel-Gaber et al., 2015; El-Siefy et al., 2017; Attia et al., 2021) or *Clarias gariepinus* (as *Clarias lazera*) (Fischthal and Kuntz, 1963b; El-Naffar et al., 1983; Negm El-Din et al., 1988; El-Naggar et al., 1993a,b; El-Seify et al., 1997; El-Mansy et al., 2011; El-Shahawy et al., 2017).

The presence of unipartite external seminal vesicles and vitelline follicles that start at the level of the ovary until they meet at the posterior end of the body distinguishes the species. The vitelline follicles are grouped in two lateral fields limited in the middle of the body, according to Bhure and Nanware (2011) and Abdel-Gaber et al. (2015). In our specimens, external seminal vesicle was not observed. Measurements of the present specimens were very compatible with those previously described, as indicated in Table 2.

### ***Glossidium pedatum* Looss, 1899:**

Looss (1899) reported *Glossidium pedatum* from *Bagras bayad* Forskal, 1775

and *B. docmac* Forskal, 1775 (Bagridae) from the Nile at Cairo. In Ethiopia, Fischthal (1973) recorded and redescribed *G. pedatum* from the intestine of *Clarias gariepinus* (as *C. mossambicus*). Bray and Hendrix (2007) recorded *G. pedatum* in Lake Malawi among *Clarias gariepinus* (as *Clarias mosambicus*). In South Africa, Matla (2012) isolated *G. pedatum* from the gut of *Clarias gariepinus*.

Characteristics of *Astiotrema lazera* El-Naffar, Saoud & Hassan, 1984 collected from the intestine of *Clarias gariepinus* (as *Clarias lazera*) from Lake Nasser at Aswan, Egypt may be a synonymy of *Glossidium pedatum*, according to Bray et al. (2006).

Mashego and Saayman (1989) noted that the features of *Afromacrodroides lazerae* Khalil, 1972, which was collected from the intestine of *Clarias gariepinus* (as *Clarias lazera*) from the White Nile near Khartoum, Sudan, were similar to those of the Plagiorchiidae, and considered *A. lazerae* a synonym of *Glossidium pedatum*. Dumbo et al. (2019) provided morphological and molecular studies on *G. pedatum* from *Clarias gariepinus* in Mozambique. They included *Afromacrodroides lazerae* Khalil, 1972 to be a member of *Glossidium* Looss, 1899 as *G. lazerae*. On the other hand, *Afromacrodroides lazerae* Khalil, 1972, has a distinct esophagus as well as a bipartite seminal vesicle. Since then, *Afromacrodroides lazerae* has been found in cichlids (Bray et al., 2006).

Further records of *Glossidium pedatum* from Egypt included those from *Clarias gariepinus* (Abdel-Gaber et al., 2016) or *Clarias gariepinus* (as *C. lazera*) (El-Shahawi and Al-Bassel 1992, Imam et al., 1991) or from bagrid fishes (Ibraheem, 2007). This is characterized by a short prepharynx and a four lobed pharynx. The ovary and testes are both in the same position. Caeca wide ends close to the posterior testis and does not extend to the posterior extremity. Vitellaria extend from the postacetabular to the posterior testis

level. As shown in Table 3, the measurements of the current specimens were comparatively consistent with those given by the previous authors.

#### ***Acanthostomum spiniceps* (Looss, 1896) Looss, 1899:**

Looss (1899) established the genus *Acanthostomum*, which included intestinal parasites of fishes and reptiles (primarily crocodiles), for the species *A. spiniceps* (Looss, 1896) and *A. coronarium* (Cobbold, 1861). In Giza, Egypt, Fischthal and Kuntz (1963a) collected *A. spiniceps* from *B. bayad*. Khalil (1963) redescribed *A. spiniceps* from *B. bayad* and *B. docmac* from the White and Blue Niles in Khartoum, Sudan. He created a new key to distinguish *Acanthostomum* species. Morsy et al. (2013) isolated this parasite from the intestine of *Lates niloticus* along the River Nile in Beni-Suef Governorate.

Further studies were provided about *Acanthostomum spiniceps* (Looss, 1896) from Egypt among bagrid fishes (Moravec, 1976; Tadros et al., 1978; Soliman and El-Damarany, 1995; Ibraheem, 2006; Mansour, 2012) and marine fishes (El-Shahawi and Al-Bassel, 1992; Abdel-Gaber et al., 2018; Tadros et al., 2020). This species was also studied by Dollfus (1932), Pogoreltseva (1952) and Fernandez et al. (2002). This species is characterized by vitellaria extend anteriorly beyond the level of the seminal vesicle and the number of circumoral spines ranges from 23-32. Measurements of the present specimens were comparatively consistent with those provided by the previous authors as given in Table 4. The River Nile in Qena Governorate constitutes a new locality to this species.

#### ***Clinostomum complantum* (Rudolphi, 1814) Braum, 1899:**

Ukoli (1966a) classified *C. complantum* metacercariae based on the following characteristics: cirrus pouch on the right margin of the anterior testis in front of the ovary; genital pore on the right

margin of the anterior testis near its middle, or occasionally displaced to its anterior margin. He also noted that the position of the genital pore is a useful character for distinguishing *Clinostomum* species, despite the fact that other authors have argued that this character can change with different fixation or flattening techniques (Baer, 1933), or during development (Vianna et al., 2003).

Li et al. (2018) identified *Clinostomum complantum* metacercariae in *Myxocyprinus asiaticus* from China. Maleki et al. (2018) found *C. complantum* metacercariae from some freshwater fishes in western Iran. Menconi et al. (2020) examined metacercariae of *C. complantum* in an Italian Subalpine Lake. Additional research was conducted on the metacercariae of *C. complantum* (Caffara et al., 2011; Simsek et al., 2018; Wang et al., 2017).

El-Shahawy et al. (2017) recorded metacercariae from some freshwater fishes in Qena Governorate, Egypt. They only studied the prevalence of infection and they didn't provide any morphological or morphometric description for their examined metacercaria. Measurements of the present specimens were remarkably similar with that given by Shini (2015) as shown in Table 5.

#### ***Euclinostomum heterostomum* (Rudolphi, 1809) Travassos, 1928:**

*Euclinostomum heterostomum* was reported among *Tilapia* spp. and *Clarias* sp in Egypt by Fischthal and Kuntz (1963c). Ukoli (1966b) provided a detailed morphological description of *E. heterostomum* from *Tilapia zilli* and *T. heudelot*. He believed that the position of the genital complex, the entrance of the uteroduct in the uterus, and the arrangement of diverticula (rather than the number of diverticula) are useful for species discrimination. He proposed the synonymy of *Euclinostomum clarias*, *E. dollfusi*, *E. bhagavantami*, *E. charmai*, *E. heptacaecum*, and *E. indicum* with *E. heterostomum*. Based on the mentioned

criteria, he recognized *E. heterostomum* and *E. multicaecum* as valid species.

Kazacos and Appel (1983) recorded *E. heterostomum* in the Aquarium Ram, *Apistogramma ramirezi* (Pisces: Cichlidae) from Hong Kong. Britz et al. (1985) reported the distribution of *E. heterostomum* metacercarial infections in *Oreochromis mossambicus*. *Euclinostomum heterostomum* was isolated from the liver, spleen, and kidney of *Channa punctata* by Sinha et al. (1988). Saad (2007) reported metacercariae of *Euclinostomum heterostomum* in the kidneys of *Oreochromis niloticus* and *Tilapia zillii* in Lake Nasser, Aswan, Egypt. He did not provide morphological or morphometric descriptions for his specimens. More research was done on *E. heterostomum* metacercariae (Donges, 1974; Jhansilakshmibai and Madhavi, 1997; Caffara et al., 2016). As shown in Table 6, the measurements of the current specimens were remarkably similar to those provided by the previous authors.

#### **Conclusion**

In this study, six species of digenean trematodes were examined from four freshwater fish species from different regions along the River Nile in Qena and Aswan governorates, Egypt. Worms were described morphologically and morphometrically using light microscopy. More scanning, transmission electron microscopy and molecular studies are recommended to justify the exact taxonomic position of these species.

#### **Conflicts of interest**

The authors declare that there is no conflict of interest.

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