

Morphological and morphometric characterization of a new digenetic trematode, *Proenenterum* sp.n., infecting the common sea bream *Pagrus pagrus* from the Red Sea in Egypt

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Abstract: In the present study, the morphology and morphometric characterization of *Proenenterum* sp. (family: Lepocreadiidae), a new digenetic trematode infecting the pyloric portion of the stomach and the middle part of the intestine of the common sea bream *Pagrus pagrus* fish, were described by light and scanning electron microscopy for the first time from the coasts of Gulf of Suez and Hurghada city of the Red Sea in Egypt. Numerous specimens were collected during the year of 2010. Out of 140, examined fish, only 46 (32.8%) were found to be naturally infected. The adult worms exhibited an elongated body with anterior pointed and posterior broad ends. The adult worm measured 2.30- 3.84 (mean 3.25 ± 0.20) mm in length and 0.42- 0.54 (mean 0.48 ± 0.02) mm in width. The present *Proenenterum* species is characterized by its smaller dimensions and the presence of a large ventral sucker, two lobed testes. The recorded parasite herein has the same general morphology of its family but with different characteristics than their members. The genus *Proenenterum* was included firstly within family Enenteridae but after that, it is separated from this genus as a separate family Lepocreadiidae due to the presence of distinct morphological criteria that specify organisms to be enenterids, these are presence of anal aperture, processes on oral suckers and an external seminal vesicle which are not present in the current study.

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Key words: *Proenenterum* sp.n. – Lepocreadiidae – *Pagrus pagrus* – Light and scanning electron microscopy.

1. Introduction

The sea bream *Pagrus pagrus* Linnaeus, 1758; Sparidae is a widely distributed fish in the Red Sea. It is an important commercial fish in Egypt with a very good prospect for aquaculture. It is a carnivorous fish feeding on crustacean and planktonic invertebrates. As a result of the significant mortalities caused by parasitism, the mass production of this species is limited (Aristizaba and Suarez, 2006), so parasites play a very important part in the life of their hosts (Dogiel, 1964). Studies on the helminth parasites of Red Sea fishes tend to be limited to short papers describing new taxa or longer works where the Red Sea is dealt with studies on the Indian Ocean. Consequently, parasitic helminths are one of the Red Sea fauna. Digenetic trematodes of marine fishes from the Red Sea received a great attention from several workers. Nagaty and his group reported many researches in this field (Nagaty, 1930, 1937, 1941, 1942, 1948, 1954, 1956, 1957; Nagaty and abdel-Aa1, 1961, 1962a,b,c, 1964, 1972). Recently, continuous works on description of several new digenitans from Red Sea fishes and revision of the taxonomy of others were recorded by Ramadan (1982, 1983, 1984, 1985, 1986, and 1988) and Ramadan Saoud and Ramadan (1985). Members of Lepocreadiidae are recognizable as worms with widely distributed vitelline follicles, a spinose

tegument, usually with a distinct I-shaped excretory vesicle (Bray 2005). Firstly, Brooks *et al.* (2000) considered just three genera in the family Enenterinae which are, *Enenterum*, *Koseiria* and *Cadenatella*. Bray and Cribb, (2001) also included the genera *Proenenterum* Manter, 1954 within family Enenterinae which were specifically rejected by Brooks *et al.* (2001). Brooks *et al.* (2001) and Bray & Cribb (2002) characterize parasites within family Enenterinae to have an oval body, spinose tegument, usually with eye spot pigment, the oral sucker is often lobed, the ventral sucker is rounded, pre-equatorial, the external seminal vesicle is absent. The caeca may unite to form a blind cyclocoel which opens through an anus. Moreover, due to some important differences between *Proenenterum* and enenterids, the former and its related species were grouped as a separate family, Leptocreadiidae, which is characterized by elongated to oval body with no eye spot pigment, and with a subglobular, subterminal oral sucker, no external seminal vesicle, no anus. Two entire or lobed testes, in a tandem position are found in the third part of the worm body. The ovary is oval or lobed-shaped, vitelline fields reach to the ventral sucker or into the forebody. The caeca may unite to form a blind cyclocoel, but with no anus.

2. Materials and methods

Samples of 140 individual sea bream fish, *Pagrus pagrus*, were collected throughout the whole year of 2010 from the coasts at the Gulf of Suez and Hurgada at the Red Sea. Fish were immediately transported in water tanks to the laboratory at the Zoology Department, Faculty of Science, Cairo University. The captured fish were kept alive in aquaria filled with the same water source to prevent the loss of mobile and temporary ectoparasites. Fish skin surfaces, fins and gills were examined, firstly, by naked eyes, then with the help of a dissecting microscope for any attached parasites, lesions or external changes. After dissection, the abdomen, the internal organs of the fish were transferred to Petri dishes with a saline solution at 0.7 % NaCl and were examined under the stereomicroscope. The flukes collected from the stomach and intestines were washed out with normal saline (0.7% NaCl). The worm washing processes are repeated several times to remove any mucus or debris from their surface, then flukes were fixed in 5% formalin, flattened by repression and stained with aceto acid carmine. The specimens were then dehydrated in an alcohol gradient series, and mounted on Canada balsam as permanent slides. Taxonomic identifications of the digenean worms were based on Yamaguti (1971). Illustrations of the presented new species were made through the aid of a drawing tube.

3. Results

Proenenterum sp.n.

3.1 Taxonomic summary

Family: Lepocreadiidae (Platyhelminths, Digenea) Odhner, 1905

Type host: Common sea bream *Pagrus pagrus* fish (Family: Sparidae, Linnaeus, 1758)

Type locality: Gulf of Suez and Hurgada city of the Red sea, Egypt.

Type habitat and infection site: The adult worm was found in the pyloric portion of stomach and the middle part of the intestine of the infected fish.

Prevalence: 46 out of 140 (32.8%) fish were found to be naturally infected.

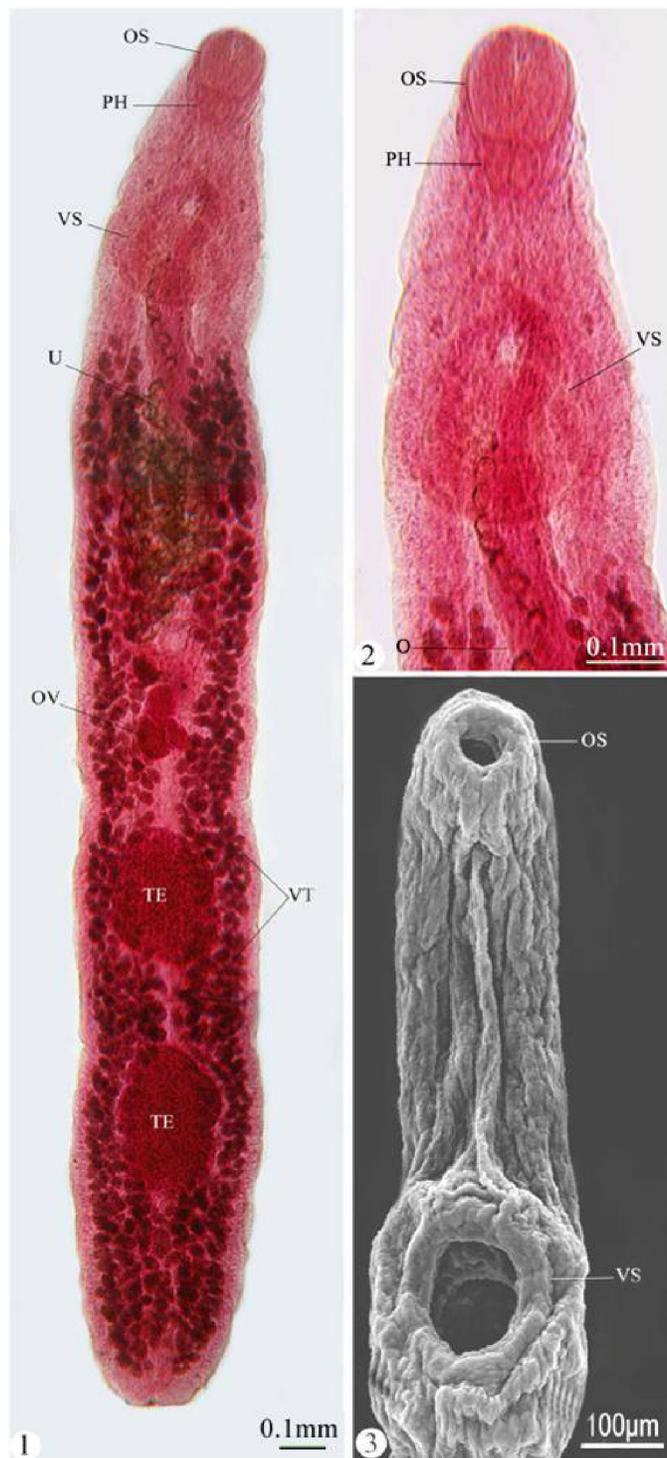
3.2 Description

The adult worm is elongated, dorso-ventrally flattened with more or less a narrow anterior and broadly rounded posterior end. It measures 2.30- 3.84 (mean 3.25±0.20) mm in length and 0.42- 0.54 (mean 0.48±0.02) mm in width (near the level of posterior testis). The oral sucker is 0.17-0.28 (mean 0.20±0.02) mm in diameter, the ventral sucker is spherical or subspherically-shaped and is larger than the oral one

measuring 0.26-0.39 (mean 0.31±0.02) mm in diameter. The pharynx is distinct, spherical to subspherical in shape measuring 0.12-0.25 (mean 0.17±0.02) mm in diameter, while the oesophagus is very short, looped and bifurcating into two long narrow intestinal caeca. It is clearly visible at the body constrictions and unites with a tubular, I-shaped excretory vesicle near the posterior end of the body to form an uroproct, while the excretory vesicle passes anteriorly terminating at a point just anterior to the posterior margin of the ovary. The anus is lacking and the excretory pore lies in the posterior extremity of the worm body. Testes are two in number, fusiform, tandem, lobed, separate and are located in the middle third of body. They are nearly equal in size measuring 0.32-0.48 (mean 0.37±0.02) mm in diameter. The cirrus sac is in the form of a large ovoid structure filling the space between the acetabulum and the intestinal bifurcation containing tubular coiled seminal vesicle. Pars prostatica is distinct, thick-walled and is surrounded by gland cells. The ejaculatory duct is short, narrow and the genital atrium is indistinct. The genital pore is located anterior to the intestinal bifurcation just adjacent to the posterior margin of oral sucker. The ovary is trilobed, entire and is found immediately in the pretesticular region. Mehlis' gland is found anterior to the ovary and the uterine seminal receptacle occupies the posterior coils of the uterus. Laurer's canal passes postero-ventrally and opens dorsally at the level of the anterior margin of the ovary. The uterus usually coils intercaecally between the anterior margin of the ovary and the posterior expanded portion of the seminal vesicle and then it passes to the genital aperture with little or no coiling. Vitellaria extend from the level of the intestinal bifurcation to the posterior end of the body locating laterally and dorso-ventral to the caeca filling the post-testicular space.

4. Discussion

Proenenterum is a genus of the digenetic trematodes belonging to family Lepocreadiidae established by Manter, 1954. Parasites within this genus are characterized by the presence of elongated aspinose body, unlobed oral sucker, with prepharynx, posteriorly united caeca, lacking of anus, gonads are tandem and close together, presence of two testes, cirrus sac largely preacetabular, lacking of the external seminal vesicle, uterus preovarian, seminal receptacle is present, eggs thin-shelled, pointed at their posterior ends and the excretory vesicle is I-shaped.



Figs.1, 2: Photomicrographs of the adult *Proenenterum* sp. infecting the common sea bream *Pagrus pagrus* fish showing the ventral sucker (VS), oral sucker (OS), testis (TE), ovary (OV), uterus (U), pharynx (PH), oesophagus (O) and the vitelline follicles (VT).

Fig.3: Scanning electron micrograph of the fore body of the adult worm showing the large-sized ventral sucker (VS) in correspondence to the oral one (OS).

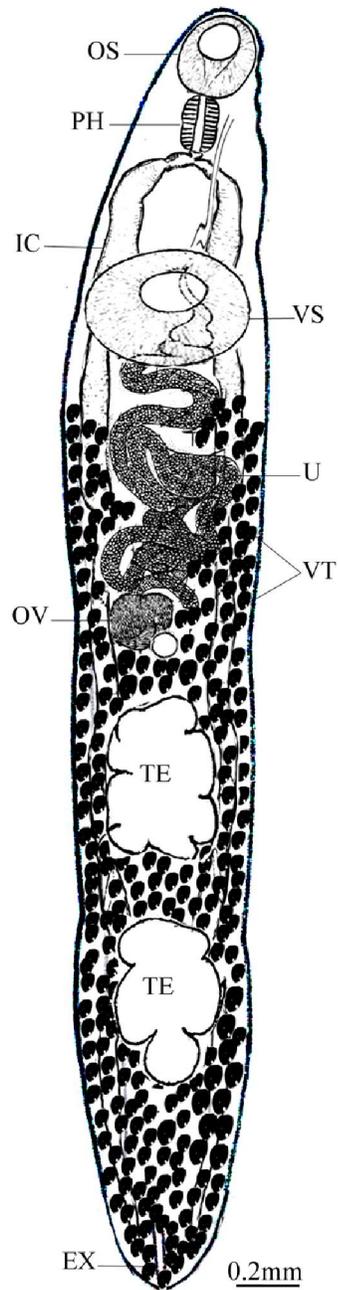


Fig.4: Line diagram of adult *Proenenterum* sp. oral sucker (OS), ventral sucker (VS), pharynx (PH), intestinal caeca (IC), testis (TE), ovary (OV), uterus (U), vitelline follicles (VT), excretory tube (Ex).

Two species of *Proenenterum* were previously reported from the intestine of *Nototheria macrocephala* fish from New Zealand, which are *P. isocotylum* and *P. ericotylum*. The present species morphology resembles the two later species, but with lower dimensions of the body parts. *P. isocotylum* is characterized by the presence of two lobed testes which is similar to the finding of the present study, but the ventral sucker is equal in dimension to the oral sucker instead of a large ventral sucker in our parasite. *P. ericotylum* differs in presence of unlobed testis but it supports the parasite under discussion in possessing a larger ventral sucker. The presence of two large testes and the cirrus sac suggests that this parasite belongs to genus *Enenterum* Linton, 1910. However, *Enenterum* sp. has an anus and an oral sucker with many processes, besides an external seminal vesicle. *Proenenterum* species has similar characters presented in members belonging to its family and more or less connects the unusual *Enenterum* and the related genera in the family Lepocreadiidae. Nagaty, 1948 has discussed new genera related to *Enenterum* including *Spiritestis*; *Hairana*; *Cadentella* and *Jeancaadenatra*. The latter two genera were elevated from subgenera of *Dollfus* 1946; these genera show a number of characters uncommon among trematodes as lobbing of the oral sucker, accessory ventral suckers, caecal union, anus, and the presence of a single testis. Only *Hairana* resembles *Proenenterum* in the absence of oral lobes, but it differs markedly in that, its caeca do not unite, it has a single testis and a pouch. All genera in the group have a well developed cirrus sac but the external seminal vesicle may be present or absent. In *Hairana* sp. no seminal vesicle was detected (Nagaty, 1948). According to these observations we consider the present parasite as first record from the common sea bream *Pagrus pagrus* fish.

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