World Applied Sciences Journal 26 (8): 1046-1052, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.26.08.8146

New Digenean Parasite Infecting the Freshwater Fish, *Clarias gariepinus* with Special Reference to its Tegumental Ultrastructure

[1]Elsayed, M. Bayoumy, [2]Sayed Abd El-Monem and [3]M.S. Abd El-Atti

[1]Hydrobiology Researches Department, National Research, Cairo, Egypt
[2]Zoology Department. Faculty of Science Cairo University, Cairo, Egypt
[3]Zoology Department Faculty of Science Zagazig University, Egypt

Submitted: Oct 11, 2013; **Accepted:** Nov 18, 2013; **Published:** Nov 30, 2013

Abstract: The following Lepocreadiid species are described. Fifty *Clarias gariepinus* fish samples were collected from Giza Province, Egypt, during the period from October 2010 to January 2011. It is found that, 12 out of 50 (24.5 %) fish were found to be naturally infected with a new species (*Pseudoholorchis clarii*) that is named according to the host harboring this parasite which is distinguished from other species of the same genus by some interspecific variations. The tegument of the present species consists of an outer syncytial layer which is folded into a series of ridges and grooves. The syncytial layer (external layer) is connected to the nucleated internal region across the fibrous discontinuous middle layer. The folds are interrupted by numerous cuticular spins; each one is enclosed at the apex and at the base by the corresponding unit membrane. The inner nucleated layer is consisted of epidermal cells arranged in two levels. The cells are conical in shape and have a large nucleus with prominent nucleoli. Large vacuoles are also observed between the epidermal cells containing granules of different shapes and sizes.

Key words: Parasites · Digenea *Pseudoholorchis clarii* · Transmission-Electron Microscope · Freshwater Fish · *Clarias gariepinus*

INTRODUCTION

There are three main groups of activities that contribute to food production: agriculture, aquaculture and fisheries. Parasites reduce fish production by affecting the normal physiology of fish and can result in mass mortalities of fish [1&2]. African mud catfish, *Clarias gariepinus* is the most sought after farmed fish species in Africa [3 & 4].

A few species of the digenean parasites have been reported from family Lepocreadiidae [5]. There are two major subfamilies in the Lepocreadiidae, the Lepocreadiinae and the Lepidapedinae. Bray & Gibson [6] pointed out that there were two main types of cirrus-sac in the lepocreadiids, with quite distinct morphologies. They called these the 'Opechonatype', haracterising the subfamily Lepocreadiinae and the 'Lepidapedon-type', characterising the Lepidapedinae. Further studies have shown that, in most cases, it is relatively easy to observe this distinction. Other characteristics often, but not invariably, co-vary with these features. For example, eye-spot pigment is usually found in the Lepocreadiinae, but rarely, if ever, in the Lepidapedinae. The basic distinction between the subfamilies is, however, the cirrus-sac structure. An 'Opechona-type' cirrus-sac has an internal male duct consisting of an oval to subglobular (occasionally sinuous), relatively thin-walled internal seminal vesicle, an oval to subglobular, vesicular pars prostatica lined with anuclear cell-like bodies and a relatively long, narrow, coiled or straight (often fairly thick-walled) ejaculatory duct which is rarely seen everted as a cirrus. A 'Lepidapedon-type' cirrus-sac has an internal male duct with a thick to very thick wall (except at the proximal extremity), a narrow and rectilinear internal seminal vesicle, a subglobular to oval pars prostatica embedded in the thick wall (lined with filamentous structures) and a short, narrow ejaculatory duct forming short conical cirrus. These cirrus-sac types have been

Corresponding Author: Elsayed M. Bayoumy, Hydrobiology Researches Department, N. R. C., Giza, Egypt.

described and figured by Bray & Gibson [6]. Utilising phylogenetic estimates inferred from molecular sequences, the superfamily Lepocreadioidea Odhner, 1905 is re-organised, with the major family, the Lepocreadiidae, split into three separate families, the Lepocreadiidae Odhner, 1905, Aephnidiogenidae Yamaguti, 1934 and Lepidapedidae Yamaguti, 1958. These families have been widely recognised as subfamilies. On the other hand, members of the family Lepocreadiidae have a gross morphology similar to members of the superfamily Allocreadioidea [7]. Here, we try to diagnose, describe and classify the parasite under investigation depending on its morphological recognition.

MATERIALS AND METHODS

Collection and Identification of Fish Samples: Fifty *Clarias gariepinus* fish samples were collected from the fish market at Giza Province (Elbahr Elazam area) in Cairo, Egypt, during the period from October 2010 to January 2011. They were examined within 24 hours after capture. The investigated fishes identified according to Bishai and Khalil [8]. They were found belonging to the Family: Clariidae.

Parasites Collection: The alimentary canal of each fish was opened using a fine scissors and a pair of forceps in a saline solution (0.7 %) and examined for 10 minutes in a Petri-dish with light shaking. Changing the physiological saline was repeated to clarify the parasites. The latter were washed again with the same saline to free from the adherent mucous or any other fibers. A brush or plastic pipette was used for this purpose. A binocular dissecting microscope was used in the helminthological examination.

Preparing Permanent Slides: Helminthic parasites were passed through the known technique applied for preparing permanent slides. They are relaxed according to Stoskoff [9], fixed with formalin acid alcohol. After fixation, the collected parasites washed several times for 15 minutes to remove the excess fixative and processed for staining in Acetic acid alum Carmine (for 1-2 hours); dehydrated in an ascending series of ethyl alcohol (30, 50, 70, 90, 96% and absolute alcohol) leaving the specimens for 3-5 minutes in each grade. The specimens were then cleared in Xylene, mounted in Canada balsam, covered with a cover glass and were left to dry in an oven at 40 °C.

Measurements: After the specimen was mounted on a slide, its outer borders and inner organs were measured. All measurements are in mm and were done after microscope calibration using a special slide with an additional ocular micrometer.

Transmission Electron Microscopy: The whole parasite was fixed in 3% Glutaraldehyde in 0.1 M Sodium cacodylate buffer (PH 7.3 – 7.4) for at least 4 hours at 4°C. After 4-5 washing in the same buffer, 10-15 minutes for each. Samples were postfixes with 2% (W/V) Os O4 for 1-2 hours, then washed again 4-5 times in the previous buffer, 20 minutes for each. A dehydration process was carried out in a graded series of Ethanol (10 minutes) for each. After that the parasites were treated with absolute Ethanol two times for each one for 10 minutes, then treated with Propylene oxide, two times for 15 minutes. Then samples were filtered with the solvent, embedded in Araldite mixture and finally incubated two days at 60 °C.

Semi-thin and ultra-thin sections were cut on a Reichert ultra cut RMC (MT 6000- XL, ultra microtome), with glass knives. Then the sections were stained with Tolidine blue for light microscope examination. While, ultra-thin sections were mounted on Copper graids, stained with saturated Alcoholic uranyl acetate for 20 minutes and Alkaline lead citrate for 1.2 minutes [10] and finally examined with JEM-100 S Jeol transmission-electron microscope.

RESULTS

Taxonomic Summary: Family: Lepocreadiidae (Platyhelminths, Digenea) Odhner, 1905

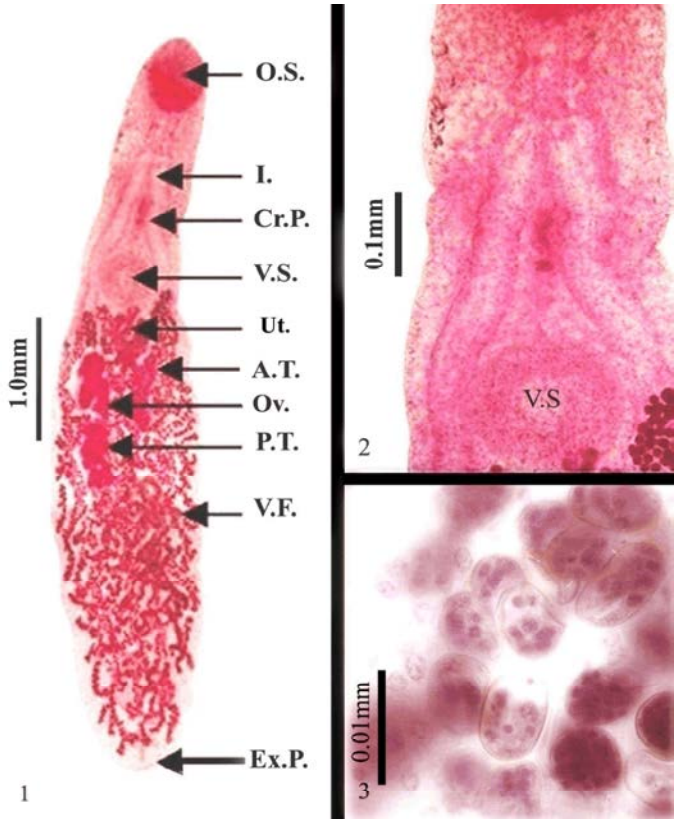
Type Host: *Clarias gariepinus*

Type Locality: Giza Province (El-Bahr El-Azam area) South Cairo, 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.

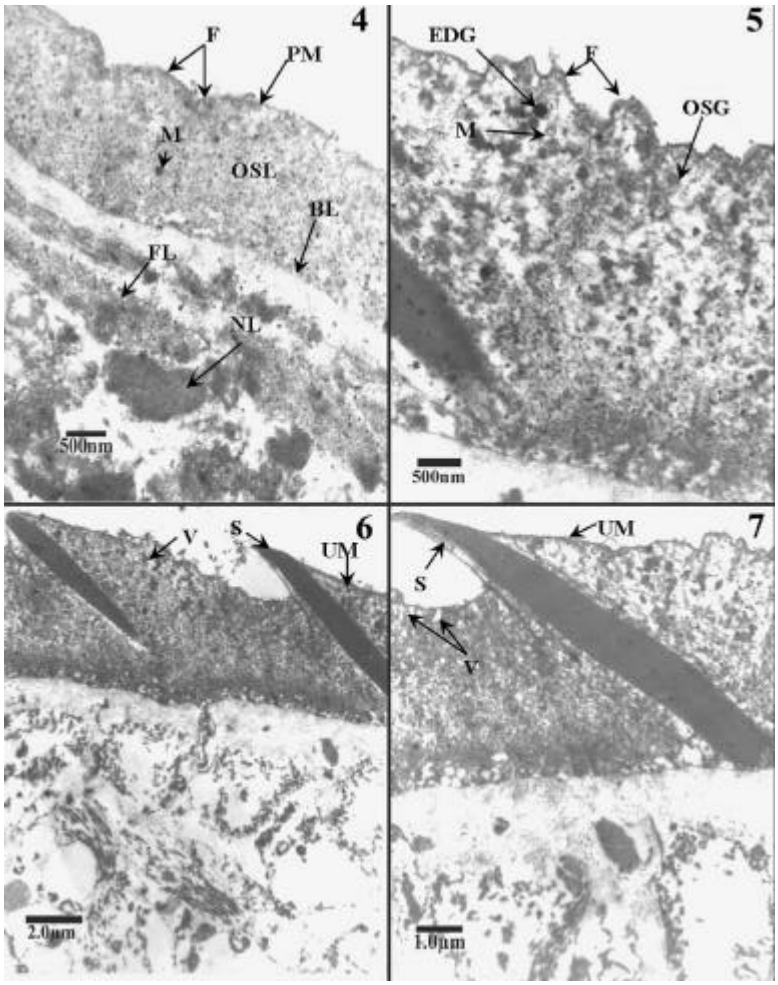
Prevalence: 12 out of 50 (24.5 %) fish were found to be naturally infected.

Etymology: This species (*Pseudoholorchis clarii*) is named according to the host harboring this parasite and it is deposited in the parasitological collection of the Zoology Department, Faculty of Science, Cairo University, Egypt under the number 1407.



Figs. 1-3: Photomicrographs of *Pseudoholorchis clarii* infecting *Clarias gariepinus*: 1. Whole mounted *Pseudoholorchis clarii* showing the ventral sucker (VS), oral sucker (OS), anterior testis (AT), posterior testis (PT), ovary (OV), uterus (Ut) and the vitellaria (VF).; 2. Higher magnification at the acetabular region showing the intestinal bifurcation and 3. Higher magnification of the eggs inside the uterus.

Description: The studied parasite appeared elongated, dorso-ventrally flattened with more or less a narrow anterior and broadly rounded posterior end. It measures 5.9 (5.20- 6.63) mm in length and 1.13 (0.94-1.35) mm in width (Fig. 1). The oral sucker (OS) measures 0.31×0.24 (0.26-036×0.18-0.31) mm in diameter. The ventral sucker (VS) is spherical or subspherically-shaped (Fig. 2) and is larger than the oral one measuring 0.44×0.42 (0.38-0.48×0.34-0.45) mm in diameter. The pharynx is distinct, spherical to subspherical in shape measuring 0.085×0.069 (0.078-0.091×0.062-0.075) mm in diameter, while the oesophagus is very short, looped and bifurcating into two long narrow intestinal caeca (I). It is clearly visible that there is 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 (Ex.P.) lies in the posterior extremity of the worm body. Testes are two in number; anterior testis, AT and a posterior one, PT. They are fusiform, tandem and lobed, separated from one another and are located in the middle third of body. They are nearly equal in size measuring 0.70×0.27 (0.67-0.73×0.24-0.3) mm. The cirrus pouch (Cr.P.) 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 (Ov); 0.50×0.22 (0.46-0.54×0.18-0.25) mm; is tri-lobed, 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 (Ut). 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. It is filled with eggs (Fig. 3) and then it passes to the genital aperture with little or no coiling.



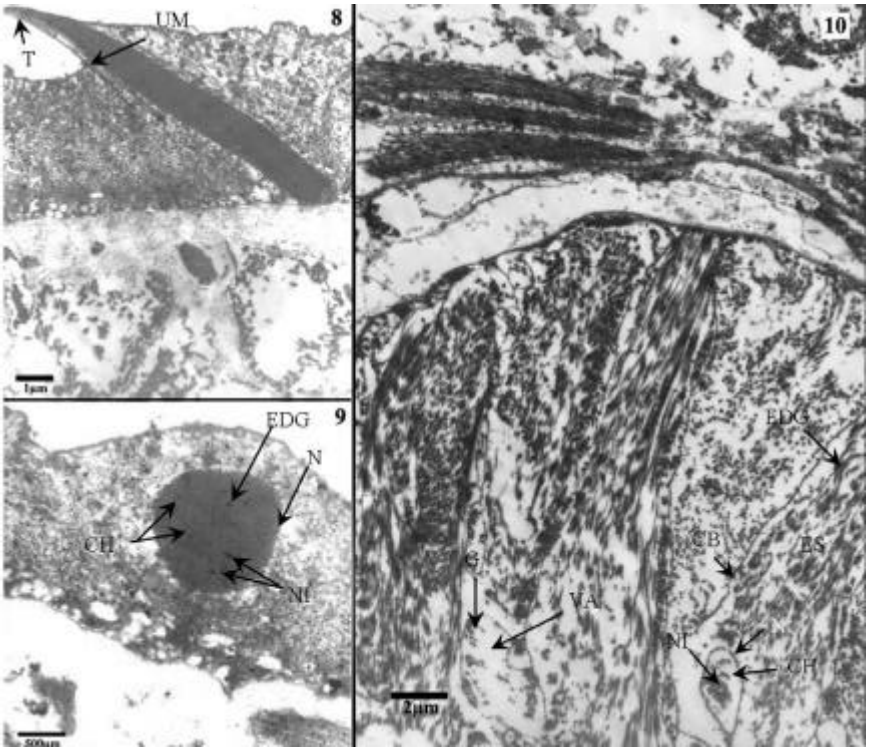
Figs. 4-7: Transmission electron micrographs of *Pseudoholorchis clarii* tegument.

Vitellaria (VF) 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, (Figs. 1, 2 &3).

Ultrastructure of Body Wall of the Parasite: The tegument of the present species consists of an outer syncytial layer (OSL) folded into a series of ridges and grooves (F). This is limited externally by a plasma membrane (PM) and by a basal lamina (BL) internally. The syncytial layer (external layer) is connected to the nucleated internal region (NL) across the fibrous (FL) discontinuous middle layer (Fig. 4). It separates the outer syncytial layer from the very thick transversely arranged discontinuous fibrous layer which represents the middle layer. The syncytial layer is characterized by the presence of numerous small mitochondria (M) and membrane-bound vesicles (V) with mainly electron-opaque fibrous contents, as well as several inclusions. This layer is connected to an inner nucleated layer (Figs. 5, 6). The outer syncytial layer bears numerous scattered folds and containing numerous mitochondria, as well as two types of secretory inclusions (Fig. 5). The first type is an electron-dense granules (EDG) which are numerous and scattered among marginal folds, while the 2nd type is an opaque secretory granules (OSG) in the form of irregular bodies.

The folds are interrupted by numerous cuticular spins, S (Fig.6). Each one is enclosed at the apex and at the base by the corresponding unit membrane (UM) (Figs. 7& 8). In all instances where contract was made, the tip (T) of the spine (Fig. 8) was bent indicating that the spine possess considerable flexibility.

The inner nucleated layer consisted of epidermal cell (ES) bodies (Fig. 10). These epidermal cells are arranged in two levels. Thus, there is an outer superficial layer and an inner inferior layer which are separated by cytoplasmic bridges (CB). The cells are conical in shape



Figs. 8-10: Transmission electron micrographs of *Pseudoholorchis clarii* tegument.

and contain a large nucleus (N) (Fig. 9) with prominent nucleoli (NI). They also contain electron dense granules (EDG) in the form of large spherical and disc-shaped granules. The nuclei of these cells are irregular in shape containing some dense chromatin patches (CH) (Fig. 9). Large vacuoles (VA) are observed between the epidermal cells which contain granules (G) of different shapes and sizes (Fig. 10).

DISCUSSION

The present work denoted that the present parasite is easily distinguished from other species of the same genus by the following interspecific variations including the body shape which varied greatly from oval to subspherical; the oral sucker may be equal or larger than the acetabulum; the oesophagus was short and the intestinal furca have a T-shaped appearance; the ovary and testes were preacetabular in position; the ovary was lobed (about 3 lobes) and was partly overlapping the right testis and the vitellaria that extended halfway between the oral and ventral suckers.

Lepocreadiidae: Lepocreadiinae. Body elongate-oval. Tegument spinose. Oral sucker subglobular, subterminal. Ventral sucker rounded. Prepharynx short. Pharynx oval. Oesophagus distinct. Caeca long, blind; reaching close to posterior extremity. Testes tandem, in mid-hindbody; lobed. External seminal vesicle, long, coiled. Cirrus-sac present. Internal seminal vesicle oval. Pars prostatica vesicular, subglobular. Ejaculatory duct long, coiled. Cirrus often everted. Genital pore median, at level of pharynx. Ovary lobed, pretesticular, separated from anterior testis, adjacent to posterior margin of ventral sucker. Canalicular seminal receptacle and Laurer's canal present. Uterus pre-testicular, intercaecal, mostly postovarian. Eggs tanned, operculate, without filament. Vitellarium follicular, reaches to about level of ventral sucker. Excretory pore terminal; vesicle reaches to posterior testis. Comparing the characteristics of the parasite under investigation, it is clear that there is a close similarity with Pseudoholorchis pulcher previously described by Manter [11] from *Latridopsis ciliaris*, but with some measurement differences. As designed by Woo [12] &Cohen *et al*.[13], the digenean integument consists of an external layer of cytoplasm joined by cytoplasmic bridges, with nucleated masses of cytoplasm lying internal to the body musculature. The syncytial tegument confers a number of paramount advantages on parasitic organisms. First, the absence of cell boundaries means it is less ulnerable to attack and breakdown by host agents, such as digestive enzymes, detergent bile acids and

components of the immune system. Secondly, it means that movement of substrates and ions is possible without restriction. The sunken nucleated region is distant from any adverse influence of the host. The separation of the nucleated regions, although attached to the same cytoplasmic mass, allows regional differentiation and specialization [14].

The tegument of the parasite under discussion is formed of an outer syncytial layer, while the most inner part of the tegument is characterized by the presence of epidermal cells in which the nuclei are located. Thus, the nuclei did not occur in the surface coat of the cytoplasm. These results indicated that the tegument of the present trematodes resembles that of monogenean; endoparasitic digeneans and cestodes so far described [15-18]. The outer layer of the present parasite bears scattered folds, while that of *Euclinostomum ardeolae* described by Ahmed *et al*. [18] is folded into a series of ridges and grooves which agreed with observations reported by many authers [16, 17,19]. The function of these folds may be either for support or to present an extended area of the body surface [20].

Mitochondria are present in the outer syncytial layer but they are few in number in the tegument of *Cynodiplostomum duboisi* [18] and they are numerous in *Euclinostomum ardeolae* of the previous authors. The few mitochondria suggested that the tegument is largely protective in function and probably has limited absorptive function suggested by Dunn *et al*. [16]. On the other hand, the tegument of the presented worm bears numerous cuticular spines which agreed with the results reported by Burton [20] for *Haematoloechus medioplexus*, Morris and Threadgold [15] for *Schistosoma mansoni* and Shaw [19] for *Diplectanum aequans*. Moreover, the tegument is characterized by the occurrence of numerous secretory bodies which agreed with similar observations obtained by Dunn *et al*. [16] for *Gigantocotyle explanatum*; *Gastrothylax crumenifer* and *Srivastavaia indica*. Tegumental secretory bodies have been ascribed several diverse functions [22]. Thus, Dunn *et al*. [16] have suggested that tegumentary secretory bodies have an immunoprotective function for flukes against host immunological attack.

From the above description it is clear that the parasite under investigation can be identified as *Pseudoholorchis clarii*, belonging to Family Lepocreadiidae Odhner, 1905, subfamily Lepocreadiinae and the genus: *Pseudoholorchis* Yamaguti, 1958.

REFERENCES

1. Eissa, I.A.M., V.H. Zaki, N.G. Ali, M. Zaki and I.M. Aboyadak, 2013. Gut digeneaiasis in African catfish *Clarias gariepinus* with estimating the efficacy of some anthelmintics. Life Sc. J., 10(3): 1605-1609.
2. Hassan, A.A., B. Akinsanya and W.A. Adegbaju, 2012. Impacts of Helminth Parasites on *Clarias gariepinus* and Synodontis clarias from Lekki Lagoon, Lagos, Nigeria. Report and Opinion, 2(11): 42-48.
3. Amisah, S., M.A. Oteng and J.K. Ofori, 2009. Growth performance of the African catfish, *Clarias gariepinus*, fed varying inclusion levels of Leucaena leucocephala leaf meal. J. Appl. Sci. Environ. Manage., 13(1): 21-26.
4. Olumuji, O.K. and M.K. Mustapha, 2012. Induced Breeding of African Mud Catfish, *Clarias gariepinus* (Burchell 1822), using Different Doses of Normal Saline Diluted Ovaprim. J. Aquacult. Res., 3(4): 1-3.
5. Bray, R.A. and T.H. Cribb, 1997. The subfamily Aephnidiogeninae Yamaguti, 1934 (Digenea: Lepocreadiidae), its status and that of the genera Aephnidiogenes Nicoll, 1915, *Holorchis* Stossich, 1901, *Austroholorchis* n. g., *Pseudaephnidiogenes* Yamaguti, 1971, *Pseudoholorchis* Yamaguti, 1958 and Neolepocreadium Thomas, 1960. Sys. Parasitol., 36: 47-68.
6. Bray, R.A. and D.I. Gibson, 1989. The Lepocreadiidae (Digenea) of fishes of the north-east Atlantic: review of the genus *Neolepidapedon* Manter, 1954, with a description of N. smithi n. sp. Sys. Parasitol., 13: 11-23.
7. Bray, R.A. and T.H. Cribb, 2012. Reorganisation of the superfamily Lepocreadioidea Odhner, 1905 based on an inferred molecular phylogeny. Sys. Parasitol., 83: 169-177.
8. Bishai, H.U. and M.T. Khalil, 1997. Freshwater fishes of Egypt Publication of National Biodiversity Unit. No. 9.
9. Stoskoff, M.K., 1992. Fish Medicine. W. B. Saunders Company, Philadelphia, pp: 882.
10. Reynolds, E.S., 1963. The use of lead citrate at high PH as an electron-opaque stain in electron microscopy. J. Cell Biol., 17: 208-212.
11. Manter, H.W., 1954. Some digenetic trematodes from fishes of New Zealand. Tran. Royal Soc. New Zealand, 82: 475-568.

12. Woo, P.T.K., 2006. Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections Second Edition. UK by Biddles, King's Lynn.
13. Cohen, S.C., A. Kohn, M. Batista-Farias and F. Diniz, (2001). Ultrastructure of the tegument of *Saccocoelioides godoyi*. J. Helminthol., 75: 15-21.
14. Halton, D.W., 1997. Nutritional adaptations to parasitism within the Platyhelminthes. Inter. J. Parasitol., 27: 693-704.
15. Morris, G.P. and L.T. Threadgold, 1968. Ultrastructure of the tegument of adult *Schistosoma mansoni*. J. Parasitol., 54: 15-27.
16. Dunn, T.S., R.E.B. Hanna and W.A. Nizami, 1987. Ultrastructural and cytochemical observations on the tegument of three species of paramphistomes (Platyhelminthes: Digenea) from the Indian water buffalo, *Bubalus bubalis*. Inter. J. Parasitol., 17: 1153-1161.
17. Mattison, R.G., S.M.A. Abidi, W.A. Nizami and R.E.B. Hanna, 1994. Preliminary studies in numerical systematics of Paramphistomoidea (Digenea: Trematoda) from domestic Artiodactyla of Northern India. Sys. Parasitol., 27: 81-103.
18. Ahmed, A.K., G.A. El-Shahawi, S. Abd El-Monem and A.R. Bashtar, 1998. Electron microscopic study of metacercaria cyst wall and adult tegument of *Cynodiplostomum duboisi* (Diplostomatidae Poirier, 1886) and *Euclinostomum ardeolae* (Clinostomatidae Luhe, 1901). Egypt. J. Zool., 30: 337-351.
19. Shaw, M.K, 1980. The ultrastructure of the epidermis of *Diplectanum aequans* (Monogenea). J. Parasitol., 8: 9-21.
20. Lyons, K.M., 1970. The fine structure and function of the adult epidermis of two skin parasitic monogeneans, *Entobdella soleae* and *Acanthocotyle elegans*. Parasitol., 60: 39-52.
21. Burton, P.R., 1964. The ultrastructure of the integument of the frog lung-fluke, *Haematoloechus medioplexus* (Trematoda: Plagiorchiidae). J. Morphol., 115: 305-18.
22. Lumsden, R.D., 1975. Parasitological review: surface ultrastructure and cytochemistry of parasitic helminths. Exp. Parasitol., 37: 267-339.