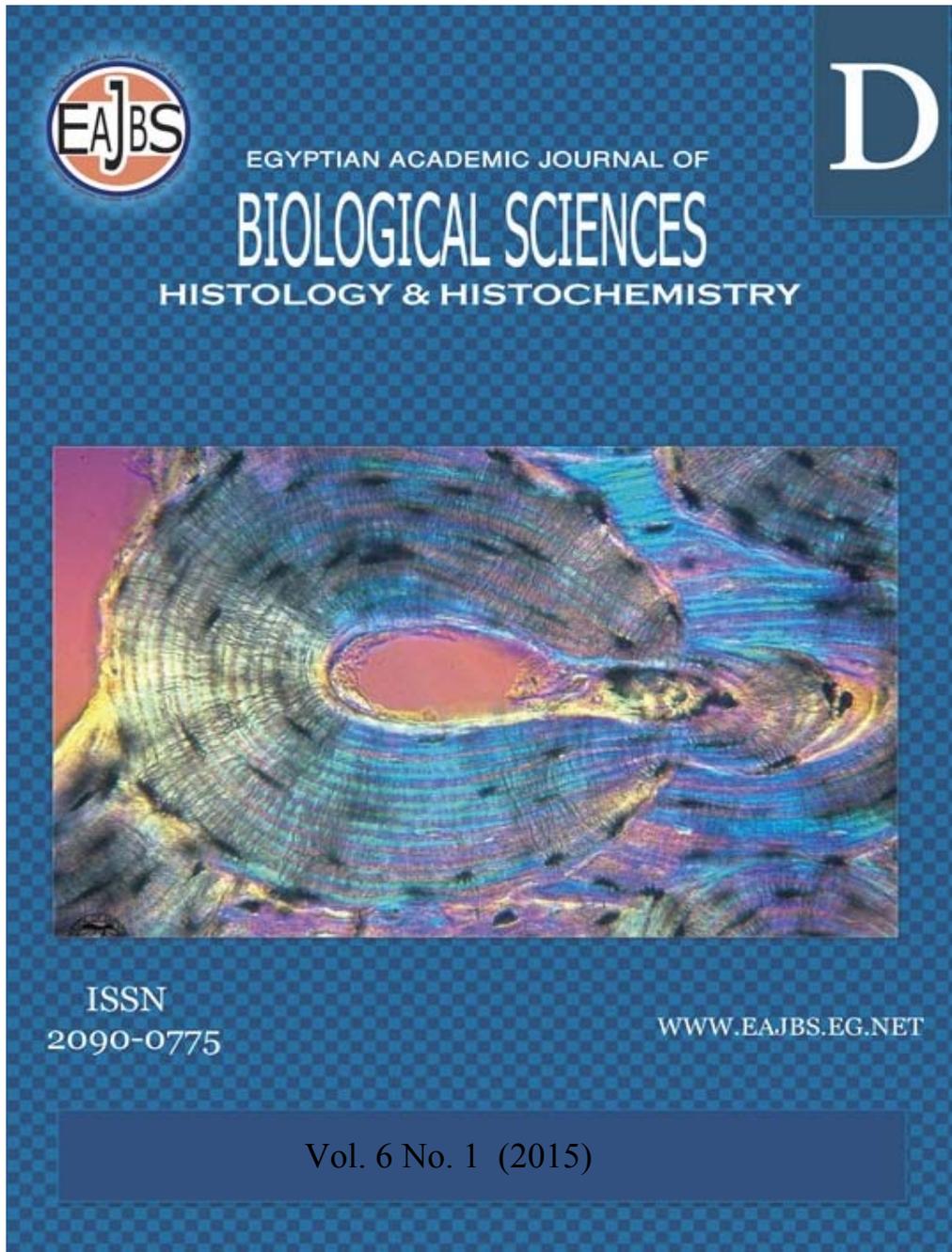


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences ,Department of Entomology ,Faculty of Sciences Ain Shams University .

Histology& Histochemistry Journal include various morphological, anatomical, histological, histochemical, toxicological , physiological changes associated with individuals, and populations. In addition, the journal promotes research on biochemical and molecular-biological or environmental, toxicological and occupational aspects of pathology are requested as well as developmental and histological studies on light and electron microscopical level, or case reports.

www.eajbs.eg.net



SEM Study on Morphology and Surface Topography of *Wenyonia minuta* Woodland 1923 (Cestoda: Caryophyllidea) and the histopathological Consequences on *Synodontis filamentosus* (boulenger, 1901) from Lekki Lagoon, Lagos – Nigeria.

Akinsanya, B.¹; Hassan, A.A.². and Adeogun, A. O.².

1- Department of Zoology, University of Lagos

2- Department of Zoology, University of Ibadan

ARTICLE INFO

Article History

Received: 22/1/2015

Accepted: 25/3/2015

Keywords:

SEM

Morphology

Surface topography

Wenyonia minuta

Histopathological analysis

ABSTRACT

The SEM study on morphology and surface topography of *Wenyonia minuta* was undertaken. The parasite was recovered from the intestine of *Synodontis filamentosus*. A total of two hundred and forty five specimens of the fish host were examined. The female specimens (n=88) has a prevalence of 69.3% while the male specimens (n = 157) had a lower prevalence of 46.4%. The SEM description of the caryophyllidea cestode revealed the scolex to be fluke – like with four shallow longitudinal groove and flat pointed anterior end. The overall length of the scolex is 104250.97µm with a breadth of 653451.20µm. The furrow longitudinal groove were only observed on the dorsal side of the scolex. External protuberances were also reported in this study. The histopathological analysis shows matting, lymphocytic infiltration of the lamina propria, goblet cell hyperplasia while some sections with no significant pathological changes were also observed.

INTRODUCTION

The caryophyllaeidae cestodes are parasitic in the intestine of freshwater teleost fishes, mainly cyprinids, catfishes and the catostomids. A few of them also parasitise the coelom of freshwater oligochaetes.

Mackiewicz (1994) reported that tapeworms of the family caryophyllaeidae have been reported in Africa, Europe, Australia and North America. The genus *Wenyonia* was created by Woodland 1923 based on their peculiarity differing from all other members of the caryophyllaeidae by the possession of the sexual apertures in the anterior half of the body and a uterus whose longitudinal extent is at least equal to that of the testes.

The genus is known and reported by several authors in Africa such as Akinsanya *et al.* 2008, Ukoli 1968, Ugwuzor 1987, Origbo 1987, Otebele 1989 and Okaka, 1991. The genus being found in the siluriform fishes use tubificid oligochaetes and small fishes as intermediate hosts.

The fish host, *Synodontis filamentosus* is of great commercial value in Africa. Willoughby (1974) and Berra (1981) reported that the fish species is restricted to water systems within the tropics. They also feed mainly on chironomids and are caught mainly in inshore waters (Willoughby 1974). Gosse (1986) reported that *S. filamentosus* are dioecious fertilization external, oviparous and show distinct pairing during breeding.

Parasitic diseases of fish constitute one of the major problems confronting modern fish culturists. The pathological conditions arising from such infections assume a high magnitude causing significant mortalities among infected fish species. There is limited information on the fish parasite ultrastructure in any Nigerian freshwater body. The aim of this study was to obtain data on the surface topography of the helminth parasites using SEM in order to demonstrate congruency with existing descriptions or to provide unreported morphological features based on the limitations of light microscope.

The present study focuses on the ultrastructure of *Wenyonia minuta* and pathological effects of the parasite on the fish hosts.

MATERIALS AND METHODS

Study Area

Lekki Lagoon supports a major fishery in Nigeria. The lagoon is located in Lagos State Nigeria and lies between longitudes 4°00' and 4°15'E and between latitudes 6°25' and 6°37' N. It has a surface area of about 247km² with a maximum depth of 6.4m; a greater part of the lagoon is shallow and less than 3.0m deep. The Lekki lagoon is part of an intricate system of waterways made up of lagoons and creeks that are found along the coast of south-western Nigeria from the Dahomey border to the Niger Delta stretching over a distance of about 200km. It is fed by the River Oshun and Saga discharging into north-western parts of the lagoon.

Lekki lagoon experiences both dry and rainy seasons typical of the southern part of Nigeria. The vegetation around the lagoon is characterized by shrub and *raphia* palms, *Raphia sudanica* and oil palms, *Elais guinensis*. Floating grass occur on the periphery of the lagoon while coconut palms *Cocos nucifera* are widespread in the surrounding villages. The rich fish fauna of the lagoon includes *Heterotis niloticus*, *Gymnarchus niloticus*, *Clarias gariepinus*, *Malapterurus electricus*, *Synodontis clarias*, *chrysichthys nigrodigitatus*, *Parachanna obscura*, *Mormyrus rume*, *Calabaricus calamoichthys*, *Tilapia zilli*, *Tilapia galilaeus*, *Hemichromis fasciatus* and *Sarotherodon melanotheron*. Fig. 1 shows map of Lekki lagoon, Lagos, Nigeria

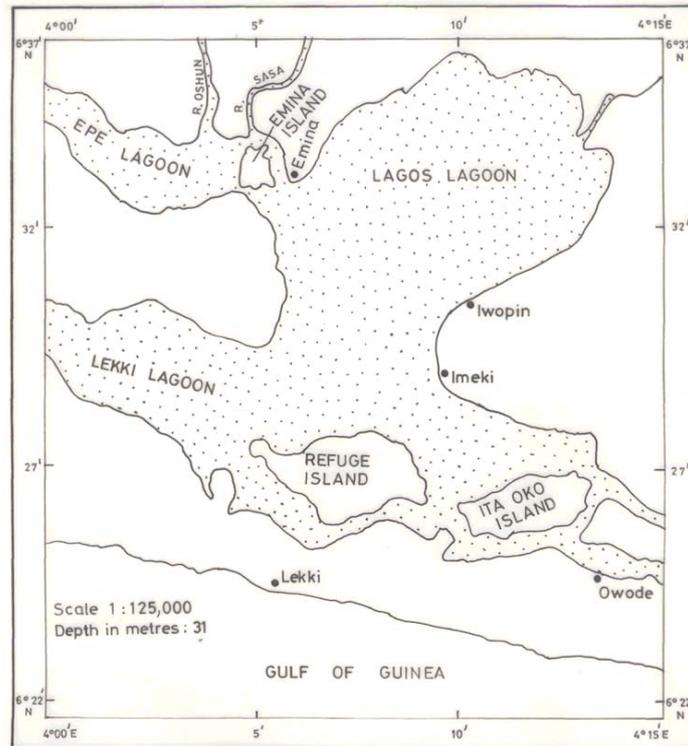


Fig. 1: The map of Lekki Lagoon, Lagos, Nigeria.

Collection and Examination of Specimens

A total of two hundred and forty-five specimens of *Synodontis filamentosus* were subjected to parasitologic investigations. The fish species were procured at Oluwo Market Epe. They were allowed to stabilize initially and were later examined for the presence of parasites. One hundred and fifty seven male ($n = 157$) and eighty eight female ($n = 88$) of the fish specimens were examined. The fish specimens were later dissected for the isolation of the parasites. The presence of parasites is observed by their wriggling movement. They were removed and fixed in 70% alcohol. The parasites were later sent to Natural History Museum, United Kingdom for their identification to species level.

Histopathological Analysis

The infected fish tissues of *Synodontis filamentosus* were fixed in Bouin's fluid for 7 hours and the fixative were later decanted and replaced with 10% phosphate buffered formalin. The

dehydration of the tissues took place in increasing concentrations of alcohol and then twice in absolute alcohol at 30 minutes interval. All the recovered parasites were from the intestinal tissues and were then embedded in paraffin wax, blocked, sectioned and stained with haematoxylin and eosin. The stained tissues were washed off in tap water and the over stained ones destained in 1% acid alcohol. The tissues were mounted using DPX mountant, dried and examined under the microscope.

Scanning Electron Microscopy

Worms to be scanned were washed in phosphate buffered solution at 15 minutes interval to remove debris and to hold pH (7.4) steady during the fixation process after being fixed in 2.5% glutaraldehyde. Post fixation was done accordingly in 1% osmium tetroxide (O_5O_4) for 2 hours. This is followed by dehydration through series of alcohol in increasing concentrations (50 – 100% washes), critical point dried and sputter-coated with gold. They were then examined using the Tescan SEM VEGA

of Rhodes University, Grahamstown, South Africa. Worms with scoleces were deposited at Natural history Museum, parasitic worm division, United Kingdom.

RESULTS

Prevalence of infections in the fish species

The overall prevalence in *Synodontis filamentosus* was 54.7%. The female specimens had a prevalence of 69.3% while the male specimens had a prevalence of 46.4%. A total of sixty one and seventy three female and male specimens were infected respectively.

Wenyonia species have been parasitizing the fish species of the family mochokidae. This study employed the use of scanning electron microscope to describe the surface topography of the parasites. A total of one thousand, eight hundred and twenty parasites were recovered from the fish species.

SEM Description of *Wenyonia minuta*

Wenyonia minuta (Woodland 1923) Syn. *Wenyonia* McConelli Ukoli, 1972.

Specimens of *Wenyonia minuta* (Woodland, 1923) was recovered from the intestine of *Synodontis filamentosus* from Epe lagoon, Lagos, Nigeria.

The following description is based on examination of whole specimen of the Caryophyllidea cestode.

The scolex is fluke like with four shallow longitudinal furrow (Fig. 2 A) or groove and with short neck. (Fig. 2 B) The length of the scolex is 1042450.97 μm with a breadth of 653451.20 μm . Scolex broad at the middle and pointed (Fig. 2 C) towards the anterior end. Tegument rough towards the posterior end of the cestode (Fig. 2 D-E) and smooth at the middle (Fig. 2 F). Scolex with three cephalic alae on dorsal side with lateral cephalic papillae. (Fig. 2 G) The circumference of the scolex measures 470571.70 μm^2 . The scolex is arrow

shaped with flat pointed anterior end. The tegument width immediately behind the short neck is 671312.06 μm .

The measurement of the tegument width towards the caudal region are 658561.88 μm , 661878.11 μm and 591618.12 μm respectively. This implies that the tegument width varies from the short neck towards the posterior end. The whole worm length measures 10189235.11 μm . Tegument smooth at the cephalic end, and rough towards the middle to the caudal end. The excretory pore lateral and circular and has a breadth of 1964.86 μm with a lateral papillae near it. There is a conspicuous left lateral diagonal groove with a length of 32783.55 μm . There are five consecutive lateral longitudinal papillae on the right side of the tegument.

The caudal region curved inward on the left side with inconspicuous curve on the right side. A single caudal tegumental alae also present close to the end of the body. Fig. 2 A-F shows the SEM photomicrograph of *Wenyonia minuta*

Histopathology Consequences of *Wenyonia minuta* on *Synodontis filamentosus*

The results of the histopathological consequences of *Wenyonia minuta* on *Synodontis filamentosus* shows different pathological effects. The sections shows diffuse necrosis of surface epithelium with matting and shortening of intestinal villi. Mild lymphocytic infiltration of the lamina propria was also observed.

Denudation of the intestinal epithelium with marked goblet cell hyperplasia was also observed. Hyperplasia of lymphoid aggregates within the submucosa was also evident. Diffuse and multifocal infiltration of epithelium with numerous inflammatory cells was also seen. In some other sections, well structured villi with intact epithelium coupled with no significant pathological changes were also observed. Fig 3 A - D shows the

pathological changes observed in the fish intestine.

DISCUSSION

The Scanning Electron Microscopy study and the attendant effects of *Wenyonia minuta*, on *Synodontis filamentosus* was carried out. Akinsanya and Otubanjo (2006) had earlier recovered *Wenyonia* species from *Clarias gariepinus* and Akinsanya and Otubanjo (2008) also reported *Wenyonia* sp from *Synodontis clarias*.

Documentation by Khalil and Pooling (1997) reported the presence of *Wenyonia minuta* in *Chrysichthys auratus*. (Geoffroy Saint, Hilaire) (Siluriformes: claroteidae). This *Caryophyllaeid cestode* in this study was not however reported by Akinsanya *et al.* (2007) on helmeted bioload of *Chrysichthys nigrodigitatus*.

Woodland (1923) found the genus *Wenyonia* to differ remarkably from all members of the *Caryophyllaeidae* by the possession of the sexual apertures in the anterior half of the body and a uterus whose longitudinal extent is at least equal to that of the testes.

Ukoli (1972) on the occurrence, morphology and systematic of cestodes of the genus *Wenyonia* also recovered *Wenyonia McConelli* which he confirmed to be the same with *W. minuta*. The description of Ukoli (1972) on *W. minuta* that the body is fluke is also in conformity with this study which also reported the fluke-like nature of the scolex and a pointed anterior end of the apical introversion.

Ukoli (1972) and Bjoern *et al* (2011) reported 7 – 8 and 21 – 39 shallow longitudinal grooves respectively. The *Wenyonia minuta* described in this study differ remarkably from the description of Ukoli (1972) and Bjoern *et al* (2011) in having between 1 – 4 conspicuous longitudinal grooves only on the dorsal side of the scolex. Longitudinal grooves were not seen or

observed on the ventral side of the described *Caryophyllaeidae cestode* as reported by Ukoli, (1972). The extent of the deepness of the shallow longitudinal groove on the scolex is also not reported in this study. The SEM description of *Wenyonia minuta* in this study reported some external protuberances such as papillae on both lateral and dorsal region of the cestode. These structures were not reported by Ukoli (1972) and Bjoern (2011). *Wenyonia McConelli* and *W. minuta* were reported to differ in the shape of the scolex by Bjoern (2011) and that *W. minuta* is a distinct species, well separated morphologically and genetically from *W. McConelli*.

The posterior end of the described *W. minuta* in this study showed an inward side curved on both sides which is more pronounced on the left lateral side. There exist some variations in the morphological details of Nigerian freshwater fish parasites to the already described ones in different geographical locations.

Histopathological observations in the intestinal mucosa of the fish hosts shows different pathological effects. Akinsanya (2007) on histopathological study on the parasitized visceral organs of some fishes of Lekki Lagoon reported various pathological conditions in the examined fishes. In this study several pathological conditions of the intestinal mucosa were also reported.

Banhawy *et al.*, (1975) also reported degenerative changes in the gut wall, liver, and pancreas of *Synodontis schall* as a result of *Wenyonia virilis* infection. Mitchell and Hoffman (1980) also reported severe damage to the intestine (obstruction and enteritis) caused by *Caryophyllidean cestode* infecting cultured European and Chinese carp. This is in agreement with this present study which also reported several pathological changes such as matting and shortening of intestinal villi, mild lymphocytic infiltration of the lamina

propria and most importantly goblet cell hyperplasia. Adeyemo and Agbede (2008) on histopathology of tilapia tissues harbouring *Clinostomum tilapiae* parasites reported proliferation of eosinophiles at the secondary lamellar of the gills. *Clinostomum* species were not however reported in the present study. Different authors such as Imam (1971) Tadros *et al.*, (1979) and Nassef (1988)

reported several histopathological alterations in the intestinal wall of fishes due to parasitic helminthes. The histopathological effects reported in this study agreed with the report of Longshaw (2012) on Lymphocytic infiltration of Lamina propria, goblet cell hyperplasia and hyperplasia of lymphoid aggregate within the submucosa.

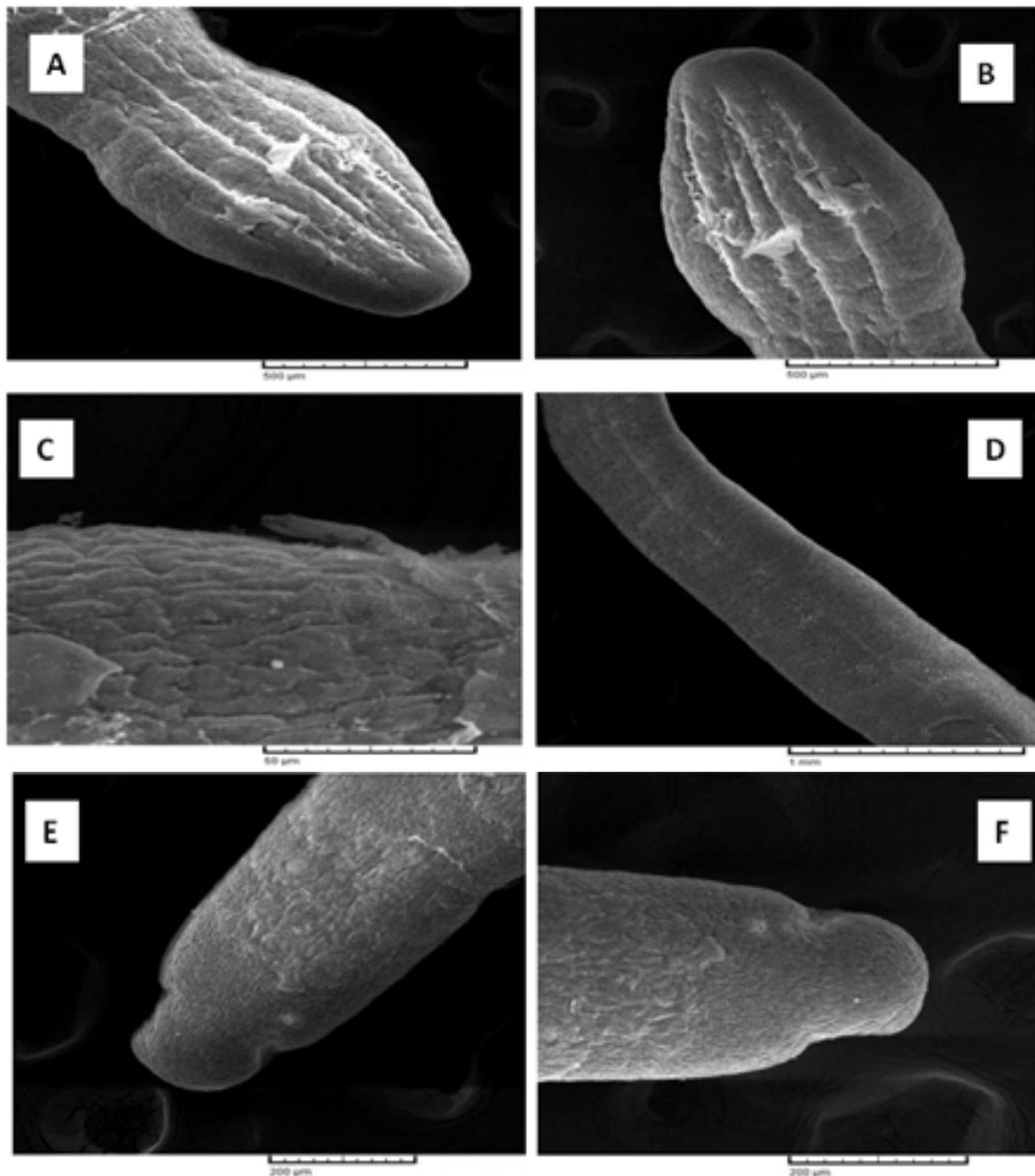


Fig. 2: SEM micrographs of *wenyonia minuta*. **A** - Cephalic region of *wenyonia minuta* longitudinal furrow, **B** - Anterior region showing the short neck, **C** - lateral papillae in *Wenyonia minuta*. **D** - Tegument of *Wenyonia minuta*, **E** - Shows the pointed anterior end, **F** - Sublateral view of posterior end of *Wenyonia minuta*, Posterior extremity, and ventral view.

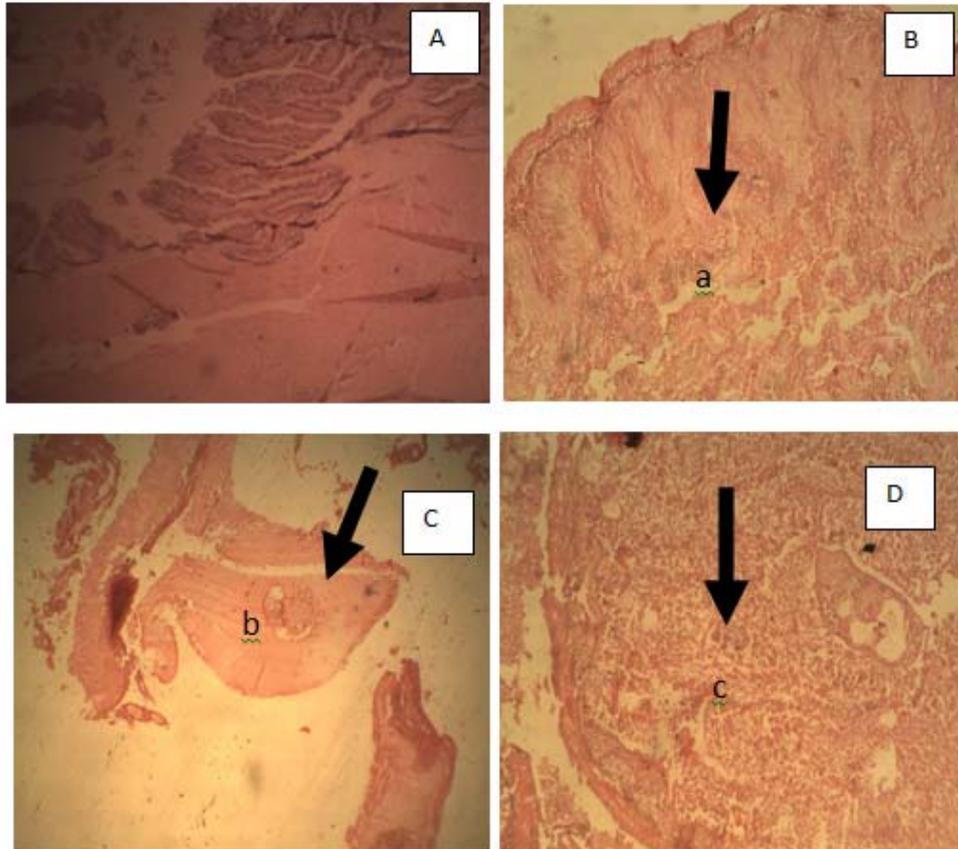


Fig. 3A - D: Section through the Intestine of *Synodontis filamentosus* showing different Pathological conditions. (A), Normal section. (B), a- Marked thickening and hyperplasia of intestinal mucosa with sloughing of the epithelium and accumulation of necrotic debris within the lumen. (C), b - Multifocal necrosis of smooth muscle of intestinal wall. (D), c - Intraluminal nematode parasite surrounded by thick sloughed epithelial cells mixed with inflammatory debris X 100

REFERENCES

- Adeyemo, A.O. and Agbede, S.A. (2008). Histopathology of *Tilapia* tissues harbouring *Clinostomum tilapiae* parasites. *African Journal of Biomedical Research*. 11: 115-118.
- Akinsanya, B., & Otubanjo, O. A. (2006). Helminth parasites of *Clarias gariepinus* (Clariidae) in Lekki Lagoon, Lagos, Nigeria. *Revista de Biología Tropical*, 54, 93–99.
- Akinsanya, B., Hassan, A. A., & Adeogun, A. O. (2008). Gastrointestinal helminth parasites of the fish *Synodontis clarias* (Siluriformes: Mochokidae) from Lekki Lagoon, Lagos, Nigeria. *Revista de Biología Tropical*, 56: 2021–2026.
- Akinsanya, B., Hassan, A.A. and Otubanjo, O.A. (2007a). A comparative study of the parasitic helminth fauna of *Heterotis niloticus* (Osteoglossidae) and *Gymnarchus niloticus* (Gymnarchidae) from Lekki Lagoon, Lagos, Nigeria. *Pakistan J. Biology* 10(3): 427-432.
- Banhawy, M. A., Anwar, I. M., & El-Naffar, M. K. (1979). Histochemical studies on the ileum and liver of the silurid fish, *Synodontis schall* infected with the tapeworm, *Wenyonia virilis*.

- Proceedings of the Egyptian Academy of Sciences, 29: 59-66.
- Banhawy, M. A., Saoud, M. F. A., Anwar, I. M., & El-Naffar, M. K. (1975). The histopathological effects of the parasitic tapeworm *Wenyonia virilis* on the ileum and liver of the silurid fish *Synodontis schall*. *Annals of Zoology*, 11: 83-101.
- Berra, T. (1981). An atlas of distribution of the freshwater fish families of the world. University of Nebraska. Lincoln, Nebraska. 191pp
- Gosse, J.P. (1986). Mochokidae. In: J. Dagel, J.P. Gosse and Thys D.F.E. Van den Audenacrd (eds.) Checklist of the freshwater fishes of Africa (CLOFF A). ORSTOM, Paris. 2: 105-152.
- Imam, E.A. (1971). Morphological and biological studies on the enteric helminthes infecting some of the Egyptian Nile fishes particularly *Polyonchobothrim clarias* of *Karmol clarias lazera* and *Clarias anguillaris*. Ph.D. Thesis, Parasitology. Cairo University.
- Longshaw, M (2012) Anisakis larvae ("herringworm"; Neematoda) in fish, 1-5. In *ICES identification leaflets for diseases and parasites of fish and shellfish*.
- Mackiewicz, J. S. (1994). Order Caryophyllidea van Beneden in Carus, 1863. In: Khalil, L. F., Jones, A., & Bray, R. A. (Eds) Keys to cestode parasites of vertebrates. Wallingford: CAB International, pp. 21-43
- Mitchell, A.J. and Hoffman, G.L. (1980). Important tapeworms of South American freshwater fishes. US Dept. Int. Fish and Wildlife Serv. Fish Diseases Leaflet. 59: 17pp.
- Nassef, T. M. N. (1988). Morphological studies on some gastrointestinal parasites of freshwater fishes. Vet. Thesis, Cairo Univ., Egypt.
- Origbo, K.B. (1987). Studies on the parasites of fish of Okhuaihe river. B.Sc. dissertation, University of Benin, Unpublished data.
- Otebele, O.N. (1989). Studies on Parasites of cultured fish in Ogba fish farm. B.Sc. dissertation, University of Benin, Unpublished data
- Okaka, C.E. (1991). A survey into helminth parasities of fishes of Asa river and its dam at Ilorin, Nigeria. *Journal of Experimental and Applied Biology*. 3: 12-18.
- Tadros, G.; Iskandar, A. R. and Wassef, N. A. (1979). On an intestinal cestode and acanthocephalan from the Nile and Red Sea fishes with histopathological study of their habitat. *J. of Egypt. Parasitol.*, 1(9):143-15
- Ukoli, F. M. A. (1972). Occurrence, morphology and systematic of caryophyllaeid cestodes of the genus *Wenyonia* Woodland, 1923 from fishes in River Niger, Nigeria. *Journal of the West African Science Association*, 17:49-67
- Ugwuzor, G.N. (1987). A survey of the Helminthic parasites of fish in Imo river. *Nigeria Journal of Applied Fisheries and Hydrobiology*, 2: 25 - 30.
- Ukoli F.M.A. (1968). Occurrence, morphology and Systematics of Caryophyllaeid cestodes of the genus *Wenyonia* Woodland, 1923 from fishes in River Niger, Nigeria. *Journal of West African Scientific Association* 17: 49 - 67.
- Willoughby, N.G. (1974). The ecology of the genus, *Synodontis* (Pisces: *Siluriodei*) in Lake Kainji, Nigeria. Ph.D. Thesis, University of Southampton, U.K. 288p.
- Woodland, W. N. F. (1923). On some remarkable new forms of Caryophyllaeidae from the Anglo-Egyptian Sudan, and a revision of the families of the Cestodaria. *Quarterly Journal of Microscopical Science*, 67: 435-47