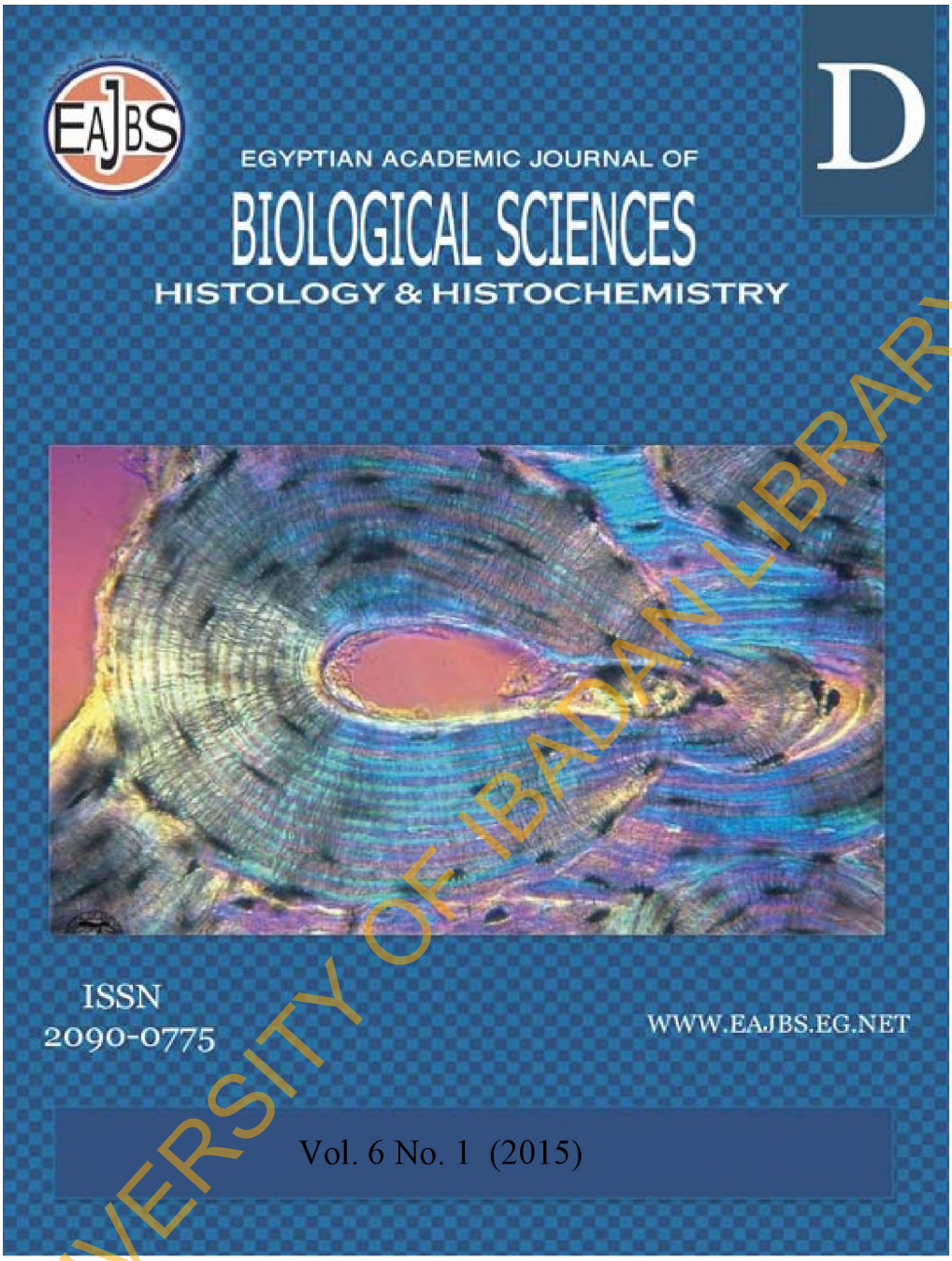

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SEM Study on Morphology and Surface Topography of W*enyonia minuta* Woodland 1923 (Cestoda: Caryophyllidea) and the histopathological Consequences on S*ynodontis filamentosus* (boulenger, 1901) from Lekki Lagoon, Lagos – Nigeria.

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ABSTRACT

The SEM study on m orphology 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 fi ve specimens of the fish host were exam ined.The fem ale specim ens (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 longitudina l 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 m atting, lym phocytic 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 caryophyllaedae have been reported in Africa, Europe, Australia and North Am erica. The genus W enyonia was created by W oodland 1923 based on their peculiarity differing from all other members of the caryophyllaeidae by the posse ssion 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 sm all fishes as intermediate hosts.

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

Parasitic diseases of fish constitute one of the m ajor problem s confronting modern fish culturists. The pathological conditions arising from such infections assume a high m agnitude causing significant m ortalities am ong infected fish species. There is lim ited informations on the f ish parasite ultrastructure in any Nigerian freshwater body. The aim of this study was to obtain data on the surface topography of the helm inth parasites using SEM in order to dem onstrate congruency with existing descriptions or to provide unreported m orphological 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 m ajor 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 247km 2 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 m ade up of lagoons and creeks that are found along the coast of south-western Nigeria from the Dahom ey 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 palm s *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 m ap of Lekki lagoon, Lagos, Nigeria

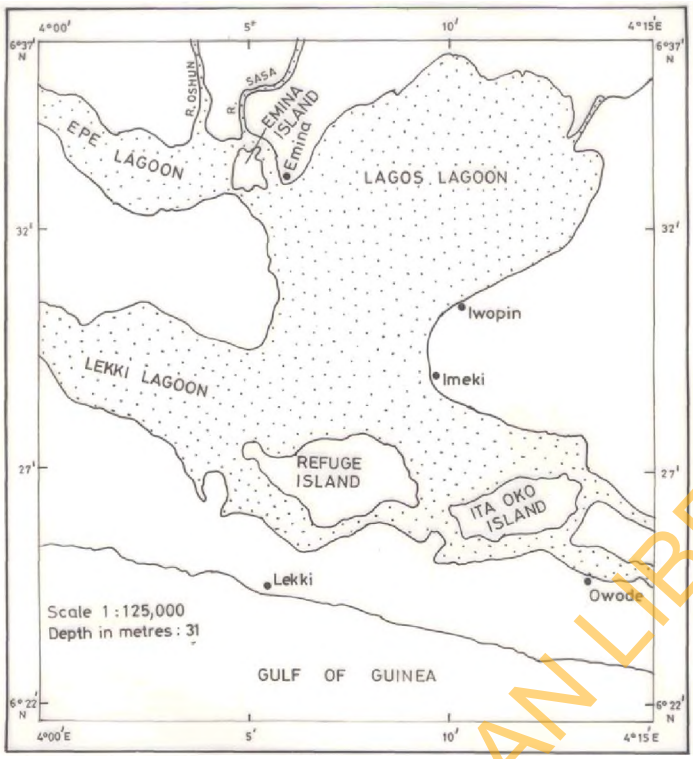

Fig. 1: The map of Lekki Lagoon, Lagos, Nigeria.

Collection and Examination of Specimens

A total of two hundred and forty-five specim ens 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 exam ined for the presence of parasites. One hundred and fifty seven m ale (n = 157) and eighty eight fem ale (n = 88) of the fish specimens were exam ined. The fish specimens were later dissected for the isolation of the parasites. The presence of parasites is observerd 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 Bouins fluid for 7 hours and the fixative were later decanted and replaced with 10% phosphate buffered form alin. 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 f rom the intestinal tissues and were then em bedded in paraffin wax, blocked, sectioned and stained with haematoxylin and eosin. The stained tissues were washed of f in tap water and the over stained ones distained in 1% acid alcohol. The tissues were m ounted using DPX m ountant, dried and examined under the microscope.

Scanning Electron Microscopy

Worms to be scanned were washed in phosphate buffered solution at 15 minutes interval to rem ove debris and to hold PH (7.4) steady during the fixation process after being fixed in 2.5% glutaldehyde. Post fixation was done accordingly in 1% osm ium tetraoxide (O_sO_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, Graham stown, 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 specim ens had a prevalence of 69.3% while the m ale specim ens had a prevalence of 46.4%. A total of sixty one and seventy three fem ale and m ale specimens were infected respectively.

Wenyonia species have been parasitizing the fish species of the family mochokidae. This study em ployed the use of scanning electron m icroscope 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 (W oodland 1923) Syn. W enyonia 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 specim en 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 1042450.97µm with a breath of 653451.20µm. Scolex broad at the middle and pointed (Fig. 2 C) towards the anterior end. Tegum ent rough towards the posterior end of the cestode (Fig. 2 D-E) and sm ooth 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 m easures 470571.70µm^2. The scolex is arrow shaped with flat pointed anterior end. The tegum ent width im mediately behind the short neck is 671312.06µm.

The m easurement of the tegum ent width towards the caudal region are 658561.88µm, 661878.11µm and 591618.12µm respectively. This im plies that the tegum ent width varies from the short neck towards the posterior end. The whole worm length m easures 10189235.11µm. Tegum ent sm ooth at the cephalic end, and rough towards the middle to the caudal end. The excretory pore lateral and circular and has a breadth of 1964.86µm with a lateral papillae near it. There is a conspicuous left lateral diagonal groove with a length of 32783.55µm . 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 photom icrograph 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 m atting and shortening of intestinal villi. Mild lymphocytic infiltration of the lamina propria was also observed. Denudation of the intestinal epithelium with m arked goblet cell hyperplasia was also observed. Hyperplasia of lym phoid aggregates within the subm ucosa was also evident. Dif fuse and m ultifocal infiltration of epithelium with num erous 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*. (Geof froy Saint, Hilaire) (Siluriformes: claroteidae) .This *Caryophyllaeid cestode* in this study was not however reported by Akinsanya *et al.* (2007) on helm inth bioload of *Chrysichthys nigrodigitatus*.

Woodland (1923) found the genus *Wenyonia* to dif fer rem arkably f rom 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 system atic of cestodes of the genus *Wenyonia also recovered Wenyonia McConelli* which he confirmed to be the sam e 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 rem arkably 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 dif fer in the shape of the scolex by Bjoern (2011) and that *W. minuta* is a distinct species, well separated m orphologically 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 m ore pronounced on the left lateral side. There exist som e variations in the morphological details of Nigerian freshwater fish parasites to the already described ones in different geographical locations.

Histopathological observations in the intestinal m ucosa of the f ish 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 Hoffm an (1980) also reported severe dam age to the intestine (obstruction and enteritis) caused by *Caryophyllidean cestode* infecting cultured European and Chinese carp. This is in agreem ent with this present study which also reported several pathological changes such as m atting and shortening of intestinal villi, m ild lymphocytic infiltration of the lam ina

propria and m ost im portantly goblet cell hyperplasia. Adeyem o and Agbede (2008) on histopathology of tilapia tissues harbouring *Clinostomum tilapiae* parasites reported proliferation of eosinophiles at the secondary lam ellar of the gills. Clinostom um species were not however reported in the present study. Different authors such as Im am (1971) Tadros *et al*., (1979) and Nassef (1988) reported several histopathological alterations in the intestinal wall of fishes due to parasitic helm inthes. The histopathological effects reported in this study agreed with the report of Longshaw (2012) on Lym phocytic infiltration of Lamina propria, goblet all hyperplasia and hyperplasia of lym phoid aggregate within the submucosa.

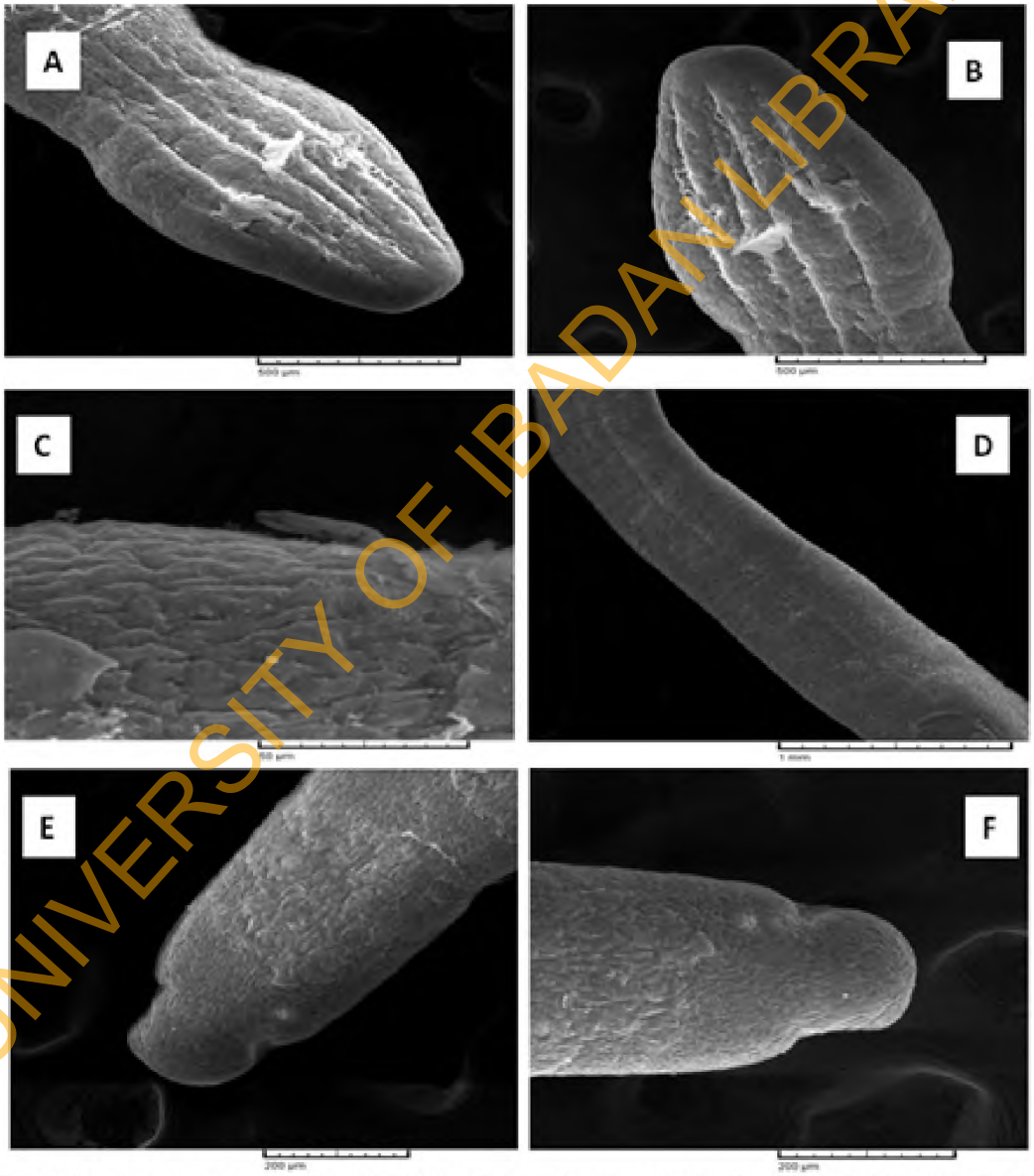

Fig. 2: SEM Pho tomicrograph of *Wenyonia minuta*. **A** - Cephalic reg ion of *Wenyonia minuta* showing *longitudinal furrow*, **B** - Anterior r egion sh owing th e sh ort n eck, **C** - lateral p apillae in *Wenyonia minuta*. **D** - Tegument of *Wenyonia minuta*, **E** - Shows the pointed anterior end, **F** - Sublateral view of posterior end of *Wenyonia minuta*, Posterior extremity, and ventral view.

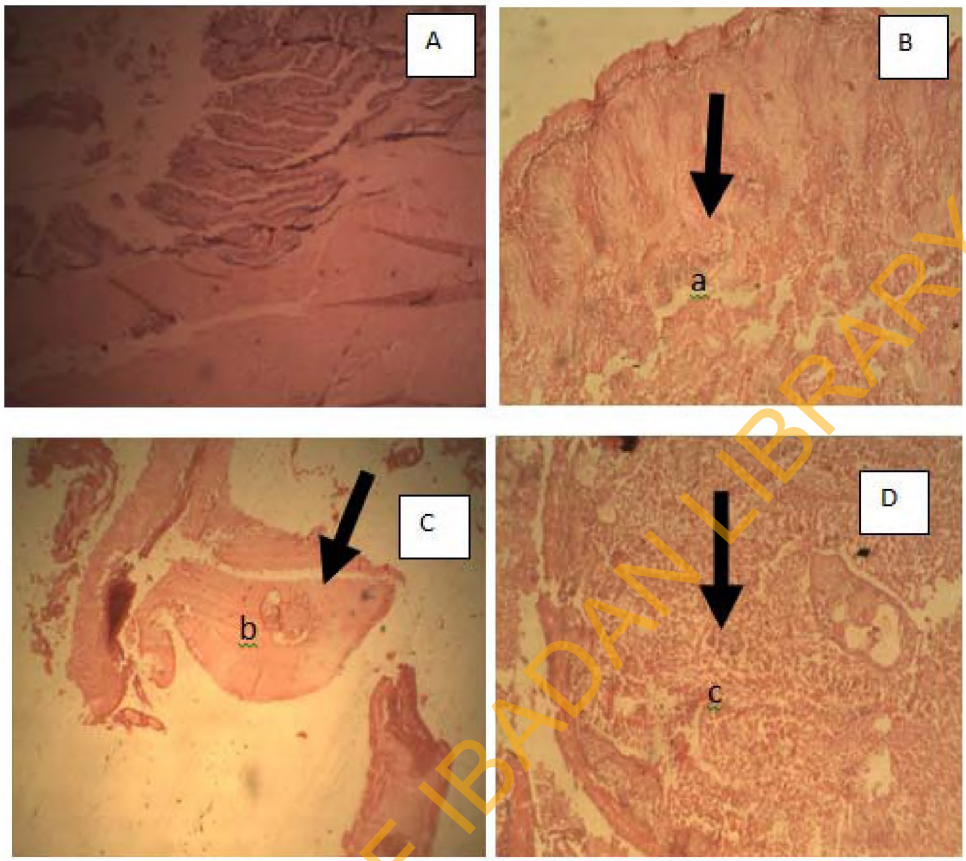

Fig. 3A - D: Section through the Intestine of *Synodontis filamentosus* showing different Pathological conditions. (A), Normal section. (B), a- Marked thickening and hyperplasia of intestinal mucosa with sloughing of epithelium and accumulation of necrotic debris within the lumen. (C), b - Multifocal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

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