Studies on *Cucullanus* sp. (Nematoda: Cucullanidae) parasitic in *Tilapia zillii* (Gervais, 1848) from Lekki Lagoon, Lagos, Nigeria.

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ABSTRACT

Based on the parasitological examination of three hundred and twenty one specimens of *Tilapia zillii* (n=321), thirty three nematode species of the genus *Cucullanus* Muller 1777 were recorded. The fish species had overall prevalence of 9.6%. The female specimens had a prevalence of 11.8% (n=110 (18) 11.8%), while the male specimens had a slightly lower prevalence of 8.5% (n=211 (18) 8.5%).

The nematode *Cucullanus* sp. was collected from the intestine of the fish host. SEM description of the recovered *Cucullanus* sp. shows some distinguishing features which place it apart from most of its congeners. The submedian papillae were located on the cuticle, single amphid in between the two upper submedian papillae, short spicule and 23 upper teeth and 34 lower teeth on the dorsoventrally elongate mouth. The thistological sections of the parasitized host revealed marked sloughing of cells into the lumen and intraluminal adult nematode mixed with necrotic debris.

Keywords: *Cucullanus* sp., *Tilapia zillii*, Lekki Lagoon, Nigeria.

INTRODUCTION

Tilapia zillii, (Gervais, 1848) occurs mostly in freshwater, venturing occasionally into brackish waters (Riehl and Baensch, 1991). El-Zarka, (1956) described *T. zillii* as euryhaline fish, tolerating salinity up to 29.0 ppm. The fish species are substrate brooders with formation of stable pairs with both parents participating in nest building and defense of the young. Bousso (1991) reported that the investigation of fish helminthes is very important for public health and hygienic purposes.

Poulin and Morand (2004) reported that parasites are an important component of global biodiversity. In Nigeria, therefore the parasitic fauna of freshwater fishes has been studied by a number of workers (Aderounmu and Adeniyi 1972; Okaka 1991; Akinsanya and Hassan 2002a; Akinsanya and Otubanjo 2006; Akinsanya *et al*. 2007a; Bichi and Ibrahim 2009; Usip *et al*., 2010; Akinsanya and Hassan 2011).

Luque and Poulin 2007 reported that parasites play an important role in natural ecosystems and that the identification of hot spots of high parasite diversity as well as low parasite density is important for understanding the function of the biosphere. Otitoloju *et al.*, 2010, 2011, 2013 and Saliu *et al*. 2014 have reported the threatened nature of biodiversity of freshwater ecosystems in Nigeria, mainly by environmental problems resulting from the degradation of the ecosystems.

The study of parasite biodiversity is therefore very important because of their roles in the regulation of the density of host populations, stabilization of the food webs and the structuring of animal communities. Therefore, a good knowledge of parasite diversity is crucial for environmental management and conservation (Luque and poulin 2007). Chubb (1982) reported that the health of a fish is affected by nutrition and that the presence of a large population of fish provide ample habitat for

parasites and the stress condition associated with such crowding will affect the health and subsequent susceptibility of the fish to parasites.

The genus *Cucullanus* Muller 1777 has an uniform morphology and many similarities (Moravec *et al*., 1993). Caspeta-Mandujano *et al*. (1999) reported that family Cucullanidae contains more than 100 species compressed to three genera: *Cucullanus* Mueller 1777, *Dichylyne* Jagerskiold 1902 and *Neocucullanus* Travassos, Artigas and Pereira 1928 (Petter 1995).

The aim of this study is to provide morphological data of a newly reported species of *Cucullanus*; parasitic in *Tilapia zillii* from Lekki Lagoon, Lagos, Nigeria and its pathological effects on the fish host.

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 shrubs and *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 a map of Lekki lagoon, Lagos, Nigeria.

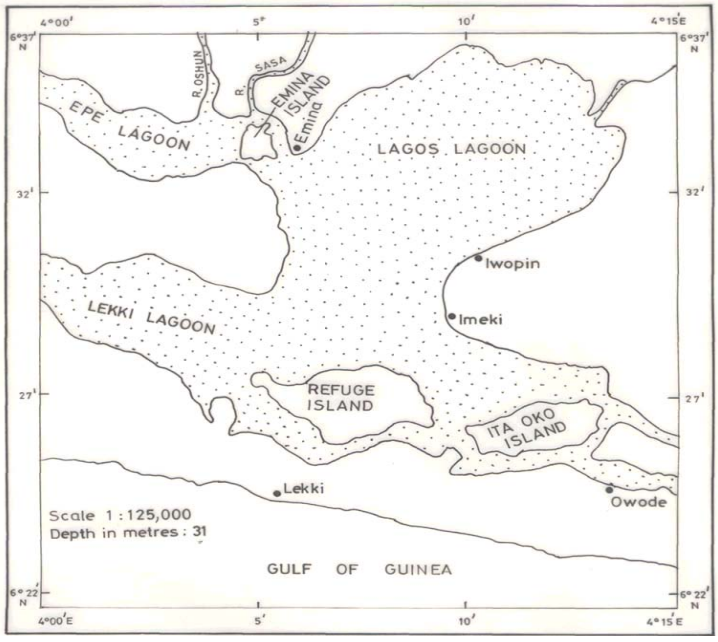

Fig. 1: The Map of Lekki Lagoon, Lagos, Nigeria.

Collection and Examination of Specimens

Tilapia zillii specimens were collected from Oluwo Market, Epe, Lagos – Nigeria. Three hundred and twenty one specimens were subjected to parasitological examination; 110 female and 211 males.

The collected specimens of *Cucullanus* sp. were fixed in 2.5% glutaldehyde solution in 0.1M phosphate and kept in a refrigerator. Some of the recovered parasites were also fixed and sent to the parasitic worms Division, Natural History Museum, United Kingdom for their identification.

Scanning Electron Microscopy (SEM)

For scanning electron microscope the cold temperatures were used to decrease extraction and provides a slower rate of autolysis. The nematode specimens were later rinsed in the same buffer solution 3 times at 15 minutes interval to remove debris and to hold pH (7.4%) steady during the fixation process. Post fixation was done in 1% osmium tetra oxide for 2 hours. The samples were later dehydrated through a graded ethanol series. After dehydration, the nematodes were dried to the critical point in liquid CO_2. The CO_2 were brought above critical point (31.1C and 1,073Psd) so as to become dense vapour phase. The samples were later sputter coated and examined with the scanning electron microscope of the Central Michigan University, U.S.A.

Histopathological Analysis

For histopathological analysis, infected tissues of the fish host were fixed in Bouins fluids for 7 hours. They were later preserved in 10% phosphate buffered formalin and 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 destined in 1% acid alcohol. The tissues were mounted using DPX mounting, dried and examined under the microscope.

RESULTS

The *Cucullanus* species in this study was collected from the intestine of *Tilapia zillii*.

Order: Ascaridida
Super family: Seuratoida
Family: Cucullanidae Cobbold, 1864
Genus: *Cucullanus* Muller, 1777

These are medium sized nematodes with slender body and smooth cuticle. The oral aperture is dorsoventrally elongate and surrounded by membranous collaret in which there are 23-34 teeth. The teeth are triangular in form. There are fount submedian cephalic papillae of which two are conspicuously located on top of the cuticle in between them is a pair of amphids. The two erect cephalic papillae were on top of the cuticle with the left measures 17.6µm in length and 15.1µm in breadth. While the right measure 15.6µm and 15.8µm in length and breadth. The dorsoventrally elongate mouth measures, 123.1µm and 9.4µm in length and breadth respectively. Amphids measures 3.3µm and 2.9µm in length and breadth respectively. A slight depression near the cephalic region is observed with a papillae, 4.2µm and 3.3µm in length and breadth. Four alaes were also located on top of the cuticle with lengths of 10.0µm, 9.1µm, 8.2µm, 3.3µm and breadth of 6.5µm, 5.3µm, 6.0µm and 3.6µm respectively. Two papillae lies, diagonally on the cuticle with lengths 5.3µm and 4.5µm with breadth of 4.2µm and 2.7µm. The male organ is lateral, 16.5µm length and 18.3µm breadth with an erect papillae on top of the cuticle, length 5.8µm and breadth 7.3µm.

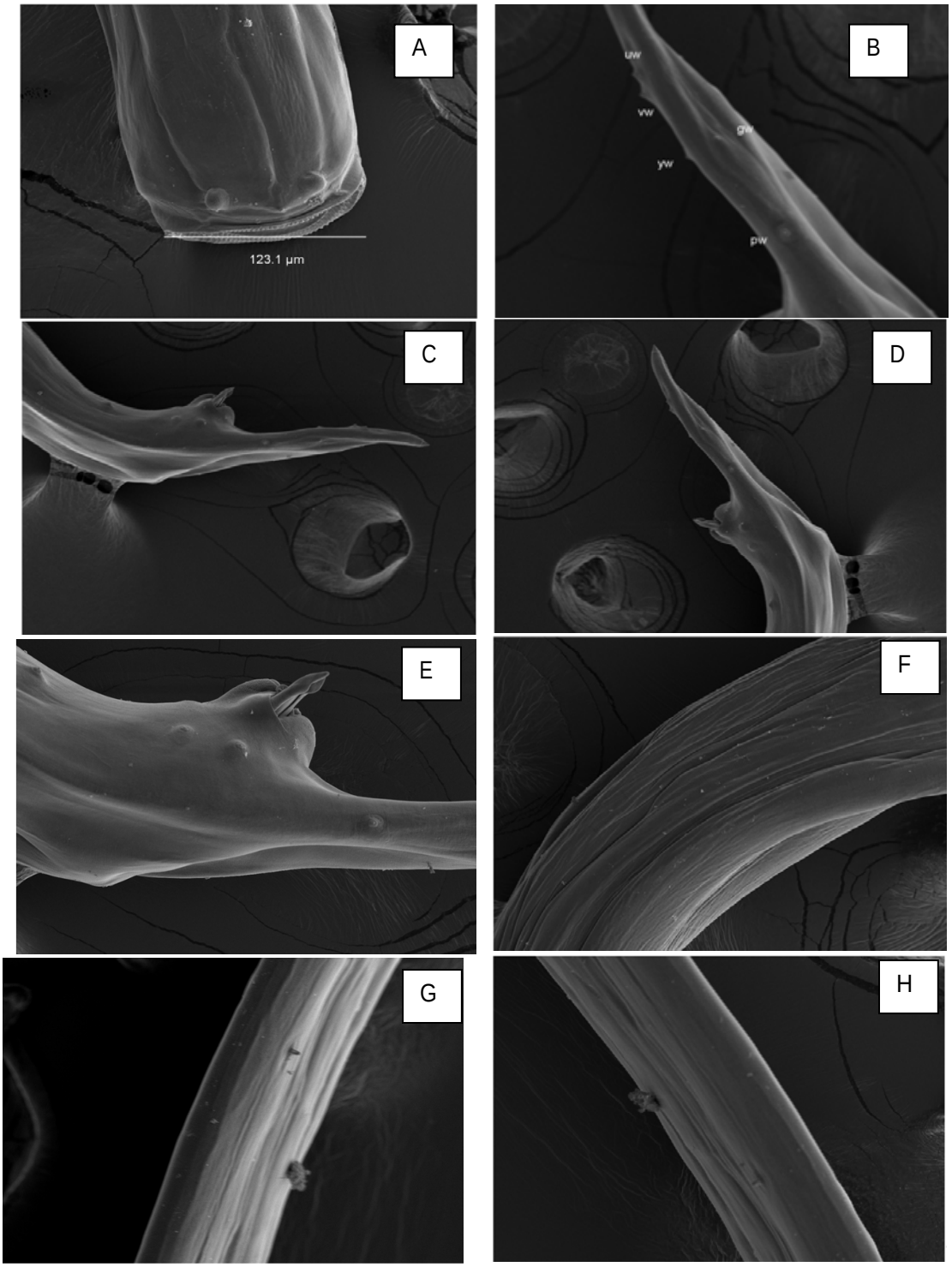

Fig. 2: SEM Micrograph of *Cucullanus* sp.*;* **A.** Cephalic end showing triangular teeth; **B.** Caudal region showing the alae; **C.** Caudal end showing the short spicule; **D.** Caudal end showing the long tail; **E.** Caudal end showing the pre-anal papillae; **F.** Cuticular body of the nematode; **G.** Cuticular body showing the male organ and an erect papillae; **H.** Lateral cuticular body of the nematode.

The nematode is characterized with numerous compressed and longitudinal alae and papillae, on the cuticle. A structure that looks like deride is located at the lateral region with 28.1µm and 34.5µm in length and breadth respectively.

The caudal end bears the spicules which are equal and the tail spicule enclosed with a length of 38.7µm. The posterior end measures 106.5µm. The distance of the

spicule from the posterior end is 247.3µm. The tail is conical and measures 11.8µm and 12.2µm in length and breadth respectively. The short terminal end of the tail is 35.4cm. The cuticular body at the middle measures 100.8µm. There is a single papillae located laterally at the caudal region with more than eleven alae on top of the cuticle. Excretory pore located near the end of the posterior region. While the plasmid is located laterally

Histopathology investigation

The photos of tissue sections of the intestine of *Tilapia zillii* as a result of the infection by *Cucullanus* sp. revealed some pathological consequences. They show marked sloughing of cells into the lumen with moderate necrosis of the mucosa. There was loss of surface epithelium as a result of the mass sloughing of cells into the lumen. There was section of intraluminal adult nematode parasite mixed with necrotic debris. Fig. 3 shows the results of the histopathology of the parasitized intestinal mucosa of the fish host.

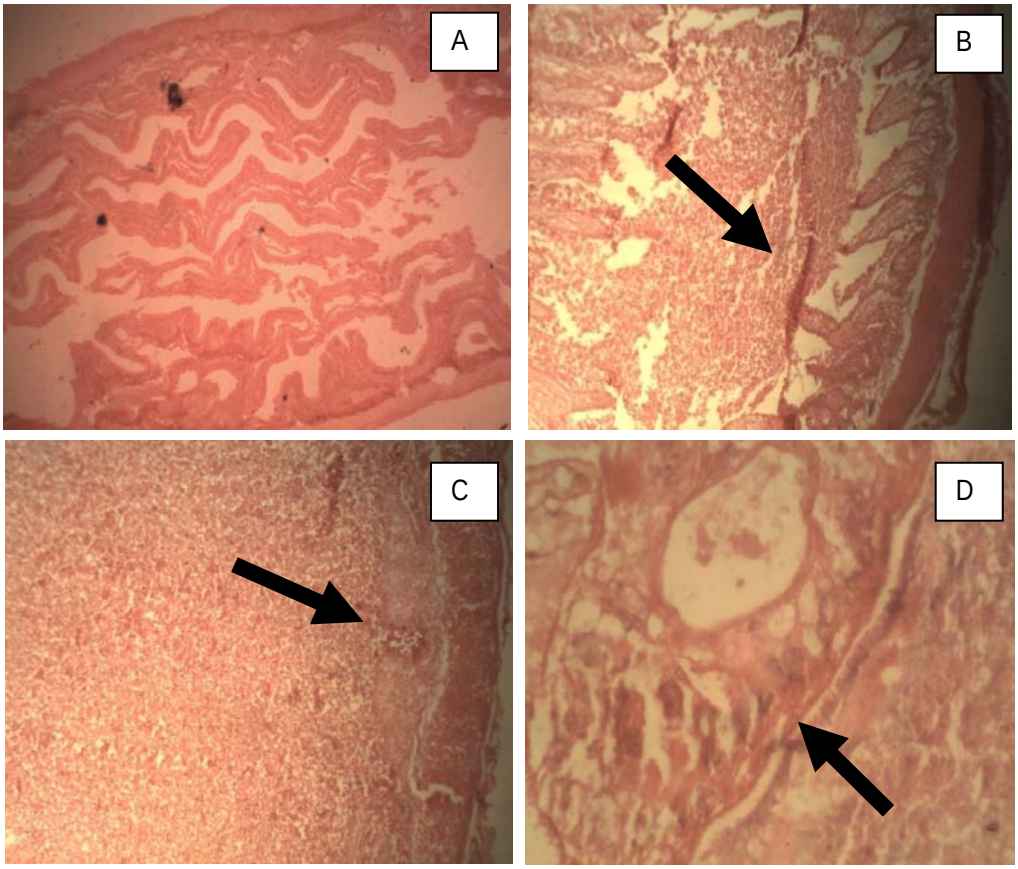

Fig. 3: Section through the Intestine of *Tilapia zillii* showing different Pathological conditions. (A), Normal section. (B), Denudation of surface epithelium with marked sloughing of cells into the lumen. (C), remnant of nematode parasite embedded within the mucosa mixed with thick inflammatory and necrotic debris within the lumen. (D), Intraluminal nematode parasite mixed with inflammatory debris X 100

Prevalence and Intensity of Infections

The overall prevalence of infections in *Tilapia zillii* is 9.6%. The females had a slightly higher prevalence of 11.8% than the males with a prevalence of 8.5%. The intensity of infections was also low with a total of seventy parasites recovered from the fish species.

DISCUSSION

The genus *Cucullanus* Muller 1777 has been reported by Moravec *et al*. (1993) to include several species that have many similarities and their original descriptions being very poor making comparisons between them very difficult. Moravec (1994) reported that *Cucullanus* sp. is mainly parasitizes fishes and rarely other animal groups. This is confirmed by Gonsalez – solis *et al*. (2007) who said that the genus has more than 100 species parasitized in different orders of fishes and that at least 70 species have been described from the marine and brackish waters. Moravec *et al*. (2005) also emphasized that the morphology of the genus *Cucullanus* is uniform and that some of them have been inadequately described which make detailed description and comparison among them almost impossible. This then make authors to describe this parasite based on host groups.

This is the first SEM description of *Cucullanus* sp. from Nigerian fresh waters. Petter (1995) reported several *Cucullanus* species already described such as *C. pinnai* Travassos; *C. zungaro* (Vaz and Pereira 1934); *C. pauliceae* Vaz and Pereira, 1934; *C. mogi*; *C. colossomi* Diazlengria, 1968 and *C. oswaldocruzi*. Moravec *et al*. (1977) reported however that those Cucullanus sp. not reported by Peter 1995 should be considered as inquirendae species.

The present *Cucullanus* species described from Lekki Lagoon, Lagos – Nigeria, conforms to the most important morphological features to allocate a nematode within the genus *Cucullanus* (Petter, 1994).

The morphology and the measurement of this present SEM description of Nigerian *Cucullanus* species agree with that description of Petter and Sey (1997).

The present *Cucullanus* species which is considered new record in this location, differs from the already described species in having much shorter spicules of 38.7µm. Joong and Moravec (2008) reported that ten *Cucullanus* sp. have been described from anguilliform fishes and that the morphology of all the species are rather similar. They emphasized that *C. muracnesocis* and *C. murenophidis* differ considerably from *C. robustus* in possessing much shorted spicules that is less than 500µm long. The present species have equal size spicules but very short and farther to the end of the conical tail. Narcisa *et al* (2000) also reported that the main distinguishing characters of *C. heliomartinsi* are markedly short spicules that correspond to 2.5% of the total body length with deirids and excretory pore situated posterior to the oesophago-intestinal junction. The excretory pore of the present species is located very close to the end of the posterior region.

Narcisa *et al*. (2000) reported the presence of 25 to 28 small triangular teeth from a new *Cucullanus* sp., *C. heliomartinsi*, parasitizing *parauchenipterus striatulus*. This is not in agreement with this present description of 23-34 small triangular teeth.

Moravec *et al*. (1997) also reported dorsoventrally elongate mouth surrounded by narrow flange (collarette) but not specific in the number of small teeth in their description of *Dichelyne hartwichi*. They also reported slender, equal spicules in the parasite. Dichylyne was considered by Akram and Khatoon (1999) as a synonym of *Cucullanus* with the designer of keys to three subgenera *Pakdacnitis*, *Truttaedanilis* and *Campanarougettina*.

The present *Cucullanus* species in this study showed two conspicuous sub-median papillae instead of four. The location of the sub-median papillae is on top of the cuticle. They lie erect on top of the cuticle. John (1997) in his description of *Cucullanus palmeri* also reported 60 – 80 teeth, 4 small inner papillae, with 4 large

outer papillae, 2 lateral amphids as all structures external to collarette. In this study only a single amphid is seen in between the two large cephalic papillae. The number of caudal papillae also makes the species in the present study differ remarkably. There are more than 11 caudal papillae, single phasmid and conical tail but not curved ventrally as in male species. The possession of much shorter spicules of less than 40µm, the arrangement of caudal papillae, position of the sub-median cephalic papillae, and the number and shape of the numerous minute teeth on the dorsoventrally elongate mouth makes the *Cucullanus* sp. in this present study new to the genus.

This species, which is named as *Cucullanus sheilanensis* from Lekki Lagoon, Lagos Nigeria was recovered from *Tilapia zillii* in the water body. The present study revealed some pathological consequences on the intestinal wall of the fish host as a result of infection by *Cucullanus* sp*., such as,* sloughing of cells into the lumen and necrosis of the mucosa top the common histopathological consequences of the parasite against the fish host. Moravec and gut (1982) reported that massive infections by *Capillaria* (*Pseudocapillaria*) and *Capillostrongyloides* which attach to and feed on the intestinal mucosa caused emaciation and mortalities in aquarium reared neotropics Cichlids and Siluroids. The pathological effects of parasites on the fish hosts are however dependent on the intensity of the parasites.

Moravec *et al*. (1984) reported that there was no pathological implication of the introduction of *Caplillostrongyloides brevispicula* to carp reared in warm water ponds in Israel. In this present study necrosis and some pathological consequences were observed. Necrosis is almost always detrimental and can be fatal due to the fact that cells which die due to necrosis do not usually send the same chemical signals to the immune system that cells undergoing apoptosis do. This consequently prevents nearby phagocytes from locating and engulfing the dead cells.

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