

**SCANNING ELECTRON MICROSCOPY (SEM) OF  
NEOECHINORHYNCHUS SP. AND ECHINORHYNCHUS SP  
(ACANTHOCEPHALA: NEOECHINORHYNCHIDAE AND  
ECHINORHYNCHIDAE), IN THE BLACK CHINNED TILAPIA,  
SAROTHERODON MELANOTHERON (RUPELL, 1852)  
FROM CULTURED AND OPEN LAGOON IN GHANA**

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**ABSTRACT:** Increased interest in fish culture has increased awareness of and experience with parasites that affect fish health, growth, and survival. The majority of fish parasites can only be identified by the use of a microscope. In this study the Scanning electron microscope (SEM) was used to identify for the first time, *Neoechinorhynchus* sp. and *Echinorhynchus* sp. in *Tilapia* from aquaculture and open lagoon in Accra. The genus *Neoechinorhynchus* occurs mostly in freshwater fishes while the *Echinorhynchus* sp. occurs in both fresh water and marine habitat. These parasites were isolated from the intestines of collected fish samples. The mean length of the trunk of the *Neoechinorhynchus* is 1701  $\mu\text{m}$ . The proboscis is short and subcylindrical measuring 51  $\mu\text{m}$  in length and 36.7  $\mu\text{m}$  in diameter near the base. The proboscis has three rings of curved spines each with six spines. The anterior spines are long measuring a mean of 12.36 $\mu\text{m}$  in length. The body surface lacks spines and consists of a series of folds. The *Echinorhynchus* on the other hand has a much smoother body with no spines. It is distinguished by small size, low number of hooks, and by marked sexual dimorphism of the trunk. Proboscis is cylindrical. These are characteristics typical of the genera.

**Keywords:** *Acanthocephala*, *Neoechinorhynchus*, *Echinorhynchus*, SEM.

## INTRODUCTION

Acanthocephala are readily recognised by their evaginable proboscis crowned with several rows of recurved hooks and are similar to the Nematoda in appearance. (Soulsby, 1982) In the encased larval stage, in tissues, the spiny proboscis is retracted. The worms are sac-like, containing lemnisci connected to the proboscis and genital organs opening posteriorly. The number and arrangement of the hooks on the proboscis are the main criteria for differentiation of species. A wider range of anatomical details are considered for determination of higher taxa (Kabata, 1985). The phylum Acanthocephala includes approximately 1,150 species of relatively small vermiform endoparasites, the adults of which feed on the intestinal walls of vertebrates, especially in freshwater and marine fishes (Ruppert and Barnes, 1994)

There are relatively few studies of the acanthocephalans and general parasitic infestations of fish in Ghana. Thus

the acanthocephalans of water bodies in Ghana are rarely known. Van Cleave's, 1940 description of species of Acanthocephalans were used as a guide for classification. The acanthocephalan genus *Neoechinorhynchus* is a relatively large genus with approximately 70 – 75 known species (Ching, 1984). But Amin (2002) studied 107 species of *Neoechinorhynchus* and recognised 88 to be valid. The Acanthocephala occurs throughout the world and is typically found in freshwater fishes (Nickol and Thatcher, 1971), although it occurs in other invertebrates as well.

In this paper, a description of the fine structure of the species *Neoechinorhynchus* and *Echinorhynchus* found in the intestine of the black chinned tilapia, *Sarotherodon melanotheron* (Rupell, 1852) was carried out. This is a common fish found in most fresh and brackish water bodies in Ghana. The scanning electron microscope was used for the first time in Ghana, to enhance the details of these species by bringing out clearly the arrangement of the proboscis, thus helping in easier identification. This study

contributes to the knowledge of the genus *Neoechinorhynchus* and *Echinorhynchus*, as well as to the knowledge of the acanthocephalans of fishes in Ghanaian waters.

## MATERIALS AND METHODS

### Study Site

The parasites were isolated from the intestine of *Tilapia* specimen purchased from the Sakumo II lagoon and Ministry of Fisheries pond in Accra, Ghana. A total of 102 fish samples were collected from both sites on the same day.

### Parasite Isolation

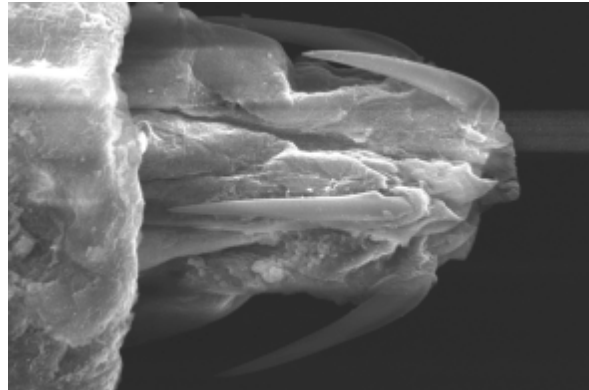
Fish samples were screened for any ecto parasites on the field. Fish were then collected on ice and brought to the laboratory to be dissected. Parasites were teased out of the gut region. A total of 226 acanthocephalans were collected. These were identified to genus using the Meyer-Van Cleave System for identification of classes, orders, families and subfamilies of acanthocephalans (Van Cleave, 1936), and some keys to species of the genus *Neoechinorhynchus* for freshwater fishes. Worms were placed in saline solution prior to being processed and then placed in cold distilled water to allow for relaxation. Some were cleared with glycerin, and mounted on a slide using Canadian's balsam. The worms fixed on slides were observed under the Nikon eclipse E200 light microscope and grouped according to their morphological characteristics.

### SEM study

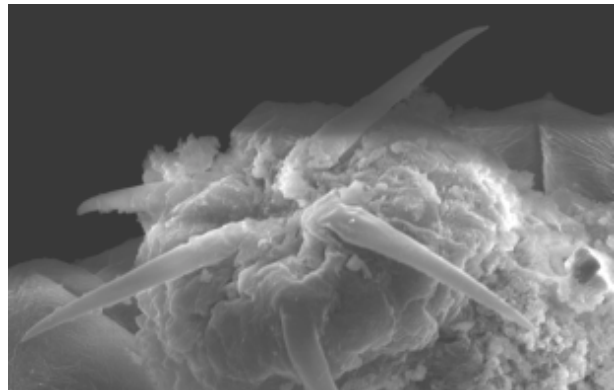
Worms to be scanned were fixed in 3% glutaraldehyde, in 0.1M sodium cacaldehyde buffer, pH 7.4 for 2 hours. These are then washed in 0.1M sodium cocaldehyde buffer, pH 7.4. They are secondarily fixed in 1% osmium tetraoxide (OsO<sub>4</sub>) for an hour and then washed again in cocaldehyde buffer. This is followed by dehydration through series of alcohol in increasing concentrations (50-100% washes). Samples were freeze dried and coated with platinum. They were then observed and photographed with a Joel-6390LV Scanning Electron Microscope (SEM), with an acceleration voltage of 15 KV.

## RESULTS

The study employed the use of the electron microscope to help in the further classification of the Acanthocephalans which happened to be the most prevalent parasite in the fish screened.



**Figure 1: SEM photograph of the Globular proboscis with hook rings and anterior part of *Neoechinorhynchus sp.* Ultra structural appearance of the largest hooks located in the anterior ring of hooks. This is a key taxonomic feature for identification of *Neoechinorhynchus* genera.**



**Figure 2: A SEM photograph of another *Neoechinorhynchus sp.* with most parts of the proboscis inverted. The proboscises here are enrooted within the proboscis.**

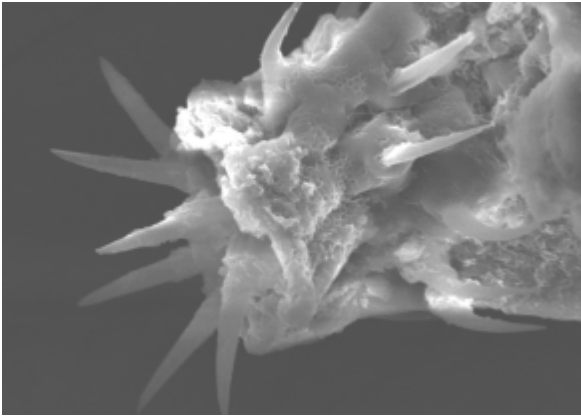


Figure 3: A SEM photograph of echinorhynchus sp. with proboscis almost completely extended.



Figure 4: A light microscope view of *Neoechinorhynchus* sp. identified



Figure 5: Head region of the *Neoechinorhynchus* sp. (Van Cleave, 1920)



Figure 6



Figure 7

Figures 6 and 7 are the anterior and posterior regions respectively of a male species of the *Neoechinorhynchus* sp.



**Figure 8**



**Figure 9**

Figures 8 and 9 show the posterior and anterior region of the male *Echinorhynchus sp.* (Muller 1776) with proboscis sucked in.



**Figure 10: A light microscopic view of the female *Echinorhynchus sp.***

### Characteristics features *Neoechinorhynchus*

The adults are parasitic and exclusively in fish. Its proboscis has comparatively small numbers of hooks. Their larva is parasitic in crustaceans. They have 2 lemnisci, one short and one long. Their testes are oval to elliptical, and more elongated. The anterior part of trunk is not narrow. Has delicate body structure. Proboscis is small, rounded, with spiral rows of hooks. Has short neck. The proboscis had 18 hooks, in 6 spiral rows of 3 each. (This was detected using the Scanning Electron Microscope).

### Distinguishing Characteristics of the genus *Echinorhynchus*

Cement gland is divided into two or more lobes; proboscis usually with large number hooks. Body small to medium sized with hypodermic nuclei small and numerous. Lucunar system consisting of lateral main vessels and reticular anastomoses. Proboscis long, cylindrical, directed ventrad, with 9-26 longitudinal rows of 5-16 hooks each; root of hook simple, becoming smaller towards base of proboscis where it disappears. The proboscis receptacle of worms in this genus are cylindrical to claviform. Proboscis is double-walled, with ganglion near its middle. Their testes are oval to elliptical, tandem, contiguous or not, in middle third trunk. There are six or more cement glands, more or less compact, one behind another.

## DISCUSSION

This study documents the occurrence of the genus *Neoechinorhynchus* and *Echinorhynchus* in an acultured pond and an open lagoon in Accra, specifically as an intestinal parasite of the black chinned *Tilapia*. The characteristics described of the specimens studied, such as the shape and size of the proboscis, number and position of the spines on the proboscis, and the form of the body, clearly place these specimens within the genus *Neoechinorhynchus* and *Echinorhynchus*. The *Echinorhynchus* sp. was identified from fish from the Sakumono lagoon. This species from literature is mostly found in the marine environment. They may be found in the brackish system if salinity is high (Nickel et al., 1971).

The *Neoechinorhynchus* sp. was identified from the aquaculture as well as the lagoon. This species occurs throughout the world and is typically found in freshwater (Anim, 1985). In the classification of acanthocephala by Van Cleave and Yamaguchi (1963), the body of

*Echinorhynchus* was small or middle-sized in the acanthocephala, the proboscis was cylindrical in shape and the hooks on the proboscis were arranged in 9-26 longitudinal rows, with 4-16 hooks per row and became smaller towards the base of the proboscis. Male worms had two testis arranged linearly in the middle or posterior portion of the body with six cement glands arranged linearly in the posterior portion of body.

Presently, little is known about the life history of this species of *Neoechinorhynchus*. In this study, the number of acanthocephalus per fish increased as the size of the fish increased. This finding has been reported previously; for example, Amin, 1985 noted the progressive increase in the number of acanthocephalans of the genus *Echinorhynchus* in older brown trout and whitefish of North America. Acanthocephalans of the class Eoacanthocephala, to which the genus *Neoechinorhynchus* belongs, typically use crustaceans as intermediate hosts (Schmidt, 1971). Therefore, intermediate host for this species is a crustacean found in this water environment. Remains of a crustacean yet to be identified were found in the digestive tract of one of the fishes dissected; this could be the intermediate host for the infective cystacanth stage.

*Tilapia* is a delicacy by many in Ghana. It is mostly gilled and often left half cooked. Humans are therefore likely to be infected with this species of *Neoechinorhynchus*. Acanthocephalans have been known to infect or potentially infect human beings in other areas (Castro, 2004), and the genus *Neoechinorhynchus* has been reported to infect mammals occasionally (Van, 1953).

## CONCLUSION

Two acanthocephalans species isolated from black chinned *Tilapia* from the lagoon and aquaculture farm have been identified using the SEM. They were the *Neoechinorhynchus* and *Echinorhynchus*. The *Echinorhynchus* was found in the lagoon while the *Neoechinorhynchus* was isolated from both the lagoon and aquaculture.

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