

ULTRA-STRUCTURAL STUDIES ON *CUCULLANUS BARBI* (BAYLIS, 1923) – ANEMATODE PARASITE OF *MORMYRUS KANNUME* WITH EMPHASIS ON SEASONAL VARIATION AND BIOREMEDIATION FOR HEAVY METAL IN THE MUSCLES

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Received 27/ 10/ 2015

Accepted 20/ 12/ 2015

Abstract

A total of 202 *Momyrus kannume* specimens were randomly collected monthly from River Nile, Demietta branch at Meet Ghamer cregion, Dakahleia Governorate, Egypt during the period from April 2012 to March 2013. All collected fish were subjected to clinical, postmortem, and parasitological examinations. Also, based on light of scanning diction microscopically observation of *Cucullanus barbi* (Nematoda; Cuncullanidae) are described from the intestine of kanumme, *Mormyrus kannume*, Nile-inhibiting fish species. The prevalence of *Cucullanus barbi* infestation in *Mormyrus kannume* was 2.9%. Seasonal prevalence was highest in autumn (6.5%) and the lowest prevalence was in winter (2.3%). *Cucullanus barbi* is mainly characterized by awide pseudobuccal capsule, the absence of ventral sucker, long sclerotized spicules which measure from 449.1- 441.5µm and Triangular elevated anal opening. Scanning electron microscopy revealed several importances, as previously unreported morphological differences in the number of pre and post-cloacal papillae of the male worm. This result revealed that the occurrence and characteristic features of *Cucullanus barbi* had indirect relation with evaluated levels of some heavy metals as Fe, Cu, Zn, Mn, Pb, Cd in muscles.

INTRODUCTION

Egyptian's markets preferred Tilapia fish and the most consumers don't know much more about many types of freshwater fishes and the main catch of the Nile River of the Nile tilapia (*Oreochromis niloticus* and an others species).

Therefore, it's necessary to introduce new types of Nile fish to markets to fill the gap of protein shortage.

The Mormyrid species (*Mormyrus kannume*) (Elephant-snout fish) occupies the lowest commercial importance relative to major dominant species in the catch and has a wide distribution in Africa, found in Uganda, Nigeria, Blue Nile and Lake Victoria, and other African lakes and rivers (Khallaf and Authman, 2010). It was reported to be distributed in the River Nile and Lake Nasser, and it was described as a common species but gradually decreasing (Bishai and Khalil, 1997) and present Abundant during June and July.

The catch fish industry has decreased significantly due to several factors which include fish diseases. Parasitic diseases constitutes as one of the major factors in the limiting of fishes production (Woo, 1995).

In spite of the intensive studies conducted on the biology and infection of several Nile fishes, it was found these studies on *Mormyrus kannume* in Egyptian water were very scarce (El-Etreby, 1985, Al-Bassel, 2003 and Ahmed, 2007).

There is no pathognomonic abnormalities showed in infestation by Cucullanid nematodes as infested fish revealed emaciation and P.M lesions showed anemic with enlargement and congestion in internal organs (Bassiony, 2002).

Cucullanid nematodes were studied from the deep-sea of marine fishes (Bayoumy *et al.*, 2008; Yooyen *et al.*, 2011; Timi *et al.*, 2009; Moravec & Justine, 2011; Pereira and Vieira; 2014 and Abdel-Ghafar *et al.*, 2014) and from freshwater fishes (Al-Bassel, 2003 and Caspeta-Mandjano *et al.*, 2010). The nematode genus *Cucullanus* are characterized by a highly developed buccal cavity formed by the oesophagus (Berland, 1970) and males with or without a precloacal sucker and harboring 10-15 pairs of caudal papillae (Maggeanti, 1971) most of the described species of *Cucullanus* have been described from marine or brakish-water fishes(Gonzalez-Solis *et al.*, 2007).

Fish are often at the top of the aquatic food chain and metals are accumulated in itto concentrations much time higher than that present in water and sediment. Fish canabsorbheavy metals through epithelial or mucosal surface of the skin, gills and gastrointestinal tract (Jovanovic *et al.*, 2011). The impact of heavy metals residue on fish flesh affects directly or indirectly human health (NWQCU, 1995). The most toxic heavy metals of particular concern to aquatic animals are cadmium (Cd), lead (Pb) and mercury (Hg) that have the way to fish flesh mainly via gills and liver (Bishai& Khalil, 1997).

The aim of the our present study was to supplement the existing morphological data with those obtained from the light microscopy and for the first time, scanning electron microscopy and through the light on seasonal variation, densityof parasite, evaluate the monthly variation of some heavy metals residues; Fe, Cu, Zn, Mn, Pb and Cd contents in muscles of *M. kannume* collected from Domiatte branch from Nile River in Meet Ghamr, Dakahlya Governorate, Egypt.

MATERIAL AND METHODS

A total of 202 *M. kannume* specimens were collected monthly by fishermen, using (Trammel nets) between April 2012 to March 2013 from River Nile, Demietta branch at Meet Ghamer region, Dakahleia Governorate. The total length of collected fish varied from 13 to 60 cm and the average body weight varied from 18.6 to 1390.6 gm. Then transported after catching to the laboratory{Central Laboratory for Aquaculture Research, Abbassa, Abou Hammad Sharkia, Egypt (CLAR)} for dissection and examined.

Clinical picture and Postmortem Examination:

Clinical signs and postmortem examined in live or freshly dead fishes for detection of any clinical abnormalities according to the methods described by Woo (1995).

Parasitological examination and Identification of parasites:

Macroscopic examination was adopted according to Lucky (1977) for the detection of any external and internal parasites. The isolated parasites were preserved and processed as described by Lucky (1977) then examined microscopically and identification was done according to Baylis, 1923.

Light and scanning electron microscope:

The Nematode species were removed from intestine by aid of stereomicroscope. The worms were washed in physiological saline and fixed in 10% cold buffered formalin and cleared in lactophenol and mounted with polyvol for morphological study and counted.

For scanning electron microscopy, specimens were fixed in 3% buffered Glutaraldehyde, washed in cacodylate buffer and dehydrated in ascending series of ethanol. Critically point dried in liquid CO₂ and sputter coated with gold – palladium and examined with JEOL scanning electron microscope (E. M. Unit, Mansoura University). Taxonomic identification of the isolated parasites were based on Baylis, 1923 (*Cucullanus barbi*).

Heavy metal analyses:

The musculature were collected for detection of accumulated of heavy metals (mg/kg. dry wt.). This methods described by AOAC (1996) by using Atomic absorption spectrophotometer (model Thermo Electron Corporation)

Statistical analysis:

One-way ANOVA and Duncan multiple range test were used to evaluate the significant differences of the concentration of metals in musculature of examined fish. Significant differences are stated at $P < 0.05$ (Bailey, 1981).

RESULTS

Clinical and postmortem examination of naturally infected fish:

The clinical signs in the infected fishes *M. kannoma* revealed no pathognomic clinical abnormalities. Some infected of *M. kannoma* showed abdominal distension with slight emaciation, while *M. kannoma* showed ulcer on the skin, scaleless and hemorrhage on based of pectoral and abdominal fins (Figs.1 and 2)

The total length varied from 13 to 60 cm and the average total body weight varied from 18.6 to 1390.6 gm Table (1). Fish was divided into groups according to length with 5 cm interval.

Table 1. The total length and the total body weight of *Mormyrus kannume* from River Nile, Demietta branch, Meet Ghamer region.

Fish No.	Length		Weight	
	Size group	Mean length	Size group	Mean weight
17	13-17	15.2	18.6 - 45.9	27.7
15	17.1-21	19.7	38.7 - 87.7	65.1
43	21.1-25	23.4	71.8 - 180.3	110.1
50	25.1-29	27.3	124 - 255.4	177.9
35	29.1-33	30.9	187.6 - 344.8	253.8
20	33.1-37	35.5	244.6 – 548	412.1
7	37.1-41	39.4	365.7 – 668	540.6
7	41.1-45	42.9	356 – 757	605.4
3	45.1-49	48.3	727.8 - 912.1	846.7
1	49.1-53	50.0	1110	1110
2	53.1-57	54.5	779.4 - 1449.4	820.2
2	57.1-61	60.0	1012.7 - 1390.6	1201.7

P.M. examination of freshly dead fishes revealed general congestion in internal organs of infected fishes. In some cases showed enlargement, swelling and variable degrees of congestion in liver, excessive mucus secretion and swelling in intestine due to attachment of adult nematodes to wall (Figs.3 and 4).

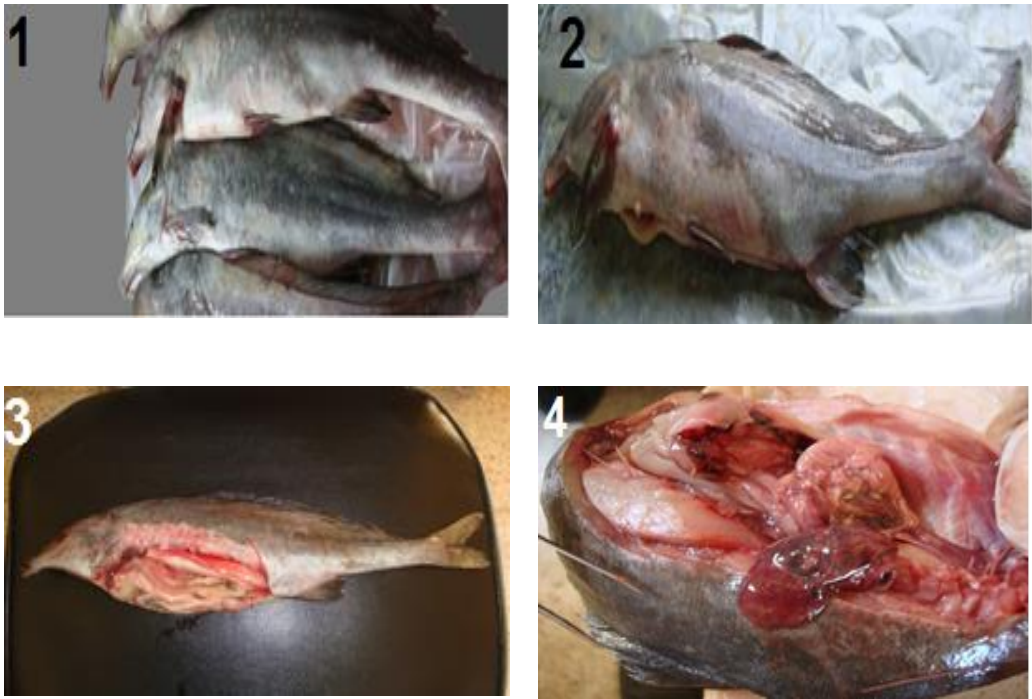


Fig. 1 & Fig. 2: Infected *M. kannoma* showing ulcer on the skin, scaleless and hemorrhage on based of pectoral and abdominal fins.

Fig. 3& Fig. 4: Dissection of Infected *M. kannoma* showing enlargement, swelling and variable degrees of congestion in liver, excessive mucus secretion and swelling in intestine.

Parasitological examination and Identification of nematode parasites:

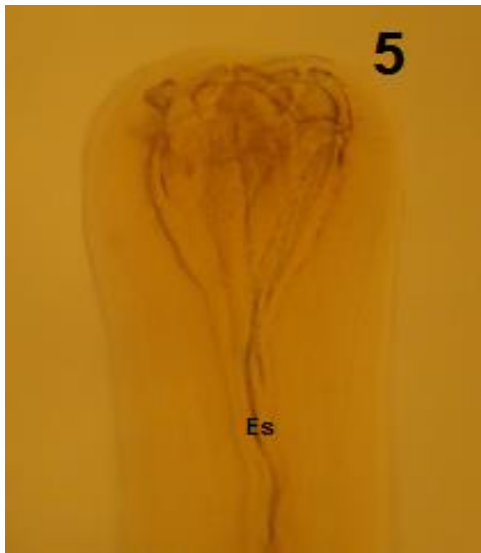
Parasite description:

Fish specimens were examined parasitologically (morphologically and microscopically) and identified according to its morphometric measurements as follows: *Cucullanus barbi* was recovered from the anterior of the intestine of *M. kannoma*. In general, the nematode parasite was whitish and long-sized with very thick cuticle and transversally fine striations (Figs., 17&18). The anterior extremity forms a rounded slit-like oral opening surrounded by a collarete armed with arrow of teeth-like structures (Figs., 5, 6, 12 & 13) head with two pairs of cephalic papillae & one pair of amphids (Figs., 7, 14, 15 & 16)

pseudobuccal capsule formed of the esophagus & buccal cavity which lined with cuticle. The females were 9.70-14.30 (12.0) mm long by 0.43-0.56 (0.49) mm in width and males were 8.4-12.5 (10.45) mm long by 0.32 -0.41 (0.36) mm in width. Its long- sized nematode. Both male and female has a conical posterior extremity ending with a micron (Figs., 8, 9, 19 & 22).

Anterior end of intestine at junction with esophagus usually extends into two variably short ceca directed anteriorly with small vulva part contain Ripe eggs embryonated (Figs., 10& 11).

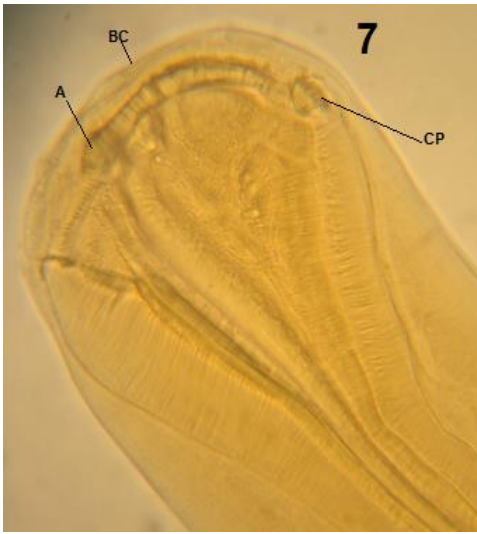
As the anal opening looks like an elevated rim (Figs., 20 & 21), the male sclerotized spicules are curved ventrally and measured 449.1 – 441.5 μm in length (Fig., 23).



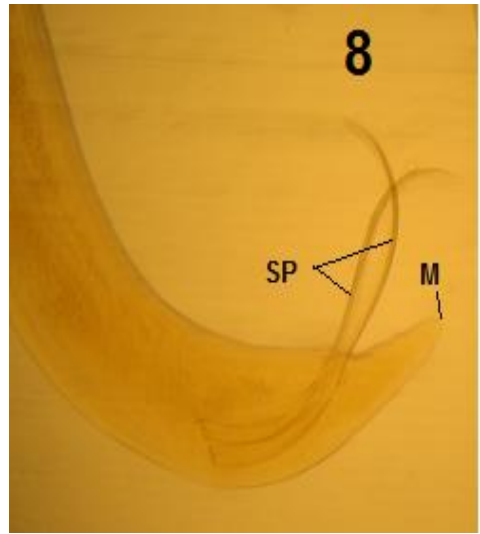
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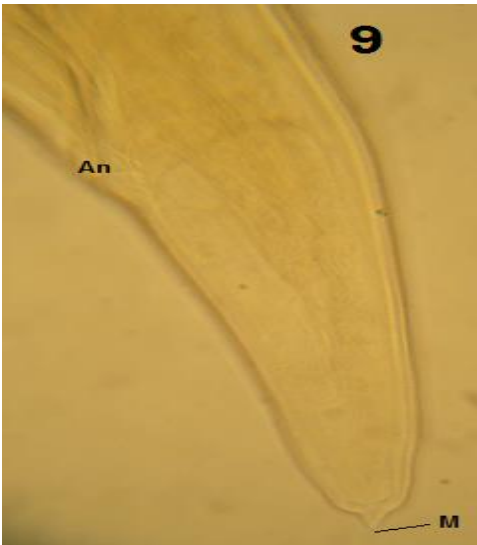
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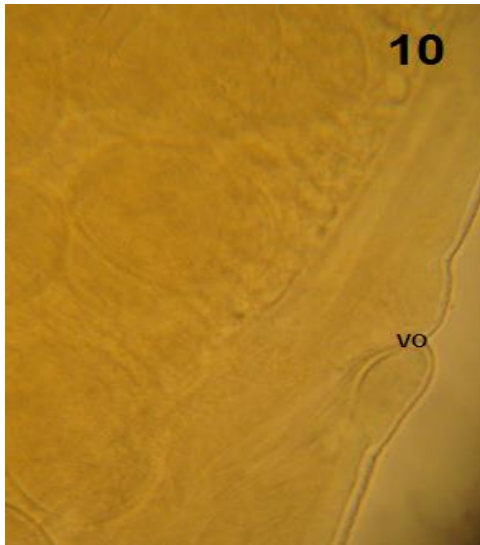
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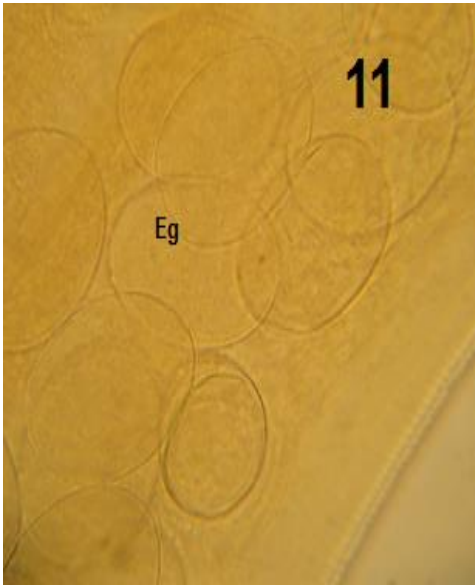
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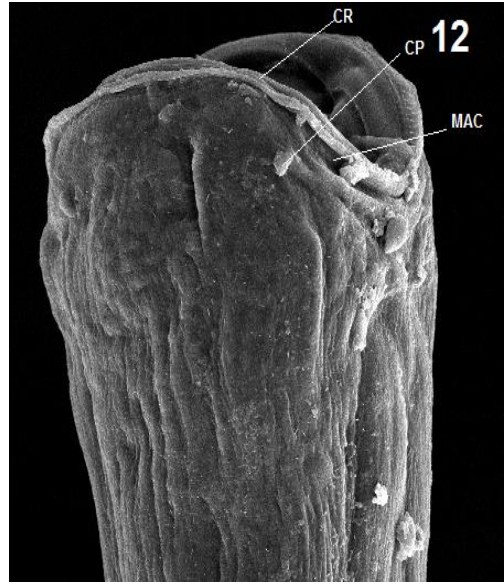
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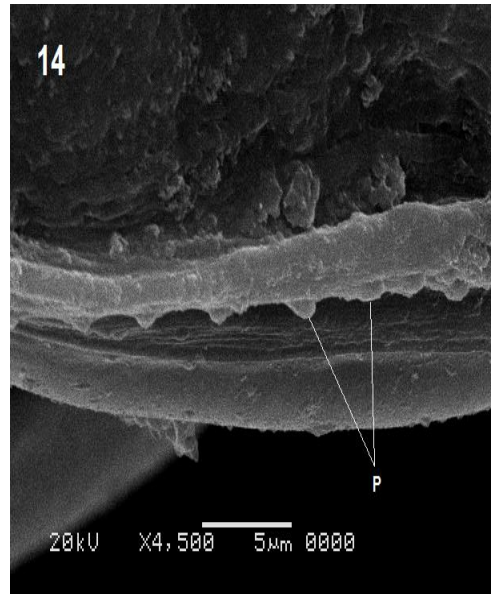
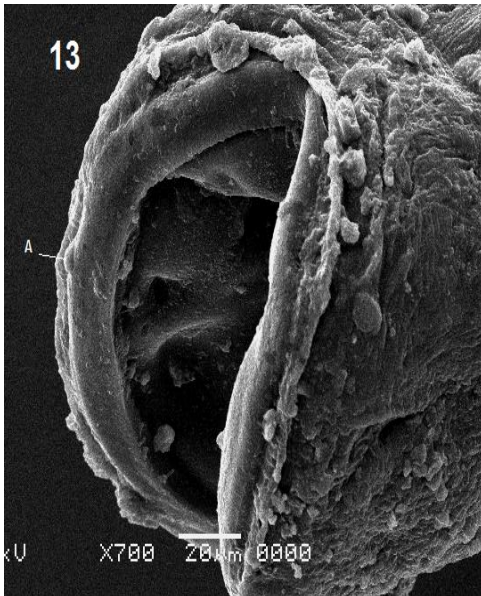
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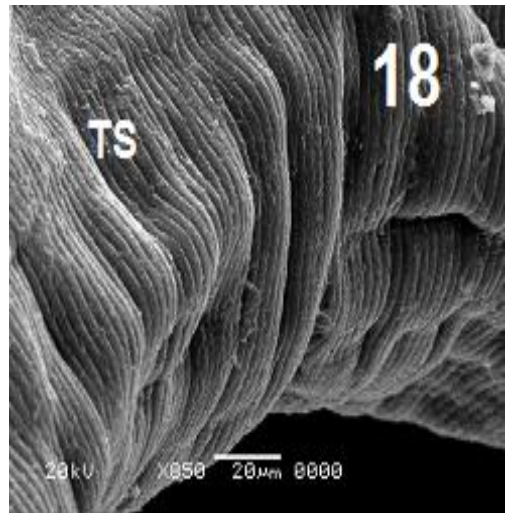
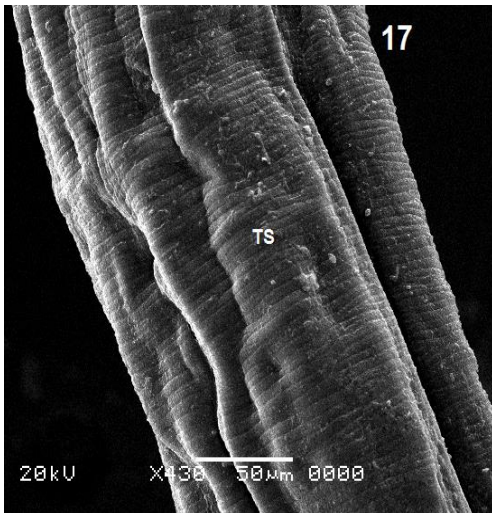
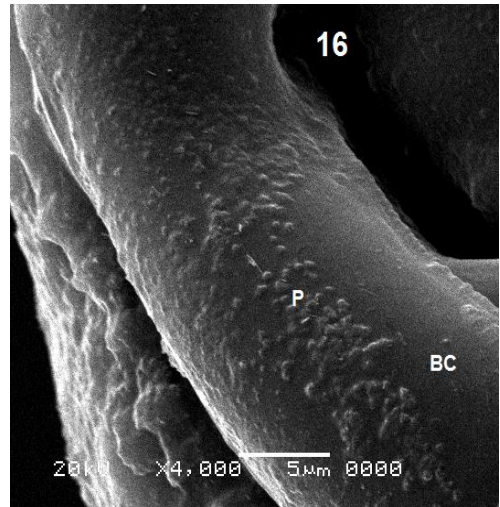
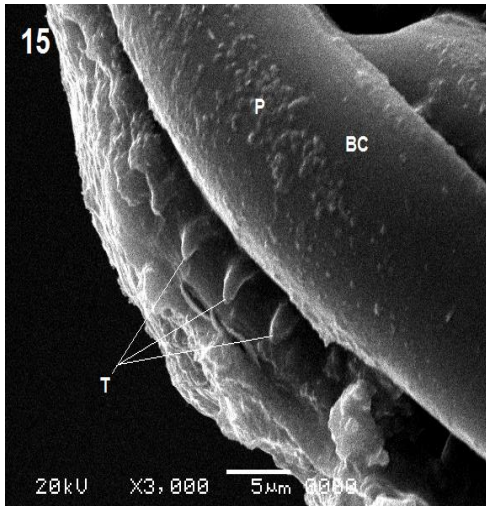


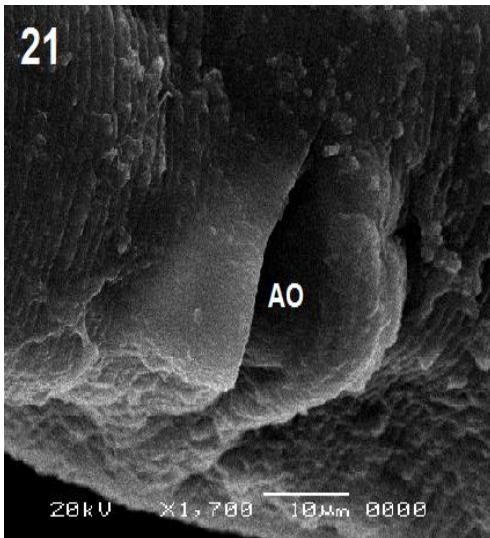
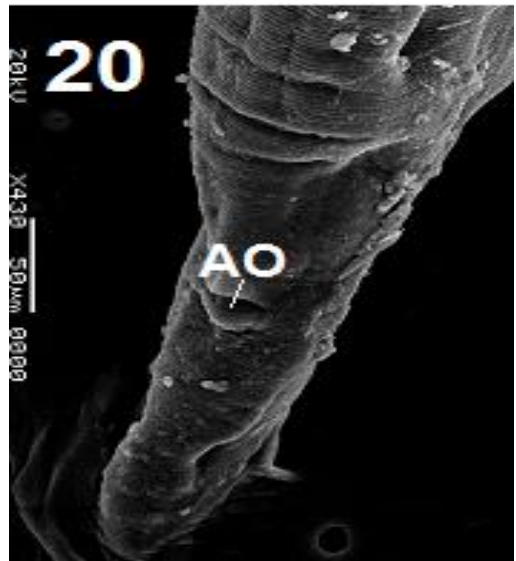
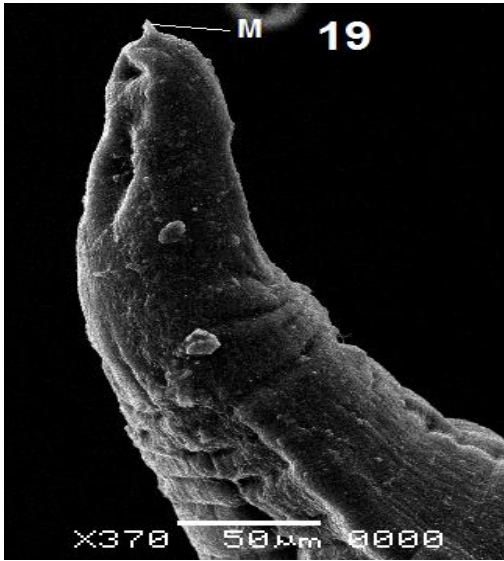
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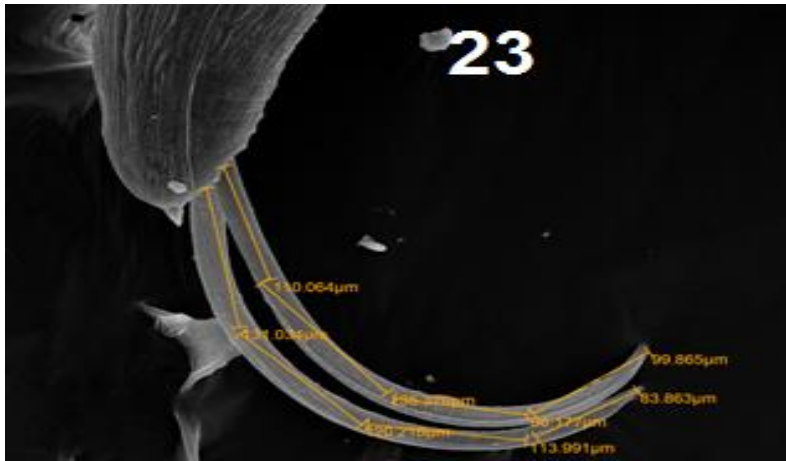
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X200 ____ 100μm

Figure 5: Anterior end of *Cucullanus barbi* (dorsoventral of buccal capsule).

ES: esophagus.

Figure 6: Anterior end of *Cucullanus barbi* (ventral of buccal capsule).

ES: esophagus.

Figure 7: Anterior end of *Cucullanus barbi* (A: Anphid/ BC: Buccal capsule/ CP: Cephalic papilla).

Figure 8: Caudal end of male of *Cucullanus barbi* (SP: Spicules/ M: Micron)

Figure 9: Posterior end of female (An: Anal opening / M: Micron).

Figure 10: Region of vulva (VO: Vulval opening).

Figure 11: Vulva part of gravid female of *Cucullanus barbi*. (Eg: Egg).

Figure 12: Dorsal view of the head capsule (CP: Cephalic papillae/C R: cephalic ring /M A C: Membranous alae collarette).

Figure 13: Ventral view of the head capsule (A:Amphid "arrows")

Figure 14: Higher magnification of labial edge showing papilla "arrows"(P: Papillae)

Figure 15: Higher magnification of labial edge showing (BC: Buccal capsule/
P: papillae/T: Teeth).

Figure 16: Higher magnification of buccal capsule showing papillae (P:
papillae/ BC: Buccal capsule).

Figure17: Body surface of the middle region showing transverse striation (T S:
Transverse striation).

Figure 18: Higher magnification of body surface showing transverse striation
(TS: Transverse striation)

Figure 19: Tail region of female showing micron (M: Micron).

Figure 20: Tail region of Female showing anal opening (AO: Anal opening).

Figure 21: Higher magnification of Anal opening of female showing a
characteristic elevated rim (Arrows)

Figure 22: Posterior extremity of male showing spicules and characteristic 3
pairs of pre- cloacal papillae and one pair of post cloacal ventral
papillae (P C P: pre- cloacal papillae/ P C V P: post cloaca lventral
papillae/ S P: spicules/ M: Micron.

Figure 23: Posterior extremity of male showing measurement of spicules.

Table 2. Total and Seasonal prevalence of *Cuculanus barbi* infested *M. Kannoma*:

Seasone	<i>M. kannoma</i>	No. of Infected	%
Summer	37	-	-
Autumn	61	4	6.5
Winter	56	2	3.6
Spring	48	-	-
Total prevalence	202	6	2.9

Concerning the intensity of infection among the examined fishes, the present results showed that *Cuculanus barbi* was recorded from 1-3 individual parasite / one fish.

Heavy metals concentration:

Data in Table (4) recorded the concentration of Fe in muscle ranged between 7.7 ± 0.799 mg / kg in September to 58.977 ± 4.93 mg / kg in December.

The values of Cu recorded in muscle were high in June 5.43 ± 0.90 mg / kg and low in March 0.41 ± 0.049 mg / kg.

Zinc were lowest values in March 1.3 ± 0.19 mg / kg while the highest were 8.51 ± 0.92 mg / kg in December.

Manganese were recorded the highest values in November 0.69 ± 0.176 mg / kg while the lowest were 0.033 ± 0.006 mg / kg. The Mn was not detectable in June month.

The concentration of Pb and Cd were not detectable for both elements except in a few cases for Pb in January to March in muscles and the highest Pb recorded 0.37 ± 0.05 mg/kg in February and lowest 0.197 ± 0.047 mg/kg in March.

Table 3. Concentrations (mg/L) of some heavy metals in muscles of *Mormyrous kannume* collected from Damietta branch Nile River in Meet Ghamr, Dakahlia, Egypt for a year.

Parameter / Date	April	May	June	July	August	September	October	November	December	January	February	March
Fe	10.1 7± 0.99	10.5 5± 0.67	20.9 57± 1.84 0	21.8 8± 0.91	10.5 14± 1.17 6	7.7± 0.79 9	54.6 3± 4.80	45.5 9± 3.57	58.9 77± 4.93	16.7 8± 1.22 3	42.7 3± 8.74	19.2 2± 0.94
Cu	1.66 ± 0.53 4	2.06 6± 0.15	5.43 ± 0.90	4.86 ± 1.37	2.10 ± 0.20	2.07 ± 0.16 4	3.98 ± 0.41	3.36 ± 0.74	0.88 ± 0.16 5	1.37 ± 0.16 5	1.05 ± 0.23 6	0.41 ± 0.04 9
Zn	1.43 ± 0.48	1.4± 0.46	2.71 6± 0.29 7	4.2± 0.65 9	1.98 6± 0.52 6	1.48 6± 0.38	5.81 ± 2.10	7.94 ± 4.06 7	8.51 ± 0.92	3.62 ± 0.51	2.94 ± 0.50	1.3± 0.19
Mn	0.03 3± 0.00 6	0.07 7± 0.02	ND	0.02 8± 0.01 6	0.07 ± 0.02 3	0.03 7± 0.01 1	0.26 2± 0.16 8	0.69 ± 0.17 6	0.54 ± 0.39	0.26 ±0 .07	0.56 ± 0.23	0.17 ± 0.02 1
Pb	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.26 5± 0.07 7	0.37 ± 0.05	0.19 7± 0.04 7
Cd	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Means with the same letter in the same row are not significantly different at P<0.05

DISCUSSION

Members of *cucullanus* generally parasitize freshwater, brackish & marine fishes, they range from medium to large size with a characteristic thick cuticle some of the members of this genus were poorly described, these have an almost identical morphology and some of them are poorly described, making their comparison difficult.

Long studies on *cucullanus* have been done in Africa (Petter, 1974; Moravec *et al* 2005,2008 ; Gonzalez – solis *et al.*, 2007, Park & Moravec, 2008) in other areas Lafranuchi *et al.*, 2004, Cabanas – Carranza & Caspeta – Mandujana, 2007; Moravec *et al.*, 2008) . *Cucullanus barbi* Baylis 1923, *Cucullanus baylisi* Companat-Rouget 1961, *Cucullanus clarotis*.

Baylis 1923, *Cucullanus chrysophrydis* Gendre 1927, *Cucullanus dodsworthi* Barreto 1922, *Cucullanus elongates* Smedly1933, *Cucullanus gendrei* Campanant-rouget 1961, *Cucullanus hians* Dujardin 1845, *Cucullanus longicollis* Stossich, 1899, *Cucullanus mauritanicus* Gendre 1927, *Cucullanus murenophidis* Campanat-Rouget 1961, *Cucullanus pulcherrimus* Barreto 1918, *Cucullanus tripapillatus* Gendre 1927, Campanant-Rouget 1957&1961, *Cucullanu saegyptae* Abdel-Ghafar *et.al.*,2014 and *Cucullanus djilorensis* n.sp Ndew *et al.*, 2014.

Our results disagree with those of Awharitoma and Okaka 1999 who recorded a 60.8% infection rate for *Cucullanus barbi* infecting Cichlid fish from Okhuaihe River in Edo state. Cucullanids show a narrow host specificity science the great majority of species has been found in phylogenetically related fish (Gonzalez-solis *et al.*, 2007) The morphology is rather uniform an some of them have been inadequately described so that a detailed comparison is very complicated (Moravec *et al.*, 2005) so some authors prefer to deal with these parasites according to their host group or their geographical distribution (Moravec *et al.*, 1997; Caspeta-Mandujano *et al.*, 2000 and Daniel *et al.*, 2002).

In this study, the prevalence of *Cuculanus barbi* infection in different examined *M. kannume*. that agreed with (Garo, 1993) Where she noted the presence of parasitic nematodes of some locally consumed fish in Egypty, Recoded two species in fish from Lake Nasser Viz. *Cucullanus barbi* Baylis, 1923. Garo, 1993 found 42 adult worm of *Cucullanus barbi* in the intestine of fish hosts *Bobusbynni*, *Mormyrous kannume*, *Mormyrous casche* as well as *Lates niloticus*. On the other hand, (Saoud&Wannas, 1984) studied the

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Mormyrus species prefer deep water and are rarely Caught in great number. Asignificant body of studies deals with their gonad condition. (Mohammed, 2014), Growth curves, age estimation, reproductive cycles, external morphology and feeding habits (Lles 1960, Scott 1974, Gilmore 1971, Vander waal 1985, Adebisi 1987, Zaher *et.al.*, 1991, Kolding *et.al.*, 1992, Kouamelan *et.al.*, 1999, Authman and Khallaf 2009, Khallaf and Authman 2009, 2010, Mekkawy and Hassan 2012 and Kramer 2013).

The chitinous triangular teeth are probably used during penetration into the migration through the intestinal wall of the fish host, while the rows of sensitive papillae served for orientation during sexual intercourse (Khallaf, 2002).

In recent times however, the occurrence of metal contaminants, especially the heavy metals in excess of natural loads has become a problem of increasing concern. This situation has arisen as a result of the rapid growth of population, increased urbanization and expansion of industrial activities, exploration and exploitation of natural resources, extension of irrigation and other modern agricultural practices as well as the lack of environmental regulations (FAO, 1992).

Fish are notorious for their ability toconcentrate heavy metals in their tissues. The metals exist most probably as cationic complexes and accumulate in the internal organs of fish.

Iron is an important element and is necessary for the synthesis of hemoglobin, unsurpassed by any other heavy metals in the earth's crust (El-Naggaret *al.*, 2009). The lowest values of Iron were recorded in the muscle. These results agree with those examined in the previous researches in other fish species such as *Clarias gariepinus*. The results in this study of *M. kannume* (muscle) are lower than those recorded in the *Clarias gariepinus* in the previous researches (Osman and Kloas, 2010). This may be due to the feeding behavior for *M. kannume*.

Copper is commonly a natural element in water and sediment. The metal is insoluble in water, but many of its salts are highly soluble. Copper is a fundamental micronutrient to all forms of life, in enzyme activity and random rearrangement of natural proteins (Bower, 1979 and Yacoub, 2007), but very high intake of Cu can cause adverse health problems. In the present study the concentrations of copper were measured in the muscle,

similar results were obtained by (Bahnasawy and Khidr, 2011, Nwabueze & Oghenevwairhe, 2012 and Ali and Abd El- Hamed, 2014). These seasonal variations may be due to the fluctuation of the amount of agricultural drainage water, sewage effluents and industrial wastes discharged into the water (Zyadah 1995). These results agree with (Khaled, 2004; Ali and Abdel-Satar, 2005 and Ali and Abd El- Hamed, 2014) they who said that increase of metals levels in tissues of some invertebrate and fish species were observed during summer months that were related to the increased metabolism due to high temperature.

Zinc is an essential element for normal growth, reproduction and longevity of animals (Sultana and Roa, 1998) and is a common pollutant as well. Mining smelting and sewage disposal are major source of zinc pollution. Fish take it up directly from water, especially by mucous and gills (El-Naggar, 2009). The maximum permissible level (MPL) of zinc is 50 ug/g dry wt. according to Australian NHMRC (Bebbington *et al.*, 1977) and 40 ug/g dry wt. according to Food and Agriculture Organization (FAO, 1983).

Manganese is an essential constituent for bone structure, reproduction and normal functioning of the enzyme system Manganese is an essential constituent for bone structure, reproduction and normal functioning of the enzymes system (Fleck, 1976 and El-Naggaret *al.*, 2009). It becomes toxic only when present in higher amount, but at low level it is considered as micronutrient (El-Naggaret *al.*, 2009).

Lead is non-essential element and higher concentrations can occur in aquatic organisms close to anthropogenic sources. It is toxic even at low concentrations and has no known function in biochemical processes (El-Naggaret *al.*, 2009). Lead was found to inhibit the impulse conductivity by inhibiting the activities of monoamine oxidase and acetylcholine esterase to cause pathological changes in tissue and organs (Osman and Kloas 2010).

It is suggested that the low accumulation of metals in muscle may be due to lack of binding affinity of these metals with the proteins of muscle. This is particularly important because muscles contribute the greatest mass of the flesh that is consumed as food. Heavy metal concentrations reported in the present study is consistent and within the range reported in a previous study that evaluated metal contamination of African catfish sampled from Rive Nile (Osman & Kloas, 2010) and (Ali and Abd El- Hamed, 2014)

The concentrations of Fe, Cu, Zn, Mn, Pb and Cd varied according to season, locality and tissue type (Yacoub, 2007 and Ali and Abd El- Hamed, 2014). Metal accumulation in fish tissues is dependent upon environmental factors such as temperature, size and age of fish and processes of biotransformation and excretion (Zhou *et al.*, 2001).

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دراسات للتركيب الدقيق للكينكيولانس باربي (بايلز، 1923) - الطفيليات الإسطوانية
من سمكة القنومة مع التركيز على التغيرات الموسمية والمعالجة البيولوجية
للمعادن الثقيلة في العضلات

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الملخص العربي

تم تجميع 202 عينة من أسماك القنومة بطريقة عشوائية مرة كل شهر من نهر النيل، فرع دمياط عند منطقة ميت غمرتبع محافظة الدقهلية- مصر خلال الفترة من أبريل 2012 إلى مارس 2013. تعرضت جميع الأسماك التي تم جمعها للفحوصات المعملية من حيث الشكل الظاهري للسمكة، الفحص الداخلي بعد الوفاة، والفحص الطفيلي للأحشاء الداخلية. وأيضاً، إلقاء الضوء والمسح المجهرى للكينكيولانس باربي وتم وصفها وعزلها من أمعاء سمكة القنومة. ولقد أوضحت الدراسة فروق هامة وغير مثبته مسبقاً وهي إتساع فى المحفظة الفمية وكذلك الحلمات الموجودة قبل وبعد فتحة المزرق بالنسبة للذكر. عدم وجود ممص بطنى. وجود شوكتان مغلظتان طويلتان ويقدر طولهما من 449.1 إلى 441.5 ميكرون. الفتحة الشرجية مثلثة الشكل وبارزة.

كانت نسبة الإصابة والانتشار للكينكيولانس باربي في سمكة القنومة 2.9% وكانت أعلى معدل إنتشار موسمي في فصل الخريف (6.5%) وأقل معدل إنتشار في فصل الشتاء (2.3%). وكشفت هذه النتيجة أن توجد علاقة غير مباشرة مع مستويات تقييم بعض المعادن الثقيلة مثل الحديد والنحاس والزنك والمنجنيز والرصاص والكاديوم في العضلات.