

STUDIES ON CALLITETRARHYNCHOSIS AMONG MARINE BLUEFISH EUTHYNNUS AFFINIS (BALAMITA), FROM THE RED SEA IN SUEZ PROVINCE

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ABSTRACT

The present study was carried out on 80 marine scombrid bluefish which were collected from the Red Sea at Suez during the period from January 2000 to January 2001 to investigate Callitetrarhynchosis as one of the most prevalent parasitic affections that induces economical losses among the marine scombrid bluefish *Euthynnus affinis*. The fish were examined externally and necropsied. Specimens were collected from the gills, musculature, peritoneum, myocardium, liver and intestine fixed in 10% neutral buffered formalin and 5µm thick paraffin sections were prepared, stained and examined microscopically.

External examination of naturally infested fish showed no pathognomonic clinical signs, while the internal examination revealed heavy infestation of plerocercoid larvae especially in liver and musculature of the caudal peduncle, branchial cavity as well as other internal organs. The total prevalence was 51.25%; but, in summer, it was 95% with heavy intensity which ranged from 40 to 70 larvae/fish (summer), 20-34 larvae per fish (spring), 8-12 larvae per fish (autumn), and 6-10 larvae per fish (winter). Parasitological examination revealed that, the obtained larvae were plerocercoids of *Callitetrarhynchus gracilis*, which belonged to order Tetrarhynchidea. The gills, musculature, peritoneum and some internal organs showed pressure atrophy, degeneration and focal necrosis beside some circulatory disturbances and inflammatory reactions with proliferation of melanomacrophages.

INTRODUCTION

Parasitic infestation represents the majority of the known infectious diseases affecting fish. The internal parasitic ones are common especially in marine and estuarine fishes. Tapeworm infestation, among such fish, is of a great pathological significance, mostly as larvae and this is es-

pecially true for the order Tetrarhynchidea (**Overstreet, 1978, Brill et al., 1987, Sindermann, 1990, and Palm, 1997**). Plerocercoid larvae occur frequently in the viscera and flesh and can materially reduce the value of fish as a food for humans (**Arme et al., 1983 and Woo, 1995**).

The present study was planned to investigate the natural infestation of Tetrarhynchidea tape-worm-plerocercoids among marine bluefish for the first time in Egypt, from the clinical, parasitological and pathological points of view.

MATERIALS AND METHODS

Fish samples :

Eighty freshly caught wild marine bluefish (*Euthynnus affinis*), commonly named Balamita, of different sizes were randomly collected from the Red Sea in Suez Province during the period from January, 2000 till January, 2001.

Clinical examination :

Fish samples were clinically examined using the methods described by **Lucky (1977)** for determining any abnormalities on the external body surface of the examined fish.

Postmortem examination :

It was carried out on the gills, branchial cavity as well as the abdominal cavity, internal organs and musculature according to the methods adopted by **Amlacher (1970)**.

Parasitological examination:

The cysts were dissected from tissues of the fish and each cyst was precisely incised to obtain the larva. The larvae were relaxed in refrigerator for two hours, then compressed between two glass slides and fixed in 10% neutral buffered formalin. The fixed larvae were washed in tap-water, stained in Semichon's acetocarmine, dehydrated in ascending ethanol series, cleared in xylene and mounted in Canada balsam for microscopic examination (**Brooks et al., 1999**).

Histopathological examination:

Specimens from different organs and tissues of infested fish (*E. affinis*) were collected and immediately fixed in 10% neutral buffered formalin. The specimens were dehydrated using ascending concentrations of ethanol, cleared in xylene and blocked in paraffin. Tissue sections of 5 μ m thickness were obtained and stained with hematoxylin and eosin (**Drury and Willington, 1980**).

RESULTS & DISCUSSION

Identification of fish :

The examined wild marine fish belonged to family Scombridae (Blue fishes), genus *Euthynnus*, species *Euthynnus affinis* which are commonly named El Balanita in Egypt.

Clinical examination :

The naturally infested fish were apparently healthy and the external examination revealed no pathognomonic clinical signs or abnormalities.

Postmortem examination :

The infested fish showed large white fibrous cysts occupying more than half the area of the liver surface (Fig., 1). Similar picture was observed in the musculature of the caudal peduncle, which showed extreme congestion and few hemorrhagic spots with embedded encysted larvae (Fig., 2) in heavy infestation. Also, there was a pile of larvae in the branchial cavity and branchiostegal musculature (Fig., 3), while the other internal organs revealed slight congestion with presence of few attached plerocercoids.

Prevalence and intensity :

Out of 80 marine bluefish *Euthynnus affinis*, 41 (51.25%) were infested with plerocercoids of *Callitetrarhynchus gracilis*. The seasonal prevalence revealed that, the infestations were increased in summer (95%) and spring (80%) and decreased in autumn (20%) and winter (10%). The intensity of infestation was high in summer (40 to 70 larvae per fish) and spring (20 to 34 larvae per fish), while it was low in autumn (8 to 12 larvae per fish) and winter (6 to 10 larvae per fish) (Table, 1).

Parasitological examination :

Yellowish white oval-shaped cysts (7-10 x 1.5-2mm) were attached to or embedded in tissues of the fish. They were found in liver beneath the capsule, in peritoneum and mesentery and beneath the serous covering of the gut as well as in the musculature and branchial cavity. These cysts were double layered containing plerocercoid larva and turbid fluid. Microscopic examination and identification revealed that, these larvae were plerocercoid, belonging to, order Tetrarhynchozoa, family Dasyrhyngidae **Dollfus 1935**, subfamily Callitetrarhynchinae, genus *Callitetrarhynchus*, species *gracilis* (Fig., 4).

Description : The holdfast organ was long measuring 3.354 mm in length with two bothridia which were patelliform, each with posterior marginal notch and measured 0.7-0.8 mm in length. The vaginal region was long with tentacle sheath and the bulb was five time as long as

wide containing the frontal glands, which were absent from the first quarter of the vaginal region. Tentacles were long with chainette hooks, which were spaced and wingless. Satellite hooks were present but the intercalary hooks were absent and there was no special basal armature. Retractor muscles were inserted in the end of bulbar cavity and about 1 mm in length (Figs., 5, 6 & 13).

Histopathological examination:

The gills showed sloughing of the secondary lamellae, congestion and mononuclear cell infiltration in the primary lamellae as well as edema, hemorrhage, mononuclear and eosinophilic granular cells in the gill arch (Fig., 7). Numerous larvae were embedded among the muscle bundles and surrounded by fibrous connective tissue capsule and numerous eosinophilic granular cells (Fig., 8). The surrounding muscles suffered edema, pressure atrophy and hyaline degeneration. Some of the intermuscular blood vessels were slightly dilated and showed thickened wall and necrotic endothelium. Numerous immature and mature larvae were attached to the peritoneum. Marked edema and active proliferation of melanomacrophages and mononuclear cells were seen in the peritoneal wall (Fig., 9). The myocardium revealed larvae surrounded by edema, hemorrhage, melanomacrophages and mononuclear leukocytes. The surrounding cardiac muscles suffered hyaline degeneration, pressure atrophy and Zenker's necrosis (Fig., 10). The pericardium contained the larvae and showed edema, hemorrhage, mononuclear cell infiltration and fibrous connective tissue proliferation. The liver showed the parasitic larvae in the hepatic parenchyma and portal areas. A connective tissue capsule and melanomacrophages as well as mononuclear cells surrounded the larvae. The infested liver suffered intercellular edema, cloudy swelling and/or vacuolar degeneration of most hepatic cells as well as necrosis (Fig., 11). Focal mononuclear cell infiltration was seen in the necrotic areas. Numerous larvae were attached to the hepatic surface. The hepatic capsules suffered hemorrhage and perihepatitis. The parasitic larvae were evident in the intestinal lumen and wall. Marked epithelial necrosis and sloughing were seen. The lumen contained cell debris, eosinophilic granular cells and some mononuclear leukocytes. The lamina propria and submucosa showed edema, mild congestion and numerous lymphocytic and eosinophilic granular cells (Fig., 12). The kidneys revealed marked tubular nephrosis, interstitial hemorrhage and focal depletion of the hematopoietic tissue.

For the first time in Egypt, the present study is concerned with a disease of an economic importance in marine bluefish (EI-Balamita), *Euthynnus affinis* obtained from the Red Sea. It was manifested by the presence of numerous encapsulated larvae of tapeworms in the branchial cavity and internal organs especially the liver and musculature of the caudal peduncle. However, there was no evident external signs or lesions as seen in most internal diseases (Hoole and Arme, 1986). The total prevalence of the disease was 51.25% and the intensity of the infestation

ranged from 6-70 larvae per fish. The seasonal variation revealed that, high prevalence and intensities were detected in summer, 95% (40-70/fish) and spring, 80% (20-34/fish). This increase could be correlated with the elevated environmental temperature. Most plerocercoid infestation of fish occurs during summer and early autumn. Moreover, most of the tapeworms reach sexual maturity, in their definitive hosts, and release eggs during the summer and spring. At the same time, population of the invertebrate hosts (first intermediate hosts) reaches their peak. Also, the differentiation into procercoids is rapid due to high water temperature (Woo, 1995).

The identification of tapeworm-plerocercoids is very difficult than adult ones due to the absence of great deal of morphological variations in the former as no mature and gravid proglottids and the holdfast organ is still primitive. Moreover, there is no clear-cut pattern concerning host specificity of tapeworm-plerocercoids (Hoffman, 1967 and Margolis and Arthur, 1979). Based on the parasitological examinations, the obtained larvae were identified as plerocercoids of *Callitetrarhynchus gracilis* according to Schmidt (1986), Grabada (1989) and Khalil et al., (1994) who concluded that, tetrarhynchidea and tetraphyllidea could be differentiated easily by the presence of spiny proboscides in the former.

Pathologically, a variable number of the parasitic larvae were observed in the gills, musculature, peritoneum, liver and other internal organs of the infested fish. Microscopically, pressure atrophy, marked degeneration and sometimes necrosis were seen in the vicinity of the larvae together with circulatory disturbances and mild inflammatory reactions. The infiltration of eosinophilic granular cells and proliferation of melanomacrophages, in most organs, represented the body response and defense against the infection. The depletion of hematopoietic tissue as well as the atrophy, degeneration and necrosis could be due to the parasitic toxic metabolites and the pressure exerted by the plerocercoids. Similar pathological changes were observed by Arme et al., (1983), Sharper et al., (1992) and Aly et al., (1996).

Table 1 : Seasonal variation of Callitetrarhynchosis among marine bluefish *Euthynnus affinis*.

| Season | No. of examined fish | No. of Infested fish | % | Intensity of larval infesta-tion per fish |
|--------|----------------------|----------------------|-------|---|
| Winter | 20 | 2 | 10 | 6-10 |
| Spring | 20 | 16 | 80 | 20-34 |
| Summer | 20 | 19 | 95 | 40-70 |
| Autumn | 20 | 4 | 20 | 8-12 |
| Total | 80 | 41 | 51.25 | 6-70 |

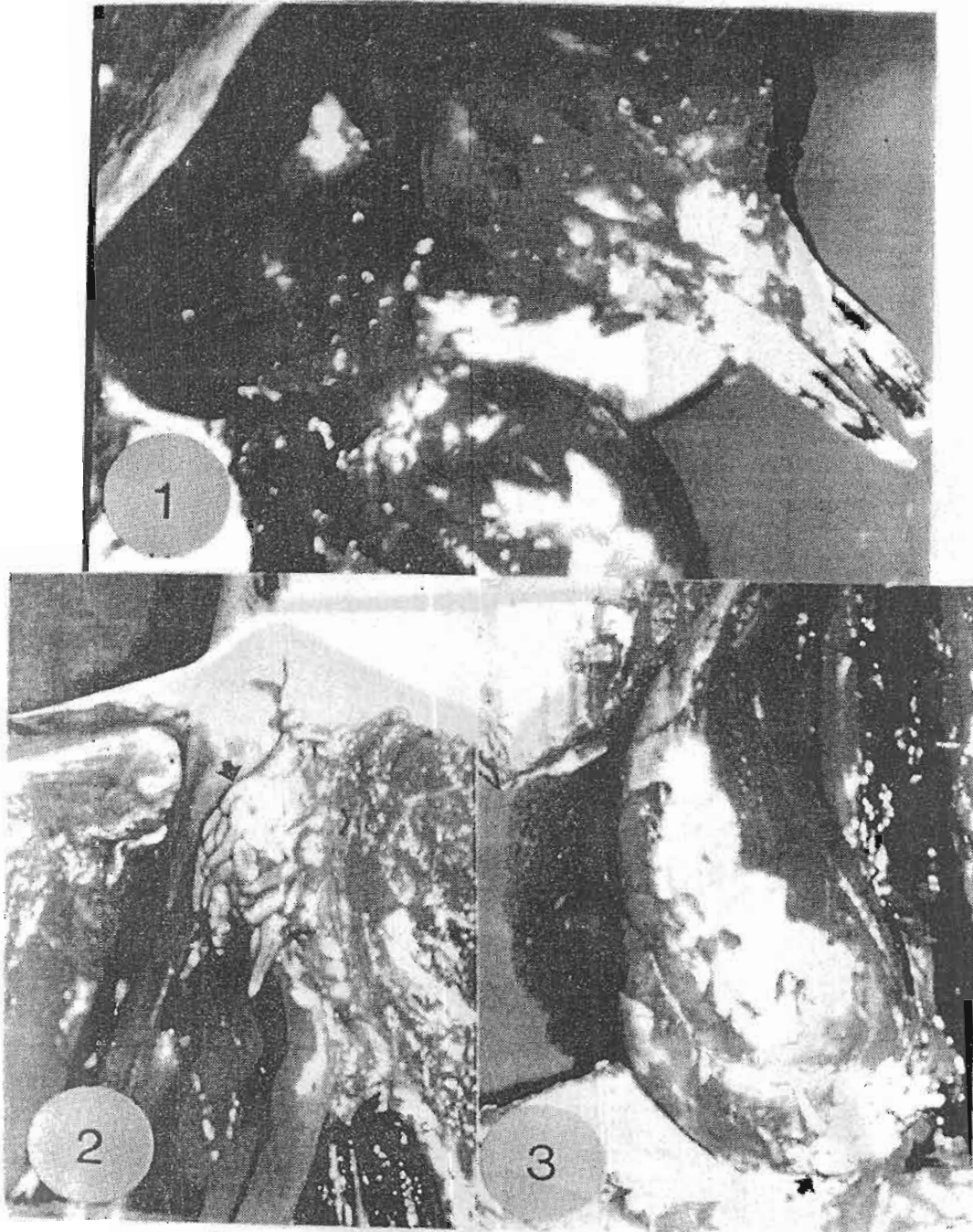


Fig. 1 : Liver infested with encapsulated plerocercoids.

Fig. 2 : Musculature of fish infested with encapsulated plerocercoids.

Fig. 3 : Gills and branchial cavity infested with encapsulated plerocercoids.

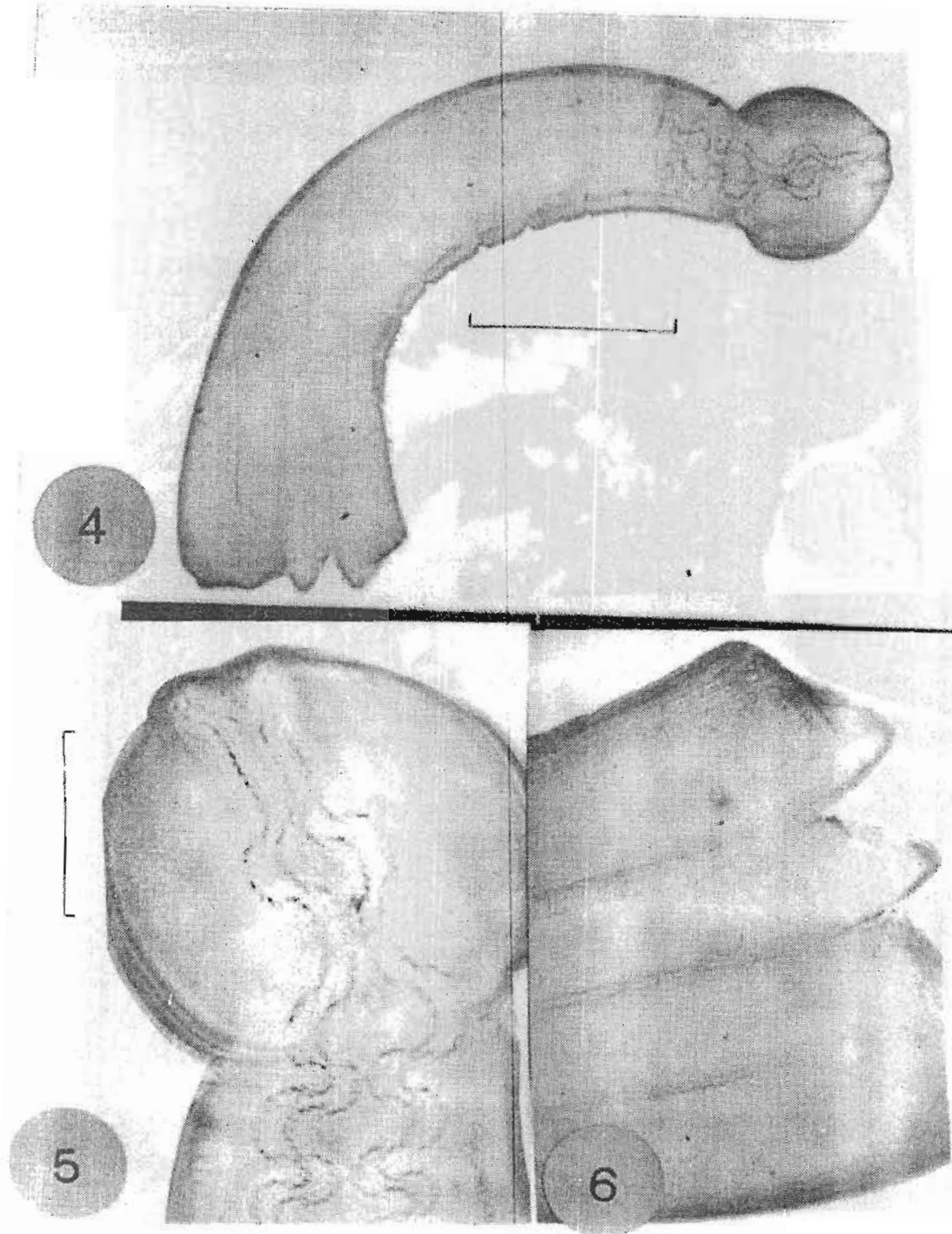
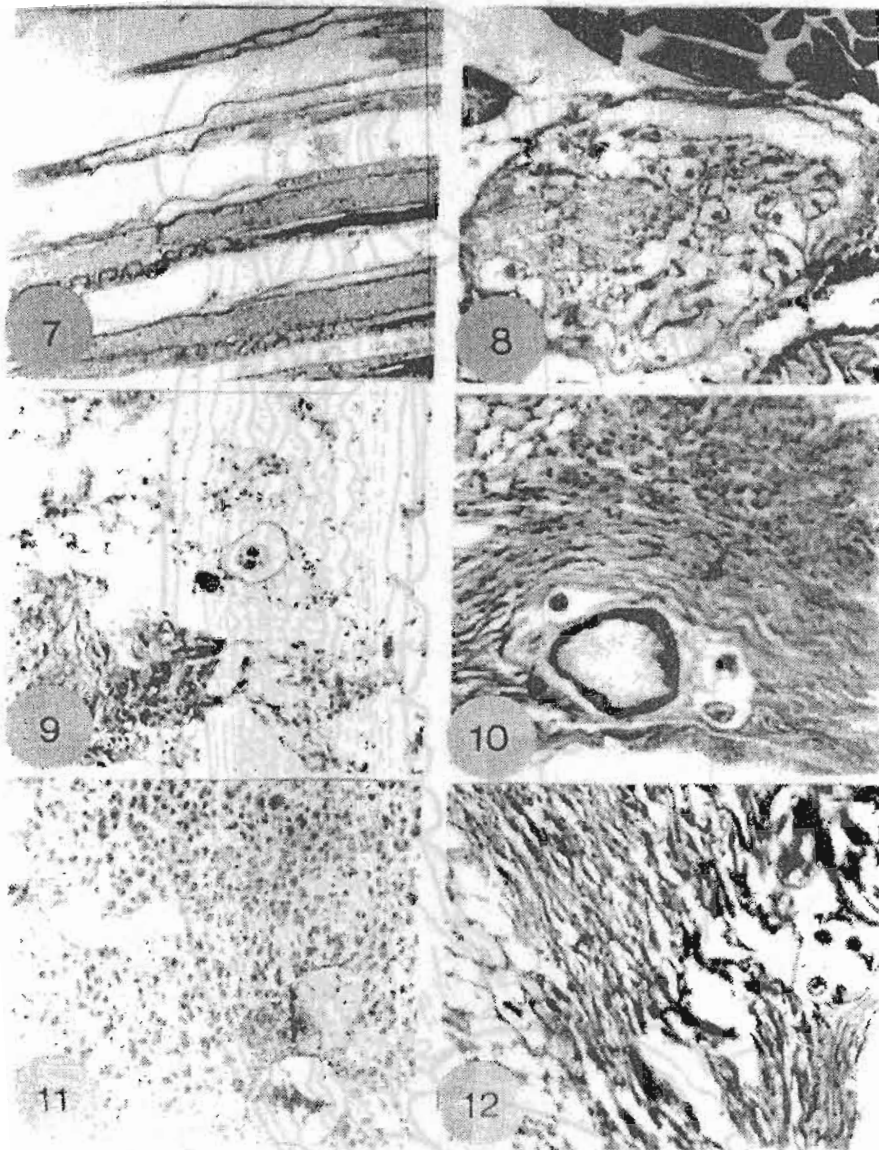


Fig. 4 : Plerocercoid of *Callitetrarhynchus gracilis*. Semichon's acetocarmine, Bar = 1mm.

Fig. 5 : Holdfast organ of plerocercoid of *C. gracilis*. Semichon's acetocarmine, Bar = 0.3 mm.

Fig. 6 : Retractor muscles of Plerocercoid of *C. gracilis*. Semichon's acetocarmine.



Figs. (7-12) : H & E, X 250. , Figs., 8-12 : Showing cross sections of *C. gracilis* plerocercoids. 7-Gills showing congestion and leukocytic infiltration in the primary gill lamellae with sloughing of the secondary lamellae. 8- Musculature showing fibrous tissue proliferation, pressure atrophy and eosinophilic granular cell infiltration. 9- Peritoneum showing edema and proliferation of melanomacrophages as well as mononuclear cells. 10- myocardium showing pressure atrophy, hyaline degeneration and Zenker's necrosis. 11-Liver showing edema, necrosis and activation of melanomacrophages. 12- Intestine showing epithelial necrosis with edema and cellular infiltration in the intestinal layers.

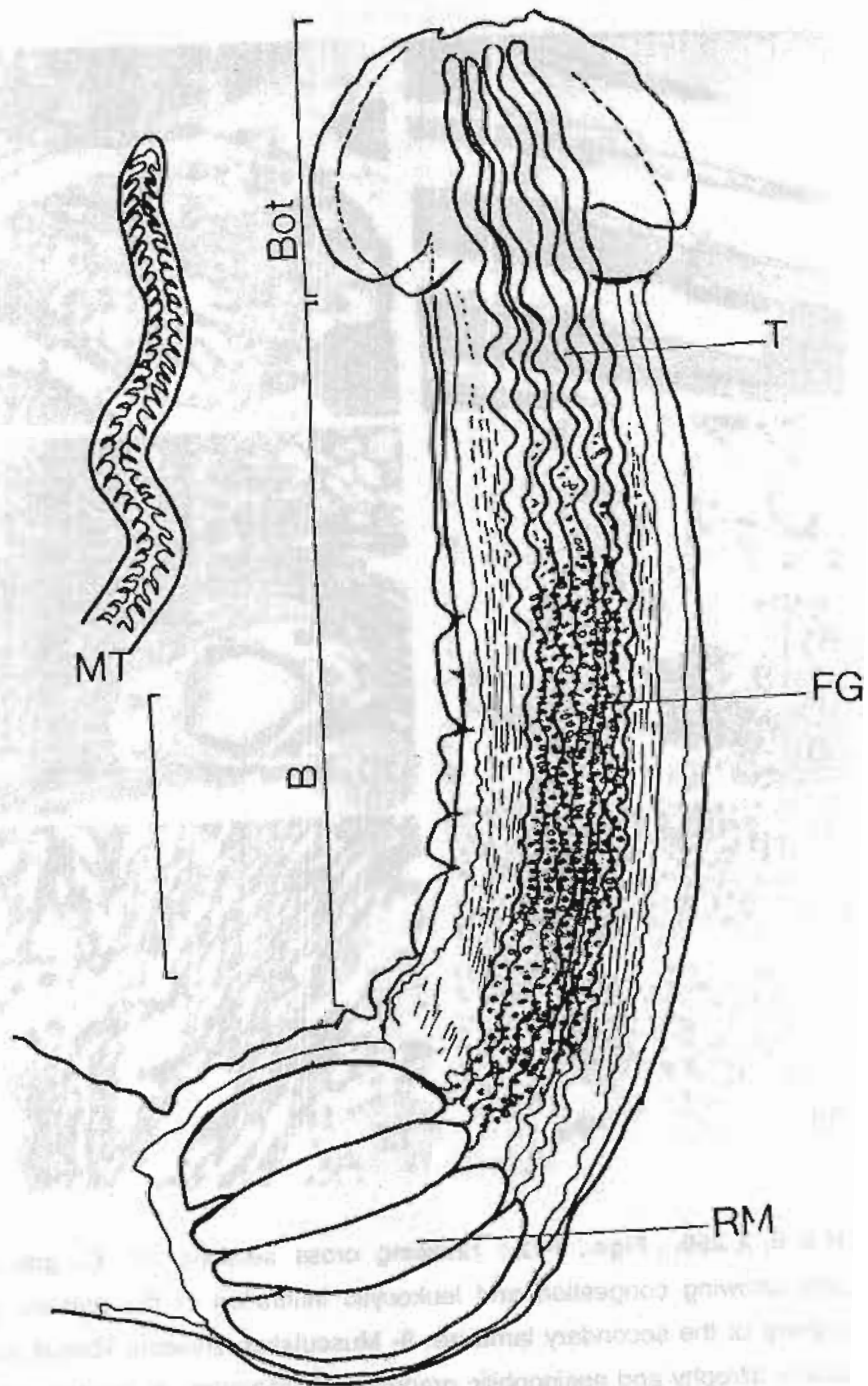


Fig. 13 : Camera Lucida drawing of *Callitetrarhynchus gracilis* plerocercoid, Bot.: Bothridia, B: bulb, FG: Frontal gland, T: Tentacles, MT: Magnified part of tentacles with chinatte hooks, RM: Retractor muscles, Bar= 1mm.

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الملخص الغربى

دراسات عن مرض الكالى تترارنكا فى السمكة البحرية الزرقاء (البلميطة)

من البحر الأحمر فى مدينة السويس

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أجريت هذه الدراسة على ٨٠ سمكة طازجة من أسماك البلميطة البحرية الزرقاء من البحر الأحمر وقد تم فحص هذه الأسماك إكلينيكياً وطفيلياً وباثولوجياً لدراسة الأعراض المرضية والآثار الباثولوجية للطور اليرقى (بليروسيركويد) للدودة الشريطية كالى تترارنكا جراسيلز. وكانت النسبة العامة للإصابة بهذا الطور اليرقى ٢٥٪. كما كانت نسبة وشدة الإصابة فى الصيف والربيع أعلى منها فى الشتاء والخريف وهذا يرجع إلى وفرة القشريات فى الصيف والربيع وهى العائل الوسيط الأول لهذه الدودة كما يرجع إلى سرعة تطور الأطوار اليرقية بسبب توفر درجة حرارة مناسبة حيث أن الأسماك من ذوات الدم البارد، وبالرغم من أن الطور اليرقى (بليروسيركويد) كان متحوصلاً فى معظم الأحشاء الداخلية للأسماك فى الكبد والعضلات والتجويف البطنى وعلى الخياشيم إلا أن الأسماك كانت سليمة ظاهرياً ولم تظهر عليها أى أعراض مميزة للإصابة، وقد أسفر الفحص الهستوباثولوجى عن وجود ضمور مع انحدار وتغير فى الأنسجة والأعضاء المصابة مع وجود تغيرات دورية دموية والتهاب بالإضافة إلى تكاثر خلايا الميلانومكروفاج. وقد تم وصف هذا الطور اليرقى وصفاً دقيقاً والاستعراف عليه وتصنيفه وهذه الدراسة تعتبر الفريدة من نوعها فى مصر والتي استهدفت هذا الطور اليرقى من الناحية الإكلينيكية والباثولوجية والطفيلية.