

## Light and Electron Microscopic Studies on Some Larvae In Marine Fishes

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### ABSTRACT

A total of 441 marine fishes consisting of 64 Red porgy (*Pagrus pagrus*), 34 Mearge (*Argyrosomus regius*), 20 Sea bass (*Morone labrax*), 58 Red mullets (*Mullus surmuletus*), 29 Narrow-barred Spanish mackerel (*Scomberomorus commerson*), 100 Mediterranean sand smelt (*Atherina*), 43 Brushtooth lizasrdfish (*Saurida undosquamis*), 9 Kawakawa (*Euthynnus affinis*), 48 Sardinellas nei (*Sardinella spp.*) and 36 Mediterranean horse mackerel (*Trachus mediterraneus*) were collected from Suez Canal area (Suez and Ismailia Provinces) during the period extended from April 2006 till March 2007. In the cestodes larvae infection, the highest prevalence rate was recorded in *Euthynnus affinis* 100%, followed by *Atherina* 86%, *Trachus mediterraneus* 19.44% and *Morone labrax* 5% with total infection rate 23.35%. While, the highest prevalence rate of larval nematodes infection was recorded in *Trachus mediterraneus* 100% followed by *Sardinella spp.* 87.5%, *Atherina* 47%, *Saurida undosquamis* 41.86% and *Mullus surmuletus* 39.65% and the total infection rate was 37.64%. Five species of cestodes larvae (*Ligula intestinalis*, *Proteocephalus sp.*, *Ophiovalipora minuta*, Tetraphyllidean larvae, Pleurocercoid larvae) and two species of larval nematodes (*Anisakis simplex* larvae and *Anisakis sp.* larvae). The morphological description using light microscope for the isolated specimens and Scanning electron microscope for *Ligula intestinalis* and *Anisakis simplex* larvae were recorded.

### INTRODUCTION

Marine fishes play an important economic role in providing increasing source of protein especially in the developing countries. There is no doubt that parasites that found in fishery product is a hazard to human health and reduce its value (1).

Numerous tapeworms problems have been recorded in aquaculture and fisheries of marine water (2). Plerocercoid larval stage of *Ligula intestinalis* infests fishes as second intermediate host and it had a widespread distribution (3- 6). Anisakid larvae (*Anisakis simplex*) have been reported worldwide in approximately 200 fish species (7). In Egypt many research workers recorded it (8-13). Larval nematodes can result in zoonotic diseases especially *Anisakis simplex* is most associated with human diseases (14). Public health problems arise only when people eat raw or lightly preserved fish. (15). Recently, Scanning electron microscope provided helpful in studying the tegument structure of

the parasites (6). Therefore, the aim of the present study was to throw the light on the morphology of some larvae of some marine fishes using the light and scanning electron microscope.

### MATERIAL AND METHODS

#### Light microscope examination

A total of 441 marine fish samples representing 10 fish species were collected from Suez Canal area (Suez and Ismailia Provinces) during the period extended from April 2006 till March 2007. The collected larval cestodes were fixed in 10% formal saline under the slight pressure of a cover glass for 24 hours, and then the specimens were stored in only 5% formalin over night. The collected larvae washed several times in tap water and transferred to distilled water for 30-60 minutes. After that they were stained with acetic acid alum carmine, dehydrated in graded series of ethyl alcohol, cleared with clove oil, then xylene and mounted in Canada balsam (9,16). While the collected larval

nematodes were washed in saline solution, then relaxed and fixed in warm solution of 70% alcohol and 5% glycerol. The worms were then cleared in lactophenol and mounted in polyvol (17).

#### Scanning electron microscope examination

The larvae specimens were fixed immediately in 2.5% glutaraldehyde solution in 0.1 phosphate buffer (pH 7.4) for 30 minutes. After washing in the same buffer solution, they were fixed in 1% osmium tetroxide buffer for 1-2 hours, washed in distilled water, dehydrated in ethanol. Samples were critical point dried with carbon dioxide dryer. Dried samples were mounted on aluminum stumps and covered by gold film and viewed on Jeol (Japan) F 840A Electron Probe Microanalyzer Scanning Electron Microscope (18).

### RESULTS

Examination of the collected fishes revealed that 23.35% of the collected fishes were infected with cestode larvae and 37.64% were infected with larval nematodes. In the cestode larvae infection, the highest prevalence rate was recorded in *Euthynnus affinis* 100%, followed by *Atherina* 86%, *Trachus mediterraneus* 19.44% and *Morone labrax* 5%. While, the highest prevalence rate of larval nematodeinfection was recorded in *Trachus mediterraneus* 100% then *Sardinella* spp. 87.5%, *Atherina* 47%, *Saurida undosquamis* 41.86% and *Mullus surmuletus* 39.65%. Taxonomy and morphological description of the detected parasites were denoted as follow:-

#### Larval cestodes

**Phylum:** Platyhelminthes Gegenabaur, 1859.

**Class:** Cestoda

**Order:** Pseudophyllidea

**Family:** Diphyllbothriidae

**Genus:** *Ligula*

**Species:** *Ligula intestinalis*

**Host:** *Atherina* and *Euthynnus affinis*

**Habitat:** Body cavity

Plate (1A&B)

#### Description

The body of these plerocercoids was elongated, solid, whitish and flat. It measured 4 -13 cm in length and 0.6-0.9 cm in width. The external segmentation of strobila was absent. Two bothridial depressions were hardly visible on the anterior end representing of scolex.

**Order:** Proteocephalidea

**Family:** Proteocephalidae

La Rue, 1911.

**Genus:** *Proteocephalus*

**Species:** *Proteocephalus* sp. Weinland, 1858.

**Host:** *Atherina* &

*Trachus mediterraneus*

**Habitat:** Intestine

Plate (1 C)

#### Description

These larvae were small, white and ovoid in shape. The body ranged from 0.65 - 1.01 mm in length and 0.26- 0.29 mm in width. The larva formed from scolex carrying four typical equal suckers ranged from 0.08 - 0.13 mm in diameter and tail which was slightly rounded. The fifth apical sucker was also present and measured 0.05 - 0.12 mm in diameter. The scolices were free from spines or hocks.

**Order:** Cyclophyllidea

**Family:** Dilepididae

**Genus:** *Ophiovalipora*

**Species:** *Ophiovalipora* minuta

Coil, 1950.

**Host:** *Atherina*

**Habitat:** Intestine

Plate (1 D)

#### Description

The larval stages of these cestodes was small, white, and ovoid with short neck. The body measured 0.8 - 1.3 mm length and 0.38 - 0.41mm width. The scolex carrying four suckers armed with tow row of crown hooks measured 0.07 - 0.11 in diameter.

**Order:** Tetraphyllidean

**Species:** Tetraphyllidean larvae

**Host:** *Atherina*

**Habitat:** Intestine

Plate (1 E)

**Description**

The larvae were white and ovoid and having short neck. The body length was 1.6 – 1.69 mm and 0.4 – 0.42 mm as maximum Scolex bearing four suckers armed with hooks each measured 0.13 – 0.15 mm long and 0.12 – 0.13 wide. The terminal sucker not armed with hooks which measured 0.07 - 0.08 mm long and 0.08- 0.09 mm wide.

**Species:** Plerocercoid larvae

**Host:** *Atherina*

**Habitat:** Intestine  
Plate (1 F)

**Description:**

The larva was small, ovoid in shape without neck. The total length was 0.09 – 1.3 mm long and 0.2 – 0.25 mm wide. This plerocercoid could not identified to genus or species because of few diagnostically useful characters.

**Larval nematodes**

**Phylum:** Nematelminthes

**Class:** Nematoda

Rudolphi, 1808.

**Order:** Ascaridida

Skrjabin et Schulz, 1940.

**Superfamily:** Ascaridoidea

Raillet et Henry, 1915.

**Family:** Anisakidae

Raillet et Henry, 1912.

**Genus:** *Anisakis*

**Species:** *Anisakis simplex*

Rudolphi, 1809.

**Host:** *Mullus surmuletus*

**Habitat:** Abdominal cavity  
Plate (2 A&B)

**Description**

The larva was slender in shape and whitish in colour. The body measured was 21 – 26 mm in length and 0.37 - 0.51 mm in width. The cuticle had fine transverse striations. The mouth opening surrounding with three lips which were two ventro-lateral and one dorsal and having a characteristic antero-laterally boring tooth at its anterior end. The excretory pore was situated between the ventro-lateral lips. The mouth led to oesophagus which

measured 1.8–2.4 mm long and provided with an elongated ventriculus measured 1.02 – 1.40 mm long. The intestinal caecum was absent.

The tail was broadly rounded and measured 0.17-0.2 mm long and provided with mucron.

**Species:** *Anisakis sp.*

**Host:** *Atherina*, *Trachus mediterraneus*, *Sardinella sp.* and *Saurida undosquami*.

**Habitat:** Abdominal cavity and muscles.  
Plate (2 C&D)

**Description**

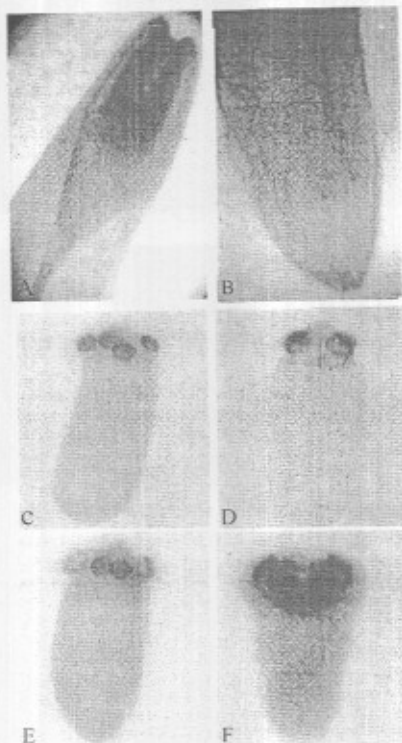
The total length of this larvae was 4.42 - 7.31 mm and its width was 0.15 - 0.24 mm. The anterior end of body characterized by the presence of prominent boring tooth. Oesophagus was cylindrical, muscular and measured 0.63 - 0.81 mm long ending in small globular ventriculus that measured 0.26 - 0.49 mm long and ventricular appendix was 0.41 - 0.69 mm long. The anus was lateral with a distance 0.15-0.18 from the tail. There was no mucron. <sup>Carus, 1863.</sup>

**Scanning Electron Microscope (SEM) of *Ligula intestinalis***

The anterior end of the plerocercoid *Ligula intestinalis* was blunt, more rounded with botherial grooves, while the posterior end was more tapering. Transverse striations were present and the magnification showed tegumental protuberances overlapped, bend and clasp each other with surface ridge system which characteristic for *ligula intestinalis* plerocercoides, Plate (3).

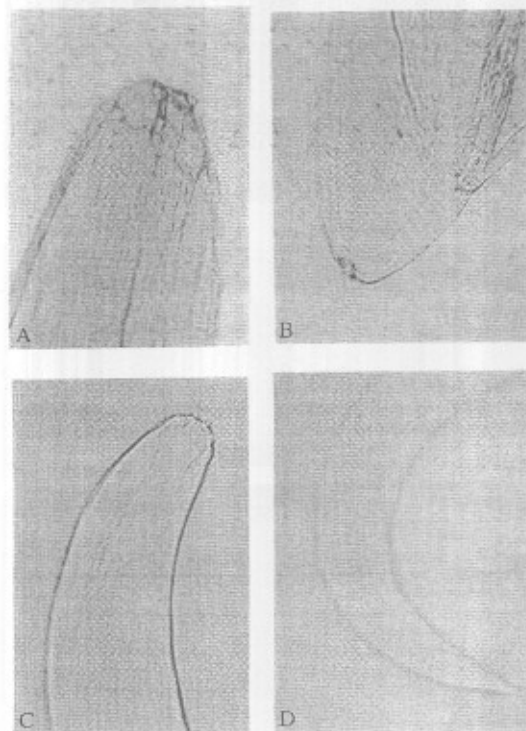
**Scanning Electron Microscope (SEM) of *Anisakis simplex* third- stage larvae**

The anterior end had a boring tooth projected anteroventrally which located ventral to the mouth opening, three lips were present one dorsal and two ventrolateral. The excretory pore was in form of a transverse slit opened between the ventrolateral lips. Transverse striations of cuticle was observed posterior to the lips. The posterior end of larvae showed a characteristic mucron, Plate (4).



**Plate (1)**

- A- *Ligula intestinalis* anterior end (X40).
- B- *Ligula intestinalis* posterior end (X40).
- C- *Proteocephalus* sp. (X100).
- D- *Ophiovalipora minuta* (X100).
- E- Tetrephyllidean larva (X100).
- F- Pleurocercoid larva (X100).



**Plate (2)**

- A- *Anisakis simplex* larva anterior end (X450).
- B- *Anisakis simplex* larva posterior end (X450).
- C- *Anisakis* sp. larva anterior end (X100).
- D- *Anisakis* sp. larva posterior end (X100).

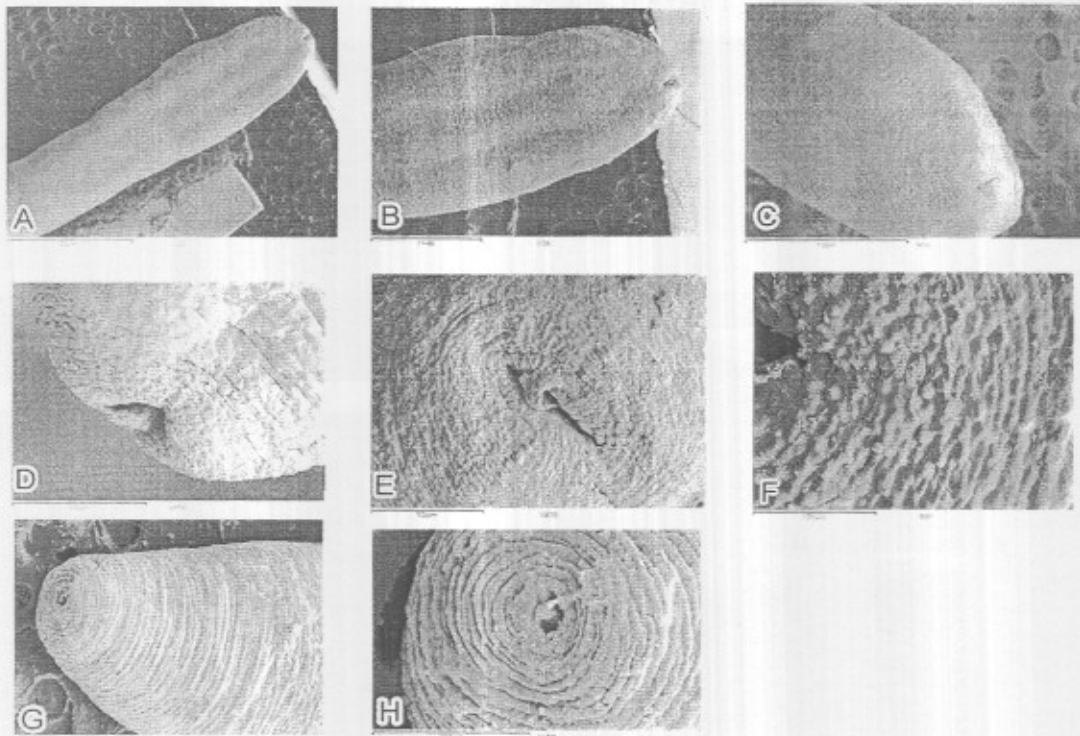


Plate (3): SEM micrograph of *Ligula intestina*

A, B & C- Showing ventral and dorsal aspects.

F- Showing magnified section showing tegumental protuberances and the ridge system.

G- Showing the posterior end.

D & E- Showing the anterior end.

H- Showing striations of the body.

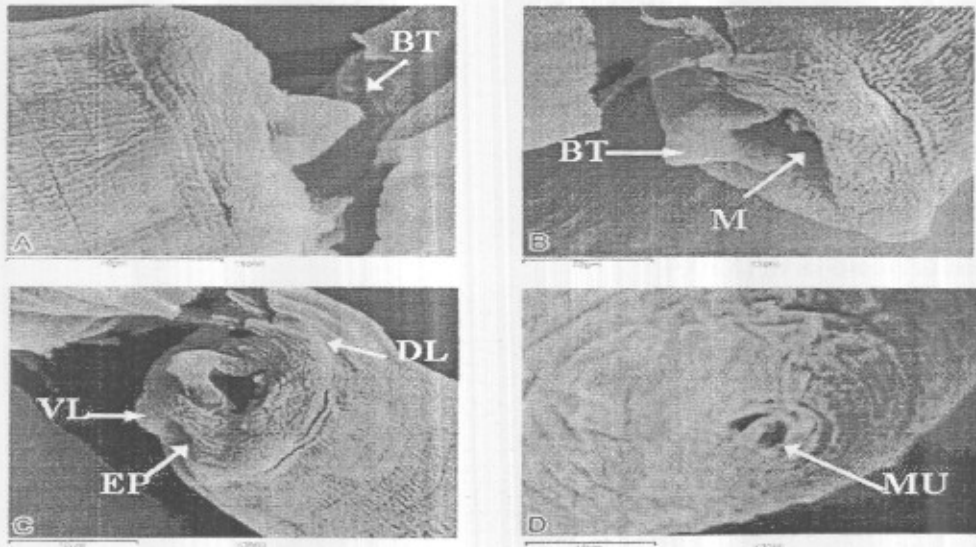


Plate (4): SEM micrograph of *Anisakis simplex* third-stage larvae

A-Showing the anterior end with the boring tooth. B-Showing the boring tooth in the mouth of the anterior end. C-Showing the boring tooth, the mouth, the dorsal lip, the excretory pore between the ventral lips and the transverse striations of the cuticle.

D-Showing the posterior end with the characteristic mucron.

## DISCUSSION

The present study revealed that the total prevalence rate of larval cestodes was 23.35%, in the Arabian Gulf fishes it was 27.16%, (19) while it was higher than cestode infection in the Mediterranean Sea fishes with prevalence rate 0.3% and 1.46%, respectively (9,20,21). Cestode infection could not be detected among the examined fishes, while it was lower than in Mediterranean sand smelt fish 100% (5).

Regarding the larval nematodes infection the total prevalence rate was 37.65%. This result was similar (22) (37.7%) but higher than (9) in the Mediterranean Sea fishes (3.96%) and (5) in Mediterranean sand smelt fish 20% and lower than in Red Sea fishes 45.9%(13).

Concerning with the morphological characters of cestodes larvae *Ligula intestinalis* which recorded from the body cavity of *Atherina*. The results coincided with those of previous studies (5,6). While *L. intestinalis* was obtained from *Barbus paludinosus*, *Rutilus rutilus* and *Tinca tinca*, respectively (4,23, 24).

*Proteocephalus sp.* was obtained from *Atherina* and *Trachus mediterraneus* (25) and recorded from *Trachus mediterraneus* (10) while it was detected from other marine fishes (8,26). All the previous authors recorded similar morphological description.

*Ophiovalipora minuta* it was detected from *Atherina* which (5,27). The simple anatomical characters coincide with those described previously (5,28-30). However, it was recorded from the Green heron (27), the eggs pass out of the heron eaten by a freshwater crustacean, possibly a copepod and develop into a precercoid, then eaten by a fish a plerocercoid develop in their organs. Finally a green heron eats the infected fish and the worm develops into the adult.

**Tetraphyllidean larvae** were collected from *Atherina* it was small, white and ovoid worm, with a short neck. The scolex had four suckers around a bulb and a terminal sucker without hooks this description similar to that previously described (5,28).

**Plerocercoid larvae** these Pleurocercoids were not highly specific but can develop in wide range of fish hosts (31). Similarly Pleurocercoid larvae was detected from *Atherina* as the present specimens (5).

Dealing with larval nematodes *Anisakis simplex* larvae (Rudolphi, 1809) it was characterized by antero-laterally directed boring tooth at its anterior end. The cuticle was provided with fine transverse striation. The tail was provided with a mucron at its tip. These structures were similar to that cited in several reports (9-13,32- 34).

*Anisakis sp.* Larvae were morphologically similar to those described by several researchers (5,19) where it was characterized by the presence of dorsal cephalic tooth, conical tail without mucron. Also it was detected from abdominal cavity and muscles of *Atherina* (5).

In the present study, scanning electron microscope (SEM) of *Ligula intestinalis* revealed a blunt anterior end more rounded with characteristic botherial grooves, while the posterior end was more tapering. Transverse striations were present. The magnification showed a characteristic tegumental protuberances overlapped, bend and clasp each other with surface ridge system. The previous features were similar to that of Mediterranean sand smelt fish (6).

In scanning electron microscope (SEM) observations of the anterior end of the third-stage larvae of *Anisakis simplex* a prominent boring tooth projecting anteroventrally and three bilobed lips were present and the excretory pore was in form of a transverse slit situated between the ventrolateral lips. Transverse striations of cuticle was observed posterior to the lips which gradually became wider at the posterior end. The posterior end of larvae showed a characteristic mucron. These observations were agreed with that obtained previously (12,32, 35).

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### الملخص العربي

دراسات بالمجهر الضوئي و الألكتروني الماسح على بعض يرقات أسماك المياه المالحة

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المعمل المركزي لبحوث الثروة السمكية- مركز البحوث الزراعية- وزارة الزراعة\*\*

أجريت هذه الدراسة على عدد ٤٤١ من الأسماك البحرية (٦٤ مورجان، ٣٤ لوت، ٢٠ قاروص، ٥٨ بربوني، ٢٩ دراك، ١٠٠ بساريا، ٣٤ مكرونة، ٩ بلاميطة، ٣٨ سردين، ٣٦ باغة) حيث تم تجميع العينات من قناة السويس (محافظة السويس و الأسماعيلية) في الفترة من أبريل ٢٠٠٦ حتى مارس ٢٠٠٧.

أوضحت الدراسة أن نسبة الإصابة العامة ليرقات الديدان الشريطية كانت ٢٣,٣٥% حيث كان أعلى معدل إصابة في أسماك البلاميطة، البساريا، الباغة والقاروص بنسبة (١٠٠، ٤٤، ١٩، ٨٦ و ٥%) على التوالي.

و نسبة الإصابة العامة ليرقات الديدان الاسطوانية كانت ٣٧,٦٤% حيث كان أعلى معدل إصابة في أسماك الباغة، السردين، البساريا، المكرونة و البربوني بنسبة (١٠٠، ٨٧,٥، ٤٧، ٤١,٨٦ و ٣٩,٦٥%) على التوالي.

تم التعرف على خمسة أنواع من يرقات الديدان الشريطية (الليجيولا إنتستيناليز، البروتوسيفيلس، اوفيوفالبيورا مينيوتا، يرقات التيترافيلايدين، يرقات البلوروسركويد) و كذلك على نوعين من يرقات الديدان الاسطوانية (الانيساكس سيمبلكس، نوع من يرقات الأنيساكيد).

و قد اشتملت الدراسة على فحص يرقات الليجيولا إنتستيناليز و الانيساكس سيمبلكس باستخدام الميكروسكوب الإلكتروني الماسح و اضافة المزيد من التفاصيل الدقيقة لهذه اليرقات.