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Studies on some digenetic trematodes paraisitising some groupers fish in Jeddah area (Red Sea Coast, Saudi Arabia)

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ABSTRACT

The present study includes description of three species related to three different families of digenetic trematodes. These species were extracted from the digenetic tracts (stomach, pyloric caeca and gut) of two species of the most commercial fish in the studied area (*Epinephelus fuscoguttatus* and *E. tauvina*). All fish samples were collected from Jeddah coast (Saudi Arabia, Red Sea), during the period from January to December 2006. In this study, 114 fish specimens were investigated searching for trematodes. The extracted endoparasites were flattened, fixed and prepared for further study. These digenetic trematodes were *Cainocreadium epinepheli, Erilepturus hamati* and *Prosorhynchus jupe*. Identification and full description of these trematodes were given using both light and scanning electron microscopy. The using of SEM in this study has revealed with precision a number of surface structures in such details. Comparisons between these parasites and the related forms were reported. The highest infection of such trematodes was detected in *E. fuscoguttatus* (21.43 %) and it was 20.69 % in *E. tauvina.* The prevalence of infection was seen to be high during spring and autumn, with slightly decrease during summer and winter.

Key words: *Epinephelus,* digenetic trematodes, endoparasites ultrastructure, Red Sea, Jeddah

INTRODUCTION

The study of fish parasites has attracted the attention of many parasitologists, since they not only cause injuries or even death to the fish[(1)], but also do some human health problems[(2)]. These parasites may cause excitement of fish, loss of weight or may introduce toxic metabolic products and depress food metabolism; moreover these hosts may act as carriers or vectors of other pathogens[(3)].

Digenetic trematodes of the Red Sea were reported by many authors[(4,5,6,7,8,9,10,11)]. However, very scanty researches have been recorded on the parasitic trematodes such as Bakriba [(12)]. Consequently, the present study derives its value and represents one of the first trials in Jeddah coast to study such helminth parasites.

During the present study, digenetic trematodes were chosen since they attain large sizes and exist in considerable numbers for general description. The obtained parasites were redescribed using light and scanning electron microscopy. The high magnification provided by using SEM has revealed with precision a number of details that were not reported before. The main target of this study is to identify and give a full description to the digenetic trematodes in the common species of groupers, which chosen for their commercial value in the Red Sea[(13)].

MATERIALS AND METHODS

One hundred and fourteen serranid fish (Groupers), namely *Epinephelus fuscoguttatus* (Forsskål, 1775) and *E. tauvina* (Forsskål, 1775), were collected weekly from the Red Sea, off Jeddah coast (Saudi Arabia) during the year 2006. The collected fishes were identified according Randall [14]. Fishes were dissected immediately after few hours from capture. The digestive tracts including stomach, pyloric caeca and intestine were isolated and searched for the digenetic trematodes.

Relaxation, fixation, staining and mounting of the collected parasites were carried out according to Pritchard and Kruse[15]. Trematodes were flattened between a slide and a cover slip, then immersed in formalin (5%) for about 2-4 hours. Specimens were washed several times in fresh distilled water, then stained by Grenacher's borax carmine stain[16]. Mounted specimens were examined and photographed using a photo-research Microscope (Model Dialux 20EB Leitz). Measurements were carried out using a graduated slide to the nearest 0.01 mm and were expressed as mean (± S.E.).

For scanning electron microscopy, fresh parasitic specimens were fixed immediately after isolation in a mixture of 1:3 gluteraldehyde (2%) and osmium tetraoxide (1%), dehydrated in graded series of alcohol, dried with carbon dioxide, mounted on aluminum stubs, and then coated with gold. Specimens were examined and photographed under scanning electron microscope (Model JEOL, ISM- 63600 LV) at 15 Kv.

Identification of parasites was done according to the morphological similarities with descriptions of Yamaguti[17], Bray *et al.*[18] and Nahhas *et al.* [19]. Collected specimens were also identified by Dr. Rod Bray, at the Natural History Museum of London,UK.

RESULTS AND DISCUSSION

***Cainocreadium epinepheli* Yamaguti [17]** (Fig. 1)

Family: Opecoelidae Ozaki (1925); Subfamily: Plagioporinae Manter (1942);
Genus: *Cainocreadium* Nicoll (1909)

Description is based on two specimens collected from the pyloric caeca of *Epinephelus tauvina* and *E. fuscoguttatus*. The body is elongated pear-shaped, tapering anteriorly and crenulate posteriorly. It measures about 4.55 mm long by 1.55 mm wide. The oral sucker (os) is nearly rounded in shape, small in size (measuring 0.275 mm in diameter). The ventral sucker (vs) is slightly oval in shape and larger than the oral one, measuring about 0.525 x 0.475 mm. The mouth aperture leads to a short and broad prepharynx (pr) measuring 0.055 mm long, then to a muscular pharynx (p) measuring 0.18 x 0.20 mm. The oesophagus (o) is cylindrical, measuring 0.32 x 0.175 mm and bifurcated into two long intestinal caeca (ic)which running blindly to the posterior extremity of the body. The ovary (ov) is measuring about 0.32 x 0.23 mm and with four rounded lobes. The uterus (u) has many coils and lies in the space between ovary and the ventral sucker. The cirrus pouch (cp) is long, lies lateral to the ventral sucker and measures 0.89 x 0.13 mm. The metraterm (me) is well developed, extending as a longitudinal tube along the cirrus pouch and contains oval embryonated eggs. The genital pore is median and lies just behind the bifurcation of the intestinal caeca. The vitellaria (v) are follicular and enormous in number, extending from the level of intestinal bifurcation along the whole sides of the body. Testes are paired,diagonal with irregular shape and located in the posterior third of

the body. The anterior testis (at) measures 0.41 mm in diameter while the posterior one (pt) measures 0.50 mm in diameter; the distance between the two testes is about 0.15 mm. A tubular median excretory vesicle (ev) appears to be extended from the level of the posterior testis till opens at the posterior end of the body by an excretory pore (ep).

Examination by scanning electron microscopy declair that the tegument is smooth, nonspinous and slightly folded, with some transverse lateral ridges as tegumental thickening around the body (Plate 1, A). The high magnification shows large number of dome-shaped papillae or scales, formed a condensed structure covering all the tegument and separated with a network of grooves (Plate 1, B).

Manter[20] pointed to the close relation between the three genera *Cainocreadium*, *Hamacreadium* and *Peracreadium*. Yamaguti[21] listed *Cainocreadium* as a synonym of *Peracreadium*. Durio and Manter[22] transferred *Hamacreadium* species with a median genital pore to the genus *Cainocreadium*. Yamaguti[17] described *Hamacreadium epinepheli* which collected in Japan from two fishes (*Epinephelus akaara* and *Lethrinus haematopterus*). He differentiated *Hamacreadium epinepheli* from *Hamacreadium mutabile* (Linton, 1910) by the position of the genital pore on the median line in the former and on the left side in the latter. Nagaty[23] believed that difference between these two species is insufficient and considered both *Hamacreadium epinepheli* (Yamaguti, 1934) and *Hamacreadium mutabile* (Linton, 1910) are syonyms. Durio and Manter[22] supported Yamaguti [17] and renamed *Hamacreadium epinepheli* to *Cainocreadium epinepheli*. This latter view was accepted by Yamaguti[24] after Parukhin[7] separated *Hamacreadium epinepheli* from *Hamacreadium mutabile*. Ramadan[5] redescribed *Hamacreadium mutabile* from many fishes collected from the Red Sea. He reported that his material differs from other species by its characteristic egg shape, which being oval, operculated at one end and with a small distinct process at the other end as well as the characteristic arrangement of the ovarian lobes; but his description based on the presence of the genital pore in the median line just behind the intestinal furca, a character which must places his specimen in the genus *Cainocreadium* and not *Hamacreadium*.

***Erilepturus hamati* Yamguti (1934)** (Fig. 2)

Family: Hemiuridae Lühe (1901);
Subfamily: Dinurinae Looss (1907);
Genus: *Erilepturus* Woolcock (1935)

Description is based on six specimens collected from the stomach of about 58 fish of *Epinephelus tauvina* species. The body is elongate fusiform, somewhat flattened dorso-ventrally. It is divided into distinct regions: a soma (body proper) and the retractile tail or ecosoma (ec). Ecosoma is partly or totally retracted into the soma. The total body length is about 4.325 mm long by 1.08 mm wide at the acetabular level. The oral sucker (os) is small, subterminal, cup-shaped, measuring 0.248 mm long x 0.268 mm wide, and preceded by a short pre-oral lobe. The ventral sucker (vs) is larger than the oral one and measures 0.606 x 0.556 mm, and lies at the middle of soma slightly towards the right side. The oral sucker is followed by a globular pharynx (p) which measuring 0.12 x 0.14 mm. The oesophagus (o) is short,

measuring about 0.050 x 0.045 mm, bifurcating into two narrow intestinal caeca extending backwards to near the ecosoma origin. The testes are slightly diagonal, situated at a short distance behind the ventral sucker. The anterior (or right) testis (at) measuring 0.245 mm long x 0.25 mm wide, while the posterior (or left) testis (pt) measuring 0.27 x 0.26 mm. The seminal vesicle (sv) is large, saccular, opposite to the posterior margin of the ventral sucker and measuring 0.585 x 0.31 mm. The ovary (ov) is ovoid, lies just behind to the posterior testis, lobed and measuring about 0.24 x 0.31 mm. The uterus (u) is long, filled with numerous small brown eggs (e); the egg measures 0.0204 x 0.0117 mm. The uterus extends anteriorly to the metraterm then to the tubular genital atrium (ga). The genital pore lies immediately behind the pharynx. The vitellaria (v) arranged in a rosette form and consist of 7-8 digitiform tubes surrounding the ovary and not extending into the ecosoma. The excretory pore lies on the posterior tip of the ecosoma.

The scanning electron micrographs (Plate 2, A) revealed that the worm is cylindrical in shape and contains two circular suckers; the tegument is thick and provided with transverse cuticular crenulations. The oral sucker (Plate 2, B) is small containing the oral aperture and preceded by a muscular pre-oral lobe, while the ventral sucker (Plate 2, C) is larger, lies ventrally, oval in shape and with a large number of sensory papillae, which scattered randomly especially on the posteriorridge of its ventral lip. Each sensory papilla (Plate 2, D) has a triangular or pear-like shape and directed posteriorly. The excretory pore (Plate 2, E) is semi-circular in shape, situated at the extreme posterior end of the worm and surrounded by a few number of sensory papillae.

Until the year 1992, about 31 species have been described under the genus *Erilepturus*. Bray *et al.*[25] reviewed that this genus including 24 species as synonyms of *Erilepturus hamati*, and the other 7 species were kept valid in the genus. Al-Yamani and Nahhas[26] described a trematode named *Clupenuroides sheemi*, closely similar to *Erilepturus hamati* but without seminal receptacle. The present study agree with that of Hassanine[26] who reported that the genus *Clupenuroides* may be a synonym to genus *Erilepturus*, perhaps because of the difficulty in observation of the seminal receptacle which was not indicated in many specimens of *Erilepturus.* The present redescription is very closely identical to the original description given by Yamaguti [17].

***Prosorhynchus jupe* Kohn (1967)** (Fig. 3)

Family: Bucephalidae Poche (1927);
Subfamily: Prosorhynchinae Nicoll (1914);
Genus: *Prosorhynchus* Odhner (1905)

Description is based on four specimens collected from the pyloric caeca and gut of *Epinephelus fuscoguttatus*. The body is subcylindrical, measuring about 2.02 x 0.295 mm. The rhynchus (rh) is in the form of funnel-shaped plug and measuring 0.145 x 0.16 mm. Mouth aperture (m) lies at about the end of the anterior third of the body length. Pharynx is small and muscular; oesophagus (o) is short; the intestinal caecum (ic) is saccular and measuring 0.345 x 0.113 mm. The two testes are entirely separated from each other; the anterior testis (at) lies immediately posterior to the ovary and measures 0.115 x 0.105 mm; the posterior testis (pt) lies at the middle of the worm and measures 0.105 x 0.12 mm. The cirrus pouch (cp) is elongated sac, lies near the posterior end, measures 0.33 x 0.08 mm and containing ellipsoidal

seminal vesicle. The genital pore lies near the posterior extremity. The ovary (ov) is rounded, measuring about 0.095 mm in diameter, nearly at the same level of pharynx. There are two vitellarian bunches (v), one anterior opposite to the ovary and the posterior one lies between the two testes. Each bunch consists of about 12-13 follicles, each follicle measures about 0.0483 mm in diameter. The uterus (u) extends from the anterior testis to the posterior end of the body. Eggs (e) are small, ovoid and measuring 0.03 x 0.023 mm. The excretory pore (ep) lies at the posterior end of the body, while the excretory vesicle was not detected.

SEM-photomicrographs (Plate 3) displayed the general shape of the body and detected that the tegument is thick layered and covered with scales on the different parts of the body (Plate 3, A & C) and on the rhynchus (Plate 3, B). Groups of scales are densely scattered randomly on the outer body surface next to the rhynchus till the posterior part of the body. These scales have serrated margins.

Ozaki[28] considered the genus *Prosorhynchus* as a synonym of the genus *Gotonius*. Bray and Justine[18] reported that there are over 70 species of *Prosorhynchus* were listed. Nahhas *et al.*[29] recorded three species of *Prosorhynchus* from the Arabian Gulf: *Prosorhynchus pacificus, Prosorhynchus manteri* and *Prosorhynchus epinepheli*. *Prosorhynchus ozakii* was reported from the *Epinephelus* sp. of the Red Sea by Parukhin[7] but he gave neither measurements nor illustration. *Prosorhynchus epinepheli* was described from *Epinephelus* fish by Yamaguti[30], Saoud *et al.*[6] and by Kardousha[31]. *Prosorhynchus atlanticus* was recorded by Manter[32], Nahhas and Short[33], Overstreet[34], Madhavi[35], Fischthal[36] and Amato[37]. *Prosorhynchus pacificus* was described by Nahhas *et al.* (2006), whom listed that the shape of the rhynchus shows some variations, especially with respect to its base. *Prosorhynchus atlanticus* was considered as a synonym to *Prosorhynchus pacificus* by many authors[32, 38, 34, 35, 39], but Siddiqi and Cable [40] and Nahhas and Cable [19] objected such opinion on the ground that egg size was variable.

Out of one hundred and fourteen serranid fish examined, twelve were infested by such studied trematodes with a prevalence of 10.5 %. The highest infection of such three studied trematodes was detected in *E. fuscoguttatus* (21.43 %) while it was in *E. tauvina* (20.69 %). It was also noticed that the incidence of infection with such digenetic trematodes was seen to be high during spring and autumn seasons, with slightly decrease during summer and winter. This may attributed to the differences in the temperature, extensive feeding of fishes and the availability of the intermediate hosts of these parasites during such seasons.

A comparison between the morphometric dimensions of the main parts of the three studied trematodes in addition to the hosts and sites of infection were listed in Table (1).

Table 1. Main morphometric dimensions (in mm) of the three studied digenetic trematodes

Measurements (Mean ± SD)	*Cainocreadium epinepheli*	*Erilepturus hamati*	*Prosorhynchus jupe*
Body length (mm)	4.280 ± 0.740	4.260 ± 0.150	2.015 ± 0.078
Body width (mm)	1.580 ± 0.460	1.000 ± 0.080	0.295 ± 0.021
Anterior testis (L)	0.411 ± 0.013	0.245 ± 0.007	0.113 ± 0.006
Anterior testis (W)	0.410 ± 0.028	0.250 ± 0.007	0.107 ± 0.006
Posterior testis (L)	0.530 ± 0.021	0.265 ± 0.007	0.103 ± 0.005
Posterior testis (W)	0.360 ± 0.000	0.255 ± 0.007	0.120 ± 0.000
Ovary (L)	0.320 ± 0.000	0.240 ± 0.014	0.096 ± 0.006
Ovary (W)	0.230 ± 0.000	0.305 ± 0.007	0.097 ± 0.005
Eggs (L)	0.070 ± 0.014	0.109 ± 0.128	0.030 ± 0.000
Eggs (W)	0.035 ± 0.021	0.012 ± 0.0007	0.023 ± 0.000
Hosts	*Epinephelus tauvina* and *E. fuscoguttatus*	*Epinephelus tauvina*	*Epinephelus fuscoguttatus*
Site of infection	pyloric caeca	stomach	pyloric caeca and gut

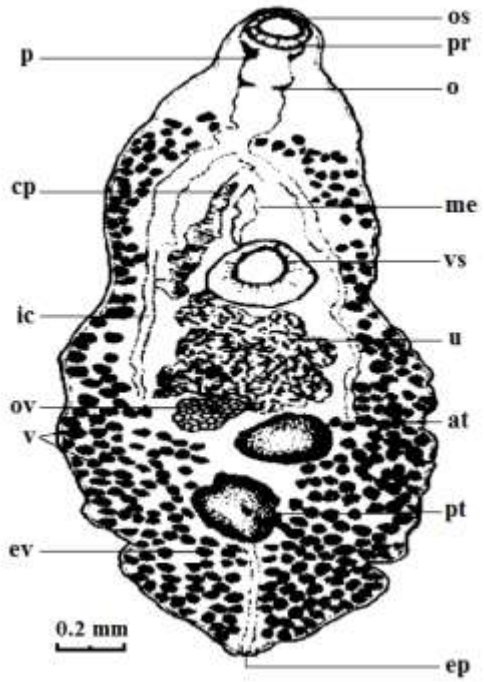

Fig. 1. Photomicrograph of adult *Cainocreadium pepinepheli* Yamaguti (1934). at, anterior testis; cp, cirrus pouch; ep, excretory pore; ev, excretory vesicle; ic, intestinal caecum; me, metraterm; o, oesophagus; os, oral sucker; ov, ovary; p, pharynx; pr, prepharynx; pt, posterior testis; u, uterus; v, vitellaria; vs, ventral sucker

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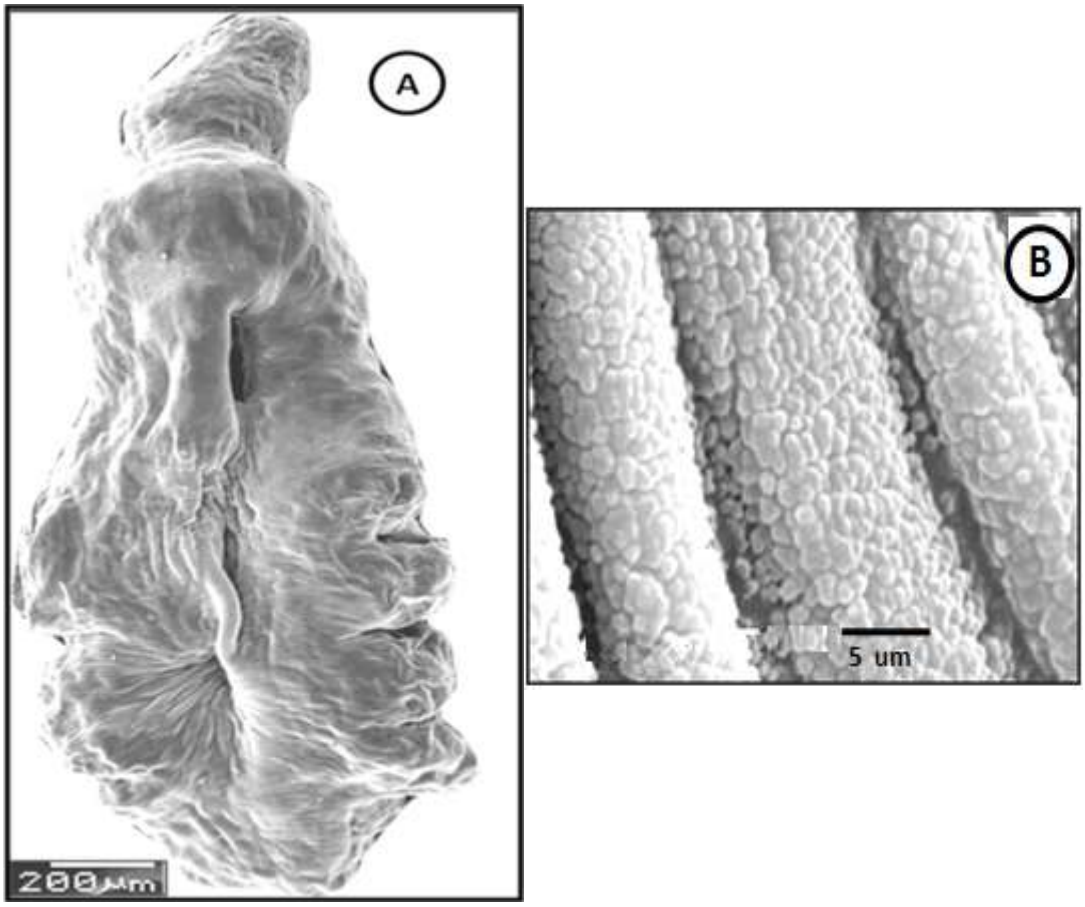

Plate 1. SEM- photomicrographs of adult *Cainocreadium pepinepheli* Yamaguti (1934). A, whole mount; B, a magnified part of scales on body tegument

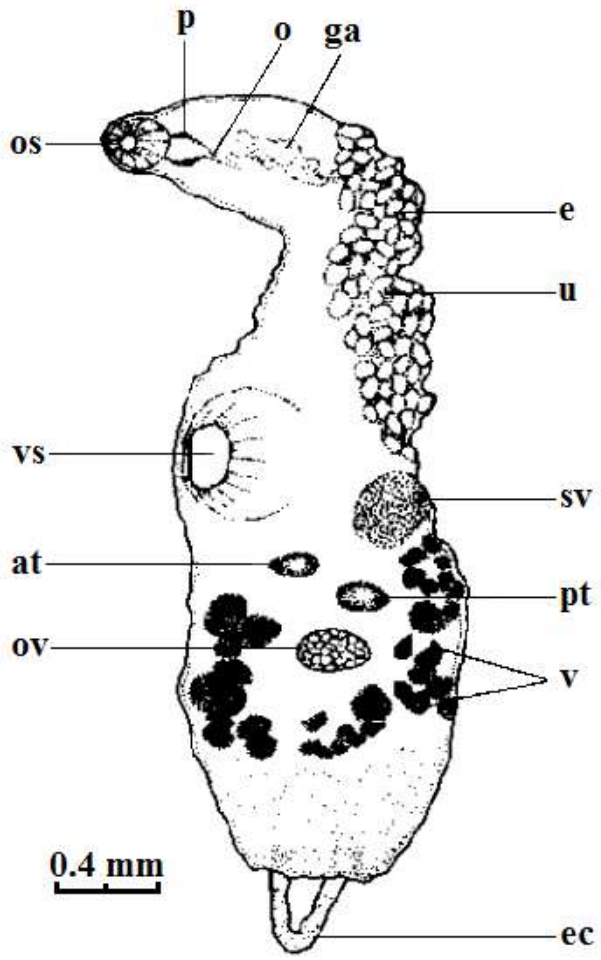

Fig. 2. Photomicrograph of adult *Erilepturus hamati* Yamaguti (1934). at, anterior testis; e, egg; ec, ecosoma; ga, genital atrium; o, oesophagus; os, oral sucker; ov, ovary; p, pharynx; pt, posterior testis; sv, seminal vesicle; u, uterus; v,vitellaria; vs, ventral sucker

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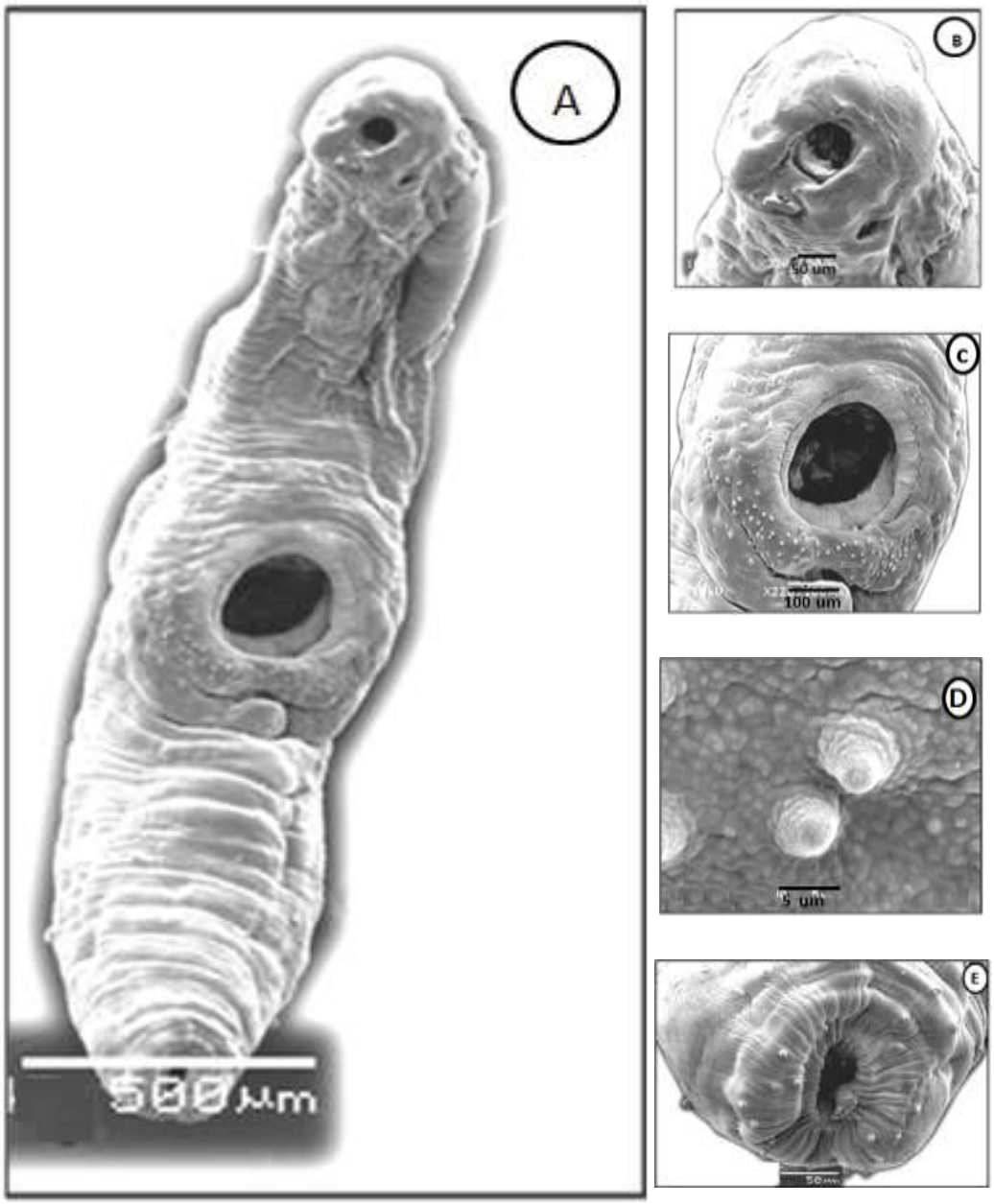

Plate 2. SEM- photomicrographs of adult *Erilepturus hamati* Yamaguti (1934); showing the whole worm (A), a magnified part of oral sucker (B), ventral sucker (C), sensory papillae on body tegument (D) and excretory pore (E)

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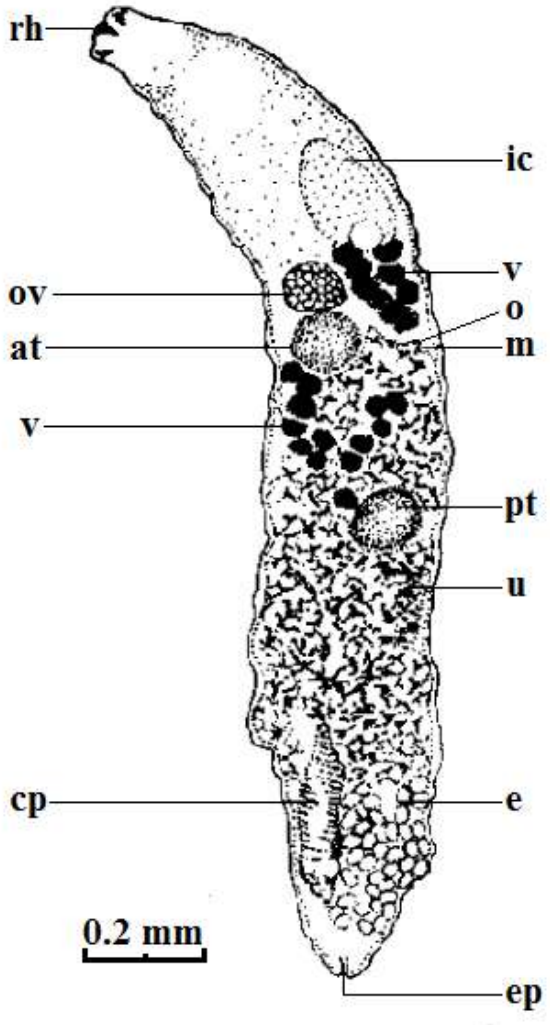

Fig. 3. Photomicrograph of adult *Prosorhynchus jupe* Kohn (1967).
at, anterior testis; cp, cirrus pouch; e, egg; ep, excretory pore; ic, intestinal caecum; m, mouth; o, oesophagus; ov, ovary; pt, posterior testis; rh, rhynchus; u, uterus; v, vitellaria

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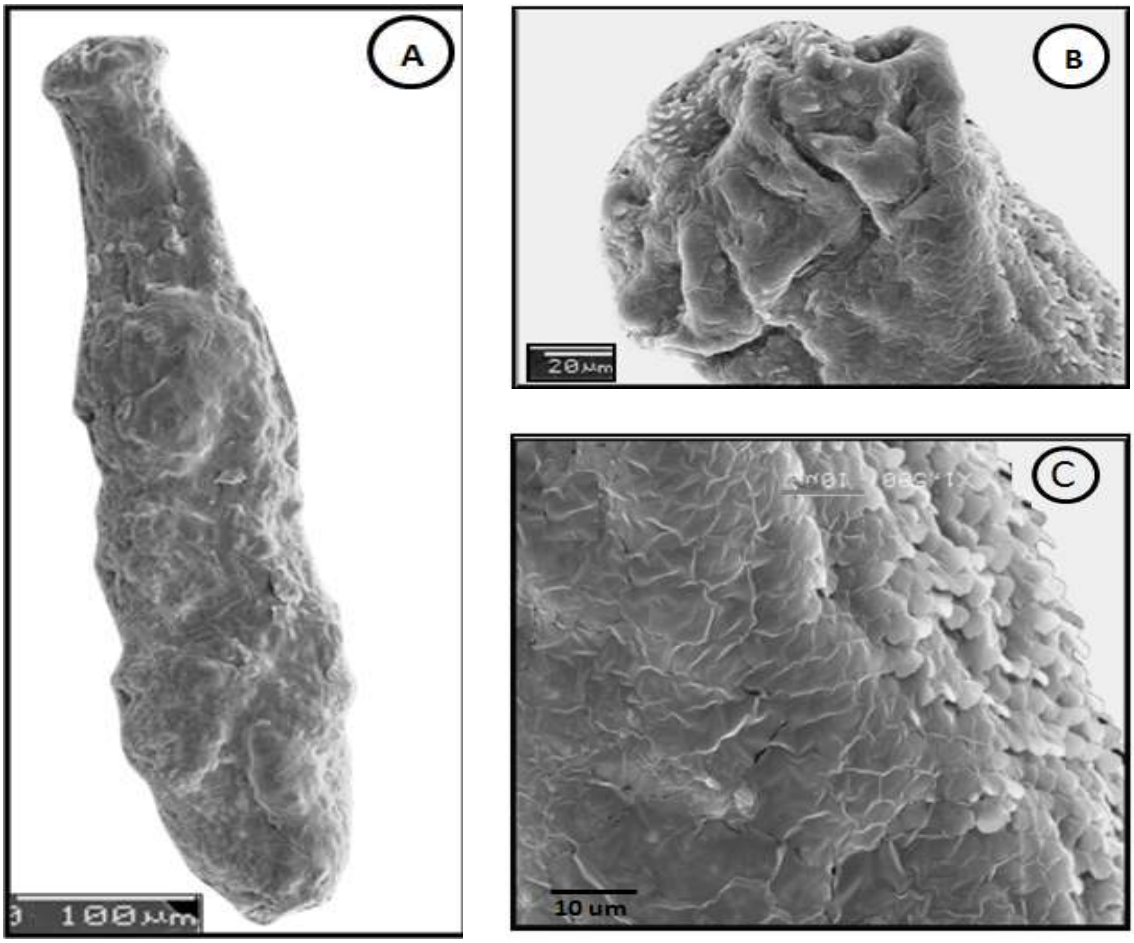

Plate 3. SEM- photomicrographs of adult *Prosorhynchus jupe* Kohn (1967). A, whole mount; B & C, magnified parts of rhynchus and scales on the tegument.

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دراسات على بعض التريماتودا (ثنائية العائل) المتطفلة على بعض أسماك الكشر فى منطقة جدة (ساحل البحر الأحمر –المملكة العربية السعودية)

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المستخلص

يشمل البحث وصف لثلاثة أنواع من الديدان االورقية تتبع لثلاثة عائلات مختلفة، وهى *كينوكريديم إبينيفيلى*، *إريلبترس هاماتى*، *وبروسورينكس جوبى*. وهذه الطفيليات تصيب نوعان من الأسماك الإقتصادية الشائعة لعائلة الكشر (الهامور) وهى *إبينيفيلس فوسكوجوتاتس*، *إبينيفيلس توفينا*. وجميع هذه الأسماك مصادة من شاطىء جدة بالبحر الأحمر- خلال الفترة من يناير وحتى ديسمبر 2006. تم فى هذه الدراسة فحص القناة الهضمية (المعدة- الردب البوابى- الأمعاء) لحوالى 114 سمكة (56 من النوع الأول، 58 من النوع الثانى) بحثآ عن الديدان الورقية بالطريقة التقليدية للفحص وبإستخدام المجهر الضوئى والمجهر الإلكترونى الماسح. وقد ساعد المجهر الإلكترونى الماسح على الوصف الدقيق لبعض التراكيب السطحية والتى ساعدت أيضآ فى التعريف الدقيق لهذه الطفيليات. وتم عمل مقارنات لهذه الطفيليات مع نظيراتها المسجلة فى الأبحاث السابقة. وقد تم تسجيل نسبة الإصابة بهذه الديدان الثلاثة فى أسماك *إبينيفيلس فوسكوجوتاتس* حوالى 21.43 % ، وفى أسماك *إبينيفيلس توفينا* 20.69 % . كما لوحظ أن نسبة الإصابة كانت عالية خلال فصلى الربيع والخريف، مع إنخفاض بسيط خلال فصلى الصيف والشتاء.