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Scanning Electron Microscope of *Sclerodistomum aegyptiaca* n. sp. (Digenea, Sclerodistomidae) from the Marine Fish *Saurida undosquamis* from the Suez Gulf, Red Sea, Egypt.

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ABSTRACT

A new trematode species *Sclerodistomum aegyptiaca* (Hemiuroidea, Sclerodistomidae) was isolated from (50%) out of 120 (50 males and 70 females) Brushtooth Lizard fish *Saurida undosquamis* collected from the Suez Gulf, Red Sea. Females fish were more infected (64.3%) than males (30%); the total mean intensity was (2.75±1.44), it was (2.26±1.33) in males and (2.9±1.45) in females. The effect of some biological factors (sex, weight) of the host on the infection rate has been studied using statistical analysis. It revealed that there is no significant difference between the infected males and females (P<0.01). Also, larger fish had more infection prevalence than smaller ones. The new species was studied using light and scanning electron microscope. It is distinct morphologically by the presence of (1) musculated esophageal bulb, (2) extremely short esophagus, (3) the position of gonopore that located very near to acetabulum than the oral sucker, (4) the ovary in the hind body nearer to the posterior extremity than acetabulum, (5) the extension of the uterus (6) the distribution and shape of vitellaria. SEM revealed the distribution of minute spines on the ventral surface of the worm except on the oral sucker and the inner rim of ventral sucker, the presence of different types of papillae, pits, tegumental folds and ridges.

INTRODUCTION

Among the animals, fish are the most important hosts for maintenance of parasites mainly helminthes. Most of fish have parasites and they not only serve as hosts of different parasites but also serve as carrier of many larval parasitic forms that affect fish health growth and survival (Moyo *et al.*, 2009). The helminthes cause economic losses by decreasing the aqua resource production, reduction in fish growth and increase susceptibility of fish to other pathogens and raising mortality rates (Cowx, 1992; Shah *et al.*, 2012). Consequently, it causes serious diseases in many vertebrates including man (Rhode, 2005; Amer, 2014). From economic point of view, helminthes cause a significant damage to their hosts and their pathogenic effects are diverse.

The infected fish by parasites have clinical signs as anemia, weakness, severe emaciation due to the absorption of a considerable quantity of nutritive substances from host through their body surfaces (Banerjee *et al.*, 2017). Some infested fish showed sluggish movement, loss of condition with paler coloration and imbalanced swimming as indicated by Eissa *et al*. (2012). The Lizard fish *Saurida undosquamis* Richardson, 1848 (Synodontidae) is a very commercially important fish species in marine aquatic water especially in Egypt with a very good prospect for aquaculture. A few authors have been manipulated the parasitic infection of *S. undosquamis* fish (Sathyanarayana, 1982; Bray, 1990; El-Naffar *et al*., 1992; Abdel-Baki *et al*., 2009 and Peyghan *et al*. 2009). *Saurida undosquamis* having a wide geographical spread, a high growth rate, resistant to handling and stress and well appreciated in a wide number of African countries.

The surface morphology using SEM technique is very useful in studying the details and for distinguishing the much closed species of trematodes that are not differentiated by light microscopy study. These details include many tegumental structures such as spines, papillae, teeth, ridges and sensory receptors. Many parasitologists have been studied the surface topography of several species of trematodes, *Shistosoma* spp. (Miller *et al.*, 1972; Sobhon and Upathum, 1990), *Fasciola* spp. (Becker *et al.*, 1980; Meaney *et al.*, 2004), *Zygocotyle lunata* (Sotillo *et al.*, 2012), *Orthocoelium parvipapillatum* (Anuracpreeda *et al.* 2016) and *Allogenarchopsis bareilliensis* (Gupta *et al.*, 2017). There is no enough information about the parasites that infect *S. undosquamis* fish and the factors that help in this process so, a further research works on these parasites are needed to explore the insufficient data on them.

MATERIALS AND METHODS

Fishing and Sampling Sites:

A total number of 120 marine fish *Saurida undosquamis* were collected from the Suez Gulf, Red Sea. The fish ranged in weight from 40-220 gm. and from 15-35 cm. in total length. The samples were collected at different seasons throughout the whole year of 2016.

Parasitological examination:

The fish were dissected immediately and the abdominal cavities were inspected for extra-intestinal helminthes. Internal organs such as alimentary canal, kidney, heart, liver, spleen and gonads were isolated from body cavity then washed several times by 0.7% saline solution. The intestine was carefully split open longitudinally to aid the emergence of helminth parasites.

Microscopy and mounting:

The collected trematodes were fixed in 70% ethyl alcohol, stained in Acetic Acid Alum Carmine, cleared in Clove oil and mounted by Canada balsam. For (SEM) study flukes were washed in saline solution (0.7%), fixed in 2% glutraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and post-fixed in osmium tetroxide (OsO_4) for 2 hours then washed in sodium cacodylate buffer, dehydrated in ascending ethanol series and CO_2 critical point dried (Abdou, 2008). The specimens mounted on stubs then coated with gold, examined and photographed by scanning electron microscope (Jeol. JSM-5400) at the Atomic Energy Agency, Cairo, Egypt.

Illustrations and measurements:

Drawings were done with the help of Camera Lucida and measurements were taken from a compound microscope with the use of a graduated slide. All measurements were taken (length x width) in millimeters unless stated otherwise. Parasite specimens were photographed by camera attached microscope.

Deposition of specimens:

Parasite specimens (Holotype and Paratypes) were deposited in the helminthes collection in the Zoology Department, Faculty of Education, Ain Shams University.

Statistical analysis: SPPS 16.0 for Windows (Paired samples test) was used.

RESULTS

The examined fish were 50 males and 70 females with different sizes. Sixty samples were found infected with trematode parasites with a percentage of (50%). Females fish were more infected (64.3%) than males (30%) (Table 1). The total mean intensity was (2.75±1.44), the mean intensity in males was (2.26±1.33) and in females was (2.9±1.45). The statistical analysis revealed that no significant difference between males and females infected by *Sclerodistomum aegyptiaca* n. sp. ($P<0.01$). The trend of infection prevalence was observed positive to host body size that have medium and large sizes and negative to those of small body sizes of the host (Table 2).

Table 1: The prevalence (%) of *Sclerodistomum aegyptiaca* n. sp. in relation to the sex of the host

Fish Sex	No. Examined Fish	No. Infected Fish	Prevalence %	Mean Int.±SD
Male	50	15	30	2.26±1.33
Female	70	45	64.3	2.9±1.45

Paired Samples Test:
Mean= 1.083, Std. Dev. = 0.573, Std. Error Mean=0.052, T =20.68, df=119
Sig. (2-tailed) =0.00

Table 2: The prevalence (%) of *Sclerodistomum aegyptiaca* n. sp. in relation to host's weight

Body Weight (g)	No. Examined	No. Infected	Prevalence %
40-100	9	0	0
101-150	49	20	40.8
151-220	62	40	64.5
Total	120	60	50

In the present investigation, a trematode species isolated from the fore intestine of *S. undosquamis* was identified as *Sclerodistomum aegyptiaca* n. sp. Recent publications present diverse opinions concerning the classification and taxonomy of trematodes and the following keys were consulted for identification the present fluke: Yamaguti (1958); McDonald (1981); Gibson *et al.* (2002) and Jones *et al.* (2005). The trematode was studied for accurate identification using scanning electron microscope.

***Sclerodistomum aegyptiaca* n. sp.**

Taxonomic summary
Family: Sclerodistomidae (Platyhelminthes, Azygiida) Odhner, 1905
Type host: *Saurida undosquamis* Richardson, 1848 (Synodontidae)
Type locality: Gulf of Suez, Red Sea**,** Egypt.
Site of infection: Fore intestine
Etymology: The parasite is named after its locality
Type material: Holotype Voucher No. HCZEF HEL 1K
Paratypes Voucher No. HCZEF HEL 2K, HEL 3K, HEL 4K, HEL 5K, HEL 6K, HEL 7K, HEL 8K. HEL 9K, HEL 10K.

Abbreviations: HCZEF Helminthes Collection of Zoology Department, Faculty of Education, Ain-Shams University.

Description: (Plate 1, Figs. A-C)

Based on the holotype and 10 paratypes. Body is delicate, aspinose, small in size, cuticle transversely folded, fore body slightly tapered, more or less pointed from its posterior end. Body length 1.6-1.9 mm by 0.13-0.2 width. Length/width ratio 12.3-9.5: 1. Oral sucker is sub terminal, globular, it measures 0.17-0.175x0.13-0.19. Ventral sucker is prominent, situated at the ending of the first third of body. It is rounded with mid-ventral aperture and measures 0.21-0.26x0.22-0.25. Oral/ventral sucker ratio is 0.59-0.718: 1. The distance between ventral and oral sucker is 0.34-0.38. Prepharynx is short. Pharynx is oval, musculated, highly developed and its diameter measures 0.18-0.2. The esophagus is very short. An esophageal bulb is present and it measures 0.6-0.75**.** The esophagus is leading to two intestinal caeca terminating blindly to the posterior extremity. Two testes, oval, tandem, post-acetabular, at the mid body, the anterior testis measures 0.187- 0.2x0.15-0.16 and the posterior one 0.19- 0.23x0.14-0.15. Genital atrium is large, post-bifurcal point of the two intestinal caeca, it contains the hermaphroditic duct and it measures 0.17-0.174x0.09-0.095. Seminal vesicle is wide tubule, very closed to acetabulum, pars-prostatica short, narrow and has thick layer of prostate cells. Ejaculatory duct is short joining metraterm at base of genital cone to form hermaphroditic duct. Gonopore is situated anterior to acetabulum between the intestinal caeca (Plate 1, Fig. B). Ovary is rounded, post-testicular, in the first third of hind body, its diameter 0.16-0.18. Seminal receptacle is absent. Uterus is strongly developed occupying all space between ventral sucker and posterior body extremity. Vitellaria are several, long winding and slender tubules with different sizes, they distribute on each side of body from the posterior edge of ventral sucker to the body end (Plate 1, Fig. A). Eggs are small, numerous and operculated, they measure 27-29x13.6-17 micron (Plate 1, Fig. C). Excretory vesicle V shaped with terminal pore.

Scanning Electron Microscopy

The surface of *Sclerodistomum aegyptiaca* n. sp. was studied by scanning electron microscope. The body is elongate, semi rounded from two extremities and has many tegumental infoldings. The body is covered ventrally by minute regularly overlapped spines with pointed ends that not observed by light microscope. The distribution of these spines decreases gradually towards the last third of the soma (Fig. A). Oral sucker is sub-terminal and has distinct thick dorsal non-spinose wall. The area posterior to oral sucker is characterized by pits, infoldings and tegumental depressions (Fig. B). At high magnification of the cephalic end, two types of irregularly distributed papillae are demonstrated, higher frequency of small sensory unciliated, and few of domed papillae (Fig. C). The ventral sucker is situated at the first third of body and the gonopore is located on an elevated tegumental extension very closed to the ventral sucker (Fig. D). The internal lip of the ventral sucker and the tegument surrounding the gonopore has few tegumental outgrowths, many sensory receptors and domed papillae (Fig. E). Numerous regularly backwardly directed spines with slightly pointed end are distributed on the outer tegument surrounding the posterior edge of ventral sucker (Fig. F).

A high magnified part of the tegumental surface at the ventromedial area posterior to the ventral sucker indicated the presence of many tegumental structures including transversally grooves, numerous blindly pointed spines arranged in rows in a regular pattern, thickening elevations as tegumental folds, transverse wrinkles interspersed in between sensory papillae and few button papillae (Fig. G). All of those structures described above decrease or disappear gradually towards the posterior extremity of the worm. Dorsally, there were found many longitudinal ridges (Fig. H). The tegumental surface around the excretory pore is characterized by the presence of 4-5 rows of small pointed spines in a circle pattern. A few distributed papillae are found on the tegument far from excretory pore, tegumental ridges and infoldings are also observable in that area (Figs. I &J).

DISCUSSION

Parasites have an essential role in the marine ecosystems by affecting population dynamics of their hosts (Rohde, 1993). The genus *Sclerodistomum* described in the present study from *S. undosquamis* marine water fish as new host record and from Egypt as new locality record. The high infection rate (50%) reported in the present investigation by the present fluke may be explained by the favorable environmental conditions or due to some biological factors related to the behavior of the host. The obtained data revealed that females fish have higher infection rate (64.3%) than males (30%) while no significant difference between them. The differences in infection between the two sexes could be due to differential feeding quantity or the quality of food eaten and as a result of different degrees of resistance to infection (Emere, 2000; Onwuliri and Mbgemena, 1987). The obtained results showed that the worm has a tendency to infect the large weights of the host. These results are similar to that introduced by Aliyu and Solomon (2012). They regarded that to the greater surface of larger fish to infection than in smaller ones or to feeding habitat.

Few species are known for the present genus and the new species *S. aegyptiaca* is mainly differs from other species of the genus described previously by possessing an esophageal bulb, the position of gonopore that located very near to acetabulum, the location of the ovary that in the hind body nearer to the posterior extremity than acetabulum, the extension of the uterus and the high distribution and various shapes of vitellaria. Also, the Scanning Electron Microscope has revealed a distribution of minute spines on the ventral surface of the worm except on the oral sucker and the inner rim of the ventral sucker. Pritchard (1963) reported *S. bravoae* as new species from the stomach of *Diodon hystrix* at Hawaii and distinguished the species by elongated body, diagonal testes that are closed together and the distribution of vitellaria in the form of seven tubes that are subdividing laterally on each side. The present species differs from the previous species in the position of the testes which are tandem and the irregular different shapes of vitellaria extending from the posterior edge of acetabulum to the posterior extremity. *Sclerodistomum diodontis* Bravo-Hollis, 1954 was recorded from the body cavity of *Diodon holacanthus* in the Mexican Pacific and differs chiefly from the present fluke in the longer hind body and vitellaria that don't reach the ending of the ceca. Also, the described new species in the present research differs from *S. italicum* (Stossich, 1893) Looss, 1912 in the position of gonopore which is more anterior between the two suckers and entirely preacetabular seminal vesicle.

The examination of the surface of *Sclerodistomum* genus was studied for the first time by scanning electron microscopy. The evaluation has provided more details that not appeared by light microscope which are helpful in the identification of the present species. These details including whole body shape, tegumental structures such as papillae, spines, ridges and sensory receptors.

The present trematode has a characteristic topography by having several types of papillae on the tegumental surface surrounding oral and ventral sucker; small sensory, domed and button like papillae. Similar tegumentary papillae are reported by Gupta *et al.* (2017) for *Allogenarchopsis bareilliensis* from *Ghanna striata* in India. Moravec (2009) studied the surface morphology of *Nicolla skrjabini* (Opecoelidae) from brown trout *Salmo trutta fario* in Czech Republic and showed several sensory papillae distributed at oral sucker and genital pore. The presence of various types of papillae may reflect variations in their functions such as helping the worm to establish and maintain itself within its host through receiving information from surrounding environment (Ip and Desser, 1984). Furthermore, papillae represent mechano-rheo-chemo receptors that serve useful functions for orientation and feeding (Halton, 2004 and Gupta *et al.* 2017). Smyth and Halton (1983) supposed that these chemoreceptors give the worm the ability for selecting site of infection for host habitat and for micronutrients. In the present study few papillae are seen on the surface near the excretory pore that supposed to be helpful in contact communication.

A heavy occurrence of small unipointed spines at the ventral side of the present described species is similar to those spines described by Crites and Jilek (1981) for *Hasstllesia tricolor* (Brachylaimidae) and Chai *et al.* (1992) for *Heterophyes nocens*. The arrangement and distribution of spines in imbricated view may help the worm in attachment in host tissue. The presence and arrangement of papillae and spines in the present trematode may help the parasite in fixing in host mucosal wall which is similar to the mode of fixing that recorded by Naem and Smythe (2015) in *Q. quinqueserialis* infecting Muskrat. Many infoldings, pits and tegumental depressions observed in the ventral area posterior to oral sucker and on the middle part of *S. aegyptiaca* n. sp. are closed to those described by Abdou (2001) for *Erilepturus hamati* (Hemiuridae) and Luiza *et al*. (2003) for *Ithyoclinostomum dimorphum* (Clinostomatidae). The tegumental infoldings are more prominent at the ventral sucker as they may aid in muscular movement of the worm within host's intestine. Further studies on the distribution, life cycle of parasites infecting economically important fish are required for saving more protection from diseases that may reach to human.

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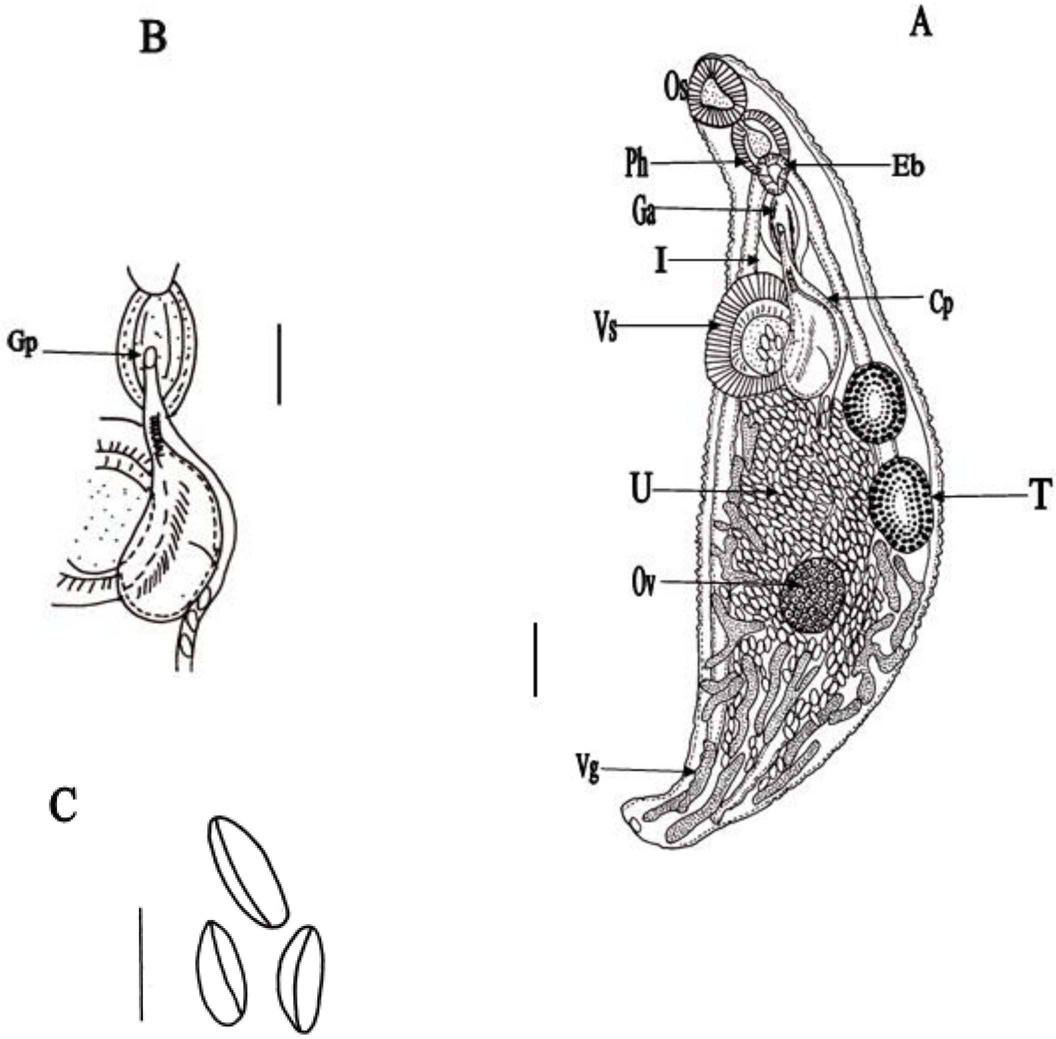
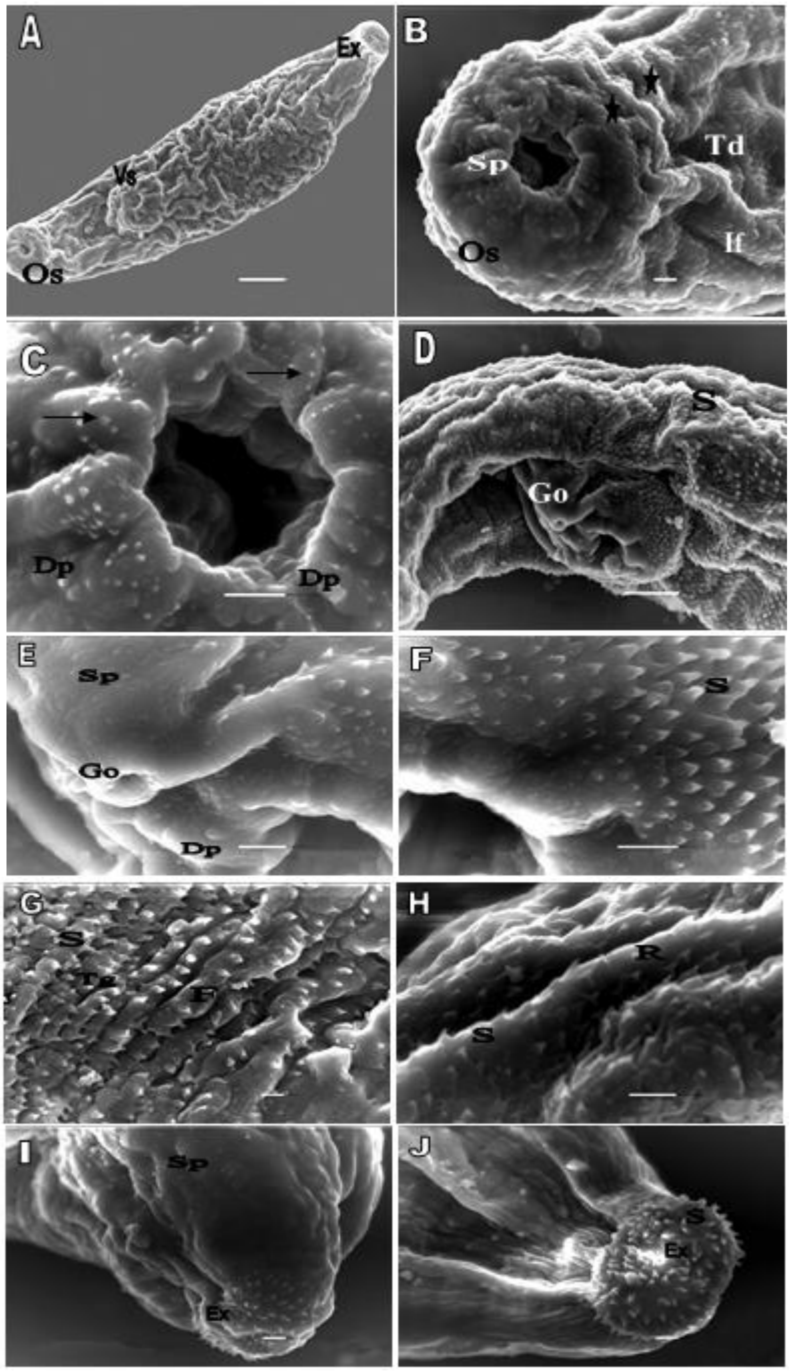
Plate 1



Figs. A-C: Light Microscope Drawing of *Sclerodistomum aegyptiaca* n. sp. (Scale=0.01). **A-** Whole mount, ventral surface (oral sucker **Os**, esophageal bulb **Eb,** pharynx **Ph**, intestine **I**, genital atrium **Ga**, cirrus pouch **Cp**, ventral sucker **Vs**, testes **T**, ovary **Ov**, uterus **U**, vitelline gland **Vg**). **B**-Terminal genitalia (Gonopore **Gp**), **C**-Eggs.

Plate 2



Figs. A-J: Scanning Electron Microscope of *Sclerodistomum aegyptiaca* n. sp. **A:** The whole body worm ventral view showing oral sucker (Os), ventral sucker (Vs) and excretory pore (Ex). **B:** The anterior end of the fluke; sensory papillae (Sp), pits (stars), infoldings (If), tegumental depressions (Td). **C:** Enlarged view of the oral sucker; sensory papillae (arrow), domed papillae (Dp). **D:** Venterolateral view of the worm showing gonopore (Go), tegumental spines (S). **E:** high magnification of gonopore (Go) exhibiting sensory papillae (Sp), domed papillae (Dp). **F:** Enlarged view of external tegument of ventral sucker showing regular distributed spines (S).

G: Magnification of tegument posterior to ventral sucker exhibiting spines (S), tranverselly grooves (Tg), tegumental folds (F). **H:** A side of dorsal body surface at middle body showing spines (S), longitudinal ridges (R). **I:** A lateral view of posterior extremity showing sensory papillae (Sp), excretory pore (Ex). **J:** Ventral view of posterior body end showing spines (S) in cicular pattern and excretory pore (Ex).

ARABIC SUMMARY

دراسة بالمجهر الألكترونى الماسح على طفيل *Sclerodistomum aegyptiaca* نوع جديد (Digenea, Sclerodistomidae) من أسماك المكرونة فى خليج السويس، البحر الأحمر، مصر.

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فى هذه الدراسة تم عزل ووصف نوع جديد من الوشائح الورقية ***Sclerodistomum aegyptiaca*** (Hemiuroidea, Sclerodistomidae) من أسماك المكرونة التى تم جمعها من منطقة خليج السويس ،البحر الأحمر، وكانت نسبة الأصابة (٥٠%). تم دراسة النوع الحالى بواسطة كلا من المجهر الضوئى والالكترونى الماسح وقد أظهر الاول أن أهم مايميز النوع الجديد وجود بصيلة مريئية عضلية حول مرىء قصير للغاية، موقع الثقب التناسلى بالقرب من الممص البطنى ، موقع المبيض فى الجزء الخلفى للجسم بالأضافة الى المساحة الكبيرة التى يشغلها الرحم وشكل وتوزيع الغدد المحية. بينما أظهر المجهر الألكترونى الماسح وجود أشواك دقيقة على السطح البطنى للدودة فيماعدا الممص الفمى والحافة الداخلية للممص البطنى بالأضافة لوجود أنواع مختلفة من الحلمات والثقوب والثنيات الجلدية. أيضا تناولت الدراسة الحالية مناقشة تأثير بعض العوامل البيولوجية الخاصة بالعائل كالجنس والوزن على معدلات الأصابة بالطفيل وتم عمل تحليل أحصائى للنتائج.