Further Histological and Ultrastructural Observations on the Cestode, Senga Mastacembeli Sp. N. with the Revesion of Genus Specieses

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Abstract: A total of 122 Mastacembelus mastacembelus were collected from Greater Zab river near Erbil city, Kurdistan region, north of Iraq, during the period from January to the end of December 2011. In the laboratory, the fish was identified and then the fishes were opened from the abdominal site and the gastrointestinal tract was removed out and examined carefully for cestodes. Live cestodes were recovered from the intestine of the infected fish and examined by compound light microscope and scanning microscope, also histological sections were prepared for some samples. The study showed a number of differential characters of Senga mastacembli sp. n. which not revealed during the first description by Rahemo (1996) also during the ultra-structural study of it by Rahemo and Mohammad. (1998). The study improved that the species S. Mastacembli is a valid species name, also the type host of it M. Simach is regarded as a synoname to M. mastacembli and the last is regarded as a type host for this cestode.

1. Introduction

The genus Senga was established with the type species S. besnardi from Siamese fighting fish, Betta splendens in an aquarium at Vinecunes, France (Dolfus, 1934). Cestodes of the genus Senga (Order: Pseudophyllidea; Family: Bothriocephalidae) are parasitizing the intestine of freshwater teloestes (Schmidt, 1986). In members of the genus Senga hooks on scolex arranged in two semicirles, bothria which are shallow and well developed, apical disc intended dorsally and ventrally. Neck absent. External metamerism present but incomplete. Proglotids acraspedote, wider than long except when gravid. Genital pores dorsal, virtually median. Testes medullary, in two lateral fields. Ovary compact, median, posterior. Vitelline follicles cortical, continous around margins of proglottid. Uterus loops forward, uterine sac opens ventrally by median pore near anterior margin of proglottid. Eggs operculate (Khalil et al., 1994).

According to Yamaguti (1959), four species of Senga reported from different species of fishes in the world. While, eight species have been recorded from Singapore (Polyakova and Kirin, 2005),

recently 27 species have been recorded from India (Bhure and Nanware, 2011). In Iraq, only one species recorded namely S. mastacembeli Rahemo, 1996 which described as a new species by from Mastacembelus simach collected from Tigris river near Musol city (Mhaisen, 2012).

The present study was planned for redescription of S. mastacembeli, including its morphology, surface ultra structure and histologic structure which parasitizes M. mastacembelus in the Greater Zab River and brief comparison of the genus Senga with its nearest genus, Polyonchobothrium.

2. Materials and Methods

A total of 122 Mastacembelus mastacembelus were collected from Greater Zab river (tributaries of the Tigris river) at Gwer district near Erbil city, Kurdistan region, north of Iraq, during the period from January to the end of December 2011. The fish specimens were collected by gill netting and cast netting by local commercial fishermen.

In the laboratory, the fish was identified according to Coad (2010); Froese and Pauly (2016), and then the fishes were opened from the abdominal site and the gastrointestinal tract was removed out from the rectum to the esophagus and opened longitudinally and examined carefully for cestodes (Amlacher, 1970; Barson, 2004).

For light microscopy (LM), a live cestodes were recovered from the intestine of the infected fish. The parasites were then washed in 0.6% saline solution, fixed in hot 4% formalin and preserved in 70% ethanol. The staining is accomplished by using Schuberg's iron hydrochloric carmine by Regressive staining method, destaining was performed with an acid ethanol (HCl/ETOH) solution and stopped by transferring the samples into 80% ethanol. Dehydration by using of graduated concentrations of ethanol. The specimens put between a coverslipe and a piece of paper to avoid coiling during dehydration, clove oil used for clearing of the specimens after dilution by graduted concentrations of ethanol. Drawings were made with the aid of a Zeiss microscope drawing attachment (Scholz & Hanzelová, 1998). Photos were taken with Olympus camera (Japanese origin), and the figures were drawn by using a camera lucida (drawing tube).

For histological study, the samples of cestodes preserved in 70% ethanol then dehydrated by graduated concentration of ethanol (80%, 90% and 96% ((twice)), then ethonol changed with aceton for 35-40 min., then transfered to toluen for one hour then to a new toluen for 1.30 hour. The specimens embbeded in Paraplast media (Paraplast Regular) manufactured by Sigma-ALDRICH co. for 24 hours then transfered to a new Paraplast for 48 hours and third paraplast for 72 hours in oven on 56C°. Then the samples were placed in a perpendicular position under a dissecting microscope, which has to be done quickly before the paraffin hardens. After 30 minutes the vial was placed in the refrigerator, to be become hard in order to remove the paraffin-enclosed sample from the vial.

After embbeding, sections where obtained by using microtome at 8-12 µm thick, streched on 40 C° water bath then fixed on slides with help of 80 C◦ hotplate. Wax were removed from sections by using xylene then descending sereis of alcohols were used for dehydration. Sections were stained using hematoxyline-eiosin, finally mounted in canada balasm (Scholz & Hanzelová, 1998).

Specimens for the Scanning Electrone Microscopy (SEM) were fixed and preserved like that used for (LM), then dehydrated completly. Chemical method, Hexamethyldisilazan was used for drying of tape worms by covering the specimens with this material for 5-10 min., then the chemical taked out totally with a droper, leave the worms to dry at room temp. Finally, when the specimens totally dried

embbeded on the target and sputter-coated with gold; they were examined using a JEOL JSM-7401F scanning electron microscope (Academy of Czech Republic - parasitology institute) at an accelerating voltage of 4 kV GB low linked to an external computer system. Each specimen was observed specially on the morphology of the scolex (Scholz & Hanzelová, 1998).

3. Results and Discussion

Fishes were surveyed for parasitic cestodes during the period of the present study. The survey showed the occurrence of one cestode belonging to the genus Senga.

Senga mastacembeli Rahemo, 1996

Host: Mastacembelus mastacembelus (Marmarej).

Prevalence of infection: 73.77%.

Mean intensity: 2.3.

Site infection: Intestine.

Locality: Greater Zab river at Gwer District.

Description: Medium sized, white yellowish worms. Body length 90-123 mm, width 2.12-2.89 mm. Scolex lancet shaped, 0.561- 0.752 mm length, 0.865-0.917 mm width (Fig. 1A; 2A; 4A). Bothria 0.653-0.739 mm length, 0.174-0.189 mm width (Fig. 1B; 1C; 2B; 4B). Rostellum 0.083-0.91 mm length, 0.168-0.183 mm width (Fig. 1B; 2B; 4B). Hooks are in one row and two semi-circles around the anterior end of rostellum, 48-50 in number, 0.023-0.031 mm length, 0.0052-0.0078 mm width (Fig. 1C; 2B; 4C; 4D; 4E). Immature proglotid, 0.351-0.405 mm length, 0.462-0.492 mm width (Fig. 1D). Mature proglotid wider than long, 0.357-0.374 mm length, 1.298-1.342 mm width (Fig. 1E; 1F; 2C). The ovary is bilobed and compact in texture, 0.299-0.345 mm in length and 0.065-0.079 mm in width (Fig. 1E; 2C), similar observations were recorded by Rahemo (1996). Oviduct, 0.059-0.065 mm length (Fig. 1E; 2C). Uterus highly curved, 0.389-0.412 mm length, 0.023-0.034 mm width (Fig. 1E; 2C). Vagina median in ventral surface of the proglotide, 0.034-0.041 mm length, 0.132-0.139 mm width (Fig. 1F; 2C). Cirrus sac semi spherical to sub spherical, alternated in proglotids measured, 0.174-0.183 mm length, 0.213-0.233 mm width and with a thick muscular wall (Fig. 1E; 2C). The testes in the present specimens varies between 65 -72 in mature segments, situated at the lateral fields, each 0.0132 -0.167 mm length, 0.0226-0.0261 mm width not reaching the ovary level, and are medullary in position (Fig 1E; 2C; 3C; 3D). Anyhow, the number of testes reported in the present specimens is less than that estimated by Rahemo (1996) it may be due to maturation status, but in both studies testes are found in medulla. As seen in (Fig 1E; 1F; 3A; 3B; 3C; 3D), most of the reproductive organs are demonstrated either grossly or in both longitudinal and cross sections. Vitelline follicles 0.0089-0.0097 mm length, 0.0078-0.0091 mm width (Fig 1F; 2C). The vitelline follicles in the examined specimens lie on both sides of the mature segments occupying extensive area (Fig 1E; 1F; 1G). Furthermore, these vitelline follicles are cortico-medularly in position but more abundant at the medulla. Such results were not reported by Rahemo (1996) and Rahemo and Mohammad (1998).

Gravid segment, 0.448-0.477 mm length, 1.821-2.45 mm width, containing an enlarged sac like uterus, 0.356-0.371 mm length, 0.734-0.762 mm width, filled with eggs (Fig. 1G; 2D; 3C). Uterus is

filled with eggs which are operculated, oval in shape 0.0023-0.0031 mm length, 0.0012-0.0013 mm width, the operculum was not seen by Rahemo (1996) and Rahemo and Mohammad (1998). Further studies are suggested to explore the factors influencing the hatching of these eggs i.e. the removal of this operculum and the emerging of the oncomiracidium of the present species which was not observed neither in this study nor in the previous studies (Rahemo, 1996; Rahemo and Mohammad, 1998). However, experimental studies may reveal the stages of the life cycle of the present worm both in Greater Zab river from which the present fishes were collected or from Tigris river from which Rahemos′ specimens were collected.

Scanning electrone microscopy of the scolex showed the presence of fine, hairy michrotichia, medium in length about 10 µm (Fig. 4F), these microtiches usually present in cestodes (Roberts and Janovy, 2005) thier function is not known but it may help in the attachement of the worms to intestinasl lining. These michrotrichis were not observed by Rahemo (1996) as he did not used SEM. The michrotrichis of the scolex (Fig. 4E) seem to be irregular, some are short others are long, some posteriorly directed while others are transversely directed. Comparing the michrotrichis of this worm with Hymenolepis nana, in the latter they are all posteriorly directed while in other cestodes, Trypanorhynch they are five to six- figered palmate michrotrichis (Roberts and Janovy, 2005). Also (Fig. 4F) show hard and well formed hooks which are posteriorly curved and pointed distally while thier proximal part are inserted in the rostellum. Furthermore, these hooks are arranged in two semicircles (Fig. 4B; 4C; 4E), these observations where not noticed by Rahemo (1996). In addition, scanning electrone micrographs show that the bothria are deep, highly musculated and with glandular tips (Fig. 4C; 4D). Anyhow, the glandular nature of these glandular tips are to be confirmed by tacking transmission electrone microscopy in order to have predection about thire function, which is likely a secretion used for attachment. As in (Fig. 4F) these microtrichis are of filamentous and canoid type which directed postriorly similar to the observations of transmission electrone microscope observation carried out by Rahemo (1998). Generally these michrotriches are similar to micrivilli present in the gut mucosa of vertebrates and invertebrates. Electrone miscroscopical study show that each michritiche have a dense distal portion set off from the base by a multilaminar plate (Roberts and Janovy, 2005). Anyhowe, Rahemo and Mohammad (2002) made a scanning electron microscopy for the scolex of this cestode but thier observation were obage and incomplete as the microscope at that time was not working properly. Neck absent (Fig. 4B).

As revealed from both longitudinal and cross sections (Fig. 3A; 3B; 3C; 3D) their are no septa betweem proglottidis which is a general style of cestode structure, such fenomenon was not recorded in the previous studies (Rahemo, 1996; Rahemo and Mohammad, 1998).

From the paraffine sections musculature not developed highly, only some logitudinal and transverce bundles were located underneath the tegument (Fig. 3C; 3D). The vitelline follicles are cortical, compact and spherical in transvers sections (Fig. 3C; 3D). Testes are medullary, arranged in two incomplet rows, starting from the uterus anterior end level and extended to the end of posterior end level of ovaries (Fig. 3A; 3C; 3D). Ovary is bilobed, compact, connected by narrwo connections with short oviducts (Fig. 3C; 3D). The most important observation in the present study is the Mehlis′ gland which is found to be large, spherical or subovoid in cross section lie just directly beneath the ovary loops, sometimes may pass to other adjacent proglottids since there are no intersegmental septa in the present worm (Fig. 3A; 3C).

It is noteworthy that most of the above observation were lacking in examination of the Senga

specimens carried out more than three decade ago.

The present study regarded as a first redescription of this species in the world including a lot of important characters like shape and position of vettilaria and testes, shape and long of oviduct, Mehlis′ gland, vagina and eggs, also it is histology and surface ultra structure (like michrotriches) characters by using scanning electron microscope. The fish host of the present cestode is M. mastacemulus, while in the previous studies, Mastacembelus simach, more likely these two names are synonemous according to fishbase (2011) and Coad (2010), in his valid book gave no report of S. simach.

Bashě and Abdullah (2010) described Polyonchobothrium magnum from the intestine of the same host (M. mastacemulus) and locality (Greater Zab river) with very similar characters and hooks arranged in two semicirles and since the hooks of Polyonchobothrium spp. are arranged in four quadrants, for this, this name regarded as a synoname for S. mastacembeli (Khalil et al., 1994).

Species revesion of genus Senga:

According to Bhure and Nanware (2011) there are 26 other species under this genus were recorded from freshwater fishes namely:

Senga ophiocephalina from Ophiocephalus argus at Taimen, China.

S. lucknowensis from Mastacembelus armatus in India.

S. malayana from Channa striata.

S. parva and S. filiformis from Channa micropeltes at Malacca.

S. pahangensis from Channa micropeltes at Tesak Bera.

S. besnardi from Ophiocephalus gachua in India.

S. visakhapatanamensis from Ophiocephalus puncatus in India.

S. khami from Ophicephalus marulius from Kham river at Aurangabad.

S. godavari from M. armatus at Nanded, M.S. India.

S. aurangabadensis was added from M. armatus at Aurangabad M.S. India.

S. paithaniensis from M. armatus.

S. raoi and S. jagannathae from Channa punctatus.

S. maharashtrii and S. gachuae from the intestine of M. armatus.

S. chauhani from Channa punctatus.

S. mohekarae from the intestine of the M. armatus, at Parli, Dist. Beed, M.S. India, S. armatusae from Mastacembalus armatus.

S. tappi from M. armatus.

S. ayodhensis from Amphinuous cuchia.

S. baghui from Rita rita.

S. jadhavae from Mastacembelus armatus.

S. chandapurensis from Mastacembelus armatus.

S. tictoi from Punctatus ticto.

S. madhavae from M. armatus.

Rego (1997) described a new species of cestodes belonged to the genus Senga from the intestine of Astyanax scabripinnis from Brazil namely: S. besnardi. The distinguishing features of this species are scolex rectangular, with an apical bilobed disc whose margins carry a row of hooks, divided in two half circles of hooks, each half has 14-15 hooks of different size and form, some vestigial and rudimentary hooks intercalated between the others, as described to S. besnardi

Polyakova and Kirin (2005) described Senga sharpiloi from Channa micropeltes from Singapore. S. sharpiloi differs from others species of genus Senga by the size strobili length, scolex is pear shaped, apical disc elongated slightly, hooks of the ends of both semicircles are smaller than the others and number of hooks (44-50 + 4), number of testes which is 22-24 in each proglottid and arrangement of them.

Bhure and Nanware (2011) described S. satarensis from M. armatus at various places of India. The worm comes closer to all the known species of this genus in general topography of organ but differs due to scolex pear shaped, tapering anteriorly and broad posteriorly, rostellum medium, rounded, bearing 28-30 rostellar hooks, mature proglottids six to seven times broader than long, testes 175-200 in numbers, scattered throughout the segment, cirrus pouch oval, ovary distinctly bilobed, vagina thin, runs posteriorly, genital pores oval in shape, vitellaria granular, uterus saccular and egg elongated.

Wankhede et al. (2012) studied the histopathological changes of the intestine of freshwater fish M. armatus infected with Senga sp. and noticed destruction and extrusion of the intestinal villi, fibroblast cells and plasma cells.

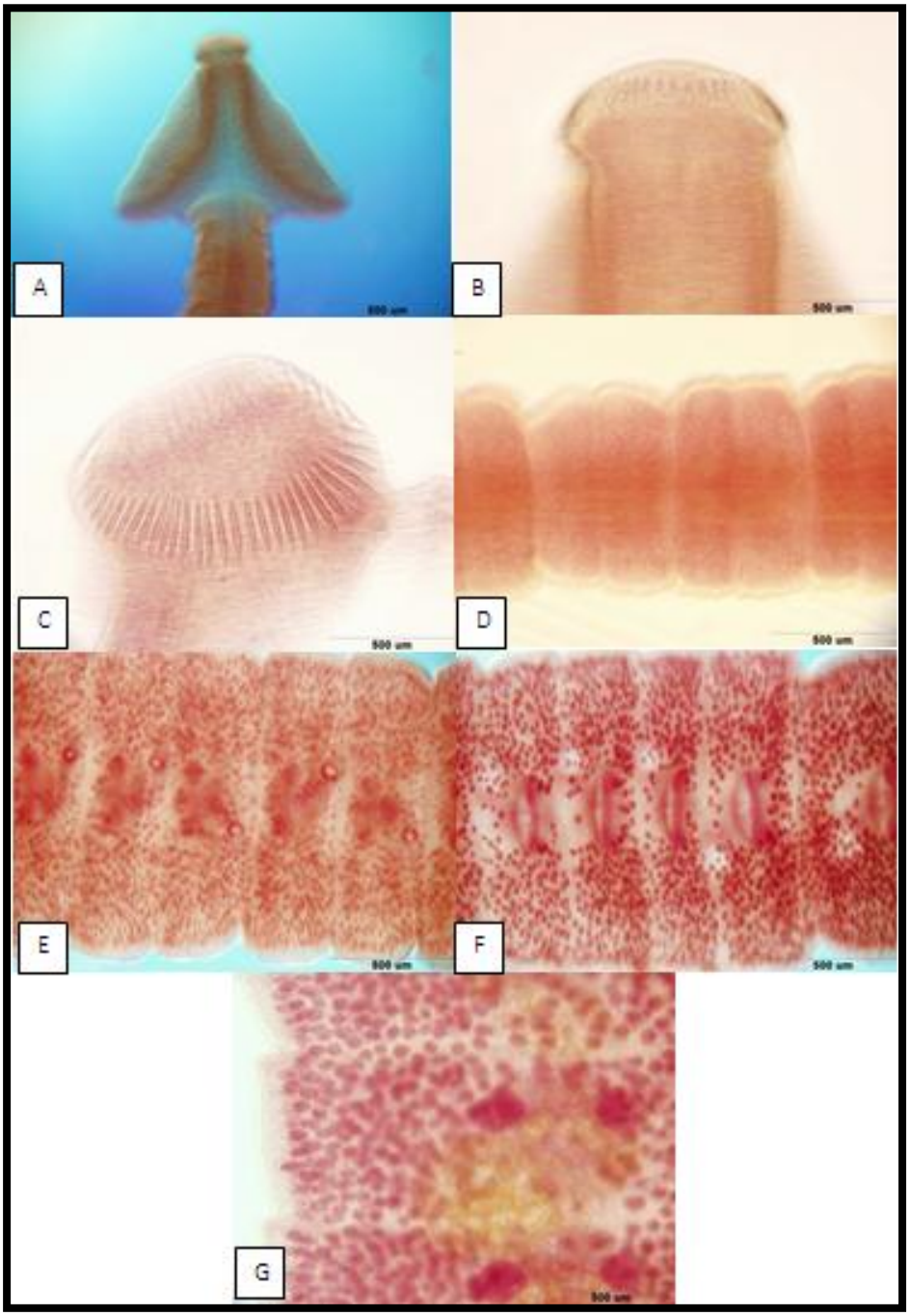

Figure (1): Photomicrograph of Senga mastacembeli.

A- Scolex, B- Rostellum, C-Pressed prososcis show hooks, D- Immature proglotid, E- Mature proglotid (Dorsal view), F- Mature proglotid (Ventral view), G- Gravid proglotid.

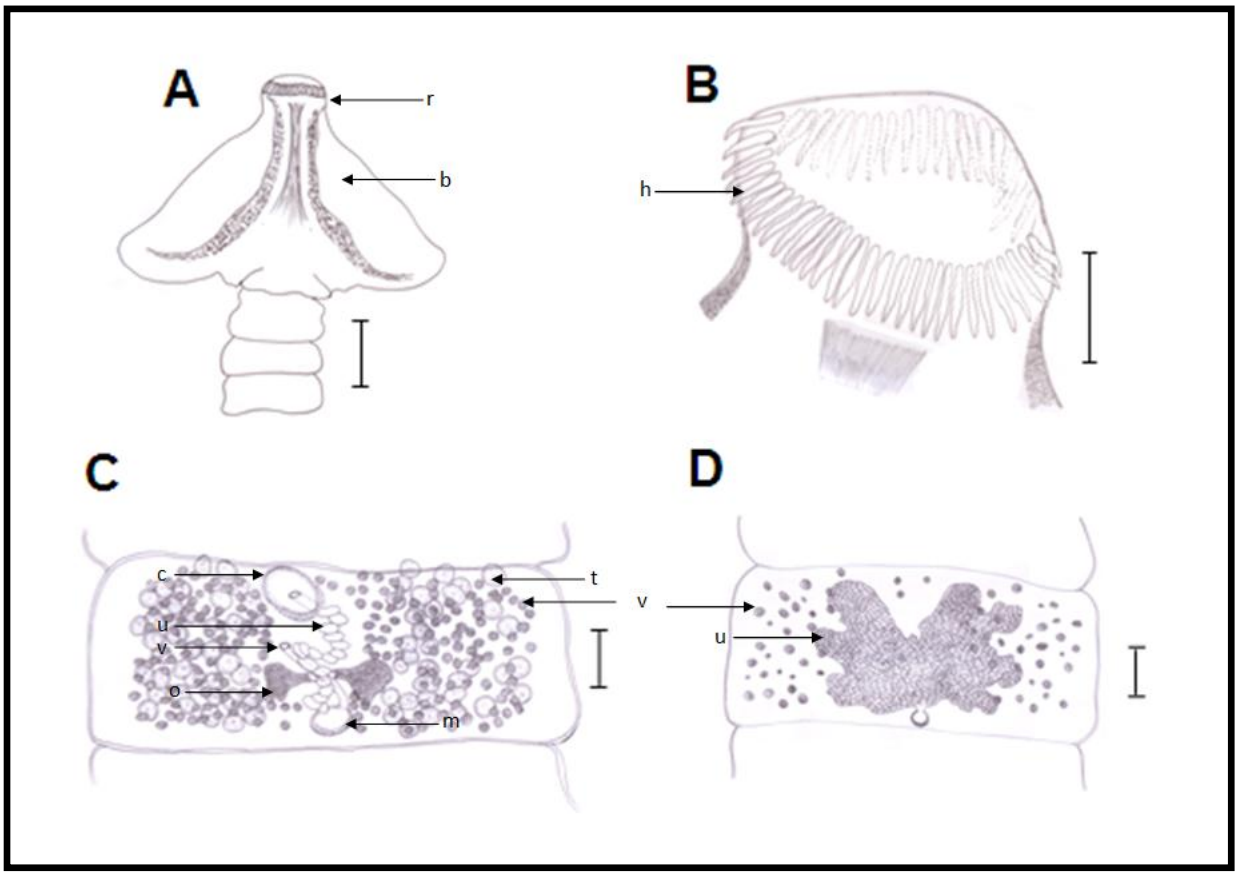

Figure (2): Lucida drawings of S. mastacembeli.

A- Scolex, B- Rostellum, C- Mature proglotid, D- Gravid proglotid.

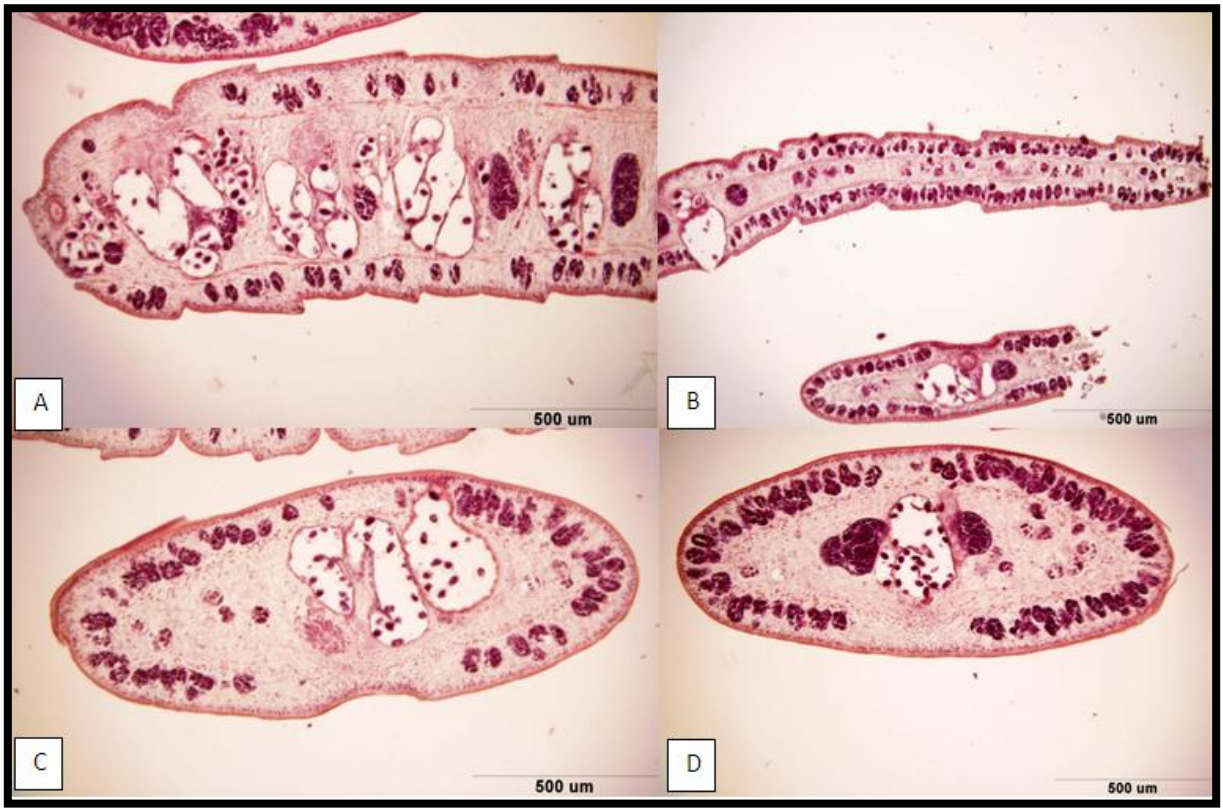

Figure (3): Photomicrograph of S. mastacembeli sections.

Longitudinal section of gravid proglotids, B- Longitudinal section of mature proglotids and cross section of mature proglotid through mehl′s gland, C- Cross section of mature proglotid through pre-ovarian region, D- Cross section of mature proglotid through ovarian region.

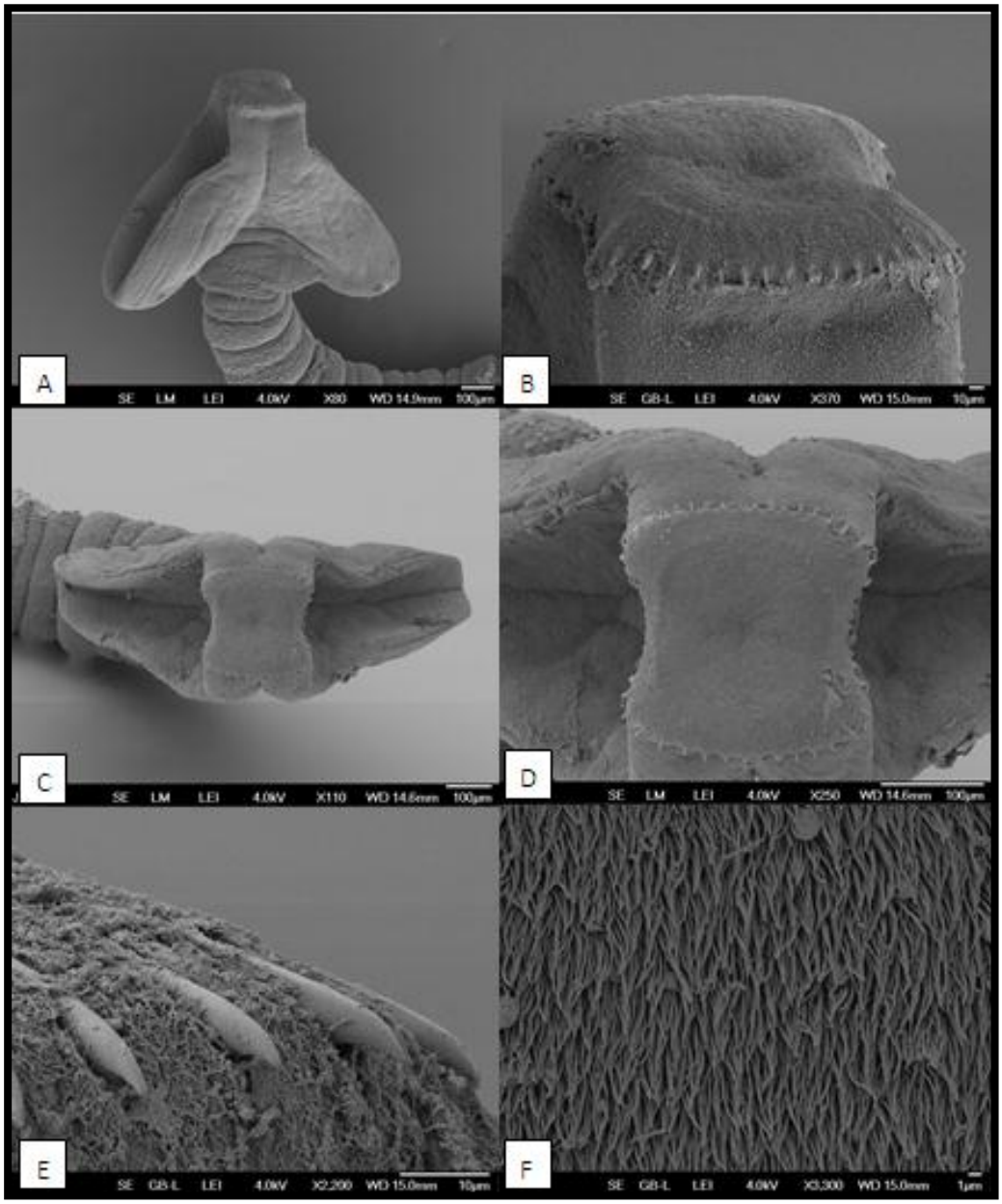

Figure (4): Scanning electron micrograph of S. mastacembeli.

Scolex (Dorsal view), B- Rostellum (Dorsal view), C- Scolex (En face view), D- Scolex (En face view) showing the hooks hemispheres, E- Hooks, F- Michrotriches from proglotide surface.

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