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Some studies on parasitic isopods of some marine fishes

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Abstract

A total number of 150 different marine fish species represented as 50Argyrosomus regius(L ute fish) from Mediterranean Sea at Damitta Province,Egypt,50 Pagrus pagrus (Morgan fish) from Mediterranean Sea at Matrouh Province, Egypt and 50Xiphias gladius(Abo saif fish) from the red sea at Hurghada city (south of Sinai province) , Egypt . The incidence of parasitic isopods among 150 marine fish was 4%. Specimens were subjected to parasitological examinations for detection of isopod infestations. The detected parasites were Anilocra leptosome and Cymothoa indica from Argyrosomus regius and Pagrus pagrus respectively while no infestation recorded in Xiphias gladius. The infestation rate with Anilocra leptosome and Cymothoa indica was4 and 8% respectively.The parasites observed in the gill chamber of the host.. The morphological characteristics of these parasites were described in details using light and scanning electron microscopy.

Introduction

Fish are considered as one of the most palatable and valuable food in most countries, beside it is also proved that it contain easily digestable protein of high nutritional value. It is rich in unsaturated fatty acids, that why it is preferred by some sick people specially those suffering from heart and circulatory disorders. Marine fishes are also preferred than fresh fishes as the former are rich in trace elements as phosphorous and iodine which are essential for cell anabolism and its use in medicaments .Fish like any other vertebrates are suffering from parasitism **(Azza, 1990)**, ectoparasite infestation not only result from direct harm to fish, but also from disfigurement which renders fish grown for food and ornamental fish unsuitable for sale, thus impose a big loss to fish industry **(Piasecki *et al.*, 2004).** Isopods considered as a large ectoparasitic crustacean group on marine fish, diverse and occur on fish worldwide. Isopoda is an order (group) of crustaceans that includes woodlice, sea slaters and their relatives. Isopods live in the sea, in fresh water, or on land, and most are small greyish or whitish animals with rigid, segmented exoskeletons (external skeletons). They have two pairs of antennae, seven pairs of jointed limbs on the thorax, and five pairs of branching appendages on the abdomen that are used in respiration.

Females brood their young in a pouch under their thorax **Rhode,** (**2005**). **Kabata (1970**) mentioned that the numbers of isopods infesting fish were expected to increase and numerous of isopod species awaited discovery, especially in the tropical and subtropical regions. Cymothoid isopod causes serious problems to host fishes .They were fed on blood and macerated tissues; several species settled in the buccal cavity of fish, others lived in the gill chambers or on the body surface including the fins**.Kabata,(1970); Woo,(2006) and Ravichandran *et al.*,** (**2007).** Little is known about the marine isopods in Egypt except those recorded by **Hassan, (2001); Eissa, (2002) ,Ali and Abo-esa (2007) , Abd el all and el Ashram (2011)** and **Eman et al (2014)** , because the species concepts are weakly established in the literatures. Therefore, the present investigation was conducted to view a light on isopoda among some marine fish from Mediterranean Sea in Matrouh and Damietta Province, and from the red sea in Hurghada city (south of Sinai) , Egypt including prevalence of infection and morphological description using scanning electron microscopy .

MATERIALS AND METHODS

A total number of 150 different marine fish species represented as 50 *Argyrosomus regius* fish from Mediterranean Sea at Damitta Province, 50 *Pagrus pagrus* fish from Mediterranean Sea at Matrouh Province, Egypt and 50*Xiphias gladiu*s fish from the red sea at Hurghada city (south of Sinai province), transferred to the laboratory and subjected to clinical and parasitological examinations according to **Amlacher (1970).** Isopods were removed from the host fish; their location and its density were noted. Also, prevalence among the examined fish was calculated. Isopod specimens were collected from the gill chambers and immediately preserved in 70% ethanol.. Preparation for scanning electron microscopy (SEM) involved dehydrating the parasites in an absolute (100%) ethanol solution followed the method outlined in **Wilson (2003)**. Drying the specimens for SEM was accomplished using carbon dioxide critical point method. Dissected parts were mounted vertically on SEM stubs using double adhesive carbon spots. Specimens were digitally imaged on a Leo 435VP using a Robinson backscatter detector. Digital images were saved for later processing.

Results and Discussions

Classification of the detected species:

Kingdom: Animalia

Class: crustacea

Order: Isopoda

Family: Cymothoidae

Genus: Ailocra

Spp: Anilocra leptosoma

Genus: Cymothoa

Spp: Cymothoa indica

Table (1) Showing the prevalence of isopoda infection among the examined fishes.

Isopoda	**Fish examined**	**No exam.fish**	**No.infect. Fish**	**% of infect. Fish**	**Density .Parasite. infect**
Anilocra leptosoma	Argyrosomus regius	50	2	4	One parasite per infect. Fish
Cymothoa indica	Pagrus pagrus	50	4	8	One parasite per infect. Fish
-	Xiphias gladius	50	0		
		150	6	4	

The overall prevalence of isopoda in different marine species was (4%) , the detected species with its prevalence were:*Anilocra leptosoma* from *Argyrosomus regius* (4%) ,*Cymothoa indica* from *Pagrus pagrus* (8%) . The intensity of infection was one parasite per fish . No infectiom recorded in *Xiphias gladiu*s fish. These results were agreed greatly with **Eman et al (2014)** who revealed that (4%) out of 150 were infected with isopods, the detected species with its prevalence were: *Anilocra meridionalis* from *sardinella* species with prevalence of (4%); *Renocila thresherorum* from *Morone labrax* with prevalence rate of (6%) and *Cymothoa exigua* from *Sciaena umbra* with prevalence of (2%).while higher prevalence with isopoda was detected by **Badawy (1994*)*** who recorded 8.62% of isopods from Mediterranean Sea at Port Said province. **Aneesh et al(2013*)*** recorded highest prevalence *of Mothocya renardi*cymothoid isopod 93.18% from *Strongylura leiura* fish and the intensity being equal to 1.71, concerning

with higher prevalence of *Cymothoa indica was detected* by several authores as **Ravi and Rajkumar (2007)** stated that the prevalence of infection of C. *indica* was 27.8% from *Oxyurichthys microlepis* in the south-east coast of India; **Costa and Chellappa,** (**2010**) recorded that the prevalence of C. *indica* from *Etroplus maculates* was15.3% *and* 11.76% for *Cymothoa spinipalpa from E. suratensis* . **Ismail and Abdel-Razec (2010)** recording *Cymothoa indica* from buccal cavity of Myripristis murdgam with prevalence rate (62.5%) and from the branchialcavity with prevalence rate (18.75 %) **Abd El Aal1 and El Ashram (2011)**, recorded that the incidence of parasitic marine isopod*(Cymothoa spinipalpa*) among *Argyrops filamentosus* fish was 9% . The intensity of infestation one per fish. **Özer(2002**) recorded 7.4% in *Nerocila bivittata* on *Parablennius sanguinolentus* in the Samsun coast **Yamauchi *et al.*, (2005)** recorded the presence of *Norileca indica* in stomach of dolphin for first time in Philippine. **Eissa, (2002) and Mousa and Tantawy, (2006)** recorded higher prevalence 47 and 40.8% in *Centropristis filamentosus* and *seabastus marinus* fish respectively **Ali and Abo-esa (2007)** recorded an isopoda, *Ovoinella obovata* in Red sea shrimp (*Penaeus semisulcatus*) but belonged to different family (Bopyridea) with higher incidence 32%. **Alas *et al*., (2008**) stated that the prevalence of infection of Livoneca *redmanii* was5.9% *from Chloroscombrus chrysurus* **Carvalho- souza *et al.*, (2009)** who mentioned that the prevalence were 11.76 and 15.38% in *Caranx crysos* and *Lutjanus synagris* fish respectivelywhile. **Rameshkumar and Ravichandran (2010)** recorded that the prevalence of *Nerocila phaeopleura* . on *Rastrelliger kanagurta.* was 6.4% . **Eissa *et al.*, (2012**) recorded the summer season as the highest infestation rate 19%, followed by autumn 17%, while spring 7% and the lowest was 4% in winter season..**Ganapathy et al, (2013)** recorded the prevalence and intensity of isopods 5.0% for *Mothocya epimerica* infestations in *Atherina boyeri*. The intensity ranged from 1 to 1.7 parasites per fish .. **Noor El-Deen et al. (2013)** mentioned that, the prevalence of *Nerocila orbignyi* infestation in European seabass during summer and spring seasons, while infestation was disappeared during autumn and winter seasons. **Alaa Abdel-Aziz et al(2014**)showed that, the annual percentage of infestation by isopods, *Nerocila bivittata* on benthic feeder, *Lithognathus mormyrus* at Abu Qir Bay, Alexandria is 3.13%.. **Tavares-Dias et al (2014)** recorded that the prevalence of *Braga patagonica* was 0.04% in Solimões River near Marchantaria Island4.28% inTarumã-Mirim Stream, State of Amazonas and the intensity of infection was 1-2 parasites/host.

Concerning the Morphology of detected isopods **Anilocra leptosoma (Bleeker, 1857).**

Site of infection: gill chamber**.**

The parasite is narrow**,** somewhat more compressed and dorsally convex with body length 15 mm by 8mm width. Pale to brown in color(fig.1and 2) as the dorsal surface with scattered chromatophores connect entreated on posterior border ofsegments. Eyes moderately large (E) fig.4. Cephalon (C) narrows anteriorly to triangular fig.4. apex folded down (ventrally) between bases of first antennae (A) fig .3 downward folded gives anterior margin of cephalon truncate appearance in dorsal aspect, cephalon not immersed in pereonite .1 .Firse antennae (Antennule Au) Fig .5 with 8 article , extending slightly beyond mid point of eye . Antennae (A). fig .5 with 9 articles , extending to middle or posterior of pereonite .2(pr) .Mouth part (Labrum .L) fig.5 containing manibule and maxilla , maxilla medial lobe small , mandibule palp with 13 brush –tipped setae on distal margin. pereon (Pr) formed of 7 pereonites (fig.3), posteriolateral angles of all pereonites evenly rounded , not extended. Coxal plat (cp) fig.7 small and compact, failing to reach posterior margins of their respective pereonites, pereopodes are seven in number , pereopodes(Prd) 2- 4 (fig.7) gradually increasing in length posteriorly , sub equal in length and ending with hock like appearance. Peleon (pl), fig .4, not immersed in pereonite.7, decreasing gradually in width posteriorly , peleon formed of 5 peleonites(Pl) fig.8 sub equal in length and five peleopods (Pld) fig .7 . Peleotelson (plt), fig.8 and peleonite 5 sub equal in width . Uropodal ramai(Ur) fig 6,8 evenly ovate reaching barely beyond posterior margin of pleotelson . Gravid female containing marcipium (Ma, fig 4) containing eggs. This description in agreement with **Bruca (1981) and Eman et al (2014)** who recorded Anilocra meridionalis female but Anilocra leptosome distinguished from them, pereopodes and pleopodes ending with hock like appearance. Ailocra spp differentiated from other cymothoid by their narrow and convex body, larg eyes and coxal plates are small and compact. This description nearly similar to that stated with **Brusca, (1981). Williams &Williams (1999)** who recoded these parasites have a wide variety of fish hosts in two classes, 10 orders and 20 families. And **Eman et al (2014)** who recorded that Anilocra meridionalis female (with body length (11- 35 mm) and width (4-6 mm). Eyes moderately large. Cephalon narrows anteriorly to triangular, t. First antenna reaching about midline of pereonite1, second antenna reaching posterior margin of pereonite 2. Cephalon not immersed in pereonite1.Pereon; posteriolateral angles of all pereonites evenly rounded, not extended. Coxal plates small and compact, failing to reach posterior margins of their respective pereonites. Pleon not immersed in pleonites 7, decreasing gradually in width posteriorly; subequal in length. Prepods gradually increasing in length posteriorly. Pleotelson and pleonite 5 are subequal in width. Uropodal rami evenly ovate, reaching barely beyond posterior margin of pleotelson.

Renocila thresherorum female was depressed, 12-30 mm length and 7 - 14 mm width. Dorsal surface with scattered chromatophores, concentrated on posterior borders of segments. Cephalon width 1.3 times length; posterior border weakly immersed in pereonite. Eyes well developed. Antenna 1 of eight articles, barely reaching anterior margin of pereonite1; maxilliped with two terminal, and one subterminal spines. Maxilla 1 with four terminal spines. Pereon: Pereonites 1 and 5 longest; 2, 3 and 7 shortest and 4, 6 subequal. Posteriolateral agle of pereonite 5 not produced, of pereonite 6 moderete and of pereonite 7 is completely produced. Pereopods increasing in length gradually posteriorly and all without carinae. Pleonites of pleon are subequal in width and length. Posterior magin of pleotelson evenly rounded and complete fusion between it and pleonite5. Uropodal endopod ovate; exopode elongate, longer than endopod; uripods extended beyond posterior margin of pleoteson.

Concerning the morphology of *Cymothoa indica* female **(Schioedte et Meinert, 1884)**

Site: gill chamber.

The females have creamy white color. The body stout , dorsum vaulted , about 25mm long by 10 mm wide ,widest at pereonite 6 , bilaterally symmetrical Cephalon 2 times as wide as long(fig 1,2) , nearly pyriform and broadly truncate anteriorly in dorsal view , not distinctly immersed in pereonite .1 Eyes small moderately distinct . Mouth part (L) of two magnification in fig12,13 , containing maxilla and mandibule . Mandibule palp without setae , maxilla with 4 terminal spine . Antennule (Au) fig .14 , stouter and sub equal in length to antenna , with 8 articles , extending to anterior margin of pereonite .1 , first three articles slightly wider than others , antenna (A) with 9 articles decreasing gradually in width (fig.14) . Pereon formed of 7 pereonites , the dorsal side of pereon containing scales(Sc) have serrated edges and cuticular depression are furnished with three to four knobs arranged in semi cuticular rows called microtrich sensilla(Ms). pereopodes have characterstic lobe on the posterior angle of the ischium called coxal crest (CC) fig .15 , pereopods (prd) fig .14 , without spines . Peleon formed of 5 peleonites (pl) fig.16, 1-4 sub equal in length , fifth slightly longer . Pleotelson (plt) fig .16, slightly wider than fifth peleonite , posterior margin broadly rounded . Uropods reaching almost distal margin of pleotelson. The morphological description similar to that stated with **Jean and Michel (2006)**, **Ravi and Rajkumar (2007**) **Ismail and abdel-Razek(2010)** but differ in the measurements . Also this parasite differ from other cymothoid in the structure of microtrich sensilla , where in this parasite microtrich sensilla formed only from knob(socket) but in other cymothoid recorded by **Khalaji,2014** formed from knob , collar , shaft and filament. **Thatcher et**

al., 2007 studied the morphology of the isopode *Cymothoa spinipalpa* ond rcorded that the body measured 11.5 by 5.1 mm and had 7 articles in antennule and 8 in antenna Ganapathy et al,2013 revealed that these species are wholly carnivorous. Result shows how they are adapted for tearing and bolting fish food material. The mouthparts consist of a labrum, paragnaths, paired mandibles, maxillules, maxillae and maxillipeds. The labrum and the paragnaths are the least developed but peculiarly the mandibles are asymmetrical, large, stout and highly modified. The analysis of gut contents indicated that *Cymothoa indica* and *Joryma brachysoma* diet consisted of 90% to 95% of animal blood. The diet of *Mothocya renardi*, *Ryukyua circularis* and *Joryma hilsae* were mainly composed of mucus (80%-90%). The stomach contents of *Nerocila phaeopleura* and *Nerocila sundaica*, were dominated by body muscles. **Abd El Aal1 and El Ashram (2011),** studied the morphological characters of *Cymothoa spinipalpa* and recorded that the females were creamy white color and mean measured 29 mm long by 14 mm wide at level of pereonite 5. Cephalon deeply immersed in the first pereonite. The anterior border of the first pereonite broadly excavated to receive cephalon. Two seseal eyes dark in color present anteriorly one on each side of cephalon. Antennule consists of 8 articles. Also, antenna consists of 8 articles but shorter and narrower than antennule clear the mouth parts at two level of magnification. Pereon, pereonite 1 longest; 2-5 sub equal in length; 5-7 shorter. Pereopodes 1-3 small; 4-7 larger with carinae. Pleon formed of five segments immersed in pereonite 7 Pleopodes all billaminated and simple Uropods with slender sub equal rami Pleotelson rounded posterioly and twice wide as long. **Şevki Kayış1,and Yusuf Ceylan (2011**) stated that the Body sizes of the parasite *Nerocila orbignyi* Females were 28.3 mm, 14.1 mm . **Eman et al (2014)** recorded that *Cymothoa exigua* female with body length 8-30 mm and its width 4-15 mm, dorsal surface without scattered chromatophores. Cephalon is moderately immersed into pereonite 1.Eyes well developed. Antenna 1 not reach to the end of anterior third of pereonite 1, antenna 2 reaching to half of pereonite 1.In peron, pereonite 1 longest; 2-4 subequal in length; 5-7 decreasing in length posteriorly and pereonite 7 is the shortest, pereonite 5&6 are the widest. All coxae fail to reach posterior margin of their respective segment. Pereopods from1 to7 without spine. In pleon, pleonites 1-5 with medial elevation; 4-5 widest and pleonite 5 is the longest. Pleotelson wider than longer. Uropodal rami narrow and elongate not extended beyond posterior border of pleotelson. **Rameshkumar and Ravichandran (2010)** studied the morphology of the female of *Nerocila phaeopleura* and stated that the Body length measured 18-21 mm, width 7-8 mm, body longer than broad, symmetrical, black with uniform distribution of chromatophores Cephalon -hemispherical with smoothly rounded anterior margin, posterior border tirsinuate, eyes dark margin, posterior border tirsinuate, eyes dark distinct set of posterio lateral aspect of cephalon; pleon –distinct

narrower than pereon and also described the male and reccorded that the Male measured 11-13 mm long, 4-6 width, body very small; eyes dark. Absence of male in this Study. **Ravichandran et al (2010)** recorded that Parasite body of Joryma brachysoma was dorso-ventrally flattened (depressed) with a head, fused with the first thoracic segment (cephalothorax), thorax and abdomen. The thorax Had seven segments and abdomen six (often fused into two to five). One pair of thoracic appendages modified into mouthparts, and seven pairs are unmodified. The abdomen has six pairs of appendages, and ends in a terminal shield (pleotelson). **Tavares-Dias et al (2014)** stated that the Females *of Braga patagonica* were: oval body; light colored (closer to vert 340). Triangular cephalon, long and rounded anterior; long maxillipeds, with side lobes with hairy bristles and relatively small eyes (Table 2). Wide pereon, highest and widest at the 5th pereonite. Narrow pleon. Prominent pleotelson, wider than long. Uropod shorther than pleotelson; elongated oval branches; exopodite longer than endopodite. Males: Smaller than females cephalon and pleon relatively larger than females in proportion to their bodies. Sexual dimorphism evident in the maxilliped, through shape and size and in the second pleopod with a slender male appendix.

In recent years, several SEM investigations have been made on isopods surface features **Guy et al (1987)** made a detailed SEM study on the gnathiid isopod *Paragnathia formica***Abd El-Aal**. **(1988)** on the land isopod Porcellio scaber **Camp (1988)** studied the morphology of the body appendages of the gnathiid isopod Bythognathia yucatunensis. **Abd El-Bar (1995)** on the marine isopod *Sphaeroma serratum* . **Shields & Ward (1998)** examined the un usual endoparasitic isopod *Tiarinion texopallium*, from the majid crab *Tiarinia sp*. and directed special attention to the description of the antennules, antennae and pereiopods related to parasitic adaptation. **Leistikow (1998)** investigated the oniscoid isopod *Pentoniscus* and described a new species with details of its mouthparts, pereiopods and pleopods. **Keable (1999**) described a new species of the cirolanid isopod *Dolicholana*, and redescribed *Dolicholana porcellana* with special reference to their mouthparts and setal types. He revealed the difference between the molar median surfaces of *Dolicholana elongata* and Natatolana corpulenta by scanning electron micrograph**. Al-Ahmadi (2001)** described the morphology of the mouthparts of *Cirolana bovina* include the mouth lobes (upper and lower lips or the labrum and labium) and paired mandibles, maxillules, maxillae and maxillipeds which are modified first pair of thoracic appendages as mouthparts. They are attached ventrally to the head.

Absence of male in this study due to short live spane and the cymothoids are patandrous hermaphrodites as mentioned by **Ravichandran et al (2009)**

In conclusion. The parasites occupy the entire branchial chamber of the host thus may produce pressure on the gill surface and thus affecting the efficiency of respiration.

Although, the infestation may cause immediate death, it will affected the normal growth of the host fishes. They may lead to economic loss of fishes.

Fig. 1: Anilocra leptosome (dorsal view).

Fig.2. Anilocra leptosome (ventral view).

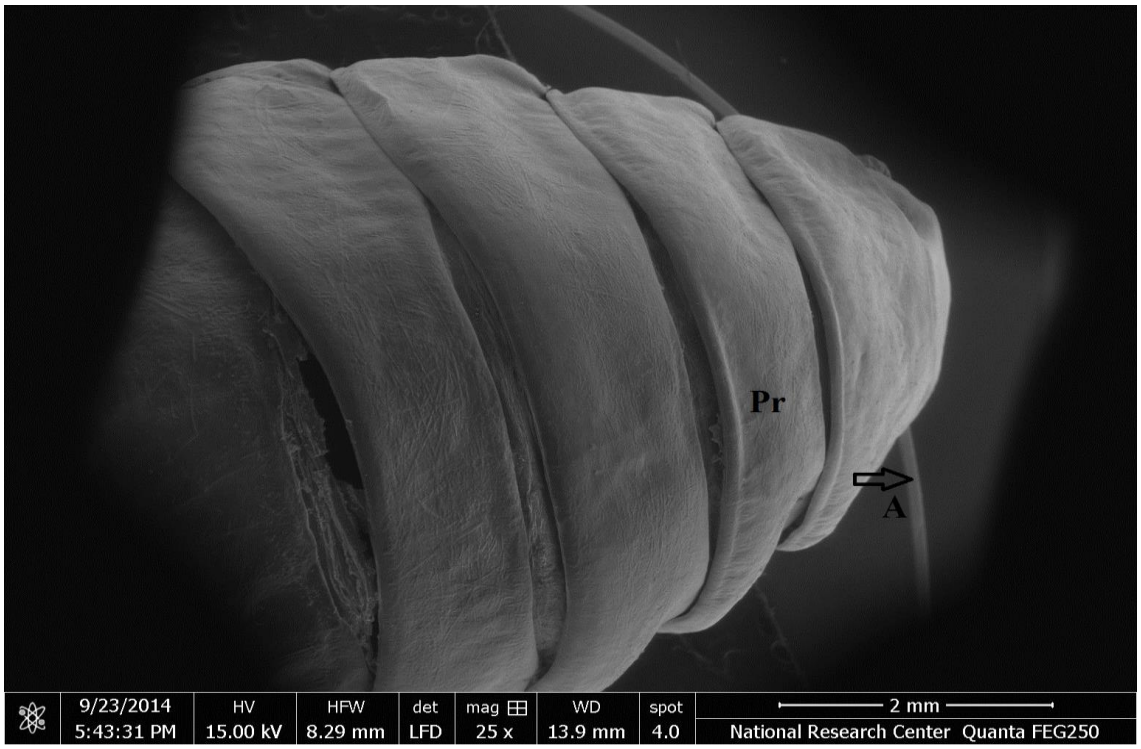

Fig.3. Anilocra leptosome Anterior dorsal surface (X25) Showing (A) Antenna and pereonites(Pr).

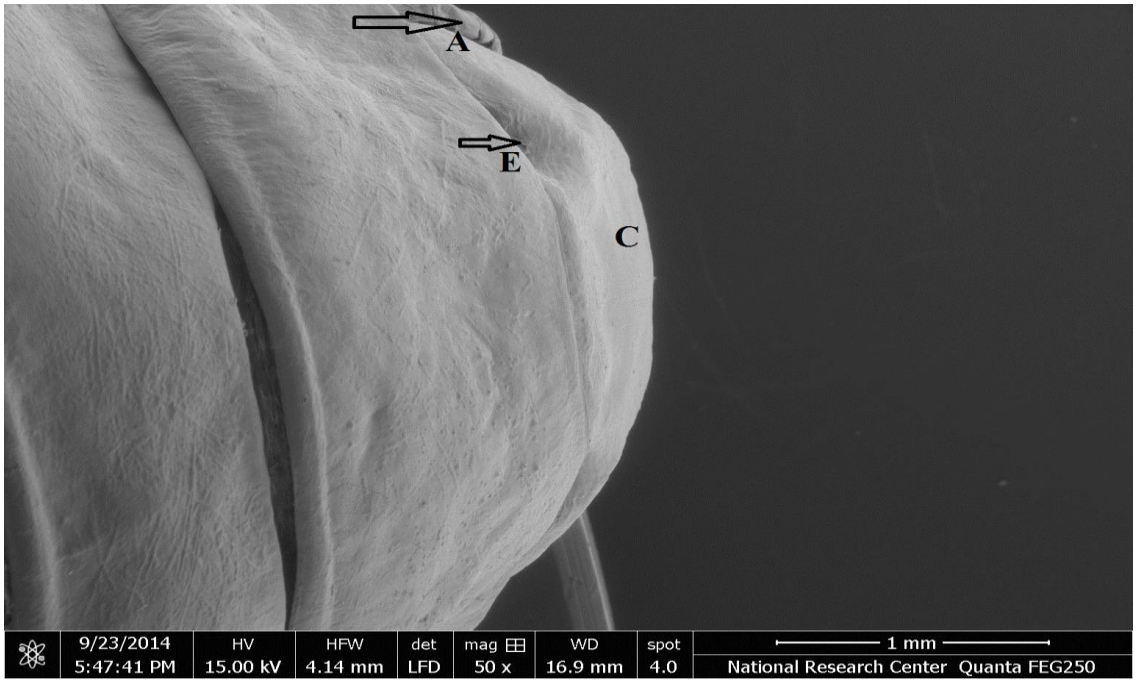

Fig.4. Anilocra leptosome (X50). Anterior dorsal surface showing (C) cephalon, 2eyes (E) and antenna (A).

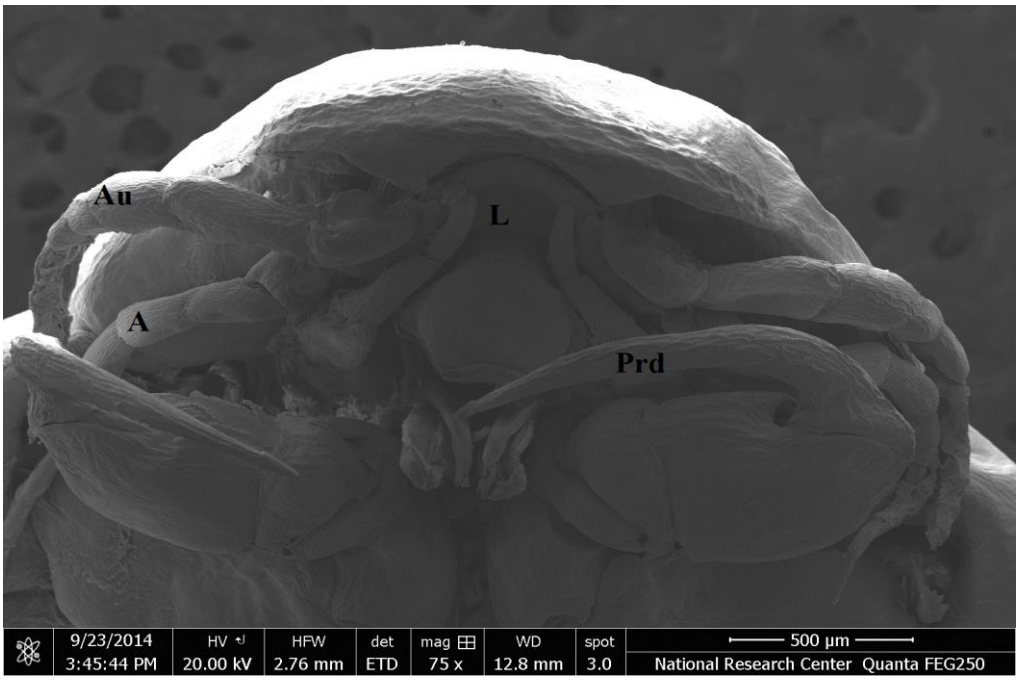

Fig .5. Anilocra leptosome (X75). Anterior ventral surface Showing mouth part (labrum, L) 2 pairs of Antenna (A)and Antennul (Au) , 1st pair of Pereopods (Prd).

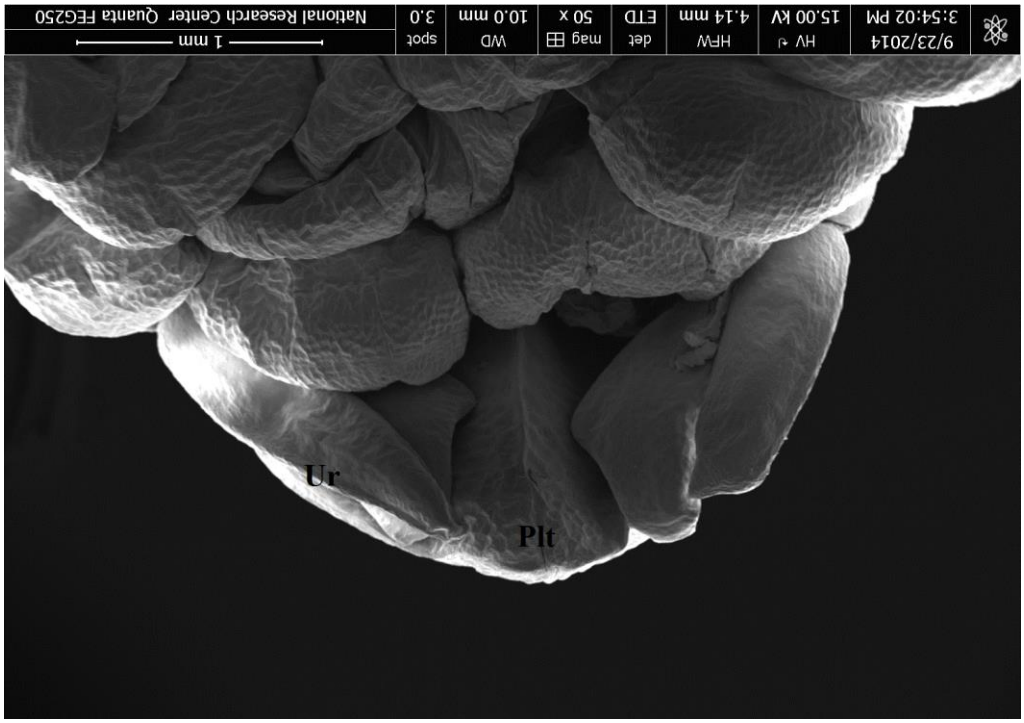

Fig.6. Anilocra leptosome (X50). Posterior ventral surface showing pleotelson(Plt) and uropods (Ur).

Fig.7. Anilocra leptosome (X12) . Ventral surface showing 2 pairs of antennul (Au) and antenna(A) ,pereopods (Pr), peleopods(Pl), Coxal crest(Cc)and Marsipium (Mu)

Fig.8. Anilocra leptosome (X40). Posterior dorsal surface showing Peleonites(Pl) ,pleotelson (Plt) and uropods (Ur) .

Fig.9: Cymothoa indica. (Dorsal view)

Fig.10: Cymothoa indica (ventral view)

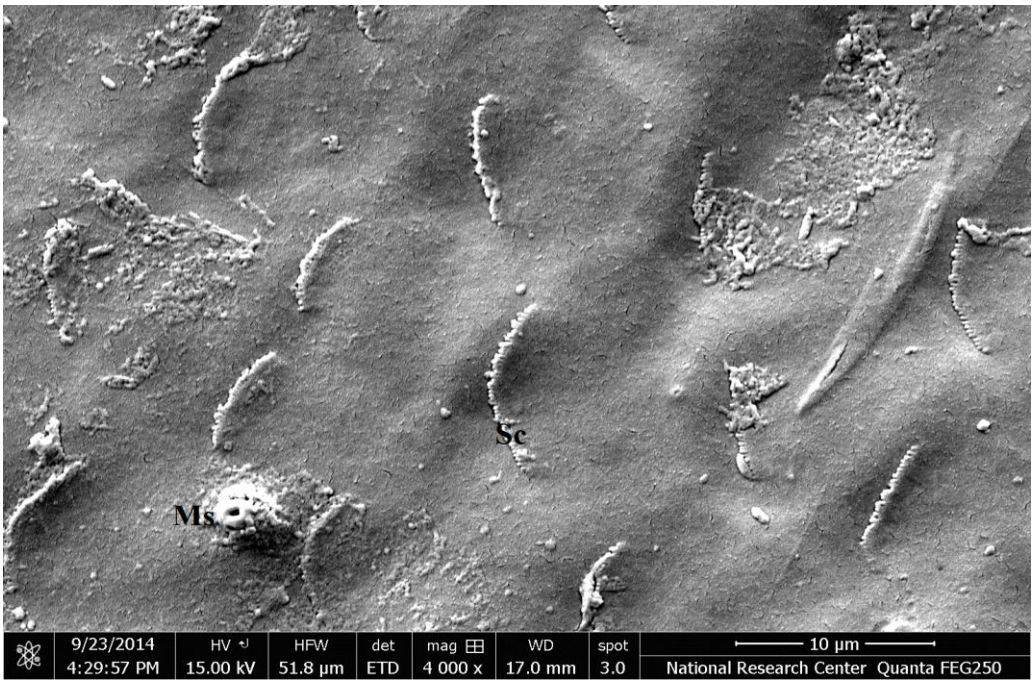

Fig.11: Cymothoa indica(X4000) . Dorsal surface of pereon showing scales (Sc) and microtrich sensilla (Ms)

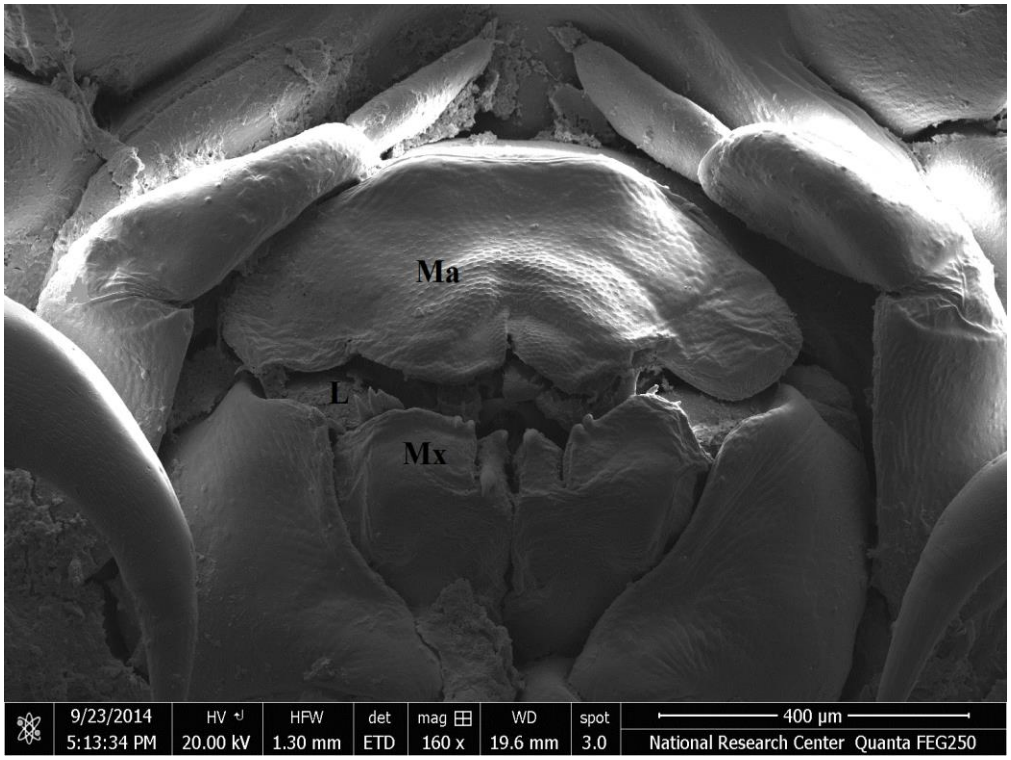

Fig . 12 . Cymothoa indica(X160) .Anterior ventral surface showing mouth part (L)with Maxilla (Mx) and Madibule(Ma) .

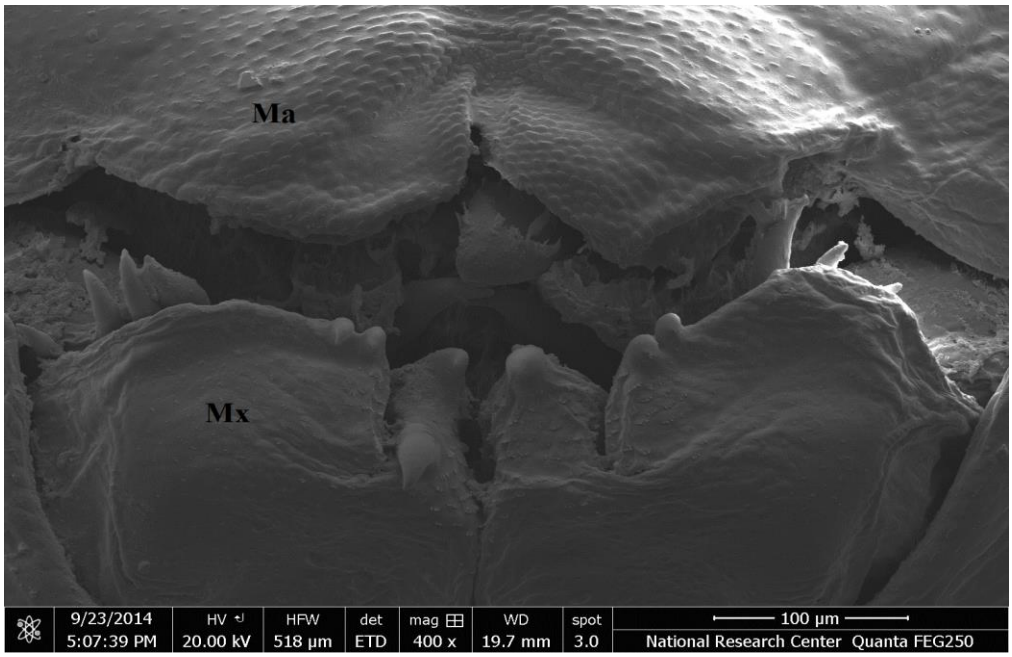

Fig .13 Cymothoa indica(X400) . Anterior ventral surface showing labrum(L) with Mandibul (Ma) and Maxilla (Mx) .

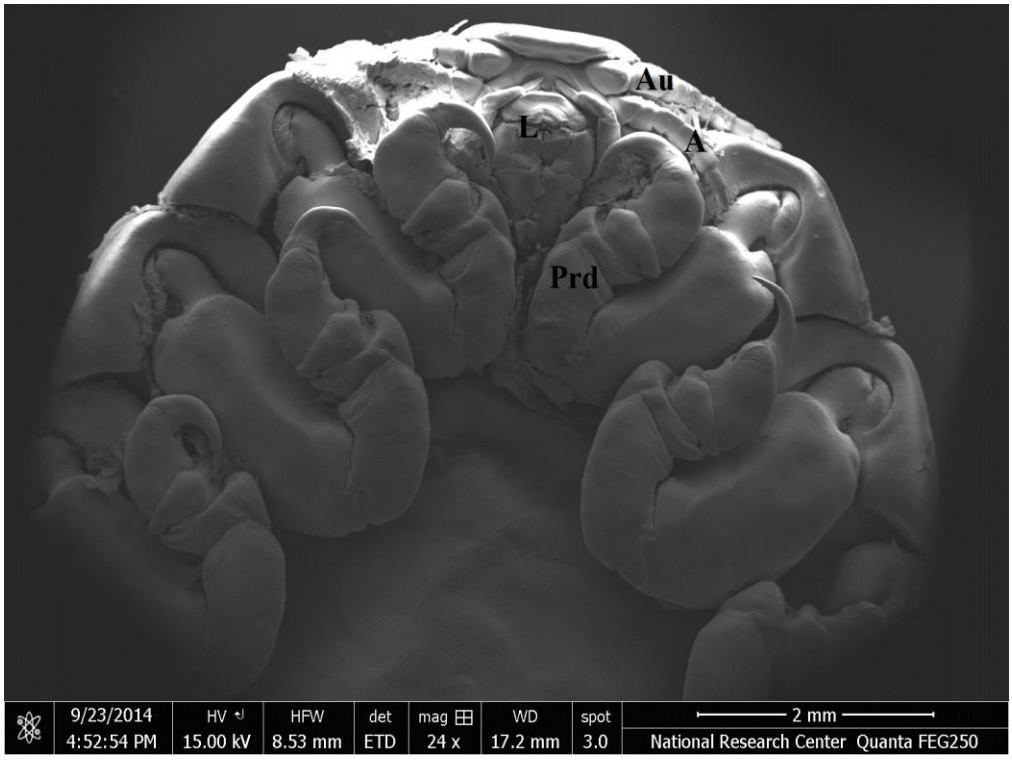

Fig.14.Cymothoa indica (X24) Anterior ventral surface showing Antennule (Au) antenna(A) and pereopods(Prd)

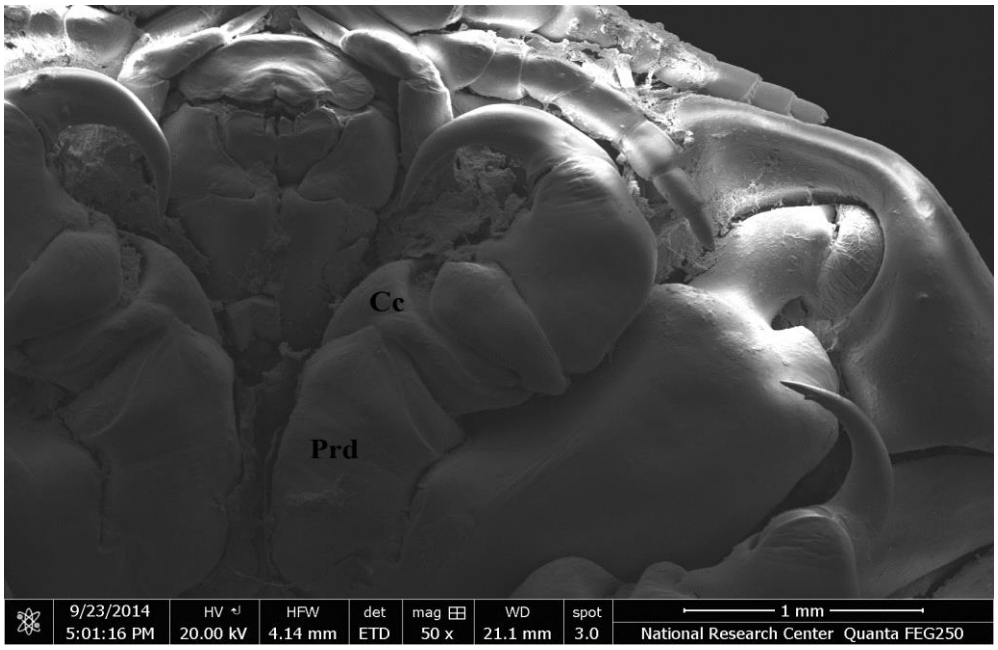

Fig .15. Cymothoa indica (X50) Anterior ventral surface showing peeeopods (Prd) with coxal crest (C).

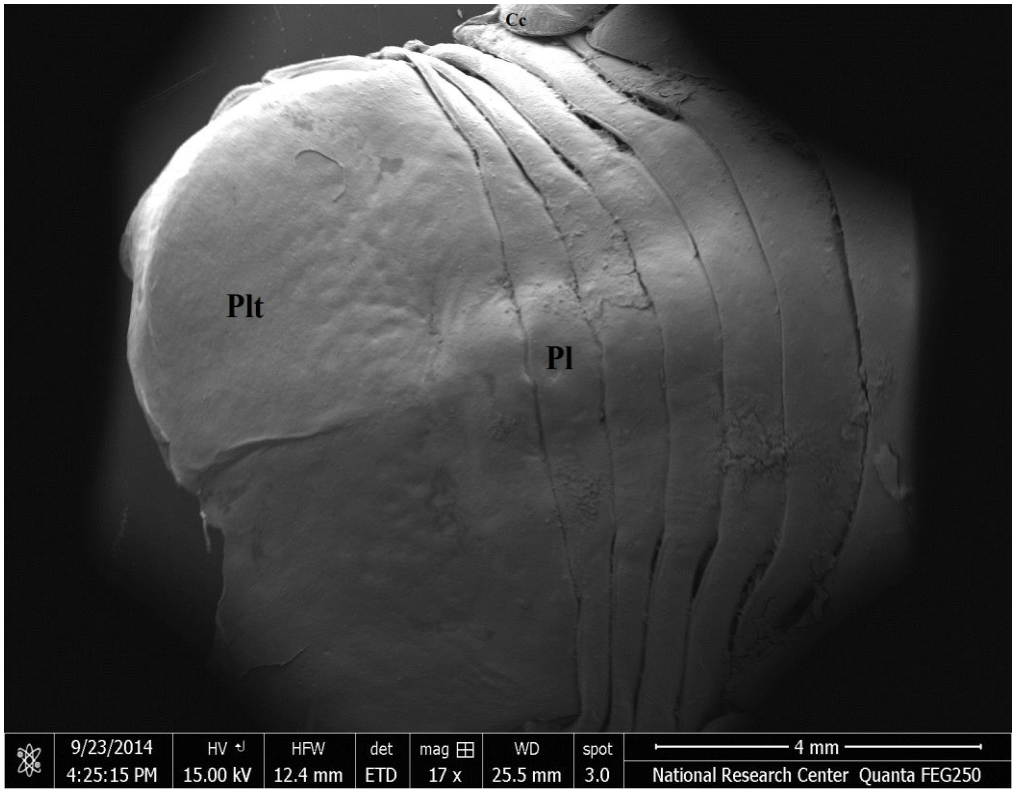

Fig. 16. Cymothoa indica (X17) Posterior dorsal surface showing coxal crest (Cc) , peleonits (Pl) and pleotelson (Plt).

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