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**FINE STRUCTURES OF ADULT FEMALE
OF *ERGASILUS SIEBOLDI* (COPEPODA:
POECILOSTOMATOIDEA) FROM EGYPTIAN
FRESHWATER FISH (*BAGRUS BAYAD*)
(With 3 Plates)**

By

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**التركيب الدقيق لأنثى أرجسيلس سيبولدي الناضجة في أسماك
المياه العذبة المصرية (البياض)**

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شملت هذه الدراسة الوصف التفصيلي الدقيق لطفيل أنثى أرجسيلس سيبولدي الناضجة في أسماك البياض في مصر باستخدام الميكروسكوب الضوئي و الميكروسكوب الإلكتروني الماسح. وقد أوضحت الدراسة أن أنثى الطفيل تتكون من 10 حلقات وطولها حوالي 1.4 ± 0.05 مم وعرضها 0.04 ± 0.01 ، وتتميز الرأس باتحادها مع الحلقة الصدرية الأولى وتسمى "سيفالوسوم" ثم أربعة حلقات صدرية حرة تتصل كل حلقة بأرجل العوم ثم يليها خمس حلقات للجسم وتعرف "بيوروسوم" وتتكون من حلقة جنسية مزدوجة (وتحتوي على زوج من الفتحات الجنسية الطويلة ومزينة بثلاثة خطوط عرضية وبها أشواك بطنية) ثم ثلاث حلقات بطنية بالإضافة إلى حلقة خامسة تساعد الطفيل على الحركة. وقد أوضح الفحص بالميكروسكوب الإلكتروني الماسح أن هناك شوكة كبيرة وكذلك أشواك صغيرة واضحة على الطبقة السطحية الخارجية من الجزء الداخلي الأول للخطاف (antenna) وكذلك شوكة مخروطية على الخطوط القدمية وأشواك طرفية على الطبقة السطحية الداخلية لقدم العوم الثانية ويعتبر هذا الوصف لأول مرة بالنسبة لهذا الطفيل بالإضافة إلى الحلقات البطنية مزينة بصف غير كامل من الأشواك الحادة.

SUMMARY

Detailed fine structures of *Ergasilus sieboldi* female from Egypt was studied using both Light microscopy (LM) and Scanning electron microscopy (SEM). The results demonstrated that adult female is cycloform with 10 segments measuring 1.4 ± 0.05 mm in length and

0.051 ± 0.04 mm in width. The first five segments formed prosome which consisted of a cephalosome and four free pedigerous somites. The other five body segments formed urosome which exhibited genital double somite, three abdominal somites and fifth pedigerous somite. The first and second antennular segments were partially fused anteriorly and distinct posteriorly. The genital double somite is wider than long bearing a pair of longitudinal genital aperture parallels the longitudinal axis of the body and ornamented with three transverse bands of spines ventrally. SEM demonstrated that there were a distinct peg seta and minute setae were found locating on the integument of first endopodal segment of antenna. A large conical seta was detected on the interpodal sternites and marginal spinules on the integument of 1st exopodal segments of 2nd swimming leg for the first description. Abdominal somites were ornamented with incomplete row of sharp spinules.

Key words : *Fine structures, Ergasilus sieboldi, fish, Copepoda*

INTRODUCTION

Ergasilidae is a large family of parasitic copepods belonging to the order Poecilostomtoidea. During their life cycle, the parasite spends a series of free living stages that terminate with adult mating and death of males. However, the fertilized females become infective and parasitized the gills of fish. So, the adult females are only parasitic to fish (Abdelhalim *et al.*, 1991).

Ergasilus sieboldi are found to be infesting over sixty fish species with a higher parasitic intensity reached over 13400 parasite individuals per gills of one fish (Abdelhalim *et al.*, 1991). This parasite causes commercially significant epizootics in both cultured and wild population fish (Woo, 1995). It is also common and endemic among African freshwater fish (Fryer, 1968) inducing damages of infested gills and subsequently mortalities of fish (Paperna, 1996).

Several studies are found dealing with the taxonomy of parasitic species of genus *Ergasilus* (Markewitch, 1976; Kabata, 1979, 1985; El-Rashidy, 1999 and El-Rashidy and Boxshall, 1999, 2002). Unfortunately, there is a paucity of detailed information either on external morphology or fine integumental structure of *Ergasilus sieboldi* infesting Egyptian fish. The only previous description among this parasite from Egyptian fish was done by light microscopy (Imam *et al.*, 1975). However, a fine structure by scanning electron microscopy (SEM) was demonstrated among British freshwater fish (Abdelhalim *et al.*, 1991 and 1993). Furthermore, several

new *Ergasilus* species were recorded in Brazil and UK depending on light microscopical studies (Montu and Boxshall, 2002 and El-Rashidy and Boxshall, 2002) Therefore, the main target of this study is to describe the fine integumental structure of *Ergasilus sieboldi* infesting the Egyptian freshwater fish (*Bagrus bayad*) using SEM.

MATERIALS and METHODS

1. Collection of Copepods

Ergasilus Copepods were collected from naturally infested freshwater fish obtained from the Nile during spring of 2001 at El-Moneib market, Giza, Egypt. The parasites were washed several times in distilled water to remove mucous and debris. The specimens were preserved and fixed in 70% ethanol.

2. Light Microscopy (LM)

Permanent specimens of *Ergasilus* species were prepared as technique described by Soulsby (1982). The cleaned washed Copepods were dehydrated in glycerin-ethanol series, cleared in lactophenol and mounted on glycerin-gelatin medium. Finally, the specimens were carefully examined, drawn by Nikon light microscopy (Japan) and identified according to Markewitch (1976) and Kabata (1979).

3. Scanning Electron Microscopy (SEM)

Fresh collected copepods were washed several times in distilled water to remove the debris. The specimens were then fixed in 2.5% glutaraldehyde, transferred to Electron Microscopy Unit, Faculty of Science, Ain Shams University and processed as technique described by Abdelhalim *et al* (1993) The specimens were washed in three changes of phosphate buffered saline, dehydrated in ascending grades of ethanol and dried at critical point drier (SPI Supplies, Canada). The specimens were glued on specimen stubs and coated with 15 nm gold in Sputter coater (Jeol, JFC-1100 E, Japan). Finally, the specimens were examined and viewed on a Scanning Electron Microscope (Jeol, JEM-1200 EX II, and Japan).

RESULTS

1. Light Microscopy (LM).

L.M. demonstrated that adult female of *Ergasilus sieboldi* is cyclopiform (Plate.1.A) measuring 1.4 ± 0.05 mm in length and 0.051 ± 0.04 mm in width. It consists of 10 body segments ; the first five segments represents prosome which consists of a cephalosome and four free pedigerous somites. The other five body segments forms urosome which

exhibits genital double somite, two abdominal somites and urosomites (anal somite and caudal rami).

Cephalosome (Plate. 1. A) is slightly constricted at its posterior third. It constitutes about two thirds of the body length excluding caudal setae. Cephalic shield is ornamented dorsally with circular and inverted T-shaped marking. Tergites of second to fourth free pedigerous somites are narrowing posteriorly.

Genital double somite (Plate.1.A) is wider than long and appeared strongly convex at the lateral margin with a higher sclerotised cuticle anteriorly than posteriorly. It bears a pair of longitudinal genital aperture dorsolaterally which parallel the longitudinal axis of the body. It is also ornamented with three transverse bands of spines ventrally at the anterior part only. Genital double somite is twice as long as the first abdominal somite. First abdominal somite appeared to be as long as second abdominal somite which exhibits a row of spines ventrally.

Urosomites (anal somite and caudal rami) are separated by zone folded cuticles (Plate.1.A). Anal somite obviously appeared smaller than second somite (caudal rami) which deeply incised medially with lateral and ventral spinules at posterior margin. Caudal rami are 1.7 times longer than anal somite and ornamented with irregular rows of spinules and pores ventrally. It is also armed with four setae; three of which are located ventrally along its distal margin and another longer seta is located dorsally. The longest caudal seta is ornamented with spinules.

Antennule (Plate.1.C) is composed of 6-segments. The 1st and 2nd segment are partially fused anteriorly and distinct posteriorly. The segmental formula is figured as follow: 2, 8, 2 + ae (aesthetasc), 2, 2, 5, ae.

Antenna ((Plate.1.B) is a powerful clawed appendage which is adapted for embracing the gill-filaments. It consists of 4 segments; a relatively long coxobasis (1st segment) and 3-segmented endopods bearing a curved claw. First segment (coxobasis) is formed from coxa and basis. Second segment (=1st endopodal segment) is highly inflated and nearly twice as long as coxobasis with a wider middle part than the proximal and distal parts of the segment. It is armed with a peg seta located at about approximately the end of the mid-third of the inner margin of the segment. Third segment (=2nd endopodal segment) is curved and armed with a spiniform seta at the concave margin. Forth segment (=3rd endopodal segment) is relatively long (=2nd + 3rd endopodal segments or 90 % of 1st segment). Terminal claw is strongly recurved, nearly about 1/4 length of 3rd antennal segment and exhibited a fossa near the tip.

Mandible (mn) is consisted of the coxa and gnathobase (Plate.1.D) with lacking any trace of true palp (basis and rami). Gnathobase is provided with three toothed blades (anterior, middle and posterior). The anterior blade appeared to be articulated at its base. The middle and posterior blades are not articulated basally.

Maxillule (ml) is typically reduced to an unsegmented lobe (Plate.1.D) exhibiting three setae; one seta is located medially and other two setae are situated distally.

Maxilla (mx) is composed of a large basal syncoxa and a small distal basis (Plate.1.D). The syncoxa is ornamented with a number of integumental pores. The basis is armed with a dense array of sharp distally located teeth.

Swimming legs 1-4 (Plate.1.E-H) are bi-ramous which branched into two rami ; an outer exopod and an inner endopod . All rami are of 3-segments except that of leg 4 exopod has only 2- segments (Fig. 1 H). Spinulation of basis are arranged in row (s) along inner margin of leg1 and over the inner anterior aspect of leg 2-4. An outer seta is located at lateral margins of all legs. Setules are situated at the outer margin of 1st-exopodal segment of all legs, and located also on inner margin of 1st-endopodal segment of only leg 1-3 and on all endopodal segment of leg 4. A large conical seta is each located at the posterior margins of interpodal sternites of 1st-exopodal segment on all swimming legs (birami). Armature formula of the rami is figured as follows:-

| | Coxa | Basis | Exopod | Endopod |
|-------|------|-------|----------------|---------------|
| Leg 1 | 0-0 | 1-0 | 1-0; 1-1; II-5 | 0-1; 0-1; 1-3 |
| Leg 2 | 0-0 | 1-0 | 1-0; 0-1; 1-6 | 0-1; 0-2; 1-4 |
| Leg 3 | 0-0 | 1-0 | 1-0; 0-1; 1-6 | 0-1; 0-2; 1-4 |
| Leg 4 | 0-0 | 1-0 | 1-0; 0-5 | 0-1; 0-2; 1-4 |

Fifth leg (Plate.1.I) is consisted of two segments; a protopodal segment with an outer seta and a free exopodal segment with two terminal setae of unequal length.

2. Scanning Electron Microscopy (SEM)

SEM clearly demonstrated that *Ergasilus sieboldi* female (Plate 2) has an expanded prosome without any trace of a functional suture separating 1st pedigerous somite from cephalosome (Plate 2, a). It exhibits a well defined boundary separating the cephalosome from 1st pedigerous somite (Plate 2.b). This boundary is defined by the space between the anterior margin of the tergite of 1st pedigerous somite and the posterior margin of the dorsal cephalic shield. It is also found that

Ergasilus sieboldi female has a true cephalothorax with 1st pedigerous somite which appears the longest pedigerous somite (Plate 2.b). Distribution of sensilla and pores of integumental glands at the body surface of prosome revealed that they are restricted to the dorsal cephalic shield and concentrated around the cephalic margin. Rostral plate is situated at the middle area of the dorsal cephalic shield anteriorly. It is demarked from the rest of the body by two deep furrows bearing nauplius eye which is visible externally as a raised integumental area (Plate 2.f).

Antennule is 6-segments. The 1st and 2nd segments are partially fused anteriorly and distinct posteriorly (Plate 2.c,d). The 2nd and 4th segments are each provided with a long seta (aesthetasc), located on its posterior margin distally (Plate 1.d). The 6th segment is triple of another antennular segment long bearing bristles or setae (Plate 2.e).

Antenna is 4-segments. The 1st endopodal segment is highly inflated with a widest area at its middle part. It is provided with two types of setae; peg setae located approximately at the end the second third of the inner margin, and other minute setae located proximally and distally at its dorsal surface (Plate 2. e). Setae distribution on 2nd 3rd and 4th endopodal segments are identical with that described by light microscopy (Plate 2. b).

SEM confirmed that genital double somite is wider than long and provided with two longitudinal genital apertures, each located at the second third of the convex side laterally. The genital aperture appeared as a longitudinal slit with a broad proximal and pointed distal ends (Plate 3.A). Posterior margin of each abdominal somite is ornamented with incomplete row of sharp spinules (Plate 3.D). Anal somite appeared smaller than 2nd abdominal somite and provided with irregular rows of sharp spinules posteriorly (Plate 3.E). Caudal rami are longer than anal somite and ornamented with pores and caudal setae (Plate 3.D).

Egg sacs are fairly long, fusiform and smooth containing a large number of eggs (Plate 3.F).

Swimming legs 2-4 pedigerous somites are narrower posteriorly (Plate 3.A). The lateral margin of both exopodal and endopodal segments of leg 2 are each provided with one row of sharp spines. The 1st exopodal segment of leg 2 is also ornamented with a large conical seta, posteriorly located at the interpodal sternites (Plate 3.B,C). The 2nd endopodal segment of leg 2 is also armed with a long setules at its inner margin (Plate 3.C). The last exopodal segment is provided with six setules (bristles) where the outer one is the longest (Plate 3.B).

DISCUSSION

The present study described in details the fine integumental structure of *Ergasilus sieboldi* infesting fresh water fish (*Bargus bayad*) in Egypt. Unfortunately, there was no previous data in the available literature dealing with scanning electron microscopy (SEM) of genus *Ergasilus* from Egypt. However, a single fine structure study by SEM was found among *E. sieboldi* from British fresh water fish (Abdelhalim *et al.*, 1993).

As reported by Fryer (1969 and 1982), Kabata (1979) and Abdelhalim *et al.* (1993), our study demonstrated that the 1st pedigerous somite is incorporated into the cephalothorax of *E. sieboldi* in Egypt. Moreover, a well defined boundary (space) was found in this study separating the cephalosome from 1st pedigerous somite. This result was in accordance with that described by Abdelhalim *et al.* (1993) among British fresh water fish.

Our descriptions demonstrated that the rostral area was limited from the rest of the cephalic shield by two deep furrows. However, no specialized rostral area is defined in *E. sieboldi* recovered from British water fish (Abdelhalim *et al.*, 1993).

The present SEM descriptions demonstrated for the first time that there was a partial distinct fusion of 1st and 2nd antennular segments of *E. sieboldi*. This statement was similar to the light microscopy description of *Ergasilus ecuadorensis* recovered from British fresh water fish (El-Rashidy and Boxshall, 2002). Contrary, complete separation between 1st and 2nd antennular segments was found in British isolates of *E. sieboldi* (Abdelhalim *et al.*, 1993). Furthermore, a segmental homogeneity of antennular segments of basic Poecilostomtoid was established (Huys and Boxshall, 1991).

The present study recorded that there was a long seta located distally at its posterior margin of 2nd and 4th antennular segments. However, Abdelhalim *et al.* (1993) recognized presence of a seta distally at the posterior margin of 4th antennular segments. Evidently, these results provided that this compound segment and the most proximal ones were located a posterior seta. Moreover, our study demonstrated a short seta at the dorsal surface of 3rd antennular segments and at 6th segments as a triple segments. Similar results were recognized by Huys and Boxshall (1991) and Abdelhalim *et al.* (1993).

The present SEM illustrated that there were minute setae and a peg seta located the dorsal surface of 1st endopodal segments. These

setae probably have a functioning dual role as a sensory organ and as a part of attachment mechanism. These results were identical with that reported by Abdelhalim *et al.*(1993) for *E. sieboldi* of British origin. Moreover, a proximal spiniform seta was found at 2nd endopodal segments of *E. sieboldi* antenna. This result was closely similar to that described for *Ergasilus ecuadorensis* antenna (El-Rashidy and Boxshall, 2002). However, it was in disagreement with description of Abdelhalim *et al.* (1993) for *E. sieboldi* of British origin, they established a small process at the 2nd endopodal segments. These variations may be due to the variety of local environments. It may be also attributed to the modification of these structures as sensory organs which may be used for attachment of the copepod to the gill filament of fish.

As reported by Abdelhalim *et al* (1993), this study illustrated that the maxillule of *E. sieboldi* is very reduced and ornamented with three setae. This setae orientation suggested probably to have a sensory role. Moreover, the fine structure of *E. sieboldi* maxilla presented here was also identical with that described by Abdelhalim *et al* (1993) for *E. sieboldi* of British origin. Furthermore, our results indicated that the inner seta of 1st endopodal segment was absent in all swimming legs. A similar result was demonstrated by Abdelhalim *et al.* (1993).

Our SEM clearly demonstrated for the first time that a distinct peg seta and minute setae on the integument of the 1st endopodal segments of antenna and the interpodal sternites of 1st exopodal segment were armed with a large conical seta. Moreover, linear sharply spinules were found to locate along the three exopodal segments of 2nd swimming leg. In addition, this study recorded also for the first time that the posterior margin of anal somite was provided with an incomplete row of sharply pointed spinules and a pore at each caudal ramous.

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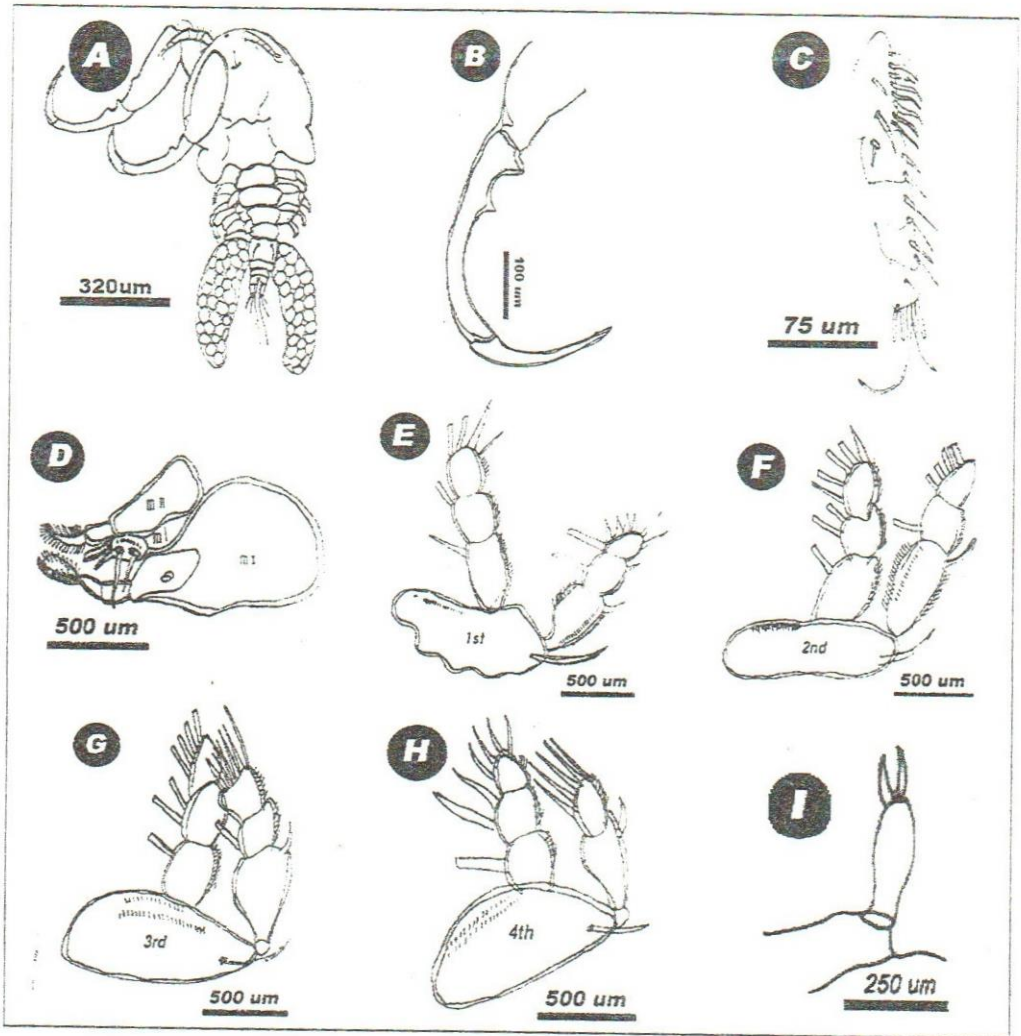


Plate 1. LM of *Ergasilus seiboldi*, Adult female

A, dorsal view; B, antenna, with inset showing detail of a spiniform seta at concave margin; C, antennule showing partially fused of its 1st and 2nd segments; D, mandible (mn), maxillule (ml) and maxilla (mx); E, 1st swimming leg, anterior; F, 2nd swimming leg, anterior; G, 3rd swimming leg, anterior; H, 4th swimming leg, anterior; I, 5th swimming leg

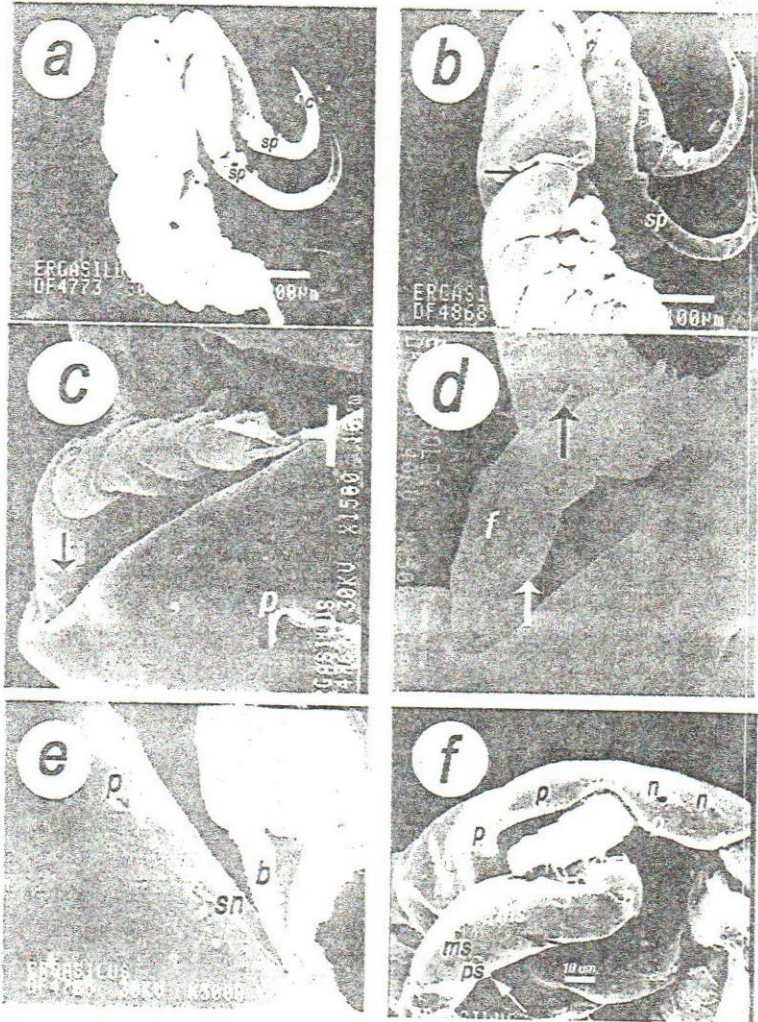


Plate 2. SEM of *Ergasilus seiboldi* , Adult female

a-b, lateral view with posterior margin of dorsal cephalic shield (black arrow) and antenna showing the spiniform (sp) on the 2nd endopodal segment, strong claws (c) at the distal part of antenna. c-e, antennule (dorsal view) showing 1st and 2nd antennular segments; partially fused (f) anteriorly and distinct posteriorly (white arrow). d, the 4th segment provided with a distal seta (black arrow). e, the 6th segment bearing bristles (b); the marginal pore (p) and sensella (sn) situating on the cephalic shield. f, rostral area of cephalic shield (dorsal view) with nauplius eye (n) showing a concentrated pores (p), the 1st endopodal segment of antenna showing peg seta (ps) [white arrow] and minute setae (ms) [black arrow].

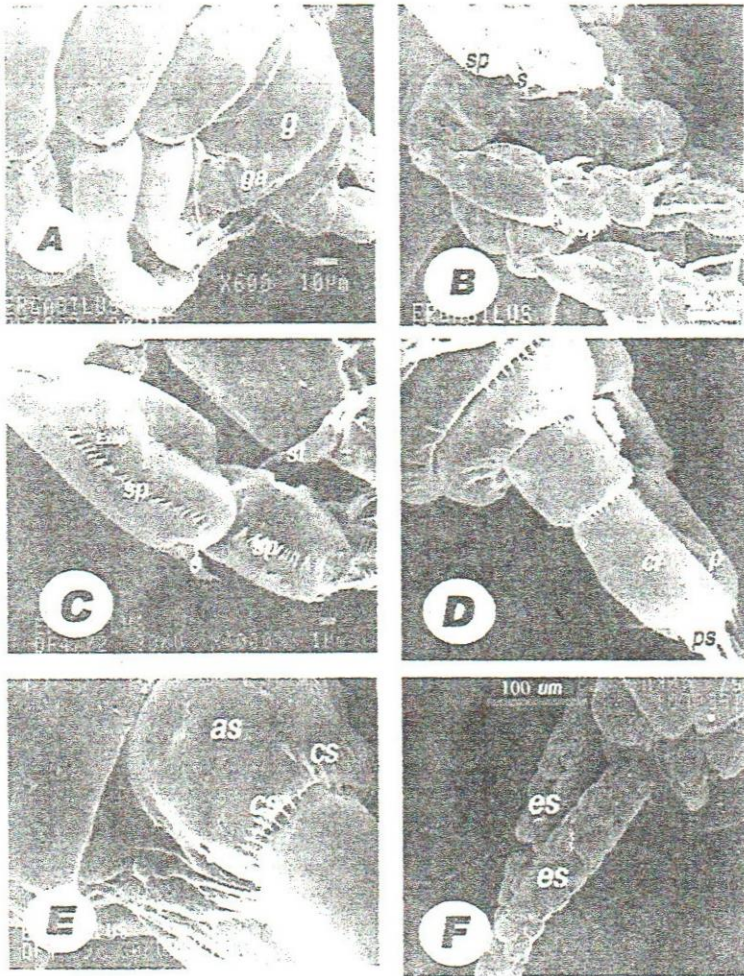


Plate 3. SEM of *Ergasilus seiboldi* , Adult female

A, Urosome (dorsal view) showing; (**g**) genital double somite, (**ga**) genital appretures. **B**, Swimming legs (ventral view) showing; (**sb**) 2nd biramous; (**s**) large seta on posterior margin of interpodal sternites, and (**sp**) a row of spinules along the outer margin; **C**, 2nd swimming leg (ventro-lateral view) showing; (**cs**) a large conical seta on posterior margin of interpodal sternites (**st**) of 1st exopodal segment, and (**sp**) a row of spinules along all exopodal segments; **D**, (**cr**) Caudal rami (ventral view) showing; (**p**) a single pore, and (**ps**) posterior setae on each caudal ramous; **E**, (**as**) Anal somite (ventral view) showing; (**cs**) an incomplete row of sharp conical spinules; **F** , Egg sacs (dorsal view) showing; (**es**) several number of eggs in each egg sac.