Proceedings of the First International Conference (Egyptian British Biological Society, EBB Soc)
Egyptian Journal of Biology, 2001, Vol. 3, pp 20-28 © Printed in Egypt. Modern Press, Cairo.

A stereoscopic study of the mouthparts of the marine isopod, *Cirolana bovina* (Isopoda: Flabellifera)

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ABSTRACT
The three-dimensional study of the mouthparts of the isopod crustacean *Cirolana bovina* is carried out for specimens collected from the Mediterranean Sea, Alexandria, Egypt, to reveal their morphology and related cuticular microstructures, especially setal types. 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 possible functions of the mouthparts, especially in feeding are discussed in light of their structure.

KEYWORDS: Crustacea, isopods, SEM, mouthparts, cuticular microstructures.

INTRODUCTION

In recent years, several SEM investigations have been made on crustacean surface features (Abd El-Aal 1988) on the land isopod *Porcellio scaber* (Schmalfus 1978) and Powell & Halcrow (1982) on some other terrestrial and marine isopods, Mayer & Rochow (1980) on the marine isopod *Glyptonotus antaracticus,* and Abd El-Bar (1995) on the marine isopod *Sphaeroma serratum.* Jones & Fordy (1973) described the mouthparts of the free-living isopod *Jaera nordmanni*. Guy *et al* (1987) made a detailed SEM study on the gnathiid isopod *Paragnathia formica*. Bruce (1981) described three genera within the cirolanid family (*Metacirolana, Neocirolana,* and *Anopsilana*) and three other new genera (*Natatolana, Politolana* and *Cartetolana*). Camp (1988) studied the morphology of the body appendages of the gnathiid isopod *Bythognathia yucatunensis*. Shields & Ward (1998) examined the unusual 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. Chu & Leong (1996) described the bopyrid ectoparasitic isopod *Orbione halipori,* and concluded that this species is specifically associated with various penaeids. They also suggested that there is a positive correlation between the female length of *Orbione halipori* and the carapace length of its penaeid host *Metapenaeus joyneri*. 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 micrographs. Leistikow (1998) investigated the oniscoid isopod *Pentoniscus* and described a new species with details of its mouthparts, pereiopods and pleopods. Muller & Salvat (1993) examined the cirolanid isopods inhabiting the French coral reefs and found three new species: namely *Cirolana paracradia, Metacirolana pigmentata* and *Metacirolana moortgati.* In the sphaeromatid isopod *Paracerceis sculpta,* Shuster (1991) studied the anatomical changes in females associated with the reproductive moult. He stated that the mouthparts of most females either become modified to circulate water beneath the oostegite or cease to function altogether and become fused to the cephalon. Bowman (1977) described the mouthparts of the cirolanid isopod *Ceratolana papuae,* and the prominent horns formed by its rostrum and frontal lamina as a diagnostic feature of that species. Lincoln & Jones (1973) described the cirolanid isopod *Anuropus branchiatus*. They stated that this large

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isopod has modified uropods for a presumed respiratory function, in addition to the pleopodal surfaces. This is probably correlated with the large size of the organism and correspondingly heavy respiratory demand. Wägele (1987), in his study of the behavior and feeding mechanism of the valviferan isopod of genus *Antarcturus* exemplified by *A. spinacoronatus*, gave detailed description of their specialized pereiopods and body surface cuticular spines. He compared the results to those of *Arcturella*, which seemed to be more specialized.

The marine isopod *Cirolana bovina* (Barnard 1940) is a member of the family Cirolanidae and is one of the largest isopods known to date. Barnard (1940) and Pillai (1967) have reported this species. Isopods of the genus *Cirolana* always prefer hard substrata and are rarely found in sand or mud (Jones 1967). Laboratory examination of the gut contents of the *Cirolana bovina* under study revealed the presence of pieces of segmented cuticle. This latter is likely to be from a gammarid crustacean species utilizing the same habitat.

None of the previous studies have examined the mouthparts of *Cirolana bovina* nor has the relationship of structure to function been explored. This work aims to examine the structure of the mouthparts and relate it to their function in the feeding process.

MATERIALS AND METHODS

For scanning electron microscopy specimens of *Cirolana bovina* were collected from Alexandria (Mediterranean Sea) at a depth of one meter. The heads were removed with a sharp razor blade to avoid any discharge that may conceal surface details. They were then fixed in 2.5% glutaraldehyde in 0.1m Sodium cocodylate buffer at pH 7.4 for 3-4 h., washed several times in cocodylate buffer and finally washed in distilled water in an ultra-sonic bath for 20 sec. to clean their surface of any extraneous matter. Glutaraldehyde- fixed mouthparts were immediately dissected under a binocular microscope and post-fixed in 2% buffered Osmium tetroxide for 2h, rinsed in distilled water changes, dehydrated in an ascending ethanol series, then in 50: 50 ethanol and acetone, each for 20 min., followed by pure acetone. The dried specimens were oriented and attached to aluminum stubs with double faced sellotape and critical point – dried in liquid CO_2. The mounted specimens were gold-coated in B sputter-coater for one minute. The coated specimens were examined in a Jeol T 100. Scanning Electron Microscope, operated at an accelerating voltage of 15KV.

RESULTS

Morphology of the mouthparts: 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. Details are given below.

The Labrum (Plate, 1- A, B): On the under side of the head there is an elongated convex frontal lamina with an anterior curved edge and a straight base, lying between the basal joints of the antennules and antennae. Behind these and in front of the mouth, the labrum presents two articulated divisions attached to the base of the frontal lamina. An anterior trapezium-shaped division with an obviously broad base is jointed to a rectangular posterior division. The latter has an arched free edge, slightly notched in the middle and fringed with close –set, soft, short setae. The labral free part is much broader than long and has a dorsal scaley surface with scattered curved spines arising from pits.

The Paragnaths (Labium, Plate, 4A, 5A): These consist of two tiny thin lobes projecting from the ventral border of the mouth. They were difficult to examine, being curled and vestigial. No surface details were detected.

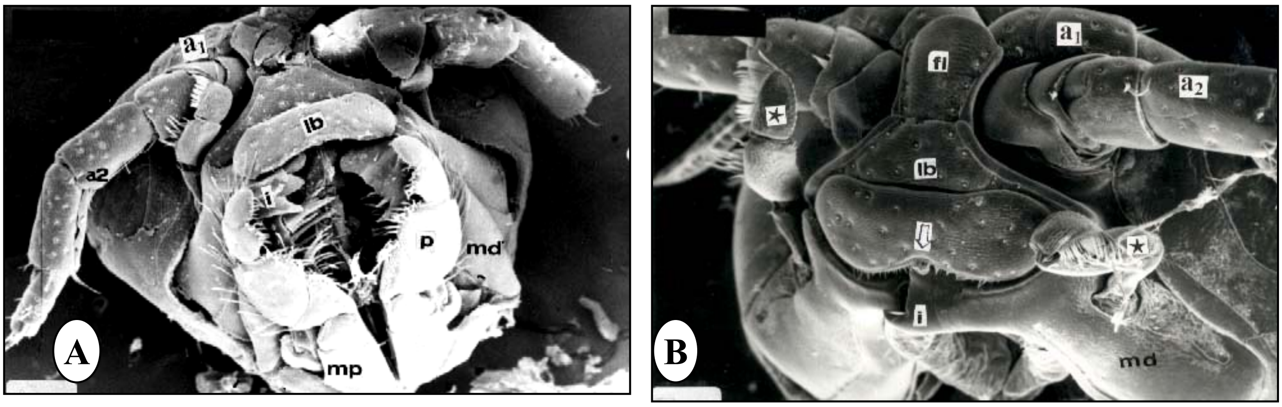


PLATE 1: Scanning electron micrographs of the underside of the head of *Cirolana bovina.* **A:** Antero-ventral view of the head showing the first and second antennae (a_1, a_2) and the mouthparts. lb-labrum, md-mandible, p-maxillipedal palp, mp-maxilliped, I-incisor processes of mandible. 50 X. **B:** Ventral view of the head showing structure of the labrum. Note the bi-partite labrum-lb, attached to frontal lamina–fl, arrow indicates labral notch; a_1, a_2 (first and second antennae). Note the free movement of mandibular palps arising from socket stars, I-incisor process of madible, md- mandible0 Note also the left mandibular palp in cleansing action. 75X.

The Mandibles (Plate, 2, 3): The two mandibles are prominent and lie laterally next to the labrum. Each mandible consists firstly of an elongated stem covering one side of the head and carrying an external lateral palp with freely movable bases and secondly, a shredding-grinding device. The latter is composed of upper incisor processes and lower median molar plate with "lacinia mobile" (Dahl & Hessler 1982) in-between. The mandibular palp consists of three subequal more or less cylindrical segments the second and third of which bear externally long strong, comb-like setae, each beset with two rows of stiff setules (comb-teeth). The asymmetrical distinctive feature between the mandibles is presented by the incisors, which are tridentate but differ in form. In more detail, the right mandible has well defined sharply pointed (canine teeth-like) incisors. Otherwise, the left mandible shows largely coalescing incisors, two of that are broad and slightly detached (molar-like) but the third incisor is ventral, curved up with a sharp edge and obviously longer than the other two (cutting tooth-like). Next to the incisors lies the lacinia mobiles that consists of two toothed parts, an upper small part with four short hooked setae arranged circularly and a large lower flattened part terminating by stout longer pointed setae " lacinia mobiles". The latter lies externally to the upper molar surface, which is a broad, rough and rounded elevated area (mill-like surface). The third part of the shredding- grinding device is the lower median molar plate (saw-like structure). It is a large, elongated triangular plate with serrated upper edge. It is attached at its base where it moves up and down and has a free apex.

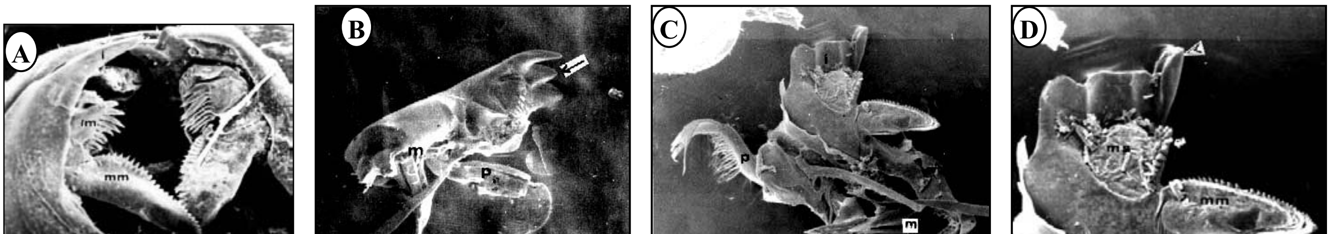
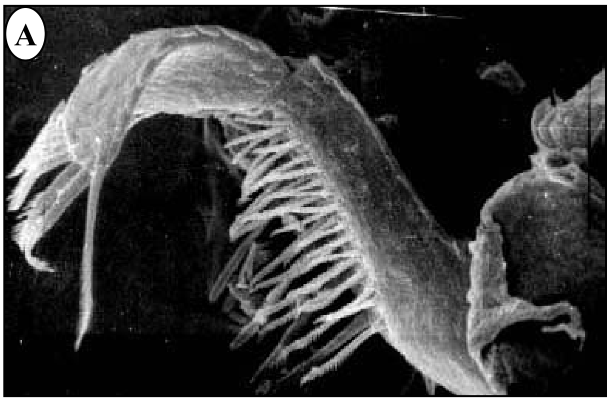


PLATE 2: Scanning electron micrographs of the mandibles (in situ) of *Cirolana bovina.* **A:** Magnified part of ventral view of the head showing right and left asymmetrical mandibles. Note I-incisor process, lm-lacinia mobiles, mm-molar median surface0 200X. **B:** The right mandible showing tridentate incisor process (arrow), mandibular palp-p, muscles-m. 75X. **C:** The left mandible is showing its different incisor processes-I, mandibular palp- p, muscles-m. 100X. **D:** Higher magnification of C showing lacinia mobilis (arrow), molar surface-ms, molar median surface-mm. Note the long tooth – like incisor (arrowhead). 200X. **E:** Higher magnification of the right mandible showing lacinia mobiles (arrow), molar median surface-mm, incisor process-I, mandibular palp-p.150X.



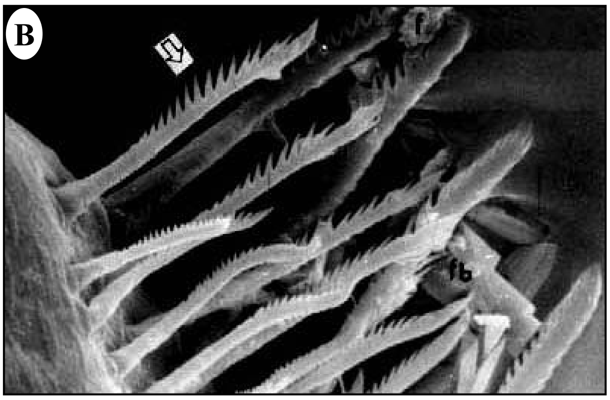


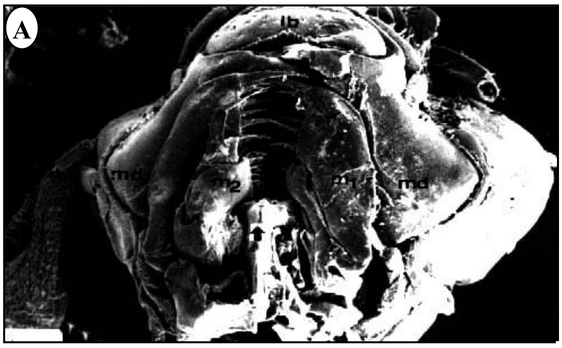
PLATE 3: Scanning electron micrographs of the mandibular palp and lacinia mobiles of mandibles of *C. bovina*

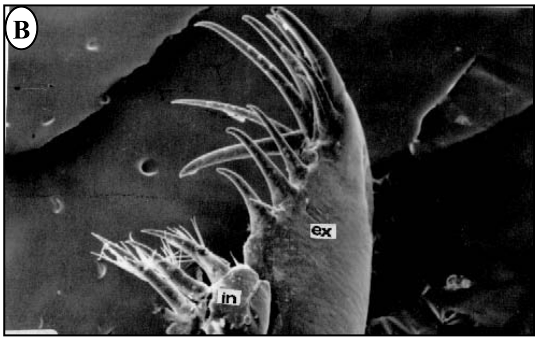
A- Mandibular palp is showing its terminal two segments carrying comb setae. 500X.

B- Higher magnification of A. Note the comb setae (arrow) trapping food particles (f) and foreign bodies (fb). 1000X.

C- Higher magnification of lacinia mobiles.Note the stout setae (double arrows) and the short hooked setae (single arrow). 750X.

The Maxillules (First maxillae, Plate, 4-A, B, C): These are a pair of flattened setose structures, each with a basal stem articulating with two endites, a large external and a small internal one. The external endite is longer and terminates with robust setae, but the internal endite carries three robust pappose setae and a few setules on its terminal edge.





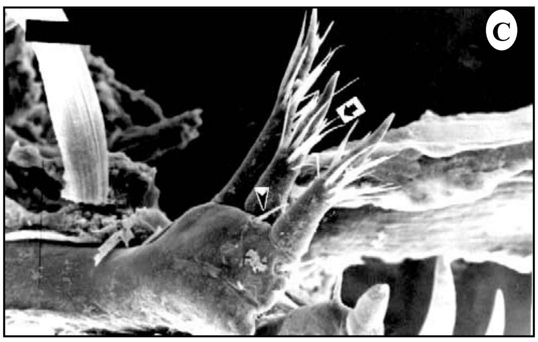


PLATE 4: Scanning electron micrographs of the head and maxillules of *Cirolana bovina.* **A:** Ventral view of the head shows the position of maxillules after removal of the maxillipeds. Note m_1 -maxillule, m_2-maxilla-, md-mandibles with crossed incisors, labrum-lb, (black arrow) indicates paragnaths. 50X. **B:** Dorsal view of maxillulary endites. Note the external endite carrying robust setae- ex and the in-internal endite with three robust pappose setae. 200X. **C:** High magnification of maxillulary internal endite showing the setules of robust pappose setae arranged in circlets (arrow). Note also the simple setae (arrowhead). 300X.

The Maxillae (Second maxillae, Plate, 5, 6): These are foliaceous smaller and softer mouthparts than the first maxillae. They lie next to the maxillules in front of the maxillipeds. The maxillae are two elbow-shaped thin and flattened structures. Each is composed of a curved cylindrical base attached to three endites. The first endite is the largest and proximal and its median side is studded with plumose setae, having from many to a few setules or none at the upper end. The second and third endites are distal and ending with long spiky serrate setae. The second endite is median and broader than the third one.

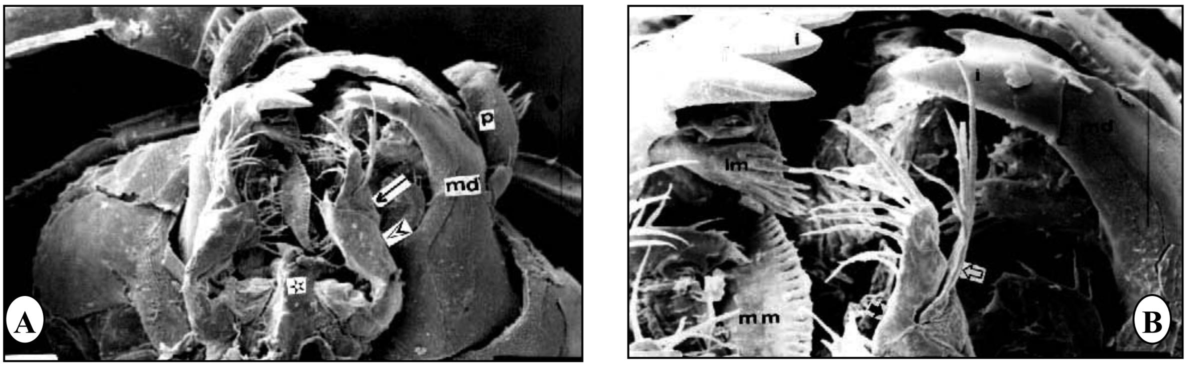


PLATE 5: Scanning electron micrographs of the maxillae of *Cirolana bovina.* **A:** Ventral view of the head shows the maxillae after removal of the maxillipds. Note the first proximal endite (arrowhead), the second and third endites (arrow), the paragnaths (star), mandibular palp- p, mandible md. 75X. **B:** Higher magnification of (A) showing the second median maxillary endite (black arrow), the third maxillary endite (white arrow), molar median surfaces-mm, lacinia mobiles-lm, mandible -md, incisor process of mandible-i. 150X.

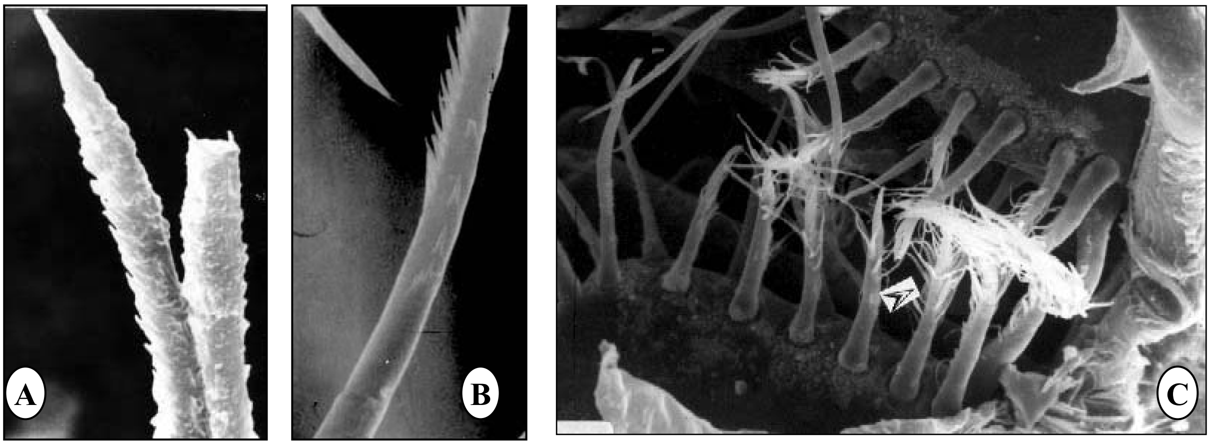


PLATE 6: Scanning electron micrographs of the setal types of the maxillary endites of *Cirolana bovina.***A:** Spiky serrate setae of maxillary third endite. 1500X. **B:** Spiky serrate setae. of maxillary second endite. 1000X. **C:** The maxillary proximal endite showing plumose setae (arrowhead). 500X.

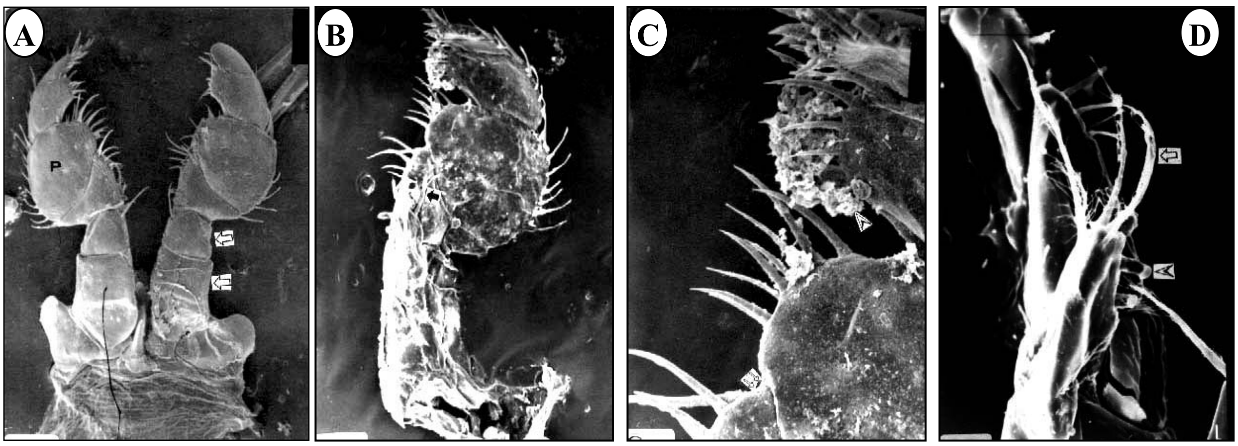


PLATE 7: Scanning electron micrographs of the maxillipeds of *Cirolana bovina.* **A:** Dorsal view of the maxillipeds showing the maxillipedal stem (arrows) and maxillipedal palp-p. 75X. **B:** Ventral view of the maxilliped shows the internal plumose setae on its tip (arrow). 100X. **C:** High magnification of the maxillipedal palp shows the entangled food particles (arrowhead) and lateral spiky serrate setae (arrow). Double arrow indicates possible openings of integumental glands. 350X. **D**: The tip of a maxilliped showing plumose setae (arrow), coupling hooks (arrowhead). 350X.

The Maxillipeds (Plate, 7, 8): Both the maxillipeds are the most external and cover the underlying mouthparts except the lateral stems of the mandibles. Each maxilliped has a roughly inverted S- shape and is a mirror image of the other. The outer maxillipedal surface appears hard, scaley and segmented. The basal segment is tri-hedral and excavated internally to receive the second, which is the largest and pentagonal but elongated in the direction of the maxillipedal length. The third maxillipedal segment is quadrate-trapazial, small and carries distally a huge palp, curved towards the midline of the body. The fourth distal article is the smallest, ventral cylindrical and terminates with a few plumose setae but its inner edge has three coupling hooks. The maxillipedal palp has four articulated and fringed laterally with spiky setae. The latter show rows of spines with swollen tips that appear bent or erected.

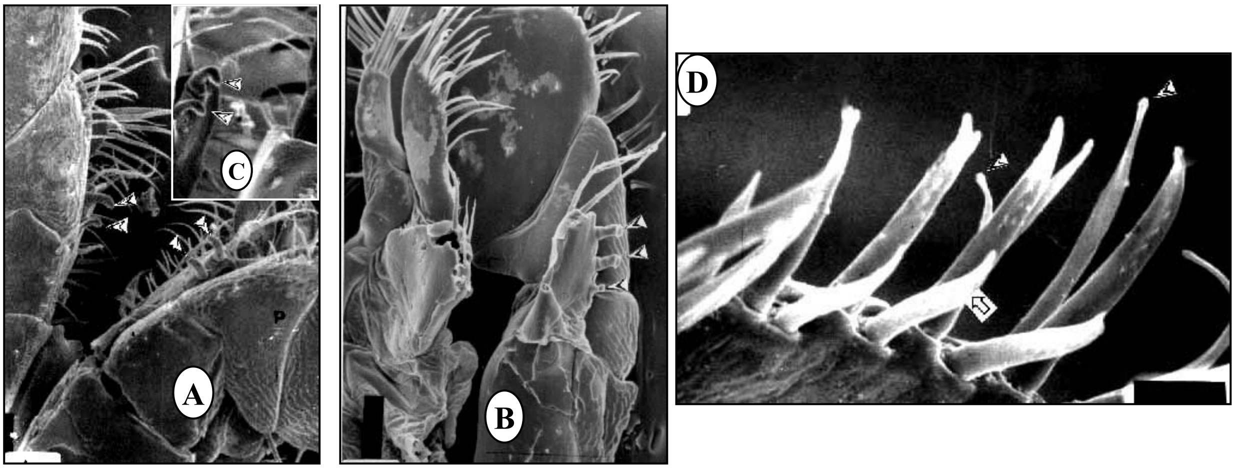


PLATE 8: Scanning electron micrographs of the maxilliped of *Cirolana bovina.* **A:** Dorsal view of the maxillipedal median margins showing the opposite position of coupling hooks (arrowheads). Note the huge maxillipedal palp- p. 150X. **B:** Ventral view of the maxillipedal tip showing the coupling hooks- (arrowheads). 150X. **C:** Lateral view of the coupling hooks (arrowheads). Note also the scaly surface of the maxillipeds. 75X. **D:** High magnification of the maxillipedal palp showing the spiky setae with swollen tips (arrowheads). Note the rows of spines (arrow). 1000X.

DISCUSSION

Isopod crustaceans exploit different habitats and can consume different foods of variable size and nature. They are also largely free living with a few groups being parasitic, such as the ectoparasitic Bobyridae, Gnathiidae and Cymothoidae while others are symbiotic (Rotramel 1975 and Marsden 1982). Some species of isopods live as symbionts on Limpets (Branch 1975) and on Chitons (Glynn 1968). Consequently, isopods have different life styles. This is reflected in the structure of their mouthparts and the setal types borne upon them. Generally, crustaceans as a lower arthropod group than insects have the mouth lobes (upper and lower lips) more or less developed or even modified when compared to those of insects. The latter are more potentially developed and highly modified to perform definite functions and have received much attention in research due to their economic and medical importance.

In the study organism *Cirolana bovina,* the labrum is attached to the frontal lamina situated at the base of the antennae. The latter's shape has been used in classification of Cirolanid genera (Monod 1976; Bruce 1981). It is structurally similar to the frontal lamina of *Cirolana sulcaticauda* (Bruce 1981). The labrum of *C. bovina* is movable and overhangs the mouth, thus forming a roof guiding the ingested food into the mouth. Meanwhile, the hairy setae fringing its free edge are likely to be gustatory for tasting food similar to those found in the marine isopod *Sphaeroma serratum* where the upper lip is different in form and shows more surface details including tricorn- like cuticular microstructures among integumental gland openings (Abd El-Bar 1995). Unlike the labrum, the labium of *C. bovina* is greatly reduced, but follows basically the general structural plan of isopods as a deeply notched outgrowth of the lower border of the mouth. This has been reported for the terrestrial isopod *Porcellio scaber,* and the marine isopod *Sphaeroma serratum* respectively (Abd El-Aal 1988, Abd El- Bar 1995). Field observations revealed that *C. bovina* lives naturally in a community dominated by gammaridean crustaceans. In addition pieces of crustacean cuticle were found in its gut contents indicating its predatory habit that involves detection, manipulation and processing of a prey item before swallowing. Detection of the prey may be visual and the mandibles are well equipped for handling the prey or large food items. The mandibular medial cuticular surface is elaborated into different forms of hard denticles as a pronounced cutting tooth, molars, and canines for shredding and masticating food; hooked teeth of lacinia mobiles, for perhaps tightly gripping the prey, serrated movable plates for sawing hard bits of prey and then crushing them between opposite grinding molar surfaces, like stones if needed. The shredded and ground food is pushed into the mouth by perhaps, the stout setae of lacinia mobiles aided by the long maxillulary robust setae as well. The mandibular palp can be twisted and moved freely to clean the head appendages, including the antennae (plate,1b) and the mouthparts with its numerous strong comb setae. This is similar in its type to the case of *Sphaeroma serratum* (Abd El-Bar 1995), where the comb setae were with close-set setules or not. Also, the strong asymmetrical mandibles of *C. bovina* are comparable to those of *Metacirolana,* especially, the molar surfaces (Muller & Salvat 1993) and the incisors are similar to those of *Catetolana* (Bruce 1981). In *C. bovina* the incisors of each mandible have three sclerotized cusps instead of four as is the case in *Metacirolana moortgati* (Bruce 1981). It is worthy to notice that Hale (1925) and Jones (1976) used the form of mandibles as a systematic basis for separating or comparing the different genera such as *Neocirolana* and *Cirolana* Shuster (1991) noted that the mandibles and maxillipeds of postmolt and gravid females of some isopods (e.g. *Paracerceis)* may lose their setae as they stop feeding during this period.

Considering the first maxillae, it is found that its inner endite in *Cirolana bovina* has three robust pappose setae as opposed to three plumose ones in *Metacirolana moortgati* and four plumose setae in *Sphaeroma serratum*. In addition, the outer endite of the maxillae is heavily chitinized and denticulate in *Metacirolana* (Muller & Salvat 1993) and in *Sphaeroma serratum* (Abd El-Bar 1995), but it is smooth in *Cirolana bovina* and may be used for pushing food into the mouth. The maxillae, being foliaceous mouthparts, carrying plumose and spiky serrate setae seem to take part in filtering the suspended food particles from the water current that is produced by the maxillipeds. The filtered particles and then get scraped off with their powerful long serrate setae. This is reminiscent of the branchiopod phyllopodia used in filter-feeding process (Abd El-Aal1988). Such feathery setae in *C. bovina* may also mesh for space-sealing, thus forming a floor and preventing food from falling out of the mouth. The maxillipedal palps are enormous directed forwards and inwards and carry short spiky setae with swollen tips. These may be bi-functional, as chemo- or mechano-receptors for detecting prey or for encrusting food on the substratum and scraping it for ingestion after being mixed with mucous secretion from the integumental glands' openings at the base of the maxillipedal palps. The maxillipedal palp of *Sphaeroma serratum* carries typical filter setae with packed soft setules, while its maxillipedal plumose setae are more numerous with dense setules (Abd El-Bar 1995). In contrast, the maxillipedal plumose setae of *C. bovina* are few and ventral and may be used as a secondary filtering net applied to the primary maxillary one for controlling the mesh size. *C. bovina* has three maxillipedal hooks ,two are distal and longer than the third . These have been examined in some marine isopods and found to be restricted to females. For instance, Holdich (1968) found one coupling maxillipedal hook in the sphaeromatid *Dynamene bidentata.* The cuticular surface of Crustacea, like that of other arthropods, shows a wide variety of microstructures as displayed by Scanning Electron Microscopy. The most complex structures are the setae, where as some cuticular structures are only ornamental and others are sensory (Holdich 1984 and Abd El-Aal 1988).

The present observations reveal a great diversity of surface microstructures, as different setal types and scattered microscales that may be species -specific fingerprint. In this respect, cuticular microstructures are heavily distributed over the body surface of terrestrial isopods in contrast to their aquatic allies. The functional interpretations are analogous to those proposed for other arthropods (Schmalfus 1978; Meyer-Rochow 1980). Previous studies on topographical features of marine isopods (Powell & Halcrow 1982) revealed poorly furnished surfaces with microscales, as it is the case in the present study. This may be advantageous for reducing friction during swimming and discouraging the hiding and settlement of microorganisms (Meyer – Rochow 1980). In the light of the hypothesis that the structures of the mouthparts are related to diet, it seems obvious that *C. bovina* is primarily a macro-feeder and mainly carnivorous. It is secondarily a micro-feeder, feeding on fine food particles filtered out of the flowing currents possibly as an additional food source while at rest.

The mouthparts of *C. bovina* provides a background for further studies of their ultrastructural features and feeding mechanisms. Moreover, the highly differentiated structure of the mandibles may be used in the construction phylogenies within the isopods, as the morphology of the mouthparts has been the basis of amphipod systematics since the time of Boech (1871).

Acknowledgment: Thanks are due to Zoology Department and the Electron Microscope unit, Faculty of Science, Zagazig University for providing research facilities. Also, we like to thank members of the Institute of Oceanography and Fisheries, Alexandria, Egypt for their guidance and help in specimen collection.

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الملخص العربى

دراسة بالمجهر اللالكتروني الماسح علي أجزاء متماثلة الأرجل البحرية "سيرولانا بوفاينا" (متماثلة الأرجل:فلابليفيرا).

الأحمدى شفيق الذهبي، منى عبد الحفيظ عبد العال وسلوى ذكى عبد البر

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أجريت هذه الدراسة بالمجهر الإلكتروني الماسح علي أجزاء الفم في متماثلة الأرجل *سيرولانا بوفاينا* التي جمعت عيناتها من شواطئ ألا سكندرية علي البحر المتوسط لدراسة شكلها الخارجي, وكذلك تفاصيل التراكيب الجلدية المرتبطة بها وبخاصة أنواع الأهداب المتواجدة عليها وإيضاح العلاقة بين شكل تلك التراكيب ووظائفها الغذائية. وقد وجد أن أجزاء الفم تتكون من الشفة العليا والشفة السفلي وزوج من كل من اللحي والفكيكى والفكي والأرجل الفكية. كما وجد أيضا آن الشفة السفلي أثرية آما اللحيان منها غير متماثلين ويتميزان بكبر حجمهما وتطورهما تركيبيا.

كما اتضح أن تركيب أجزاء الفم ومحتويات القناة الهضمية يعكسان طرق الاغتذاء في هذا الحيوان. حيث انه يتغذى أساسا بافتراس مفصليات متواجدة ببيئته الطبيعية, كما يلجا أحيانا لطريقة الترشيح باستخدام أنواع معينة من الأهداب الجلدية ربما في أوقات الراحة. وقد نوقش التركيب الوظيفي لأجزاء الفم وأنواع التراكيب الجلدية المتصلة بها.