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Light and Scanning electron microscopic investigations on gravid female *Anilocra* sp. (Isopoda: Cymothoidae) infesting *Tilapia zillii* in Qarun Lake, Egypt.

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ABSTRACT
Severe infestations of isopods on some economic fishes inhabiting Qarun Lake were observed during April 2020. The present light and Scanning electron microscopic studies have been carried out on gravid female *Anilocra* sp. parasitizing *Tilapia zillii* collected from this Lake. A stereomicroscopic study has been carried out to illustrate the morphological characteristics of this cymothoid isopod. Detailed Scanning electron microscopic investigations were achieved on surface ultrastructural modifications of ovigerous females such as cephalic sensory organs, piercing-sucking mouthparts, pereopods, pleopods, and different developmental stages of ova within the marsupium. Further molecular, taxonomic, and ultrastructural studies must be done to provide profitable knowledge helping in the identification of these deleterious isopods devouring economic fishes of Qarun Lake.

INTRODUCTION

Qarun Lake is an interior Lake located at El-Fayoum depression and is recorded as protected area back 1989 **(Hussein *et al.*, 2008)**. It has an average surface area of about 53 thousand acres, average water depth about 4 meters and draws about 470 million cubic meters of agricultural drainage and domestic waste waters through 12 incoming drains annually **(El-Sherif and Abd El-Ghafour, 2016)**. This natural Lake is subjected to intense isopod infestation in the beginning of 2015 and this problem caused critical fall of fish stock and consequently resulted in squeaky economic deprivations **(Mahmoud *et al.*, 2016)**. *Tilapia zillii* fishes live in fresh and brackish waters and play important roles in controlling aquatic weeds as well as human consumption especially in poor African countries **(Bradbeer *et al.*, 2018)**.

Few families of isopods are ectoparasitic as Cymothoidae, Bobyridae and Gnathiidae **(Marsden, 1982)**. Family Cymothoidae involved 43 genera and 358 species infecting marine, freshwater or brackish teleost fishes **(Brusca, 1981; Horton, 2000)**.

Members of family Cymothoidae have been recorded from the Mediterranean Sea, Red Sea, Adriatic Sea, Black Sea, Atlantic Ocean, India, Brazil and Australia **(Hale, 1926; Trilles, 1991; Horton, 2000; Kayis and Ceylan, 2011; Rameshkumar *et al.*, 2011; Gueretz, *et al.*, 2018; Alves *et al.*, 2019).** The ectoparasitic cymothoids are sucking the blood from the branchial chambers, fins, mouth cavities or self-wounds they created in the flesh of their fish hosts **(Hoffman, 1998)**. Cymothoids secrete an anticoagulant material from their esophageal glands during feeding **(Romestand and Trilles, 1976)**. They are reported to cause slow growth rates, tissue damages, anemia and finally death of fishes **(Espinosa and Hendrickx, 2001; Bunkley *et al.*, 2006)**. Inflammations and bacterial or fungal contaminations were often spotted around wounds inspired by this ectoparasites and may facilitate the admittance of other pathogenic microorganisms **(Brusca and Gilligan, 1983; Horton and Okamura, 2003)**. Fifty-one species in the genus *Anilocra* were listed **(Bruce, 1987)**. *Anilocra physodes* was recorded at the Red Sea fishes **(Al-Zubaidy, 2007; Al-Zubaidy and Mhaisen, 2013)**. 15 species belonging to the genera; *Anilocra, Nerocila, Ceratothoa, Emetha, Mothocya and Livoneca* are reported to infect bony fishes of Antalya Gulf, Aegean Sea , Sea of Marmara and the Black Sea **(Öktener and Trilles, 2004; Innal *et al.*, 2007; Öktener *et al.*, 2018)**. Molecular studies proved the presence of two cymothoids; *Livoneca redmanii* and *Anilocra* sp. nesting Qaroun Lake **(Geba, *et al.*, 2019)**. Cymothoids are pentandorous hermaphrodites starting their lives as males and later changing sex **(Serita *et al.*, 2017)**. The male will later transform to female owing to phermonal secretions of nearby female **(Bunkley and Ernest, 1998)**. Ultrastructural investigations on ectoprasitic isopods were rarely studied and many species still undescribed **(Al-Zahaby *et al*., 2001; El-Shahawy and Desouky, 2010)**. The present study aims to provide a brief ultrastructural redescription of gravid female *Anilocra* sp. infesting the economic fish *T. zillii* in Qarun Lake. This study may also supply useful informations which may be helpful in the taxonomy and controlling of these harmful ectoparasites.

MATERIALS AND METHODS

Collection of Tilapia zilli

The host fishes *T. zilli* were captured during April 2020 with the help of local fishermen from Helnan Auberge Hotel beach, Lake Qarun, El-Fayoum Governorate, Egypt. Fishes were transported alive in ice boxes to the laboratory of Invertebrates, Faculty of science, Zagazig University. Isopods were removed from the branchial cavities of infested fishes and placed in 70% ethanol. The mouth parts were carefully dissected under binocular stereomicroscopy using dissecting needles and forceps.

Scanning electron microscopic studies

Specimens of *Anilocra* sp. were fixed in 3% glutaraldehyde in 0.1 m Sodium cocodylate buffer at pH 7.4 for 3 hours. Specimens were washed two times in sodium phosphate buffer, post fixed in 1% osmium tetroxide in the same buffer for 2 hours at 4 ºC and then washed four times in sodium phosphate buffer. They were dehydrated in an ascending series of ethanol (70%–100%). Specimens were dried and coated in a Polaron SC 500 sputter coater with a thin layer of gold. Specimens were examined with a Joel JSM 5800 Scanning Electron Microscope at the Electron Microscopy Unit, Faculty of Agriculture, El-Mansoura University, Egypt.

RESULTS

Morphological characterizations of ovigorous female *Anilocra* sp.:
The body of *Anilocra* sp. is arched dorsally, measuring 1.4-1.7 cm in length and 0.8-1 cm in width. Its colour is yellowish brown and is characterized by scattered dark brown chromatophores concentrated dorsally on the cephalic shield, pereonites, pleotelson and basipodites of uropods while, they are found on the basis of pereopods ventrally. Its body plan consists of unsegmented cephalon, pereon (7 pereonites) and pleon (5 pleonites). The dorsal surface of *Anilocra* sp. is covered by articulating chitinous sternites. A ventral marsupium is established by a number of oostegites under the pereon (Plate I, a-d).

1-The cephalon

Scanning electron microscopic studies on gravid female *Anilocra* sp. showed that the cephalon is triangular, unsegmented, covered dorsally with a smooth cephalic shield and not immersed in the first perionite. The anterior margin of the cephalon is rounded and folded ventrally between the bases of the antennae. The posterior margin of cephalon is trilobed. The cephalon is formed ventrally of a concaved and crimped frontal lamina, convex clypeus, two smooth lateral laminae and medial piercing sucking mouth parts. A characteristic fissure is found between the frontal lamina and clypeus (Plate II, a-d).

1.1. Cephalic sense organs

- **Eyes**

The cephalon of *Anilocra* sp. is provided with two widely separated and large unstalked eyes (Plate I, a, b). Each compound eye is formed of numerous dark hexagonal ocelli. The outer boarder of the eye was rounded while the inner edge with angled edges. Its posterior margin was rounded and reached the lower margin of the cephalon (Plate II, c).

- **Antennules**

A pair of antennules with brightly white colour is observed (Plate I, d) and their basal segments are embedded in tuberculous grooves of the clypeus. Each antennule is composed of 8 articles extending beyond the posterior margin of the eye. The 2nd and 3rd articles were the largest. The peduncle of antennule is formed of the first 3 articles while the flagellum is consisted of the rest 5 slender articles. A tuft of simple setae is located on the ventral surface of the flagellar articles from the 4th to the 8th articles (Plate II, d). The external surface of the basal segment of antennule was scaly and carried three small setae (Plate II: e).The upper surface of antennule is occupied with many sensory pits and each pit is comprised of multiple small sensory papillae (Plate II: f).

- **Antennae**

The antenna of *Anilocra* sp. was longer than antennule and its basal article is inserted in specific groove of the clypeus. Each antenna was white in colour and is composed of 10 articles extending till the posterior end of first pereonite. The peduncle is formed of the first the 4 articulating articles while the flagellum is formed of the rest 6 articles. The 3rd

article of the antenna is supported with one seta while the 4th article carries 8 elongated and stout setae. A tuft of simple setae is found ventrally starting from the 5th to the 10th articles (Plate III: e). The upper surfaces of antennal articles are occupied with many cuticular sensory pits (Plate II: e, f).

1.2. Mouth parts

The ovigorous female *Anilocra* sp. is fringed with styliform mouth parts adapted for piercing and sucking the blood of their fish hosts. These mouth parts are formed of a single labrum, in addition to paired mandibles, maxillules, maxillae and maxillipeds.

- ***Labrum***

  The labrum has convex upper surface, rounded posterior end and flattened labral free edge. It is found between the bases of the paired antennae in front of the mouth opening. It consists of two articulated portions attached posteriorly to the clypeus (Plate III: a).The free labral edge of the lower portion only is confirmed with numerous palmate microtriches. These microtriches were concentrated only on the lower labral portion (Plate III: b, d).

- ***Mandibles***

  Two mandibles are found laterally guarding the mouth opening. Each mandible is formed of a mandibular palp and an incisor process. The mandibular palp consists of 3 elongated articles and its basal article was the largest without setae. The second article is supported with 5 comb-like distolateral setae. The distal article of the mandibular palp is provided with 16 distolateral comb- like strong setae. (Plate III: c).The mandibular molar process is terminated with 4 sharp canines -like incisors (Plate III: d).

- ***Maxillules***

  Each maxillule was slim, elongated and is ended terminally with 4 hook-like robust setae. Three equal robust and terminally curved setae are arranged externally while the 4th seta is much smaller and inserted in an opposite direction (Plate III: e, f).

- ***Maxillae***

  The two maxillae are located next to the maxillules and situated externally beside the maxillipeds. They are columnar, flattened and curved distally. Each maxilla is composed of three curved lobes. The basal lobe is the broadest while the median lobe is the largest. The terminal lobe is provided distally with 4 small robust setae (Plate IV: a).

- ***Maxillipeds***

  The maxillipeds were the largest part covering other mouth parts. The basal article (stem) was the broadest while the propodus was the smallest (Plate II: d). The stem of maxilliped is branched into an outer maxillipedal palp and an inner maxillipedal incisor (Plate IV: a). The palp is formed of 3 articles without setae.

The incisor is ended with one large dactyl located in front of the mouth opening (Plate III: f).

2-The pereon

The pereon of gravid *Anilocra* is found to be the largest body region and is consisted of 7 articulating pereonites (Plate I: c).The anterior border of the first pereonite is excavated to anchor the trilobed posterior margin of the cephalon (Plate II: a, c).The 5^{th} perionite was the broadest segment. Each pereonite has one pair of terminally clawed pereopods for gripping their fish hosts. The pereopod consists of a large basis, ischium, merus, carpus, propodus and a large curved dactyl. The coxae of pereopods are fused with the dorsal tergites forming epimera.

Lateral epimera are protruded posteriorly. The first three pairs of pereopods have nearly the same sizes, directed anteriorly and smaller than the rest of pereopods. The 6^{th} and 7^{th} pereopods were the tallest and are directed posteriorly (Plate IV: b, c). Pereopods of gravid females is provided with specific appendages (oostegites) which are folded forming a marsupium for incubating different developmental stages of fertilized ova. Incubated ova were small, ovate and had yellowish colour (Plate IV: d, e).

3- The pleon

The pleon of gravid female was smaller and narrower than pereon and is formed of 5 articulating pleonites. The first pleonites is deeply immersed in the 7th pereonite. Epimera were directed posteriorly and not strongly expanded. The first 4 pleonites are provided with 4 pairs of lamellar pleopods (Plate IV: b, d).

Pleopods are bi-ramous, with rounded terminal ends. Pleopods carried no setae and expedites were slightly larger than endopodites. Lateral margin of pleopods are weakly convex and broadly rounded distally. The 5^{th} pleonite is provided with a pair of uropods. Expedites of uropods were taller than endopodites. The uropods had lingual shape and extend posterior to the margin of pleotelson. The pleotelson is large with rounded posterior boarder and comprised no setae (Plate IV, f).

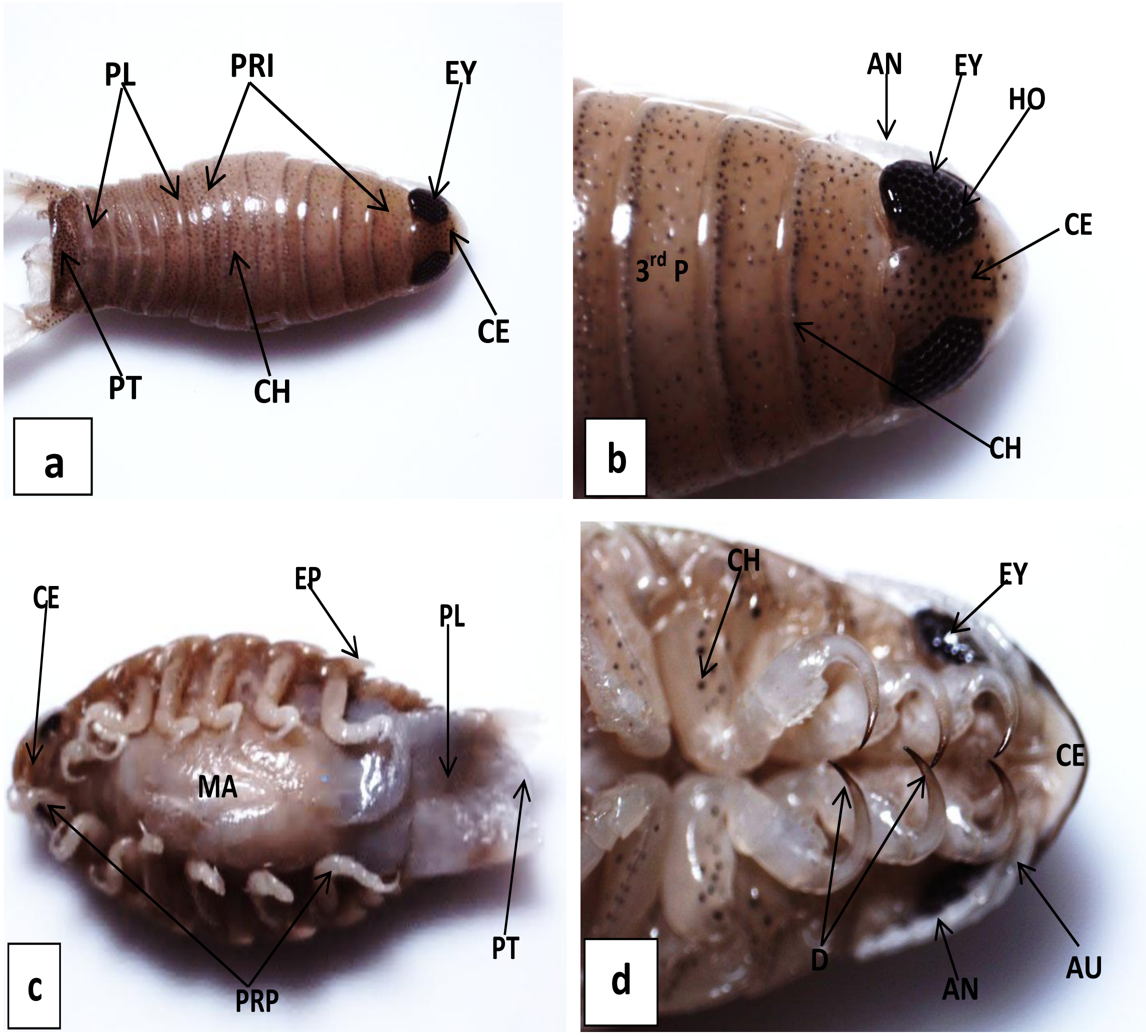

Plate I (a-d): Stereomicroscopic photographs of gravid female *Anilocra* sp. **(a):** Dorsal view showing body regions and characteristic chromatophores (Orig. mag. X 10). **(b):** Higher magnification of the dorsal surface showing the compound eyes and hexagonal dark ocelli (Orig. mag. X 20). **(c):** Ventral view of ovigorous female showing the marsupium (Orig. mag. X 10). **(d):** Higher magnification of the ventral surface showing cephalic sense organs and three pairs of pereopods (Orig. mag. X 50). *AN: Antenna; AU: Antennule; CE: Cephalon; CH: Chromatophores; D: Dactyls of pereopods; EP: Epimera; EY: Eye; HO: Hexagonal ocelli; MA: Marsupium; 3rd P: Third pereonite; PL: Pleon; PRI: Pereon; PRP: Pereopods; PT: Pleotelson.*

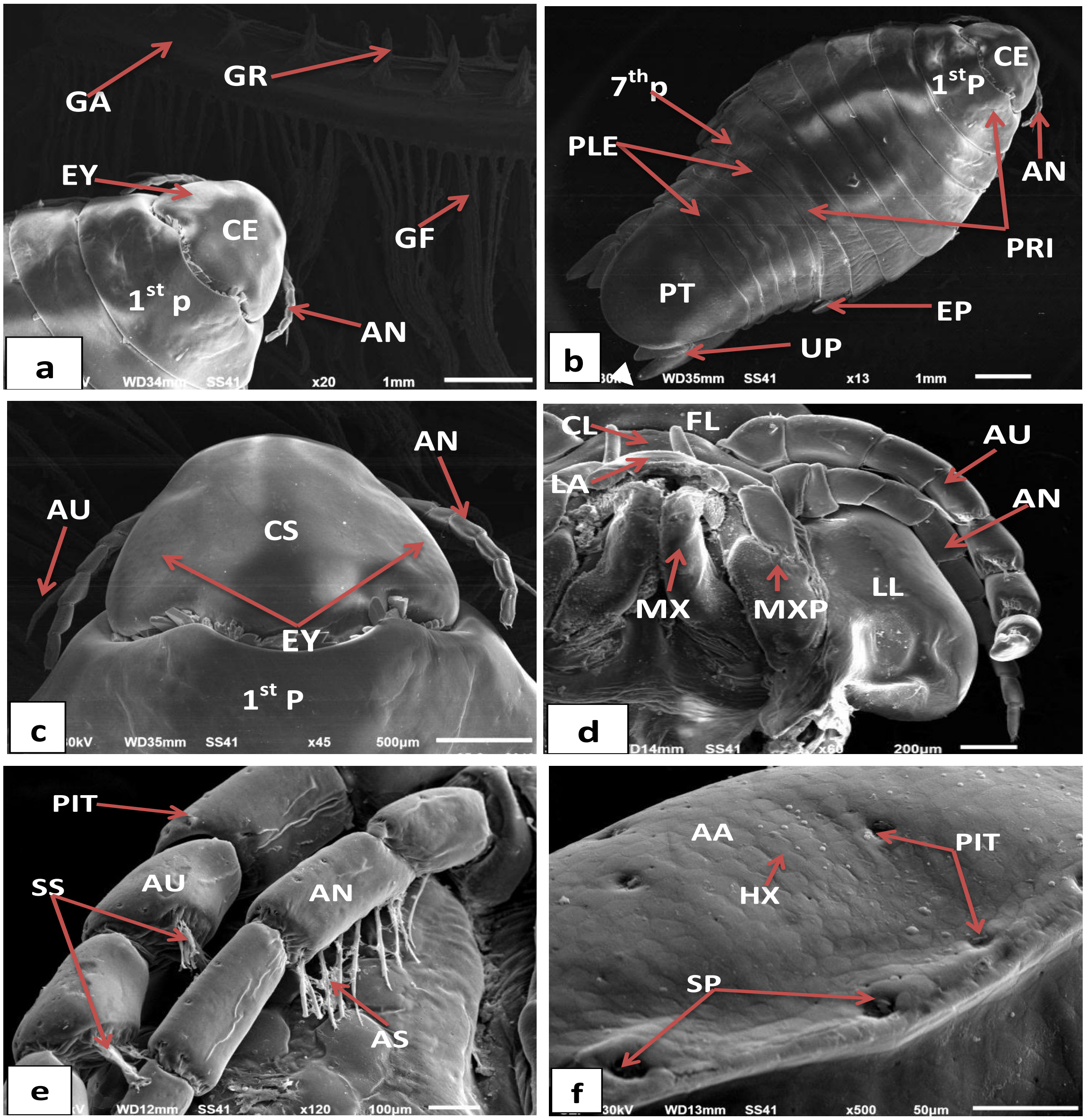

Plate II (a-f): Scanning electron microscopic photographs of gravid female *Anilocra* sp. **(a):** Dorsal view of *female Anilocra* sp. attached to gill filaments of *T. zillii* (Orig. mag. X 20). **(b):** Dorsal view showing different body regions (Orig. mag. X 13). **(c):** Higher magnification of cephalic region showing sense organs and ocelli of compound eyes (Orig. mag. X 45) **(d):** Ventral view of dissected cephalon showing antenna, antennules and superficial mouth parts (Orig. mag. X 60). **(e):** Ventral view of antennal and antennular articles showing different types of sensory setae (Orig. mag. X 120). **(f):** Higher magnification of the dorsal surface of the 2nd article of antennule showing sensory pits involving sensory papillae (Orig. mag. X 500). *AA: Antennual article; AN: Antenna; AS: Antennual setae; AU: Antennule; CE: Cephalon; CL: Clypeus; CS: Cephalic shield; D: Dactyl; EP: Epimera; EY: Eye; FL: Frontal lamina: GA: Gill Arch; GF: Gill filaments; GR: Gill raker ; HX: Hexagonal scales; LA: Labrum; LL: Lateral lamina: MX: Maxilla; MXP: Maxilliped; 1st P: First pereonite; 7th P: Seventh pereonite; PIT: Sensory pits; PLE: Pleon; PRI: Pereon; PT: Pleotelson; SP: Sensory papillae; SS: Sensory setae; UP: Uropod.*

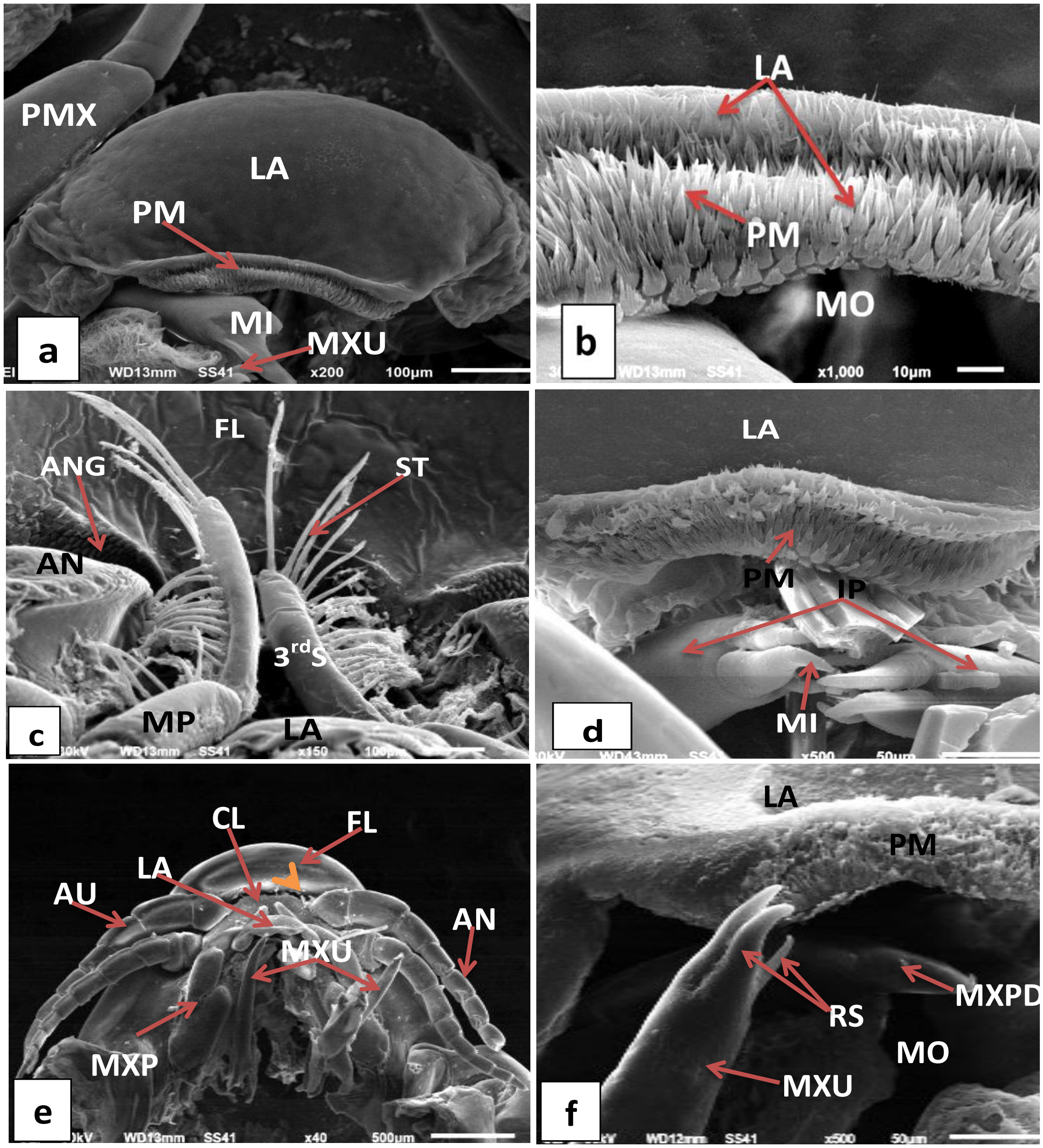

Plate III (a-f): Scanning electron microscopic photographs of mouth parts of gravid female *Anilocra* sp. **(a):** Dorsal view of the labrum showing palmate microtriches on the lower free labral margin (Orig. mag. X 200). **(b):** Higher magnification of palmate microtriches (Orig. mag. X 1000). **(c):** Dissected cephalon showing the mandibular palp setae (Orig. mag. X 160). **(d):** Dissected cephalon showing the terminal incisor process carrying 4 canines -like incisors (Orig. mag. X 500). **(e):** Dissected cephalon showing maxillules and maxillipeds (Orig. mag. X 40). **(f):** Higher magnification of the distal end of maxillule showing 4 terminal robust setae (Orig. mag. X 500). *AN: Antenna; ANG: Antennal groove; AT: Antenna; AU: Antennule; CL: Clypeus; FL: Frontal lamina; IP: Incisor process of mandibles; LA: Labrum; MI: Mandibular incisor; MO: Mouth; MP: Mandibular palp; MXP: Maxilliped; MXPD: Maxillipedal dactyl; MXU: Maxillule; PM: Palmate microtriches ;PMX: Palp of maxilliped;* RS: *4 terminal robust setae of maxillule ;3rd S: The third segment of mandibular palp; ST: Mandibular setae. Arrowhead: A fissure separating the frontal lamina and clypeus.*

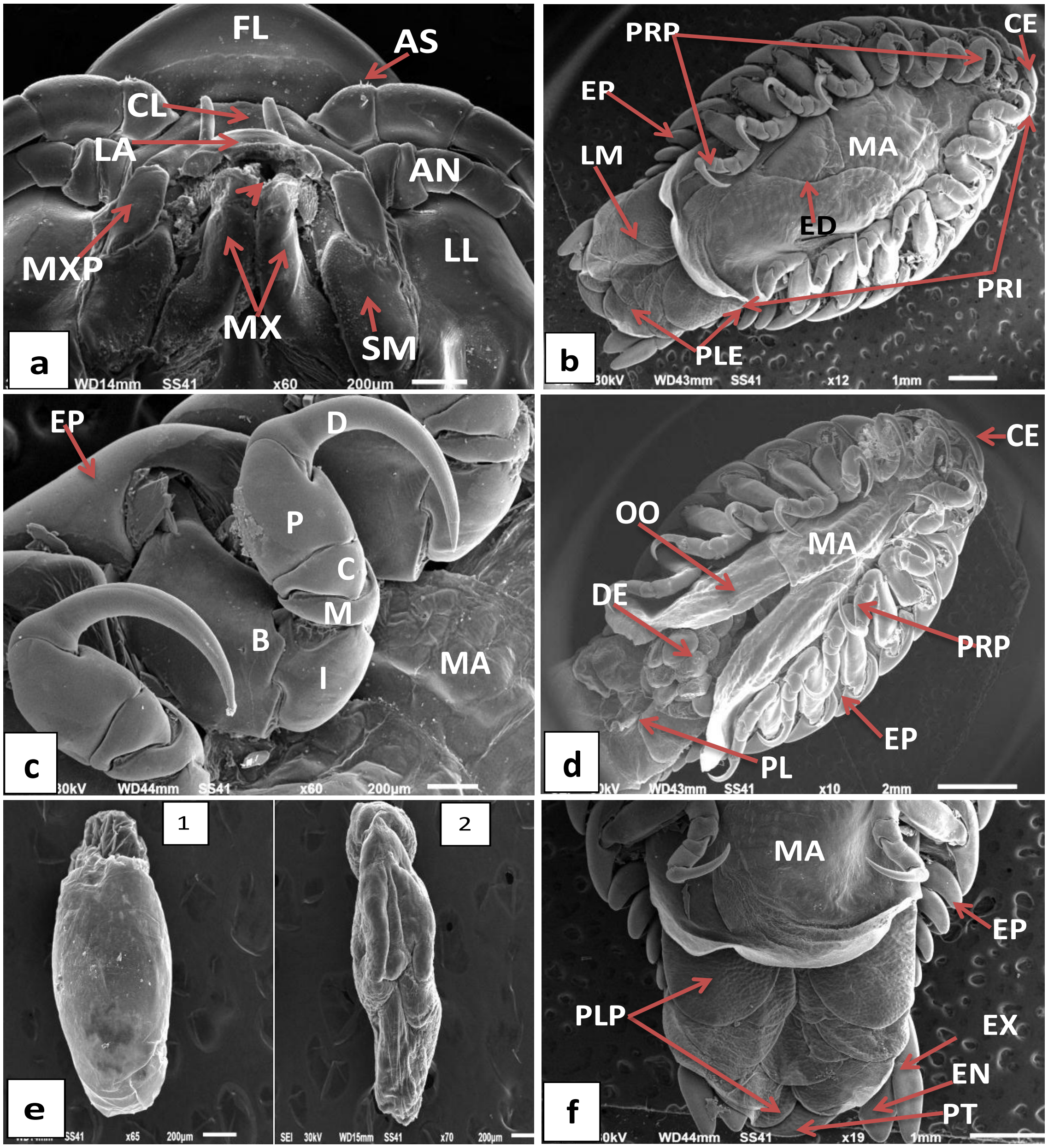

Plate IV (a-f): Scanning electron microscopic photographs of ventral surface of gravid female *Anilocra* sp. **(a):** Dissected cephalon showing maxilla and maxillipeds (Orig. mag. X 60). **(b):** Ventral view showing different body regions and the brood pouch (Orig. mag. X 12)**. (c):** High magnification of pereopods and epimera (Orig. mag. X 60). **(d):** Marsupium with removed oostegites showing incubated ova (Orig. mag. X 10). **(e** 1,2**):** Different developmental stages of ova (Orig. mag. X 70). **(f):** Posterior region showing pleopods, uropods and pleotelson (Orig. mag. X 19). *AN: Antenna ; AS: Antennual setae; B: Basis; C: Carpus; CE: Cephalon ; D: Dactyl; DE: Developing eggs; ED: Edge of oostigite ; EN: Endopodite of uropod; EP: Epimera; EX: Exopodite of uropod ; CL: Clypeus; FL: Frontal lamina; I: Ischium ; LA: Labrum; M: Merus; MA: Marsupium; MO: Mouth; MX: Maxilla; MXP: Maxilliped; OO: Oostigites ; P: Propodus; PL: Pleon; PLP: Pleopods: PRI: Pereon; PRP: Pereopods; PT: Pleotelson; SM: Stem of maxilliped. Arrowhead: Maxillary robust setae.*

DISCUSSION

Previous studies have been carried out on the identification of isopods and their hurtful effects on economic fishes dwelling Qaroun Lake; *Anilocra leptosome* and *Cymothoa indica* **(Rania and Rehab, 2015)** *Nerocila orbignyi* **(Abdel-Latif, 2016; Mahmoud *et al.*, 2016; Younes *et al.*, 2016; Shaheen *et al.*, 2017)** *Anilocra physodes* **(Mahmoud *et al.*, 2017 ; Shaheen *et al.*, 2017)** *Renocila thresherorum* **(Mahmoud *et al.*, 2016, 2017; Shaheen *et al.*, 2017; Ali and Aboyadak, 2018)** and *Levonica redmani* **(Mahmoud *et al.*, 2017)**. Recently, molecular characterizations documented that *L. redmanii* was slightly bent to the left and *Anilocra* sp. with straight body are found in Qarun Lake **(Geba, 2019)**. This study concerned with providing detailed ultrastructural redescription of the gravid female *Anilocra* sp. because, gravid females of isopods have morphological characteristics that may not be existed in juveniles or males **(Serita *et al*., 2017)**.

Cymothoid isopods are protandrous hermaphorodites, making them troublesome to identify during various developmental stages **(Cook and Munguia, 2015)**. The present Stereomicroscopic study revealed that *Anilocra* sp. has yellowish brown colour with scattered dark brown chromatophores on the cephalic shield, pereonites, pleotelson and basipodites of uropods dorsally and on the basis of pereopods ventrally. Contrary, **Mahmoud *et al*., (2016)** reported dark chromatophores concentrate only on the posterior aspects of segments only and they identified this species as *Renocila thresherorum*. The eye of *Anilocra* sp. is consisted of numerous dark hexagonal ocelli. Antennules have 8 articles carrying tufts of simple setae on articles 4 - 8, articles 2,3 are the largest and the external surface of the basal segment of antennule is scaly providing with 3 small setae and many sensory pits. The number of articles of antennules is an agreement with the finding of **Öktener *et al*, (2018)** in *Anilocra physodes*. The antenna is composed of 10 articles with numerous external sensory pits and the 3rd article has one seta while the 4th article carries 8 elongated setae. A tuft of simple setae is found on the ventral surface from the 5th to the 10th article. Contrarily, **Mahmoud *et al*., (2016)** reported the antenna is formed of 9 articles. **Rania and Rehab, (2015)** found that antennae of *Anilocra leptosome* and **Shaheen *et al.*, (2017)** in *Anilocra physodes* have 9 articles extending to middle of the first pereonite. *Trichoniscus alexandrae* males are blind isopods holding cephalic cuticular pits containing a tuft of setae that secrete materials helping in sex appreciation **(Lombardo *et al*., 2006).**Scanning and TEM studies on antennal sense organs of the isopod *Ligia oceanica* illustrated their chemical functions in testing the environment **(Alexander, 2009)**. The antennal sense organs of the isopod *Natatolana borealis* are chemosensitive, the tufted setae may take part in gathering particles while the plumose setae are mechanoreceptive **(Kaim-alka, 2010)**. The mouthparts of *Anilocra* sp. are often styliform and adapted for piercing and sucking the blood of their fish hosts. The labrum consists of two articulated portions and the lower labral portion is fringed with numerous palmate microtriches. Microtriches being unique to cestodes and appear to be ubiquitous among cestodes **(Chervy, 2009)**. These microtriches in cestodes might maintain an intimate contact with the mucosal surface of the host and serve an absorptive function **(Read, 1955)**. The mandibular palp of *Anilocra* consists of 3 articles and the third article is provided with 16 distolateral setae while mandibular molar process carries 4 canines -like incisors. The maxillule in this study has four terminal robust setae. These findings are in accordance with **Trilles, (1975)**. Contrary, only two spines only on maxillules of *Anilocra* were reported by **Kussakin, (1979)**. The mandibular palp of *A. leptosome* contains 13 brush –tipped setae located on the distal article **(Rania and Rehab, 2015)**. Maxilla in this study is provided with 4 small robust setae. Contrarily, the medial and lateral lobes of maxilla carry two spines (**Öktener *et al*, 2018)**, while median lobe has one spine and lateral lobe with 2 spines **(Montalenti, 1948)**. The maxillipedal palp in this study consists of 3 articles devoid of setae and ends with one large dactyl. **Trilles, (1975)** reported three spines on article 3 of the maxilliped of *Anilocra* ovigorous female while five spines were reported by **Kussakin, (1979)**. *Anilocra* is dressed by a number of articulating chitinous sternites for protection, pereon consists of seven pereonites and the 1st pereonite is excavated to receive the trilobed posterior margin of the cephalon. Contrarily, pereon of *Rocinela signata* has eight pereonites and coxal plates **(Alves *et al.*, 2019)**. Pereonite of *Anilocra* has long tapering dactyl for clinging onto their hosts and its lateral epimera are protruded posteriorly. Pereopods of gravid females have oostegites which are folded to form a marsupium. In mature females of isopods, the thoracic legs form oostegites which are folded to form the brood chamber that is filled with water in aquatic and terrestrial isopods **(Edward, 2004)**. Eggs of aquatic isopods hatch as mancae similar to adult stages **(Kirkim *et al.*, 2008)**. The pleon of *Anilocra* sp. consists of five articulating pleonites, the 1st pleonites is deeply immersed in the 7th pereonite. Pleopods are bi-ramous, lamellar with weakly convex lateral margins and broadly rounded distal ends. These findings are in agreement with **Rania and Rehab, (2015)** in *A. leptosome* and **Mahmoud *et al.*, (2017)** in *A. physodes*. The endopodites of pleopods are modified into lamellar appendages with thin, permeable cuticles which act as gills (**Wilson, 1989**).

CONCLUSION

The present ultrastructural investigations may provide important guidance for identifying the species of *Anilocra* infecting *Tilapia zillii* at Qarun Lake. The previously reported fish production losses from Qarun Lake may be due to high infestation rates of parasitic isopods that cause severe physiological effects on their fish hosts. Further molecular, taxonomic and ultrastructural studies must be carried out to detect the actual types of these deleterious isopods. Biological control methods may be valuable in decreasing high infestation rate of isopods at Qarun Lake.

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