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Ultrastructure of *Lamproglena pulchella* (Copepoda: Lernaeidae), a gill parasite on the freshwater fish, *Leuciscus vorax*, from Tigris River, Iraq

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ABSTRACT

A new record of Lamproglenine copepods, *Lamproglena pulchella* were collected from the gills of cyprinid freshwater fish *Leuciscus vorax* from the Tigris River, Iraq in order to investigate the morphological and functional characteristics of their appendages using scanning electron microscope. A maxilliped appears robust, and bears at its apex two subequal curving, claw-like spines. First legs were with a small protuberance on medial margin of the sympod near the base; endopod apparently unsegmented, exopod indistinctly two-segmented, with several setae on lateral margin and single stout seta on apex. Second legs have two segmented rami; endopod unarmed and exopod with single seta on lateral margin of basal segment and three setae at apex. Third and fourth pairs of appendages were similar, with alike rami and with only two setae at the apex of exopod. These appendages are thought to serve as a holdfast for attachment on the gill filament of the tigris fish. Cuticular differentiations found on the body surface of *L. pulchella* play a secondary role in the attachment of the copepod on its host. Current study microscopy shows that the maxillae of *L. pulchella* are characteristically prehensile and thus provides an efficient tool for the attachment to the host tissue.

INTRODUCTION

Copepods are the second largest group of parasites in marine fish and the third largest in freshwater hosts (Luque and Tavares, 2007). Copepods play major roles in pond ecosystems, serving as: food for small fish, micropredators of fish and other organisms, fish parasites, intermediate hosts of fish parasites, and hosts and vectors of human diseases (Piasecki *et al.*, 2004). *Lamproglena pulchella* was described for the first time on *Cyprinus jeses* in Europe by Nordmann (1832). This preliminary investigation was conducted on the serious mortalities in fish and the occurrence of *L. pulchella* in the gills of two fish species found in Balıklıgöl, Turkey. The genus *Lamproglena* is typically gill parasites of freshwater fish, and they have the capability to cause fish losses in aquaculture (Öktener *et al.*, 2008). Lernaeidae is the major family of cyclopoid parasitic copepods. Members of this family parasitize freshwater fish and some of them are of the most harmful parasites of aquaculture fishes (Ho and Kim, 1997). The Lernaeidae is comprised of ten genera and subgenera which includes the genus *Lamproglena* and genus *Lernaea* that represents the two largest genera containing 77% of the known species of this family (Ho, 1984).

Members of the subfamily Lamprogleninae are characterized by the absence of the holdfast on their cephalothorax and the possession of a pair of uniseriate egg sacs.

Most species are known from females, which are highly modified for a parasitic mode of life, whereas their males die soon after mating (Ho, 1998). According to Mhaisen (2012), among the 21 crustacean species reported from freshwater fishes of Iraq, five species belong to the family Lernaeidae: three of the subfamily Lamprogleninae: *Lamproglena chinensis* (Yü, 1937), *L. pulchella* (von Nordmann, 1832) and *Pseudolamproglena annulata* (Boxshall, 1976) and two of the subfamily Lernaeinae: *Lernaea cyprinacea* (Linnaeus, 1758), and *L. oryzophila* (Monod, 1932). Lernaeidae are parasites on freshwater teleosts and because of their morphological plasticity, they are characterised by pronounced sexual dimorphism, because their males do not undergo metamorphosis (Kabata, 1992).

The genus *Lamproglena* includes over 40 parasite species in freshwater fish families such as: Cyprinidae, Clariidae, Cichlidae and Channidae. In literature, this crustacean has been identified in Asia, Africa, Europe and South America (Stavrescu –Bedivan *et al.,* 2008). These parasites attach on gills and general body surface, causing wounds which become the sites of secondary infection by microbes (Paperna, 1975). The damage caused by these parasites is more pronounced in culture systems such as ponds and cages (Woo, 1995; Yambot and Lopez 1997). In the genus *Lamproglena*, as well as in *Lernaea*, only the adult females are gill parasites of fishes (Lester and Hayward, 2006). Female body of genus *Lamproglena* divided into cephalothorax, long sub-cylindrical thoracic neck and more or less distinct trunk like posterior section. Cephalothorax with holdfasts of various types. Neck with or without secondary holdfast. Posterior section either containing indistinguishable fused genital segment and abdomen or indistinctly divided in their two components. Antenna absent or present, other appendages cyclopoid. One to four pairs of biramous legs, fifth and sixth legs also present or absent (Kabata, 1992). Thus, the present work aims to investigate the ultrastructure morphological characters of the parasitic copepod, *L. pulchella* and its appendages, using scanning electron microscopy.

MATERIALS AND METHODS

About 9 females of the parasitic copepod *Lamproglena pulchella* were collected from the gill filaments of the cyprinid freshwater fish, *Leuciscus vorax* caught from the Tigris River, Iraq. For scanning electron microscopy observations, *Lamproglena* specimens were removed from the fish and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at (4°C). They were washed in the buffer before the post-fixation in 1% osmium tetroxide, in the same buffer, at 4°C for 1 h. Then, the specimens were dehydrated in a graded acetone series (30%, 50%, 75%, 90%, and 100%) and critical-point dried. They were then sputter-coated with gold palladium. SEM photographs were taken with a Jeol electron microscopy at an accelerating voltage of 30 kV in the Electronic Microscopy Unit, Faculty of Agriculture, Mansoura University.

RESULTS

Description of Adult female

The body of the adult female *Lamproglena pulchella* is elongated and consists of three distinct parts: cephalothorax, trunk and abdomen (Fig.1a). Cephalothorax (Figs.1&2) incorporating first leg-bearing segment, of about equal length and width, slightly wider in its posterior half, with marked indentations at about midlength of lateral margins, separated from trunk by deep, neck-like constrictions.

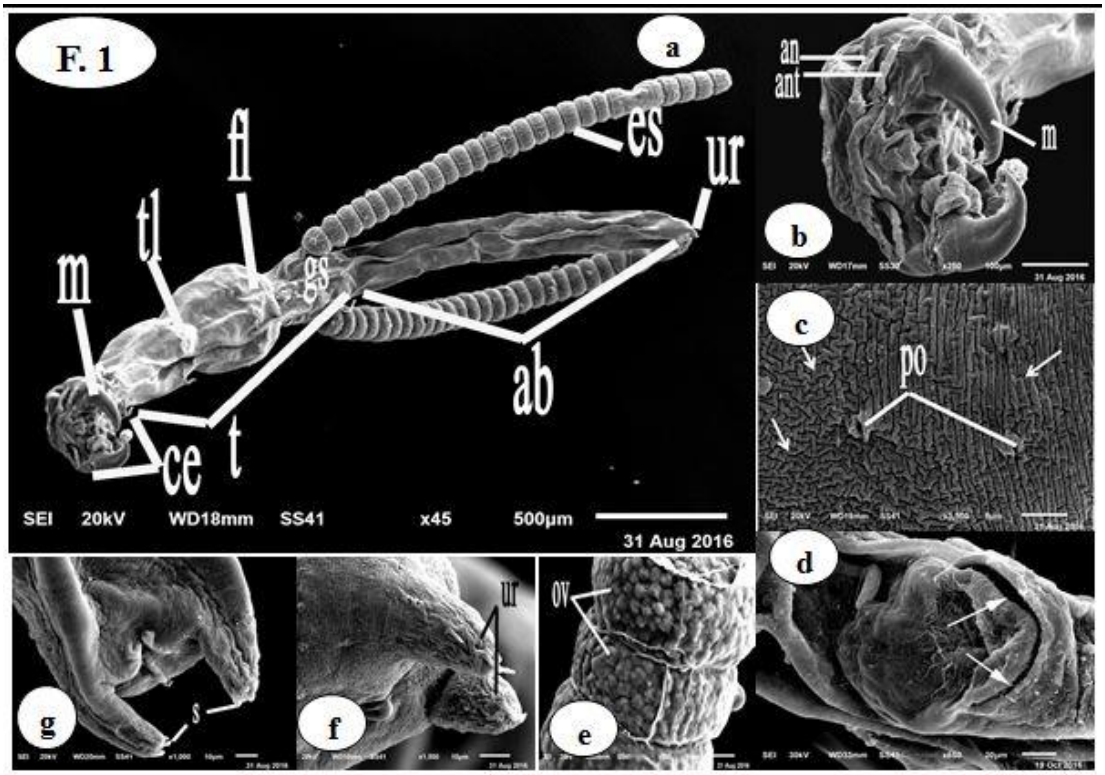

Fig. 1: Scanning electron micrographs of female *L. pulchella,* a: ventral view, showing cephalothorax (ce), trunk (t), abdomen (ab), uropod (ur), maxilla (m), third leg (tl), fourth leg (fl), genital segment (gs) and egg sac, (es). b: cephalothorax (enlarged) showing 1st antenna (an), 2nd antenna (ant) and maxilla (m). c: details of cerebral projections (arrows) and pores (po) on dorsal body surface. d: insertion site of egg sacs (arrows). e: egg sac (enlarged) showing ova (ov). f&g: uropods showing setae (s).

The body is maggot- like with two egg sacs and each comprises long uniseriate chain of eggs containing up to 20 eggs per sac and were clearly observed attached to the parasite (Fig. 1a). The trunk is oval, longer than wide and subdivided into three regions, first consisting of fused second and third leg-bearing segments, second consisting of fourth leg-bearing segment, and third (genital complex, Fig.1a) consisting of fused fifth leg-bearing segment and genital segment. Genital complex is narrower than preceding trunk regions (Fig.1a).

Abdomen distinctly three-segmented, sub-cylindrical, tapering somewhat posteriorly (Fig.1a). First antenna (Fig. 1) short, situated on ventral surface near anterior margin of cephalothorax, directed posterolaterally, segmented, armed with several very short setae. Second antenna (Figs. 1 & 2) is situated lateral to transverse ridge on ventral surface of cephalothorax, directed posteriorly, segmented with two long setae on distal segment, and armed with many setae. Maxilla (Figs. 1 & 2) is sturdy, two-segmented and subchelate. Subchela was curved inwards with a spiniform process on the inner surface close to the base. Maxilliped (Fig. 2a) carries carrying at its apex two subequal curving; claw-like spines. Uropod (Fig. 1) is short and armed armed with three small setae on or near distal margin and one on lateral margin. Scanning electron microscopy shows that the first four pairs of legs are biramous. First pair (Fig. 2c) has a small protuberance on medial margin of sympod, near base; endopod apparently unsegmented, exopod indistinctly two-segmented, with several setae on lateral margin and single stout seta on apex.

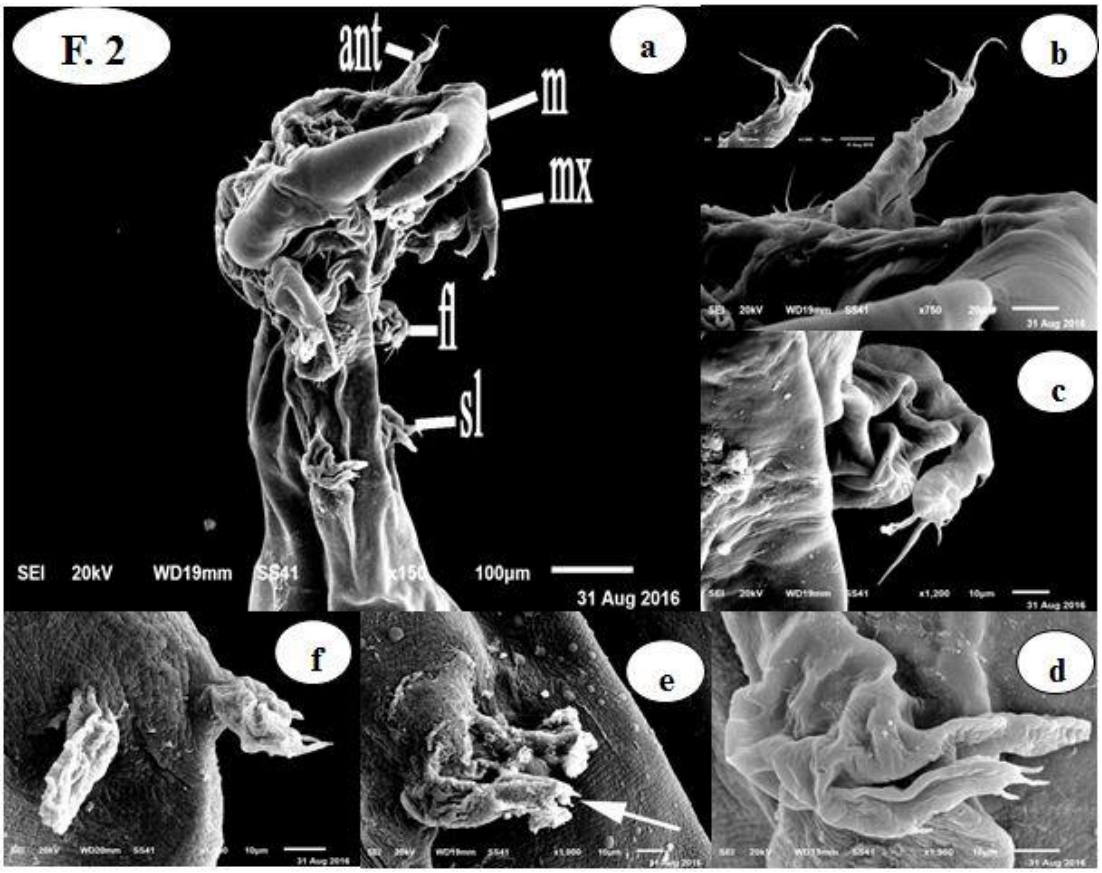

Fig. 2: Scanning electron micrographs of female *L. pulchella,* a: cephalothorax and trunk, showing 2nd antenna (ant), maxilla (m), maxilliped (mx), first leg (fl), second leg (sl). b: 2nd antenna. c: first leg. d: second leg. e: third leg. f: fourth leg.

Second leg (Fig. 2d) bears indistinctly two segmented rami; endopod unarmed, exopod with single seta on lateral margin of basal segment and three setae at apex.Third and fourth pairs (Figs. e & f) are similar with alike rami, with only two setae at apex of exopod. Dense cerebral projections identified on body surface of *L. pulchella* are involved in attachment. Also, pores observed on the surface of the body of *L. pulchella* could intervene in the capture of preys by secreting a kind of mucus.

DISCUSSION

Lamproglena pulchella von Nordmann, 1832, was recorded in Albania, France, Germany, Hungary, Italy, Latvia, Poland, Russia, Serbia and Ukraine. Recent studies reported this species also in Austria (2006) and Macedonia (2004) fauna (Stavrescu – Bedivan *et al.*, 2008). The first record of this copepod in Romanian fauna belongs to Angelescu (1974), who found a single specimen in a single *Chondrostoma nasus* specimen from the Iron Gates reservoir. The great interest manifested for *L. pulchella* can be explained by the correlation attributed to this species with the biomonitoring of the aquatic ecosystems pollution level (Stavrescu -Bedivan *et al.*, 2008). In the current work, *L. pulchella* was found attached to the gills of tigris freshwater fish. *Leuciscus vorax* in the Tigris River in Iraq. This species characterized by a huge, ventrally extended posterio-lateral part of the cephalothorax.

The current study described the ultrastructure of the cephalothoracic appendages. Sayed *et al.* (2013), observed that the presence of the compressed host

tissue in the vicinity of the claws of maxillipeds provided an evidence that this tissue is being pushed into the buccal cavity of the parasite. Sayed *et al.* (2013) suggested that the adequate musculature of the maxillae and maxillipeds allows stretching, contraction, and rolling of these appendages while feeding. Observations under electron microscopy in the current study showed that *L. pulchella* used their prehensile maxillae, maxillipeds and the four legs to attach to the gill filaments. In addition, tegument differentiations play a secondary role in the fixation and attachment. The present study indicates that maxillipeds are robust ending with two hook-like terminal spines or claws. Therefore, the maxillipeds are suggested to assist in the parasite attachment which terminates in two claws. The maxilliped of most *Lamproglena* species, including *L. hemprechii*, *L. hoi*, and *L. clariae*, previously recorded from southern Africa, bears three hook-like terminal spines or Claws (Liesl and Jo, 2007). Observations under scanning electron microscopy by Liesl and Jo (2007) showed the maxilliped of *L. hepseti* terminates in four claws. In *L. elongata*, Capart (1956) revealed that the maxilliped terminates in five spines and in *L. cornuta* (Fryer, 1965) a single spine (Fryer, 1968). Four pairs of legs were smaller and differed in their structure from the other thoracic appendages. They ended with stout seta. This structure is suggested to serve in the attachment to the adjacent secondary gill. Thus, the current study, based on SEM microscopy, revealed that only the maxilla plays a role in the primary attachment to the gill filaments and acts as primary grasping organ. *Lamproglena* species have previously been recorded from other members of characin fishes, i.e. *L. hemprichii* on *Hydrocynus forskahlii* (Cuvier) from Egypt (Piasecki, 1993; von Nordmann, 1832), on *H. vittatus* from Lake Bangweule (Fryer, 1959), on *H. brevis* (Gunther) from Niger (Dollfus, 1960; Fryer, 1964), on Brycinus nurse (Ruppel) from the Galma River in Nigeria (Shotter, 1977), on *Hepsetus odoe* (Bloch) from the Okavango River and Delta, Botswana (Liesl and Jo, 2007) and *L. monodi* on *Oreochromis niloticus* and *Tilapia zillii* (Sayed *et al*, 2013). Fryer (1968) observed that members of Lamproglena show a high degree of host-specificity, being mostly confined to members of a single fish family (Liesl and Jo, 2007). Our results confirm previous studies as *L. pulchella* was found on fresh water fish host, *Leuciscus vorax*.

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