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Abstract

Lernanthropus kroyeri (Copepoda, Siphonostomatoida: Lernanthropidae), a parasitic crustacean from the gills of *Dicentrarchus labrax*, was abundant at higher spring and summer temperatures. Accordingly, the opportunity was taken to throw light on some morphological adaptations with the aid of scanning electron microscopy to clarify the possible functions of these structures.

several kinds of tegumental structures were identified on the body surface, among which the tubular and filamentous forms were the most dominant. These tegumental components seem likely to provide the parasitic copepod with an increase of the surface area for better integumental respiration. SEM observations also revealed the existence of longer and more diverse tegumental components, which are probably involved in sensory functions of the tegument. Moreover, uniciliated sensory formations were recognized regularly across the whole tegumental surface, except on the antennae and maxillipeds. These sense organs show marked variability in distribution and morphology.

1. Introduction

Copepods are one of the most successful groups of the animal kingdom on the Earth **(Boxshall and Defaye, 2007; Rameshkumar and Ravichandran, 2012).** Their high densities in oceans make copepods one of the most abundant metazoan groups. Parasitic copepods are common fish ectoparasites and exhibit worldwide distribution in both natural habitats and pisciculture systems. These potential pathogens feed on host tissues, mucous and blood **(Johnson *et al*., 2004).** Their mode of attachment and feeding behaviour are responsible for many primary diseases and secondary infections. The possible roles that parasitic copepods play as vectors for other disease agents. Death may be caused by the development of secondary infections intensified by stress and creation of open wounds, osmoregulatory failure, and in the case of the gills, respiratory impairment **(Bowers *et al*., 2000; Kua *et al*., 2012; Hirose and Uyeno, 2014).**

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Abu Samak (2005) demonstrated the mode of attachment of the copepod *Lernanthropus kroyeri* to the gills of the sea bass fish, *Dicentrachus labrax* and reported on the histopathological impacts of this ectoparasite on the gill microhabitats. The most relevant histopathological signs were cell atrophy, fibrosis, deformity of the gill cartilage, hemorrhage, hyperplasia and excessive mucous production **(Abu Samak, 2005).**

Among the major fish ectoparasites, copepods are, most of the time, attached to the gills **(Boungou *et al.*, 2013).** There are main groups of parasitic crustaceans affecting commercially important aquaculture species, most of which are external parasites are the branchiura, copepod and isopod **(Heckmann, 2003)**. Egypt is topographically situated along side great areas of fresh and marine water. Egypt is one of the coastal countries that must take benefit from fish proteins **(Abou Zaid *et al.*, 2018).** As in many parts of the world, aquaculture production in the Mediterranean has been expanding rapidly over recent years. Parasitic copepods infect fundamentally different taxa of aquatic animals and show an amazing diversity in body form and life cycle strategies **(Boxshall and Halsey, 2004).** Gill parasitic are common on cultured and wild fish. Many of these species have long been recognized to have the potential to affect the growth, fecundity and survival of hosts **(Johnson *et al.,* 1996).**

Due to their bizarre ability to adapt to more diversified hosts in aquatic ecosystem ranging, from sponges to vertebrates, copepods occupy an advanced rank among parasites, from simple shape, such as sponges to more complex forms, such as vertebrates (Boungou *et al.,* 2013). As fish gills are specialized tissues for gas exchange, circulation, ion and acid-base balance, hormone production, and nitrogenous waste secretion **(Pelster and Bagatto, 2010).** Gill parasites attach to the gills of fish and feed on their host's blood and tissue **(Ojha and Hughes, 2001).** Research into the influence of parasites in aquatic environments has been limited to a few well-studied ecosystems **(Lafferty *et al.,* 2008).** The success of the sea-cage farming is essentially correlated with the possibility to reduce production cost; however, the difficulty of managing disease within sea-cages is regarded as a major drawback **(Ernst *et al.,* 2002).** Fishes reared in aquaculture mostly acquire ectoparasites associated with several cases of deaths in culture systems **(Mladineo 2007).** In recent years, crustacean parasitic diseases are becoming more frequent in the aquacultures and considered the more parasitic problem on cultured marine fish **(Tansel and Fatih, 2012).**

Dicentrarchus labrax now constitutes the most superior commercially fish species in the Mediterranean area **(Antonelli *et al.*, 2009).** The European sea bass *Dicentrarchus labrax* is one of the main cultured fish species in the Mediterranean area. It represents an important financial source in the Mediterranean, especially in Corsica **(Antonelli, 2010).** Disease outbreaks and mortalities caused by *L. kroyeri* are frequently observed in sea bass culture and economic losses occur as the result of reduced feed conversion, growth reduction, mortality, loss of product value and treatment costs **(Manera and Dezfuli, 2003)**. Previous studies have already highlighted the presence of this parasite species, but they have focused primarily on their general morphology and pathologies **(Toksen *et al.*, 2006; Fahmy, 2014).** The relation between the structure of appendages and their possible functions has been studied on the copepod parasite, with *Lernanthropus kroyeri* (**Khidr *et al.*, 2014**). The tegument is an important interface for interactions between host and parasite **(Antonelli, 2010)**. Therefore the current study was carried out in order to describe the body surface of *Lernanthropus kroyeri* parasite using scanning electron microscope examination on some unreported superficial structures attached with the tegument, specially, sensory structures and adherent organs. This contribution of the study of pathogenic parasitic copepod *Lernanthropus kroyeri* provides additional information to the tegument and the fine structures that found on its appendages.

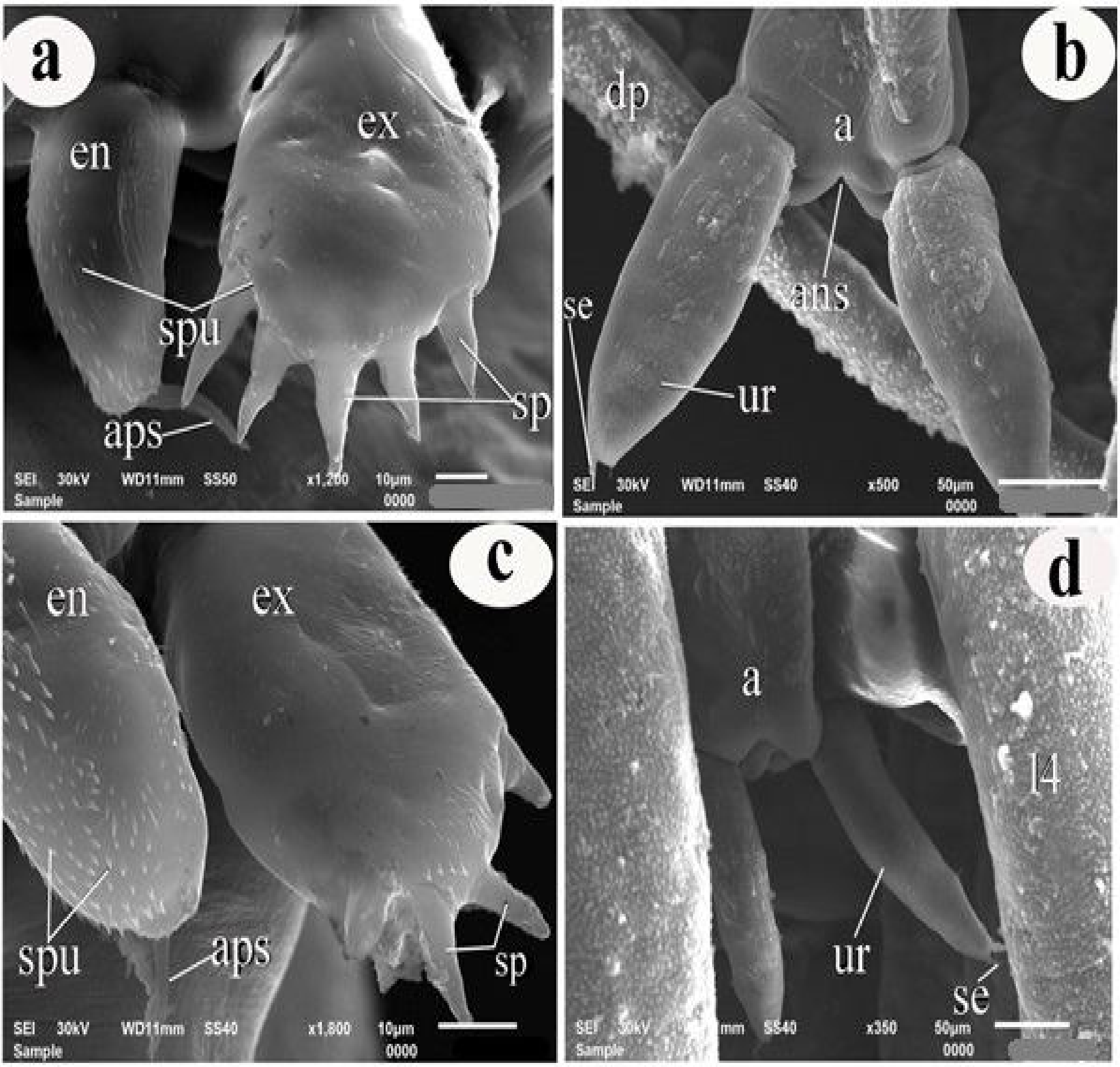
Materials and Methods

Ultrastructural studies were achieved on the gills of cultured sea bass *Dicentrarchus labrax* from the Damietta province that is naturally infested with *Lernanthropus kroyeri* van Beneden and Hesse 1851. Twenty specimens of the sea bass were examined and surveyed for copepod crustaceans. External microhabitats, comprising skin and fins, and gills, were examined in fresh host specimens under stereomicroscope to locate and isolate encountered parasites. Copepods were identified using a light microscope according to the description cited in previous studies **(Ozel *et al.,* 2004; Toksen *et al.,* 2006).** Seven specimens of *L. kroyeri* were studied using scanning electron microscopy (SEM). The copepods were removed alive from their microhabitats on the host, washed repeatedly with seawater to free them from mucus, fixed in cold (4°C) 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2, dehydrated in ascending series of ethanol (30%, 50%, 75%, 90%, and 100%), critical-point-dried, and sputter-coated with gold/ palladium. Parasites were examined under JEOL JSM-6510LV scanning electron microscope operated at an accelerating voltage of 30 kV in the Electron Microscopy Unit, Faculty of Agriculture, Mansoura University.

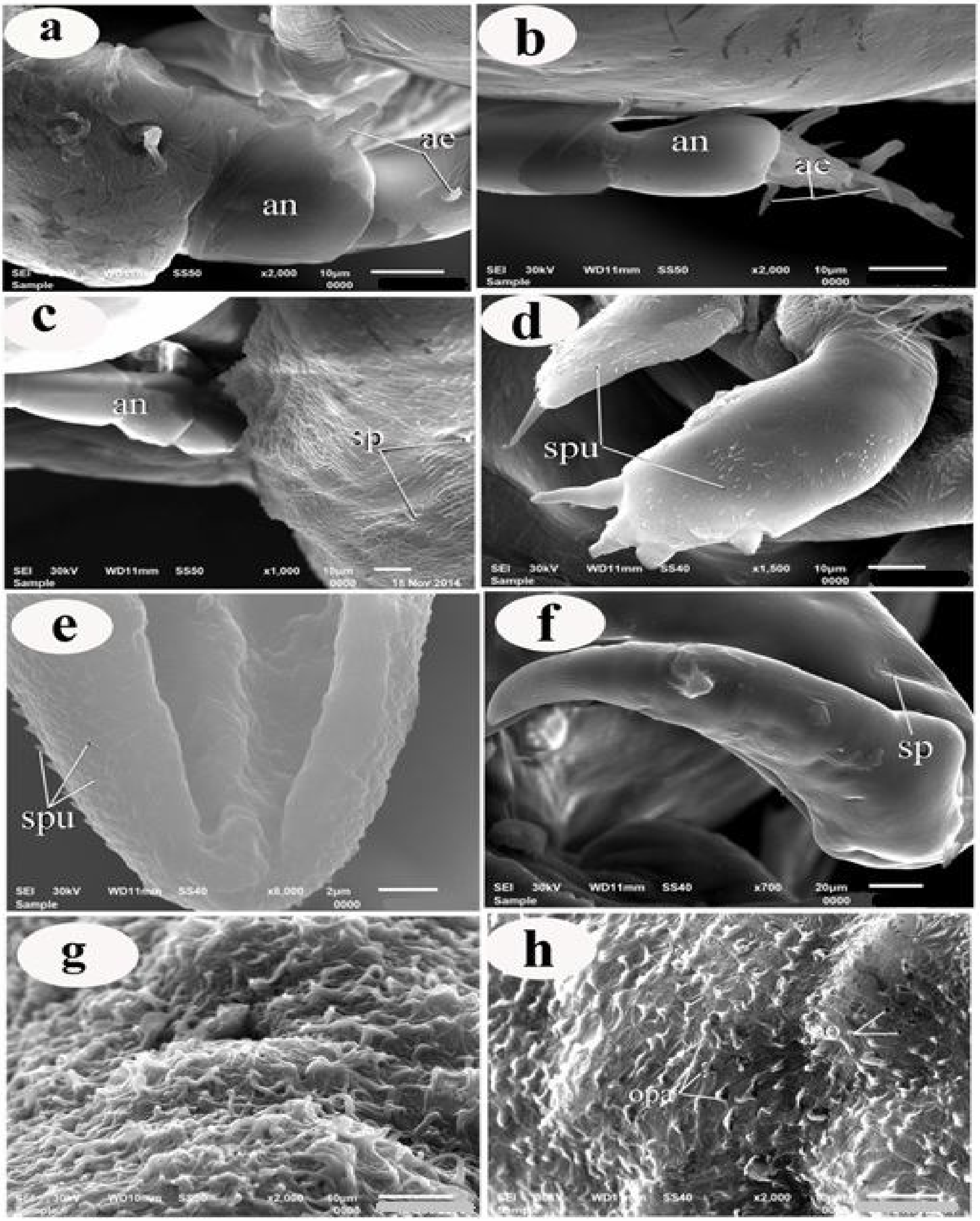
Results

Ten, out of 20 examined sea bass gill specimens, were infected with *Lernanthropus kroyeri.* Female copepods were primarily attached to gill filaments by their second antennae; maxillae, maxillipeds and thoracic legs were involved as assisting organs. The body of each copepod was extended between the hemibranchs, adhesived to the internal surface, with their axis equivalent to the primary filaments axis and with their vertical margins oriented across the gill arch.

Scanning electron microscopy observations presented useful information on the integument outgrowths of *Lernanthropus kroyeri*. Cephalothorax carries four pairs of cephalic appendages (antennules, antennae, maxillae, and maxillipeds) and two pairs of thoracic leg appendages. The trunk, composed of posterior end of the thorax and the abdomen, carries two or three pairs of legs according to sex, the genital area, and uropods. Several kinds of supposed sensory structures and epidermal extensions were recognized on the tegument of *L. Kroyeri,* Accurate tegumental protrusions such as spines, spinules, denticles, teeth and setae with different shapes and sizes were recognized on the body surface (tegument) and appendages as shown in Fig.1 (a, b, c & d) and Fig. 2 (c, d, e & f). Unique structures called Aesthetes were restricted on the antennules (Fig. 2a & b). The aesthetes consist of sensitive cells just above the surface of antennules. Uniciliated sensory formations, not fixed on papillae and released from cavities, appear regularly on all body tegument, except on the antennae and maxillipeds. These formations show variation in distribution and morphology. They may exist singly or in combination. Fine, intense, merged bristles appeared on the dorsal body surface and legs (Fig. 2g). Ornamented papilla exists on the body surface (Fig. 2h). They are principally recognized nearby the second leg. Pores were also noticed on the body surface (Fig. 2 h).



(Fig. 1): Epidermal structures of *L. kroyeri*. **a** Second leg of male. Exopod (ex) provided with spines (sp) and spinules (spu); endopod (en) with an apical seta (aps). **b** Genital area of male, abdomen (a), anal splits (ans),dorsal plate (dp), setae (se) and uropods (ur). **c** Second leg of female. Exopod (ex) provided with spines (sp) and spinules (spu); endopod (en) with an apical seta (aps). **d** Genital area of female, abdomen (a), 4th thoracic leg (l4),setae (se) and uropods (ur).



(Fig. 2): Sensory structures of *L. kroyeri***.** **a** Antennule, (an) at the base part, bearing aesthetes (ae). **b** Antennule, (an) at the distal part, (an) bearing aesthetes (ae). **c,d,e,f** details of tegument features: **c** cephalothorax with antennules (an) and spines (sp) . **d** 2nd leg, spinules (spu). **e** mouth tube, spinules (spu). **f** maxillipeds, spine (sp). **g** Details of fine, intense, and merged bristles found on dorsal body surface and legs. **h** Aspects of ornamented papilla (Opa) and pores (Po).

Discussion

Parasitic crustaceans are numerous and have worldwide distribution in fresh, brackish and salt waters (**Jithendran *et al.,* 2008).** Previous studies have reported on occurrence of *Lernanthropus kroyeri* on the gills of *Dicentrarchus labrax* **(Abu Samak, 2005; Antonelli, 2012**; **Eissa *et al.,* 2017; Abou Zaid *et al.,* 2018)**.

In the present study, SEM observations revealed some superficial structures, sensory structures and adhesive organs, on the tegument of *Lernanthropus kroyeri*. SEM observations also revealed several kinds of tegumental structures abundant ones showed a tubular or filamentous form, which assisted the parasitic copepod in the respiration process; the other type of tegumental structures consists of roughly longer and more diverse extensions which probably exhibit a sensory function. In addition, unique structures called Aesthetes were restricted on the antennules formed of sensitive cells just over the surface of the antennules.

Uniciliated sensory formations appeared regularly on all body tegument, except on the antennae and maxillipeds, and showed variation in distribution and morphology. These formations resemble those obtained by **Antonelli, (2012)** who observed numerous sensory endings on the body surface of *L. kroyeri* and suggested that they are involved in feeding and attachment. The author also showed that the copepod is provided with a considerable increase of cuticular surface for a better oxygen utilization, and so improving respiratory processes through the integument.

Moreover, **Antonelli (2012)** suggested that the cuticle of *Lernanthropus kroyeri* may provide the principal interface between the organism and its microenvironment. Amongst other functions, the cuticle acts as a defense tool against pathogens, constitutes a barrier mediating osmotic and respiratory exchanges and provides a support for the body musculature and internal organs.

Morphologically the present result was in agreement with that obtained by **Ohtsuka and Huys (2001)** who demonstrated that Aesthetes on the antennules are chemoreceptors which detect changes in hydrodynamic forces , dissolved chemical substances, monitoring of food, mate tracking, and/or chemical communication between males and females. **Gresty *et al.,* (1993**) reported that numerous sensory endings identified on the body surface of *L. kroyeri* are involved in feeding and attachment. Cuticular differentiations play a secondary role in the fixation (Fahmy *et al*., 2019). Setae occurring on the antennules are used in guiding copepod to a prospective host (Quilichini and Antonelli, 2012)**.**

Furthermore, the morphological features of *L. kroyeri* in the present study are similar to those revealed by **Poquet (1983)** who observed two types of epicuticular formations on the dorsal body surface: very abundant ones with a tubular or filamentous aspect and longer and more ramified expansions which are less abundant

Also, **Razouls, (1996**) reported that the pores, corresponding to the opening of secretary glands, observed on the surface of the body of *L. kroyeri* could intervene in the capture of preys by secreting a kind of mucus and setae occurring on dorsal body surface have been considered the most likely receptors involved in the sensing of hydrodynamic disturbance. **Heuch *et al.,* (2007)** recorded that the body surface of parasites is richly equipped with numerous receptors, most of them may be with functional significances, monitoring chemical and mechanical signals from surrounding environment**.**

Boxshall *et al.,* (1997) reported that several types of sensory structures identified on *L. kroyeri* were resembled such as previously observed in other copepods, and the form and distribution varies according to species**.**

Concerning the morphological description the present study was similar to that obtained by **Antonelli, (2012)** and **Poquet (1983)** but the later was reported that cuticular formation was longer and more ramified expansions while in the present result it was shorter extensions.

In conclusion, the differences between the current study and previous studies may be contributed to the type of host species, age of the sea bass, fish sampling site, feeding habits and sampling time.

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