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Ultrastructural features of some integumental structural of *Lernanthropus kroyeri* Van Beneden parasitic copepoda (Siphonostomatoidae, Lernanthropidae)

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Abstract

Lernanthropus kroyeri (Copepoda, Siphonostomatoida: Lernanthropidae) is a gill parasite found on the sea bass *Dicentrarchus labrax*. It was significantly present during spring and summer, agreeing with a period of increasing temperature. Due to this, it shows morphological modifications, which are studied through scanning electron microscopy. This study made it possible to reveal some superficial structures associated with the tegument, in particular, sensory structures and anchoring organs were studied to illustrate the possible functions of these structures. On the body surface, several types of epicuticular formations were identified: very plenty ones with a tubular or filamentous aspect, which provides the copepod with a considerable increase of cuticular surface in order to a better oxygen usage, and so improving respiratory processes through the integument ; the other type of cuticular formation consists of longer and more manifold expansions less abundant than the other ones , and they have mainly a sensory function.
Uniciliated sensory structures, not mounted on papillae and protruding from pits, occur regularly on all body surfaces, except on antennae and maxillipeds. These receptors show variation in distribution and morphology.

Keywords: Parasitic copepods, *Lernanthropus kroyeri*, *Dicentrarchus labrax*, integument, ultrastucture.

Introduction

Parasites play a particularly prominent role in aquatic ecosystems where they can dominate the biomass and productivity of the food webs in which they occur by Kuris *et al.,* (2008). As in many parts of the world, aquaculture production in the Mediterranean has been expanding rapidly over recent years. The continuous decline of ocean fisheries and the increasing global fish consumption have promoted the rise of aquaculture. The need for increased supplies of aquatic foods to meet the growing demands of an expanding population is well recognized (FAO 2010; Guo and Woo 2009). Parasites are often inconspicuous relative to other members of aquatic

communities 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). Cultured fish may develop higher parasite burdens than those present in wild fish populations because the development of innovative culture techniques and rearing conditions can favor parasite outbreaks resulting in significant economical losses (Ghittino *et al.,* 2003; Johnson *et al.,* 2004). Fishes reared in aquaculture mostly acquire ectoparasites associated to several cases of deaths in culture systems (Mladineo 2007).

One of better-known example is the infection of fish with gill copepods, which do exert an important ecological and economical impact on awide array of fish species (Ragias *et al.,* 2004). Human activities, including those in or adjacent to aquatic environments, can have a pronounced effect on the emergence of disease (including parasites; (Daszak *et al.,* 2000), with implications for human health (Jones *et al.,* 2008) and wildlife (Dobson and Foufopoulos 2001). Because anthropogenic activities increasingly alter the distribution and abundance of parasites (McKenzie, 2007; Morand and Krasnov, 2010; Polley *et al.,* 2010) the ability to predict and mitigate the consequences of emergent infections depends, in part, on our understanding of how human activities influence the role of parasites in ecosystems. Previous observations revealed that Caligidae or Lernanthropidae are both dominant copepods parasites affecting fish species of great economic interest under rearing conditions (Raibaut *et al.,* 1998). 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).

Lernanthropus kroyeri Van Beneden, belonging to Lernanthropidae, is commonly found in the Mediterranean caged-reared sea bass (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 (Manera and Dezfuli, 2003; Abu Samak 2004; Ozel *et al.,* 2004; Toksen *et al.,* 2006; Fahmy, 2014). Data on the pathological effects have been well documented in several studies. Investigations revealed that infections through the attachment of parasites and active feeding on mucus and epithelial cells of host fish by large populations could cause severe damages such as necroses, hemorrhages, inflammation, and mucus hyperproduction (Noga, 2000; Manera and Dezfuli 2003; Ragias *et al.,* 2004; Abu Samak, 2005; Fahmy, 2014). The relation between the structure of appendages and their possible functions have 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 made to reveal the body surface of *Lernanthropus kroyeri* parasite using scanning electron microscope examination on some unreported superficial structures associated with the tegument, in particular, sensory structures and anchoring organs.

Materials & Methods

Ultrastructural studies were carried out on the gills of cultured seabass *Dicentrarchus labrax* from Damietta province, that were naturally infested with *Lernanthropus kroyeri* van Beneden, 1851. Twenty sea bass Dicentrarchus labrax were examined for the presence of parasites. Body surface (skin, fins) and gills of the fish were examined. Gill arches were carefully removed and studied in a fresh condition. Copepods were identified using a light microscope according to the description done in previous studies **(Ozel *et al.,* 2004; Toksen *et al.,* 2006).** Seven specimens of *L. kroyeri* were studied using scanning electron microscopy. The copepods were removed alive from their hosts, 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 through a graded ethanol series (30%, 50%, 75%, 90%, and 100%), critical-point-dried, and sputter-coated with gold/ palladium. Samples were examined under JEOL JSM-6510LV scanning electron microscope operated at an accelerating voltage of 30 kV.

Results

Ten out of the 20 sea bass gills sampled were infected with *Lernanthropus kroyeri*. Adult female copepods were attached to the flat, lamellae bearing sides of the primary lamellae (gill filaments) by their second antennae and third legs. The body of each parasite was positioned between the hemibranchs, attached to the internal face, with their axis parallel to the primary lamellae axis and with their cephalic extremities oriented towards the gill arch.

Scanning electron microscopy (SEM) observations provided useful information on the integument structures of *Lernanthropus kroyeri*. Cephalothorax bears five pairs of cephalic appendages (antennules, antennae, maxillules, maxillae, and mandibles) and two pairs of thoracic leg appendages. The trunk, consisting of posterior part of the thorax and the abdomen, bears two or three pairs of legs according to sex, the genito-abdominal complex, and caudal rami.

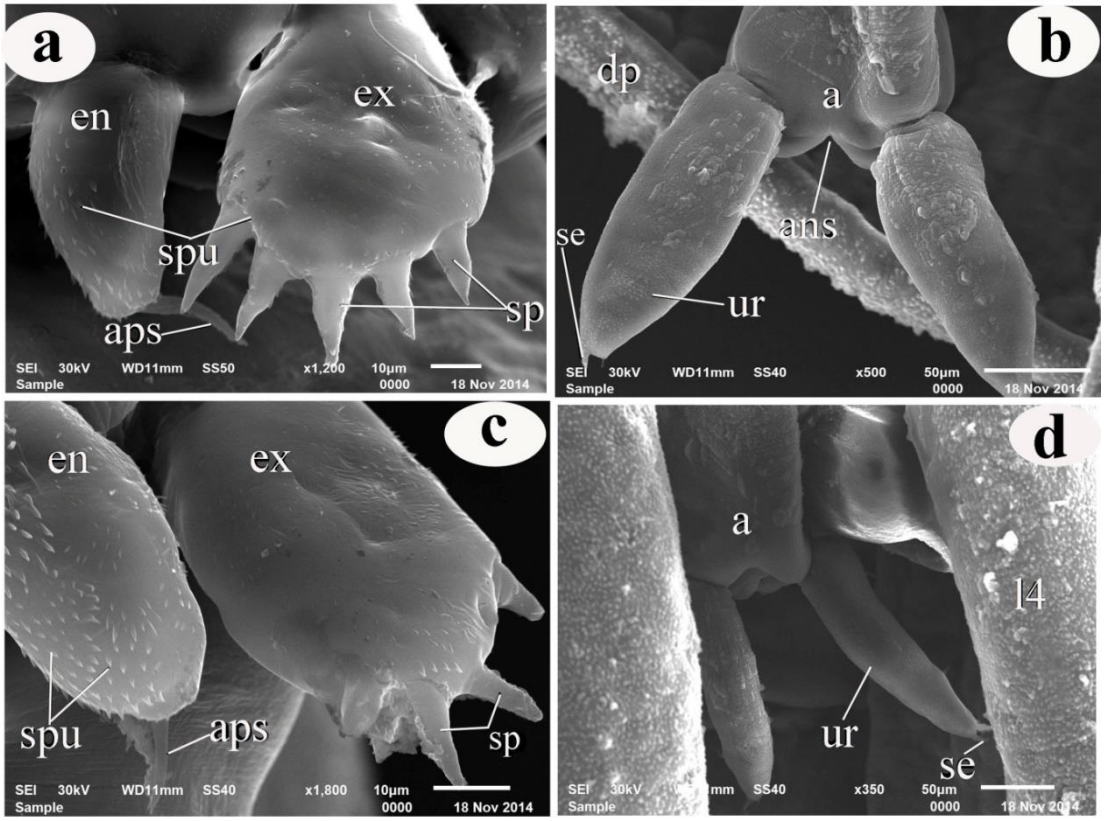

Fig. 1: Cuticular structures on both sexes of *L. kroyeri*. **a** Second leg of male. Exopod (ex) provided with spines (sp) and spinules (spu); endopod (en) with an apical seta (aps). **b** Genital area of male, abdomen (a), anal slits (ans),dorsal plate (dp), setae (se) and uropods (ur). **c** Second 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).

Tegument structures

Several types of presumed sensory structures and cuticular outgrowths were identified on the body surface of L. kroyeri.

1- Aesthetascs were identified on the antennules (Fig. 2a&b).
2- Minute tegumental structures such as spines, setules, denticles, teeth and rows with various forms and sizes were observed on the body surface and appendages (Figs.1(a,b,c,d) & 2(c,d, e&f).
3- Uniciliated sensory structures, not mounted on papillae and protruding from pits, occur regularly on all body surfaces, except on antennae and maxillipeds. These receptors show variation in distribution and morphology. They may occur singly or in groups. Fine, dense, and intermingled, they are observed on dorsal body surface and legs. The ends of these receptors appear branched out (Fig. 2g).
4- Ornamented papillae bearing cilium occur on body surface. They are mainly observed near the first and second leg. Every cilium appears branched out (Fig. 2h). Pores were also observed on tegument (Fig. 2 h).

Discussion

Comparing with other crustacean groups, parasitic copepods have the most various structures and lifestyles (Lee *et al.*, 2005). Surface morphology, especially the number, shapes, size, and distribution of various tegumental structures may be helpful to understand the life and physiological condition of parasites.

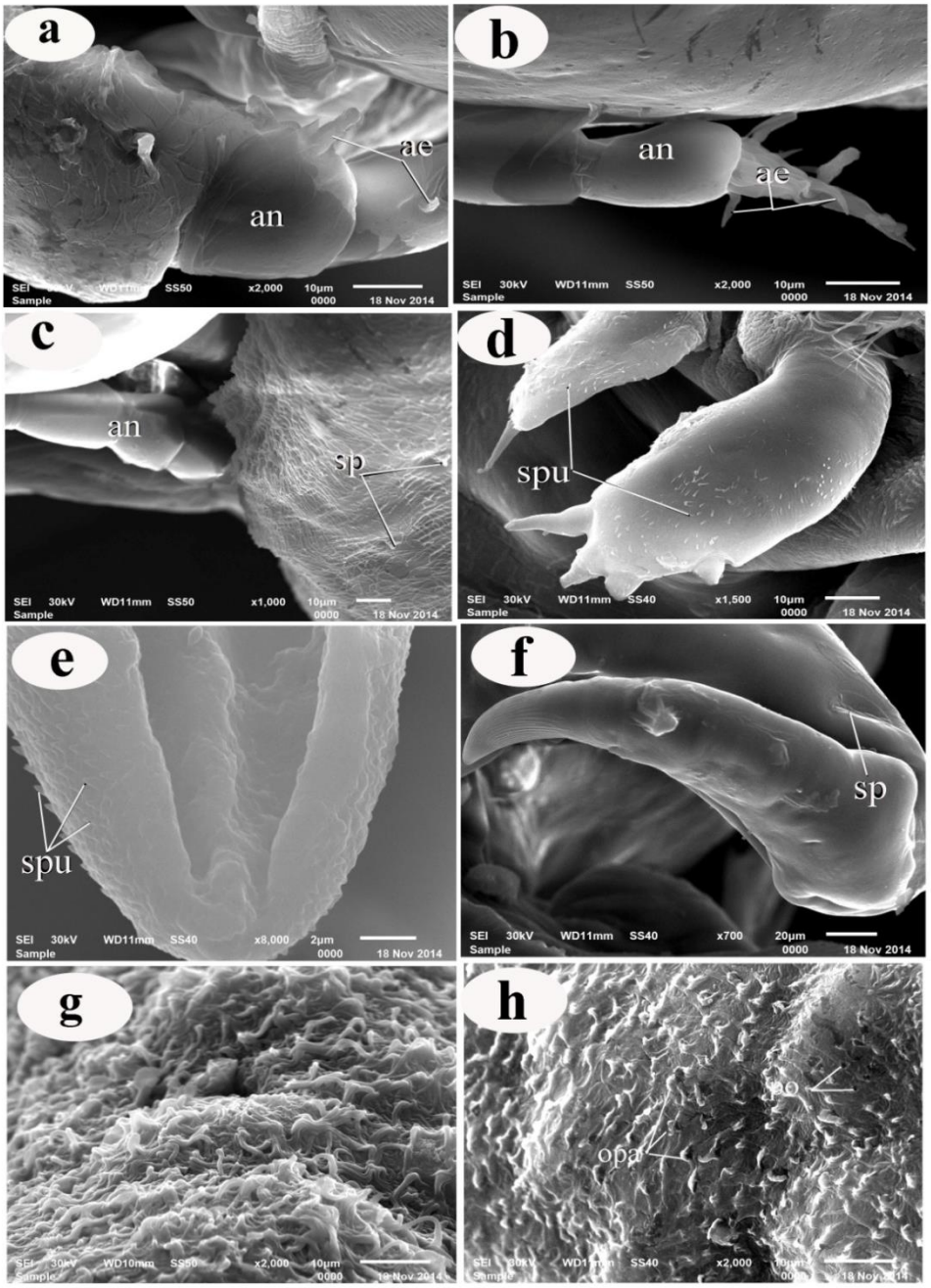

Fig. 2: Sensory structures of *L. kroyeri*. **a** Antennule, (an) at the base part, bearing Aesthetascs (ae). **b** Antennule, (an) at the distal part, (an) bearing Aesthetascs (ae). **c,d,e,f** details of tegument features: **c** cephalothorax with antennules (an) and spines (sp) . **d** 2nd leg, spinules (spu). **e** mouth tube, spinules (spu). **f** maxillipeds, spine (sp). **g** Details of fine, dense, and intermingled setae occurring on dorsal body surface and legs. **h** Details of ornamented papillae (Opa) bearing cilium observed near the first and second leg, and pores (Po).

Tegument structures

Numerous sensory endings identified on body surface of *L. kroyeri* are involved with feeding and attachment. Also, provided the copepod with a considerable increase of cuticular surface in order to a better oxygen utilization, and so improving respiratory processes through the integument

(Antonelli, 2012).

Recent studies have revealed remarkable diversity of parasitic copepod sensory abilities and behaviors. 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 such as reported with (Heuch *et al.*, 2007). Scanning electron microscopy examination of *L. kroyeri* during the present investigation made it possible to reveal some previously unreported superficial structures in this species. 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 (Boxshall *et al.*, 1997). It is now widely accepted that, for successful mate recognition (and copulation), most copepods rely heavily on sensory modalities such as mechano- and/or chemoreception. Ohtsuka and Huys (2001) mentioned that Aesthetascs present on the antennules are chemoreceptors able to detect changes in water movement, dissolved chemical substances, information about detection of food, mate tracking, and/or in the exchange of chemical information between both sexes. Numerous sensory endings identified on body surface of *L. kroyeri* are involved with feeding and attachment. Cuticular differentiations play a secondary role in the fixation. Setae occurring on the antennules are used in guiding copepod to a prospective host (Gresty *et al.*, 1993).

Most parasites copepods feed by rasping at the surface of their host using their appendages. Sensory endings present near the oral cone, on maxillules and maxillae, are presumably mechanoreceptors involved with feeding, while the length and flexibility of dense uniciliated sensory endings occurring in the dorsal body surface, or near legs of *L. kroyeri*, suggest that they may be rheoreceptors/tangoreceptors involved in the orientation of the parasite in relation to the flow of the ventilation water currents.

The cuticle of *Lernanthropus kroyeri* may provide the principal interface between the organism and its external environment. Amongst other functions it acts as a defense against pathogens / host attack, constitutes a barrier mediating osmotic and respiratory exchanges and provides a site for support / attachment of the body musculature and internal organs as reported by Antonelli, (2012).

Other minute tegumental structures such as spines, setules, denticles and teeth may provide the principal interface between the organism and its external environment. Setae occurring on dorsal body surface have been considered the most likely receptors involved with the sensing of hydrodynamic disturbance (Razouls, 1996). Previous studies on some parasite copepods species indicate that they are sensitive to hydro-mechanical signals (Poulin *et al.*, 1990). Parasitic copepods are sensitive to low-frequency water acceleration such as those produced by a swimming fish (Heuch and Karlsen, 1997). 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 (Razouls, 1996). In copepods searching for food, chemoreception and mechanoreception work in conjunction.

In conclusion, the current study appeared that the tegument layers of *Lernanthropus kroyeri* is very similar to that described by Antonelli,(2012). Cuticular differentiations play a secondary role in the fixation and they have mainly a sensory function.

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الملخص العربي

عنوان البحث: الأشكال الدقيقة لبعض التركيبات الجليدية للطفيلي مجدافي الأرجل ليرنانثروبس كرويري

شرين فهمى
قسم علم الحيوان – كلية العلوم – جامعة دمياط – دمياط - مصر

استهدفت الدراسة الحالية القيام بعمل دراسات على الطفيليات مجدافية الأرجل التي تصيب أحد الأسماك البحرية ذات الأهمية الاقتصادية وهي أسماك القاروص (دايسنتراركس لابركس) والتي تم تجميعها من المياه الساحلية لمدينة دمياط (ميناء دمياط) على البحر المتوسط. طفيل ليرنناثروبث كرويري يصيب خياشيم أسماك القاروص (دايسنتراركس لابركس) ويتواجد بكثرة في الفصول الصيفية مع ارتفاع درجة الحرارة.
اهتمت الدراسة بتناول النواحي المورفولوجية للطفيلي مجدافي الأرجل ليرنناثروبث كرويري باستخدام الميكروسكوب الالكتروني الماسح وقد بينت أن الجسم في أنثى وذكر (ليرنناثروبث كرويري) ممدود ، وتتحد منطقة الرأس مع عقل الصدر الأولى مكونة الرأسصدر ثم تتحد العقل الأخيرة من منطقة الصدر لتكون المجمع التناسلي. جدار الجسم من الخارج في طفيلي (ليرنناثروبث كرويري) يحتوي على تركيبات أو بروزات جليدية. العديد من النهايات الحسية التي تم تحديدها على سطح الجسم في طفيلي (ليرنناثروبث كرويري) يمكن أن تشارك في التغذية و عملية الإلتصاق و علاوة على ذلك تقوم النهايات الحسية بتحسين الاستفادة من الاكسجين لعملية التنفس خلال الإهاب السميك للطفيلي (ليرنناثروبث كرويري).
يوجد ثلاثة أنواع لهذه التركيبات الجليدية على سطح الجسم في طفيلي (ليرنناثروبث كرويري):

١. **النهايات الحسية على الزبيني (الزباني الأول)**
٢. **تركيبات جليدية دقيقة تتمثل في الأشواك، الشويكات و الأسنان متنوعة ومختلفة الحجم على سطح الجسم والزوائد**
٣. **تراكيب حسية غير مهدبة مثل الحلمات الحسية و النتوءات الحسية على كل سطح الجسم ماعدا الزبينيان والأرجل الفكية للطفيلي**
٤. Ultrastructural features of some integumental structural of *Lernanthropus kroyeri* Van Beneden parasitic copepoda (Siphonostomatoidae, Lernanthropidae)