Epibiotic ciliates *Scyphidia* sp. and diatoms on *Tigriopus fulvus* (Copepoda: Harpacticoida) exoskeleton

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Abstract

Several microorganisms – epibionts – can adhere to living supports taking advantage for their survival, feeding and movement. Epibiosis occurs particularly in aquatic environments, on both benthic and planktonic organisms, among which copepods and cladocerans represent an important living support. The harpacticoid copepod *Tigriopus fulvus*, living in the splashpools of rocky coasts, was studied to recognize the occurrence of epibionts on the exoskeleton surface using scanning electon microscopy techniques. The first evidence of ciliate *Scyphidia* sp. on *Tigriopus fulvus* has been described and the occurrence of algae *Cocconeis* sp. has been observed as well. Epibionts were found to adhere to antennae, a site linked to the exploitation of water currents carrying food particles to mouthparts and to swimming legs. The reason of the occurrence on swimming legs is less clear and needs further observations. Pertinent results are described and discussed and the influence of epibionts on life cycle and behavior of *Tigriopus fulvus* is considered.

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Key words: epibiosis, ciliates, *Scyphidia* sp., copepods, *Tigriopus fulvus*.

Acknowledgements: special thanks are due to Reinhardt Møbjerg Kristensen, Curator at Natural History Museum of Denmark, for his cooperation in ciliate identification and to Giorgio Bavestrello (Department of Earth, Environment and Life Sciences, University of Genoa, Italy) and Rob McAllen (University College Cork, Ireland) who provided us with the specific bibliography.

Received for publication: 23 July 2014.
Revision received: 20 November 2014.
Accepted for publication: 20 November 2014.

Journal of Biological Research 2014; 87:4600
doi:10.4081/jbr.2014.4600

Introduction

Epibiosis is an ecological relationship in which microorganisms, such as bacteria, fungi, algae, and protozoans, live attached to a living support, the basibiont, during all or single phases of their life cycle.[1,2] Even though it is considered essentially as a commensal relationship, epibiosis can cause negative effects on the host, such as decrease of survival, growth and reproduction, motion difficulties, increase of predation pressure interfering with the escape capability and swimming of host, direct damage or induction of diseases, competition for food, increasing energy demands, susceptibility to predation and faster sinking rate.[3-6] In particular, in food-limited environments ciliate epibionts can affect the survival of zooplankters,[4,7] and decrease their fitness due to raised energetic demand for locomotion.[8] Nevertheless, epibiosis can provide benefits to the basibiont, such as better availability of food and decrease of predation if epibionts are not attractive or repellent. The advantages for epibionts are mainly due to the possibility to be passively transported with obvious increase of feeding, enhancement of feeding rate[9] and food capturing efficiency,[10] and decrease of predation.[3,5]

In aquatic environments, microorganisms can survive as free forms or by adhering to surfaces.[11,12] The adhesion to living supports is thought to be extremely advantageous to epibionts which would be able to survive longer than free forms in stressful conditions.[13] The microbial colonization of an organic substratum is a complex phenomenon originating from the initial random or chemotactic collision between microorganisms and surface, which results in the development of microorganisms on the surface itself.

Attached ciliates are common mainly on benthic organisms,[14,15] but also the adhesion to phytoplankton and zooplankton as well as to suspended particles has been repeatedly reported.[10,16,17] Some ciliates may attach and detach randomly without the intervention of specialized structures,[18] but others produce stalks that allow them to better adhere to the surfaces.[19] Copepods and cladocerans, being dominant in the zooplankton, are one of the main living supports providing attachment sites for a wide range of microorganisms that find abundant available binding surfaces on their exoskeleton.[20-24] The occurrence of ciliate epibionts was recognized also on parasite copepods such as *Lepeophtheirus salmonis*, ectoparasite of marine salmonids.[25] In particular, harpacticoids seem to be very important basibionts.[2,26]

Tigriopus fulvus Fischer is a harpacticoid copepod typical of supralittoral splashpools, a semi-confined environment characterized by wide seasonal and diel fluctuations of environmental conditions.[27,28] *Tigriopus fulvus* has been extensively studied for reproduction, life cycle,[29,30] ecology[31] and at present is utilized as test-species in ecotoxicology.[32-35] This copepod is known to host bacteria *Vibrio alginolyticus*[36] as well as diatoms on the exoskeleton surface.[37] This paper describes the first scanning electron microscopy (SEM) evidence of ciliate epibionts on *Tigriopus fulvus* exoskeleton.

Materials and Methods

Tigriopus fulvus specimens were sampled from splashpools along the Eastern Ligurian riviera (Genova Nervi, Ligurian Sea, Italy) by means of sterile bottles. Specimens were transferred in sterile seawater filtered with fiberglass filters and membrane filters Gelman GN/6 (0.45 μm). In the laboratory copepods were maintained for a week at 30-32 PSU salinity, 8.0-8.3 pH, 14°C and 12:12h light:dark period in a low temperature incubator (Haeraeus BK 6160), and fed with marine microalgae *Tetraselmis suecica* cultured as reported.[38]

For SEM observation ten specimens were carefully rinsed five times (3 min each) in sterile seawater, fixed with 4% buffered seawater formalin, dehydrated in ethyl alcohol gradients, dried in a critical-point drying apparatus, coated with gold in an ion-sputtering apparatus and observed with a Vega-3 LMU SEM (Tescan, Brno, Czech Republic).

Results

Scanning electron microscopy analysis revealed the occurrence of ciliates in one of examined specimens. They were subsequently classified at genus level as *Scyphidia* sp. Ciliates were found on swimming legs and antennae of a *Tigriopus fulvus* adult male (Figures 1 and 2). On the contrary, nine specimens did not show ciliate epibionts. Several adhered diatoms, presumptively belonging to the genus *Cocconeis* sp., as well as rod-shaped and coccoid bacteria were observed on the ventral region of a *T. fulvus* adult female (Figure 3).

Discussion

The utilization of SEM techniques allowed us to recognize the occurrence of epibiont protists (algae, protozoa, and bacteria) adhered to the splashpool copepod *Tigriopus fulvus* and to classify the ciliate epibionts as belonging to the genus *Scyphidia*.

It is known that ciliates may adhere to a wide range of surfaces among which freshwater and marine crustaceans are included.[16,39-41] Some *Scyphidia* are known to occur in freshwaters and to colonize mainly copepods (57.14% of the whole population), cladocerans and rotifers.[5] Other species, such as *Scyphidia ubiquita* Hirshfield, have a littoral distribution and were recorded in the mantle cavity of snails *Littorina* sp.[42]

Ciliate adhesion has been linked to copepod gender or developmental stage. As a matter of fact, in Indian lakes peritrich epibionts were found to adhere mainly to *Mesocyclops aspericornis* females *vs* males or copepodids.[41] Copepods *Metridia longa* and *Paraeuchaeta norvegica* collected in west Spitsbergen (Svalbard) were found to carry epibiotic ciliates *Paracineta* sp. which attached only to females.[43] Utz and Coats[44] stated that calanoid copepodids were more infested than adults suggesting a preference of epibionts for the attachment to juveniles. The observations reported here do not allow us to understand if the adhesion is more pronounced on males or females or if adhesion is more prevalent on adults or larval stages. To solve this question more numerous observations are needed.

Some ciliate epibionts were found to be selective for the attachment to different body regions. For example, the prosome of *Mesocyclops aspericornis* is the preferred site of epibiont adhesion.[41] Other studies showed that the cephalothorax and the abdomen of calanoid copepods were more infested than antennae and swimming

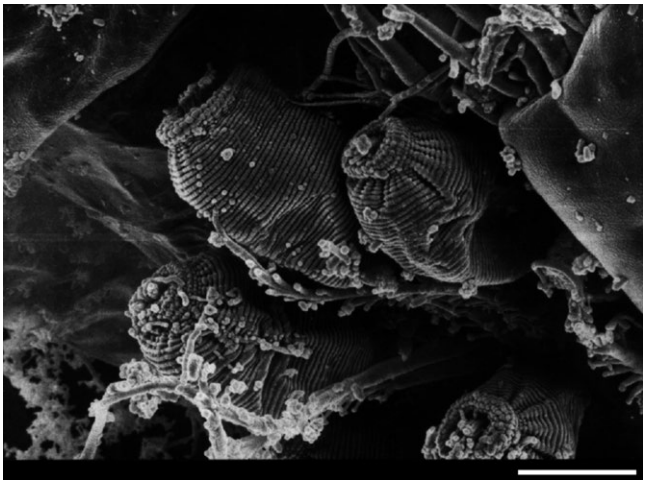

Figure 1. Ciliates *Scyphidia* sp. (n=4) on swimming legs of a *Tigriopus fulvus* adult male. Scale bar=10 μm.

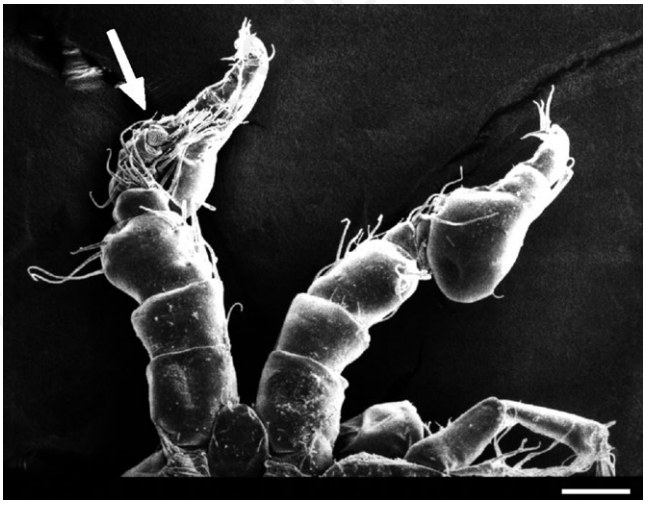

Figure 2. Ciliates *Scyphidia* sp. on antennae of a *Tigriopus fulvus* adult male. Arrow indicates the place of adhesion of the ciliate. Scale bar=40 μm.

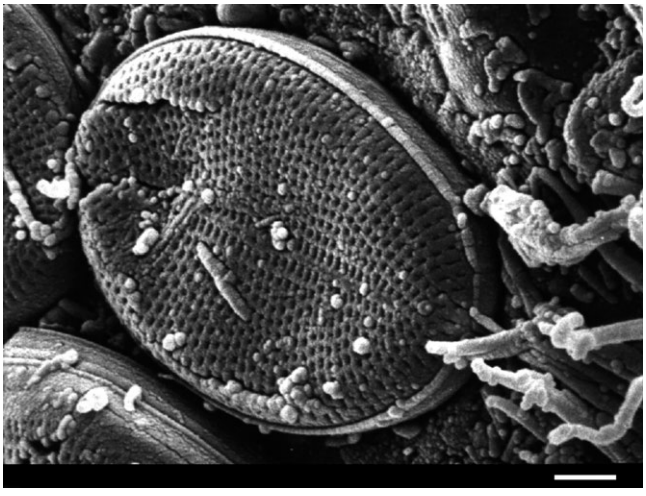

Figure 3. Pennatae diatoms (n=3) on *Tigriopus fulvus* female exoskeleton. Scale bar=2 μm.

legs indicating a preference of epibionts for less active parts of the host.[44] On the contrary, our observations show the occurrence of *Scyphidia* epibionts on swimming legs and on antennae. As the choice of the site of adhesion is presumptively linked to an advantage that a site could provide in comparison to another one, the occurrence of epibionts on antennae has a reasonable significance since epibionts could better exploit water currents carrying food particles to mouthparts with a consequent improved feeding. Conversely, the reason of the occurrence on swimming legs where food availability is presumptively lower than near other body regions is less clear and needs further observations. Our observations partly disagree from those by Yamaguchi,[45] who reported that calanoid copepodids collected in subarctic Pacific, Japan Sea and Okhotsk Sea were seen to have no epibionts on antennules, feeding appendages and caudal rami and only a scarce presence on swimming legs (5%) *vs* great numbers on the urosome (89%), metasome (60%), and cephalosome (19%). Microbial epibionts, mainly bacteria (*Leucothrix*) but also peritrich ciliates and diatoms, the latter on swimming legs and furca, were described on the exoskeleton of adult, but not on copepodids, of *Tigriopus brevicornis*, a harpacticoid copepod whose habitat is analogous to that of *Tigriopus fulvus.* They were indicated presumptively to have effects on the movement and feeding of copepods; mouthparts and swimming legs were also heavily biofouled with ciliates that presumptively here take advantage of feeding currents.[46]

Conclusions

Ciliate epibionts associated with crustacean zooplankton are widespread in aquatic systems, but their ecological roles are poorly known. Further investigation is needed to study the occurrence, coverage and distribution of epibiosis on *Tigriopus fulvus* and to know how this ecological relationship could affect life cycle and behavior of this harpacticoid copepod.

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