Ultrastructural Evidence of Luminescent Gland Openings in *Metridia gerlachei* Giesbrecht, 1902 (Copepoda: Calanoida)

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Abstract

The luminescence is a feature of several marine planktonic organisms, such as bacteria, algae, cnidaria, ctenophores, crustaceans and pyrosomes; in particular, a lot of pelagic crustacea have got luminescent organs (Ghirardelli, 1981, Gruner, 1994) useful for trophic, reproductive or defense purposes. Several calanoid copepods are luminescent and possess variously distributed epidermal glands. In this paper the occurrence of luminescent gland openings in adults and copepodids of *Metridia gerlachei* sampled during the Italian Oceanographic Campaign in Antarctica 1987-88 in Terra Nova Bay (Ross Sea) is described by using scanning electron microscopy (SEM) techniques. The openings of cephalothorax luminescent gland has been observed and described with the aim to complete what is known about this subject (Piñero de Verdinelli, 1981); the occurrence of a gland opening, not previously described, has been reported. Considering the extreme characteristics of the environment in which *Metridia gerlachei* lives and considering also that this species seems to prefer to live in low-light waters (Carl et al., 1990, 1992a) as the constant light conditions during the Antarctic summer restrict its occurrence in surface waters, luminescent structures could have an adaptive significance both for reproduction and defence.

Introduction

The luminescence is a phenomenon occurring in several organisms living in different environments (Hastings, 1996) and it can provide ecological and biological advantages, for example in inter- and intra-specific relationships, in the reproductive behaviour, in predation and in defence activities. In the marine environment the luminescence is typical not only of deep-water organisms, but also of several beings living in intermediate and surface waters: light emission is known in the bacteria *Photobacterium* spp. and *Vibrio* spp. which live in association with zooplankton and fish (Boyd, 1992; Haygood and Distel, 1993), in *Vibrio fisheri* symbiotic of squids (Boeticher and Ruby, 1990; Lee and Ruby, 1992; Ruby and McFall-Ngai, 1992), in several strains of bacteria isolated from cultured prawns (Liu et al., 1996). The occurrence of bioluminescent bacteria has been also considered as a marker to recognize the pollution state of the water (Ramaiah and Chandramohan, 1993). Bioluminescent phenomena are also recorded in several unicellular algae: luminescent dinoflagellates are abundant in epipelagic oceanic zones (Tett, 1971) and in heterotrophic species the emission of light seems to be affected by starvation (Buskey et al., 1992) and by food quality and concentration (Buskey, 1995).

Several higher marine organisms, such as cnidarians, ctenophores, cephalopods, crustaceans (ostacods, copepods, amphipods, mysids, euphausiids, decapods), and tunicates (pyrosomes) show bioluminescence. Light production due to the zooplankton (siphonophores, ctenophores, euphausiids) emission was observed in sound scattering layer (Clarke and Backus, 1956; Kampa and Boden, 1956; Barham, 1963, 1966; Sameoto, 1980; Greene et al., 1988; Widder et al., 1992). All luminescent crustaceans are marine and almost all pelagic (Gruner, 1994); some of them produce secretions forming a luminescent cloud, probably to frighten predators (Ghirardelli, 1981). Luminescent organs of Crustaceans consist of epidermal cells or glands, but also parts of the epotopancreas can produce light. Among Copepods, luminescent species belong mostly to calanoids (Clarke et al., 1962; Gruner, 1994). Light production in copepods involves epidermal glands distributed all over the body and in the appendages according to a definite scheme. The occurrence of light glands, fluorescent glands and luminous pores is also known in calanoids such as *Gaussia princeps* (Barnes and Case, 1972).

In the Antarctic region the study of the luminescence is a subject of particular concern to recognize morphological, biochemical and physiological processes and to explain...
evolutionary events and adaptive strategies accomplished in such peculiar and extreme ecosystem. Among Antarctic copepods *Metridia gerlachii* is a particularly interesting autochthonous species; it inhabits the waters around the whole continent (Farren, 1929) and was recognized as a frequent species in the Ross Sea (Carli et al., 1990, 1992a, 1992b; Zunini Sertorio et al., 1990, 1992; Pane et al., 2004). In the family Metridiidae luminescent glands consist of two spindle-shaped cells differently coloured, debouching by a common pore; it has been supposed that the first one produces luciferin and the other one luciferase; the ejected secretion forms a bright cloud or a luminous green-bluish point (Gruner, 1994). Among Metridiidae *Metridia longa* was indicated to be the most luminescent organism in the marginal ice zone of the Greenland Sea (Buskey, 1992). As at present the ultrastructural morphological characteristics of *Metridia gerlachii* have been scarcely studied (Romano et al., 1999; Mariottini et al., 2000), aim of this work is to describe the occurrence of luminescent gland openings in the adult female of *Metridia gerlachii* by means of scanning electron microscopy (SEM) techniques.

**Materials and methods**

*Metridia gerlachii* specimens were sampled between December 1987 and February 1988 during the Italian Oceanographic Campaign in Antarctica in the Terra Nova Bay (Ross Sea) by means of a BIONESS electronic multitnet (Guglielmo et al., 1990).

Specimens have been maintained in 4% formalin; in the laboratory the taxonomic identification was carried out by means of a Wild M3C stereomicroscope, a Leitz Diaplan microscope and a Zeiss ID02 invertoscope.

Samples utilized for the SEM observation were treated according to Cohen (1979); briefly, they were rinsed twice in 2.5% glutaraldehyde in cacodylate buffer 0.1M at 4°C for 24 hours and subsequently post-fixed in 1% OsO4 in cacodylate buffer at 4°C for 30 minutes, rinsed twice in the same buffer; rinsed ones in distilled water, dehydrated in ethanol of increasing concentration up to 100%; critical point dried from liquid CO2.

Specimens were mounted with small pieces of double-sided adhesive tape on aluminium stubs, coated with a 20 nm gold layer in an argon atmosphere flow discharge sputter coating-unit (Polaron E 5100) and examined by means of a ISI SS-40 scanning electron microscope operated at an accelerating voltage of 10-20 Kw.

**Results**

The arrangement of luminescent gland openings placed in the cephalothorax has been observed considering the work of Piñero de Verdinelli (1981). Scanning electron images show the occurrence of some openings near the emergence of the antennae and above the emerging of mouth appendages of the adult female (figs. 1, 2) and of the copepodid IV (fig. 3); some openings have been also observed in the appendages and in the abdomen of copepodid IV (fig. 4), and in the end part of prosome of the adult female (figs 5, 6). A detailed picture of an opening is shown in fig. 7.
Therefore, the description and distribution of lateral openings and partly of frontal and posterior ones observed by Piñero de Verdinelli (1981) are confirmed by the here reported observations. Nevertheless, during this study also the occurrence of other openings in the portion of cephalothorax placed between the anterior and the middle-hind part of cephalothorax, at mandibular level, and in abdomen and appendages has been observed.

**Discussion**

*Metridia gelrachei* is a typical Antarctic copepod, which greatly occurs in the Ross Sea (Bradford, 1971; Carli et al., 1990, 1992a, 1992b; Zunini Sertorio et al., 1990, 1992). Although its morphological description using light microscopy has been carried out by several scientists (Giesbrecht, 1902; Wolfenden, 1908; Tanaka, 1960; Bradford, 1971; Ramirez and Dinofrio, 1976; Piñero de Verdinelli, 1981), the available ultrastructural observations are scant; thus, specific studies could allow to better characterize the species and improve the morphological knowledge. Furthermore, on the whole the knowledge about luminescence of Crustaceans, and particularly of Copepods, is still scarce, since a number of luminous species live in meso- and bathypelagic waters; therefore, the occurrence, distribution, biological and physiological significance of luminescent glands in Antarctic species is to date greatly unknown. On the other hand, only by electron microscopy it is possible to obtain detailed informations about this matter. Several hypotheses exist on the aptitude of pelagic marine organisms to produce light, but direct research and observations are very scarce, so the explication of such phenomenon is often theoretical. However, it was supposed that the ability to produce bioluminescence provides with several biological and ecological advantages for the organisms in the intraspecific relationships and also as a response to the environment (Gruner, 1994).
It's well known that luminescent organs of Crustaceans are formed differently in the different taxonomic groups; among Copepods it is possible to observe such structures particularly in Calanoids, although studies concerning luminescence have been carried out also on Oncaea and Corycaeus (Poeziolostomatoida), Oithona (Cyclopoida), and Macrostetella (Harpacticoida); the species belonging to these genera are characteristic of low-light zones and make vertical diel migrations, inhabiting deep waters with daylight and rising to the surface during the night (Gruner, 1994). In Copepods luminescent glands are epidermic and disposed in well defined locations; as concerns Metridinidae each gland consists of two spindle-shaped and differently stained cells, debouching together with a pore (Clarke et al., 1962); it has been supposed that one of them produces luciferin, and the other one luciferase (Gruner, 1994). After stimulation, the "mixture" secreted is ejected in the water, where it forms a diffuse light cloud or a well defined light point, which can shine even for one minute. Subsequently, Copepods go away from the emission swimming actively; in this case the luminescent reaction is an escape defensive behaviour. Previous descriptions pointed out the typical green-blue colour of the light. The secretion is clearly sticky and traces often remain on the body near the pores. The luminescence can be also harmful for the emitting organism, owing to the possible attraction of specific predators, nevertheless, luminescent specimens have probably developed still greatly unknown fine neurophysiological adaptations and behaviours. Piñero de Verdhinelli (1981) described in Metridia gerlachei small (sensilla), mean (not defined glands) and large openings (luminescent glands); the location on prosome of luminescent gland openings was exhaustively described and these structures have been divided into three groups: the first one includes 10 openings located in the anterior region, the second is composed by 1 pair of openings for each side in the middle-hind region; finally, the third group includes 6 openings located in the hind region; luminescent glands are present also in the last thoracic segment, one on the left and two on the right side. The above mentioned arrangement has been confirmed by SEM, at least as concerns lateral openings; further studies will attempt to furnish scanning images also of dorsal structures. Nevertheless, the occurrence of other openings has been indicated.

It's noticeable that, as the above-mentioned luminescent and diel migrating species (Gruner, 1994), also Metridia gerlachei is a species living in low-light waters (Carli et al., 1990, 1992a) and its occurrence in surface waters during the Antarctic summer is highly restricted by the nearly constant light conditions. In this connection, luminescent structures could have an adaptive significance both as concerns reproductive strategies and defence behaviour.

References
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