



Coccomyxa sp. (Chlorophyta: Chlorococcales), a new pathogen in mussels (*Mytilus galloprovincialis*) of Vigo estuary (Galicia, NW Spain)

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ARTICLE INFO

Article history:

Received 20 March 2009

Accepted 11 August 2009

Available online 15 August 2009

Keywords:

Coccomyxa sp.

Green algae

Mytilus galloprovincialis

Galicia

Spain

ABSTRACT

In this work, we describe the occurrence of irregular shaped green aggregations in the mantle, gill filaments, adductor muscle, visceral mass and haemolymph of wild mussels (*Mytilus galloprovincialis*) collected from the Vigo estuary (Galicia, NW Spain). Microscopic examination of these masses revealed that they consist of intracellular green algae which are spherical to oval in shape, 5 µm in length and 3 µm in width, without flagella and with a smooth surface. The algal cells present a small single nucleus, a mitochondrion, 1–2 parietal chloroplasts and lack pyrenoids. Reproduction is by formation of 2–4 autospores or daughter cells. Pigment analysis reveals the presence of photopigments typical of green algae in addition to alloxanthin, diadinoxanthin and diatoxanthin. These carotenoids are noted for the first time in a parasitic chlorophyte. The signs of infection, together with the morphological observations, suggest that this parasitic algae may be *Coccomyxa parasitica*. However, further molecular studies are required for confirmation. This is the first report of *Coccomyxa* algae parasitizing the species *M. galloprovincialis*.

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1. Introduction

Although rare, algal pathogens have been associated with a wide variety of terrestrial and marine host taxa (Joubert and Rijkenberg, 1971; Rogers et al., 1980; Morse et al., 1981; Goldberg et al., 1984; Jones et al., 1997; Hightower and Messina, 2007). In bivalve molluscs, Wiborg (1946) reported Norwegian horse mussels *Modiolus modiolus* with a green mantle. The green colouration was caused by a small, green flagellate, approximately 12 µm in diameter, which was not described further. Naidu and South (1970) and Naidu (1971) described green mantle-tissues in scallops (*Placopecten magellanicus*) from Newfoundland. The green colour was caused by a unicellular, 1.5–5 µm diameter, round to ovoid alga, living as a parasite in the scallop. Recently, in south Norway, Mortensen et al. (2005) reported the presence of a picoeukaryote alga in the mussel *Mytilus edulis*.

Mussel farming is one of the main aquaculture activities in Galicia, ranking Spain as the second world producer after China and the first in volume of production for human consumption (Franco Leis, 2006). This is why the study of parasites and other pathologies in *Mytilus galloprovincialis* is of vital importance, not only from the scientific point of view, but also for economic reasons. In spite of the numerous studies on parasites and

parasite-induced pathologies affecting *M. galloprovincialis*, the occurrence of parasitic algae has never been described before.

In this work, we describe for the first time the presence of a parasitic chlorophyte belonging to the genus *Coccomyxa* infecting the mussel *M. galloprovincialis*.

2. Materials and methods

2.1. Mussel sample

One thousand wild mussels whose total length ranged between 31 and 65 mm, with an average height of 47.07 ± 5.94 mm, were collected between March and April 2006 from the middle and lower intertidal zone in the Vigo estuary (42° 17'N–8° 45'W).

2.2. Microscopy

For histology studies, the mantle of mussels infected with green algae were dissected under stereomicroscope and, later, the tissues were fixed in Davidson's fixative, embedded in paraffin, and the sections stained with haematoxylin and eosin (H&E).

For electron microscopy, all samples were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer at pH 7.2 and post-fixed in 1% osmium tetroxide in the same buffer. For transmission electron microscopy (TEM), the mantle fragments of 10 mussels containing green pustules were then dehydrated in increasing strength

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acetone series, embedded in an Epon–Araldite mixture and sectioned with a diamond knife using an ultramicrotome Reichert Ultracut S. The ultrathin sections were stained with uranyl acetate and lead citrate prior to examination with a Philips CM20 TEM. For scanning electron microscopy (SEM), green pustules were homogenized and the resulting suspension filtered through a cellulose acetate membrane (0.45 μm) (Iglesias et al., 2001). After fixation, the filters containing the recovered algae were dehydrated in ethanol series, critical-point dried and gold coated with a sputter coater, and observed and photographed with a Philips XL30 SEM.

2.3. Pigment analysis

In order to determine pigment composition, pigment extraction was conducted by adding 2 ml of 95% methanol to the lyophilized material and, then, the sample was homogenized using a pipette tip adapted to fit the shape of the vial. Pigment profiles were analyzed by high performance liquid chromatography (HPLC) using a Waters Alliance System, a 996 Waters photodiode array detector and a Waters Symmetry C8 column (150 $\times$ 4.6 mm, 3.5 mm particle size, 100 Å pore size). Marker pigments were analyzed and identified by spectrum and retention time (Zapata et al., 2000).

3. Results

Sixty-eight mussels (overall prevalence 6.8%) showed spherical or subspherical green¹ masses of different sizes, some of them with diameters greater than 2 mm (Figs. 1 and 2), in the gill filaments, haemolymph, adductor muscle, visceral mass and particularly in the mantle. Fresh and SEM observations showed that these masses were formed by unicellular green algae that were round to oval in shape, 5 μm in length and 3 μm in width, with smooth surface and without flagella (Figs. 3 and 4). The histological studies showed that the green masses consist of granulomatous-like foci (Fig. 5) but without apparent host response around algal aggregation (Fig. 6).

In several heavily infected mussels eroded areas were detected in regions of the inner side of the shell just in contact with the green pustules (Fig. 7). In addition, we observed a slight deformation in shell margin and a greater shell fragility at the insertion level of the adductor muscle (Fig. 8).

TEM studies confirmed that algae are intracellular (Fig. 9A). The algal cell exhibits a small single nucleus, a mitochondrion, 1–2 parietal chloroplasts and lacks pyrenoids (Fig. 9B). Reproduction is by formation of 2–4 autospores or daughter cells (Fig. 9C and D).

The morphological characteristics confirm that the parasitic algae described herein belongs to the genus *Coccomyxa* (Chlorophyta: Chlorococcales) (see Table 1 for comparison with other congeneric algae parasitizing bivalves).

No significant differences were observed in size–frequency distribution for infected and non-infected mussels ($t=1.118$, $\text{df}=6$; Fig. 10). About 56% of infected hosts ranged in length from 41 to 50 mm, whereas mussels longer than 60 mm were not found parasitized. Prevalence was below 8% in all size classes except for the smallest one (31–35 mm), where it reached 33% (Fig. 10).

Pigment profile of *Coccomyxa* sp. consisted of two chlorophyll components (chlorophyll *a* and chlorophyll *b*) as well as the carotenoids neoxanthin, violaxanthin, lutein, zeaxanthin, and β , β -carotene, all of them typical of green algae. In addition, we detected for the first time in a parasitic chlorophyte the carotenoids alloxanthin, diadinoxanthin and diatoxanthin (Fig. 11, Table 2).

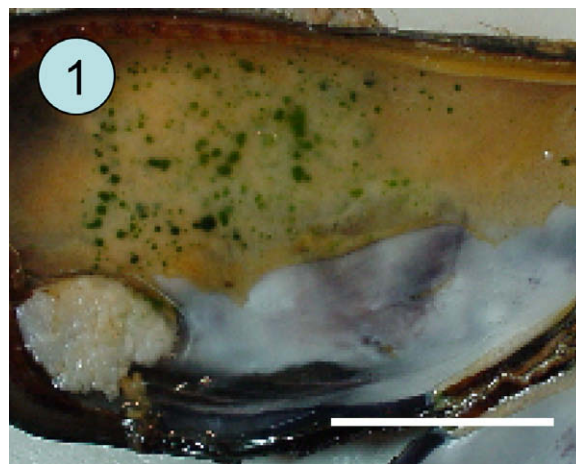


Fig. 1. Macroscopic aspect of *Mytilus galloprovincialis* mantle infected with *Coccomyxa* sp. Scale bar = 1 cm.

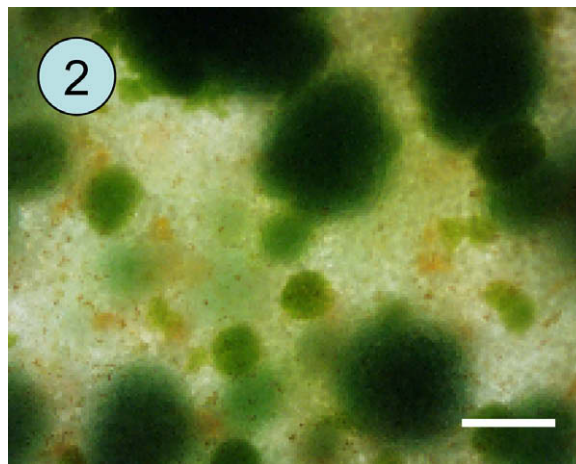


Fig. 2. High magnification of algal infection in the mantle of *M. galloprovincialis*. Scale bar = 1 mm.

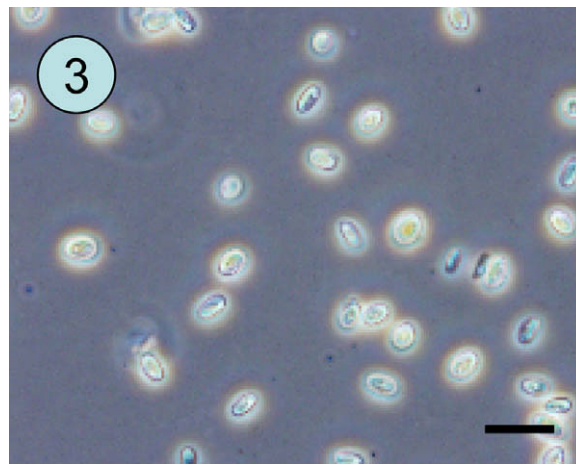


Fig. 3. Algal cells of *Coccomyxa* sp. showing round to oval form and green colour. Scale bar = 10 μm .

4. Discussion

Naidu and South (1970) and Naidu (1971) reported the presence of green algae, in Newfoundland, in *P. magellanicus*, which

¹ For interpretation of colour in Figs. 1–8, the reader is referred to the web version of this article.

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