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Ultrastructural description of an unidentified apicomplexan oocyst containing bacteria-like hyperparasites in the gill of *Crassostrea rizophorae*

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ABSTRACT: Oocysts of an unidentified coccidian are reported in this study to parasitize the gills of the oyster *Crassostrea rizophorae* (Mollusca, Bivalvia) collected near the city of Recife (Itamaracá Island, 07° 38′ 00″ S, 34° 48′ 06″ W), Brazil. Oocysts appeared as light and dense forms, both containing rod-shaped, bacteria-like hyperparasites (BL). Both light and dense oocysts were spherical, 4.3 to 4.7 µm in diameter, but denser oocysts had irregular contours. Both forms consisted of a thick dense wall (~165 nm thick) consisting of 3 layers. The outermost, a dense and irregular layer about 25 nm thick, possessed numerous bead-like structures and some slender conical projections (up to 1.5 µm long). The inner layer of the wall was formed by a dense and homogenous layer about 125 nm thick. Between these 2 layers, a thin light layer about 12 nm thick was present. Uninucleated sporocysts occupied the internal space of the oocyst and contained some rod-shaped BL and mitochondria surrounded by numerous ribosome-like particles. The dense forms of the oocysts showed the same structures described in the lighter forms and appeared to be the final maturation form of the oocysts. Free sporozoites were occasionally observed among oocysts.

KEY WORDS: Ultrastructure · Unidentified · Oocyst · Coccidian · Bacteria-like hyperparasite · *Crassostrea rizophorae*

INTRODUCTION

Parasitic and symbiotic microorganisms have been reported in a range of marine animals, mainly in mollusc bivalves, which are of considerable economic importance (Lauckner 1983, Bower et al. 1994). The major disease-causing agents found in bivalve tissues are bacteria, rickettsiae, mycoplasmas and protozoan species, all of which can sometimes cause the death of the host (Lauckner 1983, Elston & Peacock 1984, Azevedo & Villalba 1991, Azevedo 1993, Bower et al. 1994, Renault & Cochennec 1995, Chen et al. 2000, Hine & Diggles 2002). Of the protozoans, coccidian parasites have been found in different organs of aquatic animals, causing significant pathology and mortalities (Lauckner 1983, Bower et al. 1994, Gestal et al. 1999). However, little information about parasitoses and associated mortalities are available on molluscan aquatic fauna from the Brazilian Atlantic coast (Azevedo & Matos 1999, Padovan et al. 2003, Azevedo & Padovan 2004).

This paper describes some light microscopic and ultrastrucutral aspects of 2 forms of unidentified coccidian oocysts containing rod-shaped bacteria-like hyperparasites (BL) that were occasionally found in Brazilian oysters.

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MATERIALS AND METHODS

Oysters *Crassostrea rizophorae* Guilding, 1826 (Mollusca, Bivalvia) were collected from Itamaracá Island (07° 38′ 00″ S 34° 48′ 06″ W), near the city of Recife on the Northeastern Atlantic coast of Brazil. This work is part of a health program concerned with the aquatic fauna of this region. Small fragments of parasitized fresh gills containing numerous oocysts were observed under a light microscope and photographed using Nomarski differential interference-contrast (DIC) optics. Measurements were made directly on living oocysts or by means of photomicrographs obtained from living oocysts. For transmission electron microscopy (TEM), small fragments of the infected gills were fixed in 3.0% glutaraldelyde in 0.2 M sodium cacodylate buffer for 10 h at 4°C, washed overnight in the same buffer at 4°C, and then postfixed in buffered 2.0% osmium tetroxide for 3 h at the same temperature. After dehydration in a graded ethanol series and propylene oxide, the fragments of infected gills were embedded in Epon. Semithin sections were stained with methylene blue-Azur II and the ultrathin sections were double-stained with uranyl acetate and lead citrate, and observed in a JEOL 100CXII TEM, operated at 60 KV.

Infected gill fragments from oysters were aseptically removed and incubated in the filtered seawater at 20°C, without antibiotics, for 7 to 10 d in order to observe whether oocysts become liberated from the surrounding tissues.

RESULTS

Light microscopic studies

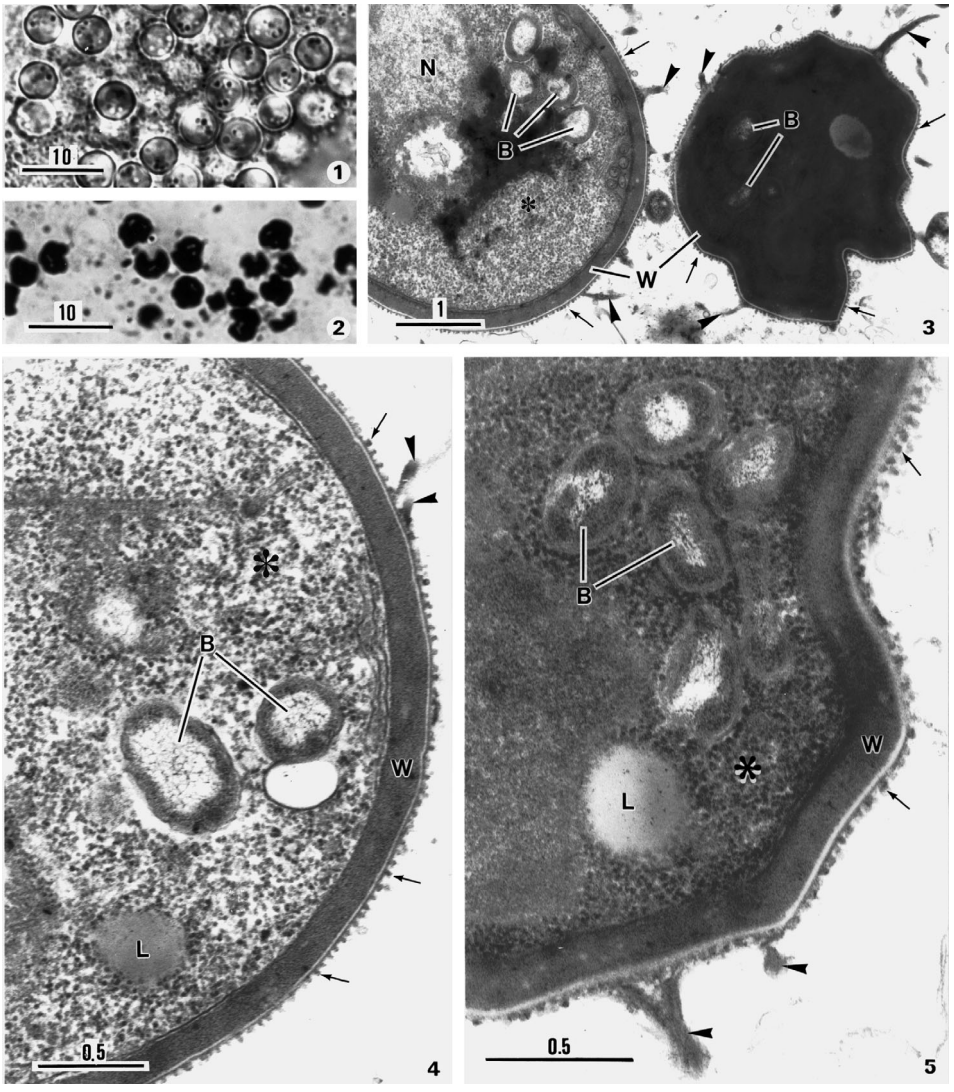
A protist represented by walled oocysts in the gill of *Crassostrea rizophorae* was observed in fresh squash preparations under light microscopy (Fig. 1). Isolated or grouped oocysts were irregularly dispersed in the sub-epithelial gill tissues (Figs. 2 & 3). In semithin sections it was observed that some irregular oocysts appeared denser (Fig. 2). Each oocyst, measuring 4.3 to 4.7 µm (n = 50) in diameter, contained a single uninucleated cell (Fig. 3). Seven of 50 (14%) oysters examined contained oocysts.

Electron microscope studies

The oocysts were represented by 2 forms, each with a different electron density (Figs. 3 to 9). The lighter forms were spherical and all oocyst components were easily observed (Figs. 3 & 4), the denser forms possessed the same components, but displayed an irregular outline and were rare (Figs. 3, 6 to 9). Both types of oocysts were formed by a wall and contained a uninucleated sporozoite (Fig. 3). Sections revealed that the oocyst wall, about 160 (153 to 169) nm (n = 50) thick, was composed of 3 layers (Figs. 3 to 6). The outermost, a dense and irregular layer 25 nm thick, possessed numerous bead-like structures surrounding the oocyst wall. These structures were regularly distributed at the periphery and were easily seen in transverse (Figs. 4 & 5) and tangential sections of the wall. Some slender projections with a conical-like configuration emerged among the small bead-like structures. Each slender conical projection, measuring up to 1.5 µm long were formed longitudinally by microfibrils (Fig. 5). Surrounding these bead-like structures was a very fine network of anastomosed microfibrils (Fig. 5). These 2 kinds of wall ornaments were continuous and appeared to originate from the outer layer of the oocyst wall (Figs. 3 to 5). The inner layer of the oocyst wall consisted of a dense homogenous layer, about 125 nm thick, that was in contact with the internal sporozoite (Figs. 4 & 5). Between the outer and inner layers was a thin light layer about 12 nm thick (Figs. 3 & 4).

In both forms, some rod-shaped bacteria-like hyperparasites (BL) were observed in direct contact with the cytoplasm of the oocysts. In ultrathin serial sections a maximum of 9 BL per oocyst (more frequently 5 to 7) were counted. The double membrane envelope of BL resembles that of a Gram-negative type. The interior was occupied by a lighter zone with fibrillar material, which probably corresponds to DNA (Figs. 3 & 4). Other cytoplasmic structures were mitochondria with several cristae, not well evident, possibly due to incomplete post-fixation and deficient osmium tetroxide penetration (Fig. 7). Mitochondria were ellipsoidal, and in favorable sections the largest were 1.5 to 1.8 × 0.3 to 0.6 µm (Figs. 6 & 9). In all ultrathin sections mitochondria were generally observed to be grouped, 4 to 6 in number (Fig. 6). Mitochondria were surrounded by numerous ribosome-like structures, regularly disposed around the external mitochondrial membranes (Fig. 9). The matrix of the oocyst cytoplasm was occupied by numerous ribosome-like granular masses (Figs. 4 to 7), among which some lipid droplets and some light circular areas were observed (Figs. 4 & 5).

After 7 to 10 d of incubation in filtered seawater, some of the oocyst wall ornamentations (mainly the slender conical projections)disappeared and the oocyst wall appeared less dense, becoming lighter thanks to the appearance of several small light areas (Fig. 10). On the other hand, some free uninucleated sporozoites devoid of any wall and containing BL were observed among the oocysts (Fig. 10). No other developmental stages were observed.

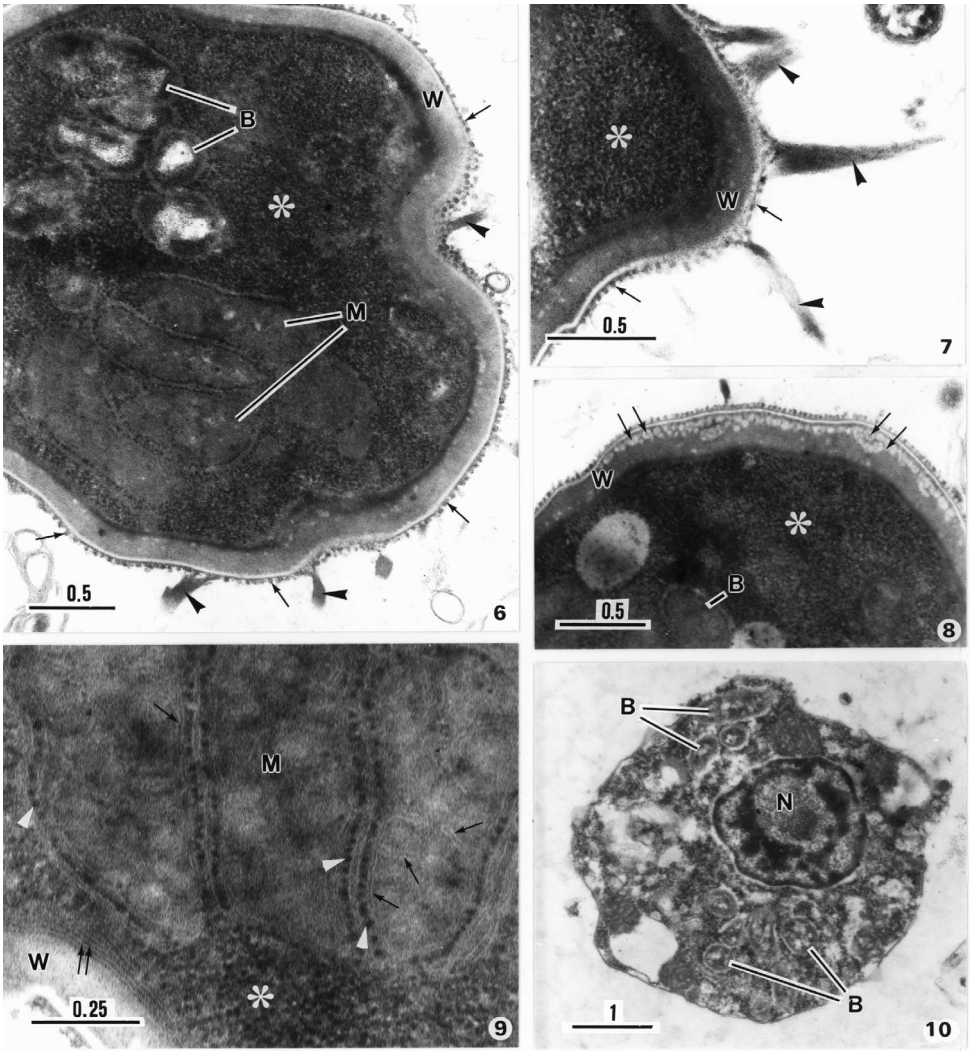


Figs. 1 to 5. Unidentified coccidian oocysts (UCO) from the gill of the oyster *Crassostrea rizophorae.* All scale bars in µm. Fig. 1. Several spherical UCOs observed by differential interference-contrast (DIC). Fig. 2. Semithin section of a group of irregular dense forms of UCO. Fig. 3. Ultrathin sections showing the ultrastructural organization of the 2 similar forms observed in Figs. 1 & 2. Features include a wall (W) with some external ornaments (slender conical projections: arrowheads; numerous bead-like structures: arrows), bacteria-like hyperparasites (B) and nucleus (N), which are located in the oocyst cytoplasm (✻). Fig. 4. Ultrathin section of a light form of UCO showing the wall (W) and the ornaments (bead-like structures: arrows; slender conical projections: arrowheads) and the cytoplasmic bacteria-like hyperparasites (B), lipid droplets (L) among numerous ribosome-like structures (✻). Fig. 5. Ultrastructural detail of the periphery of a dense form of the UCO (✻) showing the wall (W) and their ornaments (bead-like structures: arrows; slender projections: arrowheads) and some bacteria-like hyperparasites (B)

A semischematic drawing of the coccidian oocyst obtained by observation from ultrathin sections is shown in Fig. 11.

DISCUSSION

Ultrastructural observations made during previous studies of the oyster *Crassostrea rizophorae* parasitized by *Nematopsis mytella* (Padovan et al. 2003) appear similar to the present coccidian species. Coccidian species are parasites of many hosts and infect different kinds of cells (Bower et al. 1994, Azevedo & Matos 1999, Gestal et al. 1999, Scippa et al. 2000, Azevedo 2001). However, the present coccidian could not be identified because of the lack of different life cycle stages. The oocyst wall and the internal organization are similar to the oocysts of some coccidian species (Gestal et al. 1999).



Figs. 6 to 10. Unidentified coccidian oocysts (UCO) from the gill of the oyster *Crassostrea rizophorae* and a free sporozoite. All scale bars in µm. Fig. 6. Ultrastructure of an oocyst showing the wall (W) and the ornaments formed by numerous bead-like structures (arrows) and conical-like slender projections (arrowheads), mitochondria (M) and bacteria-like hyperparasites (B) among numerous ribosome-like structures (*). Fig. 7. Ultrastructural detail of the oocyst periphery (*) showing the wall (W) and the ornaments constituted of numerous bead-like structures regularly distributed (arrows) and some conical slender projections (arrowheads). Fig. 8. Ultrastructural aspects of part of an oocyst (*) after 7 d of incubation, showing the wall (W) with several light areas (arrows) and a bacteria-like hyperparasite (B). Fig. 9. Ultrastructural detail of mitochondria (M) showing some cristae (arrows), surrounded by numerous ribosome-like particles (white arrowheads). Stacks of membranous structures are visible (double arrows) in contact with the wall (W). * represents the oocyst. Fig. 10. Ultrastructural aspects of a free sporozoite without the wall, showing the nucleus (N) and some cytoplasmic bacteria-like symbionts (B)

The 2 oocyst forms, light and dense, appear to represent sequential phases of the maturation process during which the lighter form becomes dehydrated, denser and more irregular. On the other hand, the forms devoid of a wall, possibly a sporozoite, seem to represent a free cell liberated from oocysts.

Endocytobionts and endonucleobiontic bacteria or microorganisms have been described in different animal groups (Elston & Peacock 1984, Lobo-da-Cunha & Azevedo 1988, Azevedo 1989). In some observations, the endobacterial activity apparently does not cause any significant damage to the host (Lobo-da-Cunha & Azevedo 1988), while in other hosts it appears to contribute to the death of the host cell (Azevedo 1989).

In the present observation, the bacteria-like hyperparasites appear in the cytoplasm of the 2 forms of oocysts and in free cells (sporozoites). These hyperparasites seem to live in the cell for a long period of time, suggesting a parasitic relation.

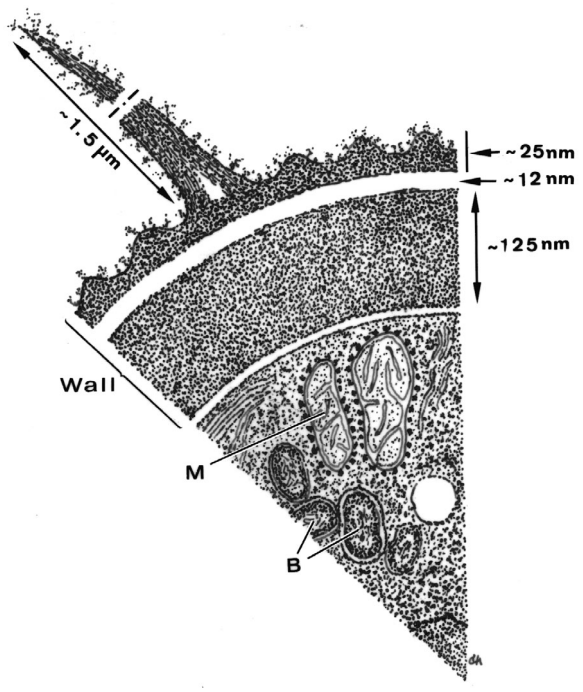


Fig. 11. Semischematic drawing of the unidentified coccidian oocyst showing the ultrastructural organization of the wall, mitochondria (M) and bacteria-like hyperparasites (B), as described in the text and illustrated in electron micrographs

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LITERATURE CITED

Azevedo C (1989) Fine structure of endonucleobiotic bacteria in gill epithelium of *Ruditapes decussatus* (Mollusca, Bivalvia). Mar Biol 100:339–341

Azevedo C (1993) Occurrence of an unusual branchial mycoplasma-like infection in cockle *Cerastoderma edule* (Mollusca, Bivalvia). Dis Aquat Org 16:55–59

Azevedo C (2001) Fine structure of sporogonic stages of *Goussia clupearum* (Apicomplexa: Eimeriidae) in the liver of infected fish (*Belone belone* L.), using light and electron microscopy. Parasitol Res 87:326–330

Azevedo C, Matos E (1999) Description of *Nematopsis mytella* n. sp. (Apicomplexa), parasite of the mussel *Mytella guyanensis* (Mytelidae) from the Amazon estuary and description of its oocysts. Eur J Protistol 35:427–433

Azevedo C, Padovan I (2004) *Nematopsis gigas* n. sp. (Apicomplexa), a parasite of *Nerita ascencionis* (Gastropoda, Neritidae) from Brazil. J Eukaryot Microbiol 5:214–219

Azevedo C, Villalba A (1991) Extracellular giant rickettsiae associated with bacteria in the gill of *Crassostrea gigas* (Mollusca, Bivalvia). J Invertebr Pathol 58:75–81

Bower SM, McGladdery SE, Price IM (1994) Synopsis of infections diseases and parasites of commercially exploited shellfish. Annu Rev Fish Dis 4:1–199

Chen MF, Yun S, Marty GD, McDowell TS and 5 others (2000) A *Piscirickettsia salmonis*-like bacterium associated with mortality of white seabass *Atractoscion nobilis*. Dis Aquat Org 43:117–226

Elston RA, Peacock MG (1984) A rickettsiales-like infection in the Pacific razor clam, *Siliqua patula*. J Invertebr Pathol 44:84–86

Gestal C, Pascual S, Corral L, Azevedo C (1999) Ultrastructural aspects of the sporogony of *Aggregata octopiana* (Apicomplexa, Aggregatidae), a coccidian parasite of *Octopus vulgaris* (Mollusca, Cephalopoda) from NE Atlantic coast. Eur J Protistol 35: 417–425

Hine PM, Diggles BK (2002) Prokaryote infections in the New Zealand scallops *Pecten novaezelandiae* and *Chlamys delicatula*. Dis Aquat Org 50:137–144

Lauckner G (1983) Diseases of Mollusca: Bivalvia. In: Kinne O (ed) Diseases of marine animals, Vol 2. Biologische Anstalt Helgoland, Hamburg, p 477–961

Lobo-da-Cunha A, Azevedo C (1988) Association between xenosomes and glycogen in the cytoplasm of the ciliate *Ichthyophthirius multifiliis*. Endocyt C Res 5:225–231

Padovan IP, Tavares LA, Corral L, Padovan PA, Azevedo C (2003) Fine structure of the oocyst of *Nematopsis mytella* (Apicomplexa, Porosporidae) parasite of the mussel *Mytella falcata* and of the oyster *Crassostrea rizophorae* (Mollusca, Bivalvia) from the Brazilian Northeastern Atlantic coast. Braz J Morphol Sci 20:121–124

Renault T, Cochennec N (1995) Chlamydia-like organisms in ctenidia and mantle cells of the Japanese oyster *Crassostrea gigas* from the French Atlantic coast. Dis Aquat Org 23:153–159

Scippa S, Ciancio A, Vincentiis M (2000) Observation on an apicomplexan microparasite from the pericardic body of *Ciona intestinalis* L. (Protochordata). Eur J Protistol 36: 85–88

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