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ULTRASTRUCTURAL ANALYSIS OF RICKETTSIA - LIKE ORGANISMS IN THE OYSTER *Crassostrea rizophorae* FROM THE NORTHEASTERN ATLANTIC COAST OF BRAZIL

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ABSTRACT

In this report, we describe the ultrastructural morphology of rickettsia–like organisms (RLOs) present in gill epithelial tissue of the oyster, *Crassostrea rizophorae*, from the estuarine region of the Parnaíba river, on the northeastern Atlantic coast of Brazil. Numerous rod-shaped RLOs formed microcolonies that were located in intracytoplasmic vacuoles up to 85 µm in diameter. These RLOs, which measured about 2 µm x 0.6 µm, had ultrastructural characteristics of prokaryotes that included a plasma membrane and a thin, Gram-negative type cell wall. Some nondividing RLOs had a transversal constriction indicative of binary fission. The disappearance of the apical cilia with concomitant lysis of the gill epithelial cells close to the parasitized cells suggested that the RLOs may be associated with mortality in these oysters. Numerous free RLOs were seen following disruption of the vacuoles during host cell necrosis and degeneration. This is the first description of a Rickettsiales-like organism in the Atlantic fauna of Brazil.

Key words: *Crassostrea rizophorae*, oyster, parasite, rickettsiae, ultrastructure

INTRODUCTION

The agents of disease and pests of mollusc bivalves include viruses, bacteria, rickettsia-like organisms (RLOs), protists and fungi [1-5,7-9,12-14,18,21-24]. Of these, RLOs, which have seldom been taxonomically identified, are reported to infect several aquatic poikilothermic groups, including bivalves [5,7,12,18,19,23,24] and crustaceans [6,8,11,13,17,20].

RLOs are small, pleomorphic, rod-shaped coccoid prokaryotes, most of which are obligate intracellular Gram-negative parasites [7,10,18]. Some RLOs have been associated with diseases in molluscs [18]. A few cases of extracellular or temporarily extracellular survival of rickettsiae have been reported [1,5].

The first RLOs were detected in the bivalve mollusc *Mya arenaria* from the Atlantic coast of the United States of America [15,16], and were later found in marine shellfish from several geographic areas [7,8,11,21,22,24]. Most RLOs have been found in dead hosts collected during investigations of invertebrate mortality [6,10,11,18,23], and have been associated with the mortality syndrome in natural populations [20,23].

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The aim of this work was to describe the ultrastructure of RLOs present in the oyster, *Crassostrea rizophorae*, on the northeastern Atlantic coast of Brazil.

MATERIAL AND METHODS

During a routine parasitological survey, 50 specimens of a natural population of the oyster, *Crassostrea rizophorae* (Mollusca, Bivalvia), were collected in the intertidal zone on the northeastern Atlantic coast of Brazil near the city of Parnaíba (02º 52′ 45″ S/ 41º 40′ 01″ W), in the State of Piauí. Small fragments of the gills were squashed and observed using Nomarski differential interference contrast (DIC) optics to assess the presence of RLOs. Transmission electron microscopy (TEM) was used to examine the ultrastructure of the microorganisms. The infected gill tissues were fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2, for 5-6 h at 4ºC, washed overnight in the same buffer at 4ºC, and post-fixed in 2% osmium tetroxide for 2 h in the same buffer and temperature. After dehydration in an ascending graded ethanol series and propylene oxide (two washes of 2 h), the fragments were embedded in Epon. Ultrathin sections were double stained with uranyl acetate and lead citrate and observed in a JEOL 100 CXII transmission electron microscope operated at 60 kV.

RESULTS

The presence of unusual intracytoplasmic vacuoles was noted in squashed preparations of the ciliated gill epithelium of *C. rizophorae*. TEM showed that these intracytoplasmic vacuoles contained numerous microorganisms identified as rickettsia-like organisms

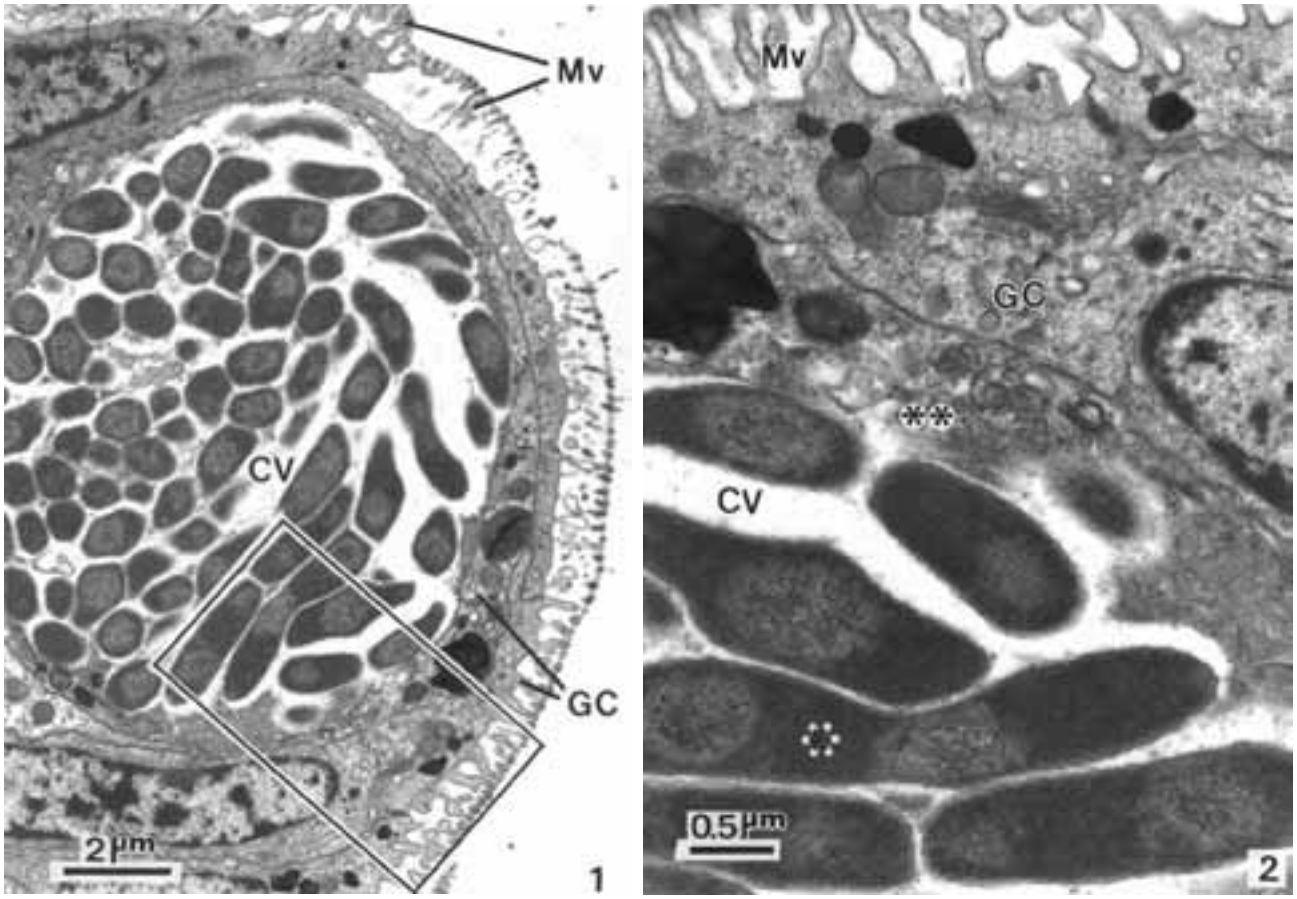


Figure 1. Intracytoplasmic vacuole **(CV)** showing numerous rickettsia-like organisms and external epithelial gill cells **(GC)** containing numerous microvilli **(Mv)**. The boxed area is enlarged in figure 2.

Figure 2 The boxed area of figure 1 showing rickettsia-like organisms, one of which is undergoing binary fission **(*)**, and the intra-cytoplasmic vacuole **(CV)** with lysed **(**)** epithelial gill cells **(GC)**.

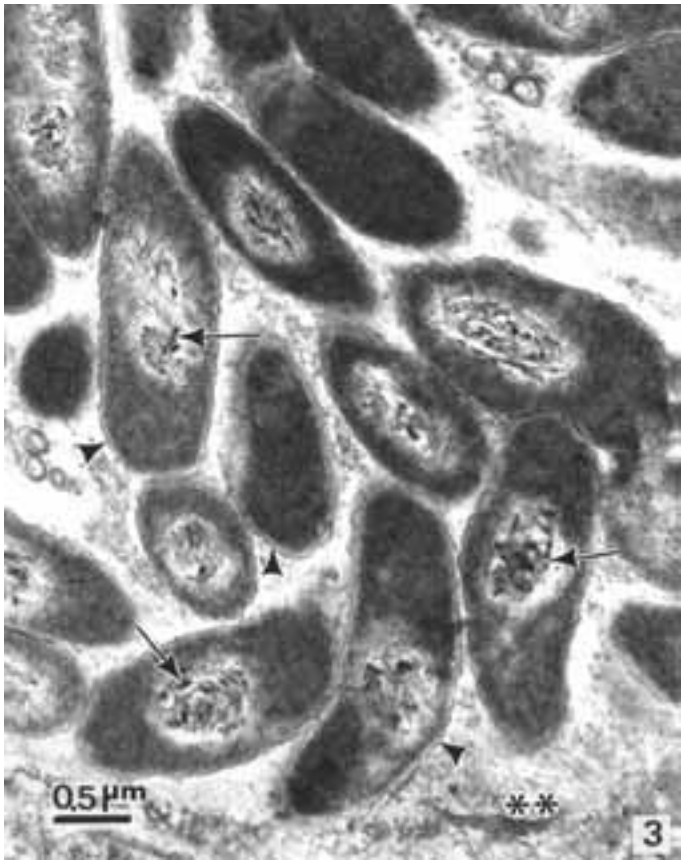
(RLOs) (Fig. 1). Of the 50 oysters examined, only four (8%) contained these vacuoles with RLOs. The RLOs formed microcolonies of ~100 specimens contained in the intracytoplasmic vacuoles of the gill epithelial cells (Figs. 1 and 2). The vacuoles, which were spherical to ellipsoidal (Fig. 1) and up to 85 µm in diameter, were surrounded by a thin layer of the host cell cytoplasm (Figs. 1 and 2), and the host cell nucleus occupied a basal position. The epithelial gill cells had surface microvilli that projected outwards and were occasionally ramified (Figs. 2 and 3) but contained no cilia (Figs. 2 and 5).

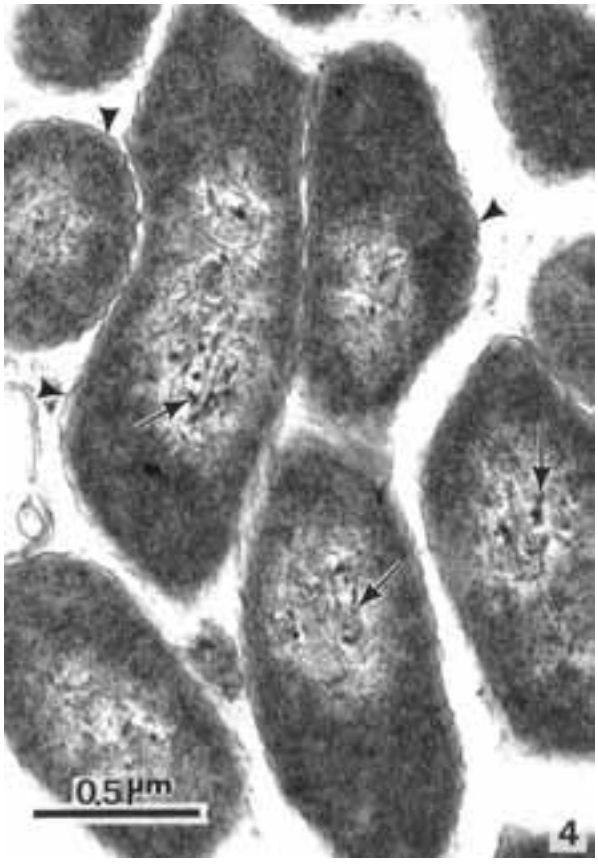
The RLOs were about 2.0 µm long (range: 1.8 – 2.3 µm) and 0.6 µm wide (range: 0.4 – 0.7 µm), had an irregular outer plasma membrane, and a thin cell wall (Figs. 3-5). Some rod-shaped forms (~4.1–4.5 µm long) with two nuclear regions and a transverse constriction were seen in nondividing RLOs (Figs. 2 and 3). In ultrathin sections, DNA strands located in the nucleoid were easily distinguishable as several intermingled dense fibrils in electron–lucent areas (Figs. 3 and 4).

The histopathological changes caused by RLOs in *C. rizophorae* included an increase in the volume of the vacuole and substantial ultrastructural modification of the host cell (Fig. 5). In advanced stages of necrosis, after rupture of the vacuole, numerous free RLOs were seen near the gill epithelial cells where hypertrophied host cells were in contact with normal cells (Fig. 6).

DISCUSSION

The ultrastructural morphology of the microorganisms reported here is the first description for intracellular prokaryotic Rickettsiae (rickettsia–like organisms or RLOs), from the Brazilian aquatic fauna. RLOs differ from bacteria by lacking a true bacterial cell wall [5,7,9,12,18], and only a few cases of extracellular survival have been reported [1,5]. The ultrastructural morphology of the RLOs from *C. rizophorae* was similar to that of RLOs in other bivalves [7,9,12,15,19,22,24], and the binary fission was also similar to that previously described for other prokaryotes [7,18]. Although the life cycle is unknown, the transmission between oysters may be direct [18].





Figures 3 and **4.** Rickettsia-like organisms showing the wall (Gram-negative type) **(arrowheads)** and the internal rickettsial organization with chromatin fibers **(arrows)**.

During the present work, we observed no relationship between the presence of RLOs and the host mortality that sometimes occurred in the laguna region in which the oysters were collected. However, since they are potentially pathogenic to man, RLOs that occur in commercially important bivalve molluscs deserve special attention because some bivalve species are habitually consumed raw [18].

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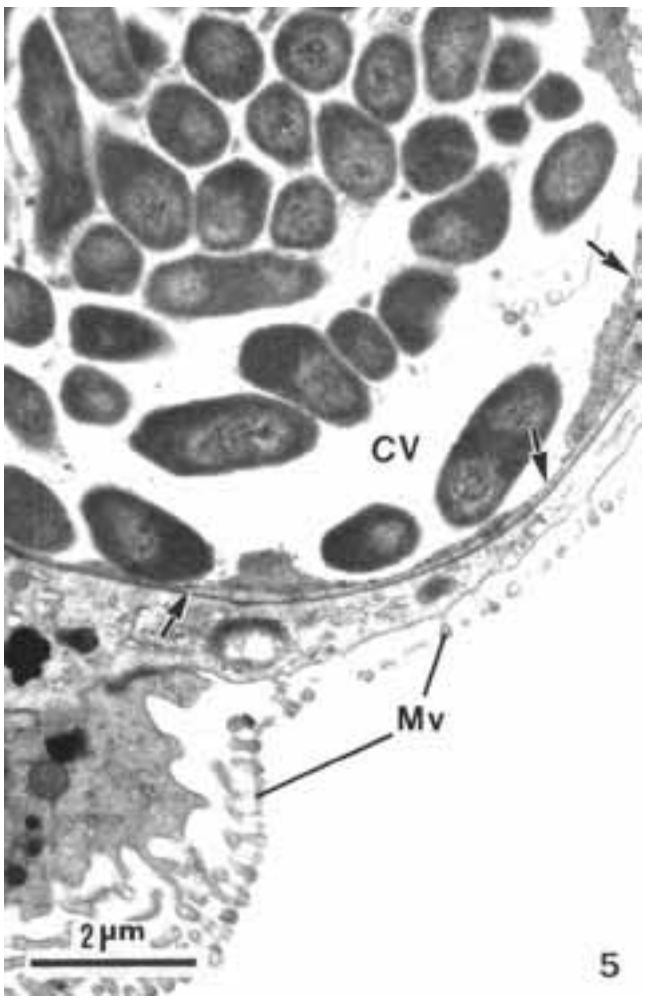

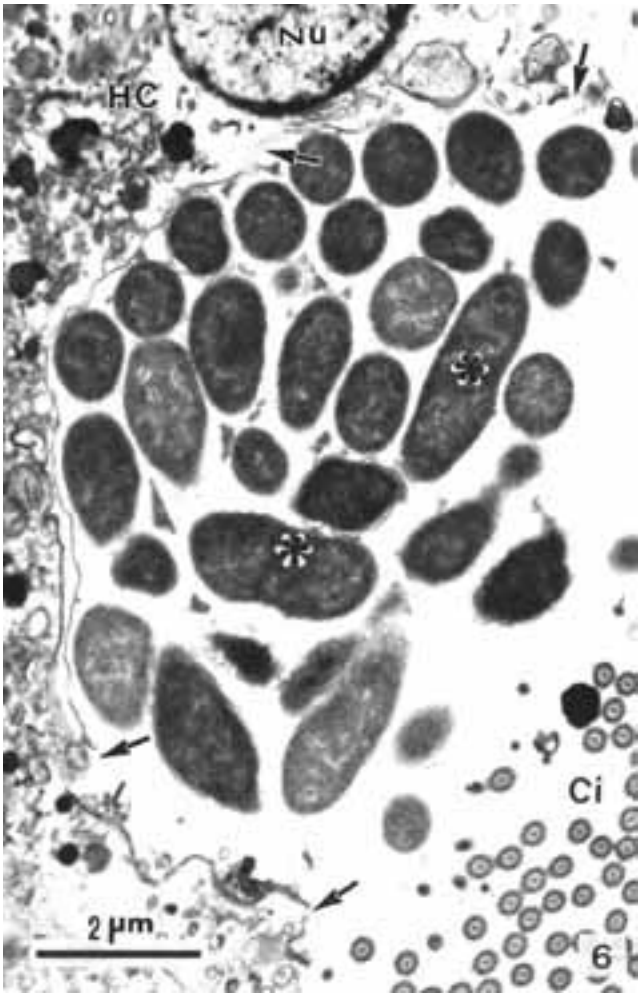

Figure 5. Intracytoplasmic vacuole **(CV)** with several rickettsia-like organisms, some of which are dividing. The thin layer of host cell cytoplasm **(arrows)** shows some signs of lysis. The periphery contains only microvilli **(Mv)**.
Figure 6. Host cell **(HC)** showing advanced lysis characterized by amorphous cytoplasmic structures, rupture of the intracytoplasmic vacuole **(arrows)** and rickettsia-like organisms. Some sections of the cilia **(Ci)**, possibly of adjacent epithelial cells, and some RLOs with signs of binary fission **(*)** are present.

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