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Ultrastructure and Cytopathology of a Rickettsia-like Organism Causing Systemic Infection in the Redclaw Crayfish, *Cherax quadricarinatus* (Crustacea: Decapoda), in Ecuador

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A study of the ultrastructural characteristics of an intracellular bacterium infecting the redclaw crayfish, *Cherax quadricarinatus,* a pathogen referred to previously as a rickettsia-like organism (RLO), revealed the presence of different developmental stages. These included a rod-shaped and uniformly electron-dense elementary body (EB) and an intermediate body (IB). The length of the EB varied between 0.48 and 0.6 μm, and the diameter was 0.3 μm. The IB was 0.75 to 1.1 μm long by 0.36 to 0.44 μm in diameter. Although the EB of this bacterium has ultrastructural characteristics similar to those of *Rickettsiella,* no information is available regarding its genetic relationship to this genus, and the intracellular bacterium should continue to be referred to as a rickettsia-like organism. The hemocytes had different levels of infection, and the RLO proliferated inside these cells. The EB appeared to be free in the cytoplasm of infected hemocytes and other cells; however, this might be a fixation artifact. The EB was also contained in membrane-bound vacuoles along with the IB. RLO colonies were observed inside small granular cells. No large granular cells were observed in the sections examined; therefore, no data were obtained regarding infection of this type of hemocyte. The fixed phagocytes on the external side of the terminal hepatic arterioles had an activated interrupted layer containing RLO bacteria. Stem cells in the hematopoietic tissue were also infected, and some cells were apparently being released into circulation.

***Key Words: Cherax quadricarinatus;* rickettsia-like organisms; hemocytes; intracellular bacteria; fixed phagocytes; infection.**

INTRODUCTION

The Australian redclaw crayfish, *Cherax quadricarinatus* (von Martens), has potential for aquaculture (Jones, 1990). Consequently, *C. quadricarinatus* has been introduced in many countries worldwide (Medley *et al.*, 1994). This species was introduced in Ecuador in 1994 (Rouse, 1994), where the most extensive development has occurred. Approximately 250 ha of commercial aquaculture ponds distributed in different farms were built in this country between 1995 and 1997 (Romero, 1997a,b).

The disease status of farmed *C. quadricarinatus* has received considerable interest in its native Australia, where several potential pathogens have been discovered and described (Owens *et al.*, 1992; Ketterer *et al.*, 1992; Anderson and Prior, 1992; Eaves and Ketterer, 1994; Edgerton *et al.*, 1994, 1995; Edgerton, 1996a,b; Edgerton and Prior, 1999).

After the introduction of *C. quadricarinatus* in Ecuador a health survey program revealed the presence of a variety of infectious and noninfectious diseases affecting pond-cultured animals (Romero and Jiménez, 1997; Jiménez and Romero, 1997, Jiménez and Romero, 1998a,b,c; Jiménez *et al.*, 1998). Some of the organisms reported in Australia have apparently been translocated with *C. quadricarinatus* and have been observed in Ecuador (Jiménez and Romero, 1997, 1998a,b).

The presence of intracellular bacteria in crustaceans from marine and freshwater environments has been reported in different species of both wild and cultured animals (Federici *et al.*, 1974; Bonami and Pappalardo, 1980; Johnson, 1984; Sparks *et al.*, 1985; Brock *et al.*, 1986; Anderson *et al.*, 1987; Krol *et al.*, 1991; Lightner *et al.*, 1992; Fryer and Lannan, 1994; Owens *et al.*, 1992; Ketterer *et al.*, 1992; Bower *et al.*, 1996; Lightner, 1996; Edgerton and Prior, 1999). Among the pathogens present in *C. quadricarinatus* is an intra-

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cellular bacterium, usually referred to as a rickettsia-like organism (RLO), and reported both in Australia (Owens *et al.*, 1992; Ketterer *et al.*, 1992; Edgerton *et al.*, 1995; Edgerton, 1996b) and in Ecuador (Jiménez and Romero, 1997). Under transmission electron microscopy (TEM) this RLO appears similar in these two countries (Jiménez and Romero, 1997).

On the basis of observations under light microscopy, Edgerton *et al.* (1995) reported that the hemocytes are infected and apparently their response to the pathogen is ineffectual. Owens *et al.* (1992) suggested that this RLO might be a member of *Rickettsiella*, a genus that previously has been reported only once in a freshwater crustacean (Federici *et al.*, 1974) and that has been described mostly in insects (Götz, 1972; Devauchelle *et al.*, 1972; Morel, 1976; Louis *et al.*, 1979; Federici, 1980; Weiss *et al.*, 1984; Henry *et al.*, 1986; Frutos *et al.*, 1994; Adams *et al.*, 1997). No descriptions of the ultrastructure or the developmental stages have been presented in the literature on infected cells in *C. quadricarinatus* (Owens *et al.*, 1992; Ketterer *et al.*, 1992; Jiménez and Romero, 1997).

This paper describes the ultrastructure of the infected hemocytes and cells of other tissues of *C. quadricarinatus* and the developmental stages of this intracellular bacterium. There is an apparent ultrastructural similarity, mainly the elementary body (EB), with some members of the genus *Rickettsiella*. There are, however, significant differences, particularly the intermediate body (IB), from existing species (Götz, 1972; Devauchelle *et al.*, 1972; Federici 1980; Weiss *et al.*, 1984). As further data, such as 16S rRNA sequence or genomic analysis, are lacking and until such information is available, the described bacterium will be referred to as an intracellular bacterium, or as a rickettsia-like organism.

MATERIALS AND METHODS

Juveniles of redclaw crayfish, *C. quadricarinatus*, were obtained from two farms in Ecuador, Pond A from Farm 1 and Pond B from Farm 2, in 1996 by retrieving them from shelters in the ponds or from edges of the ponds. Water supplying both farms came from deep wells and salinity was measured using a hand-held refractometer. During sampling, specimens were weighed in an electronic balance. Ten specimens from each pond were sampled for light microscopy and 3 for electron microscopy, 1 from Pond A and 2 from Pond B.

Davidson's AFA fixative was used to preserve all samples taken for routine histopathological study, and crayfish were processed according to the procedures described by Bell and Lightner (1988). Histological stains used included Mayer Bennet hematoxylin and eosin (H & E), Brown and Brenn tissue Gram stain (Luna, 1968), Ziehl-Neelsen (Luna, 1968), and Macchiavello (Luna, 1968). Specimens selected for electron microscopy were fixed with Karnovsky's fixative (Karnovsky, 1965). Tissues were postfixed in 1% osmium tetraoxide (OsO_4) in 0.1 M sodium cacodylate buffer for 1 h, further processed, and embedded in Spurrs resin (Spurrs, 1969). Sections were cut in a Reichert Ultracute OMU3 Leica microtome at 100-nm thickness, stained with uranyl acetate/70% methanol and lead citrate, and examined with a Philips 301 transmission electron microscope at 80 kV.

RESULTS

Clinical Signs

Affected animals presented a greenish-blue coloration. Locally, field personnel referred to affected animals as "green dwarfs." Animals reacted slowly when handled during sampling and were generally lethargic. The ponds of both farms had been stocked 4 weeks before sampling with 0.1- to 0.5-g juveniles. Sampled specimens for TEM weighed 1.0, 1.0, and 1.5 g, and the salinity of the well water in the farms was 3.5 ppt.

Light Microscopy

Basophilic colonies in some cells, apparently contained in vacuoles, were present in different tissues, indicating a systemic infection. The organs and tissues in which infection was detected under light microscopy included lamina ganglionaris, supraesophageal ganglion, supraesophageal connectives, cuticular epithelium, and connective tissue under the cuticular epithelium. Other tissues affected were the epicardium, pericardium, heart, hematopoietic tissue, the attenuated layer of fibrous connective tissue that constitutes the boundary of the antennal gland, hemocytes between the antennal gland tubules, coelomosac, connective tissue between muscle bands, muscle bands, and arterioles. No infections were observed in the epithelial cells of the hepatopancreatic tubules. The terminal arterioles in the hemolymph sinuses between the hepatopancreatic tubules conserved their architecture but basophilic colonies were present on its external side (Fig. 1). In the hypodermis of the foregut, vacuoles appeared to be released to the lumen of the stomach (Fig. 2). The basophilic colonies were gram negative, Macchiavello positive, and negative for Ziehl-Neelsen.

Electron Microscopy of Infected Cells

Electron microscopy of the affected hepatopancreas showed the presence of massive colonies of RLO; the bacteria were pleomorphic, free in the hemolymph sinuses, and contained in membrane-bound vacuoles in the hemocytes (Fig. 3). The infected hemocytes observed in the hemolymph sinuses between the hepatopancreatic tubules did not have electron-dense granules and the nucleus was reduced in size with margin-

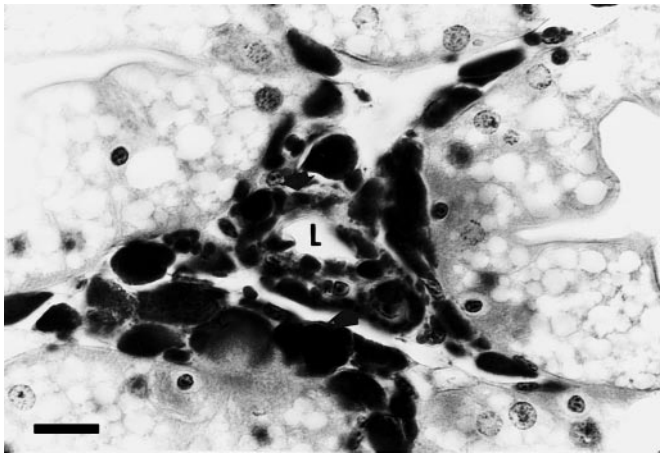

FIG. 1. Infected fixed phagocytes (arrow) on terminal arterioles in the hepatopancreas of *Cherax quadricarinatus*. Note that the architecture of the arteriole is conserved; L, lumen of the arteriole. Bar, 40 μm; H & E stain.

ated chromatin (Fig. 3). In the heavily infected hemocytes no organelles could be observed, and the membrane-bound vacuoles containing RLO reached up to 19 μm in length and 12.8 μm in width (Fig. 3).

Higher magnification of infected hemocytes showed different developmental stages of the RLO (Fig. 4), and the average EB was 0.45 μm long by 0.25 μm wide. A cell in close contact with the infected cell had spindle-shaped electron-dense granules (Fig. 4) and appeared to be a small granular cell (SGC).

In another hemocyte different forms of RLO were scattered in the cytoplasm (Fig. 5). This cell had mitochondria and a well-developed Golgi apparatus (Fig. 5). The cytoplasm in the area where the RLO was present appeared to be necrotic and electron-lucent (Fig. 5). In this cell the presence of an IB could be observed next to the EB.

Other infected hemocytes with spindle-shaped electron-dense granules in the cytoplasm had different developmental stages of the RLO in a membrane-bound, circular vacuole (Fig. 6). Small electron-lucent vesicles that ranged between 10 and 200 nm were scattered throughout the vacuole.

Endothelial cells of the arterioles in the subcuticular connective tissue had massive membrane-bound bacterial colonies (Fig. 7). A similar situation was observed

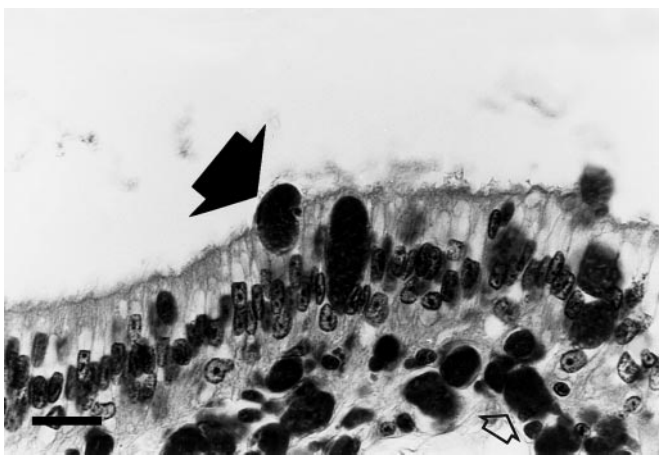

FIG. 2. Foregut columnar epithelium with membrane-bound basophilic colonies of rickettsia-like organisms (arrow) being released to the lumen. Note that the underlying connective tissue is also infected (small open arrow). Bar, 40 μm; H & E stain.

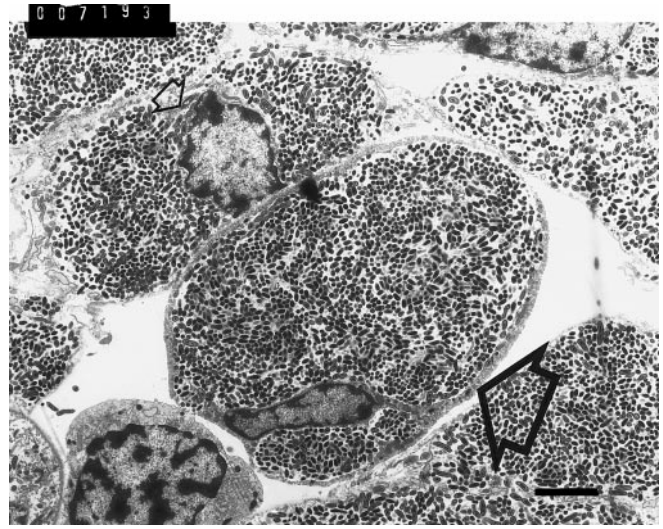

FIG. 3. Infected hemocytes in circulation through the hemolymph sinus between the hepatopancreatic tubules. One cell has a small nucleus (large open arrow) and a bacterial colony in a vacuole; a nearby cell apparently has a lower level of infection (small open arrow). Bar, 3 μm.

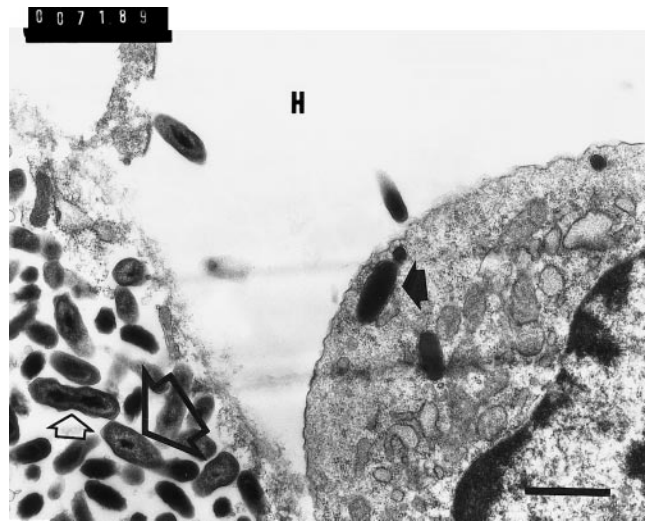

FIG. 4. Spindle-shaped electron-dense body in the cytoplasm of a hemocyte (arrow). Different stages of replication; slight separation of cell membrane (small open arrow), intermediate bodies (large open arrow); hemolymph (H). Bar, 1 μm.

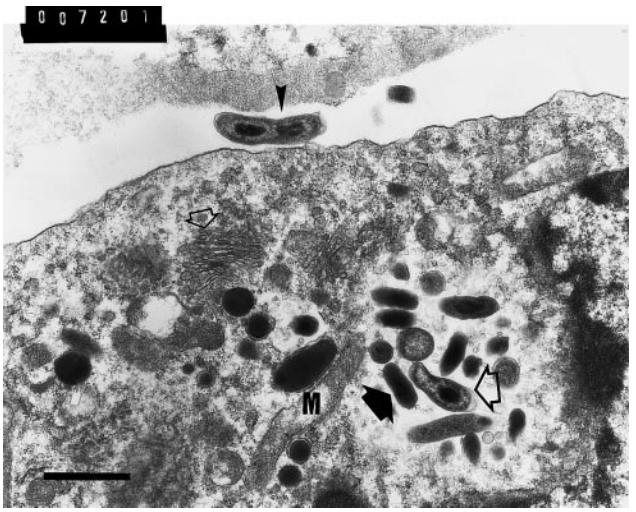

FIG. 5. EB (solid arrow) and IB (large open arrow) free in the cytoplasm of infected hemocyte. Note change of electron density in area where the colony is forming. IB elongated and with central constriction free in the hemolymph (arrowhead). Golgi apparatus (small open arrow); mitochondria (M). Bar, 1 μm.

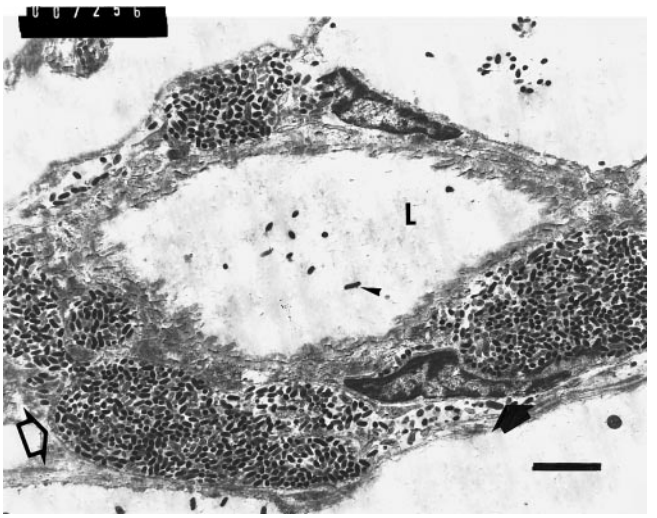

FIG. 7. Arteriole in subcuticular connective tissue infected by bacteria (open arrow). Note displaced nucleus (large arrow). Bacteria (arrowhead) are present in the lumen (L) of the arteriole. Bar, 2.5 μm.

in the hepatic terminal arterioles, but in these arterioles presumptive fixed phagocytes with activated interrupted layers were present and isolated bacteria were observed in the area between the plasma membrane and the interrupted layer (Fig. 8). The presumptive fixed phagocytes had granules of medium electron density, and in some cases bacterial colonies were present in these cells (Fig. 8).

Isolated bacteria were present in the R-cells of the hepatopancreas tubule epithelium, either in the basal area close to the nucleus or near the brush border (Fig. 9). Isolated bacteria were regularly observed in the lumen of the hepatopancreas (Fig. 9). In an area close to the muscles under the subcuticular connective tissue, a small granular cell had a massive bacterial colony which had displaced the nucleus to one side (Fig. 10). The center of this bacterial colony was occupied by EBs and the periphery by IBs (Figs. 10 and 11). The IBs had constrictions in the center of the cells, and small electron-lucent vesicles, approximately 20 nm, were present among the EBs (Fig. 11). Closer examination of other infected SGCs revealed the presence of small electron-lucent vesicles in the spaces between

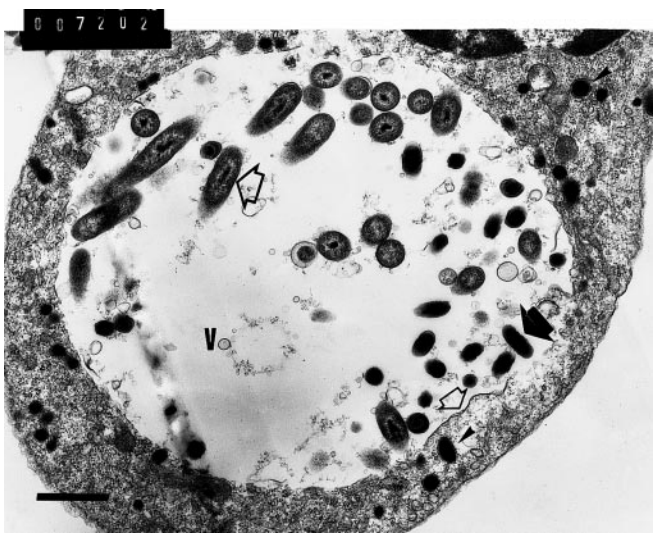

FIG. 6. Hemocyte infected by RLO colony in a membrane-bound vacuole. EB (solid arrow); IB (large open arrow); electron-lucent vesicles (V); membrane (small open arrow); spindle-shaped electron-dense bodies in the cytoplasm (arrowhead). Bar, 1 μm.

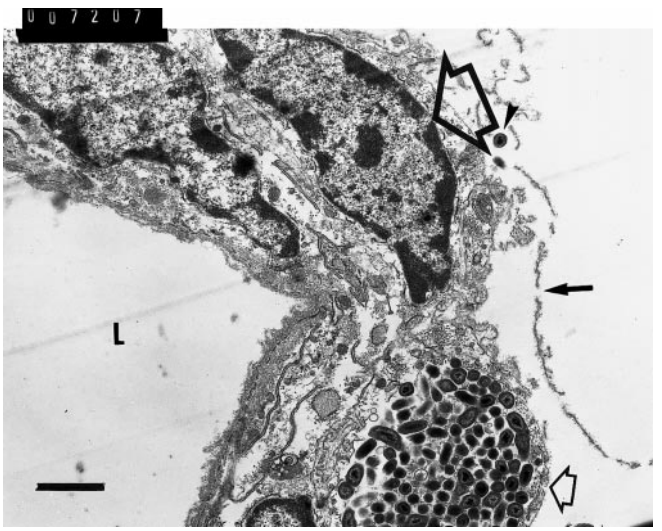

FIG. 8. Presumptive fixed phagocyte (large open arrow) and activated interrupted layer (arrow). Isolated bacterium inside interrupted layer (arrowhead) and bacterial colony in fixed phagocyte nearby (small open arrow); lumen of the arteriole (L). Bar, 1.25 μm.

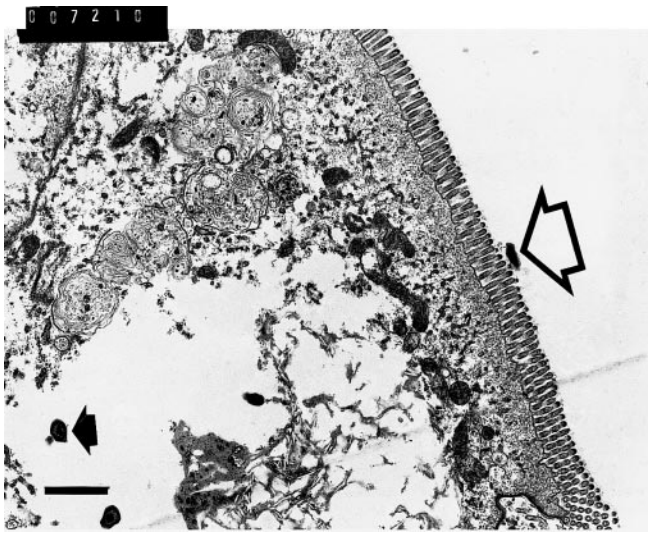

FIG. 9. Isolated EB (solid arrow) near brush border of R-cell in the hepatopancreas. Notice bacterium in the lumen of the hepatopancreas (open arrow). Bar, 1.25 μm.

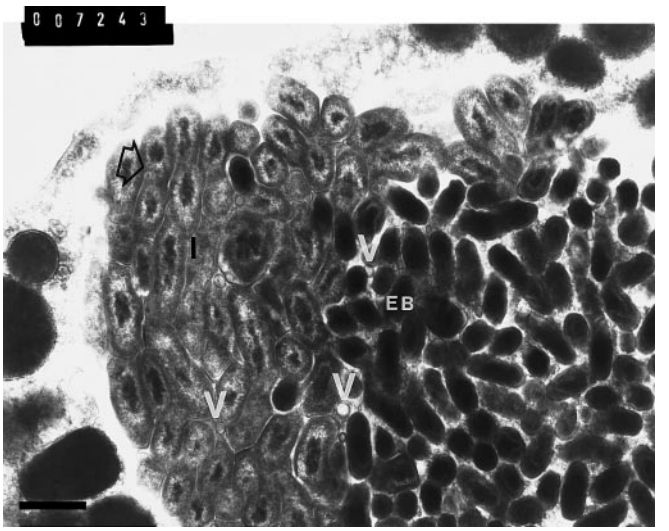

FIG. 11. Higher magnification of Fig. 10. Note that the EBs (EB) and IBs (I) are present in different areas and small vesicles (V) are among the EBs and IBs. An IB is apparently undergoing binary fission (arrow). Bar, 0.5 μm.

IBs; these IBs were apparently replicating free in the cytoplasm (Fig. 12). An area that appeared to contain a phagocytic vacuole was observed next to the IBs, but its contents could not be identified (Fig. 12). Although the EBs were present next to electron-dense granules of the host cell, no degranulation was observed in the SGCs studied (Figs. 11 and 12).

The hematopoietic tissue had bacterial infections with varying degrees of intensity in the stem cells that were characterized by a prominent nucleolus (Fig. 13). In the serial sections observed, some cells were apparently being detached from the hematopoietic tissue and released into circulation.

Epithelial cells of the labyrinth in the antennal gland, characterized by a prominent brush border and numerous mitochondria, had bacterial colonies near the base of the cell (Fig. 14). Beside these cells and in contact with the hemolymph were infected hemocytes (Fig. 14).

Ultrastructural Description of RLO

The EBs appeared rod shaped and uniformly electron dense, measuring between 0.48 and 0.6 μm in

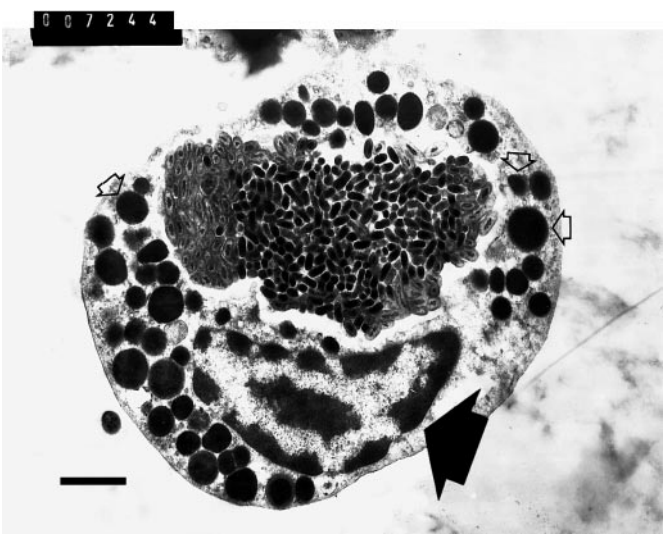

FIG. 10. Small granular cell with massive colony of bacteria. Note displacement of both nucleus (large arrow) and electron-dense granules (open arrow). Bar, 1.5 μm.

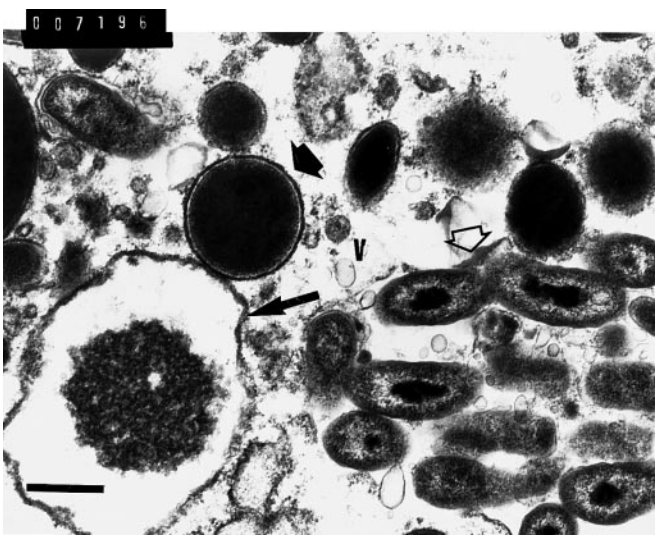

FIG. 12. Cytoplasm of small granular cell. Electron-dense granule (solid arrow). IBs with central electron-dense areas, granular periphery of cells possibly undergoing replication (open arrow), and vesicle (V) present among IBs. A vacuole with unidentified material is also present (long arrow). Bar, 0.5 μm.

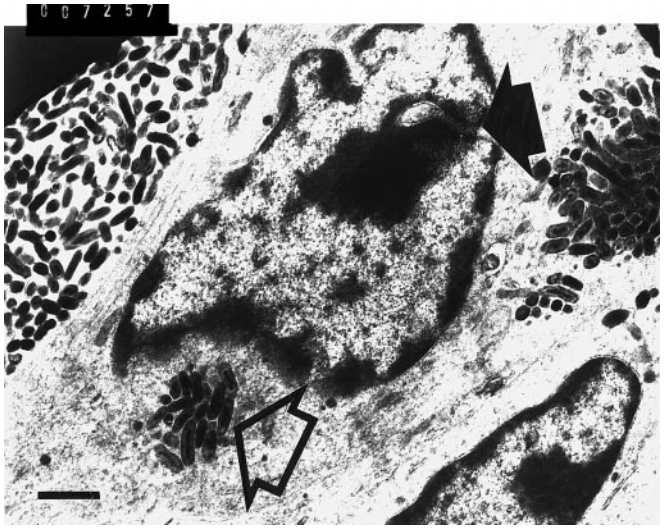

FIG. 13. Infected stem cell in hematopoietic tissue. Note prominent nucleolus (arrow) and bacterial colony with different developmental stages (open arrow). Bar, 1.5 μm.

length and 0.3 μm in diameter and surrounded by a bilayered envelope (Fig. 4). The EBs gave rise to the IBs, which appeared to be the replicative stage; the IBs measured between 0.75 and 1.1 μm in length and between 0.36 and 0.44 μm in diameter (Fig. 4). The IBs were the only cells observed to have elongations and constrictions in the center. An accumulation of electron-dense material in the periphery of the IB was accompanied by a slight separation and undulation of the cell wall, along with a prominent periplasmic space (Fig. 4). The electron density of the whole cell also decreased at this stage (Figs. 4–6).

Electron-dense material was concentrated in the center of the IBs and was observed in cross sections; the electron-dense material in the periphery appeared granular (Fig. 11). Cells apparently undergoing division had an electron-dense center (Fig. 11). No crystalline bodies were observed inside the IBs in the sections studied.

In cells in which both the EBs and the IBs were observed together, these bodies either intermingled (Fig. 8) or were grouped in masses of only EBs or only IBs in separate areas of the same cell (Fig. 11).

DISCUSSION

Light microscopy of the affected animals revealed infections in the same tissues as those described in *C. quadricarinatus* in Australia (Owens *et al.*, 1992; Ketterer *et al.*, 1992; Edgerton *et al.*, 1995; Edgerton, 1996b) and previously in Ecuador (Jiménez and Romero, 1997). However, previous publications have not reported the presence of basophilic colonies in the external areas of the terminal hepatic arterioles, which in this study were probably heavily infected fixed phagocytes (Fig. 1) or entire membrane-bound colonies apparently breaking from the foregut columnar epithelial cells (Fig. 2).

Unlike other intracellular pathogens infecting the hemocytes of crustaceans (Cornick and Stewart, 1968; Stewart *et al.*, 1983; Anderson *et al.*, 1987; Sindermann, 1988; Martin and Hose, 1992; Bower *et al.*, 1996), apparently no phagocytic vacuole was observed in some hemocytes in this study. The EBs and IBs were also observed apparently free in the cytoplasm (Figs. 4 and 5). The response of *C. quadricarinatus* to opportunistic pathogens such as *Vibrio* spp. is the formation of melanized nodules (Edgerton *et al.*, 1995; Edgerton, 1996b), which is similar to the response in other species of crustaceans with bacterial infections (Krol *et al.*, 1989; Lightner, 1996; Bower *et al.*, 1996; Vogt and Rug, 1997). In early reports of the same RLO infecting *C. quadricarinatus* in Ecuador, melanized nodules were observed (Jiménez and Romero, 1997). However, since this report such lesions have been observed only infrequently as the number of sampled animals has increased (X. Romero and R. Jiménez, unpublished).

No evidence of degranulation of SGCs was recognized during this study. This may indicate that the proPO cascade was not being initiated and, since this eventually results in melanosis (Söderhäll and Cerenius, 1992), such lack of degranulation explains the lack of encapsulation and melanization. These findings also agree with previous reports of the presence of infected hemocytes (Owens *et al.*, 1992) and the ineffectual response of these cells to this bacterium (Edgerton *et al.*, 1995).

It is possible that this bacterium either is not recognized as foreign or has an active mechanism to inter-

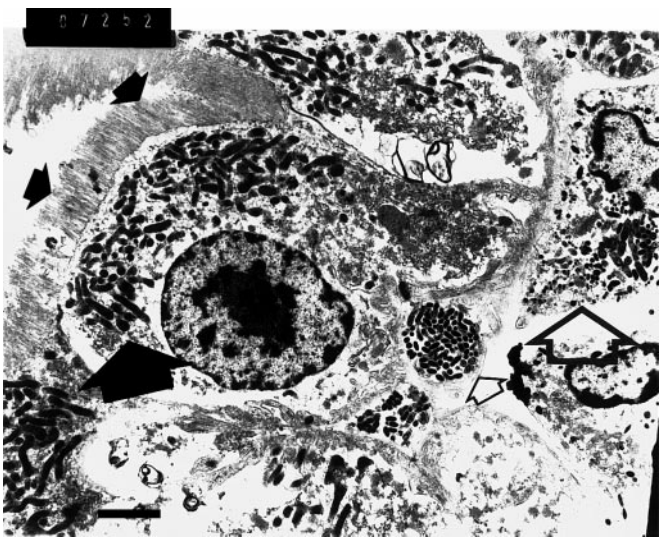

FIG. 14. Infected antennal gland tubular epithelium cell. Notice prominent brush border (small solid arrows) and mitochondria (large solid arrow). Bacterial colony at the base of the cell (small open arrow) and hemocytes nearby (large open arrow). Bar, 2.5 μm.

fere with some of the chemical pathways in the proPO system. Further research should investigate these aspects. This pathogen might also be used as an interesting model to study intracellular pathogens in crustaceans and their invasion strategies. As no large granular cells (LGC) were observed in the sections studied, it is still uncertain whether these cells are also infected by the RLO. Cells with the characteristics of a LGC were observed in other nondiseased crayfish examined under TEM in a separate study. The infected hemocytes had a size three times larger than normal hemocytes observed in noninfected *C. quadricarinatus* (Fig. 3). Members of the genus *Rickettsiella* have been observed infecting the hemocytes of its host (Devauchelle *et al.,* 1972) and existing free in the hemolymph (Federici *et al.,* 1974). Although no membrane was observed surrounding a large RLO colony in a SGC, the EBs and IBs appeared in a tightly packed formation, and it is possible that the membranes surrounding such colonies were damaged during processing (Figs. 10 and 11).

Hemocytes with spindle-shaped electron-dense bodies have been reported in the freshwater crayfish *Astacus astacus* by Vogt and Rug (1997); these authors referred to these cells as semigranulocytes. The spindle-shaped electron-dense bodies were also observed in a hemocyte that also contained larger roundish granules, and these authors referred to this type of cell as a granulocyte (Vogt and Rug, 1997). During our investigation we observed the presence of similar spindle-shaped electron-dense bodies in the cytoplasm of a hemocyte circulating next to a group of infected cells (Fig. 4) and outside a vacuole in another infected hemocyte (Fig. 6). To avoid confusion we referred to these two cells as "hemocytes" and did not classify them as any particular type of hemocyte. We did not recognize the presence of spindle-shaped electron-dense bodies in an infected hemocyte that we referred to as a small granular cell (Fig. 10). As the animals that we studied were undergoing an infective process, changes to the different types of hemocytes might have occurred. Evidently there is a need for more research on the ultrastructure of the subpopulations and types of hemocytes of different species of freshwater crayfish.

The IBs that were probably undergoing binary fission, which were observed free in the hemolymph, either could have originated from colonies in the hemocytes that had recently ruptured or might be indicative of replication free in the hemolymph (Fig. 5). It was not possible to identify whether the EB was the infective stage, as no host cells undergoing early stages of infection were observed or differentiated. Some studies have described the EB as the infective stage of other intracellular bacteria in the genus *Rickettsiella* (Devauchelle *et al.,* 1972; Weiss *et al.,* 1984; Henry *et al.,* 1986).

Among the cells in which the RLO can be found are the R-cells of the hepatopancreas, but infection did not appear to progress within these cells, since only isolated bacteria were observed (Fig. 9). Infections with an ultrastructurally similar RLO have not been reported in the hepatopancreatic tubule epithelium under light microscopy examinations in Ecuador (Jiménez and Romero, 1997). The same or a similar intracellular bacterium causing systemic infection in *C. quadricarinatus* in Australia has also not been reported infecting the R-cells or other cells of the hepatopancreas tubule epithelium (Owens *et al.,* 1992; Ketterer *et al.,* 1992; Edgerton *et al.,* 1995; Edgerton, 1996b). The rickettsia-like organism infecting the epithelial cells of the hepatopancreas of *C. quadricarinatus* recently reported by Edgerton and Prior (1999) would appear to be a different bacterium, based on histological characteristics and tissue tropism.

As many of the cells infected by the RLO were nonphagocytic (i.e., cuticular epithelium, connective tissue), this bacterium probably has a mechanism to actively penetrate and infect host cells. Rickettsiae do not kill their host by production of toxins, but rather by cell destruction (Winkler, 1990). This is consistent with the observations in the present study in which infected hemocytes were filled with the RLO until they ruptured and released the bacterium to the external medium.

Previous publications mention the presence of infected intertubular connective tissue, although no specific cell types were identified (Ketterer *et al.,* 1992; Owens *et al.,* 1992; Jiménez and Romero, 1997). Ultrastructural studies of the hepatopancreas in other decapod crustaceans describe the presence of spaces with hemolymph (i.e., hemal sinuses) between the hepatopancreatic tubules, hepatic terminal arterioles, and fixed phagocytes (Johnson, 1987; Factor and Naar, 1990; Sagrista and Durfort, 1990). These hemal sinuses have been considered as part of a connective tissue matrix consisting of an outer limiting layer, referred to as *tunica propia* by some authors, a basement membrane, contractile cells near the basal lamina of the hepatopancreatic tubules, and contractive muscular fibers surrounding the hepatopancreatic tubules (Factor and Naar, 1985; Sagrista and Durfort, 1990; Mellon, 1992; Icely and Nott, 1992). Electron microscopy work in the present investigation showed RLO infections in circulating hemocytes, the presumptive fixed phagocytes, and endothelial cells of the terminal arterioles (Figs. 3 and 8). Only isolated bacteria were found in the basal lamina, so this area of the intertubular connective tissue would appear not to be the main target of the bacterium.

The presence of the fixed phagocytes in the hepatic terminal arterioles was confirmed by the observation of an interrupted layer (Fig. 8) and as been described by other authors (Johnson, 1987; Sagrista and Durfort, 1990; Factor and Naar, 1990; Field and Appleton, 1995). According to Johnson (1987) the separation of

the interrupted layer from the plasma membrane indicates that it is activated (Fig. 8). The granules observed in the fixed phagocytes of the specimens of *C. quadricarinatus* in this study do not have the same electron density as those reported in *Homarus americanus* (Factor and Naar, 1990). The electron density of *C. quadricarinatus* fixed phagocyte granules was similar to that reported by Vogt (1996) in *Palaemon elegans* and by Johnson (1987) in the blue crab, *Callinectes sapidus*. It has been reported that in different species of decapod crustaceans particles larger than a size of 60 to 80 nm are probably trapped by the fixed phagocytes as they circulate through the hemolymph (McCumber and Clem, 1977; Clem *et al.*, 1984; Johnson, 1987; Factor and Beekman, 1990). Considering the size of the RLO EB (>300 nm), the fixed phagocytes are probably one of the first cells to be infected by freely circulating bacteria. However, only through time course studies will the roles of these cells and of the circulating hemocytes in the defense against the RLO infection in *C. quadricarinatus* be clarified. Changes in the populations of different types of hemocytes during controlled infections were observed in *H. americanus* infected by *Aerococcus viridans* (var.) *homari* (Stewart *et al.*, 1983); it is possible that a similar situation might occur in *C. quadricarinatus* infected by the RLO described in the present study. If the hematopoietic cells and the SGC are getting infected perhaps there are no cells maturing to LGC. Further work to isolate the different types of hemocytes by centrifugation, as described by Söderhäll and Smith (1983), might be attempted to establish the proportions of the three types of hemocytes in normal and infected *C. quadricarinatus*.

C. quadricarinatus has been introduced into different regions of the world (Medley *et al.*, 1994); it is possible that the RLO described in this study may prove to be as significant in these regions as it was in Ecuador, where it was the most significant pathogen of cultured *C. quadricarinatus* (Jiménez and Romero, 1998a). More information regarding this pathogen, including rates of transmission, will be necessary for health control of *C. quadricarinatus*, which is being considered for aquaculture in several areas worldwide.

The developmental stages of the RLO described in this study, mainly the size and shape of the IB, are ultrastructurally different from those described in other intracellular bacteria of the genus *Rickettsiella*, such as *Rickettsiella chironomi* (Götz, 1972; Morel, 1976; Federici, 1980), *R. popilliae* (= *R. melolonthae*) (Devauchelle *et al.*, 1972), strains of *R. grylli* (Henry *et al.*, 1986), or other *Rickettsiella* sp. (Louis *et al.*, 1979; Adams *et al.*, 1997). However, there are similarities with the ultrastructure of a rickettsia-like organism described by Federici *et al.* (1974) in a crangonid amphipod. The main difference is the absence of the elementary bodies in a paracrystalline arrangement in the present RLO. Although there is an apparent ultrastructural similarity, until further investigations using genomic DNA analysis, similar to studies of Frutos *et al.* (1994), are carried out and its relationship to other RLO's are determined, the intracellular bacterium described in this study should be referred to as a rickettsia-like organism. This will also avoid future confusion and corrections regarding the taxonomic status of the described organism.

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