

***Perkinsus* sp. (Alveolata, Perkinsidae) a Parasite of the Clam *Meretrix meretrix* (Veneridae) from Arabian Gulf: Ultrastructural Observations of the Trophozoites and the Cellular Response of the Host**

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Abstract. Genus *Perkinsus* Levine, 1978 (Alveolata, Perkinsidae) an intracellular pathogenic parasite is described from the mantle and gill filaments of a commercially important clam, *Meretrix meretrix*, collected from the Arabian Gulf, Saudi Arabia. This genus contains currently seven named species: *P. marinus*, *P. olseni* (*P. atlanticus*), *P. chesapeakei* (*P. andrewsi*), *P. mediterraneus*, *P. honshuensis*, *P. beihaiensis* and *P. qugwadi*. Meanwhile, some unnamed *Perkinsus* sp. have been described in wide variety of mollusc species. Ultrastructural features of *Perkinsus* sp. trophozoites and the host reaction are described. The different developmental stages of trophozoites appeared as single or grouped cells surrounded by amorphous material that constituted cysts or nodules randomly distributed throughout the connective tissue of the mantle. The early trophozoites were generally spherical to ellipsoidal with a circular nucleus containing a prominent central nucleolus. The cytoplasm had several small vacuoles which coalesce to form a great vacuole in the later trophozoites and the nucleus becomes eccentric. Some lysosomes were observed between the wall and the plasmalemma of trophozoites. A large number of degraded and pyknotic cell and several cellular structure with lysed aspects were encountered in the surrounding area near the cysts. Ultrastructural data showed that the lysed granular cells and the coalescence of the granules result in the cyst that encapsulates various trophozoites. In the current study, we describe for the first time the presence of *Perkinsus* sp. as well as the host reaction in clams from the Saudi Arabian coasts.

Key words: *Perkinsus*, trophozoite, encapsulation, ultrastructure, clam, *Meretrix meretrix*, Arabian Gulf.

INTRODUCTION

Parasites of the genus *Perkinsus* constitute a small group of protists that has been known to cause serious and extensive mortalities worldwide in commercially important species of marine bivalves throughout the world. The systematic position of the genus *Perkinsus* was created as new genus and simultaneously erecting a new species (*P. marinus*) (formerly described as *Dermocystidium marinus* from bivalves in USA) (Mackin *et al.* 1950) which were included in the new phylum Apicomplexa (Levine 1978). Later, it was proposed that this phylum should be transfer to a new phylum, Perkinsozoa (Norén *et al.* 1999) and more recently, in a new extensive taxonomic revision, the genus *Perkinsus* was transferred to the following groups: – Alveolata Cavalier-Smith, 1991; Protalveolata Cavalier-Smith, 1991; and Perkinsidae Levine, 1978 (Adl *et al.* 2012). Since the creation of *P. marinus*, several new species were successively erected in different countries: *P. olsenii* in Australia (Lester and Davis 1981), *P. atlanticus* in Portugal (Azevedo 1989), *P. qugwadi* in Canada (Blackbourn *et al.* 1998), *P. andrewsi* in USA (Coss *et al.* 2001), *P. chesapeakei* in USA (McLaughlin *et al.* 2000), *P. mediterraneus* in Spain, Mediterranean Sea (Casas *et al.* 2004), *P. honshuensis* in Japan (Dungan and Reece 2006), and *P. beihaiensis* in China (Moss *et al.* 2008). However, based in phylogenetic analysis, *P. atlanticus* was considered as a synonym of *P. olsenii* (Murrell *et al.* 2002) and *P. chesapeakei* was reported as junior synonym of *P. andrewsi* (Burreson *et al.* 2005). So far, only seven species of this genus have been described from bivalves, but in addition, some of non-identified *Perkinsus* species have been referred in different countries, as in Australia (Goggin and Lester 1995), in France (Goggin 1992), in Spain (Sagrìstà *et al.* 1995), in Korea (Choi and Park 1997, Park *et al.* 1999, Choi *et al.* 2002), and in the Pacific coast of North and Central America (Ralph *et al.* 2004), in Brazil (Sabry *et al.* 2009). On the other hand, it was suggested that *P. karlssoni* described from bay scal-

lop (McGladdery *et al.* 1991) should be considered as an invalid *Perkinsus* species (Goggin *et al.* 1996). Some previous studies concerning the encapsulation of trophozoites on species of the genus *Perkinsus*, as response of the infected bivalves, have been observed in several host species (Azevedo 1989; Montes *et al.* 1995, 1997; Sagrìstà *et al.* 1995, 1996; Blackbourn *et al.* 1998; Park *et al.* 1999; Casas *et al.* 2004; Villalba *et al.* 2004; Dungan and Reece 2006).

In the present study, therefore, we provide for the first time ultrastructural data of *Perkinsus* trophozoites and a description of some aspects of the host reaction from infected clams collected in the Arabian Gulf.

MATERIALS AND METHODS

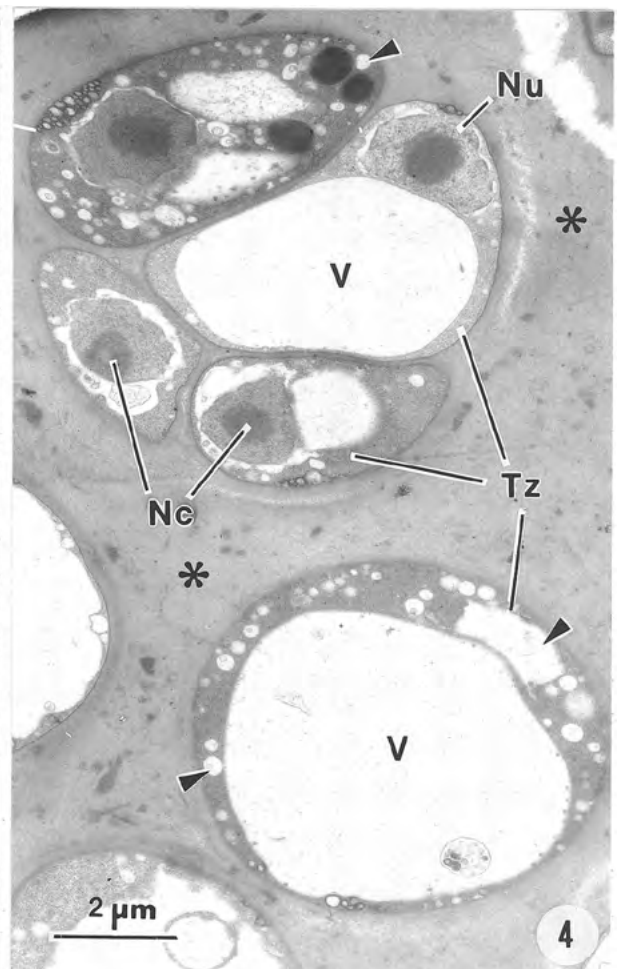
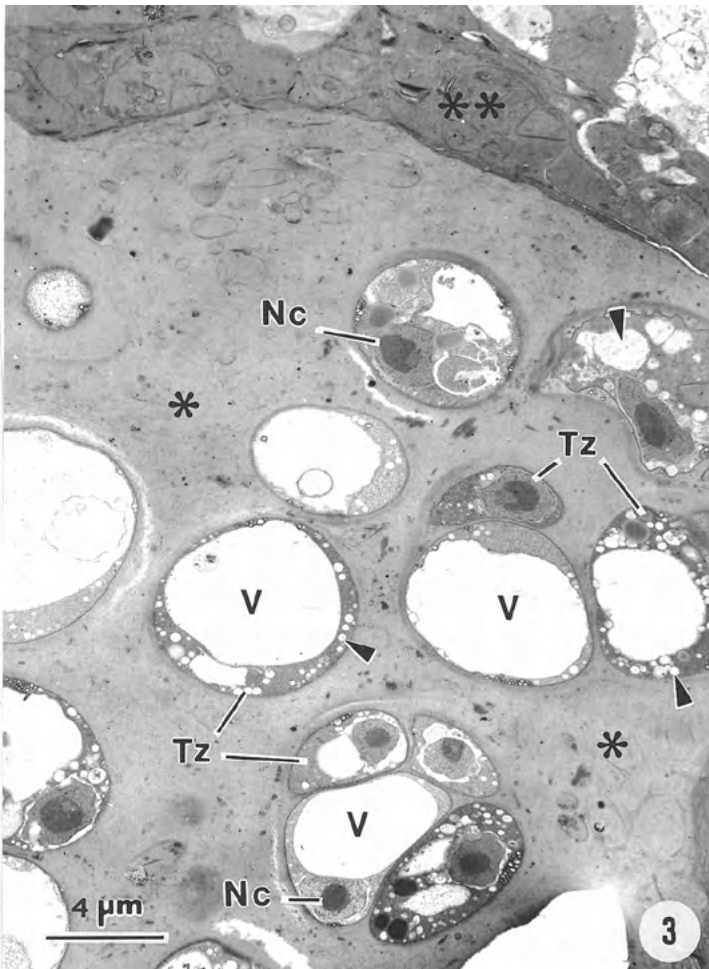
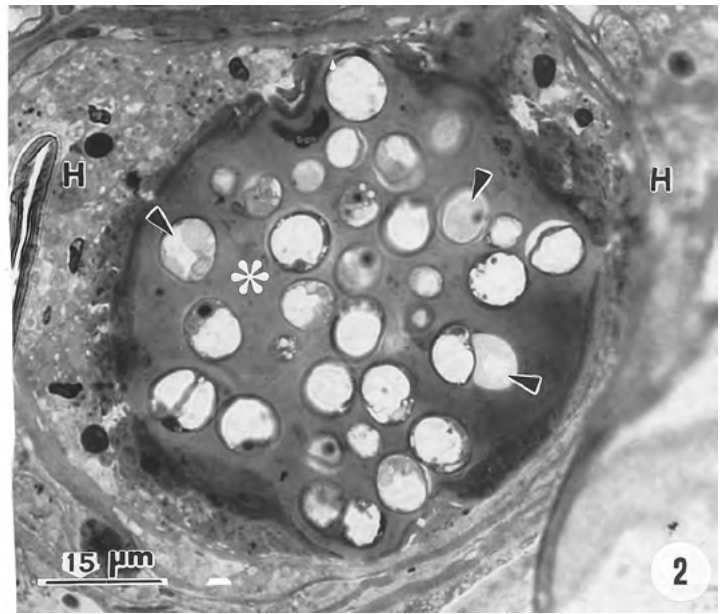
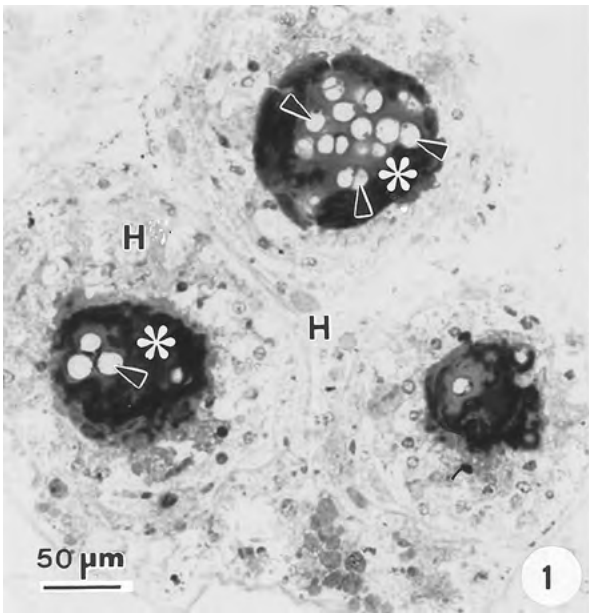
During a parasitological survey fifty living specimens of mature clams *Meretrix meretrix* Linnaeus, 1758 (Veneridae) (29–37 cm in shell length), were collected from the coastal waters of the Arabian Gulf near the city of Damamm (26°52'N, 50°02'E). Several organs and tissues were microscopically examined, but the parasite was only observed in the gills and mantle. Small fragments of infected organs were excised and examined under the light microscope with Nomarski differential interference contrast (LM-DIC) optics for the purpose of morphological study and measurement. For TEM studies, small fragments of infected tissues were fixed in 5% glutaraldehyde in a 0.2 M sodium cacodylate buffer (pH 7.2) for 24 h at 4°C, washed overnight in the same buffer at 4°C, and postfixed in 2% OsO₄ buffered with the same solution for 4 h at the same temperature. After dehydration in a graded ethanol-series followed by propylene oxide (6–8 h in each change), samples were embedded in Epon. Semithin sections were stained with methylene blue-Azur II. Ultrathin sections were cut with a diamond knife, double-stained with uranyl acetate and lead citrate, and observed in a JEOL 100CXII TEM operated at 60 kV.

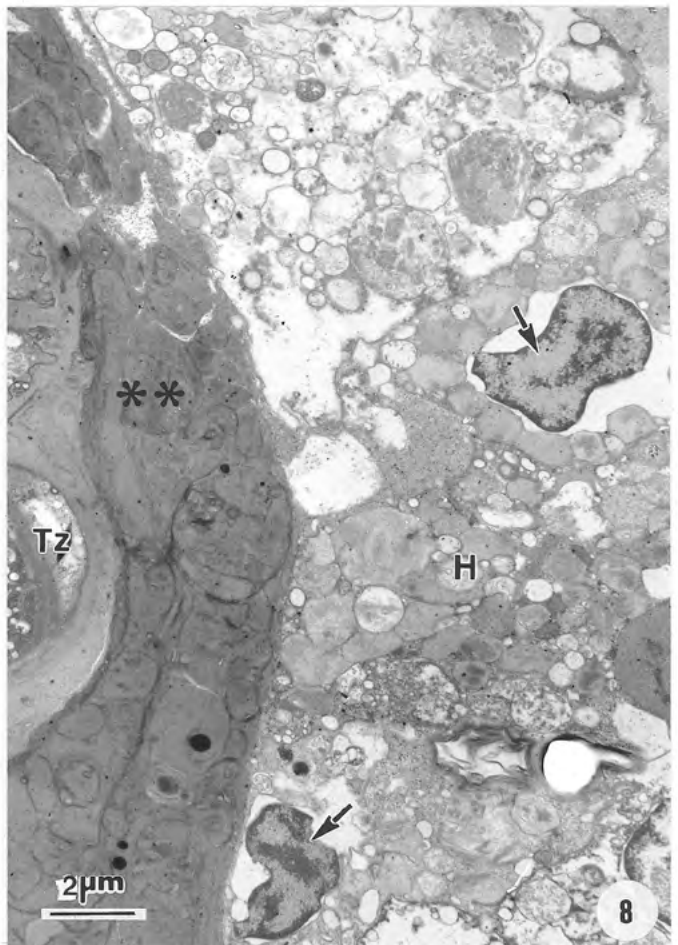
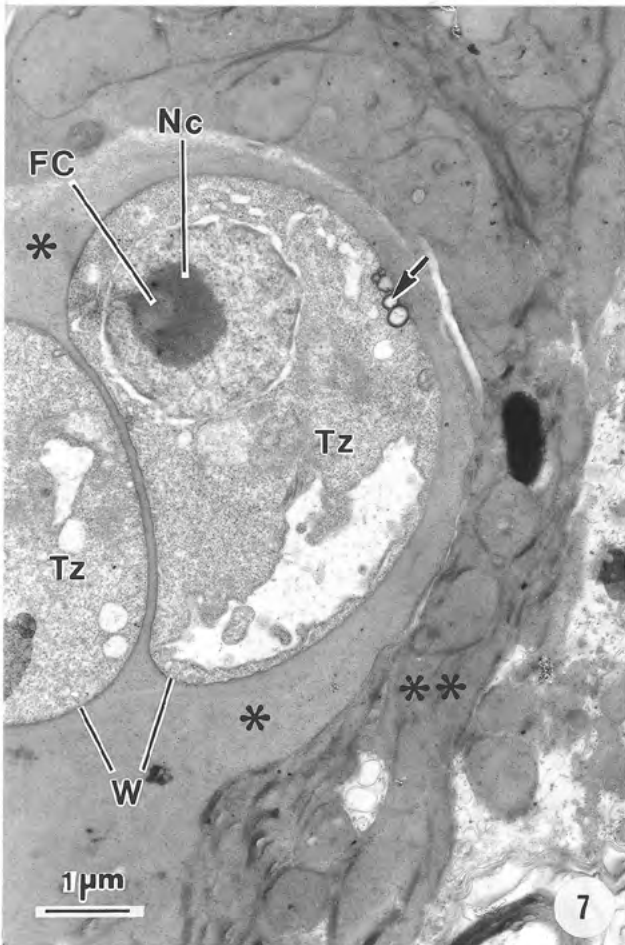
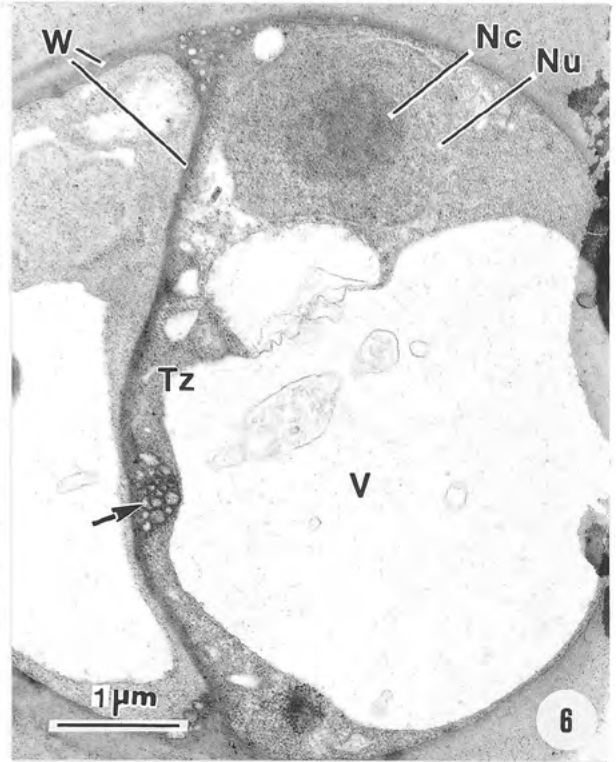
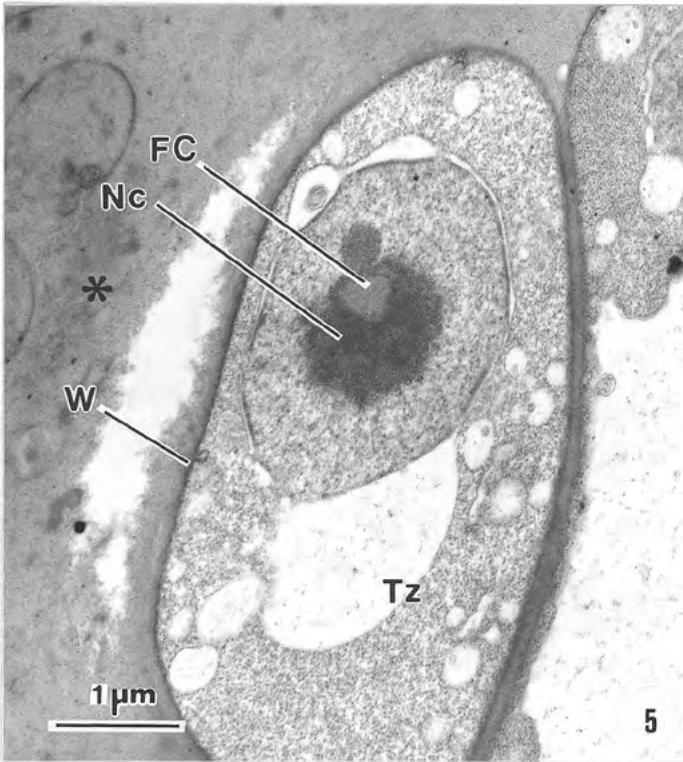
RESULTS

Macroscopic observations of the gills and mantle revealed some small milky-whitish cysts found in 48% (24/50) of examined clams. Serial semithin sections of



Figs 1–4. Light and transmission electron micrographs of *Perkinsus* sp. trophozoites (Tz) infecting the gill and mantle of the clam *Meretrix meretrix* collected from the Arabian Gulf. **1** – semithin section of trophozoites (arrowheads) encapsulated in cysts located in connective tissues of mantle (H). **2** – ultrathin section of the periphery of a cyst located. The trophozoites are isolated or grouped and both are composed by a surrounding wall, a nucleus (Nu) and a spherical prominent nucleolus (Nc) and the cytoplasm contains vacuoles (V) with several dimensions. The trophozoites are encapsulated by a homogenous amorphous substance (*) and more externally by dense cellular debris of host (**). **3** – ultrathin section of a group of trophozoites and a single trophozoite with the some morphological aspects described in the figure 2. **4** – ultrathin section of a group of two juxtaposed trophozoites separated by the adhering wall (arrowheads) and encapsulated in the homogenous mass (*). The nuclei (Nu) and the nucleolus (Nc) are present.





the infected gills and mantle, observed under the LM-DIC, showed different sized cysts and nodules in the connective tissue of the mantle were highly stained by toluidine blue (Fig. 1). Inside these cysts, various large cells (trophozoites), generally grouped, were observed (Fig. 1). At the ultrastructural level, it was observed that these cysts and large cells were delineated by a fibrogranular wall (Figs 2–4) with characteristics similar to trophozoites of the genus *Perkinsus*. Several trophozoites at different stages of maturation and varying size (up to about 18 μm diameter) were observed (Figs 2–4). Single and grouped trophozoites can be localised in cysts interior (Figs 2, 3).

The earliest trophozoites were generally spherical with a circular nucleus containing a prominent central nucleolus (Figs 2, 3). The cytoplasm was occupied by several mitochondria, ribosomes, lipid droplets and several small vacuoles. All the developmental stages of the trophozoite were surrounded by a thin wall composed of one amorphous material (Figs 2–4). The oldest stages of trophozoite maturation presented a similar general morphology, but the large vacuole occupied much of the cell volume (Figs 4, 5). These trophozoites contained eccentric nucleus with a prominent nucleolus (Figs 5–8). The cyst was internally formed of a homogenous matrix that surrounded the trophozoites located in the central zone of the cyst (Figs 4, 5) and more externally by host cells with pyknotic nuclei and numerous granules. The granules varied in size, some coalescing into dense granules (Fig. 4) and others containing membrane remains (Fig. 5). Lomosomes were observed in closed contact with the internally region of the trophozoite wall (Figs 6, 7). These granular cells exhibit some aspects of lysis (Figs 7, 8). The lysis of these granular cells and the coalescence of their granules constituted an amorphous and highly eosinophilic material that encapsulated various trophozoites (Figs 7, 8).

DISCUSSION

Microscopic features of the present microorganism indicated that the parasite cell correspond to the typical morphology of trophozoites of the genus *Perkinsus* of different described species (Azevedo 1989, Montes *et al.* 1995, Sagristà *et al.* 1995, Blackburn *et al.* 1998, Casas *et al.* 2004, Dungan and Reece 2006, Choi and Park 2010, Sanil *et al.* 2012).

The development of the cysts or nodules here reported, containing individual and grouped encapsulated trophozoites at different stages of maturation, seems to be a natural defensive reaction that results of the infiltration hemocytes. These morphological and ultrastructural aspects that have been reported in several infected organs of different host species are similar to those previously described in several hosts infected by *Perkinsus* spp. (Perkins 1976; Chagot *et al.* 1987; Azevedo 1989, 1990a, b; Montes *et al.* 1995, 1997; Casas *et al.* 2004; Dungan and Reece 2006). Unfortunately, the zoosporulation induced with fluid thioglycollate medium according to the Ray's technique (Ray 1952, 1966; Perkins 1976; Azevedo *et al.* 1990a; Casas *et al.* 2002) was not performed; however, the morphological ultrastructure of the trophozoites and the surrounding host reaction shows the evident specific organization of the presence of *Perkinsus* spp.

The host reaction observed in the present studies corresponds to the two kind of material forming the cysts. The central zone of cyst is occupied by trophozoites surrounded by a homogenous amorphous material, which is the result of a complete disaggregation of the host cellular components intermingled with hemocytes, as was previously reported in other species (Azevedo 1989, Sagristà *et al.* 1995, Blackburn *et al.* 1998, Choi *et al.* 2002, Casas *et al.* 2004, Dungan and Reece 2006, Moss *et al.* 2008). The external layer that



Figs 5–8. Transmission electron micrographs of *Perkinsus* sp. trophozoites (Tz) infecting the gill and mantle of the clam *Meretrix meretrix* collected in the Arabian Gulf. **5** – ultrathin longitudinal section of an ellipsoidal trophozoite showing the wall in contact with the homogenous amorphous mass of the cyst, the nucleus with a prominent nucleolus (Nc) and their fibrillar center (FC). The cytoplasm contains some small vacuoles (arrows). **6** – ultrathin longitudinal section of an ellipsoidal trophozoite showing the surrounding wall (W), the lomosome (arrow), the nucleus (Nu) in peripheral position and a voluminous central vacuole (V). **7** – ultrastructural details of the periphery of the cyst of *Perkinsus* sp. surrounded by the homogenous and amorphous mass (*) showing trophozoite with a prominent nucleolus (Nc) having a fibrillar center (FC) and more externally a dense layer composed of a compacted lysed host cells (**). In closed contact with the wall is observed a lomosome (arrow). **8** – the external wall layer of a cyst (**) contacting with numerous disorganized host cells (H), most of them with advanced aspects of lyses and some pyknotic nuclei (arrows).

surrounds the central zone of the cyst is denser relatively the central zone and containing several debris of reactive host cells, sometimes difficulty to identify, and several surrounding host cells, some of which with lysed aspects. These results indicate that the host reaction due to the presence of *Perkinsus* trophozoites occurring in molluscs is mainly produced by phagocytising activity (Cheng 1987; Montes *et al.* 1995, 1997; Sagristà *et al.* 1995; Casas *et al.* 2002; Villalba *et al.* 2004; Moss *et al.* 2008) by which the host cells appear to undergo lysis. Several similar descriptions of a strong host reaction in clams resulting in encapsulation of the parasite have been recorded in several species of the genus *Perkinsus* (Chagot *et al.* 1987, Gogin and Lester 1987, Azevedo *et al.* 1990b, Montes *et al.* 1997, Casas *et al.* 2004, Dungan and Reece 2006).

The lomosomes, membranous structures that differentiate between the wall and plasmalemma in different phases of the life cycle (Perkins and Menzel 1967, Perkins 1976, Azevedo *et al.* 1990a, McLaughlin *et al.* 2000, Casas *et al.* 2002), were observed in trophozoites on present study. These structures seemed not to be a well clear-cut function. Considering the reduction of the number of lomosomes at the final phase of zoosporulation, just before the opening of the discharge tube, it was suggested that these structures may interfere in the opening tube (Azevedo *et al.* 1990a). However, the appearance of these structures in most of the literature concerning *Perkinsus* spp. were not reported or, if it was cited, few suggested function have been performed.

These results here reported are the first records of a *Perkinsus* species infecting molluscs in the Saudi Arabia coasts.

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