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. chesapeaki (P. andrewsi), P. mediterraneus, P. honshuensis, P. beihaiensis and P. gugwadi. 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 lomosomes 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 *Der- mocystidium 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, Perkinsidoza (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. olseni* 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. chesapeaki* 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. olseni* (Murrell et al. 2002) and *P. chesapeaki* 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 (Sagristà 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 scallop (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; Sagristà 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...
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 fibro-granular 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 eosinophylic material that encapsulated various trophozoites (Figs 7, 8).

DISCUSSION


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, Blackbourn 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 fibrilar 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; Goggin and Lester 1987, Azevedo et al. 1990a, 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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