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Original Article

Ultrastructure of phagocytes and oocysts of *Nematopsis* sp. (Apicomplexa, Porosporidae) infecting *Crassostrea rhizophorae* in Northeastern Brazil

Ultraestrutura de fagócitos e oocistos de *Nematopsis* sp. (Apicomplexa, Porosporidae) infectando *Crassostrea rhizophorae* no Nordeste brasileiro

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Abstract

This work describes the detailed ultrastructural morphology of the phagocyte imprisoning an oyster of *Nematopsis* (Apicomplexa) found in *Crassostrea rhizophorae*, in the city of Maceió (AL), Brazil. The highly infected hosts had half-open leaflets with weak, slow retraction of the adductor muscles. Variable number of ellipsoid oocytes, either isolated and or clustered, was found between myofibrils of the adductor muscle. Each oocyst was incarcerated in a parasitophorous vacuole of host uninucleated phagocyte. The oocysts were composed of a dense wall containing a uninucleate vermiform sporozoite. The wall of the fine oocysts was composed of homogeneous electron-lucent material formed by three layers of equal thickness, having a circular orifice-micropyle obstructed by the operculum. The oocysts presented ellipsoid morphology with their wall was surrounded by a complex network of numerous microfibrils. Important details of the taxonomic value were visualized such as the ultrastructural organization of the oocyst wall and the organization of the micropyle and operculum, beyond the microfibrils that protrude from the oocyst wall only observed by transmission electron microscopy (TEM) and that may aid in the identification of the species. However, in order to clarify the systematic position of the species reported of the genus *Nematopsis*, it is important to proceed with genetic analyses.

Keywords: Oyster, microparasite, estuarine environment, mollusks, Ostreidae.

Resumo

Este trabalho descreve a morfologia ultraestrutural detalhada do fagócito encarcerando um oocisto de *Nematopsis* (Apicomplexa) encontrado em *Crassostrea rhizophorae*, na cidade de Maceió (AL), Brasil. Os hospedeiros muito infectados apresentavam valvas entreabertas com retração fraca e lenta dos músculos abdutores. Número variável de oócitos de forma elipsoide, isolados e ou agrupados foi encontrado entre as miofibrilas do músculo abdutor. Cada oocisto estava encarcerado num vacúolo parasitóforo do fagócito uninucleado do hospedeiro. Os oocistos eram compostos por uma parede densa contendo um esporozoíto vermiforme uninucleado. A parede dos oocistos finos era composta de material electron-lucente homogêneo formado por três camadas de espessura igual, possuindo um orifício circular - micrópila, obstruída pelo opérculo. Os oocistos apresentavam morfologia elipsoide, sua parede era circundada por uma complexa rede de numerosas microfibrilas. Detalhes de valor taxonômico importantes foram visualizados tais como: a organização ultraestrutural da parede do oocisto e a organização da micrópila e do opérculo, além das microfibrilas que se projetam da parede do oocisto, estrutura apenas observada em microscopia eletrônica de transmissão (MET) e que pode auxiliar na identificação da espécie. Contudo, para esclarecer a posição sistemática da maioria das espécies relatadas do gênero *Nematopsis* é importante prosseguir com as análises genéticas.

Palavras-chave: Ostra, microparasita, ambiente estuarino, moluscos, Ostreidae.

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Introduction

The microorganisms that may cause diseases or pathological conditions in several commercial bivalve species have not been investigated in detail. Many occurrences of mass mortality among farmed and wild bivalves been recently reported, and the losses to mussel and oyster farms that occur annually have primarily been attributed to poor environmental conditions, even if diseases may have caused them (BOEHS et al., 2010; BOWER et al., 1994; BRADBURY, 1994; CHENG, 1967; LAUCKNER, 1983; MOSS et al., 2007; PERKINS, 1991; POWELL & KIM, 2015; SPRAGUE, 1970).

Among the parasites infecting two types of hosts (mollusks and crustaceans) (PRYTHERCH, 1938, 1940), we highlight the genus *Nematopsis* Schneider, 1892 (Apicomplexa: Porosporidae). This is a cosmopolitan parasitic genus of apicomplexans with around 40 nominal species that have been reported in different geographic areas (ABDEL-BAKI et al., 2012; AZEVEDO & MATOS, 1999; AZEVEDO & PADOVAN, 2004; BOWER et al., 1994; OZER & GUNEYDAG, 2015; SPRAGUE, 1970; THÉODORIDÈS, 1962; TUNTIWARANURUK et al., 2004). Their vegetative and reproductive phase takes place in crustaceans (schizogonic phase), while the sporogonic phases take place in bivalves (PRYTHERCH, 1938, 1940). It has also been reported that the sporogonic phase can take place in gastropods (AZEVEDO & PADOVAN, 2004).

In Brazil, most studies have aimed to investigate the *Nematopsis* oocyst stages and have only reported on the morphology of this genus, while some have been based on histopathology, but exclusively, in light microscopy (BRANDÃO et al., 2013; BRITO et al., 2010; CEUTA & BOEHS, 2012; PINTO & BOEHS, 2008; QUEIROGA et al., 2015; SABRY et al., 2007). Few species have been described on the base of concomitant observations through both light and transmission electron microscopy (AZEVEDO & CACHOLA, 1992; AZEVEDO & MATOS, 1999; AZEVEDO & PADOVAN, 2004; MAGALHÃES et al., 2006; PADOVAN et al., 2003).

An intriguing situation exists with regard to molecular data (phylogeny). Although there is information on the 18S rDNA sequences of the *Nematopsis* sp. that have been described, it is surprising that it was not possible to obtain any information from the oocyst stage (sporogony) that would allow comparative analysis on this taxonomic group. Molecular analyses were only performed on the cephaline stages of gregarines, which were described as species of the genus *Nematopsis* (BELAFASTOVA, 1996; PRASADAN & JANARDANAN, 1996; SHANAVAS et al., 1989).

Despite various attempts to apply current standard molecular biology technologies that have been developed in our laboratory (ICBAS/UP) for phylogenetic analyses on the SSU rDNA gene sequences, we have not obtained any positive results. This is an enigmatic situation, given that it has not been possible to create a compatible sequential primer. It is curious that, although this is a cosmopolitan genus, no phylogenetic results developed from oocysts in the sporogonic phase among the species of this genus have yet appeared.

Among all the species of the genus *Nematopsis* that have been described (around 40 species), we found that a few species (e.g. *N. mytella* and *N. gigas*) have been described based only on a few comparative ultrastructural analyses on the oocyst phase (AZEVEDO & MATOS, 1999; AZEVEDO & PADOVAN, 2004; PADOVAN et al., 2003).

Considering that some undetermined species of *Nematopsis* occurring in several hosts belonging to different species (ABDEL-BAKI et al. 2012; AZEVEDO & CACHOLA, 1992; BOEHS et al., 2010; BRANDÃO et al., 2013; BRITO et al., 2010; CANESTRI-TROTTI et al., 2000; COVA et al., 2015; KUA et al., 2013; PINTO & BOEHS, 2008; QUEIROGA et al., 2015; SABRY & MAGALHÃES, 2005; SUJA et al., 2016; TUNTIWARANURUK et al., 2004, 2015), it is acceptable to think that many of the species thus described may correspond to previously described species. On the other hand, several *Nematopsis* species have been described from the gregarine stage of the schizogonic phase (CHAMBOUVET et al., 2016; CHAKRABORTI & BANDYOPADHYAY, 2010; JIMÉNEZ et al., 2002; PRASADAN & JANARDANAN, 1996, 2001; SHANAVAS et al., 1989). These descriptions have not included the corresponding stage of the oocysts (schizogonic phase). This is a confusing situation and there is no morphological comparison with other species that have previously been described.

The aim of the present study was to contribute towards better knowledge of the ultrastructural details of the oocysts of *Nematopsis mytella*, which is an apicomplexan species that has been described in marine bivalves (*Crassostrea rhizophorae*) from the Atlantic coast of Brazil, near the city of Maceió, state of Alagoas (Brazil). Additionally, the ultrastructural disorganization and disintegration of the tissues of the infected host specimens containing oocysts incarcerated in the host's phagocytes were observed and discussed.

Methodology

Four groups of ten wild *C. rhizophorae* Guilding, 1828 were collected monthly between September and December 2017 from mangroves near the city of Maceió (State of Alagoas), in the Atlantic coast of Brazil (09° 29' S, 35° 34' W). They were maintained in an aquarium with aeration for 3-5 days at a temperature of 20-25 °C and salinity of 2.5-3.5 ppm, the condition similar to their natural environment.

Oocysts in infected specimens of oysters were morphologically identified as the the genus *Nematopsis*. Some of these hosts presented gaping valves and their adductor muscles appeared to have weak contractile power. The oocysts were collected only from the adductor muscles, gill and mantle, and were prepared for common light microscopy (LM) and transmission electron microscopy (TEM) analyses.

Common light microscopy and morphological analysis on oocysts

The oocysts were examined and photographed using a Leitz microscope equipped with a Nomarski differential interference contrast (DIC) system. Morphometric analysis on the oocysts was conducted using fresh material and all measurements included the mean ± SD, range of variation and number of spores measured (range, n).

Transmission Electron Microscopy (TEM)

Samples of small infected fragments from the adductor muscle, gills, mantle and digestive gland (hepatopancreas) of *C. rhizophorae* were fixed in 4-5% glutaraldehyde that was buffered in 0.2 M sodium cacodylate (pH 7.4) for 20 to 24 h. They were then washed in the same buffer and postfixed in 2% osmium tetroxide, which was also buffered in the same solution for 3 to 4 h. All of these steps were performed at 4 °C. The samples were then dehydrated in an ascending graded series of ethanol and propylene oxide. The dehydrated samples were embedded in a series of propylene oxide and EPON mixtures, ending in EPON. Semi-thin sections were cut and stained with methylene blue-Azure II. Ultra-thin sections were cut using a diamond knife and were double-contrasted with uranyl acetate and lead citrate. The semi-thin sections were observed in LM, and the ultra-thin sections were examined and photographed using a transmission electron microscope (JEOL 100CXII; JEOL Optical), operating at 60 kV.

Results

Common light microscopy observations

Observations under the light microscope enabled identification of the presence of oocysts in several organs (adductor muscle, gills, mantle and digestive gland) of *C. rhizophorae.* The infecting oocysts were easily observed among different host cells and were identified in freshly squashed preparations as *Nematopsis* sp. (Figures 1A-B), previously described in other bivalves species. This identification was matched using semi-thin sections (Figure 1D and Figure 2A) and through ultrastructural analysis (Figures 2B-E). The phagocyte cytoplasm contained variable numbers of parasitophorous vacuoles (PVs), each containing a single oocyst (Figures 1D and 1E). These oocysts seemed to be morphologically similar with similar dimensions and similar internal organization. No measured or morphological differences between the oocysts collected from the

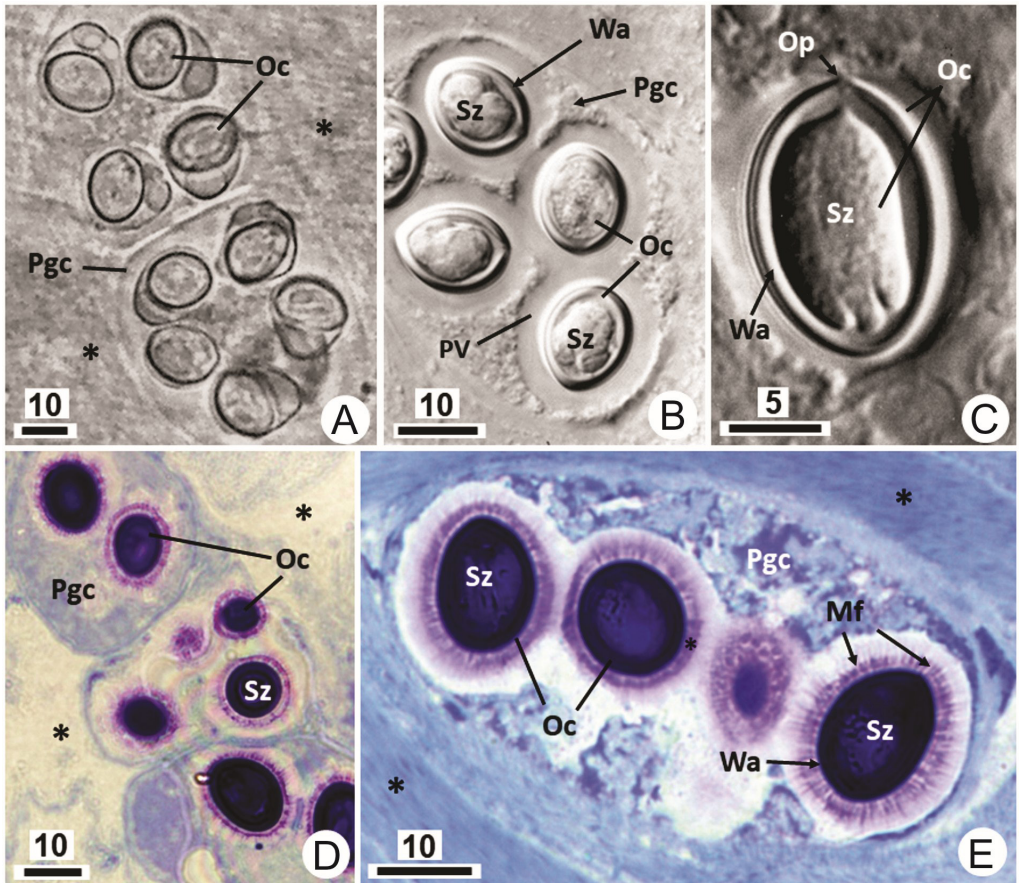

Figure 1. Different aspects of oocysts of *Nematopsis* sp. that were obtained from the host adductor muscle of *Crassostrea rhizophorae*. These oocysts were incarcerated in the host's phagocytes. Observations via light microscopy, differential interference contrast and transmission electron microscopy. A- Clusters containing some oocysts (Oc) each incarcerated in a phagocyte (Pgc), observed using DIC; B- Oocysts (Oc) observed using DIC, showing the internal sporozoite (Sz) surrounded by the oocyst wall (Wa); these oocysts were included in parasitophorous vacuoles (PVs) of the phagocytes (Pgc); C- Composition of an isolated oocyst (Oc): oocyst wall (Wa), operculum (Op) and sporozoite (Sz) observed using DIC; D- Semi-thin section through an oocyst (Oc) showing the sporozoite (Sz) and the parasitophorous vacuole of the host's phagocyte (Pgc) surrounded by other host cells (*); E- Semi-thin section showing oocysts (Oc) incarcerated by a phagocyte (Pgc): the oocysts are located among the host cells (*). Each oocyst shows an internal sporozoite (Sz), surrounded by the oocyst wall (Wa) and containing numerous microfibrils (Mf) projecting into the parasitophorous vacuole membrane. All scale bars in µm.

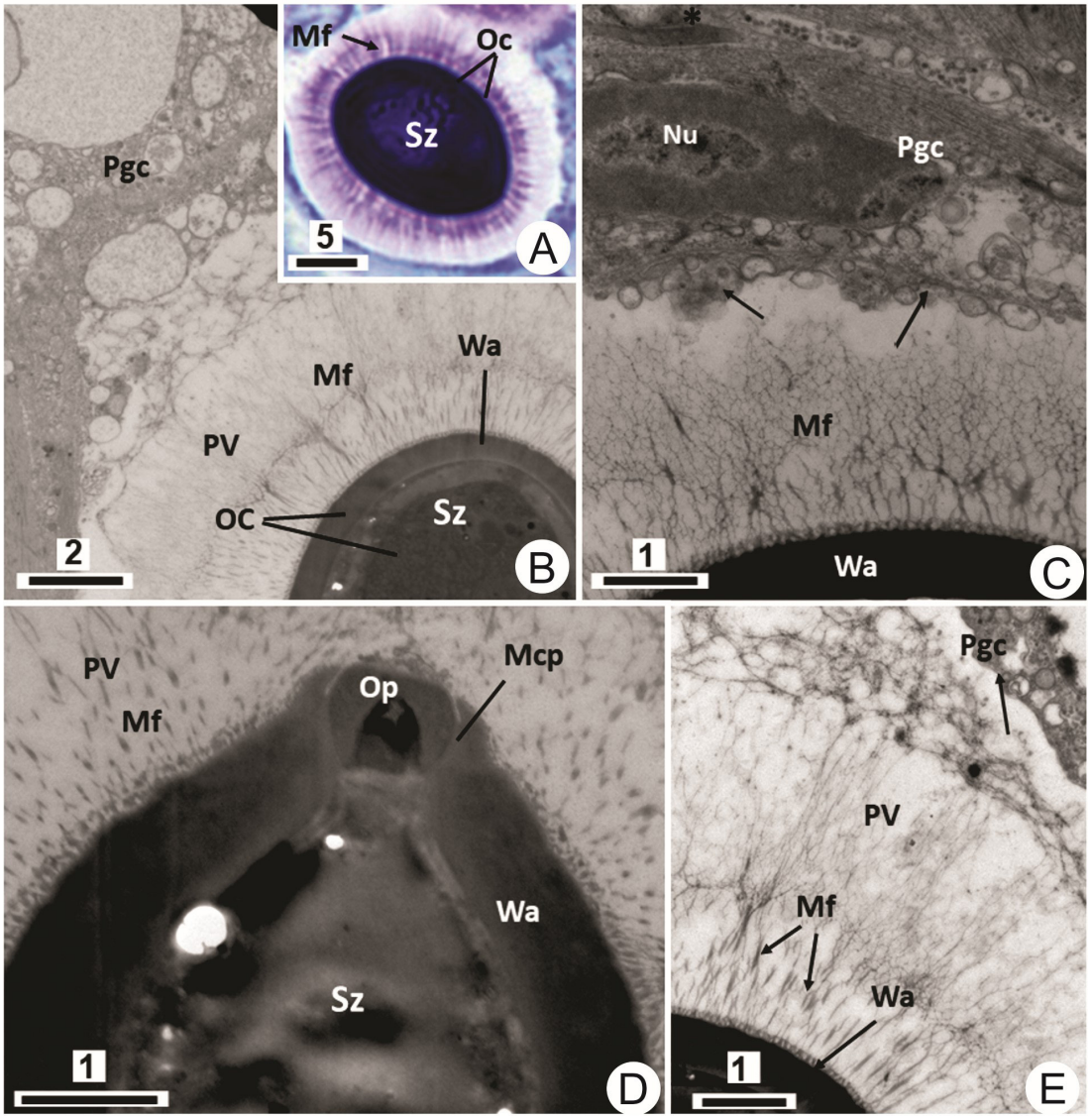

Figure 2. Some morphological aspects of the different organelles and structures of the oocytes and the surrounding host cells. A- Semi-thin cross-section through an oocyte (Oc) showing the internal sporozoite (Sz) and numerous microfibrils (Mf) projecting from the oocyst wall; B- Transverse section through an oocyst (Oc) incarcerated in a parasitophorous vacuole (PV), showing the internal sporozoite (Sz) surrounded by the oocyst wall (Wa) from which numerous microfibrils (Mf) radiate, projecting towards the phagocyte (Pgc); C- Ultrastructural detail of the peripheral zone of the oocyst wall (Wa) showing irradiation of different types of microfibrils (Mf) projecting from the oocyst wall towards the parasitophorous vacuole membrane of the phagocyte (Pgc). The phagocyte shows a pyknotic nucleus (Nu) and the parasitophorous vacuole membrane is partially destroyed (arrows); D- Ultrastructural detail of the apical region of the oocyst, showing the oocyst wall (Wa), the sporozoite (Sz) and the operculum (Op) plugging the micropyle (Mcp). Nearby, several sections of the microfibrils (Mf) are located in the parasitophorous vacuole (PV); E- Ultrastructural detail of the peripheral zone of the oocyst showing some aspects of degradation of the microfibrils (Mf) and the phagocyte periphery (Pgc) in which the membrane is in contact with microfibrils that appear to be disrupted. All scale bars in μm.

different organs were found. Thirty-one out of the 40 specimens (77.5%) of *C. rhizophorae* examined were infected by oocysts. The prevalence of oocysts varied according to the organ. It was observed that the prevalence of oocysts in the adductor muscles and in the gills were higher than the prevalence in the digestive gland and in the mantle, although it was not possible to quantify.

Our analyses based on the LM were oriented towards observing and describing exclusively the oocysts infecting the adductor muscles and, simultaneously, the host reaction due the presence of the parasite. The oocysts occurred singly or in groups, and the groups contained variable numbers of oocysts (up to 13) that were randomly dispersed throughout the adductor muscle tissue. Each oocyst was observed to be incarcerated in a phagocyte (Figures 1A-E).Single oocyst was more frequently incarcerated in individualized PVs of the host cells and these cells were identified as phagocytes (Figures 1A-E). On rare occasions, two oocysts

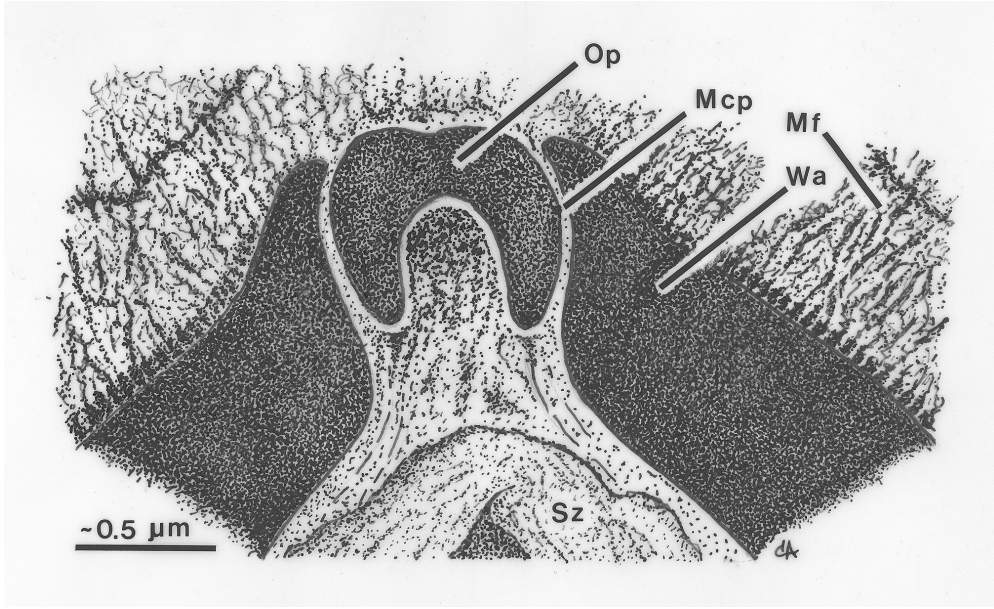

Figure 3. Schematic drawing of a longitudinal section of the operculum (Op) system of a *Nematopsis* sp. oocyst showing the operculum (Op) located in the apical region of the oocyst wall (Wa), which blocks the micropyle (Mcp). The internal part of the oocyst is occupied by the sporozoite (Sz) and the external complex system of microfibrils (Mf) projects from the oocyst wall.

were incarcerated in the same PV, as a result of contact between two neighboring PVs. The oocysts generally occupied a central position in the PV (Figures 1B-E).The oocysts were unicellular structures (15.6 ± 0.6 µm long and 11.1 ± 0.7 µm wide; n = 50) composed of an oocyst wall with an apical operculum surrounding a vermiform uninucleate cell, which was designated a sporozoite (Figures 1B-E).

Transmission Electron Microscopy (TEM) observations

Only the oocysts collected from the adductor muscles were ultrastructurally analyzed and described, as was reported. Observations on the fine structure confirmed that each PV contained a single oocyst (Figures 2B and 2C). The PV was formed by a parasitophorous membrane of irregular outline that was in close contact with the cytoplasm of the phagocyte (Figures 2B and 2C). The matrix of the PV was mainly occupied by a complicated network of numerous irregular and anastomosed microfibrils around the oocyst, projecting from the oocyst wall towards the PV membrane (Figures 2B-E). These double microfibril layers formed a complex anastomosed network projecting from the oocyst wall, in which the layer adhering to the oocyst wall had microfibrils that were thicker than those in more distant layers with more anastomosed contact with the PV membrane (Figures 2B-E).

Through observations on serial ultrathin sections, it was confirmed that each oocyst contained a single vermiform sporozoite and that the oocyst measurements (length and width) matched the LM observations (Figures 1B and 1C).The oocyst wall thickness was 0.8 ± 0.3 µm (n = 25) and this wall was formed only by homogeneous electron-dense material (Figures 2B-E). The apical region of the oocyst contained a circular micropyle (sometimes designated by a micropore) of diameter 0.9 ± 0.3 µm (n = 15). This was covered by an operculum formed by material of electron density similar to that of the wall material (Figure 2D). In favorable serial ultrathin sections, the operculum presented arcuate morphology (∩-shaped). Operculum occupied the cylindrical space of the micropyle (Figures 2D and 3)

The first signs of lysis occurred in the phagocytes when the nucleus became pyknotic (Figure 2C) and the PV membranes disappeared. The cytoplasm became lighter and numerous vesicles appeared in this region (Figures 2C and 2E). All oocysts infecting the adductor muscle, gills and mantle showed similar measurements (15.3 ± 0.6) (n=10) and ultrastructural morphology, thus suggesting that all the oocysts found in different organs of the same specimens belonged to the same species. A schematic drawing of the oocyst morphology (Figure 3) was made on observations from light and serial ultrathin sections.

Discussion

The morphology and ultrastructural analyses on the parasite oocysts and phagocytes in the infected adductor muscle, gills and mantle of the *C. rhizophorae* showed that this parasite belonging to the genus *Nematopsis* had characteristics similar to those observed in hosts from different regions worldwide (ABDEL-BAKI et al., 2012; AZEVEDO & CACHOLA, 1992; AZEVEDO & MATOS, 1999; AZEVEDO & PADOVAN, 2004; BRANDÃO et al., 2013; KUA et al., 2013; PADOVAN et al., 2003; SHANAVAS et al., 1989; TUNTIWARANURUK et al., 2004).

In Brazil, the genus *Nematopsis* has been intensively studied along the Brazilian Atlantic coast based mainly on light microscopy observations (BRANDÃO et al., 2013; BRITO et al., 2010;

PADOVAN et al., 2003; PINTO & BOEHS 2008; QUEIROGA et al., 2015; SABRY et al., 2007). The data from these studies do not allow comparative ultrastructural descriptions in relation to species that had previously been described (AZEVEDO & MATOS, 1999; AZEVEDO & PADOVAN, 2004; MAGALHÃES et al., 2006). These descriptions of undetermined *Nematopsis* species (BRITO et al., 2010; COVA et al., 2015; LUZ & BOEHS, 2015; PINTO & BOEHS, 2008; SABRY & MAGALHÃES, 2005) seem to correspond to the morphological characteristics of oocysts of the genus *Nematopsis* infecting Brazilian fauna that had previously been described.

Comparison of our results with the morphological and ultrastructural organizations of species of this genus that had previously been described showed that the oocyst organizations were highly similar. Thus, these data based on oocyst morphology confirm that the parasite described here belongs to the genus *Nematopsis* (AZEVEDO & MATOS, 1999; AZEVEDO & PADOVAN, 2004; MAGALHÃES et al., 2006), despite some morphological and ultrastructural differences. Detailed ultrastructural comparisons of the oocysts described in the present manuscript seem to confirm that they are similar to those of the species *Nematopsis mytella* that had previously been described (AZEVEDO & MATOS, 1999; MAGALHÃES et al., 2006; PADOVAN et al., 2003).

Today around 40 named species that belong to the genus *Nematopsis* have been described. In addition, several unnamed species have been attributed to this genus. Given the need for detailed morphological data and the lack of molecular data, these unnamed species may correspond to nominal species that have previously been described. Most of these unnamed *Nematopsis* were described based on LM observations and some of them were based on doubtful host specificity (ABDEL-BAKI et al., 2012; AZEVEDO & CACHOLA, 1992; BRITO et al., 2010; KUA et al., 2013; PINTO & BOEHS 2008; QUEIROGA et al., 2015; SABRY et al., 2007; SOTO et al., 1996; SUJA et al., 2016; TUNTIWARANURUK et al., 2004). Only a few of these species were identified using ultrastructural observations (AZEVEDO & MATOS, 1999; AZEVEDO & PADOVAN, 2004).

One important methodology providing an important means of diagnosing ultrastructural organizations allows details and rigor in comparing the different structures of these parasites, such as structural organization of the operculum and micropyle. Most species of the genus *Nematopsis* that have previously been described were only based on LM observations and on the parasite specificity of the hosts (BELAFASTOVA, 1996; CHAKRABORTI & BANDYOPADHYAY, 2010; JIMÉNEZ et al., 2002; KUA et al., 2013; THÉODORIDÈS, 1962; TUNTIWARANURUK et al., 2004). These observations enabled visualization of details of taxonomic value, such as the ultrastructural organization of the oocyst wall, and organization of the micropyle and operculum. The ultrastructural morphology of the micropyle and the microfibrils that adhere to *Nematopsis* oocysts seem to be important taxonomic features. However, these were not exploited to identify differences in the specificity of this genus. On the other hand, the surrounding layers of microfibrils that project from the oocyst wall towards the PV membrane, a structure only observed via TEM, may form a further characteristic that has the capacity to influence species identification. This merits exploitation in future studies.

Unfortunately, to date, no phylogenetic studies have been conducted to correlate the genomic DNA of the oocysts of some of the 40 named species based on the morphology of the oocysts. Only a few species (*N. idella* Prasadan & Janardanan, 1996; *N. annulipes* Prasadan & Janardanan, 2001; *N. messor* Prasadan & Janardanan, 2001; and *N. quadratum* Prasadan & Janardanan, 2001) have been described based on studies on the gregarine stage of the life cycle in crustacean hosts. Studies on the genomic DNA of the oocysts (sporogonic phase) during which the oocyst phase develops are of fundamental importance in relation to naming the species that are described in the schizogonic phase. This phase develops with morphological aspects that are not compatible with the morphology of the oocyst stages. No other species of the genus *Nematopsis* have been described using phylogenetic data based on genomic DNA on the oocysts stages infecting mollusks, or at least no such results have ever been published.

To clarify the systematic position of most of the reported species of the genus *Nematopsis*, detailed morphological studies on oocysts and molecular investigations on the SSU rDNA sequences based on genomic DNA isolated from oocyst stages need to be conducted.

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References

Abdel-Baki AA, Al-Quraishy S, Dkhil MA, Al-Nasr I, Oliveira E, Casal G, et al. Ultrastructural characteristics of *Nematopsis* sp. oocysts (Apicomplexa: Porosporidae), a parasite of the clam *Meretrix meretrix* (Veneridae) from the Arabian Gulf, Saudi Arabia. *Folia Parasitol (Praha)* 2012; 59(2): 81-86. http://dx.doi.org/10.14411/fp.2012.012. PMid:22779107.

Azevedo C, Cachola R. Fine structure of the Apicomplexa oocyst of *Nematopsis* sp. of two marine bivalve molluscs. *Dis Aquat Organ* 1992; 14(1): 69-72. http://dx.doi.org/10.3354/dao014069.

Azevedo C, Matos E. 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* 1999; 35(4): 427-433. http://dx.doi.org/10.1016/S0932-4739(99)80052-2.

Azevedo C, Padovan I. *Nematopsis gigas* n. sp. (Apicomplexa), a parasite of *Nerita ascencionis* (Gastropoda, Neritidae) from Brazil. *J Eukaryot Microbiol* 2004; 51(2): 214-219. http://dx.doi.org/10.1111/j.1550-7408.2004.tb00548.x. PMid:15134258.

Belafastova IP. Gregarines of the genus *Nematopsis* (Eugregarinida, Porosporidae) - parasites of the Black Sea invertebrates. *Parazitologiya* 1996; 30: 159-173.

Boehs G, Villalba A, Ceuta LO, Luz JR. Parasites of three commercially exploited bivalve mollusc species of the estuarine region of the Cachoeira River (Ilhéus, Bahia, Brazil). *J Invertebr Pathol* 2010; 103(1): 43-47. http://dx.doi.org/10.1016/j.jip.2009.10.008. PMid:19850046.

Bower SM, McGladdery SE, Price IM. Synopsis of infectious diseases and parasites of commercially exploited shellfish. *Annu Rev Fish Dis* 1994; 4: 1-199. http://dx.doi.org/10.1016/0959-8030(94)90028-0.

Bradbury PC. Parasitic Protozoa of Mollusca and Crustacea. In: Kreier JP. *Parasitic Protozoa*. California: Academic Press Inc; 1994. p. 170-180.

Brandão RP, Boehs G, Silva PM. Health assessment of the oyster *Crassostrea rhizophorae* on the southern coast of Bahia, northeastern Brazil. *Rev Bras Parasitol Vet* 2013; 22(1): 84-91. http://dx.doi.org/10.1590/S1984-29612013005000007. PMid:23538501.

Brito LO, Barros JCN, Gálvez AO, Barros FN. Presence of *Nematopsis* sp. (Protozoa, Apicomplexa) in the oyster, *Crassostrea rhizophorae* (Guilding, 1828), cultivated in the state of Pernambuco, Brazil. *World Aquac* 2010; 41(1): 60-62.

Canestri-Trotti G, Baccarani EM, Paesanti F, Turolla E. Monitoring of infections by Protozoa by the genera *Nematopsis, Perkinsus* and *Porospora* in the smooth Venus clam *Callista chione* from the North-Western Adriatic Sea (Italy). *Dis Aquat Organ* 2000; 42(2): 157-161. http://dx.doi.org/10.3354/dao042157. PMid:11023256.

Ceuta LO, Boehs G. Parasites in the mangrove mussel *Mytella guyanensis* (Bivalvia: Mytilidae) in Camamu Bay, Bahia, Brazil. *Braz J Biol* 2012; 72(3): 421-427. http://dx.doi.org/10.1590/S1519-69842012000300002. PMid:22990810.

Chakraborti J, Bandyopadhyay PK. First record of a parasitic septate gregarines (Apicomplexa: Sporozoea) in the shrimp *Peneaus monodon* in Sundarbans of West Bengal. *J Parasit Dis* 2010; 34(1): 40-43. http://dx.doi.org/10.1007/s12639-010-0002-7. PMid:21526032.

Chambouvet A, Valigurová A, Pinheiro LM, Richards TA, Jirků M. *Nematopsis temporariae* (Gregarinasina, Apicomplexa, Alveolata) is an intracellular infectious agent of tadpole livers. *Environ Microbiol Rep* 2016; 8(5): 675-679. http://dx.doi.org/10.1111/1758-2229.12421. PMid:27119160.

Cheng TC. Marine molluscs as hosts for symbioses with a review of known parasites of commercially important species. *Adv Mar Biol* 1967; 5: vii. http://dx.doi.org/10.1016/S0065-2881(08)60321-1.

Cova AW, Serafim JM Jr, Boehs G, Sousa JM. Parasites in the mangrove oyster *Crassostrea rhizophorae* cultivated in the estuary of the Graciosa River in Taperoá, Bahia. *Rev Bras Parasitol Vet* 2015; 24(1): 21-27. http://dx.doi.org/10.1590/S1984-29612015012. PMid:25909249.

Jiménez R, Barniol L, Machuca M. *Nematopsis marinus* n. sp., a new septate gregarine from cultured penaeoid shrimp *Litopenaeus vannamei* (Boone), in Ecuador. *Aquacult Res* 2002; 33(4): 231-240. http://dx.doi.org/10.1046/j.1355-557x.2002.00647.x.

Kua BC, Salleh MT, Noraziah MR. A case study of protozoan parasite gregarine *Nematopsis* spp. (Apicomplexa: Sporozoa) infection in mangrove oyster *Crassostrea belcheri* imported from Thailand. *Trop Agricult Sci* 2013; 36(3): 217-224.

Lauckner G. Diseases of Mollusca: Bivalvia. In: Kinne O. *Diseases of marine animals: 2. Introduction, Bivalvia to Scaphopoda.* Helgoland, Hamburg: Biologische Anstalt Helgoland; 1983. p. 542-548.

Luz MSA, Boehs G. Parasites in the oyster *Crassostrea rhizophorae* from farmed and natural stocks in the Bay of Camamu, Bahia, northeastern Brazil. *J Parasitol Vector Biol* 2015; 76: 120-128.

Magalhães A, Silva W, Matos E, Azevedo C. Ultrastructural morphology of *Nematopsis mytella* parasite Apicomplexa of bivalve molluscs of south of Brazil. *Rev Cienc Agrar* 2006; 46: 251-259.

Moss JA, Burreson EM, Cordes JF, Dungan CFG, Brown GD, Wang A, et al. Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay. *Dis Aquat Organ* 2007; 77(3): 207-223. http://dx.doi.org/10.3354/dao01829. PMid:18062472.

Ozer A, Guneydag S. Seasonality and host-parasite interrelationship of *Mytilus galloprovincialis* parasites in Turkish Sea coast. *J Mar Biol* 2015; 95(8): 1591-1599. http://dx.doi.org/10.1017/S0025315415000740.

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

Perkins FO. "Sporozoa": Apicomplexa, Microsporidia, Haplosporidia, Paramyxea, Myxosporidia, and Actinosporidia. In: Harrison FW, Corliss JO. *Microscopic Anatomy of Invertebrates*. New York: Wiley-Liss Inc.; 1991. p. 261-331. (Protozoa; vol. 1).

Pinto TR, Boehs G. *Nematopsis* sp. (Apicomplexa: Eugregarinida) em *Mytella guyanensis* (Lamarck, 1819) (Bivalvia: Mytillidae) da região estuarina do rio Cachoeira, Ilhéus, Bahia, Brasil. *Braz J Vet Res Anim Sci* 2008; 45(2): 95-100. http://dx.doi.org/10.11606/issn.1678-4456.bjvras.2008.26705.

Powell EN, Kim Y. The regional spatial structure of parasites and pathologies in oysters and mussels in the United States: 16 years of mussel watch. *J Shellfish Res* 2015; 34(3): 939-965. http://dx.doi.org/10.2983/035.034.0324.

Prasadan PK, Janardanan KP. *Nematopsis idella* sp. n. two cephaline gregarines from freshwater prawns in Kerala. *Acta Protozool* 1996; 35: 239-243.

Prasadan PK, Janardanan KP. Three new species of gregarines (Apicomplexa: Sporozoea: Porosporidae) in the estuarine crabs from Kerala, India. *Acta Protozool* 2001; 40: 303-309.

Prytherch HF. Life-cycle of a sporozoan parasite of the oyster. *Science* 1938; 88(2289): 451-452. http://dx.doi.org/10.1126/science.88.2289.451. PMid:17736942.

Prytherch HF. The life cycle and morphology of *Nematopsis ostrearum* sp. nov. a gregarine parasite of the mud crab and oyster. *J Morphol* 1940; 66(1): 39-64. http://dx.doi.org/10.1002/jmor.1050660106.

Queiroga FR, Vianna RT, Vieira CB, Farias ND, Silva PM. Parasites infecting the cultured oyster *Crassostrea gasar* (Adanson, 1757) in Northeast Brazil. *Parasitology* 2015; 142(6): 756-766. http://dx.doi.org/10.1017/S0031182014001863. PMid:25553815.

Sabry RC, Gesteira TCV, Boehs G. First record of parasitism in the mangrove oyster *Crassostrea rhizophorae* (Bivalvia: Ostreidae) at Jaguaribe River estuary - Ceará, Brazil. *Braz J Biol* 2007; 67(4): 755-758. http://dx.doi.org/10.1590/S1519-69842007000400024. PMid:18278331.

Sabry RC, Magalhães ARM. Parasitas em ostra de cultivo (*Crassostrea rhizophorae* e *Crassostrea gigas*) da ponta do Sambaqui, Florianopolis, SC. *Arq Bras Med Vet Zootec* 2005; 57(Supl. 2): 194-203. http://dx.doi.org/10.1590/S0102-09352005000800010.

Shanavas KR, Prasadan PK, Janardanan KP. *Nematopsis rosenbergii* n. sp. (Apicomplexa: Cephalina) from the brackishwater prawn, *Macrobrachium*

rosenbergii (De Man). *Arch Protistenkd* 1989; 137(2): 161-164. http://dx.doi.org/10.1016/S0003-9365(89)80007-7.

Soto M, Pascual S, Rodrigues H, Gestal C, Abollo E, Arias C, et al. *Nematopsis* spp. Schneider, 1892 (Apicomplexa: Gregarinida) in bivalve molluscs off Ria de Vigo (Galicia, NW Spain). *Bull Eur Assoc Fish Pathol* 1996; 16(5): 157-160.

Sprague V. Some protozoan parasites and hyperparasites in marine bivalve molluscs. In: Snieszko SF. *A symposium on diseases of fishes and shellfishes*. Washington: American Fisheries Society; 1970. p. 511-526. (Special Publ; no. 5).

Suja G, Kripa V, Mohamed KS, Shamal P, Sanil NK. *Nematopsis* sp. (Apicomplexa: Porosporidae) infection in *Crassostrea madrasensis* and its associated histopathology. *J Mar Biol Assoc India* 2016; 58(1): 29-33. http://dx.doi.org/10.6024/jmbai.2016.58.1.1890-04.

Théodoridès J. Grégarines d'invertébrés marines de la région de Banyuls. I. Eugrégarines parasites de crustacés décapodes. *Vie Milieu* 1962; 13: 95-122.

Tuntiwaranuruk C, Boonmameepool J, Noppharatarphakul N, Upatham ES. Ultrastructure of gametocyst of parasitic protozoan, *Nematopsis* sp. in Black Tiger Shrimp *Penaeus monodon* from the Gulf of Thailand. *Acta Protozool* 2015; 54(2): 137-142.

Tuntiwaranuruk C, Chalermwat K, Upatham ES, Kruatrachue M, Azevedo C. Investigation of *Nematopsis* spp. oocysts 7 species of bivalves from Chonburi Province, Gulf of Thailand. *Dis Aquat Organ* 2004; 58(1): 47-53. http://dx.doi.org/10.3354/dao058047. PMid:15038451.