Ultrastructural characteristics of *Nematopsis* sp. oocysts (Apicomplexa: Porosporidae), a parasite of the clam *Meretrix meretrix* (Veneridae) from the Arabian Gulf, Saudi Arabia

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Abstract: This paper describes the fine structure of oocysts of *Nematopsis* sp. (Apicomplexa, Porosporidae) found in the abductor muscles of seawater clams, *Meretrix meretrix* (Linnaeus, 1758) (Veneridae), collected near the city of Dammam (6°17'0"N, 50°12'0"E) in the Arabian Gulf off the coast of Saudi Arabia. Oocysts of an ellipsoidal shape were found among myofibrils of the abductor muscles of infected clams. Each oocyst is composed of an oocyst wall surrounding a single uninucleate vermiform sporozoite located in the lumen of the oocyst wall. The thin oocyst wall (0.70–0.85 µm thick) is composed of homogenous electron-lucent material formed by three layers of equal-thickness. The oocyst wall contains a plano-convex opercular-like structure about 2.5 µm in diameter and 0.75–0.90 µm thick, composed of a homogenous material with moderate electron density. The oocyst is of an ellipsoidal shape and is 15.6 ± 0.6 µm long and 11.1 ± 0.7 µm wide. Externally, the oocyst wall is surrounded by a complex dense network of numerous anastomosed microfibrils, which are attached to the oocyst wall, forming 2–3 layers and extending towards the periphery, at some points penetrating amongst the host cells. The myofibrils in some cases show evident aspects of lysis as a consequence of the appearance of lysosome-like vesicles. Lacking knowledge of a complete life cycle and/or molecular data precluded the conclusive identification of this species.

Keywords: *Nematopsis*, oocyst, ultrastructure, *Meretrix*, Saudi Arabia

Species of *Nematopsis* Schneider, 1892 (Apicomplexa) are heteroexogenous gregarine parasites commonly infecting commercially important marine molluscs as intermediate hosts having decapods as their definitive hosts (Schneider 1892, Prytherch 1938, 1940, Théodoridès 1962, Chen 1967, Sprague 1970, Lauckner 1983, Perkins 1991, Bradbury 1994, Jiménez et al. 2002). *Nematopsis* species have been reported to have monozoic oocysts (spores or sporocysts according to some authors) with a thick wall enclosing a single sporozoite (Sprague and Orr 1955, Théodoridès 1962, Sprague 1970, Desportes et al. 1977, Azevedo and Cachola 1992, Azevedo and Matos 1999, Padovan et al. 2003, Azevedo and Padovan 2004). Recently, some new *Nematopsis* spp. have been described (Azevedo and Matos 1999, Lima et al. 2001, Jiménez et al. 2002, Azevedo and Padovan 2004). Several unnamed *Nematopsis* spp. have been reported in bivalve species

from different geographic areas (Azevedo and Cachola 1992, Soto et al. 1996, Kim et al. 1998, Berrilli et al. 2000, Tuntiwaranuruk et al. 2004, 2008, Pinto and Boehs 2008). Sporogonic stages have been reported rarely in gastropods (Hatt 1927, 1931, Azevedo and Padovan 2004) and in crustaceans (Kruse 1966, Jiménez et al. 2002). In the present paper, we present light and transmission electron microscope data relating to a porosporidian parasite, *Nematopsis* sp., which infects a clam from the Arabian Gulf. This is the first description of a *Nematopsis* sp. from this region.

MATERIALS AND METHODS

Collection and isolation of specimens

Fifty adult specimens of the clam *Meretrix meretrix* (Linnaeus, 1758) (Bivalvia, Veneridae), of marketable size (~5–6 cm), were collected in May 2011 from the intertidal coast of the

Arabian Gulf near the city of Damman (26°17'0"N, 50°12'0"E), situated about 380 km from the city of Riyadh, Saudi Arabia. The living specimens were dissected under a light microscope in order to separate the infected tissues and organs.

Light and transmission electron microscopy

Only the abductor muscles were infected by oocysts, morphologically identified as belonging to the genus *Nematopsis*. Some living parasitized clams presented gaping valves and abductor muscles that appeared to have weak contractile power. The infected tissues containing some isolated oocysts were photographed using a light microscope with Nomarski differential interference contrast (DIC) optics to enable observation and measurements made directly on living oocysts or from photomicrographs obtained from living oocysts utilising image software.

For examination with the transmission electron microscope (TEM), small infected fragments of abductor muscle tissues were fixed in 5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.4) for 20 to 24 h, washed overnight in the same buffer at 4°C, and post-fixed in 2% OsO₄ for 3–4 h in the same buffer and at the same temperature. After dehydration in an ascending graded ethanol series and propylene oxide, the tissues were embedded in Epon. Semi-thin sections for light microscope examination were stained with methylene blue-Azur II. Ultrathin sections were sectioned using a diamond knife, double-stained with aqueous uranyl acetate and lead citrate and were then observed and photographed in a JEOL 100CXII TEM operated at 60 kV.

RESULTS

Light microscopy

The parasites, represented by several dispersed oocysts, each formed by an oocyst wall and internal uninucleate vermiform sporozoite, were observed in squash preparations among the myofibrils of the abductor muscle tissues of the bivalve *Meretrix meretrix*. Similar aspects of the oocysts were observed in semithin sections (Fig. 1). Among the oocysts some empty oocyst walls, without the correspondent sporozoite, were observed (Fig. 2). Phagocytes and parasitophorous vacuoles were never observed. In the squash preparations observed by light microscope 22 of 50 (44%) specimens were infected by oocysts.

Transmission electron microscopy

Each oocyst was formed by an oocyst wall surrounding a single uninucleated vermiform sporozoite accommodated in the oocyst lumen. The oocyst wall (0.70–0.85 µm thick) was composed of three equal-thickness layers of homogenous material. This structure was composed of homogenous material with structures of moderated electron density, less dense than the oocyst wall (Fig. 3). Some empty oocysts, without the correspondent sporozoite, but having a similar ultrastructural organization as the oocyst wall, were also observed (Fig. 4). By serial ultra-thin longitudinal and transversal sections it was possible to determine that the oocysts were 15.6 ± 0.6 µm long and 11.1 ± 0.7 wide (n = 20). Each sporozoite was accommodated

as a coiled vermiform containing a spherical nucleus (~2 µm in diameter) with a large nucleolus (~1 µm in diameter) and a cytoplasm with numerous small vacuoles and vesicles (Figs. 3, 5). The nucleus had a moderated homogenous electron-dense matrix without evidence of heterochromatin (Fig. 7). This matrix was in close contact with the internal face of the oocyst wall (Figs. 3, 5, 7).

The oocyst wall was in turn in close contact with a complex dense network of numerous anastomosed microfibrils, which projected from the periphery of the oocyst wall towards the surrounding host cells, with which were in direct contact (Figs. 5–8). Indeed, the close contact between the oocyst wall and the dense layers of the aggregated microfibrils caused that it was difficult to observe the outline of the oocyst wall (Figs. 3, 5, 7, 8). This complex aggregation of microfibrils was composed of two layers with different orientation and organization (Figs. 6–8). The internal layer, which was in close contact with the oocyst wall, was denser with a greater concentration in the network of the microfibrils, whereas the external layer was less dense and presented some projections among the cells of the host (Figs. 3, 5–8). These projections were in direct contact with the myofibrils and other cells of the host, and some lysosomal-like structures were observed among them (Fig. 6). The surrounding myofibrils and other cells of the host appeared degraded (Figs. 6, 8). No phagocytic cells and parasitophorous vacuoles were observed surrounding the oocyst of the parasite.

DISCUSSION

The ultrastructure of the oocyst described in the present work corresponds to that reported for sporogony of species of the family Porosporidae infecting tissues of molluscs (Sprague 1970). This family contains only two genera, *Porospora* Schneider, 1892 and *Nematopsis*. Between these two genera the most usual characteristic used in taxonomic distinction is the presence or absence of an oocyst wall (Hatt 1927, Sprague 1970). Species of *Porospora* are characterized by possessing a naked oocyst without a wall, while those of *Nematopsis* are defined as having oocyst with a thick oocyst wall enclosing a single vermiform sporozoite (Hatt 1931, Sprague 1970, Azevedo and Matos 1999).

Until recently, most species of *Nematopsis* were described using light microscopy or illustrated by schematic drawings based on light microscopy (Hatt 1927, Prytherch 1938, 1940, Sprague 1949, 1970, Belofastova 1996, Soto et al. 1996, Tuntiwaranuruk et al. 2004, Pinto and Boehs 2008). Only a few species have been studied by TEM (Azevedo and Cachola 1992, Azevedo and Matos 1999, Padovan et al. 2003, Azevedo and Padovan 2004). Several unnamed *Nematopsis* spp. from bivalve species of different geographic areas were reported as infecting a variety of mollusc species (Azevedo and Cachola 1992, Kim et al. 1998, Berrilli et al. 2000, Canestri-Trotti et

Figs. 1–6. Light and transmission electron microscopy micrographs of the apicomplexan gregarine, *Nematopsis* sp., a parasite found among the myofibrils of the abductor muscle of the bivalve *Meretrix meretrix*. **Fig. 1.** Light microscope (Nomarski differential interference contrast) observation of an oocyst containing a single sporozoite located among the surrounding myofibrils. **Fig. 2.** Semithin section showing an empty oocyst wall after the sporozoite excystment. **Fig. 3.** Ultrastructural transverse section of an oocyst showing the sporozoite, the surrounding dense matrix, the oocyst wall, and the external complex network of anastomosed microfibrillar layers (*). At the periphery some aspects of lysis among the host cells can be observed. **Fig. 4.** Ultrastructural aspect of the oocyst wall of an empty oocyst after the excystment of the sporozoite showing the organization of the wall and the peripheric microfibrillar organization (*). **Fig. 5.** Aspect of an oocyst section showing sporozoites and their vesicular organization, the surrounding dense matrix, oocyst wall and the external microfibrillar organization (*) closely adherent to the oocyst wall. The outline between these two structures is not well evident. **Fig. 6.** Detail of the external microfibrillar arrangement adherent to the wall and projected towards the periphery contacting directly the myofibrils of the abductor muscle (H). **Abbreviations:** H – host cell; M – matrix; Oc – oocyst; OcW – oocyst wall; Sz – sporozoite.

al. 2000, Carballal et al. 2001, Lima et al. 2001, Tuntivanuruk et al. 2004).

The most evident difference found in the present description compared to previously described species was in the complex ultrastructural organization of the network of anastomosing microfibrils that formed an adherent wall-like structure around the oocyst wall. The oocyst of *N. mytella* contained a complex network of numerous anastomosed microfibrils adherent to their oocyst wall (Azevedo and Matos 1999, Padovan et al. 2003) located in the parasitophorous vacuoles, but these structures were different in the present *Nematopsis* sp. The microfibrillar complex surrounding the oocyst wall of the present species has a very electron dense ultrastructural organization, not included in a parasitophorous vacuole, whereas *N. mytella* has a loose microfibrillar organization, externally contacting the phagocyte (Azevedo and Matos 1999, Padovan et al. 2003). When comparing the oocyst of *Nematopsis* sp. in the present study we observed that these structures surrounding the oocyst wall have not been reported in any other *Nematopsis* spp.

On the other hand, comparing the oocyst measurements, we observed that the oocysts of *Nematopsis gigas*, described from the marine gastropod *Nerita ascencionis*, are the largest, measuring $21.9 \times 11.5 \mu\text{m}$ (Azevedo and

Padovan 2004), while those of *N. duorari* measure $18.9 \times 9.8 \mu\text{m}$ (Kruse 1966), and *N. prytherch*, $19 \times 16 \mu\text{m}$ (Sprague 1949), i.e. they are somewhat smaller. The oocysts of the present *Nematopsis* sp. measuring $\sim 15.6 \times 11.1 \mu\text{m}$ are therefore one of the smallest among the species of this genus that have been previously described (Table 1), except that those of *N. mytella* (11.5×8.2) remain smaller than those of the present species.

Table 1. Comparative character of the oocysts of different *Nematopsis* spp., including measurements (in μm).

<i>Nematopsis</i> spp.	Hosts	Oocyst length $\times$ width	References
<i>N. schneideri</i>	<i>Mytilus edulis</i>	$12 \times 7-8$	Léger 1903
<i>N. prytherch</i>	Oyster	20×11 19×16	Prytherch 1940 Sprague 1970
<i>N. duorari</i>	Pelecypod molluscs	18.9×9.8	Kruse 1966
<i>N. legeri</i>	<i>Porospora galloprovincialis</i>	$13-16 \times 7-8.1$	Belofastova 1996
<i>N. portunidarum</i>	<i>Cerastoderma edule</i>	$10-11 \times 6.8-7$	Belofastova 1996
<i>N. incognito</i>	<i>Cerastoderma lamarki</i>	$15-15.7 \times$ $10.5-11.7$	Belofastova 1996
<i>N. mytella</i>	<i>Mytella guyanensis</i>	11.5×8.2	Azevedo and Matos 1999
<i>N. gigas</i>	<i>Nerita ascencionis</i>	21.9×11.5	Azevedo and Padovan 2004
<i>Nematopsis</i> sp.	<i>Meretrix meretrix</i>	15.6×11.1	Present study

Figs. 7, 8. Transmission electron microscopy micrographs of the apicomplexan gregarine, *Nematopsis* sp., a parasite found among the myofibrils of the abductor muscle of the bivalve *Meretrix meretrix* showing some details of the oocysts. Sporozoite, the nucleus and a nucleolus, the oocyst wall and the microfibrillar layer (*) in close contact (arrows) with the cells of the host, where there are some sections of the myofibrils (arrowheads). Abbreviations: H – host cell; Nc – nucleolus; Nu – nucleus; OcW – oocyst wall; Sz – sporozoite.

Fig. 9. Schematic drawing of an oocyst of *Nematopsis* sp., showing all oocystic components described on the basis of serial ultrathin sections.

In the present study phagocytic cells and parasitophorous vacuoles were not observed. The lysis activity corresponding to the presence of this parasite is clearly evident in some lysosomal aspects of the host cell, which shows a degree of ultrastructural degradation in the zone that is in close contact with the more external oocyst microfibrils.

Based on these morphologic differences, including the oocyst measurements and the presence of the complex microfibrillar network adherent to the oocyst wall, the absence of phagocytic cells encasing the oocyst, as well as the geographic host localization and host specificity, we expected the present species to be a new one. However, lack of the complete life cycle and/or molecular data precluded the complete identification of the present species. Curiously, some of the clams infected with *Nematopsis* sp. described in the present paper were simultaneously infected by cysts containing trophozoites of *Perkinsus* sp. This occurrence has been reported in some other bivalve species (Canestri-Trotti et al. 1998, 2000).

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Taxonomic Summary

Phylum Apicomplexa Levine, 1970
 Class Sporozoa Leuckart, 1879
 Order Eugregarinida Léger, 1900
 Family Porosporidae Schneider, 1899
 Genus *Nematopsis* Schneider, 1892
Nematopsis sp.

Specific diagnosis: oocysts among the myofibrils of the abductor muscles, each containing only one sporozoite (Figs. 1–3). No other organ or tissue was infected by this parasite. The oocyst is $15.6 \pm 0.6 \mu\text{m}$ in length by $11.1 \pm 0.7 \mu\text{m}$ in width ($n = 50$), each containing a single uninucleate vermiform sporozoite (Figs. 1–3). The oocyst wall is $0.70\text{--}0.85 \mu\text{m}$ thick and is covered by a plano-convex-like operculum hardly noticed ($\sim 2.5 \mu\text{m}$ in diameter and $\sim 0.75 \mu\text{m}$ thick) (Fig. 3). The oocyst wall is surrounded by a complex network of numerous anastomosing microfibrils that project from the oocyst wall towards the periphery contacting the host cells (Figs. 10, 11). These microfibrils are adherent to the oocyst wall forming a wall-like structure surrounding the oocyst wall. In many of the ultrastructural observations the outline of the oocyst wall was difficult to distinguish (Figs. 3, 5, 7, 8).

Type host: The oocysts were observed among the myofibrils of the abductor muscle tissues of the marine clam *Meretrix meretrix* (Veneridae).

Locality: Arabic Gulf coast ($6^{\circ}17'0''\text{N}$, $50^{\circ}12'0''\text{E}$) near city of Dammam of Saudi Arabia.

Site of infection: Myofibrils of abductor muscles.

Prevalence: 44% (22/50).

Material deposited: Glass slide with semithin sections is deposited in the Museum of the Zoology Department, College of Science, King Saud University, Riyadh City, Saudi Arabia with registration no. N-22-2011.

Histopathology: The myofibrils of the abductor muscles of the infected clams that are in contact with the oocysts show some aspects of lysis, and the abductor muscle seem to have weak contractile power.

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