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FINE STRUCTURE OF THE OOCYST OF *Nematopsis mytella* (APICOMPLEXA, POROSPORIDAE), A PARASITE OF THE MUSSEL *Mytella falcata* AND OF THE OYSTER *Crassostrea rizophorae* (MOLLUSCA, BIVALVIA) FROM THE NORTHEASTERN ATLANTIC COAST OF BRAZIL

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ABSTRACT

Oocysts of *Nematopsis mytella*, an apicomplexa gregarine parasite of the mussel *Mytella falcata* (fam. Mytelidae) and of the oyster *Crassostrea rizophorae* (fam. Ostreidae), were collected from Itamaracá Island and Suape, two distinct zones in the estuarine region of the northeastern coast of Brazil, near the city of Recife. The oocysts were examined by light and transmission electron microscopy. No morphological and ultrastructural differences were observed between oocysts of the two host species. The oocysts were engulfed by the cytoplasm of host phagocytes, with each phagocyte containing 1–19 oocysts (usually 3–5). Each oocyst was located in the center of a spherical to ellipsoidal parasitophorous vacuole (PV) 20–25 µm in diameter. The oocyst (13.2 µm long and 8.4 µm wide) consisted of an external wall 0.6 µm thick surrounded by an internal, uninucleated sporozoite. The apical region of the wall of each oocyst contained a circular micropyle 0.8 µm in diameter covered by an operculum. The oocyst wall was connected to the PV membrane by a complex network of anastomosed microfibrils. The PV matrix contained 1–2 dense bodies and the periphery was occupied by numerous glycogen particles. This is the first record of a *Nematopsis* sp. from the northeastern coast of Brazil and only the second from Brazil as a country.

Key words: Mussel, *Nematopsis mytella*, oocyst, oyster, parasite, ultrastructure

INTRODUCTION

The phylum Apicomplexa Levine, 1970 is a large group that contains numerous protozoan parasites, the most important of which belong to the order Eugregarinida Léger, 1900, with over 1,600 species. These parasites occur mostly in invertebrates and many have a high host specificity [7,10,12].

Several gregarine species parasitize commercially important marine molluscs, particularly bivalves [10]. The genus *Nematopsis* Schneider, 1892, which parasitizes bivalves, belongs to the family Porosporidae Labbé, 1899. For several years, there has been some confusion about the taxonomic distinction between the genera *Nematopsis* and *Porospora* [5,10,12]. To help distinguish these genera, *Nematopsis* has been reported to have monozoic resistant oocysts (spores) whereas *Porospora* has naked sporozoites [3,6,10].

Nematopsis species show host alternation, with gametogony and sporogony occurring in bivalves [8] and the vegetative stage (schizogony) occurring in crabs, the definitive host [4,10,12]. The prevalence of *Nematopsis* species varies among bivalve hosts in different geographic areas [2].

In this paper, we describe the ultrastructure of the oocysts of *Nematopsis mytella* and the host cells (phagocytes) from two host species found along the northeastern Atlantic coast of Brazil.

MATERIAL AND METHODS

Small fragments of the foot and gills of the mussel *Mytella falcata* (Orbigny, 1842) and of the oyster *Crassostrea rizophorae* Guilding, 1828 were studied. Specimens of both species were collected on Itamaracá island (07º38′00″ S, 34º48′06″ W) and at Suape (08º 21′06″ S, 34º 57′04″ W), two locations about 100 km apart close to the city of Recife in the Brazilian state of Pernambuco. Squash preparations of the infected tissues were examined using differential interference contrast (DIC) optics (Nomarski) to detect the infection.

For transmission electron microscopy (TEM), fragments of the foot and gills were fixed for 12 h at 4ºC in 3% glutaraldehyde buffered with 0.2 M sodium cacodylate (pH 7.2 - 7.4), then

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washed in the same buffer overnight at 4°C and postfixed in buffered 2% OsO_4 for 3-4 h at the same temperature. After dehydration in an increasing ethanol series and propylene oxide (6-10 h for each change), the infected fragments were embedded in Epon (10–12 h for each change). Semithin sections were stained with toluidine blue to determine the localization of the parasites. Ultrathin sections were subsequently obtained with a diamond knife, stained with uranyl acetate and lead citrate, and observed in a JEOL 100 CXII transmission electron microscope operated at 60 kV.

RESULTS

Numerous oocysts were observed with DIC (Nomarski) lighting in fresh smears and semithin sections of foot and gill tissue samples of the mussel *M. falcata* and the oyster *C. rizophorae*, respectively. The three dimensional morphology of the oocysts was easily observed with DIC optics**.** Grouped or isolated oocysts were present in the parasitophorous vacuoles (PV) of host cell phagocytes (Figs. 1 and 2). Each parasitized phagocyte contained a variable number of oocysts (1 to 19, more frequently 3 to 5) and was surrounded by a membrane (Fig. 2). The infected phagocytes with a large number of oocysts (15–19) measured 80–90 µm in diameter, while phagocytes with a smaller number of oocysts (3–7) measured 35–45 µm (Figs. 1 and 2). The number of oocysts per phagocyte was confirmed in semithin sections (Fig. 3, inset). Each PV measuring 23 µm (range: 20-25 µm) in diameter contained a single ellipsoidal oocyst, 13.2 (12.9–13.4) µm long and 8.4 (8.0–8.6) µm wide (n = 40) (Figs. 2 and 3, inset).

TEM of serial ultrathin sections showed that each PV contained a single oocyst and each oocyst contained only one uninucleated sporozoite (Fig. 3). No morphological or ultrastructural differences were observed between the oocysts of the two host species. The phagocytes showed some lysis, and several spherical to ellipsoidal PV contained a single oocyst (Fig. 3). All of the infected phagocytes were surrounded by an amorphous granulo–fibrillar matrix with no clearly organized structures (Fig. 3). The oocyst wall was 0.60 (0.57–0.62) µm thick and consisted of a homogeneous electron dense material (Figs. 4 and 5). Externally, the wall had a short, fine, uniform layer (about 60 nm thick) of anastomosed microfibrils that were closely adhered to the wall (Figs. 4 and 5). More externally, in the PV matrix, there was an anastomosed network of numerous microfibrils projecting from the oocyst wall towards the PV membrane (Figs. 6 and 7). The oocyst wall contained a circular micropyle ~ 0.8 µm in diameter covered by an opercular system. The apical portion of the opercular system was bound to the PV membrane by an amorphous mass. Each oocyst contained a single vermiform uninucleated sporozoite (Fig. 4).

The infected phagocytes were packed in the connective tissue of the foot of the mussels (Fig. 1) and in the gill tissues of the oysters (Fig. 3, inset). The phagocytes were uninucleated cells, and some of them had a lysed cytoplasm and nucleus (Fig. 3). In advanced stages of lysis, the PV membranes were degraded and the cytoplasmic matrix was in direct contact with the PV matrix (Figs. 4 and 6). The phagocyte cytoplasm was occupied mainly by numerous glycogen particles which were sometimes in direct contact with the PV microfibrils (Fig. 6). The glycogen particles were more numerous in the phagocytes of *M. falcata* than in those of *C. rizophorae*.

In some sections, 1–2 dense bodies and groups of agglutinated microfibrils were seen in the PV matrix (Fig. 6). Free uninucleated sporozoites containing a circular nucleus with evident masses of chromatin and cytoplasmic vesicular structures were observed among the oocysts (Fig. 9).

There were no ultrastructural differences in the shape and dimensions of the oocysts recovered from the hosts *Mytella falcata* and *Crassostrea rizophorae*.

DISCUSSION

The ultrastructural morphology of the oocysts described here agrees with the well-known sporogony that occurs in the family Porosporidea Labbé, 1899, which belongs to the phylum Apicomplexa Levine, 1982 [7].

The first species of *Nematopsis* Schneider,1892 to be described (*Nematopsis* sp.) was found in the mantle of the razor clam *Solen vagina* in France [9], and several species are now known to infect bivalve hosts in different geographic regions. The taxonomic and systematic status of *Nematopsis*, sometimes considered as a synonym of *Porospora* [4], has caused some confusion [10]. Despite this taxonomic uncertainty, the ultrastructural findings for the oocysts described here were similar to those reported for the genus *Nematopsis* in general [1,6] and for the species *N. mytella* [2].

Nematopsis legeri (de Beauchamp, 1910) is the best known species of *Nematopsis*, although, the type species is *N. schneider* Léger, 1903 found in the gills of the mussel *Mytilus edulis*. Host mortality is an important common factor among some species of *Nematopsis* [1,10], although, several species are con-

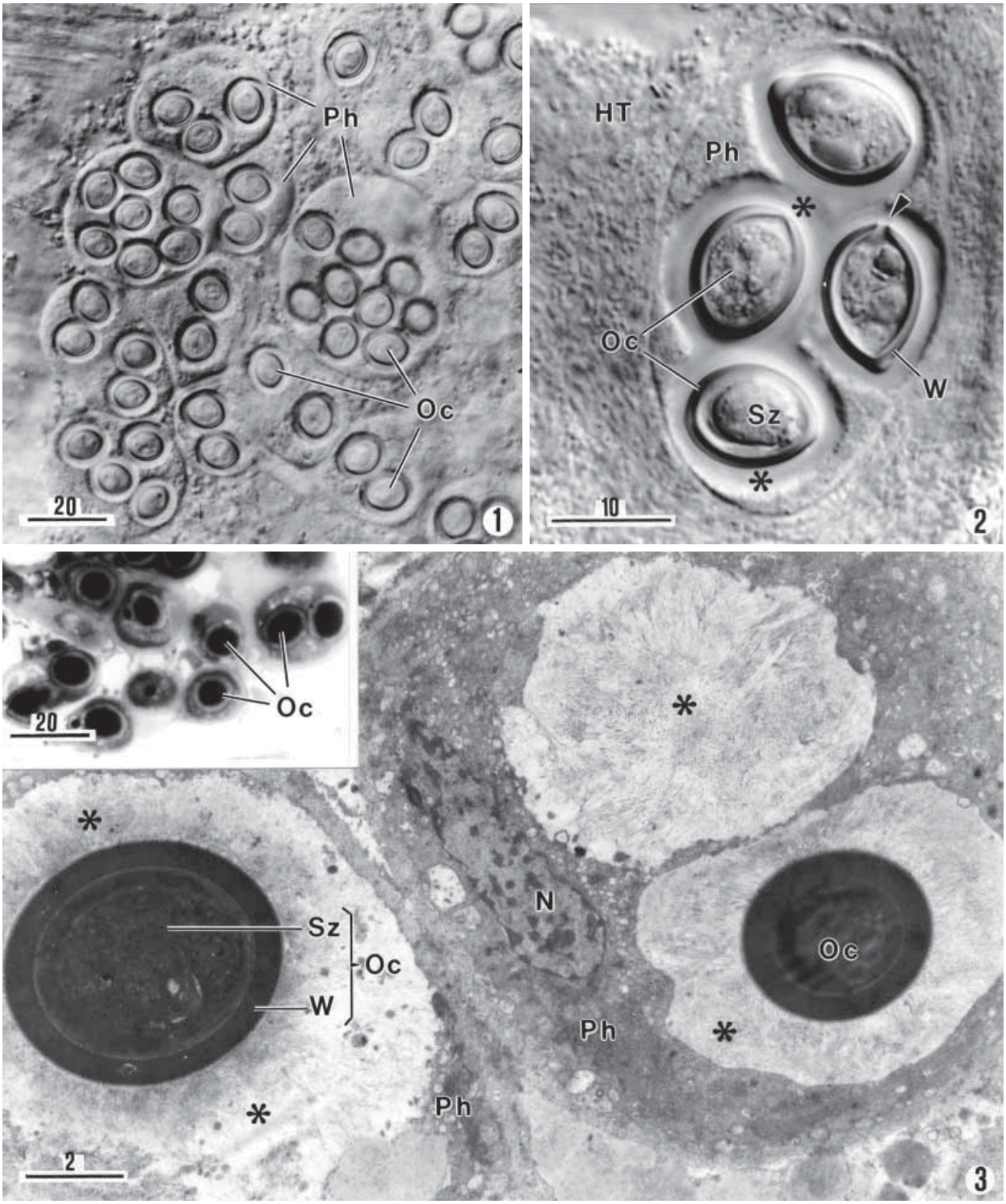

Figure 1. Squash preparation of infected foot tissue from the mussel *Mytella falcata* observed in DIC. Note the host cell phagocytes (**Ph**), each with a variable number of oocysts (**Oc**). Scale bars in μm.
Figure 2. Detail of a phagocyte (**Ph**) of *Crassostrea rizophorae* showing four oocysts (**Oc**), each one in a parasitophorous vacuole (*). One oocyst has a micropyle opening (**arrowhead**). The phagocyte is surrounded by host tissue (**HT**). Each oocyst consists externally of a wall (**W**) and an internal, uninucleated sporozoite (**Sz**).
Figure 3. Ultrathin section of the host tissue of *M. falcata* showing two adjacent phagocytes (**Ph**) containing oocysts (**Oc**). Each oocyst was formed by the oocyst wall (**W**) surrounding an internal, uninucleated sporozoite (**Sz**). The parasitophorous vacuoles were partially occupied by numerous microfibrils (*). The nucleus (**N**) of a phagocyte can be seen. Inset. Semithin section of phagocytes, containing oocysts (**Oc**) in infected gill tissue of *C. rizophorae*.

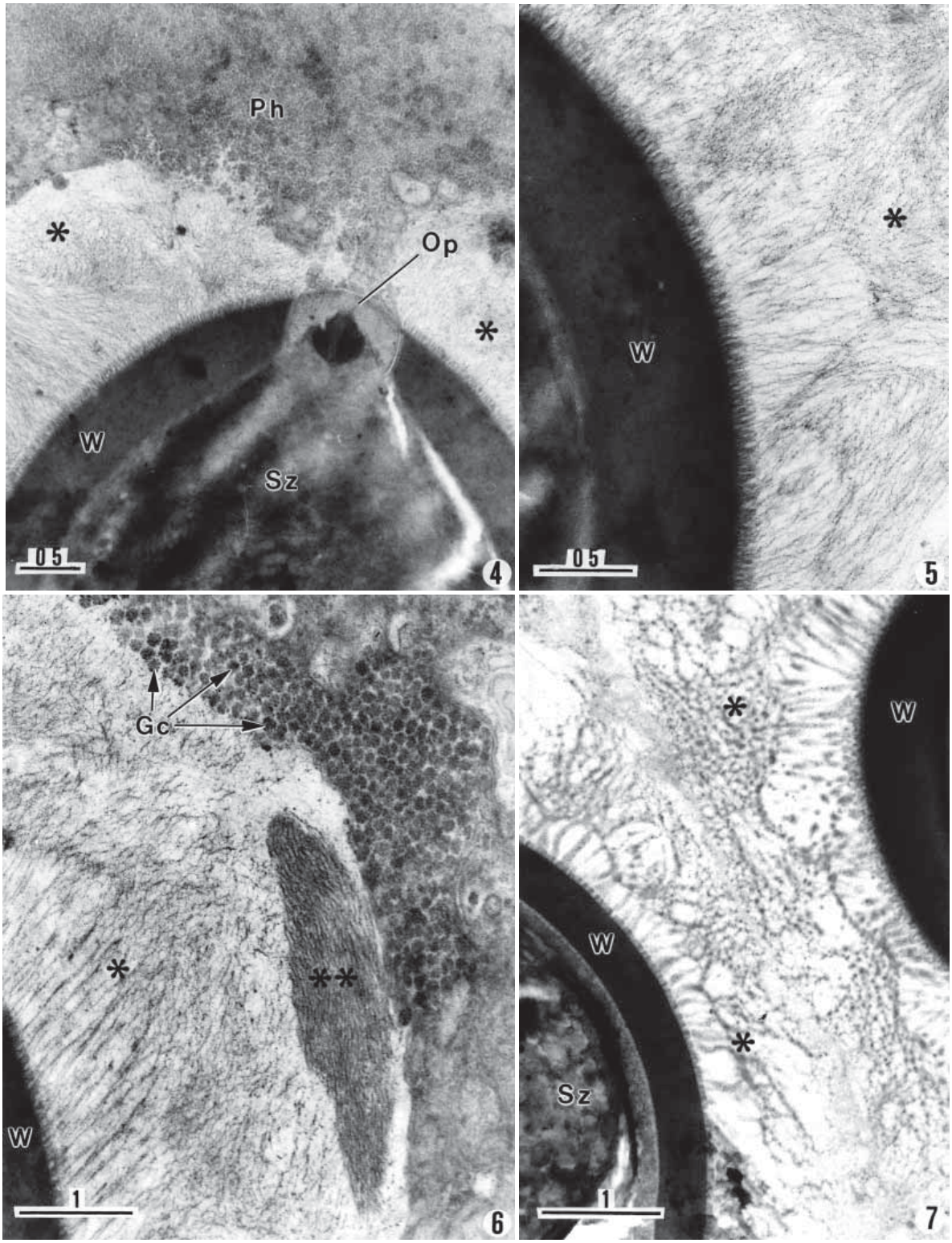

Figure 4. Ultrathin section of a phagocyte (**Ph**) of *C. rizophorae* showing the apical region of one oocyst, with a close view of the oocyst wall (**W**) and the operculum (**Op**). The matrix of the parasitophorous vacuole (*****) contained numerous anastomosed microfibrils. The oocyst contained a sporozoite (**Sz**).
Figure 5. Detail of the oocyst wall (**W**) and its attached microfibrils (*****) in *C. rizophorae*.
Figure 6. High magnification of an internal portion of a phagocyte of *M. falcata* showing the oocyst wall (**W**), the adherent microfibrils (*****), a group of agglutinated microfibrils (******) and numerous glycogen particles (**Gc**).
Figure 7. Detail of the space between two fused parasitophorous vacuoles (*****) of *M. falcata* showing the complex network of anastomosed microfibrils adherent to their walls (**W**). Part of the sporozoite (**Sz**) can be seen in one of the oocysts.

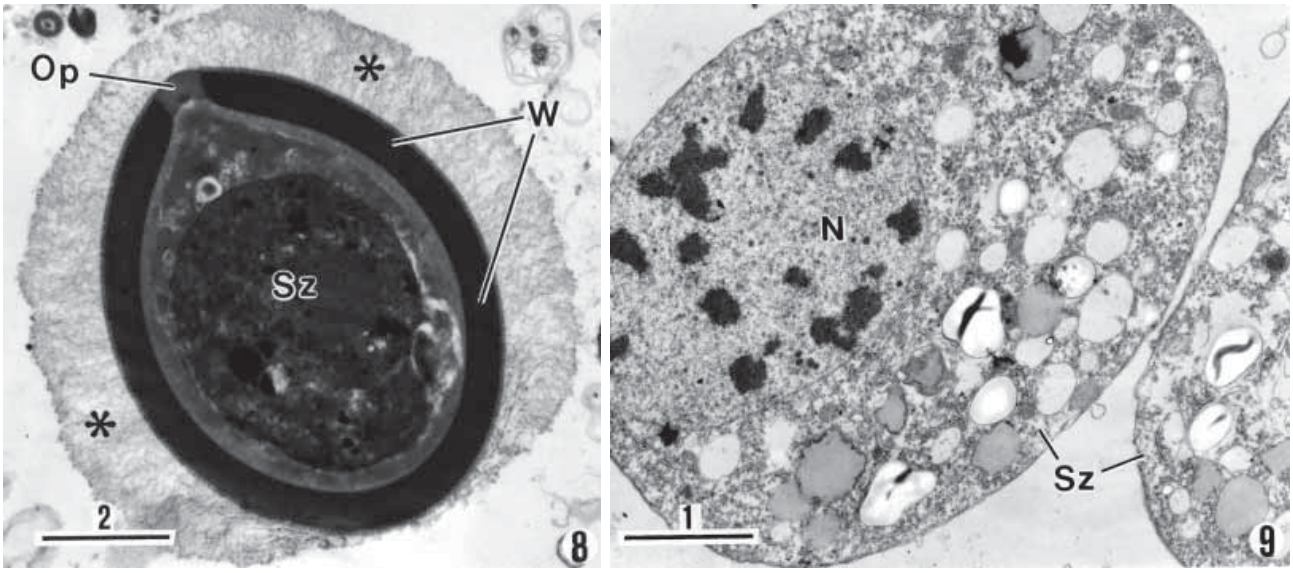

Figure 8. Ultrathin section of an oocyst of *M. falcata* showing the wall (**W**), the sporozoite (**Sz**) and the surrounding network of anastomosed microfibrils (*) following release from the parasitophorous vacuole. The operculum (**Op**) of the oocyst wall is visible.
Figure 9. Ultrathin section of two, free, uninucleated sporozoites (**Sz**) of *M. falcata*, showing the nucleus (**N**) and some cytoplasmic vesicular structures.

sidered not to be pathogenic [1,8]. Most cases of *Nematopsis* occurring in bivalves have been reported from the Atlantic coast of the United States. The first report involved *N. ostrearum* which was discovered in the oyster *Crassostrea virginica*, although this parasite also infects other host species [8,11].

Nematopsis mytella was first recorded in Brazil in the Amazonian mussel *M. guyanensis* [2] and, as shown here, also occurs in the molluscan fauna of northeastern Brazil. Light and transmission electron microscopy showed that the oocystes of *Nematopsis mytella* recovered from the mussel *M. falcata* and the oyster *C. rizophorae* were similar in morphology to those found in *Mytella guyanensis*, a recently described species from the Amazon estuary [2]. Additional ultrastrutural studies are needed to determine the relationship of this parasite to its hosts.

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