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OBSERVATIONS ON THE INTRACYTOPLASMIC MICROSPORIDIAN *Steinhausia mytilovum*, A PARASITE OF MUSSEL (*Mytella guyanensis*) OOCYTES FROM THE AMAZON RIVER ESTUARY

Edilson Matos[1], Patricia Matos[2] and Carlos Azevedo[3,4]

[1]Carlos Azevedo Research Laboratory (LPCA), Federal Rural University of Amazonia (UFRA), Montese, Belém, PA, Brazil, [2]Laboratory of Histology of Aquatic Animals (LHAA), Center for Biological Sciences (CCB), Federal University of Pará, Belém, PA, Brazil, [3]Department of Cell Biology, Institute of Biomedical Sciences (ICBAS-UP), University of Porto, [4]Laboratory of Protoparasitology, Centre for Marine and Environmental Research (CIIMAR-UP), Porto, Portugal.

ABSTRACT

Microsporidians (Microsporidia) can parasitize commercially important marine mollusks, including bivalves. In this report, we provide a brief description of the ultrastructure of the microsporidian *Steinhausia mytilovum* that occurs in the oocyte cytoplasm of the mussel *Mytella guyanensis* (Mollusca, Bivalvia, Mytillidae) from the Amazon river estuary. Mussel ovaries were fixed, stained and examined using differential interference contrast optics (DIC). The parasite developed in an intracytoplasmic vacuole containing a variable number of spores (up to 14). Mature spores were 2.3 ± 0.3 μm long and 1.7 ± 0.3 μm wide (n = 25 each). Transmission electron microscopy revealed two types of intracytoplasmic vacuoles, one containing spores with a light (less dense) cytoplasm that corresponded to the maturation phases, and the other containing mature, dense, granular spores that showed specific microsporidian structures. The anchoring disc and the anterior zone of the polar filament were surrounded by the polaroplast. The polar filament was isofilar and consisted of a double (rarely triple) coil with 9-10 turns. The ultrastructural morphology of these spores suggested that they belonged to *S. mytilovum*.

Key words: Bivalvia, microsporidia, *Steinhausia mytilovum*, ultrastructure

INTRODUCTION

Microsporidians are common parasites of several animal groups and are generally pathogenic to their hosts because of their ability to cause cellular damage [4,7,11,13]. Only a few microsporidians that parasitize marine bivalves have been described [2,5,8,9,11,14]. The genus *Steinhausia* Sprague, Ormières & Manier, 1972, which contains three species – *S. ovicola* [6], *S. mytilovum* [1,2,12,16] and *S. brachynema* [15] – is the only genus reported to parasite bivalve oocytes. One of the most important taxonomic characteristics of microsporidians is the multiplication phases of the parasite (merogony and sporogony) and, consequently, the final number of spores produced [14]. The shape and size of the spores, the host tissue affinity and the arrangement of the spores in the host cells are the main characteristics used to discriminate among microsporidian species [4,7,15,16].

Correspondence to: Edilson Matos, MSc.
Laboratório de Pesquisa Carlos Azevedo (LPCA), Universidade Federal Rural da Amazônia (UFRA), Av. Tancredo Neves, 2501, Montese, Belém, PA, Brazil. Tel: (55) (91) 3210-5207. E-mail: edilson.matos@ufra.edu.br

In this report, we describe the morphological and ultrastructural aspects of spore maturation in the microsporidian *Steinhausia mytilovum*, which parasitizes the oocyte cytoplasm of the mussel *Mytella guyanensis* (Mollusca, Bivalvia) found in the Amazon river estuary.

MATERIALS AND METHODS

Thirty mussels were collected in the Amazon river estuary in March, 2005, near the city of Augusto Correa (01^{0}01′ 45″ S/ 46^{0}38′ 57″ W), in the State of Pará, Brazil. For transmission electron microscopy (TEM), small fragments of the ovaries were fixed in 5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) for 5 h at 4°C, washed in the same buffer for 5 h at 4°C, and post-fixed in 2% OsO_4 buffered with the same solution for 2 h at the same temperature. After dehydration in an ascending ethanol series and propylene oxide, the samples were embedded in Epon. Semithin sections were stained with methylene blue-Azur II. Ultrathin sections were obtained with a diamond knife and, after being stained with uranyl acetate and lead citrate, were observed in a JEOL 100CXII transmission electron miscroscope operated at 60 kV.

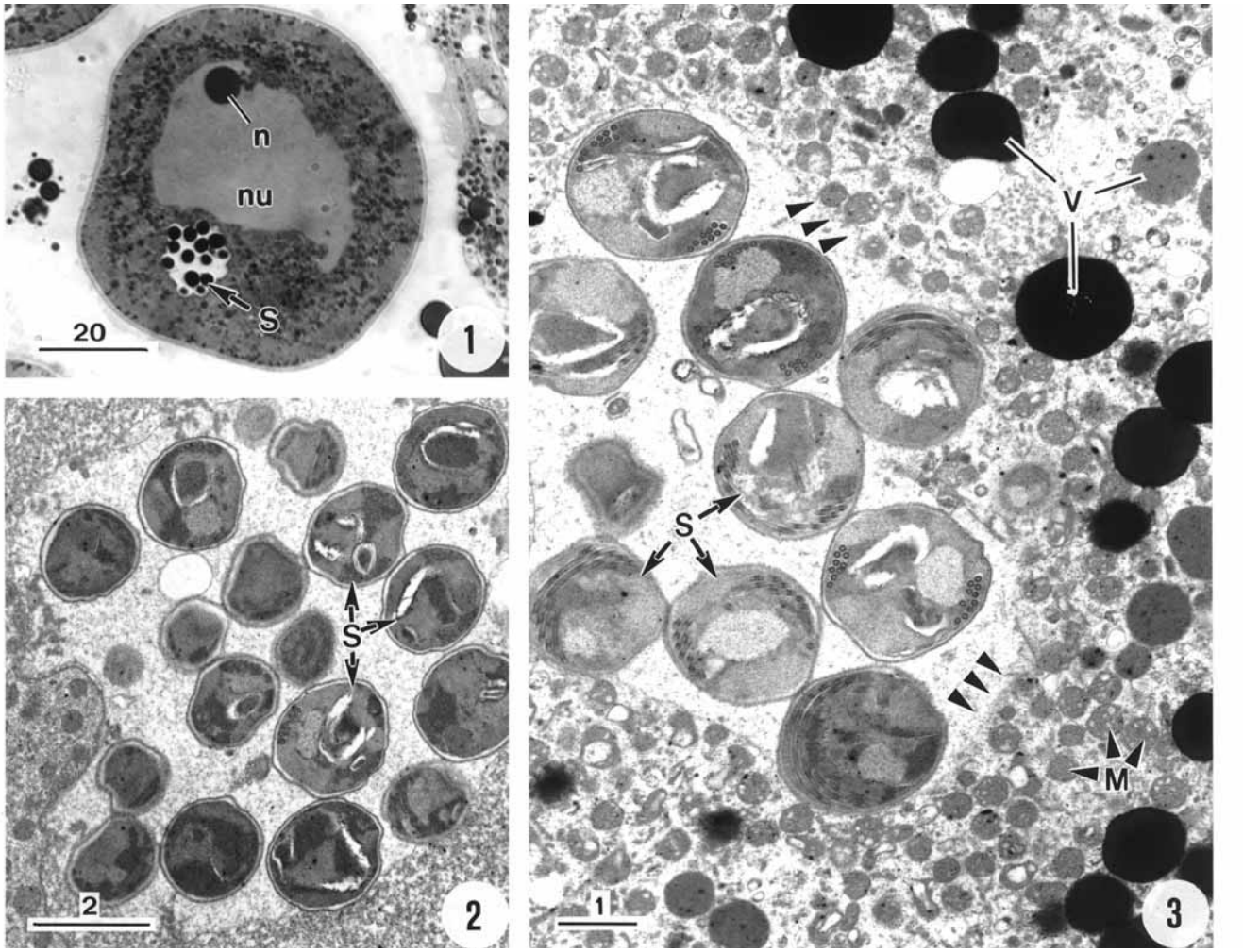
RESULTS

Light microscopy

Two out of 30 (6.6%) mussels contained parasites and only a small number of oocytes were parasitized. The parasites occurred as ellipsoidal spores enclosed in a vacuole located in the oocyte cytoplasm. One or two vacuoles per oocyte contained a variable number of spores (up to 14). Measurements done in semithin serial sections showed that the spores were 2.3 ± 0.3 μm long and 1.7 ± 0.3 μm wide (n=25) (Fig. 1).

Electron microscopy

TEM revealed the different stages of spore maturation and identified the spores as being microsporidian (Figs. 2-5). Most of the spores were located in vacuoles embedded in the host oocyte cytoplasm (Figs. 2 and 3), although a few isolated spores were seen embedded in the cytoplasm in close contact with cytoplasmic structures (Figs. 6 and 7). During maturation, the spores gradually became denser (Fig. 5) and, in the final phase of maturation, the spores were ellipsoidal, with the internal organization becoming difficult to see (Fig. 5). Some mature spores showed a less dense cytoplasm that probably resulted from artefactual empty spaces (Figs. 2-7). The spore wall was 110 ± 3 nm thick (n=25) and consisted of two layers of the same thickness. The external layer was denser than the inner layer, which was in close contact with the plasmalemma (Fig. 5). The extrusion apparatus consisted of the apical anchoring disc and the polar sac that overlaid the manubroid portion of the polar filament (Fig. 4). The posterior part



Figures 1-3. Stages of *S. mytilovum* spores maturation. **1.** Semithin section of an oocyte showing a central nucleus (**nu**), a prominent nucleolus (**n**) and an intracytoplasmic vacuole containing several spores of *S. mytilovum* (**S**); **2.** Ultrastructural aspect of cytoplasm containing a vacuole with immature spores (**S**); **3.** Ultrastructural details of immature spores (**S**) within a vacuole. The vacuole membrane shows some lysis (**arrowheads**), and the oocyte cytoplasm contains numerous mitochondria (**M**) and oocyte vitellar droplets (**V**). All scales bars in μm.

of the spore contained a coiled, double layer of the polar filament between the posterior vacuole and the spore wall. The polar filament was isofilar with 9-10 coils arranged in two, or rarely three, layers (Figs. 4 and 5).

The anterior part of the polar filament was completely surrounded by the polaroplast (Fig. 5). The two diplokaryon nuclei were surrounded by dense cytoplasm containing a barely visible hellicoidal

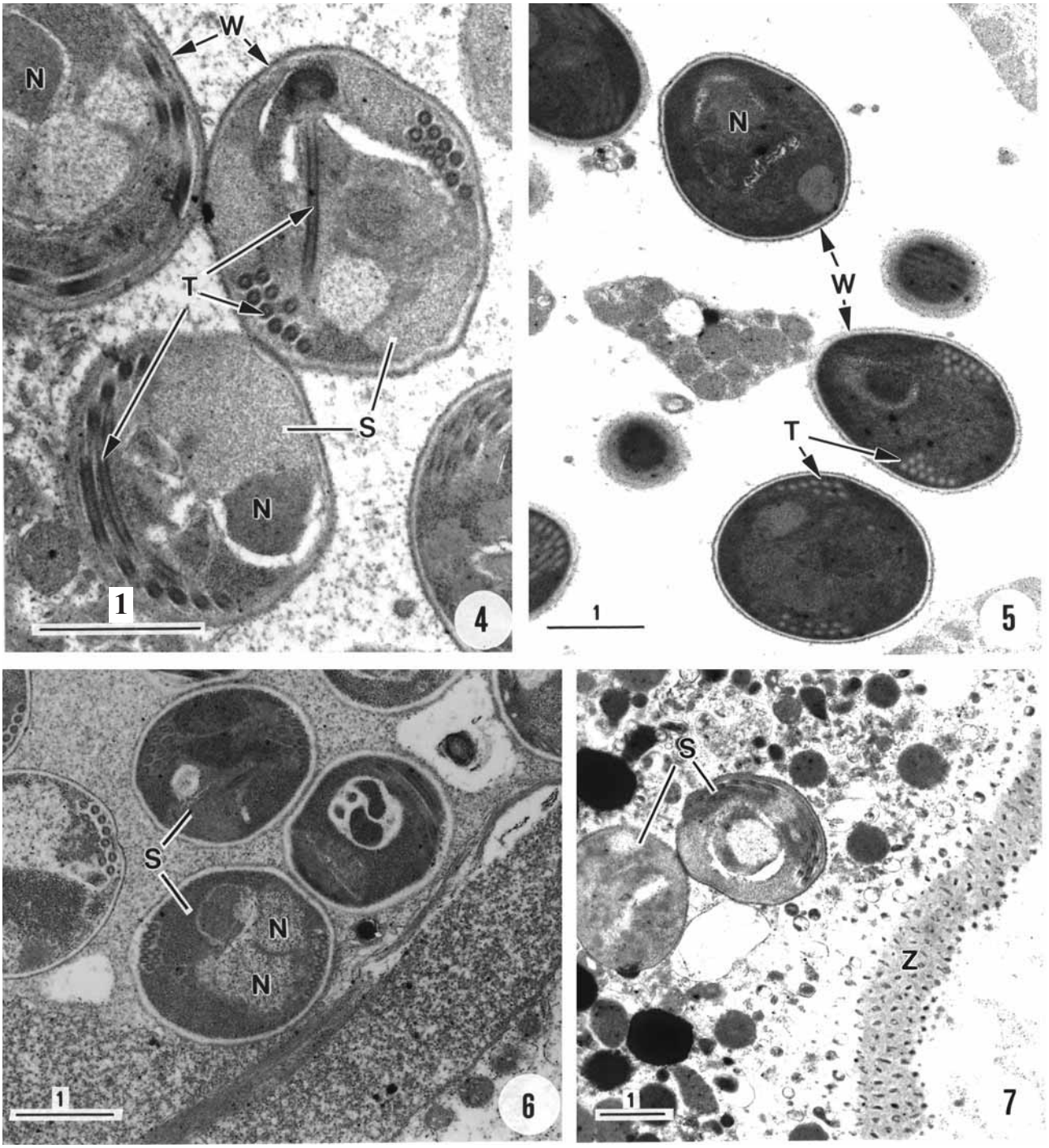


Figure 4-7. Ultrastructural details of *S. mytilovum.* **4.** Immature spores (**S**) showing the typical microsporidian structure, including the spore wall (**W**), polar filament (**T**) and its coils. Diplokaryon nuclei (**N**) are also present; **5.** Mature spores with poorly visible internal structures, except for the wall (**W**) and polar filament (**T**); **6.** Ultrathin section showing some spores (**S**), one of which has a diplokaryon (**N**); **7.** Vacuoles showing some lysis, with the spores (**S**) intermingled with the cytoplasmic contents of the oocytes. This arrangement was seen at the oocyte periphery, near the zona pellucida (**Z**). All scales bars in μm.

polyribosome (Figs. 4 and 5). In advanced stages of lysis, the vacuole membrane disappeared and the spores appeared to be in direct contact with the cytoplasmic structures (Figs. 6 and 7). Numerous mitochondria and vitellar globules were seen intermingled with spores that were displaced to the periphery of the oocyte near the zona pellucida; in these cases, the cytoplasm appeared to have been destroyed (Figs. 6 and 7).

DISCUSSION

The ultrastructural organization seen in the spores examined here corresponded to that of the phylum Microsporidia [7,13,14,16] and the genus *Steinhausia* [1,3,10,15]. This genus, which contains three species, has been reported in the oocytes of marine bivalves from different geographic areas. *Steinhausia ovicola* is a parasite of *Ostrea edulis* [6], *S. mytilovum* of *Mytilus edulis* [1,2] and *Mytilus galloprovincialis* [10], and *S. brachynema* of the snail, *Biomphalaria glabrata* [15]. Since bivalve culture is an important economic activity in many areas of the world, the damage caused to oocytes by microsporidians may adversely affect the reproductive capacity of infected mussels an have serious economic consequences.

The genus *Steinhausia* has not previously been reported in the South America fauna, and this is the first description of an intracytoplasmic microsporidian parasitizing oocytes in Brazil. Mussels from the Amazon river estuary showed a very low prevalence of this parasite. Studies of other *Steinhausia* spp. have suggested that the rate of infection shows a seasonal pattern that is directly related to water temperature, with lower temperatures resulting in a higher prevalence and a larger number of spores per mussel [1,3,10]. This pattern remains to be confirmed for the species studied here.

The ultrastructural morphology and spore size seen here were very similar to those described for *S. mytilovum* in various host species [1,3,6,10,12,15]. Based on these similarities, we conclude that the specimens examined here belonged to *S. mytilovum*, and represent the first microsporidian species to be recorded from the northern Atlantic coast of Brazil.

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