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Characterization of Minchinia sp Spores (Ascetospora: Haplosporidiidae) Infecting Teredo navalis L and Placopecten magellanicus Von Martens (Mollusca: Teredinidae) in the Western North Atlantic

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CHARACTERIZATION OF *Minchinia* sp. SPORES (ASCETOSPORA: HAPLOSPORIDIIDAE) INFECTING *Teredo navalis* L. AND *Teredo furcifera* VON MARTENS (MOLLUSCA: TEREDINIDAE) IN THE WESTERN NORTH ATLANTIC

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Master of Arts

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APPROVAL SHEET

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I'd especially like to thank my family, my husband Jack, my brother Bert and my parents, for their friendship, love and support.

LIST OF FIGURES

ABSTRACT

Spores of a haplosporidan infecting Teredo navalis and T. furcifera have been described as morphologically indistinguishable from spores of Haplosporidium nelsoni, the oyster pathogen commonly referred to as MSX. A colloidal gold immunoassay was used to test the hypothesis that H. nelsoni and the haplosporidan infecting Teredo spp. are conspecific. Additionally, antigenic characteristics of spores of the haplosporidan found in Teredo spp. were compared to spores of other local haplosporidan species, H. costale infecting Crassostrea virginica and H. louisiana infecting Panopeus herbstii. The immunoassay demonstrated that the haplosporidan infecting Teredo spp. is not conspecific with H. nelsoni, H. costale or H. louisiana.

Electron microscopy was utilized to further characterize spores of the haplosporidan infecting Teredo spp. and revealed four distinct membrane-bound extensions, one apical, opposite the opercular hinge, one terminal and two opposing lateral extensions. These extensions were not continuous with the spore wall, but contained microtubule-like structures and degrading epispore cytoplasm. Parasites in the family Haplosporidiidae are separated based on the type of epispore ornamentation into two genera, Haplosporidium and Minchinia; however, there has been some debate in the literature over the correct assignment of species to these genera. At present, species whose spores are ornamented by spore wall filaments and those ornamented by wrappings are placed in the genus Haplosporidium. Haplosporidan species with epispore cytoplasm extensions and species with unornamented spores are assigned to Minchinia. Therefore, the haplosporidan infecting Teredo spp. is placed in the genus Minchinia based on the possession of four epispore cytoplasm extensions with similar composition to the extensions found on spores of the type species, M. chitonis.

CHARACTERIZATION OF *Minchinia* sp. SPORES (ASCETOSPORA: HAPLOSPORIDIIDAE) INFECTING *Teredo navalis* L. AND *Teredo furcifera* VON MARTENS (MOLLUSCA: TEREDINIDAE) IN THE WESTERN NORTH ATLANTIC

INTRODUCTION

A haplosporidan has been reported to infect three species of shipworms, Teredo navalis L., T. bartschi Clapp and T. furcifera von Martens from Barnegat Bay, New Jersey (Hillman 1978, 1979, 1980; Hillman et al. 1982). This parasite was discovered while analyzing the effects of outflow from the Oyster Creek Nuclear Generating Station on local shipworm populations in Barnegat Bay, New Jersey. From 1975 through 1980, monthly prevalences of infection determined by histological examination were recorded and pooled for all stations sampled. Infected Teredo spp. were found throughout the year with prevalence peaks in the fall of each year. The most commonly occurring species of shipworm in the 20 stations sampled throughout Barnegat Bay was Bankia gouldi; however, this species was never found to be infected by the haplosporidan (Hillman et al. 1982). Of the three species of Teredo found, two of them, T. bartschi and T. furcifera are subtropical species, probably introduced to the area by wooden boats and able to survive in the warm water effluent of the power station. Hillman et al. (1982) hypothesized that T. bartschi may have developed some disease resistance to the haplosporidan parasite, as evidenced by increasing numbers of individuals through 1980 and decreasing parasite prevalence. Alternatively, the population of T. furcifera declined through the sampling period. Teredo furcifera was the most abundant Teredo species in 1974

(Hoagland and Turner 1980) but the number of individuals collected decreased through 1980 perhaps due to mortality caused by high parasite prevalence.

Based on light microscopy, Hillman (1979) assigned the organism parasitizing Teredo spp. to the family Haplosporidiidae (Phylum Ascetospora, Class Stellatosporea, Order Balanosporida) and discussed similarities in size and shape of its spores to those of Haplosporidium nelsoni Haskin, Stauber and Mackin, the oyster pathogen commonly referred to as MSX. MSX has been implicated in mass mortalities of Crassostrea virginica Gmelin in both Delaware and Chesapeake Bays over the last thirty years, yet the life cycle of this parasite is unknown. Investigators have suggested existence of a reservoir host, a species other than C. virginica which serves as a source of MSX from which oysters become infected, because of the lack of correlation between disease severity and oyster abundance (Ford and Haskin 1982, Andrews 1984). In addition, spores of H. nelsoni are rarely seen in adult oysters; however, a recent study by Kanaley and Barber (1989) indicates that MSX spores are more common in oyster spat (36% of 234 spat examined June 1988 were in some stage of sporulation). At present, the possibility of a reservoir host for MSX still cannot be ruled out. Hillman (1979) acknowledged the unlikelihood of Teredo spp. being a reservoir host for MSX since few Teredo spp. are found in MSX endemic areas; however, similar spore morphologies and the abundance of the spore stage in infections of Teredo spp. warranted further investigation.

Therefore, a study was undertaken to examine the hypothesis that the haplosporidan infecting Teredo spp. is conspecific with the oyster pathogen H. nelsoni and to compare the haplosporidan infecting Teredo spp. to other local species of the family Haplosporidiidae. The objectives of this study were as follows:

1. Compare antigenic characteristics of spores of the haplosporidan infecting Teredo spp. to spores of Haplosporidium nelsoni.
2. Compare antigenic characteristics of spores of the haplosporidan infecting Teredo spp. to spores of H. costale infecting the oyster Crassostrea virginica and to H. louisiana infecting the mudcrab Panopeus herbstii.
3. Examine spore morphology of the haplosporídan infecting Teredo spp. with paraffin histology, scanning electron microscopy and transmission electron microscopy.
4. Determine generic assignment of the haplosporidan infecting Teredo spp.

MATERIALS AND METHODS

Specimen collection:

In April of 1987 and 1988, pine planks for collection of shipworms were submerged beneath the dock at the Virginia Institute of Marine Science laboratory on the Atlantic coast in Wachapreague, Virginia. Teredo navalis were collected in October 1987 and October 1988 from planks exposed for six months and in February 1989 from planks exposed for ten months. Teredo furcifera were collected in October 1988 from planks exposed for six months and in February 1989 from planks exposed for ten months. Additionally, planks suspended from the dock on 24 April 1988 were sampled monthly from June 1988 through February 1989 to ascertain prevalence and intensity of infection.

Shipworms were removed from planks with scalpel and forceps. An effort was made to remove intact worms because pallets are necessary for species identification (Turner 1966); however, in some cases infected worms were obtained without pallets. Smears of Teredo spp. gills were examined by light microscopy to determine presence of haplosporidan spores.

Paraffin histology:

Pieces of infected T. navalis tissue were preserved in Davidson's AFA (30% (v/v) 95% EtOH, 20% (v/v) formalin, 20% (v/v) acetic acid and

20% (v/v) glycerin) for 24-48 hours. Tissues were then dehydrated in a graded ethanol series and embedded in Tissue Prep paraffin (Fisher Scientific, Fair Lawn, NJ) using an automatic tissue processor (Auto-Technicon, Technicon Corp., Tarrytown, NY). Tissue blocks were cut at 6 um on a rotary microtome (American Optical, Buffalo, NY). Resulting paraffin ribbons were floated on a warm water bath and picked up on glass slides coated with Szombathy's adhesive (0.01% (w/v) gelatin, 0.15% (v/v) glycerin in distilled water). Slides were allowed to dry overnight in a 45^{o}C oven and then stained with Harris' Haematoxylin and Eosin (HH&E) according to routine staining procedures.

Rabbit Immunization:

Spore suspensions for rabbit immunization were obtained by placing pieces of infected T. navalis collected in October 1987 in beakers of high salinity water (32 ppt). Supernatant was changed daily for approximately seven days until all T. navalis tissue decayed. Prior to immunization, spores were incubated one hour in a saturated solution of N-Acetyl-L-Cysteine to dissolve all remaining tissue. Spores were washed in 0.22 um filtered sea water, sonicated gently with a Sonifier Cell Disruptor (Heat Systems-Ultrasonics, Inc., Plainview, NY) to remove clumps, followed by fixation for one hour in Davidson's AFA. Fixative was removed by two additional washings in 0.22 um filtered sea water. An emulsion of 2 x 10^{7} intact spores in 1.0 ml RAS (Ribi adjuvant system: Monophosphoryl lipid A, Trehalose dimycolate and cell wall skeleton, Ribi ImmunoChem Research, Inc.,

Hamilton, MT) was injected into a New Zealand white rabbit (SUJO Rabbit Farm, Gloucester, VA) according to the following schedule:

Day 0 - 5.0 ml blood taken for pre-inoculation serum via heart puncture, 0.25 ml RAS emulsion injected subcutaneously (sc) in each of four sites along back.

Day 10- Boosted with 0.25 ml RAS emulsion sc in each of four sites along back.

Day 20- Boosted as on Day 10.

Day 29- 10.0 ml blood taken via heart puncture.

Blood was allowed to clot one hour at room temperature and overnight at 5°C. Serum was removed and aliquots of 0.10 ml were frozen at -18°C until time of assay.

IGSS assay:

Paraffin blocks of P. herbstii infected with H. louisiana and C. virginica infected with H. nelsoni or H. costale were obtained from the oyster disease archive at VIMS. These tissues had been stored in paraffin for variable periods of time. Panopeus herbstii infected with H. louisiana was preserved as described for T. navalis but stored in paraffin blocks for five years prior to immunoassay. Two of the H. nelsoni infected oysters had been preserved for 48 h in Woods' AFA (2.5% (v/v) acetic acid, 6.5% (v/v) formalin, 48.5% (v/v) 95% EtOH) and held in paraffin for 12 years. A third oyster was preserved exactly as described for T. navalis and stored in paraffin for six months prior to immunoassay. Of the three H. costale infected oysters

assayed, two had been stored in paraffin for four years and the third was held in paraffin for six years.

Tissue blocks were cut as previously described (see "paraffin histology"); however, slides with sections were left unstained for immunoassay.

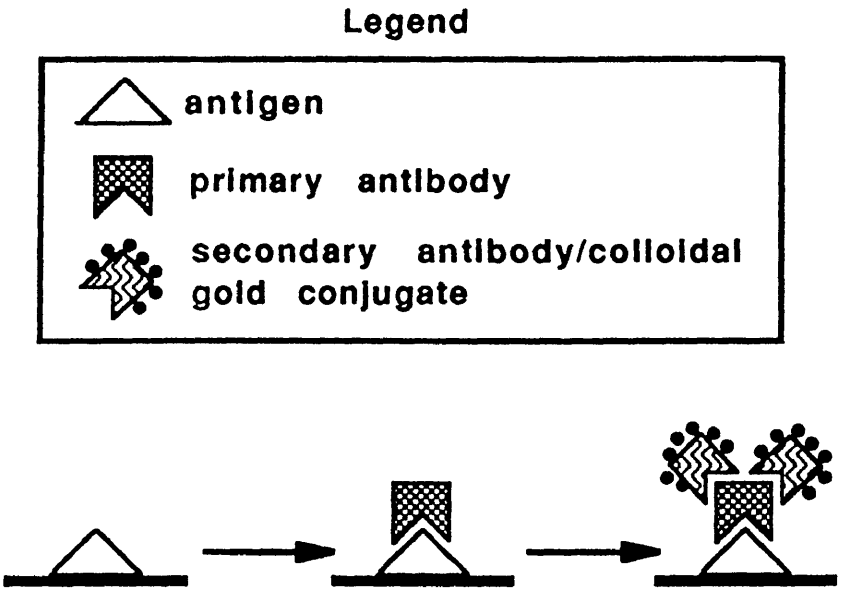
Auroprobe LM Immunogold Silver Staining (Janssen Life Science Products, Piscataway, NJ) was used as described by the manufacturer. Rabbit polyclonal antiserum made specific for spores of the Teredo spp. haplosporidan was applied to paraffin sections of spores to be tested (Fig. 1). If the spore in the section under consideration had the same antigenic properties as the Teredo spp. haplosporidan, the antibody molecules attached to the antigenic determinants on the spore coat. These antigen-antibody complexes were then tagged by the addition of goat anti-rabbit IgG coated on 5 nm colloidal gold. The signal was enhanced by precipitation of metallic silver on the gold particles yielding a dark brown to black signal at the site of each antigen-antibody complex when viewed with light microscopy.

Initially, five dilutions (1/50, 1/100, 1/200, 1/400 and 1/800 in PBS with 0.5% (w/v) bovine serum albumin and 0.5% (w/v) sodium azide) of rabbit antiserum to haplosporidan spores from T. navalis (primary antiserum, hereafter referred to as rabbit anti-HS) were tested against infected T. navalis and C. virginica infected with MSX. Sections were subsequently incubated one hour in a 1/40 dilution of secondary antibody, affinity purified goat anti-rabbit IgG conjugated to 5 nm colloidal gold particles (AuroProbe LM), and eight minutes in

silver enhancement reagents (IntenSE II, supplied with kit). Pre-treatment with Lugol's iodine and modifications in incubation times of primary antiserum and silver enhancement reagents were attempted to increase contrast between positively reacting spores and background (DeMey et al. 1986). A 30-minute incubation of the 1/100 dilution of rabbit anti-HS was chosen as optimal because background was minimal and a positive reaction was easily seen. Subsequent assays were therefore performed at this concentration. Lugol's iodine was not used except on an initial sample because pretreatmént with Lugol's increased background thereby decreasing contrast.

Cross sections through the gill regions of five infected T. navalis and the visceral mass of one infected T. navalis were tested in duplicate against rabbit anti-HS using IGSS. In addition, three oysters infected with H. nelsoni, three oysters infected with H. costale and one mudcrab infected with H. louisiana were assayed in duplicate. As negative controls, sections from each organism were assayed substituting rabbit pre-inoculation serum and commercial normal rabbit serum (Cooper Biomedical, West Chester, PA) as primary antisera. Sections of uninfected T. navalis and C. virginica were also assayed with rabbit pre-inoculation serum and rabbit anti-HS serum. In addition, paraffin sections of infected Teredo spp. from Barnegat Bay, New Jersey provided by R. E. Hillman were tested against rabbit anti-HS to confirm that the haplosporidan infecting T. navalis in Wachapreague is the same as that described by Hillman (1978). A negative control of Hillman's infected Teredo spp. using rabbit pre-inoculation serum as primary antiserum was not performed due to lack

Fig. 1. Diagrammatic representation of immunogold silver staining assay illustrating antigen, primary antibody (rabbit anti-haplosporidan spores from *T. navalis*) and colloidal gold conjugated secondary antibody.


Legend
antigen
primary antibody
secondary antibody/colloidal gold conjugate

1

of tissue.

SEM:

Sections through gill regions of infected worms were preserved for four hours in 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH = 7.4) for SEM of sporocysts. Preserved cross-sections were washed three times in 0.1 M sodium cacodylate buffer and post-fixed for two hours in 1% (w/v) OsO_4 in 0.1 M sodium cacodylate buffer at 5^oC. Following three additional washes in 0.1 M sodium cacodylate buffer, samples were dehydrated in a graded series of EtOH and transferred to 100% acetone for critical point drying in liquid CO_2. Once dried, tissue sections were mounted on support stubs using colloidal graphite in isopropanol and coated with gold-palladium (60% : 40% (w/w)) by vacuum evaporation.

For SEM study of epispore ornamentation, spores were separated from sporocysts, prior to fixation, by three different methods. In October 1987, spores were obtained from decayed T. navalis tissue as described for rabbit immunization. These spores were then washed, sonicated gently and resuspended in filtered sea water. In method two, gills from heavily infected T. navalis and T. furcifera collected in October 1988 were dissected and teased to release sporocysts. Sporocysts were then disrupted by gentle sonication to yield a suspension of spores. No effort was made to separate spores from the two species of shipworms. In the third method, sporocysts teased from

gills of T. navalis and T. furcifera were punctured with needle probes to release spores. There was no sonication in the third method.

Spores were prepared for SEM by adhesion to poly-l-lysine coated 12 mm round glass coverslips. Spores obtained by all three methods were washed three times in 0.22 um filtered sea water and fixed in 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate (pH = 7.4) for one hour at room temperature (RT). Spore suspensions were added as a puddle onto each coated coverslip and allowed to settle for one hour. Coverslips were placed in snap cap vials, washed in three 10 minute changes of 0.1 M sodium cacodylate buffer and post-fixed in 1% (w/v) OsO_4 in 0.1 M sodium cacodylate buffer for two hours at RT. Samples were again washed in sodium cacodylate buffer followed by dehydration in EtOH. Coverslips were then transferred to 100% acetone, critical point dried, mounted on stubs and coated as previously described.

TEM:

Minced gills of heavily infected T. navalis and T. furcifera were prepared for TEM by three hour fixation in 0.1 M sodium cacodylate (pH = 7.4) buffered 3% (v/v) glutaraldehyde at $5^{o}C$. Following three 20 minute washes in 0.1 M sodium cacodylate buffer, tissue was post-fixed for two hours in 1% (w/v) OsO_4 buffered in 0.1 M sodium cacodylate at $5^{o}C$. Samples were again washed in buffer, dehydrated through a graded series of EtOH and infiltrated over six days with Spurr's low viscosity embedding media (Spurr 1969). Blocks

were polymerized overnight in a 58°C vacuum oven at -15 psi. Tissue sections were cut at approximately 800 nm, stained 20 min with saturated uranyl acetate in 50% (v/v) EtOH and stained five minutes with Reynolds' lead citrate (Reynolds 1963). In addition, spores obtained in October 1988 by needle puncture of sporocysts were negatively stained with uranyl acetate. Spores were settled onto Formvar-coated grids, fixed 45 seconds in a 2% (w/v) OsO_4 chamber and stained for five minutes in 0.8% (w/v) uranyl acetate. Sections and whole mounts were viewed with a Zeiss CEM 902 transmission electron microscope.

RESULTS

Teredo spp. with haplosporidan infections were found from early September 1988 through February 1989, with heaviest infections occurring in October. Since boards were initially exposed in April, an accurate assessment of haplosporidan prevalence in May through August was not made. Very few shipworms were found in boards sampled prior to September, because it takes approximately two months for shipworms to colonize newly exposed boards and grow to a size easily detectable during board dissection. Shipworm boards should be set out several times during the year so that newly exposed boards are not relied on for haplosporidan prevalence estimates.

Shipworms with heavy infections were characterized by numerous white and brown pinpoints throughout the gills and mantle which were visible to the unaided eye. Light microscopy revealed that these pinpoints were sporocysts. The spore stage of the haplosporidan infecting Teredo spp. was the most commonly occurring stage of the parasite. Sporocysts were present in gills of infected Teredo spp. in the blood spaces, efferent branchial vein, afferent branchial vein, water tubules, epibranchial cavity and mantle cavity (Plate I, Fig. 2,3). Deterioration of the gill epithelium because of heavy haplosporidan infection was observed and is presumed to be responsible for release of sporocysts into the water tubules and mantle cavity. Sporocysts ruptured by slight coverslip pressure released

numerous spores, 6-7 um in length, ornamented by four epispore extensions (Plate II, Fig. 4,5).

IGSS Assay:

Sections of T. navalis, C. virginica and P. herbstii with heavy haplosporidan infections including spores were chosen for immunoassay comparison. Morphological similarities at the light microscope level were seen in HH&E-stained sections of H. nelsoni spores (averaging 5 x 7 um) in the digestive diverticula epithelium of C. virginica (Plate III, Fig. 6) and haplosporidan spores (5 x 6.8 um) in the gills of T. navalis (Plate III, Fig. 7). Spores of both species were slightly elongated with a thick wall surrounding a darkly-stained sporoplasm and covered by an operculum. Haplosporidium louisiana spores, averaging 6.3 um x 8 um, found in the connective tissue of P. herbstii, were much larger than the other haplosporidans assayed. Spores of H. costale in the connective tissue of C. virginica were smaller, averaging 2.5 um x 3.5 um.

In all specimens of haplosporidan-infected T. navalis tested with IGSS, immature spores reacted positively indicated by the black color of the spores (Plate III, Fig. 8). Mature spores and plasmodial stages showed little or no reaction. Spores and tissue of infected T. navalis sections assayed with rabbit pre-inoculation serum and normal rabbit serum developed a brown color (Plate III, Fig. 9). Spores reacted slightly more strongly than gill tissue although not with the intensity of spores assayed with rabbit anti-HS serum. This slight reaction demonstrated the need for negative control testing; the

amount of color development seen with pre-inoculation serum was regarded as negative background. Uninfected T. navalis showed no positive reaction when assayed with rabbit anti-HS or pre-inoculation serum. The negative background that developed in the immunoassay of infected T. navalis tissue was also seen in the assay of uninfected tissue.

Haplosporidium nelsoni spores from C. virginica did not react with rabbit anti-HS serum (Plate III, Fig. 10). Assayed sections of oyster tissue contained both mature and immature spores. Brown color development of the spores was the same as that seen for sections assayed with rabbit pre-inoculation serum and normal rabbit serum and therefore dismissed as negative background (Plate III, Fig. 11). In one of the oysters preserved in 1976 a seemingly positive reaction of MSX spores which stained dark brown to black when assayed with rabbit anti-HS serum was accompanied by dark brown to black-stained oyster tissue. This apparent reaction of MSX spores was regarded as negative because there was no difference between spores and oyster tissue in intensity of the reaction. The same oyster yielded similar results when assayed with rabbit pre-inoculation serum indicating that the reaction seen when assayed with rabbit anti-HS serum was unusually high background. Uninfected C. virginica showed no reaction.

A high background was seen in sections of P. herbstii infected with H. louisiana when assayed with rabbit antiserum or pre-inoculation serum (Plate IV, Fig. 12, 13). Nuclei of digestive tubule epithelial cells and some immature spores reacted with both types of serum producing a dark brown color. These reactions were regarded as

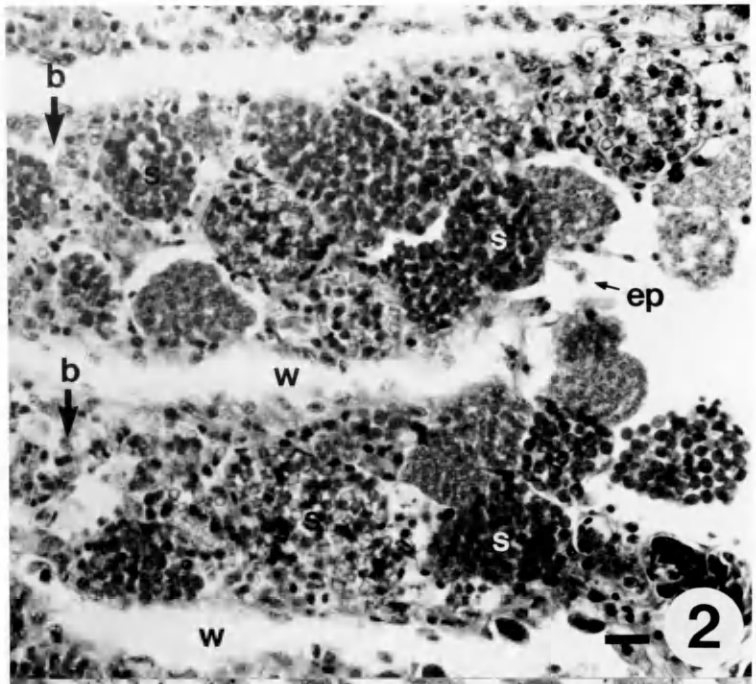
negative because the cell nuclei and spores reacted with the same intensity to both the antiserum and control pre-inoculation serum.

Spores of H. costale stained dark brown when exposed to rabbit anti-HS serum (Plate IV, Fig. 14). Spores in sections exposed to pre-inoculation serum also developed a brown color (Plate IV, Fig. 15), though not as dark as those reacted with antiserum.

Sections of infected Teredo spp. provided by R. E. Hillman were tested only against rabbit anti-HS serum due to lack of sufficient material (Plate IV, Fig. 16). Spores stained dark brown; however, this reaction was not as intense as the black-stained spores seen when infected T. navalis from Wachapreague was assayed with rabbit anti-HS serum. In addition, nuclei of some unidentifiable cells along the periphery of gill lamellae in Hillman's samples reacted with a strong black signal. No such reaction was seen in Wachapreague samples.

PLATE I

Fig. 2,3. Harris' Haematoxylin and Eosin stained paraffin sections of Teredo navalis. 2. Histological section of T. navalis gill lamellae showing sporocysts (s) in blood spaces (b), deterioration of gill epithelium (e) and sporocysts (s) in the water tubules (w). Bar = 22 um. 3. Histological section of heavily infected T. navalis gill showing sporocysts (s) in the afferent branchial vein (a) and blood spaces (b). Bar = 50 um.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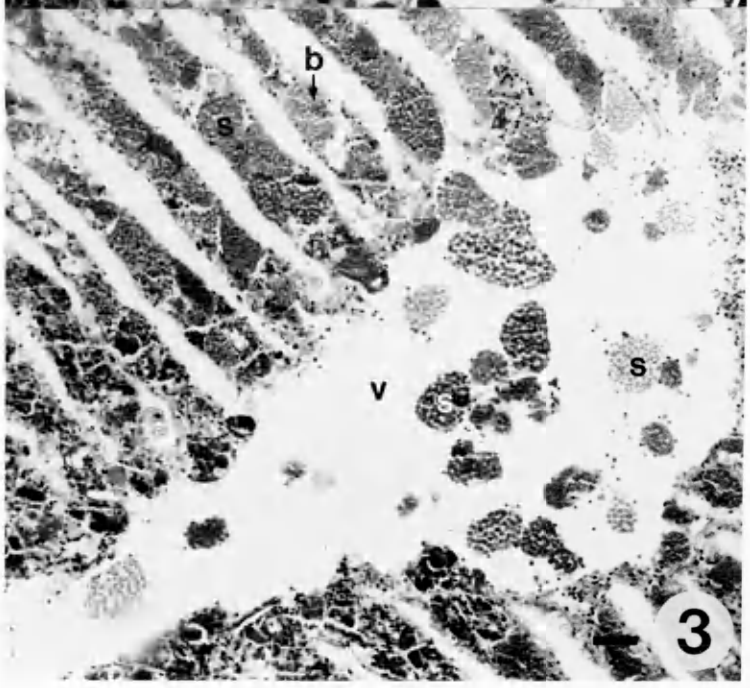
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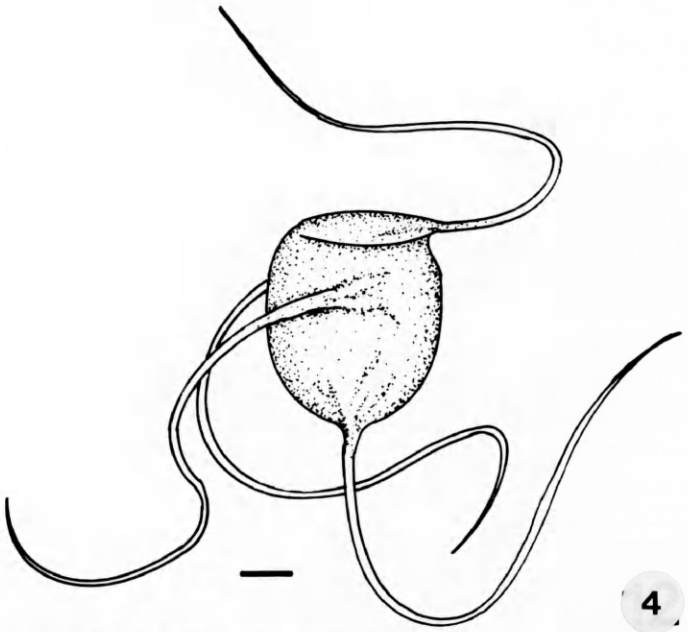
PLATE II

Fig. 4,5. Position of epispore cytoplasm extensions on Minchinia sp. spores. 4. Diagrammatic illustration of Minchinia sp. spore illustrating position of epispore extensions. Bar = 1 um. 5. Light micrograph of live Minchinia sp. spores showing epispore extensions. Bar = 5 um.



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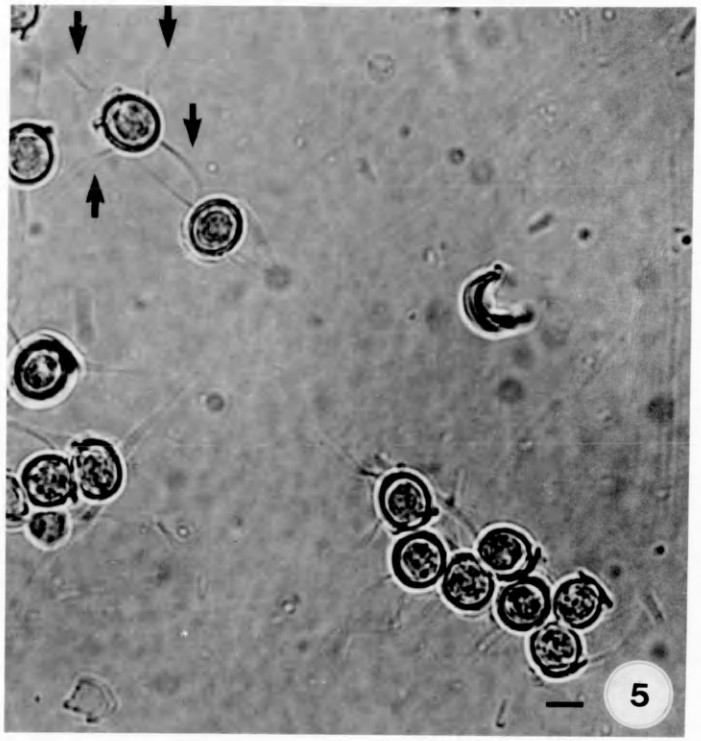

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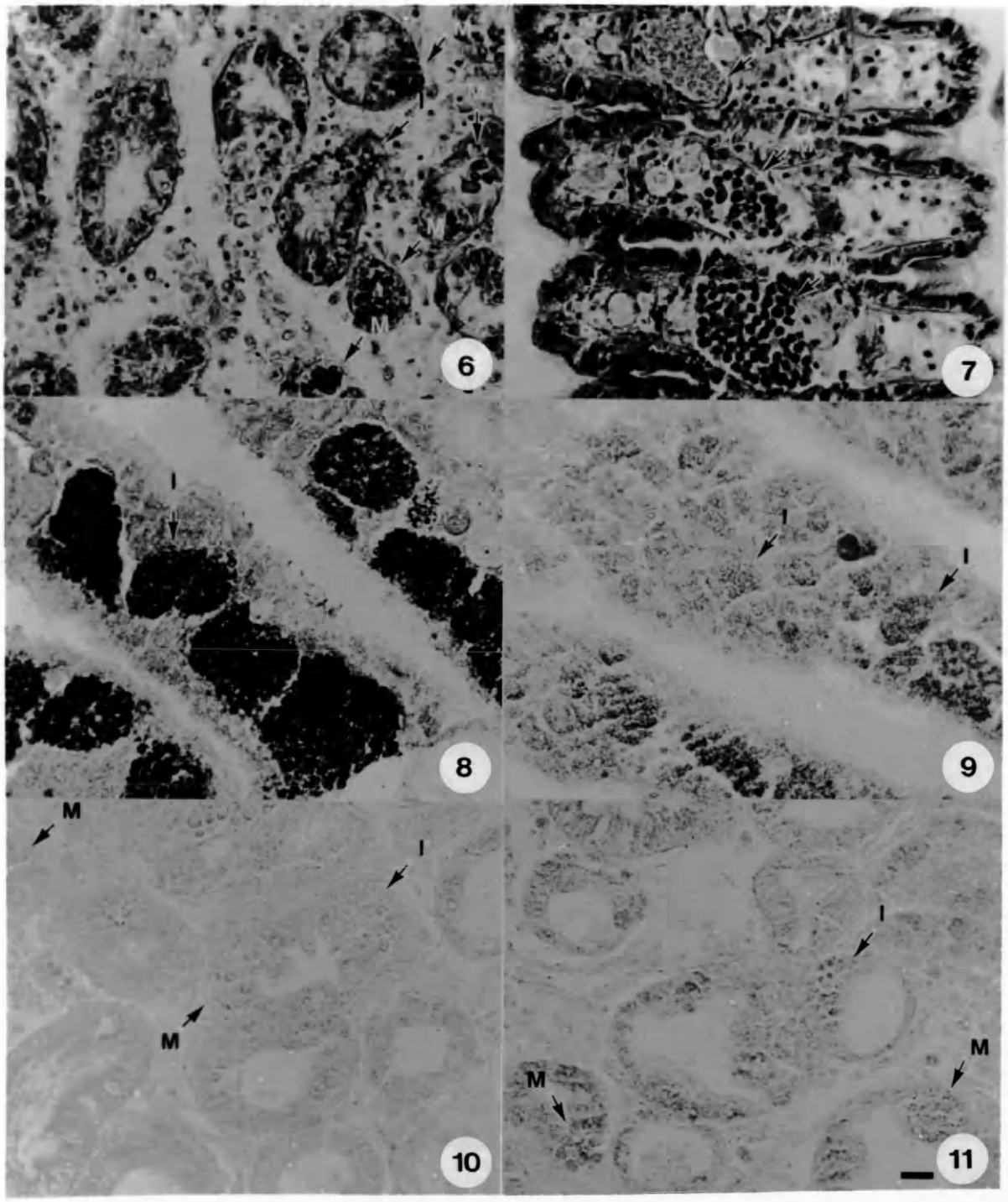
PLATE III

Fig. 6-11. IGSS of haplosporidan spores (arrows: M=mature, I=immature) in C. virginica and T. navalis. (The bar in Fig. 11 represents 12 um and applies to all figures in this plate.) Figures 6, 7 were stained with HH&E. Figures 8-11 were assayed by IGSS and counterstained with Fast Green. 6. MSX spores in digestive diverticula of C. virginica. 7. Spores in gills of T. navalis. 8. Teredo navalis gills treated with rabbit anti-HS serum. 9. Teredo navalis gills treated with rabbit pre-inoculation serum. 10. Crassostrea virginica treated with rabbit anti-HS serum. 11. Crassostrea virginica treated with rabbit pre-inoculation serum.


I
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8
I
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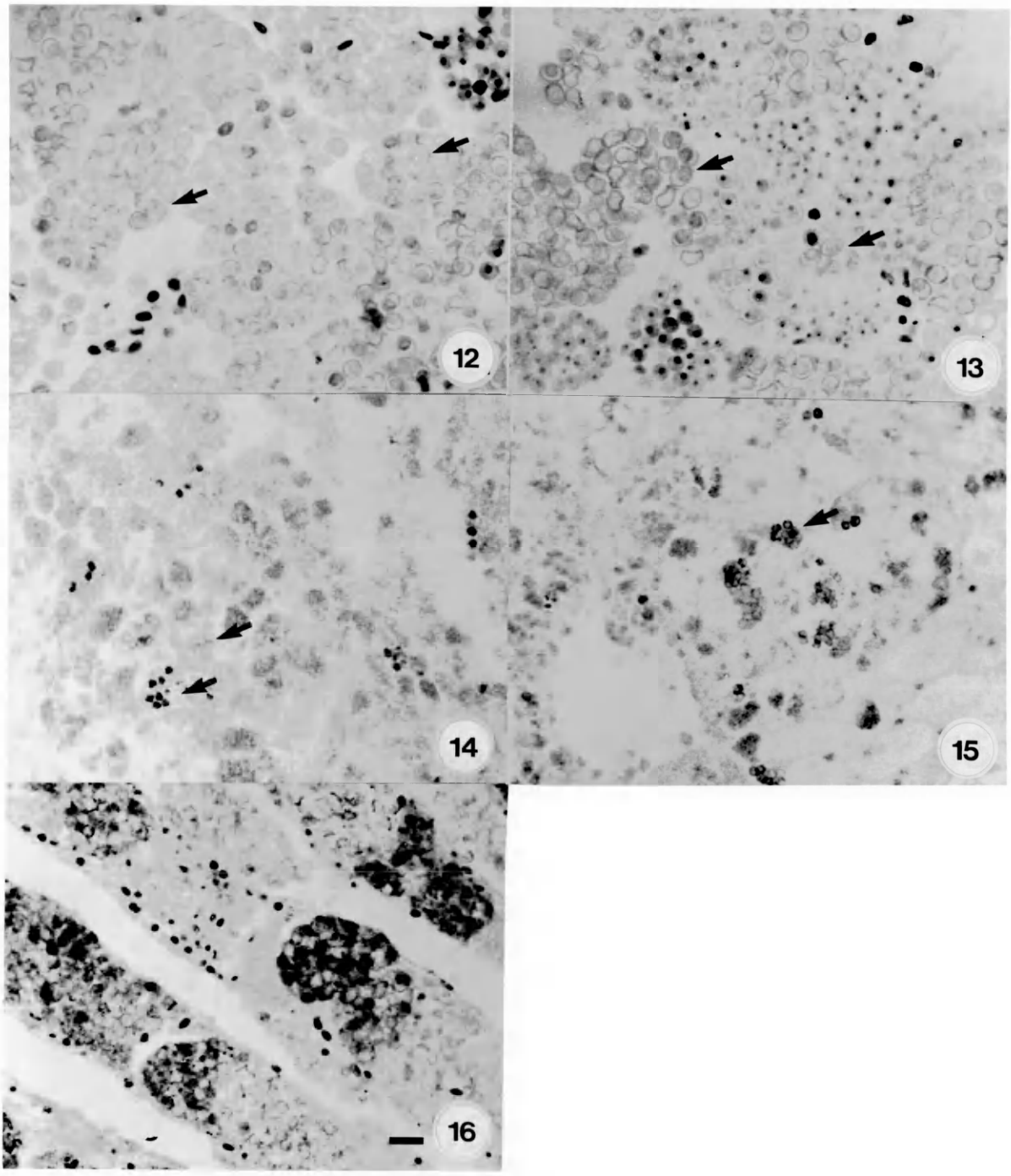
PLATE IV

Fig. 12-16. Haplosporidan species compared to Minchinia sp. by immunogold silver staining. (The bar in Fig. 16 represents 12 um and applies to all figures in this plate.) **12.** Haplosporidium louisiana spores (arrows) in P. herbstii assayed with rabbit anti-HS serum. **13.** Haplosporidium louisiana spores (arrows) assayed with rabbit pre-inoculation serum. **14.** Haplosporidium costale spores (arrows) in C. virginica assayed with rabbit anti-HS serum. **15.** Haplosporidium costale spores (arrows) assayed with rabbit pre-inoculation serum. **16.** Haplosporidan spores from Barnegat Bay Teredo spp. assayed with rabbit anti-HS serum.


12
13
14
15
16

Electron Microscopy:

When viewed with SEM, numerous closely packed sporocysts, approximately 30-60 um in diameter, were evident within the blood spaces adjacent to the food groove of Teredo spp. (Plate V, Fig. 17, 18). Additionally, sporocysts emerging from the water tubules between lamellae were seen along the gill ventral surface. Spores were represented by bulges of the opaque membrane bounding each sporocyst.

Thin sections through sporocysts revealed developing spores within membrane-bound epispore cytoplasm (Plate V, Fig. 19). Spore wall formation was initiated as nodes of wall material evenly spaced around the sporoplasm. These nodes gradually merged to form a continual wall, five to seven layers thick in mature spores (Plate VI, Fig. 23; Plate VII, Fig. 24). The spore orifice was covered by a hinged operculum composed of wall material. Within the sporoplasm of these developing spores, the spherulosome, nucleus, mitochondria and formative inclusions containing haplosporosomes were clearly visible (Plate V, Fig. 19). Near the sporoplasm membrane adjacent to the spore wall, a single layer of microfilament-like structures was evident (Plate V, Fig. 20). The epispore cytoplasm of developing spores contained microtubule-like structures immediately beneath the epispore membrane and degenerating mitochondria. (Without an immunoassay using anti-tubulin serum, it cannot be said definitively that these structures are microtubules and therefore, "microtubule-like structures" is used throughout). These microtubule-like structures, approximately 25 nm in diameter, were present around the

spore at 35-50 nm intervals, usually forming an irregular band (Plate V, Fig. 20).

Several sporocysts with tears in the sporocyst membrane 10-40 um in length were found in gill cross sections (Plate VI, Fig. 21). Spores with intertwined extensions were visible through these tears. SEM examination of individual spores isolated by needle puncture of sporocysts (Plate VI, Fig. 22) and by decay of shipworm tissue (Plate VII, Fig. 25) revealed four distinct epispore cytoplasm extensions from 10-30 um in length, one apical, opposite the opercular hinge, one terminal and two opposing lateral extensions (Plate II, Fig. 4). Cross-sections through these extensions demonstrated that they were membrane-bound and contained microtubule-like structures and degenerating epispore cytoplasm (Plate VI, Fig. 23). The number of microtubule-like structures comprising the extensions varied from approximately 135 near the base of the extension to 55 near the tip.

Thin sections of spores at varying stages of development revealed the structure of epispore cytoplasm extension formation. The band of microtubule-like structures present within epispore cytoplasm of immature spores appeared to coalesce as spores developed accompanied by progressive degradation of the cytoplasm. Mature spores were therefore surrounded by a complete membrane covering a thin layer of tightly-packed microtubule-like structures adjacent to the spore wall (Plate VI, Fig. 23; Plate VII, Fig. 24). The membrane and microtubule-like structures extended to form the tapering extensions in four distinct locations around the spore. Because of the impervious nature of the spore wall, the sporoplasm of mature spores

did not fix and infiltrate well. The sporoplasm was therefore pulled out during sectioning leaving a hole surrounded by spore wall, epispore membrane and extensions; however, these structures were well-fixed and demonstrated that the extensions were neither composed of spore wall material nor firmly attached to the wall. These findings were supported by the negatively stained whole mounts which did not reveal any periodic substructure of the extensions.

In spores collected in October 1987 that were held in sea water for seven days, the epispore membrane and extensions appeared as thin, often loose coverings sometimes partially lysed and pulled away from the spore at the operculum as if being shed (Plate VII, Fig. 25, 26). Spores with no extensions or epispore membrane were observed in the same preparation (Plate VII, Fig. 27).

PLATE V

Fig. 17-20. Minchinia sp. from Teredo spp. **17.** SEM micrograph of transverse section through shipworm gill lamellae showing abundant sporocysts (s), symbiotic ciliates (c), a food groove (f) and the afferent branchial vein (v). Bar = 100 um. **18.** SEM micrograph of sporocysts from shipworm gill lamellae illustrating individual spores within the sporocysts. Bar = 10 um. **19.** Transmission electron micrograph of immature spore within epispore cytoplasm (e). Visible within the sporoplasm are the spherulosome (sp), nucleus (n) and haplosporosome formative inclusions (h). The epispore cytoplasm contains a supporting substructure of microtubule-like structures (m). Bar = 500 nm. **20.** Enlarged view of the same spore as shown in Fig. 19 illustrating the microtubule-like structures (m) in the epispore cytoplasm and the microfilament-like structures (f) within the sporoplasm. Bar = 250 nm.

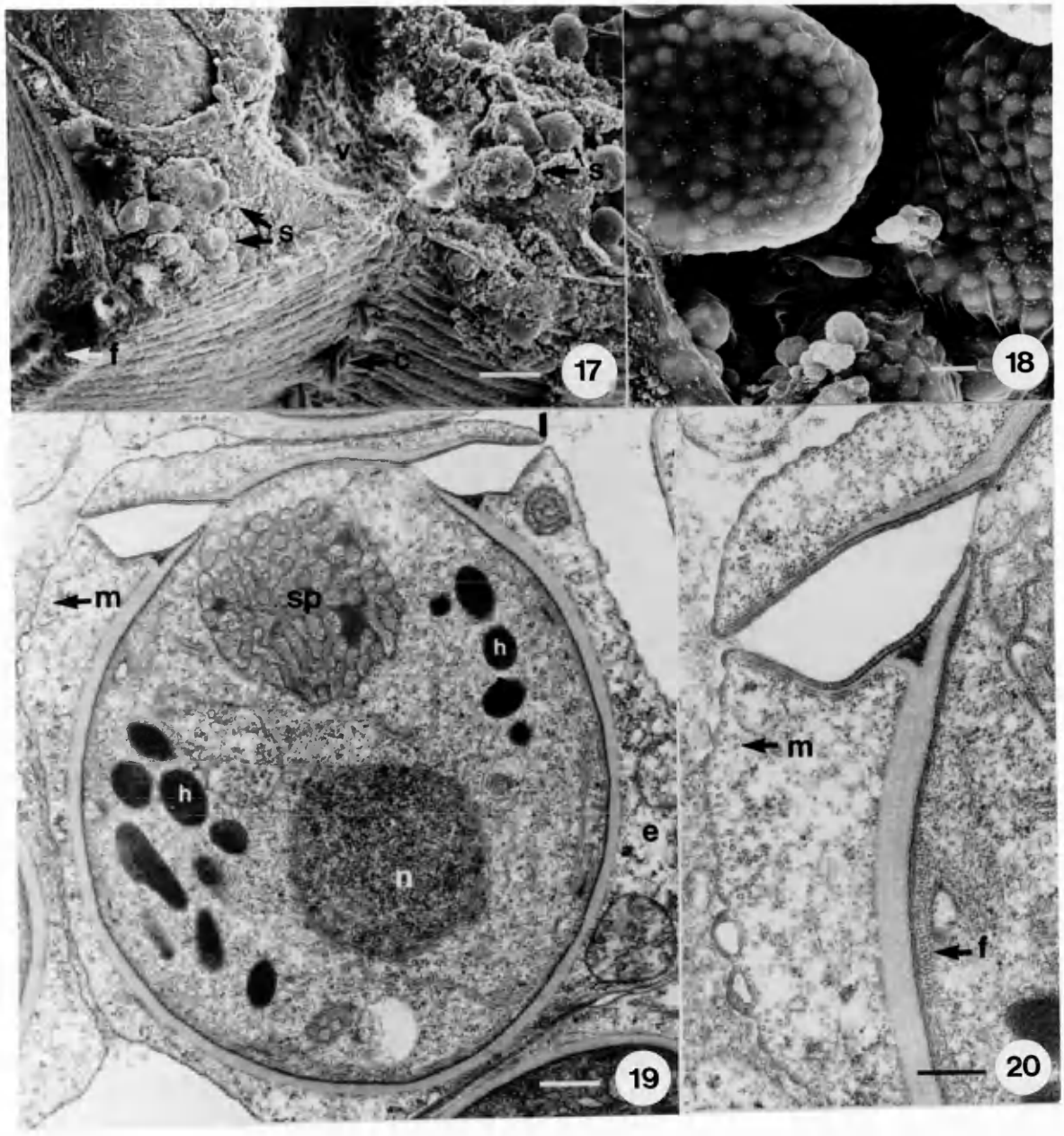

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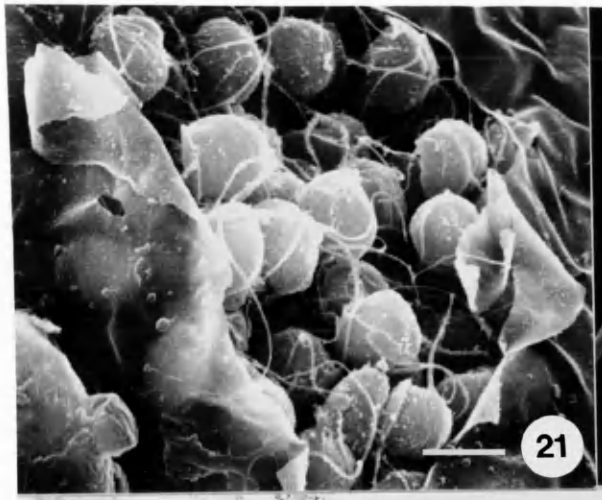
Plate VI

Fig. 21-23. Minchinia sp. spores from Teredo spp. **21.** SEM micrograph of spores with extensions observed through a tear in the sporocyst membrane. Bar = 5 um. **22.** SEM micrograph of individual spore isolated by needle puncture of sporocysts showing three of the four extensions. Bar = 1 um. **23.** Electron micrograph illustrating a longitudinal section through the base of an extension showing spore wall (w), disintegrating epispore cytoplasm (e) and microtubule-like structures (m). Also seen are an extension in transverse section (t) showing microtubule-like structures surrounded by a thin membrane, and the tip of an operculum (o) with sheath composed of microtubule-like structures (m). Bar = 350 nm.



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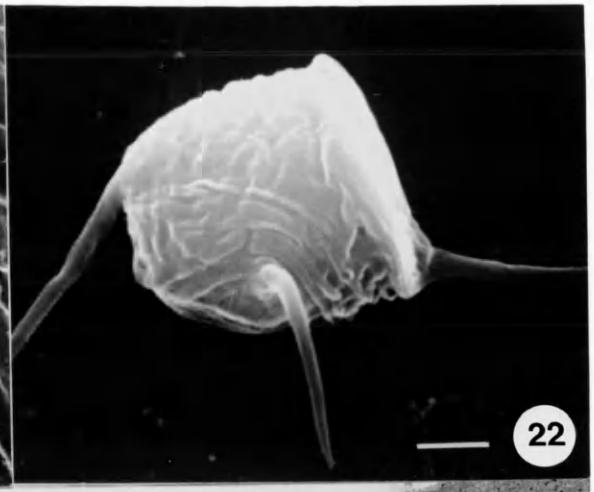

22

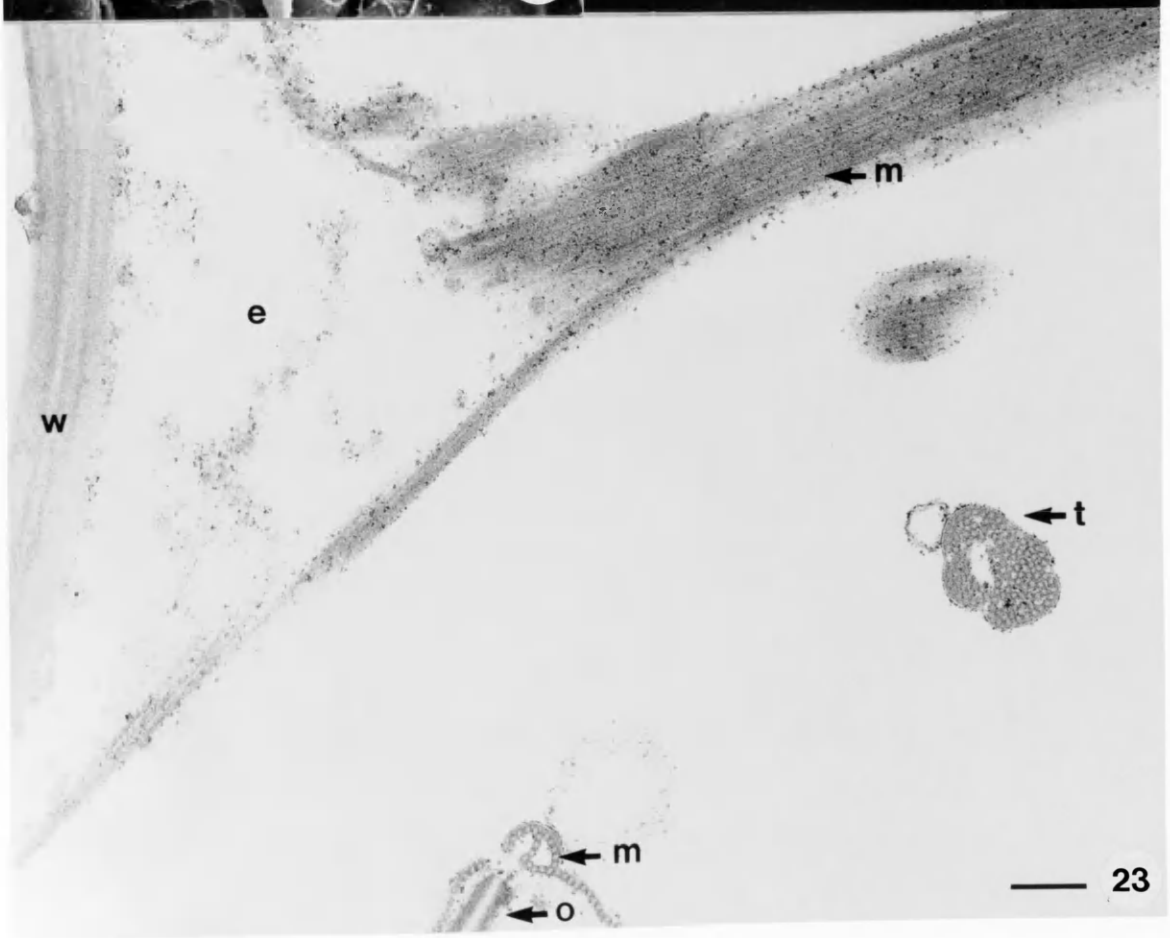

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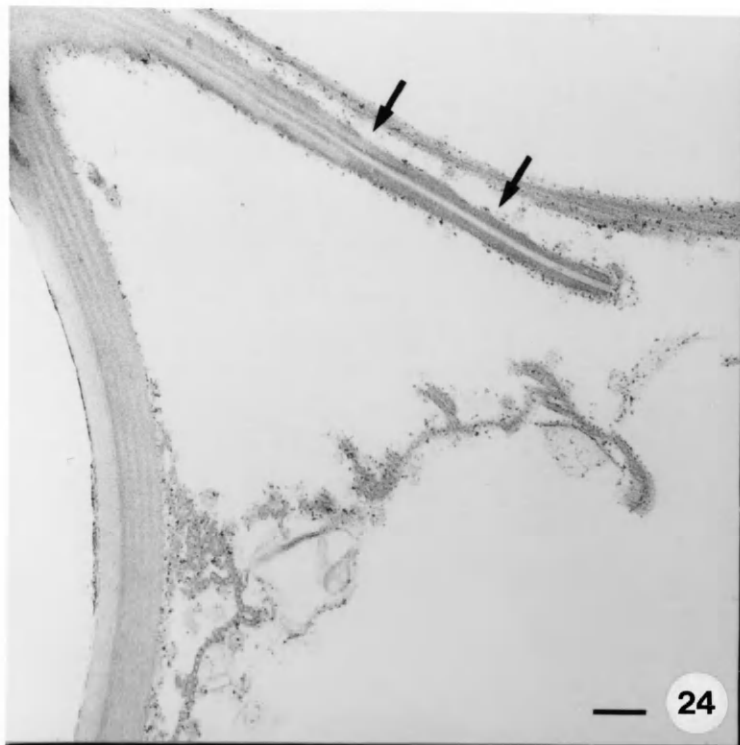
Plate VII

Fig. 24-27. Minchinia sp. spores with degrading epispore cytoplasm. **24.** Electron micrograph of base of opercular extension showing that microtubule-like structures and membrane are not attached to spore wall (arrows). Bar = 350 nm. **25,26.** Spores isolated by disintegration of shipworm tissue illustrating loosely fitting epispore membrane and extensions. Bar = 1 um. **27.** Unornamented spores following complete lysis of epispore membrane. Bar = 1 um.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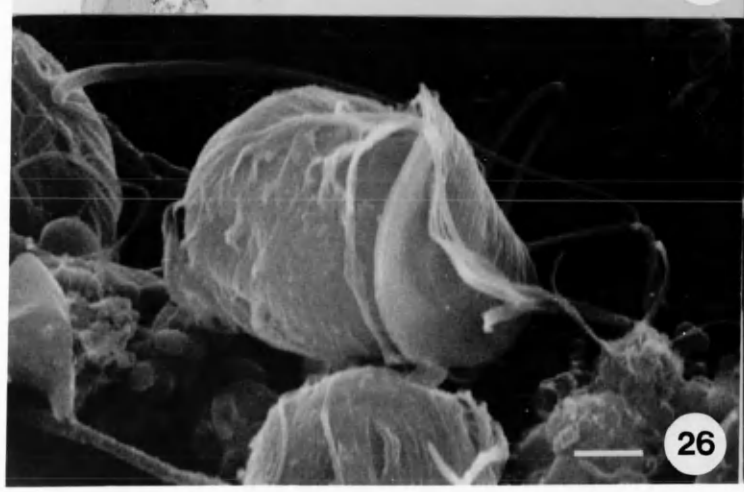
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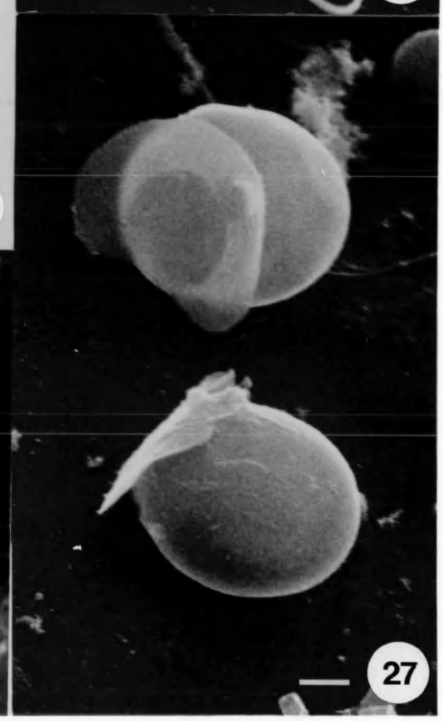
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DISCUSSION

In order to understand the results of the immunogold silver staining assay, it is important to understand antiserum specificity and cross-reactivity. Antiserum specificity results from the action of a population of individual antibody molecules directed against different determinants on the antigen molecule (Roitt, Brostoff and Male 1985). Therefore, antiserum raised against spores of the haplosporidan infecting Teredo spp. reacted specifically with several antigenic sites on the same spores. Additionally, spores of another species may have some shared antigenic sites with the Teredo spp. haplosporidan spores and thus the antiserum to Teredo spp. haplosporidan spores may cross-react by binding only to the sites that the two species have in common. In an immunoassay, the specific binding of an antiserum produces a strong positive reaction. In the case of immunogold silver staining, the specific reaction is a black reaction because of the number of antibody molecules bound and hence the amount of gold available for silver enhancement. Cross-reactivity is expressed by brown color development because fewer antibody molecules bind and there is less color development.

The fact that the antiserum raised against spores from T. navalis reacted specifically with spores of T. navalis and did not react with spores of H. nelsoni indicates that these two haplosporidan species are antigenically distinct and thus different species. These results

demonstrate that Teredo spp. is not a reservoir host for H. nelsoni. Additionally, the lack of reaction of H. louisiana spores with the rabbit anti-HS serum indicates that the haplosporidan infecting Teredo spp. and H. louisiana are also antigenically distinct. The negative background seen in sections of infected C. virginica and P. herbstii assayed with rabbit pre-inoculation serum is attributable to cross-reaction of naturally occurring rabbit antibodies with spores and tissue.

The dark brown color of H. costale spores when assayed with rabbit anti-HS serum can be explained by cross-reactivity. Immunoassay results indicate that spores of the Teredo spp. haplosporidan have some antigenic sites in common with spores of H. costale. It would seem logical that spores of different haplosporidan species would have some shared antigenic determinants; however, spores of H. louisiana and H. nelsoni did not cross-react with the antiserum to spores of the Teredo spp. haplosporidan. Therefore, the shared antigenic sites between SSO spores and spores of the Teredo spp. haplosporidan may be due to environmental conditions since both species are found on the Eastern Shore of the Chesapeake Bay. The important result in this immunoassay is that there was no specific reaction of the rabbit anti-HS serum and SSO spores. Haplosporidium costale is a separate species from the haplosporidan found in Teredo spp. These results are supported by light microscopy where differences are seen in spore size and in location of sporulation, considered by Andrews (1984) to be species specific. Sporulation of H. costale occurs in the connective tissue and yields smaller spores

than those of the Teredo spp. haplosporidan found in the blood spaces of the gill. Additionally, when viewed with TEM, H. costale spores were described by Perkins (1969) as possessing spore wall wrappings. Such wrappings are distinct from the extensions found in the haplosporidan infecting Teredo spp.

The fact that rabbit anti-HS serum reacted only with immature spores in paraffin sections of infected T. navalis indicates that the antiserum is specific for immature spores. This may be attributed to the presence in immature spores of membrane-bound epispore cytoplasm which degenerates later in spore development revealing naked spores (Burreson and Robinson 1988) as seen in SEM preparations of spores held in sea water. Antiserum made to spores with intact epispore cytoplasm would therefore not react with spores in which the cytoplasm had lysed and disappeared. Scanning electron microscopy preparations of the same material used for rabbit immunization showed spores with intact epispore cytoplasm and naked spores. Immunoassay results of the parasite stage specificity of the rabbit anti-HS serum indicate that the immature spores were greater in number or simply more strongly antigenic to the rabbit.

The antiserum to spores of the haplosporidan infecting Teredo spp. from Wachapreague did not react specifically with spores of the haplosporidan infecting Teredo spp. from Barnegat Bay. This may possibly be explained by differences in fixation or age of sections as Hillman's samples were preserved in Bouin's fluid from 1975-1980. Wachapreague samples were preserved in Davidson's AFA and embedded in paraffin six months prior to immunoassay. Fresh samples from Barnegat

Bay need to be preserved in Davidson's AFA and embedded in paraffin using the same techniques as described for the Wachapreague samples.

The discovery of spores with four epispore cytoplasm extensions further supports the immunoassay results (McGovern and Burreson 1989) that the haplosporidan infecting T. navalis is not conspecific with H. nelsoni whose spores are ornamented by wrappings. Perkins (1968, 1979) described spore wall ornamentation in H. nelsoni as developing in the epispore cytoplasm. According to Perkins (1968, 1979), the cytoplasm then dispersed as spores matured leaving threads or ribbons attached to the wall. Ornamentation of the Teredo spp. haplosporidan is clearly distinct from that of H. nelsoni spores. Rather than developing within epispore cytoplasm, the extensions of the Teredo spp. haplosporidan are composed of cytoplasm which has degraded during spore maturation causing coalescence of microtubule-like structures. These microtubule-like structures probably add support to the extensions and epispore membrane which surround the spore. It is clear from the EM micrographs that these extensions, the microtubule-like structures and membrane are at no time continuous with the spore wall. In fact, as spores develop further, the membrane and extensions are shed yielding unornamented spores.

Spores of the haplosporidan infecting Teredo spp. have epispore ornamentation that is thus far unique to any species in the Balanosporida. The spore extensions are similar to those of Minchinia chitonis (Lankester) Labbe (Labbe 1896, 1899); however, spores from Teredo spp. possess four extensions while spores of M. chitonis have only two extensions. The extensions of spores of both species are

composed of epispore cytoplasm and microtubule-like structures. Ball (1980) described short microtubule-like structures strengthening the epispore cytoplasm and extensions of M. chitonis. In mature spores, he observed a coalescence of these microtubules similar to that discovered in spores of the Teredo spp. haplosporidan. Unornamentated spores have not been described for M. chitonis; however, Ball (1980, 1981) only studied spores within host tissue.

Spores of Urosporidium jiroveci (Order Balanosporida, Family Anurosporidiidae) possess a single epispore cytoplasm extension with supporting microtubules similar in longitudinal section to those of the haplosporidan infecting Teredo spp. (Ormieres et al. 1973). However, Urosporidium spp. spores differ from the operculated Haplosporidiidae spores in that the former possess an internal flap or lingula for closure of the spore orifice (Perkins and van Banning 1981).

Haplosporidan parasites of the family Haplosporidiidae have been traditionally separated into two genera, Haplosporidium Caullery and Mesnil and Minchinia Labbe. Species of both genera have spores with an orifice closed by a hinged operculum that overhangs the spore wall except along the hinge (Perkins 1989) and ornamentation consisting of a wide variety of structures variously described by different authors as wrappings, ribbons, threads, filaments or tails. The nature of the ornamentation has recently been determined for many species of Minchinia and Haplosporidium through electron microscopy and has led to conflicting definitions of structures and confusion as to the proper generic allocation of many species.

Sprague (1982) characterized the genus Haplosporidium including such species as H. nelsoni and H. costale Wood and Andrews by threads (wrappings) wound around the spore coat while Minchinia spores, exemplified by M. chitonis and M. armoricana van Banning, were defined by anterior and posterior extensions. Sprague (1982) did not specify the origin of these threads or extensions.

In his description of H. parisi spores, Ormieres (1980) presented alternative criteria for distinguishing these two genera. Extensions of the spore wall such as the two long filaments arising from the posterior region of spores of H. parisi were differentiated from extensions of epispore cytoplasm, a distinction which Sprague (1982) did not recognize. Based on his interpretation of the original type species descriptions of M. chitonis and H. scolopli Caullery and Mesnil (Caullery and Mesnil 1905), Ormieres (1980) described Minchinia spores as possessing tails defined as extensions of epispore cytoplasm and Haplosporidium spores as possessing filaments, defined as extensions of the spore wall that persist after degradation of epispore cytoplasm. Ormieres' (1980) definition of filaments appears to have been based on a sketch by Caullery and Mesnil (1905) of H. scolopli spores with two posterior extensions. In the description of this figure, Caullery and Mesnil (1905) referred to "a delicate external membrane which is often only recognizable in some debris". Based on present knowledge, this discussion seems to pertain to membrane-bound epispore cytoplasm. It is not clear whether the extensions of H. scolopli are a part of this membrane and therefore

should be classified as epispore cytoplasm tails or are spore wall derived filaments surrounded by the membrane.

Spores with paired posterior filaments similar to those of H. parisi have been described for H. lusitanicum Azevedo (Azevedo 1984) and possibly H. comatulae La Haye et al. (La Haye et al. 1984) and H. tumefacientis Taylor (Taylor 1966). This group of species with spore wall extensions is clearly distinct morphologically from spores with epispore cytoplasm extensions such as are found in the Teredo spp. haplosporidan and M. chitonis.

Perkins (1988, 1989) generally agreed with Sprague's (1982) generic distinctions and grouped spores with prominent extensions, either epispore cytoplasm tails or spore wall filaments, in the genus Minchinia and spores lacking such extensions in Haplosporidium. Spores with epispore cytoplasm tails like those of M. chitonis were considered congeneric with M. armoricana ornamented by anterior and posterior extensions and M. parisi (= H. parisi) possessing posterior paired spore wall filaments; however, Perkins (1988) generic reallocations of H. parisi and H. lusitanicum to Minchinia may not be valid since the type species of the genus Minchinia, M. chitonis, possesses spore ornaments that are composed entirely of epispore cytoplasm and are not attached to the spore wall (Lankester 1885; Labbe 1896, 1899; Ball 1981).

Lauckner (1983) in a long footnote to a discussion of M. chitonis, stated that Minchinia is a nomen nudum because lifecycle stages of two different organisms were included in the original description. Labbe (1896, 1899) combined spore stages of a

haplosporidan in Lepidochiton cinereus with sporozoan stages of a coccidian (Pseudoklossia chitonis Debaisieux) from Acanthochiton fascicularis. Therefore Lauckner (1983) placed all species in Haplosporidium. However, the spore Labbe (1896) described was clearly a haplosporidan enabling subsequent species, with spore morphology similar to that of M. chitonis, to be assigned to the genus Minchinia. Thus Lauckner's (1983) conclusion has not gained wide acceptance.

Morphology of the M. armoricana extensions and subsequent generic assignment of this species have been a source of confusion in the literature. In his original description of M. armoricana, van Banning (1977) described anterior and posterior extensions of the epispore cytoplasm. Pichot et al. (1979) described, but did not name, a haplosporidan from Ostrea edulis that resembled van Banning's (1977) description of M. armoricana except that the spores were ornamented by filaments arising from the spore wall in extensions of the epispore cytoplasm. Perkins and van Banning (1981), studying spores held in sea water for one year, reported the presence of anterior and posterior filaments on M. armoricana consisting of bundles of fibers originating from several points on the spore surface. It is possible that the filaments described by Pichot et al. (1979) and Perkins and van Banning (1981) are either developing spore wall filaments surrounded by epispore cytoplasm or are supporting structures for epispore cytoplasm tails similar to the microtubule-like structures described here for the haplosporidan infecting Teredo spp. Bachere et al. (1987) referred to a haplosporidan from Ostrea angasi as

Haplosporidium sp. and compared it to H. armoricana but did not provide clear evidence for the composition of the epispore extensions.

In recent papers (Bachere and Grizel 1983; Desportes and Nashed 1983; Bachere et al. 1987; Chagot et al. 1987), the generic distinctions proposed by Ormieres (1980) and his definitions of tails and filaments have been followed; however, the term wrappings is still not clearly defined. According to Perkins (1968), the wrappings of H. costale are formed in the epispore cytoplasm as tubular elements and are left in contact with the spore wall after lysis of the cytoplasm. The spore wrappings of H. louisiana were described by Perkins (1975) as forming in vacuoles of the epispore cytoplasm. These ornaments are not formed until the spore wall is complete around the sporoplasm (Perkins and van Banning 1981). Following degradation of the cytoplasm, these strands were found either fused to the spore wall or wrapped loosely around it. The wrappings of H. costale and H. louisiana seem to be distinct from the spore wall filaments of H. parisi and H. lusitanicum which are attached to the wall at a single point and are formed as the spore wall is forming prior to lysis of the epispore cytoplasm (Ormieres 1980; Azevedo 1984); however, species possessing spore wall filaments and those ornamented by wrappings are presently placed in the genus Haplosporidium. Further research into the composition of wrappings is necessary to determine if they are more similar to the ornamentation of H. scolopli, the type species of the genus Haplosporidium, or to M. chitonis, the type species of Minchinia.

Haplosporidan spore ornamentation should be placed in three categories: spore wall filaments, epispore cytoplasm extensions and wrappings. Filaments, as found on spores of H. parisi, are composed of wall material and are formed as the spore wall is forming. Epispore cytoplasm extensions are more ephemeral and may be shed after spores are released from the host as has been shown herein for the Teredo spp. haplosporidan. Wrappings, exemplified by spores of H. costale, are formed in epispore cytoplasm and adhere to the spore wall following lysis of the cytoplasm.

An additional problem in classification of the Haplosporidiidae is the lack of accurate type species descriptions. The type species of the genus Haplosporidium, H. scolopli has not been studied with electron microscopy and the origin of its epispore extensions is uncertain.

Spores of haplosporidans should be more closely studied at all stages of development in order to better define the genera of this family. Minchinia dentali (Arvy) (Desportes and Nashed 1983) and M. tapetis (Chagot et al. 1987) spores have been described as unornamented. In light of the present study in which epispore cytoplasm and tails are shed at some stage of development, it seems correct to assign mature spores without ornaments to the genus Minchinia. Further research into the morphology of these unornamented spores could reveal some type of epispore cytoplasm ornamentation at an earlier stage of development.

Based upon the present understanding of the taxonomy of the Haplosporidiidae and the morphology of M. chitonis, the genus

Minchinia contains those species whose spores possess epispore cytoplasm extensions. Spores of the haplosporidan infecting Teredo spp. bear four extensions composed of epispore cytoplasm supported by microtubule-like structures enabling placement of this organism in the genus Minchinia.

LITERATURE CITED

Andrews, J. D. 1984. Epizootiology of diseases of oysters (Crassostrea virginica), and parasites of associated organisms in eastern North America. Helgolander Meeresunters., 37:149-166.

Azevedo, C. 1984. Ultrastructure of the spore of Haplosporidium lusitanicum sp. n. (Haplosporida, Haplosporidiidae), parasite of a marine mollusc. J. Parasitol., 70:358-371.

Bachere, E. and Grizel, H. 1983. Mise en evidence d'Haplosporidium sp. (Haplosporida-Haplosporidiidae) parasite de l'huitre plate Ostrea edulis L. Rev. Trav. Inst. Peches Marit., 46:226-232.

Bachere, E., Chagot, D., Tige, G. and Grizel, H. 1987. Study of a haplosporidian (Ascetospora), parasitizing the Australian flat oyster Ostrea angasi. Aquaculture, 67:266-268.

Ball, S. J. 1980. Fine structure of the spores of Minchinia chitonis. (Lankester, 1885) Labbe, 1896 (Sporozoa: Haplosporida), a parasite of the chiton Lepidochiton cinereus. Parasitol., 81:169-176.

Ball, S. J. 1981. Spore structure of Minchinia chitonis. Mar. Fish. Rev., 43:5-8.

Burreson, E. M. and Robinson, M. E. 1988. An SEM study of haplosporidan spores from Teredo navalis. J. Shellfish Res., 7:215.

Caullery, M. and Mesnil, F. 1905. Recherches sur les Haplosporidies. Arch. Zool. Exp. Gen., 4:101-181.

Chagot, D., Bachere, E., Ruano, F., Comps, M. and Grizel, H. 1987. Ultrastructural study of sporulated instars of a haplosporidian parasitizing the clam Ruditapes decussatus. Aquaculture, 67:262-263.

De Mey, J., Hacker, G., De Waele, M. and Springall, D. 1986. Gold probes in light microscopy. In: Polak, J. and van Noorden, S. (ed.), Immunocytochemistry, 2nd ed., Wright-PSG, Bristol, England, pp. 71-88.

Desportes, I. and Nashed, N. 1983. Ultrastructure of sporulation in Minchinia dentali (Arvy), an haplosporean parasite of Dentalium entale (Scaphopoda, Mollusca); taxonomic implications. Prostistologica, 19:435-460.

Ford, S. E. and Haskin, H. H. 1982. History and epizootiology of Haplosporidium nelsoni (MSX), an oyster pathogen in Delaware Bay, 1957-1980. J. Invert. Pathol., 40:118-141.

Hillman, R. E. 1978. The occurrence of Minchinia sp. (Haplosporida, Haplosporidiidae) in species of the molluscan borer Teredo from Barnegat Bay, New Jersey. J. Invert. Pathol., 31:265-266

Hillman, R. E. 1979. Occurence of Minchinia sp. in species of the molluscan borer Teredo. Mar. Fish. Rev., 41:21-24.

Hillman, R. E. 1980. Life cycle stages of Minchinia sp. in Teredo navalis. Amer. Zool., 20:961.

Hillman, R. E., Maciolek, N. J., Lahey, J. I. and Belmore, C. I. 1982. Effects of a haplosporidian parasite Haplosporidium sp. on

species of the molluscan woodborer Teredo in Barnegat Bay, New Jersey. J. Invert. Pathol., 40:307-319.

Hoagland, K. E. and Turner, R. D. 1980. Range and extensions of teredinids (shipworms) and polychaetes in the vicinity of a temperate-zone nuclear generating station. Mar. Biol., 58:55-64.

Holgate, C., Jackson, P., Cowen, P. and Bird, C. 1983. Immunogold silver staining: new method of immunostaining with enhanced sensitivity. J. Histochem. Cytochem., 31:938-944.

Kanaley, S. and Barber, R. 1989. Recent observations on the sporulation of Haplosporidium nelsoni (MSX) in the American oyster Crassostrea virginica. NJAES Publ. No. K-32901-1-89. p.74.

Labbe, A. 1896. Recherches zoologiques, cytologiques et biologiques sur les coccidies. Arch. Zool. Exp. Gen., 4:533-608.

Labbe, A. 1899. Sporozoa. In: Das Tierrich. Friedlander, Berlin, 5:1-180.

La Haye, C. A., Holland, N. D. and McLean, N. 1984. Electron microscopic study of Haplosporidium comutalae n. sp. (Phylum Ascetospora: Class Stellatosporea), a haplosporidian endoparasite of an Australian Crinoid, Oligometra seripinna (Phylum Echinodermata). Protistologica, 20:507-515.

Lankester, E. R. 1885. Protozoa. In: Encyclopaedia Britannica, 9th ed. Encyclopaedia Britannica, London, 19:830-866.

Lauckner, G. 1983. Diseases of Mollusca: Amphineura. In: Kinne, O. (ed.), Diseases of Marine Animals, Vol. II: Introduction,

Bivalvia to Scaphopoda. Biologische Anstalt Helgoland, Hamburg, 963-975.

McGovern, E. R. and Burreson, E. M. 1989. Immunoassay comparison of haplosporidan spores from Teredo navalis and Haplosporidium nelsoni spores from Crassostrea virginica. J. Protozool., **36**:289-292.

Ormieres, R. 1980. Haplosporidium parisi n. sp., Haplosporidie parasite de Serpula vermicularis L. etude ultrastructurale de la spore. Protistologica, **16**:467-474.

Ormieres, R., Sprague, V. and Bartoli, P. 1973. Light and electron microscope study of a new species of Urosporidium (Haplosporida), hyperparasite of trematode sporocysts in the clam Abra ovata. J. Invert. Pathol., **21**:71-86.

Perkins, F. O. 1968. Fine structure of the oyster pathogen Minchinia nelsoni (Haplosporida, Haplosporidiidae). J. Invert. Pathol., **10**:287-307.

Perkins, F. O. 1969. Electron microscope studies of sporulation in the oyster pathogen, Minchinia costalis (Sporozoa: Haplosporida). J. Parasitol., **55**:897-920.

Perkins, F. O. 1975. Fine structure of Minchinia sp. (Haplosporida) sporulation in the mud crab Panopeus herbstii. Mar. Fish. Rev., **37**:46-60.

Perkins, F. O. 1979. Cell structure of shellfish pathogens and hyperparasites in the genera Minchinia, Urosporidium, Haplosporidium and Marteilia--taxonomic implications. Mar. Fish. Rev., **41**:25-37.

Perkins, F. O. 1988. Parasite morphology, strategy and evolution: structure of protistan parasites found in bivalve molluscs. Am. Fish. Soc. Spec. Publ., 18:93-111.

Perkins, F. O. 1989. The Haplosporidia. In: Margulis, L., Corliss, J. D., Melkonian, M. and Chapman, D., (ed.). Handbook of Protoctista. Jones and Bartlett, Boston. pp. 19-29.

Perkins, F. O. and van Banning, P. 1981. Surface ultrastructure of spores in three genera of Balanosporida, particularly in Minchinia armoricana van Banning, 1977--the taxonomic significance of spore wall ornamentation in the Balanosporida. J. Parasitol., 67:866-874.

Pichot, Y. Comps, M. and Deltreil, J. 1979. Recherches sur Haplosporidium sp. (Haplosporida--Haplosporidiidae) parasite de l'huitre plate Ostrea edulis L. Rev. Trav. Inst. Peches Marit., 43:405-408.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol., 17:208-212.

Roitt, I., Brostoff, J. and Male, D. 1985. Immunology. The C. V. Mosby Company, St. Louis.

Sprague, V. 1982. Ascetospora. In: Parker, S. (ed.), Synopsis and Classification of Living Organisms. McGraw-Hill, New York, 599-601.

Spurr, A. R. 1969. A low-viscosity resin embedding medium for electron microscopy. J. Ultrast. Res., 26:31-43.

Taylor, R. L. 1966. Haplosporidium tumefacientis sp. n., the etiologic agent of a disease of the California Sea Mussel, Mytilus californianus Conrad. J. Invert. Pathol., 8: 109-121.

Turner, R. D. 1966. A survey and illustrated catalogue of the Teridinidae (Mollusca: Bivalvia). The Museum of Comparative Zoology, Cambridge, 265p.

van Banning, P. 1977. Minchinia armoricana sp. nov. (Haplosporida), a parasite of the European flat oyster, Ostrea edulis. J. Invert. Pathol., 30:199-206.

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