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Light and ultrastructural observations on a microsporidium in the hydrozoan *Halammohydra intermedia* (Cnidaria)

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SARSIA



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A microsporidium has been observed in the interstitial hydrozoan *Halammohydra intermedia* Clausen, 1967. The ultrastructure of the spores has been studied. The oval spores (c. $1.7 \times 1.0 \mu\text{m}$ in unfixed smear preparations) have an isofilar polar tube deposited in four to six coils in one or two layers beneath the spore wall. There appears to be octosporoblastic sporogony; however, the precise systematic position is uncertain, as prespore development and nuclear conditions are unrevealed. This is the second report of a microsporidium in the Cnidaria, and the first in a marine cnidarian.

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Keywords: Microspora; Microsporidium; spore; ultrastructure; Cnidaria; Hydrozoa; *Halammohydra*.

INTRODUCTION

The parasitic phylum Microspora has a wide range of hosts, from protozoans to mammals. The present finding appears to be the only second record of a microsporidium in the Cnidaria (see Sprague 1977). The first record (see Spangenberg & Claybrook 1961) was made from a laboratory stock of *Hydra littoralis*. The present finding also comes from the Hydrozoa, the interstitially living species *Halammohydra intermedia* Clausen, 1967 (Fig. 1). The parasite was first detected in EM-sections, and later also found with a light microscope.

MATERIAL AND METHODS

The host specimens forming the basis of this study were collected from the upper four to five cm of shell gravel at 3 m depth at Raunane, Raunefjorden ($60^{\circ}15.80'N$, $5^{\circ}10.80'E$) in the Bergen area, Norway. The samples were taken in January 1988 for EM studies, and in November 1989 for light microscopical studies. The haptic host specimens were isolated by means of the anaesthetization-decantation technique (see Pfannkuche & Thiel 1988). Live studies (three host specimens) were made by means of phase contrast and Nomarski interference contrast optics. One smear was stained in Giemsa's solution. For the EM study, host specimens were fixed at $4^{\circ}C$ for 2 hours in 2.5 % glutaraldehyde and 1 % paraformaldehyde in 0.05 M phosphate buffer with 7 % sucrose and traces of CaCl_2 (pH 7.3, 1000 mosmols); osmification (1 % at $0^{\circ}C$) for 1 hour in 0.05 M phos-

phate buffer with 1.5 % NaCl, and dehydration in an ascending series of buffer-acetone to absolute acetone. The material was embedded in Epon, sectioned with a diamond knife on an LKB ultramicrotome, stained with uranylacetate and lead citrate, and examined in a Jeol 100 CX electron microscope operated at 80 kV. Examined EM material: three host specimens.

Additional material of *H. intermedia* was collected from Høylandsskjær in Korsfjorden ($60^{\circ}11.40'N$, $58^{\circ}09.80'E$) in September 1998.

OBSERVATIONS

OCCURRENCE AND PREVALENCE

In the Raunane material local aggregations of the parasite occurred in all the three host specimens examined live. In the TEM sections the parasite was found in all of three studied host specimens, and also in this case was found in local aggregations. It occurred both in the epidermis and in the endodermis, at the tentacle bases and the aboral cone. Three host specimens of the material from Høylandsskjær were also examined live and searched for the parasite, and all proved to be more or less heavily infected with the microsporidium. The two visited localities lie eight kilometres apart, and are isolated from each other with respect to horizontal dispersal of the interstitial halammohydras via the sediment. It is assumed, therefore, that this host/parasite relationship is established in a much wider area than the investigated one.

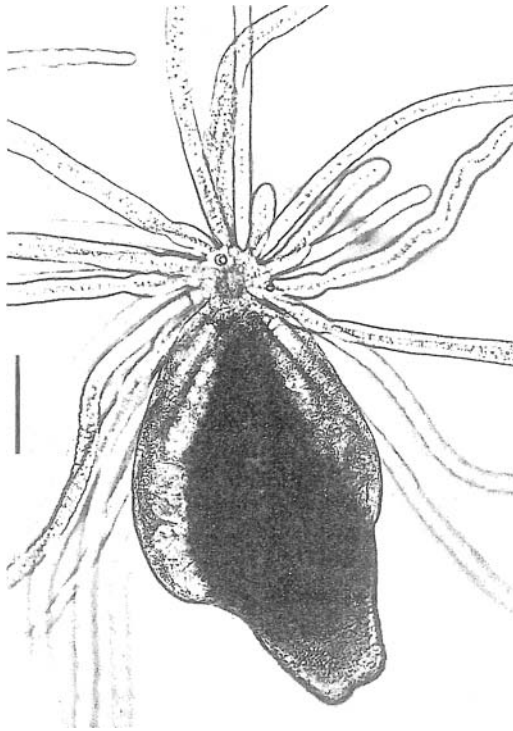


Fig. 1. *Halammohydra intermedia*, LM graph. Habitus of an adult male with two gonads. Scale line, 0.1 mm.

GENERAL FINDINGS

The spores are oval or slightly bean-shaped and measured fresh $1.6-1.8 \times 0.9-1 \mu\text{m}$ (Figs 2-4). By LM studies of the live host they can be seen only with difficulty in squeezed specimens; thus they were first observed with the electron microscope.

The spores are enclosed in vesicles (probably sporophorous) with a thin and loose wall in the host cell cytoplasm. Other stages of the parasites life cycle have not been found. In sections, small vesicles with a single spore and larger vesicles up to $3 \times 4 \mu\text{m}$ and with up to seven spores were seen. In the light microscope the highest number of spores contained in a vesicle was eight (Fig. 2C). Unfortunately, the staining with Giemsa was unsuccessful in revealing nuclei in the spores, and neither could spore nuclei be detected in TEM sections.

Several presumed sporophorous vesicles occurred in the urn-shaped adhesive organ. All of them occurred near the periphery of the organ, i.e. in the basal part of the cells (Fig. 3). Some of the vesicles also contained apparent degenerative stages of spores with a vacuolated sporoplasm (Fig. 5C).

SPORE FINE STRUCTURE

The spores (Figs 4, 5) have a smooth wall consisting of a moderately electron-dense (c. 18 nm) exospore, an electron-lucent (c. 30 nm) endospore, and the plasma membrane. The polaroplast extends from near the apical pole to the centre of the spore. Viewed in nearly transverse sections, some places seeming laminae are seen to con-

Figs 2-5. Microsporidian parasite of *Halammohydra intermedia*. Labels used in the figures: ao = adhesive organ; ci = cilia in lumen of hosts adhesive organ; en = endospore; ex = exospore; gr = membrane-bound secretion granula; mi = mitochondrion of host cell; ne = nematocyst; nu = nucleus of adhesive organ cell; pm = plasma membrane; pp = polaroplast; pt = polar tube; pv = posterior vacuole; sv = sporophorous vesicle; tb = tentacle base.

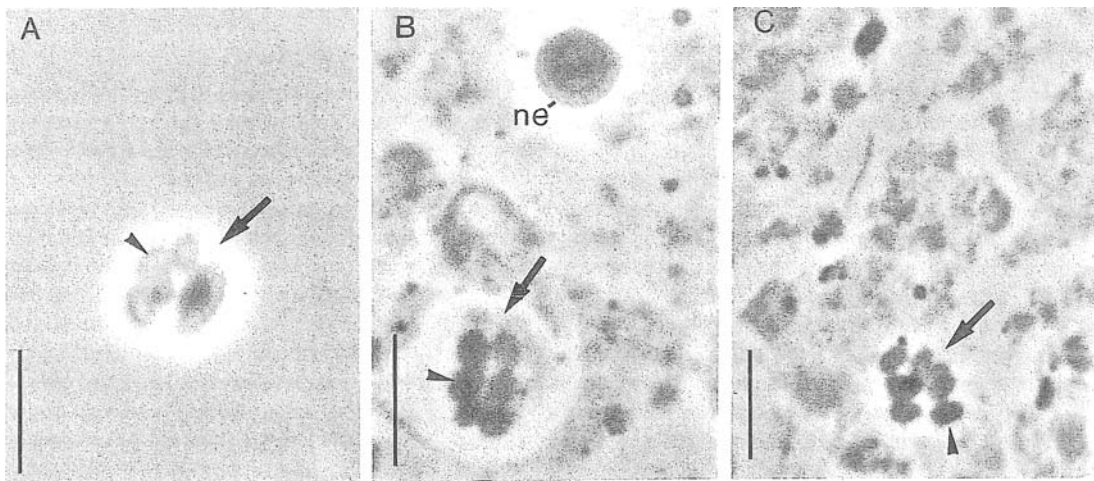


Fig. 2 A-C. LM graphs (phase contrast) of sporophorous vesicles (arrows) with different numbers of spores (arrowheads). Smears. Scale lines, 5 μm .

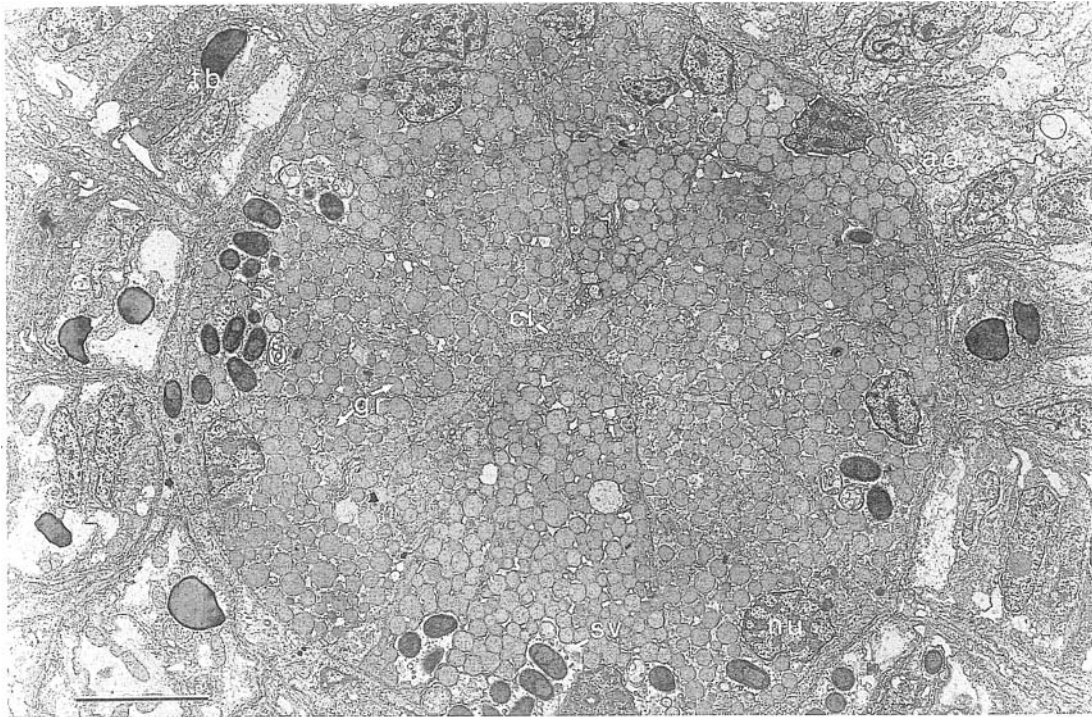


Fig. 3. TEM graph of hosts adhesive organ (transverse section) containing several sporophorous vesicles. Scale line, 5 μm .

sist of two electron dense membranes; at intervals the two membranes are less apposed, giving the impression of beaded strings. In other places the polaroplast might rather be interpreted as zigzag profiles (Fig. 5B). No strict sagittal sections of the spore were obtained; thus a comparison of the anterior and the posterior part of the polaroplast could not be made.

The polar tube is 100 nm wide at its base, and narrows gradually before it is rolled up in four to six coils of even thickness (about 60 nm) in the middle to posterior part of the spore; it is best characterised as isofilar. The coils either lie in line near the plasma membrane, or a few coils may also form an inner layer of coils. The angle of tilt of the first tube coil varies much, from c. 50 to 80 degrees to the long axis of the spore. Calculated length of the polar tube within the spore is 15–18 μm . A small, lobate (about 0.25 μm maximum diameter) posterior vacuole in one section appeared as if connected with the distalmost coil of the tube (Fig. 5A); there is no posterosome inclusion. Ribosomes occur in arrays between the spore wall and polaroplast and beyond, more scattered in the posterior part of the sporoplasm.

REMARKS

The occurrence of octosporoblastic sporogony would place the parasite within the order Meiodiaphasida Sprague, Becnel & Hazard, 1992 (see Sprague & al.

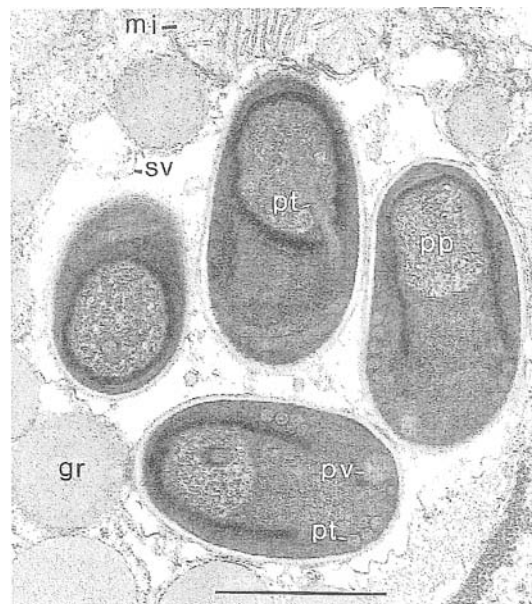


Fig. 4. TEM graph of four spores within a sporophorous vesicle in the adhesive organ. Scale line, 1 μm .

1992). The generic affiliation can not be settled until details of pre-spore development and nuclear conditions are known. The *Hydra* parasite was tentatively referred

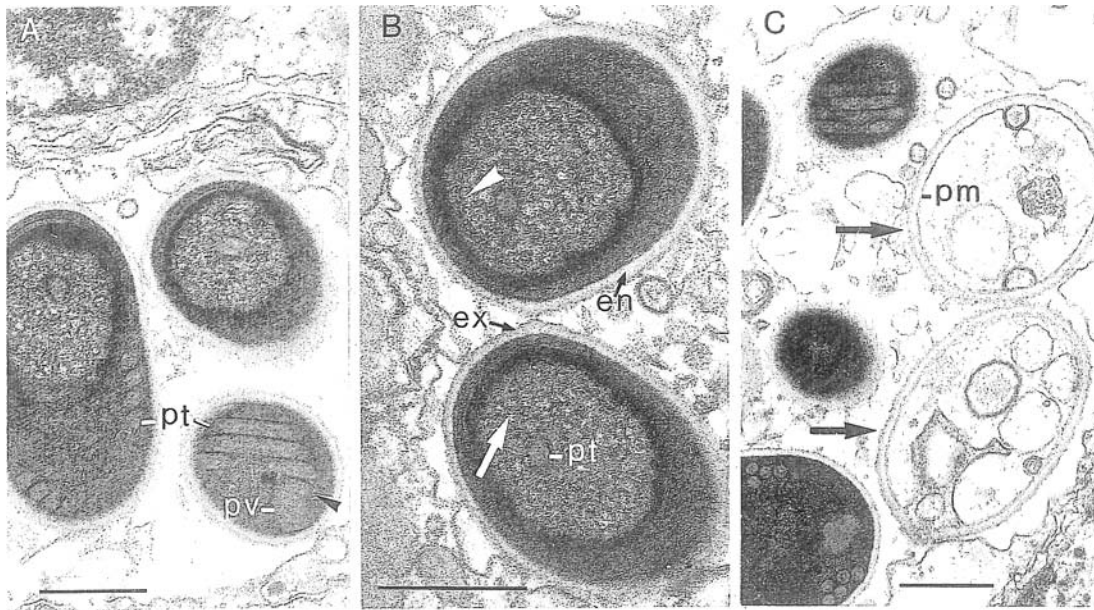


Fig. 5. TEM graphs of spores. A. Three sectioned spores. The lower spore to the right shows the posterior vacuole which could possibly be connected with the polar tube (arrowhead). B. Oblique section through two spores. Arrowhead points to a seemingly lamellar part of the polaroplast, arrow points to a zigzag-like profile. C. Part of a sporophorous vesicle. Spore to the lower left shows a bilayered coiling of the polar filament. Arrows point to presumably degenerative spores. Scale lines, 0.5 μ m

to *Pleistophora* on account of the spore structure (Spangenberg & Claybrook 1961); however, no figures were given, and the affiliation may be doubtful.

For an evaluation of the seemingly strange and aberrant polaroplast, it is desirable to obtain clearer pictures of it; other fixation procedures than the single one used might be tried.

Although infections with a microsporidium may be fatal to the host, little effect was noted in adults of *Hydra*, and no noticeable effects were detected in *Halammohydra intermedia*.

Three more species of *Halammohydra* occur in the Bergen surroundings. It is intended to examine also these species with respect to microsporidia.

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