JOURNAL OF INVERTEBRATE PATHOLOGY 38, 67–70 (1981)

A New Microsporidium from the Oyster *Ostrea lutaria* in New Zealand

J. B. JONES

Fisheries Research Division, Ministry of Agriculture and Fisheries, P.O. Box 19062, Wellington, New Zealand

Received August 11, 1980

Microsporidium rapuae n. sp. (Microsporida) forms cysts within the connective tissue surrounding the gut epithelium of the oyster *Ostrea lutaria*. Except for encapsulation of the cysts by fibroblasts no pathogenic effects were observed. Each cyst contained a large number of spores possessing long well-developed polar filaments. This is the third Microspora recorded from Bivalvia (Mollusca), and the first which does not belong to the family Chytridiopsidae (Minisporida).

KEY WORDS: *Microsporidium rapuae* n.sp.; *Ostrea lutaria*.

INTRODUCTION

Two microsporans have previously been recorded from bivalve mollusks. The first was *Chytridiopsis ovicola* Léger and Duboscq, 1917 which was found in the ova of the European oyster, *Ostrea edulis*. *Haplosporidium mytilovum* Field, 1924 was described from the ova of the mussel *Mytilus edulis*, but Sprague (1965), after studying the ultrastructure of this parasite, transferred it to the genus *Chytridiopsis*. These two species of *Chytridiopsis*, together with a third, *C. brachynema* (Richards and Sheffield, 1971), a parasite of the gut epithelium of the aquatic gastropod *Biomphalaria*, were later transferred to a new genus *Steinhausia* Sprague, Ormières, and Manier, 1972 within the new family Chytridiopsidae. All the members of this family are characterized by spherical to elliptical spores with a tendency toward minimum development of the spore organelles. The polar filament is short, the polaroplast is poorly developed, and there is little or no endospore (Sprague et al., 1972).

During 1973–1974, stained sections of tissue from 132 dredge oysters, *Ostrea lutaria*, were examined for parasites. Approximately 10% of the 109 oysters examined from Foveaux Strait, in which are New Zealand's main commercial dredge oyster beds, were found to be infected with a sporozoan parasite. All 23 oysters examined from Wellington Harbour were found to be free of infection (Jones, 1975).

The spores of the parasite occurred in small cysts in the connective tissue surrounding the gut, with each cyst containing in excess of 100 spores. From the size and shape of the spores, the parasite was tentatively placed in the phylum Microspora, but all attempts to demonstrate a polar cap using the periodic acid–Schiff technique were unsuccessful. Despite the lack of firm evidence that the parasite was a microsporidium, Sprague (1978) placed it in the "collective group" *Microsporidium*.

In 1980, Weiser (pers. commun.) suggested reembedding cysts, which had been located and trimmed from previously sectioned paraffin blocks, for electron microscopy. It was possible using this technique to recognize cysts containing spores possessing a polar filament, polar cap, and well-developed endospore. This confirmed the systematic position of the parasite, which is described for the first time in this study.

METHODS

A 3-mm thick slab of tissue was cut transversely through the stomach and hepatopancreas of each oyster examined,

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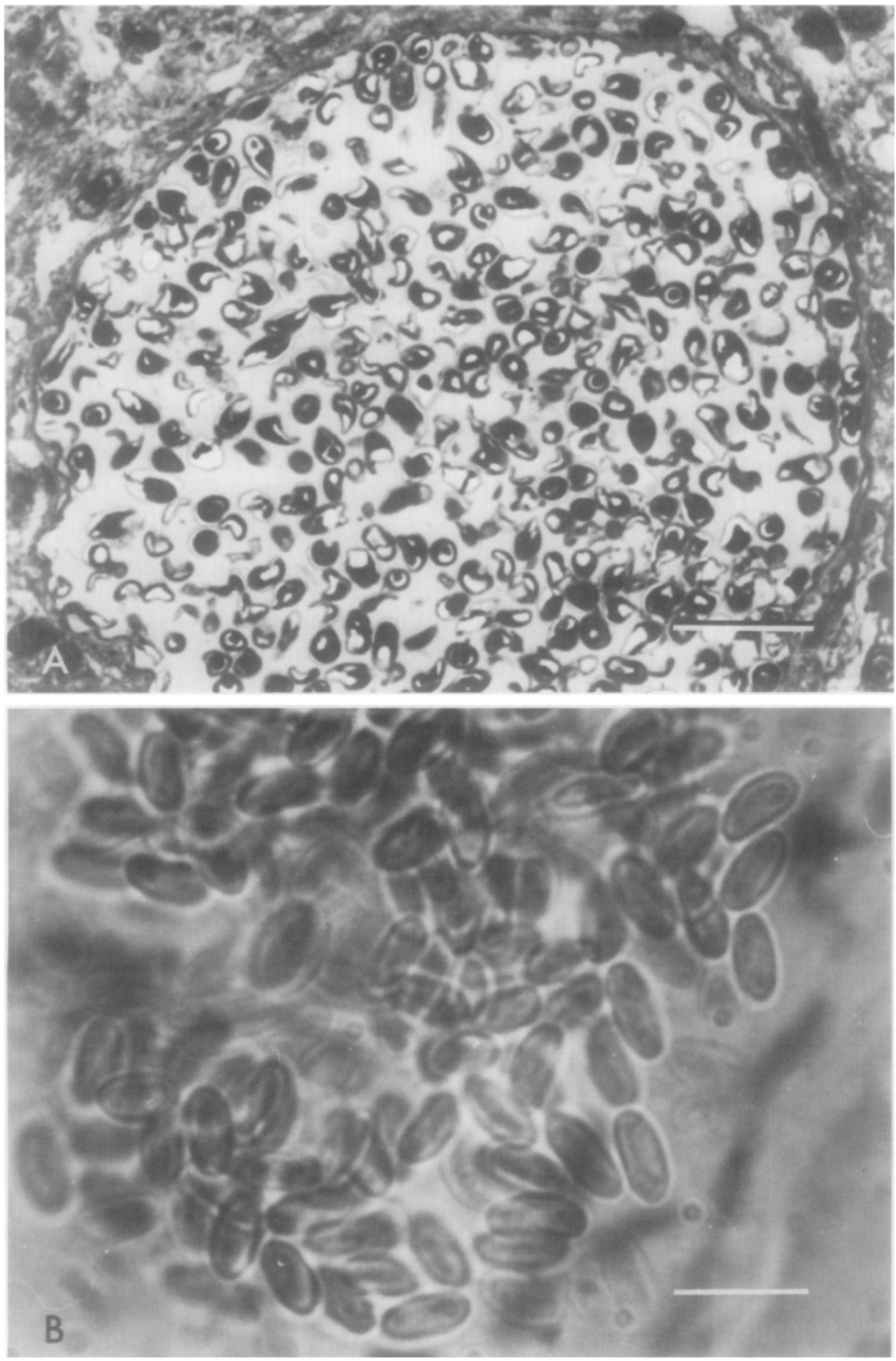

FIG. 1. (A) *Microsporidium rapuae* n.sp. cyst containing spores in tissue of *Ostrea lutaria*. Methyl blue, Araldite section. Scale bar = 12 μm. (B) *Microsporidium rapuae* n.sp. spores in oyster *Ostrea lutaria*. Frozen section, aqueous mount, phase contrast. Scale bar = 8 μm.

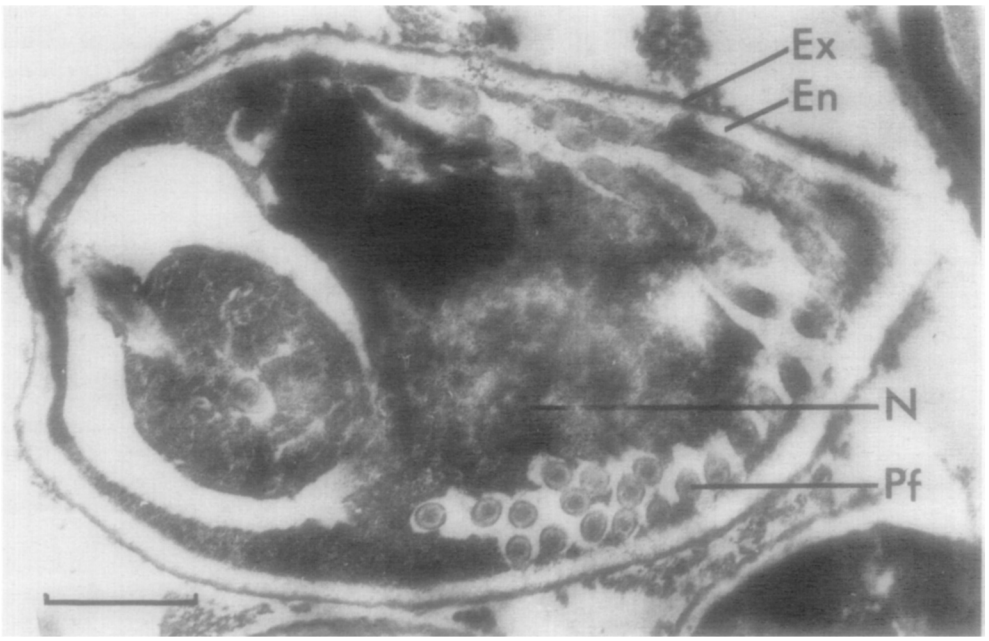

FIG. 2. *Microsporidium rapuae* n.sp. longitudinal section through spore. Scale bar = 60 nm. (Ex) Exospore; (En) endospore; (Pf) polar filament.

and was fixed in 10% EM grade formalin buffered with filtered sea water. After 3 days, the tissue was embedded in paraffin using standard techniques, and sectioned at 5 μm. Sections were stained with Giemsa's stain and examined under a compound microscope for cysts. When a cyst was located, the slide was marked and oriented over the paraffin block to locate the position of the cyst in the block. The block containing the cyst was then trimmed down to 1 mm^3 before the paraffin was removed with xylene. The tissue was rehydrated, postfixed in 2% aqueous osmium tetroxide, and reembedded in Araldite using standard procedures.

Sections were cut at 1 μm using a Reichert Ultracut and each was examined as a wet mount under phase contrast until the cyst was reached. Ultrathin sections then obtained were stained with uranyl acetate and lead citrate before examination in a Zeiss EM9A microscope.

RESULTS

No gross signs of infection were observed in the oysters. In histological sections, the cysts were oval or spherical and varied from 20 to 70 μm in long axis. Each cyst (Fig. 1A) was packed with large numbers of spores, and was surrounded by more or less concentric layers of fibrous elements intermingled with leukocytes and fibroblasts. No other pathological changes were seen. The matrix of the cyst, containing the spores, was electron transparent with no recognizable inclusions or membranes remaining, probably as a result of the postfixation procedures.

Each spore was oval, circular in cross section, and refractive (Fig. 1B). Spores from frozen sectioned tissue in aqueous mount measured $5.20 \pm 0.35 \times 2.57 \pm 0.28$ μm (mean $\pm$ 1 SD; $n = 50$). Spores embedded in Araldite measured $4.70 \pm 0.39 \times 2.39 \pm 0.28$; $n = 25$. Spore ultrastructure (Fig. 2) showed a polar cap present at the spore apex, with an attached long polar filament of 19–22 coils, subtending angle of 67° to spore axis. The polar filament, 110-nm diameter, was multilayered; the nucleus apparently single. The spore wall was of three layers, the exospore electron dense, wrinkled, 20–25 nm thick, bounded

on the inner surface by a double membrane enclosing cell contents.

DISCUSSION

With a long tubular polar filament, oval spore, and well-developed endospore, this microsporidan is quite distinct from the other Microspora described from bivalve mollusks. It may also be distinguished on spore size from *Microsporidium aplysia,* the only microsporidan recorded from a nonbivalve marine mollusk. This occurs in the neurons of the gastropod *Aplysia californica,* and has a spore size of only 1.3 × 0.7 μm (Krauhs et al., 1979). Creation of a new species is therefore justified. However, as sporogenesis has not been seen and the number of spores formed within the pansporoblast membrane is unknown, the parasite cannot be assigned to a family and must for the moment be retained within the "collective group" *Microsporidium.*

Microsporidium rapua n. sp.

Type host. Ostrea lutaria Hutton, 1873.

Site of infection. Connective tissue beneath gut epithelium.

Type locality. Foveaux Strait, New Zealand (46°40′S 168°20′E).

Type material. Holotype slide deposited in New Zealand National Museum (Prot. 23). Paratype slide in NZNM (Prot. 24). Both collected from Foveaux Strait on 26 March 1980. Other material collected from Foveaux Strait between 1973 and 1980 in collections of author; V. Sprague (University of Maryland); J. Weiser (Institute of Entomology, Czechoslovakia).

The name chosen is derived from the Maori verb "rapu" which means "to look for" or "to search."

ACKNOWLEDGMENTS

I am indebted to Prof. V. Sprague (University of Maryland); and Mr. A. Hoverd (Victoria University, New Zealand) who both tried unsuccessfully to demonstrate a periodic acid–Schiff-positive polar cap in material supplied by me; to Dr. J. Weiser (Czechoslovakia) for his suggestion of the reembedding technique used; and Mr. M. Loper (Victoria University) for making the electron microscope available.

The initial survey of oysters was carried out during tenure of an NRAC doctoral fellowship.

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