

Electron microscopical study of a new genus and new species of microsporidia in the gills of Atlantic cod *Gadus morhua* L.*

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Abstract. A microsporidium, forming xenoparasitic complexes (xenomas) of the cell-hypertrophy tumour type, was found in the gills of the Atlantic cod *Gadus morhua* L. and studied with the electron microscope. It seemed to be similar to *Nosema branchiale* Nemeczek, 1911, in the haddock *Melanogrammus aeglefinus* (L.) except in size of spore. The xenoma (cyst) was basically like the *Glugea* cyst and the parasite had some other characters in common with *Glugea*; it was apansporoblastic, unikaryotic, disporoblastic and underwent partial development in parasitophorous vacuoles. It differed from *Glugea* in lacking plasmodial stages, producing usually 1 or 2 spores (rather than 16) in a vacuole, showing no distinct tendency for different stages to occur in different zones of the cyst and having tubules in the parasitophorous vacuoles. The name *Loma morhua* n. g., n. sp. (type) was proposed and the genus assigned to the family Glugeidae Thélohan, 1892. The parasite of haddock gill was transferred to the new genus, becoming *L. branchialis* (Nemeczek, 1911) n. comb.

Introduction

Only three species of microsporidia have been described in gills of fish. These are *Nosema branchiale* Nemeczek, 1911 [transferred to genus *Glugea* Thélohan by Lom & Laird (1976) and by Sprague (1977)], in haddock *Melanogrammus aeglefinus* (L.), *Plistophora* (= *Pleistophora*) *salmonae* Putz, Hoffman and Dunbar, 1965, in the rainbow trout *Salmo gairdneri* Richardson and *Ichthyosporidium hertwigi* Swarczewsky, 1914, in *Crenilabrus pavo* (= *C. tinca* L.). These species were described only by means of light microscopy and rather superficially. In no case was the generic identity clearly established. Occurrence of *P. salmonae* and *I. hertwigi* has not been reported again but parasites identified as *N. branchiale* were later reported by Kabata (1959) and Lom & Laird (1976) in the original host; by Dogiel (1936), Fantham, Porter & Richardson (1941) and Shulman & Shulman-Albova (1953) in different subspecies of cod *Gadus morhua* L.; and by Bazikalova (1932) in both haddock and cod. Kabata (1959) pointed out that spore sizes reported by different

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authors do not agree but fall into two distinct groups whether in haddock or in cod. He concluded: 'the possibility of the existence of more than one species in what has hitherto been described as *N. branchiale* should also be considered'.

During routine examination of Atlantic cod we frequently found microsporidian cysts in the pseudobranchs and gills. We undertook an electron microscopical study of these parasites in an attempt to supplement the present meagre knowledge of microsporidian parasites in gills of fish. A preliminary note on microsporidia in the gills of cod, haddock and brook trout *Salvelinus fontinalis* Mitchell has been submitted (Morrison & Sprague 1980).

Materials and methods

Atlantic cod were caught in the approaches to Halifax Harbour in January and February, 1979, and were brought in alive. The fish were weighed, measured and sexed. The right pseudobranchs of one catch of 41 fish and the right pseudobranchs and first right gill raker of another catch of 50 fish were excised and fixed in 10% neutral buffered formalin. These samples were then dehydrated in alcohol, cleared and embedded in paraffin. Seven- μm sections were stained with Harris' haematoxylin and eosin (H&E) or Fontana-Masson stain (Armed Forces Institute of Pathology 1960) and observed and photographed with a Zeiss photomicroscope. Samples of gills from the second catch of fish were also fixed in a 0.1 M phosphate buffer containing 4% formaldehyde and 1% glutaraldehyde overnight, then 1½% OsO₄ in 0.1 M phosphate buffer for 1.5 h. They were dehydrated in acetone, embedded in TAAB resin (obtained from Marivac Co. Ltd.) and sectioned with an LKB Ultratome. Thick sections (0.5 μm) were stained with toluidine blue and photographed with a Zeiss photomicroscope. Spore measurements were made from the photographs. Thin sections were stained with 25% uranyl acetate in methanol (Stempack & Ward 1964) and lead citrate (Reynolds 1963) and observed and photographed in an Hitachi 9 electron microscope.

Results

Prevalence and intensity of infection

Seven of 41 of the first catch of cod had microsporidian cysts in the pseudobranchs. In the second catch three of 50 cod had the parasite in the pseudobranchs and three in the gills. The infection was not found in pseudobranchs and gills of the same fish. In all, about 14% of the 91 fish examined were infected.

No quantitative study of the intensity of infection was made but it obviously varied greatly from one fish to another. While some fish had few or no cysts, the gills or pseudobranchs of others had large numbers.

General effect of parasite on host

No gross signs that could be attributed to the microsporidia were observed in the infected fish.

The lesion

The cysts were spherical or oval and measured about 50–150 µm (Figs 1, 2 & 4). Those in the gills were embedded in the respiratory lamellae or in the gill filament at the base of the lamellae, in close contact with the pillar system (Figs 1–4). The infected

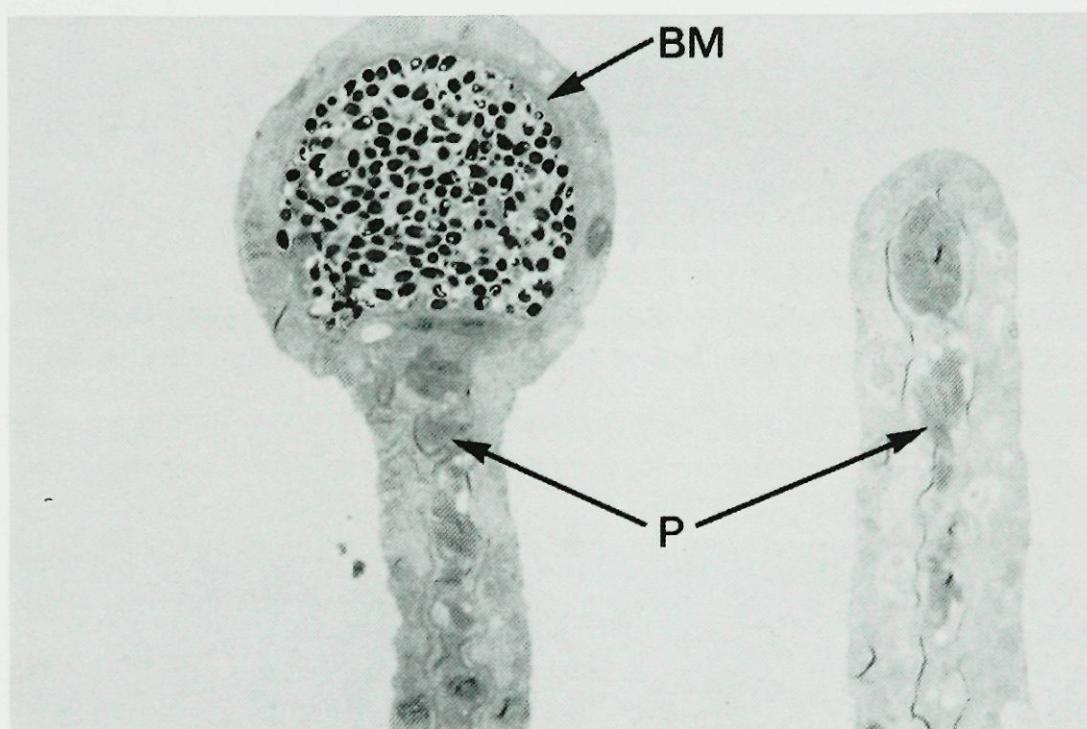


Figure 1. Tips of two gill lamellae, one normal and the other with cyst in the pillar system (P) covered by the basement membrane (BM) (semi-thin section, toluidine blue, $\times 574$).

host cell was enclosed by the basement membrane (BM) of the pillar system (Figs 1–3), this membrane constituting the thick outer part of a two-layered cyst wall. Inside that membrane and interdigitating with it was the second layer, consisting of the modified plasmalemma of the infected host cell (Fig. 3). One cyst in a gill lamella had a very thin outer layer of its wall (presumably basement membrane material from the pillar system) and was encapsulated by a thick layer of fibroblasts (Figs 4 & 5).

Cysts in the pseudobranchs were closely associated with the capillary network of the gland (Fig. 6). They were encapsulated by fibroblasts. Since samples of pseudobranchs were not prepared for electron microscopy details of the structure of these cysts were not revealed.

Mature cysts, like those of *Glugea* and *Ichthyosporidium*, lost their integrity and their contents were no longer insulated from the defensive reaction of the host cells.

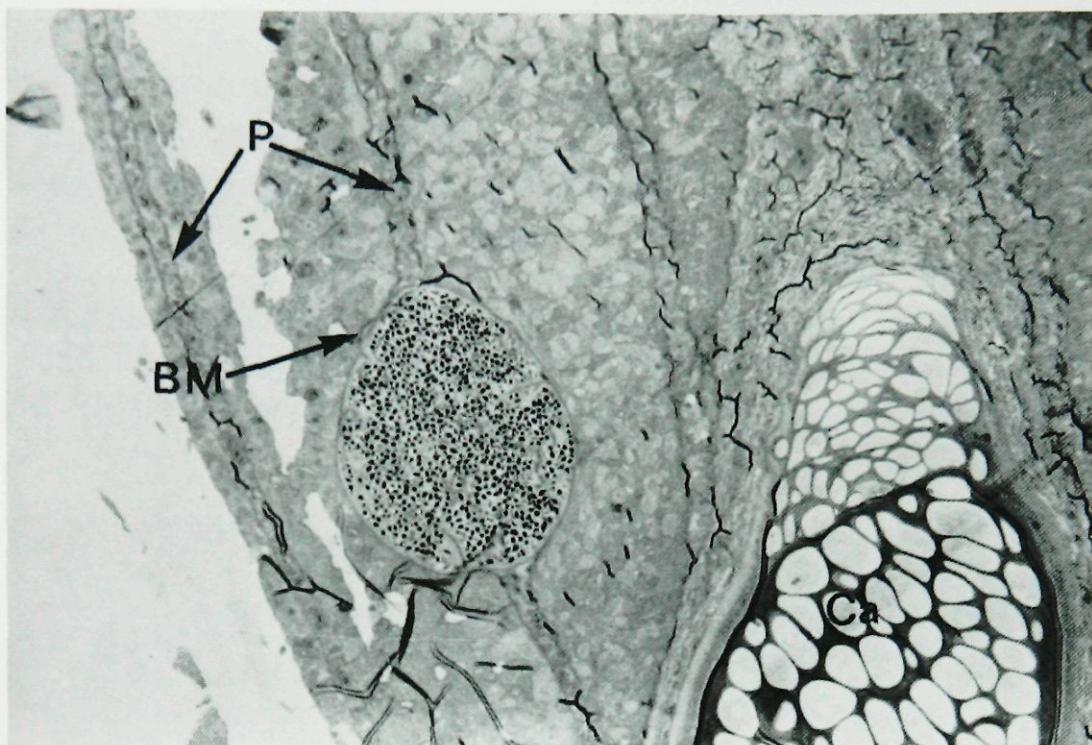


Figure 2. Basal parts of gill lamellae, one with cyst in the pillar system (P) and covered by the basement membrane (BM); Ca, cartilage of gill filament (semi-thin section, toluidine blue, $\times 203$).

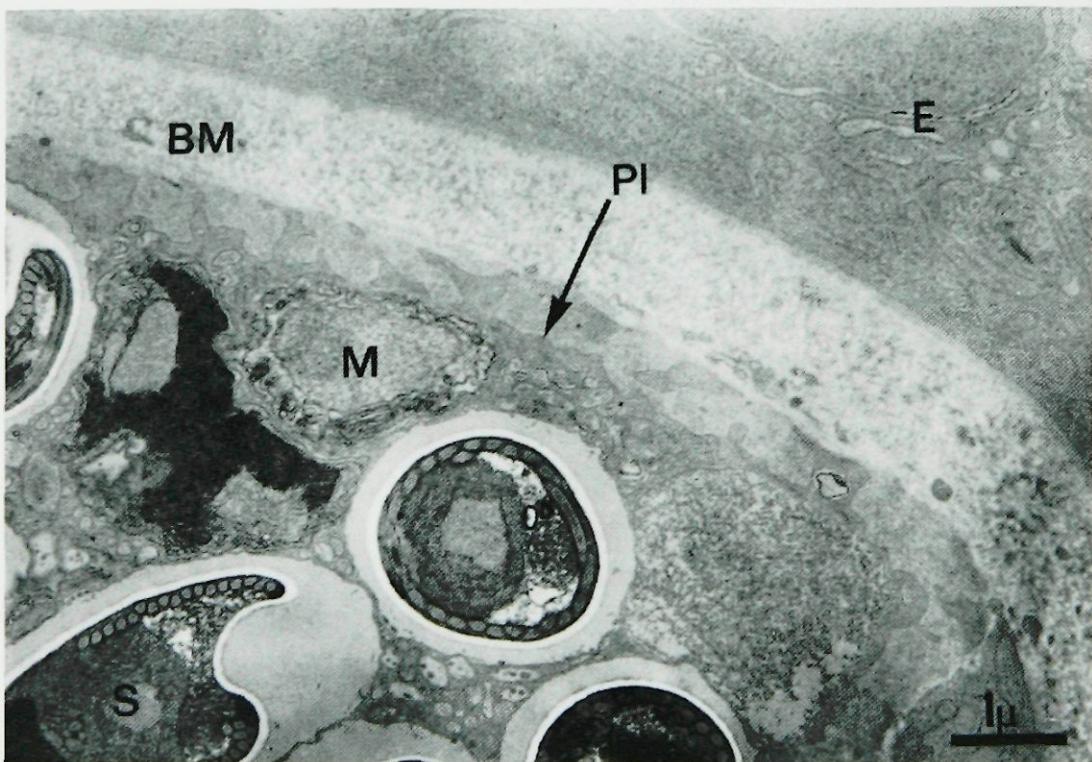


Figure 3. Electron micrograph of margin of same cyst as in Fig. 1, showing structure of cyst and surrounding tissue. E, epithelium of gill lamella; BM, basement membrane of pillar system, serving as a capsule around the cyst or outer layer of cyst wall; Pl, plasmalemma of infected host cell, modified as inner layer of cyst wall; M, meront; S, spore. Scale in this and the following figures represents 1 μm .

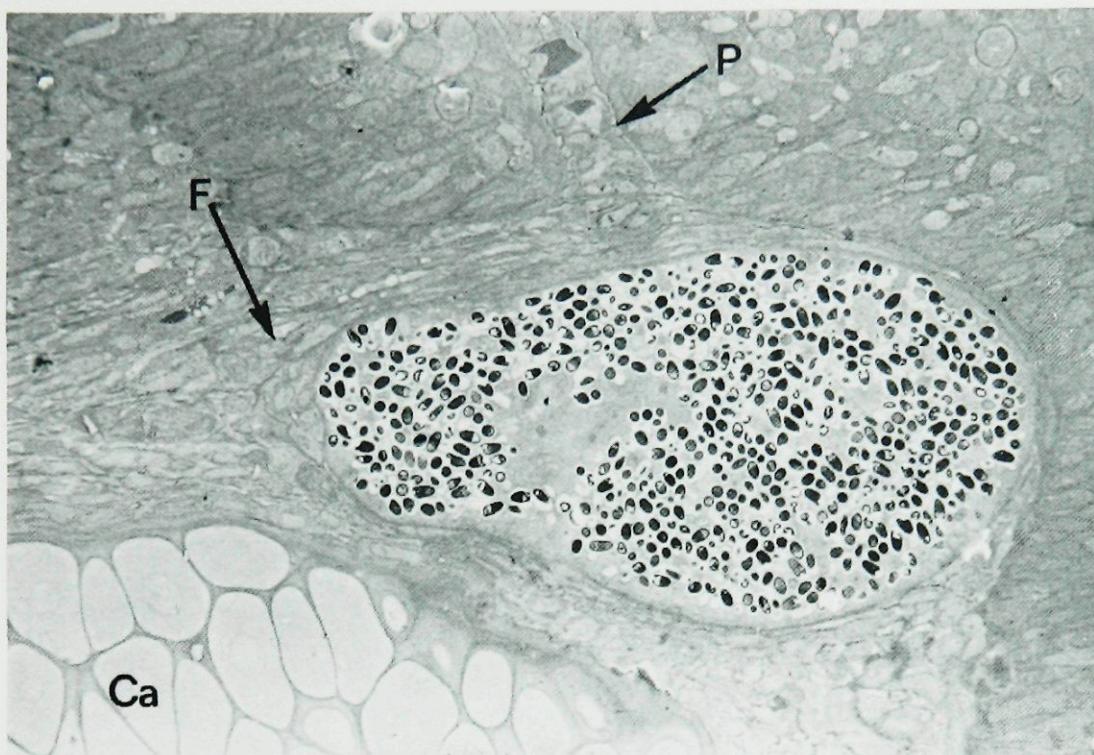


Figure 4. Cyst in connective tissue near base of gill lamella, not clearly in pillar system (P) but encapsulated by fibroblasts (F); Ca, cartilage of gill filament (semi-thin section, toluidine blue, $\times 518$).

The consequence has already been observed by Lom & Laird (1976). Phagocytes invaded the spore mass (Fig. 7) and the spores, either phagocytosed or free, infiltrated the surrounding tissue. At the same time the host tissue reacted by forming fibrous nodules to include the spores (Figs 8–10).

Both nucleus and cytoplasm of the infected host cell (cyst) were hypertrophic and otherwise modified, much as in the *Glugea* cyst. The nucleus (Fig. 11, HN) was highly branched (and possibly fragmented) while there were many vacuoles in the cytoplasm containing tubules and vesicles of endoplasmic reticulum (Figs 3 & 12) and granules.

The parasite

The cysts we observed consisted of two components, host cell and parasite, forming what Chatton (1920) has termed a 'complex xéno-parasitaire' and Weissenberg (1968) a 'xenoma'. It was essentially similar to the *Glugea* cyst, which Weiser (1976) included among the 'neoplastic xenomas'. 'Le complexe ainsi formé', said Chatton, 'est si intime qu'il apparaît morphologiquement et physiologiquement comme un tout indivis, comme un organisme autonome, dont la connaissance du développement seule peut montrer la dualité'. Nevertheless, it is convenient to describe the parasite component separately.

Since the cysts observed were clearly rather far advanced in their development, they contained mostly mature spores. Development stages of the parasite showed no distinct tendency, as in *Glugea*, to be situated progressively from the outer to inner zones of the host cell cytoplasm but were rather well mixed up (Figs 1–4 & 13).

The earliest stages seen, presumed to be meronts, were uninucleate bodies (Figs



Figure 5. Electron micrograph of margin of same cyst as shown in Fig. 4; Pl, the modified plasmalemma of the infected host cell. The cyst, not clearly inside the pillar system, has little if any of the basement membrane covering; instead, it is encapsulated by loosely packed fibroblasts, F.

3 & 13) embedded in the host cell cytoplasm in close association with the host cell mitochondria. Their most conspicuous feature was a series of perinuclear cisternae. Some showed traces of the spindle plaque on the nuclear membrane and the associated polar vesicles (Fig. 13). We could not distinguish meronts that were destined to repeat the merogony from those destined to undergo sporogony.

Sporulation stages observed were always in large parasitophorous vacuoles in the host cell cytoplasm (Figs 11–14). The vacuoles often contained many tubules and superficially resembled pansporoblastic cavities containing tubular 'secretions' from

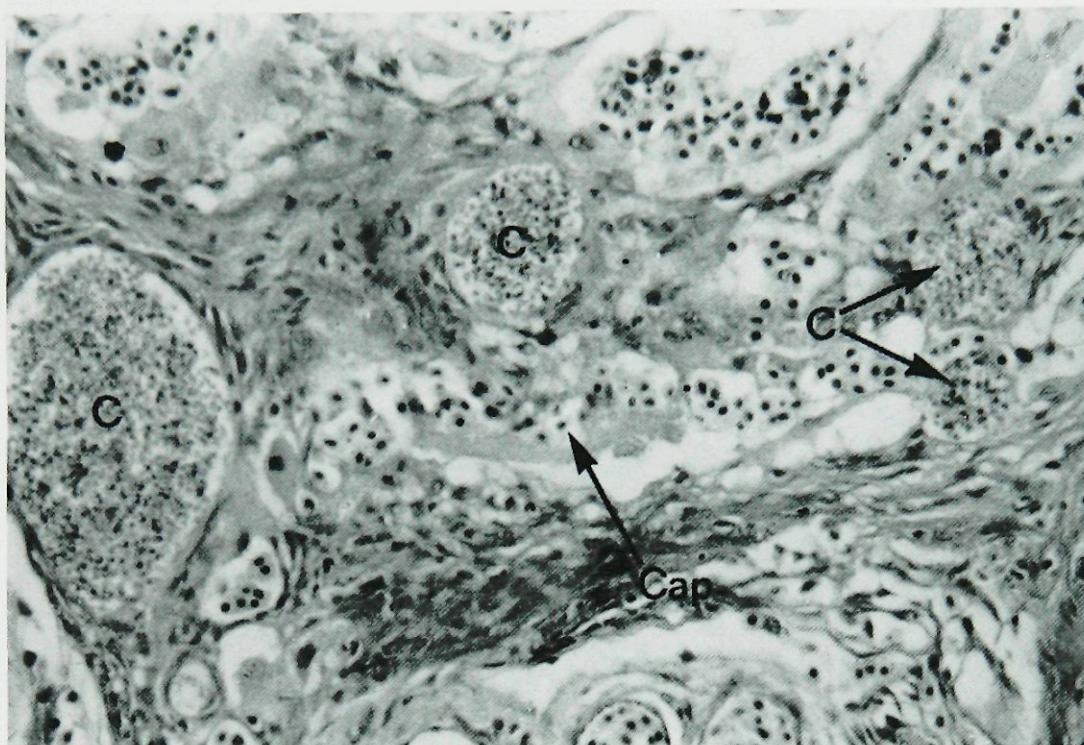


Figure 6. Cysts (C) in pseudobranch near capillary (Cap), one cyst surrounding the capillary. Cysts have fibrous capsules (H&E, $\times 322$).

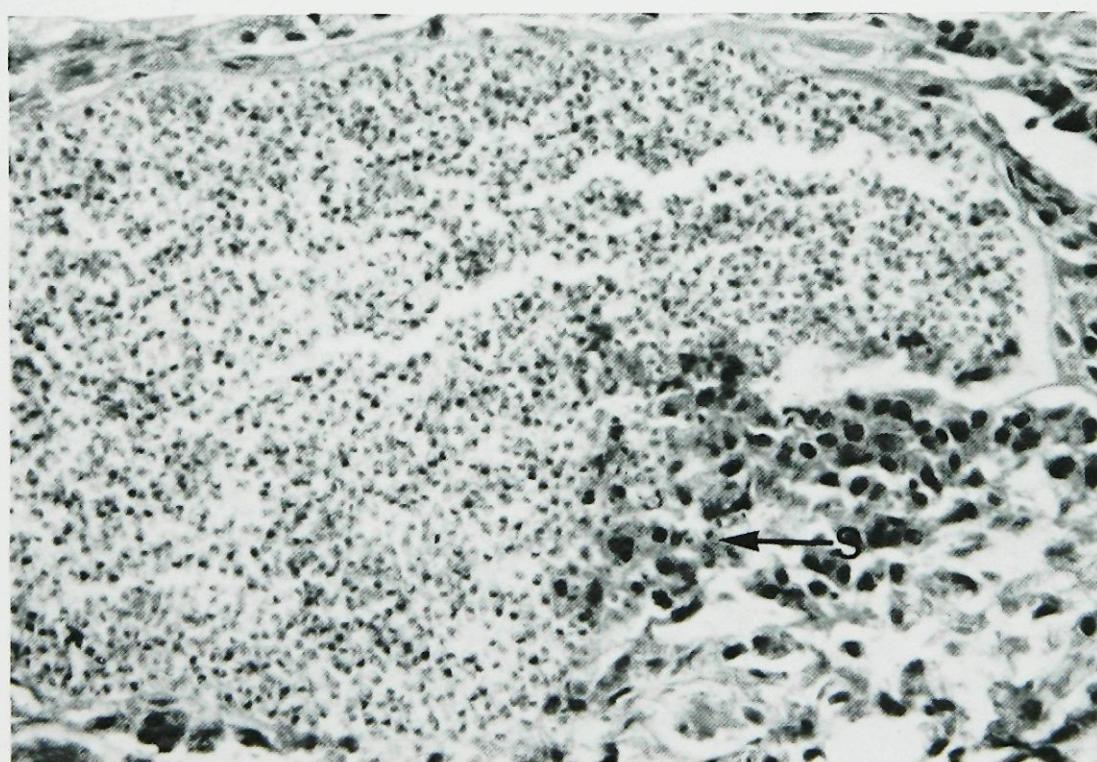


Figure 7. Disintegrating mature cyst in gill filament. Host cells, probably phagocytes, invading cysts and attacking parasites; S, spore (H&E, $\times 518$).



Figure 8. One gill lamella club-shaped due to presence of an old lesion. The cyst wall has been destroyed and the xenoma replaced by fibrous tissue. The proximal part of the pillar system (P) is still intact (H&E, $\times 203$).

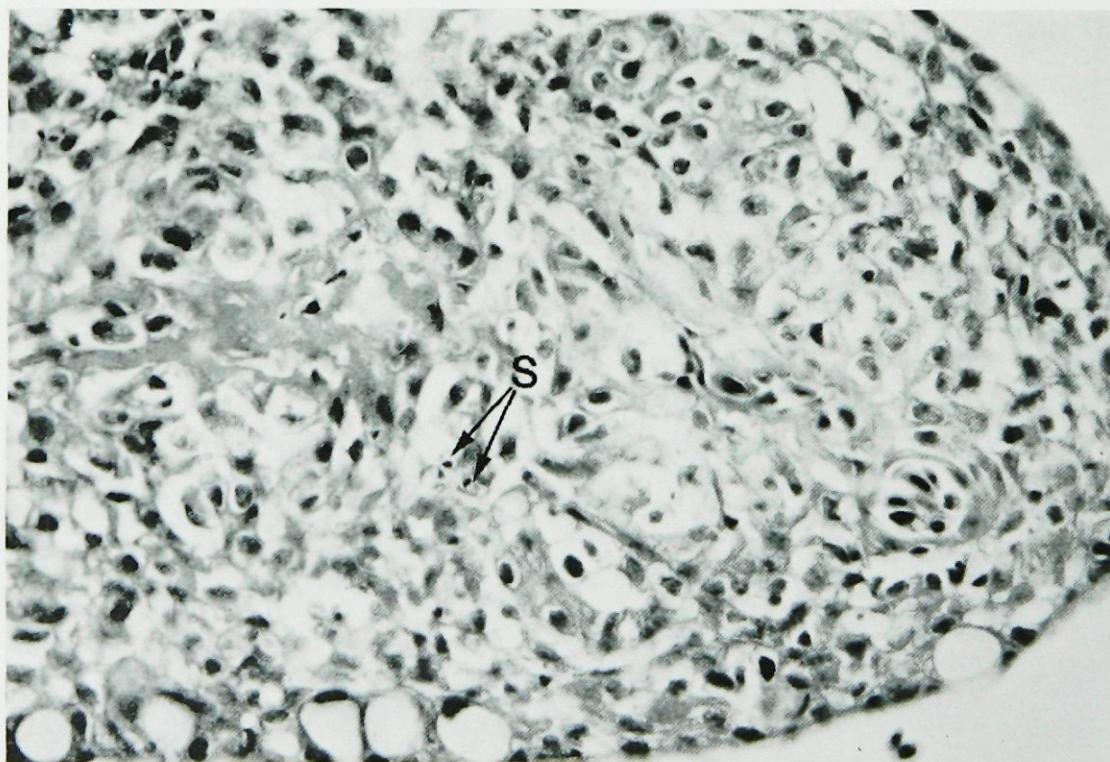


Figure 9. The same section as in Fig. 8, at higher magnification. A few spores (S) remain in the fibrous tissue (H&E, $\times 518$).

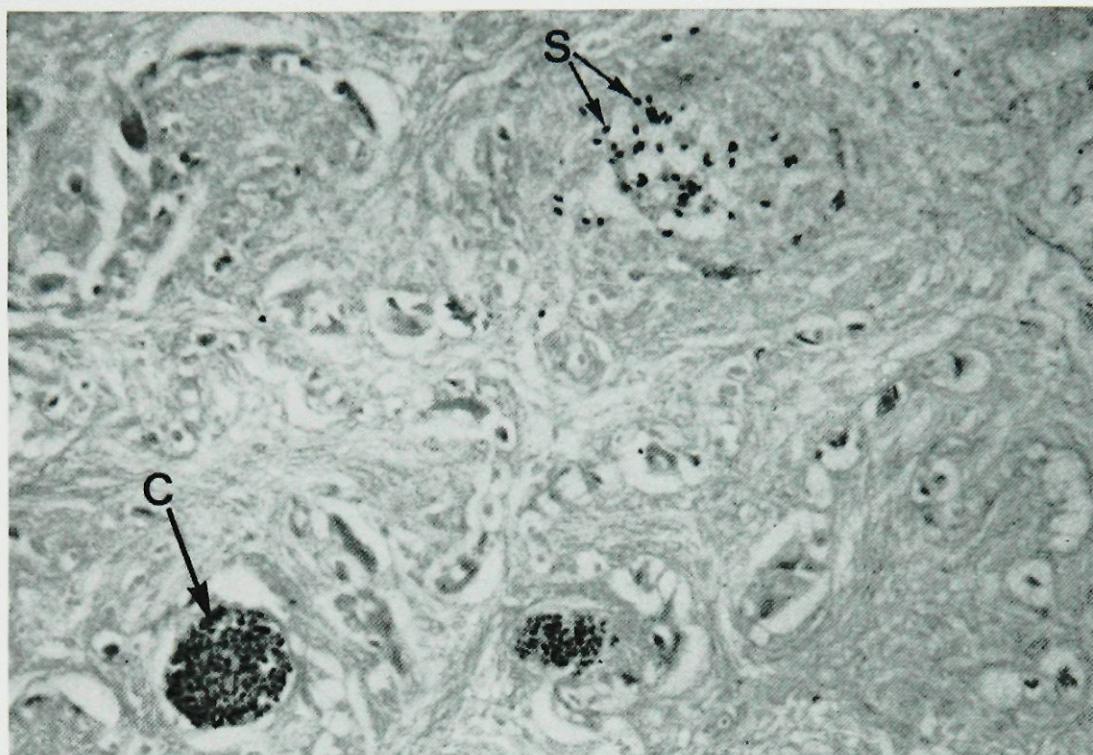


Figure 10. Section of pseudobranch with intact cysts (C) encapsulated by fibroblasts and isolated spores from disintegrated cyst (S) in fibrous nodules (Fontana-Masson, $\times 245$).

the parasites. Their bounding membranes, however, were identical in appearance to the endoplasmic reticulum of the host cell (Fig. 12). There was no evidence that the membranes differentiated from sporogonial plasmodia as do pansporoblastic membranes. The included tubules resembled fragments of endoplasmic reticulum seen in smaller vacuoles throughout the host cell cytoplasm, and sections of the tubules were connected to both the exospore and the vacuolar membranes. The tubules appeared to be most numerous during the middle stages of sporulation (Fig. 13) and they tended to disappear as the spore matured.

Sporogony was apparently a process of binary fission, resulting in 2 uninucleate sporoblasts (Figs 12 & 13) which developed into 2 uninucleate spores. Two spores were sometimes seen in a vacuole (Fig. 13 & 14) but more often they appeared single, presumably due in part to the plane of section but possibly due also to other factors.

The mature spore (Fig. 14) was elongate-ovoid and (in photographs of stained sections) measured roughly $4.2 \times 2.0 \mu\text{m}$. Its structure was like that of most members of the order Microsporida but involved some noteworthy details. Both exospore and endospore were relatively thin and often collapsed (Fig. 14,C) into the posterior vacuole. The polar filament, which originated antero-laterally, was isofilar and consisted of a single coil with about 16–17 turns in a plane perpendicular to the long axis of the spore. The sporoplasm, centrally located, had a single oval nucleus and its cytoplasm was packed with ribosomes. The ribosomes were in linear or circular arrangement, depending on the plane of section. The polaroplast showed the usual two zones, an outer lammate and an inner vesiculate one. The posterior vacuole

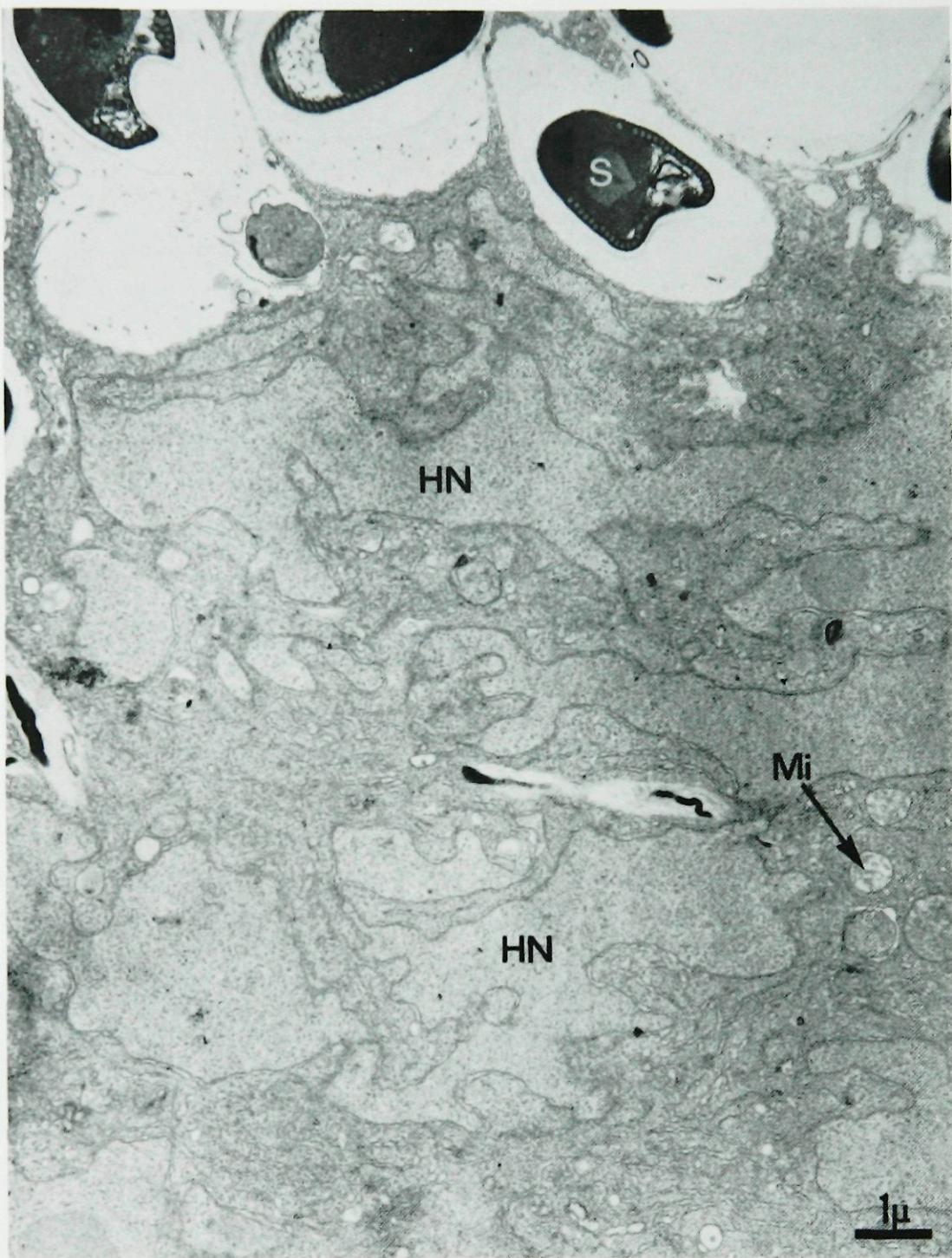


Figure 11. Internal area of same cyst as in Figs 4 & 5, showing highly branched host cell nucleus, HN; Mi, host cell mitochondria; S, spore.

was large, extending to the middle of the spore. The sporoplasm extended into the anterior part of this vacuole producing a conspicuous convexity already pointed out by Lom & Laird (1976) in similar spores found in haddock. Two morphologically different kinds of membranous inclusions were present in the posterior vacuole. One was a dense mass of membranes appearing as concentric circles (Fig. 14, ELP) and the other was a reticulum (Fig. 14, EVP).

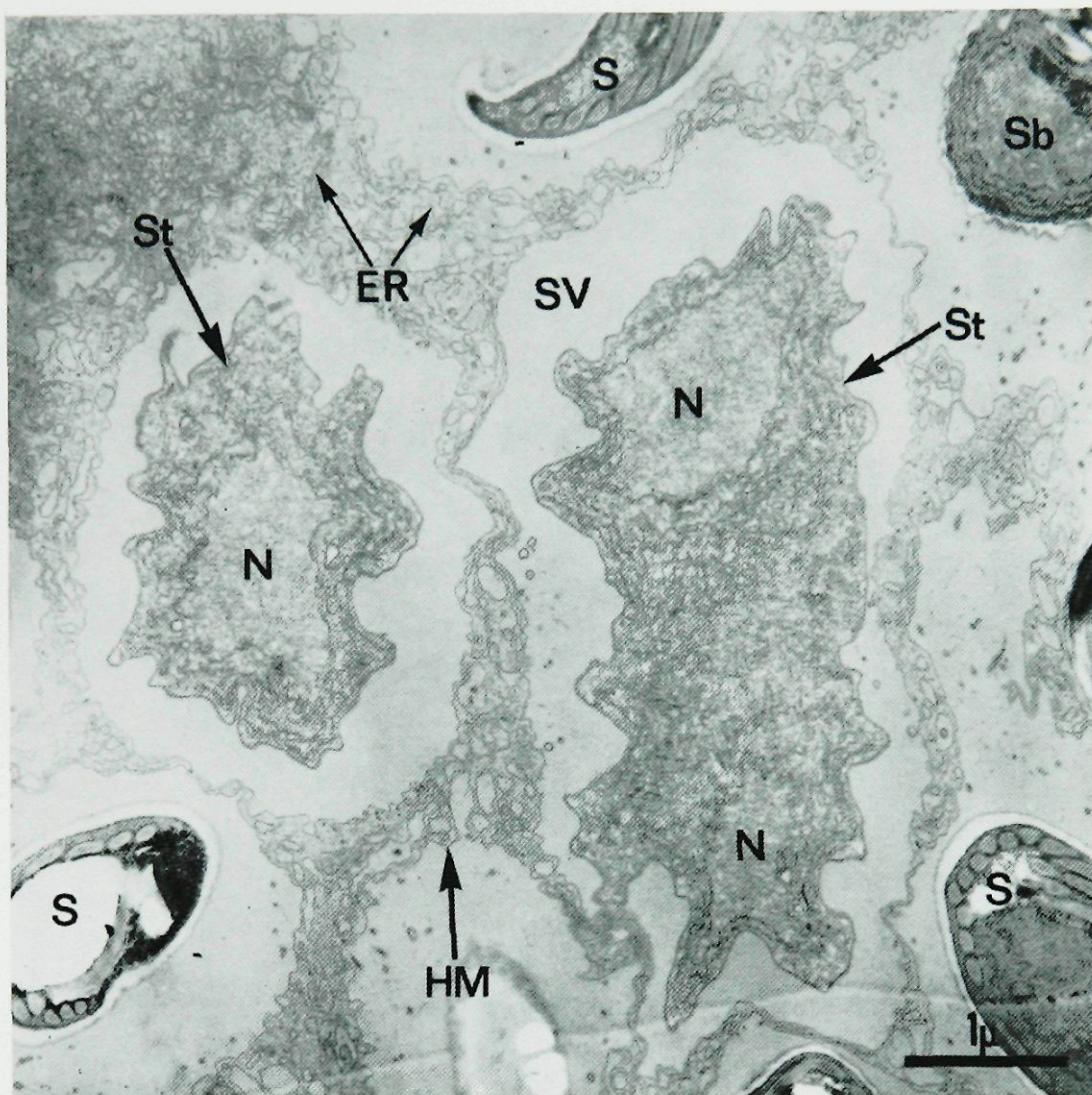


Figure 12. Two sporonts dividing (sporogony) inside vacuoles in host cell cytoplasm, one in cross-section and the other in longitudinal section; St, sporont; N, nucleus of parasite; S, spore; Sb, sporoblast (rather advanced); SV, sporogony (parasitophorous) vacuole; ER, endoplasmic reticulum of host cell; HM, host cell membranes surrounding the vacuole.

Discussion

Infection was found in about 14% of the 91 fish examined, but the true prevalence rate may have been higher. Possibly small cysts were overlooked on the one gill and/or pseudobranch examined from each fish and probably many of the gills not examined were infected.

While no general effect of the infection on the host was detected, definite pathological conditions were observed with the microscope. These were mainly the reactions of the host cells to parasites escaping from ripe cysts. These reactions, no doubt, require expenditure of energy and put some stress upon the host. Transport of spores from old cysts to new sites in the same host probably serves as a mechanism of auto-

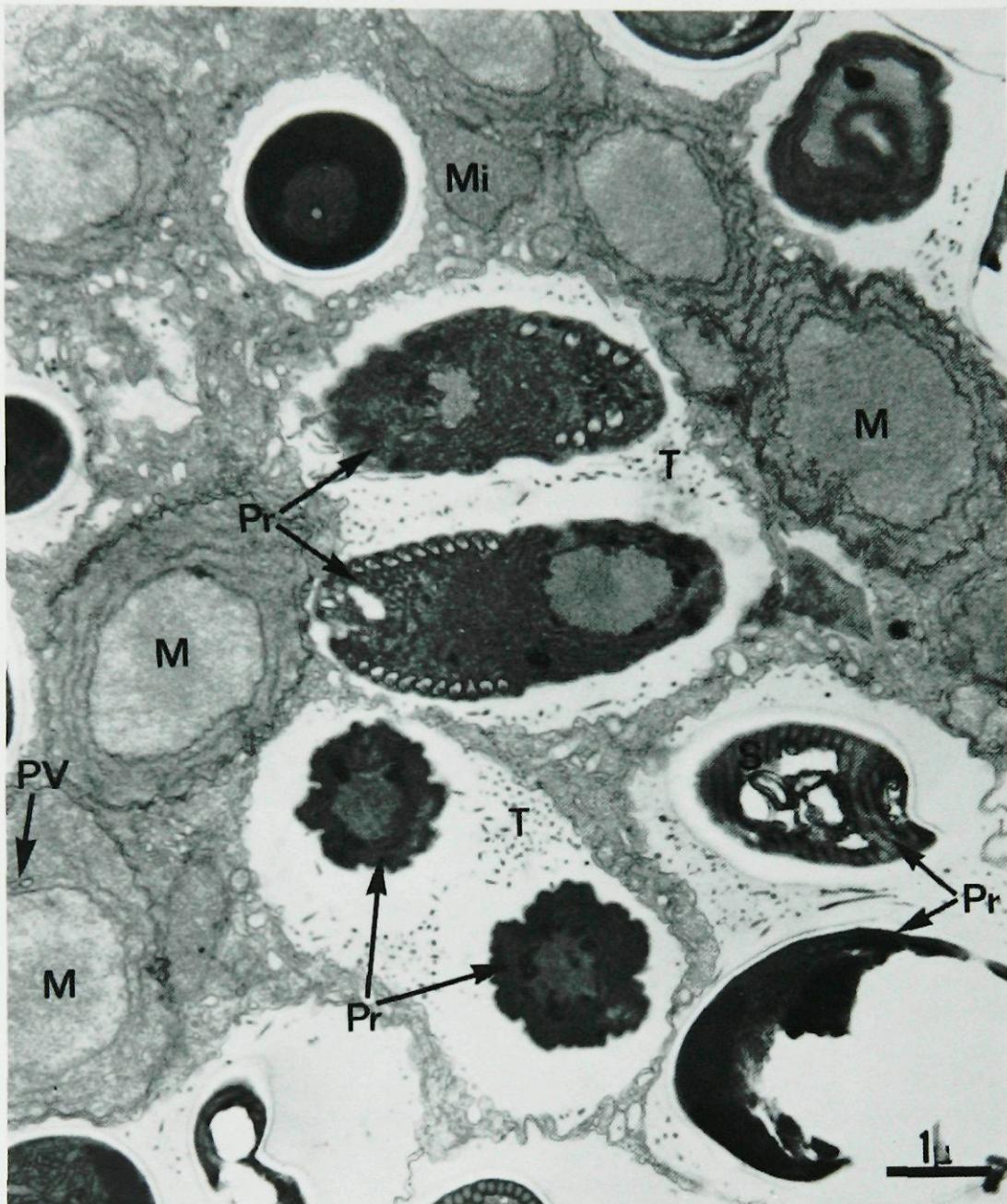


Figure 13. Pairs (Pr) of late sporoblasts or immature spores and mature spores (S) in parasitophorous vacuoles and uninucleate meronts (M) embedded in host cell cytoplasm; Mi, mitochondria of host cell. Tubules (T) are abundant in the sporogony vacuoles at this stage. Some tubule sections are continuous with the host cell membrane that surrounds the vacuole and others are continuous with the exospore. Similar tubules appear throughout this host cell cytoplasm. Polar vesicles (PV) can be seen near the nucleus of one meront.

infection, since this mechanism was rather well documented by Sprague & Hussey (1980) in fish infected with *Ichthyosporidium*. The consequence of auto-infection would be an increase of the parasite burden with an accompanying increase of stress. At the same time, heavy infection of the gills might cause some impairment of the respiratory function. Heavy infection might also impair the function of the pseudo-branch, which is still uncertain but possibly related to the choroid plexus of the eye (Wittenberg & Haedrich 1974). Although the disease caused by this infection is accompanied by an unknown and possibly small amount of stress, this may conceiv-

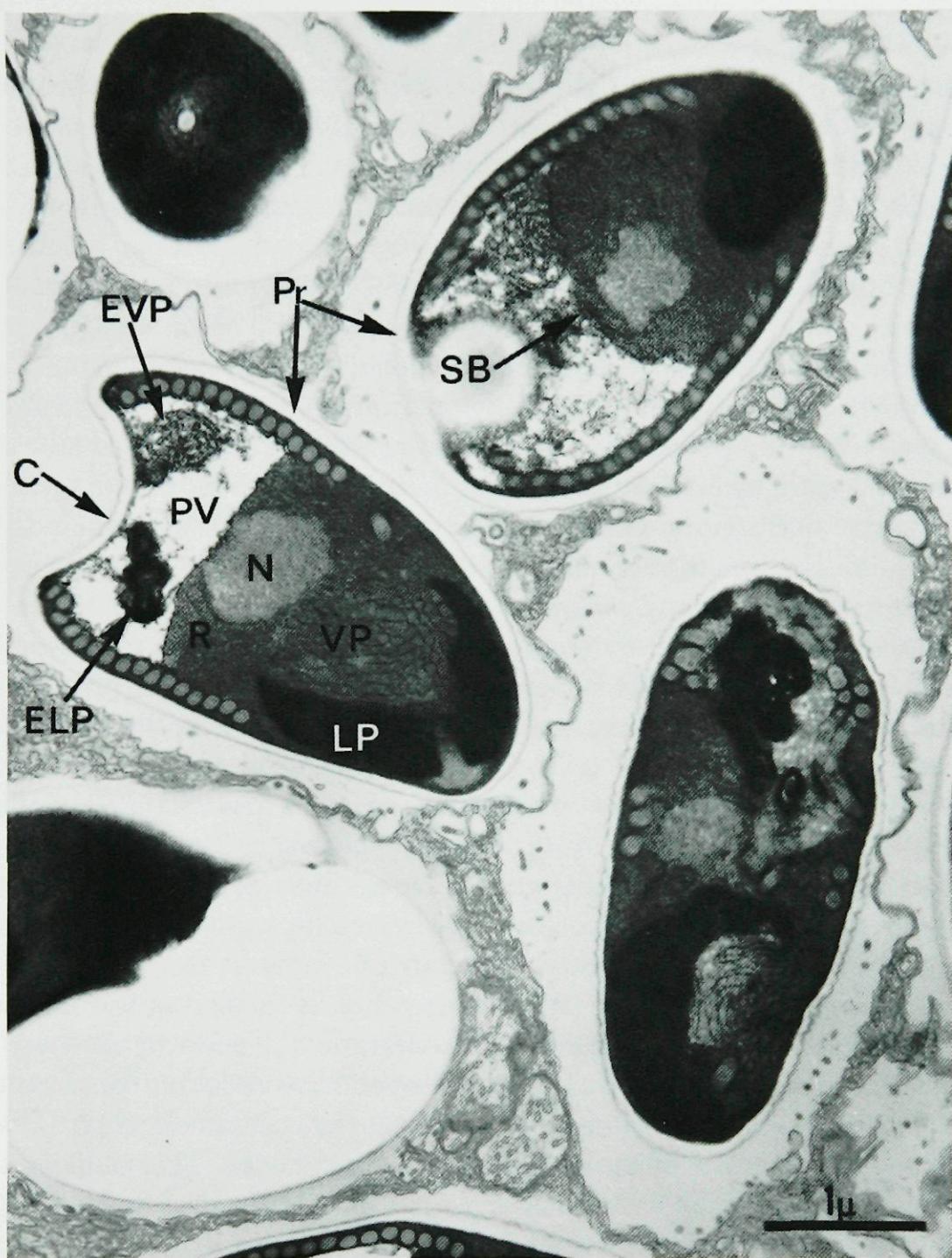


Figure 14. Mature spores in parasitophorous vacuoles. One vacuole contains a pair (Pr) but most appear to contain single spores. One spore (upper left) is rather immature and atypical in arrangement of the polar filament; N, nucleus of sporoplasm; R, ribosomes in cytoplasm of sporoplasm; LP and VP, lammate and vesiculate parts of polaroplast; PV, posterior vacuole; C, portion of spore wall collapsed into posterior vacuole; ELP, concentric layers of membranes believed to be extension of laminated part of polaroplast; EVP, reticulated body believed to be Golgi remnant associated with developing polar filament and vesiculate part of polaroplast. Part of sporoplasm (SB) extends backwards and produces a characteristic convexity in the posterior vacuole.

ably contribute to fish mortality in combination with other adverse factors in the environment.

Disintegration of ripe cysts, while liberating spores into the surrounding host tissue, presumably liberates some also into the water and makes them available for initiating infections in new host individuals.

The parasite, being apansporoblastic, having some developmental stages in parasitophorous vacuoles and being unikaryotic and disporous, has the essential characters of the family Glugeidae Thélohan, 1892. According to Sprague (1980) this family already contains three genera. They are: *Glugea* Thélohan, 1891, parasitic in fish; *Encephalitozoon* Levaditi, Nicolau & Schoen, 1923, in mammals and birds; and *Baculea* Loubes & Akbarieh, 1978, in Cladocera. The present species differs sharply from *Glugea* in lacking plasmodial stages and producing only one or two spores in a vacuole instead of 16 or 32. It differs from *Encephalitozoon* by inducing development of a cell hypertrophy tumour and producing one or two spores in a vacuole instead of several. It is unlike *Baculea* which undergoes both merogony and sporulation in parasitophorous vacuoles and produces cylindrical spores. We, therefore, conclude that the present species represents a new genus in the family Glugeidae.

Since the evidence strongly suggests that sporogony was a process of binary fission it may be a little surprising that only one spore was usually seen within a parasitophorous vacuole instead of two. Presumably, the plane of section sometimes revealed only one spore when two were actually present in a vacuole but this may not explain the fact that the majority of the spores appeared to be isolated. We think it is probable that the sporogonial division was sometimes completed and the division products (sporoblasts) isolated before the vacuole was formed. Consequently, developing and mature spores frequently occurred singly in the vacuoles.

The origin and function of the tubular elements in the sporophorous vacuole are matters of interest. Their resemblance to membrane elements in the host cell cytoplasm and their connection both to the exposure and the vacuolar membrane suggests that the parasite maintained by means of tubules a direct connection with the host cell-cytoplasm after formation of the parasitophorous vacuole. This apparent relationship further suggests that the tubules may have functioned to transport materials across the vacuolar space between the host cell cytoplasm and the developing parasite. Similar tubules were included by Vavra (1976) and Vavra & Sprague (1976) among the various 'secretions' of the microsporidia, some of which were granular secretions. We suggest that such tubules, whether in parasitophorous vacuoles in the host cell cytoplasm or in pansporoblastic cavities, are not comparable with the true granular secretions and should not be categorized as secretions.

Evidence from previous studies indicates that the two types of membranes included in the posterior vacuole of the spore were parts of the polaroplast-polar filament complex and derived from the Golgi apparatus. The reticulate membrane is like that which, as Vavra (1976) pointed out, has frequently been seen in association with the developing polar filament. Johnston, Vernick & Sprague (1978) found a similar reticulate stage in the developing polaroplast. Vernick, Sprague & Krause (1977) have referred to this type of membrane as the 'reticulate phase of the Golgi apparatus'

or, simply, 'Golgi reticulum', the English equivalent of the 'reticulum golgien' of Takizawa, Vivier & Petiprez (1975). The laminated membranes appearing as concentric circles in the posterior vacuole were extensions of the laminated outer part of the polaroplast. Similar membranes were illustrated but not mentioned by Johnston *et al.* (1978, Fig. 13) and, as Vivares & Sprague (1979) pointed out, by Sprague & Vernick (1969, Fig. 4). The inclusion most common in the posterior vacuole of microsporidia, called 'secretion granule' by Vernick *et al.* (1977) and many other names by various authors, was not clearly demonstrated in our material but presumably occurs in this species.

All the microsporidia previously reported in gills of the Gadidae were generally quite similar and were identified as *Nosema branchiale* Nemeczek, 1911, originally found in haddock. As Kabata (1959) pointed out, however, the different reports were not in agreement on spore size. Spores fell into two distinct size groups, *N. branchiale* as originally described ($6.3 \times 3.5 \mu\text{m}$) being in the large group. Spores of the present species, being about $4.2 \times 2.0 \mu\text{m}$, are in the smaller group. Its possession of smaller spores and its occurrence in a different host provide a questionable basis for regarding it as new. In spite of some reservation, we propose to treat it as new because it is the type of a new genus. By so doing we can avoid the possibility of designating a misidentified nominal species as type, with the consequent need to refer the case to the Commission (Stoll, Dollfus, Forest, Riley, Sabrosky, Wright & Melville 1961, Art. 70).

We acknowledge the fact that the apparent occurrence of large and small categories of spores is a matter needing confirmation. Possibly as Sprague (1977) pointed out, it is merely a reflection of different techniques used. If the difference is real it could have more than one explanation; it could signify the occurrence of a single dimorphic species rather than two or more distinct species.

Although we do not have enough information to form a firm opinion regarding the specific identities of all microsporidia (whether with small or large spores) reported in gills of haddock and cod, there is no reason to doubt that they are all congeneric. Therefore, we propose establishing a new genus, in the family Glugeidae, to include the microsporidia thus far reported in the gills of the Gadidae. We suggest that, provisionally, those with large spores be regarded as the species described by Nemeczek and those with small spores be regarded as identical with the one we are describing. Microsporidia in gills of the Salmonidae will be considered in a later paper.

Loma n.g.

Apansporoblastic.

Unikaryotic throughout life cycle (provisional for early merogony).

Without plasmodial stages (provisional for merogony).

Merogony while parasite embedded in host cell cytoplasm.

Sporogony disporoblastic, immediately preceding or accompanying differentiation of a parasitophorous vacuole.

Sporoblasts transform into spores within vacuole, typically one but sometimes two in a vacuole.

Parasitophorous vacuole contains tubules.

Elicits formation of xenoparasitic complex of the cell-hypertrophy tumour type.

Developmental stages not in progression from outer to inner zones of xenoma but mixed (provisional).

Sporoplasm projects as convexity into posterior vacuole of spore (provisional).

Intracellular parasites of gills of fish.

Type species *L. morhua* n. sp.

The genus includes also *L. branchialis* Nemeczek, 1911, n. comb.

Derivation of name: after Dr Jiří Lom, noted Czechoslovakian protozoologist and specialist in protozoan parasites of fish.

Loma morhua n. sp., *type species*

With characters of the genus.

Spore elongate oval, $4.2 \times 2.0 \mu\text{m}$ (mean of 50 measurements from light micrograph of resin section stained with toluidine blue).

Polar filament isofilar, with single coil turned about 16–17 times in plane perpendicular to long axis of spore.

Type host *Gadus morhua* L.

Distinguishing characters: spores smaller than those of the other species *L. branchialis*. Type host different from that of *L. branchialis*.

Type locality: approaches to Halifax Harbour, Nova Scotia, Canada.

The specific name *morhua* is the Latin name for cod.

Disposition of type materials

Two slides in International Protozoan Type Slide Collection, Division of Echinoderms and Lower Invertebrates, National Museum of Natural History, Smithsonian Institution, Washington D.C. 20560, U.S.A., nos USNM 245556 (72-2) and USNM 245557 (2300).

Two slides in National Museum of Natural Sciences, Ottawa, Canada. Catalogue No. N.M.C.I.C.(P) 1980-43.

Other slides in Histology Unit of Population Biochemistry and Histology Group, Fisheries and Environmental Sciences Division, Resource Branch, Department of Fisheries and Oceans, Box 550, Halifax, Nova Scotia, Canada B3J 2S7.

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