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Ultrastructure of *Myxidium trachinorum* sp. nov. from the gallbladder of the lesser weever fish *Echiichthys vipera*

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Abstract *Myxidium trachinorum* sp. nov. is described from the gallbladder of the lesser weever fish *Echiichthys vipera*. Pseudoplasmodia attach themselves to the gallbladder epithelium by filose processes, which are inserted between host cells. Pseudoplasmodia undergo endogenous cell formation at the secondary and tertiary levels. In the proliferative cycle, primary and endogenous cells are packed with digestive vacuoles formed by phagocytosis. In the sporogonic cycle the pseudoplasmodium becomes a pericyte enclosing two secondary cells (lacking digestive vacuoles) in a vacuole. These give rise to five cells each – two valvogenic, two capsulogenic and a binucleate sporoplasm, which mature into spores. Comparison of the disporic *M. trachinorum* with polysporic species of *Myxidium* revealed significant differences in plasmodial ultrastructure, especially their attachments to host cells, surface characteristics and mode of nutrition, and in formation of generative cells. These suggest that the genus *Myxidium* may require revision.

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Introduction

Over 150 species of *Myxidium* Bütchli, 1882 have been reported from marine and freshwater fish, several having been named since the genus was reviewed by Jayasri and Hoffman (1982), and Lom and Dyková (1992) reported 149 species. Ultrastructure studies conducted on several of these species, particularly *M. lieberkuehni* (Lom and de Puytorac 1965; Lom et al. 1989), *M. gasterostei* (Uspenskaya 1969), *M. giardi* (Hulbert et al. 1977; Paperna et al. 1987; Azevedo et al. 1989), *M. rhodei* (Dyková et al. 1987) and *M. gadi* (Feist 1995), have demonstrated biological differences in the nature of the plasmodia, including surface characteristics, size and nuclear complement, some being multinucleate and others, uninucleate. The mode of attachment of plasmodia to host epithelia and the mode of formation of the generative cells, which give rise to spores, are also variable. Multinucleate plasmodia are polysporic, producing many pansporoblasts, typically by enclosure of a generative cell in a pericyte. Pansporoblasts are typically disporic. Small uninucleate plasmodia (pseudoplasmodia) are also typically disporic, but the pseudoplasmodium itself acts as the pericyte. These differences may be of a fundamental nature, indicating that the genus *Myxidium* is a heterogeneous collection of species. We examined the host parasite interface and sporogenesis of a species of *Myxidium* from the gallbladder of lesser weever fish *Echiichthys vipera* and report particularly on its ultrastructure. We compared this *Myxidium* sp. with others, using characters of the plasmodia, the size and shape of spores and the presence or absence of striations on spore valves. We also looked at its hosts and geographical distribution and conclude that it does not conform to existing descriptions. We propose that it be named *M. trachinorum* sp. nov.

Materials and methods

Weever fish were collected by bottom trawling off Plymouth and Weymouth in the South-west and South of England. Fish were

killed by immersion in MS222 and dissected. Gallbladders were examined for the presence of infection, then transferred as quickly as possible to Karnovsky's fixative in 0.1 *M* cacodylate buffer. Specimens were washed in buffer, post-fixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol and embedded in Agar 100 resin (Agar Scientific). Spores were measured in seawater and in Giemsa-stained smears.

Results

Light microscopy

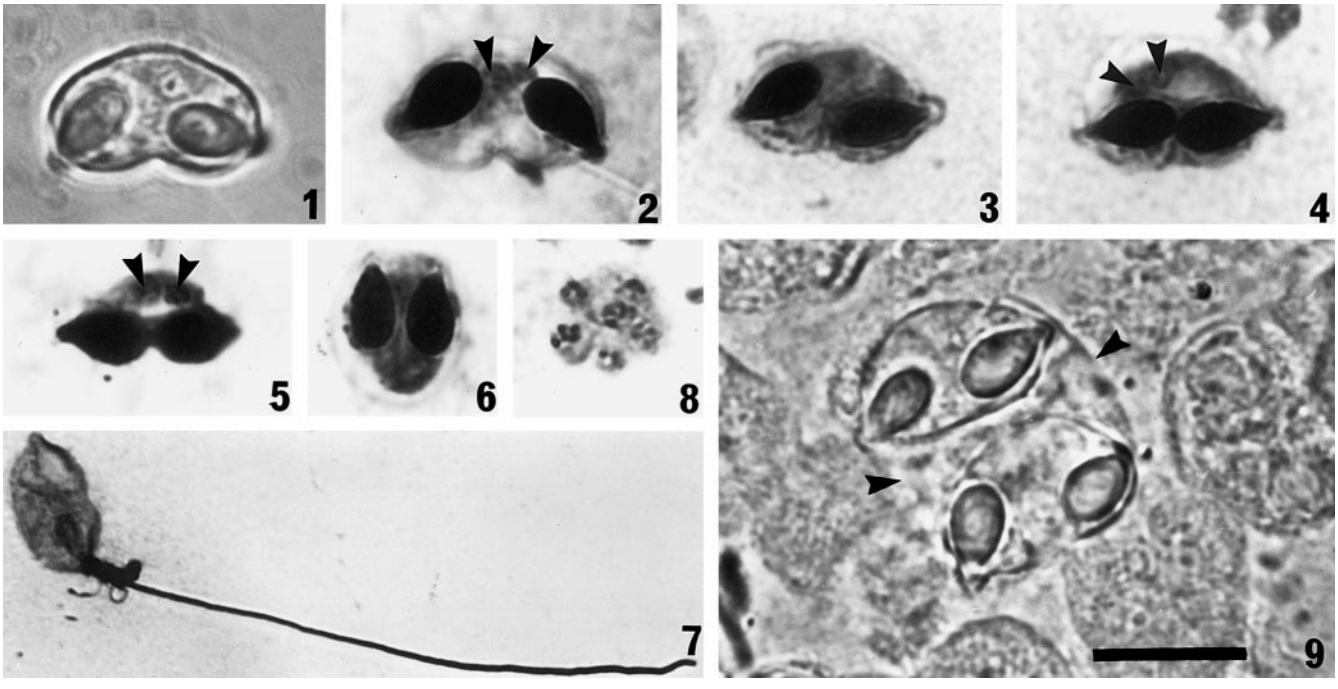
Spores are elongate, measuring 17.2 ± 0.29 × 8.8 ± 0.32 μm fresh and 15.4 ± 0.15 × 8.8 ± 0.3 μm in stained smears (means and standard errors). One surface is convex and the other usually has a prominent kink in the middle (Fig. 1). The positions of the two polar capsules are variable; sometimes they are angled such that both point slightly towards the concave surface (Fig. 2) or such that one opens towards the convex surface and the other, towards the concave surface (Fig. 3), and sometimes the capsules are arranged in a straight line with the exits directly opposite one another (Figs. 4, 5). In the latter arrangement the capsules, which are about equal in size and measure 6.8 ± 0.15 × 3.7 ± 0.08 μm, almost touch one another in the centre of the spore, the openings being slightly subterminal. The two nuclei of the sporoplasm lie in the central region towards the convex side and the sporoplasm itself usually occupies much of the space between and around the capsules. Occasionally, ovoid spores were seen in which the two polar capsules faced in the same direction (Fig. 6). These were considered to be anomalous or immature. Extruded polar filaments (Fig. 7) measured 55–82 μm (mean 65.3 μm). Rounded plasmodia with several nuclei representing stages in the development of spores measured from 5 to 10 μm in diameter before signs of polar capsule formation (Fig. 8). Disporous plasmodia were rarely seen in stained smears but were readily observable in fresh squashes of gallbladder tissue (Fig. 9).

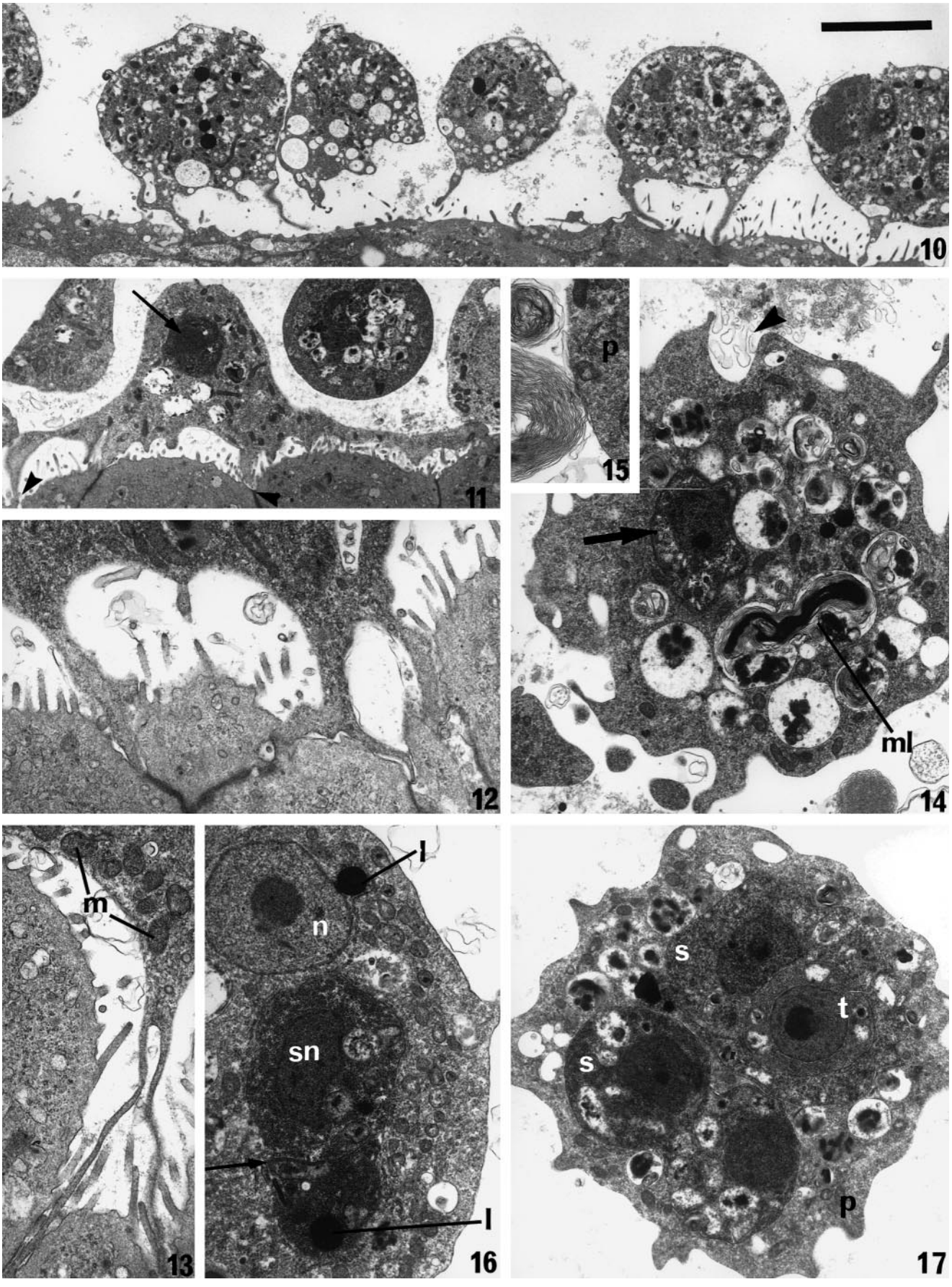
Electron microscopy

Plasmodial growth and early development

All plasmodia were uninucleate except during formation of secondary cells and are therefore referred to as pseudoplasmodia. The gallbladder epithelial cells bore a sparse array of microvilli and in well-fixed preparations showed a normal complement of organelles. In one heavily infected piece of tissue the epithelial cells were highly vacuolated (Fig. 10), but as cytoplasmic fixation, especially membrane fixation, of parasites was also poor, it was thought that this particular piece of tissue had been damaged by delayed fixation. In general there was one myxozoan pseudoplasmodium per epithelial cell in the plane of section, each with two or three tapering adhesion processes, which insinuated themselves into the junction between two cells and separated the cell

Figs. 1–9 *Myxidium trachinorum*. Light micrographs. The *bar* in Fig. 9 (10 μm) applies to all figures
Fig. 1 Unstained spore
Figs. 2–6 Giemsa-stained spores showing different orientations of polar capsules. *Arrowheads* point to nuclei of sporoplasm
Fig. 7 Spore showing one of the polar filaments fully extruded
Fig. 8 Plasmodium with six nuclei (two valvogenic, two capsulogenic and two belonging to the sporoplasm)
Fig. 9 Compressed gallbladder epithelium of a weever fish, showing a pericyte (*arrowheads*) enclosing two spores with two polar capsules each

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Figs. 10–17 *M. trachinorum*. Electron micrographs of stages in the gallbladder of *Echiichthys vipera*. The *bar* in Fig. 10 applies to all figures

Fig. 10 Several pseudoplasmodia in contact with host epithelial cells. Note that each plasmodium spans about one cell and is attached by insertion of fine adhesion processes between host cells. *Bar* 5.0 μm

Fig. 11 Pseudoplasmodium with a secondary cell (*arrow*), which has spread out to span several epithelial cells, but attachments remain characteristically at host cell junctions (*arrowheads*). *Bar* 4.0 μm

Fig. 12 Attachment of a pseudoplasmodium to several cell junctions, showing that the adhesion organelles can be branched. *Bar* 1.6 μm

Fig. 13 Attachment of a plasmodium by an adhesion process of which the two branches cause superficial separation of epithelial cells. The tips of the branches do not penetrate beyond tight junctions (*m* mitochondria). *Bar* 1.25 μm

Fig. 14 Free floating plasmodium with a secondary cell (*arrow*) and abundant phagocytic vacuoles containing a multilaminate body (*ml*) in the process of digestion and smaller aggregates of membranes with partially digested products. Surface invagination (*arrowhead*) is suggestive of phagocytosis of membranes from the bile. *Bar* 2.1 μm

Fig. 15 Concentric membranes in bile close to the surface of a pseudoplasmodium (*p*). *Bar* 1.6 μm

Fig. 16 Part of a proliferative plasmodium showing a primary cell nucleus (*n*) and a secondary cell, which has divided (*arrow*) within the endogeny vacuole membrane (*sn* secondary cell nucleus). Lipid vacuoles (*l*) are also present in primary and secondary cells. *Bar* 1.6 μm

Fig. 17 Primary pseudoplasmodium (*p*) with secondary cells (*s*), one of which has produced a tertiary cell (*t*). All belong to the proliferative phase as indicated by the presence of phagocytic vacuoles of decreasing size from the primary to the tertiary cell. *Bar* 2.2 μm

membranes to a depth beneath which tight junctions linked the two host cells (Figs. 11, 12). The adhesion processes were sometimes undivided (Fig. 10) and sometimes branched (Figs. 12, 13), but the tips of the branches were always inserted between cells. Occasionally a pseudoplasmodium stretched over several epithelial cells (Fig. 11) and several blunt processes extended towards the epithelial surface, but almost invariably it was only those associated with the cell junctions that actually made contact. The microvilli of the host cells remained intact in the space between the pseudoplasmodial surface and the host cell and some of them touched the pseudoplasmodia, but there was no cytoplasmic continuity.

Young pseudoplasmodia were uninucleate and had a population of elongate or rounded mitochondria that were especially abundant at the periphery (Fig. 13). The nucleus had a prominent nucleolus (Fig. 16). There were also vacuoles of various sizes containing electron-dense globules within a fibrillar matrix or multilaminate bodies or other membranous debris. Sometimes these vacuoles were of such abundance that they obscured other developmental processes within the plasmodium (Fig. 14). Invaginations at the surface containing extraneous membrane were highly suggestive of phagocytosis (Fig. 14), and concentric membranes similar to the multilaminate bodies in the vacuoles were present in the bile at the surface of the pseudoplasmodia (Fig. 15).

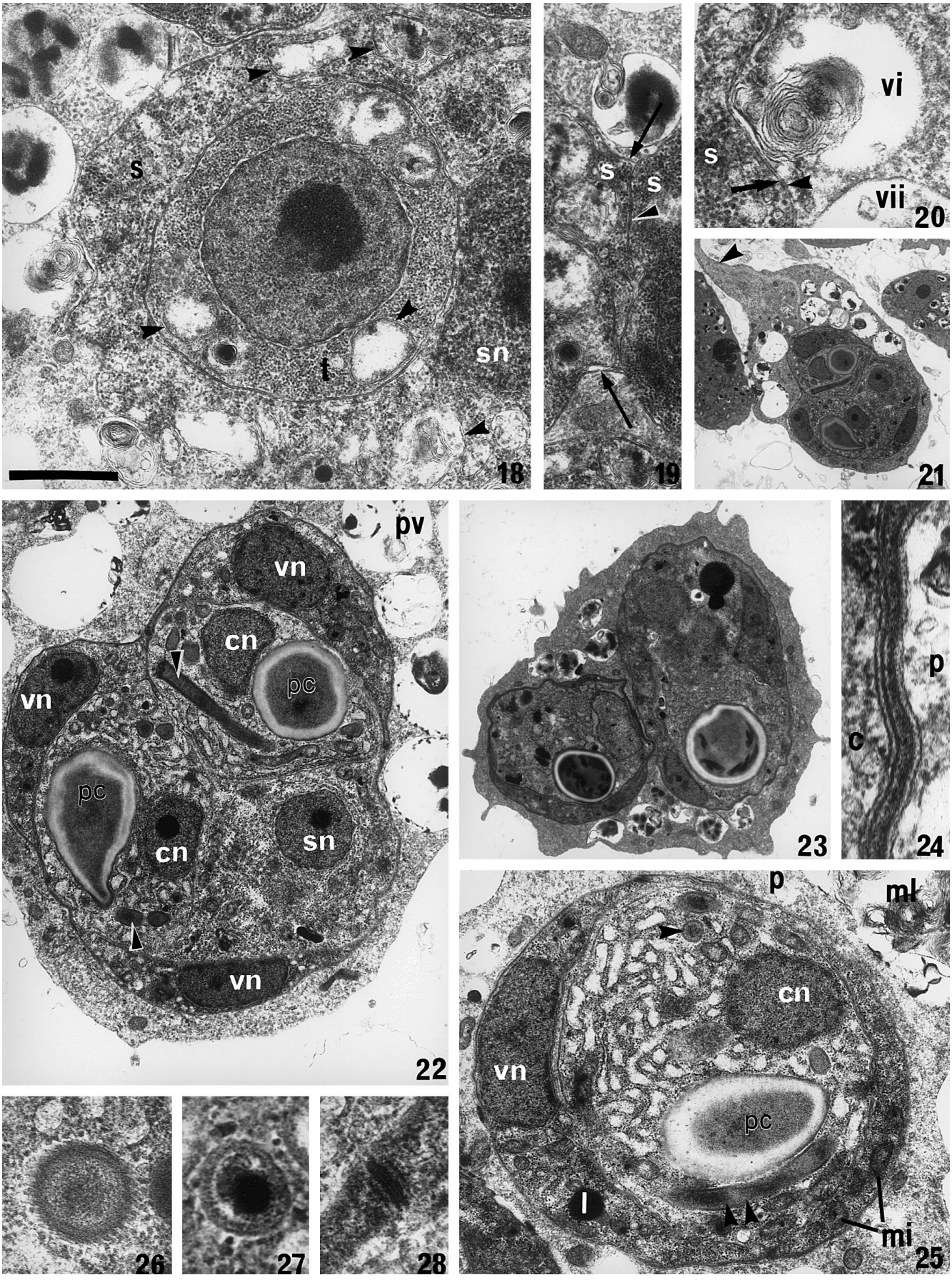
After nuclear division, while still attached to the epithelium, the pseudoplasmodia formed secondary cells by enclosure of a nucleus and some of the cytoplasm within a vacuolar membrane (Fig. 11). In some sections one secondary cell and the nucleus of the primary cell were visible in one plane (Fig. 16); in other sections, two secondary cells were visible (Fig. 17). The secondary cells could divide within a common vacuole (Figs. 16, 19) or, in turn, could undergo endogeny to achieve a cell-with-cell organization at the tertiary level (Fig. 18). The secondary and tertiary cells also contained vacuoles with dense globular or membranous contents (Fig. 18). These vacuoles were smaller than those in the primary cells and were enclosed by two membranes, whereas those in the primary cells had only one membrane. Some images suggested that there was breakdown of the membrane of the digestive vacuole in the primary cell and phagocytosis of the contents through the two membranes at the surface of the secondary cell (Fig. 20). After growth, the secondary cells were probably liberated from the primary cells to form new pseudoplasmodia, which would attach to epithelial cells and repeat the endogenous formation of new cells.

Plasmodial development into spores

Sporogony was initiated in pseudoplasmodia that remained attached to the epithelium (Fig. 21) and continued in free-floating pseudoplasmodia. In both cases the pseudoplasmodia continued to grow to accommodate the development of a spore from each of two secondary cells (disporic sporogony; Fig. 23). These secondary cells differed from those of the proliferative phase that produce tertiary cells, as their cytoplasm, containing densely packed ribosomes, was completely free of the large digestive vacuoles. Some sections showed large osmiophilic inclusions, probably lipid globules (Figs. 23, 25, 29, 32). The two sporogonic secondary cells gave rise to five cells each, which differentiated typically as two valvogenic cells, two capsulogenic cells and a sporoplasm (Figs. 22, 29), all within a close-fitting vacuolar membrane (Figs. 22, 25). The sporoplasm may have been uninucleate at first but was binucleate in mature spores. All nuclei contained a prominent nucleolus.

The cell types were easily distinguished. Capsulogenic cells (Figs. 22, 25) were packed with expanded rough endoplasmic reticulum, and there were a few mitochondria, some vacuoles containing lipid globules and a polar capsule with its external tube. The capsule had the normal structure of granular contents within a wall, of which the density increased towards the periphery. The external tube was constructed of a granular core surrounded by a more lucent layer, then a dense layer. Microtubules were not seen surrounding the capsular formations in transverse or longitudinal section, but in one slightly oblique section of the external tube, microtubules were visible at the periphery (Fig. 26).

Valvogenic cells had very little endoplasmic reticulum but abundant mitochondria in the region of the nucleus (Figs. 22, 25). These cells narrowed sharply away from the nucleus to form a thin layer, covering the capsu-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Figs. 18–28 *M. trachinorum*. Electron micrographs of proliferative and sporogonic plasmodia. The *bar* in Fig. 18 applies to all figures

Fig. 18 Part of Fig. 17, enlarged to show a secondary cell (*s*) and its nucleus (*sn*) enclosing a nucleated tertiary cell (*t*). Note the presence of two membranes around the secondary and tertiary phagocytic vacuoles (*arrowheads*). *Bar* 0.86 μm

Fig. 19 Part of Fig. 17, enlarged to show the division plane (*arrowhead*) between two secondary cells (*s*) within the closely fitting endogeny vacuole membrane (*arrows*). *Bar* 0.86 μm

Fig. 20 Part of Fig. 17, enlarged to show a digestive vacuole (*vi*) of the primary pseudoplasmodium-containing membranes in a position for phagocytosis through the endogeny vacuole membrane (*arrowhead*) and plasma membrane (*arrow*) of the secondary cell (*s*). The digestive vacuole (*vi*) itself appears to have lost its membrane (compare with the adjacent vacuole, (*vii*). *Bar* 0.46 μm

Fig. 21 Sporogonic pseudoplasmodium in which spore development is well advanced, continuing to show the attachment process (*arrowhead*) that holds it to the gallbladder epithelium. *Bar* 7.2 μm

Fig. 22 Enlargement of part of Fig. 21. The spore is anomalous in having three nucleated valvogenic cells (*vn*) but shows the two capsulogenic cells with nuclei (*cn*), polar capsules (*pc*) and external tubes (*arrowheads*). One of the nuclei of the sporoplasm (*sn*) is also visible. Note the complete absence of digestive vacuoles in the sporogonic cells and their abundance in the primary pseudoplasmodium (*pv*). *Bar* 2.2 μm

Fig. 23 A sporogonic plasmodium showing disporous sporogony. *Bar* 3.2 μm

Fig. 24 Part of a valvogenic cell in contact with the primary pseudoplasmodium (*p*) and capsulogenic cell (*c*) after it has spread out to enclose the capsulogenic cells and sporoplasm. *Bar* 0.3 μm

Fig. 25 A capsulogenic cell filled with expanded endoplasmic reticulum, showing its nucleus (*cn*), polar capsule (*pc*) and external tube in longitudinal section (*double arrowhead*) and in transverse section (*arrowhead*). The capsulogenic cell is completely surrounded by the valvogenic cell, which displays its nucleus (*vn*) mitochondria (*mi*) and lipid (*l*). Note the multilamellar structure (*ml*) in the surrounding bile in a position to be phagocytosed by the primary pseudoplasmodium (*p*). *Bar* 1.4 μm

Fig. 26 Slightly oblique section of the external tube of a polar capsule, showing surrounding microtubules. *Bar* 0.2 μm

Fig. 27 Double-walled structures with dense contents. Several of these were present in a sporoplasm that showed signs of degeneration. *Bar* 0.16 μm

Fig. 28 Junction of two valvogenic cells at the time when these slim cells had just made contact. *Bar* 0.32 μm

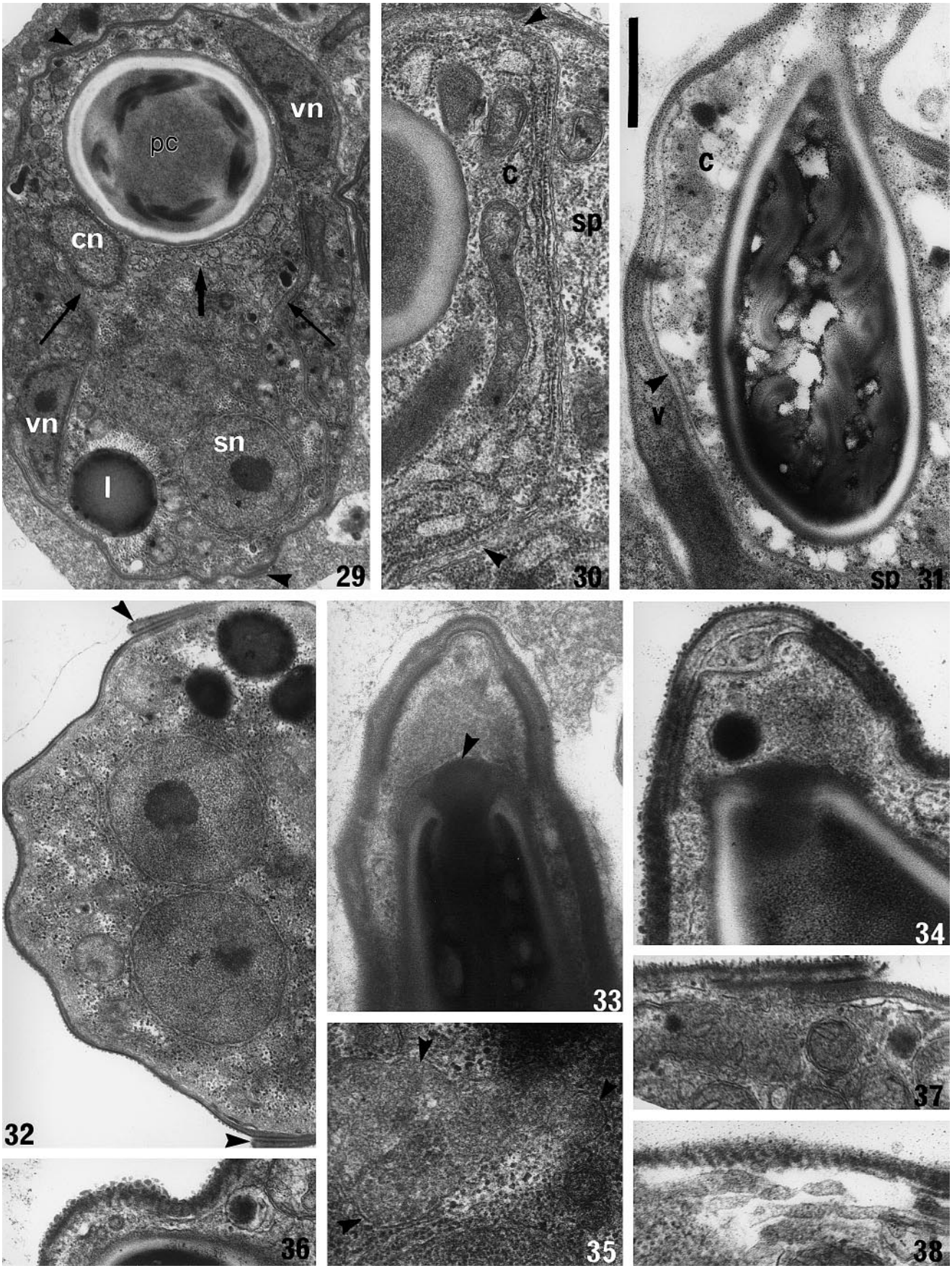
logenic cells and sporoplasm (Fig. 24). Initially the valvogenic cells met at a tight junction (Fig. 28), then overlapped for about 0.5 μm (Figs. 29, 32, 37).

Sporoplasms (Figs. 22, 32) showed one or two nuclei, depending on the plane of section; prominent mitochondria; and, frequently, some large lipid globules. The body of the sporoplasm with the nuclei occupied the centre of the spore but put out fine cytoplasmic extensions between and around the capsulogenic cells (Figs. 29–31). As the capsulogenic cell cytoplasm degenerated the arms of the sporoplasm spread further around the capsules and, in some sections, appeared to occupy all the space around the capsules. In several sections a large vesicular organelle or inclusion was present, taking the form of masses of small vesicles (possibly densely packed, coiled tubules) within a single membrane (Fig. 35). In one mature spore the sporoplasm contained spherical bodies with a thick double wall and amorphous contents (Fig. 27). Mitochondria in this cell were acristate and the nuclear envelope was inapparent; hence, the spherical bodies were considered to be abnormal.

In mature spores the capsulogenic cell cytoplasm was degenerate; the external tube had retracted into the capsule, forming several coils of the polar filament in a matrix contained by a bilayered wall. The polar filament appeared to form a loose coil down one side of the capsule and up the other side, and even down again, rather than forming a single coil close to the wall of the capsule (Fig. 31). The opening of the capsule was closed by a plug continuous with the outer layer of the capsule wall and with the polar filament (Fig. 33). The opening was situated subterminally in the spore. The valvogenic cell cytoplasm had disappeared, leaving the spore valves, the external sides of which were raised as minute papillae (Fig. 36) about 20 nm high, which in oblique sections of the wall could be seen as regular rows (Fig. 38). The papillae were present on the outer valve, where there was overlap, but not on the inner valve (Fig. 37). The plasmodium with intact organelles and food vacuoles persisted during formation of the spore walls but finally disintegrated to liberate the mature spores. The suture composed of the overlapping valves was variable in position, sometimes lying centrally in the region of the sporoplasm (Fig. 32) and sometimes being close to the poles in the region of the polar capsules (Fig. 29).

Discussion

Few of the many known species of *Myxidium* have been studied at the ultrastructural level, but available data indicate considerable diversity in their organization. Most of the species studied by electron microscopy have large multinucleate plasmodia, which produce many spores that differentiate at different times during the growth of the plasmodia. Some are histozoic, i.e. embedded securely in host tissue. The position of the coelozoic species necessitates that they develop a means of preventing premature exit with body fluids. Thus, the plasmodia of *M. gasterostei* in the gallbladder of sticklebacks, *Gasterosteus aculeatus* and *Pungitius pungitius*, are attached by a single tapering process, inserted between host cells (Uspenskaya 1969). Coelozoic plasmodia of *M. giardi* in the urinary bladder of eels, *Anguilla anguilla*, are attached by branching rhizoids, which are deeply indented into the epithelial cells but do not interrupt the cell membranes (Paperna et al. 1987). The attachment organelles of *M. gadi* to the gallbladder of pollack, *Gadus pollachius*, and saithe, *Gadus virens*, are broad extensions that are flattened against the epithelial cells, making a zone of contact similar to a gap junction (Feist 1995). The entire surface of the coelozoic plasmodia of *M. rhodei* located in Bowman's capsules of the kidney of roach, *Rutilus rutilus*, is folded to form regularly spaced ridges, but these do not make contact with the host epithelial cells. The attachment organelles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Figs. 29–36 Sporogonic stages of *M. trachinorum*. The *bar* in Fig. 31 applies to all figures

Fig. 29 Immature spore within a primary pseudoplasmodium, showing the overlap of valvogenic cells (*arrowheads*), valvogenic cell nuclei (*vn*), capsulogenic cell nucleus (*cn*), polar capsule (*pc*), sporoplasm nucleus (*sn*) and large lipid globule (*l*) in the sporoplasm. *Fine arrows* point to extensions of the sporoplasm that are beginning to surround the capsulogenic cell. The *short arrow* indicates the boundary between the sporoplasm and the capsulogenic cell. *Bar* 1.5 μm

Fig. 30 Junction of the sporoplasm (*sp*) and the capsulogenic cell (*c*), showing "arms" of sporoplasm (*arrowheads*) beginning to encompass the capsulogenic cell. *Bar* 0.7 μm

Fig. 31 An almost mature polar capsule with a coiled polar filament within a degenerating capsulogenic cell (*c*). An "arm" (*arrowhead*) of the sporoplasm (*sp*) has almost completely encircled the capsulogenic cell. The valvogenic cell (*v*) is also degenerating. *Bar* 0.9 μm

Fig. 32 Part of the sporoplasm, showing two nuclei and lipid globules. The spore valves are fully formed with surface papillae and overlap each other (*arrowheads*). *Bar* 1.0 μm

Fig. 33 Extremity of a mature spore, showing a subterminal polar capsule, with the plug in the opening (*arrowhead*) being continuous with the layers of the wall. *Bar* 0.5 μm

Fig. 34 Extremity of a mature spore cut obliquely to the opening of the polar capsule. The material filling the space in front of and around the polar capsule is the sporoplasm with a lipid globule. *Bar* 0.4 μm

Fig. 35 An organelle of unknown function in the sporoplasm. It is membrane-bound (*arrowheads*) and contains closely packed vesicles or tubules. *Bar* 0.5 μm

Fig. 36 View of the spore wall, showing the wall covered with papillae. *Bar* 0.6 μm

Fig. 37 Overlap of spore wall valves, showing papillae on the outer side of the outer valve and their absence within the overlap. *Bar* 0.4 μm

Fig. 38 Tangential section of the spore wall, showing the arrangement of papillae in rows. *Bar* 0.4 μm

that most closely resemble those of *M. trachinorum* are those of the young plasmodia of *M. lieberkuehni* in the urinary bladder of pike, *Esox lucius*. These have several fine, tapering processes inserted between host cells (S.W. Feist, personal communication). However, the large multinucleate mature plasmodia of this species have only one attachment process, rather like that of *M. gasterostei*, with the main part of the body floating in the urine (Lom and de Puytorac 1965).

The attachments of *Zschokkella mugilis* pseudoplasmodia to the gallbladder epithelium of several species of mullet are also similar to those of *M. trachinorum* (Sitjà-Bobadilla and Alvarez-Pellitero 1993), but some of the larger plasmodia of *Z. mugilis* can span as many as five cells, with attachments being inserted between each cell. The genus *Zschokkella* is similar to *Myxidium*, being distinguished only by having ellipsoidal spores with nearly spherical polar capsules, both opening on one side, as opposed to fusiform spores with pointed ends and pyriform polar capsules typically opening in opposite directions. As there is considerable overlap in these characters, a reassignment of some species may become necessary when further ultrastructural and molecular data are available.

Different modes of nutrition have been observed in *Myxidium* spp. The large multinucleate plasmodia usually develop a microvillus border, presumably to increase the absorptive surface, and at the base of the microvilli, pinocytotic activity occurs to a greater or lesser extent (Lom and de Puytorac 1965; Uspenskaya 1969). The long, retractable processes terminating in rhyzoids extending between *M. giardi* and host cells are not equivalent to microvilli (Paperna et al. 1987), but there is prolific pinocytotic vesicle formation at the plasmodial surface between the processes. In *M. gadi* the surface away from the host epithelial cells is covered with microvilli, again suggesting an increase in absorptive surface (Feist 1995). Phagocytic activity at the parasite surface close to host cells was observed in *M. lieberkuehni*, involving ingestion of amorphous, flocculent material. However, to our knowledge there has been no previous demonstration of such abundant digestive vacuole formation as was seen in *M. trachinorum* nor of the transfer of such vacuoles to secondary cells. The bile was packed with flocculent material and membranes in groups or in concentric bundles. The irregular outlines of many plasmodia, the partial enclosure of membranous material from the bile (Fig. 14) and the presence within vacuoles of closely packed concentric membranes similar to those observed at the surface (Fig. 15) provided strong evidence for nutrition by phagocytosis. Each vacuole in a primary cell is enclosed by a single membrane, but those in secondary cells are surrounded by two membranes. This indicated that selected vacuoles, mainly a smaller population, had been phagocytosed by invagination at the secondary cell surface. It would be necessary for one of the membranes to be lost during this process, and there was some indication that the membrane of the food vacuole is the one that is lost (Fig. 20). Some vacuoles containing parallel or concentric membranes have been observed in Myxozoa, e.g. the myelinic figures in *Henneguya psorospermica* (Lom and de Puytorac 1965) and *Z. mugilis* (Sitjà-Bobadilla and Alvarez-Pellitero 1993), but their role in nutrition has not been discussed.

With an abundant food supply from the bile being available to *M. trachinorum*, there would be little need to deplete the host cells for this purpose, and, indeed, the gallbladder epithelium appeared normal. We conclude that the function of the tapering processes pushing between the epithelial cells simply involves attachment to prevent the developing plasmodia from being carried out in the bile.

Two distinct cycles of development, proliferative and sporogonic, were observed in the pseudoplasmodia of *M. trachinorum*. In the proliferative cycle, each pseudoplasmodium produces two secondary cells by endogeny and these, in turn, could produce tertiary cells. After division of the primary cell nucleus the secondary cells are probably formed by envelopment of a nucleus and some cytoplasm by endoplasmic reticulum (er), the inner membrane becoming the plasma membrane of the secondary cell and the outer er membrane remaining as a vacuolar membrane. Division of a secondary cell within the vacuolar membrane was observed, implying that

division of the vacuolar membrane follows to produce the two separate cells, unless the secondary cells are released by breakdown of the primary cell. This is the most likely mode of formation of pairs of secondary cells, as has been found by Sitjà-Bobadilla and Alvarez-Pellitero (1993) for *Z. mugilis*. However, it could not be determined unequivocally that pairs of secondary cells are not also formed by separate enclosures within primary cells. Possibly both methods are employed by the pseudoplasmodium. Although a primary cell nucleus could not always be seen, it is probably retained to maintain pseudoplasmodial integrity during growth of the secondary cells. Release of the secondary cells, their attachment to host epithelium as new primary cells and repetition of the process would lead to the heavy infections observed.

The second cycle in which pseudoplasmodia of *M. trachinorum* are involved is sporogonic. The pseudoplasmodium becomes a pericyte, within which each of two secondary cells differentiates further to form the five cells (two valvogenic, two capsulogenic and one sporoplasm) that form the spore (disporic). This corresponds to the enclosure of generative cells by pericytes in the species that have multinucleate plasmodia, such as *M. lieberkuehni* (Lom and de Puytorac 1965) and *M. rhodei* (Dyková et al. 1987), in which the pansporoblasts, which are repeatedly formed by association of pericytes and generative cells, give rise to two spores each (disporic pansporoblasts). However, multinucleate species do not always form their spores in this way. Pericyte formation was never observed by Hulbert et al. (1977) in *M. zealandicum* (= *M. giardi*) in *Anguilla rostrata*, although enveloped cells of *M. giardi* were seen in *A. anguilla* by Azevedo et al. (1989). Also, in *M. gadi* the precursor cells, destined to become a spore, were more commonly formed by clustering of generative cells within the plasmodium than by division within a pericyte (Feist 1995). In *M. trachinorum*, only the primary cell in the sporogonic cycle contains digestive vacuoles, possibly because these large structures would interfere with the specialised functions of the sets of sporogonic cells. Nutrients for the growth of the sporogonic cells might be obtained by transport across the vacuolar and plasma membranes from the primary cells. Development of the polar capsules and spore valves followed the usual pattern for Myxozoa, but it is noteworthy that the spore valves lack ridges and are covered by rows of minute papillae and that the valves overlap at the suture, which was seen in variable positions.

There are several points of interest about the binucleate sporoplasms. They contain large lipid globules and organelles containing densely packed small vesicles or tubules, but the function of the latter is unknown. Organelles resembling sporoplasmosomes were not seen, though such organelles are present in *M. gadi* in the form of membrane-bound, rod-shaped or ring forms with homogeneous contents and in *M. giardi* as organelles consisting of two membranes around a core of dense material with a peripheral lucent crescent (Azevedo et al. 1989). As in *M. gadi*, the sporoplasm produces "arms" that almost surround the capsulogenic cells during their maturation, and the sporoplasm appears to fill the spore cavity once the capsulogenic cells have regressed.

None of the named species of *Myxidium* has a combination of the characters of *M. trachinorum* listed below. Some are polysporic and, for the few species for which ultrastructural data are available, the attachment processes to host cells are different. Spores of named species are either too small or too large or, if the size range is comparable, they differ in shape (bluntly rounded ends, twisted, or markedly elongate), in the presence of striations on the spore wall or in the size and position of the polar capsules (usually much smaller). *Echiichthys vipera* has not previously been reported as a host. Comparison of plasmodial and sporogonic characteristics of several species of *Myxidium* has revealed differences that may be important at the generic level. Future molecular-level studies of *Myxidium* spp. should clarify whether the genus needs revision.

Taxonomic summary

Myxidium trachinorum sp. nov.

Type host: *Echiichthys vipera*, the lesser weever fish. The specific name is derived from the family name of the weevers, Trachinidae.

Type locality: South coast of England, trawled from waters near Plymouth and Weymouth.

Site of infection: the gallbladder, attached to epithelium or free in bile.

Plasmodia: small presporogonic forms measuring up to 10 μm in diameter that undergo endogenous secondary and tertiary cell formation. They are packed with digestive vacuoles, in many cases with multimembrane contents. Sporogonic plasmodia, measuring up to 20 μm in diameter, are typically disporous.

Spores: broadly elongate with pointed ends, 17.2 × 8.8 μm (fresh), with one surface convex and the other with a prominent kink, unstriated; polar capsules are equal and flask-shaped, opening subterminally directly opposite one another, or with both slightly inclined towards the concave surface, or with one inclined towards the concave surface and the other, towards the convex surface. Measuring 6.8 × 3.7 μm (stained), they are often large enough to meet in the centre of the spore. Valves, overlapping centrally or close to the polar capsules, are unstriated but covered with rows of minute papillae. The polar filament is up to 82 μm long when extruded.

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