FOLIA PARASITOLOGICA 43: 189–202, 1996

Notes on the ultrastructure of two myxosporean (Myxozoa) species, *Zschokkella pleomorpha* and *Ortholinea fluviatilis*

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Key words: Myxosporea, ultrastructure, *Zschokkella*, *Ortholinea*

Abstract. Two myxosporean species, *Zschokkella pleomorpha* Lom et Dyková, 1995 (Zp) and *Ortholinea fluviatilis* Lom et Dyková, 1995 (Of) from the kidney of *Tetraodon fluviatilis* were studied by transmission electron microscope. Coelozoic sporogonic plasmodia of both species use pseudopodia-like projections for attachment to the epithelial cells of renal tubules. These projections either attach to host microvilli forming an interface reminiscent of septate junction (Zp) or are embedded into the epithelial cell surface (Of) or are inserted into gaps between epithelial cells (Zp, Of). Zp produces spores only by direct division of generative cells while in Of pansporoblasts prevail over direct division of generative cells. Sporogonic plasmodia of Zp greatly differ in size and in the variety of cytoplasmic constituents. A special feature in capsulogenesis is a transient envelope encasing the capsular primordium; there are fine fibres on the surface of the nascent filament spaced at 11 nm. In Of, vegetative nuclei of the plasmodium adhere to generative cells in a way reminding of sporoplasmic plasmodium of actinospores. In Of plasmodia, several unusual cytoplasmic structures were observed (membrane bound bodies with fuzzy radial contents or with a central dense inclusion, and endoplasmic reticulum cisternae forming a scalloped network). Of may also form intracellular coelozoic sporogonic plasmodia in the epithelial cells of renal tubules; these stages do not seem to constitute an important part of the life cycle.

An increasing attention has been recently paid to myxosporeans mainly in view of their pathogenicity, of the life cycle of astonishing complexity unravelled a short time ago (Wolf and Markiw 1984, El-Matbouli et al. 1995) and of the recent assignment of Myxozoa to Metazoa (Smothers et al. 1994, Schlegel et al. 1996). Ultrastructural studies, initiated by Grassé (1960), have been elucidating myxosporean cell structures, unique in many respects. Recently, ultrastructural findings confirm that the actinosporean and myxosporean stages do constitute together a single, myxozoan life cycle (Lom 1995) and are strongly in favour of metazoan nature of myxozoans (e.g., presence of desmosomes – Desportes and Théodoridès 1982, Desser et al. 1983a). Ultrastructural evidence also helps to understand the interaction of myxosporeans with host cells (Lom et al. 1989a) or tissues (Current and Janovy 1976, 1978) and hence the myxosporean pathogenicity.

Thus far, myxosporeans of 17 genera and 78 species have been examined with the transmission electron microscope. The genera most studied were *Ceratomyxa* Thélohan, 1892 (e.g., *C. globulifera* – Desportes and Théodoridès 1982 [these are also the authors and the date of description]), *Henneguya* Thélohan, 1892 (e.g., *H. adiposa* Minchew, 1977 – Current 1979), *Myxidium* Bütschli, 1882 [e.g., *M. gadi* (Gérgevitch, 1916) – Feist 1995], *Myxobolus* (e.g., *M. cerebralis* Hofer, 1903 – El-Matbouli et al. 1995), and *Sphaerospora* Thélohan, 1892 (e.g., *S. testicularis* – Sitjà-Bobadilla and Alvarez-Pellitero 1993a [these are also the authors and date of description]). Although the essential features of myxosporean structures and their morphogenesis have been established, many structures and phenomena have not been understood to our satisfaction and additional data are needed. In this paper, we present the ultrastructures of *Zschokkella pleomorpha* and *Ortholinea fluviatilis*, myxosporean species from *Tetraodon fluviatilis*, which were recently established by Lom and Dyková (1995).

MATERIALS AND METHODS

Ornamental fishes of the species *Tetraodon fluviatilis* (Teleostei, Tetraodontidae) were supplied by pet fish stores in České Budějovice, being declared as direct imports from breeders in Southeast Asia. Kidney samples taken from specimens killed by an overdose of MS 222 were fixed in cold, 2 % osmic acid in 0.1 M cacodylate buffer and embedded in Epon-Araldite. Ultrathin sections, double stained with uranyl acetate and lead citrate, were observed in Philips EM 420 and JEM 100B electron microscopes at 80 kV accelerating voltage.

RESULTS

Zschokkella pleomorpha Lom et Dyková, 1995

Plasmodia. Small plasmodia (Figs. 1, 2) were attached to microvilli of epithelial cells of the renal tubules.

Plasmodial surface was smooth, except for finger-like pseudopodial extensions, wedged among the villi, and attached to them by junctions reminiscent of septate desmosomes (Fig. 3). The spaces between the pseudopodium and microvillus membrane were spanned by fibrous bridges regularly spaced at 19 nm. Other villi, compressed into bundles between the pseudopodia were closely attached with their electron dense tips to the surface of plasmodia. In tubule segments, where the microvilli were short, pseudopodial extensions were attached directly to the boundaries between two epithelial cells (Fig. 4). In renal tubules replete with small plasmodia, those in the centre were unattached and had no pseudopodia-like surface extensions. Plasmodia contained – in addition to sporogonic cells – one or more own nuclei with rather eccentric nucleoli. A variety of vesicles, phagosomes, lipid inclusions, cisternae of rough endoplasmic reticulum, free ribosomes, β-glycogen particles and bodies with irregular stacks of membranes reminiscent slightly of multilamellar bodies (Fig. 5) was found in the plasmodial cytoplasm. Typical Golgi bodies were absent.

Small plasmodia had one to several inner secondary cells, in fact sporogonic cells located singly or forming groups squeezed together within a common vacuole in the plasmodium cytoplasm. They produced spores without pansporoblast formation. Regularly, one or two tertiary cells could be observed within one secondary cell (Fig. 6). The sequence of division of these cells during sporogenesis could not be followed.

Large plasmodia (Fig. 7) had a much greater variety of cytoplasmic constituents, especially of various vesicles, large vesicle-filled lucent vacuoles and dense vesicles. Mitochondria seemed much more numerous, cristae occupying only a small part of their volume.

Sporogenesis. It followed the pattern similar to that in many other myxosporea lacking pansporoblast formation. Sporogonic cells divided to form a group of closely adhering cells, transforming then into the future capsulogenic, valvogenic and sporoplasmic cells while gradually assuming their final position.

Morphogenesis of capsulogenic cells corresponded to that of most other myxosporeans. Only some specific features will be mentioned. The external tube with up to 8 coils (Fig. 8) around the spherical capsular primordium was much longer than the mature twisted polar filament. The end of the tube was free, facing no rudiment of the future filament discharge pore which could only be found in front of the maturing capsule (Fig. 10). The tube begun inverting into itself while much of it still contained the opaque granular material found also in the centre of the primordium (Fig. 12). After the coils of the filament have appeared within the primordium (Fig. 9), the lucent layer of the capsule wall was separated from the opaque granular inner matrix. The primordium then became encased with a thin membrane which enveloped it at a greater or closer distance within the capsulogenic cell (Fig. 11). The nascent filament revealed distinct oblique fibres spaced at 11 nm on its surface (Fig. 13). The cytoplasm of capsulogenic cells revealed swollen cisternae of the RER which gradually transformed into a mass of small vesicles eventually disappearing and leaving only free ribosomes around the maturing capsule.

The sporoplasmic cell had a pair of nuclei lying close to each other with adjacent RER cisternae and developed spherical to oval dense sporoplasmosomes (Fig. 14).

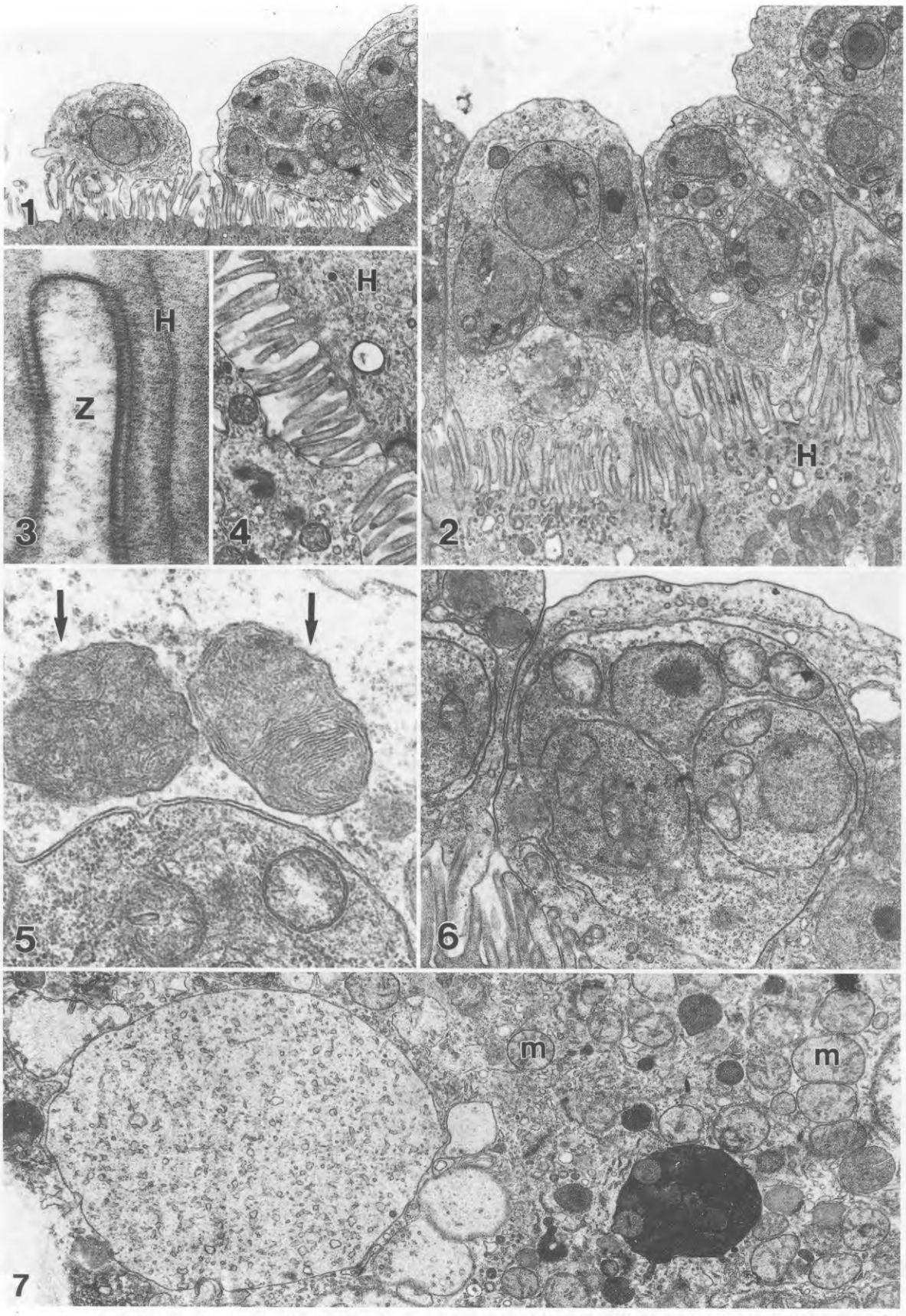
In the developing sporoblast, capsulogenic cells lay at first side by side at one end, facing the sporoplasmic cell. The bulk of the valvogenic cells was behind the sporoplasm from where they spread as very thin sheets around the capsulogenic cells where they contacted each other (Fig. 15). Eventually, the thin valvogenic cells became a sheet of two unit membranes subtended by a layer of opaque material (Fig. 16) with resorbed nodes of residual cell contents (Fig. 14). Along the sinuous course of the suture, the thickened brims of the valvogenic cells either overlapped (Fig. 17) or abutted on each other (Fig. 18). A few microtubules could be seen at the suture.

Mitochondria in the transforming sporoblast cell differed from those in the plasmodial cell. In valvogenic and sporoplasmic cells they were generally more opaque, in capsulogenic cell the lucent inside of the cristae was in strong contrast to the dark matrix.

Ortholinea fluviatilis Lom et Dyková, 1995

Plasmodia. They were of variable size (18 to 55 μm) and appearance; large and small forms do not differ in their characters too much. In heavily infected renal tubules, small plasmodia often filled completely the lumen without any attachment to the epithelium. In less heavily infected tubules, surface projections of the plasmodium contacted exactly and only the gaps between

→ **Figs. 1–7.** *Zschokkella pleomorpha*. **Figs. 1, 2.** Small plasmodia with secondary and tertiary cells attached to the epithelial cells of the renal tubules. Cytoplasmic extensions are interlocked between the host cell microvilli; × 3500 and × 7500, respectively. **Fig. 3.** Detail of the projection (Z) of plasmodium forming a junction with the host cell (H) microvilli; × 75800. **Fig. 4.** Cytoplasmic projection of the plasmodium (bottom) attached to the desmosome between two epithelial cells of the host (H); × 15300. **Fig. 5.** Bodies with membraneous contents next to a secondary cell in plasmodium cytoplasm; × 14000. **Fig. 6.** Secondary cell with two tertiary cells within a small plasmodium; × 9900. **Fig. 7.** Part of the cytoplasmic contents of a large plasmodium with mitochondria (m), lucent vacuoles with different contents and a variety of vesicles and dense bodies; × 9500.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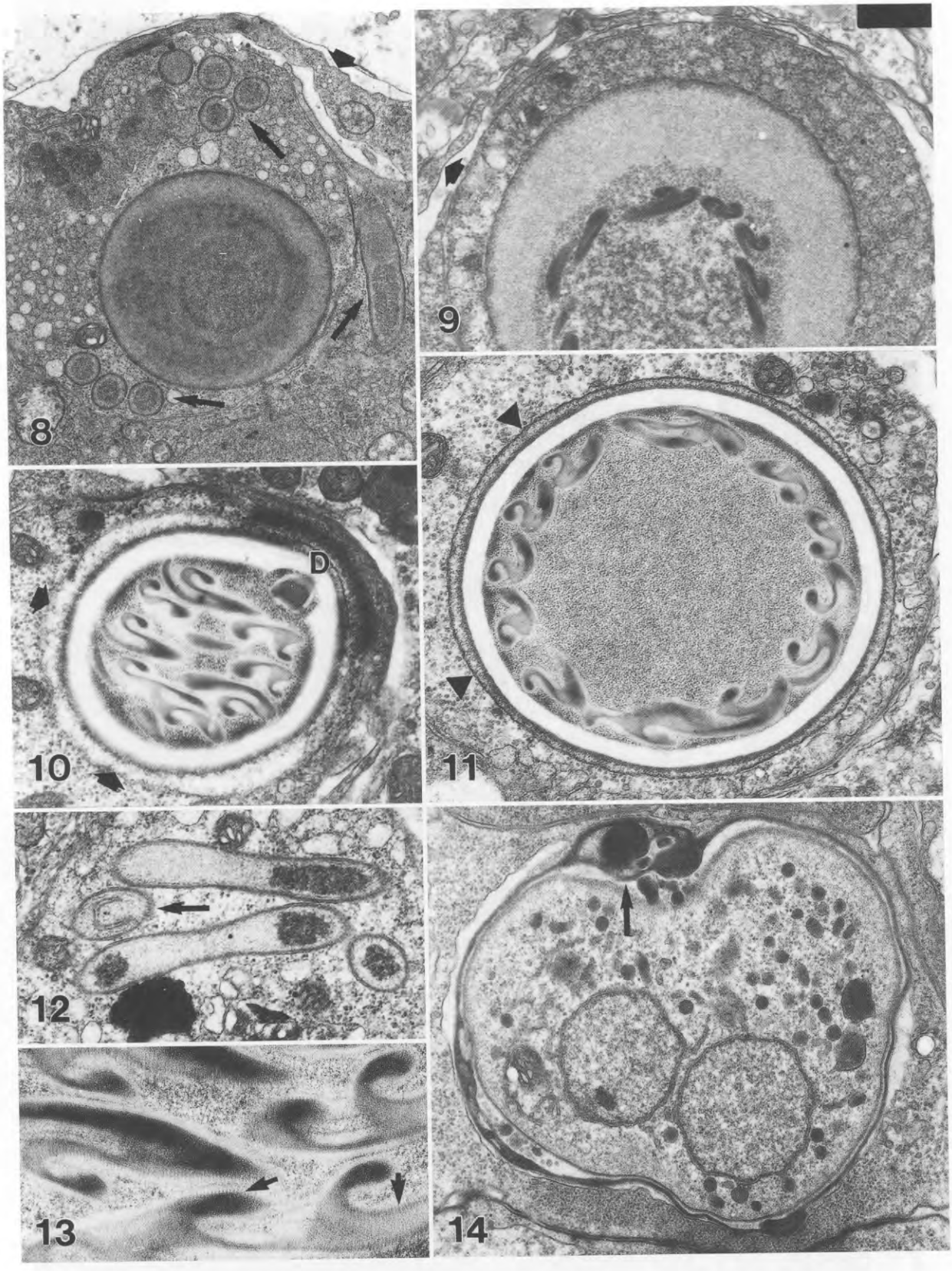
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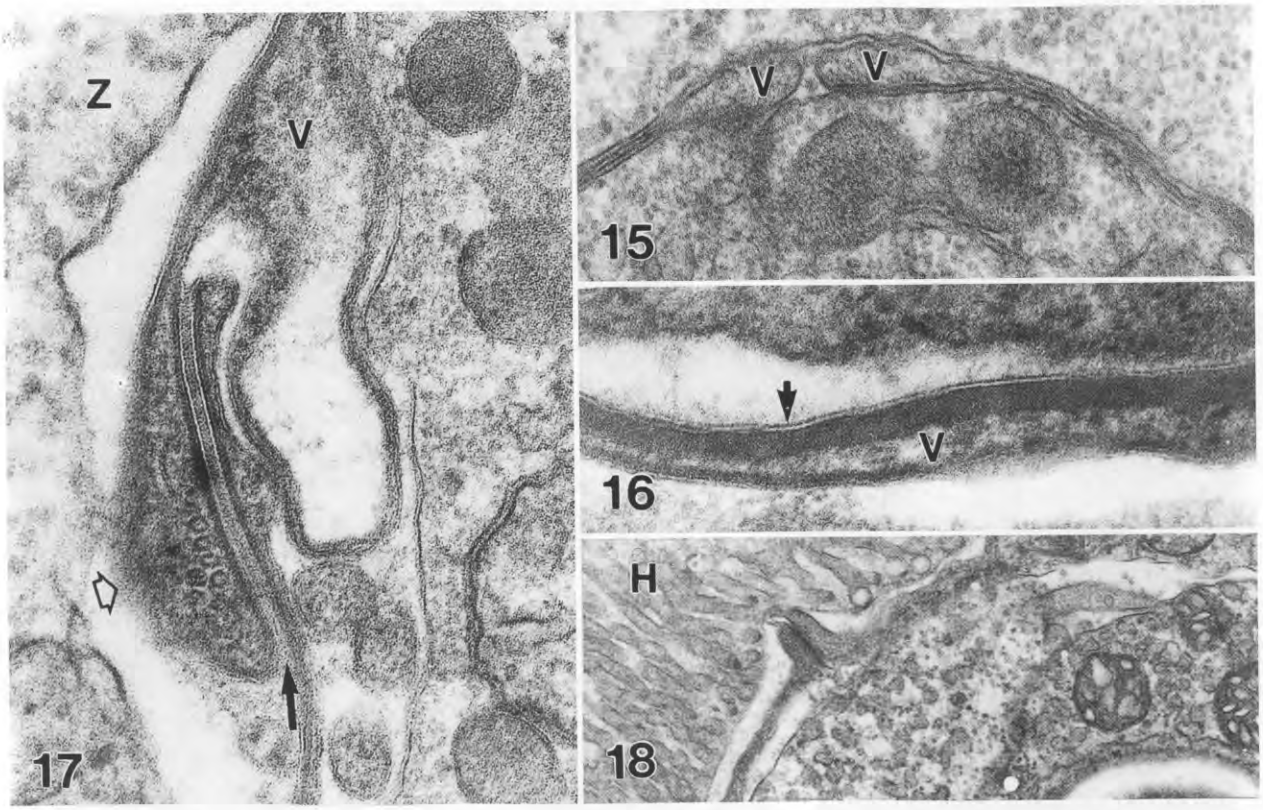


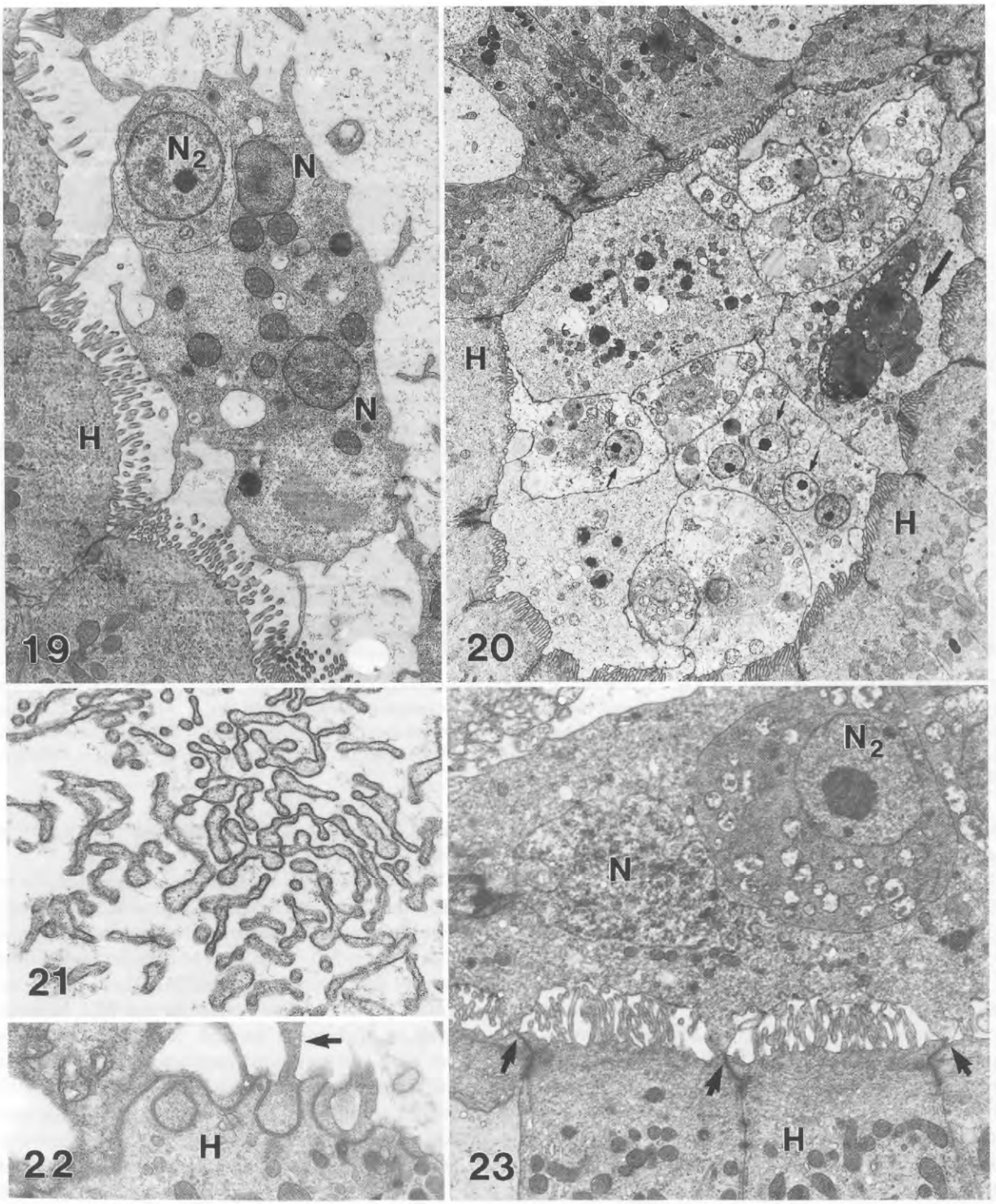
Fig. 15–18. *Zschokkella pleomorpha.* **Fig. 15.** Mutually approaching borders (V) of the very thin valvogenic cells; × 47000. **Fig. 16.** Thick electron dense layers subtending the unit membrane of the developing valvogenic cell (V); × 114800. **Fig. 17.** Dense substance filling the space of the cell junction (arrow) between the valvogenic cells (V). Empty arrow – microtubules within one of the two brims of the suture. Z – cytoplasm of the plasmodium; × 85500. **Fig. 18.** Transverse section of the suture formed by abutting valve borders. H – host microvilli; × 18000.

epithelial cells (Fig. 20), the microvilli being pressed down by plasmodium surface. If there is enough space, small plasmodia may also have pseudopodial projections (1.4 to 3.5 μm) all over their circumference, some of them being fixed again to the gaps between epithelial cells (Fig. 19).

Larger plasmodia, too, had holdfast projections attached only to the epithelial gaps (Fig. 23). The projections, holdfast and others, could be all over the plasmodium surface (Fig. 21). Plasmodium in urinary tubules in segments without detectable microvilli, had projections embedded in the cytoplasm of epithelial cells (Fig. 22). Some plasmodia spanned the whole tubular lumen (Fig. 24). There were also junctions between the dense tips of the microvilli and plasmodium surface (Fig. 25) with accumulation of dense material beneath both membranes at the points of contact.

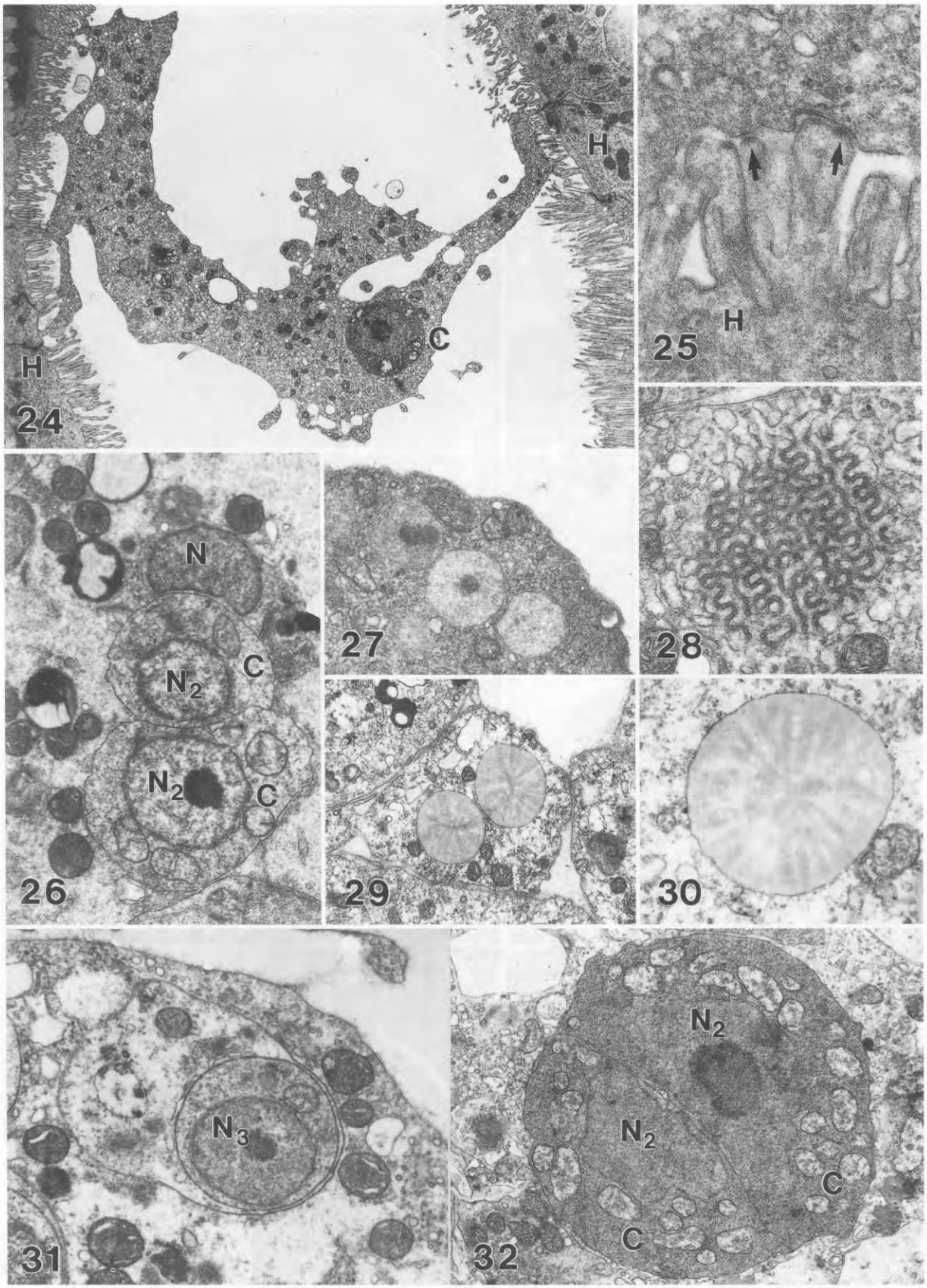
There was a variety of cell constituents which was far greater in larger plasmodia. Plasmodial nuclei were often closely attached to generative cells both in small and large (Fig. 26) plasmodia. Mitochondria had often extremely dark matrix (Figs. 19, 26). There was a considerable amount of free ribosomes and RER cisternae as well of α-glycogen particles and their aggregates. There were various simple membrane-bound vacuoles. Some seemed to be fluid-filled, looking empty, some contained granular substance and a central dense core looking like nuclei with nucleoli (Fig. 27). Others contained faint, fuzzy substance arranged radially (Figs. 29, 30), some were filled with indiscriminate dark substance. Sometimes the type of inclusions and large areas

← **Figs. 8–14.** *Zschokkella pleomorpha.* **Fig. 8.** Capsulogenic cell with sections of capsular primordium and external tubes (arrows); arrowhead points at the thin layer of the mother plasmodium containing the sporoblast; × 9800. **Fig. 9.** Capsulogenic cell, already encased with thin valvogenic cells (arrowhead); polar filament in formation within a central granular mass of the primordium; × 19300. **Fig. 10.** Not yet fully mature polar capsule with grazing section of its discharge pore (D); the dense line above it is the cell junction between the valvogenic and capsulogenic cell. Arrowheads point at the membrane around the capsule; × 15500. **Fig. 11.** Transverse section through an immature polar capsule; triangles point at the membrane around the capsule; × 19500. **Fig. 12.** Sectioned external tube with granular contents, in one section (arrow) the nascent filament can be seen; × 13600. **Fig. 13.** Transverse ridges on the surface of a young polar filament (arrows); × 54900. **Fig. 14.** Transverse section through the spore at the level of the sporoplasm with the two nuclei. Arrow points at a clump representing the degraded nucleus of the valvogenic cell; × 13600.

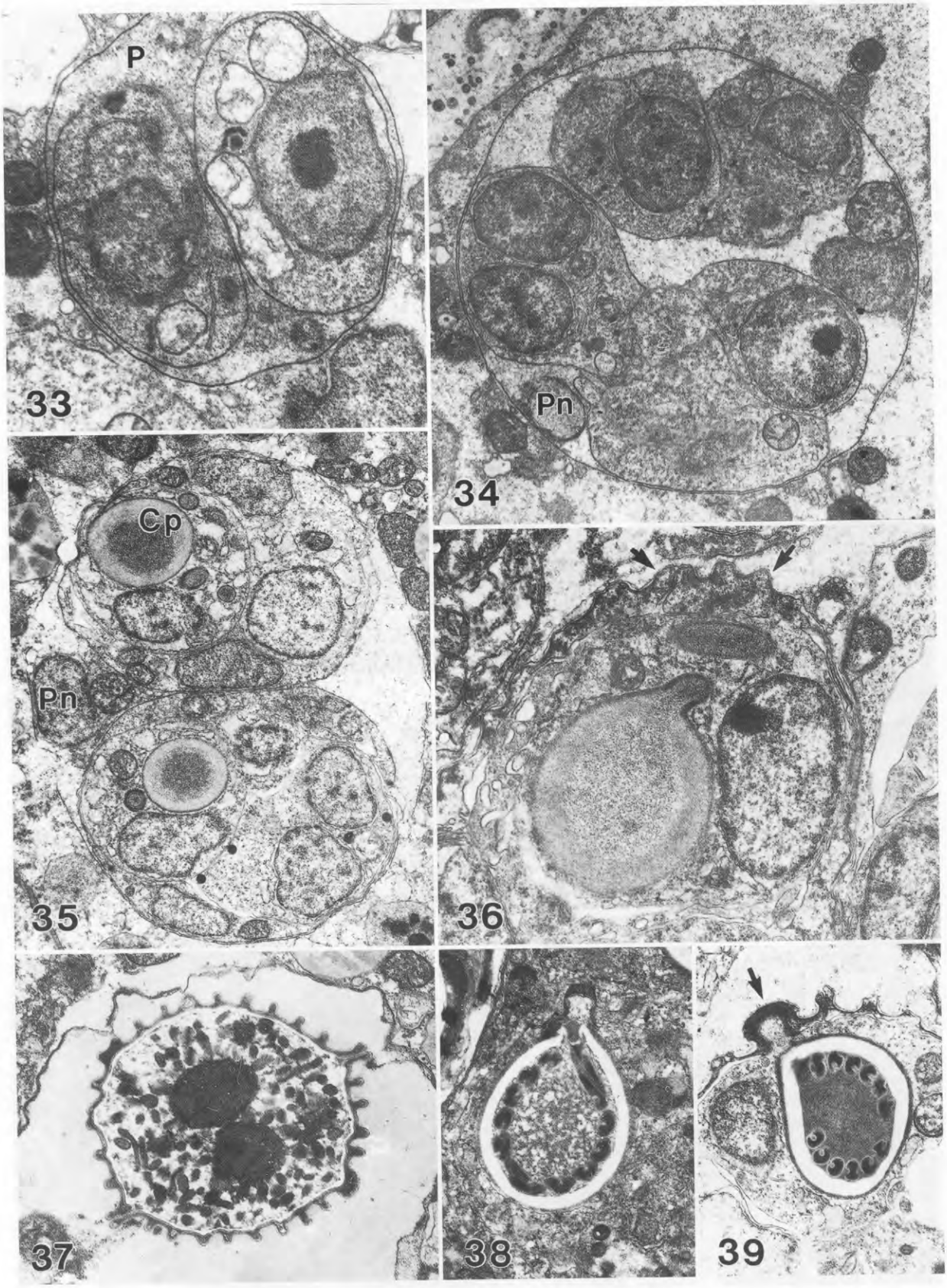


Figs. 19–23. *Ortholinea fluviatilis*. **Fig. 19.** A small plasmodium attached by finger–like projections to junctions between epithelial cells (H) of the renal tubule. N – nuclei of the plasmodium, N_2 – nucleus of the secondary cell; × 5900. **Fig. 20.** Renal tubule (H) completely occluded by plasmodia, pressing down the tubule microvilli. Small arrows point at *O. fluviatilis* nuclei, large arrow points at secondary cells; × 2200. **Fig. 21.** Grazing section of the villous surface projections of the plasmodium; × 11000. **Fig. 22.** Cytoplasmic surface projections of the plasmodium forming junctions at the surface (at left) or deeply embedded into the host cell (H); × 9600. **Fig. 23.** Part of the plasmodium with cytoplasmic projections adhering to the desmosome region of the host cells (H). N – nucleus of the plasmodium, N_2 – nucleus of the secondary cell; × 3700.

→ **Figs. 24–32.** *Ortholinea fluviatilis*. **Fig. 24.** Plasmodium spanning the lumen of the renal tubule. C – secondary cell, H – host cells; × 2000. **Fig. 25.** Junctions (arrows) between the host cell microvilli and the plasmodium surface; × 30600. **Fig. 26.** Plasmodium nucleus (N) closely adhering to a secondary cell (C). N_2 – nuclei of secondary cells; × 69000. **Fig. 27.** Cell inclusions



with a central core; × 10500. **Fig. 28.** Membranous meshwork in the cytoplasm; × 20500. **Figs. 29, 30.** Bodies with radial rodlets; × 6500 and 12000, respectively. **Fig. 31.** A cell doublet, possibly a secondary cell enclosing a tertiary one (with the nucleus N_3) or one cell enclosed within another (pericyte); × 8600. **Fig. 32.** Closely adhering secondary (= generative) cells C. N_2 – nuclei of the secondary cells; × 8900.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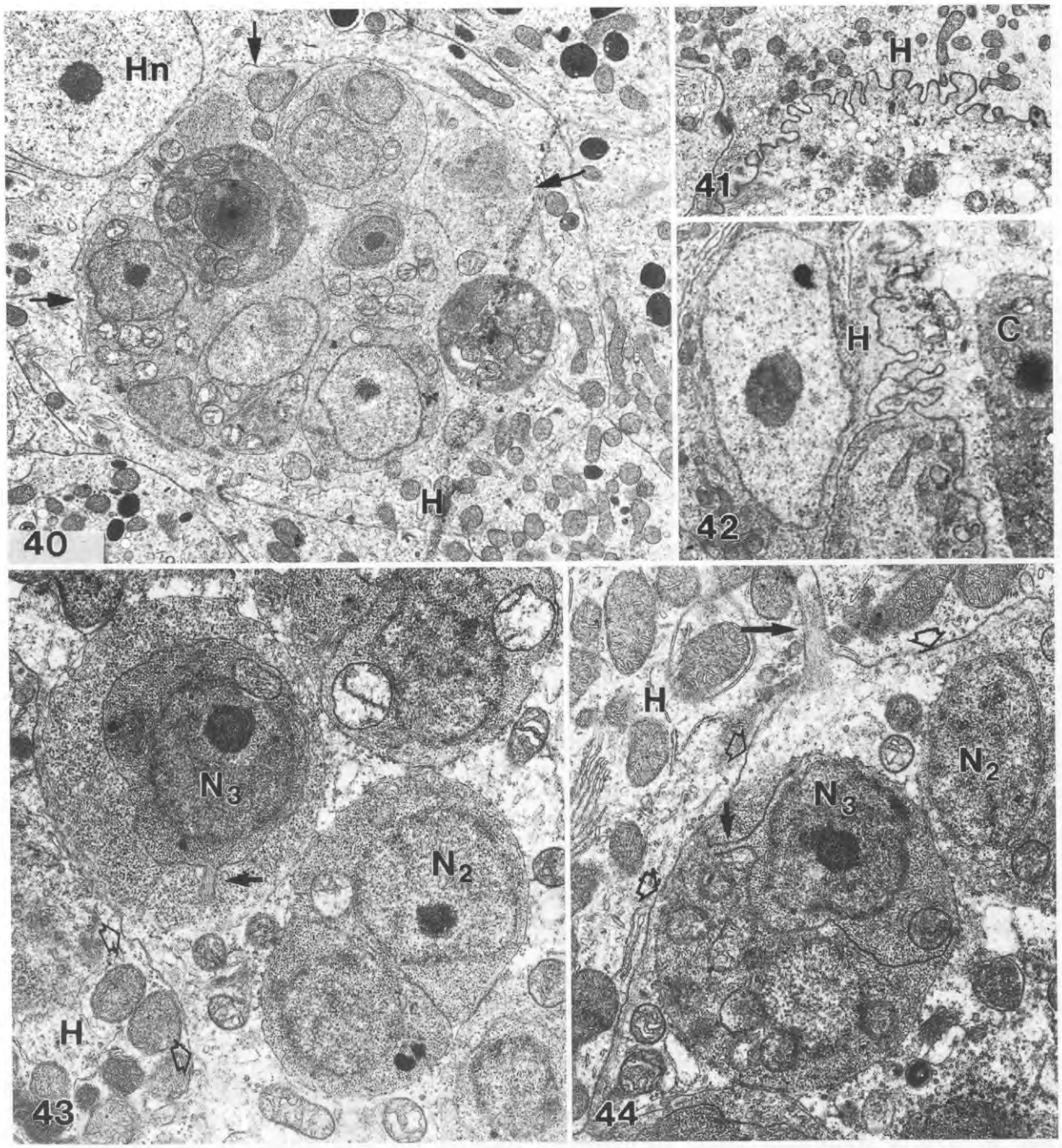

Figs. 40–41. Intracellular stages of *Ortholinea fluviatilis*. **Fig. 40.** Plasmodium with several secondary (generative) cells almost completely filling the host cell (H). Hn – host cell nucleus, arrow points at the plasmodium cell membrane; × 4900. **Figs. 41, 42.** Cell membrane of plasmodia raised in villosities. H – host cell, C – secondary cell; × 4300 and × 10100, respectively. **Fig. 43.** Part of an intracellular plasmodium. N_3 – nucleus of a tertiary cell with a projection (arrow) as a remnant of the past cytokinesis, N_2 – nucleus of one in a pair of secondary cells closely adhering together; H – host cell, arrowheads point at the boundary of the plasmodium; × 14300. **Fig. 44.** Part of a plasmodium which itself bears a projection from the past division (large arrow); the tertiary cell (nucleus N_3) with a similar projection (arrow). N_2 – nucleus of the secondary cell, arrowheads point at the plasmodium boundary; × 15400.

← **Fig. 33–39.** *Ortholinea fluviatilis*. **Fig. 33.** Early stage of pansporoblast with two cells within the pericyte P; × 18200. **Fig. 34.** Advanced pansporoblast with several cells, one of them binucleate. Pn – pansporoblast nucleus; × 6900. **Fig. 35.** Pansporoblast at the stage of formation of polar capsule primordia Cp. In the bottom sporoblast, binucleate sporoplasm can be seen; × 6500. **Fig. 36.** Capsular primordium and its external tube located in the anterior part of the spore, where the formation of ridges on the valvogenic cells starts (arrows); × 12000. **Fig. 37.** Transverse section through a mature spore at the level of sporoplasm with sporoplasmosomes; × 8100. **Figs. 38, 39.** Longitudinal and oblique section through almost mature polar capsules; arrow points at the dense substance in the shell valve in the future filament discharge canal; × 8200 and × 8700, respectively.

of cytoplasm of uniformly granular appearance (Fig. 26) seemed to reflect suboptimal state of the organism. Completely mysterious was the system of scalloped profiles, linked with cytoplasmic vesicles obviously continuous with the RER system, appearing in cross sections as a meshwork of meandering tubules (Fig. 28).

Generative cells and sporogenesis. Mitochondria of generative cells – irrespective of appearance of those in the mother plasmodium – had moderately opaque or extremely lucent matrix, the latter appearing as if swollen. Both types appeared side by side in the same plasmodium. Generative cells had a rather voluminous nucleus and a few mitochondria (Fig. 31). Some have in their cytoplasm, close to their nucleus, a microtubular bundle as the remainder of the mitotic spindle. Generative cells with a cup-like nucleus embracing closely the enclosed inner daughter cell could be seen; these formations were probably not engaged in sporogony.

Spores were formed either separately by direct division of a single generative cell (in small plasmodia), or, predominantly, in pansporoblasts. Pansporoblast formation followed the well-known pattern of a sporogonic cell developing into two spores within the pericyte. Pairs of closely adhering generative cells (Fig. 32) suggest the way how the union of sporogonic cell and pericyte can arise to produce the initial stage (Fig. 31). This was followed by the stage of two cells within the pericyte (Fig. 32) until through the next steps (Fig. 33) two sporoblasts were formed within the pericyte (Fig. 34). At this stage, the pericyte still had its nucleus while the cytoplasm became devoid of all organelles. Valvogenic cells spread around the sporoplasmic and capsulogenic cells while still retaining the appearance of a functional cell.

Differentiation of capsulogenic cells followed the usual pattern including the initial club-like structure differentiating later into subspherical capsular primordium connected with the external tube (Figs. 35, 36). The tube made two turns within the capsulogenic cell. Eventually, the primordium differentiated its inner lucent layer of the capsule wall and assumed the structure (Figs. 38, 39) of the mature polar capsule. The mouth of the inverted polar filament faced a cytoplasmic cone filling the future channel for filament discharge. A dense mass filled the adhering, lid-like space (Fig. 38) within the shell valve.

The ridges on the valvogenic cell appeared, along with accumulation of lumps of dense substance, at the late stage of capsulogenesis. In the valve sutures, borders of valvogenic cells faced each other in a junction set perpendicularly to the spore surface. Maturation of valve cells ended up with a thin, ridged, opaque shell (Fig. 37).

The sporoplasm contained two nuclei situated closely side by side and a number of ellipsoidal to rod-like vesicles which gradually became opaque sporoplasmosomes. The surface of an early sporoplasm was often raised in lobose villosities.

Intraepithelial stages. They were frequent although not numerous. Some were intercellular, wedged between epithelial cells close to their apical ends. Seen in cross section, they contained one secondary cell only, which in turn harboured sometimes a tertiary cell. However, intracellular stages prevailed, ranging from smaller primary cells with one secondary cell to larger plasmodia with many vegetative nuclei and with secondary (generative) cells (Fig. 40). Sometimes, an epithelial cell contained two parasite cells. Myxosporean stages lay, as usual, within a parasitophorous vacuole with its membrane closely applied to their own cell membrane. This vacuole could be encased with an interrupted or continuous endoplasmic reticulum cisterna. The surface of some of these stages was raised in villosities (Figs. 41, 42). Elsewhere long surface projections were seen (Fig. 44) as an evidence of a recently completed division of the plasmodium. Similar projections could also be seen in tertiary cells of the plasmodium (Figs. 43, 44). The cells in larger plasmodia were uninucleate (Fig. 40), or binucleate secondary cells. The latter could contain either tertiary cells with a small amount of cytoplasm around the nucleus or full fledged tertiary cells. Finally, there were secondary cells closely attached one to each other (Fig. 43). Actually, all these cells were reminiscent of sporogonic plasmodia in tubular lumen.

Except for a certain degree of hypertrophy, the intracellular stages did not seem to inflict any obvious changes to the infected cells.

DISCUSSION

Thus far, five species of the genus *Zschokkella* have been examined with the electron microscope (*Z. nova* Klokacheva, 1914 – Lom and de Puytorac 1965, *Z. russelli* Tripathi, 1948 – Davies 1985 and *Z. leptatherinae* Su et White, 1995 – Su 1996; in two species, the ultrastructural data were included in the original descriptions, viz., *Z. icterica* – Diamant and Paperna 1992 and *Z. mugilis* – Sitjà-Bobadilla and Alvarez-Pellitero 1993b). Thus far, no ultrastructural study of any *Ortholinea* species has been reported.

Spore formation. Considerable size variation of sporogonic plasmodia of both *Zschokkella pleomorpha* and *Ortholinea fluviatilis* is a phenomenon occurring in so-called mictosporic genera and species (Auerbach 1909), i.e., in coelozoic myxosporeans forming plasmodia that may produce one, two, more or many spores each. In addition to many *Zschokkella*, this exists in *Myxidium*, e.g., in *M. lieberkuehni* Bütschli, 1882 forming small (in urinary ducts) or very large (in urinary bladder)

plasmodia (Debaisieux 1920, Lom et al. 1989a) or in *Chloromyxum*, e.g., *C. trijugum* Kudo, 1919 (Kudo 1919). Similarly, *Z. pleomorpha* can produce either one, two or several to many spores per sporogonic plasmodium.

In the suborder Platysporina, pansporoblasts are always formed and in the order Multivalvulea they never occur. However, in some of the genera having species with mictosporic plasmodia spore formation in pansporoblasts may alternate with sporogony without pansporoblasts. In the suborder Variisporina, in the genus *Zschokkella* the species differ in this respect. *Z. mugilis* (Sitjà-Bobadilla and Alvarez-Pellitero 1993b) does not form pansporoblasts, like *Z. pleomorpha*, while *Z. nova* and *Z. leptatherinae* do so (Lom and de Puytorac 1965 and Su 1996, respectively). In some species of *Myxidium*, e.g., *M. gadi*, sporogenesis can also take place both through direct cell division and in pansporoblasts (Feist 1995). In *Chloromyxum*, some species form pansporoblasts (*C. lenorae* – Lom et al. 1988), some not (*C. leydigi* – Naville 1927).

Ortholinea fluviatilis thus expands the range of species known to produce spore both ways. The actual process of pansporoblast origin (i.e., pericyte enveloping the sporogonic cell) was not clearly observed although cell doublets such as in Fig. 32 may be interpreted in this way.

Attachment. Four ways of attachment to the host cells were observed in the myxosporeans described in this paper. The most simple was the junction between the tips of the microvilli and the surface of plasmodium of *O. fluviatilis*. Such junctions are also known in pseudoplasmodia of *Sphaerospora* species (e.g., *S. renicola* – Lom et al. 1982). Plasmodia of *Z. pleomorpha* inserted their finger-like projections between the host cell microvilli and were firmly attached to them by rather elaborate junctions of a type reminiscent of junctions found in *Hoferellus gilsoni* (Lom et al. 1986). In the complex host-parasite interface of this species, there are areas of contact between the plasmodium- and epithelial cell membranes in form of septate junctions, the gaps between membranes being spanned by bridges spaced at a similar distance, 17 nm (compared to 19 nm in *Z. pleomorpha*). Junctions of plasmodial projections in *Myxidium gadi* and host cell microvilli are similar but lack this septate appearance and have an interposed layer of amorphous material (Feist 1995). In other species (*Sphaerospora truttae* – McGeorge 1995) the projections were reported to simply interdigitate with the microvilli. In other myxosporean species the plasmodial projections are attached directly to the epithelial cells. They simply adhere to the host cell surface in either *Leptotheca* or *Ceratomyxa* (Desportes and Théodoridès 1982). Surface indentations in host cells, occupied by plasmodial projections similar to those observed in *O. fluviatilis* were observed in *Myxidium giardi* (Paperna et al. 1987). In the latter case the area of contact was actually represented by attachment points spaced at regular intervals rather then being flat.

In both *Z. pleomorpha* and *O. fluviatilis*, additional holdfasts occurred in form of cytoplasmic projections attached or inserted into the gap between neighbouring epithelial cells. This was reported before (El-Matbouli and Hoffmann 1994) in *Sinuolinea tetraodoni* occupying renal tubules of *Tetraodon palembangensis*, a fish which is congeneric with the host of *O. fluviatilis*. Similar attachment to junctions between epithelial cells was illustrated in pictures (although not mentioned) of *Zschokkella mugilis* by Sitjà-Bobadilla and Alvarez-Pellitero (1993b) and quite recently in plasmodia of *Hoferellus carassi* attached to the epithelium of the urinary bladder of goldfish (Trouillier et al. 1996).

Thus it is obvious that a given type of attachment is by no means typical of a certain myxosporean species or genus. The reasons for such a variety of ways of attachment (or no attachment at all as in *Z. leptatherinae* – Su 1996) can perhaps be interpreted in terms of adaptation to conditions prevailing in each given case (type of the host surface at the site of infection, developmental stage) rather than as a feature of the parasite.

Cytological structures. Cell structures of plasmodia as well as of developing spores confirm the great variability of myxozoan cells. The wide diversity of cytoplasmic constituents in large plasmodia is opposed to the simple structure of secondary or tertiary cells in which the nucleus is surrounded by just a thin layer of cytoplasm with a few mitochondria and small fragments of endoplasmic reticulum.

In mitochondria, there is a great variability in the shape of cristae and in the density of mitochondrial matrix. While mitochondria in the inner (generative) cells have a lucent matrix, the matrix tends to be dark in e.g., capsulogenic cells of *Z. pleomorpha* and especially in large plasmodia of *O. fluviatilis*. These states can be compared with what has been designated as orthodox and condensed mitochondrial structure. Mitochondria with equally striking dense matrix were reported in *Myxidium lieberkuehni* and *Chloromyxum cristatum* (Lom and de Puytorac 1965), *Myxidium giardi* (Paperna et al. 1987), *Z. icterica* (Diamant and Paperna 1992) or *M. gadi* (Feist 1995). Transformations of mitochondrial structure are obviously associated with not yet understood changes in metabolic activity and ion traffic at different stages of myxosporean development.

Although there are no microtubular structures in *Z. pleomorpha* and *O. fluviatilis* plasmodia (at variance with e.g., *Myxidium lieberkuehni* – Lom and de Puytorac 1965, *Sphaeromyxa sabrazesi* – Grassé and Lavette 1978, *Kudoa lunata* – Lom and Dyková 1988 or *Leptotheca/Ceratomyxa* – Desportes and Théodoridès 1982)

there is a rich variety of vesicles, vacuoles and dense bodies. These organelles are supplemented by several types of rather unusual inclusions. First there are bodies with stacks of membranes in plasmodia of *Z. pleomorpha* (Fig. 5). They are to some extent reminiscent of multilamellar bodies in plasmodia of *Myxidium gadi* (Feist 1995) rather than of myeline figures in *Z. mugili* plasmodia mentioned by Sitjà-Bobadilla and Alvarez-Pellitero (1993b). Further riddles are found in the cytoplasm of *O. fluviatilis*: there are spherical inclusions either with a dark centre or containing fuzzy radial rods. Both types have hardly any parallel among myxosporeans studied thus far, even taking into account crystalline inclusions of *Myxidium giardi* (Paperna et al. 1987). Finally, the undulated membraneous profiles bear some resemblance to stacks of annulate lamellae; however, they are linked with RER and not the nuclear membrane; perhaps a more reasonable suggestion would be a kinship with stacks of cisternae of Golgi apparatus.

These organelles expand the list of cytoplasmic constituents of unknown functions such as lobocytes (Grassé and Lavette 1978) or generative cells mutually interlocked with each other by their cell projections (Dyková et al. 1987).

Somatic nuclei of plasmodia, adhering to the surface of generative cells in *O. fluviatilis* plasmodium strongly resemble similar complexes in the sporoplasm of some actinospores. In *Neoactinomyxon eiseniellae*, somatic nuclei of the sporoplasmic plasmodium are similarly attached to the actual infective cells inside it (Marques 1984). Similar coupling exists in the sporoplasm of *Aurantiactinomyxon* sp. actinospore from *Branchiura sowerbyi* (Lom and Yokoyama, unpublished). Such association also exists in the pair of sporoplasmic cells of *Kudoa lunata* (Lom and Dyková 1988), the nucleus of the outer cell is closely attached to the surface of the inner sporoplasmic cell.

Although the functional significance of this coupling is unknown and may differ in the myxosporean plasmodium and actinospore sporoplasm it clearly shows general patterns common to both myxosporean and actinosporean phase of the life cycle (Kent et al. 1994).

In the course of capsulogenesis in both *O. fluviatilis* and *Z. pleomorpha*, the primordium and external tube have the appearance found in most myxosporeans studied, i.e., are filled with finely or coarsely granular dense substance, without containing peculiar dense structured formations such as those found in *Myxobolus funduli* (Current et al. 1979) or *M. cotti* (Lom et al. 1989b). Within the external tube, the dense granular lumps are eventually replaced by nascent filament profiles. In *Z. pleomorpha*, the almost mature capsule is encased with a dense envelope set at a certain distance from the capsule surface. This boundary was not found in any other myxosporeans.

The fine fibres on the surface of the almost mature polar filament have also been found in *Sphaerospora angulata* (actually *S. renicola*) (Desser et al. 1983a) where the fibres are set apart at a period of 11 nm; according to data in Lom et al. (1982) the period is 12 nm in this species. In *Sphaeromyxa* cf. *magna* (Lom 1969) the period is 15 nm. The size range of these fibres, including *Z. pleomorpha* (11 nm), seems to be very similar. They obviously constitute a basic element in filament morphogenesis. They tend to disappear in all species observed before the filament transforms into a mature one with a dark core inside a lucent outer layer (continuous with the chitinous (Lukeš et al. 1993) inner layer of the capsule). At that time the matrix of the capsule also appears uniformly dense and structureless.

Morphogenesis of the polar filament proceeds from simple S-shaped double walled profiles first seen within the external tube to more massive, dense and twisted profiles and, eventually, to submature dumbbell- or 8-shaped profiles with denser core and lucent envelope within capsular primordium. The latter are seen in this paper and are quite well exemplified in Desser et al. (1983b). The origin of the fine fibres on the surface of the submature polar filament may be linked with the growth of this organelle, or they perhaps may be related to very fine fibres spaced at the inner side of the wall of the external tube where they face – across the tube wall – the microtubules running on the outer surface of the tube. These fibres were recorded e.g., in *Sphaerospora testicularis* (Sitjà-Bobadilla and Alvarez-Pellitero 1983a), *Henneguya adiposa* (Current 1979) or *S. angulata* (Desser et al. 1983b). It is feasible to imagine that after the external tube has inverted inside itself and then eventually into the primordium (as postulated by several authors, see Lom and Dyková (1992)) the inner surface of the tube becomes the outer surface of the filament.

Actually, similar fibres associated with nascent filament surface while it is inside the external tube can be seen e.g., in *Thelohanellus nikolskyi* (Desser et al. 1983a, inset of Fig. 18). The final decision will only be reached with understanding of events – presently unknown – involved in the filament morphogenesis.

Intracellular stages of *Ortholinea fluviatilis*

They do not differ from stages in the tubular lumen, their proliferation being indicated by cytoplasmic bridges, remnants of the preceding division. The surface of intracellular stages was sometimes raised in villosities perhaps to increase the flow of nutrients. The lack of obvious pathogenic action does not compare with changes inflicted by intracellular stages such as the extrasporogonic stages of *Myxidium lieberkuehni* (Lom et

al. 1989a) or by multivalvulid species (Moser and Kent 1994). This also indicates that they were not extrasporogonic stages as suggested by incipient sporogony and confirmed by light-microscope observations of mature spores occurring, although rarely, in these stages. These intracellular stages may not be indispensable in the life cycle of *Ortholinea fluviatilis*. They may be later released back to the tubular lumen, some may complete the sporogenesis inside the tubule wall. Intracellular existence in myxosporea has to be taken as a regular phenomenon, since we know (El-Matbouli et al. 1995) that even typical intercellular myxoboli have obligatory intracellular stages as postulated long ago by Doflein (1898).

The finding, in plasmodia of both *O. fluviatilis* and *Z. pleomorpha*, of tertiary cells within secondary ones, can be interpreted as steps in cell proliferation necessary to produce cells staying together as a group giving rise to spores (Lom et al. 1982, Sitjà-Bobadilla and Alvarez-Pellitero 1993b) or as internal budding. Internal buds can be sequestered from mother plasmodia without destroying their integrity (light microscopic observation of large buds, Shulman 1966) or after the mother cell has disintegrated. The latter case can be seen in extrasporogonic stages in *Sphaerospora* (e.g., Bucsek and Csaba 1984, Molnár 1988). The release of internal buds in form of separate cell complexes can easily be assumed in plasmodia with a potential to form spores.

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Received 22 February 1996

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Accepted 21 March 1996