A comparative study of zoospore cytoskeleton in *Symphytocarpus impexus*, *Arcyria cinerea* and *Lycogala epidendrum* (Eumycetozoa)

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Summary

The ultrastructure of zoospores of the myxogastrids *Symphytocarpus impexus* B. Ing et Nann.-Bremek. (Stemoniida), *Arcyria cinerea* (Bull.) Pers. (Trichiida), and *Lycogala epidendrum* (L.) Fr. (Liceida) is reported for the first time, with special reference to flagellar apparatus. The cytoskeleton in all three species includes microtubular and fibrillar rootlets arising from both basal bodies of the flagellar apparatus. There is a similarity in the presence and position of the rootlets N1-5 between our species and other studied species of myxogastrids and protostelids. Thus, general conservatism of cytoskeletal characters and homology among eumycetozoa have been confirmed for these taxa. At the same time there is variation in the details of flagellar rootlets' structure in different orders and genera. The differences concern the shape of short striated fibre (SSF) which is an organizing structure for microtubular rootlets r2, r3 and r4. Its upper strand is more prominent in *L. epidendrum*. The fibrillar part of rootlet 1 is connected to basal body 1 in *S. impexus*, to basal body 2 in *A. cinerea*, and to both basal bodies in *L. epidendrum*. The rootlets r4 and r5 are very conservative and consist, correspondingly, of 7 and 2 microtubules in all three species. The comparative analysis of all investigated mycetozoon zoospores and *Hyperamoeba* is presented. Variations of flagellar apparatus structure are ascribed not only taxonomic but also phylogenetic value.

Key words: Eumycetozoa, myxogastrids, protostelids, zoospore, phylogeny, *Symphytocarpus impexus*, *Arcyria cinerea*, *Lycogala epidendrum*, *Hyperamoeba*, comparative ultrastructure
Introduction

Current systematics and phylogeny of protists in general, and of myxomycetes in particular are based on ultrastructural data related to the cytoskeletal structure (mainly flagellar apparatus) (Spiegel, 1981a, 1991, 1996; Barr, 1992; Karpov, 1997, 2000), and on molecular data (Cavalier-Smith, 1993, 1998, 2002). Many myxomycetes still remain relatively unexplored in both these aspects (Spiegel et al., 1995).

In particular, there is not much information about the most evolutionary advanced group of myxogastrids (Myxogastria) numbering about 800 species. Due to their cryptic life style and almost complete absence of meaningful taxonomic plasmodial characters, field studies of myxomycetes have invariably focused on the reproductive, spore-producing stage in the life cycle (sporocarps), and the morphological species concept dominates in myxomycete taxonomy (Clark, 2000; Gray and Alexopoulos, 1968; Schnittler and Mitchell, 2000).

The DNA sequence data on myxogastrids species are limited to the studies on Didymium iridis, Physarum polycephalum (Johansen et al., 1992), D. nigripes, Echinostelium minutum, Lycogala epidendrum, L. flavofuscum, P. didermoides, Stemonitis flavogenita and confirm that the lines leading to Physarum, Didymium and Stemonitis diverged from a common ancestor approximately at the same time, while Lycogala and Echinostelium minutum diverged from this common ancestor earlier (Miller et al., 1999, 2002). In rRNA gene sequence analysis Physarum polycephalum appears early at the tree with a low bootstrap value indicating that its position is unstable. The phylogenetic analysis of EF-1α amino acid gene sequences of Dictyostelium, Physarum and Planoprotostelium suggests that the Mycetozoa are monophyletic and branched late (Baldauf and Doolittle, 1997). The combined data set on gene sequences of four proteins (centrin, EF-1, α-tubulin, and β-tubulin) also shows that dictyostelids and Physarum form a well supported sister branch to the Metazoa+Fungi (Baldauf et al., 2000).

The ultrastructure of flagellated cells (zoospores) of 8 species from 4 orders of myxogastrids (Echinosteliida, Stemoniida, Trichiida, and Physarida) have been studied: Echinostelium minutum (Haskins, 1978); Stemonitis nigrescens (Aldrich, 1968; Gray and Alexopoulos, 1968); S. pallida (Ishigami, 1977); S. virginensis (Mims, 1973; Mims and Rogers, 1973); Trichia varia (Gottsberger, 1967); Physarum flavicolum (Aldrich, 1968); P. polycephalum (Wright et al., 1979); P. cinereum, Didymium iridis (Aldrich and Daniel, 1982); D. nigripes (Schuster, 1965). Unfortunately, the majority of these studies were carried out without modern techniques of fixation or reconstruction from serial sections, and these data are now incomplete. The most complete investigation is that of the isolated flagellar apparatus of P. polycephalum (Wright et al., 1979; Mir et al., 1983), where the terminology of cytoskeletal structures was established, which is still accepted for all myxomycetes (Spiegel, 1981a, 1982, 1991; Spiegel et al., 1995).

It is assumed that the structure of zoospore cytoskeleton may be used in the taxonomy and phylogenetic reconstructions of myxomycetes in general, especially because the structure of flagellar apparatus of mycetozoa is highly conservative (Spiegel et al., 1995).

The results of ultrastructural investigations of zoospores and resting spores of Symphytocarpus impexus B. ing et Nann.-Bremek. (Stemoniida), zoospores of Arcyria cinerea (Bull.) Pers. (Trichiida), zoospores, amoeboid cells and resting spores of Lycogala epidendrum (L.) Fr. (Liceida) are presented here for the first time, as well as a comparative analysis of rootlet structure and homology among all mycetozoa including the unusual mycetozoa incertae sedis Hyperamoeba.

Material and methods

The specimens of sporocarps of Symphytocarpus impexus (LE 49074; 23.09.1995; Russia, Valaam Isl.), Arcyria cinerea (LE 49107; 03.08.1995; Russia, Norilsk), and Lycogala epidendrum (LE 48975; 04.08.1995; Russia, Norilsk) used in the study are deposited in the herbarium of Komarov Botanical Institute (LE) of the Russian Academy of Sciences.

To obtain high concentrations of zoospores in culture, the method of alternate wetting and drying of spores (Gilbert, 1928, 1929; Smart, 1937, 1938a, 1938b; Elliott, 1949) was modified. The spore suspension in water was dried on the slide during 24 h and was rewetted with distilled water. The optimal temperature for spore germination of S. impexus was found to be 24°–26°C; its spores from our collection showed almost 100% germination within 72 hours, those of Arcyria cinerea, 32 hours and those of Lycogala epidendrum, 84 hours. After that period, the percentage of spore germination decreased sharply. Swarm cells of S. impexus as well as those of A. cinerea and L. epidendrum correspond to completely flagellated type of life cycle according to Ross’ classification (Ross, 1957). In our cultures swarm cells of S. impexus were registered even up to two weeks after the start of cultivation. After 4–5 days of cultivation, some of the zoospores transformed into amoebae but could resume their original shape and flagella after addition of fresh distilled water to the culture. Swarm cells of A. cinerea and L. epidendrum were present in the
cultures for up to 3-6 days and all zoospores transformed into amoebae during this period. After that the transformation of amoebae into zoospores did not occur.

For light microscopy the living zoospores have been fixed in OsO4 vapour for 1 minute and observed with Peraval-Interphako microscope (Zeis, Germany) equipped with a video-camera.

For transmission electron microscopy (TEM), zoospores were allowed to swim in a thin layer of water on a slide after their release from spores. They were fixed first with OsO4 vapour for 15 minutes, then concentrated by centrifugation at 1000g. Further fixation was carried out with a mixture of 2% glutaraldehyde on 0,05M phosphate buffer (pH 7.2) and 1% OsO4 on the same buffer within 1 hour on ice. The fixed pellet of cells was then rinsed twice for 15 min in distilled water (this leads sometimes to cell destruction, but makes the cytoskeletal elements better visible), dehydrated in an ethanol series and embedded in Epon. Blocks were serially sectioned with a diamond knife on a Reichert Ultracut ultramicrotome, mounted on formvar-coated slot grids, and post-stained with uranyl acetate and lead citrate. Sections were viewed on a JEM 100S electron microscope operating at 80 kV. For 3-dimensional reconstruction of the cytoskeleton, serial sections of at least 6 cells of each species were examined.

Conventions of cell orientation, rootlet nomenclature, and basal bodies numbering are those of Wright with co-authors (Wright et al., 1979, 1980; Wright, 1982).

**ABBREVIATIONS**

- ag - Golgi apparatus, bb - basal body, bb1 - basal body 1 (of the anterior flagellum), bb2 - basal body 2 (of the posterior flagellum), co - fibrilar core in mitochondrial matrix, cv - contractile vacuole, ff - fibrillar feet, fl - flagella, fr - fibrillar ring, or diaphragm, gr - granule, m - mitochondrion, mtoc - microtubular and fibrillar structures are connected with the cytoskeletal elements associated with the Golgi apparatus.

**Results**

The exsporulation of each species produced flagellates with 2 flagella, as shown in Figs 1 and 2. The flagella are formed inside the spore (Figs 3, 4). After 1-2 days, flagellates start to transform into amoebae. They lose first one flagellum to become uniflagellated cells, and then after loosing the 2nd flagellum transform into amoeboid cells (Fig. 5). At higher magnification, flagellates can be seen to have a prominent nucleus with a nucleolus in the anterior part of the cell, and a contractile vacuole at the posterior end (Figs 1, 2). There is a granule located between the nucleus and flagellar basal body, which corresponds to the cytoskeletal elements associated with the Golgi apparatus.

The general organisation of the cell is very similar in all three species. There are two basal bodies at the apical end of the cell, producing two smooth flagella. The nucleus is normally located in the vicinity of the flagellar bases (Figs 3-5). The latter also occur in amoeboid cells without flagella (Fig. 5). There are normally 1 or 2 dictyosomes of the Golgi apparatus between flagellar bases and the nucleus (Figs 3, 4). Mitochondria with branched tubular cristae are scattered through the cytoplasm. Some small food vacuoles can be seen at the periphery of amoeboid cell (Fig. 5).

The cytoskeleton of zoospores includes fibrillar and microtubular elements that are connected to flagellar basal bodies. It has many common features in all three species. The most complete set of these structures was found in *Symphytocarpus impexus*.

**Symphytocarpus impexus** (Figs 1-22)

Flagellates have two flagella (Figs 1, 2). Spores are covered by the spore wall composed of thick homogeneous material with short conical projections (Figs 3, 4). Mitochondria have tubular branched cristae without any inclusions either in the matrix or in the cristae (Figs 4, 5). Small dictyosomes of the Golgi apparatus are located near the nucleus and flagellar basal bodies (Figs 3-5).

Flagella contain a standard ‘9+2’ axoneme that emerges from the basal bodies. There are 2 basal bodies at the apical end of the cell. The anteriorly directed flagellum is motile; the second flagellum is shorter, normally passive and directed posteriorly. The microtubular and fibrillar structures are connected with two basal bodies, that is, they are flagellar rootlets. The basal bodies are located at an angle less than 90° to each other, and the basal body of posterior flagellum (bb2) attaches to the distal half of basal body of anterior flagellum (bb1) (Figs 3, 19).

Transitional fibres connect the distal part of basal bodies to the flagellar membrane at the level where the transverse plate crosses the axoneme (Fig. 15). Close to the proximal end of each basal body, there is a diaphragm-like thickening, or fibrillar ring (Figs 14, 15, 19). Fibrillar threads extend from the fibrillar ring of bb1 towards the nucleus, ending in a microtubule-
Figs 1-5. Light and electron micrographs of *Symphytocarpus impexus*. 1-2 - light microscopical images (DIC) of living zoospores (second flagellum on fig. 1 is out of focus) and spore, 3-4 - ultrastructure of mature zoospore inside the spore, 5 - structure of amoeboid cell after loss of the flagella. For abbreviations see Material and methods.
organizing centre (MTOC) (Figs 14, 15, 21). This MTOC gives rise to microtubules of rootlet 1 (r1). The fibrillar ring is also present at the base of bb2, and the connectives from this ring join it to the lateral surface of bb1 (Figs 11, 15, 19).

A structure that is important in understanding the cytoskeleton arrangement is the short striated structure (SSF). It is located on the dorsal-right surface of bb1 and composed of 8 prominent fibrillar strips crossing the triplets at right angles (Figs 6-11, 14, 15, 17-19). The most prominent upper strip of SSF initiates the microtubules of r2, radiating posteriorly from it like a fan and forming a broad arc of microtubules (Figs 6-9, 16-18). More than 30 microtubules of r2 begin closely packed in a ‘c’ shape with a very long left arm (Figs 10-12), more or less parallel to bb1, being also connected with other strips of SSF, and fan out to surround the dorsal and lateral sides of the nucleus. The right wing of SSR extends to the posterior parakinetosomal structure (PPKS) that

Figs 6-15. Structure of flagellar apparatus in zoospores of *Symphytocarpus impexus*. 6-12 - series of consecutive cross sections through the basal body 1 (view from the tip of the flagellum to the base); Arrow shows 2 deviated microtubules of r5. 13-15 - series of selected longitudinal sections through the flagellar apparatus (direction from the left to the right side of the cell). For abbreviations see Material and methods.
joins to bb2 (Figs 6-12, 17). The left part of SSR gives rise to the microtubular band r3 (Figs 6-12). Rootlet 3 is composed of 6 microtubules, goes to the left side of the cell and anteriorly (Figs 6-15, 20, 21). Thus, SSF acts as a microtubule organizing centre for r2 and r3.

The posterior parakinetosomal structure (PPKS) is very well-developed, and is composed of at least 4 dense layers of fibrillar material, which are spaced by 3 thick layers of the more electron translucent material of thin filaments (Figs 11, 12, 15-17). It covers the upper and right side of bb2, and its lowest fibers are associated with microtubules of r4 (Figs 10-12, 15-17). The rootlet r4 follows bb2 from its right side as a band composed of 7 microtubules (Fig. 10).

Two microtubules of r5 rootlet originate from the proximal end and under bb2. They pass under bb2 turning to the left and continuing along the left side of bb2 as a 2nd microtubular band (Figs 10, 11, 19). The

Figs 16-21. Serial longitudinal sections of zoospore flagellar apparatus of *Symphytocarpus impexus* (direction from the right to the left side of the cell). For abbreviations see Material and methods.
microtubules of both r4 and r5 rootlets continue posteriorly and terminate just past the nucleus. The general scheme of this flagellar apparatus structure is presented in Fig. 22.

LYCOGALA EPIDENDRUM (FIGS 23-39)

A distinctive feature of this species is the spore wall with flat outer thorns on the surface (Fig. 23), very different from those of S. impexus. The cytoplasm of the spores is highly vacuolised.

The amoeboid cells have a weakly developed endoplasmic reticulum (Fig. 24). The nuclear heterochromatin is not so conspicuous as in zoospores, and a characteristic well-developed nucleolus is present. The mitochondria in amoebae, flagellates and spores of L. epidendrum have a dense core in the matrix, oriented along the longitudinal axis of mitochondrial body (Figs 23-24).

The basal bodies of L. epidendrum zoospores lie at angles less than 90° to each other, bb2 attaching to the proximal part of bb1 (Fig. 28). There are no transverse plates in flagellar transition zones, and the fibrillar ring is absent at the base of both basal bodies (Figs 26-29, 34). The transitional fibers are well developed and supported by dots of dark material (Figs 26, 31). Rootlet 1 has 2 slightly striated fibrillar feet which connect the MTOC of r1 to the lateral sides of both basal bodies (Figs 28-29).

The SSF has a distinctive appearance. Its right and dorsal part is composed of 4-5 strands covering the proximal half of bb1 (Figs 26-28). The upper strip is rather prominent and looks like a fibrillar collar attached to the middle part of bb1 at an angle of 45° (Figs 35, 36). The distal end of this collar reaches the level of the distal end of bb1 and almost totally surrounds this basal body (Figs 31-34). The microtubules of r2 radiate posteriorly from the distal ridge of that collar (Figs 31-36). The most dorsal part of SSF reveals the usual striation, and continues posteriorly even below the proximal end of bb1 (Figs 27, 34). The left end of SSF gives rise to 5 microtubules of r3, which is associated with the fibrillar collar (Figs 25-27, 36). The plane of r3 lies, unusually, at an angle of approximately 45° to the longitudinal axis of bb1 and orthogonal to the fibrillar collar (Fig. 36). The r3 passes to the left part of zoospore and then down towards the nucleus. The right end of SSF is connected to the PPKS which covers the upper-right side of bb2 (Figs 28-30, 37-38) like in S. impexus.

Fig. 22. A general scheme of zoospore flagellar apparatus (dorsal view) of Symphytocarpus impexus (A), and a scheme of the features of this species (B). For abbreviations see Material and methods.
Figs 23-38. The ultrastructure of *Lycogala epidendrum*. 23 - general structure of spore; 24 - general structure of amoeboid cell; 25-30 - series of selected longitudinal sections through the flagellar apparatus (direction from the left to the right side of the cell); 31-34 - series of selected cross sections through basal body 1 (view from the tip of the flagellum to the base). Arrows show a separate strand of ssf; arrowheads in fig. 33 show r3; 35 - disposition of the upper strand of ssf (arrow), 36-38 - serial sections showing the disposition of r3 and upper strand of ssf (arrow) (36), and rootlets of basal body 2 (37, 38). For abbreviations see Material and methods.
The bb2 gives rise to rootlet r4 with 7 microtubules passing from its right side beneath but close to PPKS. Rootlet r5 has 2 microtubules and passes from the left side of bb2 (Figs 27, 37, 38). There are profiles of 2 more microtubules close to r5 (Fig. 38). Their origin is not clear (Figs 37-38). Figure 39 shows those features of the cytoskeleton of *L. epidendrum* zoospores that are different from those of *S. impexus* and *L. epidendrum*.

**Arctria cinerea** (Figs 40-51)

The general structure of the spores, amoeboid and flagellated cells of *A. cynerea* is similar to that of *L. epidendrum*. The mitochondria contain tubular branched cristae with an electron dense core oriented along the longitudinal axis of mitochondrion (Fig. 40). The basal bodies are normally oriented at approximately 120° to each other (Figs 41, 43, 44). The transverse plate is inconspicuous (Figs 41, 44). There is no fibrillar ring at the proximal end of bb1. Rootlet 1 arises at bb2. Its fibrillar feet are very short, and the inconspicuous MTOC produces a few microtubules (Figs 43, 44, 50).

The SSF has 5 strands applied to the dorsal surface of bb1, and looks less well developed than in *S. impexus* (Figs 42, 44). The uppermost strand forms almost a complete circle around and close to the bb1 and gives rise to the microtubules of r2 (Figs 48-49). There are about 20 microtubules and they form a more narrow fan than in two previous species. The SSF continuation on the right side of flagellar kinetosome serves as a fibrillar connective between both basal bodies, and also forms a prominent fibrillar protrusion down the bb1 (Figs 42, 44). The left end of SSF gives rise to the 4 microtubules of r3 which passes parallel to bb2 (Figs 48, 50).

PPKS is located at the same place as in *S. impexus* and *L. epidendrum*. Rootlet r4 composed of 6-7 microtubules passes beneath the bb2 from its right side, and r5 of 2 microtubules originates from the left side of bb2 (Figs 42, 43, 50). The microtubules of r4 are rather long and make contact with the nucleus (Fig.41). Figure 51 shows the features of cytoskeleton of *A. cinerea* zoospores that are different from those of *S. impexus* and *L. epidendrum*.

**Discussion**

Common organelles of flagellated and amoeboid cells (nucleus, dictyosomes and endoplasmic reticulum) are the same in all three species. The differences concern mitochondria and flagellar apparatus.

**Mitochondria.** In all three species investigated there are mitochondria with tubular branched cristae as in other mycetozoa (Patterson, 1999). *A. cynerea* and *L. epidendrum* have a fibrillar core in the mitochondrial matrix. It looks like a filamentous nucleoid and may be mitochondrial DNA. This structure is not common for the protists but occurs in the myxogastrids *Physarum cinereum* and *Didymium nigripes* (Schuster, 1965) and in *Hyperamoeba* (Karpov and Mylnikov, 1997; Zaman et al., 1999; Walker et al., in press). Similar structures have been seen in other eukaryotes having tubular or vesicular cristae in mitochondria, e.g., in *Acanthamoeba* species (Carosi et al., 1977) and the bicosoecid *Siluania monomastiga* (Karpov et al., 1998).

**Flagellar apparatus.** This study has confirmed that the flagellar apparatus of the three species studied here is similar to that observed in other myxogastrids such as *Physarum polycephalum* (Wright et al., 1979), *Echinostellium bisporum* (Spiegel, 1981a) and *Semicorula liquescens* (Haskins and McGuiness, 1988). Therefore, it may be accepted in general that the flagellar apparatus structure in myxogastrids is highly conservative, as it has been established earlier (Spiegel et al., 1995; Karpov, 1997), and which is true of most monophyletic protist groups. There are some minor differences which might be useful in determining evolutionary scenarios at species and genus level.

The protostelids have the same elements of flagellar apparatus, which, together with similarities in other characters (life-cycle, spore structure, plasmodium organisation), leads to the conclusion that the Eumycetoza (Protostelia + Myxogastria) as a whole are a monophyletic group (Spiegel and Feldman, 1989). This opinion is widely accepted (Karpov, 1990; Patterson, 1993, 1999; Cavalier-Smith, 1995, 2002; Spiegel et al., 1995; Baldauf et al., 1997). *Hyperamoeba flagellata* (Karpov and Mylnikov, 1997; Zaman et al., 1999) shares most flagellar apparatus characters with
Myxomycetes, and in spite of its inability to produce fruiting bodies has been placed within the Myxomycetes incertae sedis (Karpov, 1997, 2000). We compare below the characters of all investigated myxogastrids, protostelids and Hyperamoeba (Table 1).

**Basal bodies.** The orientation of the basal bodies relative to other elements of the flagellar apparatus is similar in all mycetozoa. Normally their placement to each other is nearly orthogonal (Spiegel, 1991). In Physarum polycephalum this angle is about 60° (Wright et al., 1979). It is less than 90° in Lycogala and Symphytocarpus (present paper), and in Cavostelium bisporum (Furtado and Olive, 1970). In Arcyria this angle varies from 120 to 100° (present paper). Hyperamoeba has basal bodies at oblique (> 90°C) angles to each other (Karpov and Mylnikov, 1997; Walker et al., in press), as do the myxomycete-like protostelids Clastostelium recurvatum and Ceratiomyxa fruticulosa (Nelson and Scheetz, 1975; Spiegel, 1981a; Spiegel and Feldman, 1988) and the myxogastrid Stemonitis pallida (Ishigami, 1977). The posterior basal

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1 It is known from other groups of protists (i.e. green algae) that the angles may change in cell cycle, depending on how the cell moves or matures, but this has not been shown for myxomycetes.

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Figs 40-50. The ultrastructure of Arcyria cinerea. 40 - general structure of amoeboid cell; 41 - structure of the anterior half of zoospore; 42-45 - series of consecutive longitudinal sections of flagellar apparatus (direction from the left to the right side of the cell); 46-49 - series of transversal sections through basal body 1 (view from the tip of the flagellum to the base); 50 - oblique section of basal body 2, showing all main flagellar rootlets. For abbreviations see Material and methods.
body is absent in Planoprotostelium aurantium (Spiegel, 1982).

The substructure of the basal bodies is quite conservative, but there are some structural differences at the proximal and distal ends.

The diaphragm-like structure, or fibrillar ring at the base of bb1, from which the fibrillar feet of r1 normally arise, is present in the myxogastrids Physarum polycephalum (Wright et al., 1979), Stemonitis pallida (Ishigami, 1977), Symphytocarpus impexus (present paper), and in Semimorula liquescens (Haskins and McGuiness, 1988). Among the protostelids, it was found in Cavostelium bisporum (Furtado and Olive, 1970) and Planoprotostelium aurantium (Spiegel, 1982). In the latter species the microtubules of r1 arise from this fibrillar ring. This structure is present in Hyperamoeba (Figs 8, 12 in: Karpov and Mylnikov, 1997; Walker et al., in prep). In P. polycephalum (Wright et al., 1979, 1980) the bb2 has a ring structure from which the fibrillar material extends to bb1. It occurs in S. impexus (present paper), and may reflect the developmental destiny of the posterior basal body which is to become the bb1 in the next cell generation (Wright et al., 1980).

The fibrillar feet that end in the MTOC and from which the microtubules of r1 arise, may be connected with one or both basal bodies. They may have prominent striated structure, or inconspicuous non-striated one. The presence/absence of striation was considered to be significant for myxomycetes phylogeny (Spiegel and Feldman, 1988). These characters vary among the myxomycetes. The feet are striated in myxomycete-like protostelids Clastostelium recurvatum and Protosporangium articulatum (Spiegel et al., 1986; Spiegel and Feldman, 1988), and Hyperamoeba dachnaya (Walker et al., in press). The feet are normally connected to bb1 in the majority of myxomycetes, including Hyperamoeba (Table 1). They are connected to both basal bodies in the myxogastrid Lycogala epidendrum (present paper) and myxomycete-like protostelids Clastostelium recurvatum and Protosporangium articulatum (Spiegel et al., 1986; Spiegel and Feldman, 1988). In one case (Arcyria cinerea) the feet attaches to the bb2 (present paper).

In all cases (with the exception of Echinostelium minutum, which has more than 2 basal bodies), if the fibrillar feet are connected with bb1, it is: (a) not prominent and striated, and (b) connected with bb1 via the fibrillar ring (Table 1). At the same time, the striation of fibrillar feet is present in all cases if they are connected with both basal bodies. Hyperamoeba dachnaya is unusual in this respect. It has striated feet, connected with bb1 (Walker et al., in press). On this background the connection of the feet to bb2 in Arcyria cinerea looks very unusual.

**Rootlet system.** Rootlet 1 radiates from the MTOC microtubules in the majority of myxomycetes (Table 1). It is missing entirely from Cavostelium apophysatum (Spiegel, 1981a), Ceratiomyxa fruticulosa (Spiegel, 1981b), and Ceratiomyxa tahiensis (Furtado and Olive, 1971; Spiegel, 1981a). The number of microtubules varies, as does the shape and size of the MTOC. The microtubules of r1 always radiate from the MTOC as singlets or pairs. The lateral microtubules are normally short, and the posteriorly directed ones are longer and often connect to the nuclear surface.

Rootlet 2 is a curtain of microtubules arising laterally to, and running parallel to bb1 in all investigated myxogastrids (Haskins, 1978; Wright et al., 1979, present paper), protostelids (Spiegel, 1981a,b, 1982, 1988; Spiegel et al., 1986; Spiegel and Feldman, 1988; Haskins and McGuiness, 1988), and Hyperamoeba (Karpov and Mylnikov, 1997; Walker et al., in press) (Table 1). The microtubules appear from the upper strand of SSF, but are also connected to bb1 via the other strands at the most dorsal and right side of bb1. The upper strand may make an almost complete circle around bb1 as in Lycogala and Arcyria and produce comparatively few microtubules, or may have a long left wing of the upper strand with many microtubules as in Physarum, Symphytocarpus, Stemonitis or Semimorula. This upper strand can arise from the distal part of bb1 as in most myxogastrids, or from the middle of bb1 as in Lycogala and Protosporangium. In the latter two genera the upper strand may be unusually long, forming a collar-like structure around the bb1 (Lycogala).

Rootlet 3 is composed of two or about seven microtubules arising laterally and running perpen-
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<th>PKS</th>
<th>SSR (number of microtubules)</th>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td><em>Stemonitis pallida</em></td>
<td>&gt;90</td>
<td>+</td>
<td>myxox</td>
<td>ns</td>
<td>bb1</td>
<td>+</td>
<td>9-10</td>
<td>+</td>
<td>+</td>
<td>4-5</td>
<td>7-8</td>
<td>?</td>
<td>?</td>
<td>Ishigami, 1977</td>
</tr>
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<td><em>Symphytocolus cumptus</em></td>
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<td>+</td>
<td>myxox</td>
<td>ns</td>
<td>bb1</td>
<td>+</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>+</td>
<td>present paper</td>
</tr>
<tr>
<td><em>Lycogala epidendrum</em></td>
<td>&lt;90</td>
<td>-</td>
<td>myxox</td>
<td>str</td>
<td>bb1</td>
<td>+</td>
<td>5-6</td>
<td>+</td>
<td>+</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>present paper</td>
</tr>
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<td><em>Arcinia cinerea</em></td>
<td>&gt;90</td>
<td>-</td>
<td>myxox</td>
<td>ns</td>
<td>bb2</td>
<td>+</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>4</td>
<td>7?</td>
<td>2?</td>
<td>?</td>
<td>present paper</td>
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<td>90</td>
<td>-</td>
<td>myxox</td>
<td>prot</td>
<td>bb1</td>
<td>+</td>
<td>5-6</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>6-7</td>
<td>2+2</td>
<td>+</td>
<td>Spiegel et al., 1986</td>
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<tr>
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<td>-</td>
<td>myxox</td>
<td>prot</td>
<td>bb1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>2+</td>
<td>?</td>
<td>Spiegel, Feldman, 1988</td>
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<tr>
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<td>-</td>
<td>myxox</td>
<td>prot</td>
<td>bb1</td>
<td>-</td>
<td>+</td>
<td>3+</td>
<td>-</td>
<td>+</td>
<td>2</td>
<td>6</td>
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<td>Nelson, Scheetz, 1975; Spiegel, 1981</td>
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<td>sbb</td>
<td>+**</td>
<td>prot</td>
<td>ns</td>
<td>bb1</td>
<td>+</td>
<td>4-5</td>
<td>+</td>
<td>+</td>
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<td>5-6</td>
<td>-</td>
<td>-</td>
<td>Spiegel, 1982</td>
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<tr>
<td><em>Cavostelium bisporum</em></td>
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<td>+</td>
<td>prot</td>
<td>ns</td>
<td>bb1</td>
<td>+</td>
<td>+</td>
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<td>6</td>
<td>+</td>
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<td>+</td>
<td>Furtado, Olive, 1970</td>
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<td>uncertain</td>
<td>ns</td>
<td>bb1</td>
<td>+</td>
<td>7-8</td>
<td>+</td>
<td>+</td>
<td>5</td>
<td>7</td>
<td>2</td>
<td>?</td>
<td>Karpov, Myshnikov, 1997</td>
</tr>
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<td><em>Hyperamoeba dachnaya</em></td>
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<td>+</td>
<td>uncertain</td>
<td>str</td>
<td>bb1</td>
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<td>8</td>
<td>+</td>
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<td>10</td>
<td>2</td>
<td>+</td>
<td>Walker et al., in press</td>
</tr>
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</table>

**Notes:** “+” – present, “-” – absent, “?” – questionable, > - more, < - less, sbb – single basal body, bb – basal bodies, myxox – myxogastriads, ns – nonstriated, prot – protostelids, str – striated, * - 3 or more, ** - microtubules of r1 originate from the ring. Digits for r3-r5 show the number of microtubules in a rootlet.
diccular to bb1 in *Physarum, Stemonitis, Symphytocarpus* and in some other mycetozoa (Table 1). It may be located at an angle of 45° to bb1 in *Semimorula, Lycogala, Planoprotostelium, Cavostelium,* and *Hyperamoeba* (Table 1). These differences may be a consequence of the position of the microtubular fan of r2 that passes parallel to the dorsal part of bb1. If they are not connected to bb1 by the SSF at its left side, the microtubules deviate from the strict parallel direction and form an angle of approximately 45° to bb1. Where the r3 microtubules are close to the microtubules of r2 (one could even think that the latter may be the secondary microtubules of r3), they follow the same angle of deviation. The small number of microtubules (2) in r3 was interpreted as an apomorphic character for myxomycete-like protostelids (Table 1) (Spiegel and Feldman, 1988). The extended data show that it may be really essential, as in other myxomycetes their number is more than 5, varying from 5 to 7 (Table 1).

The number of microtubules in r4 and r5 associated with bb2 is very conservative. It corresponds in general to the formula 7+2 in most of species (Table 1). The deviations from this formula may be associated with poor study (like in *Physarum flavicomum*) or with the fact that microtubules in different parts of the rootlet were counted. The latter consideration concerns the phenomenon of fusion of two microtubules of r4 to 2 microtubules of r5 after leaving the bb2. As a result, the r5 has at its distal end 4 microtubules instead of original 2 (Fig. 22). This microtubular fusion was demonstrated for 6 species (Table 1) of different mycetozoa. One more set of 1-2 microtubules, also considered as r5 (Spiegel, 1991) appears to be from the bb1 or MTOC. These microtubules was not shown for many species, therefore we don’t discuss them here.

Rootlet 4 is always closely associated with the right side of bb2, but its origin is connected with PPKS. This has been shown for all well-investigated myxogastrids and protostelids (Spiegel, 1981a; Wright et al., 1979; present paper). PPKS and r4 are present even if bb2 is absent (Spiegel, 1982, Table 1). Rootlet 5 is absent in those species with one basal body in which PPKS is connected with the right wing of SSF (Spiegel, 1981a; Wright et al., 1979, present paper). This leads us to conclude that r4 is a rootlet of bb1, and not of bb2.

The SSF is the most essential element of the rootlet system in all myxomycetes. It gives rise to 3 microtubular rootlets: r2, r3 and r4. This MTOC occurs in all investigated zoospores of myxomycetes and in *Hyperamoeba* (Table 1). The presence of SSF and PPKS are the 2 most conservative characters of the group, and may prove to be apomorphic ones for the Eumycetozoa as a whole.

The taxonomy and phylogeny of myxomycetes is traditionally based on the morphology of the sporangial body (sporophores) (Martin and Alexopoulos, 1969; Martin et al., 1983). Detailed investigations of morphogenesis and the sporophore ultrastructures have challenged traditional views (Eliasson, 1977; Collins, 1979; Clark, 2000; Novozhilov and Goodkov, 2000). At present the relationships of the representatives of five basic groups: *Echinosteliida (= Echinosteliales), Trichiida (= Trichiales), Stemonitida (= Stemonitales), Physarida (= Physarales) and Liceida (= Liceales) are unresolved.

Despite the fragmentary nature of the data on the flagellar apparatus of zoospores, it is normally accepted to be based upon the same plan in all myxogastrids (Aldrich, 1968, 1969; Ishigami, 1977; Haskins, 1978; Wright et al., 1979, 1980; Spiegel, 1981a, 1981b, 1982, 1988, 1991; Spiegel et al., 1986; Spiegel et al., 1995), even though this structure strongly varies even within the same genus, as illustrated in our work. Differences in rootlet structure and fibrillar connections may help to characterise certain genera and may be used alongside with their traditional characters. The similarities of composition, positional arrangements, and associations with other organelles support the assertion that the rootlets in myxomycetes are homologous and the differences that we have observed contain potentially significant phylogenetic value.

References


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