A review of the taxonomy, biology and infection strategies of "biflagellate holocarpic" parasites of nematodes

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This review discusses the taxonomy, patterns of sporogenesis and modes of infection of a group of little studied holocapic pathogens of bactivorous nematodes (and rotifers) from terrestrial and marine habitats. These holocarpic obligate parasites have been traditionally placed within the "lagenidiaceous oomycetes" although *Haptoglossa* had been placed in the Saprolegniales. The nematode pathogens that will be discussed fall within the genera *Clamydomyzium*, *Gonimocheate*, *Haptoglossa* and *Myzocytiopsis*. The patterns of asexual and sexual sporogenesis will be described in detail in the light of recent ultrastructural studies that we have undertaken. We conclude by discussing the main infection strategies employed by these organisms which we categorise into active and passive types. In the former, zoospores actively locate their host (by chemotaxis) and encyst on the host surface immediately prior to infection. In the latter types, the zoospores or aplanospores rapidly germinate to form either specialised adhesive structures (in Myzocytiopsidalean species) or specialised infective cells (in *Haptoglossa*). These "primed spores" attach to or fire in response to host contact. From these studies we conclude that these groups are probably fairly diverse and it is likely that their taxonomic status will have to be substantially revised in the light of ongoing ultrastructural and molecular studies.

Key words: *Clamydomyzium*, *Gonimochaete*, *Haptoglossa*, *Myzocytiopsis*, nemotophagous fungi, oomycete, pathogenesis, taxonomy, ultrastructure, zoosporogenesis.

Introduction

These zoosporic nematophagous pathogens are holocarpic and infect mostly bacteriophagous nematodes (and rotifers) in habitats rich in organic material and where bacterial and nematode populations are high. Whilst the majority of known species occur in "wet" terrestrial habitats a number of species have been isolated from littoral marine habitats, where they infect marine nematodes (Newell *et al.*, 1977). Only *Gonimochaete latitubus* Newell, Cefalu and Fell has been exclusively described from marine hosts, whilst *Myzocytiopsis vermicola* (Zopf) M.W. Dick and *Haptoglossa heterospora* Drechs. have been reported from both marine and terrestrial ecosystems (Newell *et al.*, 1977). This scant record of occurrence of these organisms in

marine nematodes, however, may be due to the paucity of surveys in this habitat and use of unsuitable isolation techniques. A more systematic search for these pathogens in amongst populations of marine nematodes would almost certainly prove rewarding.

These pathogens produce simple holocarpic thalli within the host body that absorb the body contents as they grow. Some species appear only to reproduce asexually, by means of aplanospores, zoospores or chlamydospores, whereas others also produce sexual oospores. They are generally considered to be primitive organisms, although it is not known whether the simplicity of their host-dependent life cycles is due to early evolution or to reduction caused by their parasitism. The asexual spores produced from the sporangium may go through several changes to produce the final infective spore and these will be documented and discussed later in this review. Highly efficient infection strategies have evolved to ensure successful infection of moving animal hosts. This review will focus on the nematophagous pathogens. It is our intention to highlight the main features of this little known group of pathogens and to assess the diversity in spore development and infection strategies. We will summarise some of our recent ultrastructural studies on thallus and spore development within members of this group, for which little detailed cytological information is currently available. It is hoped that this account will increase the general awareness of these little studied organisms.

Taxonomic perspectives

A synoptic list of the species of zoosporic nematode and rotifer parasites that have so far been described is given in Table 1, using the recent taxonomic nomenclature proposed by Dick (1995, 1997). Traditionally most of these fungi were considered as members of the Lagenidiales within the biflagellate oomycetes (Sparrow, 1973).

The first biflagellate zoosporic nematode pathogen was described by Zopf (1884) and was placed in the genus *Myzocytium* (established by Schenk in 1858 for parasites of algae). Indeed this first isolate, *M. proliferum* var. *vermicola* (Zopf, 1884), was initially thought to be a variety of an algal parasite, but was later given a separate binomial, *M. vermicola* and recognised as an obligate parasite of nematodes (Fischer, 1892). Subsequently most of the biflagellate parasites of nematodes and rotifers were placed within the genera *Myzocytium* and *Lagenidium*, although the distinguishing criteria separating these two genera were rather ill defined and often contradictory. In an attempt to clarify this confused situation, recently all biflagellate parasites of nematodes and rotifers were separated from the algal parasites and combined in a new genus, *Myzocytiopsis* within the new order Myzocytiopsidales (Dick,

Table 1. Summary of species of parasites of nematodes and rotifers within the "oomycete fungi" using the taxonomic schemes proposed by Dick (1995, 1997).

Species		Species	
Myzocytiopsis bolata	n	*Chlamydomyzium anomalum*	n, z
M. distylae		*C. aplanosporum*	
M. elegans		*C. internum*	
M. fijiensis		*C. oviparasiticum*	
M. glutinospora	n, z	*C. septatum*	
M. humama		*C. sphaericum*	n, z
M. humicola	n, z	*Gonimochaete horridula*	n
M. indica		*G. latitubus*	n
M. intermedia	n, z	*G. lignicola*	n
M. lenticularis	n, z	*G. pyriforme*	n
M. microspora		*Haptoglossa dickii*	n, z
M. oophila		*H. elegans*	
M. osiris	n	*H. erumpens*	n
M. papillata	n, z	*H. heteromorpha*	n
M. parthenospora		*H. heterospora*	n
M. subuliformis	n	*H. humicola*	
M. vermicola	n, z	*H. intermedia*	
M. zoophthora		*H. mirabills*	
		H. zoospora	n, z

n = nematophagous; z = zoosporic

1997). The nematophagous species, *M. lenticularis* (G.L. Barron) M.W. Dick, was made the type species. A number of nematophagous *Myzocytiopsis* species have now been described (Barron and Percy, 1975; Barron, 1976a,b; Dick and Glockling, 2000) in addition to a number of species recorded only from adult rotifers (Sparrow, 1936; Karling, 1944) or their eggs (Sparrow, 1939; Karling, 1944). A total of nine species of *Myzocytiopsis* are nematophagous and seven of these produce zoospores (Table 1).

At the same time as *Myzocytiopsis* was erected, Dick (1997) created another new genus, *Chlamydomyzium*, from former members of the Lagenidiales, to accommodate species which produced chlamydospores (Fig. 27), rather than oospores, as their only "resting stage". The type species, *C. anomalum* (G.L. Barron) M.W. Dick, and *C. septatum* (Karling) M.W. Dick were transferred to this new genus from *Myzocytium*, whilst the rotifer egg parasite, *C. oviparasiticum* (G.L. Barron) M.W. Dick, was transferred from *Lagenidium*. Two further aplanosporic species parasitic on rotifers, *C. internum* Glockling and *C. aplanosporum* Glockling and a zoosporic nematode parasite, *C. sphaericum* Glockling (Glockling and Dick, 1997; Dick and Glockling, 2000) have also been recently added to this genus, making a total of six species (Table 1).

The genus *Gonimochaete* was initially considered to be a primitive member of the *Entomophthoraceae* in the Zygomycetes (Drechsler, 1946), but was later considered to be a lagenidiaceous fungus by Newell *et al.* (1977). Up to that time only further asexual *Gonimocheate* species had been described (Barron, 1973), but an oospore producing species, *G. lignicola* G.L. Barron, was subsequently added (Barron, 1985). There are four species in the genus (Table 1), all of which are parasitic on nematodes. The type species, *G. horridula* Drechs., has short truncate aplanospores (Drechsler, 1946), but in other species aplanospores are pyriform (Figs. 18, 19).

Finally, the genus *Haptoglossa* was cautiously placed in the Saprolegniales by Drechsler (1940). Inspite of its many unusual features, this genus continues to be placed in the oomycetes with Dick (1995) including it in the *Ectrogellaceae* in the most recent edition of the Dictionary of Fungi. The type species, *H. heterospora*, is aplanosporic but subsequently both aplanosporic and zoosporic species have been added to this genus (Davidson and Barron, 1973; Barron, 1988, 1989, 1990; Glockling and Beakes, 2000a,b) (Table 1). It seems likely that the current taxonomic grouping of these organisms outlined in Table 1 may need re-evaluation in the light of ongoing fine-structural and molecular studies.

Biology and development

Asexual sporulation in zoosporic Myzocytiopsidalean species

Although strictly host-dependent in the wild, a few species of *Myzocytiopsis* have been maintained in pure culture in artificial liquid medium, and the reinfection of nematodes from pure culture has been achieved (Glockling and Dick, 1997). In *Myzocytiopsis*, infection occurs after an attached spore penetrates the nematode integument with a narrow germ tube (Figs. 2, 11, 43). Initially thallus development takes the form of a continuous narrow hyphal-like system within the nematode body. Later, septa are laid down at intervals (Fig. 22 arrowed) and the divided compartments often swell and disarticulate into short segments (Fig. 28) which develop into the sporangia in which the asexual spores are formed (Figs. 1, 13, 14, 30-31, 34-36). These are released from sporangia via an evacuation tube or papilla which ruptures the host cuticle (Figs. 3, 5, 37). In species such as *M. intermedia* (G.L.Barron) M.W. Dick and *M. lenticularis* the sporangia release their contents as an undifferentiated viscous mass via this evacuation tube into a fine transparent vesicle. The cytoplasmic mass differentiates into fully cleaved biflagellate zoospores within this vesicle over the course of 10-15 min (Figs. 1a,b, 3, 4, 6 and 23). Ultrastructural examination of the protoplasm of *M. intermedia* in the

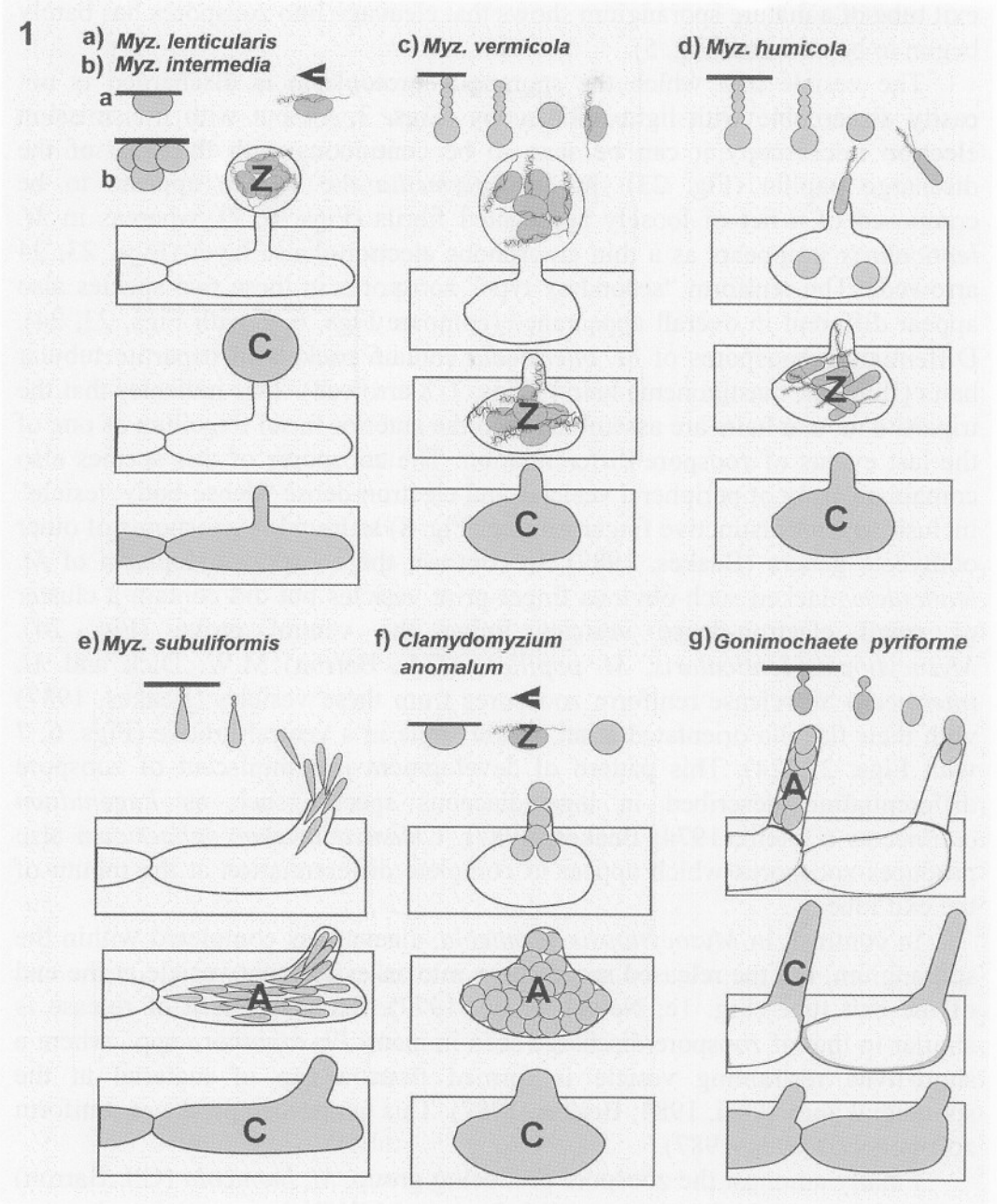


Fig. 1. Schematic diagram summarizing the variation in spore formation, release and development in **a.** *Myzocytiopsis lenticularis*; **b.** *M. intermedia*; **c.** *M. vermicola*; **d.** *M. humicola*; **e.** *M. subuliformis*; **f.** *Chlamydomyzium anomalum*; **g.** *Gonimochaete pyriforme*. Abbreviations: A = first appearance of aplanospores; C = cytoplasmic cleavage; Z = first appearance of motile zoospores.

exit tube of a mature sporangium shows that cleavage into zoospores has barely begun to be initiated (Fig. 5).

The vesicle into which the sporangial protoplasm is discharged is not easily discernable with light microscopy (Figs. 3, 4), but with transmission electron microscopy it can be seen to be continuous with the wall of the discharge papilla (Fig. 23). In *M. intermedia* the vesicle appears to be composed of a net of loosely aggregated fibrils (Figs. 6, 7), whereas in *M. lenticularis* it appears as a thin amorphous electron-dense layer (Figs. 23, 24 arrowed). The reniform "secondary type" zoospores in these two species also appear different in overall appearance (compare Figs. 6, 7 with Figs. 23, 24). Diffentiating zoospores of *M. intermedia* contain packets of tripartite tubular hairs (flimmer, mastigoneme hairs) (Figs. 7, 8 arrowed). This indicates that the tripartite tubular hairs are assembled onto the anterior tinsel flagellum as one of the last events of zoospore differentiation. The zoospores of this species also contain an array of peripheral vesicles and electron-dense "dense-body vesicle" inclusions with distinctive fingerprinting (Fig. 8) as found in zoospores of other oomycete genera (Beakes, 1989). In contrast, the zoospore cytoplasm of *M. lenticularis* lacked such obvious finger-print vesicles but did contain a cluster of small electron-dense vesicles lining the ventral grove (Fig. 24). *Myzocytiopsis lenticularis*, *M. papillata* (G.L. Barron) M.W. Dick and *M. intermedia* all release reniform zoospores from these vesicles (Beakes, 1987) with their flagella orientated at an obtuse angle in a ventral groove (Figs. 6, 7 with Figs. 23, 24). This pattern of development is reminiscent of zoospore differentiation described in lagenidiaceous species such as *Lagenidium callinectes* (Gotelli, 1974; Beakes, 1987). *Chlamydomyzium sphaericum* also produces zoospores which appear to complete differentiation at the mouth of the exit tube.

In contrast, in *Myzocytiopsis vermicola*, cleavage is completed within the sporangium, but the released spores flow into an evanescent vesicle at the end of the exit tube (Fig. 1c; Newell *et al.*, 1977). Such a pattern of release is similar to that of zoospore discharge seen in many *Phytophthora* spp., where a short-lived restraining vesicle is formed from a cap of material at the sporangial apex (Gisi, 1983; Beakes, 1987). This genus also produces reniform zoospores (Beakes, 1987).

Finally amongst the zoospore producing group, *M. humicola* (G.L.Barron) M.W.Dick and *M. glutinospora* (G.L.Barron) M.W.Dick are species showing direct release of motile zoospores (Fig. 1d) similar to that observed in the water mould *Saprolegnia* (Gay and Greenwood, 1966). The zoospores of these species are also of interest in that they are rather pyriform with flagella inserted sub-apically and have a basal concentration of storage vesicles (Fig. 14). These

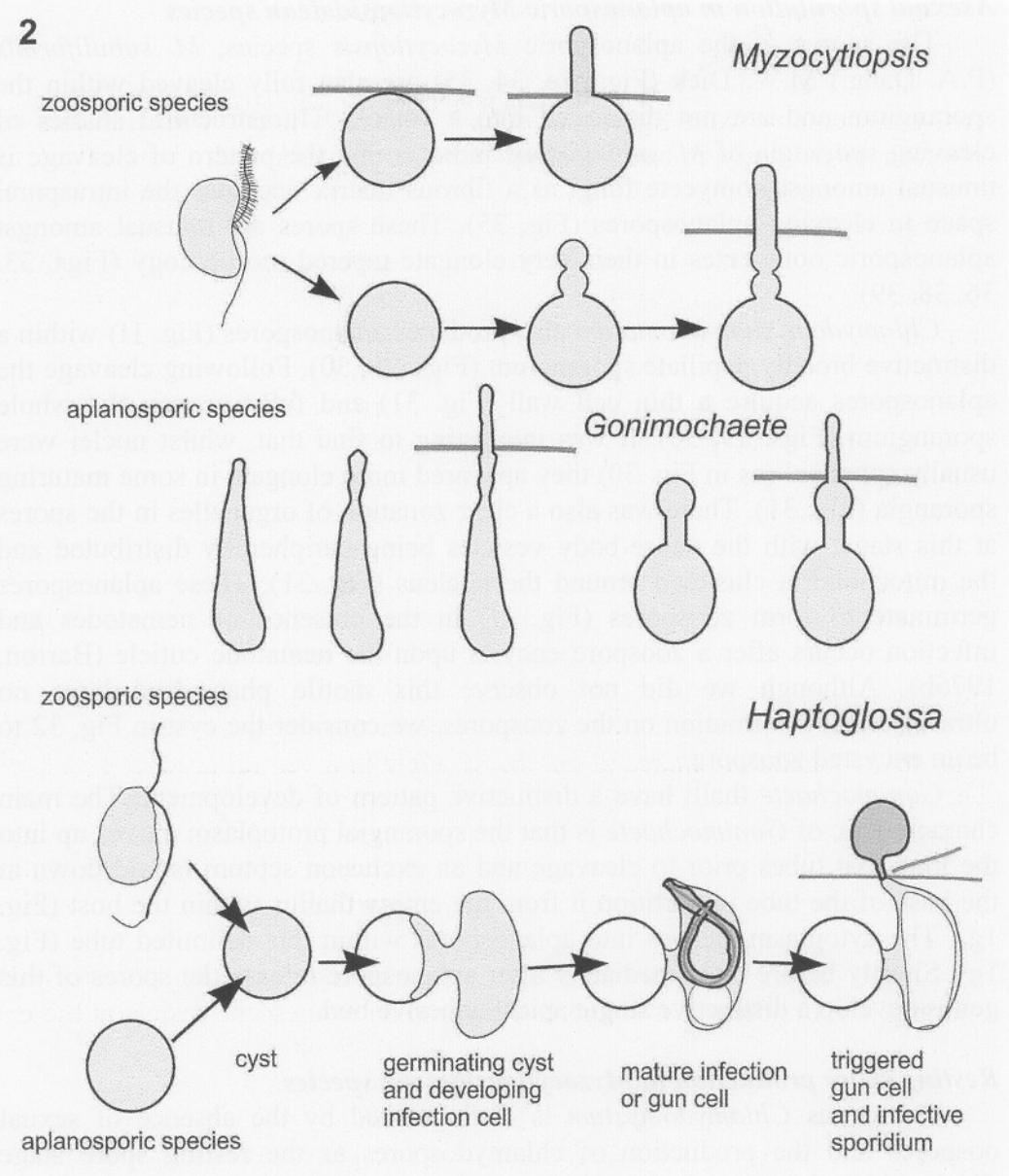


Fig. 2. Schematic diagram summarising nematode infection strategies in *Myzocytiopsis*, *Gonimochaete* and *Haptoglossa*.

zoospores consequently appear morphologically similar to the "primary type" of zoospores documented in *Saprolegnia* (Holloway and Heath, 1977; Beakes, 1987).

Asexual sporulation in aplanosporic Myzocytiopsidalean species

The spores of the aplanosporic *Myzocytiopsis* species, *M. subuliformis* (P.A. Dang.) M.W. Dick (Figs. 1e, 34, 35) are also fully cleaved within the sporangium and are not dicharged into a vesicle. Ultrastructural studies of cleaving sporangia of *M. subuliformis* indicate that the pattern of cleavage is unusual amongst oomycete fungi as a fibrous matrix occupies the intrasporal space in cleaving aplanospores (Fig. 35). These spores are unusual amongst aplanosporic oomycetes in their very elongate tapered morphology (Figs. 33, 36, 38, 39).

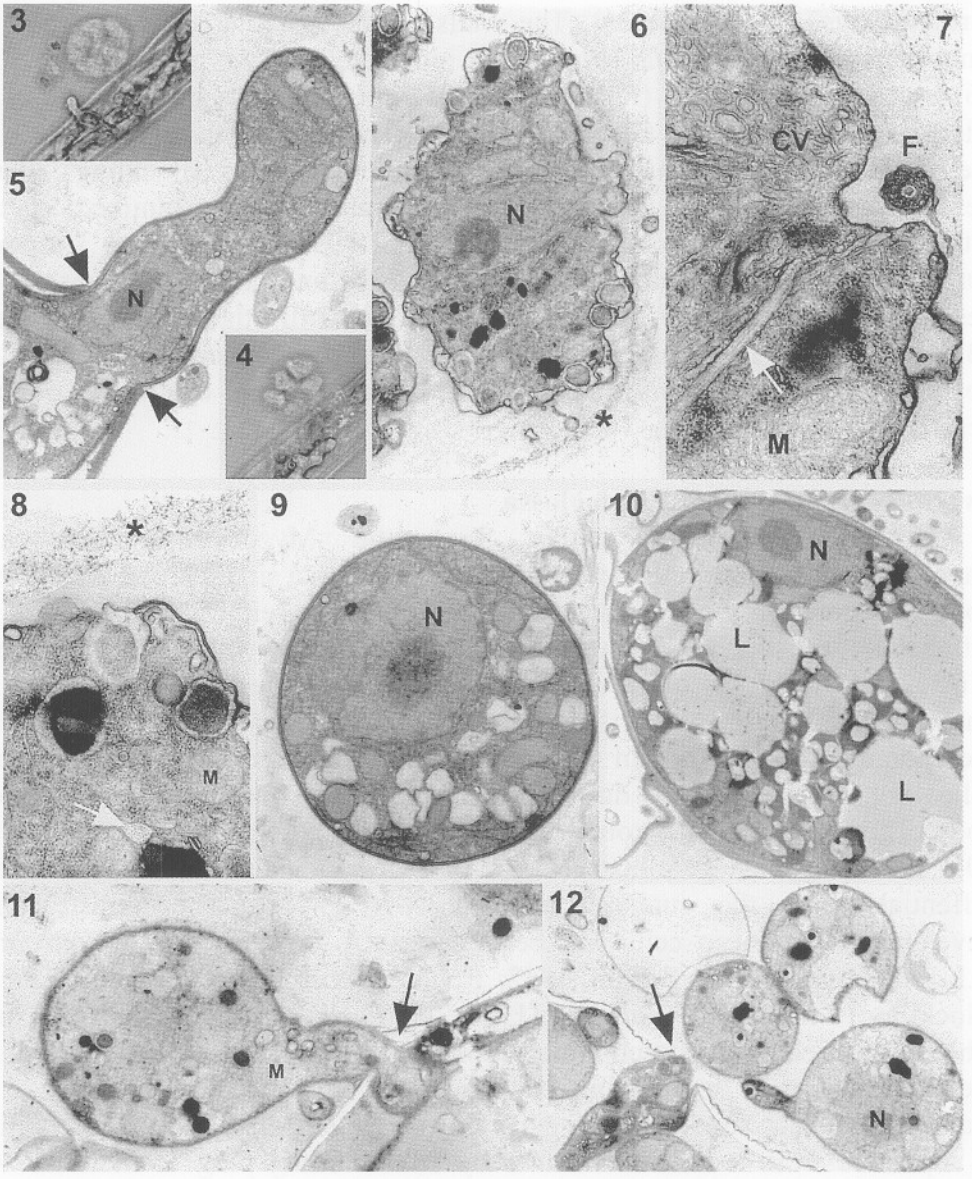
Chlamydomyzium anomalum also produces aplanospores (Fig. 1f) within a distinctive broadly papillate sporangium (Figs. 29, 30). Following cleavage the aplanospores acquire a thin cell wall (Fig. 31) and fully occupy the whole sporangium (Figs. 29, 30). It was interesting to find that, whilst nuclei were usually spherical (as in Fig. 30) they appeared more elongate in some maturing sporangia (Fig. 31). There was also a clear zonation of organelles in the spores at this stage, with the dense-body vesicles being peripherally distributed and the mitochondria clustered around the nucleus (Fig. 31). These aplanospores germinate to form zoospores (Fig. 1f) in the presence of nematodes and infection occurs after a zoospore encysts upon the nematode cuticle (Barron, 1976b). Although we did not observe this motile phase and have no ultrastructural information on the zoospores, we consider the cyst in Fig. 32 to be an encysted zoospore.

Gonimochaete thalli have a distinctive pattern of development. The main characteristic of *Gonimochaete* is that the sporangial protoplasm moves up into the long exit tubes prior to cleavage and an exclusion septum is laid down at the base of the tube to partition it from the empty thallus within the host (Fig. 1g). The cytoplasm cleaves into aplanospores within this delimited tube (Fig. 1g). Shortly before or immediately after aplanospore release the spores of this genus develop a distinctive single apical adhesive bud.

Resting spore production in Myzocytiopsidalean species

The genus *Chlamydomyzium* is distinguished by the absence of sexual oospores and the production of chlamydospores as the resting spore stage (Dick, 1997). In *C. anomalum* chlamydospores were formed abundantly in the nematode body (Fig. 27), apparently by sudden deformation of the thallus wall whereas in *C. septatum* these chlamydospores form by internal septation.

Sexual reproduction in these Myzocytiopsidalean fungi is achieved when pairs of neighbouring thalloid segments become differentiated into an antheridium and oogonium. The antheridial protoplasm then migrates into the oogonial cell, karyogamy occurs between the two nuclei and an oospore is



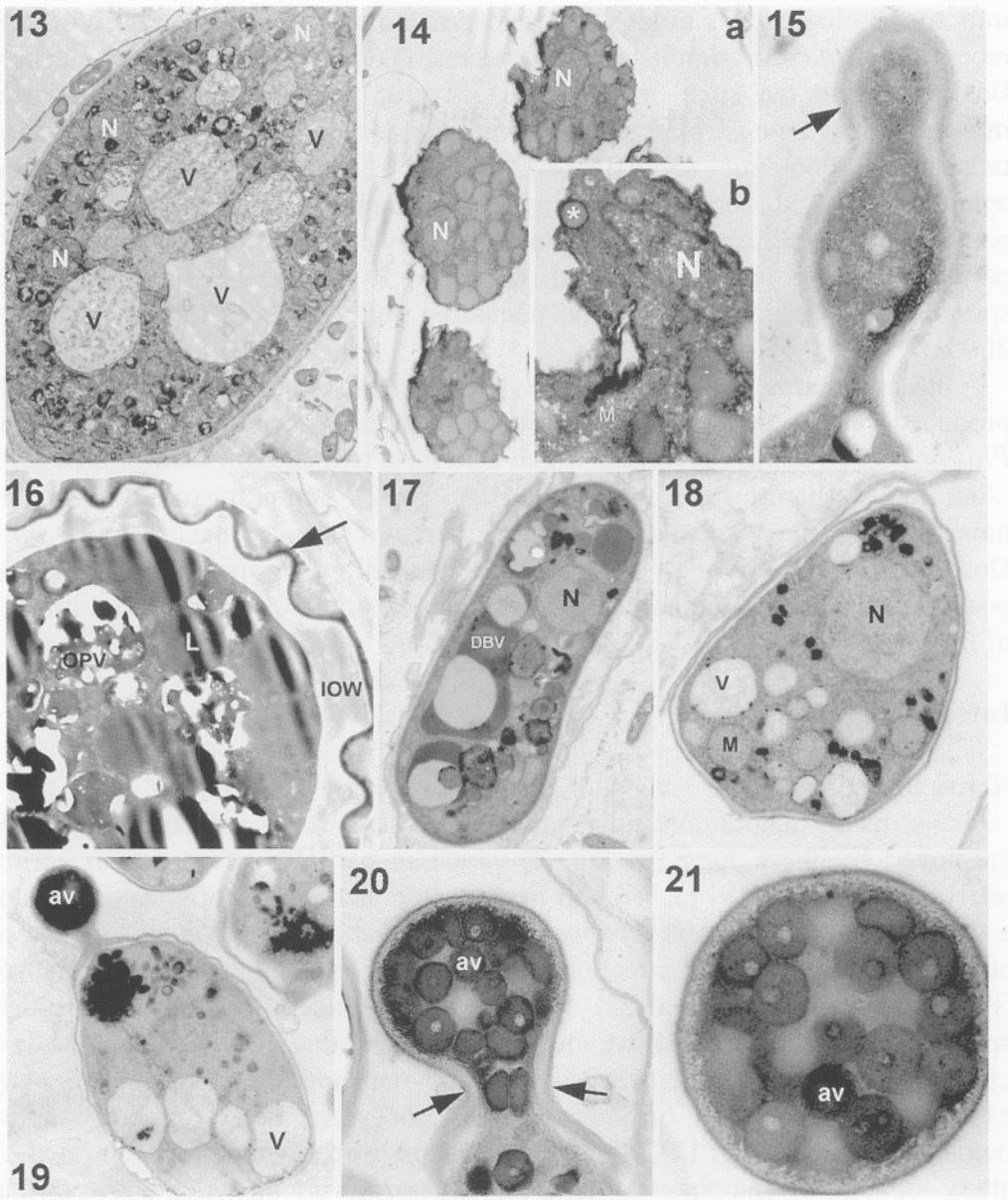
Figs. 3-12. *Myzocytiopsis intermedia.* Abbreviations: CV = contractile vacuole; F = flagellum; L = lipid; M = mitochondrion; N = nucleus. **3.** Nematode body containing mature sporangia with evacuation tubes and released mass of partially differentiated spores. **4.** Individual spores inside vesicle. **5.** Evacuation tube grown through nematode cuticle (arrowed) showing undifferentiated cytoplasm . **6.** Zoospore inside vesicle. **7.** Zoospore showing packet of tripartite tubular hairs (arrowed) near the site of flagellar attachment. **8.** Zoospore with peripheral vesicles and dense-body inclusion vesicles with fingerprinting and tripartite tubular hairs (arrowed). **9.** Encysted zoospore. **10.** Developing (post-fertilization) oospore. **11.** Encysted zoospore germinating to penetrate the nematode cuticle (arrowed). **12.** A cluster of encysted zoospores apparently penetrating the nematode cuticle at the same area (arrowed).

formed (Figs. 10, 16, 25, 26). The walls of the resulting oospores may be smooth as in *M. intermedia* (Fig. 10) or reticulate as in *M. glutinospora* (Fig. 16) and *M. lenticularis* (Figs. 25, 26). A few species such as *M. osiris* Glockling and *M. bolata* Glockling form oogonia apparently apogamously in the absence of an obvious male gametangium (Dick and Glockling, 2000). Sexual reproduction in *Myzocytiopsis* has rarely been examined at the ultrastructural level (Glockling, 1994; Dick, 1995). Developing oospores contain a single fusion nucleus and contain lipid and coalescing dense-body vesicles (Figs. 10, 16, 26) as described in oospores of other oomycete genera (Beakes, 1989). The lipid globules were largely peripheral in developing oospores of *M. glutinospora* as the spore contained large coalescing ooplast vesicles (Fig. 16).

***Asexual development in* Haptoglossa**

Haptoglossa species differ from the Myzocytiopsidalean and Lagenidialean species in that each infection produces a single broad thallus that does not become septate. Such thalli give rise to either aplanospores or biflagellate zoospores, which have whiplash flagella with long acronemes (Figs. 46, 47 and 48) quite unlike any other oomycete fungus. There is no known sexual phase in the life cycle. Cleavage in *Haptoglossa* occurs inside the sporangium (Fig. 49). In zoosporic species, there is a brief motile phase following spore release that disperses the spores away from the spent nematode body. The zoospores then round up, retracting their flagella, and encyst (Fig. 50). The cysts then germinate (Fig. 51) to form the infection spore which diffentiates into the gun cell at maturity (Fig. 52). The ultrastructural development of these cells has been described in detail for *H. mirabilis* G.L.Barron (Robb and Barron, 1982; Robb and Lee, 1986a,b) and *H. dickii* Glockling (Beakes and Glockling, 1998). Gun cells contain an inverted tube which is partially walled and which runs from the cell apex and coils around the nucleus before terminating back near the apical region (Fig. 52). At maturity, this tube fuses with the gun cell wall and opens to form a bore which is separated from a needle chamber by an occluding plug of material. An ultrastructural comparison of six species of *Haptoglossa*, 3 aplanosporic (Figs. 52, 53 a-c) and 3 zoosporic (Figs. 52, 53 d-f), showed that, despite great variation in gun cell size and shape, the internal structure was essentially similar (Fig. 52).

The aplanosporic species so far described are also heterosporic, producing two distinct sizes of aplanospore from separate, but morphologically similar sporangia (Drechsler, 1940; Davidson and Barron, 1973; Barron, 1989; Glockling and Beakes, 2000a). These different sized spores usually develop



Figs. 13-16. *Myzocytiopsis glutinospora*. Abbreviations: IOW = inner oospore wall; L = lipid; M = mitochondrion; N = nucleus; OPV = ooplast vesicle; V = vacuole. **13.** Young sporangium. **14a.** Zoospores inside sporangium. **14b.** Zoospore apex showing nucleus and peripheral vesicle (asterisk). **15.** Apical buds with adhesive coating (arrowed) from a germinated cyst. **16.** Mature oospore. **Figs. 17-21.** *Gonimochaete pyriforme*. Abbreviations: av = dense "apical vesicles" associated with infective bud; DBV = dense-body vesicles; M = mitochondrion; N = nucleus; V = vacuoles. **17.** Young thallus. **18.** Aplanospore inside evacuation tube. **19.** Aplanospore with apical bud. **20.** Spore apex showing site of bud formation (arrowed) and electron-dense bud vesicles. **21.** TS detail of vesicle-filled apical bud.

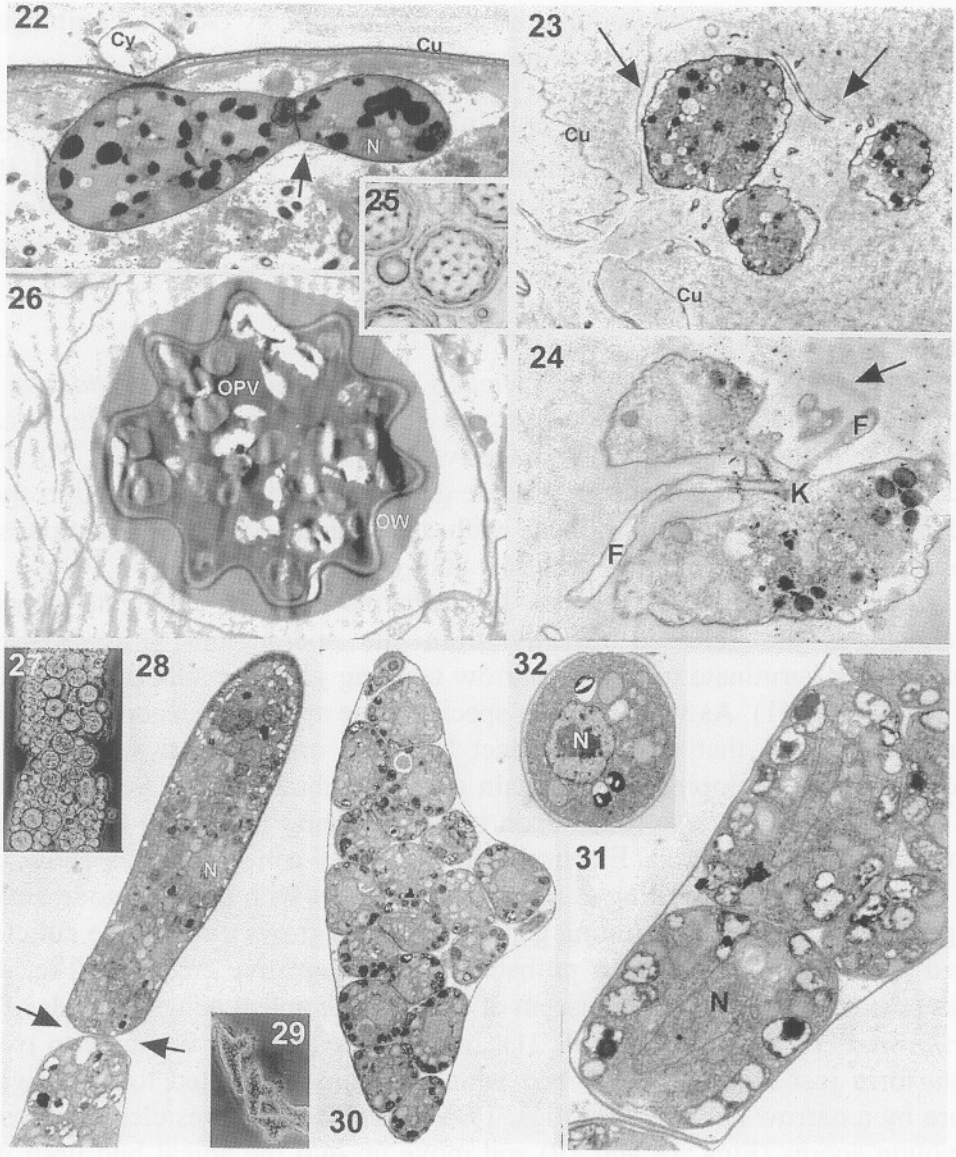
into morphologically similar large and small infection gun cells. We have recently discovered an aplanosporic species, *H. heteromorpha* Glockling and Beakes, where the small aplanospores develop into a completely different morphological type of infection cell whose mode of infection as yet remains unknown (Glockling and Beakes, 2000b). Most species release their aplanospores and zoospores from the sporangium through exit tubes or papillae. *Haptoglossa erumpens* Glockling, however, is unusual as it lacks a papilla. The spores are released when the wall of the highly swollen sporangium breaks and splits the nematode cuticle (Glockling and Beakes, 2000a).

Infection gun cells have often been compared to the infection cells produced by the plant-parasitic Plasmodiophoromycetes such as *Plasmodiophora* and *Polymyxa*, which also have two smooth whiplash flagella (Aist and Williams, 1971; Barr and Allan, 1982). Our ultrastructural studies have shown that whilst *Haptoglossa* shares many cellular features with Oomycetes and relatively few with Plasmodiophoromycetes, the genus possesses sufficient unique features to suggest it probably belongs to a lineage of its own.

Infection strategies

Active infection

We have diagrammatically summarised the main infection strategies employed by these fungi in Fig. 2, which is partially based on an earlier analysis by Barron (1977). We will first consider active infection. Zoospores of a few species, such as *Myzocytiopsis intermedia* and *M. lenticularis*, appear to actively locate a host and infect directly following encystment upon the cuticle (Figs. 1 a,b,f, 2, 11, 12 and 22). It is not known exactly how such zoosporic species locate a suitable host, but it is thought that the zoospores follow chemotactic stimuli from substances in the nematode secretions. This may explain why zoospores of some species encyst in large numbers at the nematode orifices (Fig. 12), where exudates are probably most concentrated. A study of the nematophagous chytrid, *Catenaria anguillulae*, showed that zoospores were attracted to nematode orifices and expressed consistent orientation of encystment and cyst germination (Deacon and Saxena, 1997). In *Myzocytiopsis intermedia*, the zoospores congregetae and encyst en masse around the vicinity of the nematode orifices, producing narrow germ tubes which grow towards and through the nematode cuticle (Figs. 11, 12). Barron (1976a) suggested that encysted zoospores of *M. intermedia* may only be able to penetrate through such natural orifices, but our ultrastructural studies have



Figs. 22-26. *Myzocytiopsis lenticularis*. Abbreviations: Cu = cuticle; Cy = cyst; F = flagellum; K = kinetosome; OPV = ooplast vesicle; OW = oospore wall. **22.** Empty hemispherical cyst on cuticle and septate thalli within host body. **23.** Zoospores which have been released into vesicle (arrowed). **24.** Tangential profile of a zoospore showing ventrally inserted flagella. The edge of the vesicle is arrowed. **25.** Mature oospores showing distinctive reticulate wall. **26.** Maturing oospore showing thick multilayered wall. **Figs. 27-32.** *Chlamydomyzium anomalum* Abreviation: N = nucleus. **27.** A mixture of smaller aplanospores and larger chlamydospores inside nematode cuticle. **28.** Pre-cleavage sporangium showing diarticulating thallus (arrowed). **29.** Nematode body containing mature sporangia with dome-shaped exit papillae. **30.** Fully cleaved mature sporangium containing walled aplanospores **31.** A thick-walled cyst. **32.** Aplanospores showing elongate nuclei and peripheral dense-body vesicles.

shown that they penetrate through the cuticle presumably using a combination of enzymic digestion and physical pressure (Figs. 11, 12).

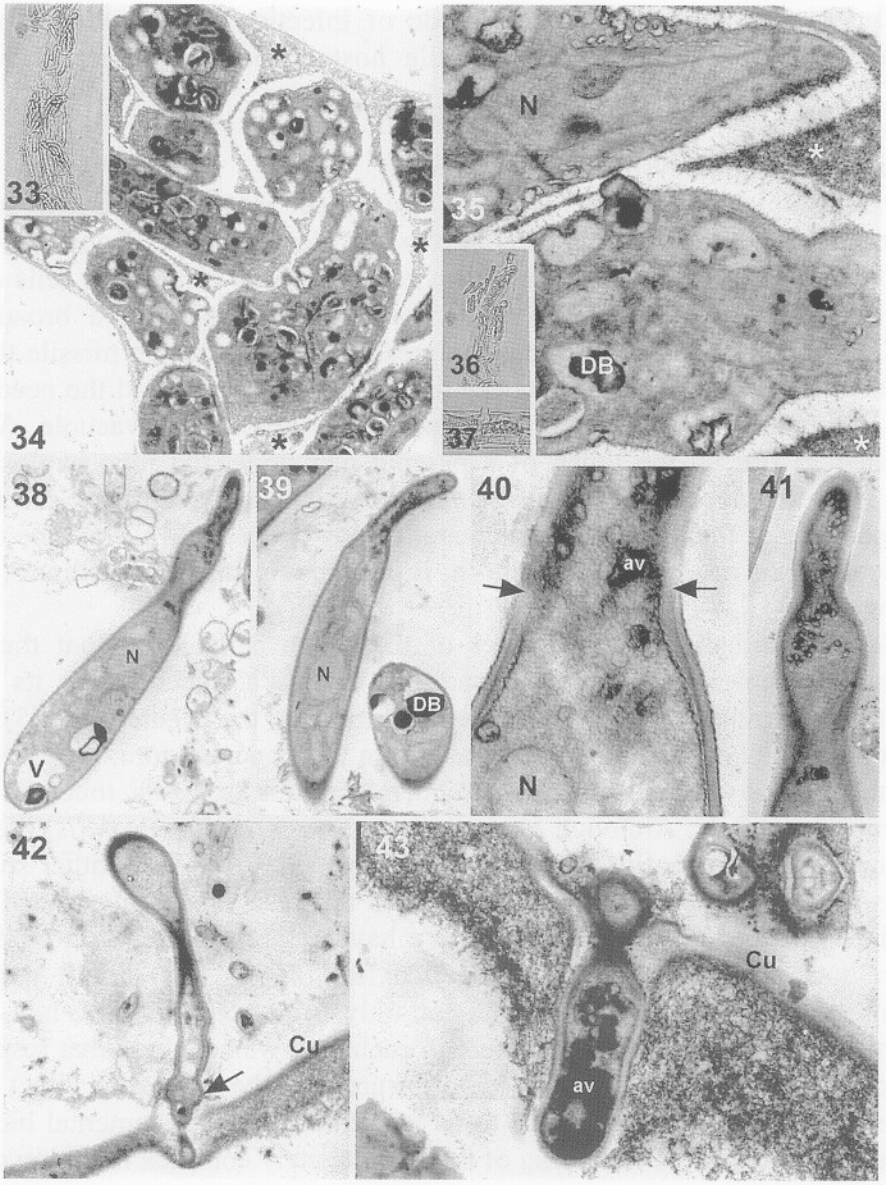
If a suitable host is not located, the zoospores will enyst directy upon the substrate (Fig. 9). In contrast in *M. lenticularis*, the zoospores usually encyst singly, flattening against the nematode cuticle into a hemisphere and then penetrating the cuticle to form a thallus (Fig. 22). *Myzocytiopsis papillata* is another species whose zoospores actively locate their nematode host and upon encystment flatten against the cuticle.

Passive adhesive infection

The zoospores of many *Myzocytiopsis* species, such as *M. vermicola*, *M. glutinospora* and *M. humicola*, encyst and germinate immediately after release to produce a specialised bud-like adhesive extension (Figs. 1c,d, 2, 15). This bud is in effect a specialised determinate germ tube. This is the infection spore and, if contact is not quickly made with a nematode, the spore continues to proliferate, forming a chain of spherical buds (Fig. 15).

In the aplanosporic species *M. subuliformis*, the elongate tapered aplanospores are released directly from the sporangium (Fig. 33) and immediately germinate to form a narrow tapering globular adhesive tip (Figs. 38, 39, 40 and 41). As with budding species these apical extensions arise from a new wall layer that is quite distinct from the original cyst wall (Fig. 40 arrowed). The developing buds contain small membrane bound vesicles (Figs. 40, 41). This species also relies upon its spores being picked up by a passing nematode for propagation. Electron microscopy of adhered spores show that the spore develops a swelling at the point of contact with the nematode cuticle (Fig. 42), and that the penetrating germ tube which grows through the cuticle is filled with dense vesicles that probably contain digestive enzymes (Fig. 43). These dense vesicles are reminiscent of those in the apical adhesive buds of *G. pyriforme* G.L. Barron (Figs. 20, 21). In *Gonimochaete* the elongate to ovoid cysts form just a single apical bud which remains connected to the original spore by a narrow neck region (Figs. 19, 20). These dense vesicles are present in young spores (Figs. 18 and 19) and move up into the apical bud until it is packed with them to the exclusion of all other cytoplasmic components (Figs. 20, 21). Saikawa and Anazawa (1985) showed how a narrow germ tube growing from the apical knob penetrated the nematode cuticle following adhesion. The thallus then grew inside the nematode body (Fig. 17). Although there are clear morphological differences, the bud-like germ tubes in *Myzocytiopsis* species and *Gonimochaete* seem to have a common adhesive function.

In all of these examples, the germinated "adhesive spores" are primed waiting for a healthy nematode to make contact with them (Figs. 1d,e, 15, 19,



Figs. 33-43. *Myzocytiopsis subuliformis*. Abreviations: av = electron-dense vesicles in adhesive pegs and penetration germ tubes; Cu = nematode cuticle; DB dense-body granules; N = nucleus; V = vacuole. **33.** Nematode body containing mature sporangia. **34.** Aplanospores differentiating within sporangium showing matrix between spores (asterisk). **35.** Detail of aplanospore initials showing fibrillar material in the cleavage planes (asterisk). **36.** Aplanospores being released from mature sporangium. **37.** Discharged thallus showing open evacuation tube. **38.** Aplanospore with apical extension. **39.** Aplanospore with elongated tip. **40.** Region of spore elongation showing discontinuity of wall layers (arrowed). **41.** Small dense vesicles in spore apex. **42.** Spore infecting nematode showing swollen area at point of contact (arrowed). **43.** Germ tube penetrating through nematode cuticle.

38 and 39). We consider that this mode of infection is a passive form of infection in that it relies upon a motile host encountering these "primed spores".

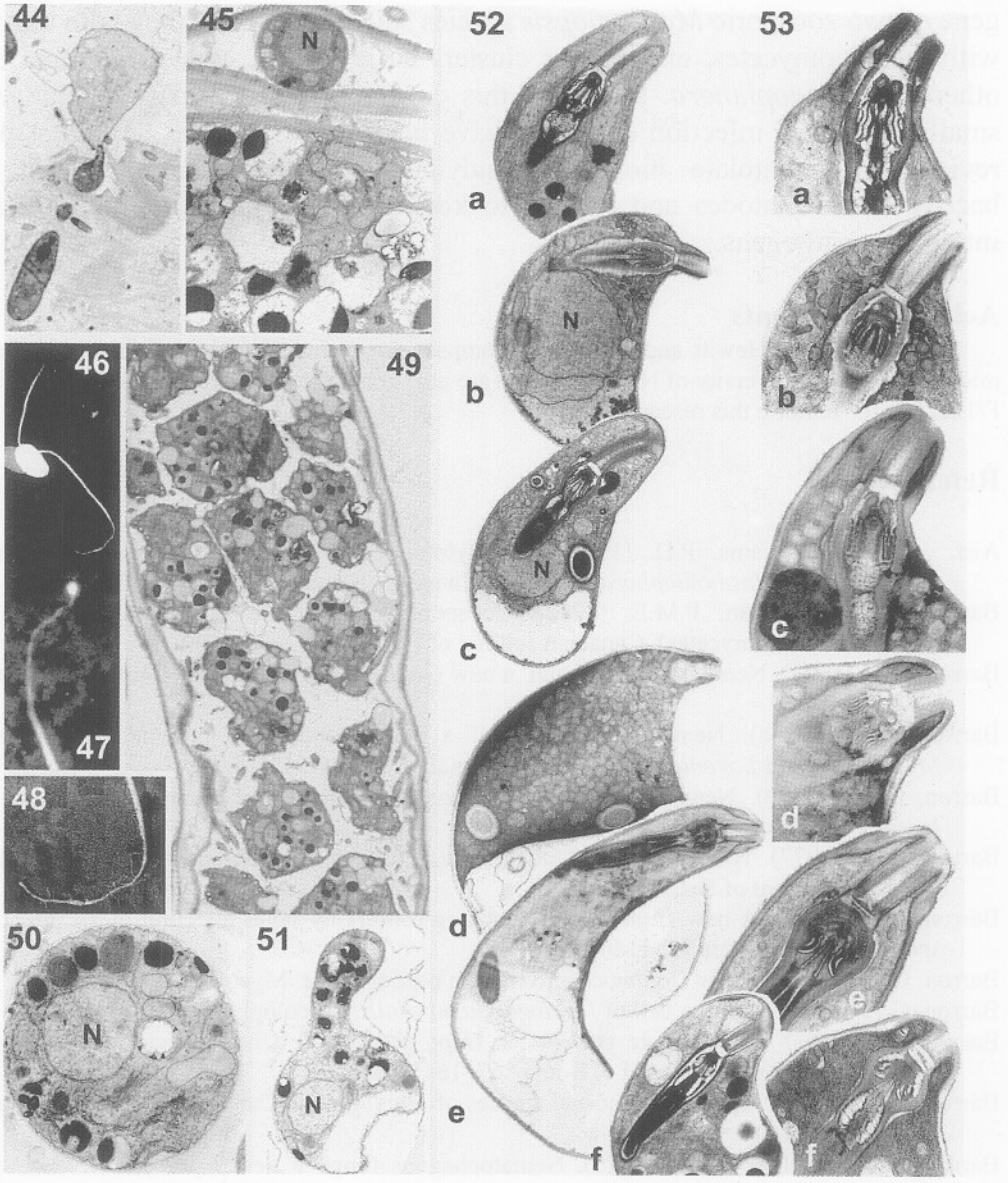
The passive injection system of Haptoglossa

The complexity of the infection gun cell was first shown in an ultrastructural study of *H. mirabilis* (Robb and Barron, 1982; Robb and Lee, 1986a,b) and a comprehensive study was recently made of the gun cells of *H. dickii* (Beakes and Glockling, 1998). The needle chamber is a broadened walled region of the tube that contains a needle-like penetration missile that is surrounded by several overlying cones that may function to hold the needle in place. The spore is kept under pressure by a large basal vacuole. When stimulated to fire by the touch of a passing nematode, the tube explosively everts, firing the needle through the nematode cuticle, and everting to insert the lower portions of the tube into the nematode body (Figs. 2, 44). The tube tail is unwalled and, as organelles rush into it, it expands to form a sporidium (Figs. 44 arrowed, 45).

Close examination of the apices of these gun cells show that they all contain a complex penetration apparatus, but each species has its own characteristic arrangement of the cones encompassing the needle (Fig. 53). The infection gun cell of *Haptoglossa* is passive in that, once mature, it lies on the substrate ready to respond to the particular stimulus of being touched by a swimming nematode. Although in some species such as *H. mirabilis*, the gun cells can be induced to fire by pressure (Barron, 1980, 1987), in other species such as *H. dickii*, the firing mechanism does not respond readily to artificial stimuli (Beakes and Glockling, 1998). We have recently proposed a model to explain gun cell firing (Beakes and Glockling, 1998), but it still requires experimental support. As the gun cell can only fire once, and there is a large investment of complex cell architecture in each cell, we conclude that response to specific stimuli must be involved to ensure that the gun cells infect their required target. There is still much to be learnt about the fundamental biology relating to the formation and firing of these exquisitely complex infection cells.

Conclusions

The significance of these various developmental and structural differences associated with asexual sporulation which we have summarised in Fig. 1 is not yet clear, but certainly indicates that *Myzocytiopsis* may be a heterogeneous group showing morphological convergence. These preliminary ultrastructural studies clearly indicate that the genus *Myzocytiopsis* is a diverse and probably unnatural grouping. Interestingly, sequencing of the small ribosomal subunit



Figs. 44-53. *Haptoglossa* spp. Abbreviation: N = nucleus. **44.** Fired gun cell of *H. heteromorpha* showing infective sporidium inside nematode body. **45.** Sporidium of *H. dickii* in sub cuticular space and developing thallus below. **46.** Shadowed zoospore of *H. dickii* showing two smooth flagella. **47.** Tip of anterior flagellum showing acroneme segment. **48.** Tip of posterior flagellum showing longer curved acroneme segment. **49.** Cleaved thallus of *H. dickii* showing zoospores inside sporangium. **50.** Encysted zoospore of *H. dickii*. **51.** Germinating cyst of *H. dickii*. **52.** Differentiating gun cells and **53** details of corresponding mature gun cell apices of the following zoosporic species: **a.** *H. dickii*; **b.** *H. northumbrica*; **c.** *H. zoospora*; and aplanosporic species: **d.** *H. heteromorpha*; **e.** *H. erumpens*; **f.** *H. heterospora*.

gene of two zoosporic *Myzocytiopsis* species show that, whilst they both cluster with other oomycetes, one species clusters more closely with *Achlya* and the other with *Phytophthora*. Inspite of this developmental diversity a relatively small number of infection strategies have been identified. We hope that this review will stimulate biologists studying ecosystems that are rich in bactivorous nematodes and rotifers to keep an observant eye out for these interesting pathogens.

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