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Fungi from the Rhynie chert: a view from the dark side

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ABSTRACT: The exquisite preservation of organisms in the Early Devonian Rhynie chert ecosystem has permitted the documentation of the morphology and life history biology of fungi belonging to several major taxonomic groups (e.g., Chytridiomycota, Ascomycota, Glomeromycota). The Rhynie chert also provides the first unequivocal evidence in the fossil record of fungal interactions that can in turn be compared with those in modern ecosystems. These interactions in the Rhynie chert involve both green algae and macroplants, with examples of saprophytism, parasitism, and mutualism, including the earliest mycorrhizal associations and lichen symbiosis known to date in the fossil record. Especially significant are several types of specific host responses to fungal infection that indicate that these plants had already evolved methods of defence similar and perhaps analogous to those of extant plants. This suggests that mechanisms underlying the establishment and sustenance of associations of fungi with land plants were well in place prior to the Early Devonian. In addition, a more complete understanding of the microbial organisms involved in this complex ecosystem can also provide calibration points for phylogenies based on molecular data analysis. The richness of the microbial community in the Rhynie chert holds tremendous potential for documenting additional fungal groups, which permits speculation about further interactions with abiotic and biotic components of the environment.

KEY WORDS: Early Devonian, host responses, microbial interactions, mutualism, parasitism, saprophytism.

The discovery of plant remains in the Rhynie chert by Mackie (1914) and subsequent detailed accounts by Kidston & Lang (1917, 1920a, b, 1921a, b) provided a wealth of information that has served as a baseline for our understanding of early terrestrial life. Although the focus of the majority of these studies has been on the above-ground components of the ecosystem, a remarkable and critical component of Early Devonian terrestrial life occurred in the rhizosphere on or beneath the surface of the substrate. In their extraordinary account of the Rhynie chert flora, Kidston & Lang (1921b) provided the first report of the microbial components in the ecosystem in the form of fungal hyphae, thick-walled resting spores, and clusters of cells that they interpreted as bacteria. Of perhaps greater biological significance was their recognition of the role that the Rhynie chert fungi played in nutrient cycling and as biotrophs that interacted with the macroplants. These authors also suggested that some of the fungi may have formed symbiotic associations with one or more of the land plants. Since the original description of fungal remains in the Rhynie chert, there have been numerous reports that have carefully documented the structure, morphology, and, in some instances, the biology of the Rhynie chert organisms, many of them summarised in this symposium volume. As a result, we know a great deal about the individual organisms that existed in this ancient environment and can now begin to explore some of the interactions that served as drivers for the Rhynie chert ecosystem.

Recent ultrastructural and molecular data have expanded the definition of fungi and their systematic relationships with other heterotrophic organisms and, as a result, the 'fungi' are regarded as a polyphyletic and heterogeneous group estimated to be comprised of more than 1·5 million species, of which fewer than 100,000 have been described and named to date (Hawksworth 1991). They occupy almost every ecological niche and are involved in a variety of different interactions in terrestrial, aquatic, and marine environments. They form associations that can be harmful and/or of benefit to organisms that make up the ecosystem (Fig. 1A), as well as to the stability of the ecosystem as a whole (e.g., nutrient cycling). In modern ecosystems, fungi decay organic debris and occur as parasites, pathogens, and mutualists of both plants and animals. Some have entered into complex symbiotic associations with land plants, whilst others have partnered with algae and cyanobacteria to form lichens (Fig. 1A). Fossil fungi possess a limited number of useful characters with which to identify taxa; we have relied on the traditional classification that defines four phyla: Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota (McLaughlin & McLaughlin 2001), but also recognise the Glomeromycota, a monophyletic group that includes the arbuscular mycorrhizae and related forms. A listing of the fungi that have been described from the Rhynie chert to date and the hosts with which they are associated is provided in Table 1. We have also included the modern analogues if these were suggested by the authors.

An appreciation of the microbial community in fossil ecosystems has been especially slow to develop, since the focus of research has generally been directed at the macroplants and animals, rather than at the microorganisms (bacteria, cyanobacteria, microalgae, fungi, zoo- and phytoplankton). In the case of fungi, palaeontologists may not be adequately trained to distinguish these organisms in samples or recognise indirect evidence of their presence. Most researchers generally tend to collect specimens that are complete and well-preserved, rather than those that are degraded due to the activity of saprophytic and parasitic microorganisms. As a result, the fossil microbial world is typically overlooked and does not become part of the diversity record. Finally, information about fossil fungi is often published in non-palaeontological journals, which has not only resulted in a perception that little of the fungal record is preserved, but also that there is insufficient knowledge of

Table 1 Rhynie chert microbial taxa and associated organisms

	Associated macroplant sporophytes	Associated macroplant gametophytes	Algal/bacterial associates	Fungal associates	Suggested modern equivalent taxa	Reference
Chytridiomycota						
Krispiromyces discoides			*Palaeonitella cranii*		*Phlyctochytrium*, *Catenochytridium*	Taylor *et al.* 1992a
Lyonomyces pyriformis			*Palaeonitella cranii*		*Rhizophydium*	Taylor *et al.* 1992a
Milleromyces rhyniensis			*Palaeonitella cranii*		*Endochytrium*, *Entophlyctis*, *Sorodiscus*, *Woronina*	Taylor *et al.* 1992a
Paleoblastocladia milleri	*Aglaophyton major*				*Allomyces*, *Blastocladiopsis*	Taylor *et al.* 1994; Remy *et al.* 1994a
Unnamed holocarpic and eucarpic forms	*Horneophyton lignieri*, *Aglaophyton major*	*Lyonophyton rhyniensis*, *Aglaophyton major*	*Palaeonitella cranii*, *Pediastrum*-like alga	Fungal spores and vesicles	*Entophlyctis*, *Hyphochytrium*, *Nowakowskiella*, *Olpidiopsis*, *Olpidium*, *Spizellomyces*, *Triparticalcar*	Illman 1984; Taylor *et al.* 1992b; Hass *et al.* 1994
Zygomycota						
Glomites rhyniensis	*Aglaophyton major*				*Glomus*	Taylor *et al.* 1995
Palaeomyces agglomerata	*Rhynia gwynne-vaughanii*					Kidston & Lang 1921b
Palaeomyces asteroxylii	*Asteroxylon mackiei*					Kidston & Lang 1921b
Palaeomyces gordonii	*Asteroxylon mackiei*, *Horneophyton lignieri*, *Rhynia* sp.					Kidston & Lang 1921b
Palaeomyces gordonii var. *major*	*Rhynia gwynne-vaughanii*					Kidston & Lang 1921b
Palaeomyces horneae	*Horneophyton lignieri*					Kidston & Lang 1921b
Palaeomyces simpsonii	*Rhynia gwynne-vaughanii*					Kidston & Lang 1921b
Palaeomyces vestita	*Asteroxylon mackiei*					Kidston & Lang 1921b
?*Winfrenatia reticulata* mycobiont	Lichen		Cyanobiont			Taylor *et al.* 1997
Ascomycota						
Unnamed perithecial ascomycete	*Asteroxylon mackiei*				Pyrenomycetes	Taylor *et al.* 1999
'**Oomycota**'						
Unnamed mycelia	?*Prototaxites taiti*				*Apodachlya pyrifera*	Harvey *et al.* 1969
Cyanobacteria						
Archaeothrix contexta					*Oscillatoria*	Kidston & Lang 1921b
Archaeothrix oscillatoriformis					*Oscillatoria*	Kidston & Lang 1921b
Kidstoniella fritschii					Stigonemataceae	Croft & George 1958
Langiella scourfeldii					Stigonemataceae	Croft & George 1958
Rhyniella vermiformis					Scytonemataceae	Croft & George 1958
Rhyniococcus uniformis					Chroococcaceae	Edwards & Lyon 1983; Tappan 1980
Winfrenatia reticulata cyanobiont	Lichen			Zygomycete mycobiont	*Gloeocapsa*, *Chroococcus*, *Chroocccidiopsis*	Taylor *et al.* 1997

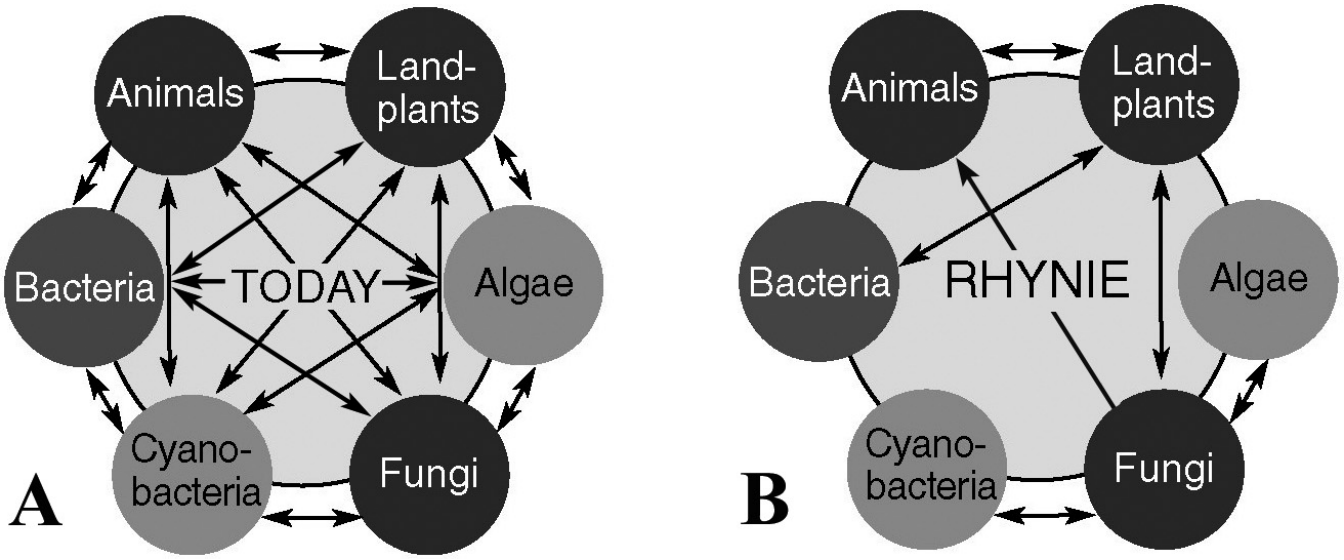


Figure 1 Comparison of interactions among organisms in modern ecosystems (A) with those that are known to date from the Lower Devonian Rhynie chert (B).

fungal interactions with other fossil organisms. Our understanding of the fossil record of fungi and other microbes has greatly increased in the past decade, in large part due to the exquisite preservation and diversity of organisms in the Rhynie chert. The focus of this paper is to review the life history biology of fungi that have been identified to date from the Rhynie chert and to document interactions, host responses, and the impact of fungi on the 'dark side' of this Early Devonian community (Fig. 1B). In comparing the Rhynie chert with modern ecosystems (Fig. 1A), we further speculate on a variety of interactions that may yet await discovery.

1. Material and methods

More than ten fossiliferous beds have been identified in the Rhynie chert deposits (Trewin & Rice 1992) and the organisms are preserved as siliceous permineralisations. As a result, specimens must be studied in petrographic thin sections. Photomicrographs were made using oil immersion objectives directly on the rock surface. Slides are kept in the collection of the Forschungsstelle für Paläbotanik, Westfälische Wilhelms-Universität, Münster, Germany. Slide numbers are included with the figure captions.

2. Microbial interactions in the Rhynie chert ecosystem

A large number of microbial interactions have been documented from the Rhynie chert and many yet remain to be deciphered (Fig. 1A, B). Within these deposits are a variety of examples of fungi occurring as saprophytes of plants and animals and as parasites of macroplants, algae, and other fungi. It is likely that many of the macroplants in the Rhynie chert were associated with fungi in an endomycorrhizal symbiosis, but to date, this relationship has only been demonstrated in a single taxon. It has been presumed that this symbiosis was mutually beneficial to both the host (*Aglaophyton major*) and the fungus; however, the presence of a glomeromycete in *Aglaophyton* does not necessarily indicate that a beneficial relationship existed between these two organisms. For example, some mycorrhizal interactions may be mutualistic or parasitic, depending on the physiology and environmental conditions experienced by the plant (Redman *et al.* 2001). We may not be able to untangle all of the dynamics of the interactions of fungi in the community structure of the Rhynie chert, since a single fungus can be pathogenic to one type of organism whilst providing benefit to another, as well as acting as a decomposer of still others (Redman *et al.* 2001). Nevertheless, the interactions that have been described to date offer the opportunity to more fully understand the microbial world in the Early Devonian.

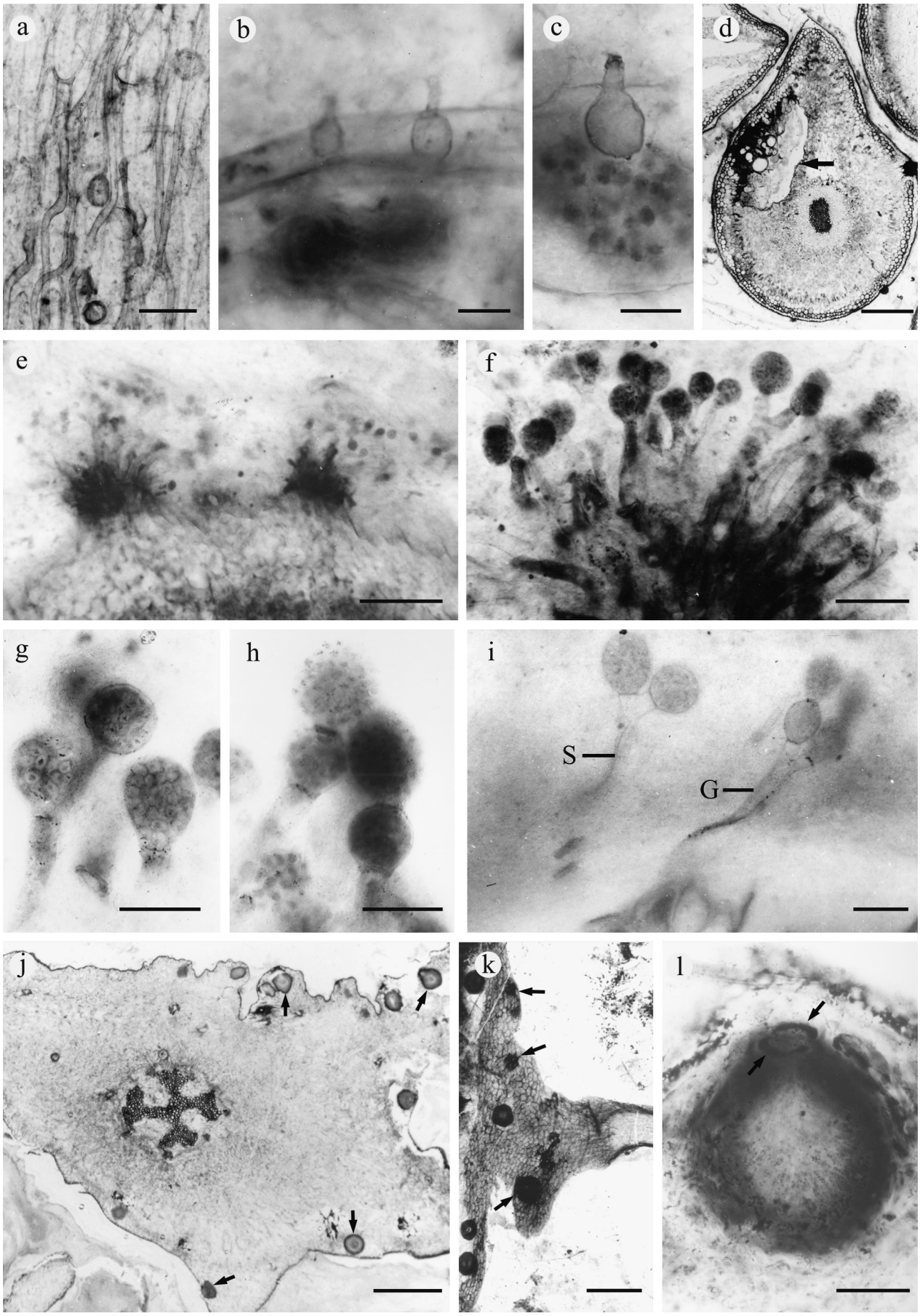
2.1. Saprophytes

To date, most of the documented fungal interactions with plants in the Rhynie chert occur through the activities of saprophytes. These include fungal hyphae ramifying through degrading plant tissues as well as the presence of chytrids (Chytridiomycota) (Fig. 2a–i) and ascomycetes (Ascomycota) (Fig. 2j–l, 5a–c). These organisms, together with bacteria, have contributed to the large amount of degraded plant biomass in the chert that cannot be classified. Kidston & Lang (1921b) described and illustrated numerous examples of septate and aseptate hyphae showing various patterns of branching, some terminating in thin-walled vesicles and others forming thick-walled resting spores (Fig. 2a). Since these fungi occur in association with all of the Rhynie chert organisms, they were obviously a critical component of the microbial world at that time.

2.1.1. Chytridiomycota. Chytridiomycetes are well represented in the Rhynie chert and include unicellular and multicellular forms that display saprophytic and/or parasitic interactions with the macroplants (Illman 1984; Taylor *et al.* 1992a, b, c; Remy *et al.* 1994a; Taylor *et al.* 1994). Members of this group were probably the most common microbial element and may have been the principal decomposers of organic material in the ecosystem. Modern chytrids are primarily aquatic and occur in fresh-water habitats, although a few may be marine; others are known from a wide range of terrestrial ecosystems including bogs, dry soils, or even desert sands. Most exist as saprophytes, but some are parasitic on algae, other fungi, various types of animals, and underground parts of plants. Some chytrids occur in the guts of herbivores as rumen symbionts. They act as important elements of modern terrestrial ecosystems by serving as cellulose decomposers.

Clusters of chytrids including individuals with well-developed zoosporangia have been reported from a variety of different Rhynie macroplant types (Taylor *et al.* 1992c). Some

occur in cells as saprophytes and parasites; others are present along plant stems and on the surface of various plant organs. Whilst their small size sometimes makes it difficult to accurately characterise the types present, their ubiquitous occurrence has provided an opportunity to detail stages of the complete life history of some forms (Illman 1984; Taylor *et al.*



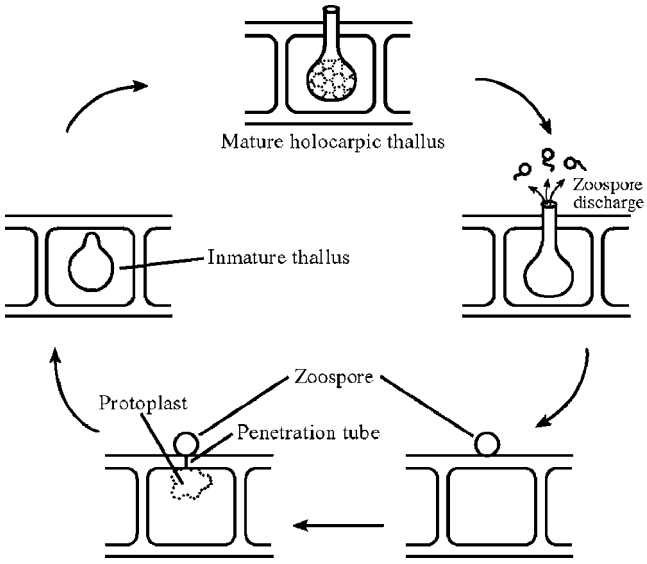


Figure 3 Suggested stages in the life history of a fossil chytrid based on information collected from the Rhynie chert.

1992c). In one common type, zoosporangia develop within thin-walled algal cells and form a zoospore discharge tube that extends through the cell wall (Fig. 2b, c). Zoospores exit the algal cell, form cysts, and subsequently infect new cells to repeat this simple life cycle (Fig. 3). Other chytrids in the Rhynie chert have infected softer cortical regions of stems and can be identified based on the presence of degraded cells and tissue systems (Fig. 2d). The presence of several different morphological types of chytrids on the same host has important implications, since species interactions may influence damage to the host and these interactions may be synergistic or competitive (Takenaka 1995).

Not all saprophytic chytrids in the Rhynie chert were unicellular. One unique form is *Paleoblastocladia milleri*, which to date has been found only associated with *Aglaophyton* (Table 1; Remy *et al*. 1994; Taylor *et al*. 1994). The fungus occurs as compact tufts of non-septate hyphae that arise through stomata or between the cuticle and epidermis of the host (Fig. 2e, f). There are three different types of reproductive structures that occur on two types of thalli of *P. milleri*. The most common form consists of bulb-shaped zoosporangia (Fig. 2f, g) that occur on thalli with dichotomously branching hyphae (sporothalli); most of these are terminal, range up to 30 μm in diameter, and contain zoospores that are spherical to hexagonal in face view, each with an opaque inclusion (Fig. 2g). Also present on some of these sporothalli are thick-walled structures that have been interpreted as resting sporangia, which produced spores that germinated into separate new thalli (gametothalli). The third type of reproductive structure consists of globose structures that typically occur in chains on the gametothallus (Fig. 2h); these have been interpreted as gametangia. Because there is sufficient material at different stages of development, it is actually possible to trace the life history biology of this unusual fungus, a rare opportunity in fossil fungi. Based on the presence of separate thalli producing either zoospores or gametes, *Paleoblastocladia* has been interpreted as possessing a distinct isomorphic alternation of generations (Fig. 4), a feature that is almost unknown in modern fungi, with the exception of some members of the Blastocladiales. It is interesting that both sporothalli and gametothalli are closely associated on the same *Aglaophyton* axes (Fig. 2i). Other than the fungus disrupting the cuticle on the surface of the *Aglaophyton* axes, no specific host response has been observed to date. We have interpreted *Paleoblastocladia* as a saprophyte because of the similarity of its morphology and life history to modern members of the Blastocladiales (e.g., *Allomyces*, *Euallomyces*, and *Blastocladia*). However, without a specific host response it is difficult to accurately interpret the type of interaction between the fungus and host. It is interesting to note that in modern ecosystems, *Blastocladia* displays enhanced growth in the presence of increased CO_2 (Held *et al*. 1969). The high CO_2 levels postulated as occurring during the Devonian (Berner & Kothavala 2001) may represent a selective pressure responsible for structural and life history complexities seen in *Paleoblastocladia*.

2.2. Parasites

Parasites obtain nourishment from other living organisms. Many parasitic fungi, however, are also capable of growing on dead organic matter and thus are termed facultative parasites or facultative saprophytes. When examining a fossil, the presence or absence of a host response can provide information about the level of interaction, but it is not always definitive. This may be further complicated by the fact that some fungi are endophytes and thus provide no observable host response symptoms (Schulz *et al*. 1999).

2.2.1. Chytridiomycota. Necrotic regions of opaque materials in some of the macroplants suggest that some chytrids parasitised their hosts (Fig. 2d; Table 2), however, the actual chytrids have not been identified in these areas of the stem. Parasitic chytrids have been described associated with several plants in the Rhynie chert (Fig. 5d–k), particularly with the green macroalga *Palaeonitella cranii* (Table 1; Taylor *et al*. 1992a, b, c). *Krispiromyces discoides* is a unicellular chytrid consistently found on stems of *Palaeonitella* (Table 1). This fungus is characterised by a saucer-shaped zoosporangium that remains on the outside of the cell wall, whilst a rhizomycelium extends through the wall into the cell lumen (Fig. 5e). There is always a host response in this association that occurs between chytrid and alga; infected cells display a hypertrophic response in which they may be up to ten times larger than normal nodal cells (Fig. 5d; Table 2; Taylor *et al*. 1992a). This pattern of host cell enlargement is identical to that seen in the extant charophyte *Chara*, an alga that is believed to be closely related to *Palaeonitella*, when it is parasitised by a chytrid (Karling 1928).

Rhynie chert chytrids were probably parasites of green algae that occurred in small colonies in the cortical cells of degraded macroplants (Fig. 5f, g; Table 1; Taylor *et al*. 1992a). Some of

Figure 2 (a) Thin section showing hyphae and swollen vesicles. Slide P1463, scale bar = 100 μm; (b) Rhizoidal cell of *Palaeonitella cranii* showing 2 chytrid zoosporangia with discharge papillae extending through cell wall. Slide P1478, scale bar = 25 μm; (c) Chytrid zoosporangium with neck extending through cell wall. Slide P1478, scale bar = 15 μm (from Taylor *et al*. 1992a); (d) Section of Rhynie chert macroplant stem showing disruption of cortex (arrow) and associated opaque necrotic area. Slide P1816, scale bar = 1 mm; (e) Two tufts of *Paleoblastocladia milleri* on stem surface of *Aglaophyton major*. Slide P2057, scale bar = 200 μm; (f) Well developed tuft of *P. milleri* showing terminal zoosporangia. Slide P2056, scale bar = 50 μm; (g) Zoosporangium with well developed zoospores. Slide P2056, scale bar = 25 μm; (h) Two pairs of gametangia; note distinct size difference. Slide P2054, scale bar = 25 μm; (i) Portion of sporothallus (S) and gametothallus (G) showing differences in reproductive structures. Slide P2084, scale bar = 25 μm (from Remy *et al*. 1994a); (j) Transverse section of *Asteroxylon* stem showing several fungal ascocarps (arrows) just beneath stem epidermis. Slide P3404, scale bar = 1 mm; (k) Base of an enation with several ascocarps (arrows). Slide P3469, scale bar = 750 μm; (l) Longitudinal section of perithecium showing central cavity containing asci. Note guard cells (arrows) surrounding ostiole. Slide P3435, scale bar = 100 μm.

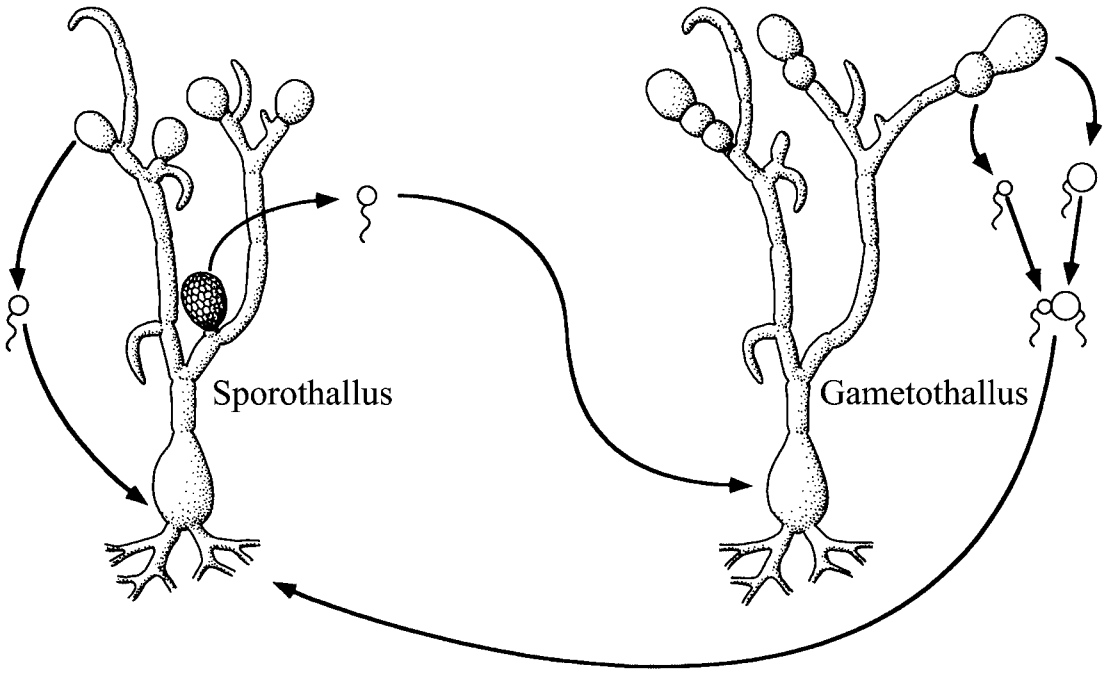


Figure 4 Stages in the life history of *Paleoblastocladia milleri*: the sporothallus produces terminal zoosporangia and intercalary, thick-walled resting sporangia that each produce zoospores; zoospores from the resting sporangia in turn develop into the gametothallus that produces gametes from gametangia.

these *Pediastrum*-like algal cells are up to 12 μm in diameter and arranged in loose clusters of several dozen cells. Chytrid zoosporangia occur inside these cells and display a small discharge tube that extends through the cell wall (Fig. 5g, h), through which zoospores were liberated. Other chytrids are common both inside and on the surface of spores of various macroplants (Fig. 5i, j, k). Some of these are associated with germinating gametophytes; others are present within the lumen of the spore.

In a few instances, it has been possible to identify swellings along the underground parts of various Rhynie chert plants. Axes of *Nothia aphylla* possess horizontal, subterranean rhizomes, from which extend multicellular rhizoids. Spherical, nodule-like structures occur on some of these rhizoids and appear to represent an outgrowth of the rhizoid (Fig. 5l). One possible explanation is that these structures represent an infection point for a parasitic fungus, such as a chytrid, since thin threads can be traced into the rhizoid. Bulges and swellings may also represent another potential host response (hyperplasia) which involved chytrids as the causal agent (Fig. 5m; Table 2). Hyperplasia causes an abnormal proliferation in the number of cells and contributes to an increase in the size of an organ. There are various cortical regions in the macroplants within the chert that show aggregations of cells that may represent this host response.

2.2.2. Ascomycota. Ascomycetes today are significant saprophytic components of modern ecosystems. They occupy a wide range of habitats and enter into a variety of interactions with other organisms in the environment, including pathogenic as well as beneficial and mutualistic relationships; many form symbiotic associations with arthropods. Current classifications subdivide the ascomycetes into three groups: Archaeascomycetes and Hemiascomycetes (the yeasts and assorted taxa), which are primarily unicellular and lack an ascoma (sporocarp that contains sexually-produced spores), and Euascomycetes, which consists of filamentous forms that produce ascomata that contain specialised cells (asci) that produce a specific number of sexually-produced haploid spores (ascospores) (Alexopoulos *et al*. 1996). Asexual reproduction is carried out by various methods that result in the formation of spores. The fossil record of the ascomycetes is poorly understood. Fossil evidence has been reported from Upper Silurian rocks in the form of macerates and consists solely of specialised, asexual cells (phialides) (Sherwood-Pike & Gray 1985). Other fossils suggested as having affinities with ascomycetes have been reported in the Devonian and Carboniferous (Taylor 1995).

The best example to date of a perithecial ascomycete interpreted as a parasite is now known from the Rhynie chert (Table 1; Taylor *et al*. 1999, in press). Beneath the epidermis and on the enation bases of *Asteroxylon mackiei* stems are spherical, opaque structures (Fig. 2j, k). Detailed examination shows that these represent flask-shaped reproductive structures (perithecia) of an ascomycete (Fig. 2l). Perithecia are up to 400 μm in diameter and characterised by short, ostiolate necks that typically protrude from the epidermis through stomatal openings of the host plant. Lining the interior of the perithecium are elongate, thin-walled filaments (paraphyses) interspersed with asci that contain ascospores (Fig. 5a). Ascospores are typically unicellular, but in some asci, and interspersed in the locule of the perithecium, spores may be one to five times septate (Taylor *et al*. 1999). Some ascospores possess a germ tube arising from one end of the spore. Small, specialised tufts of hyphae (conidiophores) that bear chains of spores at the tips occur along the axes of the host (Fig. 5b) and are also associated with immature perithecia. These typically occur in shallow depressions on the stems and may extend up to 150 μm from the stem surface. Conidiophores are typically smooth, branch occasionally, and form chains of arthrospores. Mature arthrospores are cube-shaped with slightly rounded ends and serve as reinfection agents.

One possible reason that fossil perithecial ascomycetes have not previously been described is the fact that when observed at relatively low magnifications, perithecia appear similar in size and general organisation to chlamydospores that are common in the macroplants (Fig. 5c), and thus may have been overlooked in a cursory examination. However, chlamydospores are generally more deeply embedded within the host tissues,

whilst perithecia are distributed just beneath the epidermis of the host.

2.2.3. Mycoparasites. Some fungi in the Rhynie chert obtained carbon by parasitising other fungi. Some of these mycoparasites were illustrated by Kidston & Lang (1921b, plate V) in the form of thick-walled fungal spores (chlamydospores) with smaller spores inside. They termed the smaller spores intrusive fungi and thus fully appreciated the concept of mycoparasitism. Several parasitic fungi are now known from the Rhynie chert and include forms that developed in the spore lumen of the host, between specific wall layers of the host, and on the surface of the spores (Fig. 6a–e) (Hass *et al*. 1994). Still other spores are packed with hyphae. Each of these sites is generally occupied by a different type of chytrid, suggesting that some level of host specificity was in existence in the Early Devonian. In addition, in several instances, the host response to invading parasites is analogous to that found today (Table 2). For example, Boyetchko & Tewari (1991) described projections termed callosities, lignitubers, and papillae extending from the inner surface of the chlamydospore wall in certain mycohosts; these are formed when the living protoplast of the parasitised chlamydospore continues to synthesise wall material as the parasite attempts to penetrate the spore wall. In modern chlamydospores, it is possible to see thin canals that bisect the length of similar papillae; these canals were formed by the chytrid as it tunnelled through the new spore wall. This same host response is present in the Rhynie chert spores (Fig. 6f, g). In observing this biological 'arms race' in the fossil record between the chytrid and the parasitised chlamydospore, what is fascinating is that the molecular and genetic signalling mechanisms in this interaction were apparently in existence at least 400 million years ago and are essentially unchanged compared with those found in certain groups of fungi today. It should be noted, however, that infection canals that appear similar may also be the result of bacteria and amoeba-like organisms (Table 2). Further complicating our understanding of the role of certain organisms in this ecosystem is the fact that some modern mycoparasites from arbuscular mycorrhizal spores are reported as facultative with some saprophytic ability, and thus are not entirely dependent on the spore for a carbon source (Paulitz & Menge 1984).

Filamentous ascomycetes have also been described in healthy and dead spores of the modern arbuscular mycorrhiza *Scutellospora* (Hijri *et al*. 2002). Using both molecular sequence data and transmission electron microscopy, the existence of these fungi was confirmed in the spores; however, their function remains equivocal. Since many fossil spores contain other fungi, especially the thick-walled spores so common in the Rhynie chert, this modern association may also be ancient.

There are numerous examples of hyphae that are in direct contact with other hyphae in the Rhynie chert, both in the matrix and in degraded tissues. Some of these may be haustoria, whilst others contain knobs and swellings and could represent mycoparasite appressoria (Table 2), which are specialised hyphal structures that adhere to the host and penetrate the plant epidermis. In modern fungi, this interaction directly involves some recognition between the parasite and host. It may include chemotrophic growth of the parasite toward the host, recognition, attachment, excretion of extracellular enzymes, and exploitation of the host (Chet *et al*. 1997). Examples of this interaction are difficult to identify in the fossil record, because a specific host response typically involves cytoplasmic disruption and reduced hyphal growth in the parasite. Nevertheless, it is important to document structures suggestive of appressoria.

An additional example of mycoparasitism occurs in ascomycete perithecia from the Rhynie chert (Fig. 6h). Hyphae that occur extensively in degraded perithecia are of varying diameters, suggesting that more than one single mycoparasite was present. Several perithecia contain smaller spores (20 μm) that may represent yet another mycoparasite.

2.3. Mutualists

It is estimated that many extant land plants maintain more or less intimate relationships with fungi in a variety of different interactions involving obligately biotrophic fungi (Bonfante 2003). Whilst some associations are highly specialised and confined to a relatively small group of plants, others, like the arbuscular mycorrhizae, are estimated to occur in approximately 90 % of extant plant species, including not only gymnosperms and flowering plants, but also bryophytes and pteridophytes (Read *et al*. 2000). Arbuscular mycorrhizae (AM), characterised by a mostly coenocytic mycelium and the production of chlamydospores, are now included in the monophyletic phylum Glomeromycota (Schüßler *et al*. 2001). Such fungi in modern ecosystems are known to be involved in defining ecological niches and determining plant community composition (Francis & Read 1995), as well as playing major roles in soil fertility and plant nutrition. It has also been suggested that they contribute to weathering processes in the soil (Boucot & Gray 2001). As a result of the nutritional modifications provided by the symbiosis, AM fungi influence plant growth in multiple ways; some of these include protection against some plant diseases (Blee & Anderson 2000), as well as having a negative effect on certain herbivores (Vicari *et al*. 2002) and affecting the composition of arthropod communities on plants (Gange *et al*. 2002). These fungi may also help promote the coexistence of plant species in a variety of ways (Hart *et al.* 2003).

Kidston & Lang (1921b) initially suggested a possible biotrophic relationship between some of the fungi in the Rhynie chert and the macroplants; this interaction has now been fully documented (Taylor *et al*. 1995). Whereas arbuscular mycorrhizal symbioses in the fossil record may be deduced based on the presence of hyphae, vesicles, and spores within the fossil plant tissues, the structure that defines the mutualism is an intracellular, highly branched hyphal network termed the arbuscule. In this interaction, the fungus has the ability to penetrate the macroplant cell wall without rupturing the plasmalemma to form the arbuscule. It is this structure through which nutrient transfer occurs between the host and the fungus. The first arbuscules from the Rhynie chert plants have been reported only recently (Remy *et al*. 1994b). *Glomites rhyniensis* is known from *Aglaophyton* sporophytes, as well as young gametophytes of this plant (*Lyonophyton rhyniensis*) (Table 1; Remy *et al*. 1994b). A continuous dark band in the cortex of *Aglaophyton* (Fig. 6i) contains the arbuscules of the mycorrhizal partner (Fig. 6j, k).

The report of arbuscular mycorrhizae in at least one of the Rhynie chert macroplants has important implications with respect to the evolution of this fungal interaction and the dynamics of the ecosystem during the Early Devonian. The similarity of the arbuscules of the fossil *Glomites* to the extant arbuscular mycorrhizal fungus *Glomus* is striking, and suggests that this symbiosis has operated relatively unchanged for at least 400 million years. Research on modern arbuscular mycorrhizae generally ascribes the function of increasing phosphorus uptake to the host organism via the extended hyphal network of the fungus (Smith & Read 1997). We might speculate that this Early Devonian ecosystem lacked sufficient quantities of readily available minerals such as phosphorus, and thus the capacity of the symbiosis to increase mineral uptake would have been an important selective pressure in expanding the ecosystem. Another hypothesis is that the

absence of well-developed roots in these early plants provided the selective pressure that favoured mycorrhizal associations. Moreover, the loose arrangement of the tissues in these plants provided the necessary conduit for a highly ramifying mycelium in the cortical tissues, such as that found in certain (*Arum*-type) arbuscular mycorrhizae (Brundrett 2002).

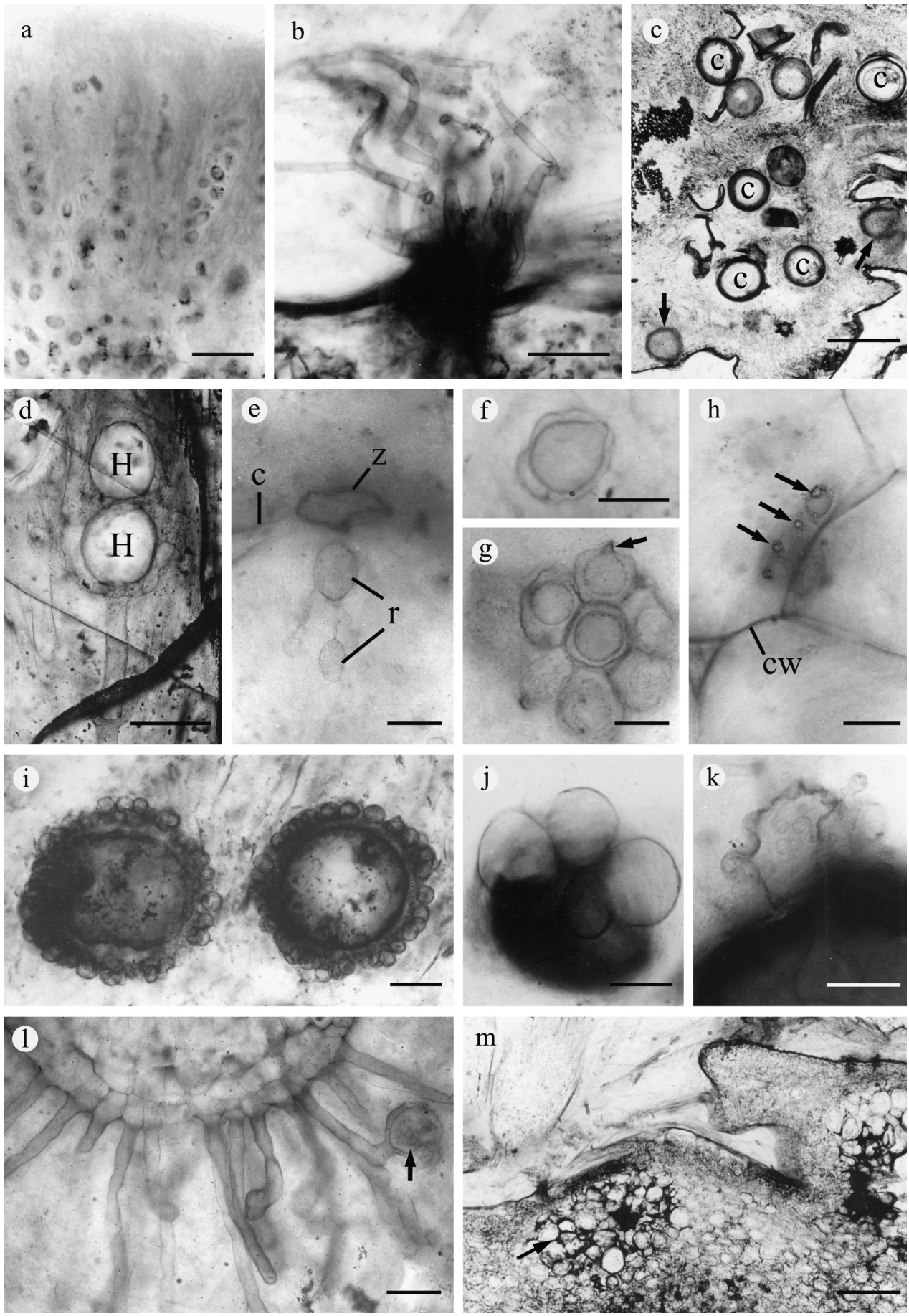


Table 2 Host responses in Rhynie chert organisms

Host	Host response	Occurrence	Causal agent	Symptom
Glomites rhyniensis	papillae	chlamdyospores	chytrids, bacteria, amoebae	synthesis of new spore wall material
All macroplants	hyperplasia	cortical tissue	unknown	increased cell production
Palaeonitella cranii	hypertrophy	internodal cells	chytrids	abnormal growth
All macroplants	necrotic areas	axes, cortex	unknown	opaque areas
Aglaophyton major	cell wall thickenings	cells with arbuscules	Zygomycete	new wall material
Various macroplants	appressoria	certain hyphae	other hyphae	hyphal enlargement, hyphae adpressed

As a result of the detailed studies by Remy & Remy (1980a, b) and Remy & Hass (1991a, b, 1996), we now know that the life histories of the Rhynie chert plants included a free-living gametophyte phase, and that fungi have been observed within the tissues of the gametophyte *Lyonophyton*. Arbuscules occur early in the development of the gametophyte, which is still attached to the spore coat. Hyphae and vesicles are present in immature gametophytes, which lack distinct tissues; the first recognisable arbuscules occur in young aerial axes. As research with other Rhynie chert macroplants continues, it will be interesting to see how many of these have entered into mycorrhizal relationships. Hass & Kerp (2003) indicate that five of the macroplants from the Rhynie chert are variously associated with glomalean-type chlamydospores, suggesting the widespread existence and biological significance of endophytic fungi in this Early Devonian ecosystem. However, the nature of most of these endophytic fungus–land plant associations cannot be established with certainty, since the fungi do not display structural similarities to extant mycorrhizal fungi, such as the arbuscular mycorrhizae (AM) that occur in the axes of *Aglaophyton*. It is possible that some of the other endophytic fungi in Rhynie chert plants may simply have been asymptomatic space-endophytes, which had neither an adverse nor a beneficial effect on their hosts. Others may represent precursor states of true endomycorrhizal associations, beneficial for the host to a certain degree, but not yet having established an interaction as intimate as that seen in extant AM fungi.

It is now known that arbuscular mycorrhizae may impact plants in a variety of ways, including both beneficial and antagonistic interactions. Further contributing to a lack of certainty as to exactly how AM fungi benefit a plant is the fact that different species of a mycorrhizal symbiont can have differential effects on the growth of plants (van der Heijden *et al*. 1998). Francis & Read (1995) demonstrated that some hosts respond mutualistically to AM, whilst other uninfected species lose fitness. Although it has been hypothesised that a shift in fungal interactions from parasitism to mutualism was an initial impetus in the colonisation of land by charophycean algae (Church 1921; Pirozynski & Malloch 1975; Atsatt 1988), it is doubtful that the fossil record will ever substantially contribute to deciphering this transition. Recent estimates based on molecular sequence data extend the age of the zygomycete (glomeromycete) fungi back to at least 600 Ma (Berbee & Taylor 2001) or even earlier – to 1000 Ma ago (Heckman *et al*. 2001). Whilst this does not demonstrate the age of the arbuscular mycorrhizal symbiosis, it does underscore the fact that the Rhynie chert symbiosis is perhaps not the earliest occurrence of this interaction. Current ideas regarding the earliest terrestrial plants suggest that they were small, green, upright axes that bore terminal sporangia and that probably possessed a prostrate anchoring and absorbing structure. These plants, termed rhyniophytoids (Edwards & Edwards 1986) or cooksonioids (Taylor 1988), are believed to represent a bryophytic grade of evolution. Although some of these specimens are known to contain a minute central strand of cells that probably functioned in conduction, other tissue systems (e.g., cortex) that might provide clues as to whether these plants were mycorrhizal are not preserved. Because these organisms are preserved as compressions and impressions, no information is available as to whether they contained fungi and thus entered into a fungal symbiosis.

Another critical component in the life history of the AM fungus is thick-walled resting spores (chlamydospores) that germinate and grow in the absence of host roots, but are not able to produce an extensive mycelial network. Recent research with modern AM fungi suggests that when no host is available the hyphae produced by the resting spores undergo growth arrest and resource reallocation (Giovannetti 2002). Some of the Rhynie chert chlamydospores with short hyphae that appear in the matrix may represent germinating spores, which suggests the existence of this life history strategy. They may also represent indirect evidence of some animal predator that grazed on mycelia in the rhizosphere, leaving behind the thick-walled spores and short hyphal attachments. Structures interpreted as oogonia with attached hyphae have been reported from the Rhynie chert (Fig. 6l) and compared with the oomycete *Apodachlya*, but these probably represent small chlamydospores or vesicles rather than oomycete reproductive structures (Table 1; Harvey *et al*. 1969).

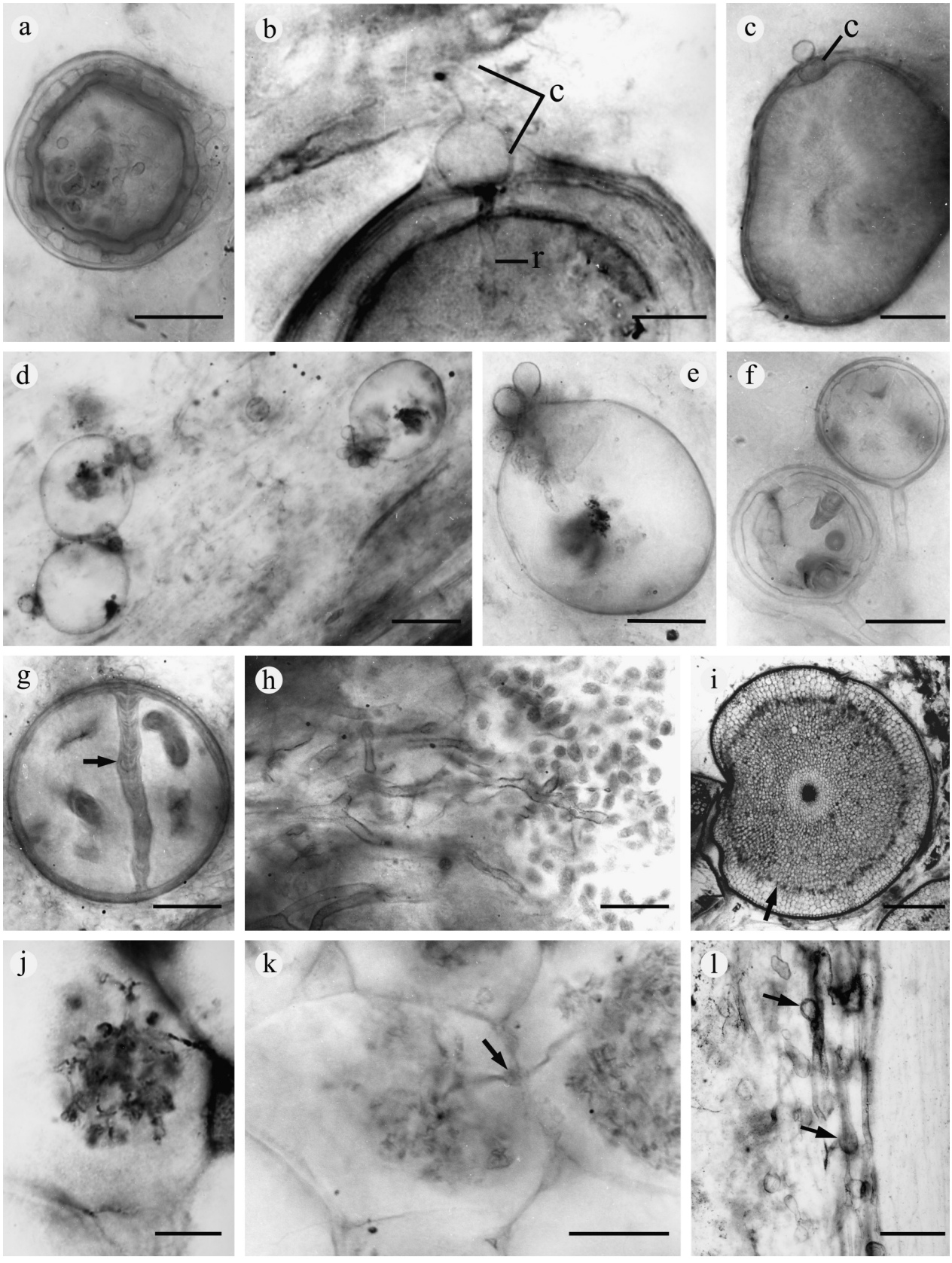
In modern ecosystems, mycoparasites can have an adverse effect on the development of the arbuscular mycorrhizal community, which thus affects the next generation of plants. Since chlamydospores infected by mycoparasites do not germinate to form the interradical hyphae which invade the underground

Figure 5 (a) Several asci containing ascospores from Rhynie chert ascomycete. Slide P3431, scale bar = 15 μm; (b) Tuft of conidiophores erupting from epidermis of *Asteroxylon* stem. Slide P3445, scale bar = 25 μm; (c) Section of *Asteroxylon* stem showing perithecia (arrows) interspersed with chlamydospores (c) Slide P3412, scale bar = 500 μm; (d) Longitudinal section of *Palaeonitella cranii* showing several nodes of branches and two hypertrophied (H) cells. Slide P1385, scale bar = 300 μm; (e) Chytrid zoosporangium (z) extending through cell wall (c) with well developed rhizomycelium (r) inside cell lumen. Slide P1359, scale bar = 25 μm (from Taylor *et al*. 1992a); (f) *Pediastrum*-like algal cell infected by chytrid zoosporangium. Slide P1483, scale bar = 10 μm; (g) Cluster of several algal cells, each containing a chytrid zoosporangium. Slide P1399, scale bar = 10 μm (from Taylor *et al*. 1992c); (h) Several algal cells attached to cortical cell wall (cw) of macroplant. Note circular openings (arrows) in zoosporangia for discharge. Slide P2496, scale bar = 15 μm (from Taylor *et al*. 1992c); (i) Two thick-walled fungal spores, each with the outer surface covered by epibiotic chytrid thalli. Slide P1935, scale bar = 50 μm; (j) Macroplant spore with three chytrid thalli protruding from the trilete suture. Slide P1347, scale bar = 15 μm; (k) Chytrid zoosporangium on macroplant spore with several discharge tubes. Slide P1687, scale bar = 15 μm; (l) Base of *Nothia* axis bearing numerous rhizoids. Note thickening (arrow) that may represent either the causal agent or symptom of fungal interaction. Slide P2868, scale bar = 100 μm; (m) Section of macroplant showing tissue disruption in the cortex. Slide P3445, scale bar = 500 μm.

parts of plants and ultimately form arbuscules in viable host tissue cells, the level of community fitness is reduced. Therefore, a high population of mycoparasites attacking chlamydospores can impact the number of mycorrhizal infections and may result in host plants that are less competitive in nutrient uptake. Although we don't know all of the complexities of the mycorrhizal relationships in the Rhynie chert, it is possible that mycoparasitism may have similarly impacted the macroplant community.

2.4. Lichens

It is estimated that approximately 20 % of extant fungal species enter into obligate symbiotic associations with cyanobacteria, green algae, or both, to form lichens. Lichens have continued



to provide challenges relative to their evolutionary history. The lichen life strategy, in which a fungus (mycobiont) and alga (phyco- or photobiont) live in a close symbiotic association, is now suggested to have arisen many times and has involved a number of algal groups over the course of geologic time (Gargas *et al*. 1995; Lutzoni *et al*. 2001). Lichens today are primarily associated with ascomycetes and the occurrence of a relatively advanced ascomycete in the Rhynie chert suggests that it is possible that this symbiosis was present, but has not yet been identified.

Although lichens have been reported from Precambrian rocks (e.g., Hallbauer & van Warmelo 1974; Retallack 1994), these have been discounted for a number of reasons (Cloud 1976; Waggoner 1995). Perhaps the most convincing fossil example of a Palaeozoic lichen symbiosis occurs in the Rhynie chert (Fig. 7a–f; Table 1; Taylor *et al*. 1997). *Winfrenatia reticulata* consists of a thin mycelial mat at least 10 cm long constructed of interwoven, aseptate hyphae. Along the upper surface are numerous, shallow, relatively uniform depressions (Fig. 7a, b). Within many of these depressions are spherical, coccoid unicells that are morphologically similar to certain cyanobacteria (Fig. 7c, d); also present are clusters of the same cells within mucilaginous sheaths that are interpreted as stages in the life history of the cyanobacterium (Fig. 7e). Hyphae of the fungus extend into the depressions and become intertwined with the bacterial cyanobionts (Fig. 7c). The range of features observed in *Winfrenatia*, including size of the thallus and number of depressions on the surface, has allowed the authors to speculate on the life history strategy, which included the production of new cells of the cyanobiont to maintain the symbiosis and support the fungus, and at the same time, an increase in the size of the fungal mycelial mat (Fig. 8; Taylor *et al*. 1997). It has been suggested that *Winfrenatia* is not a true cyanolichen, but rather represents an unstable association in which a fungus parasitised a cyanobacterial colony (Poinar *et al*. 2000). Whilst deciphering the physiological stability within a symbiotic association in the fossil record may never be fully realised, the definition of a lichen as a controlled parasitism rather than a strict mutualism is perhaps more accurate, as it underscores the varying degrees of lichenisation that appear in modern ecosystems (Ahmadjian & Jacobs 1981; Hyvärinen *et al.* 2002). In that context, *Winfrenatia* may quite accurately be used to define an Early Devonian lichen (Honegger 2001). Interestingly, extant cyanobacterial lichens have been found to show higher nitrogen levels and increased photosynthetic activity relative to green algal lichens (Palmqvist *et al*. 1998). Perhaps the *Winfrenatia* symbiosis functioned in a similar manner in the Rhynie chert ecosystem.

Although the fungal partner in most modern lichens is an ascomycete, the mycobiont in *Winfrenatia* has not been conclusively identified. The presence of aseptate hyphae and certain thick-walled, sculptured spores (Fig. 7f) associated with mycelial mats has been used to suggest that the fungal affinities may lie closer to the zygomycetes/glomeromycetes (Taylor *et al*. 1997). This is especially interesting since there is one modern example of a lichen with a glomeromycetous mycobiont and cyanobiont, *Geosiphon pyriforme*, this symbiotic association is found on the upper surface of wet soils that are poor in nutrients, especially phosphorus (Schüßler & Kluge 2001). In this unique symbiosis, the cyanobacterium *Nostoc* becomes encapsulated in a pear-shaped bladder that is formed by the fungus. The physiological exchange between the fungus and cyanobacterium takes place in this structure. It is interesting that *Geosiphon* is now included in the monophyletic group Glomeromycota, which also includes the arbuscular mycorrhizae formerly placed with the Zygomycota (Schüßler *et al*. 2001). This modern symbiosis demonstrates the structural and physiological aspects of such interactions; the association observed in *Winfrenatia* demonstrates that a glomeromycotan/cyanobacterial partnership may have existed in the Early Devonian.

2.5. Fungal/animal interactions

Since the first report of organisms in the Rhynie chert, numerous examples of extraordinarily well-preserved fossil animals have been identified among the plants. These include members of several arthropod groups, including trigonotarbid arachnids, mites, crustaceans, myriapods, and collembolans, amongst others (see review in Shear & Selden 2001). Indirect evidence of animal diversity in the chert includes various coprolites that have been distinguished on the basis of size, shape, and composition (Habgood *et al.* 2004). Some of these are made up of a heterogeneous complex of plant-derived materials (e.g., cuticle, spore fragments, conducting elements, and bits of parenchyma cells). Others suggest that the animals were more specific feeders; for example, some coprolites contain a very high percentage of spore fragments. In still others, the major constituent of the coprolites are fragments of fungal hyphae (Fig. 7g), suggesting that the animal was a fungivore.

As further work continues on aspects of the Rhynie chert fungal community, it will be interesting to see whether some of these microbes entered into fungal–animal symbiotic associations similar to those present in some modern ecosystems. The large diversity of animal remains in the Rhynie chert holds exceptional promise for the discovery of other fungal–animal interactions in addition to feeding. Within the zygomycetes are several groups of fungi which have been reported as obligate endophytes and can occupy the hindguts of various arthropod taxa (Lichtwardt 1986). The wide-ranging distribution of these modern symbioses has been used as evidence to support the hypothesis that this is an ancient interaction. To date, the only fossil example is a putative trichomycete reported by White & Taylor (1989) from the Triassic of Antarctica that consists of what is interpreted as an animal cuticle lined with elongate trichospores. The large number of major fungal taxa, including zygomycetes, and the diversity of well-preserved animals in the Rhynie chert offers the intriguing possibility that these rocks may also contain evidence of obligate symbiotic relationships similar to those with the Trichomycetes. For example, based

Figure 6 (a) Thick-walled fungal spore containing well-developed internal mycoparasite. Slide P2762, scale bar = 25 μm; (b) Mycoparasitic chytrid (c) that has penetrated thick-walled fungal spore with rhizomycelium (r) extending into cell lumen. Slide P1693, scale bar = 10 μm (from Hass *et al*. 1994); (c) Chytrid (c) that has developed between spore wall layers as a mycoparasite. Slide P2761, scale bar = 15 μm (d) Several thin-walled vesicles in the matrix with both endobiotic and epibiotic mycoparasites. Slide P1698, scale bar = 50 μm; (e) Vesicle with epibiotic chytrid and well-developed rhizomycelium in spore lumen. Slide P1698, scale bar = 25 μm (from Hass *et al*. 1994); (f) Thick-walled chlamydospores; note papillae extending into spore lumen. Slide P1671, scale bar = 25 μm (from Hass *et al*. 1994); (g) Chlamydospore with several papillae; note the concentric layering and infection canal in the papilla that bisects the spore lumen (arrow). Slide P1698, scale bar = 20 μm; (h) Hyphae of mycoparasite within an ascocarp like that in Figure 2f. Slide P3415, scale bar = 25 μm; (i) Transverse section of *Aglaophyton major* axis showing the continuous dark band (arrow) in cortex that contains the arbuscules of the mycorrhizal partner. Slide P1828, scale bar = 1 mm (from Taylor *et al.* 1995); (j) Detail of *Glomites* arbuscule showing the highly branched organisation. Slide P1703, scale bar = 10 μm; (k) Two cortical cells from band in Figure 6i, each containing an arbuscule. Arrow indicates slight thickening where arbuscule trunk has penetrated host cell wall. Slide P1827, scale bar = 50 μm (from Taylor *et al.* 1995); (l) Several thin-walled vesicle-like structures in chert matrix. Slide P1540, scale bar = 150 μm.

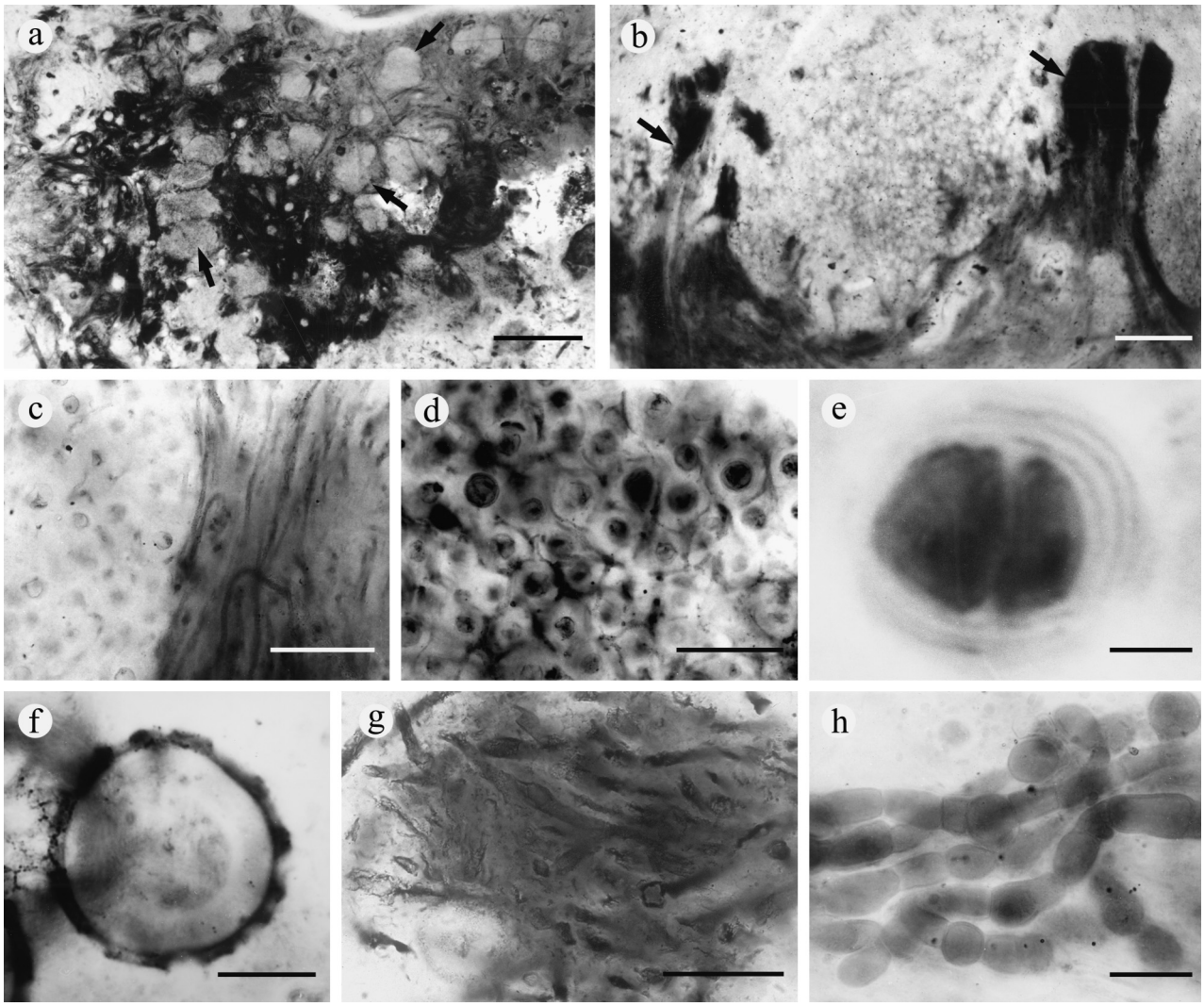


Figure 7 (a) Section of cyanolichen thallus showing depressions (lighter areas at arrows) that contain cyanobacteria surrounded by more opaque zone that represents the fungal partner. Slide P1604, scale bar = 750 μm; (b) Section of lichen at right angles to thallus in Figure 7a, showing depression and opaque walls (arrows) formed of fungal hyphae. Slide P1323, scale bar = 150 μm (from Taylor *et al.* 1997); (c) Detail of wall formed by fungal hyphae and depression containing cyanobacterial cells. Slide P1388, scale bar = 50 μm (d) Detail of unicells from depression in thallus showing association with fungal hyphae. Slide P1386, scale bar = 50 μm (from Taylor *et al.* 1997); (e) Two daughter cells of lichen cyanobiont showing remnants of several sheaths. Slide P1380, scale bar = 5 μm; (f) Thick-walled, reticulate spore found associated with hyphae in cyanolichen. Slide P1374, scale bar = 25 μm; (g) Coprolite composed of fungal hyphae. Slide P1919, scale bar = 50 μm; (h) Detail of several filaments believed to be cyanobacteria. Slide P1680b, scale bar = 20 μm.

on molecular analyses, it has recently been noted that Trichomycetes is a highly polyphyletic group (Cafaro 2003), and that at least one genus is not even a fungus, but rather a protozoan (Benny & O'Donnell 2000). Thus the possibility exists that not only are there various fungal groups that formed associations with diverse animals early in the development of a terrestrial ecosystem, but also that such associations involved other non-fungal groups as well.

All of the modern fungal groups that have been identified in the Rhynie chert to date have members that are saprophytes of various arthropods and animals. For example, modern insects have coevolved with fungi in a variety of patterns that benefit the insect in the acquisition of nutrients and metabolites, protection, and habitat conditioning. Fungi in turn may benefit from propagule dispersal and protection from grazing (Murrin 1996). Although there are reports of entomophthoralean and saprophytic fungi associated with various fossil arthropods (Poinar & Thomas 1982; Stubblefield *et al.* 1985b), none of these interactions have been reported to date from the Rhynie chert.

2.6. Bacteria

Bacteria have a major influence on the terrestrial environment in modern ecosystems because of the large number of chemical reactions they catalyse in the soil. Bacterial communities control the availability and cycling of soil nutrients, including carbon and nitrogen, and are also capable of forming beneficial and deleterious associations with plants. Some nitrogen-fixing symbioses involve specific rhizobial interactions between the microbes and plant roots and contribute significantly to the nutrition of the host; for example, they accumulate nitrogen and phosphorus in quantities similar to those in terrestrial plants (Buckley & Schmidt 2002). Although various bacteria are early colonisers in moist environments, their importance as agents of wood decay is relatively minor. Nevertheless, bacteria play some role in the degradation of cellulose. In their classic paper, Kidston & Lang (1921b) described bacteria both in the matrix and associated with some plant material; further studies dealing with microbial life in the Rhynie chert have identified additional colonies and clusters of bacterial cells (Fig. 7h) that

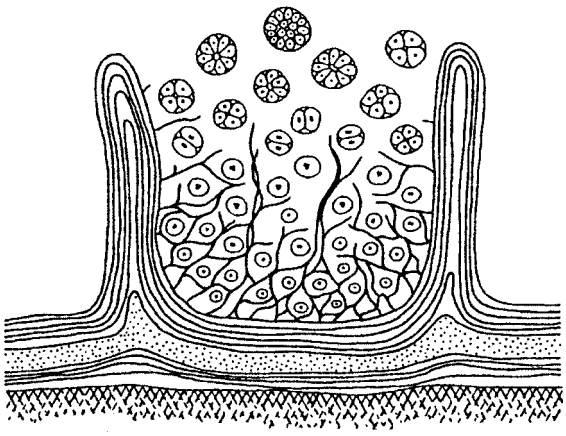


Figure 8 Detail of a single depression in the thallus of the lichen *Winfrenatia reticulata* showing walls made up of fungal hyphae that in turn surround the cells of the cyanobiont. Some of the hyphae form a net-like structure in the base of the depression; cells of the cyanobiont are undergoing division near the surface.

appear similar to cyanobacteria (Table 1; Croft & George 1958).

There are many ways that plants affect the structure of microbial communities, including the production of root exudates that can influence community composition and location. In addition, plants can impact soil microbial diversity by competing for resources. It is highly unlikely that we will ever completely know how the composition of the Rhynie chert ecosystem influenced the microbial community in the substrate. Nonetheless, a more complete understanding of the biodiversity of this complex community makes it possible to present scenarios of interactions that in turn can be compared with those in modern ecosystems (Fig. 1A). For example, could the swellings on the rhizoids of fossil macroplants such as *Nothia aphylla* represent some form of bacterium/root interaction (e.g., rhizobia) that was perhaps involved in nitrogen fixation, rather than representing a symptom of a parasitic chytrid? The fact that Early Devonian fungi can penetrate host cell walls to form mycorrhizal arbuscules and not rupture the plasma membrane indicates that an endosymbiosis with other organisms, including bacteria, was certainly possible.

3. Discussion

3.1. Evolution of fungal groups

Traditionally, the Kingdom Fungi includes the Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota; recently, the Glomeromycota has been introduced for arbuscular mycorrhizae. Together, these form a group of walled, heterotrophic organisms that are thought to have evolved from zooflagellated protozoans (Cavalier-Smith 1998). An alternative classification includes the chytrids, zygomycetes, and allomycetes (Blastocladiales and Colemomycetales) in the subkingdom Eomycota, and ascomycetes and basidiomycetes in the Neomycota (Cavalier-Smith 2001). All fungi are absorptive heterotrophs with multinucleate hyphae and chitinous spores that lack plastids. In recent years, numerous authors have used 18S rDNA sequence data to estimate divergence times of various major fungal groups (e.g., Berbee & Taylor 1993, 2001). Among the fungi, the chytrids are the only group that possesses flagellated cells, which supports the basal position of this group. This position is in turn further supported by ultrastructural, biochemical, and additional morphological features. The diversity in chytrid morphology and life history biology of the forms described to date in the Rhynie chert also support this hypothesis. Based on rDNA sequences, James *et al*. (2000) suggest that the Chytridiomycota is not a monophyletic group, and that the Blastocladiales interestingly cluster with the zygomycetes. This finding would support the ancient divergence of these two groups, which is also suggested by the structural and life history features found in the fossil *Paleoblastocladia*.

In modern ecosystems, zygomycetes, glomeromycetes, ascomycetes, and basidiomycetes are typically associated with land plants. Based on molecular sequence data, Berbee & Taylor (2001) suggest that the Glomeromycota, which today are involved in arbuscular mycorrhizal symbioses and characterised by a coenocytic thallus and the production of asexual spores, first occurred more than 600 Ma ago. This is several hundred million years earlier than previous molecular clock estimates based on rDNA sequences (Simon *et al*. 1993; Simon 1996). Fossil evidence that may contribute to elucidation of the age of the first endomycorrhizae has recently been reported from Upper Ordovician rocks (Redecker *et al*. 2000, 2002). Specimens of *Palaeoglomus grayi* include terminally-borne, globose spores up to 95 μm in diameter associated with aseptate hyphae. These specimens occur isolated in the matrix and are not associated with macroplants; however, if they are examples of glomeromycotan fungi, they help to provide a calibration point that underscores the antiquity of the arbuscular mycorrhizal symbiosis.

Ascomycetes comprise the largest group in the Fungi and are characterised by septate hyphae and a dikaryotic phase in their life cycle. One group, the filamentous ascomycetes, has enclosed ascocarps of several different morphologies, including flask-shaped perithecia. This type of ascocarp is associated with *Asteroxylon* (Figs 2j, k, 5c) and provides the earliest fossil evidence of an ascomycetous sporocarp that contains sterile paraphyses and thin-walled asci that probably forcibly discharged the ascospores. The apparently modern appearance of this fungal component of the Rhynie chert offers compelling evidence for the antiquity of this group, which can be traced back to the Upper Silurian. What is perhaps most interesting about the discovery of ascomycetes in the Rhynie chert concerns the age of the group, which had previously been assumed to be younger, based on the supposed association of leaf-inhabiting ascomycetes with early angiosperms in the Cretaceous (Berbee & Taylor 2001). What is important about this fossil is that it provides an important set of features that can be used in polarising characters and also to calibrate molecular clocks. Although there are conflicting points of view regarding the evolution of groups within the Ascomycota, Berbee & Taylor (2001) indicated that the pyrenomycetes (filamentous ascomycetes), including the experimental organism *Neurospora*, appear at the base of the Euascomycete clade.

The Basidiomycota is the second largest group in the Kingdom Fungi and possesses meiospores (basidiospores) formed on club-shaped cells. Molecular data suggest that the Ascomycota and Basidiomycota diverged from each other approximately 100 Ma earlier than the time of the Rhynie chert ecosystem (Berbee & Taylor 2001). Of the four major groups of fungi recognised today, only the basidiomycetes have yet to be conclusively demonstrated from the Rhynie chert ecosystem. This is interesting, because evidence of basidiomycetes in the form of solution troughs along the inner walls of tracheids have been reported from Upper Devonian progymnosperms (Stubblefield *et al*. 1985a).

We remain cautious as to the significance of the absence of the basidiomycetes, since the first fossil ascomycete has only recently been reported and is of a type that may be considered to be highly evolved. Basidiomycetes today include parasites

of plants as well as the principal lignin and cellulose decomposers in modern ecosystems. If current ideas regarding the divergence of Ascomycota and Basidiomycota based on molecular phylogenies are accurate, then one might expect that these two groups co-occurred at the time of the Rhynie chert ecosystem. The absence of this group in the Rhynie chert to date may be related to the apparent lack of lignin in the macroplants and/or the fact that other groups of fungi (e.g., chytrids) may have been the primary decomposers in this ecosystem. Perhaps a more plausible explanation for the absence of the group is that they simply have not yet been identified.

All of the other fungal associations that have been described from the Rhynie chert appear similar to those found today. These include epibiotic and endobiotic forms of chytrids, as well as those that have colonised highly specialised habitats between certain wall layers in chlamydospores, and others that appear to be restricted to certain hosts (Figs 5d–k, 6a–g). In modern microbial interactions there are specific signalling mechanisms that are present in both the environment and potential hosts. These complex genetic, molecular, and biochemical elicitors have been identified in some living fungi and only now are beginning to be fully understood. Whilst this level of molecular and genetic inquiry will be impossible to carry out in the Rhynie chert organisms, the host responses that have been demonstrated (Table 2) help to underscore that the relationships between fungi and other organisms are very ancient and biochemically highly complex.

3.2. Fungal distribution in plants

Another important avenue in the study of fossil fungi concerns their distribution in and on the plant. During Rhynie chert time, none of the macroplants possessed true leaves and thus there was minimum surface area for fungi to colonise in an aerial habitat. There were, however, various types of epidermal appendages, including enations in *Asteroxylon* that may or may not have been photosynthetic, and irregular surfaces on other aerial axes (e.g., *Nothia*). The presence of enations and the development of a more complex vascular system certainly represented adaptations that allowed greater photosynthetic activity and, presumably, larger size. As the macroplants became more complex and provided increased variability in microhabitats for colonisation, fungi such as the perithecial ascomycetes expanded into new niches. Not only do the perithecia occur along the stems, but also extend some distance out along the bases of enations (Fig. 2j, k). Ascomycetes of this type occur as leaf and stem parasites and are important pathogens of many extant plants. We are uncertain as to whether the Rhynie chert ascomycete was a parasite, pathogen, or saprophyte, but there is some minor evidence in the form of necrotic areas on the stems suggesting a host response to a parasite. If this ascomycete was in fact a parasite, it is problematic as to why other examples of this group have not been found on the phylloplane of slightly younger groups of vascular plants that dominated early ecosystems. Perhaps the absence of this group of potential leaf-borne fungi is the result of plant defences, rather than the failure of the fungi to exploit new niches in the ecosystem. On the other hand, it is possible that these fungi had not yet evolved either the physical adhesion properties (glycoproteins) to attach to a leaf or laminar structure, or the chemical communication system that would have been necessary in the host/fungus relationship. It is also possible that these fungi simply have not been recognised in the fossil record.

At least one other group of Rhynie chert fungi (glomeromycetes) that entered into a mutualistic symbiosis with macroplants in the ecosystem appears to have exploited a very high percentage of host cells. Whilst most modern arbuscular mycorrhizae develop arbuscules that are confined to the underground and absorptive parts of the plant, the arbuscules in *Aglaophyton* are developed in both the aerial and subaerial parts of the plant. In addition, it appears that the hyphae that form the arbuscules extend all the way to the tips of all axes. Thus, in this symbiosis, the fungus may be viewed as encapsulating a major portion of the living tissues of the sporophyte, a relationship that does not appear in modern herbaceous plants. The high correspondence of fungal physiological exchange sites (arbuscules) and living cells in the host adds support to the hypothesis that fungi were a necessary component in the transition to a terrestrial habitat (Pirozynski & Malloch 1975). However, recent ribosomal sequence data suggest that the symbiosis between green plants and fungi may have evolved prior to the colonisation of land (Tehler *et al.* 2003).

The basic types of structural and morphological features of arbuscular mycorrhizae have been termed the *Arum* and *Paris*-types, since it was from these genera of plants that mycorrhizae were originally described (Gallaud 1905). In the *Arum*-type, there is an extensive intercellular hyphal network and arbuscules are formed as terminal structures; in the *Paris*-type, extensive intercellular hyphae are absent, but a hyphal network is present in the form of coils of hyphae, with arbuscules formed as intercalary structures (Smith & Smith 1997). The *Arum*-type is less frequently found and occurs in some ferns and angiosperms (Read *et al.* 2000). Although there are exceptions, it appears that many gymnosperms, bryophytes, and so-called 'lower' vascular cryptogams, including some ferns, have AM structures of the *Paris*-type (Brundrett 2002). Based on the structural features that are used to distinguish these two types of arbuscular mycorrhizae, those that have been identified to date in the fossil record (in *Aglaophyton*) are distinctly of the *Arum*-type (Fig. 6j, k). Whilst both the physiological necessity of the plant and anatomical structure of its tissues may play important roles in the type of mycorrhizae found in plants, these distinctions may also be related to the degree of fungal infection in the plant body, as well as physiological efficiency of the arbuscules. It should be noted, however, that the same fungal species can produce both the *Arum*- and *Paris*-types, depending on the anatomy of the root, which indicates that control resides with the plants (Jacquelinet-Jeanmougin & Gianinazzi-Pearson 1983). Whilst arbuscules have been reported in the gametophytes of *Aglaophyton* (*Lyonophyton*), there remain questions as to the degree and extent of the fungal infection in this phase. In the living plant *Lycopodium*, there appears to be some variation in structural organisation in fungal endophytes of gametophytes and sporophytes (Duckett & Ligrone 1992), with no arbuscules produced in the gametophyte; arbuscules are also lacking in the moss *Hypopterygium* (Jakucs *et al.* 2003). Since *Lycopodium* possesses the same type of basic life history as that of *Aglaophyton*/*Lyonophyton*, it will be interesting to see whether this variation is also present in the Rhynie chert macroplants.

3.3. Future research directions

What types of interactions and associations will be revealed in the microbial world of the Rhynie chert in the years ahead? Certainly one series of important questions concerns whether other macroplants in the ecosystem were mycorrhizal. Whilst chlamydospores of the glomalean type have been found in tissues and associated with all of the other macroplants, this in itself is not conclusive proof that these plants contained mutualistic endosymbionts. Of equal importance will be whether the free living gametophytes were all mycorrhizal, and

if so, the extent of interaction as compared with that found in the sporophyte *Aglaophyton*, where a large number of arbuscules were formed.

Bacteria were another important biological component of the microbial world during Rhynie chert times. The resolving power available with transmitted light microscopy, and perhaps the actual preservation of bacterial cells, may not be sufficient to provide further details about these microbes. However, their occurrence in cells, tissue systems, and various other organs in the chert may provide indirect evidence of certain types of interactions. For example, in a recent study, bacteria are reported in the cytoplasm of chlamydospores of modern AM fungi, which suggests that a more complex set of interactions may exist between the fungus and the host, as well as the endobacterium and the fungus, although the complexities of this endosymbiosis are not yet fully understood (Bonfante *et al*. 2002; Bonfante 2003). Other symbionts in the form of nitrogen-fixing microbes are diverse and present in modern arthropod guts (Nardi *et al*. 2002) and may have occupied similar habitats in some of the Rhynie chert arthropods. Additionally, there is some evidence that suggests that bacteria may provide improved plant nutrition in soils low in phosphorus (Bonfante 2003). Based on this, it will be interesting to see if bacteria are directly associated with any of the Rhynie plants. Today bacteria exist primarily as free-living forms in the soil, but a few form symbiotic relationships with plants. Nodules containing bacteria have been reported from a large number of extant plants. Some of the macroplants in the Rhynie chert have swellings on the rhizoids that are of uncertain origin and may have hosted endobacteria.

Cyanobacteria form a variety of symbiotic interactions with fungi and other organisms, including lichens (Rai *et al*. 2002), such as that documented from the Rhynie chert. Other symbioses today include cycads, selected flowering plants, aquatic ferns, sponges and certain colonial animals, diatoms, and various bryophytes. Since several cyanobacteria are known from the Rhynie chert (Kidston & Lang, 1921b; Croft & George 1958), and the macroplants appear to have their closest affinities to the bryophytic grade of organisation, it will be challenging to see if cyanobacterial symbionts are also associated with any of the terrestrial components of the ecosystem.

Another potentially rewarding avenue of research within the Rhynie chert is the documentation of interactions between microbes and animals. We have already commented on the value of examining certain animals that have been described from the chert for fungal/arthropod symbiosis similar to those found in Trichomycetes today. A wide variety of ascomycetes are also associated with modern arthropods (Spatafora 2002) and the demonstration of ascomycetes in the cherts suggests another interaction that may also exist in the Rhynie chert.

Future Rhynie chert microbial research might involve potential pathogens of the macroplants. An interesting question is whether all of the necrotic areas and pustules on the surfaces of axes and enations were caused by fungi or other organisms. For example, the modern green alga *Cephaleuros* is an epiphyllous form that has typically been misidentified as a glomalean fungus (Reynolds & Dunn 1984; Chapman & Henk 1985). The filamentous alga grows under the cuticle on the upper surface of leaves, killing the tissue beneath. Tufts of reproductive structures (sporangiophores) bear zoosporangia and appear similar to fungal conidiophores; however, the presence of quadriflagellate zoospores and the life history biology positively identify this pathogen as an alga. Green algae have already been identified in the Rhynie chert, which suggests that further study of epidermal lesions may yet reveal other groups preying on the macroplants.

Further expansion of inventory of the diversity of microbes present is necessary, in order to more accurately define this specialised ecosystem and potentially offer hypotheses about patterns of nutrient cycling. Careful description of both teleomorphic and anamorphic fungal states, as well as the structures that make up the fungi, will be especially important in providing calibration points for divergence times that can be measured against phylogenies based on molecular data analysis. As the studies that are reported in this volume clearly demonstrate, the Rhynie chert ecosystem is highly complex, both physically and biologically. Despite the extensive research that has been accomplished to date, these complexities are only partially understood. Admittedly, documenting some of the potential interactions noted in this paper will be difficult and perhaps impossible. Nevertheless, the minuscule and sometimes partially opaque window into the Early Devonian that is represented by the Rhynie chert provides a level of biological inquiry that was probably not envisioned when Kidston & Lang published their extraordinary studies. Discoveries from the Rhynie chert have been exciting and challenging, both in terms of documenting the existence of the organisms present and interpreting their biological importance, yet we believe that the discoveries that remain to be made will be even more exciting and will have a still greater impact on ideas about the evolution of terrestrial ecosystems.

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