Microbial life in the late Paleozoic: new discoveries from the Early Devonian and Carboniferous

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Summary

Microorganisms are critical in the bio- and geosphere today, and certainly performed similar functions in ancient ecosystems. Bacteria, cyanobacteria, microalgae, and various microfungi and fungus-like organisms constitute a substantial component of these ancient communities, and have been responsible for the evolution and sustainability of ecosystems functions ranging from decomposition to catalysis in nutrient cycles. In spite of these profound contributions, fossil microorganisms have only relatively recently received focused attention directed at their role in ancient ecosystems.

The success of documenting fossil microorganisms and their associations with other ecosystem components relies on the manner in which the microorganisms and their host(s) are preserved. Cherts represent the most important source of evidence for fossil microorganisms *in situ* because they provide exquisite preservation of both microorganisms and host(s), and the only matrix that can be used to extract information about these life forms within the context of ecosystem complexity, versatility, and dynamics.

Perhaps the most famous chert is the Early Devonian Rhynie chert (~400myb), in which there are structurally preserved early land plants associated with a variety of microorganisms. The Rhynie chert has contributed substantially to our conception of the roles that microorganisms have played in early continental ecosystems. However, this conception is based on a relatively small number of microorganisms (mostly fungi) involved in specific interactions that have been described in detail and directly compared to modern analogues; numerous other forms and consistent associations in the Rhynie chert have not received a sufficient level of scholarly attention. Another interesting chert deposit comes from the upper Visean (~330myb) of central France, and reflects a structurally preserved flora composed of lycopsids, sphenopsids, and ferns associated with a largely unrealized diversity of microorganisms. Because of the multiple levels of association/interaction, a precise knowledge about the diversity, morphology, and ecology of microorganisms in the Rhynie and Visean cherts represents an important component of fully understanding the roles that microbial life played in continental late Paleozoic ecosystems.

The twelve scientific papers included in this thesis contribute substantially to a body of knowledge that focuses on the morphology and biology of microorganisms from the Rhynie and Visean paleoecosystems.

Photosynthetic microorganisms have rarely been described from the Rhynie chert, despite the fact that cyanobacteria and algae are common elements of aquatic environments today. The cyanobacterium *Croftalania venusta* occurs in the Rhynie chert in several distinct growth forms and may also form complex microbial mats, in which it co-occurs with various

other microorganisms. These mats provide an interesting perspective on the evolution of cyanobacterial associations with other organisms. Another interesting Rhynie chert microfossil has been identified as a phycoma assignable to the prasinophycean algal genus *Cymatiosphaera*. This discovery represents the earliest evidence of this group of green algae in a freshwater deposit.

The Rhynie chert also contains several examples of interfungal associations. *Globicultrix nugax* and *Kryphiomyces catenulatus* are two exquisitely preserved microfungi that live in the interior of glomeromycotan spores. Microfungi associated with glomeromycotan spores are particularly interesting with regard to better understanding the dynamics within the Rhynie paleoecosystem because, if they were parasites, they most likely impacted the number of viable glomeromycotan spores, and thus reduced the number of mycorrhizal inoculations and therefore altered the structure of this early land plant community.

Endophytic cyanobacteria occur in some axes of the Rhynie chert land plant *Aglaophyton major*, and represent the earliest direct evidence for a land plant-cyanobacterial association. *Aglaophyton major* is endomycorrhizal, and the cyanobacterial filaments are particularly abundant close to the mycorrhizal arbuscule zone. This may suggest that there was some level of interaction between the cyanobacteria and mycorrhizal fungi. Another example for a microorganism-land plant interaction in the Rhynie chert has been discovered from the land plant *Nothia aphylla*, in which three different fungal endophytes, including a putative endomycorrhizal fungus, concurrently colonize the subterranean rhizomes, but enter into qualitatively different relationships with the host. Although the Rhynie chert endomycorrhizae are well-understood today, the reproductive biology of the fungi involved in these symbioses remains largely unknown. New data on the morphological diversity of glomeromycotan spores from the Rhynie chert suggest that the Glomeromycota were well established as a group and relatively diverse by Rhynie chert time, even before true roots evolved since all of the Rhynie chert plants and many other early land plants at the time lacked roots.

The Visean cherts from central France are less well studied than the Rhynie chert with regard to the microbial component. An assemblage of probably saprotrophic microfungi and fungus-like microorganisms occurs in *Lepidodendron* xylem and periderm from the Visean cherts of central France. Since the organisms were abundant and diverse, they obviously played an important role in the ecology of this paleoecosystem. In addition, the evidence for chytrids and chytrid-like remains of uncertain affinity preserved in the Visean cherts is surveyed. Although these fossils do not provide a conclusive comparison with chytrids in modern ecosystems, they offer the opportunity to advance hypotheses as to the ecology of this microfungal community. Also present in several specimens of Visean lycophyte peridem is a

highly unusual intracellular endophyte, *Combresomyces cornifer*, which is interpreted as a peronosporomycete.

This thesis represents a small segment of the total level of microbial diversity and associations/interactions with other ecosystem components that existed in the Devonian and Carboniferous. Nevertheless, the extraordinary preservation has made it possible to examine the microorganisms and their hosts in great detail. The papers published to date and those in press and preparation demonstrate the value of new discoveries in more accurately depicting the individual components of fossil ecosystems, even those from the well-known Rhynie chert, and further underscore how new specimens can contribute to a more sharply focused concept of ancient ecosystem complexity. Finally, the thesis provides reference points that allow direct comparisons to be made between the Devonian and Carboniferous micro-organisms and the changing floral elements at two especially interesting points in geologic time.

1. Introduction

1.1. Definition of the term „microorganisms“

It is estimated that there are more than five million different kinds of organisms on Earth today, most of which are extremely small (e.g., May 1988). These minute life forms are commonly termed “microorganisms” or “microbes”. The collective terms „microorganisms“ (from Greek μικρός, *mikrós*, "small" and οργανισμός, *organismós*, "organism") and „microbes“ summarize all pro- and eukaryotic life forms regardless of their biological affinities that are not or just barely visible to the naked eye, and therefore can only be examined with a microscope; in general, bacteria, cyanobacteria, microfungi and fungi-like microorganisms, microalgae, and protists are included (Madigan et al. 2008).

Microorganisms are highly variable physiologically, and thus can be found in nearly every natural habitat, even in the most inhospitable environments such as the polar ice, desert sand, geysers, rocks, and the deep sea. Microorganisms play important roles in the biosphere, e.g., as primary producers of organic material at the beginning of food chains and as decomposers at the end of the nutrient cycle where they are responsible for the return of nitrogen and other substances back to the environment (Staley 2002). Due to their various and extensive interactions with other organisms, microorganisms act as selective forces in eco-system dynamics, and influence the evolution as parasites, pathogens and disease causative agents, and partners in mutualistic relationships (e.g., Goodman and Weisz 2002).

Because of their extraordinary importance in ecosystem functioning, various groups of microorgansims, as well as their interactions with other organisms, have received considerable scientific attention, and today are studied intensively with regard to their morphology, physiology, molecular biology, and genetics. During the last twenty five years, one of the most profound achievements of microbiological and ecological research is the increasingly detailed documentation of how microbial life is involved in the various processes that characterize complex modern ecosystems, and how microorganisms drive the evolution and sustainability of these ecosystems (e.g., Staley and Reysenbach 2002, and references therein). Because of the interrelationships of these organisms within the bio- and geosphere today, understanding their role in the evolution and sustainability of life is a critical theme that can only be addressed from the fossil record.

1.2. Fossil microorganisms

Although microorganisms certainly played an equally important role in ancient ecosystems, knowledge about the fossil record of these life forms remains incomplete. There are several possible reasons for this lack of data on fossil microorganisms, and microbial associations and

interactions with other organisms in ancient ecosystems. Perhaps the most important of these is the small size of the organisms and the lack of specific diagnostic features that can be resolved at the level of transmitted light. In addition, information about fossil microorganisms is based almost exclusively on the dispersed record, in which these life forms generally are not preserved *in situ* (e.g., Kalgutkar and Jansonius 2000). Moreover, the life history of many microorganisms (especially fungi) is often rather complex, and in fossil representatives cannot generally be fully reconstructed because the record is typically composed of isolated stages such as (zoo-)sporangia, cysts, and (resting) spores (e.g., Krings et al. 2009a,b). In spite of these obstacles it is possible today using various levels of inquiry and involving multiple levels of collaboration to address numerous questions about fossil microorganisms. This is especially true of questions that focus on ecosystem interactions and community structure. Often in the study of fossil microbial life there is a historical separation between those scholars with interests exclusively focusing on extinct organisms (and perhaps their value as stratigraphic markers or index fossils), and those who have the necessary knowledge about the biology and diversity of modern microorganisms. Another reason for the under-representation of descriptions of microorganisms in the paleontological literature certainly has been the general lack of exquisitely preserved fossils like those of various animals and plants that immediately captured the attention of the scientific community. Related to this aspect was the inherent collection bias in which only the most complete and showy specimens were brought to the attention of the paleontologists, while the fragmented and scrappy remains – those with potential evidence for microbial activities – were left behind.

Despite the problems noted above, there are a few remarkable early reports of exquisitely preserved late Palaeozoic microorganisms and microbial associations/interactions with other elements of ancient ecosystems (e.g., Renault 1896a, 1900; Kidston and Lang 1921b). However, these studies are based on material preserved in a siliceous chert matrix, a very special mode of fossilization (see below) in which even the most delicate structures and finest details may be faithfully preserved. Because fossiliferous cherts were locally restricted and distinct from other fossil sites, the organisms contained therein became widely sought-after curiosities that immediately attracted the attention of several prominent palaeontologists at the time.

Historically, the increasing number of reports of Precambrian microbial life (surveyed in Taylor et al. 2009) also initiated a more general paleobiological interest in evidence of microbial activities from other, geologically younger paleoecosystems. Today the importance of microbial and especially fungal remains as a major constituent of ecosystem functioning and evolution is a primary focus of many disciplines (e.g., McArthur 2006). As a result, there has been a paradigm shift in the appreciation of the microbial world in time and

space, including microbial associations and interactions with other organisms in ancient ecosystems.

The scientific attention that *in situ* preserved fossil microorganisms and their associations/interactions are receiving today from palaeontologists reach far beyond simple descriptions, and also focus on understanding the interrelationship that existed between the different components of fossil biota. These in turn are directed at assessing paleoecosystem complexities and dynamics. Moreover, some scholars have begun to incorporate interactions between fossil microorganisms and their "hosts" into phylogenetic considerations (e.g., Redecker and Raab 2006). This increasingly important aspect of the scientific work with fossil microorganisms relates to our knowledge of the evolutionary history of complex systems and processes in ancient and modern ecosystems.

A key requisite for being able to accurately identify and document the morphology, internal structure, and diversity levels of fossil microorganisms, as well as their associations/interactions with other components of the ecosystems, is the three-dimensional and structural preservation. This preservational context is necessary to place both the microorganisms and host(s) within the same microhabitat so that both can be evaluated in detail. There are several different modes of preservation that may produce exquisite fossils of microorganisms. For example, amber may preserve even the most delicate structures of some microbes in great detail (e.g., Schmidt et al. 2007; Girard et al. 2008). Moreover, amber is transparent, and thus can often be examined directly after (simple) polishing without time-consuming preparation. However, fossils preserved in amber are commonly no older than the Triassic, and thus there in nothing is known from closely related Palaeozoic forms. Moreover, pieces of amber typically are relatively small and inclusions always accidental, and thus in general, the ecological context within the community in which these organisms lived is less completely known.

A second form of three-dimensional and structural preservation occurs in carbonate coal balls. This preservation mode has been responsible for a considerable amount of detailed information about the morphology and anatomy of the plants that grew in the vast Carboniferous forest ecosystems (the so-called coal swamp forests) of Europe and North America (e.g., Phillips and DiMichele 1999). However, coal balls have yielded relatively few studies of microorganisms to date. Although there are various reasons for this paucity of attention to the microbial component of the Carboniferous coal ball floras, certainly one important reason is the commonly used cellulose acetate peel technique (for details on methodology, refer to Galtier and Phillips 1999). Since this technique relies on the acid digestion of the coal ball matrix, many of the microorganisms embedded in the matrix are lost

during preparation. In spite of this, there exist a few studies on Carboniferous microorganisms that suggest an enormous, yet largely unrealized microbial diversity during this period of time (see Taylor and Krings 2005).

1.3. Cherts

Chert deposits are among the most important sources of information about fossil micro-organisms and interactions with other organisms in ancient ecosystems (see Taylor and Krings 2005). Aspects of microbial diversity, biology, and ecology (e.g., life history features, habitat preferences), as well as specific details of microbial associations and interactions (e.g., infection pathways, spatial distribution of the microbes on or within the host, host responses), can often be demonstrated on a consistent basis using multiple examples (e.g., Remy et al. 1994; Krings et al. 2007b,c).

Chert deposits occur at various points in geologic time and typically represent an extremely dense microcrystalline or cryptrocrystalline type of sedimentary rock. Some chert deposits are formed through the accumulation of the siliceous remains of certain organisms such as diatoms or sponges. Most cherts, however, originate from the chemical precipitation of silica-supersaturated, hydrothermal water (e.g., from hot springs and geysers). As soon as the hydrothermal solution percolates to the surface, it cools and water begins to evaporate. Due to the decrease in temperature and loss of water, amorphous silica precipitates in the form of sinter that encloses all (organic) material present on the surface at the time of sinter formation. Other factors may affect the precipitation process as well, including the presence of other dissolved minerals or organic matter (Konhauser et al. 2001). During the precipitation of (amorphous) silica, monosilic acid, which is the main soluble form of silica in nature, may permeate organic tissues and cell walls. With increasing concentration of amorphous silica in the solution, monosilicic acid polymerises and forms amorphous silica that precipitates not only at the surface of the organisms, but extends into the cells and openings between cell walls. The histological character is thus “copied” by the silica deposition within a few days to a number of years (Leo and Barghoorn 1976; Jones et al. 2003). This rapid and pervasive silicification occurs prior to significant cellular decay and therefore may lead to exquisite preservation of organisms. Over time, the amorphous silica phase of the primary/initial sinter becomes unstable and gradually changes to a more stable, crystalline form of silica, which is then called a chert. The process of sinter formation can still be observed today (i.e. *in statu nascendi*) in several modern sites that are currently being investigated (e.g., the Yellowstone National Park, U.S.A., and the Taupo Volcanic Zone in New Zealand) with regard to the precise taphonomic processes involved in the preservation of animals, plants, and microorganisms (Channing and Edwards 2009). As a result of this mode

of formation, some cherts are exceptionally rich in fossils that demonstrate not only three-dimensional and structural preservation (sometimes even *in situ*), but often also remarkable details of individual cells and subcellular structures (e.g., multilayered cell walls, flagella, chromosomes, nuclear caps; see Taylor et al. 2004).

Based on the fidelity of fossil preservation, cherts provide an ideal matrix from which to extract information about microorganisms and their associations/interactions with other components of the ecosystem. Cherts provide the only source of direct evidence of the microbial world within the context of ecosystem complexity, versatility, and dynamics. Although other types of silicification exist (e.g., silicified wood) that may also contain well preserved microorganisms (compare e.g., Stubblefield et al. 1985), they do not normally provide direct information about the environment and ecosystem in which the microorganisms lived.

1.4. Thesis context

Although fossiliferous cherts containing well preserved microorganisms and microbial associations/interactions with other organisms are relatively rare, there are several important late Paleozoic chert deposits that provide detailed insights into the evolutionary history and paleoecology of microorganisms. One of these is the famous Early Devonian Rhynie chert, in which there are several types of structurally preserved early land plants associated with a wide variety of microorganisms, including bacteria, cyanobacteria, and fungi (Taylor and Krings 2005). Another, geologically younger (Carboniferous) chert deposit comes from the upper Visean (Mississippian) of central France and reflects a diverse flora composed of lycopsids, sphenopsids, and ferns. Although to date distinctly less well studied than the Rhynie chert, there are a few well-documented examples of microorganisms (mostly fungi and fungi-like organisms) from this paleoecosystem (Renault 1896a; Krings et al. 2007a). Both chert deposits have been known for more than 100 years, and the geological setting, history, as well as the fauna and flora of these sites are well-documented today.

In contrast to the Rhynie chert land plants, which all had a basic morphological uniformity (i.e. they were relatively small, generally naked, rootless, clonal, and only produced primary tissues), the Visean flora was more diverse with reference to size, morphology, and reproductive biology of the plants, and more complex in community structure. It therefore would seem obvious that the deposits also contained an increase in the diversity of microorganisms associated with these floras, as well as the number of specific plant-microbe associations/interactions.

In order to test this basic research hypothesis, a research project (funded in part by the DFG, NSF, and Alexander von Humboldt-Foundation) at the Bayerische Staatssammlung für

Paläontologie und Geologie (Munich), and in close cooperation with Hans Kerp and Hagen Hass of the Westfälische Wilhelms-Universität Münster (Germany), Thomas N. Taylor of the University of Kansas (U.S.A.), Jean Galtier of the University of Montpellier (France), and Reinhard Agerer of the Department Biology I at LMU, is documenting the morphology, biology, and biodiversity of the microorganisms preserved in the two cherts, and focuses on the biological interactions between different types of microorganisms and between microorganisms and terrestrial plants.

The doctoral thesis presented here represents an integral part of this research project, and contributes to the understanding of the microbial diversity in the Early Devonian Rhynie and Visean ecosystems, and provides details about the morphology and reproductive biology of various microorganisms that lived in these two continental paleoecosystems. The new data provide a series of reference points that allow direct comparisons between the micro-organisms and the changing floral elements at two especially interesting points in geologic time. Moreover, the inventory of fossil microorganisms is critical to subsequent studies that are aimed at understanding how these organisms functioned as integral parts of ecosystems; some of them may also contribute to a more accurate assessment of the evolutionary history of specific plant-microorganism associations and interactions, and how these relationships may have functioned as driving forces in plant evolution.

The organisms described in this thesis come from two classical localities (i.e. Rhynie in Scotland and the Massif central in France), which have been studied intensively with regard to both geological setting and development since more than 100 years, and thus are today well-known among geologists and paleontologists. Moreover, the organisms studied have been prepared according to standard procedures known to every paleontologist. As a result, the Material and Methods sections of the original papers included in this thesis have been kept brief to save space, and mostly do not represent more than tidied-up references to the original literature. Since part of the esteemed audience of this thesis may not be acquainted with the paleontological methodology and geological literature, I have added here short chapters that provides some insights into the geology of the study sites and basics of the methods used to prepare and study the organisms.

1.5. The Rhynie chert

1.5.1. Geological setting, paleogeography, and paleoenvironment

The Rhynie chert Lagerstätte is located in the northern part of the Rhynie outlier of Lower Old Red Sandstone in Aberdeenshire, Scotland, within a sequence of sedimentary and volcanic rocks (Fig. 1). The cherts occur in the upper part of the Dryden Flags Formation, in

the so-called Rhynie Block, a few hundred metres northwest of the village of Rhynie (Fig. 2). Another, similar but less well known chert deposit, which has also produced exquisitely preserved fossils, is the Windyfield chert that occurs some 700 m away from the Rhynie chert location. The cherts do not occur in natural surface exposures, but rather as loose 'float' blocks in the soil. The chert has been located *in situ* by trenching at various spots, and more recently also by drilling and coring. The deposit consists of at least 10 fossiliferous beds containing lacustrine shales and cherts that are interpreted as a series of ephemeral fresh water pools within a hot spring environment. Siliceous sinters were deposited by multiple episodes of hot spring activity, and are variably interbedded with shales, sandstones and minor tuffs in a unit about 35 m thick (Rice et al. 2002).

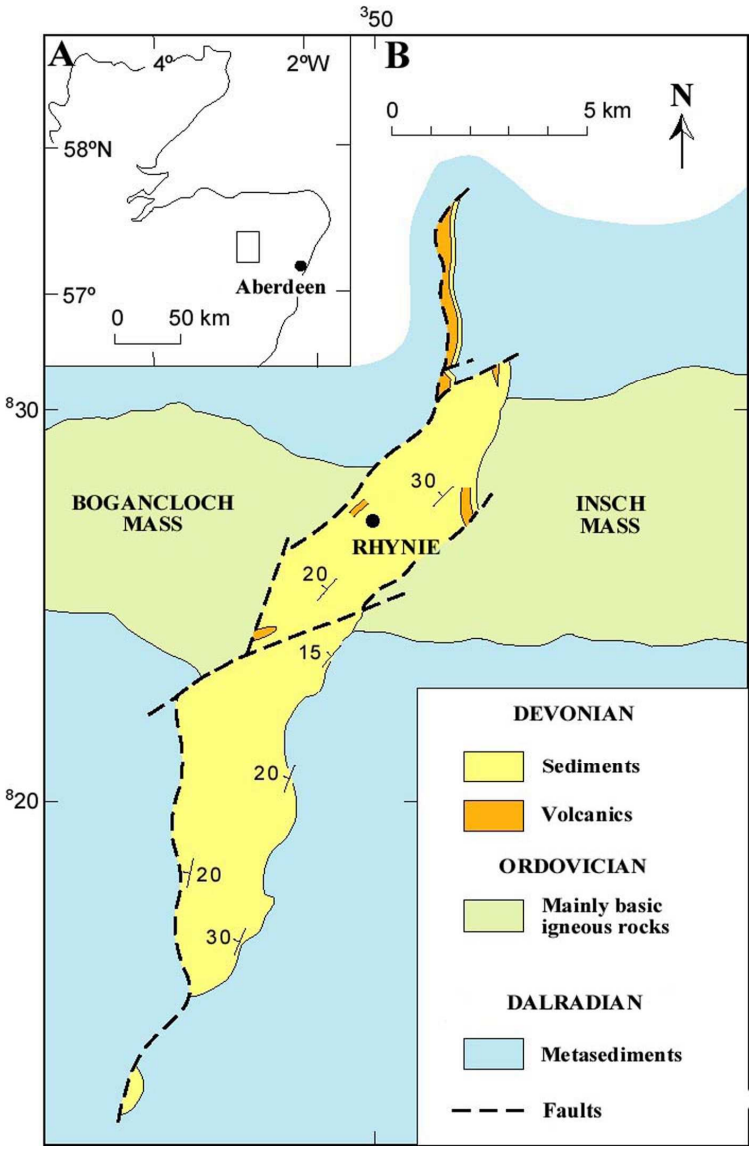

Figure 1: Geological setting of the Rhynie chert (Rice et al. 2002)

Figure 2: Location of the Rhynie chert, ©google maps 2007

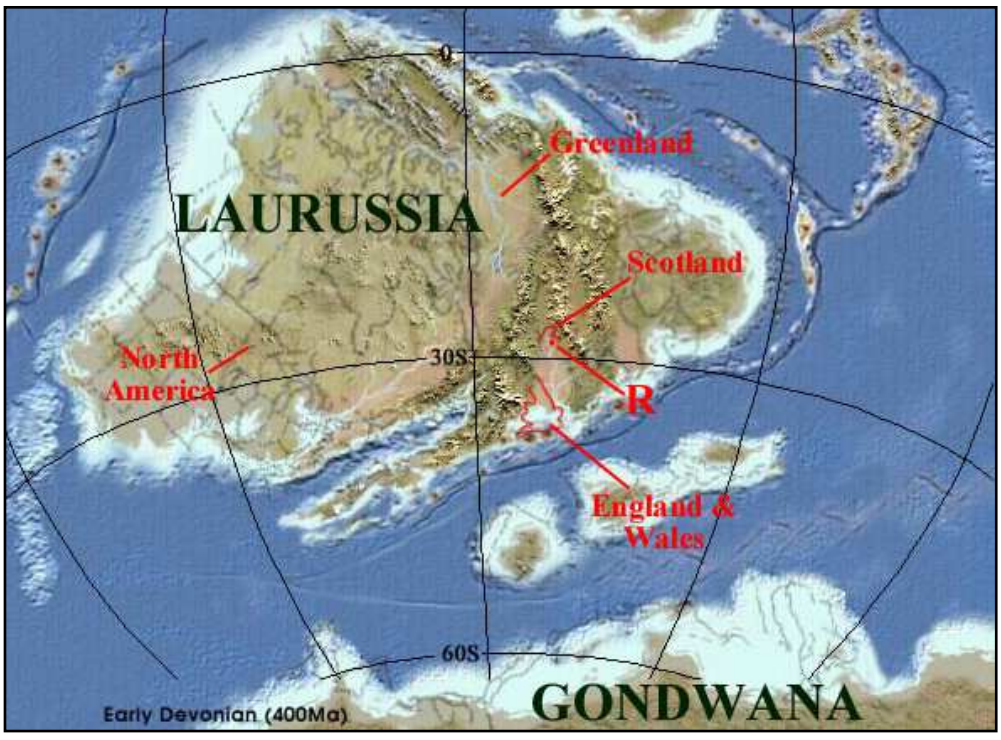

Figure 3: Paleogeographical map, ©Dr Ronald Blakey, Northern Arizona University

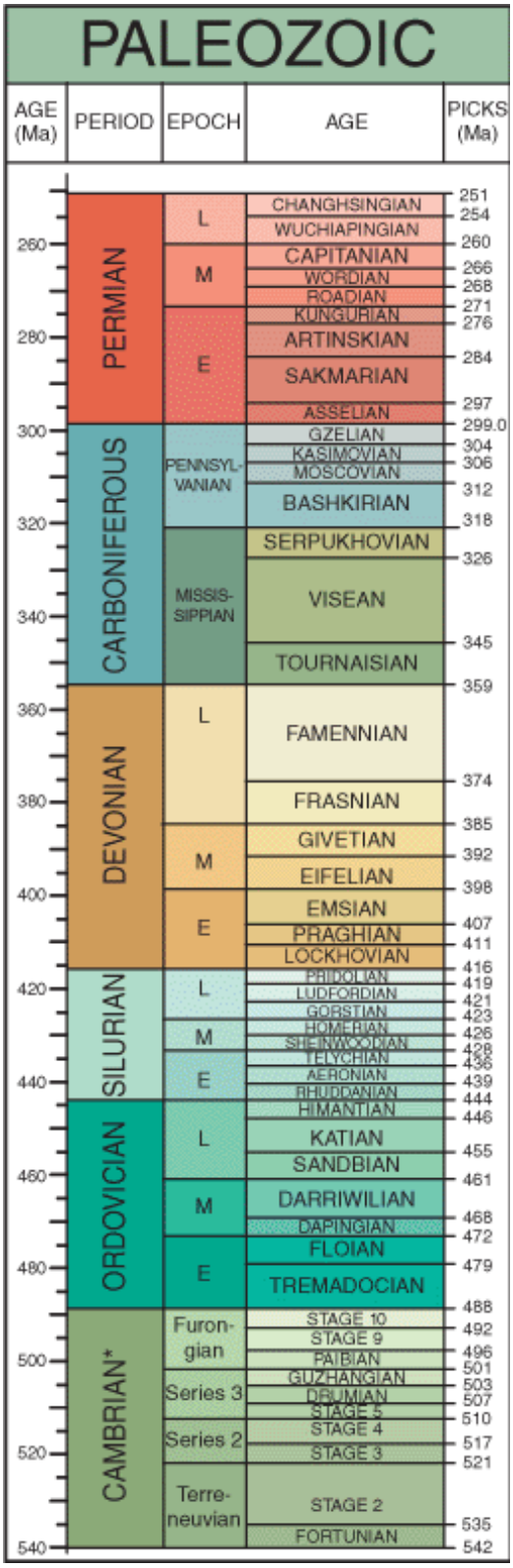

Figure 4: Geological time scale, ©IUGS 2009

At the time the Rhynie and Windyfield cherts were formed, the area around Rhynie was located approximately 28° south of the equator, and was part of a large continent that is today called Laurussia (Rice et al. 2002; Fig. 3). Based on dispersed spore assemblages and redefinition of the Pragian-Emsian boundary by the IUGS, Wellman (2006) and Wellman et al. (2006) have dated the cherts as Pragian-?earliest Emsian (Early Devonian). Radiometric dates based on 40Ar/39Ar isotopes from the cherts indicate an absolute age of 396 ± 8 million years (Rice et al. 1995; Fig. 4). Additional information about the geological setting, sedimentology, and development of the Rhynie chert Lagerstätte can be found in Rice et al. (2002), and Trewin and Rice (2004).

Preserved in the cherts are both aquatic facies and subaerial systems around the pools, i.e. soil and litter horizons with *in situ* plants, which have been studied intensively. Seven different types of land plant sporophytes and gametophytes are known from the Rhynie chert (surveyed in Kerp and Hass 2004), and an additional land plant sporophyte has more recently been described from the Windyfield chert (Powell et al. 2002). Moreover, various arthropods such as mites (Hirst 1923), trigonotarbids (Hirst 1923, Fayers et al., 2004), and crustaceans (e.g. Scourfield 1926), nematodes (Poinar et al. 2008), algae (Kidston and Lang 1921b; Kelman et al. 2004), and a diverse assemblage of microorganisms, including bacteria, cyanobacteria, microalgae, and fungi, are known to have lived in the Rhynie paleoecosystem some 400 million years ago (see Taylor and Krings 2005).

1.5.2. Research history

The discovery of the Rhynie chert dates back to 1912 when the Scottish geologist William Mackie recognized the extraordinary fossil content of these rocks. The material obtained during a systematic excavation slightly later was forwarded to the eminent paleobotanists of the time Robert Kidston and William Henry Lang for study. In a series of five benchmark papers, these authors described most of the land plants, various fungi, cyanobacteria, and nematophytes (Kidston and Lang 1917, 1920a,b, 1921a,b), with several additional papers subsequently published on the fauna (e.g., Hirst 1923; Scourfield 1926; Hirst and Maulik 1926). For the next 50 years, however, there was relatively little activity (e.g., Croft and George 1959; Lyon 1957, 1962) on the Rhynie chert biota. Then, in 1975, Kris Pirozynski and David Malloch published an influential theoretical paper in which they suggested that fungi were a critical component in the transition of plants onto the land. This hypothesis remained unsupported until Winfried Remy, Renate Remy, and Hagen Hass (all Münster, Germany) started to study the alternation of generations of the Rhynie chert plants in the 1980's (e.g. Remy and Remy 1980a,b; Remy and Hass 1991; Remy et al. 1993). During this work, they also discovered that some of the Rhynie chert land plants entered into a highly specific endomycorrhizal interaction with certain microfungi (Remy et al. 1994). This discovery stimulated a renewed interest in the role that fungi and other microorganisms played in the transition of plant life onto land, and triggered a more systematic analysis of the microbial element in the Rhynie ecosystem that is still ongoing, and today includes research groups in several countries.

1.6. The cherts from central France

1.6.1. Geological setting and environment

Mississippian (Lower Carboniferous) cherts occur in the northern part of the Massif Central in central France, where they can be found as loose blocks in cultivated fields or in stream sections. Associated with the blocks are series of rhyolitic lavas and anthracites that bound the northern margin of the large Stephanian-Autunian coal basin of this region. Radiometric dates of the lavas indicate that the cherts are late Visean in age (328 ± 5 my) (Vialette 1965; Fig. 4).

The cherts come from several localities in the vicinity of the villages of Combres and Lay, situated approximately 12 km east of Roanne, and from the Autun basin at the locality of Esnost, 10 km north of the city of Autun (Fig.5). The geological setting and Visean paleo-ecology of the localities are regarded as comparable to each other (Galtier 1971). The Visean ecosystem at Esnost has been reconstructed by Rex (1986), who indicates that the landscape was a wetland dominated by small ponds and lakes surrounded by open, lycophyte-dominated

swamp forest vegetation. Active volcanism with periodic lava streams repeatedly affected the area, so that the ecosystem was a dynamic and rather unstable system that regularly changed.

Figure 5: Location of the Visean cherts, ©google maps 2007

The Visean paleoecosystems are characterized by a diverse flora consisting of an unusually high percentage of ferns, as well as several lycopsids and sphenopsids (see Scott et al. 1984). With the exception of coprolites, remains of any animals have not been discovered to date (Rex and Galtier 1986). Microorganisms occur in virtually every plant remain preserved in the chert matrix, but only a few forms had been described when this dissertation project was initiated (Renault 1896a, 1900; Taylor et al. 1994; Grewing et al. 2003; Krings et al. 2005).

Many of the land plant fossils contained in the chert blocks are fragmented, which may suggest that they were transported into the depositional environment, i.e. the pools in which they became silicified. As a result, the Visean cherts appear to contain a relatively high number of saprotrophic microorganisms.

1.6.2. Research history

The cherts from the upper Visean of central France are known since the late 19th century. The French paleobotanist Bernhard Renault was one of the first scientists to systematically study the cherts, and he even included a few descriptions of microorganisms (bacteria, fungi, and

microalgae) in his benchmark publication (Renault 1896a). This publication was noted by the renowned American botanist, palaeontologist, and sociologist Lester Ward as "a superb work on a very difficult, but at the same time very important subject" (in Andrews 1980). In a series of shorter papers published between 1894 and 1903, Renault described several other microorganisms and gave exact information as to where these organisms occurred on or in land plants (Renault 1894a,b, 1895a,b,c, 1896a,b,c, 1900, 1903). More than half a century later, Jean Galtier revisited the cherts and provided new insights into the morphology and anatomy of the vascular plants (e.g., 1964, 1970a,b, 1971, 1980). The microorganisms, however, did not receive any subsequent attention, with the exception of a study by Taylor et al. (1994), who detailed several fungal remains. The re-assessment of *Lageniastrum macrosporae*, a colonial *Volvox*-like alga originally described by Renault that lives in the interior of lycopsid megaspores (Grewing et al. 2003; Krings et al. 2005) then initiated a systematic re-investigation of the microbial elements preserved in the Visean cherts.

1.7. Thin sections

The analysis of microorganisms that are preserved in a chert matrix heavily relies on thin sections that are prepared from rock samples. Some of the thin sections included in this thesis were manufactured by the author, but most come from large (historical) thin section collections kept at various other institutions. With regard to the Rhynie chert, the largest collection of thin sections is housed at the Westfälische Wilhelms-Universität in Münster, Germany. Additional sections come from Max Hirmer's teaching collection kept in the Bayerische Staatssammlung für Paläontologie und Geologie in Munich, Germany. Thin sections of the Visean cherts from France are from the historical Renault and Roche slide collections that are housed in the Muséum national d'Histoire naturelle de Paris, France.

Thin sections prepared by the author:

In order to prepare thin sections of both the Rhynie and Visean cherts, small pieces of rock were cut with a diamond-coated rock saw blade into thin chert wafers. The wafers were polished on one side with silicon carbide powder in water, and the polished side subsequently cemented to a microscope slide with a two-component epoxy resin (XW 397, Araldit). The wafer cemented to the glass slide was then ground on a glass panel with silicon carbide powder in decreasing grain size dispersed in water and dishwashing detergent until the wafer was thin enough for examination in transmitted light. Since none of these thin sections contained new microorganisms or interesting associations/interactions, most of the sections were discarded after microscopic analysis, although some were included in the teaching collection of the Department of Earth- and Environmental Sciences, LMU Munich (without number).

Thin sections prepared by Hagen Hass, Münster:

The Rhynie chert thin sections referred to in papers II, III, VI, VIII and X were prepared by Hagen Hass, a research scientist and laboratory technician at the Forschungsstelle für Paläobotanik, Westfälische Wilhelms-Universität Münster, according to procedures outlined above and in Hass and Rowe (1999). A special mode of cementing the chert wafer to a microscope slide with thermoplastic cement (No. 70C Lakeside Brand) was used that makes it possible to remove the wafer from the slide if necessary, turn over, and re-cement. As a result, the wafer can be ground from both sides in order to achieve the best possible resolution of the objects preserved in the chert. Specimens from the Münster Rhynie chert collection were examined and photographed using oil immersion objectives directly on the rock surface without a cover slip. Thin sections deposited in the collection of the Forschungsstelle für Paläobotanik am Geologisch-Paläontologischen Institut, Westfälische Wilhelms-Universität, Münster (Germany) have accession numbers preceded by PBO.

Thin sections from the Renault and Roche slide collections, Paris:

The historical thin section collections of Visean cherts referred to in papers V, VII and XI were prepared by B. Renault and his co-workers during the late 19th and early 20th centuries. These chert wafers were cemented to microscope slides with Canada balsam, ground to sufficient thickness, and subsequently sealed with a glass cover slip. Thin sections from the Renault and Roche slide collections in the Muséum national d'Histoire naturelle ('Laboratoire de paléontologie') in Paris (France) have accession numbers preceded by REN and ROC, respectively.

Thin sections deposited in Bayerische Staatssammlung für Paläontologie und Geologie, Munich:

The Rhynie chert thin sections containing the organisms described in papers I, IV and XI belong to the teaching collection of the late Prof. Dr. Max Hirmer kept in the Bayerische Staatssammlung für Paläontologie und Geologie, Munich (Germany), and have accession numbers preceded by BSPG.

2. Results

Chapter I: Germination shields in *Scutellospora* (Glomeromycota: Diversisporales, Gigasporaceae) from the 400 million-year-old Rhynie chert.

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ORIGINAL ARTICLE

Germination shields in *Scutellospora* (Glomeromycota: Diversisporales, Gigasporaceae) from the 400 million-year-old Rhynie chert

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Abstract Glomeromycotan spores from the Lower Devonian Rhynie chert provide the first evidence for germination shields in fossil fungi and demonstrate that this complex mode of germination was in place in some fungi at least 400 million years ago. Moreover, they represent the first direct marker relative to the precise systematic position of an Early Devonian endomycorrhizal fungus. In extant fungi, germination shields occur exclusively in the genus *Scutellospora* (Glomeromycota: Diversisporales, Gigasporaceae). These structures are regarded as a derived feature within the phylum Glomeromycota, and hence their presence in the Rhynie chert suggests that major diversification within this group of fungi occurred before the Early Devonian.

Taxonomical novelties
Scutellosporites Dotzler, M. Krings, T.N. Taylor and Agerer
Scutellosporites devonicus Dotzler, M. Krings, T.N. Taylor and Agerer

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Keywords Arbuscular mycorrhiza · Evolution · Germination · Pragian (Early Devonian) · Spore wall

Introduction

The Early Devonian Rhynie chert has provided a wealth of insights into the diversity of fungal life some 400 million years ago. As a result, members of the Chytridiomycota, Zygomycota, Glomeromycota and Ascomycota are today known in great detail from this palaeoecosystem; many of the Rhynie chert fungi have also been demonstrated in various interactions with other organisms (summarised in Taylor et al. 2004 and Taylor and Krings 2005). Among these interactions are several examples of arbuscular mycorrhizae (e.g. Remy et al. 1994; Taylor et al. 1995, 2005). The fungal partners consist of aseptate hyphae that enter the rhizomatous axes of various land plants and extend through the intercellular system of the outer cortex. The formation of intracellular arbuscules within a well-defined region of the cortex (termed the 'mycorrhizal arbuscule zone') substantiates these fungi as endomycorrhizal. The mycorrhizal fungi from the Rhynie chert have been assigned to the Glomeromycota based on structural similarities to extant representatives in this phylum of fungi and corresponding mode of mycorrhiza formation (cf. Taylor et al. 1995; Helgason and Fitter 2005); however, to date, none has been classified in greater detail and related to a particular modern taxon.

Glomeromycota is a monophyletic phylum that includes the arbuscular mycorrhizal (AM) fungi (Schüßler et al. 2001; Helgason et al. 2003; Corradi et al. 2004). It is estimated that more than 80% of vascular plants today live in symbiosis with these fungi (Smith and Read 1997). Molecular clock estimates based on amino acid sequences suggest that the Glomeromycota separated from other

fungal groups ~1,200 myBP (Heckman et al. 2001); more conservative estimates place the divergence at about 600–700 myBP (Berbee and Taylor 2001). Despite the proposed antiquity of the Glomeromycota, early fossil representatives are rare. Thick-walled spores suggested to be those of Glomeromycota have been reported from Precambrian sediments (Pirozynski and Dalpé 1989 and references therein). Similar spores are known from the Ordovician of North America (Redecker et al. 2000, 2002). In none of these accounts, however, is there any information regarding associations with plants. Glomeromycotan spores in tissues of late Palaeozoic land plants have been described from the Upper Devonian of Canada (Stubblefield and Banks 1983) and the Carboniferous of North America (Wagner and Taylor 1982). Moreover, hyphae or hyphae-like structures, aggregated in cortical tissues of the underground parts of several Carboniferous plants have variously been interpreted as arbuscules (e.g. Weiss 1904; Osborn 1909; Halket 1930; Agashe and Tilak 1970); however, most of these reports have later been questioned and the structures re-interpreted as non-fungal (coalesced cell contents) or non-mycorrhizal (cf. Stubblefield and Taylor 1988). The earliest persuasive evidence for glomeromycotan mycorrhizae in seed plants occurs in the form of non-septate hyphae, vesicles, arbuscules and clamydospores in silicified roots of the Triassic cycad *Antarcticycas schopfii* Smoot, T.N. Taylor and Delevoryas (Stubblefield et al. 1987; Phipps and Taylor 1996). Thus, the Rhynie chert mycorrhizae represent the earliest fossil evidence for Glomeromycota in symbiosis with land plants (Remy et al. 1994; Redecker 2002).

Also present in the Rhynie chert are different types of fungal spores. However, these remains have been largely neglected as a source of information about the diversity of fungi in this palaeoecosystem. In this study, we describe glomeromycotan spores from partially degraded axes of the Rhynie chert land plant *Asteroxylon mackiei* Kidst. and W.H. Lang that display a complex mode of germination involving the formation of a germination shield. The fossil spores can be directly related to a particular modern taxon because, in extant fungi, this mode of germination is restricted to species in the genus *Scutellospora* C. Walker and Sanders (Glomeromycota: Diversisporales, Gigasporaceae).

Materials and methods

The Rhynie chert Lagerstätte, an in situ silicified Early Devonian palaeoecosystem, is located in the northern part of the Rhynie outlier of Lower Old Red Sandstone in Aberdeenshire, Scotland. The cherts occur in the upper part of the Dryden Flags Formation, in the so-called Rhynie Block, a few hundred metres northwest of the village of Rhynie. The Lagerstätte consists of at least 10 fossiliferous beds containing lacustrine shales and cherts that are interpreted as a series of ephemeral fresh water pools within a hot springs environment. The chert-bearing formation is Pragian in age and has been radiometrically dated to 396±12 Ma. Detailed information about the geology and palaeontology of the Rhynie chert Lagerstätte can be found in Trewin and Rice (2004).

Spores were identified in petrographic thin-sections prepared by cementing a thin wafer of the chert to a glass slide and then grinding the rock slice with silicon carbide powder until it becomes sufficiently thin for examination in transmitted light (cf. Hass and Rowe 1999). Slides are deposited in the Bayerische Staatssammlung für Paläontologie und Geologie, Munich (Germany), under accession numbers BSPG 1964 XX 625 and 631, and BSPG 1964 XX 31.003, 31.005 and 31.006. For comparison, spores of *Scutellospora castanea* C. Walker (BEG 1: produced on onion, in neutral soil) were fixed and embedded in PVLG (Polyvinyllactoglycerol) according to a procedure outlined in Walker (1979) and studied in transmitted light.

Description

Spores with germination shield occur in cortical tissues of partially degraded axes of the early lycophyte *Asteroxylon mackiei*; a total of 12 specimens of this type of spore have been discovered to date. Spores are globose to subglobose, 260–350 μm in diameter and possess a non-ornamented surface (Fig. 1a). The spore wall is subdivided into two wall groups. The outer wall group is well preserved, up to 18 μm thick and two- or three-layered. A distinct dark layer (~3 to 4 μm thick; cf. arrow in Fig. 1a) occurs on the inner surface of the outer wall group. This layer either represents the inmost part of the outer wall group or the outmost layer of the inner wall group. The original thickness and composition of the inner wall group are difficult to estimate. A translucent region, up to 30 μm thick, occurs between the dark layer and outer surface of the spore lumen. It is not entirely clear, however, whether this region represents the original thickness of the inner wall group or is the result of shrinkage of both the inner wall group layers and spore lumen during fossilisation. The germination shield extends along the inner surface of the dark layer (Fig. 1a–e); the original location of the shield is difficult to reconstruct due to the lack of details about the composition of the inner wall group. The germination shield is round or oval in outline, ~140 μm in diameter and up to 15 μm high. It is distinctly lobed, with each of the lobes 25–33 μm wide, or displays a complex infolding along the margins (appearing in section to consist of compartments,

cf. Fig. 1a,b). In one of the spores, the connection of the germination shield to the spore lumen is apparent (Fig. 1a–c). Another specimen shows what we interpret as a germ tube that is formed by the germination shield and penetrates the outer wall group (Fig. 1e). Unfortunately, none of the spores with germination shield displays a subtending hypha or suspensor-like base. However, in one of the *A. mackiei* axes, a structurally similar but somewhat smaller (i.e. 190×150 μm in diameter) spore without germination shield occurs that is attached to a slightly bulbous subtending hypha (Fig. 1f), which is up to 18 μm in diameter and remotely resembles the characteristic suspensor-like base seen in extant *Scutellospora* species (e.g. Fig. 1g).

Relationships

The fossil spores are similar to spores produced by species in the extant genus *Scutellospora* (Glomeromycota: Diversisporales, Gigasporaceae). *Scutellospora* consists of some 20 species of arbuscular mycorrhizal fungi, all of which

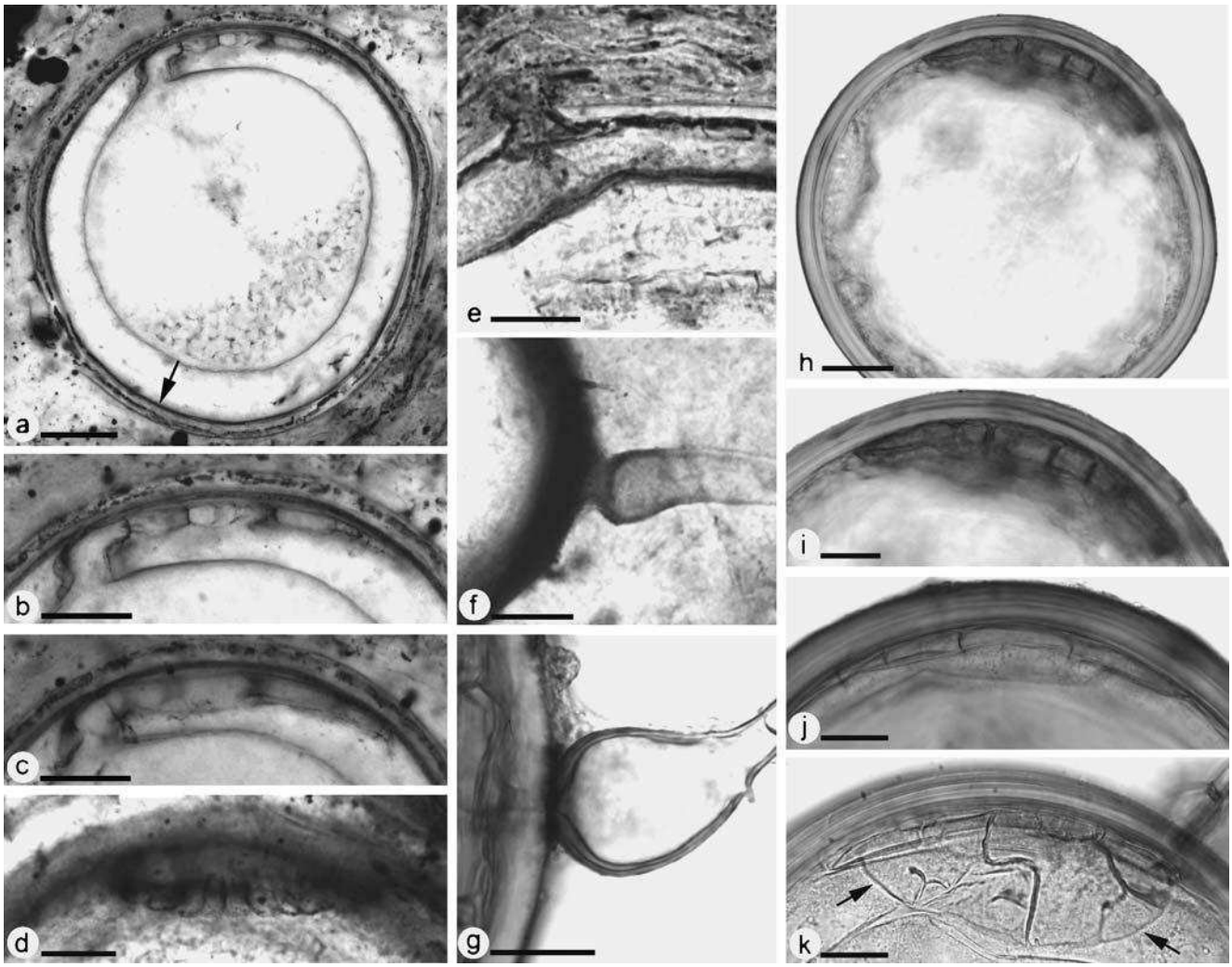

Fig. 1 *Scutellosporites devonicus* Dotzler et al. from the Rhynie chert (**a**–**f**) and spores of the extant *Scutellospora castanea* C. Walker (**g**–**k**). **a** Section through a fossil spore with germination shield. *Arrow* indicates the distinct dark layer that either represents the inmost portion of the outer wall group or outmost layer of the inner wall group. Bar=50 μm. **b** Detail of **a**, focusing on the germination shield. Bar=35 μm. **c** Germination shield in near median longitudinal section. Bar=35 μm. **d** Germination shield in oblique surface view, showing lobes/infoldings along the margin. Bar=20 μm. **e** Germ tube penetrating the outer wall group. Bar=30 μm. **f** Slightly bulbous base of a smaller glomeromycotan spore in *Asteroxylon mackiei*. Bar=20 μm. **g** Same as **f**, but from the extant *S. castanea*. Bar=30 μm. **h** Spore of *S. castanea* with germination shield. Bar=50 μm. **i** Detail of **h**, focusing on the germination shield. Bar=30 μm. **j** Germination shield of a second *S. castanea* spore in optical longitudinal section. Bar=30 μm. **k** Germination shield in oblique surface view. *Arrows* indicate the margins of the shield. The infoldings are visible as *narrow dark lines*. Bar=30 μm

produce large spores (between 120 and 640 μm in diameter) with multi-layered walls. Germination includes the formation of a germination shield, which is a specialised structure that distinguishes *Scutellospora* from the closely related genus *Gigaspora* Gerd. and Trappe (Walker and Sanders 1986) and all other members of the Glomeromycota. There are several other genera within the Glomeromycota, e.g. *Acaulospora* Gerd. and Trappe (Diversisporales, Acaulosporaceae) and *Pacispora* Oehl and Sieverd. (Glomerales, Glomeraceae), in which spore germination also includes the formation of a specialised structure between two layers of the spore wall (e.g. Stürmer 1998; Stürmer and Morton 1999; Oehl and Sieverding 2004). However, this structure, termed the 'germination orb', is more delicate, usually smaller (e.g. 14–26×20–38 μm in *P. franciscana* Oehl and Sieverd., cf. Oehl and Sieverding 2004), less complex and clearly distinguishable morphologically from the germination shields produced by the Rhynie chert spores and extant *Scutellospora*. It is still being debated whether germination orbs and germination shields are heterologous structures or synapomorphies of the Diversisporales lineage.

For comparison of the fossils with extant representatives of *Scutellospora*, specimens of *Scutellospora castanea* C. Walker were analysed (Fig. 1h–k). A complete description of *S. castanea* is provided in Walker et al. (1993) and we restrict our discussion to a brief characterization of the germination shield: In *S. castanea*, this structure is oval in outline (Fig. 1k) and occurs on the inner spore wall group. It is up to 210 μm long, 185 μm wide, 10–15 μm high and characterised by a complex infolding along the margins (appearing in optical section to consist of compartments, cf. Fig. 1h–j). Optical longitudinal sections through shields of *S. castanea* are virtually indistinguishable from sections through the fossil germination shields (compare Fig. 1a,b with Fig. 1i,j).

Based on the striking similarities in germination shield morphology between the fossils and *S. castanea*, as well as other species of *Scutellospora* detailed in the literature (e.g. Koske and Walker 1986; Walker and Sanders 1986; Walker and Diederichs 1989; Walker et al. 1998; Herrera-Peraza et al. 2001), we interpret the fossils as belonging to an early member of the genus *Scutellospora*. However, the fossil spores only provide an incomplete picture of this fungus. For example, none of the spores with germination shields display a subtending hypha or specialised base. Because extant *Scutellospora* spores are always borne on a characteristic bulbous, suspensor-like base (Fig. 1g), documentation of this feature would strengthen the proposed affinities of the fossil spores. A somewhat smaller fossil spore (lacking germination shield), which co-occurs with the large spores with germination shield, displays a slightly bulbous base (Fig. 1f). We cannot establish at present whether this spore belongs to the fungus that produced the spores with germination shield. As a result, we refrain from including the fossil spores with germination shield in *Scutellospora*, but rather introduce a new genus, for which the name *Scutellosporites* is proposed.

Taxonomy

Glomeromycota C. Walker and A. Schüßler
Diversisporales C. Walker and A. Schüßler
Gigasporaceae J.B. Morton and Benny
Scutellosporites Dotzler, M. Krings, T.N. Taylor and Agerer, gen. nov.

Derivation of generic name. The name underscores the similarity to the extant genus *Scutellospora*; the ending *-ites* is used to designate a fossil taxon.

Generic diagnosis. Spores globose to subglobose, up to 350 μm in diameter, with non-ornamented surface; spore wall composed of two wall groups; outer wall group >15 μm thick, two- or three-layered; distinct dark layer present on inner surface of outer wall group; germination by means of germination shield extending along inner surface of dark layer; shield round or oval, >100 μm long and >10 μm high, distinctly lobed or with infolded margins.

Type species. *Scutellosporites devonicus* Dotzler et al.

Scutellosporites devonicus Dotzler, M. Krings, T.N. Taylor and Agerer, spec. nov. Fig. 1a–f

Specific diagnosis. As for the genus

Derivation of specific epithet. Indicating the geologic age of the fossil.

Holotype. BSPG 1964 XX 631 (Fig. 1a in this paper)

Type locality. Rhynie, Aberdeenshire, Scotland, National Grid Reference NJ 494276

Age and stratigraphic position. Early Devonian (Pragian, ~400 myBP)

Remark. Glomeromycotan spores that resemble *Scutellosporites devonicus* have been described from degraded tissues of various Rhynie chert plants by Kidston and Lang (1921) as *Palaeomyces gordoni* Kidst. and W.H. Lang. However, in none of these spores is a germination shield obvious. Because it is most likely that there existed more than one taxon of mycorrhiza-forming glomeromycotan fungi in the Rhynie chert, we refrain from assigning the spores with germination shields specifically to *P. gordoni*.

Discussion

One of the remarkable discoveries in the Early Devonian Rhynie chert is the presence of arbuscular mycorrhizae that are strikingly similar to mycorrhizae today and were produced by the same group of fungi, i.e. members of

the Glomeromycota (Remy et al. 1994; Taylor et al. 1995; Helgason and Fitter 2005). Despite the detailed analyses that have been carried out on these ancient mycorrhizae, an exact systematic placement of the fungal partners has not been possible to date, due primarily to the fact that diagnostic features necessary in establishing the affinities of a glomeromycotan fungus (e.g. spore wall structure and colour, auxilliary cells) could not be determined with the fossils.

The prominent germination shields described in this study correspond to germination shields produced by the extant Gigasporaceae genus *Scutellospora*, and thus represent the first direct diagnostic marker that can be used to determine the systematic position of one of the Rhynie chert mycorrhizal fungi. In extant Glomeromycota, prominent and well-recognizable germination shields are known to occur exclusively in *Scutellospora*. Similar pre-germination structures (germination orbs) found in genera such as *Pacispora* and *Acaulospora* are much more delicate and become rarely visible, even in broken specimens or after specific preparations of the inner wall (Spain 1992; Oehl and Sieverding 2004). Members of *Scutellospora* display a complex mode of germination, in which, before germ tube formation, a germination shield is developed between two layers of the spore wall. The position of the germination shield varies between species of *Scutellospora* and may occur between the individual layers of the inner wall group (e.g. in *S. scutata* C. Walker and Dieder., cf. Walker and Diederichs 1989) or on the surface of the inmost wall layer (e.g. in *S. castanea*, cf. Walker et al. 1993). At maturity, the germination shield produces one to several germ tubes that penetrate the outer portion of the spore wall (Walker and Sanders 1986). A satisfactory interpretation with regard to the nature and function of the germination shields has not been published to date. One interpretation is that they are either sexual or parasexual, or perhaps asexual vestiges of some previously sexual structure (C. Walker, personal communication).

Because germination shields represent complex structures that consistently occur between distinct layers of the spore wall, this feature is regarded as derived within the Glomeromycota (Bentivenga and Morton 1996). As a result, the presence of spores with germination shield in the Rhynie chert suggests that major diversification within this group of fungi occurred before the Early Devonian. It is interesting to note, however, that the derived state of the germination shield in the family Gigasporaceae (i.e. *Gigaspora* and *Scutellospora*) has been questioned based on molecular studies (Simon et al. 1993; Redecker 2002). These authors hypothesise that *Gigaspora* is an advanced rather than a plesiomorphic genus; species in *Gigaspora* form a very narrow clade compared to the large variation within *Scutellospora* (Schwarzott et al. 2001). It has also been suggested that *Scutellospora* may be paraphyletic (Redecker 2002). Berbee and Taylor (2001) estimate the divergence time between *Gigaspora* and two species in the genus *Glomus* Tul. and C. Tul. at approximately 300 myBP based on a nucleotide substitution rate of 1.26%. Although *Scutellospora* was not included in their data set, the occurrence of spores with germination shields in deposits that are 100 million years older than the estimated divergence of *Gigaspora* from other Glomeromycota supports the hypothesis that the germination shield is an ancestral feature within the Gigasporaceae. If in fact *Gigaspora* is advanced, the mode of germination involving a germination shield was lost during the evolution of this genus (Redecker 2002). In addition, the complex system of spore walls composed of one to several wall groups seen in *Scutellospora* was also lost because members of *Gigaspora* display a much simpler wall organisation (Walker and Sanders 1986). The inner wall group in *Scutellosporites devonicus* is not preserved, and thus its original thickness is difficult to estimate. However, we suggest that the inner wall group was quite massive because one of the spores (the holotype specimen) clearly shows that the germination shield does not occur close to the surface of the spore lumen, but rather appears stalked (Fig. 1a). This suggests that the shield had to pass through a massive inner wall group before extending along the inner surface of the dark layer. The fact that the surface boundary lines of both the spore lumen and erect portion of the shield ('stalk') are not wrinkled or otherwise unnaturally distorted (cf. Fig. 1a–c) indicates that the erect portion of the shield does not represent a preservational artefact. As a result, this feature substantiates that the inner wall group was of considerable thickness because the 'stalk' of the germination shield could not be explained if the inner wall group were only a few micrometre thick. In extant representatives of *Scutellospora* the inner wall group is usually only 0.6–2 μm thick (INVAM homepage). However, for a few species, up to 18 μm thick inner wall groups have been recorded, but these are based on material mounted in PVLG, which results in expansion of the wall (e.g. from 2 to 15 μm within a few minutes in *S. spinosissima* C. Walker and Cuenca, cf. Walker et al. 1998). The considerable thickness of the inner wall group of *S. devonicus* in comparison to that seen in extant *Scutellospora* suggests that perhaps the inner wall group was gradually reduced and eventually lost in *Gigaspora*.

Time estimates for the appearance of individual lineages and taxa within the Glomeromycota are typically based on molecular and genetic studies of modern taxa. In many cases the more general results from these studies are supported by the fossil record (e.g. Simon et al. 1993; Helgason and Fitter 2005; Taylor and Krings 2005). At a finer scale of resolution, however, the fossil

record has to date mostly failed in producing suitable evidence in support for or against hypotheses based on molecular data, due primarily to the inherent incompleteness of the fossil record. As a result, *Scutellosporites devonicus* from the Rhynie chert is an important discovery because it displays the first direct marker that can be used to establish the precise systematic position of an Early Devonian mycorrhizal fungus. As the molecular phylogeny of the Glomeromycota is continuously refined, it will be interesting to see how the characters attributed to *S. devonicus* fit character states based on molecular data.

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Chapter II: Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses.

New Phytologist

Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses

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Summary

• The Early Devonian Rhynie chert has been critical in documenting early land plant–fungal interactions. However, complex associations involving several fungi that enter into qualitatively different relationships with a single host plant and even interact with one another have not yet been detailed.

• Here, we studied petrographic thin sections of the Rhynie chert plant *Nothia aphylla*.

• Three fungal endophytes (co)occur in prostrate axes of this plant: narrow hyphae producing clusters of small spores; large spherical spores/zoosporangia; and wide aseptate hyphae that form intercellular vesicles in the cortex. Host responses on attack include bulging of infected rhizoids, formation of encasement layers around intracellular hyphae, and separation of infected from uninfected tissues by secondarily thickened cell walls.

• A complex simultaneous interaction of *N. aphylla* with three endophytic fungi was discovered. The host responses indicate that some of the mechanisms causing host responses in extant plants were in place 400 million yr ago. Anatomical and life history features of *N. aphylla* suggest that this plant may have been particularly susceptible to colonization by fungi.

Key words: endomycorrhiza, interaction, *Nothia aphylla*, parasitism, Pragian-earliest Emsian (Early Devonian), Rhynie chert.

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Introduction

Terrestrial fungi and fungi-like microorganisms have been reported from rocks as old as the Precambrian. Although these reports are important in identifying the minimum age of various fungal groups and lineages, in none are the fungi described in association with other organisms that inhabited the ecosystem. By far the most extensively studied fossil fungi showing various associations and/or interactions with other organisms are those from the famous Rhynie chert, an *in situ* silicified Early Devonian hot springs environment dominated by ephemeral freshwater pools. Representatives of three fungal phyla (i.e. Chytridiomycota, Glomeromycota, Ascomycota), along with a member of the Peronosporomycetes (= Oomycota) (Taylor *et al.*, 2006), are known in great detail from this paleoecosystem (reviewed in Taylor *et al.*, 2004; Taylor & Krings, 2005). The only groups of true fungi not reported from the Rhynie chert to date are the Zygomycota and Basidiomycota.

The Rhynie chert is important because of the extraordinary preservation of many delicate land plants, animals, and microorganisms (bacteria, cyanobacteria, microalgae, and fungi) (Kerp & Hass, 2004), but also because many of these organisms were fossilized so that different stages of their life history

and associations with other organisms can be directly examined. This is especially true of the fungi, which, as heterotrophs, are intricately involved with other organisms in saprotrophic, parasitic, and/or mutualistic interactions in this paleoecosystem. As a result, the Rhynie chert provides the earliest direct fossil evidence of land plant/fungal associations and interactions in a terrestrial paleoecosystem.

Several one-to-one saprotrophic, parasitic, and mutualistic land plant–fungal interactions have been documented from the Rhynie chert to date (Taylor *et al.*, 2004). On the other hand, more complex types of interactions involving several fungi that enter into qualitatively different relationships with a single host plant and even variously interact with one another have not yet been detailed. As a result, knowledge about the degree of complexity attained by land plant–fungal interactions in this early terrestrial ecosystem remains incomplete, and thus the roles that such systems have played in early land plant and terrestrial ecosystem evolution continue to be difficult to assess based on the fossil record.

In this paper we report three different types of fungal endophytes that colonize rhizoids and prostrate (rhizomatous) axes of the Rhynie chert land plant *Nothia aphylla* Lyon ex El-Saadawy et Lacey. The spatial distribution of the individual endophytes within the axes and several characteristic cell and tissue alterations and host responses are documented. Hypotheses are advanced as to the nature of the associations between the individual endophytic fungi and land plants, and why *N. aphylla* may have been more susceptible to colonization by fungi than other Rhynie chert plants.

Materials and Methods

The Rhynie chert Lagerstätte is located in the northern part of the Rhynie outlier of Lower Old Red Sandstone in Aberdeenshire, Scotland, within a sequence of sedimentary and volcanic rocks. The cherts occur in the upper part of the Dryden Flags Formation, in the so-called Rhynie Block, a few hundred metres northwest of the village of Rhynie. The Lagerstätte consists of at least 10 fossiliferous beds containing lacustrine shales and cherts that are interpreted as a series of ephemeral freshwater pools within a hot springs environment. Preserved in the cherts are both aquatic facies and subaerial systems around the pools (i.e. soil/litter horizons with *in situ* plants); the latter became preserved as a result of temporary floodings of silica-rich water, or by groundwater percolating upwards. Based on dispersed spore assemblages and redefinition of the Pragian/Emsian boundary by the IUGS, Wellman *et al.* (2006) and Wellman (in press) have dated the cherts as Pragian-earliest Emsian. Detailed information about the geological setting, sedimentology, and development of the Rhynie chert Lagerstätte can be found in Rice *et al.* (2002), and Trewin & Rice (2004).

A total of 250 different axis segments from a succession of *in situ* stands of *N. aphylla* preserved in a single chert block (10 cm long, 9 cm wide, and 7 cm high) have been studied. All specimens were identified in series of petrographic thin sections prepared by cementing a piece of chert to a glass microscope slide and then grinding the rock to a thickness sufficient to examine in transmitted light (cf. Hass & Rowe, 1999). Host plants and fungi were examined and photographed using oil immersion objectives directly on the rock surface. Slides are deposited in the paleobotanical collection of the Geologisch-Paläontologisches Institut, Westfälische Wilhelms-Universität, Münster (Germany); accession numbers are noted in the figure captions.

Nothia aphylla

Nothia aphylla is a small sporophyte with possible affinities in the Zosterophyllophyta. The plant consists of an aerial system of dichotomously branching orthotropic axes that arise from prostrate rhizomatous axes (Fig. 1a–c). Like most of the Rhynie chert land plants, an entire *N. aphylla* plant was < 20 cm tall. The aerial axes are up to 2.5 mm in diameter and unevenly covered by slightly elongate emergences. In cross-section, the prostrate axis is bilaterally symmetrical with a median ridge (rhizoidal ridge) that extends the length on the ventral surface (Fig. 1b, R). Extending from this rhizoidal ridge are numerous unicellular and unbranched rhizoids, each up to 1.5 mm long (Fig. 1b–d). Mature rhizoids are characterized by a rounded tip. The anatomy of the prostrate axis is simple: most of the axis consists of a parenchymatous cortex, surrounded by hypodermal tissues and epidermis. The central stele is formed by a narrow zone of phloem-like tissue that surrounds a strand composed of fibrous conducting cells. The internal anatomy of the ventral rhizoidal ridge (Fig. 1d) includes a rhizoid-bearing epidermis (E), several hypodermal layers composed of relatively large and radially arranged cells (H), files of thin-walled parenchymatous cells (the so-called connective) (C) that connect to the stelar body, and individual extra-stelar conducting ('xylematic' in Kerp *et al.*, 2001) elements (X). Detailed information on the growth habit, morphology, and anatomy of *N. aphylla* can be found in Kerp *et al.* (2001) and Daviero-Gomez *et al.* (2005); the male gametophyte, *Kidstonophyton discoides*, has been described by Remy & Hass (1991).

Results

Fungi associated with *N. aphylla*

Remains of three different fungal endophytes have been discovered in rhizoids and prostrate axes of *N. aphylla*. Because we lack definitive characters to determine the systematic affinities of the fungi, they are informally referred to as fungus no. 1 (Figs 1e–g, 2a–f, 3a–d), fungus no. 2 (Figs 2a,d, 3e–h), and fungus no. 3 (Figs 3a,c, 4a–j). Longitudinal sections and cross-sections demonstrate not

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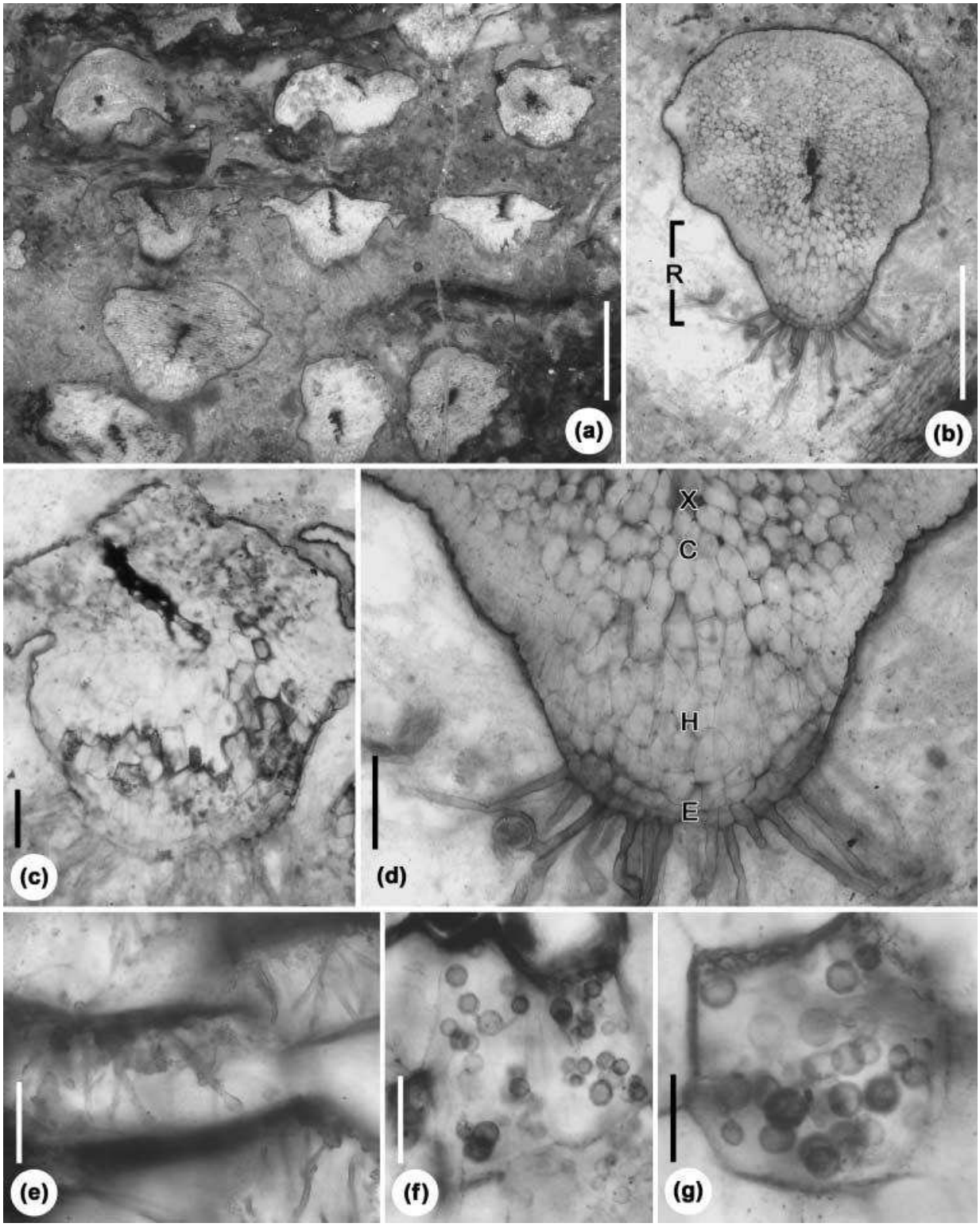

Fig. 1 Morphology and anatomy of the *Nothia aphylla* prostrate axis, and fungus no. 1. (a) Section of peat block showing slightly compressed prostrate axes in cross-section. Note that the axes occur at three different levels. Slide P2860. Bar, 1 mm. (b) Uncompressed prostrate axis in cross-section showing the typical form and ventral rhizoidal ridge (R). Slide P2868. Bar, 1 mm, (c) Slightly compressed axis showing rhizoidal ridge infected with fungus no. 1. Slide P2826. Bar, 180 μm. (d) Detail of (b) – tissues of the rhizoidal ridge: E, rhizoid-bearing epidermis; H, radially arranged hypodermal cells; C, parenchymatous cells of the connective; X, extra-stelar conducting element. Bar, 250 μm. (e) Hyphae of fungus no. 1 in extra-stelar conducting elements. Slide P2827. Bar, 15 μm. (f, g) Details of (c) – spore clusters in hypodermal cells. Bars, 55 μm (f), 30 μm (g).

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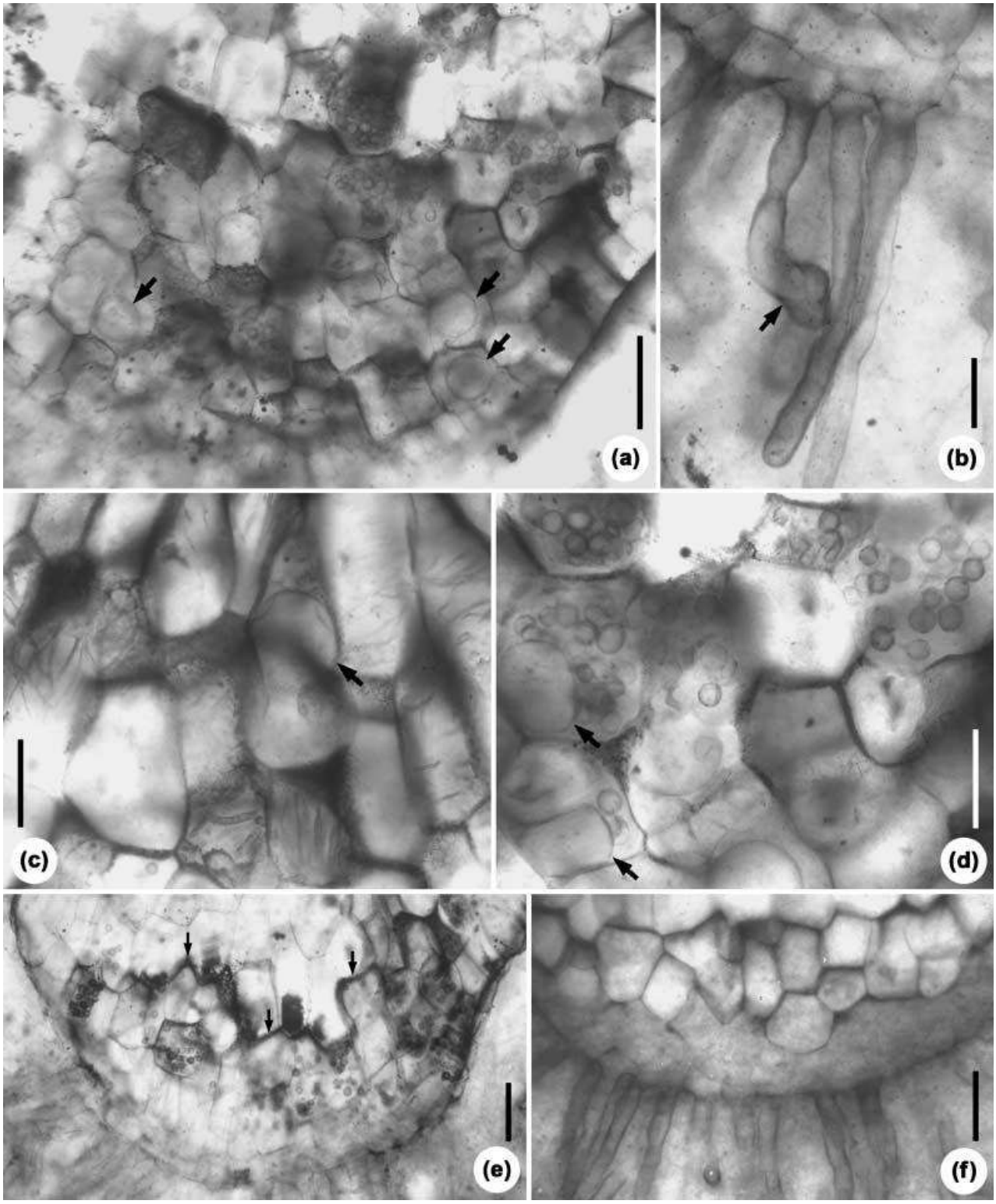

Fig. 2 Fungus no. 1 and fungus no. 2. (a) Hypodermal tissues of a rhizoidal ridge infected with fungus no. 1 and fungus no. 2 (arrows) in transverse section. Slide P3940. Bar, 100 μm. (b) Detail of Fig. 1(b) – rhizoids extending from the rhizoidal ridge. The arrow indicates a small bulge that formed on attack by fungus no. 1. Bar, 60 μm. (c) Fungus no. 1 passing through extra-stelar conducting cells of the rhizoidal ridge, and fungus no. 2 (arrow). Slide P2827. Bar, 20 μm. (d) Detail of (a) – peripheral hypodermal tissues of a rhizoidal ridge infected with fungus no. 1 and fungus no. 2 (arrows). Bar, 50 μm. (e) Detail of Fig. 1(c) – rhizoidal ridge infected with fungus no. 1. Note the zigzag boundary line (arrows) that extends across the hypodermis. Bar, 90 μm. (f) Prostrate axis showing a void where hypodermal cells have been degraded. Slide P2854. Bar, 60 μm.

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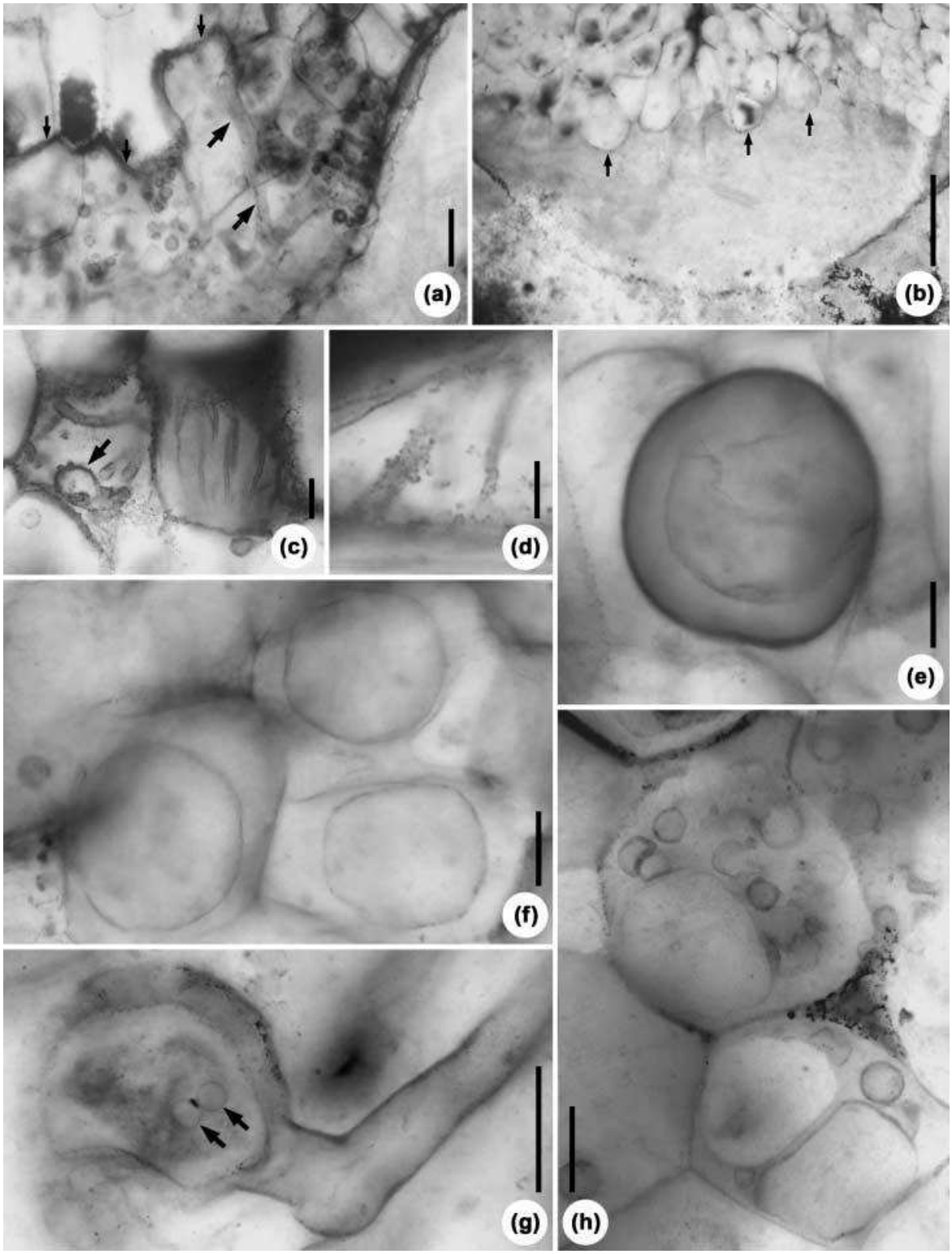

Fig. 3 Fungus no. 1, fungus no. 2, and fungus no. 3. (a) Detail of Fig. 1(c), focusing on some of the secondarily thickened hypodermal cell walls (small arrows). Note that fungus no. 1 only occurs in cells below the boundary, but a hypha of fungus no. 3 (large arrows) passes through the boundary. Bar, 60 μm. (b) Prostrate axis showing void and slightly enlarged hypodermal cells (arrows). Slide P2854. Bar, 250 μm. (c) Detail of Fig. 2(c) – fungus no. 1 and fungus no. 3 (arrow) in extra-stelar conducting cells. Bar, 10 μm. (d) Granular material deposited on fungus no. 1 in a conducting cell. Slide P2827. Bar, 7.5 μm. (e, f) Fungus no. 2 in hypodermal cells. Slides P2808 (e) and P3940 (f). Bars, 25 μm. (g) Detail of Fig. 1(d) – inflated rhizoid tip containing fungus no. 2. Bar, 60 μm. (h) Detail of Fig. 2(a) – co-occurrence of fungus no. 1 and fungus no. 2 in hypodermal cells. Bar, 25 μm.

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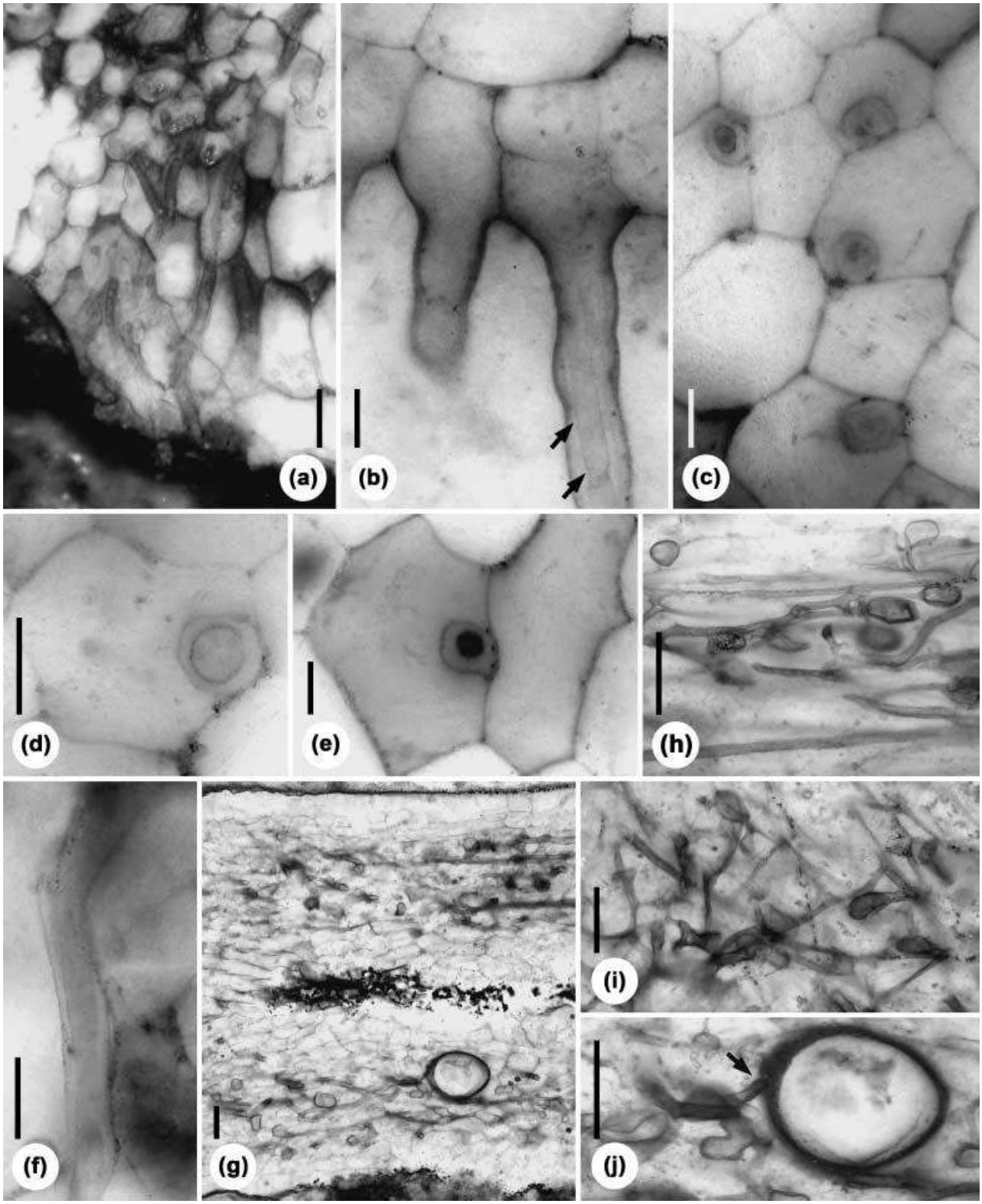

Fig. 4 Fungus no. 3. (a) Hyphae passing through the epidermis and peripheral layers of the hypodermis of a rhizoidal ridge. Slide P2835. Bar, 50 μm. (b) Detail of Fig. 1(b) – a single hypha (arrows) entering the axis through a rhizoid. Bar, 30 μm. (c–e) Transverse sections through hypodermal cells of a rhizoidal ridge, showing encasements around intracellular hyphae. Slide P3032. Bars, 20 μm. (f) Detail of Fig. 1(c) – hypha in longitudinal section. Note the encasement layer. Bar, 30 μm. (g) Longitudinal section through the dorsal portion of a prostrate axis. Intercellular hyphae, vesicles, and large spore in the cortex. Slide P3031. Bar, 100 μm. (h, i) Intercellular hyphae and vesicles in the cortex. Slides P3032 (h, longitudinal section) and P3031 (i, cross-section). Bars, 100 μm. (j) Detail of (g), focusing on the spore. Arrow indicates subtending hypha. Bar, 100 μm.

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only that these fungal endophytes are present, but also that all three endophytes may co-occur in the same host individual. Fungus no. 1 is present in some 15% of the axis segments studied, while fungus no. 2 occurs in 25% and fungus no. 3 in > 95% of the segments; all three endophytes co-occur in 5% of the segments. None of the 250 axis segments is completely free of endophytic fungi. Axes infected by fungus no. 2 and fungus no. 3 occur in all stands of the succession, while axes infected by fungus no. 1 have only been observed in three of the stands.

Fungus no. 1. This is an intracellular endophyte. The hyphae (Figs 1e,f, 2c) are smooth-walled, aseptate, 1.0–3.5 μm in diameter, and occasionally branched. In hypodermal cells, the fungus produces clusters of spherical spores borne singly on short branches (Figs 1f,g, 2a,d,e, 3a). The spores are 6–15 μm in diameter and either thin-walled and colorless or, at maturity, thick-walled and opaque. Fungus no. 1 is abundant in all tissues of the rhizoidal ridge, including rhizoids, hypodermis, and conducting cells of the ridge and connective. In the epidermis and cortical tissues on the dorsal side and lateral flanks of the axis, fungus no. 1 rarely occurs.

Fungus no. 1 enters the axes through either the tip of the rhizoid or the rhizoid base. Typically it is the rhizoid tip that is infected and, apparently as a result of the infection, forms a characteristic bulge (Fig. 2b, arrow). As the fungus spreads, the lumina of hypodermal and connective cells become filled with hyphae and spores (Fig. 2a,c,d); in the most heavily infected *N. aphylla* specimen, approx. 95% of the cells of the rhizoidal ridge contain hyphae and/or spores of this endophyte. In cross-sections of the most heavily infested axes, a distinct zigzag pattern of secondarily thickened anticlinal and outer periclinal cell walls is visible that extends across almost the entire hypodermis of the rhizoidal ridge (Figs 1c, 2e, arrows; Fig. 3a, small arrows) and separates infected from uninfected tissues. Moreover, in two of the infected rhizoidal ridges, the peripheral layers of the hypodermis (and, in one, also the ventral epidermis) are disintegrated (Figs 2f, 3b), with some of the remaining hypodermal cells slightly elongated so that they fill part of the empty space (Fig. 3b, arrows). In conducting cells of the rhizoidal ridge and connective, hyphae of fungus no. 1 extend through the cell lumina in a more or less parallel orientation (Figs 1e, 2c, 3c); branching of the hyphae and formation of spores are rare. Many of the hyphae in conducting cells are covered by a layer composed of granular material (Fig. 3c,d).

Fungus no. 2. This is represented by large spherical to ellipsoid intracellular structures (Fig. 2a (arrows), 2c (arrow), 2d (arrows); Fig. 3e–h) that are 50–120 μm in diameter or 80–150 μm long and 40–60 μm wide, and may represent resting spores (chlamydospores) or zoosporangia. The structures are thin-walled and translucent (Fig. 3f,h) or, at maturity, have slightly thicker walls and are nearly opaque (Fig. 3e). Most of the structures are empty; however, a few specimens contain a granular mass, while in others, remains of a slightly smaller thin-walled sphere are seen (Fig. 3e). Still other specimens contain one to several small spherules (Fig. 3g, arrows), *c.* 10 μm in diameter. Fungus no. 2 occurs in the rhizoids (Fig. 3g) and all tissues of the rhizoidal ridge and connective, but is most abundant in the hypodermis of the ridge (e.g. Fig. 2a). Fungus no. 2 is rare or absent in the cortical tissues of the dorsal side and lateral flanks of the axis. Usually there is one structure per host cell (Fig. 3f), but rarely up to four spores/zoosporangia of this fungus may be present in a single host cell (Fig. 3h). Often the infected rhizoids and host cells are distinctly inflated (Fig. 3e,g). In the infected host tissues, the cells normally contain either fungus no. 2 or fungus no. 1; only in a few instances do both endophytes co-occur in a single cell (Fig. 3h).

Fungus no. 3. Also present in the prostrate axes are large, repeatedly branching aseptate hyphae (Fig. 4a), 10–15 μm in diameter, that occur as intracellular endophytes in rhizoids (Fig. 4b, arrows) and the rhizoidal ridge (Fig. 4c–f). Moreover, they are abundant in intercellular spaces of the cortex of both the prostrate and proximal portions of aerial axes. In the intercellular system of the cortex, hyphae produce globose to irregularly shaped vesicles, up to 120 μm long and 75 μm wide, on short branches (Fig. 4g–i) and scattered thick-walled spores, up to 180 μm long and 150 μm wide (Fig. 4g,j).

Fungus no. 3 enters the axes via the rhizoid tip or base; infected rhizoids are not altered morphologically (Fig. 4b). In hypodermal cells, hyphae of fungus no. 3 become sheathed with an encasement layer composed of cell wall material, and thus separated from the host cell protoplast (Fig. 4c–f). In conducting cells of the rhizoidal ridge and connective, hyphae are usually covered by granular material (Fig. 3c, arrow) similar to that seen on hyphae of fungus no. 1 in conducting cells. Cell and tissue alterations or host responses associated with fungus no. 3 in the intercellular system of the cortex have not been observed.

Discussion

The fungal endophytes in *N. aphylla* are associated with characteristic cell and tissue alterations and host responses, which are either specific (i.e. associated with only one of the fungi) or unspecific (i.e. associated with two or all three fungi), and thus provide hints as to the nature of the associations between the individual fungi and land plant. Both fungus no. 1 and fungus no. 3 invade the axis through the rhizoids. While the rhizoids show a characteristic host response to attack by fungus no. 1 (Fig. 2b), they do not display host response when attacked by fungus no. 3. This suggests that the nature of the association between fungus no. 1 and the land plant differs from that between fungus no. 3 and the plant. Fungus no. 1 was probably an endoparasite.

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This hypothesis is supported by the fact that other host responses of *N. aphylla* are associated with the presence of this fungus: the most heavily infected rhizoidal ridges are characterized by a hypodermal zigzag line composed of secondarily thickened cell walls (Figs 1c, 2e, 3a). This line marks the outer boundary of cells containing fungus no. 1, and hence is interpreted as a host response that was effective in separating infected from uninfected tissues. Moreover, two of the infected rhizoidal ridges contain peripheral regions that are devoid of cells (Figs 2f, 3b). It is possible that tissue degradation was the result of the breakdown of cell walls by fungus no. 1. However, in one of these specimens, the remaining layers of the hypodermis display secondarily elongated cells (Fig. 3b, arrows) that fill part of the empty space, probably in response to tissue degradation. This suggests that tissue degradation may also have been effective as a defense mechanism. It is known that in some extant plants, phytopathogenic microorganisms may be deterred by controlled cell death around the infected areas that inhibit the microbe from spreading (Hammond-Kosack & Jones, 1996; Veronese *et al.*, 2003; Glazebrook, 2005).

Although the infection pathway of fungus no. 3 parallels that seen in fungus no. 1, the distribution of the latter endophyte within the axes differs considerably from that of the former, because fungus no. 3 also regularly occurs in the cortex of the prostrate axes, and even spreads into the proximal portions of the aerial axes. Moreover, < 5% of the axis segments studied lack fungus no. 3. The morphology of fungus no. 3, abundance, and distribution in *N. aphylla* closely correspond to those seen in the *Aglaophyton major* (Kidst. et W.H. Lang) D.S. Edwards endomycorrhizal fungus *Glomites rhyniensis* T.N. Taylor, W. Remy, Hass et Kerp (Glomeromycota) (cf. Remy *et al.*, 1994; Taylor *et al.*, 1995). However, the infection pathway of fungus no. 3 in *N. aphylla* is distinctly different from that seen in the endomycorrhizal fungus of *A. major*: While *G. rhyniensis* occurs as an intercellular endophyte in *A. major* prostrate axes that becomes intracellular exclusively within a distinct zone of the cortex (i.e. the mycorrhizal arbuscule zone), where it forms arbuscules, fungus no. 3 enters *N. aphylla* as an intracellular endophyte and remains intracellular until it reaches the cortex where it becomes intercellular and forms vesicles. A particularly intriguing host response associated with fungus no. 3 are encasement layers consisting of cell wall material (Fig. 4c–f) that exclusively form around hyphae of this endophyte in hypodermal cells of the rhizoidal ridge. The encasements may have functioned in inhibiting the extraction of nutrients from the hypodermal cells by the endophyte. That way, perhaps fungus no. 3 is 'guided' through the ridge, without adversely affecting the host, and into the cortex where intracellular penetration no longer occurs. In the intercellular system of the cortex, fungus no. 3 produces vesicles (Fig. 4g–i) that are similar to the vesicles produced by the endomycorrhizal fungus *G. rhyniensis*, and thick-walled spores (Fig. 4g,j) reminiscent of the spores seen in many extant Glomeromycota. This suggests that fungus no. 3 may be an endomycorrhizal fungus assignable to the Glomeromycota. On the other hand, arbuscules, which are a characteristic of the *A. major* endomycorrhiza (Taylor *et al.*, 1995), have not been observed in *N. aphylla* to date.

The distribution in *N. aphylla* of fungus no. 2 is similar to that seen in fungus no. 1. However, it is difficult to determine the infection pathway of fungus no. 2 because only spores/zoosporangia are preserved (Figs 2a,d, 3e–h). We hypothesize that fungus no. 2 entered the axes via rhizoids based on the spatial distribution of this endophyte both below and above the hypodermal zigzag boundary line (Fig. 2e). Cells in the area below this boundary contain numerous remains of fungus no. 2, while infected cells above the boundary are rare or lacking. The restricted abundance of fungus no. 2 in the rhizoidal ridge can only be explained if this fungus entered the axis through cells in the area below the boundary line, spread out from this point, and eventually became confined at the boundary line. Conversely, infection via epidermal cells positioned above the boundary line would result in continued infection of the lateral and dorsal portions of the cortex, but this has not been observed in any of the axes. It is interesting that, in most infected cells, either fungus no. 1 or fungus no. 2 is present, while co-occurrence of both endophytes in a single cell is rare. This suggests that fungus no. 1 might have suppressed the infection of cells by fungus no. 2, or vice versa. Often the rhizoids and axis cells that are infected by fungus no. 2 are inflated (Fig. 3e,g). This feature does not represent a true host reaction (i.e. hypertrophy), but rather is a mechanical effect caused by growth of the spores/zoosporangia in the interior of the cell. Since host reactions specifically associated with fungus no. 2 have not been observed, the nature of the association between this endophyte and *N. aphylla* cannot be assessed.

An unspecific 'host reaction' in *N. aphylla* is the presence of a granular material that coats many of the hyphae occurring within the extra-stelar conducting elements of the rhizoidal ridge and connective (Fig. 3c,d). We presume that this coating was deposited by the host cell protoplast. However, it is impossible at present to determine whether this deposition occurred specifically in response to the presence of the fungi, and thus represents a true host reaction, or whether it is related to the normal progressive build-up of secondary walls in these cells.

The land plants and microorganisms from the Rhynie chert have been studied intensively for nearly 100 yr. However, simultaneous interactions of land plant axes with several endophytic fungi have, to date, only been discovered in *N. aphylla*. Although other Rhynie chert plants may ultimately show fungal infections similar to those reported here, there are several characteristics of *N. aphylla* that suggest why this plant may have been more susceptible to colonization by fungi than other land plants in the same paleoecosystem. One reason

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may be the anatomy of the rhizoidal ridge (Fig. 1d), which is a feature that does not occur in other Rhynie chert plants. The dense tissues that make up the rhizoidal ridge are directly associated with the rhizoids and are arranged in radial files. The individual cells are thin-walled and generally larger than other hypodermal and cortical cells, and are interpreted as increasing the absorptive function (Kerp *et al.*, 2001). The radial arrangement of cells, along with the absence of large intercellular spaces, may have provided the ability for intracellular fungal endophytes to move more easily into the tissues of the axis than would be possible if the cells were smaller and less well organized. In addition, the prostrate axis of *N. aphylla* is unique because extra-stelar conducting elements are scattered within the ventral portion, rather than occurring in a distinct core. This arrangement of conducting elements may have reduced the opportunity for endophytes to move to other parts of the plant through a well-defined vascular system, and thus resulted in concentration of endophytes in tissues close to the point of entry. Finally, *N. aphylla*, like most of the Rhynie chert land plants, possessed vegetative reproduction strategies that allowed the plant to spread rapidly into new niches (Daviero-Gomez *et al.*, 2005). Sections through blocks of Rhynie chert (Fig. 1a) show closely spaced prostrate axes at several levels that indicate rapid growth of the underground parts. Sedimentological information indicates that the prostrate axes of *N. aphylla* were subterranean and perennial, and grew in sandy substrates containing a variety of microorganisms (Kerp *et al.*, 2001). This growth pattern is in contrast to other Rhynie chert plants that possessed rhizomatous axes, which grew along the substrate surface, and thus may have been less susceptible to attack by soilborne microorganisms.

Concluding remarks

Fungal endophytes are known to affect plant diversity and structure within modern communities (Sanders, 2004). This can come about as a result of both parasitic and mutualistic fungi selectively altering the asexual and sexual reproduction of the host (Koide, 2000), and also by the partitioning of carbohydrate resources to new ramets (Cheplick, 2004). One can at present only speculate as to the selective pressure that fungal endophytes may have imposed on early land plants and ecosystems such as that of the Rhynie chert. However, several specific host responses associated with the fungal endophytes in *N. aphylla* indicate that some aspects of the complex molecular systems causing host responses in extant plants were in place by the Early Devonian. This suggests that (phytopathogenic) fungi affected the early evolution of land plants, and probably also imposed selective pressure on the plants. As more information is obtained about the diversity and complexity of associations and interactions between early land plants and fungi, it may become possible to advance more detailed hypotheses that can be used in concert with those being developed with modern communities to more accurately depict the role of fungi and their associations with other organisms in the early evolution of terrestrial plants and ecosystems.

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Chapter III: An alternative mode of early land plant colonization by putative endomycorrhizal fungi.

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Addenda

An Alternative Mode of Early Land Plant Colonization by Putative Endomycorrhizal Fungi

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Aglaophyton major, endomycorrhiza, Glomeromycota, *Nothia aphylla*, Early Devonian, Rhynie chert

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Addendum to:

Fungal Endophytes in a 400-million-yr-old Land Plant: Infection Pathways, Spatial Distribution and Host Responses

M. Krings, T.N. Taylor, H. Hass, H. Kerp, N. Dotzler and E.J. Hermsen.

New Phytologist 2007; 10:1469.

ABSTRACT

Rhizomatous axes of *Nothia aphylla*, a land plant from the 400-myr-old Rhynie chert, host a fungus that closely resembles *Glomites rhyniensis* (Glomeromycota), the endomycorrhizal fungus of the Rhynie chert plant *Aglaophyton major*. However, *G. rhyniensis* is an intercellular endophyte that becomes intracellular exclusively within a well-defined region of the cortex, while the fungus in *N. aphylla* initially is intracellular but later becomes intercellular in the cortex. We hypothesize that *N. aphylla* displays an alternative mode of colonization by endomycorrhizal fungi, perhaps related to the peculiar internal anatomy of the lower portion of the rhizomatous axis, in which the radial arrangement of cells, along with the virtual absence of intercellular spaces, provides no intercellular infection pathway into the cortex.

The Early Devonian (*c.* 400 Ma) Rhynie chert is an in situ silicified hot springs environment that has become significant in our understanding of the complexity of life in early terrestrial ecosystems because of the extraordinary preservation of plants, animals, and microorganisms.[1] Moreover, various associations and interactions between different organisms can be directly examined,[2] including the earliest fossil examples of arbuscular endomycorrhizae.[3,4] The Rhynie chert land plant *Aglaophyton major* is characterized by arching, stomatiferous prostrate axes that grow along the substrate surface, and form rhizoid-bearing bulges, usually around stomata, upon contact with the substrate. Extramatrical hyphae of the mycorrhizal fungal enter the axes through these stomata, and spread out through the intercellular system of the hypodermis and cortex, subsequently penetrating individual cells within a well-defined region of the cortex (i.e. the mycorrhizal arbuscule-zone) to form arbuscules.[4]

A recently published study[5] reports on three fungal endophytes that (co-)occur in the Rhynie chert plant *Nothia aphylla*. This plant consists of upright aerial axes arising from a system of non-stomatiferous, subterranean rhizomatous axes characterized by a prominent ventral rhizoidal ridge.[6,7] The rhizoidal ridge, which is unique among Rhynie chert land plants, consists of a rhizoid-bearing epidermis, a multi-layered hypodermis, files of parenchyma cells that connect to the stele, and extra-stelar conducting elements (Fig. 1A); intercellular spaces are virtually absent.

One of the fungal endophytes in *N. aphylla* closely resembles *Glomites rhyniensis* (Glomeromycota), the endomycorrhizal fungus of *A. major*.[4] In *N. aphylla*, this fungus occurs as an intracellular endophyte in rhizoids and tissues of the rhizoidal ridge. Moreover, it is abundant in the intercellular system of the cortex of both prostrate and proximal portions of aerial axes. The fungus enters the axes through rhizoids (Fig. 1B). Once in the hypodermis, hyphae become sheathed by cell wall material (Fig. 1C). In the cortex, the fungus produces intercellular vesicles (Fig. 1D) and thick-walled spores (Fig. 1E). Based on the presence of vesicles that are similar to those of *G. rhyniensis*, and spores like those in extant Glomeromycota, we hypothesize that this fungus is an endomycorrhizal member of the Glomeromycota; however, arbuscules have not been observed to date.

If this interpretation is accurate, *N. aphylla* displays an alternative pattern of colonization by endomycorrhizal fungi. Although the morphology of the fungus and distribution in *N. aphylla* correspond to that of *G. rhyniensis* in *A. major*, the infection pathway is distinctly different. While *G. rhyniensis* is an intercellular endophyte that penetrates individual cells exclusively within the mycorrhizal arbuscule-zone,[4] the fungus of *N. aphylla* enters the plant as an intracellular endophyte, and remains intracellular until it reaches the cortex. The host plant apparently does not respond to the invading fungus because infected rhizoids are not altered morphologically. Once in the hypodermis, however, hyphae become separated from the host cell protoplast. This feature suggests a shift from

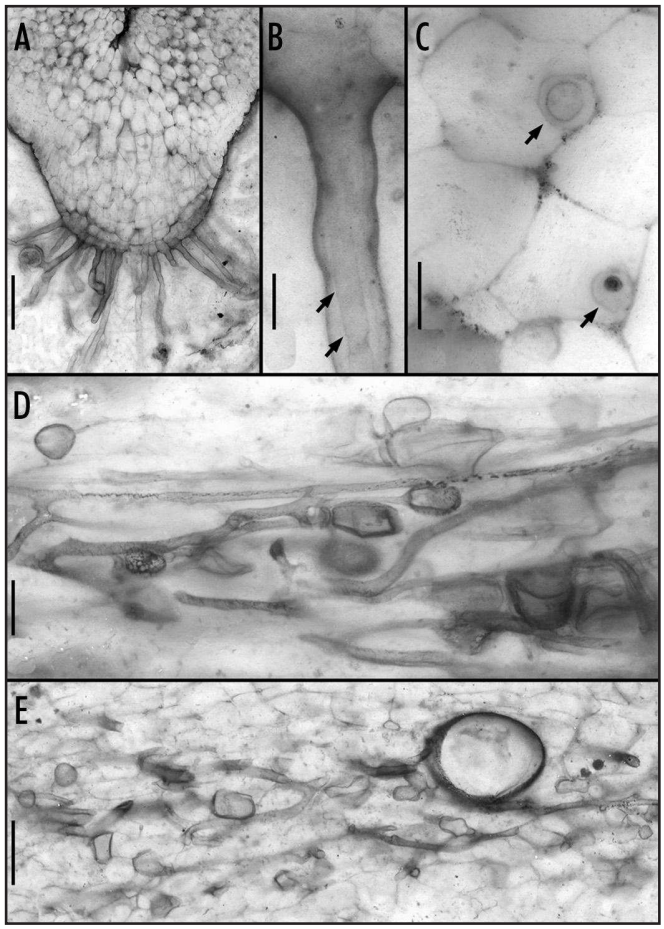

Figure 1. *Nothia aphylla* from the Lower Devonian Rhynie chert. (A) Ventral portion of a rhizomatous axis with rhizoidal ridge (cross section); bar = 250μm. (B) Fungal hypha [arrows] entering the axis through a rhizoid; bar = 30μm. (C) Sheathed intracellular hyphae [arrows] in hypodermal cells (transverse section); bar = 20μm. (D) Intercellular hyphae and vesicles in the cortex (longitudinal section); bar scale = 50μm. (E) Hyphae, vesicles, and a thick-walled spore in the cortex (longitudinal section); bar = 100μm. All images from the original paper; reproduced with permission.

(i) uncontrolled intracellular occurrence of the fungus in the rhizoids, to (ii) controlled intracellular occurrence in the rhizoidal ridge, to (iii) intercellular occurrence in the cortex.

The fact that the rhizomatous axes of *N. aphylla* are subterranean, along with the peculiar internal anatomy of the rhizoidal ridge, may have provided the selective pressure for an alternative mode of colonization by endomycorrhizal fungi. The fungus probably enters the plant through rhizoids because the axes are non-stomatiferous. Moreover, the morphology and radial arrangement of cells in the rhizoidal ridge, along with the virtual absence of intercellular spaces, perhaps does not provide an intercellular infection pathway into the cortex. We speculate that *N. aphylla* tolerated intracellular penetration in the rhizoids and within the tissues of the rhizoidal ridge in order to become inoculated. Tolerating (or even facilitating) intracellular penetration within a limited area of the axis may simultaneously have provided the plant with a means of recognizing and subsequently distinguishing the endomycorrhizal fungus from potentially harmful parasites (e.g., by surface features of the hyphae or chemical signals). Once recognized, the endomycorrhizal fungi become sheathed and "guided" through the ridge without being able to extract nutrients from the host, and into the cortex where intracellular penetration is not longer possible. The parasites, once recognized, are confined in the tissues of the rhizoidal ridge by specific or unspecific host responses, e.g., secondarily thickened cell walls.[5] Conversely, if the endomycorrhizal fungus entered the plant through surface openings, and spread out exclusively through the intercellular system, the mechanisms that might confine simultaneous parasite infections were probably much more limited.

Endomycorrhizal relationships are believed to have evolved from parasitic interactions.[8] It has been postulated that modern enodomycorrhizal fungi in some way control parasites because both compete for the same resources.[9] It may be that, during the evolution of fungal endophytism, the initial benefits of mycorrhizae included protection of the host from pathogenic fungi.[10] *Nothia aphylla* from the Lower Devonian Rhynie chert adds support to this hypothesis, and may demonstrate that more than a single pattern of colonization by endomycorrhizal fungi occurred during the early evolution of land plants.

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Chapter IV: A prasinophycean alga of the genus *Cymatiosphaera* in the Early Devonian Rhynie chert.

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A prasinophycean alga of the genus *Cymatiosphaera* in the Early Devonian Rhynie chert

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Abstract

Spherical phycomata of prasinophycean algae are preserved in the Early Devonian Rhynie chert. Because they display a surface reticulum composed of muri oriented perpendicular to the vesicle surface, they are assigned to the genus *Cymatiosphaera* (Cymatiosphaeraceae, Pyramimonadales). We interpret the size range of the phycomata (24 to 48 μm in overall diameter) as reflecting continued metabolic activity and growth of the alga during the phycoma stage. The phycomata from the Rhynie chert represent the earliest account of the genus *Cymatiosphaera* in a freshwater paleoecosystem, and broaden our knowledge about the ecological diversity of Early Devonian prasinophycean algae.
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Keywords: *Cymatiosphaera*; green algae; permineralization; phycoma; Early Devonian; Prasinophyceae; Pyramimonadales

1. Introduction

The Early Devonian Rhynie chert (*ca.* 400 Ma) has preserved a diversity of microorganisms in a non-marine paleoecosystem. Present in the cherts are bacteria (Kidston and Lang, 1921), cyanobacteria (Croft and George, 1959; Krings et al., in press), microalgae (Edwards and Lyon, 1983), peronosporomycetes (Taylor et al., 2006), and a variety of fungi (Taylor et al., 2004). Whereas the fungi have received considerable attention, the other microorganisms from the Rhynie chert remain understudied. However, detailed knowledge about the diversity of the minute life forms is important in fully assessing the complexity of this ancient ecosystem.

Documentation of algae from the Rhynie chert is particularly rare; it includes the charophyte *Palaeonitella cranii* (Kidston & W.H. Lang) J. Pia (Kelman et al., 2004) and several planktonic unicellular and filamentous microalgae. However, only two of the microalgae (i.e. *Mackiella rotunda* and *Rhynchertia punctata*) have been formally described (Edwards and Lyon, 1983). To a large degree, this is because a number of features critical in defining the systematic affinities of microalgae (e.g., flagellar apparatus, pigmentation, life history) are difficult, or even impossible, to document from the fossil record.

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In this paper we present an addition to the Rhynie chert microalgal flora that occurs in the form of prasinophycean phycomata assignable to the genus *Cymatiosphaera* Wetzel ex Deflandre (Cymatiosphaeraceae, Pyramimonadales). The Prasinophyceae is a poly- or paraphyletic group of single-celled, flagellate green algae hypothesized to have diverged early in chlorophytan phylogeny (Lewis and McCourt, 2004). The group consists of four (according to Nakayama et al., 1998) or six to seven (according to Teyssèdre, 2006) distinct clades, one of which is the Pyramimonadales. The life history of several species of Pyramimonadales includes a unique non-motile stage that is characterized by the formation of a cyst-like structure termed the phycoma. Unlike cysts, however, the alga remains metabolically active within the phycoma, and eventually undergoes vegetative reproduction. As a result, the phycoma increases in size over time (Knoll et al., 1991; Teyssèdre, 2006).

Prasinophycean phycomata are resistant, and thus often well-preserved as fossils; geologically they can be traced into the Precambrian (cf. Teyssèdre, 2006). Most fossil phycomata have been described from marine or brackish water strata (Colbath and Grenfell, 1995; G.L. Mullins, pers. commun., 2007), but there are also several reports from freshwater deposits (e.g., Doubinger, 1967; Clausing, 1993; Zippi, 1998). The phycomata from the Rhynie chert represent the earliest evidence of the genus *Cymatiosphaera* in a freshwater paleoecosystem.

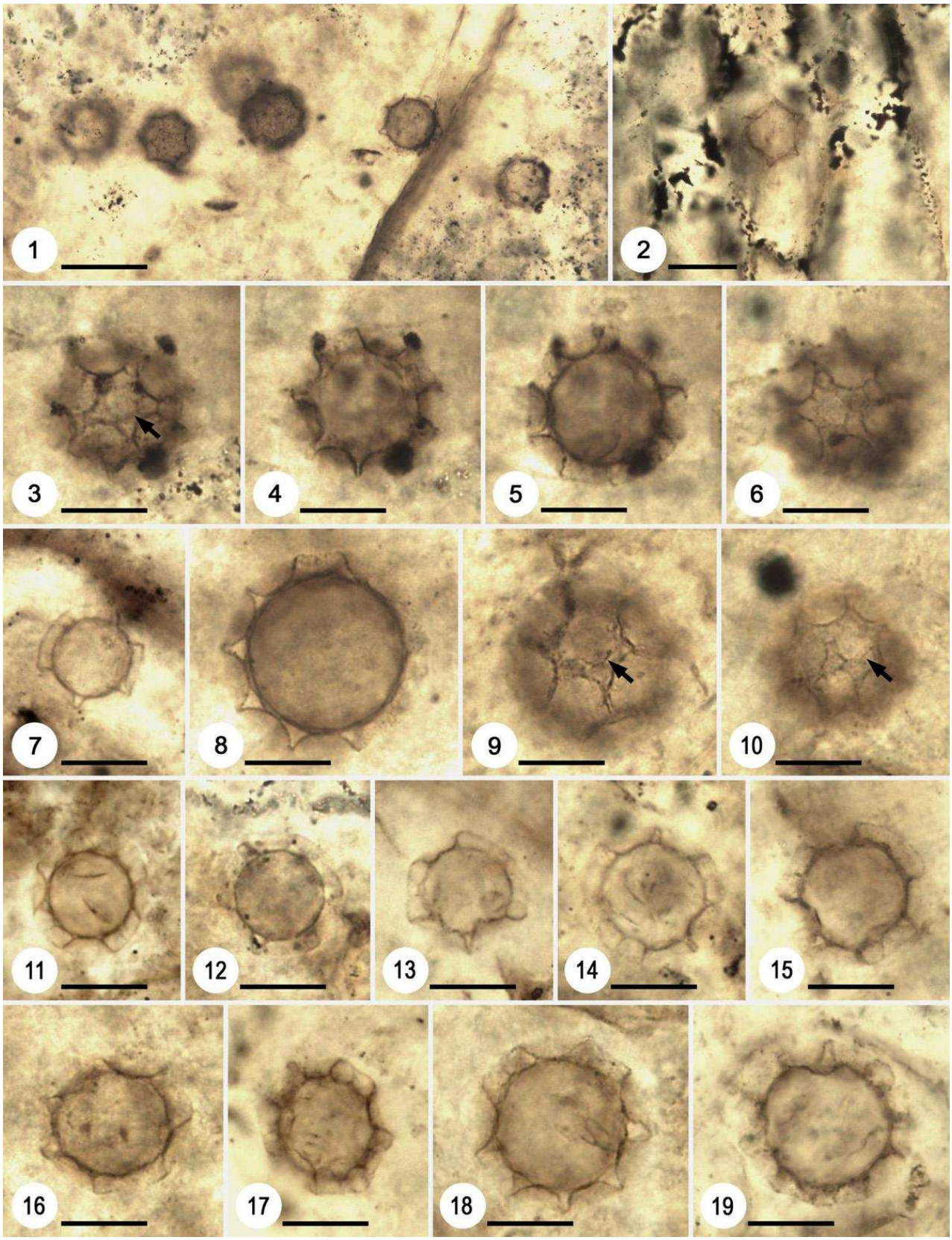
2. Materials and methods

The Rhynie chert Lagerstätte is located in the northern part of the Rhynie Outlier of Lower Old Red Sandstone in Aberdeenshire, Scotland, within a sequence of sedimentary and volcanic rocks. The cherts occur in the upper part of the Dryden Flags Formation, in the so-called Rhynie Block, a few hundred metres northwest of the village of Rhynie. The Lagerstätte consists of at least 10 fossiliferous beds containing lacustrine shales and cherts that are interpreted as a series of ephemeral freshwater pools within a hot springs environment (e.g., Rice et al., 2002). Preserved in the cherts are both aquatic (freshwater) facies from the pools and subaerial soil/litter horizons with *in situ* plants around the edges of the pools; the latter became preserved as a result of temporary flooding of silica-rich water, or by silica-rich groundwater percolating upwards. Based on dispersed spore assemblages and redefinition of the Pragian/Emsian boundary by the IUGS, Wellman et al. (2006) date the cherts as Pragian–?earliest Emsian. Detailed information about the geological setting, sedimentology, and development of the Rhynie chert Lagerstätte can be found in Rice et al. (2002), and Trewin and Rice (2004).

The phycomata were identified in several different thin sections prepared from a single large chert block that was collected from a cultivated field during the 1960s. Pieces of chert were cemented to a glass slide and then ground until the slice was thin enough to be examined in transmitted light (cf. Hass and Rowe, 1999). Slides are deposited in the Bayerische Staatssammlung für Paläontologie und Geologie, Munich (Germany), under accession numbers BSPG 1964 XX 203–209. Terminology used in the description and discussion is from Williams et al. (2000).

3. Description

A total of 115 phycomata were examined (Plate I,1–19). Most of the specimens occur singly or loosely clustered in groups of 2–10 individuals (e.g., Plate I,1) within the chert matrix where they are associated with a fine accumulation of degrading land plant fragments; a few have been found inside degrading land plant tissues (Plate I,2). Phycomata are preserved as spherical vesicles ranging from 24 to 48 μm in overall diameter (muri included). The vesicle surface is regularly ornamented with prominent laevigate muri, which are between 4 and 6 μm high, positioned perpendicularly to the surface, and have sharp outer margins oriented parallel to the surface of the vesicle (Plate I,7,11–15). Muri give the phycoma a reticulate appearance by forming polygonal fields (lacunae) that are more or less equally sized in one specimen, and generally between 15 and 20 μm in diameter. The lacunae are usually pentagonal, but hexa- and heptagons may also occur (Plate I,3,6,9,10). The intersections (corners) of the polygons are generally thickened and proximally widened, and thus appear as elongate-triangular columns in lateral view (e.g., Plate I,5,7,8,11,12,16,18). Although the size of the lacunae defined by the muri remains relatively consistent between specimens, their number varies between 12 in the smallest (e.g., Plate I, 7) and >20 in the largest phycomata (e.g., Plate I,8). The lacunae floors are up to 1 μm thick and usually laevigate. However, a few of the floors appear to have a microreticulate surface ornamentation, but this is probably a preservational artefact. Seven of the specimens show what appears to be a circular or oval orifice, between 8 and 9 μm in diameter, positioned in the center of one of the polygonal fields (arrows in Plate I,3,9,10). Neither surface pores nor an excystment structure in the form of a preformed, slit-like opening or rupture (dehiscence line) has been observed.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4. Discussion

Two features of the microfossils detailed above underscore their prasinophycean affinity: (1) The surface reticulum composed of muri that partitions the vesicle surface into regular polygonal fields. This pattern of surface ornament is typical of many fossil and extant prasinophycean phycomata (Colbath and Grenfell, 1995). (2) Considerable differences in vesicle diameter (i.e. from 24 to 48 μm) and the number of polygonal fields (i.e. from 12 to >20) among the specimens. Intrapopulation differences in phycoma diameter, which range from 10 to >100 μm in some forms (Tappan, 1980), have been recorded for many extant and fossil prasinophyceans. In modern forms, the phycoma increases in size as a result of continued growth, and eventually vegetative reproduction, of the alga within the phycoma. This has also been interpreted in fossil forms (e.g., Knoll et al., 1991; Teyssèdre, 2006). We are not aware of any study specifically addressing the mechanisms underlying the enlargement of the phycoma during maturation (e.g., by expansion of the envelope or by addition of new material), and thus cannot explain as to how the number of polygonal fields increases with the size of the phycomata. Perhaps the increase in size was not uniform, but rather resulted from a series of pulses, in which, during the quiescent phase, tension was reduced, which resulted in more fields being produced once growth resumed.

The surface reticulum of the Rhynie chert phycomata corresponds closely with that of *Cymatiosphaera* (Cymatiosphaeraceae). In *Cymatiosphaera*, the external vesicle surface is divided into polygonal fields by prominent laevigate muri perpendicular to the surface, but without displaying a system of equatorial differentiation of fields (Deflandre, 1954; Mullins, 2001). Additional diagnostic features of *Cymatiosphaera* that are in congruence with the fossils from the Rhynie chert include 1) a vesicle surface that is laevigate and without pores, spines or horns; 2) thickened intersections (corners) of the muri that look like rods or small columns in lateral view; and 3) sharp outer margins of the muri that are parallel to the vesicle surface. A second possible repository for the Rhynie chert phycomata is *Dictyotidium* Eisenack, in which the external vesicle surface is also subdivided into polygonal fields by muri that are perpendicular to the surface. However, the muri are typically lower (e.g., only 1 μm high in *D. faviforme* Schultz and up to 2 μm in *D. biscutulatum* Kiryanov; cf. Mullins, 2001) than those seen in *Cymatiosphaera* and the Rhynie chert phycomata, and thus we include the Rhynie chert fossils in *Cymatiosphaera*. Because virtually all Paleozoic age species of *Cymatiosphaera* known to date are described from macerated samples and not thin sections, it is difficult, if not impossible, to determine as to whether the organisms observed belong to a new species or have already been described based on "classical" palynological preparations. We therefore include the Rhynie chert phycomata in open nomenclature as *Cymatiosphaera* sp.

Cymatiosphaera has been documented from the Cambrian to Neogene throughout the world (e.g., Tappan, 1980; Fuxing and Qiao, 1987), with forms found in marine, brackish, and freshwater habitats. However, we are not aware of any report from a strictly freshwater paleoenvironment older than Pennsylvanian–Early Permian (i.e. Clausing, 1993). As a result, the phycomata from the Rhynie chert represent the earliest evidence of the genus *Cymatiosphaera* in a freshwater paleoecosystem.

Prasinophycean phycomata typically dehisce by rupture along a preformed line of weakness within the outer wall layer (excystment structure). Spherical phycomata rupture along a great circle, while in discoidal forms with a marginal flange dehiscence occurs across the face of the central vesicle (Colbath and Grenfell, 1995). Nevertheless, a dehiscence line or evidence of a rupture was not observed in any of the specimens from the

Plate I. Prasinophycean phycomata from the Early Devonian Rhynie chert.

1. Six phycomata loosely clustered in the chert matrix; slide BSPG 1964 XX 208; bar scale=50 μm.
2. Phycoma in degrading land plant tissue; slide BSPG 1964 XX 204; bar scale=30 μm.
3–6. Four optical sections through one of the specimens; arrow in Pl. I, 3 indicates circular structure (?opening); slide BSPG 1964 XX 206; bar scales=20 μm.
7. A small phycoma; slide BSPG 1964 XX 203; bar scale=20 μm.
8. A large phycoma; slide BSPG 1964 XX 208; bar scale=20 μm.
9–10. Phycomata showing what appears to be a circular opening or orifice [arrows] in one of the lacunae floors; slides BSPG 1964 XX 206 (Pl. I, 9) and BSPG 1964 XX 204 (Pl. I, 10); bar scales=20 μm.
11–19. Phycomata from the chert matrix; slides BSPG 1964 XX 203 (Pl. I, 11–15,17,19) and BSPG 1964 XX 204 (Pl. I, 16,18); bar scales=20 μm.

Rhynie chert. Although this might argue against assigning the fossils to *Cymatiosphaera*, it is possible that none of the Rhynie chert phycomata were mature at the time of fossilization, which might explain the absence of excystment structures. On the other hand, seven of the specimens display what appears to be a circular opening or orifice (arrows in Plate I,3,9,10). Whether this structure represents an exit pore or a preservational artefact cannot be determined. Some species in *Cymatiosphaera* (e.g., *C. mariae* Cramer, Díez, Rodriguez & Fombella) display a circular, raised wheel-like structure in the center of each field (G.L. Mullins, pers. commun., 2007). However, these structures are positive, and thus distinctly different from the openings seen in the Rhynie chert phycomata. If, however, the circular structures represent exit pores, the possibility exists that another group of organisms produced the phycomata.

Spherical excystment apertures occur in many dinoflagellate cysts where they may range from tiny pores to openings that equal in width the cyst diameter (Tappan, 1980). Moreover, among dinoflagellates are several taxa that produce cysts with a surface reticulum, some (e.g., *Calciodinellum operosum* Deflandre, *Pentadinium lophophorum* Benedek) superficially resemble that of *Cymatiosphaera*. However, the dinoflagellate reticulum does not partition the surface into similar fields, but rather forms a specific pattern of unequal and equatorially differentiated plates (Montresor et al., 1997). In addition, in organic-walled reticulate dinoflagellate cysts the excystment pore is usually not circular but rather polygonal, and the reticulum in calcareous forms consists of rod-shaped crystals, while membranaceous expansions (muri) characterize the Rhynie chert phycomata. As a result, it is very unlikely that the Rhynie chert fossils represent dinoflagellate cysts.

Some of the Rhynie chert specimens have been found inside degrading plant tissues (e.g., Plate I,2), which suggests that their origin may be with yet another group of organisms. The endoparasitic chytridiomycete *Olpidium brassicae* (Woronin) P.A. Dangeard produces resting spores in plant cells that are characterized by regularly distributed, warty projections formed by the exosporium. Such resting spores may appear star-like in lateral view, and thus similar to the Rhynie chert fossils and other *Cymatiosphaera* species. However, *O. brassicae* resting spores are easily distinguishable from *Cymatiosphaera* because they have massive walls, and the exosporium is covered by a delicate membranaceous sheath (Singh and Pavgi, 1977; Raghavendra Rao and Pavgi, 1980). Moreover, the endosporium is usually well-recognizable as a distinct thin layer along the inner surface of the exosporium. None of these structural characteristics are seen in the Rhynie chert phycomata, and thus we suggest that the phycomata were accidentally washed into the decaying plant remains, or motile cells entered the tissues and subsequently formed phycomata.

It is interesting that none of the phycomata recovered from the Rhynie chert are infected by parasitic fungi, although other small structures in the Rhynie chert matrix, e.g., land plant and fungal spores, frequently display parasite infections, predominantly by chytrids (Taylor et al., 1992, 2004; Taylor and Krings, 2005). This raises the question as to whether the phycomata were in some way immune to attack by parasitic fungi. Perhaps the phycoma stage did not last for an extended period of time, and thus these structures were not suitable hosts for parasites. This hypothesis seems plausible because, in extant prasinophyceans, the phycoma stage usually lasts from two weeks to nearly four months, depending on environmental conditions (Graham and Wilcox, 2000). Thus, phycomata are distinctly shorter-lived than most fungal and land plant spores. On the other hand, since the alga remains metabolically active during the phycoma stage, it might have been more difficult for parasites to colonize the organism. Finally, the phycoma wall is composed of a highly resistant biopolymer (Knoll et al., 1991; Versteegh and Blokker, 2004), which may have been sufficient to deter parasitism.

Although the Rhynie chert has been studied for more than 80 yr, new species and life forms are continuously being discovered. The phycomata detailed in this contribution add to our knowledge about the biodiversity of Rhynie chert green algae. Moreover, they indicate that organic-walled algal resting stages are preserved in the cherts. We anticipate that other types will be discovered as work on this paleoecosystem continues. This will lead to a more accurate picture of the microalgal flora because the systematic affinities of these organisms as fossils are far easier to document based on characteristic resting stages than on motile unicells. Moreover, it will help to more fully understand the ecology and community biodiversity of the Rhynie chert ecosystem, and will provide a framework with which the biological dynamics can be evaluated in a freshwater ecosystem that existed some 400 Ma ago.

Acknowledgments

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Chapter V: A microfungal assemblage in *Lepidodendron* from the upper Viséan (Carboniferous) of central France

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Systematic palaeontology (Palaeobotany)

A microfungal assemblage in *Lepidodendron* from the Upper Visean (Carboniferous) of central France

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Abstract

A diverse assemblage of microfungi and fungi-like microorganisms, composed of several types of hyphae, putative reproductive structures (sporangia), and a variety of spores, occurs in permineralized *Lepidodendron* xylem and periderm from the Upper Visean of central France. Some of the remains can be attributed to the Chytridiomycota and Peronosporomycetes (Oomycota) with some degree of confidence. We suggest that this assemblage represents a community of saprotrophic organisms that colonized the tissues post mortem and participated in the decay process. The permineralized *Lepidodendron* tissues from France offer a rare direct insight into the diversity of microfungi and fungi-like organisms in a Carboniferous terrestrial paleoecosystem. ***To cite this article: M. Krings et al., C. R. Palevol 6 (2007).***
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Résumé

Une association de champignons microscopiques dans des *Lepidodendron* du Viséen supérieur (Carbonifère) du Massif central francais. Une association variée de champignons microscopiques et de microorganismes de type champignon, composée de plusieurs types d'hyphes, de structures reproductrices (sporanges) et de diverses spores, est présente dans le xylème et le périderme de tiges de *Lepidodendron* perminéralisés du Viséen supérieur du Massif central français. Certains restes peuvent être attribués aux Chytridiomycota et aux Peronosporomycetes (Oomycota), avec un degré certain de confiance. Nous suggérons que cette association représente une communauté d'organismes saprotrophiques qui colonisaient les tissus post mortem et contribuaient au processus de décomposition. Les tissus de ces *Lepidodendron* perminéralisés de France offrent l'exemple rare d'un aperçu direct de la biodiversité des champignons microscopiques et des organismes de type champignon dans un paléoécosystème terrestre du Carbonifère. ***Pour citer cet article : M. Krings et al., C. R. Palevol 6 (2007).***
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Keywords: Chert; Chytridiomycota; Combres; Hyphae; *Lepidodendron rhodumnense*; Peronosporomycetes; Saprotrophism; Spore; Sporangium; France

Mots clés : *Chert* ; Chytridiomycota ; Combres ; Hyphes ; *Lepidodendron rhodumnense* ; Peronosporomycetes ; Saprotrophisme ; Spore ; Sporange ; France

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1. Introduction

Microorganisms were critical components of ancient ecosystems, and entered into a wide variety of associations and interactions with other organisms. However, only recently have they received increased scholarly attention based on the fossil record [23]. This is because most information on ancient microorganisms comes from indirect evidence and dispersed specimens, rather than from those preserved in the precise environment in which they lived (in situ). Adding to the paucity of documented fossil microorganisms in situ has been the historical belief that these life forms are too delicate to be adequately preserved, and, if they are preserved, cannot be analyzed properly with the techniques available.

One of the first scholars to systematically document Late Paleozoic microorganisms preserved in situ was the French paleobotanist Bernard Renault [9–19]. Renault's success in detailing fossil microbial life was directly attributed to the exceptional preservation of the fossils in a siliceous chert matrix, his ability to produce high quality thin sections of the cherts, and his obvious understanding and appreciation of details of the microbial world. Although Renault discovered microorganisms in a variety of cherts and other rocks of Carboniferous and Permian age, the majority of these organisms were reported from Late Visean (~330 Ma) cherts of Combres/Lay and Esnost in central France. Particularly interesting are several forms (bacteria, fungi or fungi-like organisms, and microalgae) that occur in association with land plants. Unfortunately, Renault's extraordinary work and insights into the microbial realm in ancient ecosystems was not followed after his death; only three papers detailing various levels of biological interaction have been published since (i.e. [5,7,24]).

Renault was meticulous in documenting the morphology of the individual microorganisms and, in some instances, their distribution within host plant tissues. Moreover, he was concerned with the natural affinities of these organisms, and in fact was correct in many of his taxonomic identifications. He was less interested, however, in elaborating on the complexity attained by some of the land plant/microbial associations preserved in the cherts. This was certainly due to a large degree by the fact that such topics were not of particular scientific interest at that time. Today, however, the levels of complexity attained by land plant/microbial associations represent key areas in ecological and ecophysiological research. This modern focus has initiated questions at several levels regarding the origin of land plant/microbial associations, and hypotheses that explore how they may have evolved (e.g., [4]). Answers have primarily come from molecular analyses of modern systems, whereas the fossil record has been used only in a limited sense. However, where preservation of microorganisms permits detailed macro- and microscopical documentation, the fossil record is becoming increasingly important as the only method of documenting complex land plant/microbial associations within an evolutionary context [8].

Here, we report on a diverse fossil assemblage of microfungi and/or fungi-like microorganisms consisting of hyphae, reproductive structures, and spores that occurs within the xylem and endophelloderm (i.e. the inner portion of the periderm) of *Lepidodendron rhodumnense* Renault from the Upper Visean of central France.

2. Material and methods

The cherts containing the infected *Lepidodendron rhodumnense* xylem and periderm come from the Upper Visean (Mississippian [= Lower Carboniferous]) of Combres (approximately 12 km south of Roanne), Massif Central, central France. They occur as loose blocks within rhyolitic tuffs, and were collected in cultivated fields or in stream sections. The geological setting and paleoenvironment of the Late Visean in the Roanne area have been interpreted as analogous to that in the Autun basin at the locality of Esnost, about 10 km north of Autun, Massif Central, central France [3]. Information on the geological setting of the Esnost locality can be found in [21]; for details on the preservation of fossils and a paleoecological reconstruction of the Visean wetland ecosystem at Esnost see [20].

The microfungi were identified in two thin sections (i.e. 1. xylem and endophelloderm, radial section, slide No. B49/1118; 2. endophelloderm, tangential section, slide No. B50/1137) prepared by cementing a wafer of chert to a glass slide and then grinding the wafer to a thickness sufficiently thin to be examined in transmitted light. The thin sections were prepared by B. Renault and co-workers during the late 19th and early 20th centuries, and are today housed in the 'Muséum national d'histoire naturelle' ('Laboratoire de paléontologie') in Paris (France).

3. Description of microfungi and fungi-like microorganisms

The permineralized *Lepidodendron* tissues from the Upper Visean of Combres contain abundant remains of intracellular microfungi and fungi-like microorganisms in the form of three distinct types of hyphae, several putative reproductive structures (sporangia), and a variety of

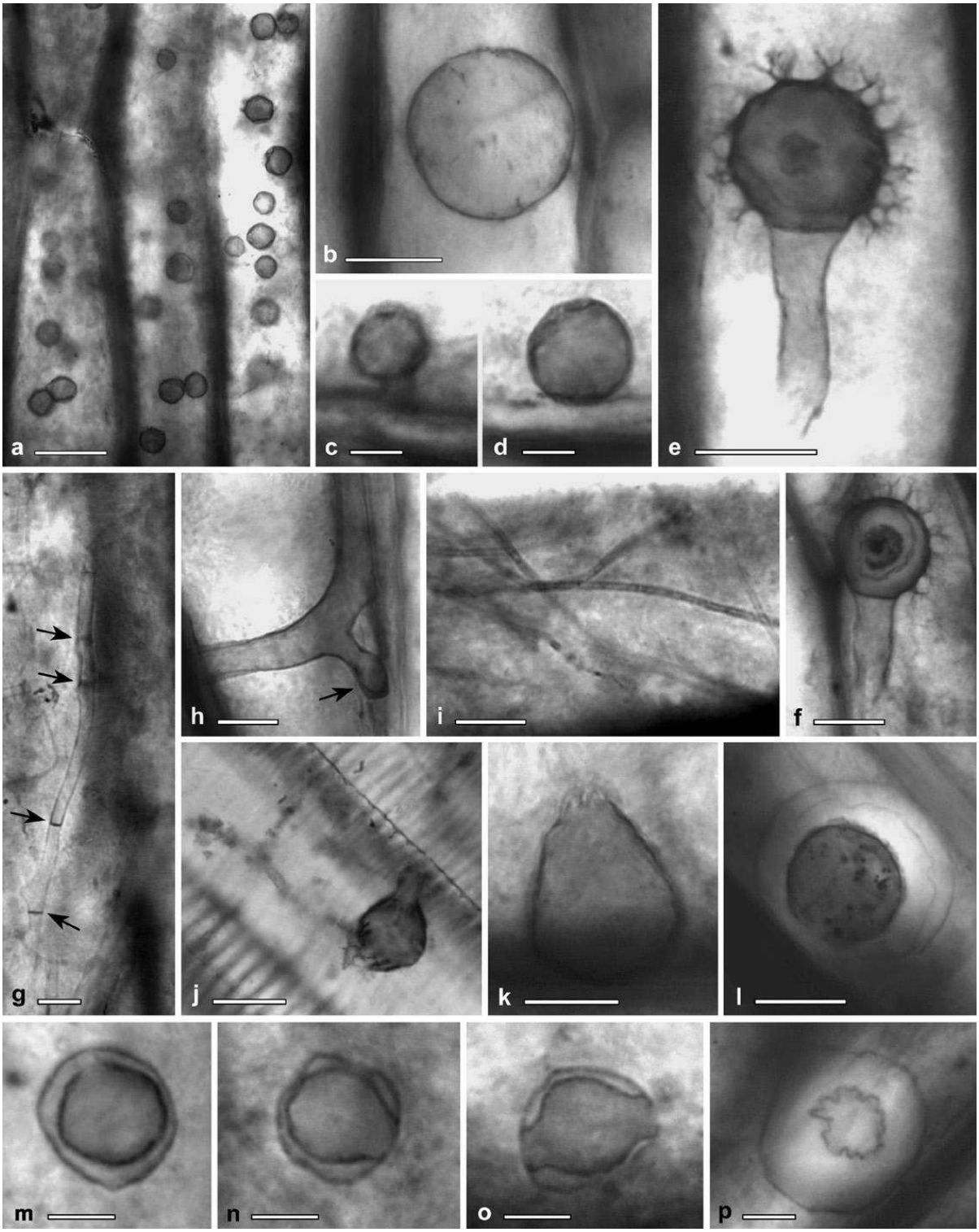

Fig. 1. Intracellular microfungi and fungi-like microorganisms in *Lepidodendron* from the Upper Visean of central France. (**a**) Clusters of small spherical spores in the endophelloderm. Slide B49/1118; bar = 20 μm. (**b–d**) Various types of intracellular spherical spores or sporangia: (**b**) unattached, large, and without orifice; (**c**) attached, small, stalked, and with distal orifice; (**d**) attached, small, unstalked, and with distal orifice. Slide B50/1137; bars = 20 μm (**b**) and 5 μm (**c,d**). (**e,f**) Oosporangia with repeatedly forking surface extensions and wide, aseptate subtending hyphae. Slide B50/1137; bars = 20 μm. (**g**) Medium-sized, irregularly septate hypha; arrows indicate simple septa. Slide B50/1137; bar = 10 μm. (**h**) Wide aseptate hypha with short, terminally swollen lateral branch (arrow). Slide B50/1137; bar = 10 μm. (**i**) Narrow aseptate hyphae. Slide B50/1137; bar = 10 μm. (**j**) Pear-shaped ?zoosporangium with narrow, aseptate subtending hypha growing along the inner surface of a tracheid. Slide B49/1118; bar = 40 μm. (**k**) Pear-shaped ?zoosporangium with apical cleft, attached to a cell wall. Slide B50/1137; bar = 10 μm. (**l**) Large,

spores. Some of the organisms are attached to cell walls, while others appear in the cell lumen. Although the thin sections come from two different chert blocks, the microfungal assemblages found within the tissues are largely composed of the same forms of organisms.

The most abundant fungal remains in the endophelloderm are three distinct types of hyphae, including (1) loose meshworks of narrow, septate, and rarely branching hyphae (Fig. 1i), between 0.9 and 1.1 μm wide, that occur in ~55% of the cells; (2) medium-sized, septate, and usually unbranched hyphae, between 2.5 and 3.5 μm wide (Fig. 1g) that have been observed in <5% of the cells; and (3) large, aseptate or occasionally septate hyphae, between 4.5 and 12 μm wide, that occur in >75% of the cells and give off medium-sized, typically aseptate hyphae. Most of the large hyphae extend parallel to the long axis of the elongate cells; rarely do they extend perpendicular to the orientation of the cells. Wide hyphae sometimes produce short lateral branches that are terminally swollen (Fig. 1h [arrow]). All hyphal types appear to extend from one cell to another through the pits in the cell walls; host responses to fungal penetration have not been observed. Hyphae also occur in the xylem, but are less well preserved.

Also present in the tissues are numerous forms of spores. In many cells, they occur in large number (e.g., Fig. 1a) and are associated with narrow hyphae. Most spores are spherical, between 5.0 and 15 μm in diameter, and possess thin and translucent or relatively thick and opaque walls. One spore type (Fig. 1m–o) is characterized by a thick but translucent wall and two, usually oppositely positioned, circular openings (Fig. 1o). Other spherical structures in the tissues cannot be identified as to whether they are fungal spores or sporangia. These include (1) large, thin-walled spherical bodies (Fig. 1b), between 30 and 35 μm in diameter, that are borne on narrow hyphae and do not display any surface ornamentation or what can be termed preformed openings; (2) tiny spherules, up to 8.0 μm in diameter, that are attached to the cell walls by a short stalk, up to 3.0 μm long, and characterized by a distal circular orifice, 2.0 μm in diameter (Fig. 1c); (3) medium-sized spherical bodies, up to 15 μm in diameter, that are similar in shape to the previously described structures but lacking stalks (Fig. 1d); (4) large, double-walled structures, 25–30 μm in diameter, containing a single, usually dark-walled sphere that may be ornamented (Fig. 1l); and (5) large, relatively thin-walled spheres, up to 35 μm in diameter, in which the contents have a wrinkled surface and are variously shaped (Fig. 1p).

We interpret two types of structures as probably representing some form of fungal sporangium. One of these is borne terminally on aseptate hyphae that grow along the inner surfaces of cell walls (Fig. 1j). This structure is pear-shaped, up to 60 μm high, and composed of a relatively thick-walled basal sphere (30–40 μm in diameter) to which is distally attached a thin-walled tube. The second form is also pear-shaped, between 45 and 55 μm high, but to date not found in organic connection to a subtending hypha. Rather, this structure is attached directly to the host cell wall; most of the specimens possess an apical cleft embracing a small opening (Fig. 1k).

The most conspicuous structures occur exclusively in cells of the endophelloderm. These are thick-walled spheres, between 25 and 35 μm in diameter, characterized by repeatedly forking, antler-like surface extensions, each up to 6.0 μm high. These spheres are always borne on prominent, aseptate subtending hyphae (Fig. 1e and f), up to 15 μm wide. Most specimens contain a single opaque globe, 10–25 μm in diameter, surrounded by a relatively thin, wrinkled wall (Fig. 1f).

4. Discussion

Bernard Renault's systematic analysis of Visean microorganisms preserved in cherts from central France represents a benchmark contribution to our understan-

double-walled sporangium containing a single dark-walled, ornamented spore. Slide B49/1118; bar = 20 μm. (**m–o**) Small thick-walled spores with two oppositely positioned openings (best seen in Fig. 1o). Slide B50/1137; bars = 5 μm. (**p**) Large ?resting spore. Slide B49/1118; bar = 10 μm.

Fig. 1. Champignons microscopiques et microorganismes de type champignon intracellulaires dans un *Lepidodendron* du Viséen supérieur de France. (**a**) Groupes de petites spores sphériques dans l'endophelloderme. Lame B49/1118; échelle = 20 μm. (**b–d**) Types variés de spores ou sporanges sphériques intracellulaires : (**b**) non attaché, grand et sans orifice ; (**c**) attaché, petit, pédicellé et avec orifice distal ; (**d**) attaché, petit, sessile et avec orifice distal. Lame B50/1137 ; échelles = 20 μm (**b**) et 5 μm (**c,d**). (**e,f**) Oogones/Oosporanges avec des expansions superficielles plusieurs fois ramifiées, terminant une large hyphe non septée. Lame B50/1137 ; échelles = 20 μm. (**g**) Hyphe de taille moyenne, irrégulièrement septée ; les flèches désignent des cloisons simples. Lame B50/1137 ; échelle = 10 μm. (**h**) Large hyphe sans cloison, avec une courte ramification à terminaison renflée (flèche). Lame B50/1137 ; échelle = 10 μm. (**i**) Hyphes étroites non septées. Lame B50/1137 ; échelle = 10 μm. (**j**) ?Zoosporange pyriforme à l'extrémité d' une hyphe étroite, non septée, qui s'étend le long de la face interne d'une trachéide. Lame B49/1118 ; échelle = 40 μm. (**k**) ? Zoosporange pyriforme avec une ouverture apicale, attaché à une paroi cellulaire. Lame B50/1137 ; échelle = 10 μm. (**l**) Grand sporange, à double paroi, contenant une seule spore à paroi sombre et ornementée. Lame B49/1118 ; échelle = 20 μm. (**m–o**) Petites spores à paroi épaisse avec deux ouvertures diamétralement opposées (bien visible en Fig. 1o). Lame B50/1137 ; échelles = 5 μm. (**p**) Large spore ? non fonctionnelle. Lame B49/1118 ; échelle = 10 μm.

ding of the diversity of microbial life in Late Paleozoic non-marine ecosystems. The significance of Renault's work parallels that of Kidston and Lang's study of microscopic life in the Early Devonian Rhynie chert [6]. Unlike the Rhynie chert microorganisms, however, the existence of exquisitely preserved minute life forms in Visean cherts from France has largely been forgotten since Renault's death. Nevertheless, his meticulous work and detailed studies represent another chapter in the investigation of microorganisms in ancient ecosystems, and constitute a largely untapped source of information about microbial life some 330 Ma ago.

The microfungal remains discovered in the *Lepidodendron* tissues indicate that these life forms were abundant and diverse, and therefore played an important role in the ecology of the Visean ecosystem at this site. It is difficult at present to accurately determine the systematic affinities of most of the microfungal remains because they consist of isolated parts and propagule, or stages of the life cycle in which critical features required in microfungal systematics are lacking. Only a few of the specimens documented here can be referred to a particular group of microfungi or fungi-like microorganisms with some degree of confidence based on comparisons to extant and other fossil forms. For example, the specimen illustrated in Fig. 1k probably represents an empty chytrid zoosporangium. There is some morphological resemblance to *Lyonomyces pyriformis* T.N. Taylor, Hass et W. Remy described as a parasite of the green alga *Palaeonitella cranii* (Kidston et W.H. Lang) J. Pia from the Rhynie chert [25]. An organism described from Carboniferous lycophyte tissues by Renault [14] as *Oochytrium lepidodendroni* consists of narrow hyphae and spherical or spindle-shaped sporangia. The narrow hyphae depicted in Fig. 1i and the spore-like structures illustrated in Fig. 1m–o might belong to this organism, which we interpret as a peronosporomycete (oomycete). Other structures described by Renault [14] from lycophyte tissues closely resemble the fossils illustrated in Fig. 1c and d, and probably also represent peronosporomycetous oospores/oosporangia. Similar structures have been found in the Carboniferous seed-like structure *Nucellangium glabrum* (Darrah) H.N. Andrews from North America, and interpreted as oogonia/oosporangia of an *Albugo*-like microorganism [22].

The most conspicuous microfossils occurring in the *Lepidodendron* endophelloderm are the specimens illustrated in Fig. 1e and f. This fossil is abundant in both samples (more than 60 individuals), and thus makes it possible to provide a detailed description and assessment of the organism that produced these structures. The structures most closely resemble the oosporangia produced by extant peronosporomycetes. This hypothesis is based principally on the presence of several specimens displaying a laterally adpressed antheridial hypha with terminal antheridium that resembles antheridia seen in modern members of the Peronosporomycetes. While the oosporangia in most extant peronosporomycetes are thin-walled and non-ornamented, some forms are characterized by a relatively thick wall, which may be variously ornamented [1]. However, we are not aware of any extant peronosporomycete that produces antler-like surface ornamentation. Within the extant peronosporomycetes, the number of oospores contained in an oosporangium is variable, ranging from one to several. The fossils consistently contain a single oospore. Extant pernonosporomycetes producing oosporangia with a single oospore are found in the Peronosporaceae, Pythiogetonaceae, Verrucalvaceae, and in some members of the Leptomitales and Rhipidiales [2]. It is possible that the fossil oogonia/oosporangia were borne on the wide hyphal type (Fig. 1h) based on the corresponding diameters of these hyphae and the subtending oogonial hyphae. The possibility exists that the short, terminally swollen lateral branches produced by the wide hyphae (Fig. 1h [arrow]) represent the initial stage in oogonium formation. If this hypothesis is accurate, then the process of oogonium formation in this Carboniferous organism resembles that of the Early Devonian peronosporomycete *Hassiella monospora* T.N. Taylor, M. Krings and Kerp, which includes short, terminally swollen hyphal branches that also represent the initial stage in oogonium formation [26].

It is equally difficult to determine the nature of the associations between the individual microorganisms and the *Lepidodendron rhodumnense* plant based on the fossils. The microorganisms may represent space endophytes or true parasites that entered the host plant while it was alive and subsequently colonized the interior tissues. On the other hand, host reactions have not been observed, which may indicate that the fungi were saprotrophs that entered the tissues post mortem and participated in the decay process. This interpretation, however, is somewhat problematic since some of the infected cells are tracheids, which are non-living at maturity, and thus not capable of producing any structural alteration in response to an invading parasite. Adding support to the hypothesis that the microorganisms represent saprotrophs colonizing the tissues after death of the plant is perhaps their abundance and ubiquitous occurrence in the samples. In addition, some of the structures discovered inside the tissues have also been observed in other thin sections where they occur in the chert matrix, associated with an accumulation of small degrading

plant fragments. Moreover, the cauline system of *L. rhodumnense* is to date known only from narrow twigs, up to 2.0 cm in diameter, which probably were positioned high up in the plant, and thus in vivo not easily accessible for soil-borne microbial endophytes. Additional material will be required to more accurately define the systematic affinities of the specimens, and to more fully understand the diversity and nature of this complex community of microfungi and fungi-like microorganisms that lived inside the tissues of *L. rhodumnense*.

The initial work by Renault in describing some of these microorganisms has opened a new window into the microbial world that existed during the Late Paleozoic. Documenting these minute life forms and the organisms in which they occur makes it possible to infer the existence of various levels of biological interaction. Renault's work clearly indicates that he understood the significance of the biological interactions represented in the fossils he prepared and examined. It is our intent to completely analyze the slides from the 'collection Renault' and other collections in order to fully document the microbial diversity and associations and interactions with other organisms in the Visean ecosystems from central France. We believe that continuing the work that Renault initiated more than 100 years ago will help to underscore the incredible genius of this paleobotanist.

Acknowledgments

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Chapter VI: A filamentous cyanobacterium showing structured colonial growth from the Early Devonian Rhynie chert.

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A filamentous cyanobacterium showing structured colonial growth from the Early Devonian Rhynie chert

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Abstract

This paper describes a new aquatic filamentous-colonial fossil cyanobacterium from the Early Devonian Rhynie chert that grows on sediment and submerged plant parts. It is associated with the formation of microbial mats, and occurs in structured colonies, in which the individual filaments are aligned more or less parallel into flat, irregular stands, or united radially into hemispherical aggregates; it may also form elongate, fan-shaped tufts. Individual filaments are ~3 μm in diameter, and consist of uniseriate trichomes composed of barrel-shaped cells enveloped in a thin but distinct sheath. Heterocysts and akinetes have not been observed, which suggests that the cyanobacterium belongs to the cyanobacterial subsection III (Oscillatoriales). This is the first account for sessile, structured colonial growth in cyanobacteria from the Rhynie chert.
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Keywords: colonial growth; *Croftalania venusta* nov. gen. et sp; cyanobacteria; filament; microbial mat; Rhynie chert; subsection III (Oscillatoriales)

1. Introduction

Cyanobacteria are critical constituents of many marine, brackish and freshwater ecosystems, not only in producing oxygen, but also in serving as one of the primary producers of organic matter at the base of the food chain (e.g., Whitton and Potts, 2000a). They also may play an important role in the nitrogen cycle by converting inert atmospheric N_2 into a metabolically accessible form such as nitrate or ammonia (Newton and Orme-Johnson, 1980). Cyanobacteria are regarded as one of the most successful groups of prokaryotic organisms in Earth history based on a fossil record that is among the oldest for any group of organisms (Golubic and Seong-Joo, 1999). Geochemical evidence from the Precambrian indicates that cyanobacteria were responsible for the transition in the Earth's atmosphere from its primordial, anaerobic state to its current, aerobic condition (Giovannoni et al., 1988; Tomitani et al., 2006).

Despite the extensive fossil record of cyanobacteria, documentation of these organisms from non-marine paleoenvironments is rare. The earliest putative fossils believed to represent cyanobacteria from a strictly continental biota come from the Early Silurian Passage Creek in Virginia, U.S.A. (Tomescu et al., 2006). Although such reports may be important in tracing the earliest

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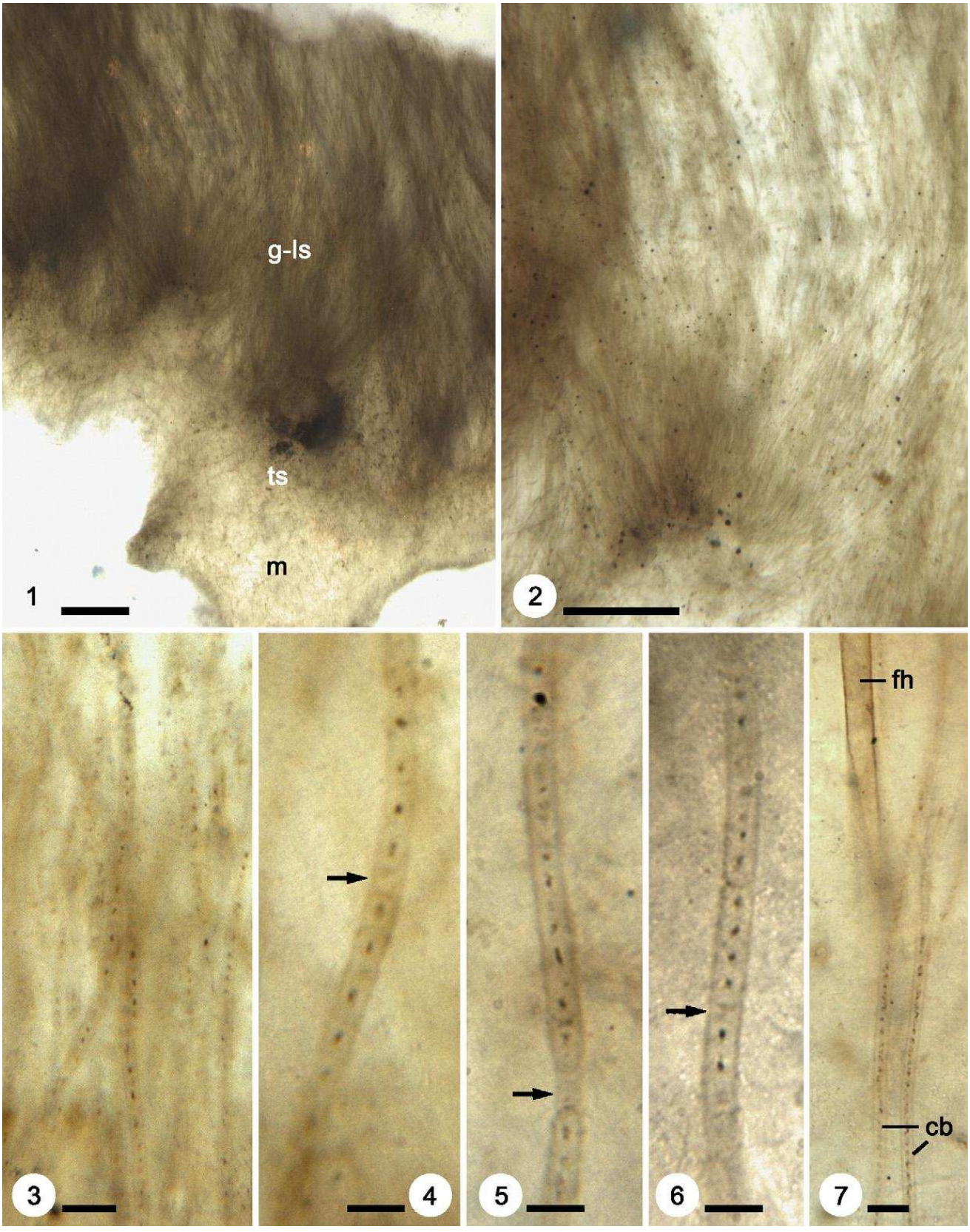
appearance of various cyanobacterial lineages in non-marine environments, and support the hypothesis that cyanobacteria were among the earliest colonizers of continents (cf. Knoll, 1985), they do not provide an accurate account of the diversity of cyanobacterial life in early non-marine ecosystems.



The Early Devonian Rhynie chert provides an exceptionally detailed account of the diversity of life on the continents approximately 400 million years ago, and to date represents one of only a few direct sources of information available about cyanobacterial diversity in early non-marine aquatic environments. Other evidence for diverse cyanobacterial life in a Paleozoic non-marine ecosystem comes from Late Devonian or Early Carboniferous thermal spring deposits in the Dummond Basin in Queensland, Australia (Walter et al., 1996, 1998).

The record of cyanobacteria from the Rhynie chert includes two coccoid forms, i.e. the *Gloeocapsomorpha*-like photobiont of the cyanolichen *Winfrenatia reticulata* T.N. Taylor, Hass et Kerp (Taylor et al., 1997) and *Rhyniococcus uniformis* D.S. Edwards et Lyon (Edwards and Lyon, 1983), and five filamentous forms, i.e. *Archaeothrix contexta* Kidston et W.H. Lang, *A. oscillatoriformis* Kidston et W.H. Lang, *Kidstoniella fritschi* Croft et George, *Langiella scourfieldi* Croft et George, and *Rhyniella vermiformis* Croft et George (Kidston and Lang, 1921; Croft and George, 1959). Recently there has also been a report of a filamentous *Archaeothrix*-like cyanobacterium that occurs as an endophyte in prostrate axes and partially degraded sporangia of the land plant *Aglaophyton major* (Kidston et W.H. Lang) D.S. Edwards (Taylor and Krings, 2005). With the exception of the photobiont of *W. reticulata* and the endophyte in *A. major*, the cyanobacteria from the Rhynie chert are motile benthic or planktonic organisms. Croft and George (1959) suggest, however, that *L. scourfieldi* may have produced a basal creeping system, with erect branches on the side opposite to the substrate. Strictly sessile forms that grow on the sediment surface, participate in the formation of microbial mats, or settle epiphytically on other organisms have not been documented from this paleoecosystem.

Here, we describe a new filamentous cyanobacterium from the Rhynie chert that is associated with the formation of microbial mats and develops hemispherical to tuft-like colonies on the sediment surface and submerged plant parts. The fossil provides the first evidence for various levels of structured colonial growth in cyanobacteria from the Rhynie chert paleoecosystem.

2. Geological setting, material, and methods

The Rhynie chert Lagerstätte is located in the northern part of the Rhynie outlier of Lower Old Red Sandstone in Aberdeenshire, Scotland, within a sequence of sedimentary and volcanic rocks. The cherts occur in the upper part of the Dryden Flags Formation, in the so-called Rhynie Block, a few hundred metres northwest of the village of Rhynie. The Lagerstätte consists of at least 10 fossiliferous beds containing lacustrine shales and cherts that are interpreted as a series of ephemeral fresh water pools within a hot springs environment. Preserved in the cherts are both aquatic facies and subaerial systems around the pools (i.e. soil/litter horizons with *in situ* plants); the latter were preserved as a result of temporary floodings of silica-rich water, or by groundwater percolating upwards. Based on dispersed spore assemblages and redefinition of the Pragian/Emsian boundary by the IUGS, the cherts are dated as Pragian–?earliest Emsian (Wellman, 2006; Wellman et al., 2006). Detailed information about the geological setting, sedimentology, and development of the Rhynie chert Lagerstätte can be found in Rice et al. (2002), and Trewin and Rice (2004).

The new cyanobacterium was identified in two different thin-sections prepared from a single chert block by cementing a thin wafer of the chert to a glass slide and then grinding the rock slice with silicon carbide powder until sufficiently thin for examination in transmitted light (cf. Hass and Rowe, 1999). The specimens were examined and photographed using oil immersion objectives directly on the rock surface without a cover slip. Slides are deposited in the collection of the Forschungsstelle für Paläobotanik, Geologisch-Paläontologisches Institut, Westfälische Wilhelms-Universität, Münster (Germany); accession numbers are included in the figure captions.

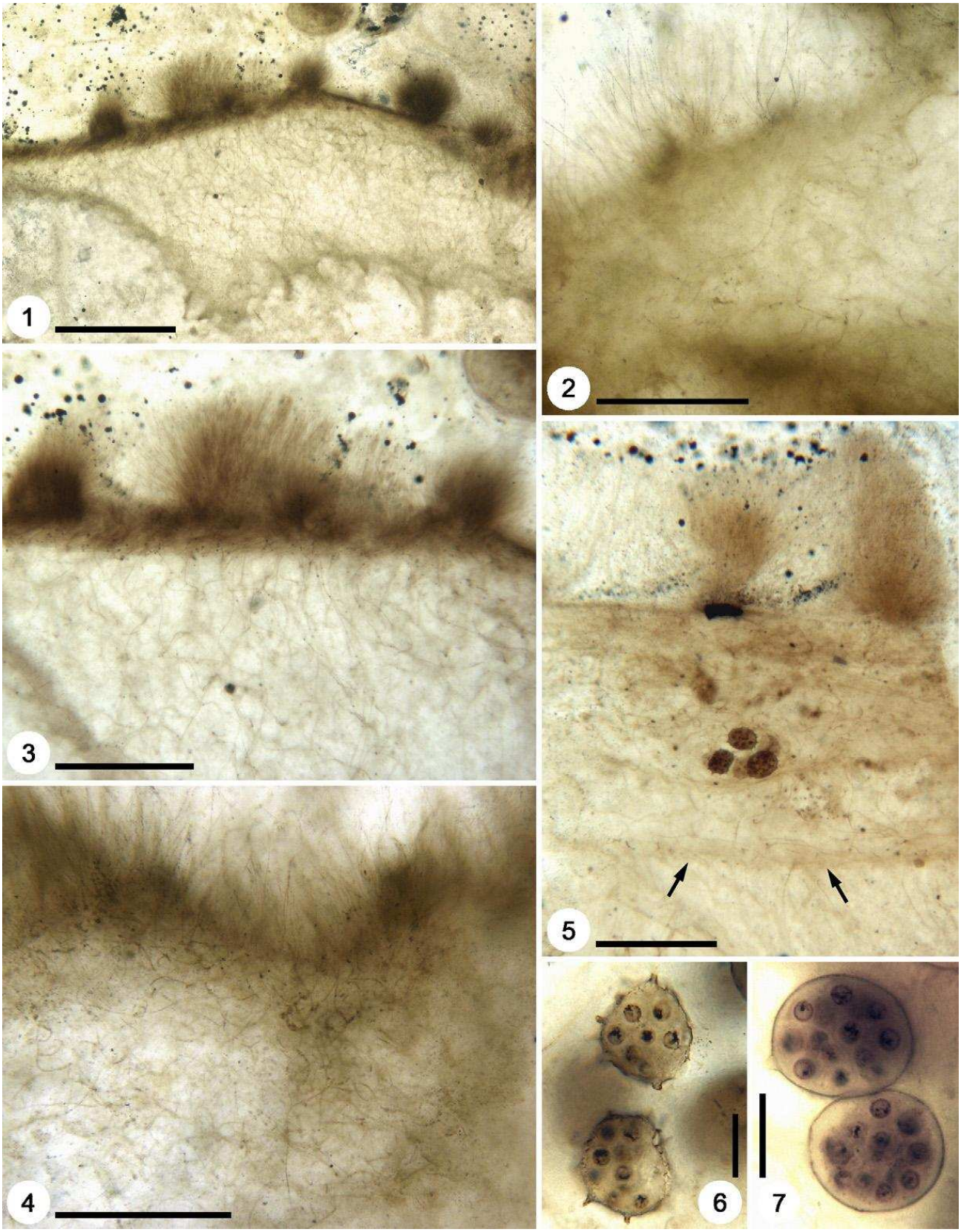
3. Systematic paleobotany

Cyanobacteria (Chloroxybacteria, Cyanoprokaryota)
Subsection III (Oscillatoriales)
Incertae sedis
Genus Croftalania M. Krings, Kerp, Hass, T.N. Taylor et Dotzler, nov. gen.

Plate I. *Croftalania venusta* nov. gen. et spec.; filament structure.

1. Grass-like stand of filaments (g-ls) growing on the top surface (ts) of one of the mats (m). Slide P2181; scale bar= 100 μm.
2. Detail of Plate I, 1, showing the arrangement of filaments in the stand; scale bar= 50 μm.
3. Detail of Plate I, 1, focal plane on some of the filaments. Scale bar = 20 μm.
4. Detail of Plate I, 2; segment of a single filament. Arrow indicates cell-free region in the filament. Scale bar = 5 μm.
5, 6. Filaments from other grass-like stands. Note the slight constrictions or folds of the sheath at the cross wall positions. Arrows indicate (narrower; e.g., in Plate I, 5) cell-free regions in the filaments. Slides P2180 (Plate I, 5) and P2181 (Plate I, 6); scale bars = 5 μm.
7. Two cyanobacterial filaments (cb) and a fungal hypha (fh) from the interior of a microbial mat. Slide P2180; scale bar = 10 μm.

Generic diagnosis: Aquatic, filamentous-colonial form growing on sediment and submerged (decaying) plant parts; trichomes uniseriate, straight to sinuous or slightly curved, and enveloped in a thin distinct sheath; trichome/filament-branching, heterocycsts, and akinetes absent; individual cells uniform in size and shape, barrel-shaped,



usually ~3.0 μm long and ~3.0 μm wide; sheath unconstricted to slightly constricted or folded at cross walls; filaments associated with formation of microbial (sometimes stratified) mats, or densely aligned vertically into irregular, "grass-like" stands, or radially into hemispherical colonies, or arranged into elongate, fan-shaped tufts.

Type species: *Croftalania venusta* M. Krings et al.

Species *Croftalania venusta* M. Krings, Kerp, Hass, T.N. Taylor et Dotzler, nov. spec.

Holotype: Specimen illustrated in Plate II, 1 from slide P 2180

Paratypes: Colonies illustrated in Plate IV, 1 (from slide P2180) and Plate V, 1 (from slide P2181)

Repository: Forschungsstelle für Paläobotanik, Geologisch-Paläontologisches Institut, Westfälische Wilhelms-Universität, Münster, Germany

Specific diagnosis: As for the genus.

Etymology: The generic name *Croftalania*, a combination of the surname 'Croft' and the given name 'Alan', is proposed in honour of William N. Croft and Eric Alan George for their contribution to the knowledge of cyanobacteria from the Rhynie chert; *venustus,-a,-um* (Lat.) = charming, lovely.

Locality: Rhynie, Aberdeenshire, Scotland, National Grid Reference NJ 494276

Age: Early Devonian (Pragian–?earliest Emsian according to Wellman et al., 2006 and Wellman, 2006)

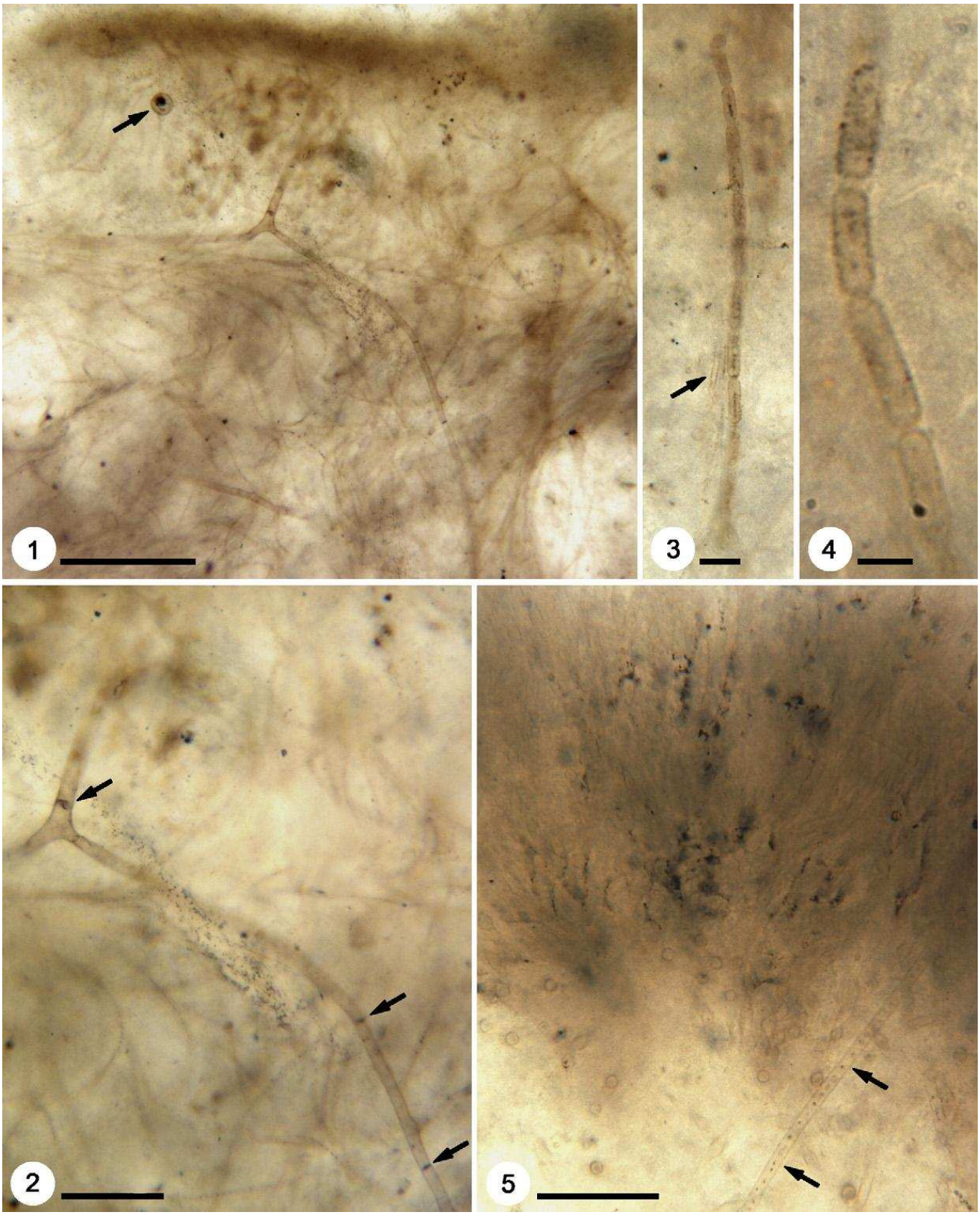
Description: Croftalania venusta is a sessile filamentous aquatic cyanobacterium that forms several distinct types of aggregates or colonies, some making up microbial mats. Individual trichomes (Plate I, 3–7) are uniseriate, cylindrical, more or less straight, slightly bent, or sinuous to curved, up to 1.5 mm long and consistently ~3.0 μm wide, and enveloped in a thin but distinct sheath. At low magnification, the filaments appear to gradually taper toward the apex (e.g., Plate II, 2; Plate V, 2), but this is probably an optical artefact; higher magnification reveals that distal filament portions are not preserved. Many of the filaments are characterized by a single line of dark globules in the interior (Plate I, 3–7; Plate III, 5 [arrows]). Since cyanobacteria, unlike eukaryotic microorganisms, produce peptidoglycan cell walls that are directly exposed to osmotic pressure, we interpret the globules as remains of both the collapsed cell walls and cell contents. As a result, what we see as fossils are only the sheaths since they are not part of the osmotic system. This concurs with observations reported from Proterozoic cyanobacteria, in which the actual cells rarely preserve and the remaining tubes are usually the empty sheaths (cf. Knoll and Golubic, 1992 and references therein). Branching is apparently absent in *C. venusta*; heterocycsts and akinetes have not been observed. Some of the specimens are represented by simple hollow tubes (sheaths) that lack any indication of the position of cross walls between individual cells (e.g., Plate V, 3,5,6); however, in others the former positions of cross walls are (partially) recognizable as external constrictions or folds of the sheath (e.g., Plate I, 3–7). These specimens indicate that the individual cells were relatively uniform in size and shape, and isodiametric or slightly longer than wide (barrel-shaped). Several filaments display short segments (up to 5.0 μm long), mostly slightly narrower (between 2.0 and 2.5 μm) in diameter than elsewhere in the filament (arrows in Plate I, 4–6), that are devoid of cells. It appears that these segments represent interruptions of the trichome within the sheath.

Croftalania venusta displays various levels of colonial growth, in which the aggregates or colonies are composed of large numbers of loosely or densely spaced filaments that are separate or connected to one another in their most proximal parts; rarely do the filaments occur singly or in isolated groups of a few. Transition from one type of colonial growth to another is gradual. The cyanobacterium may form, or represent a component of, microbial mats that cover the substrate (Plate I, 1,2; Plate II, 1–5). Mats are generally up to 2 mm thick and characterized by narrow but distinct upper (top) and lower (bottom) surfaces (up to 70 μm

Plate II. *Croftalania venusta* nov. gen. et spec.; microbial mats, colony formation on the top surface of the mats, and spiny spheres (Plate II, 6,7) from the interior of the mats.

1. Microbial mat formed by the cyanobacterium; note distinct top and bottom surface composed of densely spaced filaments. Holotype. Slide P2180; scale bar = 500 μm.
2. Margin of one of the microbial mats with "grass-like" stand of erect filaments on top surface. Slide P2180; scale bar = 200 μm.
3. Detail of Plate II, 1, focal plane on the top surface and interior. Scale bar = 200 μm.
4. Interior and top surface of microbial mat. Slide P2180; scale bar = 200 μm.
5. Stratified microbial mat; arrows indicate layer of more densely spaced filaments (=former top surface); note small cluster of spiny spheres in the interior of the mat. Slide P2180; scale bar = 200 μm.
6, 7. Spiny spheres from the interior of microbial mats containing small bodies. Slide P2180; scale bars = 25 μm.

thick) composed of more or less horizontally or obliquely arranged, densely spaced filaments within extracellular matter that appears greenish-beige (bottom surface) or brown (top surface) in thin sections. The interior consists of loose spacing of straight, bent or sinuous to curved filaments (Plate II, 1–4; Plate III, 1).



In most specimens, the bottom surface is less well-developed (Plate II, 1). Filament spacing is typically denser toward the top surface (e.g., Plate II, 1,3); in one specimen, however, the transition from loose to dense spacing is more gradual (Plate II, 4). Some of the mats are stratified and display two to several horizontal layers of densely spaced filaments (Plate II, 5 [arrows]) that probably represent former surfaces. The mats are not composed exclusively of *C. venusta* filaments, but also contain rare distinctly larger (i.e. >5.0 μm in diameter) filamentous microorganisms (Plate III, 3,4), which may either represent another type of cyanobacterium or a green alga. Also present are septate fungal hyphae (Plate III, 1,2), algal unicells or spores (Plate III, 1 [arrow]), and (clusters of) spiny spheres, each up to 35 μsm in diameter, that contain numerous small bodies (Plate II, 5–7). On the top surface of the stratified and non-stratified mats, straight and densely spaced cyanobacterial filaments, up to 1.0 mm long, are aligned vertically into irregular, "grass-like" stands (Plate I, 1,2; Plate II, 2,4,5; Plate III, 5) or united radially into more or less hemispherical or slightly elongated colonies (each up to 0.5 mm in diameter) (Plate II, 1.3.5; Plate IV,5). Similar hemispherical colonies also occur directly on the substrate (Plate V, 1–6). Submerged and decaying axes of the land plant *Nothia aphylla* Lyon ex El-Saadawy et Lacey also serve as substrate for *C. venusta*. Here, the cyanobacterium forms hemipherical (Plate IV, 3,4) to elongate, fan-shaped tufts (Plate IV, 1,2,6), up to 2.5 mm high. Some of the larger, elongate tufts show a faint stratification (Plate IV, 1,2). The fossils display a distinct difference in coloration between the central and peripheral portions of the hemipherical and tuft-like colonies (e.g., Plate II, 1,3; Plate IV, 2,5; Plate V, 1,2,4): While the peripheral regions appear yellowish, (greenish-)beige or light brown in thin sections, the central portions of the colonies are dark brown. This feature appears to be a result not only of differences in the spatial density of filaments and coloration of the individual filaments, but also of the chert matrix containing the filaments (e.g., Plate V, 6).

Remark: Because the two basic types of growth displayed by *Croftalania venusta* are fundamentally different, it might be argued that the cyanobacteria forming grass-like stands and hemispherical or tuft-like colonies and those associated with microbial mat formation represent two different species; one species colonizes substrate surfaces and decaying plant parts, but may also settle on microbial mats formed by the second species once these structures cease growth and the top surface consolidates. However, we have not found any morphological differences between the filaments involved in mat- and colony-formation that could be used to discriminate two species. Since structurally identical filaments dominate mats and colonies, we interpret both mat and colony formation as the result of the activities of a single (morpho-)species.

4. Discussion

Croftalania venusta contributes to our understanding of the structural versatility and ecological diversity of cyanobacteria in late Paleozoic non-marine aquatic ecosystems. In contrast to the previously described filamentous cyanobacteria from the Rhynie chert (Kidston and Lang, 1921; Croft and George, 1959; Edwards and Lyon, 1983), including the endophyte of *Aglaophyton major* (Taylor and Krings, 2005), *C. venusta* represents a sessile organism that grew attached to a substrate and displays various levels of structured colonial growth.

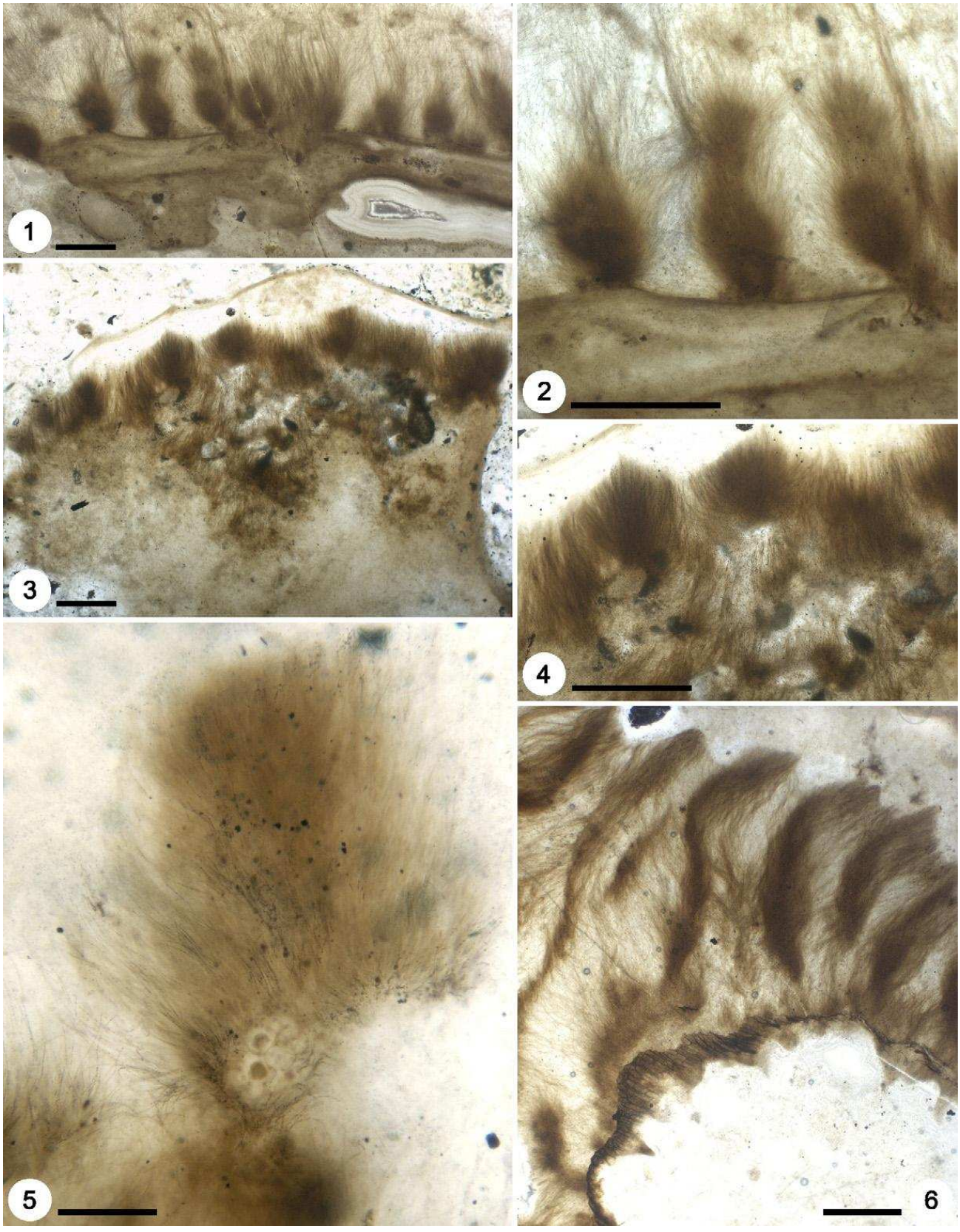
4.1. Affinities

Despite the exceptional preservation of *Croftalania venusta*, determining the systematic affinities and possible phylogenetic relationships with modern cyanobacteria remains difficult. This is because essential features required to establish the systematic position of a cyanobacterium (e.g., mode of cell division, process of reproduction, molecular and genetic data) cannot be determined from the fossils. Given that these features are critical in modern cyanobacterial systematics (Whitton and

Plate III. *Croftalania venusta* nov. gen. et spec.; components of the microbial mats (Plate III, 1–4), top surface (Plate III, 5).

1. Portion of one of the mats, showing the top surface composed of densely aligned filaments and interior composed of loosely arranged filaments and fungal hyphae. Arrow indicates algal unicell or spore. Slide P2181; scale bar = 100 μm.
2. Detail of Plate III, 1, focal plane on one of the septate fungal hypha. Arrows indicate simple septa. Scale bar = 50 μm.
3. Larger filamentous microorganism from the interior of one of the mats. Arrow indicates a fragment of a typical *C. venusta* filament that demonstrates the difference in size between the two types of filaments. Slide P2181; scale bar = 10 μm.
4. Close-up on one of the larger filamentous microorganisms. Slide P2180; scale bar = 5 μm.
5. Top surface of one of the mats, showing the transition into a grass-like stand. Arrows indicate one of the typical *C. venusta* filaments. Slide P2180; scale bar = 30 μm.

Potts, 2000b) the incompleteness of the fossil record places serious constraints on interpretation of fossil cyanobacteria. As a result, hypotheses relating to the affinities of *C. venusta* must be formulated solely on comparisons of the basic filament and colony structure to that seen in modern filamentous cyanobacteria.



Extant cyanobacteria are currently subdivided into five subsections (subsection I–V; cf. Rippka et al., 1979; Castenholz et al., 2001; Henson et al., 2004); subsections I (Chroococcales) and II (Pleurocapsales) consist of unicellular strains, while subsection III (Oscillatoriales) contains strains that are filamentous, non-heterocystous, lack akinetes, and reproduce by trichome fragmentation. Subsections IV (Nostocales) and V (Stigonematales) are composed exclusively of heterocystous forms that reproduce by hormogonia and may develop akinetes. Since *Croftalania venusta* is filamentous, and neither akinetes nor heterocysts have been detected, the fossil may be referred to subsection III (for details on subsection III see Castenholz, 1989).

The oldest microfossils interpreted as cyanobacteria belonging to subsection III come from the ~3.5 Ga-old Apex chert of northwestern Australia (Schopf, 1993, 2000), and thus it seem reasonable to conclude that this subsection had evolved into a structurally and ecologically diverse group of organisms by the Early Devonian. Two of the previously described filamentous cyanobacteria from the Rhynie chert, i.e. *Archaeothrix contexta* and *A. oscillatoriformis* (Kidston and Lang, 1921), also lack heterocysts or akinetes either, and hence may also be included in subsection III.

Among the extant genera recognized within subsection III, the filament portions of *Croftalania venusta* in which cell size and shape can be determined (e.g., Plate I, 3–7; Plate III, 5 [arrows]) are similar in basic structure to filaments of extant members of *Geitlerinema* (Anagnostidis et Komárek) Anagnostidis, *Heteroleibleinia* (Geitler) Hoffmann, *Leptolyngbya* Anagnostidis et Komárek, and *Symploca* Kützing ex Gomont (cf. Komárek and Anagnostidis, 2007). Some of these also display short interruptions of the trichome within the sheath (e.g., Komárek and Hauer, 2004) that are similar to the interruptions observed in *C. venusta* (arrows in Plate I, 4–6). Moreover, grass-like stands and/or structured colonies ('bushes') are known to be formed by modern members of *Heteroleibleinia*, *Homeothrix* (Thuret ex Bornet et Flahault) Kirchner, and *Schizothrix* Kützing ex Gomont. In addition, a change in growth like that in *C. venusta* has been reported from some *Symploca* species (Fritsch, 1959: p. 832) where the filaments at first form a dense prostrate weft, later giving rise to erect tufts, which are often visible to the naked eye. On the other hand, the overall appearance and filament arrangement in some of the hemispherical *C. venusta* colonies (e.g., Plate IV, 2) also resembles that seen in colonies of some extant members of the Rivulariaceae (subsection IV), especially species in the genus *Gloeotrichia* J. Agardh ex Bornet et Flahault. These cyanobacteria in subsection IV are generally heterocystous, and many forms produce distinct akinetes. The absence of both akinetes and heterocycsts in *C. venusta* argues against a closer relationship of the fossil to members of subsection IV. Both akinetes and heterocysts evolved long before the Early Devonian; the earliest fossil evidence for these structures is 1.6–1.4 Ga old (Golubic and Seong-Joo, 1999; Tomitani et al., 2006). We find it unreasonable therefore to propose that *C. venusta* represents a "primitive" member of subsection IV that had not yet evolved heterocysts and akinetes.

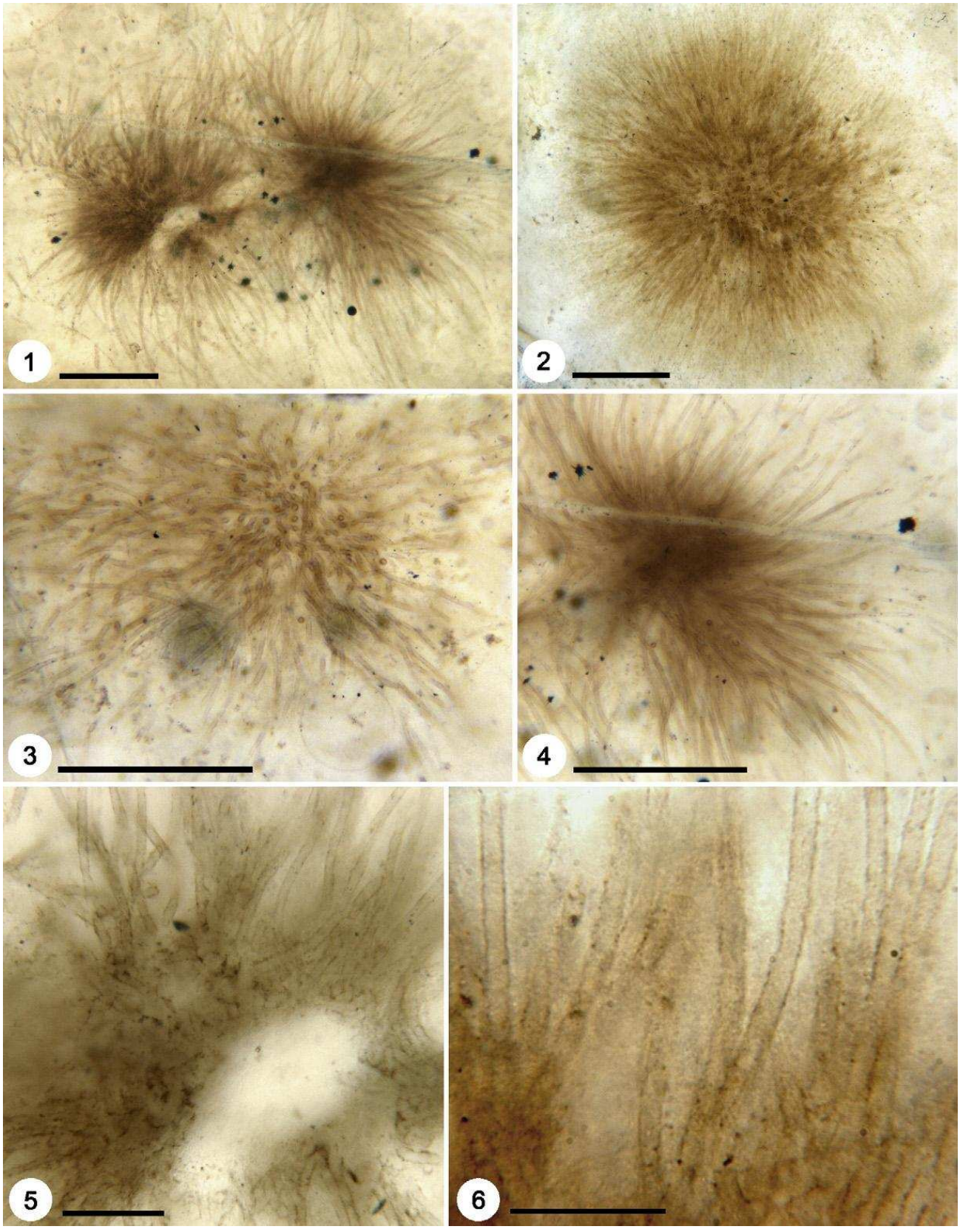
4.2. Paleoecology

Croftalania venusta was a versatile organism, capable of forming distinct types of aggregates and colonies. Among these, microbial mats (Plate I, 1; Plate II, 1–5; Plate III, 1) represent the most interesting biological aspect because, besides *C. venusta*, other microorganisms are integrated in these structures. The top and bottom surfaces of the mats are defined by densely spaced, horizonally or obliquely arranged cyanobacterial filaments. Differences in coloration of both the filaments and surrounding chert matrix between the surfaces and interior of the mats (Plate III, 5) suggest that the cyanobacterial filaments produced some sort of gelatinous or slimy substance (perhaps exopolysaccharides) to strengthen and/or consolidate the surfaces. In the interior, cyanobacterial filaments are relatively loosely arranged and interwoven with at least one other type of filamentous microorganism (Plate III, 3,4) and septate fungal hyphae (Plate III, 1,2). In addition, a characteristic type of spiny sphere containing numerous small bodies occurs in the interior of the mats (Plate II, 5–7) that may represent chytrid zoosporangia or some other type of fungal reproductive structure. Since these

Plate IV. *Croftalania venusta* nov. gen. et spec.; colonies on the land plant *Nothia aphylla* (Plate IV, 1–4,6), and on the upper surface of a microbial mat (Plate IV, 5).

1. Elongated, tuft-like colonies growing on an axis of *N. aphylla*. Paratype. Slide P2180; scale bar = 500 μm.
2. Detail of Plate IV, 1, focal plane on some of the colonies. Scale bar = 500 μm.
3. Densely spaced colonies on a *N. aphylla* axis. Slide P2180; scale bar = 500 μm.
4. Detail of Plate IV, 3. Scale bar = 500 μm.
5. Elongated colony from the upper surface of one of the microbial mats. Slide P2180; scale bar = 200 μm.
6. *Nothia aphylla* axis in cross section with several cyanobacterial colonies. Slide P2180; scale bar = 500 μm.

spheres have not been observed elsewhere in the Rhynie chert to date, it is possible that the source organism was restricted to the interior of microbial mats. Whether there was some form of mutual interaction between the organisms living in the mats, or the second filamentous microorganism and the fungi entered these structures to



gain access to a more stable environment and exploit the higher nutrient levels available in the interior, cannot be determined.

An interesting feature regarding microbial mat formation concerns the stratification present in some of the specimens (e.g., Plate II, 5 [arrows]). In these assemblages, the top surfaces of the microbial mats were consolidated and subsequently overgrown by new strata of the same morphology. This suggests that growth of the mats was dependent on various abiotic factors that continued to change (e.g., water level, nutrient load, pH). It is also interesting to note that not all of the mats are stratified. Some consist of a single layer with a distinctly different appearance of the cyanobacterium on the top surface. In these specimens, the top surface is overgrown either by vertically aligned filaments that form "grass-like" stands (Plate I, 1,2; Plate II, 2,4) or hemispherical (Plate II, 1,3,5) to slightly elongate (Plate IV, 5) colonies. We are uncertain as to why some of the *C. venusta*-dominated mats are stratified and display a history of repeated consolidations of flat surfaces, while others apparently change growth form, since both morphologies occur in the same thin section or are even located next to one another. However, it is unlikely that the colonial growth types on the top surface of the mats eventually also develop into new mat strata since filament spacing in these structures is much denser than in the interior of the mats. One possibility is that the other microorganisms within the mats played a role in determining the growth of *C. venusta*. It is possible to envision that these microorganisms somehow have induced mat formation by a cyanobacterium that, in the absence of the microorganisms, forms hemisperhical or tuft-like colonies.

Croftalania venusta forms variously shaped colonies or aggregates on the sediment surface and on submerged parts of the land plant *Nothia aphylla*. One interesting feature of these colonies is that the fossils show coloration differences between the peripheral and central portions, which include both the filaments and surrounding chert matrix. Although this feature probably is largely the result of the more dense arrangement of filaments in the center of the colonies, the fact that the filaments themselves and chert matrix surrounding the filaments are darker in the central areas (e.g., Plate V, 6) may suggest that *C. venusta* colonies were surrounded by a mucilage envelope (=colonial slime) that is similar to mucilage envelops seen in numerous extant filamentous-colonial cyanobacteria, including *Gloeotrichia* (Komárek and Anagnostidis, 2007).

5. Concluding remarks

The discovery of the filamentous cyanobacterium *Croftalania venusta* adds to our understanding of the diversity of organisms in the Rhynie chert, and thus contributes to a more sharply focused concept of the complexity of this ancient ecosystem. Nevertheless, many more specimens and forms will be necessary to fully assess the Rhynie chert cyanobacterial diversity. Cyanobacteria as components of freshwater ecosystems have received relatively little attention in the fossil record because of their small size and lack of morphologically distinct characters. In spite of this we believe that these organisms can be documented and interpolated into a broader understanding of fossil ecosystem dynamics than ever before. Moreover, as with any organism, the fossil record affords the only method of determining associations and interactions between different organisms within an evolutionary context. As a result, the *C. venusta*-dominated microbial mats provide an interesting perspective on the evolution of cyanobacterial associations that in turn can be assembled to help defining the complexity of interactions in ancient non-marine microbial communities.

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Plate V. *Croftalania venusta* nov. gen. et spec.; hemisperical colonies on the sediment surface.

1. Two small colonies on the sediment surface; note coloration difference between central and peripheral portion of the colony. Paratype. Slide P2181; scale bar = 100 μm.
2. Larger colony from the sediment surface displaying coloration difference. Slide P2180; scale bar = 100 μm.
3. Arrangement of filaments in a small hemisperical colony. Slide P2181; scale bar = 100 μm.
4. Detail of Plate V, 1, focal plane on one of the colonies. Scale bar = 100 μm.
5. Detail of Plate V, 1, focal plane on the central portion of one of the colonies. Scale bar = 30 μm.
6. Detail of Plate V, 5, focal plane on the proximal portions of some of the filaments; note the difference in coloration of both the filaments and surrounding chert matrix between the central [lower portion of image] and more peripheral [upper portion of image] parts. Scale bar = 20 μm.

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Chapter VII: *Combresomyces cornifer* gen. sp. nov., a peronosporomycete in *Lepidodendron* from the Carboniferous of central France.

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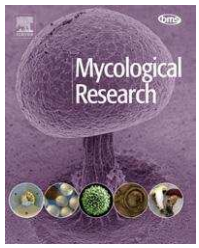

Combresomyces cornifer gen. sp. nov., an endophytic peronosporomycete in *Lepidodendron* from the Carboniferous of central France

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ABSTRACT

Structurally preserved periderm of the lycophyte *Lepidodendron rhodumnense* from the Visean (Mississippian) of central France contains a peronosporomycete (*Combresomyces cornifer* gen. sp. nov.) that occurs in the form of pyriform to subglobose terminal oogonia. On the surface is a conspicuous ornamentation, which may have formed through condensation of a mucilaginous extra-oogonial wall secretion. Some oogonia contain thin-walled spherules, which may represent (walled) oospheres or spores of an endoparasitic fungus (?chytrid), whereas single, large spheres in the interior are interpreted as oospores. Antheridia adpressed to several of the specimens are clavate and paragynous. This discovery sheds light on the morphology and biology of peronosporomycetes in a terrestrial ecosystem some 330 My ago. Although the organism occurs exclusively in the periderm of *L. rhodumnense*, it is not known whether it represents a symptomless endophyte, pathogen, or saprotroph.

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Introduction

The *Peronosporomycetes* (*sensu* Dick 2001b; *Oomycota* in older treatments) are aquatic and terrestrial microorganisms that thrive as saprotrophs and facultative or obligate parasites of plants, animals, and other fungi. The group includes 900–1500 extant species, but there may be a large, yet unexplored diversity in marine ecosystems (Dick 2001a). Although these

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organisms were initially believed to be related to the algae, and later treated as a separate group of fungi or pseudofungi; today they are included within the kingdom *Straminipila*.

Peronosporomycetes were likely among the first eukaryotes on Earth, and there are several descriptions of fossil coenocytic hyphae, oogonia-like structures, and/or various types of spores, as well as trace fossils in animal remains dating back to the Precambrian that have been interpreted as peronosporomycetes (surveyed in Tiffney & Barghoorn 1974; Pirozynski 1976). Although many of these fossils are today regarded as inconclusive at best (Johnson *et al.* 2002: http://dl.uncw.edu/digilib/biology/fungi/taxonomy%20and%20systematics/padgett%20book/), some appear to be authentic (Blackwell & Powell 2000). In spite of this, persuasive examples of fossil peronosporomycetes remain sparse because today these organisms are defined by molecular data, and ultrastructural, physiological, and life history characters that are difficult or even impossible to resolve with fossils (Dick 2001a). The only morphological features that might be used to distinguish fossil peronosporomycetes from other microbial and fungal remains are the characteristic oogonium/antheridium complexes and the encapsulated, thick-walled oospores (Dick 1995).

One rare example of a fossil peronosporomycete displaying both vegetative and reproductive features is *Hassiella monospora* from the Early Devonian Rhynie chert (Taylor *et al.* 2006). This organism is characterized by an ornamented oogonium attached to coenocytic hyphae, and when mature, the oogonium contains a single large oospore. The features seen in *H. monospora* suggest affinities with the extant genus *Pythium* (*Pythiaceae*). Success in documenting the details of *H. monospora* and other organisms from the Rhynie chert (e.g. Kerp & Hass 2004; Taylor *et al.* 2004; Taylor & Krings 2005) is directly attributed to the extraordinary preservation of the fossils in a silicious chert matrix.

Less well studied than the Rhynie chert, but also containing extraordinarily well-preserved land plants and microorganisms, are the Visean (Mississippian; ~330 Ma) cherts from the Massif Central in France. Although the terrestrial plants preserved in these deposits are well-documented (e.g. Renault 1879, 1896; Galtier 1970, 1971), the microorganisms remain understudied. Several bacteria, fungi, and microalgae, including those demonstrating various associations with land plants, were described more than 100 y ago by the French paleobotanist Bernard Renault (1896, 1900). Unfortunately, neither a comprehensive study of the Visean microbial material, nor Renault's own observations, were pursued after Renault's death. Only three papers addressing various levels of biological interaction (i.e. Taylor *et al.* 1994; Grewing *et al.* 2003; Krings *et al.* 2005) and a brief survey on the microfungi and fungi-like microorganisms inhabiting the wood and periderm of *Lepidodendron rhodumnense* (Krings *et al.* 2007) have been published since.

Here we present a conspicuous peronosporomycetous sexual reproductive structure (oogonium) from the Visean cherts, which occurred in periderm of the lycophyte *Lepidodendron rhodumnense*. Extending from the surface of the reproductive structure are prominent, antler-like extensions. On several specimens are laterally adpressed antheridia. This discovery provides important details about the morphology and reproductive biology of peronosporomycetes in a terrestrial ecosystem some 330 My years ago.

Material and methods

The cherts containing the infected *Lepidodendron rhodumnense* periderm come from the upper Visean [Mississippian (=Lower Carboniferous)] of Combres (sometimes also spelled Combre), situated approximately 12 km east of Roanne, Massif Central, central France. They occur as loose blocks within rhyolitic tuffs, and were collected in cultivated fields or in stream sections. The geological setting and paleoenvironment of the late Visean in the Roanne area have been interpreted as analogous to that in the Autun basin at the locality of Esnost, about 10 km north of Autun, Massif Central, central France (Galtier 1971). Information on the geological settings of the Roanne and Esnost localities can be found in Scott *et al.* (1984); for details on the preservation of fossils and a paleoecological reconstruction of the Visean wetland ecosystem at Esnost see Rex (1986).

The peronosporomycetous reproductive structures were identified in thin sections (radial and tangential) of five different *Lepidodendron rhodumnense* periderm specimens that were prepared by cementing a wafer of chert to a glass slide, and then grinding the wafer to a thickness sufficiently thin to be examined in transmitted light (for details about the methodology, see Hass & Rowe 1999). The thin sections were prepared by Bernard Renault and co-workers during the late 19th and early 20th centuries, and are now housed in the Muséum National d'Histoire Naturelle (Laboratoire de Paléontologie) in Paris (France) under accession numbers B49/1104, B49/1105, B49/1106, B49/1118, and B50/1137.

Results

The reproductive structures occur in structurally preserved (silicified) periderm of *Lepidodendron rhodumnense* (Fig 1A), a lycophyte originally described by Renault (1879). Extending from the surface are prominent antler-like extensions that make the specimens conspicuous and relatively easy to identify within the plant tissue. The reproductive structures are present in both the normal peridermal cells (i.e. cells of the phelloderm) [Fig 1A (centre)] and cells of the radially oriented, multi-storied ray-like secretory canals [Fig 1A (right)]; more than 100 specimens have been discovered from thin sections of five different periderm samples. They have not been detected in any other plant tissue preserved in the chert, nor have they been found in the chert matrix.

The structures are pyriform to subglobose in shape and 25–37 μm in diam (including surface extensions). They occur at the tip of a relatively short (up to 40 μm long) segment of the parental hypha (Fig 1K); a simple septum is present between the reproductive structure and subtending hypha (Figs 1C–F, I–L, 2C). The parental hypha is 8–14 μm wide, lacks surface ornamentation, and usually is slightly wider immediately below the septum (Fig 1E, K). The wall of the reproductive structure is *ca* 1 μm thick and more opaque than the wall of the subtending hypha. Although the inner surface of the wall is smooth, prominent antler-like extensions occur on

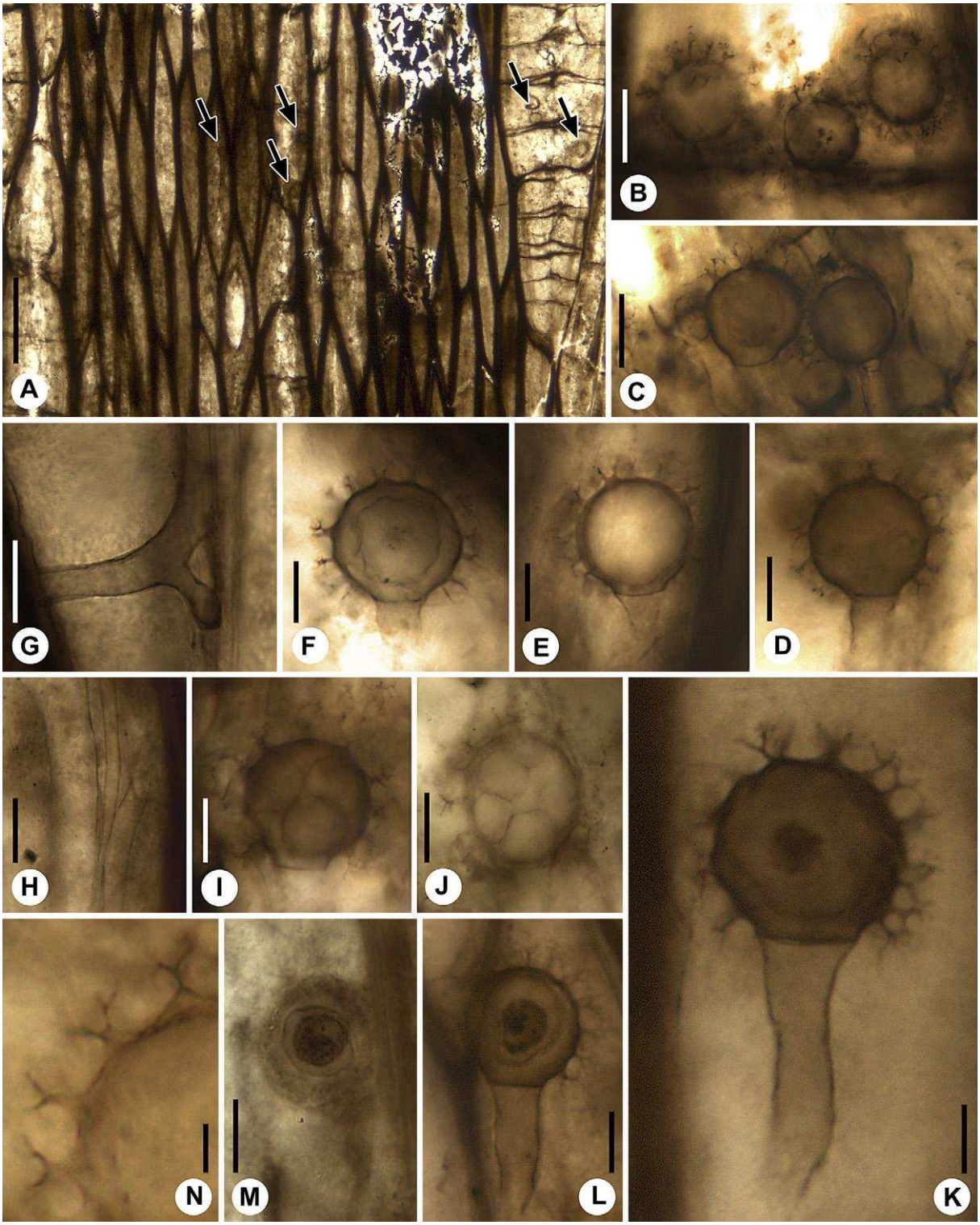

Fig 1 – *Combresomyces cornifer* gen. sp. nov. from the upper Visean of central France. (A) Periderm of *Lepidodendron rhodumnense*, tangential section, showing normal peridermal cells [phelloderm (centre of image)] and radially oriented, multi-storied ray-like secretory canals [left and right margins of image]; arrows indicate position of *C. cornifer* oogonia; slide B50/1137. (B–C) Oogonia clustered inside peridermal cells; slide B50/1137 (B) and B49/1106 (C). (D) Empty oogonium; slide B49/1106. (E–F) Oogonia containing single oospores; note accumulation of opaque material in the centre of the oospore in Fig 1F; slide B50/1137. (G) Vesicle-like swelling of a wide hypha associated with *C. cornifer*; slide B50/1137. (H) Large and medium-sized hyphae in a peridermal cell; slide B50/1137. (I–J) Sections through oogonia containing small spheres; Slide B49/1106. (K) Oogonium containing a single oospore; holotype; Slide B50/1137. (L) Oogonium containing a single oospore; note well-defined region of opaque material in the centre of the oospore; slide B50/1137. (M) Mature oospore surrounded by the degrading remains of the oogonium and oogonial stalk; slide B50/1137. (N) Close-up on some of the antler-like surface extensions borne on hollow, column-like or broadly triangular papillations of the oogonial wall proper; slide B49/1106. Bars (A) = 200 μm; (B, C, J) = 25 μm; (D–I, L) = 15 μm; (K, M) = 10 μm; (N) = 5 μm.

the exterior surface. They are regularly distributed over the entire surface except the neck region, and are positioned on hollow, column-like or broadly triangular papillations of the oogonial wall (Fig 1N). The extensions apparently do not arise directly from the oogonial wall proper, but rather consist of substance deposited onto the wall. Each extension dichotomizes once or twice, with the entire structure (including papillation) extending from the surface up to 7 μm; the distal ends are <1 μm in diam. The distal perimeter of the ornamentation boundary relative to the oogonial wall decreases in the vicinity of the oogonial septum.

Some of the reproductive structures appear to be empty, whereas others contain a single, slightly or considerably smaller sphere, up to 30 μm in diam (Figs 1C, E, F, K, L, 2C). In most, but not all, of these latter specimens, the interior sphere has a wrinkled wall and contains a well-defined opaque central area (4–7 μm in diam; Fig 1F, K–M). Other specimens contain several (usually 3–7) thin-walled spheres, each up to 13 μm in diam (Fig 1I, J). One specimen appears to represent a degraded reproductive structure in which the overall morphology is still recognizable, but well-defined walls are no longer present except for the interior sphere, which has remained intact (Fig 1M). Isolated spheres containing central accumulations of opaque material have not been observed in any of the tissue samples. Another interesting feature occurs in the form of narrow to medium-sized hyphae, 4–6 μm wide, with enlarged, club-shaped tips, which are adpressed laterally to some of the ornamented reproductive structures (Fig 2A–C). Septa separating the club-shaped tips from the subtending part of the hypha are not visible.

Vegetative hyphae or mycelia in organic connection to the reproductive structures have not been found, but co-occurring in the same cells are large, aseptate or sporadically septate hyphae, between 4.5 and 12 μm wide (Fig 1G, H). These produce medium-sized (*ca* 2.5–7 μm wide), typically aseptate hyphae. Most of the hyphae extend parallel to the long axis of the elongate cells; rarely do they extend perpendicular to the orientation of the cell. The large hyphae sometimes produce short lateral branches that are terminally swollen (Fig 1G). Hyphae appear to extend from one cell to another through the pits in the cell walls; host responses to fungal penetration have not been observed in any of the tissue samples. Similar hyphae also occur in xylem elements of *L. rhodumnense*, but are less common and have not been found in association with antler-bearing reproductive structures.

Taxonomy

Combresomyces cornifer Dotzler, M. Krings, Agerer, Galtier, and T. N. Taylor, **gen. sp. nov.**

MycoBank nos: MB 511499 (for the generic name *Combresomyces*) and MB 511500 (for the binominal *Combresomyces cornifer*)

Etym.: The generic name *Combresomyces* indicates that the fossil comes from the vicinity of the village of Combres (or Combre) in the Massif Central, central France. The specific epithet *cornifer* (Latin: *corniferus* = bearing antlers) refers to the antler-like surface extensions arising from the oogonium.

Peronosporomycetes M. W. Dick 2001, *incertae sedis*

Diagnosis: Pyriform to subglobose terminal oogonium, <40 μm in diam, thin-walled, subtended by wide hypha (oogonial stalk); simple septum present between oogonium and oogonial stalk; stalk up to 15 μm wide, slightly wider immediately below oogonium; oogonium with prominent antler-like surface extensions positioned on hollow, column-like or broadly triangular papillations of the oogonial wall proper; extensions once or twice dichotomized, up to 7 μm high (including papillations), densely spaced and regularly distributed on entire surface except in neck region; oogonium empty, sometimes containing several (usually 3–7) small spherules (?oospheres) up to 13 μm in diam or a single aplerotic or nearly plerotic oospore <30 μm in diam; antheridium clavate, paragynous, antheridial hypha 4–6 μm wide; endophytic in *Lepidodendron*, intracellular in periderm.

Typus: Slide no. B50/1137 (P-Laboratoire de Paléontologie – holotypus [illustrated in Fig 1K]).

Age: Late Visean (Mississippian; approximately 330 My).

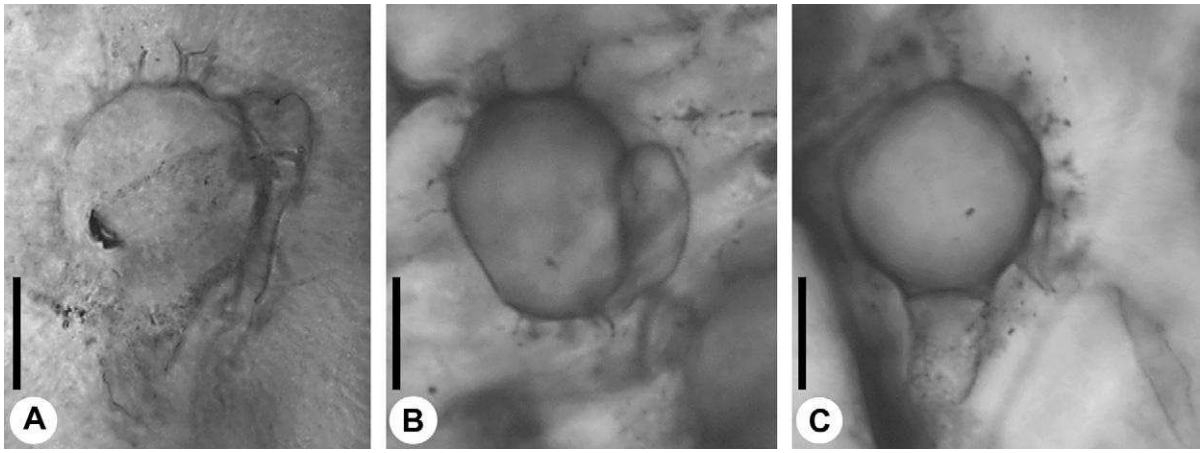

Fig 2 – *Combresomyces cornifer* gen. sp. nov. from the upper Visean of central France. Oogonia with laterally adpressed (paragynous) antheridia. (A) Slide B50/1137. (B) Slide B49/1106. (C) Slide B50/1137. Bars = 15 μm.

Discussion

The reproductive structure *Combresomyces cornifer* exemplifies the exquisite preservation of minute life forms in the Visean cherts from central France. Although the fossils recorded to date do not provide a complete picture, comparisons with extant microorganisms provide the opportunity to offer several hypotheses as to the biology and ecology of this highly unusual organism.

Morphology, reproductive biology, and affinities

We interpret these reproductive structures as oogonia produced by a member in the *Peronosporomycetes* based principally on the presence of specimens displaying laterally adpressed hyphae with enlarged, club-shaped tips (Fig 2A–C), which are structurally identical to paragynous antheridia seen in modern members of the *Peronosporomycetes* (see Dick 1969). We are uncertain as to whether the fossil antheridia were androgynous, monoclinous, or diclinous because the origin of the antheridial hyphae remains unknown. Antheridia and oogonia were perhaps produced on the large and medium-sized hyphae that occur in many of the cells (Fig 1G–H) based on the corresponding diameters of these hyphae and the subtending oogonial stalks and antheridial hyphae. It may be that the short, terminally swollen lateral branches produced by these hyphae (Fig 1G) represent the initial stage in oogonial development. If this is correct, then the process of oogonium formation in *Combresomyces cornifer* resembles that of the Devonian peronosporomycete *Hassiella monospora* from the Rhynie chert, which includes short, terminally swollen hyphal branches that also represent the initial stage in oogonial development (Taylor *et al.* 2006).

Some specimens of *Combresomyces cornifer* appear to be empty (Fig 1D), and perhaps represent undeveloped or immature oogonia, whereas others contain several (usually 3–7) small spherules bounded by a delicate but well-defined wall (Fig 1I, J). It is tempting to interpret these spherules as unfertilized oospheres based on size, shape, and the absence of internal differentiation. However, the oospheres of virtually all extant peronosporomycetes are naked structures, bounded only by a membrane (Dick 2001b), and thus it is unlikely that oospheres would become preserved in a recognizable form. There are only two extant genera (i.e. *Apodachlyella* and *Eurychasmopsis*) for which the possibility of walled oosphere production has been suggested (Dick 1986, 2001b). As a result, it is improbable, but nevertheless possible, that the small spheres in the *C. cornifer* oogonia represent (walled) oospheres. Another interpretation is that they represent spores of an endoparasite, perhaps a chytrid. Still other *C. cornifer* specimens contain single spheres (Fig 1C, E, F, L), which either fill the oogonium (plerotic) or are distinctly smaller than the exterior structure (aplerotic), and probably represent oospores. Adding support to this interpretation is the specimen illustrated in Fig 1M, which indicates that the interior sphere remains intact during degradation of the surrounding structure. We suggest that, in this fossil, the oogonia typically contain single oospores, which initially are nearly plerotic but become aplerotic with age. Many of the interior spheres are characterized by a globular accumulation of opaque material in the centre (Fig 1F, K–M). These accumulations may represent cell contents of the oospores that coalesced during fossilization. Conversely, in oogonia of extant *Rhipidiaceae*, *Pythiaceae*, *Albuginaceae*, and *Peronosporaceae*, the protoplasm is typically denser in the centre during one stage in oosporogenesis, and forms a so-called coenocentrum that is surrounded by less opaque, vacuolate cytoplasm (Dick 1969). As this developmental stage is short-lived, it is unlikely that multiple specimens would have become fossilized at exactly that stage of development. Alternatively, the opaque central regions may represent the ooplast, a large, plasmamembrane-bound, phosphate-rich structure that is present in the oospores of virtually all peronosporomycetes (Dick 2001a). The irregular, wrinkled nature of many of the oospore walls may represent a preservational artefact caused by the osmotic loss of water during fossilization.

Perhaps the most interesting feature of *Combresomyces cornifer* is the prominent ornamentation. Morphologically similar ornaments occur on the zoosporangia of certain extant chytrids (e.g. *Rhizophydium keratinophilum*) (see Karling 1946); however, the lack of exit papillae and the presence of antheridia adpressed to several of the specimens argue against *C. cornifer* being a member of the *Chytridiomycota*. There are also several acritarchs (e.g. species in *Multiplicisphaeridium*) (e.g. Tappan 1980) that possess surface extensions resembling those in *C. cornifer*. However, they differ from *C. cornifer* in lacking a subtending hypha and antheridia. Moreover, the endophytic occurrence argues against inclusion of this organism in the acritarchs. Although the oogonia of most extant peronosporomycetes lack ornamentation, some forms (e.g. species in the genus *Pythium*) (see Middleton 1943; Robertson 1979; Paul 1999, 2006; Paul *et al.* 1999) may be variously ornamented, ranging from small verrucae and papillae to prominent spines (Dick 1969, 2001b). Although the surface projections extending from the oogonia of a few members in the *Pythiaceae* (e.g. *Pythium polypapillatum* and *P. uncinulatum*) and *Saprolegniaceae* (e.g. *Aphanomyces stellatus* and *Newbya pascuicola*) may sometimes fork (van der Plaats-Niterink 1981; Khulbe 2001), we are not aware of any extant species that produces surface ornaments identical to *C. cornifer*.

The surface extensions of *C. cornifer* appear to consist of substance deposited onto the oogonial wall (Fig 1N). This may suggest that the ornament was not formed entirely by the oogonial wall proper, but rather that the distal, antler-like portions result from condensation of a mucilaginous extra-oogonial wall exudate at some stage during development. In several extant peronosporomycetes (e.g. *Aplanopsis terrestris*, *Newbya pascuicola*, and *Pythiopsis cymosa*), development of the oogonial initial is accompanied by a secretion of mucilage, which initially forms a spherical envelope with sharply defined outer surface around the developing oogonium, but subsequently becomes irregular, and finally collapses entirely or in part on the oogonium wall proper (Dick 1969; Johnson *et al.* 2002: http://dl.uncw.edu/digilib/biology/fungi/taxonomy%20and%20systematics/padgett%20book/). As a result, some of the ornaments seen on these oogonia represent remnants of the mucilaginous envelopes, rather than projections of the oogonial wall proper. Adding some support to the hypothesis that the *C. cornifer*

extensions represent condensed extra oogonial-wall secretions is the fact that the distal perimeter of the *C. cornifer* ornamentation boundary relative to the oogonial wall decreases in the vicinity of the oogonial septum, precisely as figured for *Aplanopsis* by Dick (1969).

Within extant peronosporomycetes, the number of oospores produced in an oogonium is variable, ranging from one to several. The fossil oogonia reported here consistently contain a single oospore (e.g. Fig 1F, K, L). Extant species producing single oospores are found in the *Peronosporomycetidae* and *Rhipidiomycetidae*, but single oospores also occur sporadically in *Saprolegniomycetidae* (Dick 2001b). The latter group is generally characterized by thick-walled oogonia (Dick 2001b), whereas the walls of the fossil oogonia are relatively thin (*ca* 1 μm). Thin-walled oogonia represent a typical feature of the *Peronosporomycetidae*, especially the order *Pythiales*, and some members of the *Peronosporales* (Dick 2001b). The consistent occurrence of single oospores and thin oogonial walls could be used to suggest that the affinities of *C. cornifer* are with the *Pythiales*. Conversely, mucilage secretion by oogonial initials has, to date, only been recorded for members in the *Saprolegniomycetidae*. Despite these similarities it is impossible to determine the exact systematic position of *C. cornifer* because essential features, including molecular data (e.g. ITS sequences), and physiological and certain life history characters (e.g. asexual reproduction and zoospore production), cannot be traced in the fossils.

Ecology

It is equally difficult to determine the nature of the association between *Combresomyces cornifer* and the *Lepidodendron rhodumnense* plant. The restricted occurrence in periderm might suggest that this organism represents a host-specific parasite that specifically colonized this interior tissue. Conversely, host reactions such as wall thickening or intracellular plugging of the invaded cells (e.g. Le Floch *et al.* 2005), have not been observed in any of the specimens, and this may indicate that *C. cornifer* was a saprotroph. However, many extant endophytic microorganisms are symptomless, and are known to colonize their hosts (for extended periods of time) without producing any damage or triggering any host response (e.g. Kulik 1984; Carroll 1988; Wilson 1993, 1995; D'Amico *et al.* 2008).

Adding support for the hypothesis that *C. cornifer* represents a saprotroph is its abundance and ubiquitous occurrence in the samples. As a diverse assemblage of microfungi (e.g. chytrids) and other fungi-like microorganisms co-occurs with *C. cornifer* in the periderm of *Lepidodendron rhodumnense* (Krings *et al.* 2007), it seems unlikely that this association was established when the plants were living. In addition, the cauline system of *L. rhodumnense* is, to date, known only from axis segments up to 5 cm in diam (Renault 1879; Galtier 1970). If *L. rhodumnense* was arborescent and similar in architecture to other members in *Lepidodendron*, these axes represent twigs positioned high up in the plant and are thus unlikely to have been colonized *in vivo* by so many different microbial endophytes. Arguing against a saprotrophic nature of *C. cornifer* is perhaps its apparent host specificity. However, extant lignicolous fungi that enter their host prior to, or during, the initial stages of the decay process are often highly specific, due probably to secondary compounds in the bark and wood and specific defense mechanisms that follow fungal infection (Pearce 1996).

Regardless of whether *C. cornifer* was a symptomless endophyte, parasite, or saprotroph, the question remains as to how propagation of the oospores was achieved. In many extant endophytic peronosporomycetes, the oospores are liberated into the soil upon decay of the host tissue. The absence of isolated *C. cornifer* oospores in the periderm of *L. rhodumnense*, along with the fact that a segment of the subtending hypha (oogonial stalk) is still attached to virtually all *C. cornifer* oogonia, suggests that these structures represent functional units, which remained intact upon maturity of the oospore and decay of the parental mycelium, and eventually became liberated and dispersed upon degradation of the host tissue. In a reconstruction of the Visean ecosystems of central France, Rex (1986) depicts pools and lakes surrounded by open forest vegetation scattered across the landscape. Thus, both water and wind might have served as vectors in the dispersal of *C. cornifer*. Oospores of certain extant peronosporomycetes are known to become airborne and distributed by wind (e.g. Bock *et al.* 1997; Rubin *et al.* 2001), and in a few forms not only oospores but also entire oogonia containing oospores appear to function as propagules (e.g., Vesely & Hejdánek 1981: http://www.freepatentsonline.com/4259317.html; Voglmayr *et al.* 1999). In *C. cornifer*, the surface ornamentation may have functioned as a floating or flying device in an analogous manner to the oogonial outgrowths produced by the extant aeroaquatic peronosporomycete *Medusoides argyrocodium* (*Pythiogetonaceae*) (Voglmayr *et al.* 1999). However, the oogonia of *Medusoides* are not produced within plant tissue, but rather on the surface of moist substrate exposed to the air. Alternatively, the surface ornament of *C. cornifer* may have facilitated mucilaginous attachment of the propagules to debris as a dispersal mechanism in a terrestrial environment, in a similar manner as described for the extant *Aplanopsis* and *Newbya* (Dick 1969, 2001a), or they may have functioned in the initial attachment of the dispersal units to the surface of a host. Conversely, degradation of the reproductive structure within the host cell has been observed in one instance (Fig 1M), and this seems to argue against the hypothesis that these structures represent dispersal units. However, it is likely that intracellular degradation does not represent a regular occurrence, but rather is a rare incident caused by some extraordinary circumstances (e.g. parasite attack). This conclusion would appear to be supported by the fact that, among the more than 100 specimens discovered to date, only a single degraded oogonium was observed.

Microorganisms are only now beginning to be understood within the context of the role they played in ancient terrestrial ecosystems. In modern ecosystems there are multiple levels of interaction, including a wide array of physical and biological associations, which help to define the complexities that exist in the modern world. Studies that have been completed to date on fossil microorganisms document that many of the biological interactions in ancient ecosystems are identical to those occurring in ecosystems today. As the fossil record continues to be examined in detail it is not surprising that other microorganisms will be described and illustrated that are previously unknown. Some of these, like *C. cornifer*, possess

unique combinations of characters that not only make placing them in a systematic context difficult, but also challenge the functional nature of certain features. Although *C. cornifer* shows characteristics that are consistent with the *Peronosporomycetes*, a group that, to date, is not well known from the fossil record, the unique ornamentation of this organism remains an enigma. As additional microorganisms from the Visean cherts of central France are discovered, and where possible discussed in the context of extant organisms, we believe that these fossils will greatly contribute to our understanding of the timing and significance of numerous biological interactions in the late Paleozoic microbial world.

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Chapter VIII: Endophytic cyanobacteria in a 400-million-yr-old land plant: a scenario for the origin of a symbiosis?

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Endophytic cyanobacteria in a 400-million-yr-old land plant: A scenario for the origin of a symbiosis?

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ABSTRACT

Direct evidence for the origin and evolution of land plant/cyanobacterial symbioses is virtually absent from the fossil record. Here we report on rare occurrences of prostrate mycorrhizal axes of the Early Devonian land plant *Aglaophyton major* that host a filamentous cyanobacterium, which enters the plant through the stomata and colonizes the substomatal chambers and intercellular spaces in the outer cortex. In dead ends of the intercellular system, the filaments form loops and continue growth in reverse direction. Some filaments penetrate parenchyma cells close to and within the mycorrhizal arbuscule-zone and form intracellular coils. This discovery represents the earliest direct evidence for cyanobacteria growing inside land plants, and offers a model for the types of associations that may have preceded the evolution of mutualistic land plant/cyanobacterial symbioses.

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1. Introduction

Symbioses of cyanobacteria with non-vascular and vascular land plants are relatively restricted today, but occur in most major lineages of land plants. As epiphytes and endophytes of various liverworts and mosses, hornworts, the fern genus *Azolla* de Lamarck, cycads, the angiosperm *Gunnera* L., and various orchids, cyanobacteria have evolved an array of more or less intimate relationships with their hosts (e.g., Adams, 2000; Rai et al., 2002; Tsavkelova et al., 2001, 2003a,b; Bergman et al., 2007; Usher et al., 2007; Adams and Duggan, 2008). In most of these symbioses, the primary benefit to the plant is fixed nitrogen originating from N_2-fixation of the cyanobacteria, while the cyanobacterium gains access to a stable environment and the wider nutrient source available from the host (Werner, 1987; Rai et al., 2000).

The fossil record of cyanobacteria is extensive and dates back to the Archean (e.g., Golubic and Seong-Joo, 1999; Schopf, 2000; Altermann and Kaźmierczak, 2003); by the Neoproterozoic, there is also evidence of cyanobacteria in symbiosis (Yuan et al., 2005). However, there are relatively few fossil examples of cyanobacterial associations with other organisms (Raven, 2002). This is especially true of associations with non-vascular and vascular land plants, with the possible exception of two reports on the occurrence of microbial endophytes (bacteria, cyanobacteria, or algae) in gymnospermous roots from the Jurassic and Cretaceous of India (Sharma and Suthar, 1989; Banerji and Ghosh, 2002). As a result, the origin, evolution, and significance of plant/cyanobacterial symbioses in terrestrial paleoecosystems remain an enigma (Osborne, 2005).

The famous Rhynie chert Lagerstätte, an *in situ* silicified Early Devonian (~400 Ma) hot-springs environment characterized by ephemeral fresh-water pools, has preserved a diverse assemblage of land plants, animals, macroalgae, and various microorganisms (surveyed in Kerp and Hass, 2004; Taylor et al., 2004; Taylor and Krings, 2005), including several free-living coccoid and filamentous cyanobacteria (Kidston and Lang, 1921; Croft and George, 1959; Edwards and Lyon, 1983; Krings et al., 2007a) and the *Gloeocapsomorpha*-like photobiont of the cyanolichen *Winfrenatia reticulata* T. N. Taylor, Hass et Kerp (Taylor et al., 1997).

In this paper, we present evidence from the Rhynie chert of a filamentous cyanobacterium that colonizes prostrate mycorrhizal axes of the land plant *Aglaophyton major* (Kidston et W. H. Lang) D. S. Edwards. Although the occurrence of cyanobacteria in axes of Rhynie chert land plants had already been noted by Kidston and Lang (1921),

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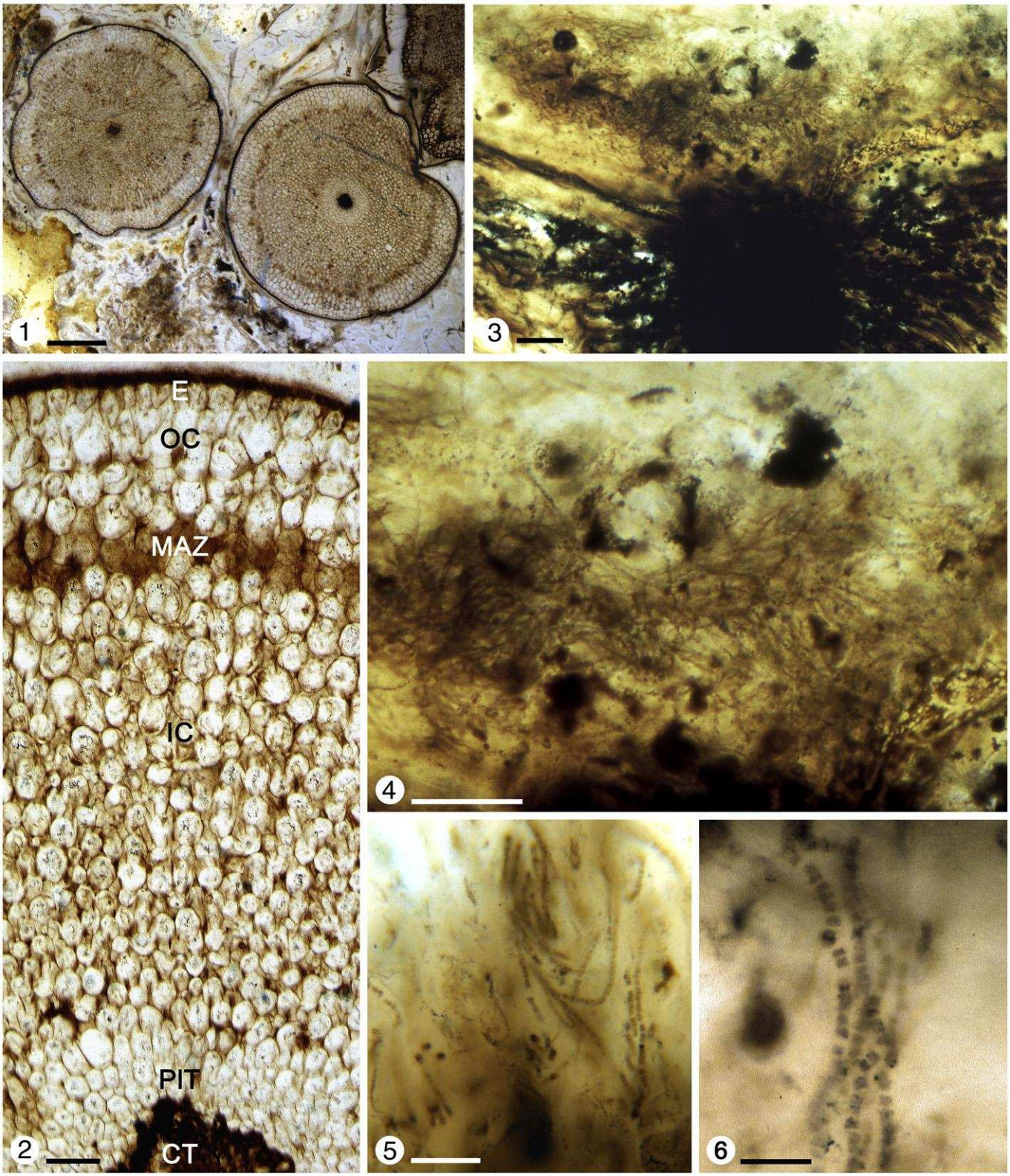

Plate I. Anatomy of *Aglaophyton major*, and filamentous cyanobacteria associated with the prostrate axes of *A. major*.

1. Transverse section through two typical axes showing the simple internal organization; slide P1828; bar = 1 mm.
2. Anatomy of the prostrate mycorrhizal axis (E = epidermis; OC = outer cortex; MAZ = mycorrhizal arbuscule-zone; IC = inner cortex; PIT = phloem-like tissue; CT = conducting tissue); slide P1612; bar = 150 μm.
3. Dense aggregate of cyanobacterial filaments in an area where the axis is injured and has exuded some type of wound secretion (opaque mass); slide P1289; bar = 100 μm.
4. Detail of Plate I, 3, showing part of the cyanobacterial aggregate; bar = 100 μm.
5. Intercellular cyanobacterial filaments near the mycorrhizal arbuscule-zone of the cortex (darker tissue in lower third of image); slide P3652; bar = 50 μm.
6. Group of filaments passing through the intercellular system of the outer cortex; slide P3652; bar = 20 μm.

this discovery was not further investigated. The new specimens reported here add to our understanding of the origin and early evolution of mutualistic land plant/cyanobacterial symbioses because they demonstrate one possible scenario leading to the development of an initial association between these two groups of organisms.

2. Material and methods

The Rhynie chert Lagerstätte is located in the northern part of the Rhynie outlier of Lower Old Red Sandstone in Aberdeenshire, Scotland, within a sequence of sedimentary and volcanic rocks. The cherts occur in the upper part of the Dryden Flags Formation, in the so-called Rhynie Block, a few hundred metres northwest of the village of Rhynie. The Lagerstätte consists of at least 10 fossiliferous beds containing lacustrine shales and cherts that are interpreted as a series of ephemeral fresh-water pools within a hot-springs environment. Preserved in the cherts are both aquatic facies and subaerial systems around the pools (i.e. soil/litter horizons with *in situ* plants); the latter were preserved as a result of temporary floodings of silica-rich water, or by groundwater percolating upwards. Based on dispersed spore assemblages and redefinition of the Pragian/Emsian boundary by the IUGS, the cherts are dated as Pragian–?earliest Emsian (Wellman, 2006; Wellman et al., 2006). Detailed information about the geological setting, sedimentology, and development of the Rhynie chert Lagerstätte can be found in Trewin and Rice (1992, 2004), Rice et al. (2002), and references therein.

The endophytic cyanobacteria were identified in thin sections prepared from two different chert blocks by cementing a thin wafer of the chert to a glass slide and then grinding the rock slice with silicon carbide powder until it is sufficiently thin for examination in transmitted light (Hass and Rowe, 1999). The specimens were examined and photographed using oil immersion objectives directly on the rock surface without a cover slip. Slides are deposited in the collection of the Forschungsstelle für Paläobotanik, Geologisch-Paläontologisches Institut, Westfälische Wilhelms-Universität, Münster (Germany); accession numbers are included in the figure captions.

3. Aglaophyton major

Aglaophyton major, a small clonal land plant sporophyte with affinities in the Rhyniophyta, is a common constituent of the Rhynie chert flora (Remy and Hass, 1996). Plants grow up to 18 cm high, and consist of a system of naked, stomatiferous, more or less cylindrical, and sinuous mycorrhizal (paramycorrhizal *sensu* Strullu-Derrien and Strullu, 2007) prostrate axes (up to 6 mm in diameter), which are loosely lying on the substrate surface and function as rhizomes. The prostrate axes dichotomise repeatedly, locally turning upwards and passing into orthotropous, fertile axes that produce terminal sporangia (Edwards, 1986). The anatomy of the axes is simple (Plate I, 1, 2): Most of the axis consists of a parenchymatous cortex, which is subdivided into an outer (Plate I, 2[OC]) and inner (Plate I, 2[IC]) cortex. The outer cortex is composed of densely packed elongate cells with narrow intercellular spaces. Cells of the inner cortex are more loosely spaced and exhibit a well-developed intercellular system. The outer cortex is surrounded by hypodermal tissues and the epidermis (Plate I, 2[E]). Between the outer and inner cortex is a well-defined region of cortical tissue (i.e. the mycorrhizal arbuscule-zone; Plate I, 2[MAZ]) where the mycorrhizal fungus forms intracellular arbuscules (for details on the *A. major* mycorrhiza, see Taylor et al., 1995). The central stele consists of a narrow zone of phloem-like tissue (Plate I, 2[PIT]) that surrounds a strand composed of thicker-walled conducting cells (Plate I, 2[CT]).

4. Results

Several segments of *Aglaophyton major* prostrate mycorrhizal axes preserved *in situ* host abundant cyanobacterial filaments in the subepidermal and cortical tissues. The infected axes are generally well-preserved; the central stele, cuticle, and epidermis are intact (Plate II, 1), but the parenchymatic cortical tissues show signs of (beginning) disintegration in the form of areas where cell walls are not distinct (e.g., Plate II, 1). The cyanobacterial filaments inside the *A. major* axes (Plate I, 5,6; Plate II, 1–10; Plate III, 1–7) closely resemble the free-living Rhynie chert cyanobacterium *Archaeothrix oscillatoriformis* described by Kidston and Lang (1921), and consist of strictly uniseriate strings of relatively uniform, discoid to barrel-shaped cells, 2–5 5 μm long and up to 5 5 μm wide (Plate I, 6; Plate II, 3–8), surrounded by a distinct colorless investment or sheath (Plate II, 4 [arrow],5–10); external constrictions or folds of the sheath at cross walls may occur (e.g., arrows in Plate II, 7,8) but are not consistently present. Branching, heterocysts, and akinetes have not been observed in any of the filaments.

Cyanobacteria enter the axes through the stomatal pores, and initially colonize the substomatal chambers (Plate III, 1). From the substomatal chambers, cyanobacterial filaments spread throughout the outer cortical tissues and mycorrhizal arbuscule-zone by growing through the intercellular system of the axes (Plate III, 2,3); in many instances, groups of up to 15 parallel filaments occur in the intercellular spaces (Plate I, 6; Plate II, 3; Plate III, 5). Since the cyanobacteria (CB in Plate III, 2) are often found next to hyphae of the mycorrhizal fungus *Glomites rhyniensis* T. N. Taylor, W. Remy, Hass et Kerp (FH in Plate III, 2), it seems reasonable to conclude that the two endophytes used the same means to extend throughout the plant. In dead ends within the intercellular system, cyanobacterial filaments form characteristic loops and continue growth in reverse direction (Plate III, 3,4). Cyanobacteria are most abundant close to and within the mycorrhizal arbuscule-zone of the axis (Plate I, 5; Plate II, 1[arrows], 2). Moreover, near the arbuscule-zone, some of the filaments penetrate the walls of individual parenchyma cells and become intracellular endophytes (Plate II, 2[arrow]; Plate III, 6,7). We have not been able to detect any structural alterations of the host cells in response to the presence of the cyanobacteria (arrow in Plate III, 6), e.g., in the form of thickened cell walls or papilla formation around the filaments. Within the parenchyma cells, the cyanobacterial filaments form coils (Plate II, 2[arrow]; Plate III, 7). Intracellular cyanobacterial filaments passing through the cell walls have not been observed, which suggests that filament growth ceases within the confines of the cell, at some point after the coil has formed. Filaments within the cells are similar in size to the filaments in the intercellular spaces, and also lack heterocysts. Whether the intracellular filaments lack sheaths, as has been reported from intracellular cyanobacteria in *Cycas revoluta* Thunberg coralloid roots by Obukowicz et al. (1981), cannot be determined.

Cyanobacterial filaments identical to those seen in the prostrate axes occur in the chert matrix around the axes; especially interesting are dense aggregations of filaments that thrive in areas where the axes are locally injured and, as a result, have exuded some type of wound secretion, which appears as opaque masses (Plate I, 3,4). Single cyanobacterial filaments sporadically occur on the surface of axes that show no evidence of wounding. In addition, a detached, empty and partially-degraded sporangium of *Aglaophyton major* has been detected in which cyanobacterial filaments occupy a void in the sporangial wall.

Although approximately 2500 thin sections containing *Aglaophyton major* from the extensive Rhynie chert collection kept at the University of Münster (Germany) have been screened for infected plant parts, colonization by cyanobacteria has to date only been observed in sections obtained from two different chert blocks. It is interesting to note that these blocks preserve a wet facies of the Rhynie paleoecosystem, in which *A. major* co-occurs with aquatic organisms, including various free-living cyanobacteria and microalgae, as well as *in situ* specimens of the charophyte *Palaeonitella cranii* (Kidston et W. H. Lang) J. Pia. The two chert blocks contain a total of four infected and nine uninfected prostrate axis segments of *A. major*.

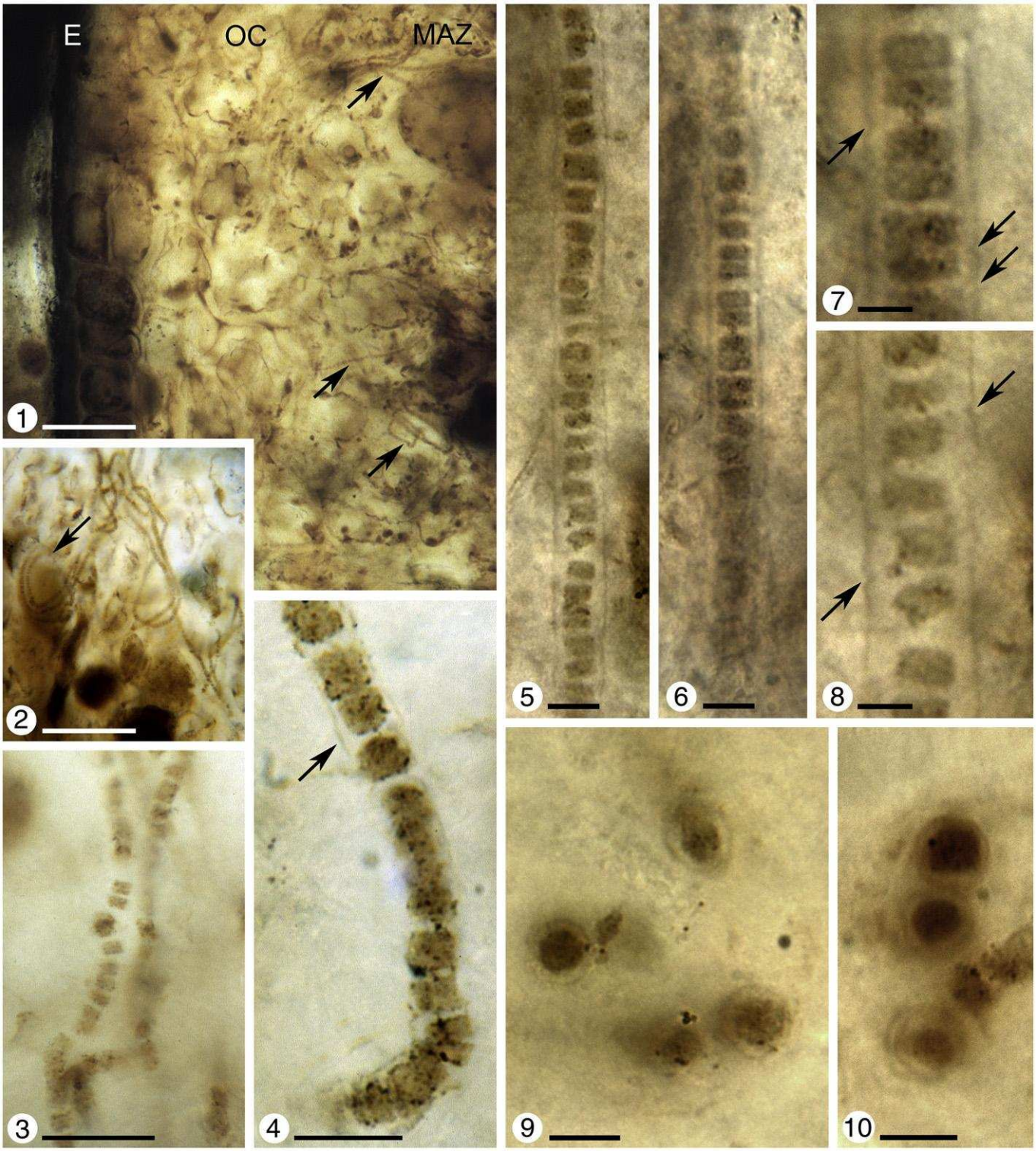

Plate II. Endophytic filamentous cyanobacteria in *Aglaophyton major* prostrate axes.

1. Cross-section through an axis, showing the epidermis (E), outer cortex (OC), mycorrhizal arbuscule-zone (MAZ), and cyanobacterial filaments (arrows); slide P3652; bar = 100 μm.
2. Cyanobacterial filaments within the arbuscule-zone; slide P3652; bar = 100 μm.
3. Cyanobacterial filaments in the intercellular system; slide P3652; bar = 25 μm.
4–6. Cyanobacterial filaments showing the morphology of the individual cells and the distinct colorless sheath (arrow in Plate II, 4); slide P3652; bars = 10 μm (Plate II, 4) and 5 μm (Plate II, 5,6).
7–8. Details of Plate II, 5,6, focusing on some of the cyanobacterial cells. Note external constrictions of the sheath at cross walls (arrows); bars = 2.5 μm.
9–10. Parallel cyanobacterial filaments (cross-sectioned) in intercellular spaces of the outer cortex; slide P3652; bars = 5 μm.

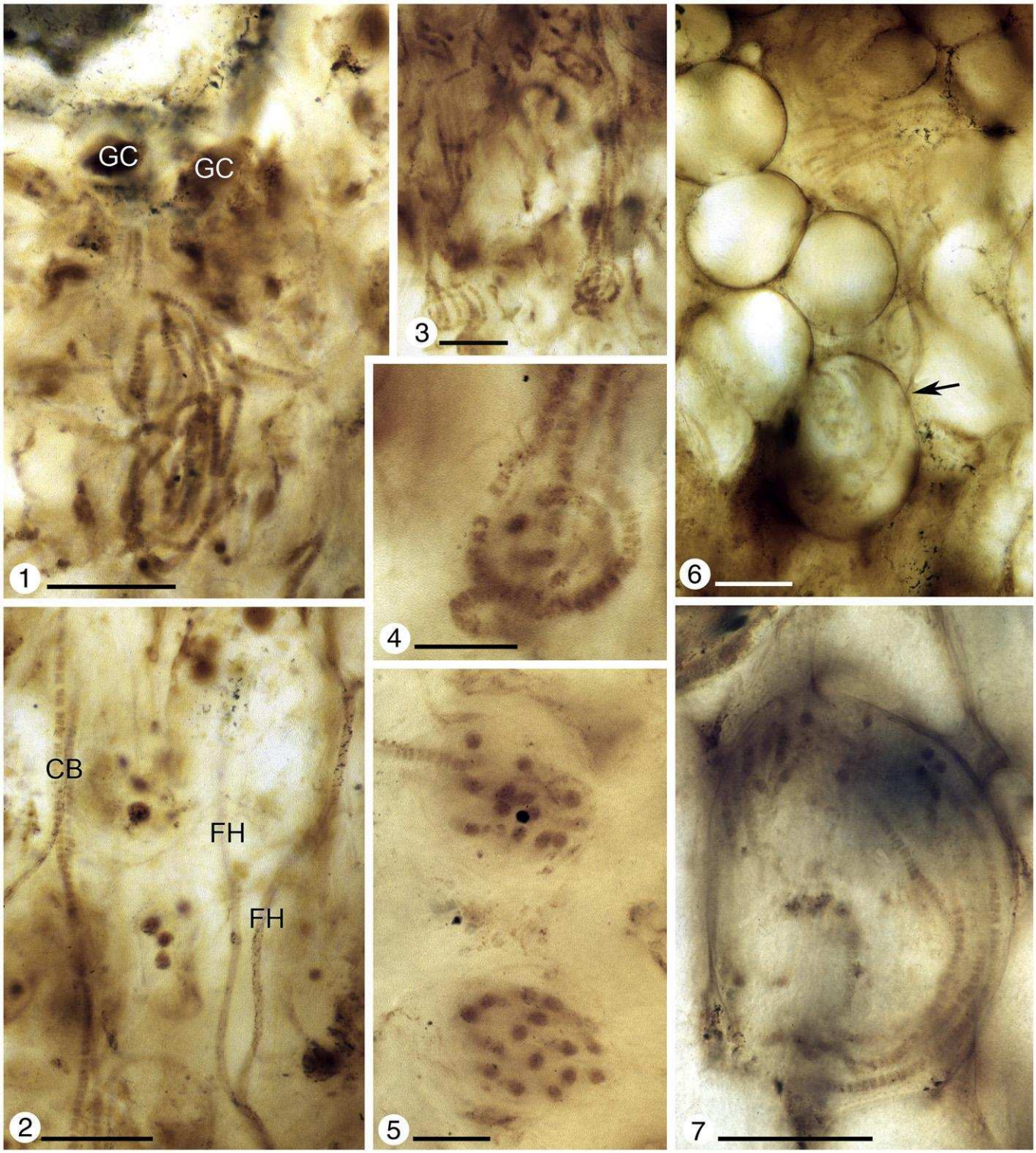

Plate III. Endophytic filamentous cyanobacteria in *Aglaophyton major* prostrate axes.

1. Filaments entering the plant through the stomatal pore (GC = guard cells) and colonizing the substomatal chamber; slide P3652; bar=50 μm.
2. Cyanobacterial filaments (CB) and hyphae of the mycorrhizal fungus (FH) in the intercellular system of the cortex; slide P3652; bar=50 μm.
3. Cyanobacterial filaments forming loops in dead ends of the intercellular system; slide P3653; bar=50 μm.
4. Detail of Plate III, 3, showing one of the loops; bar=25 μm.
5. Parallel cyanobacterial filaments (cross-sectioned) in narrow intercellular spaces of the outer cortex; slide P3653; bar=25 μm.
6. Cyanobacterial filaments in a substomatal chamber, which is surrounded by parenchyma. Note the filament that becomes intracellular by penetrating a parenchyma cell (arrow) positioned near the mycorrhizal arbuscule-zone; slide P3651; bar=50 μm.
7. Cyanobacterial coil in a parenchyma cell; slide P3651; bar=50 μm.

5. Discussion

The filamentous cyanobacteria in prostrate axes of *Aglaophyton major* represent the earliest direct fossil evidence for cyanobacterial endophytes in land plants, and the only persuasive example to date for this type of association. Although the fossils do not provide a fully documented picture, they do offer some comparisons to extant land plant/cyanobacterial associations such that it is possible to use the fossils in the development of hypotheses as to the nature and significance of this Early Devonian association.

5.1. Comparisons

In extant vascular plants, associations with endophytic filamentous cyanobacteria are found in cycads (Costa and Lindblad, 2002; Lindblad and Costa, 2002) and the angiosperm genus *Gunnera* (Bergman et al., 1992; Bergman, 2002). More recently there have also been reports of cyanobacteria occurring in the aerial root velamen of epiphytic orchids (e.g., Tsavkelova et al., 2001, 2003a,b); however, it is not well understood what role these micoorganisms play in the biology of the orchids. In cycads, cyanobacteria occur in the coralloid roots where they live as intercellular endophytes in a distinct zone composed of elongated cortical cells; for some cycad species intracellular location of the cyanobacteria has also been reported (e.g., Nathanielsz and Staff, 1975b; Obukowicz et al., 1981). In *Gunnera*, the symbiont exists as an intracellular endophyte in specialized regions or nodes within the cortex of the stolons or rhizomes (Rai et al., 2000; Bergman, 2002; Parsons, 2002).

In cycads, *Gunnera*, and *Aglaophyton major*, cyanobacteria enter the host via openings in the surface, spread throughout the plant via the intercellular system, and later may become intracellular within a well-defined region of the cortex. The infection of the cycad coralloid roots may occur at any stage of development of the root; however, the infection process (e.g., via the papillose pre-coralloid, local injuries, lenticells, or breaks in the dermal layer; see Nathanielsz and Staff, 1975a) is still not completely understood (see Costa et al., 1999; Lindblad and Costa, 2002). In *Gunnera*, cyanobacteria produce hormogonia after being attracted to mucilage-secreting glands at the base of the petioles. The hormogonia then move into the gland interiors (Bergman, 2002; Bergman et al., 2007). The *A. major* cyanobacterial endophyte uses a different infection pathway, i.e. stomatal pores (see Plate III, 1). Perhaps filament fragments or propagules washed into the substomatal chambers, and subsequently initiated growth into long filaments; or some of the free-living filaments in the vicinity of the axes simply grew into the chambers. Because aggregations of cyanobacteria are found close to small surface injuries (Plate I, 3,4), it is also possible that some filaments entered the plant post-trauma through wounds in the axis, perhaps in response to wound secretion.

The infection of *Aglaophyton major* prostrate axes via stomatal pores and initial colonization of the substomatal chambers has parallels with the entry of cyanobacteria and colonization process seen in extant hornworts (Adams and Duggan, 2008). In these non-vascular plants, the cyanobacterial symbiont usually occurs in slime cavities that are connected to the ventral surface of the thallus via stomata-like pores (mucilage clefts) (e.g., Adams, 2002). The cyanobacteria (in the form of motile hormogonia) enter the thallus through the mucilage clefts and subsequently colonize the cavities. In the hornwort *Leiosporoceros dussii* (Stephani) Hassel, however, the cyanobacterial symbiont (*Nostoc* sp.) is not restricted to slime cavities, but rather spreads within the thallus by extending through schizogenous, mucilage-filled canals, and eventually forms an integrated network throughout the plant (Villarreal and Renzaglia, 2006). This is somewhat reminiscent of the later stages in the colonization process in *A. major*, in which the cyanobacterial filaments extend through the intercellular system of the outer cortex.

One major difference between the cycad, *Gunnera*, hornwort, and *Aglaophyton major* cyanobacterial association concerns heterocyst formation. Cycads, *Gunnera*, and hornworts are associated with heterocystous cyanobacteria belonging to subsection IV (Nostocales) that display increased numbers of heterocysts in the endophytic filaments (e.g., Adams, 2000; Meeks, 2003; Bergman et al., 2007), whereas heterocysts have not been observed in any of the fossil filaments (e.g., Plate II, 4–6), neither those living outside nor those within *A. major*, which suggests that these cyanobacteria belong to subsection III (Oscillatoriales). This challenges the comparability of the fossil and extant land plant/cyanobacterial associations, and raises the question as to the nature of the fossil association.

5.2. Nature of the Aglaophyton major/cyanobacterial association

Endophytic cyanobacteria in *Aglaophyton major* prostrate axes have been observed in four axis segments from two different chert blocks. Since *A. major* represents one of the most common elements in the Rhynie chert flora, the association with endophytic cyanobacteria may be regarded as a very rare occurrence. This suggests that the *A. major*/cyanobacterial association was not an established mutualistic interaction (symbiosis), which would be expected in a much larger number of axes. The alternative is that colonization of *A. major* by cyanobacteria was a chance occurrence, and that no mutual dependency existed between the two organisms. Unfortunately, the condition of the axes at the time of colonization by cyanobacteria, i.e. alive and fully functional or in the process of dying, remains unclear. Support for the former condition is the fact that intact apices of *A. major* occur in the same chert blocks. Conversely, the outer cortex of the axes often shows stages of disintegration, which are suggestive of the latter condition. However, it cannot be determined as to whether this disintegration started before or after the cyanobacteria entered the axes, or is a preservational artifact.

In spite of this, cyanobacterial distribution within the axes is not entirely random, but rather displays a consistent pattern. The filaments enter the axes through the stomata (and perhaps small surface injuries) and extend through the substomatal chambers and intercellular system of the outer cortex (Plate I, 6; Plate II, 1; Plate III, 1–3,5). Thus far, filament growth appears to follow the simplest path in the interior of the axis. Deeper within the cortex, however, some of the filaments actually penetrate individual cell walls and become coiled within the cells (Plate II, 2[arrow]; Plate III, 6,7). Moreover, filament growth apparently ceases within the confines of the cell, at some point after the coil has formed. It is interesting that intracellular penetration is restricted to the tissue that contains the mycorrhizal arbuscule-zone. Elsewhere in the cortex, penetration of cells has not been observed; here, the host cell walls appear to represent impenetrable barriers (e.g., in dead ends of the intercellular system) that cause the filaments to form loops and continue growth in reverse direction (Plate III, 3,4). We suggest that this pattern of endophytic distribution cannot be fully explained by the architecture of the *A. major* internal tissues, but hypothesize that there may have been some level of interaction. It seems implausible that accidental endophytic growth of a cyanobacterium would result in complex features such as selective penetration of cells and subsequent formation of coils. Nevertheless, we can provide no direct evidence of interaction, e.g., in the form of a host response that may have been effective in reducing the attack or encapsulating the intruder. In other Rhynie chert plants, various host responses have been documented as a result of microbial infections (Taylor et al., 2004; Krings et al., 2007b).

Unfortunately, the quality, if any, of the interaction between *Aglaophyton major* and the cyanobacteria cannot be determined based on the fossils. If the axes were alive during colonization one could speculate that once cyanobacteria entered the tissues they were provided some benefit in the form of protection against desiccation by the homoiohydric mechanisms of the plant (see Sprent and Raven,

1985; Raven and Sprent, 1989). An alternative, although highly speculative premise at this time, is that the cyanobacterium in *A. major* entered into some level of interaction with the mycorrhizal fungus. This hypothesis is strengthened by the fact that cyanobacteria are most abundant close to and within the mycorrhizal arbuscule-zone (see Plate II, 1). Adding some support to this is the suggestion that, in extant cycads, mycorrhizal fungi may promote the fixing of nitrogen by the cyanobacteria (Fisher and Vovides, 2004), similar to the positive effect that mycorrhizal fungi have on the nitrogen fixation rates of certain bacteriorrhizal legumes (e.g., Bethlenfalvay, 1992; Herrmann, 1995).

5.3. Towards the establishment of a symbiosis?

The Early Devonian Rhynie paleoecosystem has been reconstructed as a complex and diverse environment inhabited by a variety of aquatic and terrestrial plants and animals (Kerp and Hass, 2004; Trewin and Rice, 2004). The land plants are believed to have grown around ephemeral pools, with those closest to the water exposed to temporary flooding (e.g., Remy et al., 1997). Based on the fact that most plant/cyanobacterial symbioses today thrive in moist or wet habitats (see Osborne and Sprent, 2002), Usher et al. (2007) suggest that warm and generally wet environmental conditions may have stimulated the evolution of cyanobacterial symbioses via a close and constant association of partners. The two chert blocks containing the *Aglaophyton major*/cyanobacterial association preserve a wet facies of the Rhynie paleoecosystem. In these blocks, the land plants co-occur with aquatic organisms, including cynaobacteria, microalgae, and *in situ* specimens of the charophyte *Palaeonitella cranii*. The presence of *in situ* macroalgae indicates that the infected *A. major* axes were submerged for extended periods of time, which resulted in a close and constant co-occurrence with cyanobacteria, precisely as postulated by Usher et al. (2007). Although the *A. major*/cyanobacterial association was probably incidental based on its rare occurrence to date, it demonstrates that, in this particular habitat, cyanobacterial filaments did enter land plants through surface openings, and that endophytic cyanobacterial growth was not a singular and short-lived incident, but rather happened multiple times and in several axes. In addition, the *A. major*/cyanobacterial association indicates that the endophytic filaments were alive in the axes, and even displayed a constant spatial distribution and pattern of growth within the host. As to whether the cyanobacteria were somehow attracted to the plants cannot be determined. It is interesting, however, that surface wounds in the axes apparently resulted in the production of some substance that attracted cyanobacteria based on the exclusive occurrence of dense accumulations of filaments around wound secretions (Plate I, 3,4).

Usher et al. (2007) suggest that an on-again–off-again association between land plants and cyanobacteria may not cause the necessary (and initially costly) evolutionary changes to occur in the partners that would lead to a successful partnership. Since endophytic cyanobacterial growth in *Aglaophyton major* probably represented an on-again–off-again association that only formed when the habitat was flooded, and since the cyanobacterial endophyte apparently was non-heterocystous, it is improbable that this association represented a direct precursor to the evolution of mutual land plant/cyanobacterial relationships. Nevertheless, the *A. major*/cyanobacterial association demonstrates that the two groups of organisms came into contact as early as the Early Devonian, and that this contact consisted of more than just constant and close co-occurrence. We would suggest that such an association may be viewed as a model for the types of associations that preceded, and perhaps ultimately set the stage for, the evolution of mutual land plant/cyanobacterial symbioses. In this context, the fossils described here add evidence to the assumptions of Usher et al. (2007) with regard to the environment in which the actual initial steps in the evolution of land plant/cyanobacterial symbioses probably occurred.

6. Conclusions

Hypotheses based on indirect evidence suggest that symbioses of land plants with cyanobacteria are ancient associations that evolved *ca.* 500 Ma ago (Raven, 2002; Usher et al., 2007), and represented a significant component of many terrestrial paleoecosystems (Osborne, 2005). Direct evidence, however, is lacking. The colonization of the early land plant *Aglaophyton major* by cyanobacteria based on exceptionally well well-preserved specimens is interpreted as an on-again–off-again incidental association linked to temporary flooding of the habitat. While this association of organisms cannot be determined to be mutualistic, the physical association of an early vascular land plant and cyanobacteria may represent a model as to how precursory or initial stages of such a mutualism may have developed. The fact that early land plants and microorganisms had already evolved a diverse array of complex mutulistic associations by the Early Devonian (e.g., arbuscule-forming endomycorrhizae) suggests that the genetic mechanisms and biochemical pathways necessary for such associations to function were already in place. While the fossil record can never conclusively document degrees of mutualism between organisms, it can place species within the context of time and space, and thus provide a framework with which to discuss evolving patterns of biological interaction. We view the association of cyanobacteria within the tissues and cells of *A. major* as such a framework, and await additional evidence that will substantiate or refute our hypotheses.

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Chapter IX: *Globicultrix nugax* nov. gen. et nov. spec. (Chytridiomycota), an intrusive microfungus in fungal spores from the Rhynie chert.

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Globicultrix nugax nov. gen. et nov. spec. (Chytridiomycota), an intrusive microfungus in fungal spores from the Rhynie chert

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Abstract

Fungal spores from the Lower Devonian Rhynie Chert are known to harbor a wide variety of parasitic and saprotrophic microfungi. However, only a few of these intrusive organisms have been documented in detail. This paper describes a previously unknown microfungus contained in fungal spores from the Rhynie chert; it consists of tenuous branched filaments and terminal, globose, usually apophysate sporangia with a single discharge pore or papilla. This complement of features is similar to the rhizomycelial and zoosporangial morphology seen in certain extant polycentric chytrids, and thus the fossil is provisionally placed in the Chytridiomycota.

Key words: Apophysis, Chydridiomycota, fossil fungi, Early Devonian, Rhynie chert, zoosporangium

Zusammenfassung

Pilzsporen aus dem unterdevonischen Rhynie chert beherbergen ein Reihe parasitischer und saprotropher Mikropilze, von denen allerdings nur wenige bislang detailliert dokumentiert worden sind. In dieser Arbeit wird ein bislang unbeschriebener Mikropilz aus Pilzsporen im Rhynie chert vorgestellt, der aus zarten verzweigten Filamenten und endständigen, kugeligen, meist apophysaten Sporangien mit einer einzelnen Austrittspore oder -papille besteht. Die Morphologie dieses fossilen Pilzes ähnelt der des Rhizomyzels und der Zoosporangien einiger moderner polyzentrischer Chytridien; auf Grund dessen wird das Fossil vorläufig zu den Chytridiomycota gestellt.

Schlüsselwörter: Apophyse, Chytridiomycota, fossile Pilze, Rhynie Chert, Unterdevon, Zoosporangium

1. Introduction

The Early Devonian Rhynie chert has preserved a diversity of microorganisms, including bacteria (Kidston & Lang 1921), cyanobacteria (e.g., Croft & George 1959; Krings et al. 2007, 2009), microalgae (Edwards & Lyon 1983; Dotzler et al. 2007), peronosporomycetes (Taylor et al. 2006), and fungi (surveyed in Taylor et al. 2004). Many of these life forms were fossilized so that associations and interactions with other organisms can be directly examined. This is especially true of the fungi, which, as heterotrophs, are intricately involved with other organisms in saprotrophic, parasitic, and/or mutualistic associations (Taylor & Taylor 2000). While some of the fungal associations from the Rhynie chert are known in great detail (e.g., endomycorrhizae; see Taylor et al. 1995, 2005), others continue to be incompletely understood because some of the morphology, life history, spatial distribution, systematic affinities, and/or diversity levels of the fungal partner(s) cannot be reconstructed in sufficient detail or demonstrated on a consistent basis.

One of the more frequently encountered fungal associations in the Rhynie chert are microfungi inhabiting the spores of other fungi. Several examples of these interfungal associations have been described (Kidston & Lang 1921; Taylor et al. 1992; Hass et al. 1994), one of which consists of glomeromycotan spores containing varying numbers of small gametangia, (resting) spores, or sporangia of other, intrusive fungi (e.g., Fig. 1). The systematic affinities of most of the intrusive fungi remain elusive. However, differences in size and wall composition of the gametangia, spores, or sporangia, together with differences in the morphology of occasionally present subtending hyphae or filaments, suggest that a wide variety of microfungi in the Rhynie paleoecosystem lived, reproduced, and/or produced resting stages inside the spores of other fungi (Kidston & Lang 1921). Thus, detailed knowledge about these organisms represents an important component of fully understanding the roles that fungi played in the Rhynie paleoecosystem.

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This paper describes a previously unknown intrusive microfungus in fungal spores from the Rhynie chert that is characterized by apophysate sporangia positioned terminally on tenuous filaments. Structural correspondences between the fossil and members of the extant genera *Cladochytrium* Nowak. and *Nowakowskiella* J. Schröt. (in Engler & Prantl) suggest affinities of the fossil with the Chytridiomycota, order Chytridiales.

2. Material and methods

The Rhynie chert Lagerstätte is located in the northern part of the Rhynie Outlier of Lower Old Red Sandstone in Aberdeenshire, Scotland, within a sequence of sedimentary and volcanic rocks. The cherts occur in the upper part of the Dryden Flags Formation, in the so-called Rhynie Block, a few hundred metres northwest of the village of Rhynie. The Lagerstätte consists of at least 10 fossiliferous beds containing lacustrine shales and cherts interpreted as a series of ephemeral freshwater pools within a hot springs environment (e.g., Rice et al. 2002). Preserved are both aquatic (freshwater) facies from the pools and subaerial soil/litter horizons with *in situ* plants around the edges of the pools; the latter became preserved as a result of temporary flooding of silica-rich water, or by silica-rich groundwater percolating to the surface. Based on dispersed spore assemblages and redefinition of the Pragian/Emsian boundary by the IUGS, Wellman (2006) and Wellman et al. (2006) date the cherts as Pragian-?earliest Emsian. Detailed information about the geological setting, sedimentology, and development of the Rhynie chert Lagerstätte can be found in Rice et al. (2002), and Trewin & Rice (2004).

The infected fungal spores were identified in a thin section prepared by cementing a piece of chert to a glass slide and then grinding the slice until it is thin enough to be examined in transmitted light. The slide is part of the Hirmer collection (accession number BSPG 1964 XX 24), which is today deposited in the Bayerische Staatssammlung für Paläontologie und Geologie (BSPG), Munich (Germany).

3. Systematic paleontology

Chytridiomycota M. J. Powell, 2007, *incertae sedis*

Morphogenus *Globicultrix* nov. gen.

MycoBank number: MB 512268 (cf. http://www.mycobank.org)

Diagnosis: Thallus polycentric; vegetative system (rhizomycelium) composed of branched filaments; zoosporangia terminal, apophysate or non-apophysate, at maturity with a single discharge pore or papilla.

Type: *Globicultrix nugax* M. Krings, Dotzler et T. N. Taylor (this paper)

Globicultrix nugax nov. spec.
(Figs 1[arrow], 2.1–9)

MycoBank number: MB 512269 (cf. http://www.mycobank.org)

Holotype: Specimen illustrated in Figure 2.1: Slide no. BSPG 1964 XX 24, reposited in the Bayerische Staatssammlung für Paläontologie und Geologie (BSPG), Munich (Germany).

Specific diagnosis: Thallus endobiotic, in spores of other fungi; rhizomycelial filaments tenuous, <1–2 µm wide, apparently ephemeral; zoosporangia spherical, up to 10(–15) µm in diameter, usually apophysate, wall non-ornamented; apophysis inconspicuous or prominent, bulb-shaped or somewhat pyriform, up to 3 µm long; discharge pore or papilla in apical or subapical position, <1.5 µm in diameter.

Etymology: The generic name *Globicultrix*, a combination of the Latin words *globus* (= globe, sphere) and *cultrix* (= dweller, occupant), refers to the occurrence of the microfungus in a large, globose glomeromycotan spore; *nugax* (Lat.) = cute.

Locality: Rhynie, Aberdeenshire, Scotland, National Grid Reference NJ 494276

Age: Pragian–?earliest Emsian (Early Devonian), according to Wellman (2006) and Wellman et al. (2006)

Description: *Globicultrix nugax* occurs in a single, subspherical glomeromycotan spore, 185 µm long and ~160 µm wide (Figs 1[arrow], 2.1), which is positioned on a somewhat bulbose base (Fig. 2.1[arrow]) of the vegetative or parental hypha. The host spore occurs in the cortex of a degraded land plant axis where it is associated with several other glomeromycotan spores containing intrusive microfungi. However, fungal remains displaying the same complement of features as *G. nugax* have not been detected in any one of the other spores, nor do they occur in the plant tissues and matrix surrounding the spores.

The fungus consists of a vegetative system composed of narrow, branching filaments <1–2 µm wide (Fig. 2.1). Septae, intercalary swellings, and rhizoids extending from the filaments have not been observed. Spherical sporangia, up to 10(–15) µm in diameter, occur terminally on the filaments. The wall of these structures is typically <0.5 µm thick, slightly darker than the wall of the subtending filament, and non-ornamented. Most of the sporangia are empty, while a few contain amorphous material that appears to have undergone shrinkage (Fig. 2.2[white arrow],4), probably as a result of osmotic water loss during fossilization. Sporangia are usually subtended by an inconspicuous or prominent swelling of the parental filament (Fig. 2.3,8,9). This subsporangial swelling is bulb-shaped or somewhat pyriform, up to 3 µm long and 1.5–3 µm wide. In some of the specimens, it appears that the swelling is separated from the parental filament by a constriction of the filament or by a septum (e.g., Fig. 2.4[white arrow]). Other sporangia lack the subsporangial swelling of the filament (e.g., Fig. 2.2[black arrow]). Many sporangia display a single circular discharge pore (<1.5 µm in diameter) or slightly elevated (<1 µm high), papilla-like orifice in apical or subapical position (Fig. 2.5–8[arrows]). One specimen appears to have a single circular

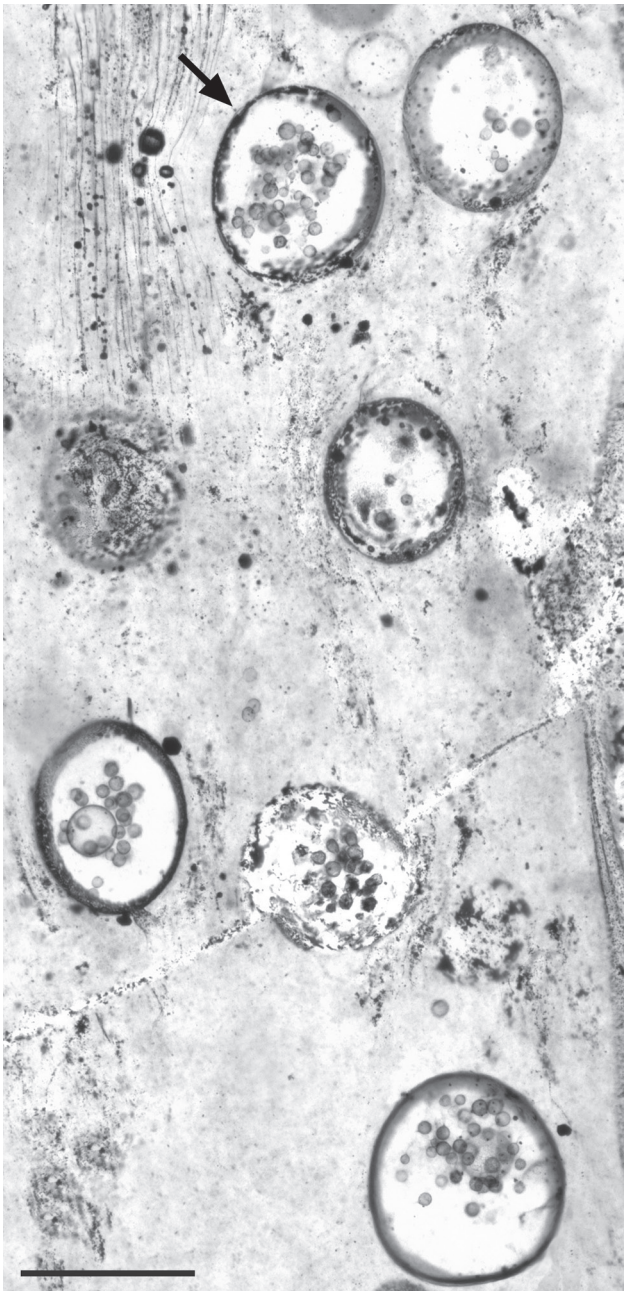

Figure 1: Large fungal (glomeromycotan) spores in the cortex of a degrading land plant axis; note that most spores contain large numbers of small gametangia, spores, or sporangia of intrusive microfungi, one of which is *Globicultrix nugax* [arrow]; slide no. BSPG 1964 XX 24; bar = 150 µm.

discharge pore and one discharge tube (Fig. 2.7[black and white arrows]), but the latter might also represent a filament fragment adhering to the sporangial wall. It is interesting to note that discharge pores or papillae have not been observed in any of the sporangia containing amorphous material. However, one of these specimens shows what appears to be an operculum (Fig. 2.4[black arrow]), but this structure might also represent an artifact of preservation. Figure 2.8 shows a swelling from which extends a well-developed sporangium with apical discharge pore [black arrow] and a smaller, bulb-like structure [white arrow] containing tiny spherules, which perhaps represents a second, immature sporangium.

Remark: Determining the exact systematic position of *Globicultrix nugax* is impossible at this time. We therefore refrain from formally referring the fossil to any one of the extant genera with which it is compared (see Discussion section below), but rather introduce a new morphogenus *incertae sedis*, *Globicultrix*, to accommodate this distinct Early Devonian microfungus until such a time that more specimens are available to increase the suite of diagnostic features.

4. Discussion

The Early Devonian Rhynie chert contains the oldest fossil evidence for microfungi inhabiting the spores of other fungi (Kidston & Lang 1921; Taylor et al. 1992; Hass et al. 1994). Especially abundant are fungal spores containing one to several small spherical gametangia, (resting) spores, or sporangia of other fungi. The most detailed account on this type of association was provided by Kidston & Lang (1921). These authors describe several types of intrusive microfungi based on the size and morphology of the gametangia, spores/sporangia, and occasionally the presence of subtending hyphae or filaments. However, none of the organisms has been formally described and named, due perhaps to the fact that the fossils do not normally display many features that could be used for comparison with modern taxa.

Globicultrix nugax is characterized by spherical sporangia that are exclusively terminal, usually positioned on a distinct swelling of the subtending filament (Fig. 2.3,8,9), and have a single apical or subapical discharge pore or papilla (Fig. 2.5–8[arrows]). The most important structural features distinguishing this form from the previously described intrusive microfungi in fungal spores from the Rhynie chert (see Kidston & Lang 1921; Hass et al. 1994) are the subsporangial swellings and the single discharge pores/papillae. However, nothing is known about the range of morphological plasticity and life history of *G. nugax*, and thus we cannot rule out that some of the specimens described by Kidston & Lang (1921) and Hass et al. (1994) are conspecific with *G. nugax*, despite the fact that they are morphologically different.

The morphology and dimensions of *Globicultrix nugax* are reminiscent of certain modern chytrids (Chytridiomycota), especially the polycentric genera *Nowakowskiella* and *Cladochytrium* (Chytridiales, *Cladochytrium* clade [cf. James et al. 2006]). *Nowakowskiella* consists of a branched rhizomycelium constructed of delicate filaments bearing operculate zoosporangia in terminal or intercalary position. Within this genus zoosporangia may be apophysate or non-apophysate, and usually are broadly or narrowly pyriform in shape, but may also be spherical. At maturity, zoospores are liberated via a short or long neck or discharge papilla (e.g., Karling, 1977; Marano et al. 2007; Pires-Zottarelli & Gomes 2007). *Cladochytrium* represents the inoperculate counterpart of *Nowakowskiella* (Karling 1977; Dayal 1997), in which zoospores are released through a discharge tube, papilla, or simple pore. Correspondences in basic structure between the rhizomycelial and zoosporangial morphology of these modern chytrids and *G. nugax* suggest that the fossil represents a polycentric chytrid in the order Chytridiales. The vegetative parts of *G. nugax* can be interpreted as remains of a branched rhizomycelium,

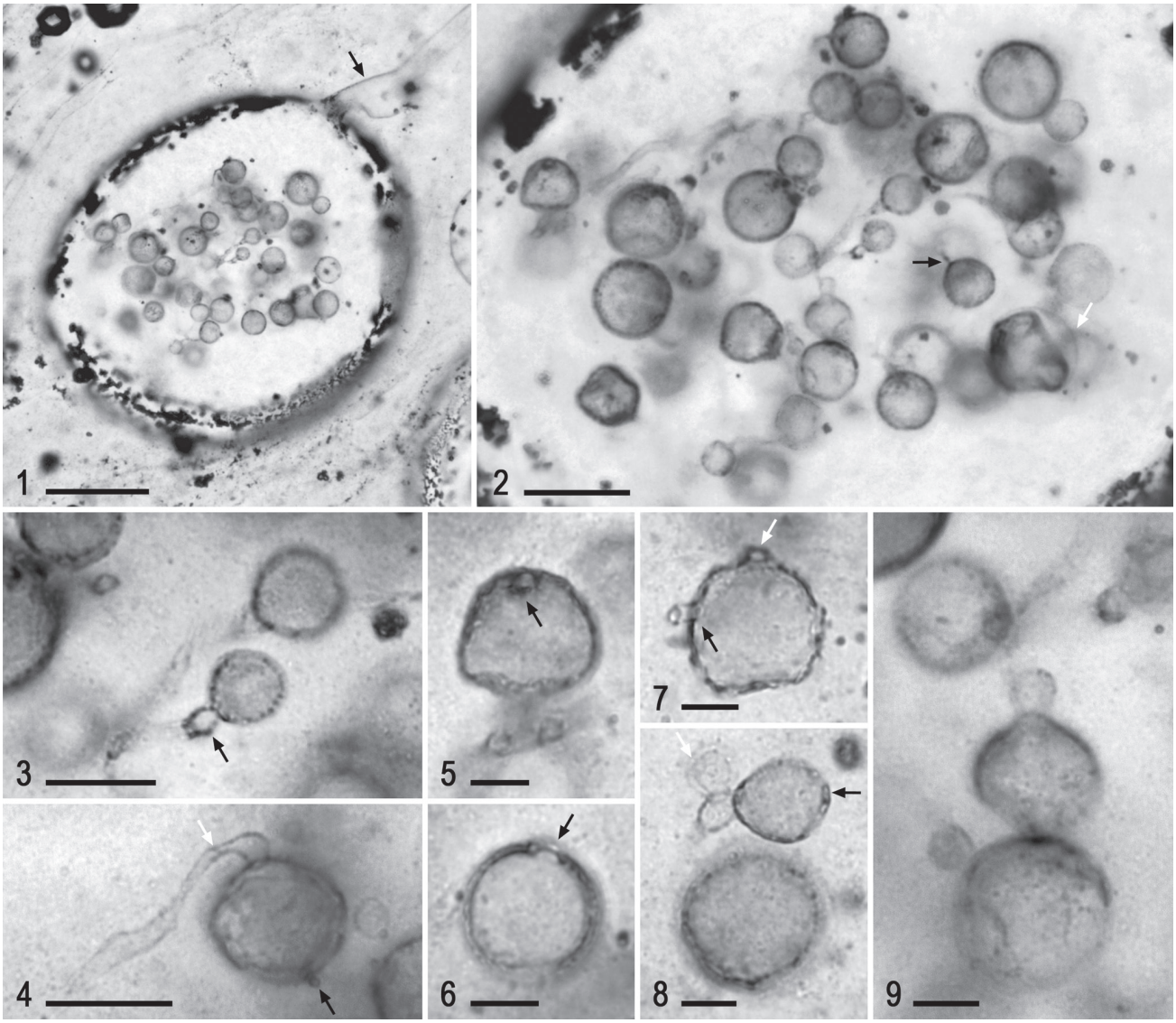

Figure 2: *Globicultrix nugax* nov. gen et nov. spec. from the Early Devonian Rhynie chert; slide no. BSPG 1964 XX 24. **2.1** Thallus within the spore (holotype); arrow points to the somewhat bulbose base of the host spore; bar = 50 µm. **2.2** Detail of Figure 2.1, focusing on the intrusive thallus; black arrow indicates a non-apophysate zoosporangium, white arrow indicates a zoosporangium showing contents; bar = 20 µm. **2.3** Filament branches bearing apophysate zosporangia [one of the apophyses is indicated by an arrow]; bar = 10 µm. **2.4** Filament with terminal, inconspicuously apophysate zoosporangium; note constriction of the filament (or septum?) immediately below the apophysis [white arrow] and what appears to be an operculum [black arrow] in apical position; bar = 10 µm. **2.5** & **2.6** Zoosporangia with discharge pores in apical position [arrows]; bars = 5 µm. **2.7** Zoosporangium with shallow discharge papilla [white arrow] and what appears to be a second, tube-like opening [black arrow]; bar = 5 µm. **2.8** Apophysate zoosporangium with discharge pore in apical position [black arrow] and bulb-like structure (perhaps a second, immature zoosporangium) extending from the apophysis [white arrow]; bar = 5 µm. **2.9** Two zoosporangia with prominent apophyses; bar = 5 µm.

the spherical sporangia as terminal zoosporangia with a single discharge pore or papilla, and the subsporangial swellings of the subtending filaments as apophyses. If these interpretations are correct, the zoosporangia with a well-developed discharge pore/papilla (Fig. 2.5–8[arrows]) may appear empty because zoospore liberation had occurred prior to fossilization. Conversely, the presence of amorphous material in sporangia lacking discharge openings (e.g., Fig. 2.2[arrow],4) may indicate that zoospores had not (yet) developed and/or been released at the time of fossilization.

An alternative interpretation views *Globicultrix nugax* as a member of the Peronosporomycetes (Oomycota). In this scenario, the sporangia represent terminal oogonia, while the swellings at the base of the sporangia are collar-like, amphigynous antheridia. However, the swellings appear to represent enlargements of the subtending filament, rather than being formed by an oogonial hypha growing through an antheridium. Moreover, oospores and parental hyphae giving rise to the antheridia have not been observed in any of the specimens. As a result, it is much more likely that *G. nugax* represents a member of the Chytridiomycota.

Although *Globicultrix nugax* is similar in basic structure to the modern chytrid genera *Nowakowskiella* and *Cladochytrium*, there are some basic differences. The rhizomycelia in both

extant taxa typically bear rhizoids and conspicuous intercalary swellings (Karling 1977; Dayal 1997). These structures have not been observed in the fossil. However, this may be due to the fact that the vegetative system is incompletely preserved in the fossil. The vegetative system of *G. nugax* was perhaps ephemeral and disintegrated rapidly upon maturation of zoosporangia. Alternatively, the main portion of the vegetative system may have been too delicate to become preserved in a recognizable manner. In addition, it cannot be determined whether the zoosporangia of *G. nugax* were operculate or inoperculate. A *bona fide* operculum has not been observed in any of the specimens, but the consistent absence of this structure may also be a result of preservation.

It is difficult to assess the nature of the association between *Globicultrix nugax* and its host. If the microfungus colonized the glomeromycotan spore while it was viable, this association would represent a form of mycoparasitism. Mycoparasites are fungi that derive the majority of their nutrients from other fungi that are alive at the time of infection (Jeffries & Young 1994; Purin & Rillig 2008). Interpreting *G. nugax* as a mycoparasite seems reasonable, as parasitic interfungal interactions appear to have been widespread in the Rhynie paleoecosystem (Hass et al. 1994). Moreover, glomeromycotan spores, which are among the largest spores known in the fungal kingdom, certainly represented particularly suitable habitats for parasitic microfungi, because they contain abundant and easily accessible nutrients. However, there is no indication of a host response in the form of structural alterations or modifications of the spore wall, which would indicate evidence of a parasitic relationship between *G. nugax* and its host. In the absence of host responses, fossil mycoparasites are difficult to distinguish from saprotrophs, which colonize and utilize dead organic matter as a carbon source (see Dix & Webster 1995). Therefore, it is also possible that *G. nugax* represents a saprotroph that colonized non-viable spores or spores that had already germinated. Most extant members of *Nowakowskiella* and *Cladochytrium* are aquatic and soil saprotrophs that thrive on/in decaying plant material (Sparrow 1960; Karling 1977), but at least one species of *Cladochytrium* (i.e. *C. aneurae* Thirum.) has been described as a parasite of liverworts from the genus *Aneura* Dumort. (Thirumalachar 1947).

The discovery of *Globicultrix nugax* in the Rhynie chert adds to our understanding of the biodiversity of late Paleozoic microorganisms, and contributes to a more sharply focused concept of the complexity of ancient non-marine ecosystems. As more information is obtained about this and other microbial life forms from the Rhynie chert, it will be possible to offer more detailed hypotheses that can be used in association with those described from modern communities to more accurately depict the role of fungi and their interactions with other organisms in the ecology and evolution of non-marine paleoecosystems.

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Chapter X: Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-yr-old Rhynie chert.

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ORIGINAL ARTICLE

Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-year-old Rhynie chert

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Abstract *Scutellosporites devonicus* from the Early Devonian Rhynie chert is the only fossil glomeromycotan spore taxon known to produce a germination shield. This paper describes a second type of glomeromycotan spore with a germination shield from the Rhynie chert. In contrast to *S. devonicus*, however, these spores are acaulosporoid and develop laterally in the neck of the sporiferous saccule. Germination shield morphology varies, from plate-like with single or double lobes to tongue-shaped structures usually with infolded margins that are distally fringed or palmate. Spore walls are complex and appear to be constructed of at least three wall groups, the outermost of which includes the remains of the saccule. The complement of features displayed by the fossils suggests a relationship with the extant genera *Ambispora, Otospora, Acaulospora* or *Archaeospora*, but which of these is the closest extant relative cannot be determined. The acaulosporoid spores from the Rhynie chert document that this spore type was in existence already ~400 mya, and thus contribute to a more complete understanding of the evolutionary history of the Glomeromycota. This discovery pushes back the evolutionary origin of all main glomeromycotan groups, revealing that they had evolved before rooted land plants had emerged.

Keywords Fossil fungi · Glomeromycota · Pragian-?earliest Emsian (Early Devonian) · Spore-saccule complex · Spore wall

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Introduction

The Glomeromycota is a monophyletic group that includes the arbuscular mycorrhizal (AM) fungi (e.g. Schüßler et al. 2001). Such apparent symbioses are known to have existed for at least 400 million years based on exquisitely preserved fossils from the Early Devonian Rhynie chert (e.g. Remy et al. 1994; Taylor et al. 1995, 2004, 2005; Krings et al. 2007). Although the Rhynie chert AM have been studied intensively, the reproductive biology of their fungal partners (AMF) remains largely unknown. Consequently, defining their exact systematic position is difficult.

Extant glomeromycotan fungi reproduce via apparently asexual spores formed singly in the soil, or in dense clusters

or sporocarps that may be above or below the ground surface. Morphology, colour, and wall composition of the spores are important features in species identification (e.g. INVAM; Walker 1983; Berch 1985; Morton 1988; Redecker and Raab 2006; Walker et al. 2007). There are three kinds of spore production among the AMF. The majority form chlamydospores by blastic inflation and thickening of a subtending hypha (glomoid). Another group produce large spores by initial production of a small bulbous base followed by blastic expansion, with or without the production of flexible inner wall components (gigasporoid). A third type of spore (acaulosporoid) is produced within an initial relatively thin-walled blastic saccule, either laterally or centrally in the narrowed saccule neck, or rarely completely filling the expanded saccule lumen.

Gigasporoid and acaulosporoid spores may possess a distinct mode of spore germination, in which germ tube formation is preceded by the development of a germination shield (INVAM; Walker and Sanders 1986; Spain 1992). There is considerable interspecific and intergeneric variation with regard to size and shape of the germination shield, which may range from small, simple coils to prominent, profoundly infolded/lobed structures (INVAM; Walker and Sanders 1986; Spain 1992; Oehl and Sieverding 2004). Both sporiferous saccules and germination shields have been used as supplementary characters in taxonomic considerations (e.g. Walker and Sanders 1986; Spain 1992; Franke and Morton 1994; Kramadibrata et al. 2000; Hafeel 2004).

While fossil evidence for acaulosporoid spores has been lacking to date, germination shields are known to occur in one fossil spore taxon from the Rhynie chert, described by Dotzler et al. (2006) as *Scutellosporites devonicus* Dotzler, M. Krings, T.N. Taylor & Agerer, and putatively related to the extant genus *Scutellospora* C. Walker & F.E. Sanders (Gigasporaceae, Diversisporales). Specimens of *S. devonicus* with a germination shield extending along the inner surface of the innermost recognisable layer of the structural spore wall were discovered in degraded aerial axes of the early lycophyte *Asteroxylon mackiei* Kidst. & W.H. Lang. The shield is subcircular or oval in outline and distinctly lobed or infolded along the margins. Subtending hyphae and other associative structures have not been observed in any of the *S. devonicus* specimens.

In this paper, we describe a second type of glomeromycotan spore with a germination shield from the Rhynie chert that occurs in axes of the rhyniophyte *Aglaophyton major* (Kidst. & W.H. Lang) D.S. Edwards. In contrast to *Scutellosporites devonicus*, however, these spores are borne laterally in the neck of a sporiferous saccule, which suggests a relationship with one of the extant acaulosporoid spore-forming genera currently named as *Kuklospora* Oehl & Sieverd. and *Acaulospora* J. W. Gerd. & Trappe (Acaulosporaceae, Diversisporales), *Otospora* Oehl, J. Panezuela & N. Ferrol (Diversisporaceae, Diversisporales), *Ambispora* C. Walker, Vestberg & Schuessler (Ambisporaceae, Archaeosporales), or *Archaeospora* Morton & Redecker (Archaeosporaceae, Archaeosporales).

Materials and methods

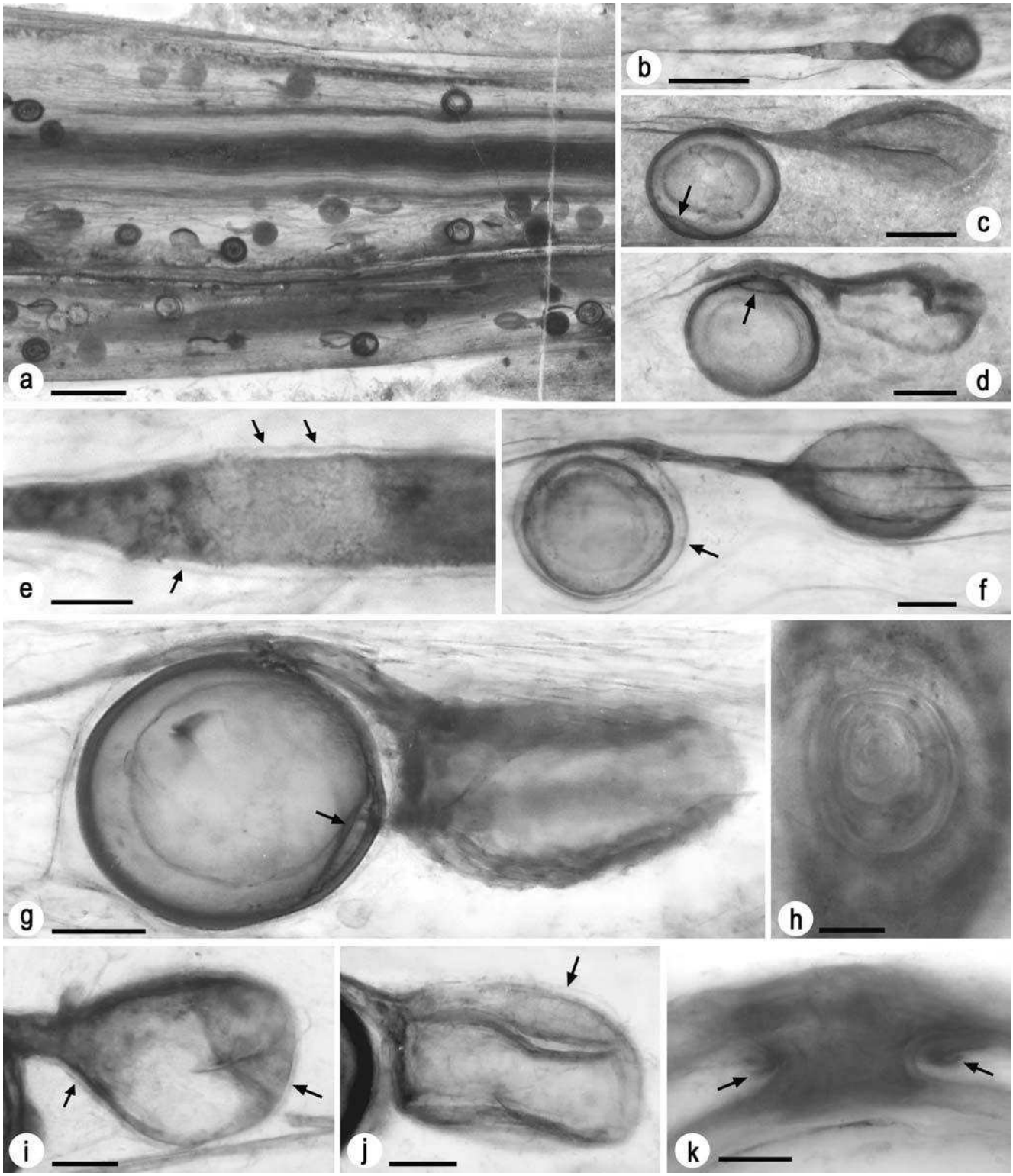
The Rhynie chert Lagerstätte is located in the northern part of the Rhynie outlier of Lower Old Red Sandstone in Aberdeenshire, Scotland, within a sequence of sedimentary and volcanic rocks. The cherts occur in the upper part of the Dryden Flags Formation, in the so-called Rhynie Block, a few hundred metres northwest of the village of Rhynie. The deposit consists of at least 10 fossiliferous beds containing lacustrine shales and cherts that are interpreted as a series of ephemeral freshwater pools within a hot springs environment. Preserved in the cherts are both aquatic facies and subaerial systems around the pools (i.e. soil and litter horizons with in situ plants); the latter became preserved as a result of temporary inundation in silica-rich water, or by groundwater percolating to the surface. Based on dispersed spore assemblages and redefinition of the Pragian–Emsian boundary by the International Union of Geological Sciences, Wellman (2006) and Wellman et al. (2006) have dated the cherts as Pragian–?earliest Emsian. Detailed information about the geological setting, sedimentology, and development of the Rhynie chert Lagerstätte can be found in Rice et al. (2002), and Trewin and Rice (2004).

Thin-sections were prepared by cementing a thin chert wafer to a glass slide and grinding it with silicon carbide powder until it could be examined in transmitted light (see Hass and Rowe 1999). Slides are deposited in the collection of the Forschungsstelle für Paläobotanik am Geologisch-Paläontologischen Institut, Westfälische Wilhelms-Universität, Münster (Germany), under accession numbers P3951–3958 and P3999.

Fig. 1 Acaulosporoid glomeromycotan spores from the Rhynie chert: Morphology. **a** Longitudinal section through a degrading *Aglaophyton major* axis containing numerous spore-saccule complexes in the cortex. Slide P3967. *Scale bar*=1.0 mm. **b** Immature saccule; note distinctly widened neck region. Slide P3967. *Scale bar*=200 µm. **c,d** Mature spore-saccule complexes with germination shield visible in the spores (*arrows*), and ridges (*white arrow* in Fig. 2c) extending along the saccule. Slide P3959. *Scale bars*=150 µm. **e** Detail of Fig. 2b, focusing on the neck region; note outer, colourless wall layer of saccule neck (*arrows*). *Scale bar*=30 µm. **f** Spore–saccule complex showing outer wall group (*arrow*) and ridges on the saccule. Slide P3966. *Scale bar*=100 µm. **g** Mature spore–saccule complex with a germination shield (*arrow*) and eccentric position of spore lumen. Slide P3968. *Scale bar*=100 µm. **h** Plan view of point of spore attachment, showing pattern of eccentric circles. Slide P3965. *Scale bar*=20 µm. **i** Saccule showing more or less intact outer wall layer (*arrow*). Slide P3966. *Scale bar*=100 µm. **j**Outer saccule wall layer appearing as bright amorphous coating (*arrow*). Slide P3968. *Scale bar*=100 µm. **k** Spore attachment showing confluence of inner neck wall layer and wc2 of outer wall group; note wc1 terminating around the spore base (*arrows*). Slide P3964. *Scale bar*=30 µm

Description

The glomeromycotan spores described below occur in large numbers in cortical tissues of several partially degraded axes of the early land plant sporophyte *Aglaophyton major* (Fig. 1a), but not in the surrounding chert matrix. Spores are borne laterally within the neck of a thin-walled, bulb-like saccule. More than 1,000 spore-saccule complexes were examined; approximately 10% of the specimens contain well-preserved germination shields. Wall structures



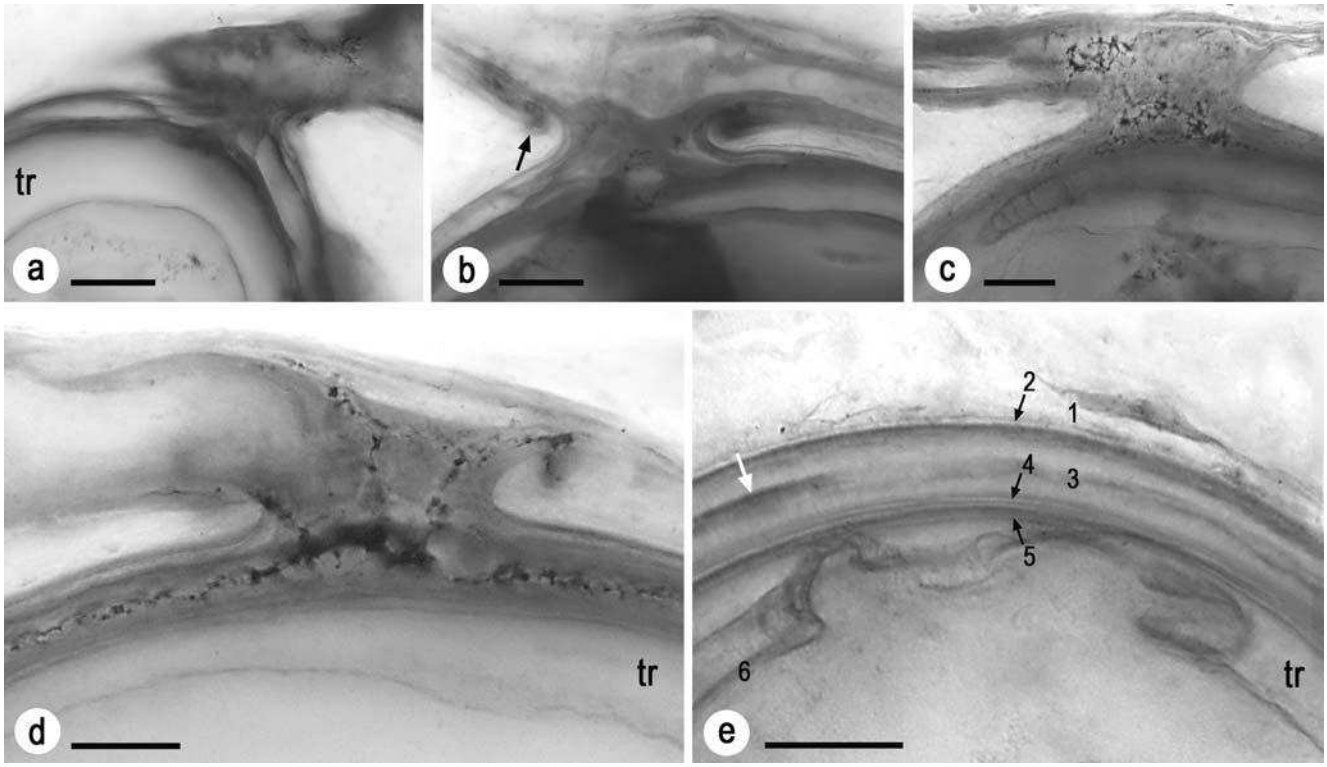


Fig. 2 Acaulosporoid glomeromycotan spores from the Rhynie chert: Morphology. **a–c** Spore attachment; note eccentric position of spore lumen in **a**, wc1 terminating around the spore base in **b** (*arrow*), and germination shield in **c**. Slides P3966 (**a**), P3961 (**b**), and P3958 (**c**). *Scale bars*=50 μm (**a**) and 30 μm (**b,c**). **d** Attachment of spore with intact outer wall group. Slide P3968. *Scale bar*=20 μm. **e** Spore with a germination shield; wc1 sloughing; *numbers* indicate the wall components and the *white arrow* indicates the irregular dark layer (possibly a split between the laminae), *tr*translucent region. Slide P3958. *Scale bar*=10 μm

are described as being composed of wall groups (WG) consisting of wall components (wc), numbered sequentially from the exterior (Walker & Vestberg 1998).

Saccule The saccules appear as long-necked balloon-shaped structures up to 700 μm long. The elongated neck is approx. 42 μm wide basally, enlarging over a distance of up to 430 μm, to 72 μm wide at the point where it expands blastically to become globose to ovoid, 200–450 μm long and 330 μm wide. Its wall is smooth, up to 7 μm thick, and is double, with a persistent inner component (wc2), up to 2 μm thick, and on about 60% of specimens a thick (5 μm) outer component (wc1), lacking or present only as an amorphous coating on the remaining specimens, and

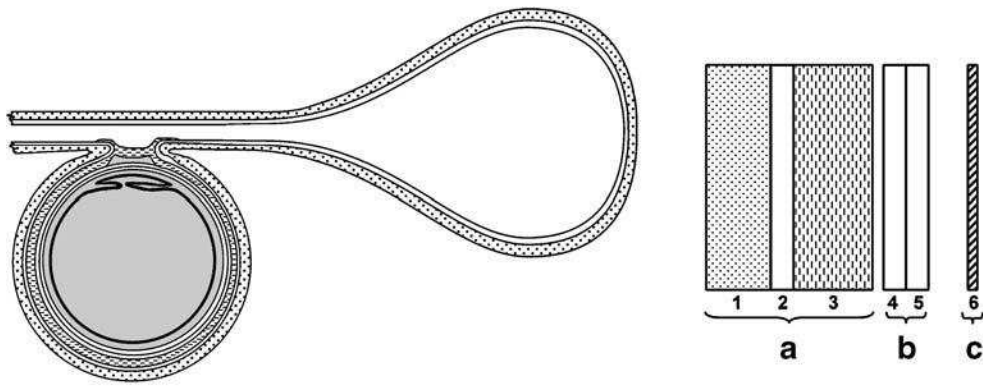


Fig. 3 Stylised illustration of the saccule and spore wall structure, and murograph of the spore wall structure interpretation from an acaulospore and sporiferous saccule from the Rhynie chert. *Shading* in the illustration is intended only to clarify the separate components. The murograph shading follows the patterns established by Walker (1983). The nature of the inner two components of WG2 could not be related to present day descriptions, and therefore have not been shaded

therefore interpreted as evanescent in the sense of Walker (1983). Most of the empty saccules are folded to various degrees where they have apparently collapsed (Fig. 1c,d,f,j). The distance between the point of spore attachment and expansion of the saccule ranges from 140 to 230 µm. The saccule wall continues, mainly seen as wc2 after wc1 has disintegrated (Figs. 1k and 2b), to become a component of the outer spore wall group.

In some specimens, the intact saccule wall components continue around the spore to form the outer component of the outer wall group (Figs. 1f and 2a), whereas in others only wc2 can be seen beyond the point of spore development (Figs. 1k - arrows, and 2b - arrow), and in yet others, little or no evidence of the saccule neck remains. The region of spore attachment forms a subcircular to oval scar in plan view, 40–55 µm in diameter, sometimes with an irregular concentric or eccentric subcircular to ovoid pattern in plan view (Fig. 1h) that is not recognizable in lateral view (Figs. 1k, 2a–c and d) and perhaps represents the individual laminae in the structural spore wall. In some specimens, there is an occlusive thickening between the saccule neck and the spore (Fig. 2b). A few specimens consist of small saccules (<200 µm long) lacking spores (Fig. 1b).

Spore The wall structure of the acaulospore is complex and cannot be determined with certainty from fossils, but we suggest that it consists of three major parts (Fig. 3). There is an outer group consisting of the lateral expansion of the saccule neck, and an adherent laminated component. This encloses a middle group of two thin, adherent components, enclosing an innermost wall group of a single thin apparently flexible component. Following current taxonomic convention, this entire complex structure (originally described by Gerdemann and Trappe 1974 as an 'azygospore') will be referred to simply as a spore (acaulospore), although its true nature (perhaps a sporangiole) remains undetermined.

The outer wall group (Fig. 3 - a) is composed of the two saccule wall components (wc1 and wc2) adherent to what appears to be the main structural spore wall (wc3). Wall component 1 (Fig. 3 - 1) is ephemeral (evanescent in the sense of Walker 1983). In some specimens (presumably immature spores), it forms a distinct layer, 5–33 µm thick (Figs. 1f - arrow, and 2a,d), which is identical to, and confluent with, the outer wall layer of the saccule neck (Fig. 2a,d). In other spores, however, it is partially disintegrated and appears as an amorphous coating (Fig. 2e). In still other spores, wc1 is lacking (interpreted as having disintegrated completely) (Fig. 2c), although remnants of it may still be present on the saccule neck (Figs. 1k and 2b). Component 2 (Fig. 3 - 2) is persistent, 2–4(–5) µm thick, and confluent with the inner wall layer of the saccule neck. The third component (Fig. 3 - 3) is up to 24 µm thick and appears to be formed of several layers (possibly laminated in the sense of Walker 1983). It appears to form de novo within the saccule wall, resulting in the thickening and persistence illustrated in Fig. 2b, d. In transmitted light, an irregular, narrow dark layer (Fig. 2e - arrow) is sometimes present within the thicker, light-coloured part of this wall component.

Wall group 2 (Fig. 3 - b) appears to consist of a pair of adherent components, wc4 and wc5 (Fig. 3 - 4 and 5), each 1–3 µm thick (Fig. 2d,e). These appear to be flexible, but it is not possible to determine their nature in relation to modern day species descriptions of members of the acaulosporoid Glomeromycota.

A translucent region (Figs. 1c,d,f,g and 2 - tr), up to 50 µm wide, occurs between the inner surface of WG2 and the outer boundary of the spore lumen formed by WG3, which consists of a single thin (<1 µm), smooth or slightly wrinkled and membrane-like component (wc6) (Figs. 1g, 2a,c–e, 3 - 6, and 4a). In most spores, the translucent region has a consistent width (e.g. Fig. 1c,d,f), but in some specimens, it is eccentric (Figs. 1g and 2a). Within the translucent region, small groups of tiny spherules (perhaps fungal spores) may occur (Fig. 4a - arrows), and in some spore, lumina, narrow hyphae are present.

Germination shield The germination shield is formed by extrusion of wc6, resulting in an apparent aperture through the membrane-like layer. It is adpressed, presumably by turgor pressure of the spore contents during life, to the outer surface of the spore lumen, and extends along the inner surface of the wc5. The aperture, more or less circular and 25–57 µm in diameter, appears to be surrounded by narrow thickened folds, each 2–4 µm wide (Fig. 4c - arrows,e). One spore has two apertures and two germination shields (Fig. 4f).

The size and shape of the germination shield, as well as the extension of the structure along the inner surface of wc5 vary. In a few specimens, the shield appears as a small, collar-like structure surrounding the aperture (Fig. 4b), while in others the collar-like structure is flared or has one to several marginal lobes and infoldings (Fig. 4d,e). In most specimens, the lobing and infolding is more profound (Fig. 4j), with one of the lobes sometimes enlarged to form an elongate, tongue-shaped extension, which is sparsely lobed along the lateral margins and deeply fringed or palmately lobed distally (Fig. 4k). In yet other specimens, only a tongue-shaped, distally lobed extension is present, but the lobed portion around the aperture is lacking (Fig. 4g–i). The largest germination shields occupy most of the inner surface of the middle wall group in plan view.

Discussion

Within the 400-million-year-old Rhynie chert are arbuscular mycorrhizae that were produced by glomeromycotan fungi

(Remy et al. 1994; Taylor et al. 1995, 2005; Helgason and Fitter 2005). An exact systematic placement of the fungal partners, however, has not been possible to date; the only glomeromycotan fungus from the Rhynie chert that has tentatively been related to a modern genus and family based on spore morphology is *Scutellosporites devonicus* (Dotzler et al. 2006). The data presented in this paper provide another opportunity to compare fossil and extant spores as a basis for evaluating the systematic affinities of an Early Devonian glomeromycotan fungus.

Morphology

The fossil spore-saccule complexes are relatively uniform with regard to overall morphology, although there is variation in wall structure and germination shield morphology.

Wall components 1 and 2 (i.e. the saccule wall) are variable in that wc1 is evident as a distinct layer in some specimens (Figs. 1e, f and 2a,d), appears to be disintegrating in others (Figs. 1j, 2e and 3 - 1), and is absent, or exists only as small fragments in still others (Figs. 1c,d and 2c). We conclude from this that it is an evanescent wall component.

Wall components 3–5 are the best preserved in all spores (Fig. 2e), representing the main structural exospore-like wall of the acaulospore and a second wall group of paired more or less flexible components forming an apparent mesospore. The nature of the dark layer seen in some specimens (e.g. Fig. 2d,e - white arrow) cannot be determined, but it may represent a split between laminae, or a preservational artefact formed during fossilisation and/or diagenesis. The two components of WG2 (Fig. 3 - b) are continuous, and appear to be tightly adherent. They thus can be considered to form a mesospore-like structure within the more rigid (protective?) structural spore wall. The translucent region (Figs. 1g and 2a,d,e - tr) between the inmost layer of the middle wall group and the spore lumen seems to be an artefact caused by shrinkage of the innermost wall component, possibly as a result of plasmolysis caused by the assumed hypertonic nature of the infiltrating mineralised water. A similar effect has been shown in a recently described species, *Diversispora celata* (C. Walker, Gamper & Schuessler) (Gamper et al., submitted), and commonly occurs when modern glomeromycotan spores are mounted in polyvinyl-alcohol lacto-glycerol (C. Walker, unpublished). The innermost wall group (Fig. 3 - c) apparently consists of a single component, and occurs as a separate entity, constituting a kind of endospore (see Walker et al. 2004 for a discussion of similar wall structure and terminology in complex glomeromycotan spores).

The variation in germination shield morphology is possibly related to their development stage in particular specimens. The apparently rod-shaped thickenings around the aperture connecting the germinations shield with the spore lumen probably are due to thickening of creases or invaginations in the wall component (Fig. 4c).

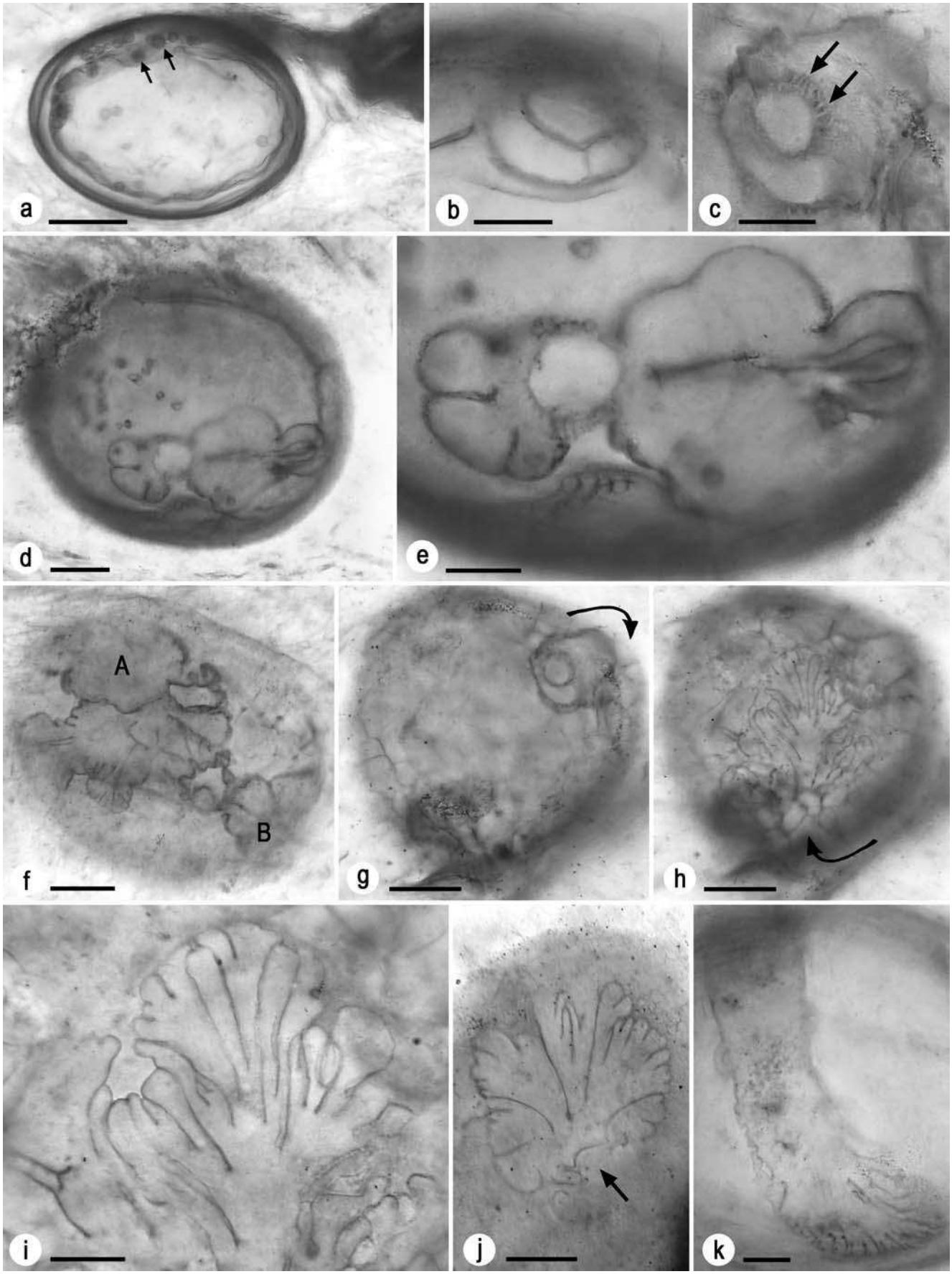
Fig. 4 Acaulosporoid glomeromycotan spores from the Rhynie chert: Germination shield. **a** Spore containing a parasite (chytrid?) in translucent region (*arrows*). Slide P3962. *Scale bar*=100 μm. **b** Small germination shield in oblique surface view. Slide P3956. *Scale bar*=30 μm. **c** Rod-shaped structures (*arrows*) around aperture (detail of **g**). *Scale bar*=30 μm. **d** Spore with germination shield; note fungal infection (small spherules) in the spore. Slide P3952. *Scale bar*=50 μm. **e** Detail of **d**, focusing on germination shield; note rod-shaped structures around aperture. *Scale bar*=30 μm. **f** Spore with two apertures and two irregularly lobed germination shields (*A* and *B*). Slide P3956. *Scale bar*=50 μm. **g,h** Tongue-shaped germination shield in two different focal planes: **g** focuses on the aperture (*arrow* indicates direction of growth), while **h** focuses on the palmately lobed, distal portion of the shield (*arrow* indicates direction of growth). Slide P3951. *Scale bars*=50 μm. **i** Detail of **h**, showing the palmately lobed portion of the shield. *Scale bar*=20 μm. **j** Lobed germination shield (*arrow* indicates aperture). Slide P3999. *Scale bar*=30 μm. **k** Distally fringed tongue-shaped portion of a germination shield. Slide P3957. *Scale bar*=30 μm

Germination shields in the fossil spores may appear as small, collar-like structures around the aperture (Fig. 4b), or as prominent, tongue-shaped structures that are fringed or palmately lobed distally (Fig. 4g–i). Other shields are plate-like and marginally lobed or infolded (Fig. 4d–f,j) in some of which one of the lobes is enlarged into a tongue-shaped extension. One particular feature is the bi-directional development of the shield as it emerges through the aperture (Fig. 4d,e). Morphological variation has been reported in shields of extant *Scutellospora* species, but no detailed study of germination shield ontogeny exists. Consequently, it is impossible to determine the cause of this variation. It is also possible that more than one species with acaulosporoid spores co-occurred in the *Aglaophyton major* axes, but the fossils do not display other structural differences from which individual species could be distinguished. The differences in spore wall structure are probably developmental or preservational, and they do not coincide with the differences in germination shield morphology. The simplest explanation is that the shield variation represents a combination of normal intraspecific variability and different stages of development.

Affinities

The most important diagnostic feature of the fossils reported here relates to the consistently acaulosporoid nature of the spores (Figs. 1c,d,f,g and 4a). This feature clearly distinguishes the spores described in this paper from *Scutellosporites devonicus*, the only other Rhynie chert glomeromyotan spore known to produce a germination shield (Dotzler et al. 2006).

Among the extant genera that contain species with a germination shield, acaulosporoid spores occur in *Kuklospora* and *Acaulospora* as well as *Ambispora* and *Archaeospora.*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The germination characteristics of the acaulosporoid *Otospora bareai* J. Panezuela & N. Ferrol, the sole species in the genus, are not described in its protologue, and the type material kindly made available for study by the curator at ETH shows no evidence of germination shield formation. However, in *Kuklospora*, the spores are produced centrally within the saccule neck, whereas in *Acaulospora*, *Ambispora* and *Archaeospora trappei* (R.N. Ames & Linderman) J.B. Morton & D. Redecker (the sole species in *Archaeospora*; see Spain et al. 2006) spore production is shifted laterally in the saccule neck (Hafeel 2004; Muthukumar et al. 2005; Sieverding and Oehl 2006), the condition most like that in the fossils. The spores of *Acaulospora* and *Archaeospora* are sessile or possess a collar or short pedicel (<27 µm long according to Walker et al. 1984), while *Ambispora* spores may arise from a distinct and persistent pedicel that can be more than 50 µm long (Walker et al. 2007). Spores of *Otospora* (Palenzuela et al. 2008), which bear a striking similarity to those of *Ac. nicolsonii* C. Walker & F. E. Sanders, are most similar, in that the saccule neck and the outer spore wall can be both contiguous and persistent. Some of the fossil spores are sessile, but others have a thickened, persistent saccule neck (Fig. 2a–d). Thus, from the manner of acaulospore development, *Acaulospora* or *Archaeospora* are least likely, and *Ambispora* or *Otospora* are most likely to be extant relatives of the fossil.

Although the fossils do not permit an exact description of the spore wall, the layering and composition appears to be quite well preserved, and some aspects are similar to those of modern acaulosporoid spores. The evanescent outer wall, which occurs in the fossil, has been described in several extant genera, including *Acaulospora*, *Archaeospora*, *Ambispora*, *Glomus* Tul. & C. Tul., *Geosiphon* (Kütz.) F. Wettst., *Otospora* and *Scutellospora* (INVAM; Walker 1983; Morton and Benny 1990; Schüßler et al. 1994; Walker et al. 1998, 2007; De Souza et al. 2005), of which *Acaulospora*, *Archaeospora*, *Ambispora* and *Scutellospora* also produce germination shields (see below), but is unlikely to be homologous among these diverse groups. In *Scutellospora*, an evanescent outer wall is only known in the ornamented species *S. spinosissima* C. Walker & Cuenca, *S. reticulata* (Koske, D.D. Mill. & C. Walker) C. Walker & F.E. Sanders and *S. cerradensis* Spain & J. Miranda (Walker et al. 1998; De Souza et al. 2005), whereas it is a typical feature of *Acaulospora*, *Archaeospora, Ambispora* and *Otospora* (Stürmer and Morton 1999; Morton and Redecker 2001; Spain et al. 2006; Palenzuela et al. 2008).

It has been suggested that *Archaeospora* and *Ambispora* differ from *Acaulospora* and *Scutellospora* in the absence of an obvious laminated wall (Spain 2003; Spain et al. 2006). However, Spain (2003) and Walker et al. (2007) have noted that this wall probably occurs in spores from all four genera. Moreover, such a wall component is clearly indicated for *Otospora bareai* and what appears to be a laminated wall is also present (Fig. 2e). In *Archaeospora*, the spore wall is thin (total wall thickness up to 6 µm) and the layering is difficult to discern, having been described as possessing up to six layers including four (presumably flexible) inner components that can be distinguished in water mounts (Spain 2003), but not in the most commonly used mountant for glomeromycotan spore studies, polyvinyl-alcohol lacto-glycerol (Walker et al 2007). Since the fossil spores are thick-walled (total wall thickness 30–60 µm, translucent region excluded), total wall thickness and wall composition argue against a closer relationship of the fossil to *Archaeospora*, at least based on the nature of the walls at the time of fossilisation. In addition, spores in *Archaeospora* are distinctly smaller (e.g. max. 100×70 µm according to Ames and Lindermann 1976; 40–80 µm in diameter according to Morton and Redecker 2001) than the fossil spores, which are up to 348 µm in diameter. A "beaded" wall, which would indicate a closer relationship of the fossil with *Acaulospora*, has not been observed in any of the specimens. However, these delicate structures would probably not be preserved in a recognisable form.

Germination shields have been recorded for members of the extant genera *Scutellospora*, *Pacispora* Oehl & Sieverd., *Acaulospora*, *Kuklospora*, *Ambispora* and *Archaeospora* (e.g. Koske and Walker 1986; Walker and Sanders 1986; Spain 1992, 2003; Oehl and Sieverding 2004; Spain et al. 2006). The germination shields of several *Scutellospora* species (e.g. *S. hawaiiensis* Koske & Gemma) and *Ambispora appendicula* (Spain, Sieverd. & N.C. Schenck) C. Walker are comparable to the multi-lobed germination shields lacking a tongue-shaped extension seen in some of the fossil spores (e.g. compare Fig. 4d,e,j with Koske and Gemma 1995: fig. 12, and Spain et al. 2006: fig. 5). Conversely, the tongue-shaped extension (e.g. Fig. 4k) present in some of the specimens appears to be similar to the initial tongue-shaped projection documented for the shield in *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders by Walker and Sanders (1986: fig. 3), and would, to a certain extent, also compare to a coiled germination shield (e.g., in *Acaulospora scrobiculata* Trappe and *Scutellospora projecturata* Kramad. & C. Walker) if they were uncoiled. There is one interesting parallel between the fossil and the extant *Archaeospora trappei* and *Scutellospora biornata* Spain, Sieverd. & S. Toro. These forms are known to occasionally produce two germination shields. In *S. biornata*, two shields have been observed together with up to six apertures (Spain et al. 1989). In *A. trappei*, the two germination shields are formed by branching of the initial outgrowth from the spore lumen (Spain 2003) in a manner similar to the bilobed shield in the fossils (Fig. 4d,e), although one fossil

spore has two germination shields formed by two separate apertures (Fig. 4f).

Although the fossil germination shields are extraordinarily well-preserved, and thus their morphology can be documented in great detail, this feature cannot at present be used to suggest affinities of the fossil spores because the nature of the variations in shield morphology cannot be fully determined. When the currently described genera are considered, the formation of acaulosporoid spores with some kind of germination shield appears to be a symplesiomorphy, as indeed do the production of evanescent and laminated wall components. However, the lack of any evident beading on the innermost wall component argues against closeness to *Acaulospora*, the majority of which have such a component. The thickened and somewhat persistent connection between saccule and spore, along with the wall structure of a main structural wall component that shows only a little sign of lamination coupled with a complex infolded germination shield, indicate a probable affinity to the archeaosporalean genus *Ambispora*. Morphologically, the spores of *Otospora bareai* are also very similar, particularly in the thickening of the saccule neck and the spore wall structure which is very similar to that of the fossil spores. However, no germination shield is reported for that species, or for the morphologically similar species, *Acaulospora nicolsonii*. The bi-lobed nature of some of the germinations shields suggests an affinity with *Archaeospora*.

Taking all the characters into consideration, it is therefore impossible to indicate a reliable affinity with any particular acaulosporoid spore producing extant group.

Conclusions

The discovery of glomeromycotan fungi associated with the earliest structurally preserved land plants does not answer the question as to when these associations first became established or the precise appearance of the ancestral fungal partner or partners. Nevertheless, the fact that the life history of some of these early land plants included two distinct generations, both associated with AMF (see Taylor et al. 2005), implies that this mutualism was both widespread and a significant factor in driving the evolution of early terrestrial ecosystems. The Rhynie chert fossils now push back the evolutionary origin of all main spore types in the Glomeromycota to a time before the evolution of true roots, and thus suggest their symbiotic nature predates root formation and the evolution of the mycorrhiza (= fungus-root). As we have noted earlier in this paper, defining the precise relationships of the early mycorrhiza-forming fungi continues to remain an important area of research. In spite of the gaps in our understanding, the discovery and documentation of features like the spore-saccule complexes and germination shields in Rhynie chert land plant-fungal associations may not only provide a benchmark with which to consider the evolution of certain fungal characters, but may also ultimately assist in framing the broader discussion about fungal diversity in time and space.

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Chapter XI: Microfungi from the upper Visean (Mississippian) of central France: Chytridiomycota and chytrid-like remains of uncertain affinity.

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Microfungi from the upper Visean (Mississippian) of central France: Chytridiomycota and chytrid-like remains of uncertain affinity

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ABSTRACT

A diverse assemblage of eucarpic and apparently holocarpic chytrids, and chytrid-like remains of uncertain affinity is preserved in late Visean (Mississippian; ~330 Ma) cherts from Combres (Roanne area) and Esnost in central France. The evidence is primarily composed of various types of (resting) spores, as well as epibiotic and endobiotic (putative) zoosporangia that occur in/on solitary unicells, peronosporomycetous oogonia, (degrading) vascular plant tissues (i.e. xylem, periderm, cortical parenchyma), and various plant and fungal spores. Vegetative parts such as tenuous filaments or rhizomycelia in organic connection are rarely preserved. Host responses possibly linked to chytrid infection occur in the form of two different types of callosities, some with a distinct penetration canal, in lycophyte xylem and periderm, as well as in fungal spores. We suggest that the majority of chytrids and chytrid-like remains preserved in the Visean cherts belonged to a community of saprotrophic microorganisms that functioned in the decomposition of organic matter. Only a few forms appear to have been parasites. The Visean cherts from France provide a rare opportunity to examine the diversity of Chytridiomycota in a Carboniferous ecosystem.

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1. Introduction

The Chytridiomycota (chytrids) is a monophyletic group of morphologically simple microfungi that reproduce with motile spores (zoospores) which typically have a single, posteriorly directed flagellum (Barr, 2001; James et al., 2006). The group is comprised of *ca.* 1250 described species (Shearer et al., 2007), which are effective as bioeroders, decomposers, parasites, disease causative agents, and mutualists (Dix and Webster, 1995), but phylogenetic studies indicate that there is a tremendous unrealized biodiversity across all lineages (e.g., Letcher et al., 2006, 2008a,b). Chytrids occur in a wide variety of habitats, ranging from dry sclerophyll forest soils (Letcher et al., 2004) to the guts of various mammals (e.g., Van der Giezen, 2002); many forms are aquatic (Barr, 2001). Understanding the biological and ecological versatility of extant chytrids, together with an appreciation of the evolutionary history and paleodiversity of these organisms, including the roles they have played in biological and ecological processes in the past, represents a critical component in elucidating the significance of fungi for the evolution and sustainability of ancient ecosystems.

Molecular clock estimates suggest that the first chytrids occurred on Earth during the Precambrian, some 1.5 Ga ago, perhaps even earlier (Heckman et al., 2001). There are several reports on Precambrian microfossils that have been interpreted as, or compared to, chytrids (e.g., Burzin, 1993; Belova and Akhmedov, 2006), but none of these are conclusive (see Butterfield, 2005). The oldest unequivocal fossil chytrids come from the Early Devonian Rhynie chert, and include a variety of holocarpic and eucarpic forms as parasites of land plants, charophytes, and other fungi (Kidston and Lang, 1921; Harvey et al., 1969; Illman, 1984; Taylor et al., 1992a,b; Hass et al., 1994; Krings et al., 2007b, in press). It is remarkable that these fossils are not only morphologically similar to modern chytrids, but also illicit the same host responses. Other fossil chytrids have been described as parasites of Carboniferous spores, pollen grains, and seeds (e.g., Renault and Bertrand, 1885; Oliver, 1903; Millay and Taylor, 1978), and a Permian *Synchytrium*-like endoparasite has been documented in permineralized plant tissues from Antarctica (García-Massini, 2007). *Rhizophidites triassicus* Daugherty is a parasite on certain Triassic spores that resembles the extant *Rhizophydium pollinis-pini* (A. Braun) Zopf, a parasite of pine pollen (Daugherty, 1941), and *Entophlyctis willoughbyi* Bradley from the Eocene has been compared to the extant *E. lobata*

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Willoughby et Townley (Bradley, 1967; but see Sherwood-Pike, 1988: 276). In addition, there are several trace fossils in the form of microborings that have been attributed to marine endolithic chytrids (e.g., Radtke, 1991; Wisshak et al., 2008). Although all these reports have been important in documenting the geologic history of Chytridiomycota, thus far only the Rhynie chert has provided insights into the diversity and significance of this group of fungi in an ancient ecosystem (Taylor and Taylor, 2000; Taylor et al., 2004).

Less well studied than the Rhynie chert, but also containing well-preserved terrestrial plants and microorganisms, including chytrids, are the late Visean (~330 Ma) cherts from Combres (Roanne area) and Esnost in central France. While the plants preserved in these deposits have been documented in detail (e.g., Renault, 1896; Galtier, 1970, 1971), most of the microorganisms remain understudied (see Taylor et al., 1994; Krings et al., 2005, 2007a; Dotzler et al., 2008). Several (putative) chytrids inhabiting land plant tissues were described by Renault (1894, 1895, 1896, 1900, 1903) and Krings et al. (2007a). Detailed illustrations, however, are lacking, and the diversity of these organisms and the roles they played in the Visean ecosystem have not been critically evaluated to date.

Here we present a (re-)evaluation and detailed photographic documentation of the chytrids and chytrid-like remains of uncertain affinity preserved in the Visean cherts from central France based on the original thin sections prepared by Renault and co-workers. These organisms are far more abundant and diverse in these deposits than originally documented, and thus the Visean of France offers a rare view of the diversity, biology, and ecology of Chytridiomycota in a Carboniferous ecosystem.

2. Material and methods

The cherts containing these organisms come from the upper Visean (Mississippian [= Lower Carboniferous]) of Combres, situated approximately 12 km east of Roanne, and from the Autun basin at the locality of Esnost, about 10 km north of the city of Autun. Both sites are located in the northern part of the Massif Central, central France. The geological setting and paleoenvironment of the late Visean in the Roanne and Esnost areas have been interpreted as analogous (Galtier, 1971). Information on the geological settings can be found in Scott et al. (1984); for details on the preservation of fossils and a paleoecological reconstruction of the Visean wetland ecosystem at Esnost see Rex (1986). Rex (1986: Fig. 3) suggests that the landscape was represented by pools and small lakes surrounded by open, lycophyte-dominated swamp forest vegetation. The environments were dominated by active volcanism, and thus it is reasonable to conclude that the pools/lakes and surrounding forests represent unstable ecosystems, which perhaps existed for relatively short periods of time. The cherts, which originally formed in the pools and lakes (Rex, 1986), occur as loose blocks within rhyolitic tuffs, and were collected in cultivated fields or in stream sections.

The microfungi were identified in thin sections prepared by cementing a wafer of chert to a glass slide, and then grinding the wafer to a thickness sufficiently thin for examination in transmitted light (for details about the methodology, see Hass and Rowe, 1999). The thin sections (>1000 slides) belong to the Renault and Roche slide collections, which were prepared by B. Renault and co-workers during the late 19th and early 20th centuries, and are today housed in the Muséum National d'Histoire Naturelle (Laboratoire de Paléontologie) in Paris (France). Accession numbers are included in the figure captions; numbers preceded by REN refer to slides from the Renault collection, whereas numbers preceded by ROC indicate slides in the Roche collection. Thin sections were analyzed by using normal transmitted light microscopy equipment (Leica); images were taken with a Leica DFC-480 digital camera.

3. Results

Chytrids and chytrid-like remains of uncertain affinity occur in the chert matrix and in association with various other organisms, including solitary unicells, terrestrial plants, peronosporomycetes, and other fungi. The record is primarily composed of isolated parts or stages of the life cycle. Despite the exceptional preservation of the fossils, determining their systematic affinities often remains difficult because the incompleteness of the record places serious constraints on interpretation. As a result, only some of the structures can be assigned to the Chytridiomycota with confidence, while the affinities of others remain equivocal because they do not display diagnostic features of sufficient clarity to allow comparisons with extant chytrids and/or with other groups of fungi and fungi-like microorganisms. Nevertheless, all of these latter fossils are somehow reminiscent of structures seen in modern chytrids, and thus are included here as 'chytrid-like remains of uncertain affinity'.

3.1. Solitary unicells

Thin-walled unicells that occur in the chert matrix in large numbers often contain a single, slightly thicker-walled globose to ovoid structure characterized by a single, narrow, usually slightly darker, tube-like projection (Plate III, 4[arrows],5,6). The unicells are up to 25 µm in diameter, and have a smooth, delicate, irregularly undulating or wrinkled wall. The internal spheres are up to 17 µm long and 14 µm wide; their wall is smooth, unornamented, and less than 1 µm thick. The tube-like projection is up to 2 µm long and 2 µm wide, and does barely protrude above the wall of the host cell.

3.1.1. Remarks

The globose to ovoid structures are interpreted as zoosporangia of an endobiotic chytrid that formed holocarpic thalli lacking specialized vegetative structures. At maturity, zoospores were released through a narrow (?inoperculate) discharge tube. The fossils may be compared with zoosporangia of the modern genus *Olpidium* (A. Braun) J. Schröter, a widespread parasite of plants and animals (e.g., Kusano, 1936; Sahtiyanci, 1962; Raghavendra Rao and Pavgi, 1980; Glockling, 1998). The affinity of the unicells containing the zoosporangia remains uncertain. They may represent unicellular algae or algal resting stages (e.g., cysts or phycomata), or possibly vascular plant cells that became detached from the source tissue (perhaps cortical parenchyma) during tissue degradation.

It is interesting to note that nearly identical associations are known from the Lower Devonian Rhynie chert where they have been

Plate I. Late Visean Chytridiomycota and chytrid-like remains in (mega-)spores.

Fig. 1 *Sublagenicula nuda*-type lycophyte megaspore containing numerous spheres and tenuous filaments [arrow]; slide no. ROC-6/126; bar = 150 µm.
Fig. 2 Detail of Plate I, 1, focusing on several spheres; black arrows indicate fragments of subtending filaments, white arrows indicate putative discharge pores; bar = 50 µm.
Fig. 3 Cluster of spheres in the interior of a *S. nuda*-type lycophyte megaspore; slide no. ROC-5/98; bar = 150 µm.
Fig. 4 Detail of Plate I, 3, showing spheres; bar = 50 µm.
Fig. 5 *Lycospora*-type spore containing a single, spherical structure that probably represents a chytrid zoosporangium; slide no. ROC-2/29; bar = 20 µm.
Fig. 6 *Granulisporites*-type spore with putative chytrid zoosporangium attached to upper surface; slide no. ROC-1/1; bar = 10 µm.
Fig. 7 *Sublagenicula nuda*-type megaspore colonized by a large chytrid [arrow] in the wall; slide no. ROC-6/115; bar = 150 µm.
Fig. 8 Higher magnification of chytrid in the megaspore wall in Plate I, 7; bar = 20 µm.
Fig. 9 Detail of Plate I, 7, focusing on the spheres in the megaspore interior; bar = 50 µm.

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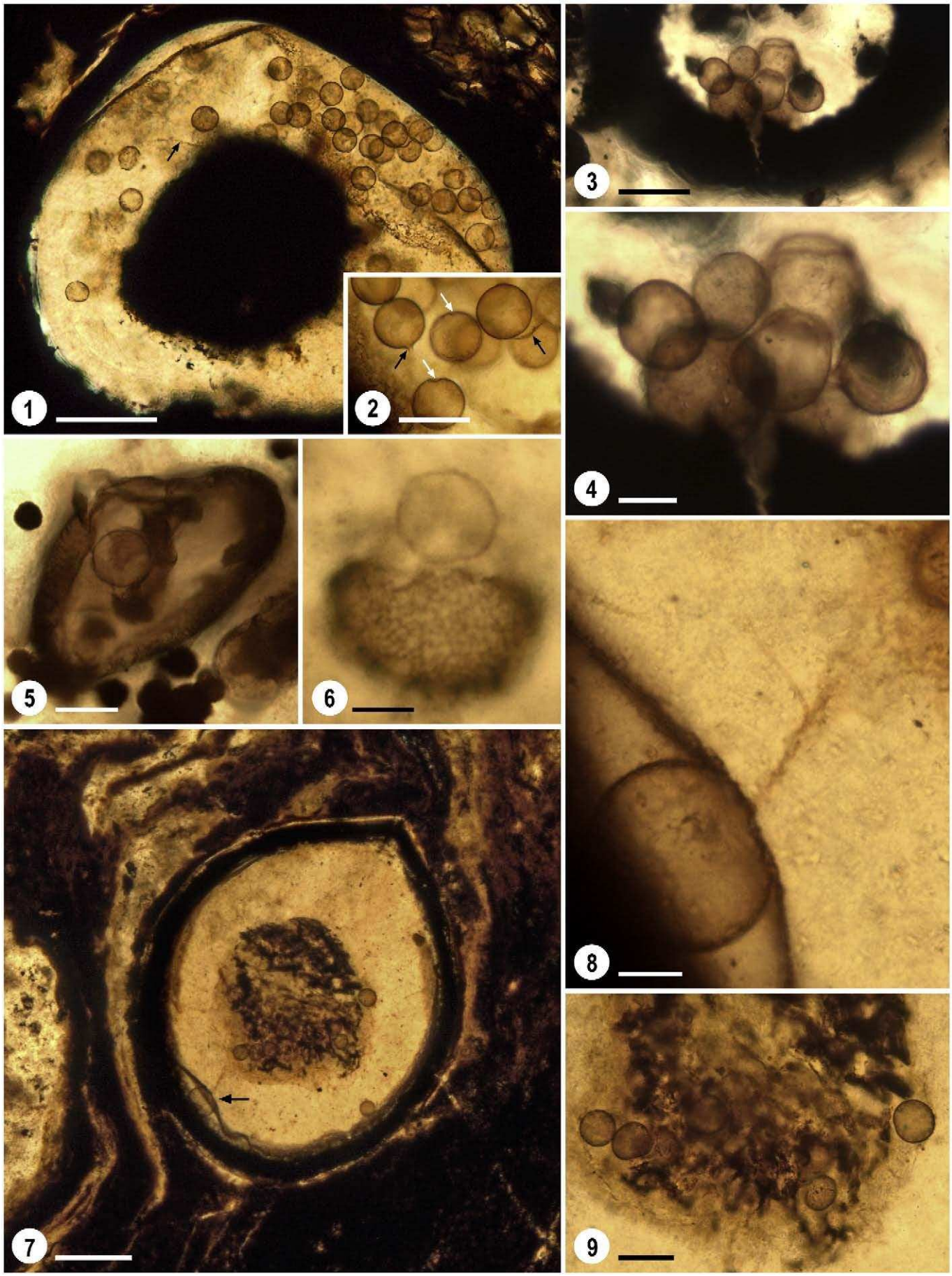

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interpreted as holocarpic chytrids in algal cells (Taylor et al., 1992b). The Rhynie chert fossils differ from the Visean specimens in that the host cells do not normally occur solitarily, but rather are arranged into *Pediastrum*-like cell clusters of two to eight cells, each infected by a chytrid. Moreover, the Rhynie chert host cells are typically characterized by distinct marginal lobes, which have not been observed in any of the Visean specimens. In addition, the Rhynie chert host cells do not occur in the chert matrix, but have been detected within aerial axes of the land plant *Horneophyton lignieri* (Kidston et Lang) Barghoorn et Darrah. The chytrid itself is nearly identical in size and shape to the forms from the Visean.

3.2. Land plant spores

Several lycophyte megaspores of the *Sublagenicula nuda*-type contain numerous spheres up to 58 µm in diameter (Plate I, 1–4); in some spores the spheres are loosely distributed in the lumen (Plate I, 1), whereas in others they are densely clustered (Plate I, 3,4). Some spheres are smooth-walled, but most display a faintly verrucose surface ornamentation. We are uncertain, however, whether this ornament represents a natural component of the spheres, or is a preservational artefact resulting from shrinkage of the spheres (and subsequent wrinkling of the wall) during fossilization. Many spheres have a short fragment of the narrow subtending filament or hypha still attached (Plate I, 2[black arrows]), and some show what appears to be a circular apical discharge pore (Plate I, 2 [white arrows], 3). Also present in the spore lumen are isolated fragments of filaments or hyphae between <1 and 2 µm wide (Plate I, 1 [arrow]).

Another *Sublagenicula nuda*-type megaspore is colonized by a relatively large chytrid (Plate I, 7[arrow]), which is one of very few examples from the Visean cherts where not only the generative portion (i.e. the zoosporangium) but also the rhizoidal system of the organism are preserved (Plate I, 8). The zoosporangium, which occurs between the separate inner wall layer and the remainder of the megaspore wall, is dorsiventrally compressed, smooth-walled, and 62 µm wide and ~50 µm high. Extending from the zoosporangium is a rhizoidal system that penetrates the inner wall layer and extends into the spore lumen (Plate I, 8). The rhizoidal system is at least 120 µm long, and appears to consist of a sparsely branched proximal portion and multi-branched distal unit. Several smooth-walled or delicately ornamented spherical structures, each up to 45 µm in diameter, occur in the megaspore lumen (Plate I, 9).

Attached to the surface of a single partially degraded fern spore (probably of the *Granulisporites*-type) is a smooth-walled spherical structure, which is 16 µm in diameter (Plate I, 6). Vegetative remains of this microorganism are not preserved. Other spores (probably assignable to the *sporae dispersae* taxon *Lycospora* J.M. Schopf, L.R. Wilson et R. Bentall) contain single spheres that are up to 20 µm in diameter and smooth-walled (Plate I, 5).

3.2.1. Remarks

Spherical structures similar to those seen in the *Sublagenicula nuda*-type megaspores (Plate I, 1–4) have previously been described from the Visean cherts of France as *Palaeomyces majus* and referred to the "Mucorinées" by Renault (1896: 441/442). We have included this form because it is also possible that they represent (immature) zoosporangia of a polycentric chytrid, perhaps a form that is comparable in basic structure to the extant *Cladochytrium* Nowakowski or *Nowakowskiella* J. Schröter (e.g., Marano et al., 2007). Spherical structures reminiscent of those in the *S. nuda*-type megaspores are also known to occur in large fungal spores from the Lower Devonian Rhynie chert; some of these spheres have been interpreted as resting stages of a peronosporomycete (Kidston and Lang, 1921), others as chytrid zoosporangia (Krings et al., in press).

The organism inhabiting the wall of the megaspore illustrated in Plate I, 7 is assignable to the Chytridiomycota with confidence based on morphology (Plate I, 8), which closely resembles that seen in many modern monocentric chytrids (e.g., species in the genera *Rhizophydium* Schenk and *Spizellomyces* D.J.S. Barr; see Karling, 1977; Chen and Chien, 1998). The smaller spheres in this megaspore (Plate I, 9) are similar to the spherical structures found in other *Sublagenicula nuda*-type megaspores based on overall shape and size (see above); the relationship between the chytrid colonizing the megaspore wall and the spheres in the megaspore lumen remains uncertain.

The sphere attached to the surface of a *Granulisporites*-type spore (Plate I, 6) is reminiscent of the zoosporangia seen in many extant chytrids that parasitize on spores or pollen grains (e.g., *Rhizophydium sphaerotheca* Zopf; see Pires-Zottarelli and Gomes, 2007: Fig. 26). The spheres inside *Lycospora*-type spores (Plate I, 5) lack diagnostic features, and thus their affinities remain uncertain.

There are several other records for fossil chytrids associated with Carboniferous megaspores and ovules/seeds. One of these is *Grilletia spherospermii* Renault et Bertrand, which occurs in the peripheral layers of the nucellus of *Sphaerospermum* Brongniart from the Pennsylvanian of Grand'Croix, France (Renault and Bertrand, 1885). Unfortunately, these authors did not provide illustrations with their description of *G. spherospermii*, and thus a comparison to our material is not possible. Chytrid-like fungi have also been recorded for *Conostoma*-type seeds from the Mississippian of Burntisland, Scotland, as well as from a variety of other Carboniferous ovules/seeds (Oliver, 1903). The precise affinities of most of these fossils cannot be determined. Fossil evidence for epibiotic chytrid zoosporangia attached to the surface of spores or pollen grains has been documented from the Devonian (Hass et al., 1994), Carboniferous (Millay and Taylor, 1978), and Triassic (Daugherty, 1941).

3.3. Plant tissues

The cherts contain numerous vegetative remains of lycophytes and ferns in various stages of fragmentation and degradation (Galtier,

Plate II. Late Visean Chytridiomycota and chytrid-like remains in lycophyte (*Lepidodendron*) xylem and periderm, including callosities in tracheids and periderm cells.

Figs. 1, 2 Cross section of primary wood showing putative chytrid zoosporangia attached to the tracheid walls [arrows in Plate II, 1]; slide no. REN-49/1106; bars = 50 µm.
Fig. 3 Detail of Plate II, 2, showing a zoosporangium; bar = 15 µm.
Fig. 4 Tracheid in cross section with hemispherical structures on the wall; slide no. REN-49/1106; bar = 20 µm.
Figs. 5 & 6 Sessile pear-shaped (conical) putative zoosporangia with a distal, cleft-like discharge opening; slide no. REN-50/1137; bars = 10 µm.
Fig. 7 Sessile putative zoosporangium with a distal discharge opening; slide no. REN-50/1137; bar = 10 µm.
Fig. 8 Stalked spherical putative zoosporangium; slide no. ROC-4/73; bar = 15 µm.
Fig. 9 Stalked (?apophysate) spherical putative zoosporangium with a distal discharge pore [arrow]; slide no. REN-50/1137; bar = 10 µm.
Fig. 10 Sessile spherical putative zoosporangium with a distal discharge pore [arrow]; slide no. REN-50/1137; bar = 10 µm.
Fig. 11 Stalked (?apophysate) spherical putative zoosporangium attached to a tracheid wall; ladder-like structures represent secondary thickenings of the tracheid wall; slide no. REN-49/1118; bar = 20 µm.
Fig. 12 Massive callosities in a periderm cell, arrow indicates central penetration canal; slide no. REN-49/1109; bar = 20 µm.
Fig. 13 Delicate [a] and large [b] callosities in periderm cells; slide no. REN-49/1109; bar = 20 µm.
Fig. 14 Variously sized callosities (in transverse section) lacking distinct penetration canals in a tracheid. Note the filament/hypha that appears to have outgrown the callosity [arrow]; slide no. REN-3/50; bar = 15 µm.
Figs. 15–17 Several views of callosities (in transverse section) in tracheids; note central penetration canals recognizable as dark spots; slide no. REN-3/50; bars = 10 µm (Plate II, 15,16) and 20 µm (Plate II, 17).

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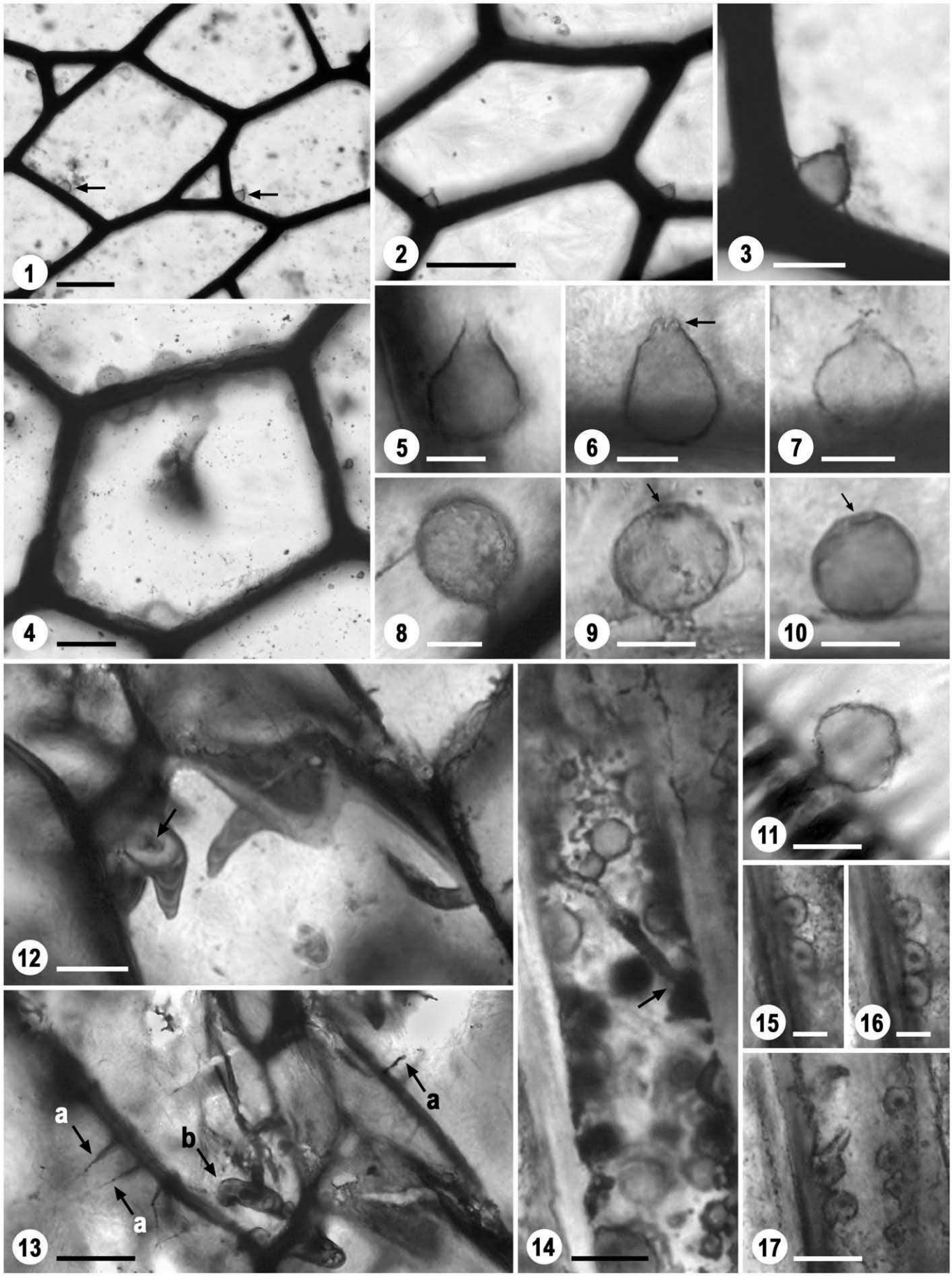

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1970). Sporadic evidence for the presence of microfungi and fungi-like microorganisms can be found associated with just about every plant fragment, but is especially abundant and diverse in lycophyte (i.e. *Lepidodendron esnostense* Renault and *L. rhodumnense* Renault) xylem and periderm (Krings et al., 2007a). Specific host responses of individual cells resulting from fungal interactions have to date only been recorded for *Lepidodendron* xylem (Plate II, 14–17) and periderm (Plate II, 12,13).

Indications of the presence of chytrids in lycophyte xylem and periderm primarily occur in the form of stalked and sessile, spherical and pear-shaped (conical) structures that are attached to the host cell walls (Plate II, 1–10; Plate III, 8). None show attachment to any extensive form of vegetative system. Spherical structures are variable in size and shape. One form is 6–20 μm in diameter, attached to the cell wall by a short stalk (or apophysis), and usually has a distal discharge pore ~2–3.5 μm in diameter (Plate II, 9[arrow], 11). In another form of comparable size and shape a stalk or apophysis is absent (Plate II, 7,10). Still another form is spherical or drop-shaped, ~30–50 μm in diameter, and attached to the wall by a relatively long (up to 20 μm) and narrow stalk (Plate II, 8; Plate III, 8). Discharge pores are absent in most of the latter structures. The second category of structures consists of unstalked, drop- or pear-shaped (conical) forms that are 10–20 μm high (Plate II, 2,3,5,6). Some appear to be closed; they usually possess a pointed tip that is relatively opaque (Plate II, 2,3). Others are open distally (Plate II, 5,6), and many possess an apical cleft surrounding the opening (Plate II, 6[arrow]). In addition, a single, relatively large pear-shaped structure, which occurs along the inside of a tracheid, extends from the tip of an aseptate filament or hypha (Plate III, 11). This structure is up to 60 μm long and composed of a relatively thick-walled basal sphere (30–40 μm in diameter) to which is attached a thinner-walled distal tube. The various types of spherical and conical structures usually are widely spaced within the plant tissue (e.g., Plate II, 1,2), but one transverse section contained a segment of xylem in which some of the cell walls were densely covered by hemispherical structures (Plate II, 4). We are uncertain as to whether these structures are microbial remains or some type of wall apposition.

Some *Lepidodendron* tracheids contain large numbers of small spore-like bodies and narrow filaments. The spore-like bodies, which appear suspended in the cell lumen (Plate III, 1) or perhaps attached to the cell wall (Plate III, 2), range from 5 to 15 μm in diameter, and possess a thick translucent wall and two, usually oppositely positioned, circular openings (Plate III, 3). The co-occurring filaments are <1 μm wide and appear aseptate.

Several tracheids (Plate II, 14–17) and periderm cells (Plate II, 12,13) in two *Lepidodendron* samples indicate host reactions in the form of conical callosities (also termed lignotubers or papillae). These structures represent inwardly directed, concentrically layered projections consisting of newly synthesized wall material that are formed by plant cells (but also by certain fungal spores; see below) in response to invading fungi. Callosities encase the invading fungal hypha or filament, and thus inhibit the extraction of nutrients from the host cell. Two types of callosities can be distinguished in the *Lepidodendron* samples: (1) a narrow form (Plate II, 13[arrows a]) that typically is straight, up to 25 μm long, proximally up to 4 μm wide, and does not show evidence of a penetration canal; and (2) a larger form (Plate II, 13[arrow b]) that may be straight or curved, up to 35 μm long by 15 μm wide, and usually contains a distinct central penetration canal, which is particularly evident in cross sections as a dark area in the centre (Plate II, 12[arrow],15–17). Longitudinal sections indicate the incremental growth of the large callosities (Plate II, 12). It is interesting to note that the narrow callosities have only been observed in periderm cells, while the larger type also occurs in tracheids. It is apparent that some of the tracheids were highly infected based on the large number and dense packing of callosities with or without a central penetration canal (Plate II, 14–17). Some specimens suggest that the fungal filaments/hyphae were able to outpace the growth of the callosity (Plate II, 14[arrow]). Finally, callosities are not uniformly distributed among the cells of the xylem and periderm, but rather occur sporadically in single cells or small clusters of adjacent cells that are surrounded by other cells lacking any evidence of structural alteration of their walls.

Other microfungal remains occur on/in degrading plant fragments that cannot be referred to *Lepidodendron* with certainty. Several structures with possible affinities in the Chytridiomycota occur on a small fragment of largely degraded (?cortical) tissue (Plate IV, 3). These structures are sessile (i.e. attached to the host cell walls), up to 25 μm wide (including rim), and 15 μm high; the wall is pustulose and appears to have been relatively robust. Basal apophyses or a rhizoidal system extending from the structures were not observed. Some are spherical to hemispherical and appear to be broadly attached (Plate IV, 3[arrow]), whereas others range from hat- to bowl-shaped, constricted at base, and have a wide distal opening surrounded by a slightly inrolled rim up to 3.5 μm wide (Plate IV, 4,5). Associated with these structures are circular, dorsiventrally compressed, saucer-shaped bodies, <20–23 μm in diameter, composed of a lense-shaped central area, ~14 μm in diameter and up to ~6 μm high. These structures possess a prominent equatorial rim (up to 9 μm wide) that is ornamented by radially oriented irregular striae (Plate IV, 6).

A single putative zoosporangium occurs on the outer surface of another largely degraded tissue fragment (Plate III, 9). The structure is broadly attached, 35 μm high and 20 μm wide, distally ruptured, and appears to have been at the time the contents were being released. The content consists of a mass of small granules. Because of the nature of the petrographic thin section, these structures could not be further examined even using oil immersion.

3.3.1. Remarks

Although both the stalked and unstalked spherical and conical structures attached to the cell walls in lycophyte wood and periderm morphologically correspond to the zoosporangia and resting spores produced by a variety of modern chytrids (see e.g., Sparrow, 1933; Sparrow and Dogma, 1972; Karling, 1932, 1968, 1977), we cannot discount that they may also represent peronosporomycetous oogonia

Plate III. Late Visean Chytridiomycota and chytrid-like remains in lycophyte (*Lepidodendron*) xylem, on degrading plant tissue, in a fungal spore, and in the matrix. Callosities in a fungal spore.

Fig. 1 Spore-like structures and narrow filaments of *Oochytrium lepidodendri* in tracheids; slide no. REN-50/1137; bar = 20 μm.
Fig. 2 Spore-like structures of *O. lepidodendri* in tracheids; slide no. REN-50/1137; bar = 30 μm.
Fig. 3 Detail of one of the spore-like structures of *O. lepidodendri*; slide no. REN-50/1137; bar = 5 μm.
Figs. 4–6 Chytrid zoosporangia in solitary unicells; arrows in Plate III, 4 indicate position of discharge papillae; slide no. REN-3/50; bars = 10 μm.
Fig. 7 Sporangium in the chert matrix; slide no. REN-3/50; bar = 15 μm.
Fig. 8 Stalked spherical putative zoosporangium attached to a tracheid wall; slide no. ROC-4/73; bar = 20 μm.
Fig. 9 Putative zooporangium (containing possible zoospores) attached to the outer surface of degrading plant tissue; slide no. ROC-5/98; bar = 20 μm.
Fig. 10 Fungal (?glomeromycotan) spore with callosities along the inner spore wall; slide no. REN-3/50; bar = 20 μm.
Fig. 11 Putative chytrid zoosporangium positioned terminally on a subtending filament attached to the wall of a tracheid; slide no. REN-49/1118; bar = 30 μm.
Fig. 12 Fungal spore containing small spherules [arrows]; slide no. REN-6/127; bar = 30 μm.
Fig. 13 Fragments of hyphae/filaments with irregular, vesicle-like swellings; slide no. REN-3/50; bar = 30 μm.

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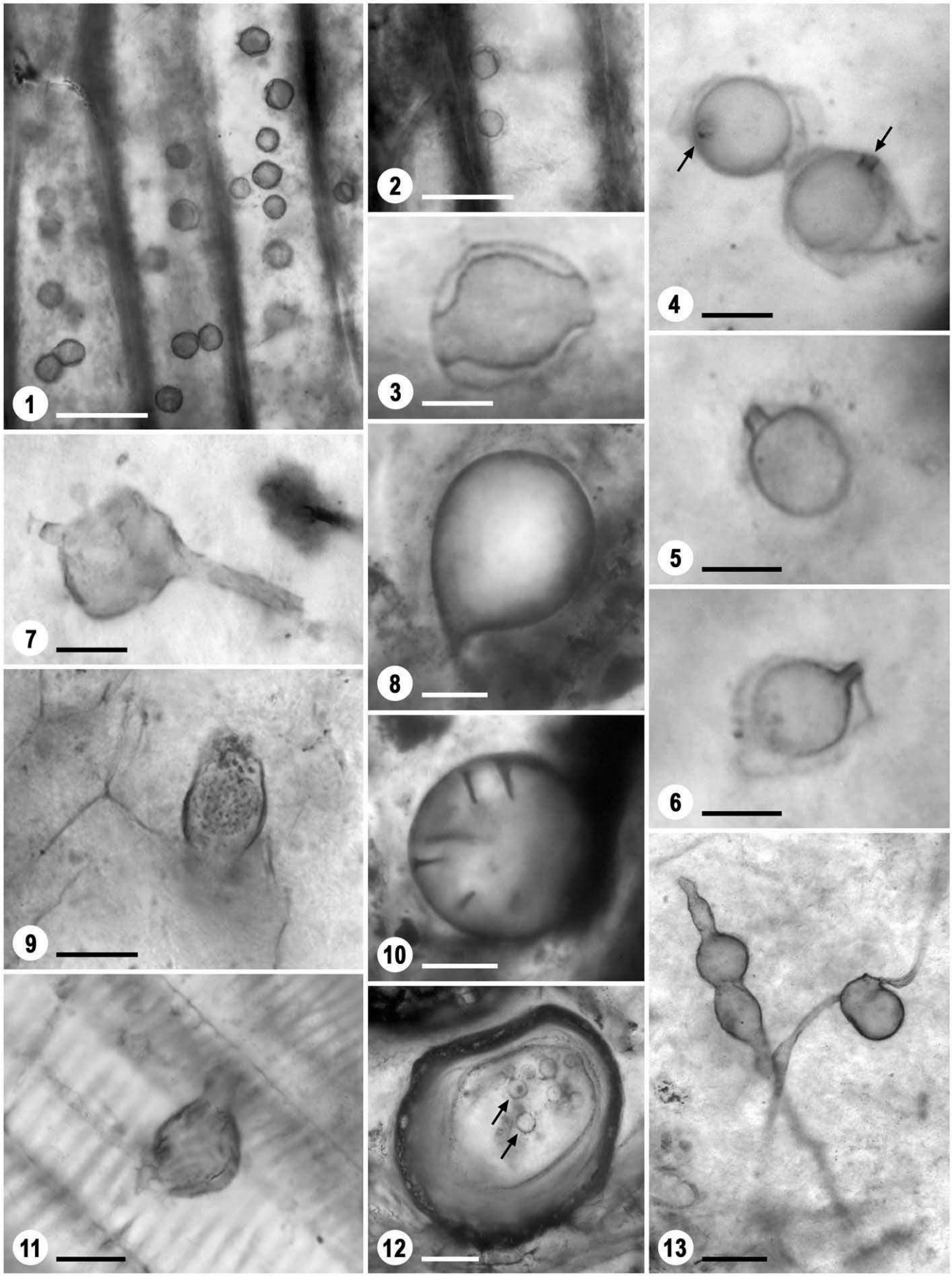

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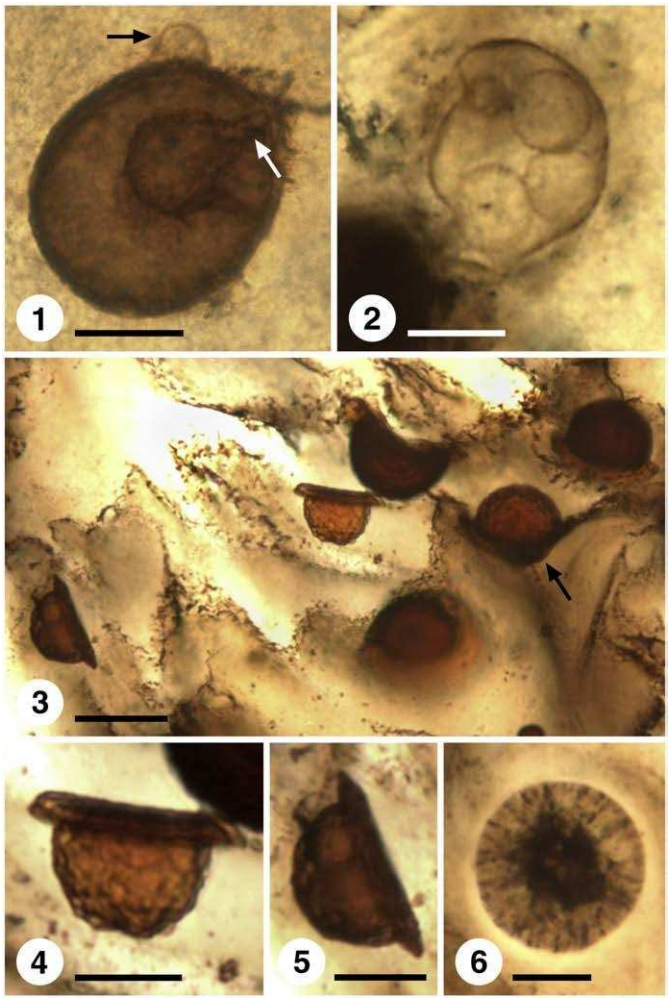

Plate IV. Late Visean Chytridiomycota and chytrid-like remains in oogonia of *Combresomyces cornifer*, on a fungal spore or oogonium, and in degrading plant tissue.

Fig. 1 Fungal spore or peronosporomycetous oogonium with chytrid zoosporangium attached to the surface [black arrow]; note the large sphere in the interior of the spore/oogonium; slide no. ROC-6/126; bar = 20 µm.
Fig. 2 Oogonium of *Combresomyces cornifer* containing several thin-walled spheres; slide no. REN-47/1081; bar = 15 µm.
Fig. 3 Degrading plant tissue with several hemispherical to hat- or bowl-shaped putative chytrid zooporangia; slide no. ROC-6/127; bar = 20 µm.
Figs. 4 & 5 Details of Plate IV, 3, showing two of the zoosporangia; bars = 10 µm.
Fig. 6 Disc- or saucer-shaped structure associated with the group of hat- or bowl-shaped zoosporangia; slide no. ROC-6/127; bar = 10 µm.

or spores/sporangia of other fungi (especially some of the spherical forms). The spore-like bodies (Plate III, 1–3) and narrow filaments/hyphae present in many of the tracheids have previously been interpreted as belonging to a single organism, *Oochytrium lepidodendri* Renault, which has been referred to the Chytridiomycota (Renault, 1895, 1896).

It remains unclear precisely what type of fungus was responsible for the formation of callosities that sporadically occur in lycophyte wood (Plate II, 14–17) and periderm (Plate II, 12,13). Nevertheless, we have included these structures here because they occur in close proximity (albeit never directly connected) to cell walls that also contain spherical and/or conical structures. If these structures represent chytrid zoosporangia, then it is quite conceivable that the developing vegetative systems of these fungi initiated the formation of callosities as the fungus moved into the lumen of the host cell.

The hemispherical–spherical structures (Plate IV, 3–5) attached to a fragment of plant tissue are interpreted as chytrid zoosporangia based on their structural correspondence with empty zoosporangia of the extant *Chytriomyces reticulatus* Persiel and *C. mammilifer* Persiel (see Persiel, 1960). The broadly attached specimens may either represent immature zoosporangia or detached mature zoosporangia that adhered to the plant tissue with their distal ends. Moreover, *C. reticulatus* produces resting spores (see Persiel, 1960: Fig. 8f) that resemble the saucer-shaped bodies associated with the fossil zoosporangia (Plate IV, 6).

3.4. Fungal spores and peronosporomycetous oogonia

There is relatively little suggestion of any association between chytrids and other fungi or fungi-like microorganisms in the Visean cherts. A single, hemispherical or dome-shaped structure (Plate IV, 1 [black arrow]), 6.2 µm high and 10 µm wide, occurs attached to the outer surface of a fungal spore or peronosporomycetous oogonium (50×43 µm in diameter). There is no vegetative system penetrating the wall of the host associated with this structure. However, a large and relatively thick-walled sphere, 18.5 µm in diameter, is visible in the interior of the host. Extending from this sphere is what appears to be a slightly thinner-walled, conical discharge tube (Plate IV, 1[white arrow]). It remains equivocal as to whether this sphere is biologically associated with the structure attached to the surface or was produced by the host.

Several apparently glomeromycotan spores show host responses in the form of callosities, up to 11 µm long and 3 µm wide (Plate III, 10). In addition, several unidentified thick-walled fungal spores associated with degrading plant material contain loose clusters of small spherules (Plate III, 12[arrows]) that are up to 10 µm in diameter.

Oogonia of the endophytic peronosporomycete *Combresomyces cornifer* Dotzler et al., an organism that appears to be consistently associated with lycophyte periderm, occasionally contain several (usually 3–7) thin-walled spheres, each up to 13 µm in diameter and bounded by a delicate but well-defined wall (Plate IV, 2). It is possible that these spherules represent unfertilized oospheres, but they may also be interpreted as resting stages of a fungal endoparasite (for details, see Dotzler et al., 2008).

3.4.1. Remarks

The single sporangium (Plate IV, 1) found attached to the outer surface of a fungal spore or peronosporomycetous oogonium likely represents a chytrid zoosporangium, while the spherules inside *Combresomyces cornifer* oogonia (Plate IV,2) may represent the resting stages of a chytrid. The callosities that occur in glomeromycotan spores are identical to the callosities formed by extant and other fossil glomeromycotan spores in response to chytrid attack (see Boyetchko and Tewari, 1991; Hass et al., 1994; Purin and Rillig, 2008).

The tiny spherules in the interior of the large fungal spores (Plate III, 12) may be resting spores of a chytrid, and thus are included here. The possibility also exists that they were produced by another type of fungus.

3.5. Preserving matrix

There are numerous microfungal remains in the chert matrix, some of which may be assigned to the Chytridiomycota. One of the more common types is an apparent reproductive structure composed of a subspherical sporangium (up to 30 µm wide) terminally positioned on a relatively wide subtending hypha. The sporangium is characterized by a prominent, apical discharge tube (Plate III,7). Another microfungal remain in the chert matrix consists of small segments of apparently aseptate filaments or hyphae (between <2 and ~6 µm wide) that have distinctly irregular, vesicle-like swellings, each up to 30 µm in diameter (Plate III,13).

3.5.1. Remark

Hyphal fragments showing distinct intercalary swellings and isolated sporangia with a prominent apical discharge tube were described

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by Renault (1896: 439/440) as *Palaeomyces gracilis*, and attributed to the "Mucorinées". However, both of these structures are also common in a variety of modern chytrids (see Karling, 1977).

4. Discussion

Although the chytrids and chytrid-like remains of uncertain affinity recorded from the Visean cherts of central France do not provide an inclusive comparison with chytrids in modern ecosystems, the fossils do provide the opportunity to advance hypotheses as to the ecology of this Mississippian microfungal community. In a reconstruction of the Visean wetland ecosystems of central France, Rex (1986: Fig. 3) suggests that the landscape was represented by pools and small lakes surrounded by open, lycophyte-dominated swamp forest vegetation. Since the cherts were formed in the pools and lakes (Rex, 1986), it is highly probable that the majority of microfungi preserved in these cherts were aquatic organisms. If the (putative) chytrids occurring on/in solitary unicells (Plate III, 4–6), and plant and fungal spores (Plate I, 1–9; Plate III, 10,12; Plate IV, 1) colonized their hosts while they were viable, then these associations would represent degrees of parasitism. Interpreting the associations as parasitic seems reasonable, as viable cells and spores represented particularly suitable host substrates and habitats for parasitic microfungi since they were available in abundance in the Visean environment. However, there is next to no conclusive evidence of any host responses in the form of structural alterations or modifications of the host cell walls, which would indicate evidence of a parasitic relationship. In the absence of host responses, it remains impossible to distinguish fossil parasites from saprotrophs, which colonize and utilize dead organic matter as a carbon source (Dix and Webster, 1995). It is therefore equally plausible that the microfungi on/in the solitary unicells and spores represent examples of saprotrophs. The only evidence indicative of a parasitic relationship occurs in the form of callosities in apparently glomeromycotan spores (Plate III, 10). Callosities of this type have been described from other fossil (e.g., from the Rhynie chert) and extant glomeromycotan spores where they represent a specific response of a viable host to chytrid parasitism (Boyetchko and Tewari, 1991; Hass et al., 1994).

The biological association of the putative chytrids with vegetative tissues of terrestrial plants remains equally difficult to resolve based on the absence of host responses directly linked with these microorganisms. It should also be noted, however, that certain types of host responses (e.g., local necroses) may not be recognizable in the fossils because the plant remains are fragmented and not preserved *in situ*, and thus it is not easy to distinguish between actual host responses and *post mortem* disintegration processes that may appear similar to pathogen-induced necroses. Nevertheless, the putative chytrids in lycophyte wood and periderm (Plate II, 1–11; Plate III, 1–3,8,11) probably represent saprotrophs because of their abundance and ubiquitous occurrence in the samples. Moreover, the high diversity of other fungi and fungi-like microorganisms inhabiting the lycophyte wood and periderm (see Renault, 1896; Krings et al., 2007a; Dotzler et al., 2008) argues against a parasitic relationship. In addition, the cauline systems of the *Lepidodendron* species preserved in the Visean cherts are to date known only from axis segments up to 5 cm in diameter (Renault 1879; Galtier 1970). If the Visean *Lepidodendron* species were arborescent and similar in architecture to other members in *Lepidodendron*, these axes would represent twigs positioned high up in the plant, and thus *in vivo* rather unlikely to have been colonized by so many different microbial endophytes (Dotzler et al., 2008). It should be noted, however, that the lycophyte tracheids are non-living at maturity, and therefore not capable of producing any structural alteration in response to an invading parasite, even if the plant as a whole was alive. It is therefore of interest that a host response in the form of callosities sporadically does occur in individual tracheids or small clusters of adjacent tracheids (Plate II, 14–17) from two of the tissue samples. This suggests that these cells were infected by a parasite during an early developmental stage, i.e. when they were still alive. The presence of two different types of callosities in *Lepidodendron* periderm (Plate II, 13) may be evidence to suggest that two different parasites, or two different types of filaments/hyphae of a single parasite were recognized by the host cell and encapsulated by the callosities. Although putative chytrid zoosporangia occur in the same tissue samples as the callosities, they have not been found in organic connection, and thus cannot be positively linked to one another.

Chytridiomycota are significant elements in modern aquatic ecosystems (e.g., Goh and Hyde, 1996; Wong et al., 1998; Gleason et al., 2008). Based on the abundance and diversity of chytrids and chytrid-like remains associated with other organisms (or with parts thereof) in the Visean cherts, we suggest that these fossil fungi also played an important role in the ecology of the Visean paleoecosystem. Gleason et al. (2008) define five roles that chytrids play in modern freshwater ecosystems: (1) chytrid zoospores are important food sources for zooplankton; (2) chytrids decompose particulate organic matter; (3 and 4) chytrids are parasites of aquatic plants and animals; and (5) chytrids convert inorganic compounds into organic compounds. None of these roles can be documented conclusively in the Visean cherts. However, based on the preceding considerations relating to the nature of the associations between the Visean chytrids and chytrid-like remains and their different hosts (see above), it is most probable that the majority of these organisms were saprotrophs decomposing organic matter; only a few (especially those inhabiting the unicells and spores) are more likely to have been parasites. Adding some support to this hypothesis is the suggestion that fungi are the principal decomposers of organic matter in many modern acidic ecosystems such as peatlands, and that they assume a more dominant role than bacteria (Thormann and Rice, 2007 and references therein).

The only other fossil locality sufficiently studied to document the diversity of chytrids in association with other organisms is the Early Devonian Rhynie chert (Taylor et al., 1992a,b; Hass et al., 1994). Some of the chytrid associations in the Rhynie and Visean paleoecosystems appear to be similar, if not identical (e.g., chytrid zoosporangia in solitary unicells; compare Plate III, 4–6 with Taylor et al., 1992b: Figs. 9, 14), while other associations are different (e.g., chytrids in xylem and periderm), which is due probably to the fact that different types of plants (e.g., lycophytes) were colonized. On the other hand, preservation of microorganisms in the Rhynie chert matrix appears to yield a more complete record and finer resolution of delicate features than that seen in the Visean cherts. As a result, the morphology, life history, spatial distribution, and diversity levels of chytrids in the Rhynie paleoecosystem can be reconstructed in greater detail and demonstrated on a more consistent basis.

5. Conclusions

Discovery of a diverse assemblage of chytrids and chytrid-like remains of uncertain affinity in late Visean cherts from central France contributes to a more sharply focused concept of the biodiversity of microorganisms in, and complexity of, Carboniferous non-marine ecosystems. We anticipate that, as more information is obtained about the microbial diversity in the Visean paleoecosystems of central France, it will be possible to provide a more detailed evaluation of the differences and similarities with not only the Early Devonian Rhynie paleoecosystem, but also modern ecosystems. The ability to track microbial biodiversity and interactions through time based on well studied terrestrial biotas represents an important data base reflecting changes in ecosystem functioning. This contribution that focuses on the Visean cherts from central France represents an initial step towards a comparison of modern ecosystems and the well understood Rhynie paleoecosystem with those that in part characterize the vast coal-forming swamps during the Carboniferous.

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Chapter XII: An unusual microfungus in a fungal spore from the Lower Devonian Rhynie chert.

Palaeontology–21.09.09 MK (revised version)

AN UNUSUAL MICROFUNGUS IN A FUNGAL SPORE FROM THE LOWER DEVONIAN RHYNIE CHERT

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Abstract: A new fossil microfungus, *Delitescimyces catenulatus* gen. et sp. nov., occurs as an endobiotic mycelial thallus in a glomeromycotan spore from the Lower Devonian Rhynie chert. The thallus consists of branched (?pseudo-)septate hyphae with numerous catenulate swellings. Some hyphal tips produce spherical reproductive structures or propagules. Hyphal morphology in *D. catenulatus* is reminiscent of that in certain extant Hyphochytridiomycota, Chytridiomycota, and even Ascomycota, but specific diagnostic features that allow assignment of the fossil to modern groups are absent. The discovery of this interfungal association broadens our knowledge about the diversity of microfungi and their intricate associations in early continental ecosystems.

Key words: catenulate swellings; *Delitescimyces catenulatus*; fossil fungi; fungal spore; interfungal association; mycelium

THE LOWER Devonian Rhynie chert has preserved a remarkable diversity of fungi and fungal-like organisms, many of which are intricately involved with other components of the ecosystem in various levels of saprotrophic, parasitic, and mutualistic relationships (reviewed in Taylor *et al.* 2004). Among the fungal relationships preserved in the Rhynie chert is the earliest direct evidence of interfungal associations and interactions, including various types of mycoparasitism (Hass *et al.* 1994).

One of the more frequently encountered interfungal associations in the Rhynie chert are fungicolous microfungi (in the broad sense of including members of the Oomycota and Hyphochytridiomycota) inhabiting spores of other fungi. Several examples of these associations have been described (Kidston and Lang 1921; Taylor *et al.* 1992; Hass *et al.* 1994; Krings *et al.* 2009), one of which consists of large glomeromycotan spores that may contain varying numbers of small, spherical gametangia, sporangia, and/or (resting) spores of intrusive microfungi. However, none of the specimens described to date (e.g. in Kidston and Lang 1921; Krings *et al.* 2009) show the remnants of the intrusive organisms organically connected to, or even co-occurring with, any extensive (rhizo-)mycelial system. Nevertheless, differences in size and wall composition of the gametangia, sporangia, or (resting) spores, together with differences in the morphology of subtending hyphae or filaments that are present, suggest that many different microfungi in the Rhynie paleoecosystem lived, reproduced, and/or produced resting stages inside the spores of other fungi (Kidston and Lang 1921). Because of these numerous levels of interaction, a precise knowledge about these organisms represents an important component of fully understanding the roles that microbial life played in this continental ecosystem some 400 Ma ago.

This paper describes *Delitescimyces catenulatus* nov. gen. et nov. sp., a nearly completely preserved mycelial thallus of uncertain affinity that occurs in a glomeromycotan spore from the Rhynie chert. The thallus is composed of (?pseudo-)septate hyphae with catenulate swellings; some hyphal tips produce spherical reproductive structures or propagules. *Delitescimyces catenulatus* provides new information about the morphology and organization of spore-inhabiting microfungi in the Rhynie paleoecosystem, and further expands our knowledge of late Paleozoic interfungal associations.

MATERIAL AND METHODS

The Rhynie chert Lagerstätte is located in the northern part of the Rhynie Outlier of Lower Old Red Sandstone in Aberdeenshire, Scotland, within a sequence of sedimentary and volcanic rocks. The cherts occur in the upper part of the Dryden Flags Formation, in the so-

called Rhynie Block, located a few hundred metres northwest of the village of Rhynie. The Lagerstätte consists of at least 10 fossiliferous beds containing lacustrine shales and cherts that are interpreted as a series of ephemeral freshwater pools within a hot spring environment (e.g. Rice *et al.* 2002). Preserved in the cherts are both aquatic (freshwater) facies from the pools and subaerial soil/litter horizons with *in situ* plants that occupied the edges of the pools; it is hypothesized that the latter became preserved as a result of temporary flooding of silica-rich water, or by silica-rich groundwater that percolated to the surface. The cherts have been dated as Pragian-?earliest Emsian based on dispersed spore assemblages (Wellman 2006; Wellman *et al.* 2006). Radiometric dates based on $^{40}Ar/^{39}Ar$ isotopes from the cherts indicates an absolute age of 396 ± 8 million years (Rice *et al.* 1995). Details about the geological setting, sedimentology, and development of the Rhynie chert Lagerstätte can be found in Rice *et al.* (2002), and Trewin and Rice (2004).

The spore containing the microfungal thallus was identified in a thin section prepared by cementing a piece of chert to a glass slide and then grinding the slice until it is thin enough to be examined in transmitted light. The slide (accession number BSPG 1964 XX 24) is part of the slide collection of the late Prof. Max Hirmer that is deposited today in the Bayerische Staatssammlung für Paläontologie und Geologie (BSPG) in Munich, Germany.

SYSTEMATIC PALEONTOLOGY

Morphogenus *Delitescimyces* nov. gen.

Type species. Delitescimyces catenulatus M. Krings, Dotzler, Longcore et T.N. Taylor, nov. sp. (this paper)

Derivation of name. The generic name, a combination of the Latin word *delitescere* (= to hide) and the Greek word *μύκης* (mýkes) (= fungus), refers to the hidden occurrence of the mycelium in the lumen of a fungal spore.

Holotype. BSPG 1964 XX 24 (Pl. 1, figs 1–9)

Repository. Paleobotanical collection of the Bayerische Staatssammlung für Paläontologie und Geologie (BSPG), Munich, Germany.

Type Locality. Rhynie, Aberdeenshire, Scotland, National Grid Reference NJ 494276

Age. Early Devonian; Pragian-?earliest Emsian (see Wellman 2006; Wellman *et al.* 2006)

Diagnosis of Delitescimyces. Thallus mycelial, composed of (?pseudo-)septate hyphae with catenulate swellings; swellings variable in size and shape, alternating with more or less pronounced constrictions; reproductive structures or propagules located on hyphal tips.

***Delitescimyces catenulatus* nov. sp.**
Plate 1, figures 1–9

Derivation of epithet. catenulatus (Lat.) = having the form of a small chain.

Diagnosis of Delitescimyces catenulatus. Endobiotic in fungal (glomeromycotan) spores; hyphae branched, with numerous catenulate swellings; swellings drop-shaped, short-elliptical, spindle-shaped, elongate-clavate, or sometimes (sub-)cylindrical, up to 20 μm long and 6 μm wide; (?pseudo-)septa mostly located in constricted areas between swellings; reproductive structures/propagules spherical, >10 μm in diameter, positioned singly on hyphal tips, subtended by short, stalk-like constriction of parental hypha, wall <1 μm thick, faintly ornamented.

Description. Delitescimyces catenulatus occurs in a single glomeromycotan spore, which is 185 μm long and ~150 μm wide. The host spore occurs in the cortex of a degraded land plant axis where it is associated with several other glomeromycotan spores, each containing intrusive microfungi (Text-fig. 1). However, fungal remains displaying the same complement of features as *D. catenulatus* have not been detected in any of the other spores, nor do they occur in the degraded plant tissues and matrix surrounding the spores.

The thallus of *Delitescimyces catenulatus* is confined to the interior of the host spore (Pl. 1, figs 1, 2). Structures that might suggest where and how the organism entered the spore have not been found, with the possible exception of an inwardly directed, papilla-like projection on the inner surface of the spore wall (Pl. 1, fig. 2 [large arrow]). The thallus consists of branched, (?pseudo-)septate[1] hyphae (or filaments) characterized by numerous

[1]We have used the term "(?pseudo-)septate" because we are unable to determine whether the structures represent actual cross-walls (septa) or just external constrictions or folds (pseudosepta) of the hypha/filament.

catenulate swellings (Pl. 1, fig. 2). Hyphae appear to branch subumbellately from a common center, with the branches diverging in a more or less open tuft that extends along the inner surface of the host spore wall. Hyphal swellings (Pl. 1, figs 3, 4, 6–8) are variable in size and shape (i.e. drop-shaped, short-elliptical, spindle-shaped, elongate-clavate, or sometimes subcylindrical), up to 20 μm long and 3–6 μm wide, and alternate with more or less pronounced constrictions, in which the hyphae range only between <1 and 2 μm wide. Septa or pseudosepta typically (but not consistently) occur in the constricted areas (Pl. 1, figs 4 [arrows], 6 [black arrow]); an aperture or pore in the center of the (?pseudo-)septa is not recognizable.

Spherical structures, 10–17 μm in diameter, occur on some hyphal tips (Pl. 1, figs 2, 6, 7). These structures, which are usually subtended by a short, stalk-like distal constriction of the parental hyphae (Pl. 1, fig. 6 [white arrow], 7), are more opaque than the normal hyphal swellings and some (i.e. the larger ones) appear to be faintly ornamented (e.g. Pl. 1, fig. 6 [lower left of image]); their wall is less than 1 μm thick. Discharge openings were not observed. In some of the spheres, a second, slightly smaller interior vesicle is present (e.g. Pl. 1, fig. 2 [small arrows]). Other spherical structures, which occur isolated in the host spore, are slightly smaller, with slightly thicker (i.e. up to 2 μm) but translucent walls, and that contain a relatively opaque, corrugated interior structure (Pl. 1, fig. 5). Another spherical structure in the host spore displays a narrow hyaline subtending hypha or filament that is unlike the hyphae forming the mycelium because it lacks swellings (Pl. 1, fig. 9 [upper sphere]). Moreover, this type of sphere contains a well delineated, distinctly smaller interior sphere. As there is apparently no physical connection between the latter two types of spheres and the thallus, it cannot be determined whether these structures actually belong to the *D. catenulatus* organism, or represent another type of intrusive microfungus that co-occurs with *D. catenulatus* in this host spore.

DISCUSSION

The Early Devonian Rhynie chert has contributed substantially to our conception of the roles that fungi, and fungal associations and interactions with other organisms have played in shaping and sustaining the early continental ecosystems (Taylor and Taylor 2000). However, this conception is based on a relatively small number of fungi involved in specific interactions that have been described in detail and directly compared to modern analogues (see Taylor *et al.* 2004); meanwhile numerous other fungal types and consistent associations in the Rhynie chert have not received a sufficient level of scholarly attention.

One of the under-studied segments of fungal life in the Rhynie paleoecosystem is interfungal associations that specifically involve various types of glomeromycotan spores containing spherical reproductive structures and/or propagules of intrusive microfungi. The benchmark study by Kidston and Lang (1921) suggests that numerous fungi and fungal-like organisms were intricately associated in this manner with glomeromycotan spores. However, only one form, *Globicultrix nugax* M. Krings, Dotzler et T.N. Taylor, has been studied in greater detail (Krings *et al.* 2009). This organism, which was discovered from the same cluster of glomeromycotan spores as *Delitescimyces catenulatus* (Text-fig. 1), is characterized by reproductive structures that resemble the apophysate zoosporangia of the extant polycentric chytrids *Nowakowskiella* J. Schröter and *Cladochytrium* Nowakowski. The principle reasons for the lack of detailed information about the microfungi inhabiting fungal spores from the Rhynie chert are the small size of the intrusive organisms and the incompleteness of the record, which does not normally permit a sufficiently detailed reconstruction of the morphology, life history, and spatial distribution of these organisms. For example, none of the specimens described to date show the remnants of the microfungi organically connected to, or even co-occurring with, any extensive (rhizo-)mycelial system. Moreover, evidence of host reaction or pathogeneity in the form of structural alterations is rare, and thus, determining the nutritional relationship between the partners remains a difficult, if not impossible task. Determining the nutritional mode(s) of these associations would be particularly interesting with regard to better understanding the functioning of the Rhynie paleoecosystem because, if the intrusive microfungi were parasites, they most likely affected the number of viable glomeromycotan spores, and thus reduced the extent of mycorrhizal inoculation and therefore may have altered the structure of the land plant community.

Although *Delitescimyces catenulatus* is more completely preserved than most other microfungi in glomeromycotan spores from the Rhynie chert, assessing the systematic affinities and biology of this organism remains difficult. This is due primarily to the nature of the spherical structures produced on hyphal tips (Pl. 1, figs 2, 6, 7) that remain equivocal with regard to their function. None show specific features such as discharge pores or papillae, or a consistent and characteristic content, and thus whether they represent gametangia, (zoo-)sporangia, or some type of resting spores remains uncertain. What is known is that these structures are well within the size range of the microfungal remnants that occur in other glomeromycotan spores from the Rhynie chert (see Kidston and Lang, 1921). What is especially intriguing is the fact that *D. catenulatus* represents the first documented case in

which spherical reproductive structures/propagules occur in organic connection with a nearly complete mycelial thallus. Moreover, the (?pseudo-)septate hyphae of *D. catenulatus* are characterized by numerous catenulate swellings that are variable in size and shape, a complement of features not previously observed from Rhynie chert fungi. While several spore-inhabiting microfungal thalli showing hyphal swellings are known from the Rhynie chert (see Hass *et al.* 1994: figs 15, 23, 27), they differ from *D. catenulatus* in having hyphae that lack pseudosepta or septa. Moreover, the hyphal swellings differ in morphology from those in *D. catenulatus*. In addition, none of the thalli figured by Hass *et al.* (1994) display hyphae in organic connection with spherical reproductive structures or propagules like those produced by *D. catenulatus*. The compound apophysis of *Krispiromyces discoides* T.N. Taylor, Hass et W. Remy (see Taylor et al., 1992: figs 22, 23), a parasite of the charophyte *Palaeonitella cranii* (Kidston et Lang) Pia, is the only fungal structure described to date from the Rhynie chert that is somewhat similar morphologically to the hyphae of *D. catenulatus*.

The vegetative system of *Delitescimyces catenulatus* has also certain similarities in basic structure with the hyphae or filaments of various extant fungi and fungal-like organisms, especially with regard to the catenulate hyphal swellings. Morphologically similar structures are known to occur in representatives of the Chytridiomycota and Hyphochytridiomycota. For example, among the Chytridiomycota (Chytridiomycetes), catenulate swellings have been described in *Catenochytridium* Berdan where they form a compound apophysis beneath the zoosporangium (e.g. Berdan 1939, 1941; Karling 1977; Barr *et al.* 1987). Some members of the Monoblepharidales (Chytridiomycota, Monoblepharidiomycetes) also produce hyphae with catenulate swellings. Especially interesting is *Gonapodya* A. Fischer, in particular *G. polymorpha* Thaxter, in which the swellings sometimes are remarkably similar in size and shape to those of *D. catenulatus* (e.g. Sparrow 1933; Johns and Benjamin 1954; Miller 1963; Karling 1977). Moreover, encysted zygotes of *Gonapodya* have been described that possess a hyaline wall (Johns and Benjamin 1954: figs 7, 8) and are comparable to the fossil sphere illustrated in Plate 1, figure 5. A distinct difference between *D. catenulatus* and *Gonapodya* is the fact that *Gonapodya* grows on the outer surface of its substrate, whereas *D. catenulatus* is endobiotic. Moreover, if *D. catenulatus* were a parasite and similar to *Gonapodya* in features of its sexual reproduction, it might be argued whether the interior of the host would have contained free water and thus a sufficient medium for the male gametes to swim to the female gametangia. *Delitescimyces catenulatus* also may be compared to *Hyphochytrium* Zopf (Hyphochytridiomycota, Stramenopila), which has catenulate swellings (e.g. in *H. catenoides* Karling; see Karling 1939; Barr 1970). Finally, hyphae somewhat similar to *D. catenulatus*

are known in *Haptospora tribrachispora* (G.L. Barron et Szijarto) G.L. Barron (anamorphic Hypocreales), an endoparasite of bdelloid rotifers. The fungus, an ascomycete, produces assimilative hyphae within the host that are composed of turbinate cells separated from one another by septa (Barron and Szijarto 1984; Barron 1991). These hyphae (e.g. Barron and Szijarto 1984: figs 4, 5, 8) are morphologically similar to some of the hyphae produced by *D. catenulatus*.

Based on the uncertainties with regard to the biology of *Delitescimyces catenulatus*, we have refrained from formally referring the fossil to any extant taxon, but rather introduce a new morphogenus to accommodate this unusual Early Devonian organism until additional specimens become available to increase the suite of diagnostic features. In a similar context we are unable to offer an analysis about the nutritional relationship between *D. catenulatus* and the glomeromycotan fungus that produced the host spore.

Research on the Rhynie chert to date has provided a wealth of information about the plants, animals, and microorganisms in this Early Devonian continental ecosystem (e.g. Kerp and Hass 2004; papers in Trewin and Rice 2004). In most other fossil ecosystems details about the microbial component of the community are lacking generally because of preservational factors and perhaps scientific curiosity. Moreover, because the diversity of organisms in later appearing continental ecosystems is extensive, and many of the land plants that form these communities were arborescent, the fossil record for the microbial component in these ecosystems remains incomplete, even in instances where preservation is exceptional. The Rhynie chert offers two unique opportunities to examine microbial interactions. One focuses on the relatively small number of plant species in this ecosystem, their small size, relatively simple organization, and surprisingly well documented biology. The second relates to the base level of information that has been generated about fungal diversity and life history biology by Early Devonian time. We believe that where these two bodies of information intersect there are numerous opportunities to test hypotheses about early land plant ecosystem interactions and to use the Rhynie chert as a platform to examine slightly younger continental ecosystems (e.g. from the Carboniferous) where plant diversity and architecture, as well as the habitat, are markedly different. As a result, the discovery of relatively simple organisms like *Delitescimyces catenulatus*, which suggests a pattern of interfungal association, represents an important step forward in helping to define patterns of resource allocation and microbial biodiversity in ancient continental ecosystems.

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Figure Captions

TEXT-FIG. 1. Glomeromycotan spores in the cortex of a partially degraded land plant axis; note that most spores contain microfungi, one of which is *Delitescimyces catenulatus* [arrow]; slide BSPG 1964 XX 24; bar = 150 µm.

EXPLANATION OF PLATE 1

Figs 1–9. *Delitescimyces catenulatus* nov. gen. et nov. sp. from the Lower Devonian Rhynie chert; all specimens from slide BSPG 1964 XX 24.

Fig. 1: Spore containing the thallus of *D. catenulatus* (holotype); bar = 50 µm.

Fig. 2: Detail of Plate 1, figure 1, showing the thallus; large arrow indicates inwardly directed projection on the inner surface of the host spore wall, small arrows point to reproductive structures/propagules containing a slightly smaller interior sphere; bar = 20 µm.

Figs 3&4: Hyphae with catenulate swellings; arrows in Plate 1, figure 4 indicate the position of (?pseudo-)septa; bars = 10 µm.

Fig. 5: Thick-walled sphere containing a corrugated structure; bar = 5 µm.

Fig. 6: Distal portion of a hypha with reproductive structures or propagules; black arrow indicates (?pseudo-)septum, white arrow points to stalk-like constriction subtending the reproductive structure/propagule; bar = 10 µm.

Fig. 7: Detail of Plate 1, figure 6 (different focal plane), showing a reproductive structure/propagule attached to hyphal tip; bar = 5 µm.

Fig. 8: Detail from the mycelium, showing the variability in size and shape of the catenulate swellings; bar = 5 µm.

Fig. 9: Two spherical structures from the interior of the host spore; the lower specimen belongs to *D. catenulatus*, whereas the upper one cannot be related to this organism with confidence; bar = 10 µm.

Text-fig. 1

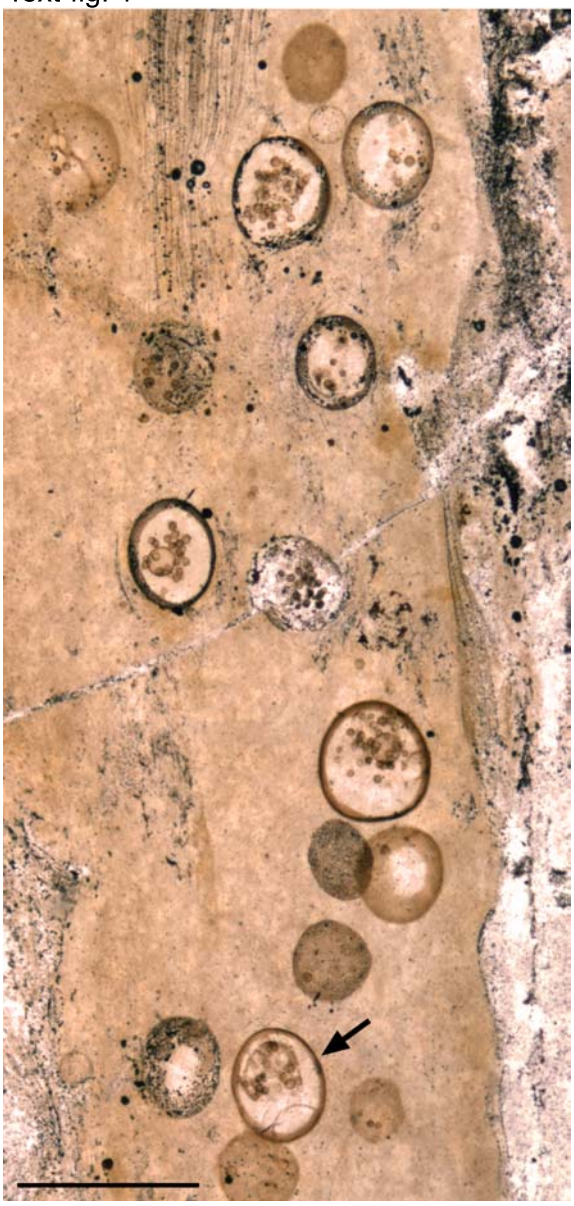
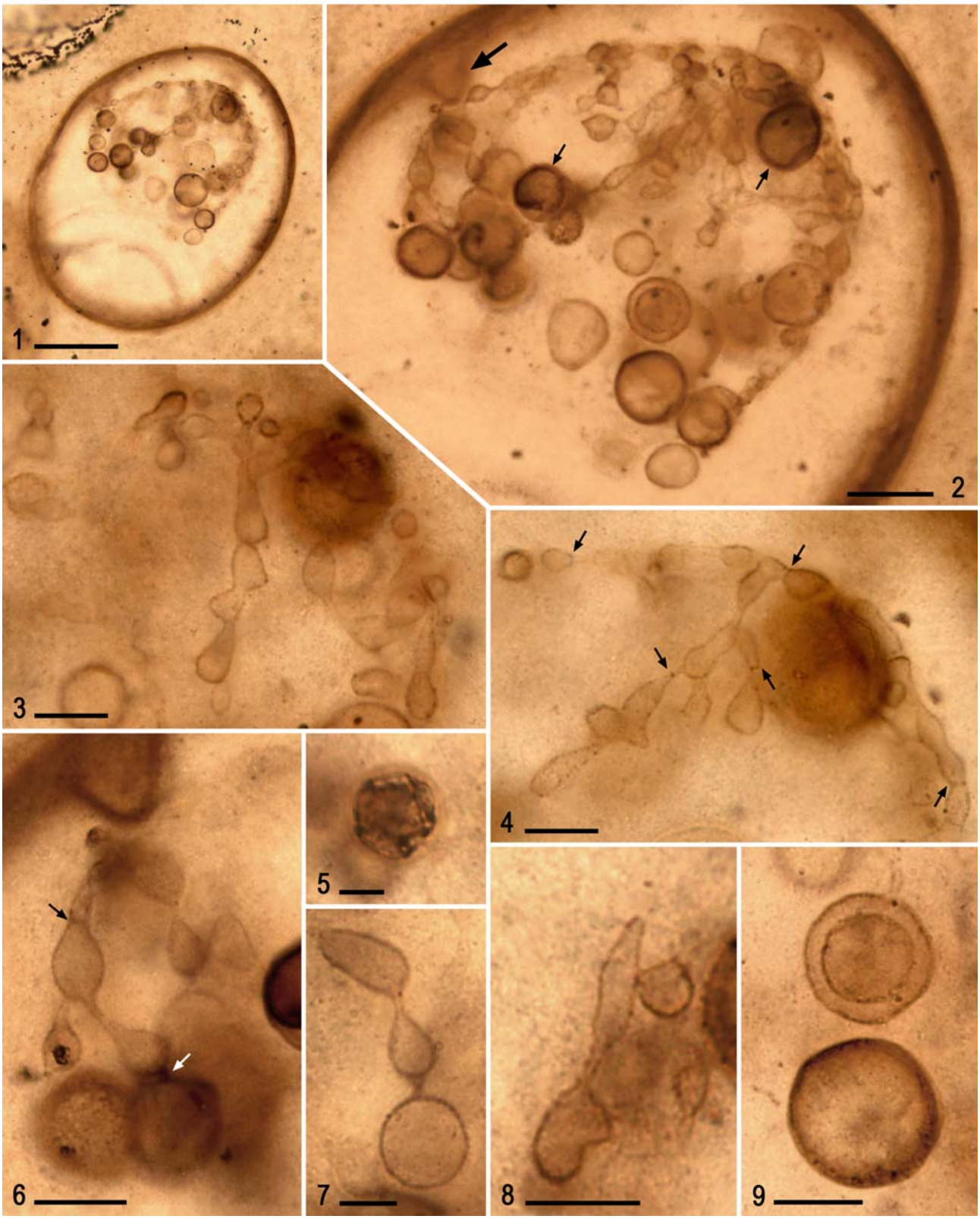


Plate 1



3. Discussion

This thesis is based on twelve papers (11 published and 1 accepted) on fossil microorganisms preserved in the Early Devonian and Visean cherts from Rhynie and central France. The papers are subdivided into two principle categories based on content. Papers in the first category (i.e. papers **I**, **IV**–**XII**) contribute to our knowledge of the morphology and biology of microorganisms from, and diversity of microbial life in, the Rhynie and Visean continental ecosystems; papers in the second category (i.e. papers **I**, **II**, **III**, **VII** and **X**) detail specific associations/interactions between land plants and microorganisms.

3.1. Rhynie chert

Although the Rhynie chert has been known and studied intensively for nearly 100 years, the microorganisms preserved in this chert (with the exception of some of the fungi) have received relatively little attention, and even less, their role with other organisms in this paleoecosystem. As a result, knowledge about the levels of biocomplexity present in this ancient terrestrial ecosystem remains incomplete, and thus the roles that these terrestrial communities played in early land plant and ecosystem evolution continues to be difficult to assess based on the fossil record. The discovery of new types of microorganisms (papers **IV**, **VI**/**VIII**, **IX**) from the Rhynie chert contributes to a more sharply focused concept of the complexity of this ancient ecosystem, including the biodiversity.

It is especially interesting to note that photosynthetic microorganisms (cyano-bacteria, microalgae) have rarely been described from the Rhynie chert, despite the fact that cyanobacteria and microalgae are common elements of virtually all aquatic environments today. Kidston and Lang (1921b), and Croft and George (1959), published one coccoid and several filamentous cyanobacteria. Moreover, two eukaryotic microalgae have been detailed by Edwards and Lyon (1983) and interpreted as members of the Chlorophyta. To a large degree, the lack of information concerning photosynthetic microorgansims from the Rhynie chert is the result of multiple features critical in defining the exact systematic affinities of these organisms (e.g., flagellar apparatus, pigmentation, life history). These features are difficult, or even impossible, to document from fossils, and thus compound the determining the affinities of the organisms, but also the deciphering of levels of interaction within the ecosystem. Recent research has shown, however, that in few instances preservation of the microorganisms is sufficient to allow detailed documentation and assignment to a group or even a genus.

Paper **VII** discusses the cyanobacterium *Croftalania venusta* that occurs in the Rhynie chert in several distinct growth forms. *Croftalania venusta* is a filamentous, non-heterocystous form that exhibits similarities to modern Oscillatoriales (cyanobacterial sub-section III; see Castenholtz 1989). This cyanobacterium grows in dense stands, attached either directly to the sediment, or to the surface of submerged land plant axes where it forms hemi-spherical or elongate, tuft-like colonies. While the colonial growth form of *C. venusta* does not appear to enter into direct relationships with other microorganisms, the cyanobacterium may also form complex microbial mats, in which it co-occurs with various other micro-organisms, including other cyanobacteria, algae, and fungal-like organisms. These mats provide an interesting perspective on the evolution of cyanobacterial associations with other microorganisms in ancient continental ecosystems.

Another fossil that adds to the inventory of Rhynie chert photosynthetic micro-organisms belongs to the prasinophycean algae genus *Cymatiosphaera* (paper **IV**). The Prasinophyceae are single-celled, flagellate green algae (Lewis and McCourt 2004). Some genera form a non-motile cyst-like structure, a so-called phycoma (Teyssèdre 2006). Unlike cysts, phycomata remain metabolically active, which results in considerable size variability within a population (Knoll et al. 1991; Teyssèdre 2006). Due to the resistant organic wall of the phycomata, these algal remains are known in the dispersed fossil record as early as the Precambrian (Teyssèdre 2006). Most phycoma-forming species have been described from marine or brackish environments (Colbath and Grenfell 1995; G.L. Mullins, pers. commun. 2007), but there are also a few forms known from fresh-water paleoecosystems (e.g., Doubinger 1967; Clausing 1993; Zippi 1998). The discovery of prasinophycean phycomata from the Rhynie chert represents the earliest evidence of this group of green algae in a freshwater deposit.

Despite the diversity of microorganisms preserved in the Rhynie chert, the fungi have received the greatest amount of attention to date for several reasons. One of these is their nutritional mode, which, as heterotrophs, requires various levels of interaction with other organisms that may be dead or alive. Because of the many ways used by fungi to obtain carbon, they are perhaps more easy to recognize as functioning components in ancient ecosystems than, for example, a cyst or phycoma of a unicellular type of planktonic alga. As a result, the Rhynie chert has contributed substantially to our understanding of the roles that fungi, and fungal associations and interactions with other organisms, have played in shaping and sustaining the early continental ecosystems (Taylor and Taylor 2000). However, this

hypothesis is based on a relatively small number of fungi involved in specific interactions that have been described in detail and directly compared to modern analogues (see Taylor et al. 2004); there are numerous other fungal types and consistent associations in the Rhynie chert that have not received a sufficient level of scholarly attention.

One of the under-studied segments of fungal life in the Rhynie paleoecosystem are interfungal associations that specifically involve various types of (putative) glomeromycotan spores containing gametangia, sporangia, and/or (resting) spores of intrusive microfungi. The benchmark study by Kidston and Lang (1921b) suggests that numerous fungi and fungus-like organisms were intricately associated with glomeromycotan spores and certainly impacted certain types of interactions (e.g., endomycorrhizal inoculation, parasitism) between these fungi and other components of the ecosystem. However, none of these intrusive microfungi has been described and analyzed in detail. The principle reasons for the lack of detailed information about the microfungi inhabiting fungal spores from the Rhynie chert are the small size of the intrusive organisms and the incompleteness of the record, which does not normally permit a sufficiently detailed reconstruction of the morphology, life history, and spatial distribution. For example, none of the specimens described to date show the remnants of the microfungi organically connected to, or even co-occurring with, any extensive (rhizo-) mycelial system. Moreover, evidence of host reaction or pathogenity in the form of structural alterations is rare, and thus determining the nutritional relationship between the partners remains a difficult, if not impossible task. Determining the nutritional mode(s) of these associations would be particularly interesting with regard to better understanding the functioning of the Rhynie paleoecosystem because, if the intrusive microfungi were parasites, they likely impacted the number of viable glomeromycotan spores, and thus reduced the extent of mycorrhizal inoculation and therefore may have altered the structure of the land plant community.

Paper **IX** details the morphology of *Globicultrix nugax,* an intrusive microfungus with possible affinities in the Chytridiomycota that occurs in glomeromycotan spores and is characterized by reproductive structures resembling the apophysate zoosporangia of the extant polycentric chytrids *Novakowskiella* and *Cladochytrium* (see Karling 1977). Another intrusive microfungus in glomeromycotan spores is *Kryphiomyces catenulatus*, a form that is distinct in that it consists of branched (?pseudo-)septate hyphae or filaments with numerous catenulate swellings (paper **XII**). These two organisms are more completely preserved than other microfungi in glomeromycotan spores from the Rhynie chert and demonstrate the diversity of types.

A second important aspect of my research concerns the complexity level(s) attained by interactions between different organisms in the Rhynie chert. One-to-one saprotrophic, parasitic, and mutualistic symbioses are known to have occurred in the Rhynie ecosystem (summarized in Taylor et al. 2004), but there is also evidence for more complex interactions involving several microorganisms that simultaneously interacted with a single host organism.

One example of this type are endophytic (inter- and intracellular) cyanobacterial filaments that are present in the prostrate mycorrhizal axes of the early land plant *Aglaophyton major* (paper **VIII**). Colonization of *A. major* axes by endophytic cyanobacteria is believed to represent a rare, and thus probably incidental, occurrence linked to temporary flooding of the habitat. Nevertheless, this association is the earliest direct evidence for a land plant-cyanobacterial association, and therefore could be a model of the precursory stages that may have preceded the evolution of mutualistic symbioses between land plants and cyanobacteria. *Aglaophyton major* is, however, not only colonized by the cyanobacteria, but also inhabited by the endomycorrhizal fungus *Glomites rhyniensis* (Taylor et al. 1995, paper **X**). This symbiosis is characterized by delicate, intracellular structures formed by the fungal partner, so-called arbuscules, that occur exclusively in a specific zone of the outer cortex (Smith and Read 2008). It is interesting to note that, in *A. major* axes containing endophytic cyanobacteria, the cyanobacterial filaments are particularly abundant close to the mycorrhizal arbuscule zone, which may suggest that there was also some level of interaction between the cynaobacteria and mycorrhizal fungi (paper **VIII**). Adding to the complexity of associations/ interactions involving the endomycorrhizal fungus of *A. major* is the fact that the spores of this fungus are frequently infected by intrusive microfungi (Kidston and Lang 1921b; Hass et al. 1994; papers **IX, XII**), some of which cause characteristic host responses, and thus indicate a parasitic relationship (Taylor et al. 2004).

Although the other land plants from the Rhynie chert (for an inventory, refer to Kerp and Hass 2004) have not been examined in sufficient detail to document the presence of mycorrhizal symbioses, there is a strong indication that Glomeromycota, which form the fungal partner of this symbiosis (see below), were in some way associated with all Rhynie chert land plants. Another Rhynie chert land plant is *Nothia aphylla*. In this plant, three different fungal endophytes, including a putative endomycorrhizal fungus, concurrently colonize the subterreanean prostrate axes (rhizomes), but each enters into qualitatively different relationships with the host (paper **II**, **III**). Each fungus causes characteristic cell and tissue alterations and/or host responses, including bulging of the infected rhizoids, inflation of

cells, secondary thickening of cell walls, cell death, and coating of the intruding hyphae with an apparent granular substance or cell wall material. Some of these host responses are remarkably similar to host responses seen in plants today, and thus indicate that certain aspects of the complex molecular systems causing host responses were in place by Early Devonian time.

The putative endomycorrhizal fungus in *Nothia aphylla* is morphologically similar to that of *Aglaophyton major*, but there are also several interesting differences between these plants. *Aglaophyton major* is characterized by arching, stomatiferous prostrate axes that grow along the substrate surface, and form rhizoid-bearing bulges, usually around stomata, upon contact with the substrate. Hyphae of the fungus enter the axes through the substrate-near stomata, extend throughout the intercellular system of the hypodermis and outer cortex, and subsequently penetrate individual cells within a well-defined region of the cortex to form arbuscules (Taylor et al. 1995). In *Nothia aphylla*, the upright aerial axes arise from a system of non-stomatiferous, subterranean rhizomatous axes. Ventrally, these axes form a prominent rhizoidal ridge that consists of a rhizoid-bearing epidermis, a multi-layered hypodermis, files of parenchyma cells that connect to the stele, and extra-stelar conducting elements. In the rhizopidal ridge of *N. aphylla*, intercellular spaces are virtually absent (see Kerp et al. 2001). Since the prostrate axes of *N. aphylla* lack stomata, the putative endomycorrhizal fungus enters the axes through the rhizoids that occur on the ventral side along the rhizoidal ridge. Because intercellular spaces are absent in the hypodermis, the fungus extends through this tissue as an intracellular endophyte until it reaches the cortex where intercellular spaces are present. In the cortex, the fungus forms an extensive intercellular network of hyphae, and produces vesicles and large, thick-walled spores. The most interesting aspect of this putative endomycorrhizal association concerns the intracellular growth of the fungus in the hypodermis, which appears to be somehow controlled by the host through the production of cell wall sheaths around the fungal hyphae. This feature suggests a shift from uncontrolled intracellular occurrence of the fungus in the rhizoids, to controlled intracellular occurrence in the rhizoidal ridge, to intercellular occurrence in the cortex. *Nothia aphylla* perhaps "tolerated" intracellular penetration in the rhizoids and within the tissues of the rhizoidal ridge because it may have simultaneously provided the plant with a parasite-recognition system (see above).

Arbuscular mycorrhizae are formed by members of the Glomeromycota, and today can be found in approximately 90% of vascular plants, as well as in some bryophytes (Read et al. 2000). Glomeromycotan fungi reproduce via large, thick-walled spores, which represent the

most important taxonomic feature of this group (e.g. INVAM-hompage, Walker and Sanders 1986; Spain 1992; Franke and Morton 1994; Kramadibrata et al. 2000; Benny et al. 2001; Hafeel 2004). Although the Rhynie chert endomycorrhizae are well-understood today, the reproductive biology of the fungi involved in these interactions remained largely unknown until recently.

Papers **I** and **X** document the morphology of several glomeromycotan spores that occur in the cortical tissues of two Rhynie chert land plants, *Asteroxylon mackiei* and *Aglaophyton major*. The spores sometimes are associated with hyphae that terminate in thin walled vesicles. In modern Glomeromycota, the spores of the various species may differ in size, coloration and thickness of the spore wall, number and thickness of individual wall layers. They may also differ in the presence/absence of certain associative structures such as bulbous swellings of the parental hypha or sporiferous saccules (e.g., INVAM). Moreover, some spores are characterized by a distinct mode of germination in which germ tube formation is preceded by the development of a germination shield (e.g., Walker and Sanders 1986). Spore morphology, color, and wall composition are important features in characterizing extant arbuscular mycorrhizal fungi, with more than 200 species described to date. Molecular studies suggest that an even larger number may be present (Redecker and Raab 2006). Only one type of glomeromycotan spore had been described from the Rhynie chert to date, and it possesses characters like the extant genus *Glomus* (Taylor et al. 1995).

In the course of this dissertation project I described a second spore type from the Rhynie chert that is similar to the extant genus *Scutellospora* with regard to the presence of a prominent, circular germination shield with a lobed margin (paper **I**), and a third type with a prominent germination shield that is usually tongue-shaped with infolded margins. Moreover, this latter spore type is borne laterally in the neck of a sporiferous saccule. This Early Devonian spore-saccule complex conforms most closely with the spore-saccule complexes present in the modern genus *Acaulospora* (paper **X**). However, the spores also show features of other modern genera within the Glomeromycota, which suggests that this fossil fungus contains a mosaic of characters that occur in several modern glomeromycotan fungi. These new data suggest that the Glomeromycota were relatively diverse by Rhynie chert time, and well established as a group even before true roots evolved since all of the Rhynie chert plants, and many other early land plants, at the time lacked roots. The recent description of an additional *Glomites* species (i.e. *G. sporocarpoides*) producing spores in sporocarps from the Rhynie chert adds further support to the early diversification of the Glomeromycota (Karatygin et al. 2004).

3.2. Visean cherts

Despite many similarities, the Visean cherts from central France differ from the Rhynie chert in several key aspects. First, the Visean cherts have been less well studied, and only a relatively small amount of information is available about the structure and complexity of the paleoecosystem. While the plants preserved in these cherts have been documented in detail (e.g., Renault 1896a; Galtier 1970, 1971), the microbial element has hardly received any attention (see Taylor et al. 1994; Krings et al. 2005).

Paper **V** reports on an assemblage of most probably saprotrophic microfungi and fungus-like microorganisms that occurs in *Lepidodendron* xylem and periderm from the Visean cherts of central France. The assemblage is composed of several types of hyphae, putative reproductive structures (sporangia), and a variety of spores. Some of the remains can be attributed to the Chytridiomycota and Peronosporomycetes (Oomycota) with some degree of confidence. The *Lepidodendron* tissues offer a rare insight into the diversity of microfungi and fungus-like organisms in a Carboniferous terrestrial paleoecosystem. Since the organisms were abundant and diverse, they obviously played an important role in the ecology of the Visean ecosystem at this site.

Chytrids (Chytridiomycota) are significant elements in most modern aquatic ecosystems (e.g., Goh and Hyde 1996; Wong et al. 1998; Gleason et al. 2008). Paper **XI** surveys the evidence for chytrids and chytrid-like remains of uncertain affinity in the Visean cherts. The evidence is primarily composed of (resting) spores, as well as epibiotic and endobiotic (putative) zoosporangia that occur in/on solitary unicells, peronosporomycetous oogonia, (degrading) vascular plant tissues (i.e. xylem, periderm, cortical parenchyma), and various plant and fungal spores. Vegetative parts such as tenuous filaments or rhizomycelia in organic connection are rarely preserved. Host responses possibly linked to chytrid infection occur in the form of two different types of callosities, some with a distinct penetration canal, in lycophyte xylem and periderm, as well as in fungal spores. Although the chytrids and chytrid-like remains recorded from the Visean of central France do not provide an easily discernable comparison with chytrids in modern ecosystems, the fossils do provide the opportunity to advance hypotheses as to the ecology of this microfungal community.

Also present in several specimens of Visean lycophyte peridem is a highly unusual intracellular endophyte, *Combresomyces cornifer*, which is interpreted as a peronosporomycete based on the presence of several specimens displaying oogonia with attached paragynous antheridia (paper **VII**). Peronosporomycota (Oomycota in older treatments) are believed to be among the oldest eukaryotes on Earth (Pirozynski 1976), however, the fossil

record of this group has remained inconclusive (Johnson et al. 2002). The characteristic oogonium-antheridium complex that occurs during the sexual reproduction process represents the only structural feature that can be used to positively identify fossil peronosporomycetes (Dick 1969). *Combresomyces cornifer* is one of only two Carboniferous microorganisms possessing this feature (see Krings et al. in press). Moreover, the oogonium of *C. cornifer* show a complex surface ornamentation composed of branched, antler-like structures that arise from small, hollow extensions of the oogonium wall. This type of surface ornamentation is unknown in extant Peronosporomycota. It is particularly interesting to note that, shortly after the original discovery of *C. cornifer* from the Carboniferous of France, specimens of *C. cornifer* were also reported from permineralized peat from the Triassic of Antarctica (Schwendemann et al. 2009). This suggests that this peronosporomycete existed morphologically unchanged for a period of nearly 90 million years, and even survived the end-Permian mass extinction event. Of further significance is the fact that, although the vegetation covers of the Carboniferous and Triassic were quite different, this peronosporopmycete obviously had the capacity to adapt to changes in host quality.

3.3. Concluding Remarks and Future Perspectives

The research results presented in this thesis about microorganisms from the famous Early Devonian Rhynie chert and a chert deposit of late Visean (Late Mississippian) age from central France are among the most detailed descriptions of late Palaeozoic fossil microorganisms *in situ* to date. The results contribute to the increasing body of information about microbial life in ancient ecosystems, and thus provide a template to interpret not only newly discovered forms with regard to their morphology and systematic position, but also the associations/interactions that occur between microorganisms and other elements of the ecosystems. This approach offers several new avenues of investigation directed at both the microorganisms and the ecosystems in which they lived. Although the Early Devonian Rhynie chert and the vast Carboniferous coal swamp forests of Europe and North America are certainly among the most intensively studied fossil ecosystems throughout geologic time, analysis of new specimens continues to broaden our understanding of the complexity and dynamics within these ancient landscapes. The research presented in this thesis demonstrates the value of new discoveries by more accurately depicting the individual components of fossil ecosystems, even such as the well-known Rhynie ecosystem, and further underscores how new specimens can contribute to a more sharply focused concept of ancient ecosystems, the biology of the organisms, and the interplay between these elements.

The results also show that, in contrast to a widely-held belief, it is not only possible to study ancient microorganisms, but also to examine their ecology and interactions with other ecosystem components, if preservation is sufficient. In contrast to the Early Devonian Rhynie chert, our knowledge about the microbial element in the Visean paleoecosystems of central France remains very incomplete. However, my research results attest to the fundamental hypothesis stated at the beginning of the thesis that the under-representation of biological interactions in the Carboniferous cherts from France does not reflect an actual paucity of associations/interactions, but rather represents a study bias that has resulted from the more intensive screening for interactions in the Rhynie chert to date.

A direct comparison of the microbial life preserved in the two cherts is difficult at present. Nevertheless, the study of chytrid-like organisms from the Visean cherts (paper **XI**) clearly shows that some forms are remarkably similar to those in the Rhynie chert, and even some of the associations/interactions appear to be similar, if not identical. Others, however, are quite different (e.g., chytrid-like remains in xylem and periderm), which is perhaps the result of different types of plant-microorganism interactions (e.g., lycophytes). On the other hand, preservation of microorganisms in the Rhynie chert matrix appears to yield a more complete record and finer resolution of delicate features than that seen in the Visean cherts. As a result, the morphology, life history, spatial distribution, and diversity levels of microorganisms in the Rhynie paleoecosystem can be reconstructed in far greater detail than ever before. Interestingly, it appears that there is some evidence to support the hypothesis that more associations between microorganisms and other elements of the ecosystem existed in the Carboniferous. For example, only three different endophytic fungi have been recorded to date in the Rhynie chert land plant *Nothia aphylla*, while the wood and periderm of *Lepidodendron rhodumnese* from the Visean of France contains >30 different structures attributable to fungal endophytes.

It is my intent to continue analyzing the Rhynie and Visean cherts in order to document the microbial diversity and associations and interactions with other organisms in these two important late Paleozoic continental ecosystems. Several groups of microorganisms have not been documented to date, despite the fact that molecular clock estimates suggest they should be present (e.g., Basidiomycota; see Berbee and Taylor 2001; Heckman et al. 2001). Moreover, the evolutionary history of several associations/interactions that are prevalent today such as ectomycorrhizae and various relationships between microbes and animals continue to remain largely unknown. In addition, there are various chert deposits from the uppermost Carboniferous and Permian, Mesozoic, and Cenozoic (e.g., the Eocene Princeton chert) that

hold great potential because of their excellent preservation and the obvious interactions that existed between the microorganisms and these more diverse floras. The same may be said for the numerous examples of silicified wood containing microbial remains that occur throughout the geologic column (e.g., Stubblefield et al. 1985; Pujana et al. in press). There are also examples of indirect evidence of plant-microorganism interactions such as remains of phytoparasitic fungi in the dung of herbivorous dinosaurs (Kar et al. 2004; Sharma et al. 2005). Although known to contain a diverse biota, including fungi, for more than 100 years, a number of fossilized types of plant resins, (sometimes collectively termed amber), in recent years have produced several interesting examples of plant-microorganism associations/interactions (e.g., Dörfelt and Schmidt 2007).

As more morphotypes of microorganisms and levels of association/interaction are described and illustrated from the fossil record, there will be increasing opportunities to relate the fossil microfungi to extant counterparts and to more precisely define the systematic affinities of the individual constituents within ancient microbial communities. Documenting the full extent of microbial diversity on/in other organisms will provide a platform with which to infer various levels of biocomplexity in ancient ecosystems, and to compare these with ecosystem complexity today.

The studies have broad implications that impact multiple areas of science. 1) They demonstrate the biodiversity of microorganisms in the geologic record. 2) They provide a direct method of examining various levels of biological interaction. 3) They provide various character states that can be used in phylogenetic studies of certain microorganisms. 4) They offer direct evidence of certain types of symbiotic associations that can be compared with modern forms. 5) They suggest evolutionary scenarios relating to the impact of parasites on life history biology and community structure. 6) They have the potential to contribute to hypotheses relating to the evolution of complex symbiotic interactions. 7) Finally, the study of fossil microorganisms opens up new areas of collaboration and interdisciplinary research directed at the bio-physical interrelationships involved in understanding the complexities of Earth history.

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5. Appendix

Papers included in this thesis

The thesis is based on the following articles, which are referred to in the text by their Roman numerals (**I–XII**, in a chronological order):

I **Dotzler, N.**, Krings, M., Taylor, T.N., Agerer, R. (2006): Germination shields in *Scutellospora* (Glomeromycota: Diversisporales, Gigasporaceae) from the 400 million-year-old Rhynie chert. – *Mycological Progress* 5, 178–18
DOI: 10.1007/s11557-006-0511-z; With permission from Springer

II Krings, M., Taylor, T.N., Hass, H., Kerp, H., **Dotzler, N.**, Hermsen, E.J. (2007): Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. – *New Phytologist* 174, 648–657
DOI: 10.1111/j.1469-8137.2007.02008.x; With permission from Wiley-Blackwell

III Krings, M., Taylor, T.N., Hass, H., Kerp, H., **Dotzler, N.**, Hermsen, E.J. (2007): An alternative mode of early land plant colonization by putative endomycorrhizal fungi. – *Plant Signaling & Behavior* 2, 125–126

IV **Dotzler, N.**, Taylor, T.N., Krings, M. (2007): A prasinophycean alga of the genus *Cymatiosphaera* in the Early Devonian Rhynie chert. – *Review of Palaeobotany and Palynology* 147, 106–111
DOI: 10.1016/j.revpalbo.2007.07.001; With permission from Elsevier

V Krings, M., **Dotzler, N.**, Taylor, T.N., Galtier, J. (2007): A microfungal assemblage in *Lepidodendron* from the upper Viséan (Carboniferous) of central France. – *Comptes Rendus Palevol* 6, 431–436
DOI: 10.1016/j.crpv.2007.09.008; With permission from Elsevier

VI Krings, M., Kerp, H., Hass, H., Taylor, T.N., **Dotzler, N.** (2007): A filamentous cyanobacterium showing structured colonial growth from the Early Devonian Rhynie chert. – *Review of Palaeobotany and Palynology* 146, 265–276
DOI: 10.1016/j.revpalbo.2007.05.002; With permission from Elsevier

VII **Dotzler, N.**, Krings, M., Agerer, R., Galtier, J., Taylor, T.N. (2008): *Combresomyces cornifer* gen. sp. nov., a peronosporomycete in *Lepidodendron* from the Carboniferous of central France. – *Mycological Research* 112, 1107–1114
DOI: 10.1016/j.mycres.2008.03.003; With permission from Elsevier

VIII Krings, M., Hass, H., Kerp, H., Taylor, T.N., Agerer, R., **Dotzler, N.** (2008): Endophytic cyanobacteria in a 400-million-yr-old land plant: a scenario for the origin of a symbiosis? – *Review of Palaeobotany and Palynology* 153, 62–69
DOI: 10.1016/j.revpalbo.2008.06.006; With permission from Elsevier

IX Krings, M., **Dotzler, N.**, Taylor, T.N. (2009): *Globicultrix nugax* nov. gen. et nov. spec. (Chytridiomycota), an intrusive microfungus in fungal spores from the Rhynie chert. – *Zitteliana A*, 48/49, 165–170

X **Dotzler, N.**, Walker, C., Krings, M., Hass, H., Kerp, H., Taylor, T.N., Agerer, R. (2009): Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-yr-old Rhynie chert. – *Mycological Progress*, 8, 9–18
DOI: 10.1007/s11557-008-0573-1; With permission from Springer

XI Krings, M., **Dotzler, N.**, Galtier, J., Taylor, T.N. (2009): Microfungi from the upper Visean (Mississippian) of central France: Chytridiomycota and chytrid-like remains of uncertain affinity. – *Review of Palaeobotany and Palynology* 156, 319–328
DOI: 10.1016/j.revpalbo.2009.03.011; With permission from Elsevier

XII Krings, M., **Dotzler, N.**, Longcore, J.E., Taylor, T.N. (accepted): An unusual micro-fungus in a fungal spore from the Lower Devonian Rhynie chert. – *Palaeontology*
DOI: 10.1016/j.revpalbo.2009.03.011; With permission from Wiley-Blackwell

Authors contribution to each paper (%)

Nora Dotzler…	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
discovered the specimens	**yes**	no	no	**yes**	**yes**	no	**yes**	no	**yes**	in part	in part	**yes**
designed and conducted the research	100	25	25	100	40	10	100	10	50	100	50	50
wrote the manuscript	90	0	0	100	25	25	90	0	25	90	25	25
prepared the artwork	100	75	75	100	100	50	100	50	100	100	100	100

I hereby confirm the above statements

_____ _____

Prof. Dr. Reinhard. Agerer Prof. Dr. Michael Krings

Taxonomic novelties in this study:

Scutellosporites devonicus Dotzler et al. paper **I**
Croftalania venusta Krings et al. paper **VI**
Combresomyces cornifer Dotzler et al. paper **VII**
Globicultrix nugax Krings et al. paper **IX**
Kryphiomyces catenulatus Krings et al. paper **XI**

Curriculum vitae

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