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Author for correspondence:
Michael Krings
e-mail: m.krings@lrz.uni-muenchen.de

Fungi and fungal interactions in the Rhynie chert: a review of the evidence, with the description of *Perexiflasca tayloriana* gen. et sp. nov.†

Michael Krings[1,2], Carla J. Harper[1,2] and Edith L. Taylor[2]

[1]Department für Geo- und Umweltwissenschaften, Paläontologie und Geobiologie, Ludwig-Maximilians-Universität, and SNSB-Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Straße 10, 80333 Munich, Germany
[2]Department of Ecology and Evolutionary Biology, and Biodiversity Institute, The University of Kansas, Lawrence, KS 66045, USA

MK, 0000-0002-6199-4235

The Lower Devonian Rhynie chert is one of the most important rock deposits yielding comprehensive information on early continental plant, animal and microbial life. Fungi are especially abundant among the microbial remains, and include representatives of all major fungal lineages except Basidiomycota. This paper surveys the evidence assembled to date of fungal hyphae, mycelial cords and reproductive units (e.g. spores, sporangia, sporocarps), and presents examples of fungal associations and interactions with land plants, other fungi, algae, cyanobacteria and animals from the Rhynie chert. Moreover, a small, chytrid-like organism that occurs singly, in chain-like, linear arrangements, planar assemblages and three-dimensional aggregates of less than 10 to $\gg$100 individuals in degrading land plant tissue in the Rhynie chert is formally described, and the name *Perexiflasca tayloriana* proposed for the organism. *Perexiflasca tayloriana* probably colonized senescent or atrophied plant parts and participated in the process of biological degradation. The fungal fossils described to date from the Rhynie chert constitute the largest body of structurally preserved evidence of fungi and fungal interactions from any rock deposit, and strongly suggest that fungi played important roles in the functioning of the Early Devonian Rhynie ecosystem.

This article is part of a discussion meeting issue 'The Rhynie cherts: our earliest terrestrial ecosystem revisited'.

1. Introduction

The Lower Devonian Rhynie chert from Aberdeenshire, Scotland, has long been recognized as one of the most important rock deposits yielding comprehensive information on early continental plant and animal life [1–6]. More recently, the Rhynie chert has also become increasingly attractive as a source of new information on the diversity of microbial life and insights into the biology and ecology of microorganisms in ancient freshwater and terrestrial environments, inspired in part by the growing awareness of the importance of the microbial component in modern ecosystems [7,8]. The documented record of microbial life from the Rhynie chert currently comprises bacteria [9], coccoid and filamentous cyanobacteria [10–13], eukaryotic algae [14–18], peronosporomycetes [19–21], fungi belonging to all major lineages except the Basidiomycota (see below), and representatives of the enigmatic nematophytes [22,23].

Fungi (in the broadest sense of including fungus-like organisms such as Peronosporomycetes and Hyphochytridiomycetes) are remarkably abundant and diverse in the Rhynie chert. Filaments, aseptate and septate hyphae, mycelial cords and a broad spectrum of different types of small propagules (e.g.

†This paper is dedicated to the memory of Thomas N. Taylor (†2016), who has set the standard in the study of Rhynie chert fungi. We honour his legacy through the continuation of the work that he so loved.

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spores) and detached reproductive units (e.g. sporangia, sporocarps) are almost ubiquitous in the chert matrix, in microbial mats and litter accumulations, and within intact and decaying land plant parts [9]. Moreover, several exquisite specimens of fungi preserved *in situ* together with their host organisms demonstrate the existence of different types of fungal associations and interactions, including parasites on algae, land plants, other fungi and possibly animals, mycorrhizas in both sporophytes and gametophytes of land plants, and saprotrophs on decaying plant parts [8].

This paper surveys the documented fungal diversity in the Rhynie chert, thereby focusing on reproductive units, which occur in nearly every thin section of the chert. Moreover, the spectrum of fungal associations and interactions that have been documented from the Rhynie chert is reviewed. However, some of the most common fungal associations in the Rhynie chert have not been particularized to date, due probably to the fact that the microbial partners are exceedingly small. The second purpose of this paper is therefore to describe *Perexiflasca tayloriana* gen. et sp. nov., an excellent example of a minute, chytrid-like Rhynie chert organism that has long been known [24], but its association with partly degraded land plant tissue, although frequently encountered in litter layers, has not been detailed to date. The Rhynie chert fungal fossils suggest that fungi were instrumental to the functioning of the Rhynie ecosystem.

2. Geological setting, material and methods

The Rhynie chert Lagerstätte is located in the northern part of the Rhynie outlier in Aberdeenshire, Scotland [25,26], and includes series of chert lenses that are principally fine grained and interpreted as having accumulated on an alluvial plain associated with ephemeral ponds and lakes. The ecosystem is interpreted as a geothermal wetland [27–29], with alkaline hot springs that were part of a complex hydrothermal system [25,30]. Both aquatic and terrestrial organisms became preserved as a result of temporary flooding with silica-rich water, or by silica-rich groundwater that percolated to the surface. The Rhynie chert biota has been regarded as early (but not earliest) Pragian to earliest Emsian in age based on spore assemblages [31,32]. An age estimate based on high-precision U–Pb dating of zircon and titanite from hydrothermally altered andesite indicates an absolute age of 411.5 ± 1.3 Ma for the Rhynie chert biota [26], while another age constraint using $^{40}Ar/^{39}Ar$ in K-feldspar from a quartz-feldspar vein that is part of the hydrothermal system responsible for the formation of the Rhynie chert yields a mean age (recalculated to be U–Pb comparable) of the fossilized biota of 407.1 ± 2.2 Ma [33]. However, the andesite cannot be fixed with certainty in the stratigraphic sequence and is certainly older than the hydrothermal alteration [34]. As a result, the date estimate in [33] probably gives a more accurate age of the hydrothermal system, and hence the age of the Rhynie chert biota. An absolute age of 411.5 ± 1.3 Ma is very close to the Lochkovian/Pragian boundary (410.8 ± 2.8 Ma), while the age suggested in [33] would correspond approximately to the Pragian/Emsian boundary (407.6 ± 2.6 Ma). For additional information on the geology and palaeontology of the Rhynie chert, refer to the other papers in this volume.

All fossils described and illustrated in this paper were identified in thin sections prepared from chert blocks by cementing wafers of the chert to glass slides and then grinding the rock slices until the sections were thin enough to transmit light. The thin sections were analysed using transmitted-light microscopy; digital images were captured with a Leica DFC-480 camera and processed in Adobe Photoshop. Most of the specimens illustrated in figures 1–5 are deposited in the Bayerische Staatssammlung für Paläontologie und Geologie (SNSB-BSPG), Munich, Germany (prefix BSPG). Additional material is housed in the Abteilung Paläobotanik, Geologisch-Paläontologisches Institut, Westfälische Wilhelms-Universität, Münster, Germany (prefix P). Accession numbers for all figured materials are included in the figure captions.

3. Fungi in the Rhynie chert: review of the evidence

(a) Vegetative remains

Fragments of fungal filaments and hyphae are frequently encountered throughout the Rhynie chert [9]. Moreover, sterile mycelia are preserved *in situ* in some sections of the chert (figure 1*a*). Hyphae may be aseptate or septate, thin- or thick-walled, tubular or irregular, branched or non-branched and some possess terminal or intercalary swellings; however, none are physically connected to reproductive structures that could be used to determine the systematic affinities of these fungi. Intermixed with filaments and hyphae are sometimes banded tubes that have been previously attributed to nematophytes, as well as branch knots comprised densely aggregated, profusely branched (banded) tubes (figure 1*c*). As to whether these structures are remnants of disintegrated *Nematoplexus* or other nematophyte thalli [22], or have formed outside the confines of a thallus or plexus remains unclear.

Mycelial cords, linear aggregations of up to greater than 50 parallel-oriented hyphae, are present in many areas of the Rhynie chert (figure 1*b*). Hyphae within one cord may vary considerably with regard to diameter (approx. 2–greater than 10 µm), wall thickness and septation; anastomoses and intrahyphal hyphae regularly occur in larger cords. Some of the smaller (i.e. constructed of less than 10 hyphae) mycelial cords in the Rhynie chert have been shown to belong to the extramatrical mycelium of the endomycorrhizal fungus *Glomites rhyniensis* [35]. Mycelial cords in fungi today aid in the exploration of the environment by facilitating long distance transport of water and nutrients (e.g. [36–39]). It is therefore plausible to assume that these structures also played important roles in Rhynie chert fungi. Unfortunately, the Rhynie chert mycelial cords have not yet been systematically analysed and documented.

(b) Reproductive units

The abundance and morphological diversity of small (less than 0.5 mm) fungal propagules and reproductive units is one of the hallmark features of the Rhynie chert. However, dealing with these remains is notoriously difficult because they usually occur detached from the systems on or in which they were produced, and thus do not provide a complete range of structural features necessary to determine their systematic affinities [40]. Only a few forms occur in characteristic configurations (figure 1*e*) or possess special features such as elaborate surface ornaments (figure 1*f*) or

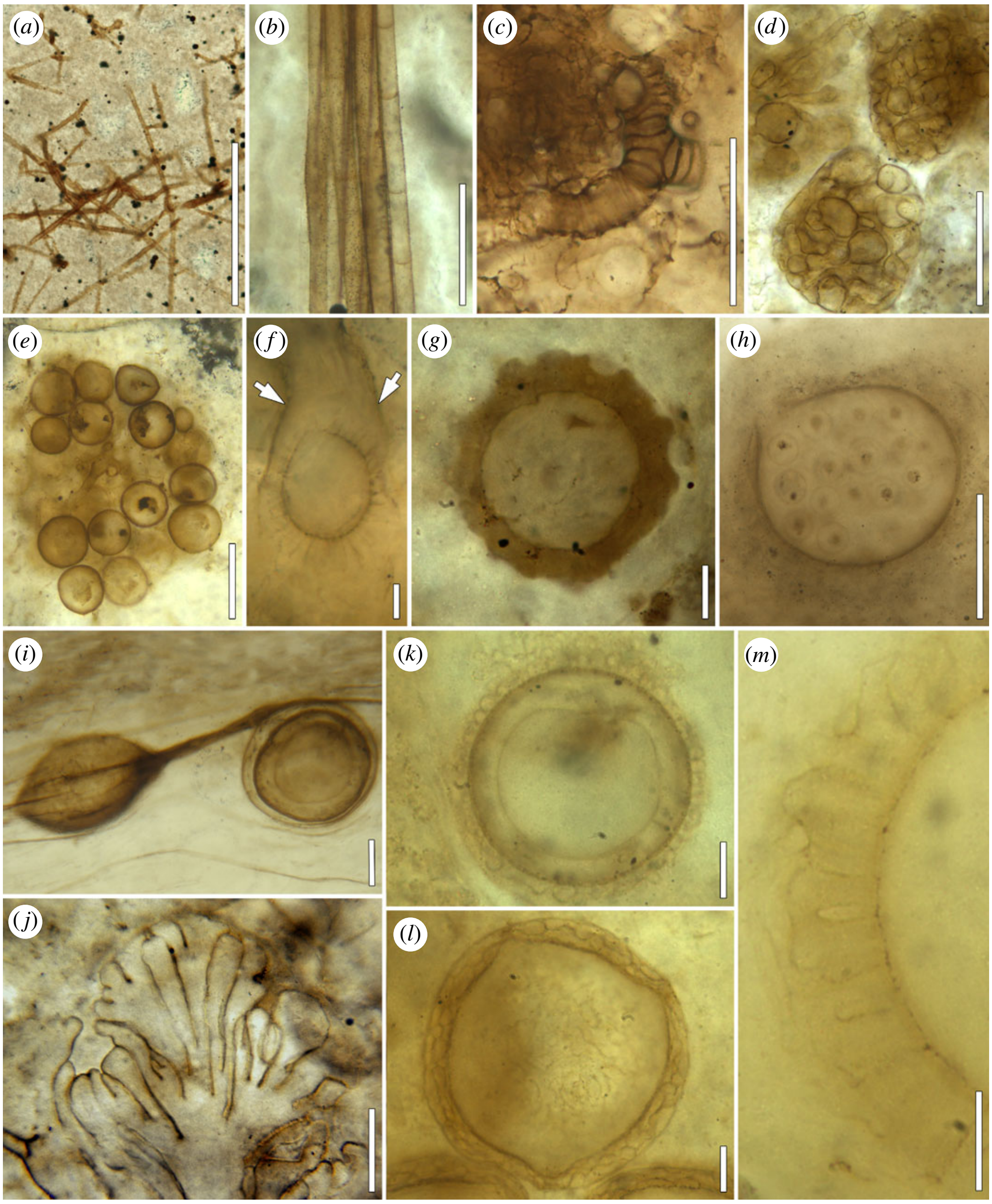

Figure 1. Fungi and fungal interactions in the Lower Devonian Rhynie chert (explanations in the text). (*a*) Mycelium in chert matrix; BSPG 2008 XVI 2; bar, 500 μm. (*b*) Mycelial cord; BSPG 2013 V 16; bar, 100 μm. (*c*) Branch knot; BSPG 2015 XVII 19; bar, 50 μm. (*d*) Vesicle clusters in land plant cells; BSPG 1965 I 295; bar, 50 μm. (*e*) Cluster of chlamydospores; BSPG 2015 XVIII 8; bar, 100 μm. (*f*) Spiny propagule surrounded by sheath (arrows); BSPG 2008 XVI 10; bar, 10 μm. (*g*) Sheathed chlamydospore; BSPG 1965 I 357; bar, 10 μm. (*h*) Oogonium containing oospores of *Frankbaronia velata*; BSPG 2013 V 50; bar, 50 μm. (*i*) Acaulosporoid glomeromycotinan spore with sporiferous saccule; P3966; bar, 100 μm. (*j*) Germination shield of acaulosporoid spore; P3951; bar, 20 μm. (*k*) *Zwergimyces vestitus*; BSPG 2013 XV 38; bar, 10 μm. (*l*) *Scepasmatocarpion fenestrulatum*; BSPG 1965 I 363; bar, 10 μm. (*m*) Two-layered hyphal investment of *H. devonica*; BSPG 2013 XV 123; bar, 10 μm.

complex wall architecture (figure 1*g*) that makes it possible to recognize distinctiveness and sometimes even determines affinities. Other forms can be attributed systematically with some degree of confidence based on a combination of structural features and content (figure 1*h*) [20,21].

Simple, spheroidal reproductive units borne terminally on hyphae are usually interpreted as glomoid glomeromycotinan spores (chlamydospores); several types were initially described and illustrated in [9] and placed in the genus *Palaeomyces*. Spore size and morphology are variable, and in some specimens, the wall is multi-layered. *Palaeomyces* is associated with several land plants, including *Aglaophyton majus* and *Asteroxylon mackiei*, as well as degraded plant material [9,41]. Other spores in *As. mackiei* have been described as *Scutellosporites devonicus* [42]. The presence of what appears to be a bulbous base in these specimens is characteristic of gigasporoid glomeromycotinan spores [43]. Moreover, a round or oval germination shield occurs within the multi-layered wall in some of the specimens. A third type of glomeromycotinan spore from the Rhynie chert develops laterally within the neck of a sporiferous saccule (figure 1*i*), and thus corresponds to present-day acaulosporoid AM fungi [44]. The wall of this spore has been suggested to consist of three major parts: a germination shield that can vary in morphology from plate-like with single or double lobes to tongue-shaped with infolded, distally fringed or palmate margins (figure 1*j*), is formed by extrusion of one of the wall components. Another acaulosporoid spore type recently discovered from the Rhynie chert is characterized by prominent fringes extending from the surface [45].

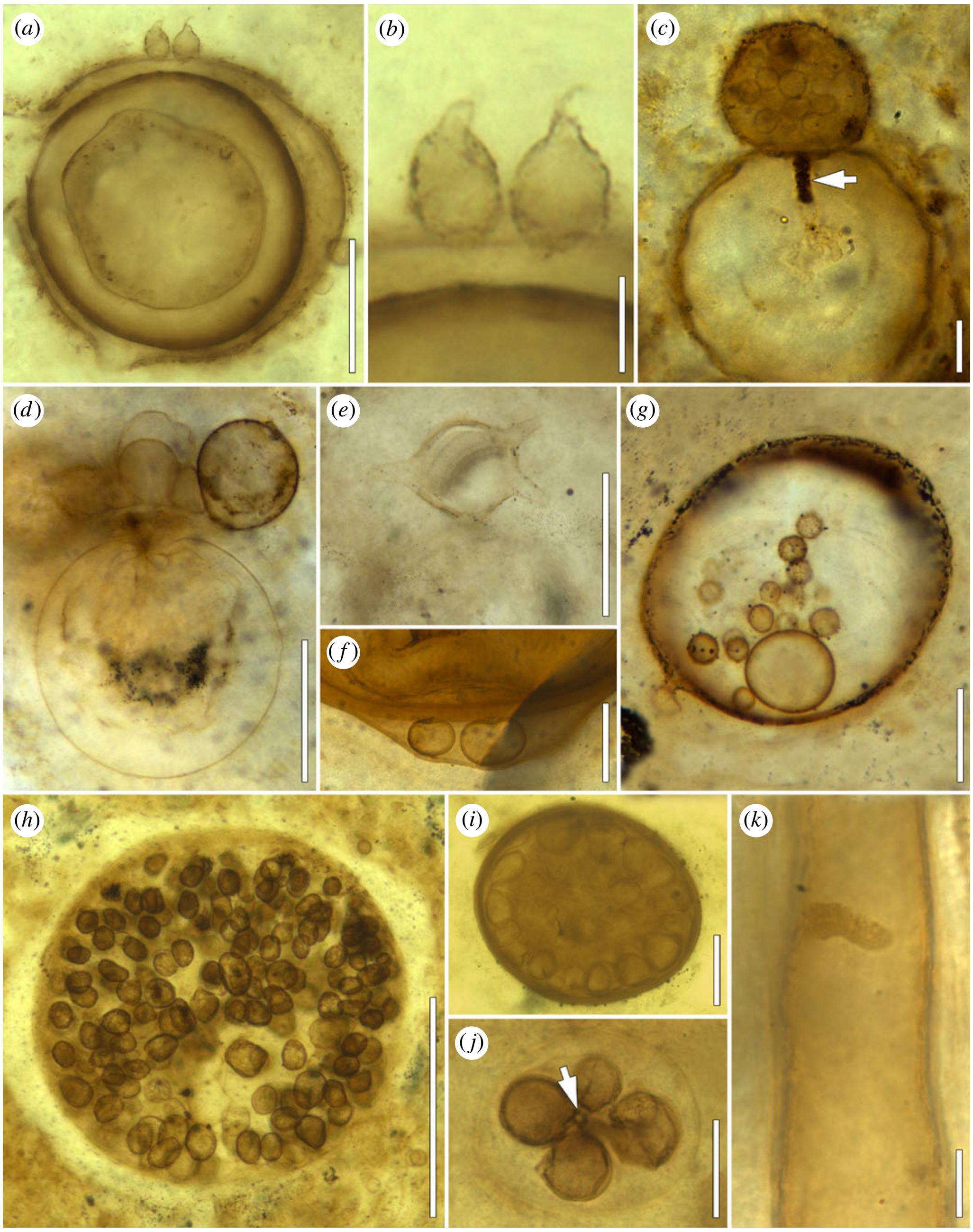

Figure 2. Fungi and fungal interactions in the Lower Devonian Rhynie chert (explanations in the text). (*a*) Partially degraded, thick-walled fungal spore with chytrid zoosporangia on surface; BSPG 2013 XIV; bar, 50 µm. (*b*) Detail of (*a*), showing zoosporangia with distal discharge papilla; bar, 10 µm. (*c*) Chytrid zoosporangium containing zoospores on fungal spore, with prominent primary rhizoidal axis (arrow) extending into host spore lumen; BSPG 2013 V 61; bar, 10 µm. (*d*) Cluster of chytrid zoosporangia extending from fungal spore; BSPG 2016 VII 6; bar, 50 µm. (*e*) *Illmanomyces corniger* on fungal spore; BSPG 2013 V 8; bar, 50 µm. (*f*) Chytrid zoosporangia between wall layers of fungal spore; BSPG 2013 XV 5; bar, 10 µm. (*g*) Putative polycentric chytrid in lumen of glomeromycotinan spore; BSPG 1964 XX 24; bar, 50 µm. (*h*) Sporocarp in large glomeromycotinan spore; BSPG 2013 XV 37; bar, 50 µm. (*i*) Sporocarp with thick peridium; BSPG 2013 XV 46; bar, 50 µm. (*j*) Tiny fungal reproductive units extending from central hypha (arrow) in lumen of glomeromycotinan vesicle; BSPG 2013 XV 125; bar, 100 µm. (*k*) Callosity in fungal hypha; BSPG 2015 XIX 92; bar, 10 µm.

Several types of Rhynie chert fungal reproductive units have been described that all possess an ancillary covering in the form of a hyphal investment or mantle. Investment morphology varies considerably among the different types, and thus renders them easy to distinguish from one another. The investment of *Zwergimyces vestitus* (figure 1*k*) consists of interlaced hyphae extending along the circumference of the structure [46]. Variations in the organization of the mantle among the specimens suggest that mantle formation took place by repeated branching of hyphae on the surface of the developing unit and by additional hyphae extending between the pre-existing mantle hyphae [40]. On the other hand, the investment of *Mycocarpon rhyniense* is two-layered, with the inner layer formed by interlaced circumferential hyphae, and the outer layer of hyphal branches that are radially oriented [47]. A two-layered investment is also present in *Helmutella devonica* [48], with the outer layer constructed of interlaced circumferential hyphae, while the inner layer consists of radially elongate elements that are closely aligned (figure 1*m*). Especially interesting is that this investment morphology closely corresponds to that seen in certain Carboniferous fungal ‘sporocarps’, including *Dubiocarpon* and *Mycocarpon* (see [49,50]). The fourth investment type is similar to *Z. vestitus*, but differs in that the

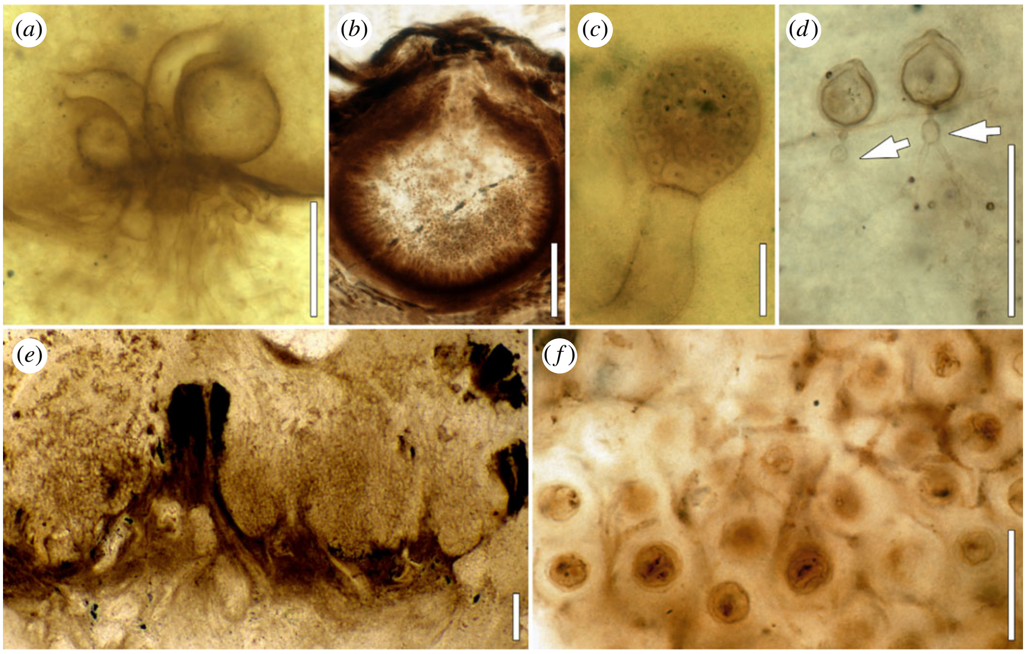

Figure 3. Fungi and fungal interactions in the Lower Devonian Rhynie chert (explanations in the text). (*a*) *Trewinomyces annulifer* extending from surface of land plant axis; BSPG 1965 I 336; bar, 50 µm. (*b*) Perithecium (longitudinal section) of *Paleop. devonicus* in *As. mackiei* (Courtesy of H. Kerp & H. Hass, Münster, Germany); P3411; bar, 100 µm. (*c*) Zoosporangium of *Paleob. milleri* (Courtesy of H. Kerp & H. Hass, Münster, Germany); P2054; bar, 10 µm. (*d*) Monocentric chytrids on *Palaeo. cranii* with endobiotic apophysis and rhizomycelium (arrows); BSPG 2016 VII 5; bar = 50 µm. (*e*) Thallus of *W. reticulata* with hyphal pockets containing cyanobacteria (Courtesy of H. Kerp & H. Hass, Münster, Germany); P1323; bar, 500 µm. (*f*) Hyphal weft of *W. reticulata* enclosing cyanobacterial cells (Courtesy of H. Kerp & H. Hass, Münster, Germany); P1386; bar, 15 µm.

investment hyphae have club-shaped tips [51]. Finally, the investment of *Scepasmatocarpion fenestrulatum* occurs in the form of a pseudotissue comprised tightly interwoven hyphae [52]. Moreover, several prominent pores extend through the investment (figure 1*l*). Krings & Taylor [46,48] and Krings *et al*. [40,47] suggest that most mantled reproductive units from the Rhynie chert have systematic affinities with the Glomeromycotina or Mucoromycotina based on similar features in modern lineages known to produce spores or sporangia with hyphal investments. One form has also been compared to the so-called 'birdsnest' condition of certain peronosporomycte oogonia [51], while *S. fenestrulatum* is reminiscent of the pycnidia and cleistothecia formed by certain modern ascomycetes [52].

(c) Associations and interactions

Several different types of fungal associations and interactions with land plants, other fungi, charophytes, animals and cyanobacteria have been described from the Rhynie chert and directly compared to modern equivalents to determine the nutritional relationship (mutualistic, parasitic and saprotrophic) between the partners in the fossils.

(i) Fungi–land plants

The most significant fungal interaction in the Rhynie chert is the endomycorrhiza (paramycorrhiza *sensu* Strullu-Derrien & Strullu [53]) that occurs in the land plant *Ag. majus* [35,54–56]. The fungal partner, *G. rhyniensis* (Glomeromycotina), produces an extramatrical mycelium composed of hyphae and mycelial cords. Individual hyphae enter the plant through stomata in the aboveground prostrate axes and spread out in the intercellular system of the outer cortex. Within the cortex, *G. rhynienis* produces vesicles and glomoid spores, as well as arbuscules within a narrow zone of tissue between the outer and middle cortex. Mycorrhizal axes of *Ag. majus* sometimes also host a filamentous cyanobacterium, which also enters the plant through stomata and invades parenchyma cells close to and within the mycorrhizal arbuscule-zone to form intracellular coils [12]. What relationship, if any, existed between the cyanobacterium and the mycorrhizal land plant remains unresolved. The endomycorrhiza in *Ag. majus* from the Rhynie chert represents one of the core pieces of fossil evidence of the evolutionary history of mycorrhizal systems [57–59]. Moreover, it substantiates the hypothesis that the establishment of plant life on land concurred with, and was profoundly influenced by, the evolution of mutually beneficial symbioses between the earliest plants and certain fungi (e.g. [60–66]).

Structures suggestive of the presence of mycorrhizal associations in other Rhynie chert plants have been reported in [41,67–71]. For example, a distinct zone of fungal colonization similar to that observed in *Ag. majus* has been described in *Rhynia gwynne-vaughanii* [67]. Moreover, various fungi occur in the prostrate axes of the land plant *Nothia aphylla*, including one that is believed to be endomycorrhizal [69,70]. As the prostrate axes of *N. aphylla* lack stomata, the mycorrhizal fungus enters the plant through rhizoids. In the host cortex, the fungus forms an extensive intercellular network of hyphae, and produces vesicles and thick-walled spores. No arbuscules have been identified in *N. aphylla*. Finally, Strullu-Derrien *et al*. [71] report on two different fungi in the land plant *Horneophyton lignieri*. *Palaeoglomus boullardii*, a member of the Glomeromycotina, occurs in the upright axes within a discontinuous zone of the outer cortex where it forms vesicles, spores and arbuscule-like structures, while *Palaeoendogone gwynne-vaughaniae*, believed to belong to the Mucoromycotina, is present in the cortex

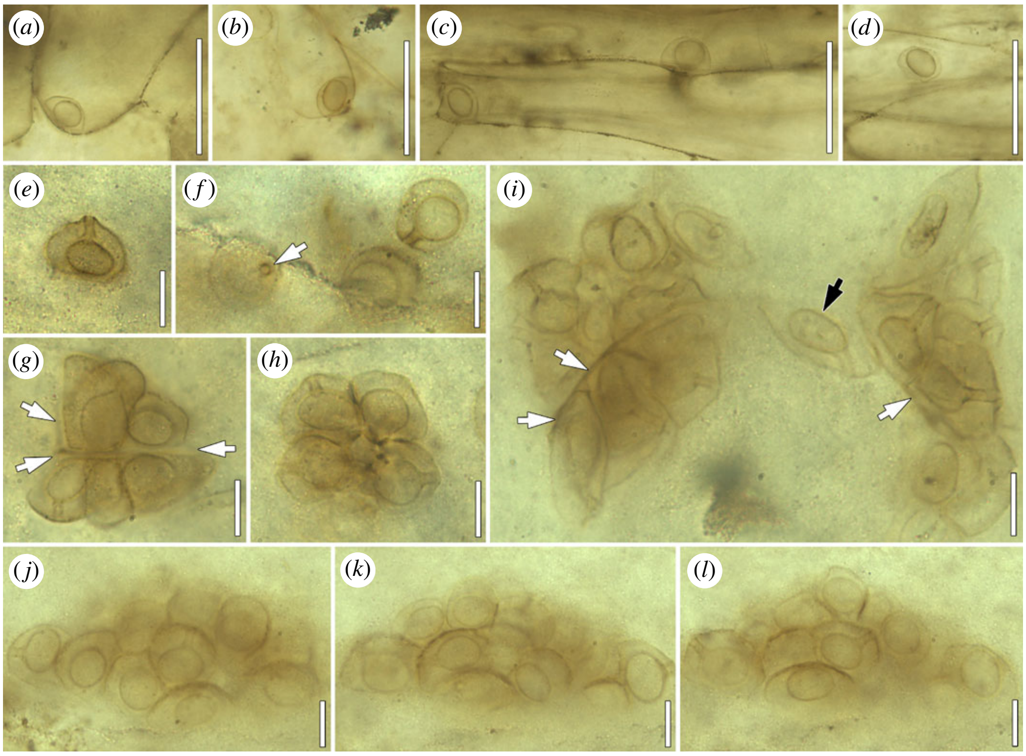

Figure 4. Fungi and fungal interactions in the Lower Devonian Rhynie chert: *P. tayloriana* gen. et sp. nov. (*a–d*) Single thalli attached to the inner surface of land plant cell walls; BSPG 2013 V 13; bars 50 µm. (*e*) Holotype. Mature thallus in the lateral view; BSPG 2013 V 30; bar, 10 µm. (*f*) Thalli in the lateral and top view; arrow points to discharge tube in surface view; BSPG 2013 V 17; bar, 10 µm. (*g*) Thalli clustered in the corner of three land plant cells; arrows indicate remains of host cell walls; BSPG 2013 V 32; bar, 10 µm. (*h*) The same as figure 2*g*, different focal plane; bar, 10 µm. (*i*) Thalli in largely degraded land plant cortical tissue; white arrows indicate remains of host cell walls, black arrow points to specimen with tiny inclusions in cavity; BSPG 2013 V 31; bar, 10 µm. (*j–l*) Optical sections of aggregate of thalli on the inner surface of plant cell; BSPG 2013 V 17; bars, 10 µm.

intercellular system in the basal part of the plant and forms intracellular coils. This discovery suggests that not only Glomeromycotina but also Mucoromycotina entered into mutualistic relationships with land plants in the Rhynie ecosystem (see [72–74]).

Evidence of fungal interactions with land plants in the Rhynie chert also occurs in the form of micrometre-sized spheroidal, obpyriform or clavate vesicles, some with one or several pores or papillae in the wall, that are attached to the outer surface of land plant spores. The vesicles are usually interpreted as chytrid zoosporangia based on correspondences in overall appearance between the fossils and certain extant chytrids colonizing spores and pollen grains [9,24,75,76]. Moreover, *Palaeozoosporites renaultii*, a Rhynie chert fungus that consists of aseptate filaments with isotomous or sympodial branching, extends through the intercellular system in the cortex of the rooting structures of the early lycophyte *As. mackiei* [77]. Arising from the filaments are globous to ovoid structures interpreted as zoosporangia and resting sporangia. The fossil resembles certain present-day polycentric chytrids, but doubts remain over the precise systematic affinity.

Another interesting fungus associated with *As. mackiei* is the perithecial ascomycete *Paleopyrenomycites devonicus*, which occurs in the axes and leaf-like appendages of this early lycophyte [78,79]. The perithecia (figure 3*b*) are characterized by short, ostiolate necks protruding from the host epidermis through stomatal openings. Lining the interior of the perithecium are thin-walled paraphyses interspersed with asci containing ascospores. Tufts of conidiophores arising from acervuli are believed to represent the anamorphic phase of the fungus. Taylor *et al*. [79] suggest that *Paleop. devonicus* might be a pyrenomycete, perhaps a member of the Sordariomycetes; however, affinities to the Taphrinomycotina and Pezizomycetes have also been discussed [80,81]. The nutritional mode of *Paleop. devonicus*, whether it was a parasite or saprotroph, remains unresolved.

An exquisitely preserved saprotrophic fungus described from the Rhynie chert is *Paleoblastocladia milleri* [82], which occurs in the form of tufts that emerge from stomata and surface ruptures in partially degraded *Ag. majus* axes. Thalli are of two nearly identical morphological types that consist of branched, intramatrical rhizoids and aseptate, erect extramatrical hyphae. On the sporothallus are terminal zoosporangia (= mitosporangia), each attached to the parental hypha by a septum (figure 3*c*). Also associated with the sporothalli are meiosporangia, or resting sporangia, characterized by a patterned surface ornament of delicate depressions or punctae. The second thallus type of *Paleob. milleri* produces barrel-shaped gametangia that are smaller than the zoosporangia and organized in pairs or stacks of three. *Paleoblastocladia milleri* shares features with certain members of the modern *Allomyces* and related species in the Blastocladiomycota [83,84]. Another saprotrophic Rhynie chert fungus that occurs in the form of tufts emerging from decaying land plant axes is *Trewinomyces annulifer* [85]. This fungus consists of a branched, intramatrical rhizoidal system and an unbranched, erect extramatrical hypha (stalk) that bears a single, terminal sporangium (figure 3*a*). The overall morphology of *T. annulifer* resembles the extant genera *Macrochytrium* (Chytridiomycota) and *Blastocladiella* (Blastocladiomycota). However, the rhizoids are septate or pseudoseptate, a feature not known in extant

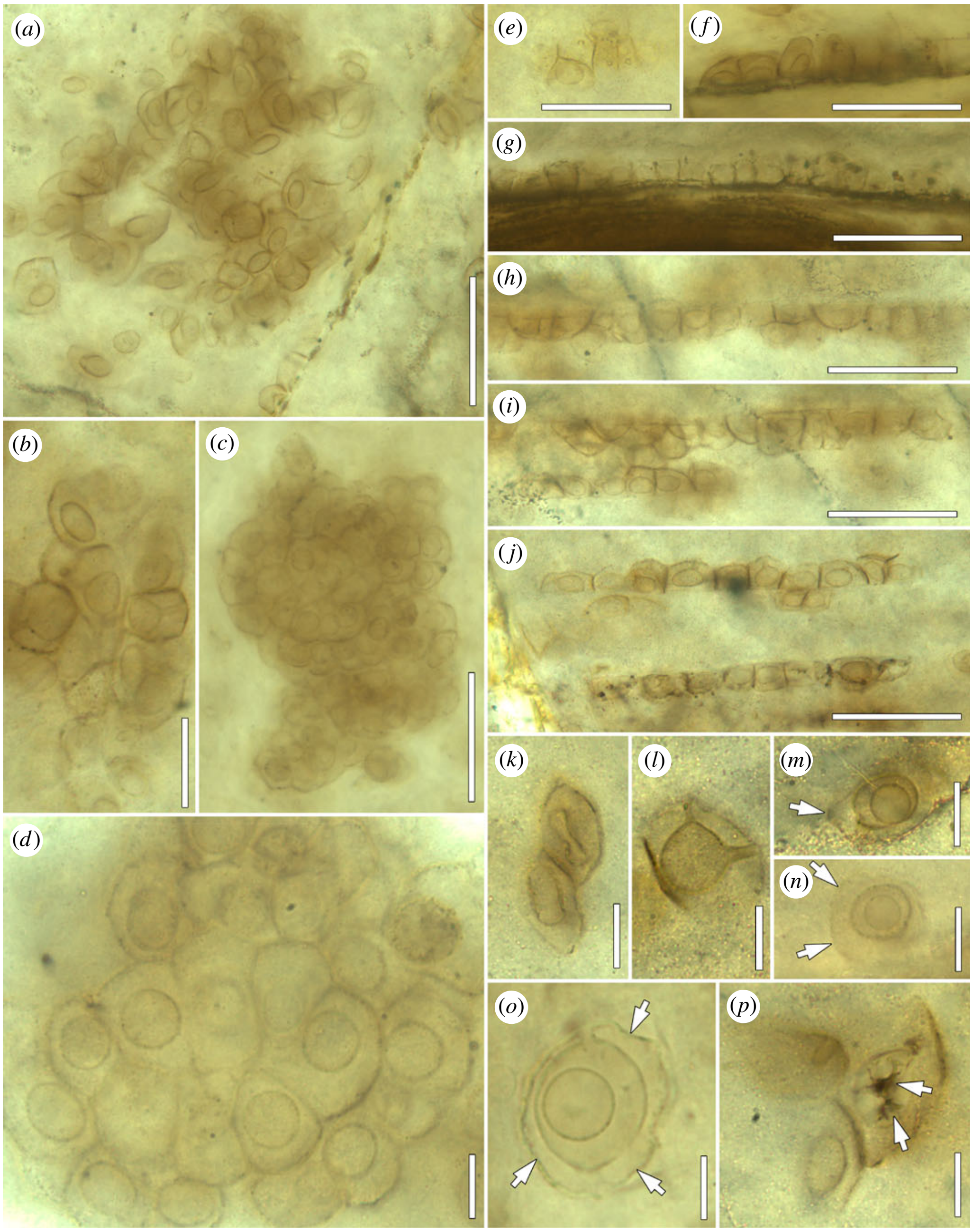

Figure 5. Fungi and fungal interactions in the Lower Devonian Rhynie chert: *P. tayloriana* gen. et sp. nov. (*a*) Aggregate in largely degraded land plant tissue; BSPG 2013 V 16; bar, 50 µm. (*b*) Group of thalli, showing variability in shape; BSPG 2013 V 34; bar, 20 µm. (*c*) Large, three-dimensional aggregate of greater than 100 thalli in chert matrix; BSPG 1964 XX 99; bar, 50 µm. (*d*) Detail of figure 3*c*, focusing on some of the thalli; bar, 10 µm. (*e*) Linearly aligned thalli in top view; BSPG 2013 V 36; bar, 50 µm. (*f–j*) Linearly aligned thalli (lateral views) in long, narrow cells close to land plant vascular strands; BSPG 2013 V 16 (*f*) and 36 (*g–j*); bars, 50 µm. (*k–p*) Morphological variants. (*k*) Thalli with elongate to dumb bell-shaped lumen; BSPG 2013 V 30; bar, 10 µm. (*l*) Thallus with two discharge tubes; BSPG 2013 V 34; bar, 10 µm. (*m–o*) Specimens surrounded by delicate sheath with one to several pores (arrows); BSPG 2013 V 17 (*m*,*n*) and 13 (*o*); bars, 10 µm. (*p*) Specimen suggestive of the presence of small rhizomycelium (arrows); BSPG 2013 V 34; bar, 10 µm.

zoosporic fungi, and this renders the systematic affinities of *T. annulifer* unresolved.

Still other fungal remains frequently associated with intact and decaying land plant axes in the Rhynie chert are intra- or intercellular vesicle clusters (figure 1*d*), thick-walled resting spores and sporocarps (figure 2*i*), wefts of hyphae and clusters of small propagules. However, the systematic affinities and nutritional modes of these fungi remain unresolved.

(ii) Fungi–fungi

Abundant evidence of interfungal associations have been reported from the Rhynie chert, including mycelia and reproductive structures inside large fungal spores (figure 2*g*) [86–88], hyphae enveloping and subsequently penetrating glomeromycotinan vesicles [89] and small fungal propagules developing in glomeromycotinan vesicles (figure 2*j*) [90]. Moreover, several examples of monocentric and polycentric chytrid-like organisms have been described as colonizers of fungal hyphae and spores (figure 1*a–e*). Most chytrid parasites of fungal spores in the Rhynie chert are characterized by epibiotic zoosporangia and rhizomycelia extending into the host spore lumen [24,86,91]. Other chytrid-like colonizers of fungal spores are found between particular wall layers of these spores or occupying the spore lumen (figure 2*f*,*g*) [9,86]. For example, *Globicultrix nugax*, a polycentric thallus comprised a rhizomycelium of branched, aseptate filaments and apophysate sporangia that are exclusively terminal, occurs in the lumen of large glomeromycotinan spores [92]. The morphology and size of *G. nugax* has been compared to extant polycentric chytrids such as *Nowakowskiella* and *Cladochytrium*, both within the order Chytridiales. Inwardly directed pegs or papillae (termed appositions or callosities) that arise from the inner surface of the host wall and encase invading fungal hyphae or filaments (figure 2*k*) represent a common host response of Rhynie chert fungi to attacks by other fungi, albeit the intrusive entity is not always preserved in a recognizable form [24,86].

(iii) Fungi–charophytes

Chytrid-like organisms have also been identified as common parasites of the Rhynie chert charophyte *Palaeonitella cranii* (figure 3*d*) [93]. One of these chytrids is *Milleromyces rhyniensis*, which is characterized by a spheroidal, endobiotic zoosporangium with a single, prominent discharge tube extending out from the host cell wall. At the base of the zoosporangium is a small rhizomycelium. Other chytrid-like organisms associated with *Palaeo. cranii* include *Lyonomyces pyriformis* and *Krispiromyces discoides*, which differ from one another in thallus morphology, but are comparable with several extant chytrid parasites of freshwater algae, including members in *Entophlyctis* and *Phlyctochytrium* (see [94,95]). The host response in *Palaeo. cranii* consists of a massive hypertrophy of cells, which grow to approximately five times the diameter of normal cells [93]. A very similar form of hypertrophy in response to chytrid parasitism has been reported in the modern genus *Chara*, a relative of *Palaeonitella* [96].

(iv) Fungi–animals

A monocentric chytrid with epibiotic zoosporangia that is quite similar morphologically to some of the forms parasitizing *Palaeo. cranii* and certain fungal spores in the Rhynie chert has been described as *Cultoraquaticus trewinii* [97]. Zoosporangia of *C. trewinii* are intermixed with spiny spherules interpreted as branchiopod resting eggs attributable to the crustacean *Lepidocaris rhyniensis*, suggesting that chytrids played important roles in the mobilization of nutrients in early aquatic food webs. Direct evidence of fungi as parts of food webs in the Rhynie ecosystem comes from coprolites containing fragments of hyphae and fungal spores [98].

(v) Fungi–cyanobacteria

A cyanolichen-like association has been described from the Rhynie chert as *Winfrenatia reticulata* [99,100]. It occurs in the form of a thallus constructed of superimposed layers of parallel hyphae. The uppermost layers are folded vertically into loops that form a pattern of ridges and circular to elliptical depressions on the surface (figure 3*e*). Extending from the walls of the depressions are hyphae that form a three-dimensional network. As a result of hyphal branching, each depression consists of lacunae that are formed by the mycobiont. The cyanobacterial photobiont consists of coccoid cells or clusters of cells, each cluster surrounded by a prominent sheath, that occur within the lacunae of the hyphal net (figure 3*f*). *Winfrenatia reticulata* most probably colonized hard substrates such as degrading sinter surfaces and may have weathered rock surfaces, thus contributing to soil formation [101].

4. Description of *Perexiflasca tayloriana* gen. et sp. nov.

Fossil genus ***Perexiflasca*** gen. nov.

Mycobank: MB 819876

Type species: *Perexiflasca tayloriana* M. Krings, C.J. Harper & E.L. Taylor, hic designatus

Diagnosis: Simple thallus comprised spheroidal, prolate or lens-shaped (i.e. dorsiventrally compressed), thin-walled cavity enveloped in prominent, translucent sheath; single discharge tube extends from cavity through sheath to surface; thalli occur singly, in planar assemblages no more than two layers high, or in tight, three-dimensional aggregates; thallus morphology variable, determined by availability of space in place of growth; single specimens typically hemispherical or pear-shaped, linearly aligned ones more or less square with adjacent sides flattened; individuals in assemblages and aggregates highly variable in size and shape depending on position within clustering.

Etymology: The name of the genus, a combination of the Latin word *perexiguus*, *-a*, *-um* (= very small) and the Medieval Latin *flasca* (= bottle, flask), refers to the small size and characteristic feature of the fossil.

Perexiflasca tayloriana sp. nov.

Figures 4 and 5

Mycobank: MB 819877

Holotype: Specimen illustrated in figure 4*e*; in slide SNSB-BSPG 2013 V 30, SNSB-Bayerische Staatssammlung für Paläontologie und Geologie, Munich, Germany

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

Age: Early Devonian; Pragian, 411.5 ± 1.3 Ma [26] or 407.1 ± 2.2 Ma [33]

Diagnosis: Thallus less than 22 μm wide, up to 20 μm high, near-spherical, hemispherical, lenticular to spindel-shaped, blunt, cubical, or pyramidal; spherical cavities up to 12 μm in diameter, prolate to lens-shaped ones 15 μm wide and 11 μm high; discharge tube 1.8 μm in diameter, length variable, erect or oblique relative to cavity floor; attached to cell walls or cell wall remains in degrading land plant tissue (rarely on fungal hyphae and spores), usually in litter layers, sometimes free-floating in chert matrix.

Etymology: In honour of the late Thomas N. Taylor, University of Kansas, USA, for his benchmark contributions to our understanding of the microbial component of the Rhynie ecosystem.

Remarks: *Perexiflasca tayloriana* was initially described (but not named) by Taylor *et al.* ([24]: figs 1–14) based on specimens in degrading *H. lignieri* rhizomes and aerial axes. The material illustrated by these authors includes several specimens with one to several prominent, papilla-like projections (referred to as 'lobes' in [24]) extending from the outer component. It is unclear whether these specimens also belong to *P. tayloriana* or represent a different organism. Support for the latter is perhaps the fact that the discharge tube in the papillate form is conical (right arrow in ([24], fig. 6), rather than tubular as in *P. tayloriana*. Moreover, no papilla-like projections have been observed in any of the greater than 1000 specimens that form the basis for the present study. We therefore refrained from including characters of the papillate specimens into the diagnoses.

Description: Most specimens occur in partially intact (senescent or dying) or degrading land plant axes, often

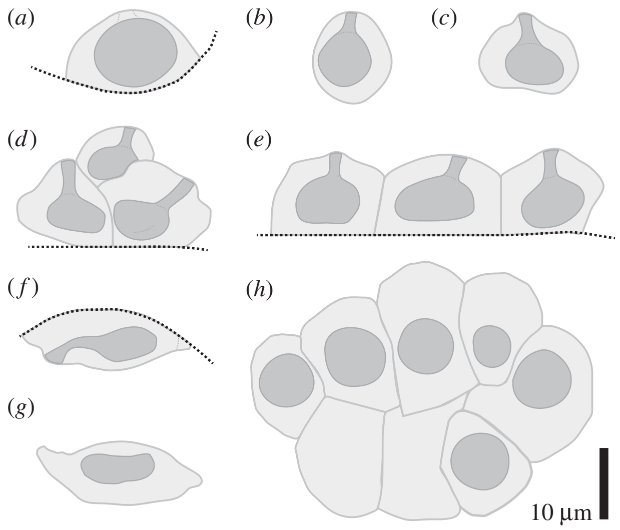

Figure 6. Fungi and fungal interactions in the Lower Devonian Rhynie chert: variability in thallus morphology of *P. tayloriana* gen. et sp. nov.; dashed lines indicate host plant cell walls; drawings are based on (*a*) figure 2*a*, (*b*) figure 2*f*, (*c*) figure 2*e*, (*d*) figure 2*i*, (*e*) figure 3*j*, (*f*,*g*) figure 2*i* and (*h*) figure 3*d*.

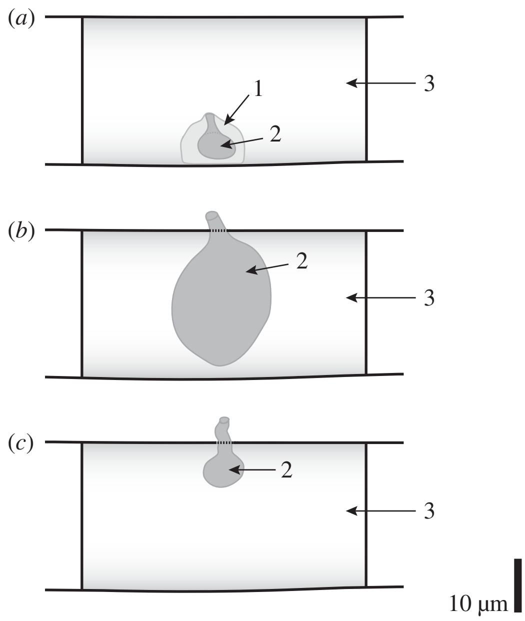

Figure 7. Comparison of *P. tayloriana* gen. et sp. nov. with *Olpidium* and *Olpidiopsis*. Basic morphology of *P. tayloriana* (*a*) suggestive of epibiotic sporangium (*a*2) surrounded by gelatinous hull or sheath (*a*1) and attached to the wall of host cell (*a*3), whereas *Olpidium* (*b*) and *Olpidiopsis* (*c*) produce endobiotic sporangia (*b*2, *c*2) within host cells (*b*3, *c*3) and release zoospores to the outside of host cell via discharge tube.

attached to cell walls or cell wall remains, but they are sometimes also found attached to fungal hyphae or reproductive units, or they occur free-floating in the chert matrix. The organism appears to be widespread in the Rhynie chert, but is most frequently encountered in litter layers comprised fragmented land plant parts (axes, sporangia) in different stages of decay, fungal hyphae, fungal and land plant spores, scattered remains of other microorganisms (e.g. cyanobacteria, algal phycomata), and to a lesser extent arthropod exuvia. More than 1000 specimens (individual thalli) have been identified in approximately 120 thin sections prepared from five different chert blocks.

Specimens consist of two major parts, which we informally call 'inner' and 'outer' component. The inner component comprises a spheroidal, lens-shaped (i.e. dorsiventrally compressed), or oblong, thin-walled cavity (on average 8.7 µm in diameter if spheroidal, and up to 15 µm wide and 11 µm high if oblong), from which extends a prominent tube approximately 1.8 µm wide (e.g. figure 4*e*,*f*,*h*). A tube is present in greater than 80% of the specimens and can be traced readily by focusing through the fossil; the remaining less than 20% of specimens lack evidence of the tube. The cavity is usually empty; however, a few specimens contain one to several tiny, opaque inclusions up to 1.7 µm in diameter (black arrow in figure 4*i*). Surrounding the inner component is the translucent outer component, which is variable in shape and thickness, ranging from near-spherical (figure 4*f*), hemispherical (figure 4*a*–*c*,*e*), lenticular to spindle-shaped (figures 3*a* and 4*i*), blunt, cubical (figure 4*j*–*f*) or pyramidal (figure 4*i*, right side of the image). The outer surface is smooth in all specimens included in this study (but see the Remarks section). The tube that extends from the cavity traverses through the outer component and connects the cavity with the environment; a collar-like rim of more opaque material may be present around the mouth of the tube (arrow in figure 4*f*).

Specimens occur singly (figure 4*a*–*e*), in chain-like, linear arrangements (figure 5*e*–*j*), in planar assemblages no more than two stories high (figures 4*g*,*i*–*l* and 5*a*,*b*), and in three-dimensional aggregates of less than 10 to very much less than 100 tightly abutting individuals (figures 4*h* and 5*c*,*d*). Large assemblages sometimes adumbrate the outlines of degraded plant cells through the pattern in which the individual specimens are arranged (figure 4*i*); in rare instances, small fragments of actual plant cell walls are enclosed in the assemblages (white arrows in figure 4*g*, *i*). Specimen morphology is variable (figure 6*a*–*h*). Single specimens *in situ* (i.e. attached to substrate) are typically hemispherical or drop-/tear-shaped (figures 4*a*–*f* and 6*a*–*c*), whereas linearly aligned ones are blunt to more or less cubical, with adjacent sides flattened (figure 5*e*–*j*). The shape of specimens occurring in planar assemblages and three-dimensional aggregates depends on the position of the specimen within the clustering (figures 4*g*–*l*, 5*a*–*d* and 6*d*,*f*–*h*). Single, free-floating specimens are variable in morphology. Tubes are mostly oriented more or less perpendicularly to the cavity floor (figure 4*e*–*h*), but may, in clustered specimens, also be oblique (figure 4*i*). Tubes in clustered specimens always extend towards a portion of the outer surface that is not blocked by plant cell walls or other specimens. This is especially well recognizable in the linear arrangements where all individuals are oriented in the same direction and have only one unblocked side (figures 5*e*–*j* and 6*e*). Conversely, individuals located deep in the interior of three-dimensional aggregates appear to lack tubes (figures 5*d* and 6*h*).

Rare (total number of specimens less than 10) variants and deviations from the normal basic morphology include specimens with a dumbbell-shaped cavity (figure 5*k*), others that possess what appears to be a second tube (figure 5*l*), and still others suggestive of the presence of a small rhizomycelium extending from the proximal side of the thallus (arrows in figure 5*p*). Moreover, several single specimens are enveloped in a delicate, sac-like structure that is variable in size and shape (arrows in figure 5*m*–*o*).

Discussion: The most characteristic feature of *P. tayloriana* is the inner component comprised a thin-walled cavity from which extends a prominent tube; cavity and tube together resemble a bellied flask, hence the genus name. It is likely that whatever developed within the cavity was liberated through the tube at maturity; specimens that lack a tube probably represent juvenile individuals. However, there is currently no evidence to determine what exactly was produced within the cavity, with the possible exception of several specimens that contain tiny inclusions (referred to as 'refractive bodies' in [24]) of unknown nature in the cavity (e.g. black arrow in figure 4*i*). Unfortunately, these inclusions are far too small to be specifically detailed.

Cavity size and tube width (if a tube is present) are relatively uniform among the specimens, but there is considerable variation with regard to the shape and thickness of the outer component, the form of the cavity and the position and length of the tube (figure 6). Shape and thickness of the outer component, as well as the form of the cavity and the position of the tube, generally appear as functions of the surrounding in which the structure develops, while the length of the tube depends on the thickness of the outer component in the area where the tube is located.

Taylor *et al*. [24] and Krings *et al*. [102] suggested that the outer component might be an algal cell or resting stage (e.g. cyst, phycoma), or perhaps a land plant cell that became detached from the source tissue during tissue degradation. The inner component was interpreted as an endobiotic zoosporangium of a holocarpic chytrid, and compared to the zoosporangia of *Olpidium*, a widespread chytrid parasite of plants and animals today [103,104]. Structurally similar to *Olpidium* are certain species in the peronosporomycete (oomycete) genus *Olpidiopsis* that are also parasites (e.g. of algae, fungi and other peronosporomycetes) and produce sporangia within host cells, with discharge tubes liberating the zoospores to the outside of the host cell [105]. However, the specimens described in this paper prompt a different interpretation, namely that the outer component represents an envelope or sheath produced by the organism itself. Support for this interpretation is the wide range of different morphologies, which result from the expansion of the developing structures into the available spaces in the respective places of growth. Especially interesting in this context are the strings of tightly abutting, blunt or cubical specimens that occur exclusively in close proximity to the central strands in certain largely degraded plant axes (figure 5*e*–*j*). This peculiar alignment results from the colonization of the limited space in the lumen of the narrow, elongate cortical cells adjacent to the strand. Moreover, several specimens suggest that a small rhizomycelium was produced by *P. tayloriana* (arrows in figure 5*p*). As a result, the complement of structural features displayed by *P. tayloriana* argues against the interpretation as a holocarpic, *Olpidium*- or *Olpidiopsis*-like organism. If *P. tayloriana* were like *Olpidium* or *Olpidiopsis*, one would expect to see a host cell containing an endobiotic zoosporangium that releases zoospores to the outside of the host cell via a discharge tube (figure 7*b*,*c*) (e.g. [95,104,105]). Rather, the basic morphology of *P. tayloriana* (figure 7*a*) is suggestive of a small, epibiotic chytrid zoosporangium attached to a substrate, possibly via a small rhizomycelium, perhaps comparable in basic morphology to certain present-day species in the genus *Rhizophydium* that are characterized by a gelatinous hull or sheath around the zoosporangium [106]. If the interpretation of the outer component of *P. tayloriana* as part of the organism itself is correct, then the rare variants shown in figure 5*m*–*o* might be specimens that are additionally surrounded by the contracted plasmalemma (arrows) of the host cell.

The nature of the relationship (i.e. parasitic or saprotrophic) between *P. tayloriana* and land plants cannot be determined, primarily because the fossils described here most certainly represent only one of several stages in the life cycle of this organism, with information on the other stages not currently available. Moreover, the life history and biology of fungi can change based on the presence or absence of a host and the type of host (e.g. [107]). Despite these limitations, we feel confident enough to advance a hypothesis on the nature of the relationship between *P. tayloriana* and land plants based on the material at hand. We suggest that the organism (perhaps in the form of motile cells or spores) infected living plants or colonized senescent or atrophied plant parts and subsequently participated in the process of biological degradation. As the decomposition of the plant progressed, the *P. tayloriana* stage of the life cycle developed and attained maturity, and ultimately, the contents (zoospores?) were released from the cavity, leading to large numbers of new individuals that further accelerated the decomposition process. The formation of assemblages and aggregates was perhaps due to dense spacing or because the contents were discharged from the cavity in the form of coherent masses [108]. Support for this hypothesis is the fact that smaller assemblages and aggregates of specimens are usually associated with plant tissue in which some of the cell outlines are still recognizable (figures 4*j*–*l* and 5*a*,*b*), while the largest specimen clusters (figure 5*c*,*d*) occur in plant parts that no longer show cell outlines. Moreover, the presence of plant cell wall fragments in several assemblages and aggregates (arrows in figure 4*g*,*i*) indicates that aggregate formation initially required the presence of a host cell wall as a substrate. The finding that specimens are common in the Rhynie chert and occur on multiple substrates suggests that *P. tayloriana* was an important contributor to the degradation of organic material in the Rhynie ecosystem, and perhaps early terrestrial ecosystems in general, that has been replaced in modern ecosystems with more efficient degraders, i.e. members of the Ascomycota and Basidiomycota.

The thalli of *P. tayloriana* appear to have been relatively resistant to degradation based on the fact that the specimens remain intact even after complete degradation of the host tissue. This explains why specimens of *P. tayloriana* sometimes appear to float freely in the chert matrix or among the severely fragmented remains of decomposed plant parts. Free-floating thalli with morphologies characteristic of thalli in assemblages and aggregates suggest that specimen clusterings eventually dissociated.

5. Summary discussion and conclusion

The fossils from the Lower Devonian Rhynie chert that are reviewed and newly described in this paper (figures 1–5 and table 1) constitute the largest body of structurally (including *in situ*) preserved evidence of fungi and fungal interactions gathered to date from any ancient ecosystem. It comes therefore as no surprise that the Rhynie chert is today widely used as a key reference for past fungal biodiversity and interactions (e.g. [110–114]). Other rocks that have been screened more systematically for fossil fungi include

Table 1. Synopsis of fungal taxa and fungus-like organisms described from the Rhynie chert.

taxon	suggested systematic affinities	occurrence/substrate	references
fungi			
Cultoraquaticus trewinii Strullu-Derrien	Chytridiomycota	epibiotic on large, spheroidal structures of uncertain affinity	[97]
Globicultrix nugax M. Krings, Dotzler & T.N. Taylor	Chytridiomycota	endobiotic in large fungal (probably glomeromycotinan) spores	[92]
Glomites rhyniensis T.N. Taylor, Remy, Hass & Kerp	Mucoromycota (Glomeromycotina)	in axes of *Ag. majus*	[35,54,55]
Glomites sporocarpoides Karatygin, Snigirevskaya, K. Demchenko & Zdebska	Mucoromycota (Glomeromycotina)	in axes of *R. gwynne-vaughanii* and *Ag. majus*	[68]
Helmutella devonica M. Krings & T.N. Taylor	Mucoromycota incertae sedis	free-floating in chert matrix	[48]
Illmanomyces corniger M. Krings, T.N. Taylor	Chytridiomycota	epibiotic on fungal (probably glomeromycotinan) spores	[91]
Krispiromyces discoides T.N. Taylor, Hass & Remy	Chytridiomycota	epibiotic on *Palaeo. cranii*	[93]
Kryphiomyces catenulatus M. Krings & T.N. Taylor	inconclusive, perhaps Chytridiomycota	endobiotic in large fungal (probably glomeromycotinan) spores	[87]
Lyonomyces pyriformis T.N. Taylor, Hass & Remy	Chytridiomycota	epibiotic on *Palaeo. cranii*	[93]
Milleromyces rhyniensis T.N. Taylor, Hass & Remy	Chytridiomycota	endobiotic in *Palaeo. cranii*	[93]
Mycocarpon rhyniense M. Krings, T.N. Taylor, E.L. Taylor, H. Kerp & Dotzler	Mucoromycota incertae sedis	free-floating in chert matrix	[47]
Mycokidstonia sphaerialoides D. Pons et Locq.	Mucoromycota (Glomeromycotina) see 45	free-floating in chert matrix	[109]
Palaeoendogone gwynne-vaughaniae Strullu-Derrien & Strullu	Mucoromycota (Mucoromycotina)	in rhizomes of *H. lignieri*	[71]
Palaeoglomus boullardii Strullu-Derrien & Strullu	Mucoromycota (Glomeromycotina)	in aerial axes of *H. lignieri*	[71]
Palaeomyces agglomeratus Kidst. & W.H. Lang	Mucoromycota incertae sedis	in aerial axes of *R. gwynne-vaughanii* and surrounding chert matrix	[9]
Palaeomyces asteroxyli Kidst. & W.H. Lang	Mucoromycota incertae sedis	in intact and decayed tissue of *As. mackiei*	[9]
Palaeomyces gordonii Kidst. & W.H. Lang (incl. *P. gordonii* var. *major* Kidst. & W.H. Lang)	Mucoromycota incertae sedis	in axes of *As. mackiei*, free-floating in chert matrix	[9]
Palaeomyces horneae Kidst. & W.H. Lang	Mucoromycota incertae sedis	in rhizomes and aerial axes of *H. lignieri*	[9]
Palaeomyces simpsonii Kidst. & W.H. Lang	Mucoromycota incertae sedis	in decayed axes of *R. gwynne-vaughanii*	[9]
Palaeomyces vestitus Kidst. & W.H. Lang	see *Z. vestitus*		
Palaeozoosporites renaultii Strullu-Derrien	zoosporic Fungi incertae sedis, perhaps Blastocladiomycota	endobiotic in rhizomes of *As. mackiei*	[77]
Paleoblastocladia milleri Remy, T.N. Taylor & Hass	Blastocladiomycota	epibiotic on partially degraded axes of *Ag. majus*	[82]
Paleopyrenomycites devonicus T.N. Taylor, Hass, Kerp, M. Krings & Hanlin	Ascomycota	in aerial axes and lateral portions of *As. mackiei*	[78,79]
Perexiflasca tayloriana M. Krings, C.J. Harper & E.L. Taylor	inconclusive, perhaps Chytridiomycota	in intact and degraded land plant tissue, on fungal spores and hyphae, free-floating in chert matrix	this paper

(*Continued*.)

Table 1. (*Continued.*)

taxon	suggested systematic affinities	occurrence/substrate	references
Scepasmatocarpion fenestrulatum M. Krings & T.N. Taylor	inconclusive, perhaps Mucoromycota or Ascomycota	free-floating in microbial mats	[52]
Scutellosporites devonicus Dotzler, M. Krings, T.N. Taylor & Agerer	Mucoromycota (Glomeromycotina)	in aerial axes of *As. mackiei*	[42]
Trewinomyces annulifer M. Krings, T.N. Taylor & H. Martin	inconclusive, perhaps Chytridiomycota or Blastocladiomycota	epibiotic on partially degraded land plant axes	[85]
Zwergimyces vestitus (Kidst. & W.H. Lang) M. Krings & T.N. Taylor	Mucoromycota incertae sedis	in intact and degraded land plant tissues, in chert litter layers	[40,46]
Lichen-like associations			
Winfrenatia reticulata T.N. Taylor, Hass & Kerp	mycobiont: Mucoromycota photobiont: Cyanobacteria	inconclusive, probably on hard terrestrial substrate	[99,100]
Peronosporomycetes			
Frankbaronia polyspora M. Krings, T.N. Taylor, E.L. Taylor, Kerp, Hass, Dotzler & C.J. Harper	Peronosporomycetes (Oomycota)	free-floating in microbial mats and litter layers	[20]
Frankbaronia velata M. Krings, T.N. Taylor, Dotzler & C.J. Harper	Peronosporomycetes (Oomycota)	free-floating in microbial mats	[21]
Hassiella monospora T.N. Taylor, M. Krings & Kerp	Peronosporomycetes (Oomycota)	free-floating in chert matrix	[19]
Nematophytes			
Nematophyton taiti Kidst. & W.H. Lang	Nematophyta	inconclusive, probably on hard substrate	[9]
Nematoplexus rhyniensis Lyon	Nematophyta	inconclusive, probably on hard substrate	[22]

Mississippian and Pennsylvanian cherts from central France, Pennsylvanian coal balls from Great Britain and North America, Permian and Triassic permineralized peat from Antarctica, and the Eocene Princeton chert from Canada (see [8] and references therein). While these deposits all have produced a variety of fungal fossils, including specimens yielding detailed information on biology and interactions (e.g. [115–119]), none come close to the quality of the Rhynie chert fossils [120].

Because of the sublime preservation, the Rhynie chert fungi have played, and continue to play a major role in shaping our perception of the diversity of fungi in ancient non-marine ecosystems and the roles that these organisms played in the biology of early plant life on land [121–126]. However, geothermal ecosystems today are remarkably rich in fungi, and plants growing in these environments often harbour diverse communities of fungi [127–130], suggesting that the fossils described to date from the Rhynie chert represent only a small segment of the fungal diversity that was actually present in the Rhynie ecosystem. The same is probably true of other microbial life (e.g. cyanobacteria, algae) in the Rhynie chert that remains generally understudied [18]. Moreover, all fungi are carbon-heterotrophic, and thus required to exploit dead organic matter and/or interact with other ecosystem constituents to obtain carbon, suggesting that the fungal interactions recorded to date from the Rhynie chert also represent only a small portion of the actual diversity [90]. The fossils of *P. tayloriana* detailed above, together with other, recently described minute life forms such as the cyanobacterium *Rhyniosarcina devonica* [13] and the alga *Hagenococcus aggregatus* [18], demonstrate that there is still tremendous unreported biodiversity in the Rhynie chert, and that it remains worthwhile to analyse the chert in search for new organisms.

Detailed descriptions of fossil fungi and fungal interactions represent valuable resources that can be used to not only assess past biodiversity and ecosystem complexity through the patterns and processes resulting from associations and interactions between individuals, populations, species and communities (e.g. [131–133]), but also define minimum ages for various lineages of fungi and calibrate molecular clocks (e.g. [134–136]). It is becoming increasingly clear that the Rhynie chert comprises different (micro-)facies characterized by communities of organisms that reflect once differing types of (micro-)habitats [30]. Drill core data suggest that there are greater than 50 fossiliferous chert layers [27,30,137], and the number of distinctive environments preserved in these layers is probably even larger. Future concerted research with all of the chert lenses will be necessary in order to catalogue the full complement of organisms and (micro-)habitats that existed in this Early Devonian hot spring ecosystem, and characterize the distinct communities and environments. Recent discoveries, including *P. tayloriana*, indicate that screening the material by using the highest possible magnification, albeit time-consuming, will open an entirely new window into the diversity of microbial life that populated the Rhynie ecosystem.

Data accessibility. All thin sections and original digital images of *P. tayloriana* are deposited in the Bayerische Staatssammlung für Paläontologie und Geologie (SNSB-BSPG), Munich, Germany. Additional material is housed in the Abteilung Paläobotanik, Geologisch-Paläontologisches Institut, Westfälische Wilhelms-Universität, Münster, Germany (prefix P).

Authors' contributions. All authors contributed equally.

Competing interests. We declare we have no competing interests.

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References

1. Kidston R, Lang WH. 1917 On Old Red Sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part I. *Rhynia Gwynne-Vaughani*, Kidston and Lang. *Trans. R. Soc. Edinb.* **51**, 761–784. (doi:10.1017/S0080456800008991)
2. Kidston R, Lang WH. 1920 On Old Red Sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part II. Additional notes on *Rhynia Gwynne-Vaughani*, Kidston and Lang; with descriptions of *Rhynia major*, n.sp., and *Hornea lignieri*, n.g. n.sp. *Trans. R. Soc. Edinb.* **52**, 603–627. (doi:10.1017/S0080456800004488)
3. Kidston R, Lang WH. 1920 On Old Red Sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part III. *Asteroxylon Mackiei*, Kidston and Lang. *Trans. R. Soc. Edinb.* **52**, 643–680. (doi:10.1017/S0080456800004506)
4. Kidston R, Lang WH. 1921 On Old Red Sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part IV. Restorations of the vascular cryptogams, and discussion of their bearing on the general morphology of the Pteridophyta and the origin of the organisation of land-plants. *Trans. R. Soc. Edinb.* **52**, 831–854. (doi:10.1017/S0080456800016033)
5. Kerp H, Hass H. 2004 De Onder-Devonische Rhynie Chert—het oudste en meest compleet bewaard gebleven terrestrische ecosysteem. *Grondboor and Hamer* **58**, 33–50.
6. Trewin NH, Rice CM (eds) 2003 *The rhynie hot spings system: geology, biota and mineralisation (Transactions of the Royal Society of Edinburgh, Earth Sciences 94)*. Edinburgh, Scotland: The Royal Society of Edinburgh Scotland Foundation.
7. Dotzler N, Krings M, Kerp H, Hass H, Agerer R, Taylor TN. 2009 Mikroorganismen vor 400 Millionen Jahren, perfekt erhalten im unterdevonischen Rhynie Chert. *Jahresber. 2008 Mitt. Freunde Bayer. Staatslg. Paläont. Hist. Geol. München e.V.* **37**, 49–62.
8. Taylor TN, Krings M, Taylor EL. 2015 *Fossil fungi*, 1st edn. Amsterdam, the Netherlands: Elsevier.
9. Kidston R, Lang WH. 1921 On Old Red Sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. *Part V. The Thallophyta occurring in the peat-bed; the succession of the plants throughout a vertical section of the bed, and the conditions of accumulation and preservation of the deposit*. *Trans. R. Soc. Edinb.* **52**, 855–902. (doi:10.1017/S0080456800016045)
10. Croft WN, George EA. 1959 Blue-green algae from the middle Devonian of Rhynie, Aberdeenshire. *Bull. Br. Mus. (Nat. Hist.), Geol.* **3**, 341–353.
11. Krings M, Kerp H, Hass H, Taylor TN, Dotzler N. 2007 A filamentous cyanobacterium showing structured colonial growth from the Early Devonian Rhynie chert. *Rev. Palaeobot. Palynol.* **146**, 265–276. (doi:10.1016/j.revpalbo.2007.05.002)
12. Krings M, Hass H, Kerp H, Taylor TN, Agerer R, Dotzler N. 2009 Endophytic cyanobacteria in a 400-million-yr-old land plant: a scenario for the origin of a symbiosis? *Rev. Palaeobot. Palynol.* **153**, 62–69. (doi:10.1016/j.revpalbo.2008.06.006)
13. Taylor TN, Krings M. 2015 A colony-forming microorganism with probable affinities to the Chroococcales (*Cyanobacteria*) from the Lower Devonian Rhynie chert. *Rev. Palaeobot. Palynol.* **219**, 147–156. (doi:10.1016/j.revpalbo.2015.04.003)
14. Edwards DS, Lyon AG. 1983 Algae from the Rhynie chert. *Bot. J. Linn. Soc.* **86**, 37–55. (doi:10.1111/j.1095-8339.1983.tb00716.x)
15. Dotzler N, Taylor TN, Krings M. 2007 A prasinophycean alga of the genus *Cymatiosphaera* in the Early Devonian Rhynie chert. *Rev. Palaeobot. Palynol.* **147**, 106–111. (doi:10.1016/j.revpalbo.2007.07.001)
16. Kustatscher E, Dotzler N, Taylor TN, Krings M. 2014 Microalgae from the Lower Devonian Rhynie chert: a new *Cymatiosphaera*. *Zitteliana A* **54**, 165–169.
17. Kustatscher E, Dotzler N, Taylor TN, Krings M. 2014 Microfossils with suggested affinities to the Pyramimonadales (*Pyramimonadophyceae, Chlorophyta*) from the Lower Devonian Rhynie chert. *Acta Palaeobot.* **54**, 163–171. (doi:10.2478/acpa-2014-0010)
18. Krings M, Kerp H, Taylor EL, Harper CJ. 2017 *Hagenococcus aggregatus* nov. gen. et sp., a microscopic, colony-forming alga from the 410-million-yr-old Rhynie chert. *Nova Hedwig.* **105**, 205–217.
19. Taylor TN, Krings M, Kerp H. 2006 *Hassiella monospora* gen. *et sp. nov., a microfungus from the 400 million year old Rhynie chert. Mycol. Res.* **110**, 628–632. (doi:10.1016/j.mycres.2006.02.009)
20. Krings M, Taylor TN, Taylor EL, Kerp H, Hass H, Dotzler N, Harper CJ. 2012 Microfossils from the Lower Devonian Rhynie Chert with suggested affinities to the peronosporomycetes. *J. Paleontol.* **86**, 358–367. (doi:10.1666/11-087.1)
21. Krings M, Taylor TN, Dotzler N, Harper CJ. 2013 *Frankbaronia velata* nov. sp., a putative peronosporomycete oogonium containing multiple oospores from the Lower Devonian Rhynie chert. *Zitteliana A* **53**, 23–30.
22. Lyon AG. 1962 On the fragmentary remains of an organism referable to the Nematophytales, from the Rhynie chert, '*Nematoplexus rhyniensis*' gen. et. sp. nov. *Trans. R. Soc. Edinb.* **65**, 79–87. (doi:10.1017/S0080456800012382)
23. Weiss HJ. 2004 (updated 2010) Enigmatic little sphere. *Rhynie chert News* 1 See http://www.chertnews.de/Pachytheca.html.
24. Taylor TN, Remy W, Hass H. 1992 Fungi from the lower Devonian Rhynie chert: *Chytridiomycetes*. *Am. J. Bot.* **79**, 1233–1241. (doi:10.2307/2445050)
25. Rice CM, Trewin NH, Anderson LI. 2002 Geological setting of the Early Devonian Rhynie cherts, Aberdeenshire, Scotland: an early terrestrial hot spring system. *J. Geol. Soc.* **159**, 203–214. (doi:10.1144/0016-764900-181)
26. Parry SF, Noble SR, Crowley QG, Wellman CH. 2011 A high precision U-Pb age constraint on the Rhynie chert Konservat-Lagerstätte: time scale and other implications. *J. Geol. Soc.* **168**, 863–872. (doi:10.1144/0016-76492010-043)
27. Channing A, Edwards D. 2009 Yellowstone hot spring environments and the palaeo-ecophysiology of Rhynie chert plants: towards a synthesis. *Plant Ecol. Divers.* **2**, 111–143. (doi:10.1080/17550870903349359)
28. Channing A, Edwards D. 2009 Silicification of higher plants in geothermally influenced wetlands: yellowstone as a Lower Devonian Rhynie analog. *Palaios* **24**, 505–521. (doi:10.2110/palo.2008.p08-131r)
29. Channing A, Edwards D. 2013 Wetland megabias: ecological and ecophysiological filtering dominates the fossil record of hot spring floras. *Palaeontology* **56**, 523–556. (doi:10.1111/pala.12043)
30. Trewin NH, Fayers SR. 2016 Macro to micro aspects of the plant preservation in the Early Devonian Rhynie cherts, Aberdeenshire, Scotland. *Earth*

Environ. Sci. Trans. R. Soc. Edinb. **106**, 67–80. (doi:10.1017/S1755691016000025)

31. Wellman CH. 2006 Spore assemblages from the Lower Devonian 'Lower Old Red Sandstone' deposits of the Rhynie outlier, Scotland. *Trans. R. Soc. Edinb. Earth Sci.* **97**, 167–211. (doi:10.1017/S0263593300001449)
32. Wellman CH, Kerp H, Hass H. 2006 Spores of the Rhynie chert plant *Aglaophyton* (*Rhynia*) *major* (Kidston and Lang) D.S. Edwards, 1986. *Rev. Palaeobot. Palynol.* **142**, 229–250. (doi:10.1016/j.revpalbo.2006.04.009)
33. Mark DF, Rice CM, Fallick AE, Trewin NH, Lee MR, Boyce A, Lee JKW. 2011 $^{40}Ar/^{39}Ar$ dating of hydrothermal activity, biota and gold mineralization in the Rhynie hot-spring system, Aberdeenshire, Scotland. *Geochim. Cosmochim. Acta* **75**, 555–569. (doi:10.1016/j.gca.2010.10.014)
34. Mark DF, Rice CM, Trewin NH. 2013 Discussion on 'A high-precision U-Pb age constraint on the Rhynie Chert Konservat-Lagerstätte: time scale and other implications'. *J. Geol. Soc.* **170**, 701–703. (doi:10.1144/jgs2011-110)
35. Taylor TN, Remy W, Hass H, Kerp H. 1995 Fossil arbuscular mycorrhiza from the Early Devonian. *Mycologia* **87**, 560–573. (doi:10.2307/3760776)
36. Thompson W, Rayner ADM. 1983 Extent development and functioning of mycelial cord systems in soil. *Trans. Br. Mycol. Soc.* **81**, 333–345. (doi:10.1016/S0007-1536(83)80085-0)
37. Cairney JWG. 1992 Translocation of solutes in ectomycorrhizal and saprotrophic rhizomorphs. *Mycol. Res.* **96**, 135–141. (doi:10.1016/S0953-7562(09)80928-3)
38. Jennings DH. 1994 Translocation in mycelia. In *The mycota, vol. 1. Growth, differentiation and sexuality* (eds JGH Wessels, F Meinhardt), pp. 163–173. Berlin, Germany:Springer.
39. Tlalka M, Bebber DP, Darrah P, Watkinson SC. 2008 Mycelial networks: nutrient uptake, translocation, and role in ecosystems. In *Ecology of saprotrophic basidiomycetes* (eds L Boddy, J Frankland, P van West), pp. 43–62. Amsterdam, the Netherlands: Academic Press.
40. Krings M, Taylor TN, Dotzler N, Harper CJ. 2016 Morphology and ontogenetic development of *Zwergimyces vestitus*, a fungal reproductive unit enveloped in a hyphal mantle from the Lower Devonian Rhynie chert. *Rev. Palaeobot. Palynol.* **228**, 47–56. (doi:10.1016/j.revpalbo.2016.01.005)
41. Sharma BD, Bohra DR, Harsh R. 1993 Vesicular arbuscular mycorrhizae association in Lower Devonian plants of the Rhynie chert. *Phytomorphology* **43**, 105–110.
42. Dotzler N, Krings M, Taylor TN, Agerer R. 2006 Germination shields in *Scutellospora* (*Glomeromycota: Diversisporales, Gigasporaceae*) from the 400 million-year-old Rhynie chert. *Mycol. Progr.* **5**, 178–184. (doi:10.1007/s11557-006-0511-z)
43. Souza T. 2015 *Handbook of arbuscular mycorrhizal fungi*. Cham, Heidelberg, New York, Dordrecht, London: Springer.
44. Dotzler N, Walker C, Krings M, Hass H, Kerp H, Taylor TN, Agerer R. 2009 Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-year-old Rhynie chert. *Mycol. Progr.* **8**, 9–18. (doi:10.1007/s11557-008-0573-1)
45. Krings M, Walker C, Harper CJ, Martin H, Sónyi S, Kustatscher E, Taylor TN. 2017 Unusual fungal reproductive units from the Lower Devonian Rhynie chert. *Zitteliana* **89**, 29–37.
46. Krings M, Taylor TN. 2013 *Zwergimyces vestitus* (Kidston et W.H. *Lang) nov. comb., a fungal reproductive unit enveloped in a hyphal mantle from the Lower Devonian Rhynie chert. Rev. Palaeobot. Palynol.* **190**, 15–19. (doi:10.1016/j.revpalbo.2012.11.008)
47. Krings M, Taylor TN, Taylor EL, Kerp H, Dotzler N. 2014 First record of a fungal 'sporocarp' from the Lower Devonian Rhynie chert. *Palaeobiodiv. Palaeoenviron.* **94**, 221–227. (doi:10.1007/s12549-013-0135-7)
48. Krings M, Taylor TN. 2014 A mantled fungal reproductive unit from the Lower Devonian Rhynie chert that demonstrates Carboniferous 'sporocarp' morphology and development. *N. Jb. Geol. Paläontol., Abh.* **273**, 197–205. (doi:10.1127/0077-7749/2014/0423)
49. Stubblefield SP, Taylor TN, Miller CE, Cole GT. 1983 Studies of Carboniferous fungi. II. The structure and organization of Mycocarpon, Sporocarpon, Dubiocarpon and Coleocarpon (*Ascomycotina*). *Am. J. Bot.* **70**, 1482–1498. (doi:10.2307/2443347)
50. Krings M, Taylor TN, Dotzler N. 2013 Fossil evidence of the zygomycetous fungi. *Persoonia* **30**, 1–10. (doi:10.3767/003158513X664819)
51. Krings M, Taylor TN. 2015 Mantled fungal reproductive units in land plant tissue from the Lower Devonian Rhynie chert. *Bull. Geosci.* **90**, 1–6. (doi:10.3140/bull.geosci.1519)
52. Krings M, Taylor TN. 2015 A fungal reproductive unit from the Lower Devonian Rhynie chert (*Aberdeenshire*, Scotland) that demonstrates an unusual hyphal investment pattern. *Scot. J. Geol.* **51**, 131–139. (doi:10.1144/sjg2014-026)
53. Strullu-Derrien C, Strullu DG. 2007 Mycorrhization of fossil and living plants. *C.R. Palevol* **6**, 483–494. (doi:10.1016/j.crpv.2007.09.006)
54. Remy W, Taylor TN, Hass H, Kerp H. 1994 Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proc. Natl Acad. Sci. USA* **91**, 11 841–11 843. (doi:10.1073/pnas.91.25.11841)
55. Taylor TN, Kerp H, Hass H. 2005 Life history biology of early land plants: Deciphering the gametophyte phase. *Proc. Natl Acad. Sci. USA* **102**, 5892–5897. (doi:10.1073/pnas.0501985102)
56. Remy W, Hass H. 1996 New information on gametophytes and sporophytes of *Aglaophyton major* and inferences about possible environmental adaptations. *Rev. Palaeobot. Palynol.* **90**, 175–193. (doi:10.1016/0034-6667(95)00082-8)
57. Harrier LA. 2001 The arbuscular mycorrhizal symbiosis: a molecular review of the fungal dimension. *J. Exp. Bot.* **52**(Suppl. 1), 469–478. (doi:10.1093/jxb/52.suppl_1.469)
58. Helgason T, Fitter A. 2005 The ecology and evolution of the arbuscular mycorrhizal fungi. *Mycologist* **19**, 96–101. (doi:10.1017/S0269-915X(05)00302-2)
59. Parniske M. 2008 Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat. Rev. Microbiol.* **6**, 763–775. (doi:10.1038/nrmicro1987)
60. Pirozynski KA, Malloch DW. 1975 The origin of land plants: a matter of mycotrophism. *Biosystems* **6**, 153–164. (doi:10.1016/0303-2647(75)90023-4)
61. Bonfante P, Selosse MA. 2010 A glimpse into the past of land plants and of their mycorrhizal affairs: from fossils to evo-devo. *New Phytol.* **186**, 267–270. (doi:10.1111/j.1469-8137.2010.03196.x)
62. Brundrett MC. 2002 Coevolution of roots and mycorrhizas of land plants. *New Phytol.* **154**, 275–304. (doi:10.1046/j.1469-8137.2002.00397.x)
63. Humphreys CP, Franks PJ, Rees M, Bidartondo MI, Leake JR, Beerling DJ. 2010 Mutualistic mycorrhiza-like symbiosis in the most ancient group of land plants. *Nat. Commun.* **1**, 103. (doi:10.1038/ncomms1105)
64. Bidartondo MI, Read DJ, Trappe JM, Merckx V, Ligrone R, Duckett JG. 2011 The dawn of symbiosis between plants and fungi. *Biol. Lett.* **7**, 574–577. (doi:10.1098/rsbl.2010.1203)
65. Delaux PM *et al.* 2015 Algal ancestor of land plants was preadapted for symbiosis. *Proc. Natl Acad. Sci. USA* **112**, 13 390–13 395. (doi:10.1073/pnas.1515426112)
66. Weiblen GD, Treiber EL. 2015 Evolutionary origins and diversification of mutualism. In *Mutualism* (ed. JL Bronstein), pp. 37–56. Oxford, UK: Oxford University Press.
67. Boullard B, Lemoigne Y. 1971 Les champignons endophytes du *Rhynia gwynne-vaughanii* K. *et L. Étude morphologique et déductions sur leur biologie*. *Botaniste* **54**, 49–89.
68. Karatygin IV, Snigirevskaya NS, Demchenko KN. 2006 Species of the genus *Glomites* as plant mycobionts in Early Devonian ecosystems. *Paleontol. J.* **40**, 572–579. (doi:10.1134/S0031030106050121)
69. Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ. 2007 An alternative mode of early land plant colonization by putative endomycorrhizal fungi. *Plant Signal. Behav.* **2**, 125–126. (doi:10.4161/psb.2.2.3970)
70. Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ. 2007 Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. *New Phytol.* **174**, 648–657. (doi:10.1111/j.1469-8137.2007.02008.x)
71. Strullu-Derrien C, Kenrick P, Pressel S, Duckett JG, Rioult JP, Strullu DG. 2014 Fungal associations in *Horneophyton ligneri* from the Rhynie chert (*c.* 407 million year old) closely resemble those in extant lower land plants: novel insights into ancestral plant–fungus symbioses. *New Phytol.* **203**, 964–979. (doi:10.1111/nph.12805)
72. Rimington WR, Pressel S, Duckett JG, Bidartondo MI. 2015 Fungal associations of basal vascular plants: reopening a closed book? *New Phytol.* **205**, 1394–1398. (doi:10.1111/nph.13221)

73. Rimington WR, Pressel S, Field KJ, Strullu-Derrien C, Duckett JG, Bidartondo MI. 2017 Reappraising the origin of mycorrhizas. In *Molecular mycorrhizal symbioses* (ed. F Martin), pp. 21–32. Hoboken, NJ: Wiley Blackwell.
74. Strullu-Derrien C, Kenrick P, Selosse MA. 2017 Origins of the mycorrhizal symbioses. In *Molecular mycorrhizal symbiosis*, pp. 3–20. Hoboken, NJ: Wiley Blackwell.
75. Harvey R, Lyon AG, Lewis PN. 1969 A fossil fungus from Rhynie chert. *Trans. Br. Mycol. Soc.* **53**, 155–156. (doi:10.1016/S0007-1536(69)80025-2)
76. Illman WI. 1984 Zoosporic fungal bodies in the spores of the Devonian fossil vascular plant, *Horneophyton*. *Mycologia* **76**, 545–547. (doi:10.2307/3793338)
77. Strullu-Derrien C, Wawrzyniak Z, Goral T, Kenrick P. 2015 Fungal colonization of the rooting system of the early land plant *Asteroxylon mackiei* from the 407-Myr-old Rhynie chert (Scotland, UK). *Bot. J. Linn. Soc.* **179**, 201–213. (doi:10.1111/boj.12307)
78. Taylor TN, Hass H, Kerp H. 1999 The oldest fossil ascomycetes. *Nature* **399**, 648. (doi:10.1038/21349)
79. Taylor TN, Hass H, Kerp H, Krings M, Hanlin RT. 2005 Perithecial ascomycetes from the 400 million year old Rhynie chert: An example of ancestral polymorphism. *Mycologia* **97**, 269–285. (doi:10.1080/15572536.2006.11832862)
80. Taylor JW, Berbee ML. 2006 Dating divergences in the Fungal tree of life: review and new analyses. *Mycologia* **98**, 838–849. (doi:10.1080/15572536.2006.11832614)
81. Lücking R, Huhndorf S, Pfister DH, Plata ER, Lumbsch HT. 2009 Fungi evolved right on track. *Myologia* **101**, 810–822. (doi:10.3852/09-016)
82. Remy W, Taylor TN, Hass H. 1994 Early Devonian fungi: a blastocladalean fungus with sexual reproduction. *Am. J. Bot.* **81**, 690–702. (doi:10.2307/2445647)
83. Emerson R. 1941 An experimental study of the life cycles and taxonomy of *Allomyces*. *Lloydia* **4**, 77–144.
84. Emerson R, Robertson JA. 1974 Two new members of the Blastocladiaceae. *I. Taxonomy, with an evaluation of genera and interrelationships in the family*. *Am. J. Bot.* **61**, 303–317. (doi:10.2307/2441610)
85. Krings M, Taylor TN, Martin H. 2016 An enigmatic fossil fungus from the 410 Ma Rhynie chert that resembles *Macrochytrium* (Chytridiomycota) and *Blastocladiella* (Blastocladiomycota). *Mycologia* **108**, 303–312. (doi:10.3852/15-224)
86. Hass H, Taylor TN, Remy W. 1994 Fungi from the Lower Devonian Rhynie chert: mycoparasitism. *Am. J. Bot.* **81**, 29–37. (doi:10.2307/2445559)
87. Krings M, Dotzler N, Longcore JE, Taylor TN. 2010 An unusual microfungus in a fungal spore from the Lower Devonian Rhynie chert. *Palaeontology* **53**, 753–759. (doi:10.1111/j.1475-4983.2010.00959.x)
88. Krings M, Taylor TN, Kerp H, Walker C. 2015 Deciphering interfungal relationships in the 410-million-yr-old Rhynie chert: Sporocarp formation in glomeromycotan spores. *Geobios* **48**, 449–458. (doi:10.1016/j.geobios.2015.09.003)
89. Krings M, Taylor TN. 2014 Deciphering interfungal relationships in the 410-million-yr-old Rhynie chert: an intricate interaction between two mycelial fungi. *Symbiosis* **64**, 53–61. (doi:10.1007/s13199-014-0302-2)
90. Harper CJ, Krings M, Dotzler N, Taylor EL, Taylor TN. 2017 Deciphering interfungal relationships in the 410-million-yr-old Rhynie chert: Morphology and development of vesicle-colonizing microfungi. *Geobios* **50**, 9–22. (doi:10.1016/j.geobios.2016.11.003)
91. Krings M, Taylor TN. 2014 An unusual fossil microfungus with suggested affinities to the Chytridiomycota from the Lower Devonian Rhynie chert. *Nova Hedwig.* **99**, 403–412. (doi:10.1127/0029-5035/2014/0205)
92. Krings M, Dotzler N, Taylor TN. 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.
93. Taylor TN, Hass H, Remy W. 1992 Devonian fungi: Interactions with the green alga *Palaeonitella*. *Mycologia* **84**, 901–910. (doi:10.2307/3760288)
94. Sparrow Jr FK. 1960 *Aquatic phycomycetes*, 2nd edn. Ann Arbor, MI: University of Michigan Press.
95. Karling JS. 1977 *Chytridiomycetarum iconographia: An illustrated and brief descriptive guide to the chytridiomycetous genera with a supplement of the hyphochytridiomycetes*. Monticello, NY: Lubrecht and Cramer.
96. Karling JS. 1928 Studies in the Chytridiales III. *A parasitic chytrid causing cell hypertrophy in Chara*. *Am. J. Bot.* **15**, 485–496. (doi:10.2307/2435797)
97. Strullu-Derrien C, Goral T, Longcore JE, Olesen J, Kenrick P, Edgecombe GD. 2016 A new chytridiomycete fungus intermixed with crustacean resting eggs in a 407-Million-year-old continental freshwater environment. *PLoS ONE* **11**, e0167301. (doi:10.1371/journal.pone.0167301)
98. Habgood KS, Hass H, Kerp H. 2003 Evidence for an early terrestrial food web: coprolites from the Early Devonian Rhynie chert. *Trans. R. Soc. Edinb. Earth Sci.* **94**, 371–389. (doi:10.1017/S0263593300000754)
99. Taylor TN, Hass H, Kerp H. 1997 A cyanolichen from the Lower Devonian Rhynie chert. *Am. J. Bot.* **84**, 992–1004. (doi:10.2307/2446290)
100. Karatygin IV, Snigirevskaya NS, Vikulin SV. 2009 The most ancient terrestrial lichen *Winfrenatia reticulata*: a new find and new interpretation. *Paleontol. J.* **43**, 107–114. (doi:10.1134/S0031030109010110)
101. Selden PA, Nudds JR. 2012 *Evolution of fossil ecosystems*, 2nd edn. London, UK: Manson Publishing Ltd.
102. Krings M, Dotzler N, Galtier J, Taylor TN. 2009 Microfungi from the upper Visean (Mississippian) of central France: Chytridiomycota and chytrid-like remains of uncertain affinity. *Rev. Palaeobot. Palynol.* **156**, 319–328. (doi:10.1016/j.revpalbo.2009.03.011)
103. Kusano S. 1936 On the parasitism of *Olpidium*. *Jap. J. Bot.* **8**, 155–187.
104. Sahtiyanci S. 1962 Studien über einige wurzelparasitäre Olpidiaceen. *Arch. Mikrobiol.* **41**, 187–228. (doi:10.1007/BF00409505)
105. Karling JS. 1981 *Predominantly holocarpic and eucarpic simple biflagellate phycomycetes*, 2nd edn. Vaduz, Liechtenstein: J. Cramer.
106. Letcher PM, Powell MJ. 2012 *A taxonomic summary and revision of Rhizophydium (Rhizophydiales, Chytridiomycota)*. Tuscaloosa, AL: University Printing, The University of Alabama.
107. Grosshart HP, Wurzbacher C, James TY, Kagami M. 2016 Discovery of dark matter fungi in aquatic ecosystems demands a reappraisal of the phylogeny and ecology of zoosporic fungi. *Fung. Ecol.* **19**, 28–38. (doi:10.1016/j.funeco.2015.06.004)
108. Hanson AM. 1944 Three new saprophytic chytrids. *Torreya* **44**, 30–33.
109. Pons D, Locquin MV. 1981 *Mycokidstonia sphaerialoides* Pons & Locquin, gen. et sp. nov., Ascomycètes fossile Dévonien. *Cah. Micropaléontol.* **1**, 101–104.
110. Stockland JN, Siltonen J, Jonsson BG. 2012 *Biodiversity in dead wood*. Cambridge, UK: Cambridge University Press.
111. Gleason FH, Karpov SA, Lilje O, Macarthur DJ, van Otgen FF, Sime-Ngando T. 2014 Zoosporic parasites of phytoplankton. In *Freshwater fungi and fungal-like organisms* (eds EBG Jones, KD Hyde, KL Pang), pp. 279–304. Berlin, Germany: Walter de Gruyter GmbH.
112. Chen JJ, Cui BK, Zhou LW, Korhonen K, Dai YC. 2015 Phylogeny, divergence time estimation and biogeography of the genus *Heterobasidion* (Basidiomycota, Russulales). *Fung. Div.* **71**, 185–200. (doi:10.1007/s13225-014-0317-2)
113. Karlsson M *et al.* 2015 Insights on the evolution of mycoparasitism from the genome of *Clonostachys rosea*. *Genome Biol. Evol.* **7**, 465–480. (doi:10.1093/gbe/evu292)
114. Tkacz A, Poole P. 2015 Role of root microbiota in plant productivity. *J. Exp. Bot.* **66**, 2167–2175. (doi:10.1093/jxb/erv157)
115. LePage BA, Currah RS, Stockey RA. 1994 The fossil fungi of the Princeton chert. *Int. J. Plant Sci.* **155**, 828–836. (doi:10.1086/297221)
116. Stockey RA, Rothwell GW, Addy HD, Currah RS. 2001 Mycorrhizal association of the extinct conifer *Metasequoia milleri*. *Mycol. Res.* **105**, 202–205. (doi:10.1017/S0953756200003221)
117. Strullu-Derrien C, Kenrick P, Rioult JP, Strullu DG. 2011 Evidence of parasitic Oomycetes (*Peronosporomycetes*) infecting the stem cortex of the Carboniferous seed fern *Lyginopteris oldhamia*. *Proc. R. Soc. B* **278**, 675–680. (doi:10.1098/rspb.2010.1603)
118. Krings M, Taylor TN, Dotzler N, Persichini G. 2012 Fossil fungi with suggested affinities to the Endogonaceae from the Middle Triassic of Antarctica. *Mycologia* **104**, 835–844. (doi:10.3852/11-384)
119. Krings M, White JF, Dotzler N, Harper CJ. 2013 A putative zygomycetous fungus with mantled zygosporangia and apposed gametangia from the

Lower Coal Measures (Carboniferous) of Great Britain. *Int. J. Plant Sci.* **174**, 269–277. (doi:10.1086/668247)

120. Locatelli ER. 2014 The exceptional preservation of plant fossils: a review of taphonomic pathways and biases in the fossil record. In *Reading and writing of the fossil record: preservational pathways to exceptional fossilization*, vol. 20 (eds M Laflamme, JD Schiffbauer, SAF Darroch), pp. 237–258. Bolder, CO, USA: Paleontological Society Papers.
121. Taylor TN, Taylor EL. 2000. The Rhynie chert ecosystem: a model for understanding fungal interactions. In *Microbial endophytes* (eds CW Bacon, JF White), pp. 31–47. New York, NY: Marcel Dekker.
122. Berbee ML, Taylor JW. 2007 Rhynie chert: a window into a lost world of complex plant-fungus interactions. *New Phytol.* **174**, 475-479. (doi:10.1111/j.1469-8137.2007.02080.x)
123. Bonfante P, Genre A. 2008 Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. *Trends Plant Sci.* **13**, 492–498. (doi:10.1016/j.tplants.2008.07.001)
124. Field KJ, Pressel S, Duckett JG, Rimington WR, Bidartondo MI. 2015 Symbiotic options for the conquest of land. *Trends Ecol. Evol.* **30**, 477–486. (doi:10.1016/j.tree.2015.05.007)
125. Li DW, Castañeda-Ruiz RF, LaMondia J. 2016 Evolution of fungi and update on ethnomycology. In *Biology of microfungi* (ed. DW Li), pp. 237–266. New York: NY: Springer.
126. Krings M, Taylor TN, Harper CJ. 2017 Early fungi: Evidence from the fossil record. In *The fungal community, its organization and role in the ecosystem* (eds J Dighton, JF White), pp. 37–46, 4th edn. Boca Raton, FL: CRC Taylor and Francis.
127. Redman RS, Litvintseva A, Sheehan KB, Henson JM, Rodriguez RJ. 1999 Fungi from geothermal soils in Yellowstone National Park. *Appl. Environ. Microbiol.* **65**, 5193–5197.
128. Henson J, Redman R, Rodriguez R, Stout R. 2005 Fungi in Yellowstone's geothermal soils and plants. *Yellowstone Sci.* **13**, 25–30.
129. Pan WZ, Huang XW, Wei KB, Zhang CM, Yang DM, Ding JM, Zhang KQ. 2010 Diversity of thermophilic fungi in Tengchong Rehai National Park revealed by ITS nucleotide sequence analyses. *J. Microbiol.* **48**, 146–152. (doi:10.1007/s12275-010-9157-2)
130. Zhou WN, White Jr JF, Soares MA, Torres MS, Zhou ZP, Li HY. 2015 Diversity of fungi associated with plants growing in geothermal ecosystems and evaluation of their capacities to enhance thermotolerance of host plants. *J. Plant Interact.* **10**, 305–314. (doi:10.1080/17429145.2015.1101495)
131. Green DG, Sadedin S. 2005 Interactions matter—complexity in landscapes and ecosystems. *Ecol. Complex.* **2**, 117–130. (doi:10.1016/j.ecocom.2004.11.006)
132. Bairey E, Kelsic ED, Kishony R. 2016 High-order species interactions shape ecosystem diversity. *Nat. Commun.* **7**, 12285. (doi:10.1038/ncomms12285)
133. Jordano P. 2016 Chasing ecological interactions. *PLoS Biol.* **14**, e1002559. (doi:10.1371/journal.pbio.1002559)
134. Beimforde C *et al.* 2014 Estimating the Phanerozoic history of the Ascomycota lineages: Combining fossil and molecular data. *Mol. Phylogen. Evol.* **78**, 386–398. (doi:10.1016/j.ympev.2014.04.024)
135. Hibbett D, Blanchette R, Kenrick P, Mills B. 2016 Climate, decay, and the death of the coal forests. *Curr. Biol.* **26**, R543–R576. (doi:10.1016/j.cub.2016.01.014)
136. Spatafora JW *et al.* 2016 A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* **108**, 1028–1046. (doi:10.3852/16-042)
137. Powell CL, Trewin NH, Edwards D. 2000 Palaeoecology and plant succession in a borehole through the Rhynie cherts, Lower Old Red Sandstone, Scotland. *Geol. Soc. London Spec. Pub.* **180**, 439–457. (doi:10.1144/GSL.SP.2000.180.01.23)