Archaeosporites rhyniensis gen. et sp. nov. (*Glomeromycota, Archaeosporaceae*), from the Lower Devonian Rhynie chert – a fungal lineage morphologically unchanged for more than 400 million years

Carla J. Harper[1,2,3,*], Christopher Walker[4,5], Andrew Schwendemann[6], Hans Kerp[7], Michael Krings[2,3,8]

[1]Botany Department, School of Natural Sciences, Trinity College Dublin, Dublin 2, Ireland; [2]SNSB-Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Straße 10, 80333 Munich, Germany; [3]Department of Ecology and Evolutionary Biology, and Natural History Museum and Biodiversity Institute, University of Kansas, Lawrence, KS 66045-7534, USA; [4]Royal Botanic Garden Edinburgh, 21A Inverleith Row, Edinburgh EH3 5LR, UK; [5]School of Agriculture and Environment, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia; [6]Department of Biology, Lander University, Greenwood, SC 29649, USA; [7]Forschungsstelle für Paläobotanik am Geologisch-Paläontologischen Institut, Westfälische Wilhelms-Universität Münster, Heisenbergstraße 2, 48149 Münster, Germany; [8]Department für Geo- und Umweltwissenschaften, Paläontologie und Geobiologie, Ludwig-Maximilians-Universität, Richard-Wagner-Straße 10, 80333 Munich, Germany

**For correspondence. E-mail charper@tcd.ie*

- **Background and Aims** Structurally preserved arbuscular mycorrhizas from the Lower Devonian Rhynie chert represent core fossil evidence of the evolutionary history of mycorrhizal systems. Moreover, Rhynie chert fossils of glomeromycotan propagules suggest that this lineage of arbuscular fungi was morphologically diverse by the Early Devonian; however, only a small fraction of this diversity has been formally described and critically evaluated.
- **Methods** Thin sections, previously prepared by grinding wafers of chert from the Rhynie beds, were studied by transmitted light microscopy. Fossils corresponding to the description of *Archaeospora* spp. occurred in 29 slides, and were measured, photographed, and compared to modern-day species in that genus.
- **Key results** Sessile propagules <85 µm in diameter, some still attached to a sporiferous saccule, were found in early land plant axes and the chert matrix; they developed, in a similar manner to extant *Archaeospora*, laterally or centrally within the saccule neck. Microscopic examination and comparison with extant fungi showed that, morphologically, the fossils share the characters used to circumscribe the genus *Archaeospora* (*Glomeromycota; Archaeosporales; Archaeosporaceae*).
- **Conclusions** The fossils can be assigned with confidence to the extant family *Archaeosporaceae*, but because molecular analysis is necessary to place organisms in these taxa to present-day genera and species, they are placed in a newly proposed fossil taxon, *Archaeosporites rhyniensis*.

Key words: acaulosporoid propagule, acaulospores, *Archaeosporites rhyniensis*, *Archaeospora* spp., *Archaeosporaceae*, *Glomeromycota*, glomeromycotan arbuscular fungi (GAF), arbuscular mycorrhizal fungi (AMF), Early Devonian, Rhynie chert, sporiferous saccule, spore wall.

INTRODUCTION

Members of the phylum *Glomeromycota* (Schüßler *et al.*, 2001; Tedersoo *et al.*, 2018; Naranjo-Ortiz and Gabaldón, 2019), the fungal lineage sometimes also referred to as *Glomeromycotina* (Spatafora *et al.*, 2016), form intimate partnerships with plants, known as arbuscular mycorrhizas. Research on the evolutionary history of these fungi includes morphological, molecular and genetic data (Redecker and Raab, 2006; Redecker *et al.*, 2013; Kamel *et al.*, 2017; Feijen *et al.*, 2018); moreover, a well-preserved fossil record has contributed greatly to our understanding of these organisms in past environments (Taylor *et al.*, 2015; Strullu-Derrien *et al.*, 2018). The Early Devonian Rhynie chert from Aberdeenshire, Scotland, which originated in a continental palaeoecosystem that became petrified through inundation with silica-rich water from hot springs (Trewin and Kerp, 2017; Garwood *et al.*, 2020), figures prominently in this context. It yields exquisitely preserved fossils of glomeromycotan arbuscular fungi in early land plants (Taylor *et al.*, 1995, 2005) that provide core fossil evidence of the early evolutionary history of mycorrhizal systems (Parniske, 2008; Martin *et al.*, 2017; Brundrett *et al.*, 2018).

The arbuscular mycorrhizal fungi (AMF) comprise the glomeromycotan arbuscular fungi (GAF) and mucoromycotinan arbuscular fungi (MAF) or 'fine root endophytes,' such as *Planticonsortium tenue* (Walker *et al*. 2018*a*). Until molecular analysis was introduced for AMF classification at the turn of the 21st century (Kramadibrata *et al.*, 2000; Morton and Redecker, 2001), AMF in general, and GAF in particular, have traditionally been defined by mode of spore formation and characteristic features, such as spore-wall architecture and ornamentation (Walker *et al.*, 2018*b*). Conversely, present-day species descriptions rely on a combination of morphological and molecular evidence, particularly as some groups, a particular example of which would be *Archaeospora*, cannot be separated to species without a phylogenetic analysis from study of the rRNA gene (Schüßler and Walker, 2019). Information on spore development and spore wall architecture is well preserved in numerous fossil glomeromycotan propagules from the Rhynie chert that are located within intact or

partially degraded land plant axes and in the chert matrix (Kidston and Lang, 1921; Taylor *et al.*, 1995; Karatygin *et al.*, 2006; Dotzler *et al.*, 2006, 2009; Krings *et al.*, 2015, 2017*a*,*b*; Harper *et al.*, 2017). The variety of glomeromycotan spores described and illustrated to date from the Rhynie chert shows that GAF were morphologically diverse, at the ordinal systematic level, by the Early Devonian. Moreover, they have pushed back the evolutionary origin of the main categories of spores in the *Glomeromycota* (i.e. glomoid, gigasporoid, acaulosporoid) to a time before the evolution of true roots (Dotzler *et al.*, 2009). Many of the Rhynie chert spores are so similar to extant fungi that, were it not for the lack of molecular evidence, they could readily be assigned to extant lineages (Brundrett *et al.*, 2018).

Among the extant lineages, the *Archaeosporaceae* is one of the earliest-diverging clades in the *Glomeromycota* (Schüßler *et al.*, 2001; www.amf-phylogeny.com). The most obvious characteristic of members of this family is the production of small (~23–110 µm in diameter), colourless, more or less globose acaulosporoid propagules, which are formed within the neck or stalk of a colourless, thin-walled, terminal balloon-like structure (Fig. 1A), termed the sporiferous saccule (Walker *et al.*, 1984). The saccule usually collapses, and normally the spore becomes detached during extraction from the soil, leaving little evidence of its developmental origins (Hafeel, 2004), though collapsed or remnant saccules may remain attached (Fig 1B).

To determine whether fossil *Archaeosporaceae*-like spore-saccule complexes occur in the Rhynie chert, we re-examined all slides used by Dotzler *et al.* (2009), as well as similar additional material. We found evidence of the frequent occurrence of *Archaeospora*-like fossils in the matrix, in decaying plant remains, and in largely intact axes of Rhynie chert land plants, occasionally alongside much larger spore-saccule complexes of the unnamed type described by Dotzler *et al.* (2009). This paper formally describes the new form as a new fossil genus and species for which the name *Archaeosporites rhyniensis* is proposed; its discovery contributes to our understanding of morphological diversity of glomeromycotan fungi in early terrestrial ecosystems.

MATERIALS AND METHODS

Geologic settings

The Rhynie chert Lagerstätte, located in the northern part of the Rhynie outlier of Aberdeenshire, Scotland includes series of chert lenses that have preserved even the finest details of early terrestrial plants, animals and a variety of microorganisms, especially fungi (Rice *et al.*, 2002; Trewin and Kerp, 2017; Garwood *et al.*, 2020). The chert lenses are interpreted as having accumulated on an alluvial plain associated with ephemeral ponds and lakes in a geothermal wetland (Channing and Edwards, 2009). The chert was formed by rapid infusion of hot, alkaline, silica-rich water that percolated to the surface and inundated the ecosystem, bringing life to an abrupt halt, and providing a remarkable fossil record of the life forms existing at the time (Powell et al., 2000; Garwood et al., 2020). The Rhynie chert biota has been regarded as early (but not earliest) Pragian to earliest Emsian in age based on spore assemblages (Wellman 2006, 2017; Wellman *et al.*, 2006). 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 (Parry *et al.*, 2011), 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 (Mark *et al.*, 2011). However, the andesite cannot be fixed with certainty in the stratigraphic sequence and is certainly older than the hydrothermal alteration. As a result, the date estimate in Mark *et al.* (2011) likely 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 by Mark *et al.* (2011) would correspond approximately to the Pragian/Emsian boundary (407.6 ± 2.6 Ma), which concurs with the dispersed spore biostratigraphy by Wellman (2006, 2017) and Wellman *et al.* (2006).

Fossil material

Fossils used in this study were identified in thin sections prepared from several different 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. 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, 3967, 3978, 3980, 3981, and 3999, and in the Bayerische Staatssammlung für Paläontologie und Geologie (SNSB-BSPG) in Munich, Germany, under accession numbers SNSB-BSPG 1964 XX 625, 633; 1965 I 330; 2013 XIV 6; 2016 XII 6, 12, 13, 18, 19, 24, 26, 31, 34, and 37–40.

All fossils were photographed with brightfield illumination through a Leica DFC-480 camera on a Leica compound microscope, and processed in Adobe Photoshop CS6, or with Canon EOS 60D or 6D cameras through a Zeiss Axioskop or a Zeiss Photomicroscope III. Depth of field limitations required the use of image stacking software (heliconsoft.com) to produce single images with the best possible focus depth. The images were displayed at 38×28 cm on a 1920×1200-pixel monitor, and the dimensions determined with the calibrated software calipers (iconico.com/caliper), each pixel representing 0.16 µm on the 1000× images, and 0.4 µm for the 400× photographs. By nature of the Rhynie chert thin sections, the measurements may be underestimates. Because they were fossilised in their natural position (*in situ*), it is not possible to be certain that the sections are exactly through the 'great circle meridian' (equator) of the specimens. Moreover, *post mortem* dehydration by silica may have caused some shrinkage (e.g. Trewin and Fayers, 2015: figs 7, 8A). For extant species, it is possible to focus through the specimens to find the best optical section so that the measurements are always at the equator.

Extant fungal material and methods

Acaulospores from extant members of the *Archaeosporaceae* were extracted from pot-culture substrate by swirling a 15–30 ml sample in water and, after allowing settling for few seconds, decanting the supernatant through a 45 µm mesh sieve. For a set of samples, the flow through the 45 µm sieving was studied, to check whether small spores with diameters below 40–45 µm are present in the cultures. No such spores could be detected. The contents were examined under a dissecting microscope with incident light. Specimens were selected with fine forceps and mounted on microscope slides in polyvinyl alcohol lacto-glycerol, with or without the addition of Melzer's reagent (5:1 v/v) and examined, after polymerisation, by transmitted light microscopy (Zeiss Axioskop and Photomicroscope III), under brightfield or Nomarski Differential Interference Contrast. Some specimens were measured directly with a calibrated eyepiece graticule, each division representing 1.6 (40× objective) or 0.6 (100× objective) µm. Staining with methyl blue, observations and subsequent descriptions follow established methods (Walker, 1983; Kramadibrata *et al.*, 2000).

Voucher specimens examined. Voucher specimens of extant species, mounted on microscope slides, are numbered (e.g., W5340) in the records of C. Walker. With the exception of *Archaeospora spainiae*, the holotype of which was loaned by Z+ZT (Universität Zürich), these are held in the herbarium of the Royal Botanic Garden Edinburgh (E). Herbarium acronyms follow Index Herbariorum (http://sweetgum.nybg.org/science/ih/). These included *A. ecuadoriana*, *A. myriocarpa*, *A. schenckii*, *A. spainiae*, and *A. trappei*. For detailed information on voucher specimens, refer to Supplementary data Table S1. The only other species currently in the genus, *A. europaea*, was published after this work was completed (Oehl et al. 2019).

Statistical methods

All statistical analysis was done in R (R Core Team, 2018). The data were first analysed by comparing the longest and shortest dimensions of the propagules across species. Data failed to meet the assumptions of multivariate normality and homogeneity of covariance matrices. Non-parametric inference of the multivariate data was made using the nonparametric comparison of multivariate samples (npmv) package (Burchett *et al.*, 2017). Propagule volumes were calculated (as an ellipsoid (oblate spheroid) body, $v = 4/3\ \pi \bullet b^2 \bullet c$) for all spores. These data also did not meet the assumptions of normality and homoscedasticity, and were analysed with the Kruskal-Wallis rank sum test.

TERMINOLOGY USED TO DESCRIBE PROPAGULE AND SACCULE MORPHOLOGY

The descriptive term 'acaulosporoid spore' was coined for propagules formed by species of *Acaulospora*, and translates to 'spore without a stalk' (Gerdemann and Trappe, 1974). Two species, *Acaulospora laevis* (the type species for the genus) and *Ac. elegans*, were initially included. Later authors added more species, some of which are now placed in different genera, orders and families. One of these, *Ac. trappei*, was transferred to the genus *Archaeospora* based primarily on analysis of the β-tubulin gene (Morton and Redecker, 2001). Later molecular analysis showed that, although having superficially similar propagule development, the *Acaulosporaceae* and *Archaeosporaceae* were widely separated phylogenetically at the ordinal level (Walker *et. al* 2007*a*). Another more closely related genus, *Ambispora* (*Archaeosporales, Ambisporaceae*), also produces acaulosporoid spores (Walker *et al.*, 2007*a*).

The spores of *Acaulospora laevis* were originally described as developing laterally *on* the 'neck' or 'stalk' of the saccule (Gerdemann and Trappe, 1974), but they actually develop internally, *within* the saccule (Mosse, 1970: fig. 16). The particular position of the spore within the saccule neck has been used to apply the terms acaulosporoid, entrophosporoid and ambisporoid, depending on whether they are produced laterally in a balloon-like protrusion, centrally in the saccule neck, or

laterally in a pedicel-like process (Figs. 2A$_{1–2}$–C$_{1–2}$). Some develop, in the manner described for *Archaeospora trappei*, laterally in a 'bud' (Fig. 2A$_{1–2}$), i.e. in the acaulosporoid manner as described for members of the genus *Acaulospora*, while others form centrally by expansion of the saccule neck (Fig. 2C$_{1–2}$), i.e. the entrophosporoid manner described for *Entrophospora infrequens* (Ames and Schneider, 1979). Still others develop a protrusion (Fig. 2B$_{1–2}$), termed a pedicel by Morton and Redecker (2001), which is similar to some members of *Ambispora*; such development could thus be called ambisporoid. The pedicel is a protrusion of the wall of a spore developing *de novo* within the saccule stalk, rather than continuous with a subtending hypha from which a chlamydospore develops, as in glomoid spores. All three of these patterns can be found within a single species of extant *Archaeospora* (e.g. *Archaeospora ecuadoriana*), and hence merely represent topological variations with no diagnostic value (Schüßler and Walker, 2019). Acaulosporoid propagules have erroneously been termed 'azygospores' in the past (e.g. Schenck and Smith, 1982). To distinguish this kind of propagule from the widely differing categories of spores in the *Glomeromycota* (Walker *et al.*, 2018*b*), the more prescriptive terminology, 'acaulospore' will be used herein.

RESULTS

Twenty-nine different Rhynie chert thin sections have yielded a total of >250 *Archaeospora*-like acaulospores (Figs. 2A$_{1–2}$–C$_{1–2}$, 3A–O, 4A–E, 4G–K, 5A, 6B), 37 of which were physically connected to a saccule (Figs. 2D$_{1–2}$–F$_{1–2}$, 3A–O, 4A, 4C, 4E, 4K, 6B). Specimens (with or without attached saccules) occur within plant tissue (decaying or otherwise) and in the surrounding chert matrix. They often co-occur with considerably larger spore-saccule complexes of the type initially described by Dotzler *et al.* (2009) in (partially) degraded axis portions of the early land plant sporophyte *Aglaophyton majus*. There seems to be no particular correlation as to whether the acaulospores formed within plant tissue or in the matrix (Table 1); however, intact saccules are more common within degraded plant axes.

Saccules

Specimens with saccules were not all in good condition. Measurements were therefore made only from those that were definitely identifiable (e.g. Figs. 3A–O, 4A, 4C, 4E, 4G, 4I, 4K). The fossil saccules appear as long-necked, balloon-shaped structures that, as measured from the distal point of the saccule to the centre of the acaulospore attachment at the saccule neck, are 31–132 µm long × 27–75 µm at the widest point (mean 83 × 41 µm), but can be globose (Figs. $2E_1$, $2F_1$), ovoid (Figs. 3B, 3K), or ellipsoid (Figs. 3E, 3G, 3H). Saccules are bound by a single, smooth wall component (C1), approximately 0.5–1 µm thick, have no obvious structural features such as laminations or ornamentation, and lack contents. In general, the saccules are far more difficult to recognize than the acaulospores; saccules have been informally termed 'ghost saccules' because they are easily overlooked (e.g. Fig. 6B). Moreover, even when spore-saccule complexes are intact within the confines of plant axes, they are usually only visible at high magnifications (i.e. 400× or 1000× total magnification) and with careful screening. Due to their small size and overall shape, if the saccules are disconnected from acaulospores (Fig. 4G), they can easily be mistaken for vesicles of other mycorrhizal fungi, and if in the matrix, saccules are typically collapsed or deformed (Fig. 4K), rendering them not recognisable; therefore, only saccules of complete spore-saccule complexes were measured.

Acaulospores

The acaulospores range from globose to subglobose, 24–83 × 24–78 (mean 51 × 44; n=248) µm. The colour of the acaulospores in nature cannot be determined, but in the fossil specimens, the saccule is grey, and the spore colourless, or yellow to brown (Figs. 3A–O). For those wall components that can be attributed with a good degree of certainty, the wall structure is composed of two or three components (Fig. 5A). The very thin, flexible nature of the saccule wall, which is an

integral, surrounding part of the propagule, is considered as wall 1, with a single component (C1) that is 0.5–2.5 µm thick. Only the outer (saccule) wall (C1) and a single wall (C2) can be seen in most instances. Component 2 is similar to a 'semi-rigid wall' sensu Morton (1990), approximately 0.5–3 µm thick (measurements rounded to the nearest 0.5 µm). Some specimens have a granular mucilage-like outer coating to which fine substrate particles adhere, lending the appearance of a thick outer wall. Other specimens have a third, innermost component (C3), which may represent a 'germinal wall' (Spain, 2003; Hafeel, 2004), but we do not rule out that it may be an artefact of microscopy due to refringence. Such differences may reflect different stages of development, or may be artefacts of preservation.

The fossils indicate that the acaulospores develop in the neck of the sporiferous saccule in either of three ways, i.e. acaulosporoid (Fig. $2D_1$), ambisporoid (Fig. $2E_1$), and entrophosporoid (Fig. $2F_1$). However, the precise details are only visible in specimens that are well preserved and ideally sectioned. The distances between the saccule base and the centre of the acaulospore attachment is variable between the acaulospore positions, e.g. acaulosporoid (45–60 µm), ambisporoid (57–65 µm), and entrophosporoid (13–15 µm). The attachment site in the acaulosporoid type, or 'lateral bud', is 5.5 µm wide; the bud is approximately 1–1.5 µm high. The pedicle in the ambisporoid type is approximately ≥10 µm by 3–4 µm long and lacks a septum. Finally, the distance between the spore attachment point and saccule neck in entrophosporoid specimens ranges from 10 to 13 µm.

Other features

In contrast to the saccules, some of the acaulospores have internal contents that range from amorphous matter (Fig. 4I) to fine particles, which may be located in the centre of the spore or along wall component C3 (Fig. 3O). None of the specimens are presented in such a way that the scar (cicatrix) where the spore detached from the saccule could be observed. The walls lacked ornamentation and were not obviously laminated (Fig. 5A). There is no evidence of dimorphic spore

production (i.e. acaulospores and glomoid spores) on the parental hyphae of the spore-saccules complexes.

SYSTEMATIC PALAEOMYCOLOGY

Fungi, Glomeromycota, Glomeromycotina, Archaeosporales, Archaeosporaceae

Fossil genus: *Archaeosporites* gen. nov. C. Walker, C.J. Harper et M. Krings

Figs. 2A$_{1–2}$–C$_{1–2}$, 3A–O, 4A–E, 4G–K, 5A, 6B

Index Fungorum: IF 556488

Etymology: The name underscores the similarity to the extant genus *Archaeospora*; the suffix *-ites* (Greek: *to indicate a close connection*) is used to designate a fossil taxon as proposed by Pirozynski and Weresub (1979).

Type species: *Archaeosporites rhyniensis* sp. nov. C. Walker, C.J. Harper et M. Krings (*hic designatus*)

Diagnosis: Fossil propagules (acaulospores), sessile, occurring singly, forming laterally or centrally within neck of sporiferous saccule; saccule <150 µm long, formed by blastic development of hyphal tip, with thin wall of one component; acaulospores globose to ellipsoid, <100 in diameter; wall of two main components, outer component (saccule wall) overlaying structural wall; outer wall component sometimes overlaid by small adherent particles.

Fossil species: *Archaeosporites rhyniensis* sp. nov. C. Walker, C. J. Harper et M. Krings **Fig. 2D$_1$**

Index Fungorum: IF 556489

Holotype: A saccule with a laterally-produced acaulospore present in thin section SNSB-BSPG 2016 XII 12 (Fig. 2D$_1$) housed in the palaeobotanical collection of the SNSB-Bayerische Staatssammlung für Paläontologie und Geologie, Munich, Germany.

Diagnosis: Spores developing within neck of balloon-shaped sporiferous saccule, formed by expansion of hyphal tip that is lost as the spore becomes detached; **saccule** approx. 41–78 × 30–78 µm (n=8), smooth, delicate, lacking protrusions, with a single wall approx. 1 µm thick; **spores**

(including remains of saccule wall) 24–83 × 24–78 (mean 51 × 44; n=248), globose to subglobose, occasionally ellipsoid, broadly ellipsoid, obovoid or ovoid, with two or three wall components, (C1–C3); **C1**, saccule wall, thin, flexible, up to 1 μm thick, sometimes lacking due to breakdown in substrate; main spore wall (**C2**) up to 3 μm thick; a very fine wall component (**C3**; germinal wall?), less than 1 μm thick, may be present.

Specimens examined: United Kingdom, Scotland, Aberdeenshire, Rhynie chert Lagerstätte. Besides the type specimen [mandated in Article 8.5 of the relevant International Code for Nomenclature (Turland *et al.*, 2018) as a single specimen], more than 50 specimens are present in the type slide. Only 8 of these specimens had recognisable saccules attached, although some had at least some evidence of the remains of the saccule surrounding the acaulospore.

Etymology: Referring to the provenance of the fossil, the Rhynie chert Lagerstätte.

Type locality: Rhynie, Aberdeenshire, Scotland, National Grid Reference NJ 494276 (57° 20′ 09.97″ N 002° 50′ 31.83″ W).

Age: Early Devonian; Pragian–earliest Emsian, 411.5 ± 1.3 Ma (Parry *et al.*, 2011), 407.1 ± 2.2 Ma (Mark *et al.*, 2011).

DISCUSSION

The Early Devonian Rhynie chert has provided astonishingly detailed insights into the early evolution of arbuscular mycorrhizal symbioses and the diversity of GAF in early terrestrial ecosystems, due to the exquisite preservation of fungal hyphae, vesicles, arbuscules, and spores that closely resemble present-day arbuscular mycorrhizas in both sporophytes and gametophytes of the early land plant *Aglaophyton majus* (Kidston and Lang, 1921; Remy *et al.*, 1994; Taylor *et al.*, 1995, 2005), and structures suggestive of the presence of similar symbioses also in other early land plants (reviewed by Krings *et al.*, 2017*a*; Brundrett *et al.*, 2018; Strullu-Derrien *et al.*, 2018; Walker *et al.*, 2018*b*). Evidence of GAF diversity in the Rhynie palaeoecosystem primarily occurs in the form of

different types of propagules that are indistinguishable from, or closely reminiscent of, propagules produced by members of extant genera such as *Glomus, Rhizophagus, Sclerocystis, Gigaspora*, and *Scutellospora* (Kidston and Lang, 1921; Taylor *et al.*, 1995; Karatygin *et al.*, 2006; Dotzler *et al.*, 2006, 2009; Krings *et al.*, 2015, 2017*b*; Harper *et al.*, 2017; Walker *et al.*, 2018*b*). Moreover, some of the enigmatic mantled fungal reproductive units from the Rhynie chert, e.g. *Helmutella devonica, Windipila spinifera*, and *W. wimmervoecksii*, may also be members of the *Glomeromycota* (Krings and Taylor, 2014; Krings and Harper 2017, 2020), albeit with hyphal investment morphologies not seen in present-day taxa, such as *Corymbiforme globiferum* (basionym *Glomus globiferum*) and *Glomus mortonii* (see Bentivenga and Hetrick, 1991; Wu and Sylvia, 1993; Błaszkowski, 1995; Koske and Walker, 1986). Taken together these fossils constitute the largest body of fossil evidence of GAF gathered to date from any ancient ecosystem. However, systematic screening of Rhynie chert thin sections recently has yielded additional types of propagules that have not been recognised previously, thus suggesting that the fossils described to date represent only a fraction of the diversity that was actually present in the Rhynie palaeoecosystem.

Affinities

The spore-saccule complexes detailed in this study are morphologically different from any type of fungal propagule previously described from the Rhynie chert. Dotzler *et al.* (2009) described an acaulospore-forming fungus from the Rhynie chert similar to present-day *Ambispora* spp. The superficial similarity of overall form (i.e. development of an acaulospore within a sporiferous saccule) may lead investigators to speculate that these two fungi might represent grossly differently-sized members of the same genus. Similar consideration of the fungi now shown to belong to three different families (*Archaeospora, Ambispora*, and *Acaulospora*) originally placed all these in the same genus, namely *Acaulospora* (e.g. *Archaeospora trappei, Ambispora nicolsonii*, and all current members of the genus *Acaulospora* initially were placed in the one genus). However biochemical

and molecular markers allowed the current placing to be made in separate genera. Although it is not possible to use these techniques on fossils, the size difference and wall structure details can be used to conclude that the two fossil acaulosporoid species mentioned above are similarly different. The organism described by Dotzler *et al.* (2009), with acaulospores 270–427 × 236–473 µm (Fig. 6A) and saccules 200–450 (–700) × 200–450 µm long (Fig. 6A), is very different from the form described in this study, which has acaulospores on average 52 × 46 µm and saccules 83 × 41 µm in size (Fig. 6B). Moreover, the wall architecture of the new form is the same as that of modern *Archaeospora* spp., whereas the acaulospores described by Dotzler *et al.* (2009) correspond to wall architectures seen in present-day *Ambisporaceae* (see below). Finally, the new spore-saccule complexes have been found in both the chert matrix and land plant axes in varying states of preservation, whereas Dotzler *et al.* (2009) report their specimens only from within partially degraded plant axes.

Development of acaulospores from a sporiferous saccule is known to occur in three different present-day families of *Glomeromycota*, i.e. the *Acaulosporaceae* (*Acaulosporales*), *Ambisporaceae* (*Archaeosporales*), and *Archaeosporaceae* (*Archaeosporales*) (Morton and Benny, 1990; Morton and Redecker, 2001; Walker *et al.*, 2007*b*). The fossils detailed in this study differ from *Acaulosporaceae* and *Ambisporaceae* primarily in the dimensions of the acaulospores, which may reach diameters of >380 µm in *Acaulosporaceae* (Schultz *et al.*, 1999) and >390 µm in *Ambisporaceae* (Spain *et al.*, 2006), but correspond to the acaulospores produced by members of the *Archaeosporaceae* that attain diameters of only 21–114 µm (Spain *et al.*, 2006). All morphological variation in the fossils is well within the range of intraspecific variability in modern *Archaeospora* spp. (e.g. Schüßler and Walker, 2019). Members of the *Acaulosporaceae*, *Ambisporaceae*, and *Archaeosporaceae* may produce two kinds of spores, i.e. acaulospores and glomoid spores (e.g. Spain 2003; Walker *et al.*, 2007*b*; Taylor *et al.*, 2014); however, no evidence of glomoid spores has been found to date in the fossils, and thus this trait cannot yet be compared. Based on overall

morphology of the spore-saccule complex, the fossils correspond well with the original descriptions of *Archaeospora trappei* (Figs. 1A, 1B, 4F, 4H) (basionym *Acaulospora trappei*) (Ames and Linderman, 1976; Morton and Redecker, 2001), *A. schenckii* (Fig. 4L) (basionym *Entrophospora schenckii*) (Sieverding and Toro, 1987; Schüßler and Walker, 2010), *A. myriocarpa* (Fig. 4D, 4J) (basionym *Acaulospora myriocarpa*) (Schenck *et al.*, 1986; Oehl *et al.*, 2011), *A. ecuadoriana* (Figs. $2A_{1-2}$–C_{1-2}, 4B) (Schüßler and Walker, 2019), and *A. spainiae* (Fig. 5B) (basionym *Palaeospora spainiae*) (Oehl *et al.*, 2015; Schüßler and Walker, 2019).

The number and nature of wall components and layers comprising the individual wall groups are used as important taxonomic characters in the *Glomeromycota* (Walker, 1983; Walker and Vestberg, 1998). The wall structure and morphological characteristics of extant and fossil material are comparable by light microscopy. The wall of the fossil acaulospores described here consists of three components (C1–3; Fig. 5A) corresponding with that of extant members of the *Archaeosporaceae* (Morton and Redecker, 2001; Fig. 5B). The number of distinguishable wall components is always three or more in the *Acaulosporaceae* and 2–4 in *Ambisporaceae* (Walker *et al.*, 2007*b*; Mosse, 1970), and in the fossils described by Dotzler *et al.* (2009), there are three wall groups which consist of 6 components. The outermost wall component (C1), if preserved, is a thin layer (≥1 µm thick) of similar thickness to many of the modern *Archaeospora* spp.; some of the fossil specimens have fine substrate particles on C1 of the saccule and acaulospore wall, which has been described as a distinct wall for *A. spainiae* (Oehl *et al.*, 2015: p. 94). As in most modern *Archaeospora* spp., the fossil acaulospore wall proper (C2) is the most prominent wall component (Fig. 5A); it appears to occur in the form of a 'semi-rigid wall' sensu Morton (1990). The innermost wall component (C3) is not present in all of the fossil specimens, and it may represent a germinal wall as described by Morton (1990), which is also present in *A. myriocarpa* (Schenck *et al.*, 1986: p. 113–114), but we do not rule out that it could be just the boundary between the plasma membrane and the rigid C2. It is impossible to determine if the fossil acaulospores are in their original

colouration, or the colour of the fossils is the result of post-inundation changes and fossilisation. Extant members of the *Archaeosporaceae* are colourless to translucent white except when moribund or dead (e.g. Ames and Linderman, 1976; Morton and Redecker, 2001). It is also impossible to know if the Rhynie organisms had pigmented acaulospores in life, but some fossil specimens appear brown (Fig. 4I). There are no coloured living acaulospores of this size in either *Archaeospora*, or other genera with smooth acaulospores, but moribund or dead spores of extant fungi in the family can become brown (Fig. 4J). It seems likely that, given the lack of pigmentation in modern species of *Archaeospora*, *Archaeosporites rhyniensis* propagules also were colourless.

Acaulospores in extant *Archaeosporaceae* develop within the neck of the saccule in one of three ways (i.e. acaulosporoid, entrophosporoid, and ambisporoid), a feature also observed in the fossils, even within the same thin section (Figs. $2D_{1–2}$–$F_{1–2}$). This characteristic is described for *A. myriocarpa*, *A. schenckii*, or *A. ecuadoriana* (Figs. $2A_{1–2}$–$C_{1–2}$), and Schüßler and Walker (2019) note that it remains unclear whether all *Archaeospora* species produce acaulospores in all three topologies.

Saccules are delicate structures and, consequently, are less persistent than the acaulospores, which have a more complex and thickened wall structure (Figs. 4K, 4L). Saccules of *Archaeosporites rhyniensis* range from 27–132 µm long, which fits well within the range of saccule length in modern *Archaeospora* spp., i.e. 22–179 µm. This is compared to the much larger saccules of the *Acaulosporaceae* (up to 360 µm long) (Błaszkowski, 1989; Palenzuela *et al.*, 2013), *Ambisporaceae* (up to 380 µm) (Spain *et al.*, 2006; Walker, 2008), and fossil saccules described by Dotzler *et al.* (2009), which reach between 200 and 700 µm in length. The saccules of the fossils described in this study do not possess lateral hyphal outgrowths, which is a typical feature of members of *Acaulospora* (Gerdemann and Trappe, 1974); rather, they are simple, smooth, balloon-shaped structures, precisely as illustrated by Ames and Linderman (1976: fig. 1A) for *Archaeospora trappei*.

Specimens of the extant *Archaeospora schenckii*, *A. trappei* and *A. myriocarpa* were measured and compared statistically with the Rhynie chert fossils, which had smaller shortest and longest dimensions (F = 520.7; df = 3.6, 346.8; $p < 0.001$), and a larger range, but fell within the overall limit given in the protologue descriptions for all species (including *A. spaniae*) of 23–114 × 21–96 µm (Fig. 7). When converted to approximate volumetric measurements, the analysis results were the same, i.e. the Rhynie acaulospores are significantly smaller (H = 484.22, df = 3, $p < 0.001$) than those of extant *Archaeospora* spp. Although one has to consider a possible shrinkage factor resulting from water loss during silicification (Fleischmann *et al.*, 2007), the differences in dimensions is still statistically so significant that we feel confident to formally describe a new fossil genus and species.

Based on morphology, the Rhynie chert fossils described herein can satisfactorily be placed in *Archaeospora* (see Table 2), but given the need for molecular evidence in modern-day taxonomy, we therefore propose to name them in the fossil genus and species *Archaeosporites rhyniensis* gen. et sp. nov. The new genus and a species are thus defined on morphology from these fossils, but with the understanding that the many specimens referred to herein could represent several morphologically similar taxa (probably at the level of species) that cannot be distinguished based on fossils.

Ecological roles of Archaeosporites and Archaeospora *species*

Based on morphological criteria, the Rhynie chert fossils described in this study are so similar to the extant *Archaeospora* that they can be placed in the *Archaeosporaceae*. By analogy with the modern species, the fossil organism may have been symbiotic with plants and formed arbuscular mycorrhizas. However, there is currently no evidence that can be used to link mycelia and arbuscules to *Archaeosporites rhyniensis*. Furthermore, it is impossible to know whether the land plant axes that contained the spore-saccule complexes of *A. rhyniensis* were alive at the time of colonization and fungal sporulation, or the acaulospores merely formed within litter layers comprised

of dead and decaying plant parts. In extant species of *Archaeospora*, the propagules are most abundant in the substrate (which may be decaying plant material, soil, or substrate in which potted host plants are grown). For example, in a sample from pot culture [Voucher W6496-5, Attempt 1289-5 of C. Walker, of *Archaeospora trappei* with *Plantago lanceolata*] there was not a single propagule in 10 cm of roots (cleared and stained with trypan blue). This extraction produced large numbers of acaulospores, 107 of which were preserved on microscope slides (W6496-1 to 6496-4). Only 8 of these acaulospores still had remnants of the saccule attached, and only a single developing saccule was found (C. Walker, Gloucester, England, UK, unpubl. res.). Propagule formation in roots is mentioned in the original species descriptions of *A. trappei*, *A. myriocarpa*, *A. ecuadoriana*, (Ames and Linderman, 1976; Schenck *et al.*, 1986; Schüßler and Walker, 2019), but not for *A. spainiae* (Oehl *et al.*, 2015); however, data on the occurrence or distribution of propagules within the soil or plants is not provided. The propagules of *A. rhyniensis* were found within plant tissue and in the surrounding matrix, and there is no evidence to suggest that one substrate was favoured for spore production (Table 1). It appears that, on average, the delicate saccules survive better when located within plant axes. Preservation of the spore-saccule complexes within the protective confines of plant tissue may have shielded the fragile structures and fine structural details against the destructive forces during fossilization in a similar manner to that suggested in other studies of fossil microorganisms (e.g. Krings and Taylor, 2012, 2015).

Members of the *Archaeosporaceae* today form mycorrhizal associations with plants (see Morton and Redecker, 2001: tab. 1). However, in comparison to other mycorrhizal fungi (e.g. *Glomus* spp.), there are relatively few experiments with plants inoculated with *Archaeospora* spp. and on the impact of *Archaeospora* mycorrhizas on plant fitness. Those investigations that did include *Archaeospora* spp. found a positive impact of the presence of the fungus on phosphorus uptake and herbivory tolerance in individual host plants (Helgason *et al.*, 2002; Bennett and Bever, 2007). Long-term ecological studies over a period of 32 years suggest that alternating mycorrhizal

symbioses with *Archaeospora* and members of the *Glomeraceae* in certain conifers increases the fitness of the individual plant, surrounding forest, and the microbial community (Lu *et al.*, 2019). In extant geothermal wetlands such as Yellowstone National Park, which has been regarded as a modern analogue to the Rhynie chert ecosystem (Channing and Edwards, 2009; Garwood *et al.*, 2020), *Archaeospora* spp. was abundantly recorded for several study sites (Appoloni *et al.*, 2008). The diversity of mycorrhizal fungi can be used as a proxy of plant fitness in extant ecosystems (Van der Heijden *et al.*, 2007; Hempel *et al.*, 2013). Bearing in mind that the Rhynie chert has yielded a considerable diversity of fossils of glomeromycotan fungi from several of the major lineages within this phylum (reviewed by Krings *et al.*, 2017*a*; Walker *et al.*, 2018*b*), and although there is no evidence of mycorrhizal associations between *Archaeosporites rhyniensis* and the plants in the Rhynie ecosystem, it seems reasonable to suggest that the availability of several different lineages of GAF may have been beneficial to the performance of the land plants in this early terrestrial ecosystem.

CONCLUSIONS

One of the intriguing palaeomycological facets of the Early Devonian Rhynie chert is the abundance and morphological diversity of arbuscular mycorrhizal fungi, especially because the early land plants that lived in this hot spring palaeoecosystem all lacked true roots. However, it becomes increasingly clear that there is still considerable undocumented diversity in the form of new specimens and hitherto unknown or unrecognized features of mycorrhizal fungi in the Rhynie chert. *Archaeosporites rhyniensis* described in this study is one excellent example of this diversity. This fossil is so morphologically similar to some extant *Glomeromycota* that, were it not for the need for molecular evidence in modern day taxonomy, it may well be placed in a modern genus, and thus suggests that the genus *Archaeospora* perpetuated for considerably more than 400 million years. Fossils such as *A. rhyniensis*, albeit incomplete (i.e. not connected to the parental mycorrhiza in the

plant), not only increase the number of calibration points that can be used in aligning molecular clock estimates with fossil evidence in the discussion of the evolution of mycorrhizal systems, but also reiterate questions pertaining specifically to the Rhynie chert mycorrhizas. For example, Strullu-Derrien *et al.* (2014) suggest that Rhynie chert land plants did not exclusively form mycorrhizas with *Glomeromycota*, but also with members of the *Endogonales* (*Mucoromycotina*), based on the presence of certain types of intercellular hyphae, hyphal segments interpreted as intracellular coils, and small propagules resembling glomoid spores in *Horneophyton lignieri*. Although this idea has found its way into the general discussion of the origin and evolution of mycorrhizal symbioses (e.g. Feijen *et al.*, 2018; Hoysted *et al.*, 2018; Bonfante and Venice, 2020), the fossil evidence is equivocal. This is in contrast with the Rhynie chert fossils of *Glomeromycota*, which are often exquisitely preserved and therefore can be assigned to modern families, orders, or genera with confidence (Brundrett *et al.*, 2018). The morphology of mucoromycotinan colonisation in plants today – from ectomycorrhizas formed by *Endogone lactiflua* (Walker, 1985) to arbuscular mycorrhizas with *Planticonsortium tenue* (Walker *et al.*, 2018*a*) and similar, unidentified fungi (Beck *et al.*, 2005) – has been illustrated in great detail, and there is also increasing literature evaluating the characteristics and evolution of these relationships based on molecular and genetic data (e.g., Chang *et al.*, 2019; Hoysted *et al.*, 2019; Rimington *et al.*, 2019, 2020). As a result, there is now a good base for seeking similarly conclusive evidence from the fossil record. There is considerable interest in the mycorrhizal component of modern ecosystems and the roles these symbioses play in ecosystem functioning, while we are only beginning to assess such interactions in the fossil record. This paper not only adds to the knowledge of *Glomeromycota* in early palaeoecosystems, but can also serve to stimulate interest in these organisms, and encourage other researchers to prepare and study suitable specimens from their collections.

ACKNOWLEDGEMENTS

The Royal Botanic Garden Edinburgh (RBGE) is supported by the Scottish Government's Rural and Environmental Science and Analytical Services Division. Thanks to R. Linderman and J. Spain (Corvallis, Oregon, USA) for *A. myriocarpa*; A. Ménendez (Buenos Aires, Argentina) and D. Redecker (Dijon, France) for *A. schenckii*; A. Schüßler (Darmstadt, Germany) for *A. ecuadoriana*. We also thank Paul Kirk (Kew Gardens, Great Britain) for a pre-submission review and supplying the IF taxon name registration numbers; the Herbarium at the University of Zürich, Switzerland (Z+ZT) for the loan of the holotype of *A. spainiae*; and Hagen Hass (Münster, Germany), Helmut Martin and Stefan Sónyi (both Munich, Germany) for technical assistance. We gratefully acknowledge two anonymous referees and handling editor S. Pressel (London, UK) for insightful comments on the manuscript.

FUNDING

This work was supported in part by funds from the U.S. National Science Foundation (EAR-0542170, and DEB-1441604 subcontract S1696A-A to M.K.) and the Deutsche Forschungsgemeinschaft (DFG grant Ke 584/13-2 to H.K. and M.K.).

LITERATURE CITED

Ames RN, Linderman RG. 1976. *Acaulospora trappei* sp. nov. *Mycotaxon* **3**: 565–569.

Ames RN, Schneider RW. 1979. *Entrophospora*, a new genus in the Endogonaceae. *Mycotaxon* **8**: 347–352.

Appoloni S, Lekberg Y, Tercek MT, Zabinski CA, Redecker D. 2008. Molecular community analysis of arbuscular mycorrhizal fungi in roots of geothermal soils in Yellowstone National Park (USA). *Microbiology Ecology* **56**: 649–659.

Beck A, Kottke I, Oberwinkler F. 2005. Two members of the Glomeromycota form distinct ectendomycorrhizas with *Alzatea verticillata*, a prominent tree in the mountain rain forest of southern Ecuador. *Mycological Progress* **4**: 11–22.

Bennett AE, Bever JD. 2007. Mycorrhizal species differentially alter plant growth and response to herbivory. *Ecology* **88**: 210–218.

Bentivenga SP, Hetrick BAD. 1991. *Glomus mortonii* sp. nov., a previously undescribed species in the Glomaceae isolated from the tallgrass prairie in Kansas. *Mycotaxon* **42**: 9–15.

Błaszkowski J. 1989. Polish Endogonaceae. VII. *Acaulospora capsicula* sp. nov. *Mycologia* **82**: 794–798.

Błaszkowski J. 1995. *Glomus corymbiforme*, a new species in Glomales from Poland. *Mycologia* **87**: 732–737.

Bonfante P, Venice F. 2020. Mucoromycota: going to the roots of plant-interacting fungi. *Fungal Biology Reviews*, in press. DOI: 10.1016/j.fbr.2019.12.003

Brundrett MC, Walker C, Harper CJ, Krings M. 2018. Fossils of arbuscular mycorrhizal fungi give insights into the history of a successful partnership with plants. In: Krings M, Harper CJ, Cuneo NR, Rothwell BW, eds. *Transformative Paleobotany. Papers to Commemorate the Life and Legacy of Thomas N. Taylor*. London, San Diego, CA, Cambridge, MA, Oxford, Elsevier/Academic Press Inc., 461–480.

Burchett WW, Ellis AR, Harrar SW, Bathke AC. 2017. Nonparametric Inference for Multivariate Data: The R Package npmv. *Journal of Statistical Software* **76**: 1–18.

Chang Y, Desirò A, Na H, *et al.* 2019. Phylogenomics of Endogonaceae and evolution of mycorrhizas within Mucoromycota. *New Phytologist* **222**: 511–525.

Channing A, Edwards D. 2009. Yellowstone hot spring environments and the palaeo-ecophysiology of Rhynie chert plants: towards a synthesis. *Plant Ecology and Diversity* **2**: 111–143.

Dotzler N, Krings M, Taylor TN, Agerer R. 2006. Germination shields in *Scutellospora* (Glomeromycota: Diversisporales, Gigasporaceae. from the 400 million-year-old Rhynie chert. *Mycological Progress* **5**: 178–184.

Dotzler N, Walker C, Krings M, *et al.* 2009. Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-year-old Rhynie chert. *Mycological Progress* **8**: 9–18.

Feijen FAA, Vos RA, Nuytinck J, Merckx VSFT 2018. Evolutionary dynamics of mycorrhizal symbiosis in land plant diversification. *Scientific Reports* **8**: 10698.

Fleischmann A, Krings M, Mayr H, Agerer R. 2007. Structurally preserved polypores from the Neogene of North Africa: *Ganodermites libycus* gen. et sp. nov. (Polyporales, Ganodermataceae). *Review Palaeobotany and Palynology* **145**: 159–172.

Garwood RJ, Oliver H. Spencer ART. 2020. An introduction to the Rhynie chert. *Geological Magazine* **157**: 47–64.

Gerdemann JW, Trappe JM. 1974. The Endogonaceae in the Pacific Northwest. *Mycologia Memoir No. 5*. Published by the New York Botanical Garden in collaboration with the Mycological Society of America. (Bronx, NY).

Hafeel KM. 2004. Spore ontogeny of the arbuscular mycorrhizal fungus *Archaeospora trappei* (Ames & Linderman) Morton & Redecker (Archaeosporaceae). *Mycorrhiza* **14**: 213–219.

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.

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous. *Journal of Ecology* **90**: 371–384.

Hempel S, Götzenberger L, Kühn I, *et al.* 2013. Mycorrhizas in the Central European flora: relationships with plant life history traits and ecology. *Ecology* **94**: 1389–1399.

Hoysted GA, Kowal J, Jacob A, *et al.* 2018. A mycorrhizal revolution. *Current Opinion in Plant Biology* **44**: 1–6.

Kamel L, Keller-Pearson M, Roux C, Ané J-M. 2017. Biology and evolution of arbuscular mycorrhizal symbiosis in the light of genomics. *New Phytologist* **213**: 531–536.

Karatygin IV, Snigirevskaya NS, Demchenko KN. 2006. Species of the genus *Glomites* as plant mycobionts in Early Devonian ecosystems. *Paleontological Journal* **40**: 572–579.

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. *Transactions of the Royal Society of Edinburgh* **52**: 855–902.

Koske RE, Walker C. 1986. *Glomus globiferum*: A new species of Endogonaceae with a hyphal peridium. *Mycotaxon* **26**: 133–142.

Kramadibrata K, Walker C, Schwarzott D, Schüßler A. 2000. A new species of *Scutellospora* with a coiled germination shield. *Annals of Botany* **86**: 21–27.

Krings M, Harper CJ. 2017. A mantled fungal reproductive unit from the Lower Devonian Windyfield chert, Scotland, with prominent spines and otherwise shaped projections extending

out from the mantle, *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* **285:** 201–211.

Krings M, Harper CJ. 2020. Morphological diversity of fungal reproductive units in the Lower Devonian Rhynie and Windyfield cherts, Scotland: A new species of the genus *Windipila*. *PalZ*, in press. DOI: 10.1007/s12542-019-00507-5

Krings M, Taylor T. 2012. Fungal reproductive units enveloped in hyphal mantle from the Lower Pennsylvanian of Great Britain, and their relevance to our understanding of Carboniferous fungal "sporocarps". *Review of Palaeobotany and Palynology* **175**: 1–9.

Krings M, Taylor TN. 2014. A mantled fungal reproductive unit from the Lower Devonian Rhynie chert that demonstrates Carboniferous "sporocarp" morphology and development. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* **273**: 197–205.

Krings M, Taylor T. 2015. Mantled fungal reproductive units in land plant tissue from the Lower Devonian Rhynie chert. *Bulletin of Geosciences* **90**: 1–6.

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.

Krings M, Harper CJ, Taylor EL. 2017*a*. Fungi and fungal interactions in the Rhynie chert: a review of the evidence, with the description of *Perexiflasca tayloriana* gen. et sp. nov. *Philosophical Transactions of the Royal Society B* **373**: ID 20160500.

Krings M, Walker C, Harper CJ, *et al.* 2017*b*. Unusual fungal reproductive units from the Lower Devonian Rhynie chert. *Zitteliana* **89**: 29–37.

Lu N, Xu X, Wang P, *et al.* 2019. Succession in arbuscular mycorrhizal fungi can be attributed to a chronosequence of *Cunninghamia lanceolata*. *Scientific Reports* **9:** 18057.

Mark DF, Rice CM, Fallick AE, *et al*. 2011. $^{40}Ar/^{39}Ar$ dating of hydrothermal activity, biota and gold mineralization in the Rhynie hot-spring system, Aberdeenshire, Scotland. *Geochimica et Cosmochimica Acta* **75**: 555–569.

Martin FM, Uroz S, Barker DG. 2017. Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria. *Science* **356**: eaad4501.

Morton JB. 1990. Evolutionary relationships among arbuscular mycorrhizal fungi in the Endogonaceae. *Mycologia* **82**: 192–207.

Morton JB, Benny GL. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, *Acaulosporaceae* and *Gigasporaceae*, with an emendation of Glomaceae. *Mycotaxon* **37:** 471–491.

Morton MB, Redecker D. 2001. Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. *Mycologia* **93**: 181–195.

Mosse B. 1970. Honey-coloured, sessile *Endogone* spores III. Wall structure. *Archiv für Mikrobiologie* **74**: 146–159.

Naranjo-Ortiz MA, Gabaldón T. 2019. Fungal evolution: diversity, taxonomy and phylogeny of the Fungi. *Biological Reviews* **94**: 2101–2137.

Oehl F, Sieverding E, Palenzuela J, Ineichen K, Silva GA. 2011. Advances in Glomeromycota taxonomy and classification. *IMA Fungus* **2**: 191–199.

Oehl F, Sánchez-Castro I, Palenzuela J, Silva GA. 2015. *Palaeospora spainii*, a new arbuscular mycorrhizal fungus from Swiss agricultural soils. *Nova Hedwigia* **101**: 89–102.

Oehl F, Sánchez-Castro I, Santos VM, da Silva GA, Palenzuela J. 2019. *Archaeospora europaea*, a new arbuscular mycorrhizal fungus from France, Germany, Italy and Switzerland, with a key to the Archaeosporaceae species. *Sydowia* **71**: 129–137.

Palenzuela J, Azcón-Aguilar C, Barea J-M, Silva GA, Oehl F. 2013. *Acaulospora pustulata* and *Acaulospora tortuosa*, two new species in the Glomeromycota from Sierra Nevada National Park (southern Spain). *Nova Hedwigia* **97**: 305–319.

Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* **6**: 763–775.

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. *Journal of the Geological Society, London* **168**: 863–872.

Pirozynski KA, Weresub LK. 1979. The classification and nomenclature of fossil fungi. In: Kendrick B, ed. *The Whole Fungus, the Sexual-Asexual Synthesis. Vol. 2. Proc. 2nd Intl. Mycol. Conf.* Ottawa, Canada: Univ. Calgary, Kananaskis, Alberta Natl. Mus. Nat. Sci., Natl. Mus. Canada and Kananaskis Foundation, 653–688.

Powell CL, Trewin NH, Edwards D. 2000. Palaeoecology and plant succession in a borehole through the Rhynie cherts, Lower Old Red Sandstone, Scotland. *Geological Society of London Special Publication* **180**: 439–457.

R Core Team. 2018. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Redecker D, Raab P. 2006. Phylogeny of the Glomeromycota. (arbuscular mycorrhizal fungi): Recent developments and new gene markers. *Mycologia* **98**: 885–895.

Redecker D, Schüßler A, Stockinger H, Strümer SL, Morton JB, Walker C. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi. (Glomeromycota). *Mycorrhiza* **23**: 515–531.

Remy W, Taylor TN, Hass H, Kerp H. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences of the United States of America* **91**: 11841–11843.

Rice CM, Trewin NH, Anderson LI. 2002. Geological setting of the Early Devonian Rhynie cherts, Aberdeenshire, Scotland: an early terrestrial hot spring system. *Journal of the Geological Society, London* **159**: 203–214.

Rimington WR, Pressel S, Duckett JG, Field KJ, Bidartondo MI. 2019. Evolution and networks in ancient and widespread symbioses between Mucoromycotina and liverworts. *Mycorrhiza* **29**: 551–565.

Rimington WR, Duckett JG, Field KJ, Bidartondo MI, Pressel S. 2020. The distribution and evolution of fungal symbioses in ancient lineages of land plants. Mycorrhiza **30**: 23–49.

Schenck NC, Smith GS. **1982**. Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida. *Mycologia* **74**: 77–92.

Schenck NC, Spain JL, Sieverding E. 1986. A new sporocarpic species of *Acaulospora* (Endogonaceae). *Mycotaxon* **25:** 111–117.

Schüßler A, Walker C. (Eds). 2010. The Glomeromycota. A species list with new families and new genera. The Royal Botanic Garden Edinburgh, The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University. Electronic edition updated 2011, retrieved from www.amf-phylogeny.com; species list updated 2013, retrieved from http://schuessler.userweb.mwn.de/amphylo/

Schüßler A, Walker C. 2019. *Archaeospora ecuadoriana* sp. nov. from a mountainous biodiversity hotspot area in Ecuador, and transfer of *Palaeospora spainiae* to *Archaeospora*, as *A. spainiae* comb. nov. *Mycorrhiza* **29**: 435–443.

Schüßler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**: 1413–1421.

Schultz P, Bever JD, Morton JB. 1999. *Acaulospora colossica* sp. from an old field in North Carolina and morphological comparisons with similar species, *A. laevis* and *A. koskei*. *Mycologia* **91**: 676–683.

Sieverding E, Toro S. 1987. *Entrophospora schenckii*: a new species in the Endogonaceae from Colombia. *Mycotaxon* **28**: 209–214.

Spain JL. 2003. Emendation of *Archaeospora* and its type species, *Archaeospora trappei*. *Mycotaxon* **87**: 109–112.

Spain JL, Sieverding E, Oehl F. 2006. *Appendicispora*: a new genus in the arbuscular mycorrhiza-forming Glomeromycetes, with discussion of *Archaeospora*. *Mycotaxon* **97:** 163–182.

Spatafora JW, Chang Y, Benny GL, *et al.* 2016. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* **108**: 1028–1046.

Strullu-Derrien C, Kenrick P, Pressel S, Duckett JG, Rioult J-P, Strullu D-G. 2014. Fungal associations in *Horneophyton lignieri* 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 Phytologist* **203**: 964–979.

Strullu-Derrien C, Selosse MA, Kenrick P, Martin FM. 2018. The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. *New Phytologist* **220**: 1020–1030.

Taylor TN, Remy W, Hass H, Kerp H, 1995. Fossil arbuscular mycorrhiza from the Early Devonian. *Mycologia* **87**: 560–573.

Taylor TN, Kerp H, Hass H. 2005. Life history biology of early land plants: Deciphering the gametophyte phase. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 5892–5897.

Taylor A, Walker C, Bending GD. 2014. Dimorphic spore production in the genus *Acaulospora*. *Mycoscience* **55**: 1–4.

Taylor TN, Krings, M, Taylor EL. 2015. *Fossil Fungi*, 1st Edition, Amsterdam, Boston, Heidelberg, London, Elsevier/Academic Press Inc.

Tedersoo L, Sánchez-Ramírez S, Kõljalg U, *et al*. 2018. High-level classification of the Fungi and a tool for evolutionary ecological analyses. *Fungal Diversity* **90**:135–159.

Trewin NH, Fayers SR. 2015. Macro and micro aspects of the plant preservation in the Early Devonian Rhynie cherts, Aberdeenshire, Scotland. *Transactions of the Royal Society of Edinburgh - Earth and Environmental Science* **106**: 67–80.

Trewin NH, Kerp H. 2017. The Rhynie and Windyfield cherts, Early Devonian, Rhynie, Scotland. In: Fraser NC, Sues HD, eds. *Terrestrial Conservation Lagerstätten. Windows into the Evolution of Life on Land*. Edinburgh: Dunedin Academic Press, 1–38.

Turland NJ, Wiersema JH, Barrie FR, *et al*. 2018. *International Code of Nomenclature for algae, fungi, and plants. (Shenzhen Code. adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017*. Regnum Vegetabile 159. Glashütte: Koeltz Botanical Books.

Van der Heijden MGA, Bardgett RD, Van Straalen NM. 2007. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11**: 296–310.

Walker C. 1983. Taxonomic concepts in the Endogonaceae: Spore wall characteristics in species descriptions. *Mycotaxon* **18**: 443–455.

Walker C. 1985. *Endogone lactiflua* forming ectomycorrhizas with *Pinus contorta*. *Transactions of the British Mycological Society* **84**: 353–355.

Walker C. 2008. *Ambispora* and *Ambisporaceae* resurrected. *Mycological Research* **112**: 297–298.

Walker C, Reed LE, Sanders FE. 1984. *Acaulospora nicolsonii*, a new endogonaceous species from Great Britain. *Transactions of the British Mycological Society* **83**: 360–364.

Walker C, Vestberg M. 1998. Synonymy amongst the arbuscular mycorrhizal fungi: *Glomus claroideum, G. maculosum, G. multisubstenum* and *G. fistulosum*. *Annals of Botany* **82**: 601–624.

Walker C, Vestberg M, Demircik F, *et al*. 2007*a*. Molecular phylogeny and new taxa in the *Archaeosporales (Glomeromycota): Ambispora fennica* gen. sp. nov., *Ambisporaceae* fam. nov., and emendation of *Archaeospora* and *Archaeosporaceae*. *Mycological Research* **111**: 137–153.

Walker C, Vestberg M, Schüßler A, 2007*b*. Nomenclatural clarifications in Glomeromycota. *Mycological Research* **111**: 253–255.

Walker C, Gollotte A, Redecker D. 2018*a*. A new genus, *Planticonsortium* (Mucoromycotina), and new combination (*P. tenue*), for the fine root endophyte, *Glomus tenue* (basionym *Rhizophagus tenuis*). *Mycorrhiza* **28**: 1–7.

Walker C. Harper CJ. Brundrett MC, Krings M. 2018*b*. Looking for arbuscular mycorrhizal fungi. (AMF) in the fossil record: An illustrated guide. In: Krings M, Harper CJ, Cuneo NR, Rothwell GW, eds. *Transformative Paleobotany. Papers to Commemorate the Life and Legacy of Thomas N. Taylor*. London, San Diego, CA, Cambridge, MA, Oxford, Elsevier/Academic Press Inc., 481–517.

Wellman CH. 2006. Spore assemblages from the Lower Devonian 'Lower Old Red Sandstone' deposits of the Rhynie outlier, Scotland. *Transactions of the Royal Society of Edinburgh, Earth Sciences* **97**: 167–211.

Wellman CH. 2017. Palaeoecology and palaeophytogeography of the Rhynie chert plants: further evidence from integrated analysis of *in situ* and dispersed spores. *Philosophical Transactions of the Royal Society B* **373**:ID 20160491.

Wellman CH, Kerp H, Hass H. 2006. Spores of the Rhynie chert plant *Aglaophyton* (*Rhynia*) *major* (Kidston and Lang) D.S. Edwards, 1986. *Review of Palaeobotany and Palynology* **142**: 229–250.

Wu C-C, Sylvia DM. 1993. Spore ontogeny of *Glomus globiferum*. *Mycologia* **85**: 317–322.

FIGURE CAPTIONS

FIG. 1. Extant *Archaeospora trappei* acaulospore development. (A) Sporiferous saccule (s) budding (arrow) prior to spore formation; cytoplasm is transferring into the enlarging bud. (B) Developed acaulospore (a) still attached to the saccule (s), showing wall components (C1, C2); W5983.

FIG. 2. Three types of acaulospore development in the neck of sporiferous saccule in extant and fossil specimens, each with corresponding graphical representation. Extant specimen, *Archaeospora ecuadoriana*, W5340 (modified from Schüßler and Walker, 2019: figs. 2a–c): ($A_{1–2}$) acaulosporoid; ($B_{1–2}$) ambisporoid; ($C_{1–2}$) entrophosporoid. Fossil specimens, *Archaeosporites rhyniensis* gen. et sp. nov.: ($D_{1–2}$) acaulosporoid; SNSB-BSPG 2016 XII 12 (D_1 = holotype); ($E_{1–2}$) ambisporoid; SNSB-BSPG 2016 XII 24; ($F_{1–2}$) entrophosporoid; SNSB-BSPG 2016 XII 12.

FIG. 3. Intraspecific morphological variability of *Archaeosporites rhyniensis* gen et sp. nov., all figures to same scale. (A) SNSB-BSPG 2016 XII 12. (B, C) SNSB-BSPG 2016 XII 13. (D–H) SNSB-BSPG 2016 XII 24. (I) SNSB-BSPG 2016 XII 31. (J–K) SNSB-BSPG 2016 XII 38. (L) SNSB-BSPG 2016 XII 39. (M, N) P3980. (O) P3981.

FIG. 4. *Archaeosporites rhyniensis* gen. et sp. nov. compared with extant species of *Archaeosporaceae*. (A-B, C-D, E-F) Acaulosporoid spores remaining attached to an easily recognisable empty saccule (s). (A) P3980. (B) *Archaeospora ecuadoriana*; W5340. (C) SNSB-BSPG 2016 XII 6. (D) *Archaeospora myriocarpa*; W5878. (E) P3980. (F) *Archaeospora trappei*; W4610. (G-H) Saccules (s) lacking acaulospore development. (G) SNSB-BSPG 1965 I 350. (H) *Archaeospora trappei*; W4743. (I-J) Acaulospores with congealed contents. (I) SNSB-BSPG 2016 XII 6. (H) *Archaeospora myriocarpa*; W5878. (K-J) Entrophosporoid spores still attached to a collapsed saccule (s). (K) SNSB-BSPG 2016 XII 26. (H) *Archaeospora schenckii*; W4389.

FIG. 5. Comparison of acaulospore wall structure (C1–3) between fossil and extant specimens. (A) *Archaeosporites rhyniensis* gen. et sp. nov. with adherent debris on the outermost wall component

(C1) and apparent third innermost wall component (C3); P3981. (B) Extant *Archaeospora spainiae*; W6437.

FIG. 6. Size comparison of Rhynie chert spore-saccule complexes. (A) Example of large spore-saccule complex described by Dotzler *et al.*, (2009); SNSB-BSPG 2013 XIV 6. (B) *Archaeosporites rhyniensis* gen. et sp. nov. spore-saccule complex, arrowhead indicates saccule; P3980.

FIG. 7. Boxplot of calculated acaulospore volumes for *Archaeospora myriocarpa*, *A. schenckii*, *A. trappei*, and *Archaeosporites rhyniensis*. Solid horizontal lines within the boxes represent the median value, the portion of the box below the median represents the lower quartile, and the portion of the box above the median represents the upper quartile. The horizontal line above the box represents the maximum, the horizontal line below the box represents the minimum, and the circles represent outliers.

TABLE 1. Distribution of intact spore-saccule complexes, detached acaulospores and detached saccules of *Archaeosporites rhyniensis* in chert matrix, within plant remains, or which cannot be determined as either in thin sections prepared from Rhynie chert block SNSB-BSPG 2016 XII.

TABLE 2. Summary of key features used to compare affinities among and between extant and fossil kinds of 'acaulospore from a sporiferous saccule'-producing fungi. Note that expansion includes any minimum or maximum measurement in any orientation (diameter, length, width, etc.). Superscript letters correspond only to references within that particular row.

TABLE 1

SNSB-BSPG 2016 XII Slide No.	Chert matrix		Plant remains		Cannot be determined	
	spore **or** saccule	spore **and** saccule	spore **or** saccule	spore **and** saccule	spore **or** saccule	spore **and** saccule
12	13	0	6	11	10	0
13	13	8	9	2	11	4
18	11	2	0	1	0	2
19	5	5	0	0	0	1
24	7	5	2	8	4	2
26	13	2	0	0	1	0
34	5	1	6	0	2	0
37	5	3	0	0	1	0
38	11	1	0	0	0	0
39	4	1	3	1	4	0
40	6	0	3	0	0	1
Total	93	28	29	23	33	10

TABLE 2

Family, specimen, or taxon	Acaulospores		Saccules		Wall organisation[e] (number of wall groups and components/layers)	Acaulospore development in saccule neck topology[f]	Reference(s)
	min. expansion (µm)[a]	max. expansion (µm)[b]	min. expansion (µm)[c]	max. expansion (µm)[d]			
Acaulosporaceae	(11–) 44	380(–520)	48	≤360	3 groups up to 7 layers	acaulosporoid, entrophosporoid	Błaszk. 1989a[a]; Palenz *et al.*, 2013[a,c]; Schultz *et al.*, 1999[b]; Gerdemann and Trappe, 1974[b]; Błaszk. 1989b[d]; Walker *et al.*, 2007*b*[e]; Schüßler and Walker *et al.*, 2019[f,] Mosse, 1970[f]
Ambisporaceae	87	>390	125	≤380	2–3 groups 3–4 components	ambisporoid	Oehl & Sieverd. 2012[a]; Spain *et al.*, 2006[b,d]; Palenz *et al.*, 2013[c]; Walker *et al.*, 2007*b*[e]; Schüßler and Walker *et al.*, 2019[f]
Archaeosporaceae	21	114	22	179	3 groups 3 components	acaulosporoid, entrophosporoid, and ambisporoid	Spain *et al.*, 2006[a,b]; Schüßler and Walker *et al.*, 2019[c–f]
Unnamed Rhynie chert spore-saccule complex[†]	236	473	200	450(–700)	3 groups 6 components	ambisporoid	Dotzler *et al.*, 2009[a–f]
Archaeosporites rhyniensis[†]	24	83	27	132	3 groups 3 components	acaulosporoid, entrophosporoid, and ambisporoid	This study[a–f]

Figure 1

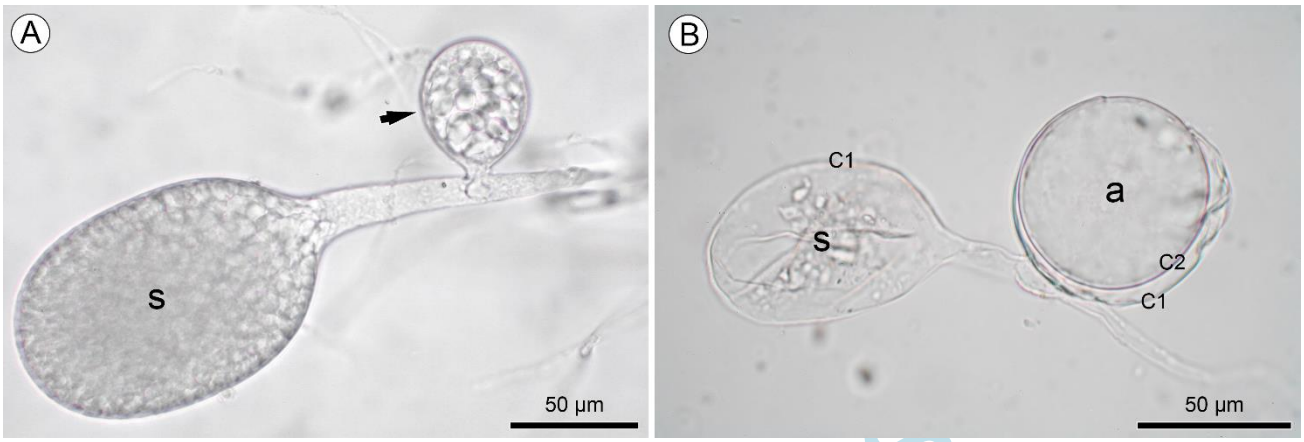

Figure 2

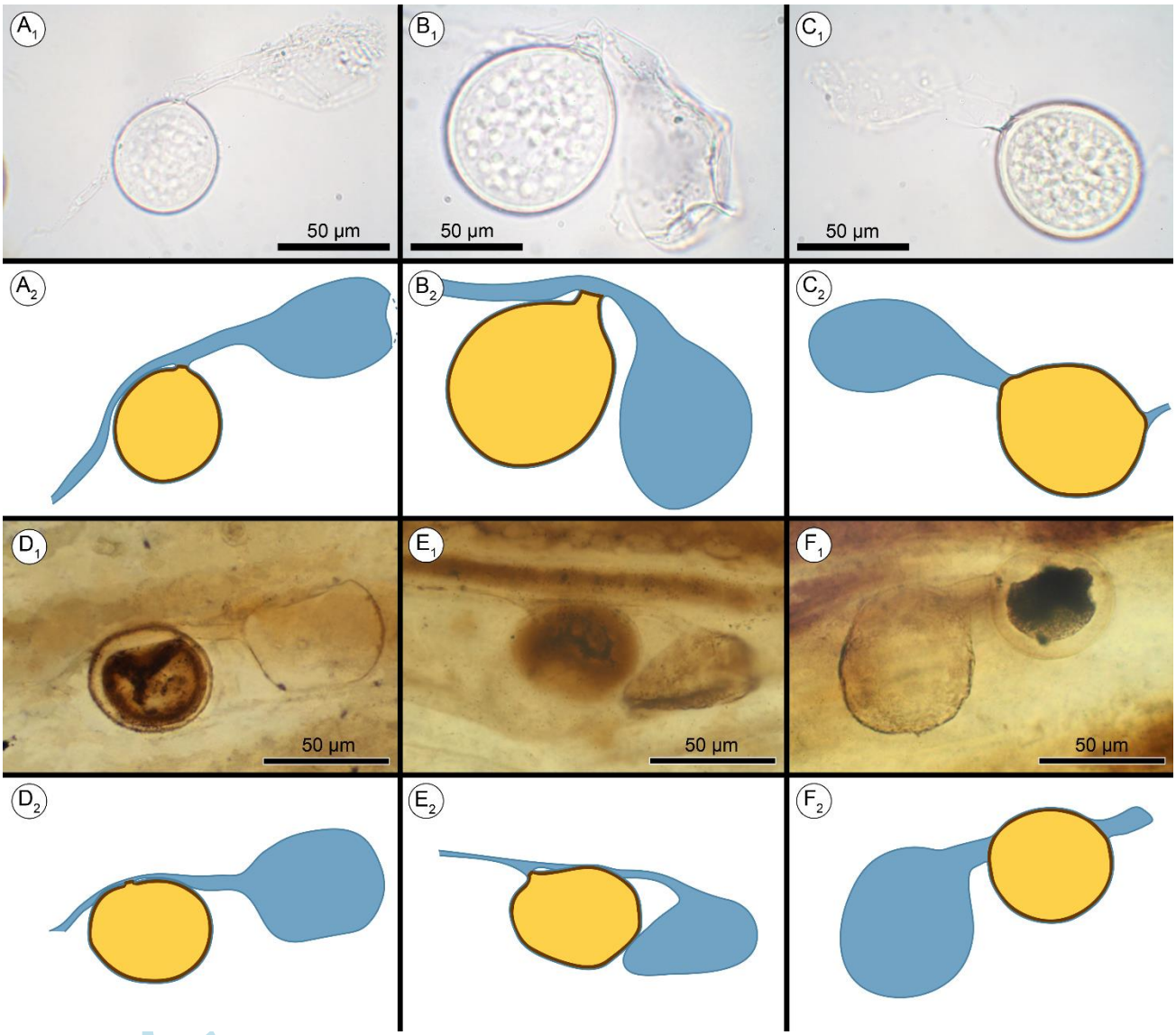

Figure 3

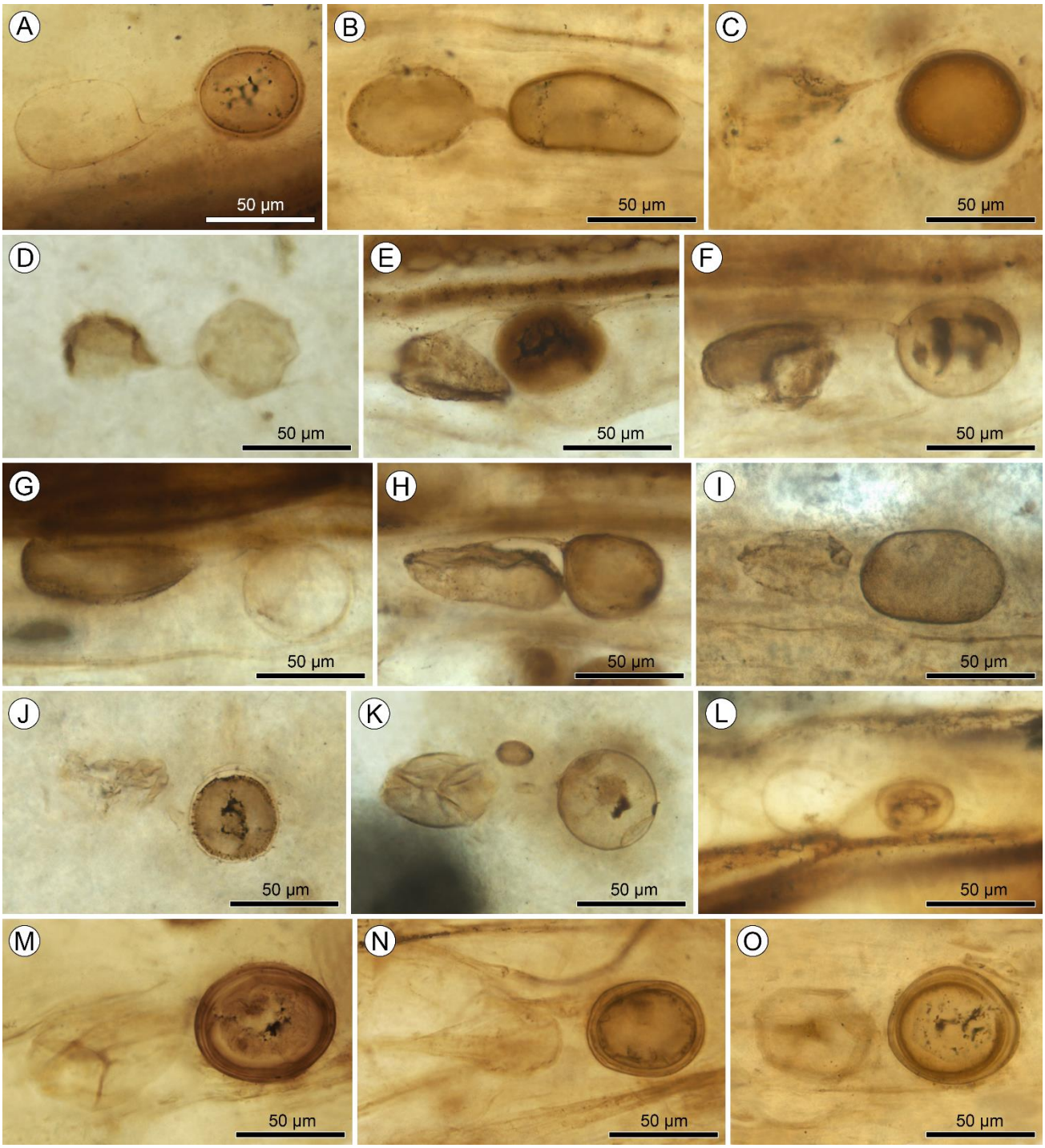

Figure 4

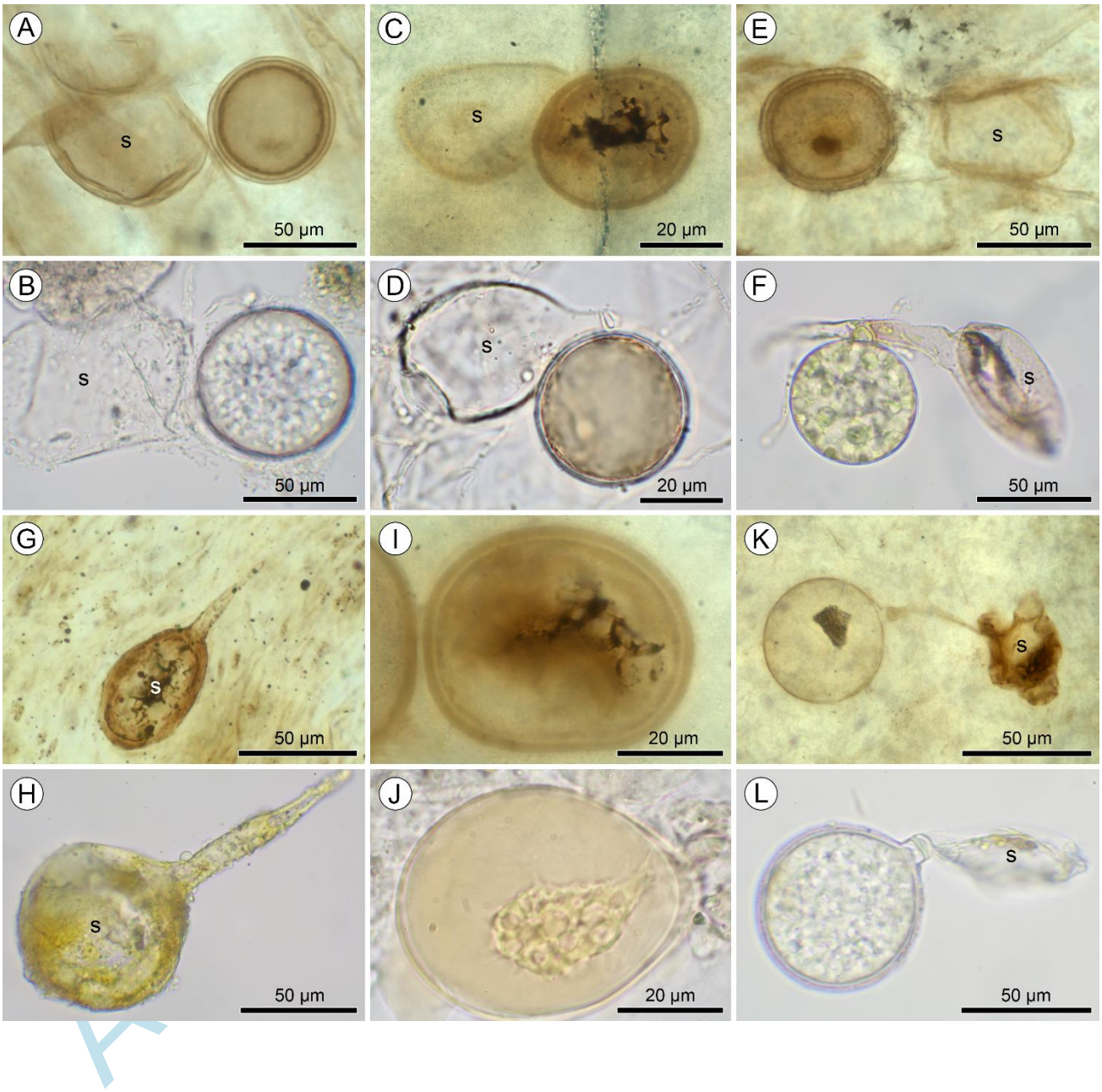

Figure 5

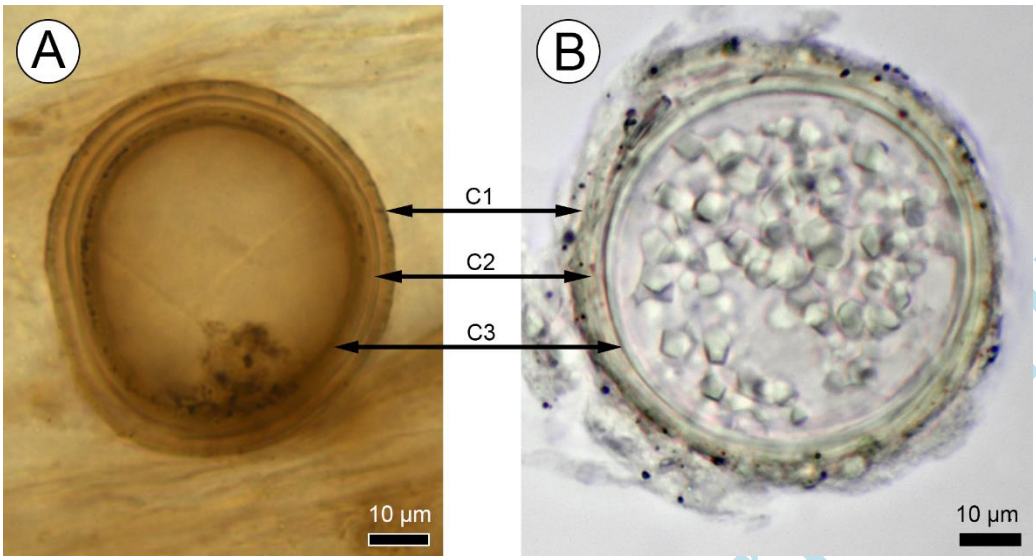

Figure 6

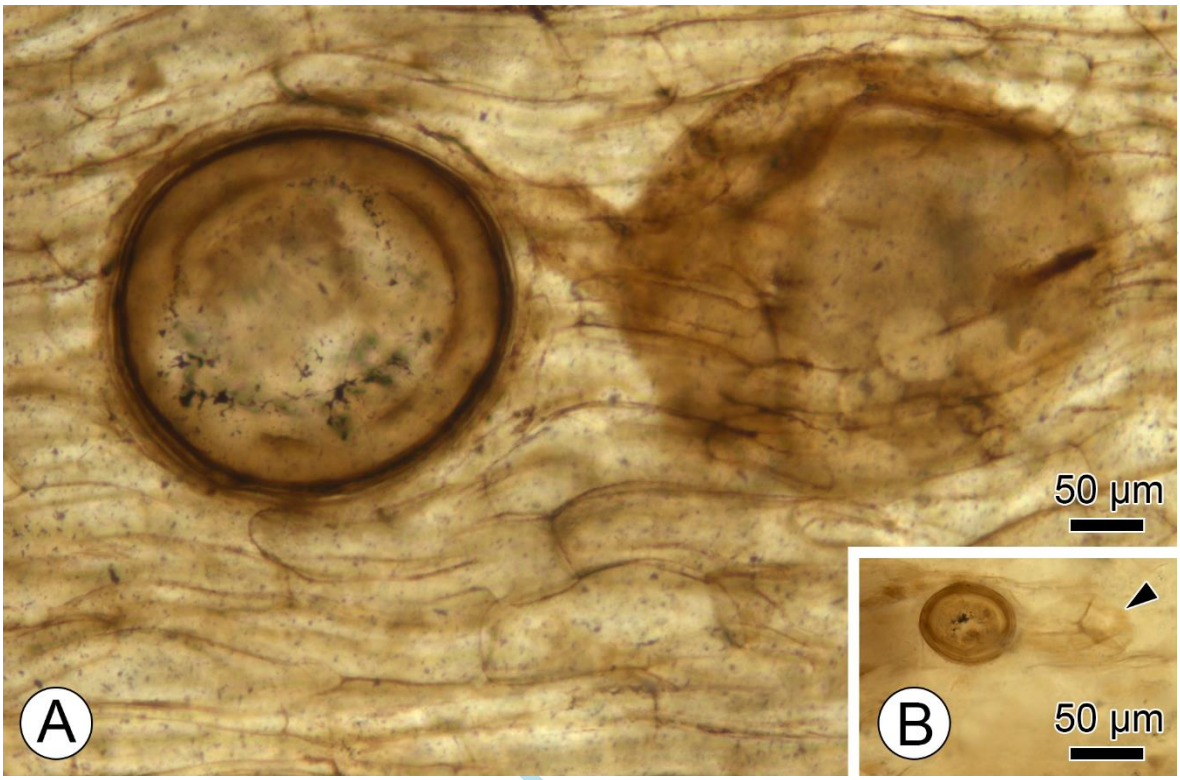

Figure 7