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2009

Dotzler, N., Walker, C., Krings, M., Hass, H., Kerp, H., Taylor, T., Agerer, R. 2009. Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-year-old Rhynie chert. Mycol Progress 8:9-18.

Published version: http://dx.doi.org/10.1007/s11557-008-0573-1

Mycol Progress (2009) 8:9–18
DOI 10.1007/s11557-008-0573-1

ORIGINAL ARTICLE

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

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Received: 4 June 2008 / Revised: 16 September 2008 / Accepted: 30 September 2008 / Published online: 15 October 2008

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

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

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Introduction

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

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

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

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

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

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

Materials and methods

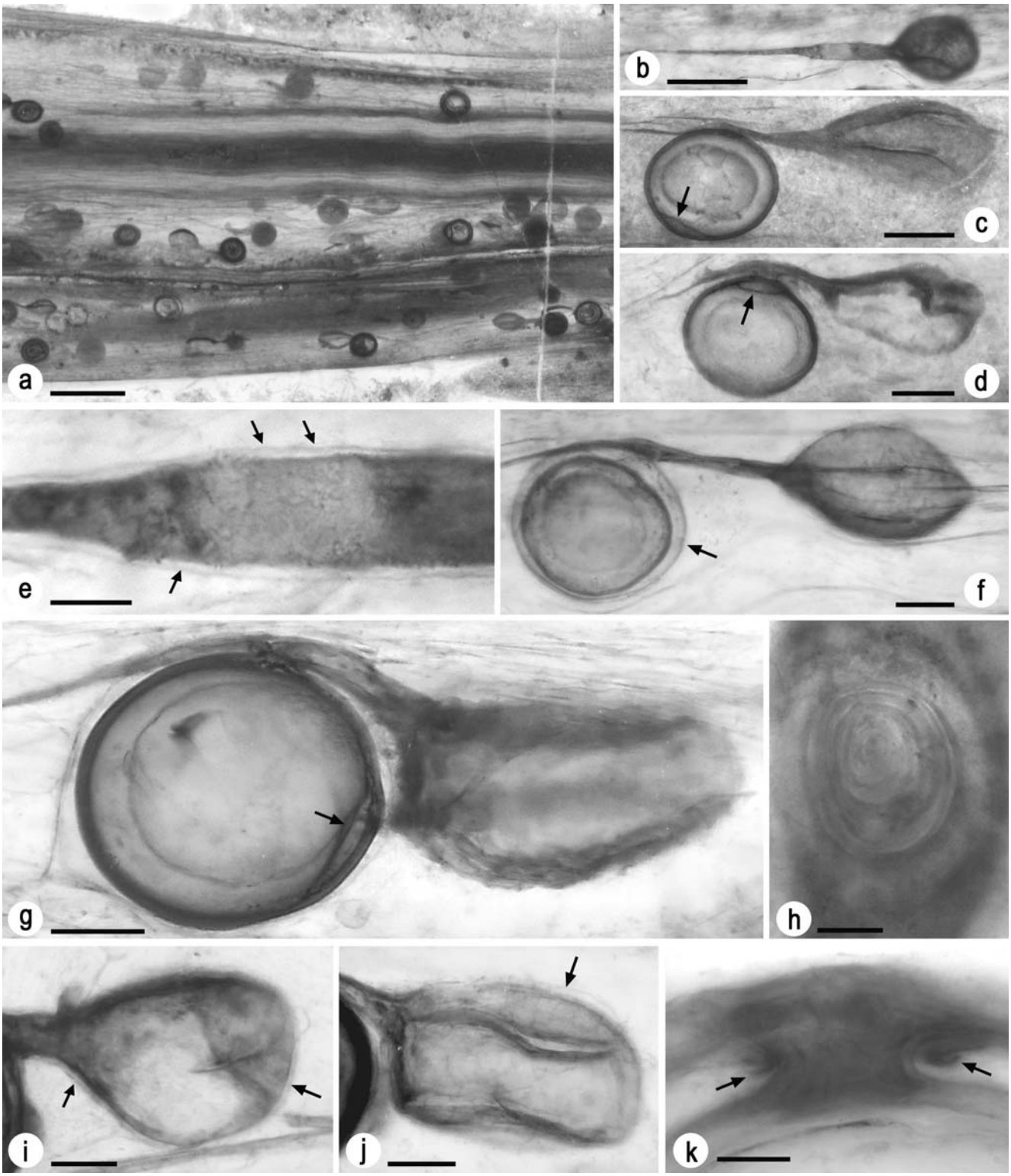
The Rhynie chert Lagerstätte is located in the northern part of the Rhynie outlier of Lower Old Red Sandstone in Aberdeenshire, Scotland, within a sequence of sedimentary and volcanic rocks. The cherts occur in the upper part of the Dryden Flags Formation, in the so-called Rhynie Block, a few hundred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

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

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

Description

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



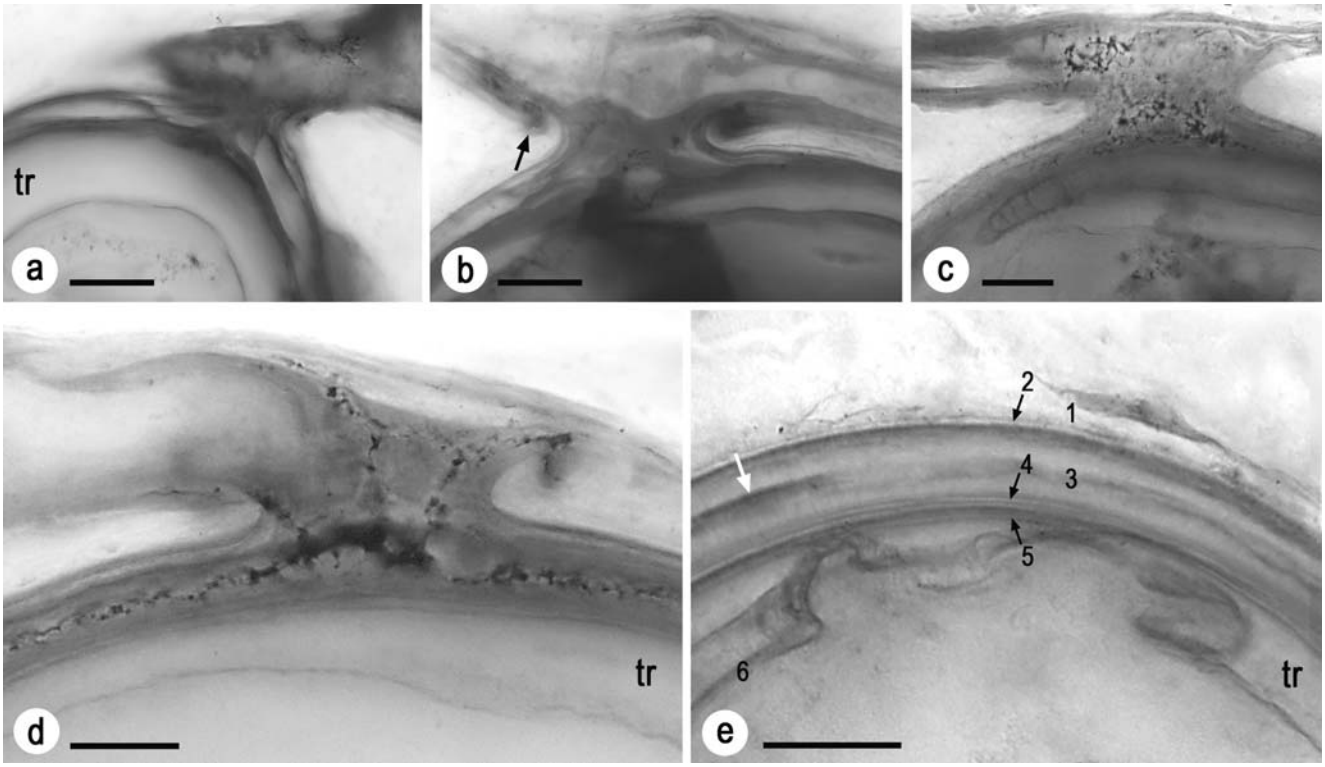


Fig. 2 Acaulosporoid glomeromycotan spores from the Rhynie chert: Morphology. **a–c** Spore attachment; note eccentric position of spore lumen in **a**, wc1 terminating around the spore base in **b** (*arrow*), and germination shield in **c**. Slides P3966 (**a**), P3961 (**b**), and P3958 (**c**). *Scale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

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

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

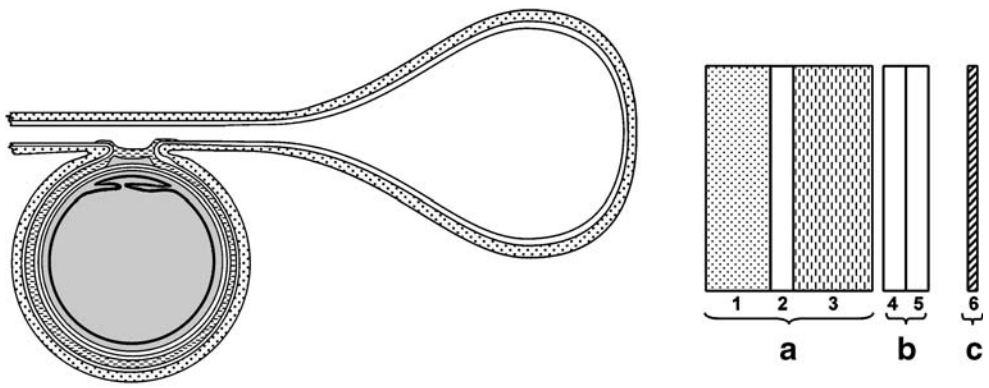


Fig. 3 Stylised illustration of the saccule and spore wall structure, and murograph of the spore wall structure interpretation from an acaulospore and sporiferous saccule from the Rhynie chert. *Shading* in the illustration is intended only to clarify the separate components. The murograph shading follows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

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

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

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

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

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

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

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

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

Discussion

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

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

Morphology

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

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

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

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

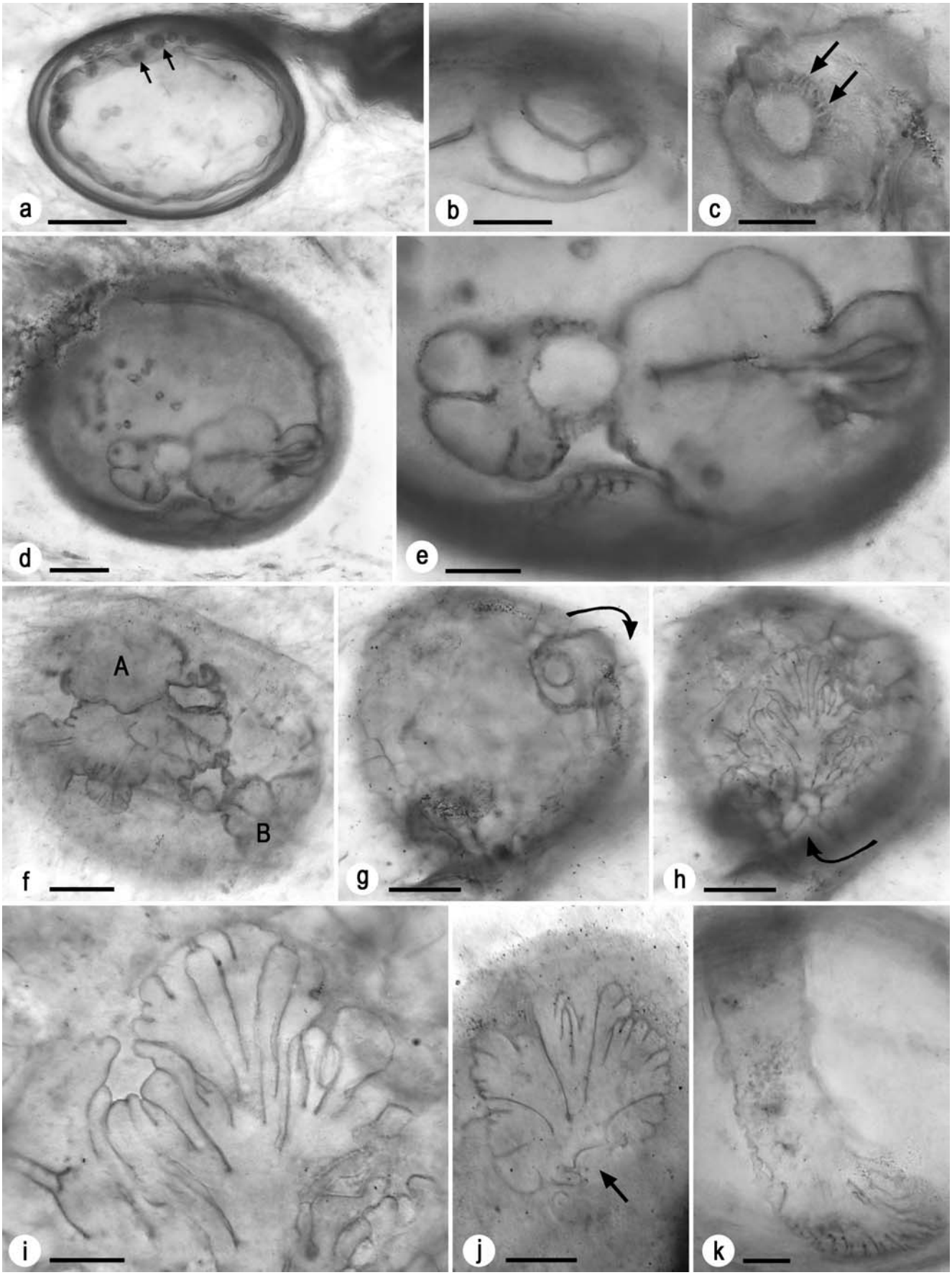
Fig. 4 Acaulosporoid glomeromycotan spores from the Rhynie chert: Germination shield. **a** Spore containing a parasite (chytrid?) in translucent region (*arrows*). Slide P3962. *Scale bar*=100 µm. **b** Small germination shield in oblique surface view. Slide P3956. *Scale bar*=30 µm. **c** Rod-shaped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

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

Affinities

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

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

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

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

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

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

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

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

Conclusions

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

Acknowledgments This study was supported in part by funds from the National Science Foundation (EAR-0542170 to T.N.T. and M.K.), the Deutsche Forschungsgemeinschaft (Ke 584/13–1 to H.K.), and the Alexander von Humboldt-Foundation (V-3.FLF-DEU/1064359 to M.K.). We acknowledge with thanks the loan of the holotype of *Otospora bareai* by the curator of the Mycological Herbarium of the ETH Zurich (ZT).

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