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Unusual fungal reproductive units from the Lower Devonian Rhynie chert

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Abstract

Small, detached fungal reproductive units are almost ubiquitous in the Early Devonian Rhynie chert; however, only a few of these fossils have been described. Three morphologically distinctive types of spheroidal reproductive units occur in litter layers from the Rhynie chert. One of these is >250 µm in diameter and has a multi-layered wall; the outer surface is prominently fringed. Because specimens are borne laterally within the neck of a sporiferous saccule, this fossil is identified as an acaulosporoid glomeromycotan spore. The second reproductive unit (80–110 µm in diameter) is characterized by elongate-conical protuberances, while the third (<45 µm in diameter) possesses a thick outer wall layer or sheath traversed by tubular canals. The systematic affinities of these last two fossils remain unresolved. The reproductive units add to the inventory of easily recognizable fungal morphologies in the Rhynie chert and contribute to our understanding of fungal paleobiodiversity in early non-marine ecosystems.

Key words: acaulosporoid spore, chlamydospore, Glomeromycota, saccule, wall structure

Zusammenfassung

Kleine, von ihren Myzelen abgetrennte Fortpflanzungseinheiten von Pilzen findet man fast überall im unterdevonischen Rhynie Chert, jedoch sind bis heute nur wenige dieser Fossilien beschrieben. Drei ungewöhnliche, sphäroidale Fortpflanzungseinheiten stammen aus Lagen verrottender Pflanzenteile. Eine Form ist >250 µm im Durchmesser, besitzt eine mehrschichtige Wand und prominente Fransen. Da das Fossil lateral aus der Nacken-Region eines Sacculus entspringt, kann es als acaulosporoide Spore eines Glomeromyzeten identifiziert werden. Die zweite Form (80–110 µm im Durchmesser) ist durch konische Auswüchse gekennzeichnet, während die dritte (<45 µm im Durchmesser) von einer massiven, von röhrenförmigen Kanälen durchzogenen Hülle umgeben ist. Die systematische Zugehörigkeit dieser beiden Strukturen bleibt unklar. Diese Funde erweitern das Inventar leicht erkennbarer pilzlicher Morphologien im Rhynie Chert, und tragen dadurch zu einem besseren Verständnis der Paläobiodiversität der Pilze in frühen nicht-marinen Ökosystemen bei.

Schlüsselwörter: Acaulosporoide Spore, Chlamydospore, Glomeromycota, Sacculus, Wandaufbau

1. Introduction

The Rhynie chert, a silicified Early Devonian hot-spring ecosystem from north-eastern Scotland, has contributed substantially to our understanding of early non-marine life (Kerp & Hass 2004). Fungi are especially abundant and diverse in the Rhynie chert. Fungal hyphae and different types of detached, small (<0.5 mm) fungal reproductive units (e.g., spores, sporangia, sporocarps) are almost ubiquitous in the chert matrix, in litter layers, and within intact and decaying land plant parts (Kidston & Lang 1921; Boullard & Lemoigne 1971; Taylor et al. 2003, 2015; Krings & Taylor 2013, 2014a,b,c, 2015a,b). Moreover, several articulated specimens demonstrate the existence of different types of fungal associations and interactions (Taylor et al. 1992, 1995, 1997; Hass et al. 1994; Remy et al. 1994; Krings et al. 2007, 2015, 2016; Krings & Taylor 2014a,b; Strullu-Derrien et al. 2014, 2015).

The abundance of fungal remains, together with the large number of morphologically different fungal reproductive units, suggests that fungi were important components of many of the vital processes that

sustained the Rhynie ecosystem. However, attempts to estimate fungal paleobiodiversity and specify what ecological roles these organisms played in fossil ecosystems are generally hampered by the fact that the vast majority of fungal remains occur detached from the systems on or in which they were produced, and thus do not provide a complete range of structural features necessary to determine their systematic affinities (Krings et al. 2016). Nevertheless, certain Rhynie chert fungal reproductive units display structural features that, albeit not diagnostic, are consistent among specimens, and thus make it possible to recognize distinctiveness (Dotzler et al. 2006, 2009; Krings & Taylor 2013, 2014a, 2015a,b). Consequently, one important first step in understanding fungal paleobiodiversity in the Rhynie chert is to report the occurrence of distinctive fungal morphologies and document their features as thoroughly as possible.

This paper describes three types of fungal reproductive units that all occur in association with decaying land plants (litter layers) in the Rhynie chert. One type represents a member of the Glomeromycota based on structural similarities to extant and other fossil representatives in this fungal phylum, while the affinities of the other types, as well as the morphology and biology of the organisms that produced these reproductive units, remain conjectural. Nevertheless, the discoveries are significant because they add to the inventory of distinctive and easily identifiable fungal morphologies in the Rhynie chert.

2. Geological setting, material, and methods

The Rhynie chert Lagerstätte is located in the northern part of the Rhynie outlier of Aberdeenshire, Scotland (Rice et al. 2002), and includes series of chert lenses that are principally fine grained and interpreted as having accumulated on an alluvial plain associated with ephemeral ponds and lakes. The ecosystem is now interpreted as a geothermal wetland (Channing & Edwards 2009), as there were hot springs which were part of a complex hydrothermal system (Rice et al. 2002). Both aquatic and terrestrial organisms became preserved as a result of temporary flooding of silica-rich water, or by silica-rich groundwater that percolated to the surface (Powell et al. 2000). 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).

The fossils were identified in thin sections that were 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. The slides are deposited in the Bayerische Staatssammlung für Paläontologie und Geologie (SNSB-BSPG) in Munich, Germany, under accession numbers SNSB-BSPG 1965 I 330, 357, 358, SNSB-BSPG 2013 V 42, 43, SNSB-BSPG 2013 XV 35, 43, and SNSB-BSPG 2015 XIX 4, 5, 15, 16, 19, 22, 39, 54, and 55. Slides were analyzed using normal transmitted light microscopy; digital images were captured with a Leica DFC-480 camera and processed in Adobe Photoshop CS6. Slide numbers for the specimens illustrated in Plates 1 and 2 are given in the figure captions.

3. Results

The reproductive units described below represent detached stages of fungal life cycles. One form clearly belongs to the Glomeromycota, but the other two are difficult to determine relative to systematic affinities. In order to adequately deal with these variables, the fossils are all informally referred to as ‘reproductive units’, but none has been formally named.

3.1 Reproductive unit 1 (Pl. 1, Figs 1–11)

Many specimens (>50) of this reproductive unit occur singly, or in small clusters of 2–3, in degrading land plant axes, but may sometimes also be found scattered in the chert matrix. Specimens are globose in shape and 250–290 µm in diameter (including fringed ornamentation; Pl. 1, Figs 1–3, 6, 9). In approx. 30% of the specimens the reproductive unit (denoted ‘Sp’ in Pl. 1, Figs 3, 4) is evidently borne laterally within the neck (denoted ‘Ne’ in Pl. 1, Figs 3, 4, 7) of a sporiferous saccule (denoted ‘Sa’ in Pl. 1, Fig. 3), thus representing an acaulosporoid glomeromycotan spore.

Spore wall structure cannot be determined with certainty because all specimens have undergone some level of physical or biological degradation (see especially specimen in Pl. 1, Fig. 2). We therefore refrained from using the Glomeromycota spore wall terminology (see Walker 1983; INVAM homepage) to describe the wall of *reproductive unit 1*, but rather have informally designated individual layers with Arabic numerals. We suggest that the reproductive unit wall consisted of three or four layers. The outer (sur-

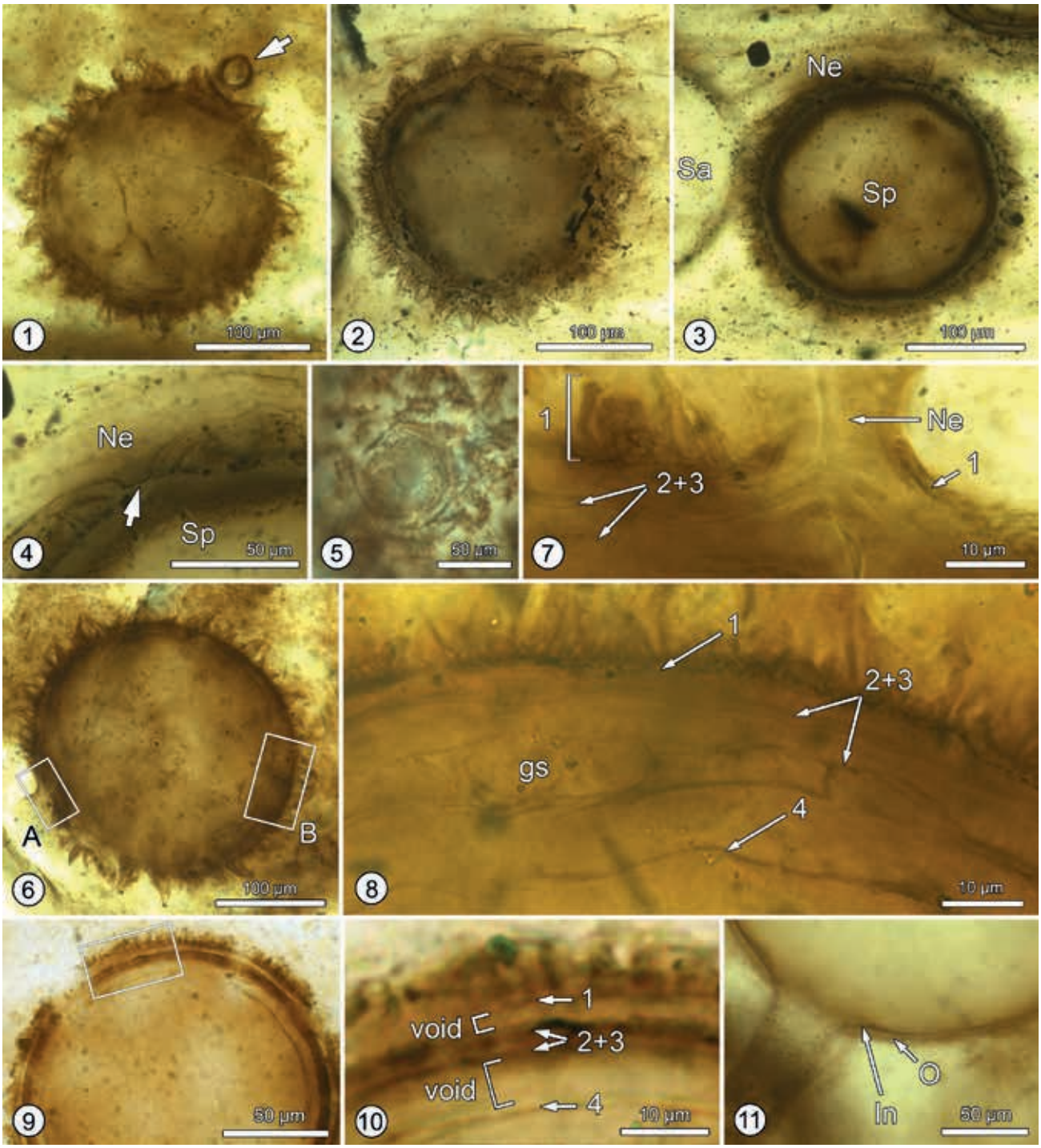

Plate 1: Unusual fungal reproductive units from the Rhynie chert: *Reproductive unit 1* (acaulosporoid glomeromycotan spore). **(1)** Largely intact spore in median cross section; arrow indicates saccule neck in cross section; slide no. SNSB-BSPG 2015 XIX 16. **(2)** Spore with largely degraded wall; slide no. SNSB-BSPG 2015 XIX 16. **(3)** Spore-saccule complex; Sa = saccule, Ne = neck, Sp = spore; SNSB-BSPG 2015 XIX 22. **(4)** Detail of Pl. 1, Fig. 3, focusing on region of spore attachment to saccule neck (arrow); Ne = neck; Sp = spore. **(5)** Plan view of point of spore attachment to saccule neck, showing delicate reticulation and irregular subcircular pattern; note arrangement of fringes into groups and short sinuous lines; SNSB-BSPG 2015 XIX 4. **(6)** Largely intact spore in median cross section; marked areas A and B magnified in Pl. 1, Figs 7 and 8 respectively; SNSB-BSPG 2015 XIX 55. **(7)** Area A of Pl. 1, Fig. 5. Transition zone between spore and neck (Ne); 1, 2, 3 = spore wall layers (see text for further explanation). **(8)** Area B of Pl. 1, Fig. 5, showing spore wall composition**;** 1, 2, 3, 4 = spore wall layers (see text for further explanation), gs = germination structure (probably a coil). **(9)** Spore with less prominent fringes; marked area magnified in Pl. 1, Fig. 10; SNSB-BSPG 2015 XIX 4. **(10)** Area marked in Pl. 1, Fig. 9. Spore wall composition; 1, 2, 3, 4 = spore wall layers (see text for further explanation). **(11)** Transition zone between neck and saccule, showing outer (O) and inner (In) layers of saccule wall; SNSB-BSPG 2015 XIX 4.

face) layer (denoted '1' in Pl. 1, Figs 7, 8, 10) bears prominent, fibrous fringes (up to 30 µm long), which appear to be arranged in groups or clusters, or short sinuous lines in plan view (Pl. 1, Fig. 5) and give the

spore a frayed appearance. Beneath this layer occur three wall layers (denoted '2, 3, and 4' in Pl. 1, Figs 7, 8, 10). The specimen shown in Pl. 1, Fig. 3 (and details of spore attachment to saccule in Pl. 1, Fig. 4) suggests that layer 1 is continuous into the saccule neck (transition zone between spore and neck denoted 'Ne' in Pl. 1, Fig. 7), while layers 2, 3, and 4 formed de novo. A prominent germination structure, probably a coil, is present between wall layers 3 and 4 in the specimen shown in Pl. 1, Figs 6 and 8 (denoted 'gs' in Pl. 1, Fig. 8). Unfortunately, size and morphology of the germination structure cannot be determined because the surface structure of the spore obstructs plan views of the inner wall layers.

The saccule, where preserved, appears as a globose structure ~270 µm in diameter. The neck is 35–38 µm wide at the point where it expands blastically (Pl. 1, Figs 3, 11). The saccule wall is up to 8.6 µm thick and appears to be two-layered, with an inner layer (denoted 'In' in Pl. 1, Fig. 11), up to 1.8 µm thick, that continues into the neck, and an outer layer (up to 7.1 µm thick) that appears as an irregular coating of the saccule and terminates around the saccule base (denoted 'O' in Pl. 1, Fig. 11). The distance between the point of spore attachment and expansion of the saccule is between 155 and 205 µm. The region of spore attachment forms a near-circular scar, 70–75 µm in diameter (diameter of actual attachment point ~20–22 µm), with a fine reticulation and an irregular concentric or eccentric subcircular pattern in plan view (Pl. 1, Fig. 5) that are not recognizable in lateral view (arrow in Pl. 1, Fig. 4).

3.2 Reproductive unit 2 (Pl. 2, Figs 1–8)

This reproductive unit occurs singly in the chert matrix within dense accumulations of degrading land plant fragments, fungal hyphae, and sediment particles; six specimens have been discovered to date.

Specimens are globose to somewhat ovoid (subglobose) in shape and 80–107 µm in diameter (Pl. 2, Figs 1, 4, 5). No subtending hyphae or other points of origin are visible, but this may simply reflect the plane of section. The wall is composed of two (or three?) layers. There is an apparent inner layer, recognizable as a dark line (0.6–1.5 µm thick) bounding the lumen (Pl. 2, Figs 2, 4, 5, 8), but this may be an artifact of microscopy or fixation of the original contents during fossilization. Surrounding this is a layer from which arise prominent, tapering, columnar protuberances (0.9–1.6 µm in diameter proximally, tips ~3.6 µm apart), which are 2–4.5 µm high (Pl. 2, Figs 2–8). Figures 6 through 8 of Plate 2 show the characteristics of a Koehler-illuminated light microscopy view of these structures: Fig. 6 focuses on the reticulate pattern formed by the abutting bases of the protuberances (some outlined by dashed lines), while Fig. 7 focuses at their tips. Plate 2, Fig. 8 shows the protuberances in side view. It appears that the protuberances penetrate through a third, probably evanescent outer wall layer, which seems to be thick in some specimens and then reduced in others.

3.3 Reproductive unit 3 (Pl. 2, Figs 9–15)

This reproductive unit has been discovered in a single, degrading land plant axis; it occurs in two clusters (together approximately 40 individuals) located in small voids that formed as a result of host tissue degradation.

Specimens are more or less globose and 29–44.5 µm in diameter. Subtending hyphae, which are visible in approximately 25% of the specimens (Pl. 2, Figs 10, 11, 14), are thin-walled, tubular, and 2.5–3.5 µm wide, with the wall thickened near the spore base to approx. 1 µm, thinning to much less than 1 µm proximally. No septum separating the reproductive unit from the subtending hypha is recognizable. The reproductive units are composed of a central cavity (<20–25 µm in diameter) bounded on the outside by a narrow wall or wall layer, which is recognizable as an opaque line 0.3–0.5 µm thick and continuous with the subtending hypha (Pl. 2, Fig. 14). Irregularities in the form of wrinkling, folding, or distortion are common (e.g., Pl. 2, Figs 9, 12). Each unit is surrounded by a prominent, relatively opaque outer wall layer, sheath, or mantle, in some specimens up to 5.5 µm thick, though the subtending hyphae lack this wall layer. The layer is usually distinctly thinner in the attachment area of the subtending hypha (Pl. 2, Figs 11, 14). The outer wall layer is uniform in texture; however, it is traversed by several tubular canals 1.1–2.1 µm in diameter (arrows in Pl. 2, Figs 11, 13) that are lined by a thin but discrete wall (Pl. 2, Figs 11, 14). In a few specimens, the outer wall layer extends outward into one to several broad-conical processes (up to 8.3 µm high) that appear to be hollow (arrows in Pl. 2, Fig. 15). The periphery of the wall may be relatively smooth (Pl. 2, Fig. 9) or irregularly wrinkled (Pl. 2, Figs 10–12, 14).

4. Discussion

Several examples of detached fungal reproductive units from the Rhynie chert have been described and illustrated by Kidston & Lang (1921) that differ from one another in size, wall thickness, and sometimes wall structure, mode of attachment to a parental hypha, and host plant preference. However, the specimens mostly lacked diagnostic characters of sufficient clarity to resolve their systematic affinities, and thus were collectively assigned to the vaguely defined fossil genus *Palaeomyces*. There are several other Rhynie chert fungal reproductive units that possess features rendering them easy to recognize and, in many instances, providing insight into their systematic affinities. For example, several types of reproductive units are surrounded by a special covering termed a hyphal mantle (Krings & Taylor 2013,

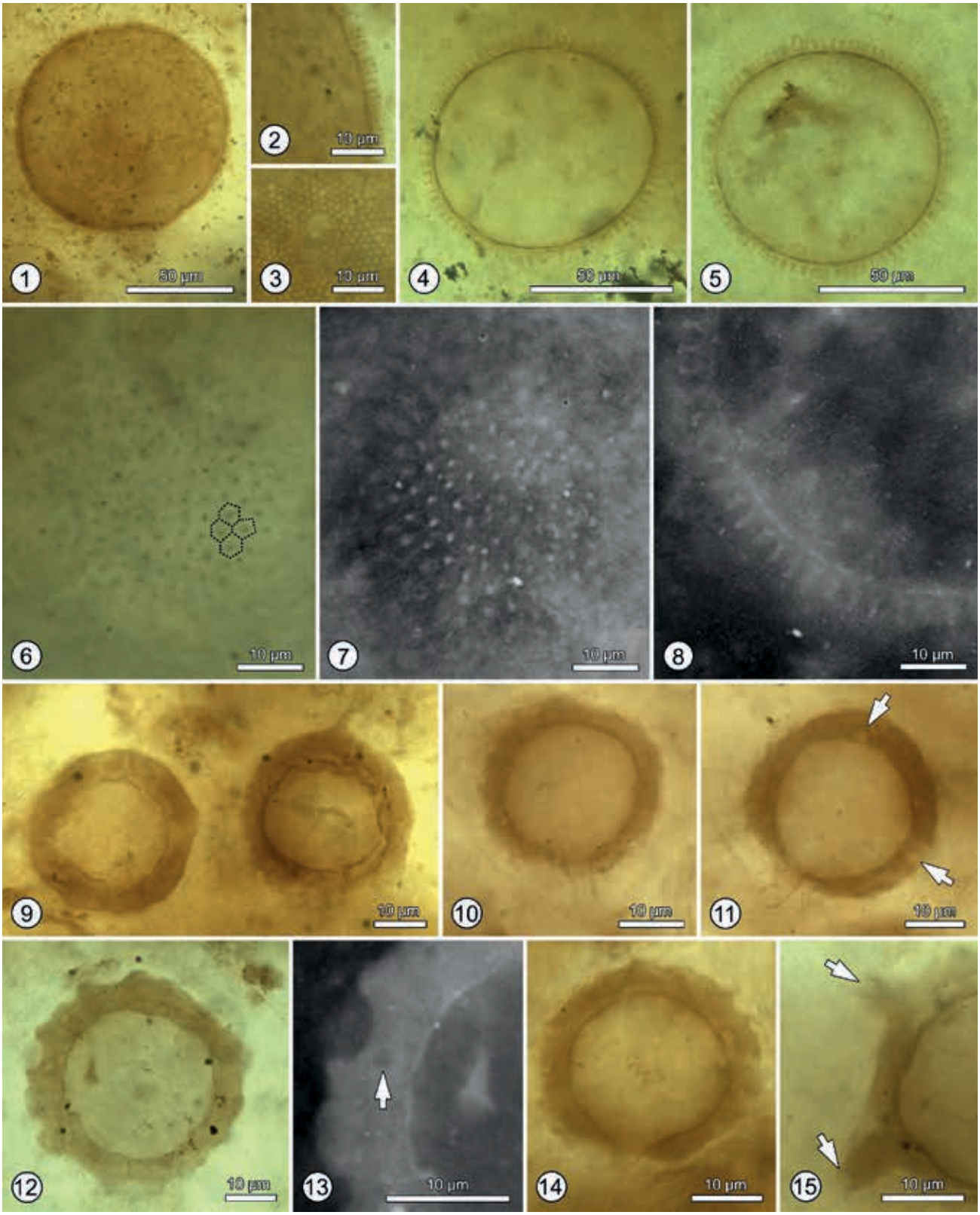

Plate 2: Unusual fungal reproductive units from the Rhynie chert: *Reproductive units 2* (Figs 1–8) and *3* (Figs 9–15). **(1, 4, 5)** Intact specimens in chert matrix, showing the conspicuous wall; slides no. SNSB-BSPG 1965 I 330 (Pl. 2, Fig. 1); SNSB-BSPG 2013 XV 35 (Pl. 2, Figs 4, 5). **(2, 3)** Details of Pl. 2, Fig. 1, focusing on wall protuberances in lateral (Pl. 2, Fig. 2) and plan (Pl. 2, Fig. 3) view. **(6)** Wall of specimen in Pl. 2, Fig. 5 in plan view, focusing on reticulate pattern formed by abutting protuberance bases (some outlined with dashed lines). **(7)** Wall of specimen in Pl. 2, Fig. 5 in plan view (reversed light), focusing on tips of protuberances. **(8)** Cross section through wall of specimen in Pl. 2, Fig. 5 (reversed light), showing putative inner wall layer and tapering protuberances. **(9)** Two specimens with relatively smooth outer surfaces; SNSB-BSPG 1965 I 358. **(10, 11, 14)** Specimens attached to tubular subtending hyphae; arrows in Pl. 2, Fig. 11 indicate canals traversing outer wall layer; SNSB-BSPG 1965 I 358. **(12)** Specimen with irregularly wrinkled outer wall layer; SNSB-BSPG 1965 I 357. **(13)** Detail of Pl. 2, Fig. 12 (reversed light), showing delicate inner wall (i.e. bright line bounding central cavity), prominent outer wall layer with canals (arrow), and diffuse outer boundary layer. **(15)** Outer wall layer extending outward into hollow processes (arrows); SNSB-BSPG 1965 I 357.

2014a, 2015a,b; Krings et al. 2014, 2016). The presence of a hyphal mantle is not in itself a diagnostic feature, but mantle morphology differs considerably among the types, and thus can be used as a structural feature in recognizing distinctiveness among these reproductive units. Another interesting Rhynie chert fungal reproductive unit is characterized by a prominent germination shield, and therefore has been directly compared to 'spores' (possibly sporangia) produced by the modern glomeromycotan genus *Scutellospora* (Dotzler et al. 2006). Yet another form conforms to propagules of the modern glomeromycotan genus *Acaulospora*, which produces spores borne laterally within the neck of a sporiferous saccule (Dotzler et al. 2009).

4.1 Reproductive unit 1

Reproductive unit 1 provides structural features indicative of systematic affinities. Several specimens show the prominently fringed sphere intimately associated with a long-necked, relatively thin-walled, globose structure. Moreover, the fringed sphere is physically connected to the neck region in some of these specimens (Pl. 1, Figs 3, 4). These features identify *reproductive unit 1* as a glomeromycotan spore-saccule complex, and accordingly, the fringed sphere is an acaulosporoid spore borne laterally within the saccule neck (see Souza 2015). This structure is similar in size and overall morphology to a fossil described previously from the Rhynie chert and interpreted as an *Acaulospora*-like glomeromycotan spore-saccule complex by Dotzler et al. (2009). However, although difficult to compare because of substantial differences in preservation, the former differs from the latter in the shape of the saccule, which is ovoid, spindle-shaped or somewhat elongate in Dotzler et al.'s (2009) specimens, but consistently spherical in *reproductive unit 1*.

The walls of the specimens described by Dotzler et al. (2009) are generally better preserved than those of *reproductive unit 1*. Nevertheless, the inner wall layer of the saccule and its neck (denoted 'In' in Pl. 1, Fig. 11) continues around the remaining inner spore wall components in both forms. An outer wall layer occurs around the saccule in *reproductive unit 1* (denoted 'O' in Pl. 1, Fig. 11), but is usually not visible around the saccule neck. It is possible that this wall layer was sloughed off from the neck during development. The layer appears to correspond to the fringe-bearing outer portion of spore wall layer 1 (Pl. 1, Figs 7, 8, 10), and might also correspond to layer 1 of the spore-saccule complexes described by Dotzler et al. (2009: fig. 3). Wall layer 1 of *reproductive unit 1* probably represents the sporangium or sporangiolum wall. Accordingly, wall layers 2 and 3 represent the main structural wall group of the acaulospore. The nature of layer 4 present in some of the specimens cannot be determined. A translucent region between the inmost wall layer and the spore lumen has been documented by Dotzler et al. (2009) and interpreted as the result of plasmolysis caused by the assumed hypertonic nature of the infiltrating mineralized water. Similar translucent regions have also been observed in the *reproductive unit 1* specimens (denoted 'void' in Pl. 1, Fig. 10).

A major difference between *reproductive unit 1* and the specimens described by Dotzler et al. (2009) relates to the outer surface of the acaulospore, which appears to be smooth in the latter but is heavily ornamented in the former form. Although there are several extant members in the Glomeromycota that produce ornamented spores (e.g., Sieverding & Toro 1987; Wu et al. 1995; Dalpé & Declerck 2002; Furrazola et al. 2011; Krüger et al. 2011; Oehl et al. 2006, 2011a,b, 2014; Palenzuela et al. 2013; Trejo et al. 2015), none possesses similar ornamentation to *reproductive unit 1*, which represents the only fossil example to date of an ornamented bona fide glomeromycotan spore. It is interesting to note in this context that ornamented spores appear to be particularly widespread among extant forms producing acaulosporoid (and entrophosporoid) spores (Sieverding & Oehl 2006; Velazquez et al. 2008; Kaonongbua et al. 2010; INVAM homepage), and thus it is perhaps not surprising that the only fossil example of a prominently ornamented glomeromycotan spore is also of the acaulosporoid type.

The nature and development of the surface ornament in *reproductive unit 1* remain unresolved. It is possible that the fringes represent a true ornament or result from the partial degradation of an ephemeral outer wall layer. An alternative, although highly speculative interpretation regards the fringes as remains of a hyphal mantle or investment consisting of hyphal segments with unevenly thickened walls. This scenario could also be used to explain the arrangement of the fringes in sinuous lines and groups (Pl. 1, Fig. 5) – they represent parts of thickened lateral walls of hyphae that extended along the surface of the spore.

4.2 Reproductive unit 2

The diagnostic feature of *reproductive unit 2* is the conspicuous wall, which is characterized by regularly spaced, elongate-conical protuberances ornamenting what appears to be the middle wall layer (Pl. 2, Figs 2, 6–8). These protuberances discriminate *reproductive unit 2* from all other spheroid objects reported to date from the Rhynie chert.

Spherical to ovoid propagules between 50 and ~500 µm in diameter and bounded with a simple or multi-layered wall are common in the Rhynie chert. Since none of these fossils provides evidence of gametangial fusion, they are commonly regarded as glomeromycotan chlamydospores (see Kidston & Lang 1921; Krings et al. 2012, 2015). *Reproductive unit 2* is 80–107 µm in diameter, and thus lies within the size range of these propagules. It may

be that *reproductive unit 2* was also produced by a glomeromycotan fungus, but the lack of any clear subtending hypha, bulbous base, or cicatrix makes it impossible to be sure. There are several extant Glomeromycota that produce spores with ornamented wall layers characterized by spines or other types of protuberances. For example, the fossil appears to be consistent with the structure of the main structural component and the disintegrating saccule neck of species of *Acaulospora* such as *A. spinosa* from the Glomeromycota (Walker & Trappe 1981). The matrix (or outer wall layer) embedding the protuberances in some of the fossil specimens could possibly be the remains of a saccule neck that surrounded them on formation. However, *reproductive unit 2* is considerably smaller than the spores of *A. spinosa*, though not of all species in the genus *Acaulospora*.

4.3 Reproductive unit 3

Reproductive unit 3 is difficult to interpret with regard to biological nature and systematic affinities. The short fragments of subtending structures seen in several of the specimens might stem from a rhizomycelium. If this is correct, then *reproductive unit 3* would be a thick-walled resting spore of a chytrid, perhaps comparable to the resting spores seen in certain members in the extant genera *Karlingiomyces* and *Rhizophydium* (Karling 1946, 1977; Blackwell et al. 2004).

Alternatively, *reproductive unit 3* might also be some type of fungal (resting) chlamydospore based on the fact that it occurs terminally on a tubular hypha (Pl. 2, Figs 10, 11, 14). The specimens in fact closely resemble small spores of the extant arbuscular mycorrhizal fungus *Glomus mortonii*, which forms blastic chlamydospores that may be surrounded by a dense hyphal mantle (e.g., Bentivenga & Hetrick, 1991). Such a mantle (sometimes called a peridium) is also formed around some spores of the species *Funneliformis mosseae*, *F. monosporus*, and *F. coronatum* (Meier & Charvat 1992; Schüßler & Walker 2010). Perhaps the mantle of *reproductive unit 3* became altered and transformed into a more or less homogeneous sheath either during fossilization or naturally during the maturation of the spore (e.g., as a result of hyphal lysis). Only a few of the mantle hyphae remained intact, and these are recognizable in the fossils as narrow canals traversing the sheath.

Alternatively, the thick outer wall layer may also have been deposited onto the outer surface of the structure as a secretion of mucilage resulting in the formation of a sheath which eventually solidified to form a protective outer layer. Support for this hypothesis is the tubular canals traversing this layer. Because most of the canals are bounded by discrete walls, these structures seem to represent narrow fungal hyphae or other microbial filaments that became enclosed in the sheath. More support for this interpretation is the observation that the outer wall layer is distinctly thinner in the attachment area (neck region) of the subtending hypha or filament (Pl. 2, Figs 11, 14). A similar decrease in the distal perimeter of the ornamentation boundary relative to the wall in the neck region has been used by Dotzler et al. (2008) to suggest that the prominent, antler-like surface extensions in the Carboniferous peronosporomycete oogonium *Combresomyces cornifer* represent condensed extra oogonial-wall secretions. It is interesting in this context that *Frankbaronia velata*, a putative peronosporomycete oogonium from the Rhynie chert, is also characterized by a sheath suggested to have formed through a secretion of mucilage (Krings et al. 2013). Although other structural features suggestive of affinities of *reproductive unit 3* to the Peronosporomycetes (e.g., antheridia, oospores) have not been found, we cannot rule out at present that this fossil represents a peronosporomycete oogonium. Yet another hypothesis is that the sheath was deposited onto the outer surface by some exterior source (e.g., within a sporangium or a meshwork of glebal hyphae). However, there is currently no strong evidence to suggest that *reproductive unit 3* developed within a larger structure which could have deposited the outer wall layer. If such were the case, then the canals, which might possibly be interpreted as remains of a hyphal meshwork that gave rise to and surrounded the reproductive units during development, must have disintegrated either on maturation or during fossilization.

5. Conclusions

Two of the three reproductive units described in this paper from the Early Devonian Rhynie chert are difficult to interpret due to their small size, incompleteness and lack of diagnostic features. In spite of these limitations, we believe it is worthwhile to record these fossils for several reasons. One involves morphological data that can be used to indirectly establish biodiversity in ancient ecosystems. This inventory of distinctive morphologies is critical to subsequent studies that are aimed at understanding how these organisms functioned as integral parts of ecosystems. A second approach therefore relates to features of the reproductive system and how these structurally (and by inference functionally) may be similar to, or differ from, the reproductive biology in modern fungi. Thirdly, the realization of additional fossils may increase the number of calibration points that can be used in aligning molecular clock estimates with fossil evidence in discussions of fungal evolution. Finally, there is considerable interest today in the fungal component of modern ecosystems and the roles these organisms played in ecosystem functioning, while we are only beginning to assess fungi in the fossil record. We hope that this paper not only adds to the growing inventory of distinctive fungal morphologies from the Rhynie chert, but also

stimulates interest in these organisms, and encourages other researchers to prepare and study suitable specimens from their collections of fossil fungi.

Acknowledgments

This article is dedicated to Dr. Winfried Werner (Munich, Germany) on the occasion of his 65th birthday. We join the community in saluting Winfried, who has always been a respected colleague. We acknowledge financial support from the National Science Foundation (EAR-0949947), the Deutsche Forschungsgemeinschaft (DFG Ke 584/13-2), and the Alexander von Humboldt-Foundation (3.1-USA/1160852 STP). We thank Mark C. Brundrett (Perth, Australia) for providing information on Glomeromycota from Kakadu National Park, Australia, and Hans Kerp (Münster, Germany) for insightful comments and suggestions.

6. References

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