Morphology and ontogenetic development of *Zwergimycetes vestitus*, a fungal reproductive unit enveloped in a hyphal mantle from the Lower Devonian Rhynie chert

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Abstract

Zwergimyces vestitus, a fungal reproductive unit enveloped in a mantle of interlaced circumferential hyphae from the Lower Devonian Rhynie chert, was formally described based on limited information obtained from six specimens. In this paper we provide additional insights into the organization of *Z. vestitus*, together with specific developmental details, from >100 new specimens. A marked expansion of the hyphal mantle may occur on one side of this reproductive unit. Moreover, the units appear to have been embedded in a loose meshwork of delicate hyphae. Variations in the organization of the mantle among the specimens suggest mantle formation took place by repeated branching of hyphae on the surface of the developing unit and by additional hyphae extending between the pre-existing mantle hyphae. What was initially described as one non-hyphal wall lining the inner surface of the mantle is now demonstrated as an outer sporangium wall, which continues into the subtending hypha, and an inner spore wall. The diagnoses for *Zwergimyces* and *Z. vestitus* are emended. Small spherules in several *Z. vestitus* specimens are interpreted as intrusive organisms. The new material of *Z. vestitus* provides the first insights into the ontogenetic development of one of the Rhynie chert mantled fungal reproductive units.

Key words: Glomeromycota; morphogenesis; Mucoromycotina; parasitism; sporangium; spore wall

1. Introduction

One of the many interesting features of the Early Devonian Rhynie chert is the occurrence of small (<0.5 mm) fungal reproductive units (e.g., spores, sporangia, sporocarps) that are almost ubiquitous in the chert matrix and in litter layers, as well as within intact and decaying land plant parts (e.g., Kidston and Lang, 1921; Boullard and Lemoigne, 1971; Taylor et al., 2003, 2015). The abundance of these remains, together with the large number of morphologically different forms, suggests that Fungi and fungus-like organisms (e.g., peronosporomycetes; see Krings et al., 2012, 2013) were important components of many of the vital processes that sustained this early non-marine ecosystem. However, attempts to estimate fungal paleobiodiversity based on the reproductive units from the Rhynie chert and specify what roles these organisms played in the ecosystem are generally hampered by the fact that the specimens almost always 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. Moreover, all specimens are usually at approximately the same stage of development, and thus there is no information about the ontogenetic development of the organisms.

Among the Rhynie chert fungal reproductive units are several types that are characterized by an ancillary surface covering in the form of a mantle or investment of tightly aligned or interwoven hyphae (Krings and Taylor, 2013, 2014, 2015a,b; Krings et al., 2014). These fossils represent the oldest evidence of hyphal mantles in fungi. It has been suggested that the mantled fungal reproductive units from the Rhynie chert may have systematic affinities with the Glomeromycota or Mucoromycotina based on similar features in modern lineages known to produce spores or sporangia with hyphal mantles (Krings and Taylor, 2014). One form has also been compared to the so-called ‘birdsnest’ condition of certain peronosporomycete oogonia (Krings and Taylor, 2015b), while another resembles the pycnidia and cleistothecia formed by certain modern ascomycetes (Krings and Taylor, 2015a).

One mantled fungal reproductive unit from the Rhynie chert with suggested affinities to the Glomeromycota or Mucoromycotina is *Zwergimyces vestitus* (Kidston et W.H. Lang) M. Krings et T.N. Taylor. It consists of a spheroid enveloped in 1–2 layers of interlaced hyphae that extend around the circumference of the structure (Krings and Taylor, 2013). The mantle is bounded on the inside by a non-hyphal wall. The original description of *Z. vestitus* was based on six specimens all of which lacked information other than on basic morphology. However, Krings and Taylor (2013) noted differences among the specimens regarding the thickness of the non-hyphal wall, and suggested that perhaps a spore formed within this reproductive unit.

In this paper, we provide a re-description and evaluation of *Zwergimyces vestitus* based on >100 additional specimens. The new material provides additional insights into the structural organization of *Z. vestitus*, together with specific developmental details, and corroborates the hypothesis that a spore with a massive, multi-layered wall formed within this reproductive unit. The discovery is significant because it provides the first detailed evidence of ontogenetic development in one of the Rhynie chert fungal reproductive units.

2. Geological setting, material, and methods

The Rhynie chert occurs within a sequence of sedimentary and volcanic rocks located northwest of the village of Rhynie in Aberdeenshire, Scotland. The Lagerstätte consists of several fossiliferous beds containing lacustrine shales and cherts that have been dated as Pragian-?earliest Emsian based on dispersed spore assemblages (Wellman, 2006; Wellman et al., 2006); high-precision age constraints on the Rhynie Lagerstätte indicate absolute ages of 407.1 ± 2.2 Ma (Mark et al., 2011) and 411.5 ± 1.3 Ma (Parry et al., 2011) for the fossilized biota. The shales and cherts are interpreted as a series of ephemeral freshwater pools and lakes within a hot spring environment (Rice et al., 2002; Rice and Ashcroft, 2003). Preserved in the chert are both aquatic (freshwater) facies from the pools and subaerial soil/litter

horizons with in situ plants that grew along the margins of the pools. For detailed information on the geology and development of the Rhynie chert Lagerstätte, refer to Powell et al. (2000), Rice et al. (2002), and Trewin and Rice (2004).

The fossils used in this study were identified in series of thin sections from several different chert blocks. These were prepared by cementing wafers of the Rhynie chert to glass slides and then grinding the rock slices until the sections were thin enough to transmit light. The slide series are deposited in the Bayerische Staatssammlung für Paläontologie und Geologie (SNSB-BSPG) at Munich, Germany, under accessions SNSB-BSPG 1965 I, 2013 V, 2013 XV, and 2015 XIX. Slides were analyzed using normal transmitted light microscopy; digital images were captured with a Leica DFC-480 camera and processed in Adobe Photoshop CS 5. Slide numbers for the specimens illustrated in Pls. I–III are given in the figure captions.

3. Systematic paleomycology

Fungi incertae sedis

Genus *Zwergimyces* M. Krings et T.N. Taylor, 2013, emend.

(MB 802281)

Emendation of the generic diagnosis: Globose to subglobose fungal reproductive unit (sporangium), <60 µm in diameter (inclusive mantle), composed of central cavity bounded on outside by narrow non-hyphal wall and enveloped in prominent mantle constructed of interlaced hyphae extending around circumference of structure; reproductive unit subtended by tubular hypha that may also be sheathed by mantle hyphae; cavity may contain single, thick-walled propagule (spore).

Zwergimyces vestitus (Kidston et W.H. Lang, 1921) M. Krings et T.N. Taylor, 2013, emend.
(MB 802282)

References:

- 1921b *Palaeomyces vestitus* (as *vestita*) Kidston et W.H. Lang: p. 869, pl. IV, figs. 36–38 [fig. 37:
lectotype]
- 2013 *Zwergimyces vestitus* (Kidston et W.H. Lang) M. Krings et T.N. Taylor: p. 15–19, pl. I, figs. 1–8
- 2014 *Zwergimyces vestitus* (Kidston et W.H. Lang) M. Krings et T.N. Taylor – Krings et al.: fig. 2a–c
- 2014 *Zwergimyces vestitus* (Kidston et W.H. Lang) M. Krings et T.N. Taylor – Krings and Taylor: fig.
2a
- 2015 *Zwergimyces vestitus* (Kidston et W.H. Lang) M. Krings et T.N. Taylor – Taylor et al.: p. 80, figs.
6.10, 6.11 [non fig. 6.9]

Emended diagnosis: Reproductive unit (28–)42–45(–60) μm in diameter (inclusive mantle), separated from parental hypha by septum; hyphal mantle (2–)4–7(–10) μm thick, constructed of thin-walled, multi-branched septate hyphae which are tubular, irregularly swollen, or more or less catenulate; peripheral mantle hyphae mostly tubular; mantle hyphae tightly aligned or abutting in fully developed units, but more loosely arranged and with spaces between individual hyphae in immature specimens; conspicuous expansion of mantle occurs on one side of structure, probably where subtending hypha was attached [rarely preserved]; non-hyphal wall (sporangium wall) recognizable as distinctive, regular opaque line extending along inner surface of hyphal mantle; immature specimens with delicate sporangium wall; second non-hyphal wall (spore wall) extending along inner surface of sporangium wall may be present; spore wall variable in thickness and appearance, ranging from a narrow line <1 μm thick to massive and >2.5 μm thick; massive spore walls uniform in texture and more or less translucent, variously differentiated into translucent and opaque bands, or stratified with distinct demarcations between individual wall components; local bulging of spore wall into

cavity lumen resulting in small void underneath sometimes present; reproductive unit surrounded by peripheral meshwork of irregularly arranged, thin-walled, probably ephemeral hyphae [rarely preserved].

Description (incorporating data from the new material and original set of specimens):

The reproductive units (Pls. I–III) occur singly or in groups (usually 2–5 individuals) in largely intact or degraded land plant tissue; specimens in intact plant tissue may occur in intercellular spaces or within individual cells (e.g., Pl. I, 9; Pl. III, 3). Other specimens are associated with accumulations of plant litter or rhizoid tufts, one specimen occurs within a large fungal spore (Pl. III, 9).

The reproductive units are globose to somewhat ovoid (subglobose) in shape and (28–)40–45(–60) μm in diameter (inclusive investment). Subtending hyphae, which are visible in c. 5% of the specimens (e.g., arrows in Pl. I, 2, 3a), are tubular and 3.5 μm wide; a septum separating the reproductive unit from the subtending hypha is recognizable in some specimens (arrow in Pl. I, 2). One specimen shows what appears to be a prominent distal inflation of the subtending hypha (labeled ‘I’ in Pl. I, 6). Krings and Taylor (2013) note opaque plaques that occur in the position where a parental hypha or filament probably was attached in two of the original specimens. However, plaques have not been observed in any of the new specimens.

All reproductive units are composed of a central cavity that is bounded on the outside by a non-hyphal wall (termed ‘inner investment element’ by Krings and Taylor, 2013) and enveloped in a prominent hyphal mantle. The distal portion of the subtending hypha may also be sheathed by mantle hyphae (Pl. I, 3a). The central cavity is 25–50 μm in diameter. The hyphal mantle is (2–)4–7(–10) μm thick, and consists of a system of relatively thin-walled, multi-branched hyphae that are tubular, irregularly swollen, or more or less catenulate, and extend around the circumference of the structure (Pl. I, 3b, 4); septa are present. Hyphae range from 1.1 to 2.1 μm wide, while hyphal swellings are variable in size and shape (i.e.

spheroidal, short-elliptical, elongate-clavate, or sometimes sub-cylindrical), and up to 6(–8) μm long and (2–)2.5–4(–>6) μm wide. Tubular or irregularly swollen hyphae 2.1–2.6(–3.2) μm wide, also extending around the circumference of the reproductive unit, may be present in the periphery (arrows in Pl. I, 5). A conspicuous expansion or proliferation of the mantle hyphae is recognizable in several well-preserved specimens (Pl. I, 10b [arrows]; Pl. II, 1b [arrows], 2a [black arrows]; Pl. III, 4). This expansion consistently occurs only on one side of the structure, probably where the subtending hypha was attached. Moreover, some of the well-preserved specimens in reversed light demonstrate that the reproductive units are embedded in a loose meshwork of irregularly arranged delicate hyphae (1.2–)1.7–2.3 μm in diameter) and hyphal fragments that appear to have undergone (partial) lysis (labeled ‘m’ in Pl. II, 1a–c). While the majority of specimens are enveloped in cohesive mantles composed of tightly aligned or abutting hyphae (e.g., Pl. I, 10a), a few appear to possess more loosely organized mantles composed of comparatively large hyphae and spaces between the individual hyphae (Pl. I, 1a–c).

The non-hyphal wall is recognizable as a distinctive, regular opaque line (<0.5–0.95 μm thick) extending along the inner surface of the hyphal mantle (e.g., Pl. I, 2 [onhw], 10c); irregularities in the form of local wrinkling or distortion may occur, but are rare. Small opaque granules sometimes occur integrated into the non-hyphal wall (Pl. III, 7). In a few specimens the non-hyphal wall appears to be partially or entirely separated from the mantle (Pl. I, 11; Pl. III, 8), perhaps as a result of turgor loss and subsequent shrinkage of the lumen during fossilization. In specimens with loosely organized hyphal mantles, the non-hyphal wall is distinctly thinner than in the other specimens (compare ‘onhw’ in Pl. I, 1c with Pl. I, 2, 10c).

Approximately 35% of the specimens show a second (inner) non-hyphal wall that extends along the inner surface of the outer non-hyphal wall. There is considerable variation among the specimens regarding the thickness and appearance of this inner wall. In a few

211 (<1%) specimens, the wall is recognizable only as a narrow line (arrow in Pl. III, 1), while in
212 the majority of units it is massive (2.8–4.4 μm thick). In specimens with a massive inner non-
213 hyphal wall, this wall may be uniform in texture and more or less translucent (e.g., arrow in
214 Pl. III, 2), variously differentiated into translucent and more opaque bands (Pl. III, 4, 5, 7), or
215 stratified with distinct demarcations between the individual layers or wall components (layers
216 ‘I–IV’ in Pl. III, 6b). However, wall stratification is generally difficult to discern; it can only
217 be observed by carefully focusing through the object at high magnification. For example, the
218 wall of the specimen illustrated in Pl. III, 6a appears to be differentiated into lighter and
219 darker bands. At higher magnification, however, it demonstrates a distinct stratification
220 pattern (Pl. III, 6b). Approximately 20% of specimens possessing a massive inner non-hyphal
221 wall also show a conspicuous shallow bulging of this wall into the cavity lumen (arrows in Pl.
222 III, 4, 5, 7) that results in the formation of a small void underneath (‘v’ in Pl. III, 4, 5, 7). The
223 void locally separates the outer and inner non-hyphal walls, thereby demonstrating two
224 discrete structures (‘onhw’ and ‘inhw’ in Pl. III, 7), rather than one entity. The absence of a
225 bulge in other specimens with a massive inner wall may simply reflect the plane of section.

226 Associated with three specimens that occur in largely intact plant tissue are mycelia
227 composed of multi-branched thin-walled, septate or pseudoseptate moniliform hyphae (Pl. I,
228 7, 8; Pl. II, 2b, 3). Individual cells in these hyphae are 2.8–9.5 μm long and wide. In one
229 specimen (Pl. II, 2a,b) there is also evidence that the reproductive unit was given off from the
230 moniliform hyphae in the form of a tubular hypha extending from the mycelium to the base of
231 the reproductive unit (white arrows in Pl. II, 2a). Another specimen located within a plant cell
232 occurs among comparatively wide (3.5–6.3 μm), more or less tubular, septate or
233 pseudoseptate hyphae (Pl. II, 4a–c). However, physical connections between the reproductive
234 units, tubular hyphae, and moniliform mycelia are not recognizable in either of these
235 specimens. Associated with one of the reproductive units described by Krings and Taylor

(2013) is a smaller structure that appears to be constructed of two bulbous components. We have not found comparable structures in any of the new specimens.

Less than 10% of the reproductive units contain evidence of intrusive microorganisms in the form of various types of solitary (Pl. III, 10, 11, 13) or clustered (Pl. III, 14) spherules, some of which characterized by a distinctly offset neck region (arrow in Pl. III, 13). The most interesting type has surface ornamentation in the form of prominent spines (Pl. III, 10, 11). Spherical microfossils with spines have not been documented to date from the Rhynie chert and their systematic affinities remain elusive. One specimen shows another organism on the mantle surface (lower right in Pl. III, 12), as well as several structures that appear to be penetration pegs (arrows in Pl. III, 12). All reproductive units colonized by other organisms are intact, but it is interesting to note that none show evidence of the inner non-hyphal wall.

Remarks: There are notable variations among the specimens of *Zwergimyces vestitus* with regard to (i) mantle coherence; (ii) whether or not a second (inner) non-hyphal wall (spore wall) is present; and (iii), if present, the thickness and composition of this inner wall. These variations cannot be interpreted as artifacts or the results of locally different preservation conditions. We cannot completely rule out the possibility that *Z. vestitus* represents several morphologically similar fungal species. However, the overall organization of the hyphal mantle, which is the single-most important feature used to distinguish between fossil genera and species of mantled fungal reproductive units in general (Krings and Taylor, 2015a; Taylor et al., 2015), is largely congruent in all specimens. As a result, we feel confident that such variations may be intraspecific, and suggest that they reflect different stages in the ontogenetic development of this reproductive unit (see Discussion section below).

It is impossible to determine whether the mycelia of moniliform (Pl. I, 7, 8; Pl. II, 2b, 3) and wide (pseudo-)septate hyphae (Pl. II, 4b, c) belong to the same organism that produced

Zwergimyces vestitus, or represent one or more different fungi that lived in the same habitat.

As a result, we have not included these features in the emended diagnosis.

4. Discussion

Zwergimyces vestitus is one of five different types of mantled fungal reproductive units described to date from the Lower Devonian Rhynie chert (Krings and Taylor, 2013, 2014, 2015a,b; Krings et al., 2014). All are spheroid-ovoid structures <100 µm in diameter that include a central cavity bounded by a non-hyphal wall, which is in turn enveloped in a hyphal mantle, but they differ from each other in size and mantle morphology. With the exception of *Scepsmatocarpion fenestrulatum* M. Krings et T.N. Taylor, which is characterized by large pores (Krings and Taylor, 2015a), all are completely closed structures. None of the specimens of any type provide information on how the reproductive units developed and what was produced in these structures.

The original description of *Zwergimyces vestitus* by Krings and Taylor (2013) is based on six specimens in a single thin section from the collection of the late Prof. Max Hirmer of Munich, Germany, and the illustrations of *Palaeomyces vestitus* by Kidston and Lang (1921b: pl. IV, 36–38). The new specimens reported here provide several structural details of *Z. vestitus* not recognizable in the original material, and thus now permit a much more precise circumscription and understanding of this Early Devonian fungal reproductive unit.

4.1 Affinities

Mantled reproductive units morphologically similar to *Zwergimyces vestitus* are known in two extant fungal lineages, i.e. the mantled zygosporangia of certain members in the Mucroromycotina (Bucholtz, 1912; Linnemann, 1941; Benjamin, 1979) and the mantled chlamydospores of certain Glomeromycota (Gerdemann and Trappe, 1974; Schenck and Smith, 1982; Bentivenga and Hetrick, 1991; Błaszkowski, 1995). Krings and Taylor (2013)

interpreted *Z. vestitus* as belonging to either of these two fungal groups, however, without further delimiting the systematic position of the fossil. While the new material does also not resolve the affinities of *Z. vestitus*, it offers one interesting feature that was not available from the original set of specimens. Several specimens show *Z. vestitus* positioned distally on a tubular hypha (Pl. I, 2, 3a), and separated from the latter by a septum (arrow in Pl. I, 2). This configuration suggests that the reproductive unit formed asexually by blastic inflation and thickening of a hypha, rather than sexually and following gametangial fusion, and therefore that *Z. vestitus* represents a glomoid chlamydospore produced by a member in the Glomeromycota, rather than a zygosporangium (see Gerdemann and Trappe, 1974; Marleau et al., 2011). Arguing against this interpretation is the specimen illustrated in Pl. II, 4a–c. The optical section labeled Pl. II, 4b depicts a configuration that is reminiscent of a zygosporangium positioned laterally between two sexual hyphae (see Benjamin, 1979; Edelman and Klomparens, 1995; Hoffmann et al., 2007). However, branches (suspensors) physically connecting the reproductive unit and hyphae have not been observed. In another specimen there is a prominent distal inflation of the subtending hypha (marked ‘I’ in Pl. I, 6). Although we interpret this inflation as a structural deformity or preservation artifact because it has only been observed in a single specimen, we cannot completely rule out the possibility that it represents a gametangium giving rise to a zygosporangium or azygosporangium (e.g., O’Donnell et al., 1977; Benjamin, 1979; Wagner et al., 2013), or a large bulbous base subtending a gigasporoid glomeromycotan spore (e.g., Khade, 2011; Oehl et al., 2011; Redecker and Schüßler, 2014). Somewhat similar to the inflated subtending hypha shown in Pl. I, 6 is the lobed structure associated with one of the original specimens of *Z. vestitus* (Krings and Taylor, 2013: pl. I, 8). However, it is uncertain as to whether this structure is at all organically connected with the reproductive unit.

Interestingly, certain structural details might also be used to suggest yet another, although at present highly speculative interpretation – the fossils represent unisporic asci of

some member of the Ascomycota. This suggestion is based in part on the fact that the mantle hyphae in *Zwergimyces vestitus* are septate. Septa have been reported in the mantle hyphae of the fossil “sporocarp” genus *Mycocarpon* S.A. Hutchinson (Hutchinson, 1955; Taylor and White, 1989; Krings et al., 2010), but do not normally occur in the mantles of Glomeromycota and Mucoromycotina (but see Buchholtz, 1912; Błaszowski, 1995). Moreover, thick-walled specimens of *Z. vestitus* are somewhat similar morphologically, although clearly on a different size scale, to the large ascospores (300–500 µm in diameter) of the extant *Glaziella aurantiaca* (Berkeley et M.A. Curtis) Saccardo (Gibson, 1984; Gibson et al., 1986), a member of the ascomycete order Pezizales (Landvik and Eriksson, 1994; Hansen and Pfister, 2006). This fungus produces cm-sized ascomata (sporocarps) which are more or less epigeous (Hansen and Pfister, 2006). Ascospores develop in unisporic asci that arise in locules from pockets of pseudoparenchyma within the ascoma wall. The mature spore wall is composed of a massive inner wall surrounded by a thin outer wall that is partially fused to the surrounding septate hyphae of the sporocarp (Gibson et al. 1986). However, if *Z. vestitus* were a unisporic ascus containing a spore, the outer non-hyphal wall which continues into the subtending hypha would accordingly be the ascus wall, rather than a thin outer spore wall, while in *G. aurantiaca* the ascus wall becomes structurally disorganized and disintegrates early in spore development (Gibson et al., 1986). Moreover, there is currently no evidence to suggest that the fossil formed within macroscopic ascomata or sporocarps.

4.2 Hyphal mantle

Several of the new specimens display a marked expansion of the hyphal mantle on one side of the reproductive unit (Pl. I, 10b; Pl. II, 1a–c, 2a; Pl. III, 4). Some of the units are additionally embedded in remnants of a peripheral meshwork of thin-walled, probably ephemeral hyphae that is barely recognizable in normal transmitted light, but appears as a diffuse plume containing hyphal fragments in reversed light (‘m’ in Pl. II, 1a–c). These two interesting

morphological features of *Zwergimyces vestitus* have only been observed in a few specimens located within intact plant cells, as well as in one specimen that occurs entangled in a dense tuft of land plant rhizoids. It is likely that the host cell walls and rhizoids have provided some level of protection (e.g., from mechanical abrasion and microbial degradation) which resulted in the near-perfect preservation of even the delicate periphery in these specimens. A similar, protective effect has been noted previously in another Rhynie chert fungal reproductive unit that occurs in clusters embedded in a loose meshwork of delicate hyphae within thin-walled land plant cells (Krings and Taylor, 2015b). The confluent hyphal meshwork in these specimens would no doubt have become dissociated (and consequently lost) along with the disintegration of the host cells.

Mantle formation in extant Glomeromycota and Mucoromycotina develops from the parental mycelium (e.g., from a gleba, the subtending hypha, or the gametangia/suspensors), or the mantle forms as an outgrowth of the developing spore/sporangium (Bucholtz, 1912; Linnemann, 1941; Błaszowski, 1995). The hyphal mantle in the vast majority of *Zwergimyces vestitus* specimens is comprised of tightly interlaced hyphae extending along the circumference of the structure. However, a few specimens possess a mantle that is more loosely organized, with spaces between the individual hyphae (Pl. I, 1a–c). Because the non-hyphal wall is delicate (e.g., compare Pl. I, 1c with Pl. I, 2, 10c), if at all recognizable, we interpret these latter specimens as ontogenetically immature. We hypothesize that the difference in mantle compactness reflects the basic pattern of mantle formation in *Z. vestitus*, and offer two explanations for the formation of the mantle in this reproductive unit. One may be that the development of the mantle is the result of a few closely associated hyphae that enclosed the initial vesicle. The envelope then grew successively more compact through repeated branching of these hyphae on the surface of the expanding vesicle (Pl. I, 3–5) and/or by additional hyphae extending between the pre-existing mantle hyphae. In this scenario, the peripheral meshwork of thin-walled hyphae formed concomitantly through some of the

hyphal branches extending outwards, perhaps in a similar manner as described in the Mississippian fungal “sporocarp” *Mycocarpon cinctum* M. Krings et al. (Krings et al., 2010). A second explanation may involve the formation of an initial, loose meshwork of thin-walled hyphae that subsequently produced branches which extended inwardly onto the surface of the developing reproductive unit, thus forming a mantle. A mantle formation pattern involving inwardly directed branches extending from circumferential hyphae in the periphery of the structure has previously been inferred in *Helmutella devonica* M. Krings et T.N. Taylor, another mantled fungal reproductive unit from the Rhynie chert (Krings and Taylor, 2014). However, the inwardly directed branches in *H. devonica* form a distinctive inner mantle layer composed of radially elongate elements. Developmental patterns concurrent with *H. devonica* have also been suggested in several Carboniferous and Triassic fungal “sporocarps” (surveyed in Krings and Taylor, 2014).

The provenance of the initial mantle-forming hyphae in *Zwergimyces vestitus* remains unclear. One possibility is that the moniliform mycelia co-occurring with two of the specimens (Pl. I, 7, 8; Pl. II, 2, 3) gave rise to the reproductive units and initial mantle-forming hyphae. Alternatively, the mantle might also have developed from hyphae at the base of the subtending hypha (as, for instance, in the extant *Mortierella globulifera* O. Rostrup; see Kuhlman, 1972) that extended upwards, sheathed the distal portion of the subtending hypha, and then proceeded to form the mantle; the conspicuous mantle expansion seen in several specimens (Pl. I, 10b; Pl. II, 1a–c; Pl. III, 4) perhaps represents a holdover from this process. However, there is currently no direct evidence to confirm either of these hypotheses.

4.3 Spore development

The non-hyphal wall is slightly to considerably more massive in ~30% of the *Zwergimyces vestitus* specimens (compare Pl. I, 2, 3a, 10c with Pl. III, 1–7). Moreover, a conspicuous local bulge of the non-hyphal wall into the central cavity (arrows in Pl. III, 4, 7), along with a void

underneath the bulge ('v' in Pl. III, 4, 5, 7), is discernible in several of the thick-walled specimens. This structural detail indicates that what appears as one cohesive non-hyphal wall in fact consists of two discrete entities, i.e. an inner non-hyphal wall ('inhw' in Pl. III, 7) that extends along the inner surface of an outer non-hyphal wall ('onhw' in Pl. III, 7). The two walls are tightly appressed to each other and distinguishable, if at all, only by slight differences in opacity (i.e. the outer wall is often more opaque; e.g., Pl. III, 2, 4, 5), except for the region of the bulge where the inner wall is lifted off and separated from the outer non-hyphal wall. This configuration suggests that a thick-walled spore formed within *Z. vestitus*. If this interpretation is correct, then the outer non-hyphal wall ('onhw' in Pl. I, 1c, 2 and Pl. III, 6b, 7), which appears to be continuous with the wall of the subtending hypha based on the specimen shown in Pl. I, 2, would represent the ascus (see section 4.1 *Affinities*) or a sporangium wall, perhaps comparable to the zygosporangium wall seen in zygomycetous fungi (e.g., Keene, 1919; O'Donnell et al., 1978; Ho and Chen, 1998) or the outmost layer(s) of glomeromycotan chlamydospore walls (e.g., the 'outer unit wall' in *Rhizophagus fasciculatus* (Thaxter) C. Walker et A. Schüßler [Walker and Koske, 1987]). None of the thick-walled specimens of *Z. vestitus* show the attachment of the subtending hypha, and it is therefore impossible to determine whether the inner non-hyphal wall (i.e. the spore wall) also continued into the subtending hypha or formed de novo within the sporangium. In *Mycocarpon asterineum* T.N. Taylor et J.F. White, a fungal "sporocarp" from the Triassic of Antarctica that also contains a single thick-walled spore, narrow hyphal branches extending from the mantle into the spore wall are interpreted as secretory structures responsible for the formation of the spore wall through continuous secretion of wall material (Taylor and White, 1989). *Zwergimyces vestitus* is characterized by a coherent outer non-hyphal wall that lines the inner surface of the mantle and remains intact throughout the subsequent development of the inner spore wall, thus arguing against spore wall secretion by the mantle. Materials secreted by the mantle after the formation of the outer wall would accumulate on the outside

of the outer wall, not on the inside. It is therefore very likely that the developing spore itself was responsible for the formation of the spore wall in *Z. vestitus*.

The bulging of the inner non-hyphal wall and formation of a void underneath may be a preservation artifact resulting from shrinkage of the reproductive unit during fossilization, or a natural feature of *Z. vestitus*, perhaps caused by the development of a pre-germination structure such as a germination shield (Walker and Sanders, 1986; Oehl et al., 2011) between the two non-hyphal walls. Some support for the latter hypothesis is the fact that prominent germination shields have been reported previously in two different glomeromycotan spores from the Rhynie chert (Dotzler et al., 2006, 2009). However, we have not found any direct evidence of pre-germination structures in *Z. vestitus*.

4.4 Paleocology

Several different types of small spherules, probably representing the reproductive units of intrusive organisms, occur singly or clustered within the lumen of *Zwergimyces vestitus*. Most of these structures closely resemble the spherical inclusions frequently encountered in other Rhynie chert fungal reproductive units (Kidston and Lang, 1921b). One distinctly ornamented (spiny) type has not been recorded previously (Pl. III, 10, 11). The organisms producing the spherules in *Z. vestitus* were probably fungi based on the presence of subtending hyphae or filaments (e.g., arrow in Pl. III, 13), but none can be assigned systematically with confidence. None of the infected *Z. vestitus* specimens show a host response (e.g., callosities; see Boyetchko and Tewari, 1991; Hass et al., 1994; Lee and Koske, 1994) or evidence of the formation of an inner non-hyphal wall, suggesting that perhaps only non-viable individuals were colonized, or that only the immature units prior to the formation of the spore wall were susceptible to infection. It is also possible that some of the intruding organisms were capable of adversely affecting spore development in *Z. vestitus*, perhaps in a similar manner as certain modern biotrophic fungi which suppress the maturation of their hosts in order to maximize the

supply of nutrients by prolonging the flow of nutrients into the immature host (White et al., 1997).

The hyphal mantle of *Zwergimyces vestitus* was a persistent structure that appears to have remained in place even upon maturation of the spore. This raises the question as to whether the mantle served a particular function in the biology of this reproductive unit. We are not aware of any study addressing the functions of hyphal mantles in extant Glomeromycota or Mucoromycotina. However, it is known that the viability and functioning of the spores of modern glomeromycotan fungi are affected by various abiotic and biotic factors, including soil moisture, temperature, and whether or not certain soil organisms such as bacteria and other fungi are present (Daniels and Trappe, 1980; Morton, 1985; Fitter and Garbaye, 1994; Walley and Germida, 1996; Xavier and Germida, 2003). It is therefore possible that the mantle of the fossil *Z. vestitus* was perhaps effective as a modulating interface or protective shield against certain (adverse) environmental influences. Moreover, the mantle might have accommodated microorganisms that were beneficial to the development of the spore or in some way stimulated spore germination (for similar interactions in modern Glomeromycota, see Mayo et al., 1986; Mugnier and Mosse, 1987; Fracchia et al., 1998). It is also possible that the mantle was capable of re-growing a regular mycelium, and hence that *Z. vestitus* concomitantly functioned as a reproductive unit and vector in vegetative dispersal. Support for this latter hypothesis is a report of mycelium development from peridium hyphae in the extant *Funneliformis mosseae* (T.H. Nicolson et Gerdemann) C. Walker et A. Schüßler (Dalpé et al., 2005).

5. Conclusions

The Early Devonian Rhynie chert has received considerable attention since nearly 100 years because it provides exceptional insights into not only the morphology and internal organization but also the life history and ontogenetic development of early terrestrial plants

(e.g., Kidston and Lang, 1917, 1920a,b, 1921a; Kerp and Hass, 2004, 2009; Taylor et al., 2005; Kerp et al., 2013). Conversely, the many different microorganisms that are preserved together with the land plants in the Rhynie chert have only recently begun to receive wider interest, and their morphology, distribution, and possible roles in ecology are now being more systematically documented (e.g., Taylor and Krings, 2015). However, depictions of different life history stages and ontogenetic development in Rhynie chert microorganisms remain rare. This is unfortunate because life history and ontogeny represent important diagnostic features that could be used to assess the systematic affinities of these organisms as fossils in the absence of molecular data. Two notable exceptions from the lack of documented evidence of microbial life history and ontogenetic development from the Rhynie chert are the chroococcalean cyanobacterium *Rhyniosarcina devonica* T.N. Taylor et M. Krings and the putative peronosporomycete *Frankbaronia polyspora* M. Krings et al., two organisms of which large sample sets of well-preserved specimens are available. Structural differences between the specimens have been used to reconstruct colony growth and expansion in the former and the process of oospore formation in the latter taxon (Krings et al., 2012; Taylor and Krings, 2015). The new material of *Zwergimyces vestitus* presented here underlines the importance of large sample sets in tracing the complements of structural features that characterize different developmental stages in the formation of the mature structure (Krings and Taylor, 2013). However, *Z. vestitus* also demonstrates that preservation quality matters, and that specimens situated in protected (micro-)environments (e.g., within intact plant tissue or in small voids within litter layers) may yield more or qualitatively superior information than specimens located outside these special environments. This knowledge can be used to refine search images and activities for the best-preserved specimens of structurally preserved fossil microorganisms in a chert or coal ball matrix, and thus can contribute to a more complete documentation and better understanding of ancient microbial life forms.

Acknowledgments

We acknowledge financial support from the Deutsche Forschungsgemeinschaft (DFG grant Ke 584/13-2), the National Science Foundation (EAR-0949947), and the Alexander von Humboldt-Foundation (3.1-USA/1160852 STP). Helmut Martin and Stefan Sónyi (both Munich, Germany) are acknowledged for technical assistance, and two anonymous referees for insightful comments and suggestions on the manuscript.

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 695

696 **Figure captions**

697

698 **Plate I:** *Zwergimyces vestitus* from the Lower Devonian Rhynie chert.

699 1a–c: Optical sections through specimen with incomplete hyphal mantle and delicate, barely
700 visible non-hyphal wall [onhw]; (a) surface view; (b) plane off center; (c) plane close
701 to center; slide SNSB-BSPG 2013 V 63; scale bars = 10 μm .

702 2: Specimen showing well-developed non-hyphal wall [onhw] and septum (arrow) separating
703 reproductive unit from subtending hypha; slide SNSB-BSPG 1965 I 327; scale bar =
704 10 μm .

705 3a,b: Optical sections through specimen with portion of subtending hypha still attached
706 (arrow); (a) plane close to center, showing distal portion of hypha enveloped in hyphal
707 mantle (arrow); surface pattern formed by hyphal investment; slide SNSB-BSPG 2013
708 V 63; scale bars = 10 μm .

709 4: Detail of Pl. I, 3b, focusing on one of the multi-branched mantle hyphae; note irregular
710 inflation of some of the lateral branches (arrow); bar = 10 μm .

711 5: Tubular peripheral mantle hypha (arrows); slide SNSB-BSPG 1965 I 327; scale bar = 10
712 μm .

713 6: Inflation [I] of the subtending hypha; arrows indicate distal constriction of subtending
714 hypha; slide SNSB-BSPG 2013 V 64; scale bar = 10 μm .

715 7: Reproductive unit (surface view) associated with hyphae composed of monilioid cells; slide
716 SNSB-BSPG 2013 V 63; scale bars = 10 μm .

717 8: Monilioid hyphae associated with specimen shown in Pl. I, 7; slide SNSB-BSPG 3013 V
718 63; scale bar = 10 μm .

719 9: Well-preserved specimen in lumen of land plant cell; slide SNSB-BSPG 2013 XV 206;
720 scale bar = 50 μm .

721 10a–c: Optical sections through specimen shown in Pl. I, 9; (a) surface view; (b) plane off
722 center; (c) plane close to center; arrows in Pl. I, 10b indicate expansion of mantle on
723 one side of unit; scale bars = 10 μm .

724 11: Specimen with non-hyphal wall partially attached to (lower right of image) and partially
725 separated (upper left of image) from mantle, perhaps as a result of shrinkage during
726 fossilization; slide SNSB-BSPG 2013 XV 23; scale bar = 10 μm .

727

728 **Plate II:** *Zwergimyces vestitus* from the Lower Devonian Rhynie chert; reversed light.

729 1a–c: Optical sections through specimen, showing mantle, mantle expansion on one side of
730 unit (arrows in Pl. II, 1b), and plume of partially degraded delicate hyphae [m]
731 surrounding the structure; (a) surface view; (b) plane off center; (c) plane close to
732 center; slide SNSB-BSPG 2013 XV 39; scale bars = 10 μm .

733 2a,b: Optical sections through reproductive unit (upper left of Pl. II, 2a) and associated
734 mycelium of delicate moniloid hyphae (lower right of Pl. II, 2b); white arrows in Pl.
735 II, 2a indicate what appears to be subtending hypha of reproductive unit, black arrows
736 show remnants of mantle expansion; slide SNSB-BSPG 2013 XV 13; scale bars = 10
737 μm .

738 3: Moniloid hyphae in cortical host cell adjacent to area depicted in Pl. II, 2b; arrows indicate
739 septa or pseudosepta; slide SNSB-BSPG 2013 XV 13; scale bar = 10 μm .

740 4a–c: Optical sections through specimen occurring in plant cell amidst wide hyphae; arrows
741 in Pl. II, 4b indicate two hyphae embracing reproductive unit, while arrows in Pl. II,
742 4c show septa; slide SNSB-BSPG 2015 XIX 4; scale bars = 10 μm .

743

744 **Plate III:** *Zwergimyces vestitus* from the Lower Devonian Rhynie chert.

745 1: Early stage in formation of second (inner) non-hyphal wall (arrow); slide SNSB-BSPG
746 2013 XV 154; scale bar = 10 μm .

- 747 2: Specimen showing opaque outer and prominent translucent inner (arrow) non-hyphal walls;
748 slide SNSB-BSPG 2013 XV 12; scale bar = 10 μ m.
- 749 3: Well-preserved specimen in partially degraded cortical land plant cell; slide SNSB-BSPG
750 2013 XV 38; scale bar = 25 μ m.
- 751 4: Detail of Pl. III, 3, showing mantle, narrow outer non-hyphal wall (opaque line), and
752 massive inner non-hyphal wall (translucent) with conspicuous bulge (arrows),
753 resulting in formation of void [v] between outer and inner non-hyphal walls; note
754 expansion of mantle on one side of structure (right side of image); scale bar = 10 μ m.
- 755 5: Massive inner non-hyphal wall and void [v] between outer and inner non-hyphal walls;
756 slide SNSB-BSPG 2013 XV 18; scale bar = 10 μ m.
- 757 6a: Specimen with massive inner non-hyphal wall; slide SNSB-BSPG 2013 V 61; scale bar =
758 10 μ m.
- 759 6b: Detail of Pl. III, 6a (different focal plane), showing outer [onhw] and inner non-hyphal
760 walls, the latter consisting of several wall layers or components [labeled I–IV]; scale
761 bar = 5 μ m.
- 762 7: Specimen in matrix, showing tattered hyphal mantle bounded on inside by narrow outer
763 [onhw] and massive inner [inhw] non-hyphal walls, and void [v] resulting from
764 bulging (arrow) of inner wall; slide SNSB-BSPG 2013 XV 208; scale bar = 10 μ m.
- 765 8: Specimen with (?outer and inner) non-hyphal wall separated entirely from mantle; slide
766 SNSB-BSPG 1965 I 322; scale bar = 10 μ m.
- 767 9: Large fungal spore containing specimen of *Z. vestitus*; slide SNSB-BSPG 1965 I 372; scale
768 bar = 25 μ m.
- 769 10: Specimen with spiny sphere in lumen; slide SNSB-BSPG 1965 I 348; scale bar = 10 μ m.
- 770 11: Detail of Pl. III, 10, focusing on spines of interior sphere; scale bar = 5 μ m.

771 12: Specimen with microorganism attached to outer surface [lower right of image] and several
772 penetration pegs extending into lumen (arrows); slide SNSB-BSPG 1965 I 373; scale
773 bar = 10 μm .
774 13,14: Specimens containing smaller spheres, probably reproductive units of intrusive
775 organisms; note distinct neck of interior sphere (arrow) in Pl. III, 13; slides SNSB-
776 BSPG 2013 XV 39 (Pl. III, 13) and 1965 I 365 (Pl. III, 14); scale bars = 10 μm .