

An unusual specimen of the enigmatic fungal reproductive unit *Windipila spinifera* from the Lower Devonian Rhynie cherts of Scotland

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Abstract

Windipila spinifera from the Rhynie cherts is a spheroidal microfossil enveloped in a hyphal mantle from which extend prominent spines and otherwise shaped projections. It is believed to be a reproductive unit of a fungus in the Glomeromycota or zygomycetes, but features to determine the systematic affinities have not hitherto been documented. This study describes a new specimen of *W. spinifera* that contains a single spherical structure from which a hypha arises that extends outside and terminates in what appears to be a sporangium. The specimen is reminiscent of germinated zygospores of the germ-sporangial type, and thus may suggest affinities of *W. spinifera* to the zygomycetes. However, the interior sphere and its outgrowth could also be a part of another organism that had invaded *W. spinifera*.

Keywords

fossil fungi, Glomeromycota, intrusive organisms, zygomycetes, zygospore germination

1. Introduction

The Lower Devonian Rhynie cherts (including the Rhynie and Windyfield cherts) of Scotland give detailed insights into fungal diversity in a terrestrial ecosystem c. 410 Ma ago (Taylor et al. 2015; Krings et al. 2017a; Strullu-Derrien et al. 2019). One important proxy indicator of this diversity is the morphological variety of fungal propagules and microscopic reproductive units, both of which are present in virtually every thin section of the cherts. However, dealing with these fossils is difficult because they mostly occur detached from the systems on or in which they were produced, and thus do not provide an inclusive comparison with present-day equivalents to determine their systematic affinities (Krings and Harper 2020).

Several types of fungal reproductive units have been described from the Rhynie cherts that all possess an ancillary covering in the form of a hyphal investment or mantle. Mantle morphology varies (considerably) among the different types, and thus renders them easy to distinguish from one another (Krings and Taylor 2013, 2014, 2015a, 2015b; Krings et al. 2014, 2016, 2017b; Krings and Harper 2017, 2018, 2020). One of these fossils is *Windipila spinifera* (Fig. 1A), a walled spheroid less than 150 µm in diameter that is enveloped in circumferential hyphae from which extend prominent, thin-walled spines and otherwise shaped projections (Krings and Harper 2017, 2018).

Most mantled fungal reproductive units from the Rhynie cherts, including *Windipila spinifera*, are believed to belong to either the Glomeromycota or the zygomycete fungi based on similar features in modern

lineages known to produce spores or sporangia with hyphal mantles (e.g., Krings et al. 2016, 2017a; Krings and Harper 2020). The occurrence of several specimens on what appear to be simple, tubular subtending hyphae has been used to suggest that *W. spinifera* developed asexually by blastic inflation and thickening of a hyphal tip, rather than sexually and following gametangial fusion, and for this reason might be a glomoid chlamyospore (Krings and Harper 2017). However, structural features to more precisely delimit the nature and systematic affinities of this fossil have not been documented to date.

This study describes a new specimen of *Windipila spinifera* from the Windyfield chert that contains a single spherical structure from which an unbranched hypha is given off that evidently passes through the wall and mantle and terminates in what appears to be a sporangium with a distal discharge papilla. While this specimen does also not clarify the affinities of *W. spinifera*, it resembles a germinated zygospore or azygospore with a tubular sporangiophore and distal germ sporangium, and thus is suggestive of affinities to the zygomycetes.

2. Geological setting

The Rhynie chert Lagerstätte is located in the northern part of the Rhynie outlier of Lower Old Red Sandstone in Aberdeenshire, Scotland, in an extensive sequence of sedimentary and volcanic rocks. The cherts occur in the Rhynie Block of the Dryden Flags Formation located northwest of the village of Rhynie. The Lagerstätte is made up of fossiliferous beds containing lacustrine shales and chert that have been interpreted as a series of ephemeral pools within a geothermal wetland, with alkali-chloride hot springs that were part of a complex hydrothermal system in a region affected by volcanic activity (Rice et al. 2002; Rice and Ashcroft 2004; Trewin and Fayers 2016; Channing 2017). The Windyfield chert site occurs ~700 m to the northeast of the original Rhynie chert site (Trewin and Rice 1992; Fayers 2003; Garwood et al. 2020). It was deposited in an area of hot-spring feeder activity based on the hydrothermally altered nature of its associated fluvio-lacustrine shales and sandstones; paleoenvironments ranged from terrestrial, vegetated aprons of laminated and brecciated sinter to low-temperature pools and marginal aquatic settings (Rice et al. 2002; Fayers 2003; Fayers and Trewin 2004). The Rhynie and Windyfield cherts are regarded as broadly coeval, i.e., 411.5 ± 1.3 to 407.6 ± 2.6 million years old according to Mark et al. (2011, 2013) and Parry et al. (2011), Pragian-earliest Emsian according to Wellman (2006, 2017) and Wellman et al. (2006). For details on the geology and development of the Rhynie chert Lagerstätte, as well as on the paleontology, refer to Trewin and Kerp (2017), Garwood et al. (2020), and the Rhynie chert volume edited by Edwards et al. (2018).

3. Material and methods

The specimen described below was identified in a thin section prepared by cementing a wafer of the Windyfield chert to a microscope slide, and then grinding the rock slice until it was sufficiently thin (i.e. c. 60–80 μm thick) to transmit light (for details on thin section preparation, refer to Hass and Rowe 1999). The slide is deposited in the SNSB-Bayerische Staatssammlung für Paläontologie und Geologie (SNSB-BSPG) in Munich, Germany, under accession number SNSB-BSPG 2016 VII 117. The specimen was studied using normal transmitted light microscopy; digital images were captured with a Leica DFC-480 camera and processed in Adobe Photoshop CS5. For comparison, another specimen of *Windipila spinifera* from the holdings of the SNSB-BSPG (accession number SNSB-BSPG 2016 VII 82) has also been illustrated.

4. Results

The specimen (Fig. 1B₁) is located in the chert matrix, within silicified litter comprised predominantly of fragmented and mostly heavily degraded land plant axes, dispersed land plant spores, unidentifiable debris, and fungal hyphae. The reproductive unit is spherical, roughly 145 μm in diameter (including mantle but excluding projections), and composed of a central cavity bounded by a non-hyphal wall up to 2 μm thick. No evidence has been found of pores or otherwise shaped orifices in the wall. The reproductive unit is enveloped in a hyphal mantle composed of 1–3 layers of tightly interlaced hyphae extending along the outer surface of the non-hyphal wall. Prominent projections, irregularly distributed over the entire surface, are given off from the circumferential hyphae in an outward direction. However, there are fewer projections in this specimen as compared to the specimens described previously (Fig. 1A_{1,2}; Krings and Harper 2017, 2018). The structure in vivo was probably positioned terminally on a lateral branch or outgrowth of a tubular hypha (see below). However, the attachment site is obscured by the mantle hyphae and most of the hyphal nexus is not recognizable due to the plane of the section through the specimen.

The reproductive unit contains a single, more or less spherical structure (henceforth called interior sphere) that is c. 55 μm in diameter, lacks structured contents, and is delineated by a smooth wall up to 0.75 μm thick. Its position within the cavity is highly eccentric in such a way that it comes into contact with, and in vivo perhaps adhered to, the inner surface of the non-hyphal wall of the reproductive unit. The interior sphere is somewhat flattened where it is in physical contact with this wall. No evidence of a subtending hypha or any other kind of parental structure has been found. However, the cavity of the reproductive unit contains shrivelled fragments of thin-walled hyphae or filaments (Fig. 1B₃). Arising from roughly the center of the flattened portion of the interior sphere is a narrow hypha 1–2 μm wide (arrows in Fig. 1C), which evidently passes through the non-hyphal wall of the repro-

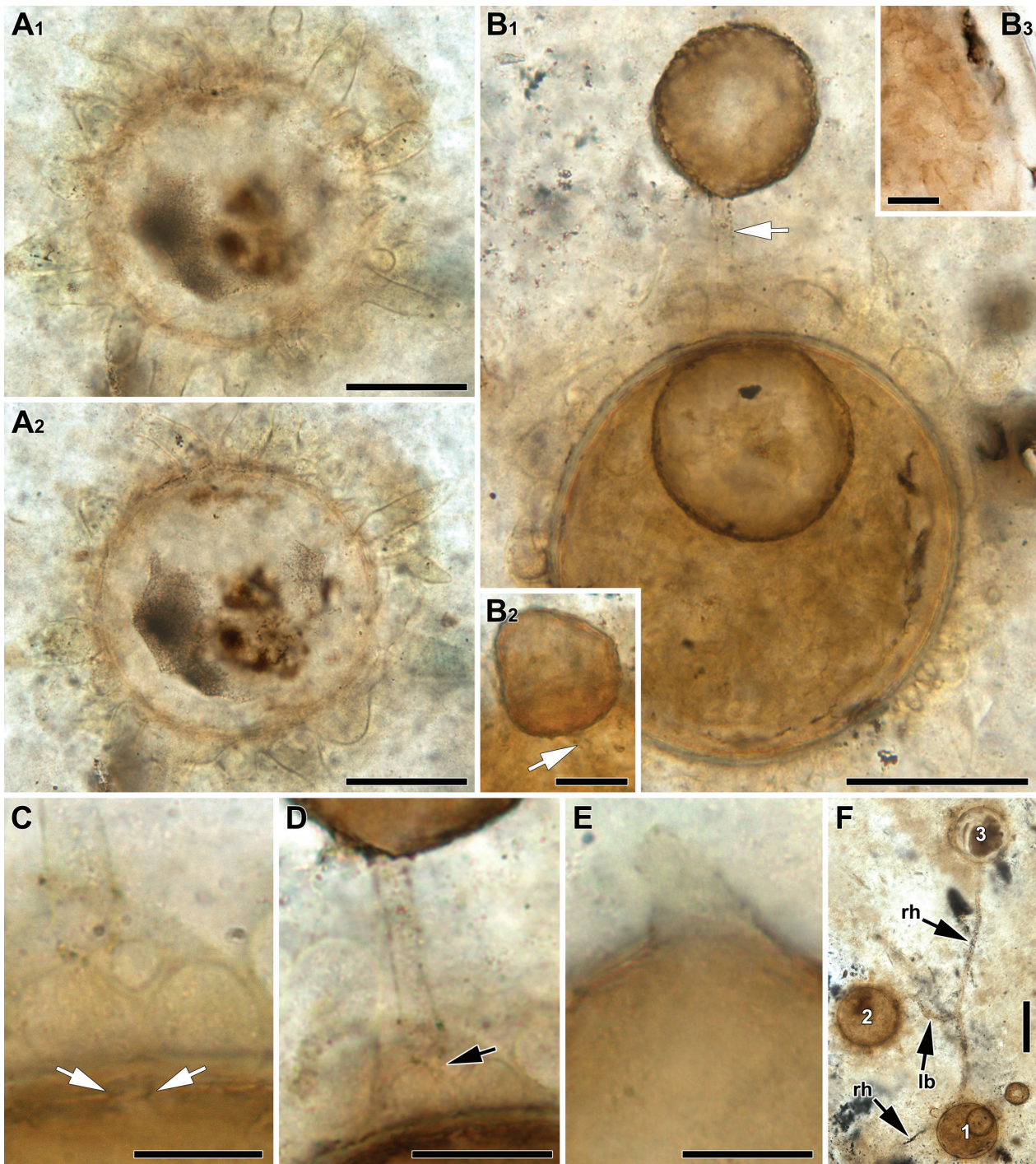


Figure 1. *Windipila spinifera* from the Lower Devonian Rhynie cherts (Windyfield chert). **A_{1,2}**, Well preserved specimen in two different focal planes (**A₁**: median optical section, **A₂**: section off center), with prominent spines and otherwise shaped projections extending from mantle (focal plane **A₁**, previously published by Krings and Harper 2018: fig. 1A). Slide SNSB-BSPG 2016 VII 82. **B–F**. New specimen. Slide SNSB-BSPG 2016 VII 117. **B₁**, Overview (median optical section), showing interior sphere in highly eccentric position and stalk-like hypha (arrow) terminating in exterior sphere. **B₂**, Second exterior structure in different plane; note attached fragment of putative parental hypha (arrow). **B₃**, Shrivelled hyphae or filaments in cavity of reproductive unit. **C**, Hypha arising from interior sphere (arrows). **D**, Stalk-like hypha becoming wider in periphery of mantle (arrow), and extending in outward direction. **E**, Papilla-like, distal putative discharge apparatus of exterior sphere. **F**, Hypha (rh) with three attached *W. spinifera* reproductive units (labeled 1–3), of which the one in the lower right of the image (labeled 1) is magnified in Fig. 1B₁; specimen 2 occurs on a lateral branch (lb) and 3 at the tip of the hypha. Scale bars: 10 μ m (**B₃**, **C**, **E**); 20 μ m (**B₂**, **D**); 50 μ m (**A₁**, **A₂**, **B₁**); 100 μ m (**F**).

ductive unit and proceeds on the outside into the mantle. Unfortunately, the exact path of the hypha through the wall and mantle is not traceable. The hypha reappears in the

periphery of the mantle where it expands to up to 6.5 μ m wide (arrow in Fig. 1D) and becomes tubular (stalk-like), and from there runs outwards c. 40 μ m into the ambience

(arrow in Fig. 1B₁). The hypha is unbranched and terminates in another spherical structure (henceforth called exterior sphere), which is 46 µm in diameter, bounded by a smooth wall, and does also not have structured contents. The exterior sphere is characterized by a single, distal, thin-walled papilla-like projection 8.5 µm high (Fig. 1E). A second exterior structure, c. 38 µm in diameter and somewhat asymmetrical or distorted, occurs in close proximity to the exterior sphere in a different plane of the specimen (Fig. 1B₂). This structure, which is partially embedded in the mantle of the reproductive unit, is subtended by a short fragment of what appears to be a hypha (arrow in Fig. 1B₂). As to whether this hypha also originates from the interior sphere cannot be determined.

The hypha (rh in Fig. 1F) that gives rise to the specimen described above (labeled 1 in Fig. 1F) bears two further *Windipila spinifera* reproductive units (labeled 2 and 3 in Fig. 1F), one of which occurs on a lateral branch (lb in Fig. 1F), while the other (partially degraded) appears to be positioned at the tip of the hypha itself. Because these latter specimens do not exhibit any new or unusual features, they will not be discussed here any further.

5. Discussion

Eight morphologically different types of mantled fungal reproductive units have so far been described from the Rhynie cherts, of which *Windipila spinifera* with its prominent mantle spines certainly is one of the more peculiar ones (Krings and Harper 2017, 2018). A similar, albeit less impressive mantle occurs in the little-known *W. pumila* (Krings and Harper 2018). A third type attributed to *Windipila*, *W. wimmervoeksii*, is characterized by mantle hyphae with vesicle-like inflations from which extend needle-like processes that connect the inner mantle tier with an outer tier of irregularly inflated and interwoven hyphae (Krings and Harper 2020).

Windipila has been suggested to have affinities to either the Glomeromycota or the zygomycete fungi (Krings and Harper 2020). One of the key features distinguishing these two groups of fungi is the sexual stage of the life cycle, which occurs in zygomycetes as a result of zygosporogenesis following gametangial fusion, but has not been observed in Glomeromycota (Benjamin 1979; Benny et al. 2001, 2012). Mature zygosporangia or zygospores with attached gametangia and/or suspensors are therefore the most important component of the zygomycete life cycle that can be used to positively identify a fossil member in this group of fungi (Krings et al. 2012, 2013). However, none of the *Windipila* species can presently be positively identified as a zygomycete because there is no evidence of gametangia and/or suspensors. Rather, several specimens of *W. spinifera* (Krings and Harper 2017: fig. 2) and *W. wimmervoeksii* (Krings and Harper 2020: figs 2d, 3a) appear to have developed on simple, tubular subtending hyphae. Moreover, hyphal anastomoses and H-branching, which are common in several lineages of

glomeromycotan fungi (Walker et al. 2018) but believed to be lacking or rare in zygomycetes (Gregory 1984; Glass and Fleissner 2006; Ivarsson et al. 2015), have been documented in *W. wimmervoeksii* (Krings and Harper 2020: figs 4a, b, 5).

The most interesting structural element of the only known specimen of *Windipila pumila* is a walled spherical structure that occurs in the cavity of this fossil (Krings and Harper 2018: fig. 2). Similar spheres have not hitherto been recorded in *W. spinifera* or *W. wimmervoeksii*, but they have been described in other Rhynie chert mantled fungal reproductive units, including *Scepasmatocarpion fenestrulatum* (Krings and Taylor 2015a: figs 1e, 2c) and *Zwergimyces vestitus* (Krings et al. 2016: pl. III, 2–8). The interior spheres have been used to speculate that these reproductive units could be sporangia or sporangiola, perhaps comparable to the zygosporangia of zygomycetes or the sporangial or sporangiolar layer(s) of certain glomeromycotan spore walls (see Walker et al. 2021: fig. 1), and the interior spheres either the zygosporangium or the sporangiospore proper (e.g., Krings and Harper 2018).

The fossil presented in this study provides the first evidence of the occurrence of interior spheres also in *Windipila spinifera*. Moreover, it exhibits several structural features pertaining to the interior sphere that have not been documented in any other Rhynie chert mantled reproductive unit. Said interior sphere gives off a hypha that exits the reproductive unit and terminates in another spherical structure, which is characterized by a papilla-like protrusion that most likely functioned as a discharge apparatus. There is a certain level of morphological correspondence between the fossil and certain zygomycetes of the order Mucorales in which zygosporangium germination entails the formation of a germ sporangium (e.g., Brefeld 1872; De Bary 1884; Blakeslee 1906; Gauger 1961: figs 1–3; Hocking 1967). During the sexual stage of the life cycle, these fungi produce zygosporangia (zspo in Fig. 2) containing single zygospores (zsp in Fig. 2) following gametangial fusion, and sometimes azygosporangia containing azygospores, which are similar to zygospores but form parthenogenetically without gametangial fusion (e.g., Benjamin and Mehrotra 1963; O'Donnell et al. 1977). The zygosporangium wall eventually ruptures and the zygosporangium germinates. If germination is of the germ-sporangial type (sensu Guo and Michailides 1998), then the zygosporangium produces a tube-like structure termed a sporangiophore or promycelium (sph in Fig. 2), on the tip of which a germ sporangium develops (spo in Fig. 2). After maturation of the spores (called germ sporangiospores), the germ sporangium ruptures and liberates the spores, which, if they fall on a suitable substrate, germinate and develop into a new mycelium (e.g., Cerdá-Olmedo 2001: fig. 1). It is conceivable that the *W. spinifera* specimen described above represents a zygosporangium or azygosporangium containing a zygosporangium or azygosporangium (i.e. the interior sphere), which has germinated and produced a sporangiophore (i.e. the stalk-like hypha) with a terminal germ sporangium (i.e. the exterior sphere). The second exterior

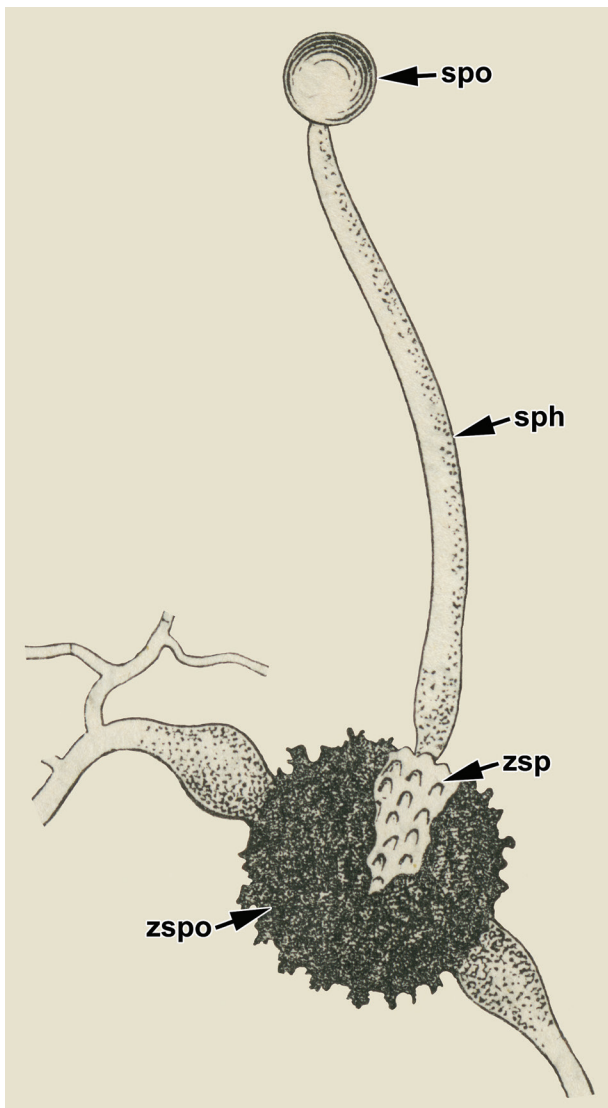


Figure 2. Germinated zygospore of *Mucor mucedo* (Mucorales); zspo = zygosporangium, zsp = zygospore, sph = sporangiophore or promycelium, spo = germ sporangium (modified from De Bary 1884: fig. 71C).

structure, which occurs partially embedded in the hyphal mantle of the reproductive unit, could be another germ sporangium. Germinated zygospores with two sporangiophores, each bearing a terminal sporangium, have also been reported in certain present-day zygomycetes (e.g., Michailides and Spotts 1988).

One might counter this comparison by pointing out that no evidence of gametangia and suspensors has been found in any of the *Windipila spinifera* specimens. It is possible, however, that these structures were small, inconspicuous, and entirely embedded in the mantle. For example, the zygosporangia of certain present-day *Mortierella* (Mortierellaceae, Mucorales) species are surrounded by extensive hyphal coverings arising from the suspensors or from hyphae at the base of the suspensors (Brefeld 1881: pl. V, figs 12, 14, 15; Kuhlman 1972; Ansell and Young 1983) that render the gametangia and suspensors very difficult to recognize. It can also be argued

that the interior sphere in *W. spinifera* is too small to be a zygospore or azygospore because zygosporogenesis involves the development of a complex, multi-layered wall (i.e. the combined sporangium and spore wall) in which newly deposited wall layers follow exactly the shape of the previously deposited layers (e.g., Brefeld 1872: pl. II, fig. 20; Hawker and Gooday 1968; Hawker and Backett 1971; O'Donnell et al. 1978). Rather, the interior sphere is somehow reminiscent of the single oil globules that are visible in the zygospores of, for instance, certain representatives of the mucoralean genera *Radiomyces* (Radiomycetaceae; e.g., Embree 1959: figs 12, 13; Benny and Benjamin 1991: figs 8, 16, 18, 29h, i) and *Dichotomocladium* (Lichtheimiaceae; e.g., Benny and Benjamin 1993: fig. 4b). However, it seems unlikely that oil globules would become preserved in a recognizable form. It can also not be ruled out that the spore proper in *W. spinifera* has shrunken during germination, and again during the silicification process. One must furthermore take into account the possibility that entirely fossil lineages of zygomycete fungi, as well as fossil representatives of present-day lineages, have existed that were characterized by traits unknown in any present-day representative. For example, the germ sporangium in present-day Mucorales ruptures at maturity to release the spores, while the fossil spores likely were liberated through a distal discharge papilla.

An alternative hypothesis views the interior sphere in *Windipila spinifera* as a part or life cycle stage of some other fungus or fungus-like organism that had invaded and colonized the reproductive unit, and reproduced by producing a sporangium (or sporangia?) outside of the host. Two lines of evidence seem to support this hypothesis. First, co-occurring with the interior sphere in the cavity of the *W. spinifera* specimen are fragments of hyphae or filaments, which indicate that, at some point, another fungus has been present in this structure, unless the mantle hyphae had invaded the cavity from the circumference (see below). Second, several different types of small spherules occurring singly or in groups, and probably representing the reproductive units of intrusive fungi, have been reported in the cavity of *Zwergimyces vestitus* (Krings et al. 2016), and thus indicate that mantled fungal reproductive units in the Rhynie paleoecosystem have been invaded by other microorganisms and used as a habitat. On the other hand, there is no evidence of a physical connection between the interior sphere in *W. spinifera* and any system on which it could have developed. This argues against the hypothesis that the sphere belongs to an intrusive organism. It is also possible that the *W. spinifera* specimen had already fulfilled its function, namely to produce a spore that subsequently germinated, and, consequently, was in the process of natural decay at the time of fossilization. If this is the case, then the hyphae inside the cavity could belong to saprotrophic fungi or even be outgrowths from the mantle hyphae that invaded the senescing reproductive unit and participated in its degradation.

6. Conclusions

The fossil record rarely gives comprehensive insights into the life history of fungi, which is unfortunate because life history stages can provide valuable information to assess the systematic affinities of fossil fungi in the absence of molecular data (Taylor et al. 2015). The affinities of the organism that produced *Windipila spinifera* mantled reproductive units remain conjectural, in spite of the fact that the fossil detailed above shares certain features with germinated zygospores of the germ-sporangial type. I hope that specimens showing these features in greater detail become available as exploration of the Rhynie cherts continues, and that these fossils will clarify the nature of the interior sphere and reveal hitherto unknown aspects, which can then be used to determine the systematic position of *W. spinifera*.

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