

Morphological diversity of fungal reproductive units in the Lower Devonian Rhynie and Windyfield cherts, Scotland: a new species of the genus Windipila

Michael Krings & Carla J. Harper

PalZ

Paläontologische Zeitschrift

ISSN 0031-0220

PalZ

DOI 10.1007/s12542-019-00507-5



Your article is protected by copyright and all rights are held exclusively by Paläontologische Gesellschaft. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".



Morphological diversity of fungal reproductive units in the Lower Devonian Rhynie and Windyfield cherts, Scotland: a new species of the genus *Windipila*

Michael Krings^{1,2,3} · Carla J. Harper^{1,3,4}

Received: 9 October 2019 / Accepted: 7 November 2019

© Paläontologische Gesellschaft 2019

Abstract

The Lower Devonian Rhynie and Windyfield cherts from Scotland contain a remarkable diversity of microscopic fungal propagules and reproductive units; however, only relatively few of these fossils are described. One of them is *Windipila spinifera*, an unusual reproductive unit from the Windyfield chert that consists of a walled spheroid (~ 100 µm in diam.) surrounded by a mantle of interlaced hyphae; prominent spines and otherwise shaped projections, produced by these hyphae, extend out from the mantle. Here, we present *W. wimmervoecksi* sp. nov., also from Windyfield, which differs from *W. spinifera* in that the mantle hyphae produce vesicle-like inflations instead of spines. Narrow processes arising from the inflations connect the persistent inner mantle tier with a probably ephemeral outer tier of irregularly inflated hyphae. There is some evidence to suggest that *W. wimmervoecksi* was a member of the Glomeromycota; however, the precise systematic affinities remain unresolved. This discovery broadens our understanding of the patterns of mantle formation that were present in fungi by the Early Devonian.

Keywords Fossil fungi · Fungal paleodiversity · Hyphal mantle · Glomeromycota · Reproduction · Subtending hypha

Introduction

The Early Devonian Rhynie chert Konservat-Lagerstätte (here including the Rhynie and Windyfield cherts) in Aberdeenshire, Scotland, has provided detailed insights into the diversity and biology of fungi in a continental paleoecosystem approximately 410 Ma old (Taylor et al. 2015). One

important proxy indicator of fungal diversity in the Rhynie paleoecosystem is the abundance and morphological diversity of microscopic propagules and reproductive units, which occur in virtually all thin sections of the chert (Kidston and Lang 1921; Krings et al. 2017a). However, dealing with these remains is notoriously difficult because they usually 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 and Harper 2017). Only a few forms occur in characteristic configurations or possess special features that make it possible to recognize distinctiveness and sometimes even reveal affinities (e.g., Dotzler et al. 2009). Consequently, one important first step in assessing fungal diversity in the Rhynie paleoecosystem is to report the occurrence of distinctive morphologies and document their features as thoroughly as possible (Krings et al. 2017b).

An ancillary covering in the form of a hyphal investment or mantle characterizes several types of Rhynie and Windyfield chert fungal reproductive units. Mantle morphology varies considerably among the different types, and thus renders them easy to distinguish from one another (Kidston and Lang 1921; Krings et al. 2014, 2016, 2017b; Krings

Handling Editor: Mike Reich.

✉ Michael Krings
krings@snsb.de

¹ SNSB-Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Straße 10, 80333 Munich, Germany

² Department für Geo- und Umweltwissenschaften, Paläontologie und Geobiologie, Ludwig-Maximilians-Universität, Richard-Wagner-Straße 10, 80333 Munich, Germany

³ Department of Ecology and Evolutionary Biology, Natural History Museum and Biodiversity Institute, University of Kansas, Lawrence, KS 66045-7534, USA

⁴ Botany Department, School of Natural Sciences, Trinity College Dublin, Dublin 2, Ireland

and Taylor 2013, 2014, 2015a, b; Krings and Harper 2017, 2018a). The most unusual of these fossils, *Windipila spinifera* M. Krings et C. J. Harper, possesses a mantle of interlaced circumferential hyphae, and prominent, thin-walled spines and otherwise shaped projections extending out from the mantle (Krings and Harper 2017, 2018a). A similar mantle morphology occurs in *Windipila pumila* M. Krings et C. J. Harper; however, this fossil is only half the size of *W. spinifera*, and the projections are less prominent (Krings and Harper 2018a).

This paper describes *Windipila wimmervoecksi* sp. nov., a reproductive unit from the Windyfield chert that corresponds to *W. spinifera* in basic organization, but differs in that the circumferential mantle hyphae are characterized by prominent, vesicle-like inflations from which may extend delicate, needle-like processes. Moreover, the longest of these processes connect the persistent inner mantle tier with a probably ephemeral outer tier of multi-branched, irregularly inflated and interwoven hyphae. The new fossil contributes to our knowledge of the morphological diversity of fungi in the Early Devonian Rhynie ecosystem.

Geological setting

The Rhynie chert Lagerstätte is located in the northern part of the Rhynie outlier of the Lower Old Red Sandstone in Aberdeenshire, Scotland, within an extensive sequence of sedimentary and volcanic rocks. The chert deposits occur in the Rhynie Block of the Dryden Flags Formation, located northwest of the village of Rhynie. The Lagerstätte consists of fossiliferous beds containing lacustrine shales and chert that have been interpreted as a series of ephemeral freshwater pools within a geothermal wetland (Trewin and Kerp 2017), with alkali-chloride hot springs that were part of a complex hydrothermal system in a region affected by volcanic activity (Trewin and Fayers 2016; Channing 2017). Preserved within the chert are both aquatic (freshwater) facies from the pools and subaerial soil/litter horizons with in situ plants that are hypothesized to have grown along the margins of the pools. Preservation of the biota is thought to have taken place as a result of temporary flooding of silica-rich water, or by groundwater high in silica percolating to the surface (Powell et al. 2000).

The Windyfield chert occurs some 700 m to the northeast of the original Rhynie chert site (Fayers 2003). 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 (Trewin and Rice 1992; 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), which is in broad agreement with spore assemblages indicating that the cherts date from the middle Pragian to lower Emsian stages of the Lower Devonian according to Wellman (2006, 2017) and Wellman et al. (2006). For details on the geology, development, and paleontology of the Rhynie chert Lagerstätte, refer to Trewin and Kerp (2017), Edwards et al. (2018), and Garwood et al. (2019).

Materials and methods

The specimens used in this study were identified in a series of nine thin sections prepared from a single block of Windyfield chert by cementing wafers of the chert to glass slides, and then grinding the rock slices until they were thin enough to transmit light but still between 90 and 120 μm thick. While this may not be ideal for the analysis of plant tissues, it is generally advantageous for the study of fungi since thicker thin sections contain a higher percentage of complete individuals that provide information on the exact size and shape of the organisms (Krings et al. 2010; Taylor et al. 2011). The block and slides are deposited in the SNSB-Bayerische Staatssammlung für Paläontologie und Geologie (SNSB-BSPG) in Munich, Germany, under accession number SNSB-BSPG 2017 XXVI. Slides were studied using normal transmitted light microscopy; digital images were captured with a Leica DFC-480 camera and processed in Adobe Photoshop CS5. For comparison, specimens of *Windipila spinifera* from the holdings of the SNSB-BSPG (accession number SNSB-BSPG 2016 VII) have also been illustrated. Slide numbers for the specimens illustrated are included in the figure captions.

Description

Context

Three of the nine thin sections prepared from Windyfield chert block SNSB-BSPG 2017 XXVI have yielded a total of 12 well preserved and > 10 incomplete and/or (partially) degraded specimens of the fungal reproductive unit that is described in the following paragraphs. Specimens occur singly or in loose groupings of 2–4 individuals (arrows in Figs. 1a, c, 2a–c) within a dense accumulation of land plant (likely *Nothia aphylla* Lyon ex El-Saadawy et Lacey) axes (“lpa” in Fig. 1a) in which the xylem strand (“xy” in Fig. 1b, c) and cuticle (“cu” in Fig. 1b) are still partially intact and in place, while the epidermis and cortical tissues (“co” in Fig. 1b) are (largely) lost. The reproductive units

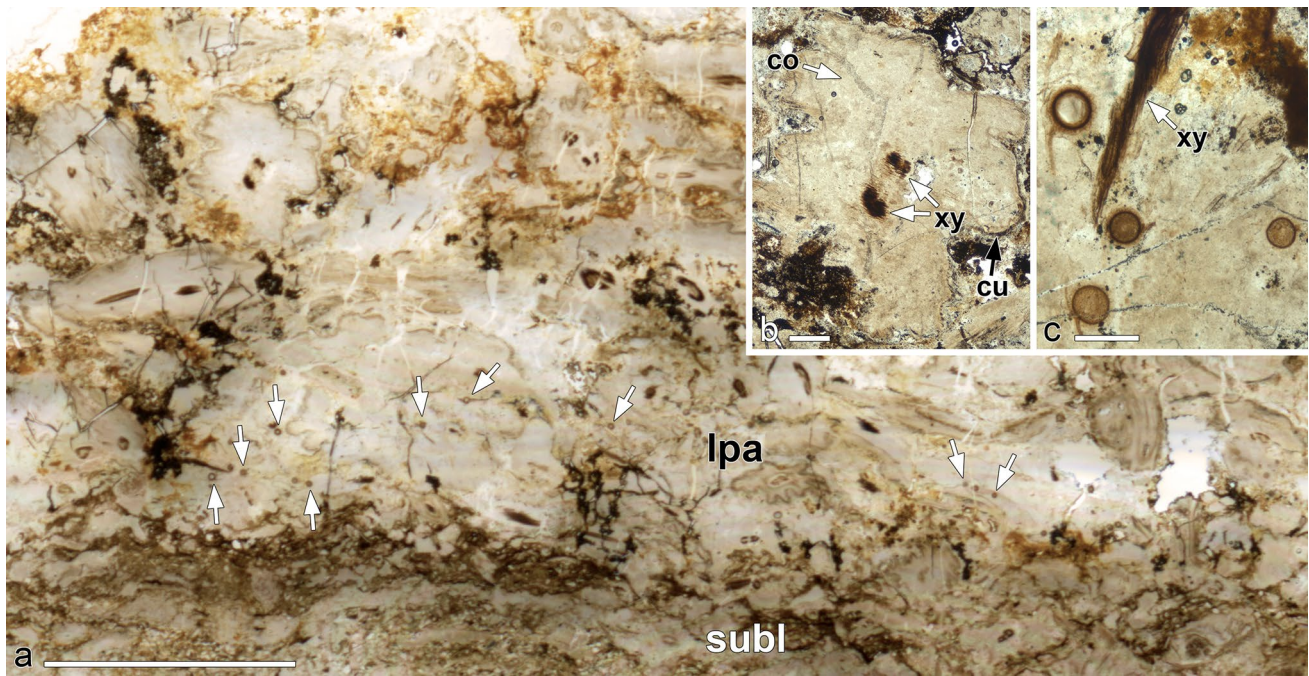


Fig. 1 Thin section SNSB-BSPG 2017 XXVI 3. **a** Accumulation of land plant axes (lpa) containing *Windipila wimmervoecksi* sp. nov. (arrows); *subl* substrate layer traversed by land plant axes; scale bar 5 mm; **b** detail of **a**, plant axis (transverse section) with xylem strand

(xy) and cuticle (cu) still partially intact and in place, and epidermis and cortical tissues (co) largely lost; scale bar 200 μ m. **c** Detail of **a**, *W. wimmervoecksi* sp. nov. in plant axis, located close to vascular strand (xy), within what used to be cortical tissue; scale bar 200 μ m

are located in the plant axes, typically in regions of the axis that used to be cortex, and often in close proximity to vascular strands (white arrows in Figs. 1c, 2a, b). Co-occurring with the land plant axes are various types of fungal hyphae, scattered land plant spores, unidentifiable organic matter, and abundant sediment particles. The axes containing the fungal reproductive units rest upon a substrate layer that also contains degraded (and usually somewhat compressed) land plant axes (“*subl*” in Fig. 1a). All fossils are preserved as three-dimensional petrifications.

Overall morphology

The reproductive units (Figs. 1c, 2a–e, g, 3a, k, l) are spheroidal, 120–160 μ m in diameter (including inflations but excluding processes and outer mantle tier; see below), and composed of a central cavity up to 122 μ m in diameter that is bounded on the outside by a wall 2–7 μ m thick (“*cw*” in Fig. 2g). The cavity is enveloped in a prominent mantle that occurs in the form of a one- or two-layered (rarely three-layered) system (up to 30 μ m thick; “*it*” in Fig. 3h, i) of interlaced septate hyphae (arrows in Figs. 2f, 3b), which all extend along the circumference of the structure; moreover, hyphal inflation and subsequent formation of prominent projections represents a major component of the mantle formation process. All specimens are empty or contain amorphous matter, with the exception of the one shown in Fig. 2g that

contains a large spheroidal body (~94 μ m in diameter) with a wrinkled and blurred outer demarcation. Degraded specimens differ from the intact ones in that the mantle is collapsed or partially missing (Fig. 4a, b).

Mantle

Circumferential mantle hyphae (Figs. 2f, 3b, c; black arrows in Fig. 3h, i) are 3–6(–6.5) μ m wide and relatively thick-walled (wall thickness up to 1 μ m); they are circular, oval, triangular, or dome-shaped in cross section if located directly adjacent to the cavity wall, and mostly circular elsewhere. Septa are relatively common (e.g., arrows in Figs. 2f, 3b). Emerging from the mantle are prominent projections in the form of blister-like vesicles (up to 23 μ m high and <10–18 μ m in diameter in top view; see Figs. 2f, 3a₁, j, k), which are derivatives of the circumferential hyphae that develop as intercalary and terminal hyphal inflations (Figs. 2e_{1,2}, 3d). Projections are loosely to densely spaced, regularly distributed over the entire surface of the reproductive unit, and highly variable in size and shape; they are predominantly near-spherical, prolate or oblate spheroidal or ellipsoidal, drop-shaped, or irregular, but some may also be rod- to column-like or, rarely, conical (black arrow in Fig. 3a₂). The conditions of the inflations vary from distended (e.g., Fig. 2e₁) to somewhat flaccid (e.g., Fig. 3l), probably reflecting different levels of turgescence of these

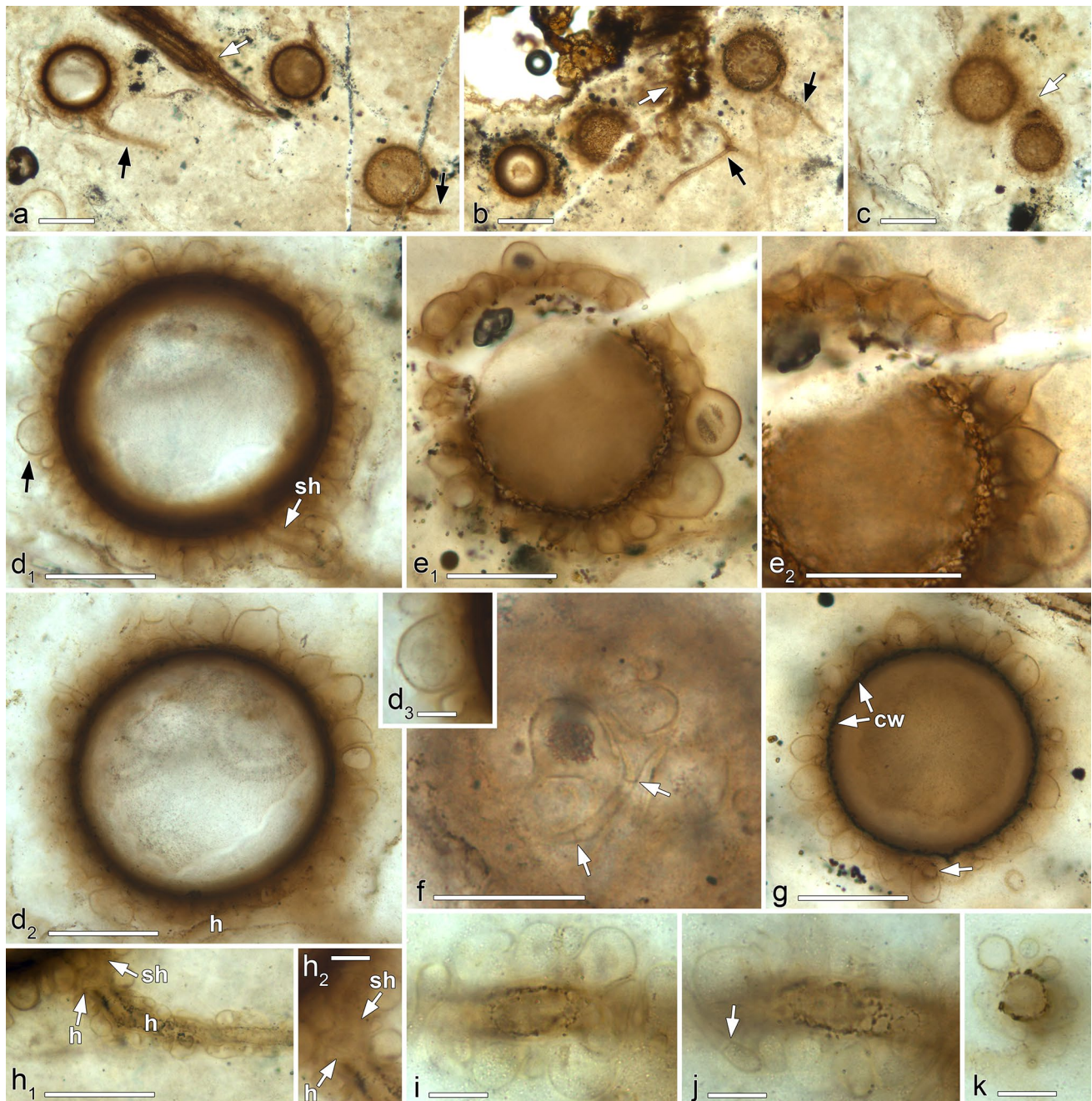


Fig. 2 *Windipila wimmervoeckii* sp. nov.—morphology; slide SNSB-BSPG 2017 XXVI 3. **a, b** Specimens in degraded land plant cortical tissue; white arrows indicate remains of vascular tissue, black arrows indicate subtending and associated hyphae; scale bars 100 μ m. **c** Specimens conjoined by hyphal meshwork (arrow); scale bar 100 μ m. **d_{1,2}** Detail of **a**, holotype in two different focal planes; *sh* subtending hypha, *h* prominent hyphal branch (details of hyphal attachment in **h_{1,2}**); black arrow indicates inflation containing ovoid inclusions; scale bars 50 μ m. **d₃** Detail of **d₁**, inflation with inclusions; scale bar 10 μ m. **e_{1,2}** Specimen with large inflations, many of which bearing distal processes (especially in **e₂**); scale bars 50 μ m.

f Top view of mantle, showing septate circumferential mantle hypha (arrows) and several vesicle-like inflations; scale bar 50 μ m. **g** Specimen showing cavity wall (cw) and mantled subtending hypha in cross section (arrow); scale bar 50 μ m. **h₁** Mantled hypha (*h*) that forks into a subtending hypha (*sh*; continuation of the branch denoted “*sh*” in **d₁**) and a prominent branch (*h*; continuation of the branch denoted “*h*” in **d₂**); scale bar 50 μ m. **h₂** Detail of **h₁**, subtending hypha (*sh*) and hyphal branch (*h*); scale bar 10 μ m. **i, j** Portions of mantled hyphae; arrow in **j** indicates septum; scale bars 10 μ m. **k** Hypha surrounded by mantle with inflations in cross section; scale bar 10 μ m

structures at the time of fossilization. Some of the inflations possess a rounded tip, while others are mucronate (Fig. 2d₂, e₂, g). Still others give off one or two distally tapering processes (prolongations), which typically are distinctly thinner-walled than the structure from which they arise (Figs. 2e₂, 3e, f); a septum usually occurs at the base of the process (e.g., arrow in Fig. 3f). Most processes are simple, straight, (slightly) bent, or kinked, needle-like structures, 2.3 µm wide proximally and up to 14 µm long; some, however, are considerably longer (up to 35 µm), one to several times septate, and may branch distally (Fig. 3g; white arrows in Fig. 3i). These latter processes in vivo gave rise to (or were part of) a second, outer mantle tier (10 to > 25 µm thick) constructed of loosely interwoven, multi-branched and irregularly inflated hyphae (“ot” in Fig. 3h, i), which were probably ephemeral because the available evidence of the outer mantle tier in these otherwise so well preserved structures is exceedingly scanty and limited to small patches in two different specimens. Two of the reproductive units that are located directly adjacent to one another are conjoined by a confluent, gleba-like hyphal meshwork extending from one mantle to the other (white arrows in Figs. 2c, 3l). The meshwork is very similar, if not identical in morphology to the outer mantle tier seen in two specimens occurring singly.

Subtending hypha

The reproductive units are positioned terminally on relatively thick-walled (wall thickness up to 2 µm), tubular hyphae, which are 4.5–12 µm in diameter beneath the spheroid (Fig. 4a; “sh” in Figs. 2d₁, h_{1,2}, 4b₄); some are characterized by a conspicuous kink (black arrows in Figs. 3l; 4a, b₄). It is often difficult to trace the point of origin and length of the subtending hypha, as well as the configuration of the hyphal nexus on which the reproductive unit is borne, because the subtending structures are buried within, and hence obscured by, the mantle (e.g., white arrow in Fig. 2d₁, g; black arrows in Fig. 3l). Several of the specimens suggest that the subtending hypha proper (e.g., “sh” in Figs. 2d₁, h_{1,2}, 3a₂) either represents a short lateral branch or outgrowth of a tubular running hypha (e.g., “h” in Figs. 2d₂, h_{1,2}, 3a_{1–3}), or gives off one prominent lateral branch immediately beneath (or at some distance away from) the terminal spheroid. In addition, one partially degraded specimen lacking part of the mantle (Fig. 4b_{1–5}) shows a subtending hypha (“sh” in Figs. 4b₅, 5a) arising from the immediate vicinity of an H-connection (“Hb” in Figs. 4b₂, 5a) and what most likely is an anastomosis (“A” in Figs. 4b₄, 5a [contact area enlarged in Fig. 5b]) between two hyphal segments (“h₁” and “h₂” in Figs. 4b_{1,3}, 5a). Subtending hyphae (e.g., “sh” in Fig. 2d₁; white arrow in Fig. 2g; black arrows in Figs. 3l, 4a), as well as their associated running hyphae (e.g., black arrows in Fig. 2a, b, h–k; “h” in Fig. 3a₃), are usually (but

not always; e.g., see “h” in Fig. 2d₂) enveloped in a mantle, which consists of tenuous, colorless hyphae that are septate (arrow in Fig. 2j) and characterized by prominent, sac- or vesicle-like inflations (Fig. 2i–k). The inflations are densely spaced, up to 16 µm high, and vary in shape from spheroidal or bulb-shaped to irregular (in section view); they are generally smaller than the inflations produced by the hyphae sheathing the reproductive unit. No evidence has been found of pointed tips or elongate processes in any of these inflations.

Additional observation

A few of the inflations (< 10) contain single specimens or small clusters of thin-walled ovoid inclusions ~4.5 × 6 in size (Fig. 2d₃; black arrows in Figs. 2d₁, 3c).

Systematic paleomycology

Fungi incertae sedis

Genus *Windipila* M. Krings et C. J. Harper, 2017 (MB 821334)

Type species. *Windipila spinifera* M. Krings et C. J. Harper, 2017 (MB 821335)

Windipila wimmervoecksii sp. nov.

Figures 1c, 2, 3, 4a, b, 5

Mycobank. MB 832439

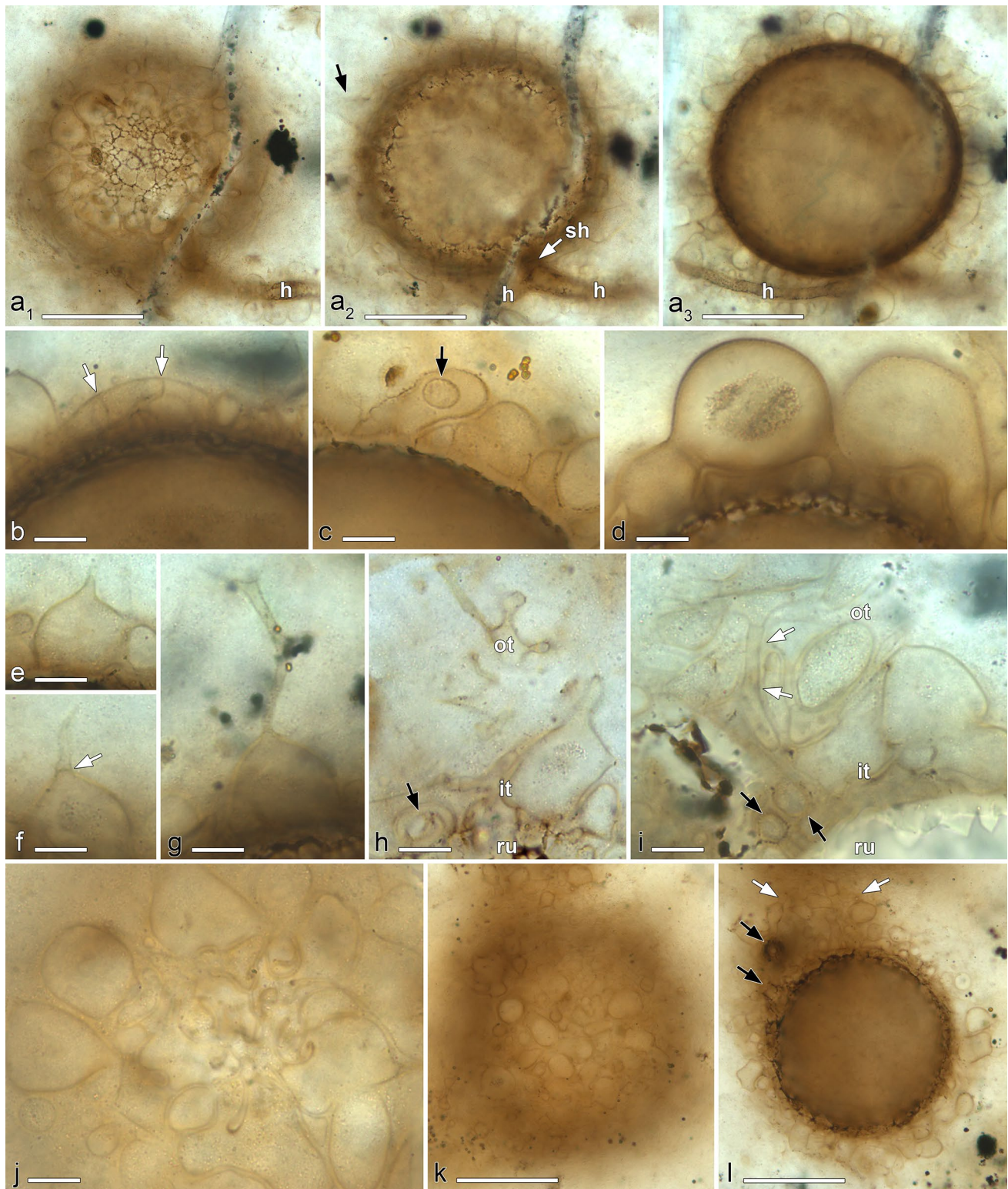
Etymology. The epithet is proposed in honor of Messrs Edgar Wimmer and Helmut Voecks of Munich, Germany, for their continued support of our work on the Rhynie chert.

Holotype. Specimen illustrated in Fig. 2d (details of hyphal attachment in Fig. 2h–j); in slide SNSB-BSPG 2017 XXVI 3, SNSB-Bayerische Staatssammlung für Paläontologie und Geologie, Munich, Germany.

Type locality. Windyfield chert site (National Grid Reference NJ 4977 2825), ~700 m NE of original Rhynie chert site of Special Scientific Interest, Aberdeenshire, Scotland (National Grid Reference NJ 494276).

Age. Early Devonian; early (but not earliest) Pragian to earliest Emsian (Wellman 2006, 2017), 411.5 ± 1.3 Ma (Parry et al. 2011), 407.1 ± 2.2 Ma (Mark et al. 2011).

Diagnosis. Reproductive unit > 100 and < 200 µm in diameter (including inner mantle tier); cavity wall distinct; inner



mantle tier up to 30 μm thick, composed of 1–2(–3) layers of interlaced, regularly septate hyphae characterized by prominent projections in the form of intercalary and terminal blister- or vesicle-like inflations; inflations loosely to densely spaced, regularly distributed over entire surface of

reproductive unit, variable in size and shape, often with one or two thin-walled, simple, straight or bent needle-like processes; longest processes septate, often distally branched, connecting inner mantle tier with probably ephemeral outer mantle tier (not normally preserved) of multi-branched,

Fig. 3 *Windipila wimmervoeckii* sp. nov.—Morphology; slide SNSB-BSPG 2017 XXVI 3 unless otherwise stated. **a**_{1–3} Specimen in three focal planes, from top view to median cross section; note mantled hypha (“h” in **a**₁) that forks into a short subtending hypha (sh) and a prominent branch (left letter “h” in **a**₂), the continuation of which is denoted “h” in **a**₃; black arrow in **a**₂ shows rare conical inflation; scale bar 50 µm. **b** Circumferential mantle hypha with septa (arrows); scale bar 10 µm. **c** Elongate inflation with ovoid inclusion (arrow), and several circumferential mantle hyphae in cross section; scale bar 10 µm. **d** Detail of Fig. 2e₁ (slightly different focal plane), circumferential mantle hypha with inflations; scale bar 10 µm. **e, f** Inflations bearing needle-like processes; note septum (arrow) at base of process in **f**; scale bars 10 µm. **g** Long, distally branched process; scale bar 10 µm. **h, i** Portions of mantle in which also the outer tier is preserved; black arrows indicate mantle hyphae in cross section, white arrows show long process connecting inner and outer mantle tiers; *ru* reproductive unit, *it* inner mantle tier, *ot* outer mantle tier; slide SNSB-BSPG 2017 XXVI 1; scale bars 10 µm. **j** Top view of specimen in **a** (magnified), showing densely spaced inflations; scale bar 10 µm. **k** Detail of Fig. 2c (different focal plane), reproductive unit in top view; scale bar 50 µm. **l** Detail of Fig. 2c (different focal plane), reproductive unit in median section view, and gleba-like meshwork (white arrows); black arrows indicate subtending hypha immersed in mantle of reproductive unit; scale bar 50 µm

irregularly inflated and interwoven hyphae; clustered specimens may be embedded in confluent hyphal meshwork; subtending hypha tubular, sometimes with a conspicuous kink; subtending hypha and their associated running hyphae usually (but not always) surrounded by mantle composed of thin-walled septate hyphae with sac- or vesicle-like intercalary inflations; pointed tips or elongate processes absent in these inflations.

Discussion

The fossil record of mantled fungal reproductive units currently includes seven different types from the Lower Devonian Rhynie and Windyfield cherts, seven or eight from the Carboniferous of Europe and North America, and at least three from the Triassic of Antarctica (surveyed in Krings et al. 2013a, 2017a; Taylor et al. 2015). Mantled reproductive units resembling these fossils in varying degrees occur in two extant fungal groups, namely in the form of invested/mantled zygosporangia (and azygosporangia) in the zygomycete fungi (e.g., Bucholtz 1912; Yao et al. 1996), and in the form of mantled chlamydospores in the Glomeromycota (or AMF = arbuscular mycorrhizal fungi; e.g., Błaszkowski 2012). Consequently, the vast majority of fossil types, including *Windipila* (see Krings and Harper 2017, 2018a), have also been interpreted as belonging to either of these groups of fungi; however, only a few (e.g., *Halifaxia taylorii* M. Krings, J. F. White, Dotzler et C. J. Harper, *Jimwhitea circumtecta* M. Krings et T. N. Taylor) have been assigned with confidence based on the presence of structures indicative of gametangial fusion (White and Taylor 1989; Krings

and Tayler 2012; Krings et al. 2012, 2013b), while most do not permit further delimitation of their systematic position. Moreover, the complex patterns of mantle formation that occur in some of the fossil types (e.g., *Helmutella devonica* M. Krings et T. N. Taylor [2014: fig. 4], *Mycocarpon asterium* T. N. Taylor et J. F. White [1989: fig. 15]) are not known in present-day zygomycetes or Glomeromycota.

Comparisons and attribution

The Windyfield chert fossils presented in this study differ in mantle morphology from all previously described fossil mantled fungal reproductive units; however, a high level of morphological congruence exists to *Windipila spinifera* (Fig. 4c–g), the largest mantled reproductive unit hitherto known from the Rhynie and Windyfield cherts (Krings and Harper 2017, 2018a). Several morphological features of the new fossil correspond to key traits defining the genus *Windipila* and its type species, *W. spinifera*, including (1) the mantle of interlaced septate hyphae extending along the circumference of the structure; (2) the prominent projections, which develop as intercalary and terminal inflations and lateral outgrowths of the circumferential mantle hyphae and represent the most important element in the visual appearance of the mantle; (3) narrow processes or branches arising from the projections; and (4) the tubular subtending hypha sheathed (at least in part) by a mantle (Table 1).

The new form differs from *Windipila spinifera* chiefly in that the projections produced by the mantle hyphae typically occur in the form of vesicle- or blister-like inflations, while they are mostly spine-like or otherwise elongate structures in *W. spinifera* (Fig. 4c–e). However, projections identical to the ones characteristic of the new form can, very rarely, also occur in *W. spinifera* (Fig. 4g; Krings and Harper 2017: fig. 1E, M) and vice versa (e.g., black arrow in Fig. 3a₂). This raises the question as to whether both forms were perhaps morphological variants of one biological species? Nothing is known about the range of morphological plasticity of fossil fungal reproductive units. However, *W. spinifera* occurs in the chert matrix (Krings and Harper 2017, 2018a), while all specimens of the new form are located within land plant axes. It is, therefore, possible that mantle morphology appears as a function of the surrounding in which the structure develops, and that the surrounding plant tissue perhaps prohibited the formation of elongate spines in the new form or made them unnecessary.

Vesicle-like inflation of circumferential mantle hyphae has also been observed in two other Rhynie chert fungal reproductive units, namely *Zwergimyces vestitus* (Kidston et W. H. Lang) M. Krings et T. N. Taylor (Krings and Taylor 2013: pl. I, 6) and an unnamed form that occurs in small clusters in land plant tissue (Krings and Taylor 2015b: fig. 11a). However, these fossils are both considerably

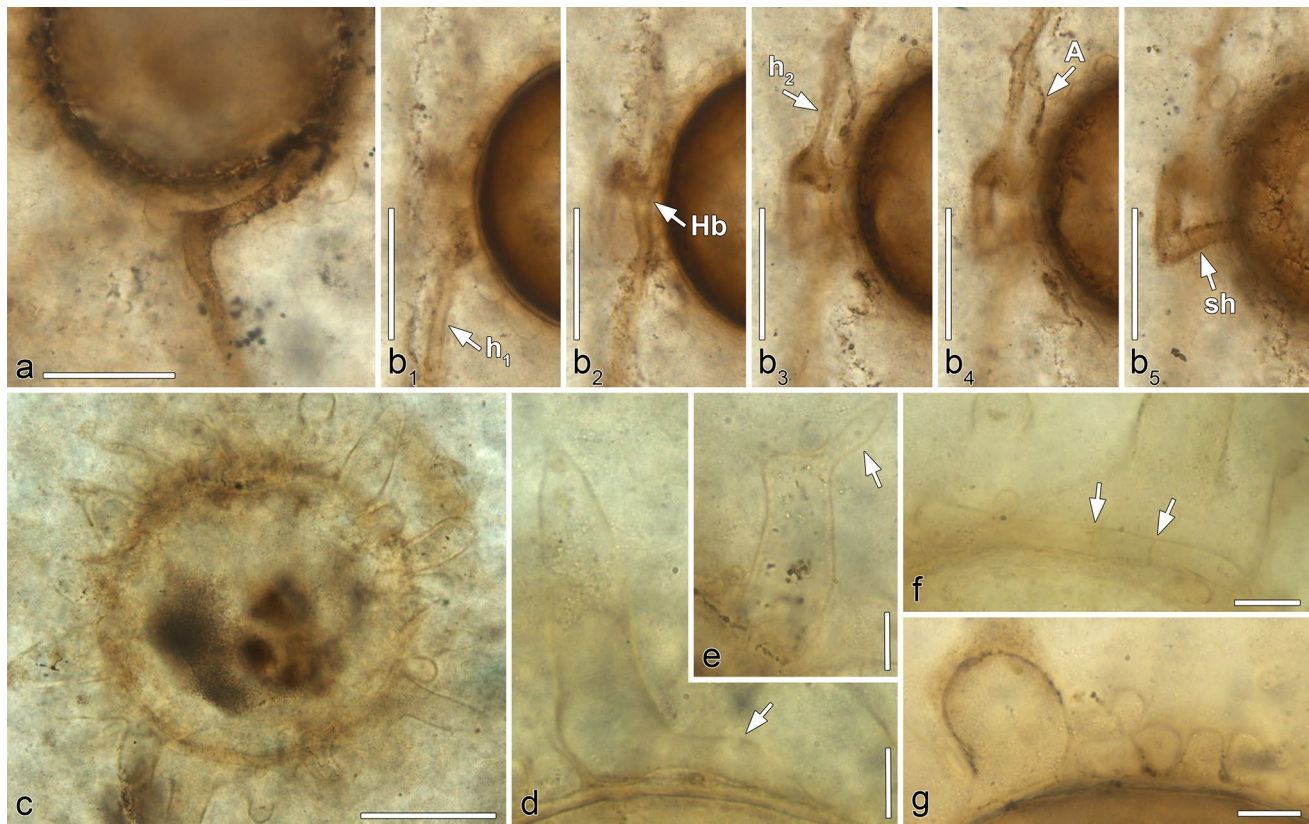


Fig. 4 *Windipila wimmervoecksi* sp. nov.—hyphal attachment; slide SNSB-BSPG 2017 XXVI 3; and *W. spinifera* M. Krings et C. J. Harper—morphology; slide SNSB-BSPG 2016 VII 82 unless otherwise stated. **a, b** *Windipila wimmervoecksi* sp. nov. **a** Detail of Fig. 2b, showing kinked subtending hypha enveloped in colorless mantle hyphae with inflations; scale bar 50 μ m. **b₁₋₅** Partially degraded specimen in five different focal planes; reproductive unit is positioned terminally on kinked subtending hypha (sh), which arises from a hyphal nexus (graphic presentation in Fig. 5a, b) composed

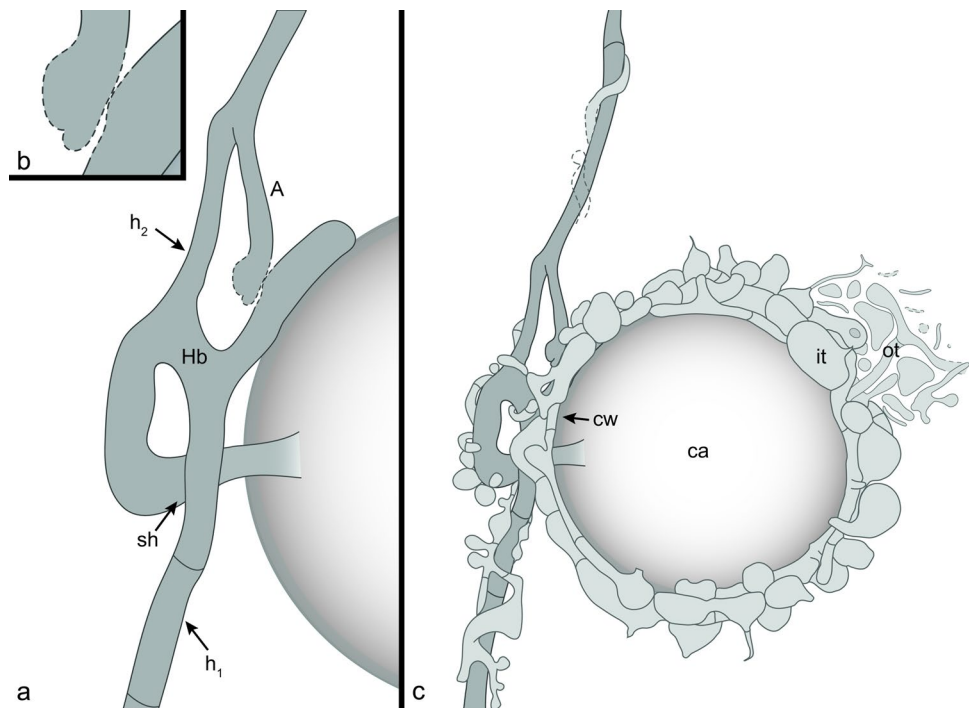
of an H-connection (Hb) between two parallel hyphae (h_1 , h_2) and a putative anastomosis (A); scale bars 50 μ m. **c–g** *Windipila spinifera*. **c** Median section view of reproductive unit, scale bar 50 μ m. **d** Mantle hypha (arrow) and spine-like projection; scale bar 10 μ m. **e** Spine-like projection with blunt apex giving off narrow, distal process (arrow); scale bar 10 μ m. **f** Mantle hypha with two septa (arrows); scale bar 10 μ m. **g** Mantle hyphae (section views) and vesicle-like inflation; slide SNSB-BSPG 2016 VII 117; scale bar 10 μ m

smaller, and the inflations in the unnamed form are exclusively terminal. Moreover, the inflations occur scattered and do not dominate the visual appearance of the mantle. Finally, there is no evidence that narrow processes extended from any of the inflations. On the other hand, *Z. vestitus* is the only fossil mantled fungal reproductive unit besides the new form and *W. spinifera* in which the subtending hypha is sometimes also sheathed by a mantle (Krings et al. 2016: pl. I, 3a).

Several specimens of the new form provide evidence of the presence in vivo of a second, outer mantle tier of interwoven and irregularly swollen hyphae (Fig. 3h, i) or a hyphal meshwork extending around and between adjacent individuals (Fig. 3l). Conversely, no evidence of additional, peripheral mantle layers or hyphal meshworks has been found in *Windipila spinifera* (Krings and Harper 2017, 2018a). It is impossible to determine whether the absence of evidence of these mantle components in *W. spinifera* is

indicative of interspecific variability, and hence a diagnostic structural difference, or is due to the ephemeral nature, and thus a low preservation potential, of the peripheral mantle portions. For example, the outer mantle tier and confluent hyphal meshwork were perhaps components of the juvenile or developing reproductive unit and normally disintegrated upon maturation. Support for the latter conjecture is the fact that ephemeral outer mantle tiers and confluent, gleba-like hyphal meshworks are occasionally present also in several other fossil mantled fungal reproductive units, including *Zwergimyces vestitus* from the Rhynie chert (Krings et al. 2016: pl. II, 1), the Carboniferous *Mycocarpon pachyderma* (Williamson) S. A. Hutchinson (McLean 1922: pl. IX, 12) and *M. flexus* B. H. Davis et Leisman (Stubblefield et al. 1983: fig. 1), as well as an unnamed form, also from the Carboniferous (Krings and Taylor 2012: pl. I, 7–10). An outer mantle layer roughly similar to the outer mantle tier of the new Windyfield form occurs in the Mississippian

Fig. 5 *Windipila wimmervoecksii* sp. nov.—reconstructions. **a** Graphic presentation of hyphal nexus figured in several focal planes in Fig. 4b_{1–5} (for explanation of abbreviations, see Fig. 4b). **b** Close-up of putative anastomosis (denoted “A” in **a**). **c** Synoptic reconstruction of reproductive unit, showing central cavity (ca), cavity wall (cw), and hyphal mantle composed of persistent inner (it) and portion of probably ephemeral outer (ot) tiers in paramedian section view, as well as kinked subtending hypha and portion of mycelium composed of hyphae partially enveloped in mantle (based on specimens figured in Figs. 2, 3, and 4a, b)



reproductive unit *Roannaisia bivitalis* T. N. Taylor, Galtier et Axsmith (Taylor et al. 1994: pl. II, 2). However, this form is up to 600 μm in diameter and characterized by what appears to be a non-hyphal outer covering, which has not been observed in any other mantled fungal reproductive unit. Moreover, the outer mantle tier in *R. bivitalis* is believed to be persistent, not ephemeral.

Based on the comparisons and reflections presented in the preceding paragraphs, we assign the new Windyfield chert fossils to the genus *Windipila* as defined by Krings and Harper (2017), and interpret them as a distinct species, for which the name *W. wimmervoecksii* sp. nov. is proposed, albeit with the understanding that they might represent the same biological species as *W. spinifera*, but which is impossible to determine from the material at hand.

Systematic affinities

Like most other fossil mantled fungal reproductive units, *Windipila wimmervoecksii* was probably produced by a member of the zygomycete fungi or Glomeromycota (see Taylor et al. 2015). 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 to date in Glomeromycota (Benjamin 1979; Benny et al. 2001; Benny 2012). Mature zygosporangia or zygosporangia with attached gametangia and/or suspensors (for structural nomenclature, see Edlmann and Klomparens 1995: fig. 1; Krings et al. 2012: fig. 3) 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. 2013a). *Windipila wimmervoecksii* cannot be positively identified as a zygomycete because there is no evidence of gametangia and/or suspensors. Rather, the occurrence terminally on a simple, tubular hypha or hyphal branch suggests that this reproductive unit, precisely as *W. spinifera* (Table 1; see Krings and Harper 2017), formed asexually by blastic inflation and thickening of a hyphal tip, rather than sexually and following gametangial fusion, and for this reason might represent a glomoid chlamydospore.

One of the partially degraded specimens of *Windipila wimmervoecksii* provides rare insights into the organization of the parental mycelium, which may also be relevant to the assessment of the systematic affinities. This fossil, figured in several focal planes in Fig. 4b, is physically connected via a kinked subtending hypha (“sh” in Figs. 4b₅, 5a) to a small hyphal nexus (graphic presentation in Fig. 5a, b) composed of an H-connection (“Hb” in Figs. 4b₂, 5a) resulting in two parallel hyphae (“h₁” and “h₂” in Figs. 4b_{1,3}, 5a), and a hyphal bridge that most likely represents an anastomosis extending from one of these hyphae to the other (“A” in Figs. 4b₄, 5a; details in Fig. 5b). H-connections (or H-branching) are typical of the mycelium in Glomeromycota, and hyphal anastomoses have been reported frequently in several glomeromycotan lineages (Walker et al. 2018). Conversely, anastomoses are believed to be lacking or rare in zygomycete fungi (Gregory 1984; Glass and Fleissner 2006; Ivarsson et al. 2015). Hyphal anastomoses play key roles in the formation of efficient, interconnected AMF

Table 1 Synopsis of key characters of Early Devonian mantled fungal reproductive units of the genus *Windipila*

Taxon	Provenance	Maximum cavity diameter	Maximum thickness of cavity wall	Maximum mantle thickness (including projections and inflations)	Mantle organization	Hyphal attachment	Reference(s)
<i>Windipila spinifera</i>	Windyfield	105 µm	3.5 µm	50 µm	Interlaced circumferential hyphae giving off prominent, outwardly directed and usually spine-like projections from which may distally or laterally extend narrow processes or hyphal branches	Tubular hypha up to 8 µm wide; distal portion enveloped by narrow hyphae	Krings and Harper (2017, 2018a)
<i>Windipila pumila</i>	Rhynie	48.8 µm	< 1 µm (wall of interior sphere 3.5 µm)	10 µm	Interlaced circumferential hyphae giving off outwardly directed, usually spine-like projections	Unknown	Krings and Harper (2018a)
<i>Windipila wimmerovoeckii</i> sp. nov.	Windyfield	122 µm	7 µm	30 µm (inner tier); > 25 µm (outer tier)	Interlaced circumferential hyphae with prominent intercalary and terminal inflations from which may distally extend narrow processes connecting the persistent inner mantle tier with an ephemeral outer mantle tier (not normally preserved) of irregularly inflated and interwoven hyphae	Tubular hyphae up to 12 µm wide, often with a kink; usually enveloped by hyphae with prominent inflations	This study

mycorrhizal networks, which are fundamental to AMF survival, to plant/soil nutrient flow, and to the maintenance of genetic diversity (e.g., Chagnon 2014; Giovannetti et al. 1999, 2015; de Novais et al. 2013, 2017). In addition, anastomoses between extraradical mycelia originating from the root systems of different host species allow for the formation of wide hyphal networks interconnecting an indefinite number of plants living in the same ecosystem (de Novais et al. 2017). Thus, the hyphal nexus co-occurring with one of the *W. wimmervoecksi* specimens supports the hypothesis that this type of reproductive unit was produced by a member of the Glomeromycota.

The third species in *Windipila*, *W. pumila*, is based on a single specimen lacking evidence of the subtending structure (Krings and Harper 2018a: fig. 2); it differs from the other species not only in regard to size, but also in that it contains a single interior sphere (Table 1). The cavity wall in *W. pumila* has been suggested to represent a sporangium wall, perhaps comparable to the zygosporangium (or azygosporangium) wall in extant zygomycetes or the outmost layer(s) of glomeromycotan chlamydospore walls, while the interior sphere would accordingly be a zygospore (or azygospore) or the chlamydospore wall proper (Krings and Harper 2018a). The spheroidal structure that occurs in the cavity of one of the *W. wimmervoecksi* specimens (Fig. 2g) is more irregular in outline, and its outer boundary is blurred, and thus probably represents a preservation artifact resulting from water loss and condensation of the cavity contents during fossilization.

Mantle function

Whether the hyphal mantles in *Windipila wimmervoecksi* and other fossil fungal reproductive units served a specific biological function is difficult to answer, due in part to a lack of knowledge of the functions of hyphal mantles in extant zygomycete fungi and Glomeromycota. Krings and Harper (2017) suggested that hyphal mantles were perhaps effective as modulating interfaces or protective shields. For example, the mantle of *W. wimmervoecksi* might have developed early and pushed the surrounding cells of the host plant cortex to the side to make space for the expanding reproductive unit. Another scenario views the mantle as a barrier against mycoparasitic fungi, which were superabundant in the Rhynie paleoecosystem (Hass et al. 1994; Krings and Harper 2018b); the living hyphae could actively have degraded invaders that penetrated into the mantle. As a matter of fact, none of the *W. wimmervoecksi* specimens show symptoms of fungal colonization of the reproductive unit, and evidence of intruders in the mantle is exceedingly rare (Figs. 2d₃, 3c). It has also been speculated that surface structures protect reproductive units and propagules from UV-B radiation and can prevent desiccation (Norros et al. 2015).

Provided that the *Windipila wimmervoecksi* hyphal mantle remained (partly) in place even upon maturation of the propagule (= spore) and liberation of the reproductive unit from its host plant, it is also conceivable that this structure accommodated certain microorganisms (Krings et al. 2016) which were beneficial to the development of the propagule, in some way stimulated propagule germination (for similar relationships in modern Glomeromycota, see Roesti et al. 2005; Bonfante and Desirò 2017), or promoted mycorrhizal activity and optimized plant performance (see Frey-Klett et al. 2007; Agnolucci et al. 2015, 2019). Support for this hypothesis is molecular evidence showing that the symbiosis between AMF and certain endobacteria dates back at least to the time when AMF formed the ancestral symbiosis with early land plants (Naumann et al. 2015; Naito and Pawlowska 2016). Moreover, dissemination of propagules together with their beneficial microorganisms (“piggyback transportation”) might have been advantageous because this strategy ensured the presence of suitable microbiota in the place of germination. One could even go so far as to speculate that these complex mantled fungal reproductive units were “seed-like” in function and served to vector the fungus around in the environment. Many modern seeds host bacteria and fungi on their surfaces and interiors that the seedling will need to establish itself in a new site, procure nutrients, and ward off disease agents (e.g., Verma et al. 2017; Shahzad et al. 2018; Verma and White 2018; White et al. 2019). Some of the hyphal inflations in *W. wimmervoecksi* could have been containers for nutrients that fed the surface-vectored bacterial community. On the other hand, the chance of reaching a suitable microhabitat for germination is an important parameter determining the likelihood for fungal dispersal units to establish new reproductive individuals (Calhim et al. 2018). It is, therefore, also possible that hyphal mantles somehow facilitated arrival of the reproductive units on a specific substrate. For example, it has been shown that fungal spore surface structures in certain zygomycetes and higher fungi dictate where in the soil column they are preferentially deposited (Calhim et al. 2018: fig. 2; Hemkemeyer et al. 2019).

Conclusions

The many different fungal propagules and reproductive units that are preserved in the Early Devonian Rhynie and Windyfield cherts have recently begun to receive attention, and their morphology and distribution are now being more systematically documented (e.g., Krings et al. 2017b; Krings and Harper 2017, 2018a). These accounts represent valuable resources that can be used to assess paleodiversity and biotic complexity, and thus contribute to our ability to ultimately understand some of the biological processes that made the

Rhynie paleoecosystem. *Windipila wimmervoecksi* represents the most recent example of the growing number of mantled fungal reproductive units from the Rhynie and Windyfield cherts that lead to speculation as to their systematic affinities and biological significance. Within these fossils there are basic similarities in size and organization that suggest most may belong to the zygomycete fungi or Glomeromycota. Like so many aspects of paleomycology, one specimen often is the single necessary segment of information that helps to clarify the nature and affinities that have remained elusive (Taylor et al. 2011). This may well also be the trajectory regarding *W. wimmervoecksi* as we continue to screen the Rhynie and Windyfield cherts for fungal remains, and new specimens are reported and studied. We hope that *W. wimmervoecksi* and other recent discoveries of fossil fungi provide a stimulus for a wider appreciation of the paleodiversity of these organisms and increase the interest in questions pertaining to the roles they played in ancient ecosystems.

Acknowledgements We thank E. Lange and H. Martin (both Munich, Germany) for technical assistance, and James F. White Jr. (New Brunswick, NJ, USA) and an anonymous referee for insightful comments that improved the manuscript.

References

- Agnolucci, M., F. Battini, C. Cristani, and M. Giovannetti. 2015. Diverse bacterial communities are recruited on spores of different arbuscular mycorrhizal fungal isolates. *Biology and Fertility of Soils* 51: 379–389.
- Agnolucci, M., L. Avio, A. Pepe, A. Turrini, C. Cristani, P. Bonini, V. Cirino, F. Colosimo, M. Ruzzi, and M. Giovannetti. 2019. Bacteria associated with a commercial mycorrhizal inoculum: community composition and multifunctional activity as assessed by illumina sequencing and culture-dependent tools. *Frontiers in Plant Science* 9: 1956.
- Benjamin, R.K. 1979. Zygomycetes and their spores. In *The Whole Fungus. II. The Sexual-Asexual Synthesis [Proceedings of the 2nd International Mycological Conference held at the Environmental Sciences Centre of the University of Calgary Kananaskis, Alberta, Canada]*, ed., B. Kendrick, 573–621. Ottawa: National Museum of Natural Sciences, National Museums of Canada, and the Kananaskis Foundation.
- Benny, G.L. 2012. Current systematics of Zygomycota with a brief review of their biology. In *Systematics and evolution of fungi*, eds. J.K. Misra, J.P. Tewari, and S.K. Deshmukh, 55–105. Boca Raton: CRC Press.
- Benny, G.L., R.A. Hamber, and J.B. Morton. 2001. Zygomycota: zygomycetes. In *The Mycota VIIA Systematics and evolution*, eds. D.J. McLoughlin, E.G. McLoughlin, and P.E. Lemke, 113–146. Berlin: Springer.
- Błaskowski, J. 2012. *Glomeromycota*, 1–303. Kraków: W. Szafer Institute of Botany, Polish Academy of Sciences.
- Bonfante, P., and A. Desirò. 2017. Who lives in a fungus? The diversity, origins and functions of fungal endobacteria living in *Mucoromycota*. *ISME Journal* 11: 1727–1735.
- Bucholtz, F. 1912. Beiträge zur Kenntnis der Gattung *Endogone* Link. *Beihefte zum Botanischen Centralblatt (Abteilung II)* 29: 147–224.
- Calhim, S., P. Halme, J.H. Petersen, T. Læssøe, C. Bässler, and J. Heilmann-Clausen. 2018. Fungal spore diversity reflects substrate-specific deposition challenges. *Scientific Reports* 8: 5356.
- Chagnon, P.L. 2014. Ecological and evolutionary implications of hyphal anastomosis in arbuscular mycorrhizal fungi. *FEMS Microbial Ecology* 88: 437–444.
- Channing, A. 2017. A review of active hot-spring analogues of Rhynie: environments, habitats and ecosystems. *Philosophical Transactions of the Royal Society London (B: Biological Sciences)* 373: 20160489.
- Dotzler, N., C. Walker, M. Krings, H. Hass, H. Kerp, T.N. Taylor, and R. Agerer. 2009. Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-year-old Rhynie chert. *Mycological Progress* 8: 9–18.
- Edelmann, R.E., and K.L. Klomparens. 1995. Zygosporogenesis in *Zygorhynchus heterogamus*, with proposal for standardization of structural nomenclature. *Mycologia* 87: 304–318.
- Edwards, D., L. Dolan, and P. Kenrick (eds.). 2018. The Rhynie cherts: our earliest terrestrial ecosystem revisited. *Philosophical Transactions of the Royal Society of London (B: Biological Sciences)* 373: 1–201.
- Fayers, S.R. 2003. *The biota and palaeoenvironments of the Windyfield Chert, Early Devonian, Rhynie, Scotland*, 1–549. PhD thesis, University of Aberdeen, Aberdeen, Scotland.
- Fayers, S.R., and N.H. Trewin. 2004. A review of the palaeoenvironments and biota of the Windyfield chert. *Transactions of the Royal Society of Edinburgh, Earth Sciences* 94: 325–339.
- Frey-Klett, P., J. Garbaye, and M. Tarkka. 2007. The mycorrhiza helper bacteria revisited. *New Phytologist* 176: 22–36.
- Garwood, R.J., H. Oliver, and A.R.T. Spencer. 2019. An introduction to the Rhynie chert. *Geological Magazine*. <https://doi.org/10.1017/S0016756819000670>.
- Giovannetti, M., D. Azzolini, and A.S. Citeresi. 1999. Anastomosis formation and nuclear and protoplasmic exchange in arbuscular mycorrhizal fungi. *Applied and Environmental Microbiology* 65: 5571–5575.
- Giovannetti, M., L. Avio, and C. Sbrana. 2015. Functional significance of anastomosis in arbuscular mycorrhizal fungi. In *Mycorrhizal networks*, ed. T.R. Horton, 41–67. Dordrecht: Springer.
- Glass, N.L., and A. Fleissner. 2006. Re-wiring the network: understanding the mechanism and function of anastomosis in filamentous ascomycete fungi. In *The Mycota, I. Growth, differentiation and sexuality*, eds. U. Kues and R. Fischer, 123–139. Berlin, Heidelberg: Springer.
- Gregory, P.H. 1984. The fungal mycelium—an historical perspective. In *The ecology and physiology of the fungal mycelium*, eds. D.H. Jennings and A.D.M. Rayner, 1–22. Cambridge: Cambridge University Press.
- Hass, H., T.N. Taylor, and W. Remy. 1994. Fungi from the Lower Devonian Rhynie chert: mycoparasitism. *American Journal of Botany* 81: 29–37.
- Hemkemeyer, M., B.T. Christensen, C.C. Tebbe, and M. Hartmann. 2019. Taxon-specific fungal preference for distinct soil particle size fractions. *European Journal of Soil Biology* 94: 103103.
- Ivarsson, M., J. Peckmann, A. Tehler, C. Broman, W. Bach, K. Behrens, J. Reitner, M.E. Böttcher, and L. Norbäck Ivarsson. 2015. Zygomycetes in vesicular basanites from Vesteris Seamount, Greenland Basin—a new type of cryptoendolithic fungi. *PLoS One* 10(7): e0133368.
- Kidston, R., and W.H. Lang. 1921. On Old Red Sandstone plants showing structure, from the Rhynie Chert bed, Aberdeenshire. Part V. The Thallophyta occurring in the peat-bed; the succession of the plants throughout a vertical section of the bed, and the conditions of accumulation and preservation of the deposit. *Transactions of the Royal Society of Edinburgh* 52: 855–902.

- Krings, M., and C.J. Harper. 2017. A mantled fungal reproductive unit from the Lower Devonian Windyfield chert, Scotland, with prominent spines and otherwise shaped projections extending out from the mantle. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* 285: 201–211.
- Krings, M., and C.J. Harper. 2018a. Additional observations on the fungal reproductive unit *Windipila spinifera* from the Windyfield chert, and description of a similar form, *Windipila pumila* nov. sp., from the nearby Rhynie chert (Lower Devonian, Scotland). *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* 288: 235–242.
- Krings, M., and C.J. Harper. 2018b. Deciphering interfungal relationships in the 410 million-year-old Rhynie chert: glomoid spores under attack. *Geobios* 51: 151–160.
- Krings, M., and T.N. Taylor. 2012. Fungal reproductive units enveloped in a hyphal mantle from the Lower Pennsylvanian of Great Britain, and their relevance to our understanding of Carboniferous fungal “sporocarps”. *Review of Palaeobotany and Palynology* 175: 1–9.
- Krings, M., and T.N. Taylor. 2013. *Zwergimyces vestitus* (Kidston et W.H. Lang) nov. comb., a fungal reproductive unit enveloped in a hyphal mantle from the Lower Devonian Rhynie chert. *Review of Palaeobotany and Palynology* 190: 15–19.
- Krings, M., and T.N. Taylor. 2014. A mantled fungal reproductive unit from the Lower Devonian Rhynie chert that demonstrates Carboniferous “sporocarp” morphology and development. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* 273: 197–205.
- Krings, M., and T.N. Taylor. 2015a. A fungal reproductive unit from the Lower Devonian Rhynie chert (Aberdeenshire, Scotland) that demonstrates an unusual hyphal investment pattern. *Scottish Journal of Geology* 51: 131–139.
- Krings, M., and T.N. Taylor. 2015b. Mantled fungal reproductive units in land plant tissue from the Lower Devonian Rhynie chert. *Bulletin of Geosciences* 90: 1–6.
- Krings, M., T.N. Taylor, J. Galtier, and N. Dotzler. 2010. Microproblematic endophytes and epiphytes of fern pinnules from the Upper Pennsylvanian of France. *Geobios* 43: 503–510.
- Krings, M., T.N. Taylor, N. Dotzler, and G. Persichini. 2012. Fossil fungi with suggested affinities to the Endogonaceae from the Middle Triassic of Antarctica. *Mycologia* 104: 835–844.
- Krings, M., T.N. Taylor, and N. Dotzler. 2013a. Fossil evidence of the zygomycetous fungi. *Persoonia* 30: 1–10.
- Krings, M., J.F. White, N. Dotzler, and C.J. Harper. 2013b. A putative zygomycetous fungus with mantled zygosporangia and apposed gametangia from the Lower Coal Measures (Carboniferous) of Great Britain. *International Journal of Plant Sciences* 174: 269–277.
- Krings, M., T.N. Taylor, E.L. Taylor, H. Kerp, and N. Dotzler. 2014. First record of a fungal “sporocarp” from the Lower Devonian Rhynie chert. *Palaeobiodiversity and Palaeoenvironments* 94: 221–227.
- Krings, M., T.N. Taylor, N. Dotzler, and C.J. Harper. 2016. Morphology and ontogenetic development of *Zwergimyces vestitus*, a fungal reproductive unit enveloped in a hyphal mantle from the Lower Devonian Rhynie chert. *Review of Palaeobotany and Palynology* 228: 47–56.
- Krings, M., C.J. Harper, and E.L. Taylor. 2017a. Fungi and fungal interactions in the Rhynie chert: A review of the evidence, with the description of *Perexiflasca tayloriana* gen. et sp. nov. *Philosophical Transactions of the Royal Society of London (B: Biological Sciences)* 373: 20160500.
- Krings, M., C. Walker, C.J. Harper, H. Martin, S. Sónyi, E. Kustatscher, and T.N. Taylor. 2017b. Unusual fungal reproductive units from the Lower Devonian Rhynie chert. *Zitteliana* 89: 29–37.
- Mark, D.F., C.M. Rice, A.E. Fallick, N.H. Trewin, M.R. Lee, A. Boyce, and J.K.W. Lee. 2011. $^{40}\text{Ar}/^{39}\text{Ar}$ dating of hydrothermal activity, biota and gold mineralization in the Rhynie hot-spring system, Aberdeenshire, Scotland. *Geochimica et Cosmochimica Acta* 75: 555–569.
- Mark, D.F., C.M. Rice, and N.H. Trewin. 2013. Discussion on ‘A high-precision U–Pb age constraint on the Rhynie Chert Konservat-Lagerstätte: time scale and other implications’ (*Journal*, Vol. 168, 863–872). *Journal of the Geological Society of London* 170: 701–703.
- McLean, R.C. 1922. On the fossil genus *Sporocarpion*. *Annals of Botany* 36: 71–90.
- Naito, M., and T.E. Pawlowska. 2016. Defying Muller’s Ratchet: Ancient heritable endobacteria escape extinction through retention of recombination and genome plasticity. *mBio* 7(3): e02057–15.
- Naumann, M., A. Schüssler, and P. Bonfante. 2015. The obligate endobacteria of arbuscular mycorrhizal fungi are ancient heritable components related to the Mollicutes. *ISME Journal* 4: 862–871.
- Norros, V., E. Karhu, J. Nordén, A.V. Vähätalo, and O. Ovaskainen. 2015. Spore sensitivity to sunlight and freezing can restrict dispersal in wood-decay fungi. *Ecology and Evolution* 5: 3312–3326.
- Novais, C.B. de, C. Sbrana, O.J. Saggin Júnior, J.O. Siqueira, and M. Giovannetti. 2013. Vegetative compatibility and anastomosis formation within and among individual germings of tropical isolates of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza* 23: 325–331.
- Novais, C.B. de, A. Pepe, J. Oswaldo Siqueira, M. Giovannetti, and C. Sbrana. 2017. Compatibility and incompatibility in hyphal anastomosis of arbuscular mycorrhizal fungi. *Scientia Agricola* 74: 411–416.
- Parry, S.F., S.R. Noble, Q.G. Crowley, and C.H. Wellman. 2011. A high precision U–Pb age constraint on the Rhynie chert Konservat-Lagerstätte: time scale and other implications. *Journal of the Geological Society of London* 168: 863–872.
- Powell, C.L., N.H. Trewin, and D. Edwards. 2000. Palaeoecology and plant succession in a borehole through the Rhynie cherts, Lower Old Red Sandstone, Scotland. *Geological Society of London Special Publication* 180: 439–457.
- Rice, C.M., N.H. Trewin, and L.I. Anderson. 2002. Geological setting of the Early Devonian Rhynie cherts, Aberdeenshire, Scotland: an early terrestrial hot spring system. *Journal of the Geological Society of London* 159: 203–214.
- Roesti, D., K. Ineichen, O. Braissant, D. Redecker, A. Wiemken, and M. Aragno. 2005. Bacteria associated with spores of the arbuscular mycorrhizal fungi *Glomus geosporum* and *Glomus constrictum*. *Applied and Environmental Microbiology* 71: 6673–6679.
- Shahzad, R., A.L. Khan, S. Bilal, S. Asaf, and I.-J. Lee. 2018. What is there in seeds? Vertically transmitted endophytic resources for sustainable improvement in plant growth. *Frontiers in Plant Science* 9: 24.
- Stubblefield, S.P., T.N. Taylor, C.E. Miller, and G.T. Cole. 1983. Studies in Carboniferous fungi. II. The structure and organization of *Mycocarpon*, *Sporocarpion*, *Dubiocarpon*, and *Coleocarpon* (Ascomycotina). *American Journal of Botany* 70: 1482–1498.
- Taylor, T.N., and J.F. White Jr. 1989. Fossil fungi (Endogonaceae) from the Triassic of Antarctica. *American Journal of Botany* 76: 389–396.
- Taylor, T.N., J. Galtier, and B.J. Axsmith. 1994. Fungi from the Lower Carboniferous of central France. *Review of Palaeobotany and Palynology* 83: 253–260.
- Taylor, T.N., M. Krings, N. Dotzler, and J. Galtier. 2011. The advantage of thin sections over acetate peels in the study of late Paleozoic fungi and other microorganisms. *Palaios* 26: 239–244.

- Taylor, T.N., M. Krings, and E.L. Taylor. 2015. *Fossil fungi*, 1st ed. Amsterdam, Boston, Heidelberg, London: Elsevier/Academic Press Inc.
- Trewin, N.H., and S.R. Fayers. 2016. Macro to micro aspects of the plant preservation in the Early Devonian Rhynie cherts, Aberdeenshire, Scotland. *Earth and Environmental Sciences Transactions of the Royal Society of Edinburgh* 106: 67–80.
- Trewin, N.H., and H. Kerp. 2017. The Rhynie and Windyfield cherts, Early Devonian, Rhynie, Scotland. In *Terrestrial Conservation Lagerstätten. Windows into the evolution of life on land*, eds. N.C. Fraser and H.D. Sues, 1–38. Edinburgh: Dunedin Academic Press.
- Trewin, N.H., and C.M. Rice. 1992. Stratigraphy and sedimentology of the Devonian Rhynie chert locality. *Scottish Journal of Geology* 28: 37–47.
- Verma, S.K., and J.F. White. 2018. Indigenous endophytic seed bacteria promote seedling development and defend against fungal disease in browntop millet (*Urochloa ramosa* L.). *Journal of Applied Microbiology* 124: 764–778.
- Verma, S.K., K. Kingsley, I. Irizarry, M. Bergen, R.N. Kharwar, and J.F. White. 2017. Seed-vectored endophytic bacteria modulate development of rice seedlings. *Journal of Applied Microbiology* 122: 1680–1691.
- Walker, C., C.J. Harper, M.C. Brundrett, and M. Krings. 2018. Looking for arbuscular mycorrhizal fungi (AMF) in the fossil record: An illustrated guide. In *Transformative Paleobotany: Papers to Commemorate the Life and Legacy of Thomas N. Taylor*, eds. M. Krings, C.J. Harper, N.R. Cúneo, and G.W. Rothwell, 481–517. London, San Diego, CA, Cambridge, MA, Oxford: Elsevier/Academic Press Inc.
- Wellman, C.H. 2006. Spore assemblages from the Lower Devonian 'Lower Old Red Sandstone' deposits of the Rhynie outlier, Scotland. *Transactions of the Royal Society of Edinburgh, Earth Sciences* 97: 167–211.
- Wellman, C.H. 2017. Palaeoecology and palaeophytogeography of the Rhynie chert plants: further evidence from integrated analysis of in situ and dispersed spores. *Philosophical Transactions of the Royal Society of London (B: Biological Sciences)* 373: 20160491.
- Wellman, C.H., H. Kerp, and H. Hass. 2006. Spores of the Rhynie chert plant *Aglaophyton* (*Rhynia*) *major* (Kidston and Lang) D.S. Edwards, 1986. *Review of Palaeobotany and Palynology* 142: 229–250.
- White Jr., J.F., and T.N. Taylor. 1989. Triassic fungi with suggested affinities to the Endogonales (Zygomycotina). *Review of Palaeobotany and Palynology* 61: 53–61.
- White Jr., J.F., K.L. Kingsley, S. Butterworth, L. Brindisi, J.W. Gatei, M.T. Elmore, S.K. Verma, X. Yao, and K.P. Kowalski. 2019. Seed-vectored microbes: their roles in improving seedling fitness and competitor plant suppression. In *Seed endophytes*, eds. S.K. Verma and J.F. White Jr., 3–20. Cham: Springer.
- Yao, Y.J., D.N. Pegler, and T.W.K. Young. 1996. *Genera of endogonales*, 1–299. Surrey: Royal Botanic Gardens.