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Hassiella monospora gen. et sp. nov., a microfungus from the 400 million year old Rhynie chert

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ABSTRACT

A new microfungus, *Hassiella monospora* gen. et sp. nov., consisting of coenocytic hyphae is associated with degraded plant material in the Early Devonian silicified Rhynie chert ecosystem. Some hyphae produce small bulb-like projections that subsequently develop into spherical, thick-walled and highly ornamented reproductive structures. Mature reproductive structures are characterized by a prominent, funnel-shaped appendage that is interpreted as an amphigynous antheridium. When combined, these features are suggestive of the oogonia/oosporangia in certain extant members of the *Peronosporomycetes* (*Oomycota*).

Introduction

The extraordinary preservation of the Pragian (Early Devonian) Rhynie chert ecosystem has provided a cornucopia of details on the diversity of microorganisms that lived in this hot springs environment more than 400 million years ago. Among the microorganisms preserved in this late Palaeozoic ecosystem, the fungi have to date received most scholarly attention. As a result, members of the *Chytridiomycota*, *Glomeromycota*, and *Ascomycota* are known in detail from the cherts; these fungi have also been demonstrated in various associations and interactions with other organisms (Taylor *et al.* 2004 and references therein). Moreover, the occurrence of *Zygomycota* in the Rhynie chert has been suggested based on isolated spores (Taylor *et al.* 1997). The *Basidiomycota* is the only group of fungi that has not been documented in this ecosystem to date. Also present in the chert matrix, and variously associated with plant debris, are numerous fungal-like fragments

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for which the precise affinities remain unknown. As a result, many of these remains have been neglected as a source of information that might further expand information about the diversity of microorganisms in the Rhynie chert.

One group of modern heterokont microorganisms that has variously been included with the algae and fungi are the *Oomycota*, syn. *Pseudofungi* (Cavalier-Smith 2001) syn. *Peronosporomycetes* (Dick 2001). These organisms may be facultative or obligate parasites to saprotrophs. While most forms occur in fresh water or terrestrial habitats, there is a largely unexplored diversity in marine ecosystems that may be considerable. Today the *Peronosporomycetes* are defined by ultrastructural, molecular, and other characters that are impossible to resolve with fossil specimens. The only feature that might be useful in identifying fossil *Peronosporomycetes* is the oogonium/antheridium complex, and the encapsulated thick-walled sexual spores termed oospores (Dick 1995). Less easy to recognize, but still potentially possible to find in the fossil record, are hyphae terminating in zoosporangia. Within the Rhynie chert are several thick-walled structures containing a single spore-like unit that we interpret as oogonia or oosporangia of a peronosporomycete (oomycete).

Materials and methods

The Rhynie chert Lagerstätte site is located in Aberdeenshire, Scotland. It consists of at least 10 fossiliferous beds containing lacustrine shales and cherts that are interpreted as a series of ephemeral fresh water pools within a hot springs environment. Detailed information about the geological setting, sedimentology, and ontogeny of the Rhynie chert Lagerstätte can be found in Rice *et al.* (2002). The cherts have been radiometrically dated as Pragian (396±12 Myr) and occur in the upper part of the Dryden Flags Formation. Specimens were identified in petrographic thin sections prepared by cementing a thin wafer of the chert to a glass slide and then grinding the wafer with silicon carbide powder until it becomes sufficiently thin enough for examination in transmitted light. The specimens were examined and photographed using oil immersion objectives directly on the rock surface without a cover slip. Slides are deposited in the collection of the Forschungsstelle für Paläobotanik, Geologisch-Paläontologisches Institut, Westfälische Wilhelms Universität, Münster (Germany), under accession number 1672.

Description

The specimens are included in an aggregation of hyphae that is several millimetres in diameter (Fig 1A). Hyphae are not extensively associated with degraded plant tissue, but rather occur within the matrix associated with a fine accumulation of plant fragments. Individual hyphae are approximately 4–6 μm diam (Fig 1B–D), aseptate throughout, and randomly oriented in the chert matrix. Hyphal tips are rounded and may be slightly swollen (Fig 1E). Branching is relatively frequent and includes numerous small, sometimes closely spaced papilla-like protuberances. These outgrowths distally swell and become bulb-like [Fig 1B, G (left)]. In most specimens, the parental hypha is constricted in the region of the bulb-like outgrowth. None of the small protuberances displays a separation from the vegetative hypha in the form of a septum. However, several larger spheres, which we interpret as developmental stages, show separation from the parental hyphae by a simple septum that occurs at the base of the short, thin-walled hyphal segment (Fig 1D, arrow). Also arising from vegetative hyphae are larger, more or less globose spheres [Fig 1F, G (right), H, I], which are typically opaque, possess a thick outer wall, and are characterized by a prominent, funnel-shaped appendage (Fig 1F, arrow). The largest of these structures range up to 28 μm diam and display two distinct wall layers. Extending from the outer wall layer are closely spaced verrucae, approximately 1.2 μm high, which form a reticulate pattern on the surface (Fig 1G, H). The most unusual feature displayed by the largest spheres is the funnel-shaped appendage. In these spheres, which we interpret as mature based on size and distinct ornamentation, the appendages possess a conspicuous thick wall. In one mature sphere (Fig 1I), two hyphal branches come in contact with the outer wall on either side; the arrow in Fig 1I points toward the tip of one of these hyphal branches extending to approximately the mid point of the sphere.

Taxonomy

Hassiella monospora T.N. Taylor, Krings & Kerp, **gen. et sp. nov.**

Peronosporacaeae (Oomycetes), incertae sedis

Etym.: The generic name *Hassiella* is proposed in honour of Mr. Hagen Hass for his extraordinary technical and scientific contributions to our understanding of the biodiversity of the Rhynie chert ecosystem. The specific epithet *monospora* acknowledges the single oosphere/oospore in the oogonium/oosporangium.

Diagnosis: Coenocytic hyphae 4–6 μm in diam and smooth walled; some producing oogonia; mature oogonia/oosporangium up to 28 μm in diam, ornamented by verrucae that form a reticulate pattern with individual elements up to 1.2 μm high, oogonia with funnel-shaped and thick-walled appendage at base (amphigynous antheridium?).

Typus: Slide no. 1672, in the Paleobotanical Collection of the Forschungsstelle für Paläobotanik. Geologisch-Paläontologischen Institut, Westfälische Wilhelms–Universität Münster (Germany); Fig 1F in this paper (—holotypus).

Age: Pragian, Early Devonian.

Discussion

Affinities with extant fungi

Although *Hassiella monospora* includes both vegetative and reproductive features, only the latter are useful in assessing the systematic position of this Early Devonian microorganism. Based on a detailed comparison of the fossil with extant fungi and fungi-like microorganisms, there are several features that correspond to those seen in modern members of the *Peronosporomycetes* (*sensu* Dick 2001; *Oomycota* in older taxonomic treatments). These include coenocytic mycelia in which septa are only produced to delimit reproductive structures.

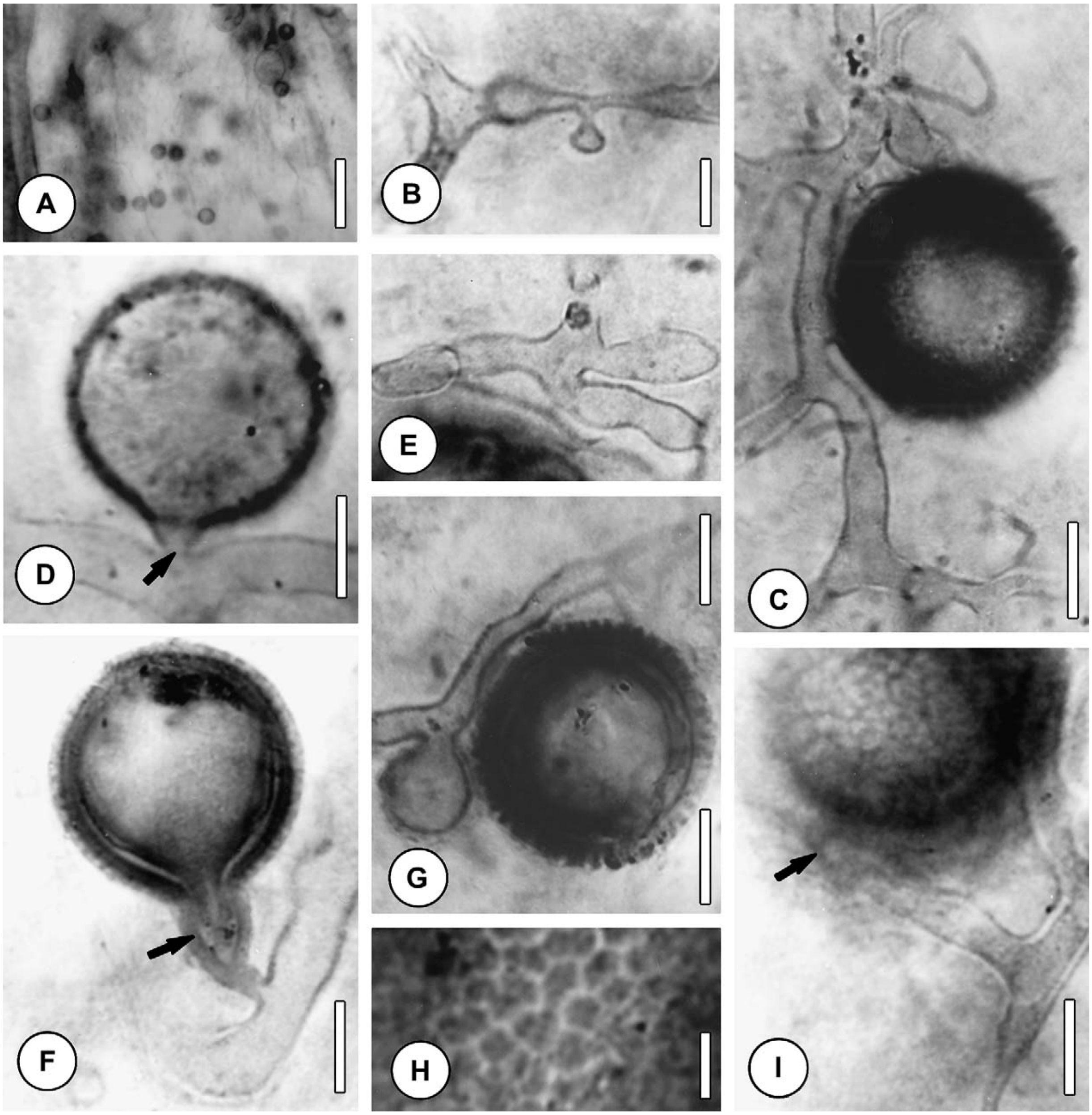

Fig 1 – *Hassiella monospora* gen. et sp. nov. from the Lower Devonian Rhynie chert. Slide no. 1672 (WWU Münster, Paleobotanical Collection). (A) Cluster of spores and hyphae associated with plant debris. Bar scale = 100 μm. (B) Swellings along hypha. Note constriction of parental hypha in the area where the swelling occurs. Bar scale = 10 μm. (C) Branching hyphae and thick-walled oogonium/oosporangium. Bar scale = 10 μm. (D) Immature oogonium. Note thin wall and septum (arrow). Bar scale = 10 μm. (E) Slightly swollen hyphal tips. Bar scale = 10 μm. (F) Mature, thick-walled oogonium/oosporangium attached to hypha by funnel-shaped appendage interpreted as an amphigynous antheridium (arrow). Bar scale = 10 μm. (G) Immature (left) and mature (right), highly ornamented oogonium with thick wall. Bar scale = 10 μm. (H) Detail of mature oogonium/oosporangium surface. Bar scale = 2 μm. (I) Mature oogonium (or immature oosporangium) with hyphal branch (paragynous antheridium at arrow) in contact with the spore wall surface. Bar scale = 10 μm.

Moreover, the thick-walled spheres described here (Fig 1C, F, G, I) most closely resemble the oogonia/oosporangia that are produced by a number of extant *Peronosporomycetes*. Although the oogonia/oosporangia in a number of extant *Peronosporomycetes* are thin-walled and relatively short lived, there are some forms in which these structures are characterized by a relatively thick wall, which may also be variously ornamented (Dick 1969). Within the extant *Peronosporomycetes*, the number of oospores contained in an oosporangium is also variable, ranging from one to several. *Peronosporomycetes* producing oosporangia with a single oospore are found in members of the *Peronosporaceae*, *Pythiogetonaceae*, *Verrucalvaceae*, and in some members of the *Leptomitales* and *Rhipidiales* (Dick *et al.* 1984). In some species characterized as producing single oospheres, abnormal oogonia may contain multiple oospheres (Anikina *et al.* 1999). Although it is inherently impossible to directly compare fossil and extant organisms, especially fungi in which the taxonomic framework is constructed on various reproductive and life history features, it is nevertheless valuable to point out similarities that may exist as a basis for establishing subsequent relationships. Two extant oomycetes that are monosporous and that possess comparable ornamented oogonia/oosporangia similar to that seen in *Hassiella monospora* are *Verrucalvus* (Dick *et al.* 1984) and *Pachymerta* (Dick *et al.* 1989). Dick *et al.* (1989) use the shape of the verrucae to distinguish *P. chaunorhiza* from *V. flavofaciens*. We are uncertain as to the range of variability in the ornamentation of *Hassiella*, however, based on the limited number of specimens and lack of information on the developmental stage, ornamentation on the fossil oogonia/oosporangia is far less distinctive than that of either *P. chaunorhiza* or *V. flavofaciens*. Surface ornamentation is an important character in extant *Peronosporomycetes* (Spring & Thines 2004) and hence may also be useful in the systematics of fossil forms once a sufficient number of specimens are available so that variability can be determined.

If our interpretation of *H. monospora* as a member of the *Peronosporomycetes* is correct, the vesicle-like spheres that are produced by the vegetative hyphae may represent stages in the development of oogonia, including: (a) an initial stage (Fig 1B); (b) two intermediary stages [Fig 1D, G (left)]; and (c) the mature stage [Fig 1F, G (right)]. We interpret the prominent funnel-like structure at the base of the oogonium/oosporangium (Fig 1F, arrow) as an amphigynous antheridium. Amphigynous or collar-like antheridia are produced by plant pathogens such as *Phytophthora* and form when an antheridium develops on the end of a hyphal branch and a second hypha penetrates it to swell and form an oogonium. In hypogynous antheridia, the antheridium forms from the oogonial stalk (Dick 1969). Conversely, if this interpretation is correct then what we have described as a thick wall at the base of the *H. monospora* oogonium is in fact the collective oogonium and antheridium walls tightly pressed together. This would also explain the continuity between the oogonium and the inflated base (Fig 1F). Another interpretation is that the thickened basal end of the mature oogonium/oosporangium is simply a structural feature at the point of attachment with the parental hypha. Because heterothallic species of *Phytophthora* generally possess only amphigynous antheridia and homothallic species paragynous antheridia, Brasier (1983) suggested that amphigyny may be a mechanism to facilitate outbreeding, however, it is becoming increasingly apparent that the mechanisms of heterothallism are extremely complex and involve a number of variables (e.g., Brasier 1992). For example, some studies indicate that in at least one heterothallic species, *P. cinnamomi*, both antheridial types may be produced (Hüberli *et al.* 1997). Although it is unlikely that at our current level of understanding it will be possible to distinguish between mating types and incompatibility systems in fossil plant pathogens, even with exceptionally well preserved material like that from the Rhynie chert, the association of structural features such as amphigynous antheridia may allow for more specific identification of certain lineages within major groups, and help to polarize character states leading to a more complete understanding of ancient reproductive strategies and ancient lineages. For example, the use of certain molecular markers, at least in the genus *Phytophthora*, suggests that amphigyny and heterothallism may be ancestral (Cooke *et al.* 2000). A far less plausible explanation is that the funnel-shaped structure is some specialized base of the oogonium. If this is accurate then the hyphal segment along the side of the oogonium in Fig 1F may represent a paragynous antheridium.

There are two other microorganisms in the Rhynie chert that bear some resemblance to *Hassiella monospora*. One of these are thick-walled spores that were initially described as *Palaeomyces* Kidston and Lang (1921). However, these spores are generally much larger (i.e. 50–500 μm in diam), lack the conspicuous surface ornament of *H. monospora*, and probably represent chlamydospores of an unknown fungus. *Hassiella monospora* also shares some superficial resemblance to some zygomycetous fungi. If *H. monospora* were a zygomycete, the structure that we interpret as an ornamented oogonium/oosporangium would represent a zygosporangium. Accordingly, the prominent funnel-shaped attachment segment would represent a suspensor. However, none of the specimens shows a second attached suspensor, or a preformed area in the sporangium suggesting that a second, short-lived suspensor was once present. Moreover, in extant zygomycetes a septum occurs between the zygosporangium and the suspensors; a feature which is absent in *H. monospora* (Fig 1F). Rather, there is only a single type of septum present in the organism. This septum is clearly discernable in relatively large spheres at the point where they are attached to the vegetative hypha (Fig 1D, arrow), although none of the early bulb-like stages show evidence of a septum at the base [Fig 1B, 1G (left)].

Fossil record of Peronosporomycetes

There are several reports of fossil coenocytic hyphae, oogonium/oosporangium-like spheres, and various types of spores, as well as trace fossils in animal remains, which have been interpreted as peronosporomycetes/oomycetes (e.g., Tiffney & Barghoorn 1974; Pirozynski 1976). In their monographic treatment of the *Saprolegniales*, Johnson *et al.* (2002) discount most of these reports as being inconclusive. This is due to the fact that the primary diagnostic features of peronosporomycete, sexual and asexual reproduction, require examination of living organisms (Dick 2001). Notwithstanding these inherent limitations, there is one report that strongly suggests the occurrence of *Peronosporomycetes* in the late Paleozoic. An *Albugo*-like microorganism is described from the interior of the seed-like structure *Nucellangium glabrum* from the Upper Pennsylvanian of North America (Stidd & Cosentino 1975). These authors describe and illustrate several thick-walled oogonia/oosporangia, each containing a single oosphere/oospore. In addition, they report a pattern of tissue disruption in the host that is similar to pathological symptoms inflicted on extant flowering plants by modern parasitic *Peronosporomycetes* of the *Albugo*-type.

Although the timing of the major radiation and diversification of the *Peronosporomycetes* remains unsettled (Brasier & Hansen 1992), it is generally hypothesized that this group evolved relatively recently (Cavalier-Smith 2001). This is the same conclusion that was initially proposed for the *Ascomycota*, which were interpreted as having co-evolved with the flowering plants in the Early Cretaceous (e.g., Pirozynski

1976). Today, however, the ascomycetes are known to extend back to the mid Silurian based on compelling fossil evidence (Sherwood-Pike & Gray 1985; Taylor *et al.* 2005), and even earlier based on sequence data used to calibrate molecular clocks (Berbee & Taylor 2001).

The fossil record continues to provide new discoveries that not only increase the age resolution for major clades of fungi and other microorganisms, but also provide information that can be used to polarize character states (e.g., ostiolate perithecia, Taylor *et al.* 2005), and is especially important in a group such as the *Oomycota* which lacks a substantial fossil record. If the major radiation and diversification of the *Peronosporomycetes* is a relatively recent event, then does this necessarily mean that ancient members of the group, or precursors that show at least some of the group-defining characters, cannot be recognized in the fossil record? We believe that they can. Although some discount reports of fossil peronosporomycetes that are older than the Cainozoic because of the absence of a complete suite of diagnostic features, others regard at least some of these fossils as authentic (e.g., Blackwell & Powell 2000). Among all of these fossils, *H. monospora* certainly displays the most convincing complement of structural similarities to the extant peronosporomycetes, and thus is perhaps the most persuasive example to date of a late Palaeozoic member of this group. The fact that *H. monospora* was discovered in the Rhynie chert comes as no surprise as this extraordinary Early Devonian ecosystem is precisely the type of habitat that one would expect to contain peronosporomycetes. As the molecular phylogeny of the *Peronosporomycetes* is continuously refined, it will be interesting to examine the morphological characters attributed to *H. monospora* and to see how they fit with character states based on molecular data.

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