Mycologia, 97(3), 2005, pp. 725–729.

Protoascon missouriensis, a complex fossil microfungus revisited

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Abstract: The Carboniferous microfungus *Protoascon missouriensis* has been interpreted variously as an ascomycete, chytridiomycete, zygomycete and oomycete. We offer a more complete interpretation based on a re-examination of the type material that suggests the fossil represents an (a)zygosporangium-suspensor complex of a zygomycete comparable to some modern members of the Mucorales.

Key words: (a)zygosporangium, Carboniferous, Mucorales, suspensor, zygomycete

Microfungi represent a loosely defined assemblage of heterotrophic organisms that have no taxonomic rank, but rather includes members of several major fungal groups (e.g. chytridiomycetes, ascomycetes, basidiomycetes, glomeromycetes and zygomycetes). In general microfungi are characterized by the microscopic nature of their sporocarps. They are found today in almost every environment, ranging from the intestines of marine animals, surfaces of macrofungi, soil of tropical rain forests, to bare rock surfaces in polar regions. Because of the heterogeneity of these organisms, coupled with the size of the sporocarps, detailed information on many microfungi and their interactions with other organisms has been slow to accumulate and the various effects they may have on their hosts continue to be incompletely documented. Nevertheless today there is a voluminous literature on these organisms that spans several disciplines (e.g. mycology, molecular biology, medicine, plant and animal pathology), which is difficult to survey in a comprehensive manner.

Although microfungi have been noted in the fossil record for a long time (Kalgutkar and Jansonius 2000), they have been largely ignored because attention in paleobotany primarily has been directed at the more common and easily recognizable land plants. Moreover paleobotanists traditionally lack sufficient knowledge of mycology and especially those fungal groups that do not display easily identifiable characteristics (e.g. large fruiting bodies). Despite these inherent limitations there have been a number of important contributions to our understanding of the paleobiology and evolutionary history of various microfungi, including monocarpic chytrids from the Lower Devonian (Taylor et al 1992), trichomycete-like fungi in the hindguts of Triassic arthropods (White and Taylor 1989) and epiphyllous micro-ascomycetes of the Tertiary (Dilcher 1965). Molecular biology clearly indicates the antiquity of all major fungal groups, with some estimates extending fungal lineages back to ca. 1 byr (Heckman et al 2001). However hypotheses based on these molecular clocks still require substantiation from compelling fossils, not only to test their validity and estimates of divergence times but also to provide a basis for examining the origin and evolution of various character states.

Batra et al (1964) report a single assemblage of some 50 peculiar microfossils from a Carboniferous permineralization (coal ball) collected from Tebo Coal of the Cabaniss Formation, Cherokee Group (Middle Pennsylvanian, Pioneer Mine, Appleton City, Missouri). Each of these microfossils consists of a pair of tiny conjoined structures 50–150 μm diam, in which the distal structure is thick-walled and ornamented, while the proximal structure is relatively thin-walled (Fig. 1). Up to 12 filamentous appendages arise from near the apex of the proximal structure and envelop the distal structure (Figs. 2, 3). Each pair of structures measures ca. 250 μm from the base of the proximal structure to the tip of the enclosing appendages. Moreover Batra et al report

Accepted for publication 14 Mar 2005.
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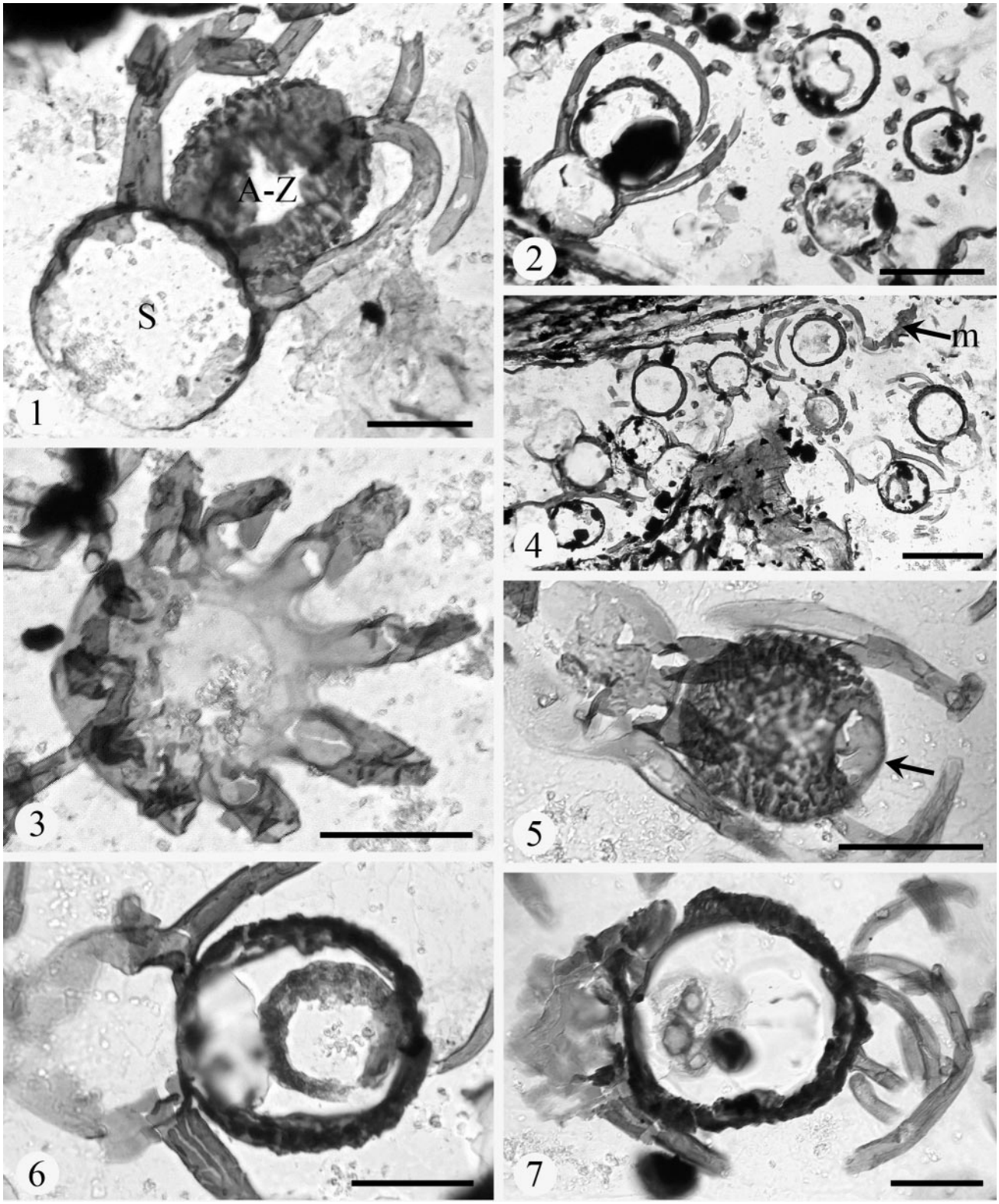

FIG. 1. Specimen showing suspensor (S) with appendages and (a)zygosporangium (A–Z). Slide #22 428. Bar = 25 μm.

FIG. 2. Specimens in longitudinal (left) and transverse sections (right) showing suspensor appendages. Slide #22 420. Bar = 50 μm.

FIG. 3. ''Basket'' of suspensor appendages separated from suspensor apparatus. Slide #22 428. Bar = 25 μm.

FIG. 4. Several specimens of *Protoascon* within megaspore membrane (m). Slide #22 421. Bar = 100 μm.

FIG. 5. (A)zygosporangium showing thin-walled region (arrow) suggestive of suspensor attachment site. Slide #22 417. Bar = 25 μm.

that these microfossils occur exclusively within the confines of the megaspore membrane of a poorly preserved specimen of the putative seed *Nucellangium glabrum* Darrah et al (FIG. 4). These authors suggest that the affinities of the fossils lay within the Ascomycota, perhaps close to the Erysiphales, in which ascocarps contain only a single 8-spored ascus, and introduce the name *Protoascon missouriensis* for the organism.

Baxter (1975) later altered his view regarding the affinities of *P. missouriensis*, pointing out that the presence of the fungus in a partly decomposed specimen of *N. glabrum* suggests that the plant part might have been submerged and subsequently colonized by a saprophytic aquatic organism. He suggests that the fossil shares more structural features with some modern oomycetes, such as members of the Saprolegniales or Peronosporales. Features that Baxter uses to support this hypothesis include a swollen hyphal base (= the proximal structure), which is similar to that found in members of the extant genus *Apodachlya* Pringsheim. The Peronosporales, however, are characterized by terminal oogonia that develop spiny and other types of thickened walls (= the distal structure). Pirozynski (1976a, b) suggests affinities with the zygomycetes and compares *P. missouriensis* to the extant *Absidia glauca* Hagem (cf. Ellis and Hesseltine 1965). The most recent interpretation is that of Johnson et al (2002) who suggest a morphological similarity between the appendages of *P. missouriensis* and that of some members of extant Spathulosporaceae (Ascomycota), such as S. *adelpha* Kohlmeyer (Kohlmeyer 1973).

We have re-examined the type specimen of *Protoascon missouriensis,* including additional preparations in the Paleobotanical Collection of the Natural History Museum and Biodiversity Research Center at the University of Kansas under accession numbers 22 415–22 429 (previously AC10038B #1, 4, 6–11, 13–19). As a result we support the zygomycete affinities as postulated by Pirozynski (1976a, b). We interpret the larger, thick-walled distal structure as either a zygosporangium or azygosporangium produced by a zygomycetous fungus, probably included within the Mucorales. Adding support to this interpretation is the fact that the wall of the distal structure is two-layered, which indicates that an inner (a)zygospore is surrounded by the ornamented wall of the sporangium (FIG. 6). The majority of *P. missouriensis* specimens possess a single well developed appendaged suspensor (i.e. the proximal structure with filamentous appendages, FIGS. 1–3), which might suggest that these sporangia are of the azygosporangium-type. Azygosporangia occasionally are produced by many Mucorales and develop from a single gametangium that does not show any evidence of sexual fusion; in a few forms, azygosporangia are known to outnumber zygosporangia. The basic structure of the fossils best can be compared with azygosporangia produced by *Mucor azygosporus* Benjamin (e.g. O'Donnell et al 1977, FIG. 18), although the suspensor in the latter does not produce appendages; however, as noted by Pirozynski (1976b), the appendages in *P. missouriensis* are remarkably similar to those of *Absidia glauca,* which has 12–20 fingerlike appendages that enclose the zygospore (Ellis and Hesseltine 1965). Batra et al (1964) interpret the appendages as septate. Our re-examination of the type material indicates that these structures in fact are aseptate. In accordance with the interpretation of these structures as (a)zygosporangia, the proximal structure (FIG. 1) is thus homologous with the suspensor and the enclosing aseptate extensions that envelop the distal structure (FIGS. 1–3) homologous with suspensor appendages (cf. Benjamin 1979).

It is interesting that several specimens of *P. missouriensis* display sporangia with a distal opening, or thinned area in the wall (FIG. 5). While this feature may reflect a preservational artefact, it is possible that the opening/thinned area in the wall represents the attachment site of a second suspensor. In at least one specimen a smaller distal structure is attached to the thick-walled sporangium that might represent a second suspensor. If this is the case, these structures represent zygosporangia. In some modern mucoralean species (e.g. *Mortierella chlamydospora* [Chesters] Plaats-Niterink), zygosporangia generally possess a single well-developed globose suspensor, while the second suspensor is poorly developed and atrophies during early development of the zygosporangium (Watanabe 1990). Thus, if the area of attachment of the small, short-lived suspensor disappears during development and maturation of the sporangium (e.g. by being covered with wall materials), it is possible that all specimens of *P. missouriensis* represent zygosporangium-suspensor complexes. In some specimens a cluster of small spherical bodies (FIG. 7) occurs within the distal spherule. These structures initially were interpreted as spores produced by *P. missouriensis* (Batra et al 1964). However we believe that

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FIG. 6. Thick-walled (a)zygosporangium showing internal spore. Slide #22 422. Bar = 25 μm.

FIG. 7. (A)zygosporangium with smaller internal structures (mycoparasites?). Slide #22 419. Bar = 25 μm.

these bodies instead represent chytrids or some other mycoparasite because each is present in only a single individual of *P. missouriensis*.

The basal region of all of the suspensors (i.e. proximal structures) in the fossil unfortunately shows no evidence of attachment to the vegetative portion of the fungus, although Baxter (1975) indicates that *Protoascon missouriensis* grows directly on the megaspore membrane and might not have produced vegetative hyphae at all. However thin-walled hyphae rarely are preserved in Carboniferous coal balls and thus it might be possible that the hyphal network of *P. missouriensis* simply did not fossilize, whereas the thicker-walled and more robust (a)zygosporangium-suspensor complexes are well preserved. As a result of our re-interpretation, we provide this emended diagnosis:

Fungi

Order Mucorales *Incertae sedis*
Genus *Protoascon* Batra, Segal et Baxter 1964, emend.

Emended generic diagnosis: Fungal reproductive unit—(a)zygosporangium-suspensor complex—in two parts, distal structure thick-walled with prominent ornamentation; proximal structure with thinner wall; up to 12 appendages symmetrically arranged in a ring, unbranched, tapering, and aseptate, appendages arise from the distal margin, curl and envelop the distal structure.
Type species: *Protoascon missouriensis* Batra, Segal et Baxter 1964

Species *Protoascon missouriensis* Batra, Segal et Baxter 1964, emend.

Emended diagnosis: Structures up to 150 μm diam, wall of distal (a)zygosporangium up to ~20 μm thick with surface ornamentation of evenly spaced verrucae, distal opening present or absent; wall of proximal suspensor smooth and less than 5 μm thick; suspensor appendages with smooth wall, some with longitudinal striation extending from base to near tip, up to 150 μm long and 20 μm diam at base; tip blunt. *Holotype:* R.W. Baxter Paleobotanical Collection, coal ball 14 417, slide #22 419 (previously AC10038B peel #8), (housed in the Paleobotanical Collection of the Natural History Museum and Biodiversity Research Center at the University of Kansas).

Most modern members of the Mucorales are saprophytes in soil and organic debris; a few are known to be pathogens (Benny et al 2001). The depositional environment in which *Protoascon missouriensis* is preserved consists of peat composed of highly fragmented and partly degraded plant material. Among the few identifiable structures within this peat is a poorly preserved seed-like structure assignable to *Nucellangium glabrum,* which represents the host of *P. missouriensis*. There is no evidence of *P. missouriensis* in the remainder of the peat. Whether this suggests that the fungus exhibited some degree of host specificity or is merely a preservational artifact or random occurrence cannot be determined based on the material at hand. Because *P. missouriensis* occurs in the interior of *N. glabrum* (i.e. within the confines of the megasporangium membrane), the possibility also exists that the fungus actively penetrated the seed and thus might have been an endoparasite and pathogen.

Protoascon missouriensis represents the oldest unequivocal fossil evidence for the Mucorales and thus demonstrates the antiquity of this group of fungi. This parallels the demonstrated fossil record for other major fungal groups, including chytrids and ascomycetes. Moreover *P. missouriensis* demonstrates that characteristic reproductive features observed in modern Mucorales were well established by the Carboniferous. In addition the presence of *P. missouriensis* in the interior of plant parts suggests that interactions with other organisms were also in place by the Carboniferous.

With increasing effort to better understand the organisms and interactions within modern ecosystems, including the roles of microfungi and other microorganisms, numerous questions arise as to how these evolved. Answers to such questions with regard to microfungi have come primarily from molecular analyses of modern organisms. The fossil record has been used in only a limited fashion. Nevertheless the (a)zygosporangium-suspensor complex *Protoascon missouriensis* demonstrates that, where preservation is sufficient to permit detailed structural and morphological analyses, the fossil record of microfungi has the potential to contribute significantly to our understanding of both the complexity that existed in ancient ecosystems and the evolutionary history of interactions between microfungi and other groups of organisms.

ACKNOWLEDGMENTS

Financial support was provided in part by the Alexander von Humboldt Foundation (Germany, V-3. FLF-DEU/1064359) and the National Science Foundation (Grant No. OPP-0229877).

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