Glomalean Fungi from the Ordovician
Dirk Redecker, Robin Kodner, Linda E. Graham

Fossilized fungal hyphae and spores from the Ordovician of Wisconsin (with an age of about 460 million years) strongly resemble modern arbuscular mycorrhizal fungi (Glomales, Zygomyctes). These fossils indicate that Glomales-like fungi were present at a time when the land flora most likely only consisted of plants on the bryophytic level. Thus, these fungi may have played a crucial role in facilitating the colonization of land by plants, and the fossils support molecular estimates of fungal phylogeny that place the origin of the major groups of terrestrial fungi (Ascomycota, Basidiomycota, and Glomales) around 600 million years ago.

Arbuscular mycorrhizal (AM) symbiosis between modern plants and fungi is ubiquitous and contributes to nutrient acquisition by most vascular plants (1). It has also been reported for hepatics and hornworts (2, 3). Arbuscular mycorrhiza may have played an important role in the success of early terrestrial plants (4, 5).

Because of the poor preservation of most fungal structures, it has been difficult to interpret the fossil record of fungi (6–8). Hyphae, the vegetative body of fungi, bear few distinctive morphological characters, and organisms as diverse as cyanobacteria, eukaryotic algal groups, or Oomycetes can be easily mistaken for them. The most convincing evidence for the presence of fungi is fungal fossils associated with plants, including arbuscular mycorrhizae (9, 10) and ascomycetous or chytrid parasites (11, 12).

The previously reported oldest fungal fossils originate nearly exclusively from a single site, the Devonian Rhynie Chert [400 million years ago (Ma)] (9, 10). Possible earlier ascomycetes were reported from the Silurian of Sweden (13).

The fossils reported here were found in the Guttenberg Formation, mid-Ordovician dolomite of Wisconsin, which was deposited in a presumed shallow marine setting between 460 and 455 Ma (14–16). The fossilized material consists of entangled, occasionally branching, nonseptate hyphae and spores (Fig. 1, A to C and E to G). The hyphae have a diameter of 3 to 5 μm and bear globose to subglobose terminal spores that are 40 to 95 μm in diameter. These spores are connected to cylindrical to slightly flared (Fig. 1, C and G) or recurved (Fig. 1E) subtending hyphae. No specialized attachment structures are visible. Where spores are not entangled in hyphae, a single hyphal connection per spore can be discerned (Fig. 1, C and E to G). We found no single spore that did not retain its subtending hypha. The spore wall apparently consists of a single layer. In their shape, size, and hyphal connection, the spores resemble those of modern glomalean fungi in the genus Glomus (Fig. 1, D and H). Similar loose spore clusters are frequently formed by present-day AM fungi but are unknown from algae, other fungi, or Oomycetes.

Species determination in the Glomales depends on the layer structure of the spore wall (17). No such layers could be discerned in the fossils. Layers may have degraded before they were deposited, over time, or during the acid treatment used to dissolve the rock. Our experiments with living extant spores showed that the spore wall layers are acid stable, but for dead extant spores, the loss of wall layers by microbial degradation has been frequently reported (17).

Our findings push back the time of the fossil record of glomalean fungi by 55 to 60 million years and also suggest that these fungi were present before the first vascular plants arose. Although hepatics and hornworts lack roots that would allow them to form a “mycorrhiza” in a strict sense, AM-like colonization of their thallus has long been known (2). Evidence that a hornwort forms an association with a defined AM fungus was only obtained recently (3). We have no evidence that these Ordovician fossil fungi were associated with plants, but fossil evidence for Ordovician land plants (18) and the well-documented capability of some extant bryophytes for AM-like interactions suggest this possibility. Alternatively, they may have formed symbioses of the Geosiphon type or have been saprobes.

Geosiphon is a nonmycorrhizal ancestral member of the Glomales, which forms an endosymbiosis with cyanobacteria (19) and evolved at approximately the same time as the deeply divergent mycorrhizal lineages (20). It also forms spores of the Glomus type (21).

The fossils reported here support time estimates of fungal phylogeny derived from ribosomal small subunit sequences (22) (Fig. 2). Using a modified data set from a previous study (22), we included the Glomales-like fungi from the Ordovician and some other recently reported fossils to date their divergences within the tree (23). Some of these fossils provide useful calibration points for the tree (Fig. 2, points a and d).

![Fig. 1](https://www.sciencemag.org/content/289/5482/1920/F1.large.jpg)  
**Fig. 1.** (A to C and E to G) Fossil hyphae and spores from the Ordovician and (D and H) spores formed by extant glomalean fungi. (A and B) Overviews of the fossilized material. (C, E, F, and G) Fossil spore details. (C) Detail of (B). (D) A spore of present-day Glomus sp. S328 with layered wall structure. In (G), the arrow shows walls of a subtending hypha in connection with the spore wall. (H) A spore of present-day Glomus leptotichum, a member of the deeply divergent glomalean lineages. Images were obtained by light microscopy (28) of the specimens in air (A, C, F, and G), differential interference contrast microscopy of the specimens in polyvinylalcohol-lactoglycerol (D, E, and H), and confocal laser scanning microscopy with the autofluorescence of the material (B). All scale bars are 50 μm.
Fig. 2. The phylogenetic tree of the fungi derived from small subunit ribosomal sequences. Letters a to e assign tree branching points to fungal fossils. The respective geological times are given as numbers on the tree. Triangles indicate that all fossils could also have been deposited later in the history of each clade, allowing the origins of the clades to be shifted back in time. Point a indicates the glomalean fossils reported in the present study; b indicates the fossil arbuscular mycorrhizae from the Rhynie Chert (400 Ma) (9); c indicates a fossil clamp connection (earliest evidence of Basidiomycetes, 290 Ma) (29); d indicates the Ascomycetes from the Rhynie Chert (400 Ma) (30); e indicates the gilled mushroom in amber (90 Ma) (68); and f indicates the arbuscular mycorrhizae of the Gigasporaceae type from the Triassic (~ 240 Ma) (31). The outgroups were Diphasoeca grandis (Choanoflagellate), Ichthyophonus hoferi, Dermocystis salari (early divergences of the animals (32)). Branches that are not supported by at least 50% bootstrap analyses are shown in gray.

whereas others apparently were formed after the origin of the clade (Fig. 2, points b, c, and e) (24). The assumption that the fossil organisms existed at the time of, or after, the divergence of the deep glomalean lineages (Fig. 2, point a) places the origin of the Ascomycete/Basidiomycete clade at 600 to 620 Ma (25), in good agreement with the date of 600 Ma from Berbee and Taylor (22). No lineage before the Glomales developed plant symbioses, but numerous later diverging clades did, with global impacts on terrestrial ecosystems.