# 9 Glomeromycota

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## I. Introduction

The Glomeromycota are a monophyletic group of fungi living as obligate biotrophs forming arbuscular mycorrhiza (AM) or (in one instance) an endosymbiosis with cyanobacteria (Schüßler et al. 2001b). Being one of the smallest of the fungal phyla, the Glomeromycota presently include only approximately 230 described species (Schüßler and Walker 2010). Taxa have been traditionally described based on the morphology of the large, multinucleate spores, which are sometimes organized in spore aggregates or sporocarps. However, due to the paucity of morphological characters, molecular data have been increasingly used for taxon description from the phylum down to species.

Within the true fungi, the Glomeromycota have been placed as a sister group to Ascomycota and Basidiomycota (Dikarya) in rDNA-based phylogenies, but they group among lineages of the paraphyletic zygomycetous fungi when protein-coding genes are used (Lee and Young 2009; Liu et al. 2009; Redecker and Raab 2006). In the case of a sister group relationship to the Dikarya, the clade uniting the two would be characterized by the ability to form mutualistic symbioses with plants or algae, which is rarely found in other clades. Zygomycetous fungi and Glomeromycota both have coenocytic (non-septate) mycelium and a certain similarity of the spores and sporocarps, but both could be shared ancestral traits.

Molecular-marker-based field studies have recently revealed a considerable diversity of AM fungi (AMF) that could not be assigned to formally described species, possibly due to a

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high proportion of species rarely or never producing spores.

Here, we review the recent state of glomeromycotan systematics. The numerous changes and revisions in AMF systematics over the last decade are presented in the context of the historical background and also of their implications for ecology and evolution.

## II. Arbuscular Mycorrhizal Symbiosis

AM is the most widespread type of mycorrhizal symbiosis, a mutualistic association between plants and fungi. The great majority of land plants, among them vascular plants, up to around 80 % of investigated species, are known to form AM (Brundrett 2009). The remainder either is nonmycorrhizal or forms one of the other types of mycorrhiza, i.e., ectomycorrhiza, ericoid, or orchid mycorrhiza. It should be noted, however, that many gymnosperms, pteridophytes, and even nonvascular plants, like liverworts and hornworts, form AM or AM-like associations (Smith and Read 2008). Thus, AMF are found ubiquitously in soils wherever their plant hosts are available.

As obligate symbionts, the fungi depend entirely on the support of reduced carbon compounds by plants. The dependence of plants on their mycosymbionts varies according to plant species and environmental conditions, but in any case AM is one of the major factors in plant nutrient uptake and nutrient cycling in the soil. Plants benefit in particular from the transport of immobile ions, such as phosphate, which are difficult for the root to reach (Smith and Read 2008). Significant transport of nitrogen has also been reported (Jin et al. 2005). The hyphae of extraradical mycelia are by an order of magnitude finer than the root hairs and therefore much more efficient in taking up ions from small soil pores and extending the volume of exploration for immobile nutrients well beyond the depletion zone found around the root. Among other benefits to plants, which may be due in part to better mineral nutrition but also to less-investigated, more specific effects, improved resistance against root and other pathogens has been reported (Azcon-Aguilar and Barea 1996).

As most crops form AM, this symbiosis also has considerable economic importance (Gianinazzi et al. 2010). However, fungal diversity in agricultural settings seems to be strongly diminished by management practices such as plowing or by fungicide application (Helgason et al. 1998; Oehl et al. 2003).

Typically, AMF form finely branched tree-shaped structures within root cells, the eponymous arbuscules. Plant and fungal cytoplasm are only separated by plasma membranes and a very thin layer of amorphous wall polymers, facilitating the exchange of nutrients between symbionts (Bonfante-Fasolo and Grippiolo 1982). In fact, an exchange of phosphate from fungus to plant across the arbuscules has been demonstrated, whereas hexoses apparently are also transferred elsewhere from the plant to the fungus (Smith et al. 2001). Some glomeromycotan families also form storage organs inside roots, the vesicles, which usually appear at later stages of the association.

The morphology of intraradical (within root) symbiotic structures in the AM has been classified into two types, the *Paris* and the *Arum* types, according to the two host plants where they were first described. In *Arum*-type colonization, the fungus proliferates along the root in the intercellular spaces and arbuscules enter into the cells from the resulting axes. In the *Paris* type, the fungi spread from cell to cell, and in many cases intracellular hyphal coils are formed instead of or together with arbuscules. Thus, in many cases plants forming AM do not necessarily show arbuscule formation. The two types, however, just represent two ends of a continuum of structures that are determined by the plant host, the identity of the fungus, or the interaction of the two (Dickson et al. 2007). Thus, they may even be present in the same root.

Arbuscular mycorrhizal fungi are found everywhere where hosts to this symbiosis occur. Non-AM plants may have other kinds of mycorrhiza, e.g., many woody species, in particular the Pinaceae, which have ectomycorrhiza, orchids and ericoid plants with their own associations, and some families typically regarded as

nonmycorrhizal, such as the Brassicaceae, Chenopodiaceae, and Cyperaceae, may still have members that form these associations (Smith and Read 2008). Therefore, the habitats of these fungi include most plant ecosystems, even submerged plants (Sondergaard and Laegaard 1977), plants in geothermal soils (Appoloni et al. 2008; Bunn and Zabinski 2003), and deserts (Stutz and Morton 1996).

## III. Morphology and Reproduction

The Glomeromycota form a coenocytic mycelium of narrow to broad (2–10 μm, sometimes up to 20 μm), often knobby hyphae. Anastomoses, resulting in an interconnected hyphal network, have been reported frequently from the Glomeraceae but do not seem to occur or are rare in the Gigasporaceae, although the latter possess the ability to form end-to-end anastomoses to bridge interrupted hyphal connections (de la Providencia et al. 2005; Gerdemann 1955a; Purin and Morton 2011). Septa are formed in senescent parts of the mycelium, when the fungus retracts the cytoplasm, or after spore formation.

Germ tubes emerge from spores in different ways, according to the taxon: through the attachment of the subtending hypha or through the spore wall (in some taxa both modes exist) and with or without the involvement of a membranous germination structure (germination shield, germination coil; see following sections for details). Spore germination may be enhanced by plant-produced factors (Bécard et al. 1995). Strigolactones have been identified as compounds inducing spore germination or hyphal branching near a prospective host, thereby maximizing the chance to colonize it (Akiyama et al. 2005; Besserer et al. 2006). On the root surface, appressoria (hyphopodia) are formed that allow the fungus to enter the epidermal cells. The formation by the plant of a prepenetration apparatus facilitates and directs the entrance and the transit of hyphae across the epidermal and cortical root cells (Genre et al. 2008).

Inside the root the fungus may form arbuscules, hyphal coils, or vesicles. Depending on physiological factors, spore formation may be triggered after some time. These spores are always multinucleate and, depending on the size, may contain between fewer than 50 and several thousand nuclei (Bécard and Pfeffer 1993; Marleau et al. 2011). The question of whether these nuclei are genetically homogeneous or constitute a mixed "population" of genotypes has been the subject of a long-standing debate [for overviews see Rosendahl (2008) and Young (2008)]. New roots may be colonized from spores after germination or in many taxa also directly by mycelia emanating from a colonized root. Exceptions to the latter again are members of the Gigasporaceae, which apparently always colonize roots starting from spores. Hyphal fragments in the soil may also act as infective propagules.

No morphological evidence for sexual reproduction has been confirmed in the Glomeromycota. Therefore, their spores, despite a certain resemblance to *Endogone* zygospores, are assumed to be formed asexually. Close examination of nuclear migration during spore formation provided no hint of sexual processes (Jany and Pawlowska 2010). However, studies combining microscopic examination and molecular genetics have provided evidence for an exchange of genetic markers between different strains and, thus, for genetic recombination (Sanders and Croll 2010), at least in the model AMF *Rhizophagus irregularis* (formerly known as *Glomus intraradices* or *Glomus irregulare*).

## IV. Dispersal and Host Relations

### A. Geographical Distribution

Due to the cryptic nature of their association with plants, data about the geographical distribution of glomeromycotan taxa are scarce. Large regions of the world have not been surveyed, even for AMF spores, which would allow at least limited insight into local glomeromycotan diversity. A number of species have

been found in only a single location and could be endemic, while others are surprisingly widespread globally. It is indeed puzzling when approximately 20 % of all described morphospecies are found in one region and approximately 12 % in a single field site (Oehl et al. 2003). Molecular field surveys confirmed the pattern of widespread (bona fide) endemism on one hand but extremely widespread dispersal of other taxa on the other (Öpik et al. 2006). However, certain species that were proposed as possibly specific to a certain environment or altitude were later detected, on the basis of molecular markers, in very different habitats (Krüger et al. 2011). Thus, it must be concluded that much remains to be discovered in this respect and that it is too early to make concise statements about the biogeography of most AMF taxa. The well-studied species *R. irregularis* has been detected in a multitude of habitats and regions, often as the dominant molecular taxon (e.g., Appoloni et al. 2008; Sýkorová et al. 2007), and disturbance-adapted species such as *Glomus mosseae* (recently renamed *Funneliformis mosseae*) are also extremely widespread, especially in agricultural soils (e.g., Daniell et al. 2001; Helgason et al. 1998, Hijri et al. 2006). Interestingly, genotypes of this species seem to be rather uniform worldwide, with no geographic structure detectable. Based on these data, Rosendahl (2008) concluded that the species probably has been relatively recently spread by agricultural practice around the world. The more thorough and defined use of molecular operational taxonomic units (MOTUs) (Hibbett et al. 2011) might facilitate a better understanding of AMF biogeography in the future, providing their clear definition (Hawksworth et al. 2011).

Dispersal has not been well studied in the Glomeromycota. Hyphal spread from colonized plants and spores transported with soil particles may be the predominant nonhuman-mediated means of dispersal, but translocation of spores by earthworms or mammals has also been reported (Gange 1993). Some sporocarpic species might also be spread through the feces of rodents (Mangan and Adler 2002).

### B. Host Specificity

Considering the relation between glomeromycotan species number and the richness of potential host plants there does not seem to be much room for host specificity in AM. Indeed, greenhouse experiments, combining single species of plant host and mycobiont, indicated almost universal compatibility (Klironomos et al. 2000). It is clear, however, that species cultivatable in the greenhouse are most likely not representative of what occurs in the field, and the diversity of cultured AMF may be strongly biased toward generalists. Molecular approaches allowed this question to be addressed in the field, and the results generally showed the absence of strict specificity. Most plants associate with several glomeromycotan species at the same time, and most glomeromycotan species are linked to different species of plants. However, a certain degree of host preferences (Helgason et al. 2002; Sýkorová et al. 2007) was demonstrated in some studies. Strict host specificity in the sense of a limited spectrum of fungal associates of a host plant was found only in mycoheterotrophic plants that parasitize the mycorrhizal association (Bidartondo et al. 2002).

## V. Development of Taxonomic Theory

The history of AM research and glomeromycotan taxonomy has been reviewed by Koide and Mosse (2004) and was described as comprising four major periods (Stürmer 2012): the discovery period (1845–1974), the alpha taxonomy period (1975–1989), the cladistics period (1990–2000), and the phylogenetic synthesis period (since 2001). Spores and sporocarps of glomeromycotan fungi had in fact been collected and described long before it became clear that these fungi formed a mycorrhizal association. Initially, nearly exclusively sporocarp-forming species were the focus, starting with the first *Glomus* species described by Charles and Edmond Tulasne (Tulasne and Tulasne 1844), other species initially placed in the genus *Endogone*, previously erected by

Link (1809), and species of *Sclerocystis* (Berkeley and Broome 1873), all of these in the family Endogonaceae.

The first observation of what may constitute an AM was reported by Nägeli (1842), who found "fungi within cells" in *Iris* roots, but by the end of the nineteenth century several researchers had published descriptions that definitely showed this type of mycorrhiza (e.g., Janse 1897; Schlicht 1889). In 1885, the term mycorrhiza was used by Frank; however, it was ectomycorrhiza that was first recognized as a mutualistic symbiosis between plants and fungi (Frank 1885). Later "endotrophic mycorrhiza" or "vesicular-arbuscular mycorrhiza" (VAM) began to receive attention (Gallaud 1905; Janse 1897; Peyronel 1923). The term vesicular eventually was dropped because it became clear that some major groups in the Glomeromycota do not form vesicles.

Hyphal connections between mycorrhizal roots and sporocarps were noticed (Peyronel 1923). To establish a causal link between sporocarps and mycorrhizal infection, i.e., to fulfill Koch's postulates, took another three decades until the work of Mosse (1953) and Gerdemann (1955b). Now it was also possible to set up cultures of a defined mycorrhizal fungus together with a host plant to propagate it separately from other species and study its biology.

After pioneering studies, such as that by Nicolson and Gerdemann (1968), describing AM fungal species within the concept of the genus *Endogone*, the monograph by Gerdemann and Trappe (1974) constituted the birth date of the taxonomy of known AMF. For the first time, these authors placed all taxa of AMF known at the time in a stringent Linnaean context. They removed all AM-forming, nonzygosporic species from *Endogone* and placed them in the genera *Glomus*, *Sclerocystis*, and (newly described) *Acaulospora* and *Gigaspora*. For the first time, the mode of spore formation, that is, the way spores are formed on hyphae (see below for details), was recognized as a taxonomically useful character. Still, sporocarpic species accounted for a large proportion of the species listed in this account, reflecting the searching strategies of early mycorrhizologists, which very much resembled truffle hunting. However, the wet-sieving and decanting method of isolating glomeromycotan spores formed singly or in small clusters in the soil had already been reported by Gerdemann and Nicolson (1963), and in the ensuing 20 years, the sporocarpic species were destined to become a relatively marginal phenomenon, so that in 1990 they only accounted for approximately 42 % of *Glomus* (including *Sclerocystis*) species, compared to 95 % in 1974.

The growing interest in AM as a potential resource for agriculture and its recognition as an ecologically important factor also raised interest in the species diversity of these fungi, resulting in numerous descriptions in the 1970s and 1980s. It must be noted, however, that up to the present the mycorrhiza formation of many species is implied by analogy and has been proven only for a subset of species by pure culture on a host plant. The spore wall structure of the glomeromycetes was recognized as a crucial character for distinguishing species. The method of visualizing its components by crushing the spores gently on a microscope slide under a cover slip in a mountant, such as polyvinyl alcohol lactoglycerol (PVLG), became common. To better describe the multitude of wall structures, Walker (1983) created a standardized system of "walls" (discernible substructures of the spore wall) and "wall groups" (arrangements of walls staying attached to each other during this treatment). This standardization was an important step forward to compare different species more efficiently.

In 1990, Morton and Benny placed the genera known by then in a hierarchical taxonomic structure, removing AMF from the Endogonacae and placing them in their own order, Glomales (the orthography of which was later corrected to Glomerales). Cladistic analyses of the characters of spore morphology were used to provide the first putatively phylogenetic framework for the Glomerales, separating two major clades, the suborders Gigasporineae and Glomineae, and the families Glomeraceae (as Glomaceae), Acaulosporaceae, and Gigasporacaceae. Another advance was the inclusion of the spore ontogeny to group the spore wall structure hierarchically in contrast to the strictly phenetic system of Walker. This

was based on the observation that there is a predictable sequence of the formation of the respective "walls" or "wall layers" in the different taxa. It had already been noticed that certain taxa possess flexible inner walls, which sometimes are involved in spore germination and bear specialized structures (germination shields, germination orbs) playing a role in this process (Morton 1995; Walker and Sanders 1986).

Despite attempts to classify them using fatty acid profiles (Bentivenga and Morton 1996), isozymes, or monoclonal antibodies, the phylogenetic position of the Glomeromycota remained the subject of much speculation. After Simon et al. (1993) provided the first DNA sequences of the nuclear small subunit (SSU) ribosomal RNA gene from three AM fungal species, it was clear that they were a lineage of the true fungi, but, due to the limited taxon sampling and the absence of DNA sequences for many other basal fungal lineages, their exact placement could not be determined. Nevertheless, these data led to the first attempts to detect AMF by molecular methods in the environment (Clapp et al. 1995). At the time, methods to study the diversity of ectomycorrhizal fungi were far ahead of those for AMF because they were easier to study and had already been used to show the discrepancy between the diversity of mycorrhizal symbionts analyzed directly from roots and the diversity of their fruiting structures (Gardes and Bruns 1993). These findings stimulated the design of molecular tools to also analyze AM fungal species' richness in nature.

Molecular DNA data then became more common in elucidating the phylogenetic relationships among glomeromycotan fungi. The fact that *Geosiphon pyriformis*, a fungus forming an endosymbiosis with cyanobacteria, belongs in a basal glomeromycotan lineage was elucidated by SSU sequences (Gehrig et al. 1996) following the recognition of the similarity of its spores with those of some AMF (Fig. 9.1i) (Schüßler et al. 1994). Nowadays this makes *G. pyriformis* an interesting model for molecular biological studies of symbiosis-related genes (Schüßler 2012; Schüßler et al. 2006).

At the same time, molecular data demonstrated that morphological characteristics previously used to distinguish higher-level taxa, such as genera and families, were poor predictors of phylogenetic relationships. As an example, the genus *Paraglomus*, a deeply diverging lineage in the Glomeromycota, has spores that are morphologically indistinguishable at the genus level from those of *Glomus*, but the two genera are separated by hundreds of million years of evolutionary history (Morton and Redecker 2001; Redecker et al. 2000b).

In the Archaeosporaceae, some taxa even produced on the same fungal thallus glomoid spore types thought to be indicative of the genus *Glomus* and spores typical for the genus *Acaulospora* (Fig. 9.1h) (Morton and Redecker 2001; Morton et al. 1997). Molecular data revealed that they belonged to neither genus but rather constituted another deeply divergent lineage. This was the case for *Archaeospora leptoticha*, later placed in the genus *Ambispora* (Redecker et al. 2000b). Similarly, acaulosporoid and entrophosporoid spore formation in *Archaeospora trappei* and *Archaeospora schenckii* does not imply a close phylogenetic relation with *Acaulospora* or *Entrophospora*. The phylogenetic position of *Entrophospora infrequens*, however, has been impossible to determine because DNA analyses from different laboratories yielded a variety of sequences, often related to *Claroideoglomus* (Rodriguez et al. 2001), a fact that has been impossible to explain up to now.

With increasing knowledge of the phylogeny and biology of AMF, similarities to zygomycetes, such as *Endogone*, appeared to be more and more superficial, and the zygomycetous fungi began to emerge as an ill-defined, paraphyletic assortment of fungal lineages. Consequently, the monophyletic Glomeromycota were separated in their own monophyletic phylum (Schüßler et al. 2001b).

The species in two recently described genera, *Diversispora* and *Redeckera*, were previously placed in *Glomus* but shown to be phylogenetically very distant (Redecker et al. 2007; Schüßler and Walker 2010; Walker and

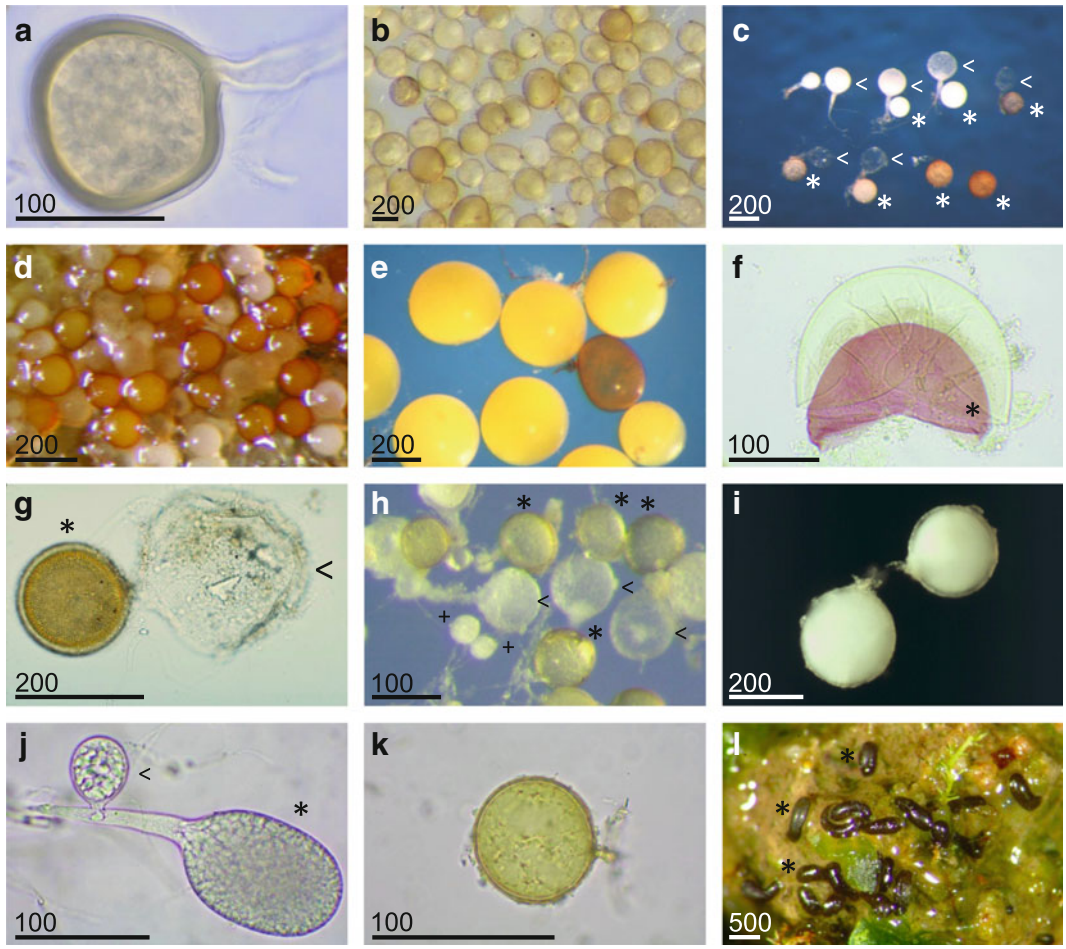


**Fig. 9.1** From *Top left* to *bottom right* (**a**) *Glomus macrocarpum* (Glomeraceae; from >150 year-old type material); (**b**) *Claroideoglomus claroideum* (Claroideoglomeraceae); (**c**) *Acaulospora spinosa* (Acaulosporaceae; *asterisk* sporiferous saccules *open angular bracket* spores); (**d**) *Diversispora epigaea* (Diversisporaceae; BEG47, a culture frequently used in AM research); (**e**) *Gigaspora gigantea* (Gigasporaceae); (**f**) *Pacispora franciscana* (Pacisporaceae; *asterisk* germinal wall stained with Melzer's); (**g**) *Entrophospora infrequens* (Entrophosporaceae; *asterisk* sporiferous saccule *open angular bracket* spore); (**h**) *Ambispora fennica* (Ambisporaceae; *asterisk* sporiferous saccules *open angular bracket* acaulosporoid spores *plus symbol* glomoid spores); (**i**) *Geosiphon pyriformis* spores (Geosiphonaceae); (**j**) *Archaeospora trappei* (Archaeosporaceae; *asterisk* sporiferous saccule *open angular bracket* acaulosporoid spore); (**k**) *Paraglomus occultum* (Paraglomeraceae); (**l**) *Geosiphon pyriformis* symbiotic bladders (*asterisk* dark vesicles, harboring cyanobacteria). *Scale bars* in micrometers

Schüßler 2004). Another example of where molecular data guided morphological analyses in defining new taxa was *Pacispora*, uniting characteristics like the glomoid spore formation and germinal walls similar to *Scutellospora* (Oehl and Sieverding 2004; Walker and Schüßler 2004; Walker et al. 2004).

Based on nuclear ribosomal DNA (rDNA) sequences, Schüßler and Walker (2010) redefined more genera in the Glomeromycota and created the new family Claroideoglomeracae for other species previously in *Glomus*. More and more species descriptions have been accompanied by molecular data, illustrating the usefulness of such data in separating taxa with few morphological

characters. The need to be able to assign these taxa to sequences from environmental studies, allowing new perspectives on their distribution and phylogeography, is also satisfied by such studies.

## VI. Classification

### A. Phylum Characteristics

The Glomeromycota are fungi that grow mostly hypogeously in association with plants; some, especially those forming sporocarps, fruit on the soil surface. They propagate generally by asexual spores, but in some groups also by hyphal fragments or colonized root pieces. Their spores are relatively large, with a diameter between less than 40 μm and more than 1,000 μm (Fig. 9.1e), containing up to several thousand nuclei and prominent lipid and protein globules. In some taxa, spores are formed within the roots. Spores are formed singly, in loose clusters, dense masses, or in sporocarps. The sporocarps formed by the Glomeromycota are agglomerations of a few to several hundred thousand spores, their size accordingly varying between less than 500 μm and greater than 4 cm. Sporocarps are sometimes covered by an outer peridium, whereas the spores can be embedded in mycelium or in some cases be radially arranged around a hyphal plexus.

### B. Morphological Criteria Used for Classification

The color, size, and shape of spores and the characteristics of hyphal attachment of the spores are important morphological criteria for determining taxa. The color, number, thickness, and consistency of wall layers have been used to distinguish species, whereas the presence or absence of flexible "germinal walls" and the morphology of the hyphal attachment (the so-called mode of spore formation) traditionally were used to determine the genus or family (Morton 1988). The staining behavior of the intraradical structures was also used to distinguish taxa, but it is variable within some families. The fact that some modes of spore formation seem to have evolved multiple times in the phylum has increased the importance of molecular phylogenetic data; in fact, some taxa, such as the orders, are mainly based on molecular phylogenies and sequence signatures. All orders presented here are monophyletic, based on nuclear rDNA data (Schüßler and Walker 2010; Schüßler et al. 2001b).

While electron microscopy (EM) has been employed widely to elucidate the intraradical exchange structures in AM, in particular the arbuscules (Bonfante-Fasolo and Grippiolo 1982), it has only sporadically been used to characterize spore wall structure. Nor have other subcellular details been analyzed broadly so far; for instance, the details of nuclear division are still not known.

### C. Orders and Families (For an Overview See Table 9.1)

1. Glomerales J.B. Morton and Benny (Sensu Schüßler et al. 2001b)

In this order, spore formation is exclusively glomoid, i.e., spores are formed by blastic expansion of a hyphal tip. The hyphae often remain attached to the spore, and the attachment is straight or recurved, but never with a bulbous sporogenous cell. The opening of the hyphal attachment may be closed by wall layers, a septum, or remain open; germination occurs through the attachment. The spore walls are often layered, comprising multiple lamellae. Ornamentation of the spore wall surface is usually absent; if present, it is relatively simple. This mode of spore formation, however, is also found in unrelated lineages.

The mycorrhizae usually stain strongly with trypan blue, chlorazol black, or acid fuchsin. Ovoid vesicles are often formed at later stages of colonization.

a) Glomeraceae Piroz. and Dalpé
Spore formation occurs singly, in roots or in soil, in loose clusters or in sporocarps (Fig. 9.1a). In some species, the formation of complex sporocarps occurs with peridium or hyphal

**Table 9.1** Classification of the Glomeromycota

| Order | Family | Approximate species number |
|---|---|---|
| Glomerales | Glomeraceae | 108 |
| | Claroideoglomeraceae | 6 |
| Diversisporales | Diversisporaceae | 10 |
| | Gigasporaceae | 53 |
| | Acaulosporaceae | 38 |
| | Pacisporaceae | 7 |
| Archaeosporales | Archaeosporaceae | 2 |
| | Ambisporaceae | 9 |
| | Geosiphonaceae | 1 |
| Paraglomerales | Paraglomeraceae | 3 |
| Familia incertae sedis | Entrophosporaceae | 3 |

plexus. No flexible inner walls are found. This family seems to contain about half of all described species of the phylum, although this could not be confirmed using molecular data for many species. Field surveys have also shown that many molecular operational taxonomic units (MOTUs) belong to this lineage, which is also known as the phylogenetic group, *Glomus* Group A [see for definition Schüßler et al. (2001a) and Schwarzott et al. (2001)].

b) Claroideoglomeraceae C. Walker and A. Schüßler

In this family, spores (Fig. 9.1b), which are usually formed singly in the soil, have walls with an ephemeral outer component that sloughs off in mature spores, a characteristic that also occurs in the Glomeraceae. A semiflexible innermost component [endospore, according to Schüßler and Walker (2010)] has been reported that may, however, be difficult to distinguish from the inner lamella of a rigid spore wall. This family corresponds to *Glomus* Group B.

2. Diversisporales C. Walker and A. Schüßler

This order contains a large variety of spore morphologies and is mainly delimited based on nuclear rDNA data.

a) Gigasporaceae J.B. Morton and Benny

Species in this family form relatively large spores (diameter 120 to >1,000 μm) that develop singly in the soil and are the only infective propagules (Fig. 9.1e). A bulbous sporogenous cell is found at the hyphal attachment (gigasporoid mode of spore formation), which is usually persistent. The mycorrhizae stain uniformly dark with standard procedures; the intraradical hyphae vary considerably in width. The arbuscules often have swollen trunks. No vesicles are formed in this family. On the extraradical mycelium, characteristic thin-walled auxiliary cells of unknown function are conspicuous. No interhyphal anastomoses are formed, whereas the fungi have the ability to bridge wounded mycelium parts by end-to-end anastomoses (de la Providencia et al. 2005).

In *Gigaspora* the spore wall does not contain flexible walls, only rigid components. Spores are brightly colored (white to yellowish green unless senescent) but never hyaline. Spores germinate directly through the spore wall with the germ tube emerging from a pustulate region at the inner layers of this rigid wall. In *Scutellospora* and *Racocetra*, spores are hyaline to dark brown and possess inner flexible germinal walls, which may color deeply pink with Melzer's reagent. On this germinal wall, a permanently present germination shield is found, from which the germ tube emerges (Walker and Sanders 1986). Spores in some species have highly complex surface ornamentations.

It has been known for quite some time that *Scutellospora* is paraphyletic with respect to *Gigaspora*, the lack of germinal walls in the latter clearly being the derived condition. Oehl et al. (2008) proposed splitting *Scutellospora* into five genera and the *Gigasporaceae* into four families based on nuclear large subunit (LSU) rDNA data and the morphology of the germination shield. Because this approach relied on the interpretation of insufficiently robust phylogenetic analyses and a single, plastic morphological character only, it was later rejected by Morton and Msiska (2010). These authors proposed a classification into three genera in a single family.

b) Acaulosporaceae J.B. Morton and Benny
Spores are formed either laterally (acaulosporoid mode) or centrally within (entrophosporoid mode) the hypha terminating in a thin-walled sporiferous saccule (Fig. 9.1c) that is formed before the spore (Gerdemann and Trappe 1974). The saccule and the sporiferous hypha usually detach at spore maturity; therefore, spores of the Acaulosporaceae are mostly sessile. At the occluded points of attachment, the detached hypha leaves one (acaulosporoid type) or two (entrophosporoid type) scars on the spore wall, which may, however, be difficult to observe. The sporiferous saccules are ephemeral with thin walls. The spore color ranges from hyaline to pale golden, orange, or dark brown to black, according to the species. The spores possess ephemeral outer layers, a rigid, often laminated, structural wall, and one or two inner germinal walls with flexible components. Depending on the species, the surface of the structural wall is often ornamented, with ridges, warts, pits, or spines. The spores germinate directly through the wall with the germ tube originating from a germination orb, a round, often spiral-shaped structure formed between the germinal walls or between the germinal and the structural wall (Stürmer and Morton 1999). The mycorrhizae in the Acaulosporaceae stain with varying intensity. The vesicles formed inside the roots may be lobed, but this is not confined to this group.

The acaulosporoid and entrophosporoid mode of spore formation were once thought to be substantial enough to warrant the separation of two genera, but in fact they are derivatives of a similar process, as the two types are also found in closely related species of the Archaeosporaceae (Kaonongbua et al. 2010), which is phylogenetically quite distant from the Acaulosporaceae.

c) Pacisporaceae C. Walker, Blask., A. Schüßler and Schwarzott
This family was established for members of the Diversisporales, with spores formed in the glomoid mode but containing germinal walls (Fig. 9.1f) and a so-called germination shield (Oehl and Sieverding 2004; Walker et al. 2004). Spores are hyaline to light brown to reddish brown; structural walls are often ornamented. The detailed mycorrhizal morphology in this group is unknown as no stable and pure cultures exist.

(d) Diversisporaceae C. Walker and A. Schüßler
Spores mostly form in the glomoid mode, singly, in aggregations or in dense spore clusters (Fig. 9.1d), or sporocarps (*Diversispora*, *Redeckera*). However, in the genus *Otospora* J. Palenzuela, N. Ferrol and Oehl spore formation on a persisting ear-shaped stalk has been reported (Palenzuela et al. 2008) and *Entrophospora nevadensis* has been placed in this family (Palenzuela et al. 2010); however, both reports require additional study to validate the placement. The Diversisporaceae are well separated by rDNA phylogenies from other glomeromycotan lineages that also form glomoid spores. Previously this phylogenetic lineage was known as *Glomus* Group C.

3. Paraglomerales C. Walker and A. Schüßler

a) Paraglomeraceae J.B. Morton and D. Redecker
The four species currently known in this family form small, hyaline glomoid spores (Fig. 9.1k). They can be separated from other lineages forming glomoid spores mainly based on molecular data, i.e., nuclear rDNA and sequences of the LSU of RNA polymerase II (*rpb*1), fatty acid profiles, and antibodies (Morton and Redecker 2001). In rDNA phylogenies the Paraglomeraceae were suggested to constitute the most deeply diverging lineage of the Glomeromycota (Redecker et al. 2000b), and this conclusion has received additional support (Krüger et al. 2012). Mycorrhizae, at least in some species, stain very faintly, so that it is difficult to determine and quantify root colonization.

4. Archaeosporales C. Walker and A. Schüßler

This order constitutes a deeply divergent lineage of the phylum, comprising three families with different modes of spore formation.

a) Archaeosporaceae J.B. Morton and D. Redecker

The spores formed by the two known species in this family are acaulosporoid (Fig. 9.1j) and entrophosporoid, with thin and, thus, semiflexible layers not reacting with Melzer's reagent (Kaonongbua et al. 2010; Morton and Redecker 2001). The mycorrhizae only stain faintly. A complex germination apparatus was reported (Spain 2003) but so far has not been independently confirmed. Also, a glomoid form has been reported.

b) Ambisporaceae C. Walker, Vestberg and A. Schüßler

This family is unique in the sense that at least some species are dimorphic, that is, spores of the acaulosporoid and the glomoid type are formed on the same fungal thallus (Fig. 9.1h). Also, some fungal isolates may form only the glomoid spore type (Morton et al. 1997). Walls of glomoid spores are usually soft and pliable; therefore, the spores do not crack under pressure from the cover slip but form folds. Sometimes they are covered with a mucilaginous coat to which soil particles tend to adhere. Acaulosporoid spores may be formed on a short pedicel that may persist on the spore, giving the false impression of a glomoid spore. Their spore wall structure is complex, with two to four layers. The thick inner walls have flexible components that do not react with Melzers's reagent and do not form germination shields or orbs. Germination occurs through the opening of the pedicel. The mycorrhizae stain very weakly; occasionally vesicles have been reported.

c) Geosiphonaceae Engler and Gilg, Emend. A. Schüßler

The only species of this family, *Geosiphon pyriformis*, is unique in the phylum because it is currently not known to form AM but an endocytobiosis with cyanobacteria of the genus *Nostoc* [for a recent review, see Schüßler (2012)]. The cyanobionts are harbored within multinucleate vacuolated fungal bladders on the soil surface, which are up to 2 mm long (Fig. 9.1l). The cyanobacteria provide photosynthates to the fungal partner, which provides all necessary mineral nutrients and water to the cyanobacteria except nitrogen, which can be fixed by the cyanobacterial heterocysts. The fungus forms whitish glomoid resting spores with layered walls, singly or in loose clusters (Fig. 9.1i).

It is unknown whether the fungus also forms AM, but its endocyanobiosis clearly represents an interesting and useful model system to better understand the symbiotic interface and nutrient exchange between the Glomeromycota and their photoautotrophic partners.

Nuclear SSU rDNA data have placed this species and family firmly in the Glomeromycota as one of the basal lineages. It has been proposed that this type of symbiosis could reflect an evolutionary precursor of AM [for a recent review, see Schüßler and Walker (2011)].

5. Familia *Incertae Sedis*

a) Entrophosporaceae Oehl and Sieverd

Into this family and its only genus, *Entrophospora*, were placed species forming entrophosporoid spores that could not be assigned to either Acaulosporaceae or Archaeosporaceae. *Entrophospora infrequens* is the generic type species, and its spores (Fig. 9.1g) have a complex and characteristic wall structure comprising rigid and semiflexible components, one wall layer having pits interlocking with projections of the layer above (Hall 1977). No pure culture of this species is available, but *E. infrequens* is rather often found in mixed cultures set up from field material (so-called trap cultures), but spore production ceases after some time. The species has presented a puzzle in molecular phylogenetic studies because very diverse, putatively contaminant-derived sequences normally representing lineages with different spore morphologies were detected (Rodriguez et al. 2001). However, the origin of the sequences is unclear, and the phylogenetic position of this family and its biological background therefore remain obscure.

### D. Species Concepts

Species have been described in the Glomeromycota usually as morphospecies. The size, shape, and color of spores are determined using a dissecting microscope, and hyphal attachments and the wall layer structure of the slightly cracked spores are examined in PVLG mounts at higher magnification. The reaction of spore wall components to Melzer's reagent also seems to be an important criterion (Morton 1988).

A major obstacle in studying the Glomeromycota has always been the inability to cultivate them separately from their plant host. Most often they have been propagated in open-pot cultures, which require several months to grow. Sometimes cultures are inoculated using single spores; thereby assuring that only a single species is present in the culture, but in this case special measures must be taken to achieve acceptable inoculation success. The purity of such pot cultures is difficult to maintain, and the harvested biological material always contains nonglomeromycotan microorganisms, complicating molecular analysis (Hijri et al. 2002; Walley and Germida 1996). Under these conditions, the degree of morphological and genetic variation within a species may be very difficult to assess. Monoxenic cultures on transformed roots (Bécard and Fortin 1988) offer a much higher security standard but are available only for a small fraction of the existing species.

Morphological characters to separate species are few and often difficult to observe; some species are apparently plastic in their morphology, depending on the culturing conditions and other factors. It must be emphasized that the majority of glomeromycotan species have been described not on the basis of pure cultures but using material collected from the field or mixed cultures set up from field material (trap cultures). In fact, many species have been described from obviously nonviable or degraded spores, resulting in misleading descriptions. In the strict sense, the ability to form mycorrhizae has therefore not been demonstrated but is assumed by analogy for many glomeromycotan species.

DNA sequences have been increasingly used to support (or reject) morphospecies concepts, but a stringent molecular species concept is difficult to establish. It has long been known that numerous variants of nuclear-encoded rDNA coexist within a single glomeromycotan spore (Sanders et al. 1995). Such variation was not found for the mitochondrial DNA (Raab et al. 2005), but for some other nuclear genes normally present as a single copy (Helgason et al. 2003; Koch et al. 2004), making it impossible to assign a single, unique sequence to a species. It has now been recognized that such intraorganism polymorphism is also found in other eukaryotes and has been underestimated in fungi, but in some species of the Glomeromycota it reaches exceptionally high levels (Stockinger et al. 2009, 2010). The possible contribution of pseudogenes to this polymorphism has not been determined systematically, but for the LSU rRNA gene most variants were also found in the transcriptome and indicated to be functional (Boon et al. 2010). For the rDNA Internal Transcribed Spacer (ITS) region (ITS1, 5.8S, ITS2) alone, which has been suggested as the primary DNA barcode for fungi (Schoch et al. 2012), it was shown that the intraspecific and intrasporal variation can be so high that closely related species are difficult or impossible to separate (Stockinger et al. 2009). In other fungi, molecular phylogenetic species concepts have been applied using coalescent analyses based on the criterion that species are reproductively isolated (Taylor et al. 1999). In the Glomeromycota, the genetic bases are still unclear for the great majority of lineages. Coalescent analyses cannot be applied to clonal lineages and require multilocus phylogenies, which are not yet available for the majority of glomeromycotan taxa.

Anastomosis formation could be another criterion for a biological concept of species delimitation. In *R. irregularis* (*Glomus intraradices*) hyphal cross bridges were observed between genetically distinguishable isolates at a frequency decreasing with genetic distance of the strains (Croll et al. 2009). However, in other species anastomoses only seem to occur within the same or very closely related isolates

(Giovannetti et al. 2003) or could not be observed at all (Purin and Morton 2011).

As the diversity of members of the Glomeromycota detected in environmental studies using molecular methods seems to greatly outnumber morphospecies, operative concepts were used to enumerate this diversity (e.g., Öpik et al. 2008). These concepts were based on cutoff values of sequence similarity, the definition of monophyletic groups by phylogenetic analyses, or a combination of both. However, many of these studies used exclusively the nuclear small ribosomal subunit as a marker gene, which was shown to be unsuitable for separating closely related species (Walker et al. 2007). It has become clear that cutoff values of sequence similarity cannot be generalized across families and orders. Nevertheless, molecular operational taxonomic unit (MOTU) estimates are, and will be (Hawksworth et al. 2011), highly useful as comparative proxies of biodiversity in field settings, but most authors recommend avoiding the usage of the term species in this context if MOTUs are not defined at this taxonomic level.

## VII. Evolution of the Phylum

The evolutionary aspects of AMF, evolution of AM, coevolution of the symbiosis partners, and the putative impact of the AM on the colonization of land by plants has recently been reviewed in this series (Schüßler and Walker 2011). Here, some of the major points are briefly discussed.

### A. Ecological Aspects

Unfortunately, not much is known about the differences in symbiotic function among the families of the Glomeromycota. Certain trends on this level were identified, for example, the differences in hyphal network architecture by the formation of anastomoses in the Glomeraceae and the absence of such networks in the Gigasporaceae (de la Providencia et al. 2005). It was also suggested that symbiotic benefits for the plant were mainly based on nutrient transport in Gigasporaceae and mainly on increased resistance against pathogens in the Glomeraceae (Klironomos et al. 2000). Different nutrient foraging behaviors have been compared among some species in the Glomerales (Jansa et al. 2005). Agricultural practice seems to have varied influence on taxon occurrence on different levels from family to species (Helgason et al. 1998; Hijri et al. 2006), which may in part be correlated with the life history strategies of species or families (Sýkorová et al. 2007).

### B. Spore Structure and Ontogeny

Concerning the evolution of spore structure, more data are available. Still, as the function of many specific components of spore formation (e.g., sporiferous saccule) is unknown, it is difficult to interpret morphological evolution of spore formation, i.e., to define derived versus ancestral morphological characters. Current knowledge of glomeromycotan phylogeny allows pinpointing the following trends:

(A) The glomoid, acaulosporoid, and entrophosporoid modes of spore formation are polyphyletic. The glomoid type is particularly widespread among unrelated lineages. Glomoid and acaulosporoid types may occur in the same species, indicating that these two types of structures are nonhomologous. The switch between entrophosporoid and acaulosporoid formation seems to require only small changes in the development pattern. This may explain why in each of two very distantly related families (Acaulosporaceae and Archaeosporaceae), closely related species sharing numerous other characteristics differ only in this respect.

(B) The presence of so-called germinal walls with germination shields/orbs is restricted to the Diversisporales, where they can be found in all four spore types, but it has not yet been conclusively demonstrated whether these structures are homologous. The loss of these structures is evident in *Gigaspora*, which is clearly a derived and not a basal genus within in the family.

### C. Evidence from Fossil Record and Patterns of Association with Plants

The earliest known, most widely recognized evidence for glomeromycotan fungi are 460 million-year-old fossilized glomoid spores and hyphae from Ordovician limestone (Redecker et al. 2000a). At this time, land plants had probably reached the morphological complexity of today's liverworts; therefore, it is not surprising that such plants are not well documented in the fossil record; thus a direct interaction of these fungi with the early land plants could not be shown up to now. Unequivocal evidence for embryophytes dates back to about 470 million years ago (mya), in the form of cryptospore assemblages (Rubinstein et al. 2010), and early vascular plants can be traced back about 420 million years (Stewart and Rothwell 1993).

Among the wealth of different early Devonian life forms that are exceptionally well conserved in the Rhynie Chert, dating back 400–412 mya, are the oldest known and most beautifully conserved arbuscules, the first evidence for the AM symbiosis itself (Remy et al. 1994). The fossils were detected in the rhizomes of Devonian plants such as *Aglaophyton*, with a much more advanced morphology than the putative Ordovician plants. These plants had not yet evolved roots but were colonized in their shoot cortex, illustrating the fact that roots came later than mycorrhiza, if interpreted by function and homology. In this sense, the term mycorrhiza obviously should not be used exclusively for associations involving root organs.

Besides early evidence for a number of fungal lineages, the Rhynie Chert also contained well-conserved spores of *Scutellospora*- and *Acaulospora*-like morphology and structures closely resembling germination shields (Dotzler et al. 2006, 2009). These fossils indicate that even 400 mya much of the glomeromycotan diversity on the order and family level may have been present already and that the deep lineages, such as Archaeosporaceae and Paraglomeraceae, may be considerably older. It may, however, also just indicate that character evolution of the glomeromycotan spore is more complex than previously thought, involving losses of characters previously thought to be indicative of an advanced state (Schüßler and Walker 2011).

The occurrence pattern of AM in extant plant groups indicates strongly that the ability to form this symbiosis is an ancestral character of land plants. Other types of mycorrhizae are clearly secondary associations of land plants, found exclusively in derived plant lineages and also much later in the fossil record (LePage et al. 1997). Many species of the deepest lineages (hornworts and liverworts) of land plants form associations with glomeromycotan fungi. Interestingly, recent findings indicate that extant bryophytes also form associations with zygomycetes from the *Endogone* containing clade (Bidartondo et al. 2011). This stimulates the discussion about mycorrhizal associations of early land plants, as *Endogone* most likely branches earlier than the AMF in the fungal tree of life. However, recent bryophytes also form close associations with ascomycetes and basidiomycetes, and an ancestral origin of such a symbiosis with *Endogone*-like fungi remains speculative.

The complete absence of mycorrhiza or mycorrhizalike symbioses or the presence of other types of associations than AM can be most parsimoniously explained by a loss or a switch from an ancestral state. In any case, the AM-specific plant genes and their functions are extremely conserved from bryophytes to vascular plants (Wang et al. 2010).

Taken together (Schüßler and Walker 2011), these data support the hypothesis that plants and AMF colonized the land masses together (Pirozynski and Malloch 1975), the fungi being potentially instrumental in the success of the colonization. In early terrestrial ecosystems before the formation of fertile soils and humic layers, the absorbing capacity of a fungal mycelium for nutrient uptake and transfer may have been even more crucial than today.

## IX. Conclusion

There have been numerous revisions of glomeromycotan taxonomy in recent years, reflecting the steadily growing knowledge about the evolutionary relationships of these fungi. Molecular data

have allowed the refinement of morphology-based approaches as well as avoidance of pitfalls by almost inevitable overinterpretation of the few morphological characters that have been used in Glomeromycotan classification. This has led to a better appreciation of the genetic diversity of arbuscular mycorrhizal fungi on all levels, from the phylum to species and populations, although a conclusive molecular species concept remains one of the major challenges for future research. There is an increasing interest in Glomeromycotan fungi as an ecologically and economically important group of organisms, for example, in the context of sustainable management of environmental resources. Future research on AM needs as a framework a robust, natural taxonomy based on the phylogenetic relationships of these fungi, which leaves space for future changes without artificial overinflation of taxa.

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