Taxonomy of Arbuscular Mycorrhizal Fungi

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ABSTRACT

Taxonomy of arbuscular mycorrhizal fungi (AMF) created problems mainly being obligate symbionts. In this review, the history and development of taxonomy of AMF is addressed. In the initial discovery period (1845-1974) the sporocarp-forming species were described and a classification for these fungi was proposed. This was followed by alpha taxonomy period (1975-1989) in which time solid morphological basis for species identification was developed resulting in description of several new species. The next cladistic period (1990-2000) resulted in the first cladistic classification of AMF based on phenotypic characters. The present phylogenetic synthesis period (2001-till date) proposed a new classification based on genetic characters using sequences of multicyclic RNA genes. In conclusion it can be said that the taxonomy of AMF belonging to the phylum Glomeromycota has generated considerable confusion and controversy among mycologists working with this important plant symbionts.

KEYWORDS: AM fungi, Classification, Glomeromycota, Taxonomy

INTRODUCTION

Arbuscular mycorrhiza (AM) is a symbiosis between fungus and the root, in which the fungus supplies the root with nutrient and the root supplies the fungus with carbon (Harley and Smith, 1983). Intensive research has been carried out throughout the world on the role of these fungi in improving growth and yield in plants. It is now well-demonstrated that these fungi can substantially increase the uptake of mineral nutrients, particularly P, drought tolerance and resistance to soil-borne plant pathogens (Bagyaraj, 2011). Formerly, all fungi forming arbuscular mycorrhizal associations were placed under the genus Endogone. The genus Endogone (Gr. Endo=inside; gone=seed) was first described by Link in 1809 which form mycorrhizal associations with higher plants and also produce hypogeous sporocarps in soil. The history of AMF taxonomy can be grouped under different periods as suggested by Sturmer (2012).

THE DISCOVERY PERIOD (1845-1974)

In 1845, Tulasne and Tulasne published a brief description of the species Glomus microcarpus and Glomus macrocarpus in a manuscript written in Latin and later transferred the two species to the genus Endogone (Tulasne and Tulasne, 1851). The genus Sclerocystis was proposed by Berkeley and Broome (1873) to encompass species forming spores in small sporocarps. Both genera were classified in the family Endogonaceae, order Mucorales. Interestingly, Glomus and Sclerocystis were described before the term “mycorrhiza” was coined by Frank in 1885. The first monograph of the family Endogonaceae treated in the order Mucorales (Zygomycetes) has been prepared by Thaxter (1922). Fungi of this family were located in four genera producing spores in sporocarps: Endogone Link: Fries, Glaziella Berk., Sclerocystis Berk. & Br., and Sphaeroecras Sacc. & Ellis. In 1935, Zycha transferred the one species of Sphaeroecras to Endogone. The existing genera included both chlamydosporic and zygosporic species. Thaxter (1922) and Godfrey (1957) considered chlamydosporic species to be anamorphs of those producing zygospores, following the finding of both types of spores in sporocarps of Glomus fasciculatum (at that time known as Endogone fasciculata and E. microcarpa).

During this period, the widely used method of wet sieving to extract sporocarps and non-sporocarpic spores from soils was developed by Gerdemann and Nicolson (1963), which led to an increase in taxonomic activity. Collaboration between Gerdemann and Trappe resulted in the classical publication “The Endogonaceae in the Pacific Northwest” by Gerdemann and Trappe in 1974. The authors in this publication proposed a classification for fungal taxa including 30 species forming arbuscular mycorrhiza. They recognized Glomus as a valid genus distinct from Endogone, as previously proposed by the Tulasne brothers, and erected two new genera, Acaulospora and Gigaspora, which had also been shown to form arbuscular mycorrhizal associations. In this classification (Table 1), the genera Glomus, Sclerocystis, Acaulospora, and Gigaspora were placed in the family Endogonaceae, order Mucorales, phylum Zygomycota, together with Endogone and two other genera, Glaziella and Modicella, which were not known to form arbuscular mycorrhizal associations. Later, Modicella was transferred to the family Mortierellaceae by Trappe (1982) and Glaziella was transferred to the Ascomycota by Gibson et al. (1986). A new genus, Complexipes, erected by Walker (1979) and tentatively placed in Endogonaceae was later transferred to Discomycetes (Trappe, 1982). The classification by Gerdemann and Trappe (1974) was important for the taxonomy of these organisms, and it provided a sound basis for systematic knowledge during several years to follow.

THE ALPHA TAXONOMY PERIOD (1975-1989)

This period contributed to the establishment of a solid morphological basis for identification and classification of glomeromycotan fungi. The 15 years are characterized by: (1) the proposal of several new genera and families, (2) a profuse description of new species, and (3) the proposal for standardization of phenotypic characters of arbuscular mycorrhizal fungal (AMF) spores to describe new species. New taxa forming arbuscular mycorrhizal associations were proposed based on living and fossil evidence. A new genus, Entrophospora, was erected by Ames and Schneider (1979) based on the observation that the formation of a “saccule” prior to spore development. Walker and Sanders (1986) differentiated between species of Gigaspora proposed by Gerdemann and Trappe (1974) based on whether spore
germination occurred through a flexible “shield” on an inner flexible wall or directly through the spore wall, and they used the former character to define a new genus, *Scutellospora*. During this period, a large number of new species were described. Walker in the UK established an interest in this group of fungi, initially publishing with Trappe (USA), but later also establishing collaboration with several other researchers, principally Koske (USA). After initial work with Nicolson (UK), Schenck (USA) also established a group describing new species, and partly stemming from work with Schenk, Spain and Sieverding published new species from their work in Colombia. Towards the end of this period, Błaszkowski (Poland) and Morton (USA) began to publish new species based on morphological characteristics.

Only 12 years after the monograph by Gerdemann and Trappe (1974), the number of described glomeromycotan species had jumped to 77 (Trappe, 1982), and 6 years later, Schenck and Pérez (1988) listed 126 species. In parallel, different keys for AMF species identification developed, such as the synoptic key of Trappe (1982), the dichotomous key of Hall and Fish (1979), and Hall (1984), and keys for groups of species (e.g., Koske and Walker, 1985). A significant step forward for those interested on AMF taxonomy and identification at the time was publication of the “Manual for the Identification of VA Mycorrhizal Fungi” (Schenck and Pérez, 1988) which compiled all summary species descriptions. All descriptions during this time were based on morphological features of spores. Spore subcellular structures, which are diverse, largely accounted for most differences between species. Walker also proposed a “muograph” that consists of a graphic representation to depict the different wall types and groups found in a spore. Berch (1986) in her treatise on the *Endogonaceae* suggested the use of the word “wall layers” instead of “wall”. The different wall layers described were laminated, evanescent, membranous, amorphous, coriaceous and germinable. Towards the end of this period, Morton (1988) critically evaluated all morphological criteria used to classify and identify AMF species and suggested some approaches to clarify taxonomy concepts.


This period is marked by a new classification and the entry of molecular biology into systematics of glomeromycotan fungi. It is characterized mainly by: (1) proposal of a cladistic classification for AMF based on phenotypic characters, (2) description of new taxa based on fossil records, (3) proposal of a spore development model with re-evaluation of terminology for spore subcellular characters, and (4) use of genetic characters to define taxa and elucidate evolutionary relationships. One of the important landmarks in these 11 years was the first cladistic analysis of glomeromycotan fungi and the proposal of a new classification. Morton (1990) proposed two main clades, one consisting of *Gigaspora* and *Scutellospora* species and the other harboring *Glomus, Sclerocystis, Acaulospora*, and *Entrophospora*. This cladistic analysis, together with additional information from spore ontogeny and mode of spore germination, formed the basis for a radical change in classification (Morton and Benny, 1990) (Table 1). Genera of AMF were removed from the order *Endogonales* and placed in the newly erected order *Glomerales* (published as *Glomales*) in the families *Glomeraeaceae* (*Glomus* and *Sclerocystis*), *Acaulosporaceae* (*Acaulospora* and *Entrophospora*), and *Gigasporaceae* (*Gigaspora* and *Scutellospora*). *Glomeraeaceae* and *Acaulosporaceae* were hypothesized to be closely related and placed in the sub-order *Glomineae* and the family *Gigasporaceae* in the sub-order *Gigasporineae*. This was the first new classification proposed since Gerdemann and Trappe (1974) had considered that AMF should remain in the phylum *Zygomycota* some 25 years earlier.

Morton (2000) proposed that the arbuscular mycorrhizal symbiosis had arisen during two distinct periods rendering the order *Glomerales* polyphyletic: *Glomineae* and *Gigasporineae* would represent two evolutionary branches. Evidence to support this hypothesis is related to the mode of spore formation (Franke and Morton, 1994), morphology of fungal mycelium (Brundrett and Kendrick, 1990), types of infective propagules (Biermann and Linderman, 1983; Jasper et al., 1989), and cell wall composition (Gianinazzi-Pearson et al., 1994). The classifications of Gerdemann and Trappe (1974) and Morton and Benny (1990) did not state clearly into which class the AMF species should be included: *Endogonales* in the former and *Glomerales* in the latter were left in the class *Zygomycetes*. Cavalier-Smith (1998) later proposed that fungal species establishing (vesicular) arbuscular mycorrhizas with plants could be grouped in a new class, the *Glomomycetes*, within a new phylum, *Archemycota*.

The number of new species described in this “cladistics period” totaled one third of that described in the previous “alpha-taxonomy” period. One of the main events during these 11 years was the use of SSU gene sequences to elucidate evolutionary relationships among taxa within the order *Glomerales*. This period ends with the identification of two ancestral clades based on rDNA sequences (Redecker et al., 2000).

**THE PHYLOGENETIC SYNTHESIS PERIOD (2001 TO TILL TODAY)**

This ultimate period is characterized by: (1) the proposal of a new classification based solely on genetic characters (SSU rRNA gene), (2) description of new taxa based on the fossil record, and (3) the creation of new taxa and a new classification based on a combination of phenotypic and genetic characters. The most important event in this period has been the naming by Schüßler et al. (2001) of a new phylum within the kingdom Fungi to group all AMF species. The proposed phylum *Glomeromycota* is based on a phylogenetic analysis of SSU rRNA gene sequences. Four new orders (*Paraglomerales, Archaeasporales, Diversisporales*, and *Glomerales*) and new families were proposed (Table 1). The term “glomerospores” was coined by Goto and Maia (2006) to denominate spores formed by fungi in the *Glomeromycota*. After the new classification by Schüßler et al. (2001), the last 10 years have been characterized by descriptions and proposals of new families and genera for both ancient and extant AMF, with some of the taxa proposed still in debate among taxonomists. A more radical expansion of genera and families in the
Glomeromycota was proposed by Oehl et al. (2008) based on
interpretation of the previous works of Walker et al. (2004),
De Souza et al. (2005), Ahulu et al. (2006), and Redecker et
al. (2007). In the past few years, two distinct classifications
have been further proposed for the Glomeromycota (Table 1),
both of which are characterized by a rearrangement of the
genus Glomus sensu lato shown previously to be polyphyletic
performed a phylogenetic analysis of glomeromycotan fungi,
based on near-full-length SSU rRNA gene sequences and
proposed a new family and three new genera. They separated
Glomus into the genera Funneliformis, Sclerocystis, and
Rhizophagus in the family Glomeraceae with the remaining
species of Glomus and Claroideoglomus in the family
Claroideoglomeraceae. Rhizophagus was first proposed by
Dangeard (1900) and synonymized with Glomus by
Gerdemann and Trappe (1974). Inspection of the protologue
of Rhizophagus populinus revealed that this fungus is an AMF
species, and it was resurrected by Schüßler and Walker (2010)
to harbour AMF species that form large numbers of spores in
the roots. Schüßler and Walker (2010) recognized that their
phylogeny is incomplete because no living material is
available for molecular analyses of many previously
described glomeromycotan species. Therefore, some species
were retained in their original genus but referred to as
“species of uncertain position.” Oehl et al. (2011) proposed a
rearrangement of species in the genus Glomus sensu lato and
erected the genera Simiglomus and Septiglomus in the
Glomeraceae, and Viscospora in the Claroideoglomeraceae.
An evidence-based consensus for the classification of AMF
(Glomeromycota) was published by Redecker et al. (2013).
The authors point out that recent publication of numerous new
taxa at all level within Glomeromycota has created confusion
and operational difficulties for those working with AMF. The
fungi being obligate symbionts pose problems not
encountered for many other groups of organisms. The
taxonomy of AM fungi thus has undergone intensive
investigation and has experienced lots of controversy and
radical transformations. However the characteristics of some
of the commonly occurring AM fungi used by majority of
mycorrhiza workers to identify them are given below.

Glomus: Spores formed blastically on subtending hyphae,
singly, in loose aggregates or in a sporocarp. Vesicles are thin
walled and ellipsoid. Intraradical hyphae rarely coiled, with
cross-connecting branched hyphae. Mycorrhiza stains darkly.
Arbuscules with flared or cylindrical trunks with incremental
narrowing of branch hyphae. Spores with spore wall formed
by a variable number of layers all originating from the
subtending hyphae, no germinal walls differentiated.
Germination through the lumen of the subtending hyphae or
through the spore wall.

Acaulospora: Spores formed laterally from the neck of a
sporiferous saccule which leaves one scar on the spore
surface. Vesicles vary in shape with knobs and concavities.
Intraradical hyphae straight or coiled near the entry points.
Mycorrhiza stains weakly. Arbuscules with flared or
cylindrical trunks with incremental narrowing of branch
hyphae. Spores with spore wall formed by three layers and
two inner germinal walls each with two thin layers that can be
adherent. The innermost germinal wall has a beaded surface.
Germination through a flexible, plate like germination orb.

Entrophospora: Spores formed within the neck of a
sporiferous saccule which leaves two scars on the spore
surface. Vesicles, arbuscules, intraradical hyphae and
mycorrhizae staining as in Acaulospora. Spores with spore
wall formed by two layers. Other spore subcellular structures
and germination identical to that in Acaulospora.

Gigaspora: Spores formed terminally on a bulbous
sporogenous cell; auxiliary cells finely papillate or
echinulate. No vesicles produced. Intraradical hyphae
frequently coiled, especially near entry points, often knobby
or with projections. Arbuscules with swollen trunks with
abrupt narrowing of branch hyphae. Spores with spore wall
formed by two permanent layers, no inner germinal walls
differentiated. At germination, a thin layer interspersed with
warts differentiate and germ tube grows throughout the spore
wall.

Scutellospora: Spores formed terminally on a bulbous
sporogenous cell; auxiliary cells almost smooth to knobby.
No vesicles produced. Arbuscules and intraradical hyphae
similar in morphology to Gigaspora. Spores with spore wall
formed by two permanent layers and 1-3 inner germinal
walls, each with two layers. Germ tube grows from flexible,
plate-like germination shield that differentiates on the surface
of the last germinal wall.

CONCLUSIONS

Taxonomic and systematic studies of AMF can be traced
back to the early works of the Tulasne brothers (1845) and
the Thaxter (1922) revision of Endogonaceae. In the last 45
years, the classification of this group of fungi has undergone
considerable transformations, from being merely
descriptive and based solely on spore morphology
(Gerdemann and Trappe, 1974) to being based on cladistic
analysis of genetic and phenotypic characters. Morton and
Benny's (1990) classification is based on the analysis of
phenotypic characters (spore morphology and mycorrhizal
characters), classifications of Schüßler et al. (2001), that of
Schüßler and Walker (2010) on genetic characters
(sequence variation of the SSU rDNA), and that of Oehl et
al. (2011) on combined genetic and phenotypic characters.
Up to 2001, these fungi were included in one class, one
order, three families, and six genera; 10 years later, with the
use of genetic characters, they are distributed into one to
three classes, four to five orders, 11-14 families, and 18-29
narrowing of branch hyphae. Spores with spore wall
formed by two permanent layers and 1-3 inner germinal
walls, each with two layers. Germ tube grows from flexible,
plate-like germination shield that differentiates on the surface
of the last germinal wall.

Table 1. (2001), that of

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Table 1: Proposals of classification of glomeromycotan fungi within the kingdom Fungi
REFERENCES


