Taxonomic Characteristic of Arbuscular Mycorrhizal Fungi-A Review

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Abstract: Arbuscular mycorrhizal fungi (AMF) have mutualistic relationships with more than 80% of terrestrial plant species. Despite their abundance and wide range of relationship with plant species, AMF have shown low species diversity. AMF have high functional diversity because different combinations of host plants and AMF have different effects on the various aspects of symbiosis. Because of wide range of relationships with host plants it becomes difficult to identify the species on the morphological bases as the spores are to be extracted from the soil. This review provides a summary of morphological and molecular characteristics on the basis of which different species are identified.

Key words: AMF · Taxonomic Characteristics

INTRODUCTION

The fungi forming arbuscules in roots of terrestrial plants always created great taxonomic problems, mainly because of difficulties to extract their spores from the soil and to maintain the fungi in living cultures. Peyronel [1] was first to discover the regular occurrence of both associations that of the spores and sporocarps of the first described family Endogonaceae from vesicular-arbuscular mycorrhizas of plants. He suggested that the fungi can be considered as the progenitors of the mycorrhizae. The first monograph of the family Endogonaceae Paol. placed in the order Mucorales (Zygomycetes) was prepared by Thaxter [2]. Fungi of this family were described in four genera which produced spores in the sporocarps. The four genera being those of Endogone Link: Fries; Glaziella Berk.; Sclerocystis Berk. and Br.; and Sphaerocreas Sacc. and Ellis. The existing genera included both chlamydosporic and zygosporic species of fungi. Thaxter [2] and Godfrey [3], however, considered chlamydosporic species to be anamorphs of those producing zygospores, following the finding of both types of spores in sporocarps of the present day Glomus fasciculatum earlier nomenclated as Endogone fasciculata and E. microcarpa.

Sizable data of the biology of fungi belonging to family Endogonaceae has been generated from their studies in pot cultures. The mode of germination of spores of these fungi, their life cycles, subcellular spore structure and the manner of colonization of roots have been recognized as the main characters [4, 5]. It has been found that some taxa are both arbuscular mycorrhizal, in the host roots, whereas other species of mycorrhizae lacked vesicles. The first taxonomic key for the recognition of the types of the endogonaceous spores has been prepared by Mosse and Bowen [6]. The Endogonaceae was revised by Gerdemann and Trappe in [7]. He maintained the family in the order Mucorales comprising, 44 species in seven genera. Amongst these, many a taxa were redefined. Two genera Acaulospora and Gigaspora and 12 species were described novo. The genus Endogone contained 11 species with zygospores arranged in sporocarps. Two of these, E. lactiflua Berk. and Broom and E. flammicorona Trappe and Gerd., are ectomycorrhizae [7-9]. Therefore, according to Gerdemann and Trappe [7], the other Endogone species were ectomycorrhizal or saprotrophic fungi.

The genus Glomus with 19 species, then with two varieties of G. macrocarpus, i.e., G. macrocarpus var. macrocarpus and G. macrocarpus var. geosporus, originally erected by Tulasne and Tulasne [10] and the genus Sclerocystis with four taxa contained species forming chlamydospores blastically at hyphal tips. In contrast to sporocarpic Sclerocystis spp., the chlamydospores of the members of genus Glomus have been considered to occur mainly in loose aggregates or singly in the soil, although the genus also
included species forming compact sporocarps with or without a peridium. Species of the genus Glomus have been found to form vesicular-arbuscular or only arbuscular mycorrhizae. The distinctive property of members of the genus Sclerocystis was the production of chlamydospores arranged in a single layer around a central, spore-free plexus. The newly erected genera Acaulospora and Gigaspora have been defined by Gerdemann and Trappe [7] as forming azygospores singly in the soil, although no parthenogenetical process of sporocarpic Glomus was observed. Acaulospora spp. produced spores laterally on the neck of a sporiferous saccule and species of the genus Gigaspora formed spores terminally at the tip of an inflated, bulbous sporogenous hypha. The genera Glaziella and Modicella Kanouse were represented by chlamydosporic and sporangial species, respectively.


Since 1984, the genera Glaziella and Modicella were not considered to be members of the family Endogonaceae. The presence of septa in fragments of sporocarps of Glaziella aurantiaca (Berk. and Curtis), Cooke has decided that the fungus has first been transferred to Deuteromycotina [21] and then to a new order, Glaziellales with one family, Glaziellaceae [22]. The genus Modicella has been transferred to the Mortierellaceae [23].

Walker and Sanders [24] separated the genus Scutellospora from the genus Gigaspora, with fungi forming spores having at least one inner germination wall and later containing species with spores lacking an inner germination wall having no physical contact with their main, structural wall.

Morton and Benny [25] transferred soil-borne fungi, forming arbuscules in roots of terrestrial plants, to a new order-Glomales (orthographically corrected to Glomerales) as G. infrequens [12]. Spores of E. infrequens were transferred to Deuteromycotina [21] and then to a new order, Glaziellales with one family, Glaziellaceae [22].

Almeida and Schenck [27] concluded that, except for Sclerocystis coremioides, a continuum of morphological properties exists between sporocarpic Glomus species and the other members of the genus Sclerocystis. As a result, the five-species genus Sclerocystis was reduced to a one having a single-species by them.

Redecker et al. [28] utilizing both morphological and molecular data, transferred Sclerocystis coremioides to the genus Glomus and, thereby, eliminated the genus Sclerocystis from the Kingdom Fungi.

Two new families of Archaeosporaceae and with order Glomales (now Glomerales) Paraglomaceae (now Paraglomerae) were erected by Morton and Redecker [29] on the basis of the molecular, morphological
and biochemical investigation. Despite similarities in mycorrhizal morphology each of these families was phylogenetically distant from each other and from other glomalean families. The family Archaeosporaceae contained one genus, Archaeospora, with three species forming a typical Acaulospora-like spores from the neck of a sporiferous saccule. Two of these species, Archaeospora and A. leptoticha, were considered to be dimorphic, since there former Glomus-like spores as well. The genus Paraglomus in the family Paraglomaceae (now Paraglomerales) consisted of two species producing spores indistinguishable from those of the species of Glomus.

The classification of arbuscular fungi presented here is basically that of Schubler et al. [26] with later amended by Oehl and Sieverding [30], Walker and Schubler [31], Sieverding and Oehl [32], Spain et al. [33], Walker et al. [34] and Palenzuela et al.[ 35]. In this system of classification, fungi producing or considered to produce arbuscular mycorrhizae are placed in the Phylum Glomeromycota and Glomeromycetes class. This class comprises four orders Archaeosporales, Diversisporales, Glomerales, Paraglomerales with a total distribution of ten families. These families include thirteen genera [26, 30-35]. The earlier relationships of the fungi with Zygomycota, as described in the preceding pages, stands revised presently, since molecular investigations relate these more closely with the members of the Phyla Ascomycota and Basidiomycota.

Glomeromycota C. Walker and Schuessler
Glomeromycetes Cavalier-Smith
Archaeosporales C. Walker and Schuessler
Ambisporaceae C. Walker, Vestberg and Schuessler
Ambispora Spain, Oehl and Sieverd
Geosiphonaceae Engler. and E. Gilg emend. Schuessler Geosiphon (Kütz.) F. Wettst.
Diversisporales C. Walker and Schuessler
Acaulosporaceae J.B. Morton and Benny
Acaulospora Gerd. and Trappe emend. S.M. Berch
Kuklospora Oehl and Sieverd.
Acaulospora Gerd. and Trappe emend. S.M. Berch
Diversisporaceae C. Walker and Schuessler
Diversispora C. Walker and Schuessler
Otospora Oehl, J. Palenzuela and N. Ferrol
Entrophosphoraceae Oehl and Sieverd.

Gigasporaceae J.B. Morton and Benny
Gigaspora Gerd. and Trappe emend. C. Walker and F.E. Sanders
Scutellerospora C. Walker and F.E. Sanders
Pacisporaceae C. Walker, Blaszk., Schuessler and Schwarzott
Pacispora Oehl and Sieverd.
Glomerales J.B. Morton and Benny
Glomeraceae Piroz. and Dalpe
Glomus Tul. and C. Tul.
Paraglomerales C. Walker and Schuessler
Paraglomerales J.B. Morton and D. Redecker
Paraglomus J.B. Morton and D. Redecker

The fungi of the order Archaeosporales either form endocytosymbioses with photoautotrophic prokaryotes (Geosiphon pyriformis) or produce mycorrhizae with arbuscules, both with or without vesicles. Their spores are colourless and do not react with Melzer’s reagent. Glomoid sporewhich are identical to those of AMF genus Glomus are formed singly or in clusters both on or under the soil surface. Acaulosporioid spores, similar to those of the members of the genus Acaulospora, develop singly but in the soil. This group of AMF differ from other arbuscular fungi by the possession of the rRNA SSU gene signature YCTATCYKYCTGGTGAKRCG, showing homology to the position 691 of the Saccharomyces cerevisiae SSU r RNA sequence J01353, with the coloured nucleotides being specific for the AMF taxon. The order Archaeosporales contains three families, Archaeosporeaceae with the genera Archaeospora and Intraspora, Ambisporaceae with the genus Ambispora and Geosiphonaceae with the genus Geosiphon.

Members of the order Diversisporales form mycorrhizae with arbuscules, frequently lacking vesicles, with or without auxiliary cells. Spores develop either inside as entrophosphoroioid spores of the genera Entrophospora and Kuklospora or laterally on the neck of a sporiferous saccule such as acaulosporioid spores of the genus Acaulospora and Otospora, also either from a bulbous base on the sporogenous hypha like gigasporoid spores of the genera Gigaspora and Scutellerospora, or blastically at the tip of a sporogenous hypha such as the glomoid spores of the genera Diversispora and Pacispora. They differ from other arbuscular fungi by the possession of the rRNA
SSU gene sequence signature YVRRYW/1-5/NGYYYGB, corresponding to homologous position 658 of the S. cerevisiae SSU rRNA sequence J01353 SSU rRNA, GTYARDYHMYY/2-4/GRADRKKYGWCRAC, corresponding to homologous position of the S. cerevisiae SSU rRNA sequence position 1346 of the S. cerevisiae SSU rRNA sequence J01353, TTATCGGTTRAATC, corresponding to homologous position 650 of the S. cerevisiae rRNA SSU sequence J01353 and ACTGAGTTMATYT, corresponding to homologous position 1481 of the S. cerevisiae rRNA SSU sequence J01353 with the coloured nucleotides being specific for the taxon. The order Diversisporales is represented by five families, Diversisporaceae with the genera Diversispora and Otospora, Acaulosporaceae with the genera Acaulospora and Kuklospora, Entrophosporaceae with the genus Entrophospora, Gigasporaceae with the genera Gigaspora and Scutellospora and Pacisporaceae with the genus Pacispora.

Fungi of the order Glomerales are usually hypogeous and rarely epigeous. They produce mycorrhizae with arbuscules, vesicles and spores. Spores are formed either blastically at the tip of a sporogenous hypha or intercalary within the hyphae. Spores occur singly or in clusters or sporocarps having a peridium. They differ arbuscular fungi by the possession of the rRNA SSU gene sequence signature YTRRY/2-5/RYYARGTYGNCACCTTTAGAGGAACTATCGGT GTYTAACCGRGG, corresponding to homologous position 1353 of the S. cerevisiae SSU rRNA sequence J01353, with the coloured nucleotides being specific for the taxon. The order Glomerales includes one genus, Glomus.

Species of the order Paraglomerales form arbuscular mycorrhizae, rarely with vesicles. Spores are glomoid and colourless. The fungi differ from other arbuscular fungi by the possession of the rRNA SSU gene sequence signature GCCAAGCGTACCATGCGCTTTAACCAGCCGT, corresponding to homologous position 703 of the S. cerevisiae SSU rRNA sequence J01353, with the coloured nucleotides being specific for the taxon. The order Paraglomerales is represented by a monotypic family, Paraglomeraseae, containing single genus, Paraglomus.

At present, the number of described species of arbuscular fungi is about 200. According to Morton et al. [36], the number of species of this group of fungi may come up to 2700.

**Morphological Characters of AMF Fungal Spores:**
The AMF spores were described and characterized on the basis of their morphological and biochemical characters by Spain et al. [33]; Morton [37] and Walker and Sanders [24].

Spores of arbuscular fungi contain structures each having a unique phenotypic property. According to Morton [37], each spore, regardless of species, forms one spore wall. Additionally, all species of the genera Acaulospora, Ambispora, Archaeospora, Entrophospora, Gigaspora, Intraspore, Kuklospora, Pacispora and Scutellospora produce spores with 1-3 inner walls called “germinal walls”. Expect for Gigaspora spp., where germinal wall is physically merged with the spore wall, the spores of other genera have germinal walls arising and then functioning independently of their spore wall.

**Spore Wall:** The spore wall in originates from the tip of sporogenous hypha whereas Diversispora, Glomus, Gigaspora, Pacispora, Paraglomus and Scutellospora spp., in Entrophospora, Intraspore, Kuklospora and some Glomus spp. produce spores intercalary. It arises from the side of the hyphae in Acaulospora, Ambispora, Archaeospora and Otospora spp. With the increasing diameter of the spore the spore wall grows, thickens and differentiates into its structures and ceases as the spore growth maximizes. Post maturity changes observed are minor viz. changes in the colour, thickness and rigidity.

The spore wall of the species of arbuscular fungi recognized consists of at least two layers while although some species many differentiate in a 4-layered spore wall. The wall of the most juvenile spores may be 1-or 2-layered, however, in Acaulospora. gerdemannii, spore wall layer 2 forms first and then an outer layer originates de novo [29]. The spore wall layers and germinal walls may differ both in phenotypic and biochemical properties.

Eight phenotypes of spore wall layers are recognized.

- **Mucilagenous layer:** This layer forms the spore surface of e. g. Glomus claroideum, Gl. mosseae and Gl. multiforum. In juvenile spores, this layer is smooth or slightly roughened.

- **Semiflexible layer:** A semiflexible layer usually is thin, somewhat less flexible than thin, flexible layers present in inner germinal walls of species of the
genera, Acaulospora and Scutellospora. In crushed specimens, the semiflexible layer frequently detaches from a layer adherent to its lower surface and does not wrinkle as a flexible layer (see, e.g., G. arenarium). When the semiflexible layer covers a laminate spore wall layer, its longevity is higher than that of a mucilaginous layer, although this layer rarely is present intact or at all in mature spores. Generally, in species having a semiflexible layer, it is smooth when not deteriorated. In Gl. multiformum, the lower surface of the semiflexible layer is ornamented producing ingrowths from the base to form pits in the upper surface of the next laminate layer.

- **Other deteriorating and sloughing layers:**
  These layers form a spore surface and are more or less persistent. In young and sometimes in mature spores, these layers resemble a unit layer. However, with time, these layers always deteriorate and frequently are absent in older spores. In some species sloughing layer is ornamented with blister-like processes that deteriorate and disappear with time for example in the species of Acaulospora, Appendicispora, Archaeospora, Entrophospora, Intraspora, Kuklospora and Otospora the sloughing layer is continuous with the wall of the neck of a sporiferous sacule.

Another type of age related deteriorating and sloughing layer is one which expands in the lactic acid-based mountants. It was originally found in the wall structure of spores of G. pansihalos [38]. This layer also occurs in spores of A. morrowiae and G. arenarium [37, 39].

- **Unit layer:** This layer is a uniform and a persistent structure. It may be colourless, as, e.g., in Acaulospora thomii, or coloured, as, e.g., in Gigaspora gigantea. In some species, this layer is ornamented as like warts in Scutellospora persica. Sometimes, the unit layer is very thin and shows similar colour as its penultimate layer in S. pellucida. This can mislead in the omission of the layer, similarly coloured as its penultimate layer, as, e.g., in. Hence, this layer may be easily omitted.

- **Granular layer:** This structure is reported uniquely in G. caledonium was revealed only in the wall structure of spores of as third layer in a four layered sporewall. It is seen as a thide, but like unit layer in mildly crushed spores.

- **Laminate layer:** A laminate layer is a consination of usually very thin, <0.5 µm, tightly adherent sublayers. It may be colourless through the entire life of a fungus, as, in Paraglomus laccatum and Glomus diaphanum, or may gradually darken because of the addition of next, coloured sublayers. Both the upper and the lower surface of the laminate layer may be smooth or ornamented with processes as seen in Entrophospora infrequens and G. pansihalos or as pits in Acaulospora cavernata, A. lacunosa, G. insculptum and G. multiformum. In species of the genera Diversispora, Gigaspora, Glomus, Pacispora, Paraglomus and Scutellospora, the laminate layer is continuous with the laminate layer of the subtending hypha or the sporogenous cell. Except for members of the genera Acaulospora and Entrophospora, the spore wall of species of the other genera of the Glomeromycota contain only one laminate layer. In most of the Acaulospora spp., their 3-layered spore wall contains two inner laminate layers. In the spore wall of E. infrequens, second layer too is finely laminated. The permanent laminate layer generally is the main structure protecting the spore inside from the influence of different abio-and biotic stresses. In E. infrequens, the second laminate spore wall layer deteriorates with age and is frequently completely sloughed in mature spores.

- **Layer:** This phenotype is a thin, ca. 0.5 µm thick and flexible structure, usually wrinkling in spores crushed in PVLG. The layer resembles flexible layers of germinal walls of spores of fungi of the genera Acaulospora, Ambispora, Archaeospora, Entrophospora, Intraspora, Kuklospora and Otospora respectively. It originates similarly, but only after the first one is completed and has no physical connection with the sporiferous sacule. This layered structure is commonly found in spores of species described in the genera Acaulospora, Ambispora, Archaeospora, Entrophospora, Gigaspora, Intraspora, Kuklospora, Otospora, Pacispora and Scutellospora. The number of germinal walls in the species described ranges from 1 to 3. Each wall consists of 1-3 layers. The first germinal wall forms after the synthesis of the spore wall is completed and has no physical connection with the spore. The next wall too originates similarly, but only after the first one is completed.

**Germinl Wall:** Germinal walls produced by the AMF of the genera Acaulospora, Ambispora, Archaeospora, Entrophospora, Gigaspora, Intraspora, Kuklospora, Otospora, Pacispora and Scutellospora. The number of germinal walls in the species described ranges from 1 to 3. Each wall consists of 1-3 layers. The first germinal wall forms after the synthesis of the spore wall is completed and has no physical connection with the spore. The next wall too originates similarly, but only after the first one is completed.
fully differentiated. The germinal layers of all species are colourless. In most of the taxa the components of the germinal walls, whenever formed, are more or less flexible. With the increasing ability of spores to germinate, the germinal wall/s also condition themselves to complete differentiation. In members of the genus Gigaspora, a germ tube directly originates from the germinal wall which is tightly adhered to the spore wall. In comparison the of other genera, forming at least one germinal wall, germ tubes grow from earlier produced pre-germination structures. Various called "germination orb" in Acaulospora and Kuklospora spp., germination shield in Pacispora and Scutellospora spp., or germination structure in A. appendicula. These pre-germination structures are placed on the upper surface of the innermost germinal wall where more than such wall is present. However, no pre-germination structures have been reported in the spores of Entrophospora, Intraspora and Otospora spp. till date.

Six phenotypes of layers are revealed in the germinal walls of the species of arbuscular fungi.

**Smooth, Thin, Flexible Layer:** Although this germinal layer is smooth, thin, ca. 0.5 µm thick, it looks wrinkled once the spores are crushed in PVLG. In most arbuscular fungi, this layer seems to be a tightly adhered second layer of almost similar properties, therefore, may be erroneously interpreted as a single layer, instead of being be layered.

**Ornamented, Thin, Flexible Layers:** Two phenotypes of ornamented, thin, flexible, layers have so far been reported; a beaded layer and a knobby layer. The beaded layer commonly is the part of the innermost germinal wall of the spores of genera Acaulospora and Kuklospora. It is thin, ca. 0.5 µm thick and covered with granular structures excrescences (beads) that frequently disperse where the spores are crushed. In spore with three layered germinal wall, the outer surface of the innermost layer is the knobby layer, for example as in A. cavernata. This surface resembles as if a plastic covering on the inner surface of the innermost germinal layer: in one layered germinal wall spores like G. gigantea, this single layer in itself show such an orientation of the knobby layer.

**Thin, Semiflexible Layer:** The semiflexible germinal layer is somewhat more rigid than the flexible layer and does not wrinkle in crushed spores. The semiflexible layer occurs alone or adheres to another semiflexible layer in some species of the genera Acaulospora, e. g. in A. mellea and A. scrobiculata and Kuklospora.

**Thick, Flexible Layer:** Walker and Sanders [24] called it "coriaceous wall". It was described as having a leather-like appearance in hypertonic solutions. While the outer and inner surfaces of the innermost germinal wall of the 3 layered spore constitute the knobby and breeded layers respectively. This part is the middle of the same layer such as in the spores of Acaulospora cavernata and Paraglomus scintillans.

**Plastic Layer:** The plastic layer was described as “an amorphous wall” by Morton [40]. This layer is highly plastic and is not disturbed by applied pressure to spores while being crushed in lactic acid-based mountants. It is always the innermost layer in the subcellular structure of spores of the genera Acaulospora, Kuklospora and Scutellospora.

**Thin, Rigid Layer:** In 1-layered germinal wall spores, for example in Acaulospora gedanensis a thin, rigid layer forms the first. Such layers also occur in the middle of the 2-layered germinal walls of the spores of example A. appendicula, A. fennica and A. gerdemannii. In A. gedanensis, Ap. fennica and A. Gerdemannii. This layer is smooth and in A. appendicula they are ornamented with a convex, alveolate reticulum, unlike all the other species of AMF, producing spores with flexible or semiflexible germinal walls, this type of layer is highly fragile than the other ones described above.

**Pre-Germination Structures:** As already mentioned three types of pre-germination structures, from which germ tubes arise, were recognized in AMF.

**Germination Orb:** A germination orb is formed by a centrifugally rolled, hyaline to light-coloured hypha. The orb is usually circular or somewhat elliptic when seen in a plane view and located on the upper surface of the innermost germinal wall when observed in a cross view. The germination orb is a transient structure and decomposes to a completely unrecognizable structure in older spores. Such orbs are reported only in a few species of the genus Acaulospora and Kuklospora.

**Germination Shield:** Except for the germination shield of spores of S. projecturata, the germination shields of the other species of the genus Scutellospora are thin, uniform, elliptic or cardioid, hyaline to coloured lobes with a more or less incurved or incised margin. They commonly occur in mature spores of the species of genus Scutellospora. The formation of this structure on the upper surface of the innermost germinal wall ends the on to genetical
development of spores of these fungi. Unlike orbs the germination shields do not deteriorate with age. Kramadibrata et al. [41] have reported the formation of shield by the coiled hyphae resembling therefore, the germination orbs of *Acaulospora* and *Kukulospora* genus, in the species *Sprojecturata*. Of the other members of the Phylum Glomeromycota, only *P. scintillans* and *P. franciscana* were found to form germination shields resembling in appearance to those of the species of Scutellospora. However, like orbs of Acaulosporathe germination shields in these species decompose with age and are usually invisible in older spores.

**Germination Structure:** Such a structure, resembling a germination shield of spores of genus Scutellospora, has been detected on the upper surface of the second germinal wall of spores of Ap. appendicula [33]. No mention of its persistency is made anywhere.

The germ tube of Glomus spp. either grows from the inner surface of the subtending hyphal wall near the spore, a septum of the subtending hypha, or from the spore wall itself. In all these cases, however, no distinct pre-germination structure as above has been reported.

**CONCLUSION**

The present review has been envisaged with an aim to provide some insight into the taxonomic characteristics of AMF. From the review it becomes clear that the taxonomic position of different genera and species are difficult to identify on the morphological basis. Molecular approach is more authentic in identify the exact taxonomic position of different genera and specie of AMF.

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