ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 4, pp: (58-71), Month: October - December 2018, Available at: www.researchpublish.com

Arbuscular Mycorrhizal Fungi: Taxonomy and its Systematics

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Abstract: **In this comprehensive review authors gave emphasis on the complexity of the taxonomy and systematics of AM fungi which are obligate in nature and form a mutually symbiotic association with the roots of higher plants since the evolution land pants. It is discussed in detail how initially the identification and classification of AM fungi was based on solid morphological characters and resulted in profuse description of new species. It is also discussed how the recent advancement in the field of molecular tools and techniques has revolutionized the taxonomy and systematics and as a result many robust classification of AM fungi has come. At the end it is also discussed that how the new classifications are based on morphological and ontogenic characters of AM fungal spores as well as consensus nucleotide sequences (SSU, ITS, LSU, β-tubulin and nrDNA).**

Keywords: **Spore morphology, Wall layers, SSU, ITS, LSU, β-tubulin, nrDNA, Ontogeny, Phylogeny.**

1. INTRODUCTION

Arbuscular Mycorrhizal (AM) fungi are found in intimate association with the roots of higher plants since the evolution of land plants. In fact, it was AM fungi, which provided nutrition to the early land plants via their hyphae (Dotzler *et al.* 2009; Bonfante and Selosse, 2010). AM fungi have been reported from the Devonian gametophytes of 400 million years ago (Taylor *et al*, 1995; Phipps and Taylor, 1996). Remy et al. (1994) demonstrated arbuscule like structure from the subterranean organs of *Aglaophyton major* collected from Rhynie Chert formation. Stubblefield et al. (1987) observed hyphae, vesicles, and spores in well-preserved roots from the Triassic. These are similar as found in extant mycorrhizal structures. After extensive analysis, it is now clear that mycorrhizal association has was established between Ordovician and Devonian (Stubblefield *et al.* 1987).

Since their origin, AM fungi have travelled through the different ages and they faced different environmental conditions. Now, it is clear that, more than 80% of vascular land plants are associated with AM fungi (Smith and Read, 2008; Brundrett, 2009). AM fungi associations are reported from all terrestrial ecosystems, including tropical to temperate forests, alpine, sand dunes, deserts, grassland, aquatic plants and agroecosystems as well as metal polluted soils.

AM fungi are obligate biotrophs. They need roots of living host to grow and complete their life stages. No synthetic medium is formulated till date, which can support the full proliferation of AM fungi in absence of living host. Scientists have made several attempts to formulate artificial culture media to support the growth of AM fungi (Hildebrandt *et al.* 2002). However, this approach is still to be worked out. The obligate biotrophic nature of AM fungi is one of the major constrains while studying the taxonomy of AM fungi. On association with living roots, AM fungi produce hyphae, arbuscules, vesicles and spores inside the roots cortex and hyphae, vesicle and spores outside the roots **(Fig. 1 A-D)**. In family Gigasporaceae AM fungi produce auxiliary cells instead of vesicles **(Fig. 1 E)**.

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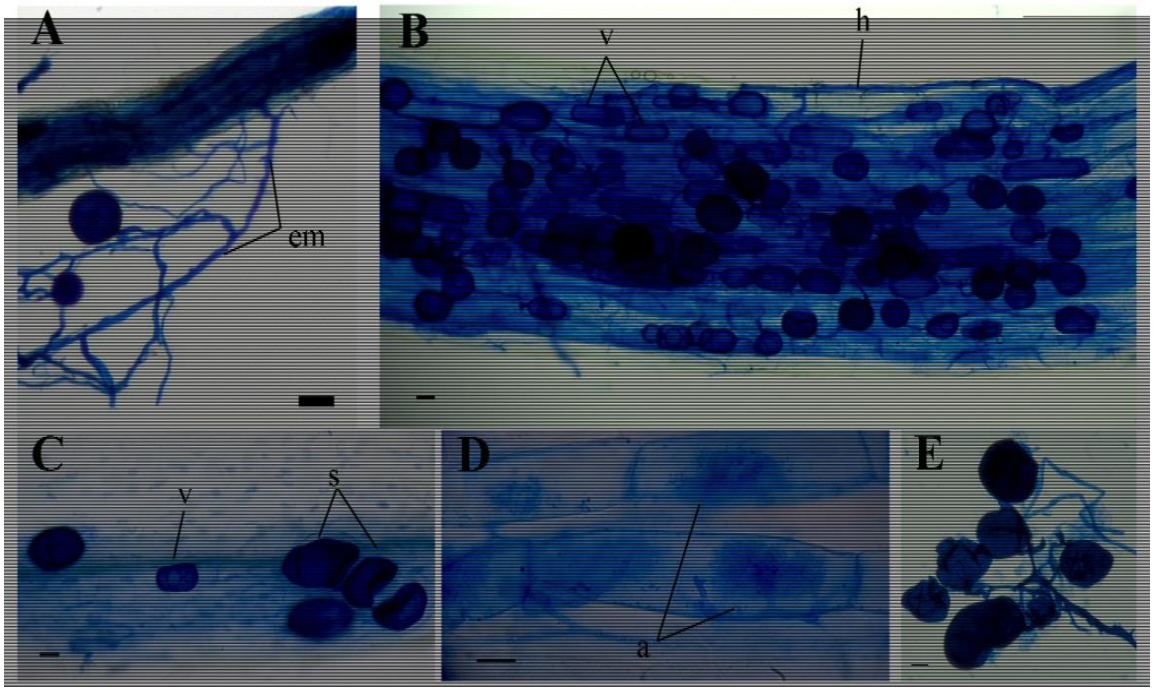

Fig. 1: Various AM fungi structures associated with roots. **A:** a root bit showing extrametrical hyphae (em). **B:** heavily colonized root bit showing intraradical hyphae (h), vesicle (v) and numerous round spores. **C:** intraradical vesicle and spores. **D:** tree-shaped arbuscules (a) **D:** auxiliary cells. Bar = 20 µm.

The early phase of taxonomy of AM fungi solely relied on few morphological parameters such as sporocarp. Later, when free and single spores have been reported, they were utilized in the identification and naming of AM fungi. Since the method of wet-sieving and decanting (Gerdemann and Nicolson, 1963) the AM fungal taxonomy gained a momentum. Later, 'wall layers' and other morphological parameters were adapted for the AM fungal species identification. Ontogeny of AM fungal spore is also established as one of the important parameters to distinguish the AM fungal species. Many other approaches like, serology, ELISA, etc., were also developed in time, but now they are out of trend. Development in the techniques of molecular biology has opened a new dimension in AM fungal taxonomy. Many conserved barcode regions such as, SSU, ITS, LSU, mtDNA, nrDNA have been identified and are being utilized to better understand the origin, evolution and phylogeny in AM fungal taxonomy.

2. EARLY PHASE OF TAXONOMY

Symbiotic association between a fungus and roots has been discovered in *Monotropa hypopitys* L. by Franciszek Kamienski (Kamienski, 1881) and term "Mycorrhiza" was coined by Frank in 1885 (Frank, 1885). However, the fungi of the Vesicular Arbuscular Mycorrhiza (now Arbuscular Mycorrhiza) were described much earlier. First description of any AM fungi has been published by Tulasane brothers (Tulsane and Tulsane, 1845). They described *Glomus* with its two species, i.e., *G. microcarpus* and *G. macrocarpus*. Because of the formation of the spores, Tulasne and Tulasne (1845) considered the genus *Glomus* phylogenetically close to *Endogone*, a genus erected by Link (1809). Later, Tulasane brothers (Tulasne and Tulasne, 1851) transferred both species of *Glomus* to *Endogone* because chlamydospores of the former were recognized to be similar to zygospores of the latter (Schüβler and Walker, 2011). The genus *Sclerocystis* was created by Berkeley and Broome (1873) for encompassing the species forming spores in small sporocarps. Both the above genera were classified in the family Endogonaceae, order Mucorales. The family Endogonaceae was initially proposed by Fries in 1849 (Fries, 1849) in the Tuberales, but later transferred to the Mucorales by Bucholtz in 1912 (Bucholtz, 1912).

Thaxter (1922) revised the Endogonaceae and included four genera *viz.*, *Endogone* Link: Fries, *Glaziella* Berk., *Sclerocystis* Berk. & Br., and *Sphaerocreas* Sacc. & Ellis. These all were sporocarpic fungi producing chlamydospores and zygospores both. After finding both type of spores in the sporocarp of *Endogone fasciculata* (now *Rhizophagus fasciculatus*) and *E. microcarpa*, Thaxter (1922) and Godfrey (1957) considered chlamydosporic species to be anamorphs of those producing zygospores.

Later, Peyronel (1923) suggested that, so called "Vesicular–Arbuscular Mycorrhizae" were formed by fungi of the genus *Endogone*. However, it was Mosse (1953) who first demonstrated that adding the sporocarps of *Endogone* species (isolated form the mycorrhizal strawberry roots) to sterile soils with strawberry seedlings resulted in a typical mycorrhizal colonization. This fungus was later described in her honor as *Endogone mosseae* (= *Glomus mosseae*, = *Funneliformis mosseae*).

Later, letter codes were used to describe the AM spore types. For example, Gerdemann (1955) described spores of types A, B, and C. Type A clearly would have been placed in *Glomus* at the time, while type B referred to a species in a yet to be named family Gigasporaceae because of the presence of a bulbous base, and type C described auxiliary cells that were considered spores and also unique to species in Gigasporaceae.

3. MIDDLE PHASE OF TAXONOMY

After the devise of wet sieving and decanting method by Gerdemann and Nicolson in 1963 for the isolation of sporocarpic and non-sporocarpic fungi, the interest in taxonomy of AM fungi increased dramatically. In 1986, Mosse and Bowen prepared the first key for the identification of endogonaceous spores. They described seven genera of vesicular arbuscular mycorrhizal fungi. Later, Endogonaceae was revised by Gerdemann and Trappe (1974). The detail outline is presented in the **Fig. 2**. In this classification, they have described 44 species under seven genera, which contained few ectomycorrizal and saprotrophic fungi also. Two new genera (*Acaulospora* and *Gigaspora*) and 12 new species were described and many taxa were redefined. The genus *Glomus* was erected from the *Endogone* (as previously merged into *Endogone* by Tulasane brothers) and established as distinct and valid genus.

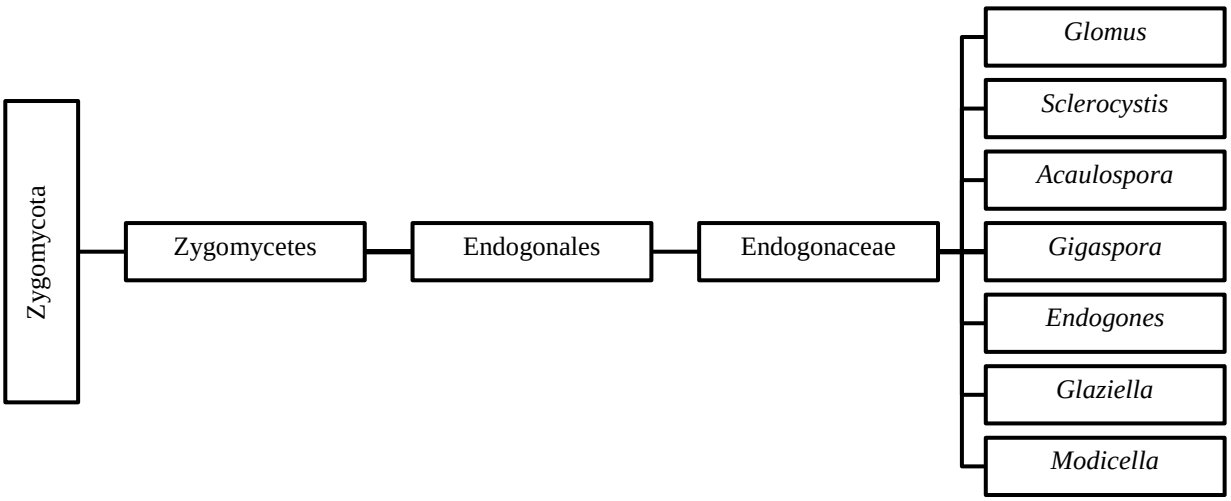

Fig. 2: Classification of AM fungi proposed by Gerdemann and Trappe (1974).

The genus *Glomus* contained 19 species and *Sclerocystis* contained 4 species. All the species of *Glomus* produced spores blastically. In some species loose to compact aggregates of spores were reported with or without peridial wall. The genus *Sclerocystis* was characterized by the presence of chlamydospores in single layer around a central plexus of hyphae. The two new genera *Acaulospora* and *Gigaspora* were characterized by the formation of spores singly in the soil. The former produced spore at the neck of sporiferous saccule and the later produced spore at the tip of bulbous sporogenous hyphae. All the seven genera *viz.*, *Glomus*, *Sclerocystis*, *Acaulospora*, *Gigaspora*, *Endogone*, *Glaziella* and *Modicella* were placed in the family Endogonaceae, order Mucorales, phylum Zygomycota. As *Glaziella* and *Modicella* were not forming arbuscular mycorrhizal association, *Modicella* was transferred to the family Mortierellaceae by Trappe (1982) and *Glaziella* was transferred to the Ascomycota by Gibson et al. (1986). This classification provided a sound basis for the taxonomy of AM fungi and used as reference for up to several years.

A new genus *Entrophospora* with *E. infrequens* was erected in the Endognaceae by Ames and Schneider (1979). The genus was earlier included in the *Glomus* as *Glomus infrequens* (Hall, 1977) which was characterized by the spore formation on sporiferous saccules (a feature of *Acaulospora*). But, the position of spore was in the neck rather than by side of the neck. This feature provided strong basis for the erection of *Entrophospora*.

In the same year, Benjamin (1979) redefined the order Mucorales. He retained in the order mucorales only saprotrophic or non-haustorial fungi which, reproduce asexually by sporangia, sporangiola, merosporangia, chlamydospores, arthrospores and yeast like cells. Consequently, he transferred the family Endogonaceae to the order Endogonales, already erected by Moreau (1953).

Walker and Sanders (1986) erected a new genus *Scutellospora* from the *Gigaspora* of Gerdemann and Trappe (1974). This separation was on the basis of germination from the 'germination shield' found in the innermost layer of *Scutellospora* and not in *Gigaspora*.

For the ease of identification of species of AM fungi, Trappe (1982) has developed a synoptic key. Later on, dichotomous key of Hall and Fish (1979), and Hall (1984) and keys for groups of species by Koske and Walker (1985) have also come. An important publication "**Manual for the Identification of VA Mycorrhizal Fungi**" has been published by Schenck and Pérez (1988) which compiled all summary species descriptions. All the above manuals and keys are now out of print but these are still being used by some laboratories as ad-on for the AM fungi species identification.

During the period of 1975-1989, a number of new species have been described and only after 12 years of monograph of Gerdemann and Trappe (1974) the AM fungi species had jumped to 77 (Trappe, 1982) and 6 years later, Schenck and Perez (1988) listed 126 species. In this duration AM fungi were being identified solely on certain morphological characters and subcellular structures of spore. Lack of a standardized terminology has often resulted in impair identification of species. To overcome this problem, Walker (1983) proposed the new terminology for the description of AM fungi. He proposed the term 'walls' for the identification of AM fungi, which can be grouped in to 'walls groups'. The 'wall types' are identified by phenotype in intact or broken spores and 'wall groups' are identified as aggregation of wall layers in broken spores. Walker also proposed the term 'murograph' for the description of walls. A murograph is graphical representation of different distinct wall layer of a spore. Walker in his original article described wall types as 'unit', 'laminated', 'evanescent' and 'membranous'. As new species were described, additional wall types were introduced: expanding (Berch and Koske, 1986), amorphous (Morton, 1986), coriaceous (Walker, 1986), notched (Koske and Gemma, 1995) and germinal (Spain *et al.* 1989). Berch (1986) in his treatise on Endogonaceae has criticized this terminology. Berch was of argument that, each wall layers for describing the characters of spore wall should be based on the knowledge of the origin of each wall layer. This view of Berch has opened the vista for studying the spore development, while determining the wall layers to understand the nature of wall layers in more precise manner. Later, Morton (1988) suggested and clarified some criteria for the identification and classification of AM spores.

Morton (1990) considered 27 phenotypic characters of spores and mycorrhiza and analyzed 57 AM fungi species. He hypothesized that all the AM fungi comprised a monophyletic group by sharing nature of mutual symbiosis and formation of arbuscules within the roots. He also proposed two clades of AM fungi, one consisting of *Gigaspora* and *Scutellospora* species and the other consisting of *Glomus*, *Sclerocystis*, *Acaulospora* and *Entrophospora*.

Later, Morton and Benny (1990) placed the arbuscule forming fungi in a new order, Glomales (now Glomerales). They divided the order glomales into three family *viz.*, Glomeraceae Acaulosporaceae and Gigasporaceae **(Fig. 3)**.

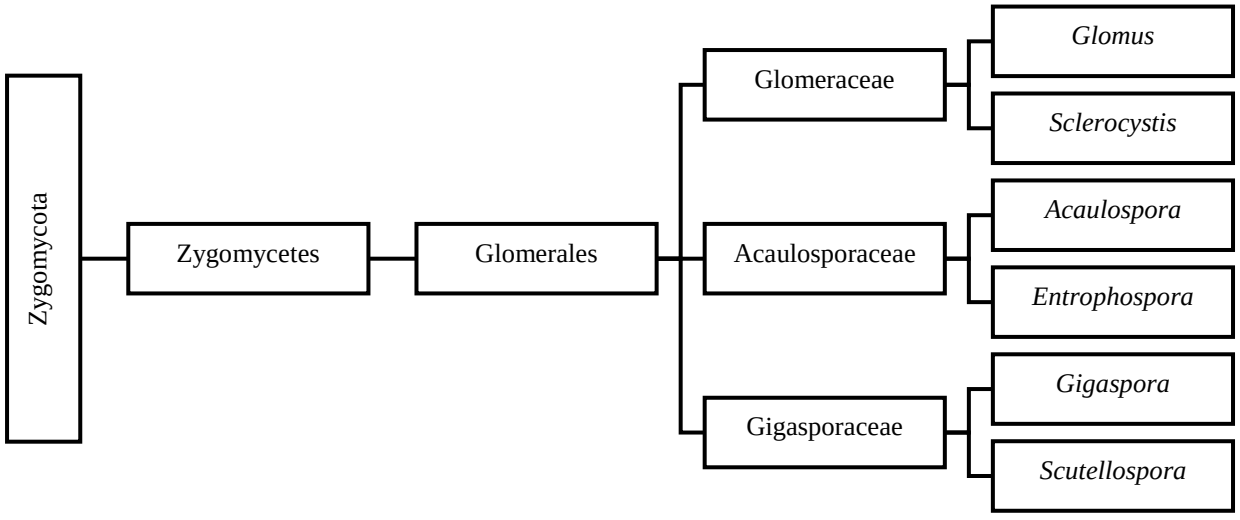

Fig. 3: Classification of AM fungi proposed by Morton and Benny (1990).

The family Glomeraceae consisted of two genera *viz.*, *Glomus* and *Sclerocystis*, Acaulosporaceae family consisted of genera *viz.*, *Acaulospora* and *Entrophospora* and two genera were included in family Gigasporaceae *viz.*, *Gigaspora* and *Scutellospora*. The taxa of family Glomeraceae were distinguished from Gigasporaceae mainly by the presence of vesicles, which are otherwise absent in family Gigasporaceae. This classification was based on the cladistic analysis, spore development and mode of spore germination.

Monophyly of *Glomus* (as suggested by Morton and Benny, 1990) was contested by Walker (1992) and later Morton (2000) questioned on the monophyletic origin of entire order Glomerales. Simon et al. (1993) also questioned about the phylogenetic relationship among the three families in the order Glomerales. Morton (2000) suggested that, Glomineae and Gigasporineae are the two evolutionary clades and had arisen at two distinct periods. This view was supported by the evidences of types of infective propagules (Biermann and Linderman, 1983; Jasper *et al.* 1989), morphology of fungal mycelium (Brundrett and Kendrick, 1990), mode of spore formation (Franke and Morton, 1994) and cell wall composition (Gianinazzi-Pearson *et al.* 1994).

Gerdemann and Trappe (1974) placed the AM fungi into the order Endogonales and Morton and Benny (1990) placed AM fungi into order Glomerales. But, both the literature did not clearly state the class of AM fungi. It was Cavalier-Smith (1998), who placed fungi forming arbuscular mycorrhizal association with plants in a new class the Glomeromycetes within a new phylum Archeomycota.

Analysis of extant species of AM fungi and the examination of fossil records led to the proposition of new taxa and the transfer of species to other genera. Taylor et al. (1995) proposed the genus *Glomites* and described *Glomites rhyniensis* from aerial stems and rhizomes of the 400-million-year-old fossil Devonian plant *Aglaophyton major*, based on extraradical and intraradical hyphae, chlamydospore-resembling spores, and arbuscule-resembling structures in the fossil plant. Phipps and Taylor (1996) proposed the genus *Gigasporites* and the species *Gigasporites myriamyces* and *Glomites cycestris* from the Triassic plant *Antarcticycas* from a siliceous chert. *Glomites* and *Gigasporites* were hypothesized to be related to the extant genera *Glomus* and *Gigaspora*, respectively.

In 1990, Almeida and Schenck found that, except for *Sclerocystis coremioides*, sporocarpic *Glomus* species and other members of *Sclerocystis* shares a continuum of morphological features. Accordingly, the five-species genus *Sclerocystis* was reduced to a single species one.

4. MOLECULAR PHASE OF TAXONOMY

Before molecular techniques, the only way to identify AM fungi was by a careful microscopic examination of the spores. Unfortunately, spores are relatively simple structures that offer only a limited number of potential discriminating features. Redecker et al. (2000) utilized both morphological and molecular data and transferred *S. coremioides* to the genus *Glomus*.

Morton and Redecker (2001) erected two new families in the order Glomales (now Glomerales), i.e., Archaeosporaceae and Paraglomaceae (now Paraglomeraceae). This was based on the data from molecular, morphological and biochemical investigations. Despite similarities in mycorrhizal morphology, each of the two families was phylogenetically distant form each other and also from other glomalean families.

Archaeosporaceae contained single genus, *Archaeospora*, with three species forming *Acaulospora* like spores from the neck of sporiferous saccule. Two of these species, *Ar. gerdemannii* and *Ar. leptoticha*, were characterized by formation of dimorphic spores, i.e., they also produce *Glomus* like spores. Paraglomaceae (now Paraglomeraceae) also contained single genus, *Paraglomus*. Later, in 2001 Schüßler et al. (Schüßler *et al.* 2001) erected the Glomarales to the phylum level presented in **Fig. 4**.

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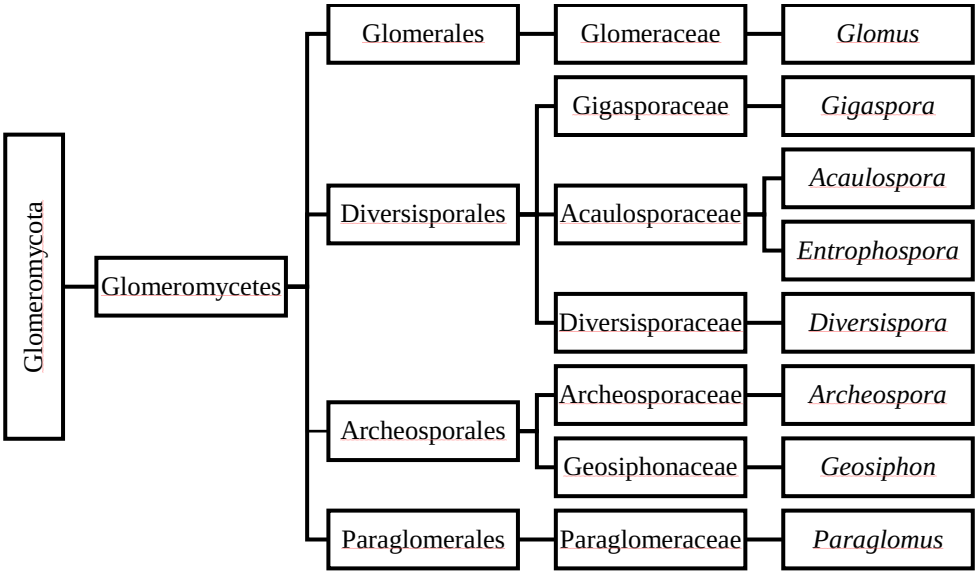

Fig. 4: Classification of AM fungi proposed by Schüßler et al. (2001).

They analyzed the AM fungi and *Geosiphon pyriformis* on basis of molecular (SSU rDNA gene), morphological and ecological characteristics and separated them from all major groups. Consequently, they removed them from the polyphyletic Zygomycota, erected them in a new monophyletic phylum, the Glomeromycota. Further, they erected three new orders i.e., Archaeosporales, Paraglomerales and Diversisporales. According to them, the Glomeromycota probably diverged from same common ancestor as the Ascomycota and Basidiomycota have. This separation gives Glomeromycota a proper and natural status.

In early June 2004, Oehl and Sieverding (Oehl and Sieverding, 2004) identified four new species and re-described two species of the *Glomus* and placed them in new genus, *Pacispora*. This newly described genus has been placed in the family glomeraceae. The genus was characterized by the terminal spore formation, as seen in *Glomus* and *Paraglomus* with germination structure as found in *Acaulospora* and *Entrophospora*. During same year, in late June 2004, Walker and Schüßler (Walker and Schüßler, 2004) described same species as *Gerdemannia*, under the family Gerdemanniaceae in the order Diversisporales. But, as per article 11.3 of the Code, *Pacispora* got priority over *Gerdemannia* and therefore, the later was rejected as it became the illegitimate name as per the article 52.1 of the Code.

In 2006, Sieverding and Oehl (Sieverding and Oehl, 2006) revised five species of *Entrophospora*. Based on differences in spore morphologies and root infection characteristics they retained only *Entrophospora infrequens* and *Entrophospora baltica* in the genus *Entrophospora*. They raised these two species with *Entrophospora* as type genus in the new family Entrophosporaceae. The other three species were organized in two new genera. *Kuklospora* gen. nov. with *Kuklospora colombiana* and *Kuklospora kentinensis* (formerly *Entrophospora colombiana* and *Entrophospora kentiniensis*) were placed into the family Acaulosporaceae and *Intraspora* gen. nov. with only one species *Intraspora schenckii* (the former *Entrophospora schenckii*) was included into the family Archaeosporaceae.

In 2006, Spain et al. (Spain *et al.* 2006) described a new genus *Appendicispora* in the family Archaeosporaceae with three species i.e., *Ap. appendicula*, *Ap. gerdemannii* and *Ap. jimgerdemannii*. *Ap. appendicula*, the type species derived from the dimorphic *Acaulospora appendicula*, a "synonym" of *Ac. gerdemannii* erroneously thought to represent the acaulosporoid form of *Archaeospora leptoticha*. *Ap. gerdemannii*, based on *Archaeospora* (*Glomus) gerdemannii. Ap. jimgerdemannii*, a nom. nov. was assigned for the former *Ac. gerdemannii*. Thus, genus *Archaeospora trappei*, remained alone as monotypic *Archaeospora*. They differentiated two genera on the basis of spore development, wall morphology, germination, and root colonization structures as well as by molecular data. The spores of *Appendicispora* were characterized by three layered wall with appendix arising laterally from the hyphal neck of a terminal sporiferous saccule.

On the basis of morphological evidences and SSU and ITS region rDNA data, Walker et al. (2007) erected and described a new genus *Ambispora* gen. nov. typified by *Ambispora fennica* sp. nov. The genus contains three species i.e., *A. fennica*, *A. leptoticha* comb. nov. (basionym *Glomus leptotichum*), and *A. gerdemannii* comb. nov. (basionym *G. gerdemannii*) known to produce both glomoid and acualosoroid spores, while another species *A. callosa* comb. Nov (basionym, *G.*

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callosum), is known to produce only glomoid spores. The new genus along with all four species were placed in a new family, the Ambisporaceae fam. nov. in order *Archaeosporales*. Later, Walker and Schüßler, (2010) proposed a classification presented in **Fig. 5**.

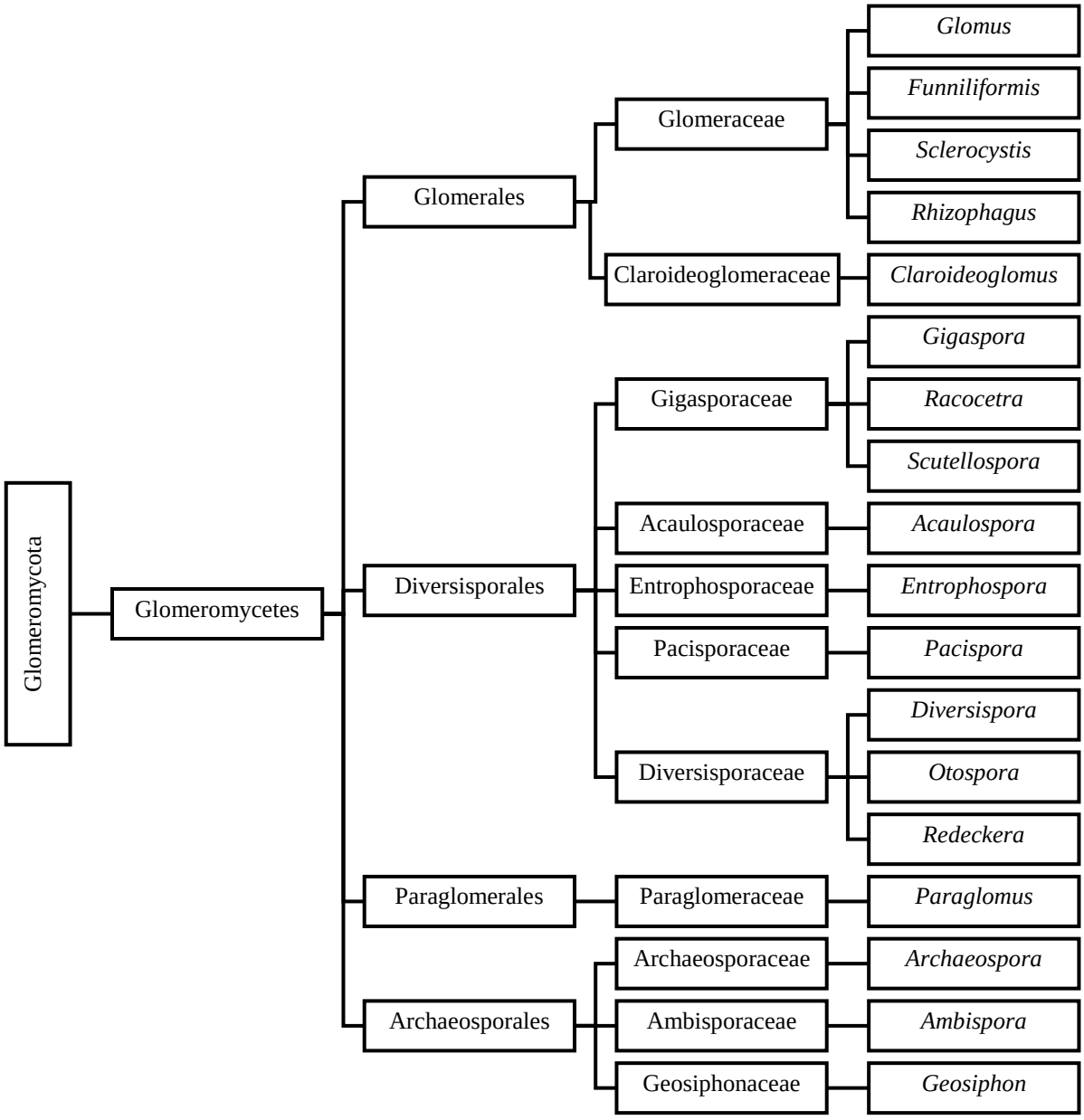

Fig. 5: Classification of AM fungi proposed by Walker and Schüßler (2010).

Oehl et al. (2011a) proposed a classification which was based on combined genetic and morphological characters. The genetic characters include partial sequences of β-tubulin, and SSU and LSU rRNA and morphological characters associated with color, shape and thickness, pore closure of subtending hyphae etc. In their classification, they divided the Phylum Glomeromycota into three classes namely Glomeromycetes, Archaeosporomycetes and Paraglomeromycetes. Class Glomeromycetes includes three orders Glomerales, Diversisporales and Gigasporales. The former two orders form vesicles and arbuscules while the later one forms arbuscules and extra radical auxiliary cells. In their classification they also rearranged species of *Sclerocystis* and *Rhizophagus* and transferred back to genus *Glomus*. In the same year, Oehl et al. (2011b) erected new genera *Simiglomus* and *Septoglomus* within the family Glomeraceae, and *Viscospora* within Claroideoglomeraceae. The authors also confirmed that Paraglomerales species germinate through spore wall as the members of Glomerales. Further, Oehl et al. (2011c) proposed new recombination of species from previously described

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Acaulospora myriocarpa, *A. undulata*, *A. nicolsonii*, and *Scutellospora nodosa* as *Archaeospora myriocarpa*, *Ar. undulata*, *Ambispora nicolsonii*, and *Cetraspora nodosa* respectively. Oehl et al. (2011d) proposed the new genus *Orbispora* within family Scutellosporaceae (Glomeromycota). In *Orbispora*, spores are formed terminally on sporogenous cells with three spore walls with single mono-lobed hyaline to subhyaline germination orbs on the inner 'germinal' wall that was identical to the orbs in *Kuklospora colombiana*, *K. kentinensis*, and a few *Acaulospora* species. These authors transferred *Scutellospora pernambucana* and *S. projecturata* within genus *Orbispora*.

In their recent, reclassification Oehl et al. (2011 f) divided the Phylum Glomeromycota into three classes, five orders, 14 families and 29 genera (**Fig. 6**). They found that the spore formation in 10 genera was exclusively glomoid, one was gigasporoid, seven were scutellosporoid, four were entrophosporoid, two were acaulosporoid, and one was pacisporoid. Whereas, in three genera bimorphic spore formation and one genus i.e., *Geosiphon* is associated with cyanobacteria.

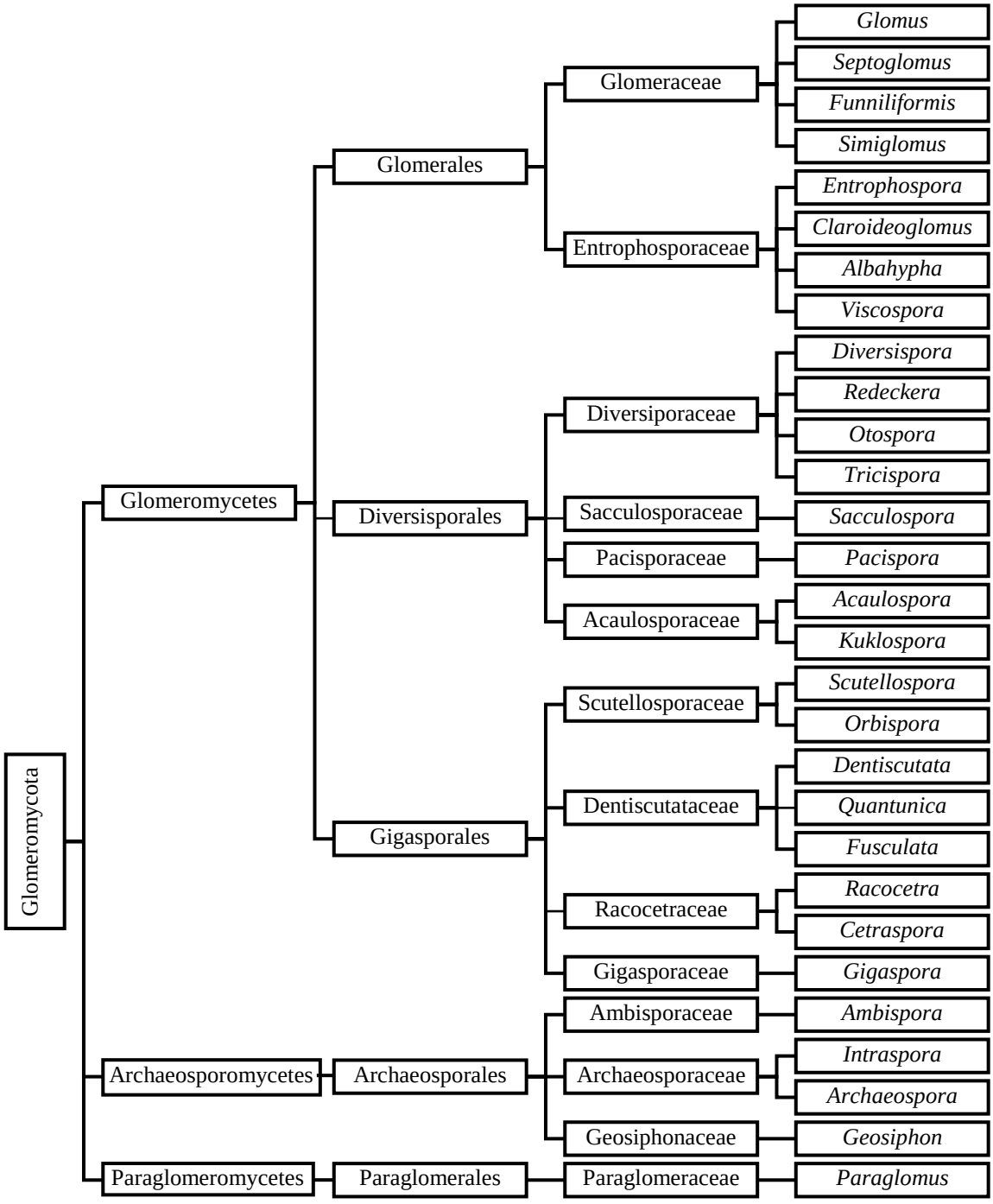

Fig. 6: Classification of AM fungi proposed by Oehl et al. (2011f).

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Goto et al. (2012) proposed a new classification based on combined molecular and morphological studies and erected a one family Intraornatosporaceae with two new genera *Intraornatospora* and *Paradentiscutata*, two new species *viz.*, *P. bahiana*, *P. maritima* and *I. intraornata* a new combination (**Fig. 7**).

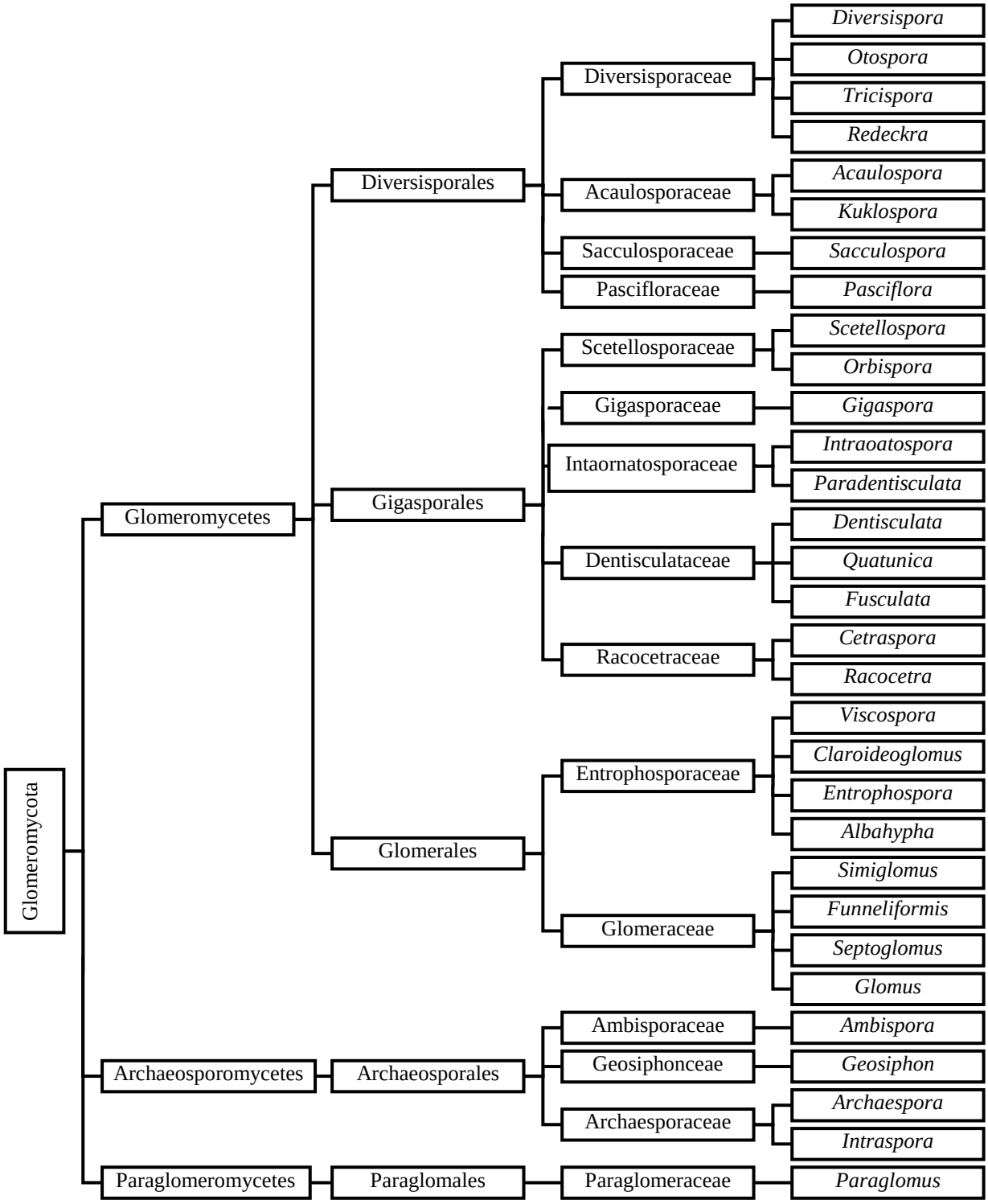

Fig. 7: Classification of AM fungi proposed by Goto et al. 2012.

This new family was placed within order Gigasporales. Spores of both the genera were characterized by introverted ornamentations on the spore wall, distinguished by spore wall structure and germ shield while the species were characterized by projection on the outer surface of spore.

In the year 2013, (Błaszkowski *et al.* 2013) described and illustrated two new species within genus *Septoglomus* (Glomeromycota) as *Septoglomus fuscum* sp. nov. and *S. furcatum* sp. nov. Based on Phylogenetic analyses of the SSU, ITS and LSU nrDNA sequences they suggested that, both new species (*S. fuscum* and *S. furcatum*) were different from previously described species. Spores of *S. fuscum* usually occur in loose hypogeous clusters, rarely singly or inside roots, brownish orange to dark brown, globose to subglobose with diameter (20-)47(-90) µm, rarely ovoid having 21-50 × 23-60 µm diameter. Spore wall consists of a semi-persistent, semi-fexible, orange white to golden yellow, rarely hyaline, outer layer, easily separated from a laminate, smooth, brownish orange to dark brown inner layer. In contrast to this, spores of *S. furcatum* are reddish brown to dark brown, globose to subglobose, (106-)138(-167) µm diameter, rarely ovoid, 108-127 × 135-160 µm, usually with one subtending hypha that is frequently branched below the spore base, or occasionally with two subtending hyphae located close together. Spore walls consisted of three layers. It is noteworthy that, spore-wall of both the species did not stained with Melzer's reagent. The authors also established single species culture using *Plantago lanceolata* as a host plant and found that both the species form arbuscular mycorrhizae.

Later, three new species were discovered from the hyper arid sandy plain of South Arabia by Symanczik, et al. (2014). Based on morphological and molecular analyses of rDNA sequences (SSU, ITS, LSU and ITS), they confirmed that the newly discovered species belong to well described genera but different from known species. Consequently, the authors described them as *Diversispora omaniana*, *Septoglomus nakheelum* and *Rhizophagus arabicus* spp. nov. in which *D. omaniana* and *R. arabicus* were isolated from the native, arid habitat, while *S. nakheelum* was isolated from a nearby irrigated date palm plantation. Interestingly, in the same year two new species were erected by Błaszkowski et al. (2014) and placed within genus *Septoglomus* (*S. jasnowskae* and *S. turnauae*) of Glomeromycota. Morphologically, the first species (*S. jasnowskae*) was characterized by small spores, pale yellow to brownish yellow colored having 2-layered spore wall and usually formed in loose clusters. While the second species i.e., *S. turnauae* formed singly and the spore having 4 wall layers. They also established single-species cultures with *Plantago lanceolata* as host plant and found that *S. jasnowskae* consisted of arbuscules, hyphae and vesicles, whereas, *S. turnauae* comprises of arbuscules and hyphae only.

Recently, Sieverding et al. (2015) erected genera, *Rhizoglomus* gen. nov. (Glomeraceae) which was typified by *Glomus intraradices* (*Rhizoglomus intraradices*). Based on phylogenetic analysis, the genus forms a separate clade within family Glomeraceae. *Rhizoglomus* gen. nov. was morphologically characterized by spores with cylindrical subtending hyphae and distinct wall layers (two or three rarely up to five). These genera consisted of 13 species *viz., R. intraradices, R. aggregatum, R. antarcticum, R. arabicum, R. clarum, R. custos, R. fasciculatum, R. invermaium, R. irregulare, R. manihotis, R. microaggregatum, R. natalense,* and *R. proliferum*. While, *Rhizophagus* (type: *R. populinus*), is excluded because it is a pathogenic genus and does not belong to the Glomeromycota. More recently, Błaszkowski et al. (2015) described two new species (*Dominikia duoreactiva* sp. nov. and *D. difficilevidera* sp. nov.) and placed within recently erected genus *Dominikia* (Glomeromycota). They placed two new species within *Dominikia* on the basis of morphology and phylogenetic analyses (SSU-ITS-LSU sequences). Morphologically the spores of *D. duoreactiva* sp. nov. were distinguished by its yellow color, spore formation in loose clusters, 30-70 µm diameter with a three wall layer of which layers 1 and 3 wall layer stained with Melzer's reagent. While, the spores of *D. difficilevidera* sp. nov., was distinguished by its hyaline color, arise singly, diameter 31-45 µm with three-layered spore wall. Further, the authors suggested that *D. duoreactiva* occurs restricted to certain place while, *D. difficilevidera* has a worldwide distribution. In the same year, Błaszkowski et al. (2015) erected a new species *Glomus tetrastratosum*. They proved that *Glomus tetrastratosum* was undescribed species of the family Glomeraceae on the basis of morphological and phylogenetic analyses of sequences (SSU) rRNA, (ITS) rDNA and (LSU) rRNA. The species was characterized by the spore formation singly and in cluster (loosely) in soil, pastel yellow to brownish yellow in color, globose to subglobose (diameter = 136 µm) and rarely ovoid (110-150 µm) with a subtending hyphae with a pore (open or occluded by a septum). The spore wall consisted of four wall layers. a mucilaginous, hyaline layer 1 which stains in Melzer's reagent, a unit, hyaline, permanent layer 2, a laminate, colored layer 3 and a flexible to semi-flexible, colored layer 4. In singles species culture *G. tetrastratosum* was found to formed structures like arbuscules and vesicles with *Plantago lanceolata*. Thus, it has been concluded that the discovery of new genera and species provide a way to understand and explore the functional significance of AM and offers a new opportunity for conservation, re-vegetation and sustainable development of ecosystem.

ACKNOWLEDGEMENTS

Authors are thankful to University Grant Commission, New Delhi, for providing financial assistance to Mr. Ovaid Akhtar as JRF. Authors are also thankful to Head, Department of Botany, University of Allahabad, Allahabad for providing library and laboratory facilities.

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