How far have we progressed in Glomeromycota taxonomy and systematics?

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Taxonomy is the discipline that names, organizes, and defines species, the fundamental units of biological classification. In recent decades we have experienced significant advances in the descriptions of fungal taxa and classification revisions, reflecting new data obtained mainly from studies using genetic tools. On average, more than 2,000 fungal species are described per year, resulting from intense work by taxonomists who recently came together to create an international consortium to discuss, organize, and establish a broad foundation for the fungal classification (Hyde *et al*. 2023). Even with these advancements, it is estimated that over 94% of fungal species have not been described yet (Antoneli *et al.* 2023). A particular challenge in fungal taxonomy is the phylum *Glomeromycota* (arbuscular mycorrhizal fungi; AMF), a group of obligate symbiont microorganisms forming arbuscular mycorrhizas in the roots of ca. 72% of vascular plants in terrestrial and aquatic environments (Meng *et al.* 2023), and with representatives (*Funneliformis* and *Rhizoglomus*) among the 100 most cited fungal genera in the world (Bhunjun *et al*. 2024).

The *Glomeromycota* taxonomy began in 1844 with the description of the first two species, *Glomus macrocarpum* and *G. microcarpum*. The discovery of these early species was possible because their spores were formed in large sporocarps, visible to the naked eye. The recognition of the symbiotic nature of this group of fungi and their ability to enhance plant growth, the techniques for extraction of spores from the soil, and the possibility of growing them in culture resulted in an increasing interest (Koide and Mosse 2004) and the description of new taxa, including species that produced spores singly, invisible without the use of a microscope (Sportes *et al.* 2021). Gerdemann and Trappe (1974), using refined spore wall characteristics, established the foundations of the AMF taxonomy and proposed the first classification, as members of the family *Endogonaceae*. Later, significant improvements were introduced including a revision of the classification, nomenclature of the spore wall components, and subcellular organization of spores (Walker 1983; Morton 1988; Morton and Benny 1990). At this time, the most significant taxonomic

novelties were the transfer of the genera *Glomus* and *Sclerocystis* from the family *Endogonaceae* to the *Glomeraceae,* and the placement of AMF in a new order, *Glomerales*.

Exceptionally valuable changes in the taxonomy of *Glomeromycota* have been made in the last two decades, mainly due to the involvement of molecular techniques. The first descriptions of AMF including morphological and molecular characteristics date back to 2000 (Declerck *et al*. 2000). Morton and Redecker (2001) proposed the first reclassification of AMF based on the phylogenetic analysis of the 18S *nuc* rDNA gene. Also in 2001, the genus *Glomus* was indicated to be polyphyletic and AMF were placed in a newly created phylum, *Glomeromycota* (Schüßler *et al*. 2001).

The studies discussed above and subsequent phylogenetic analyses have clearly demonstrated that the use of molecular data significantly sharpens the boundaries between species and increases the reliability of their classification (Oehl *et al*. 2011; Błaszkowski *et al*. 2022; Wijayawardene *et al*. 2022). Recently, phylogenomic analyses were applied to shed light on the relationship within the members of the *Glomeromycota* and their position in the fungal kingdom (Montoliu-Nerin *et al*. 2021). The taxonomic rank of this monophyletic lineage of basal fungi as Phylum or Subphylum has a long lasting debate (Spatafora *et al*. 2016; Montoliu-Nerin *et al*. 2021; Wijayawardene *et al*. 2022), but recent evidence reinforces their status as Phylum (Glomeromycota) and unveils their relationship with Dikarya (Wijayawardene *et al*. under review).

The difficulties in the study of *Glomeromycota* arise mainly from the following two reasons. First, numerous members of the *Glomeromycota* are missed in sampling due to infrequent or seasonal spore formation. Second, the lack of basic knowledge about the biology and life history strategies (e.g., requirements for germination of spores, the presence of a suitable host plant, and the conditions for growing both partners), which make it impossible to fully understand the life cycle and, consequently, the morphology of the specimens found.

Glomeromycotan species identification based on spore morphology alone requires experience and access to vouchers of the type and reference materials deposited in collections (**Fig. 1**). *Glomeromycota* members produce only one reproductive structure (glomerospores) with useful phenotypic and histochemical characters (spore ontogeny, morphometrics, subcellular structure, staining in Melzer's reagent) for morphological identification. The number of diagnostic morphological features is small - mainly for species forming glomoid spores, where these characters present phenotypic and histochemical variability. Furthermore, some members of the *Glomeromycota* may produce two different spore types (dimorphism and synanomorphism), and the recognition of only one morph may lead to incorrect taxonomic conclusions (Goto *et al.* 2008; Bills and Morton 2015; Blaszkowski *et al.* 2022; Kokkoris *et al.* 2024). Nevertheless, morphological characters remain important for differentiating groups at different taxonomic levels or for guiding the selection of fungi that may belong to new species (**Fig. 2**). For instance, the germinal shields (or orbs) and spore wall organization are important for distinguishing acaulosporoid, pacisporoid, scutellosporoid, and ambisporoid spores, and the morphology of the subtending hypha to discriminate glomoid spores (Oehl *et al*. 2011).

Glomeromycota have a complex genetic structure with multinucleate spores and a coenocytic mycelium, which is the cause of the high molecular variability and intragenomic polymorphism (Chen *et al*. 2018) that significantly complicate the classification of species and gene locus "sequencing" without cloning. Moreover, the genetic basis of the *Glomeromycota*
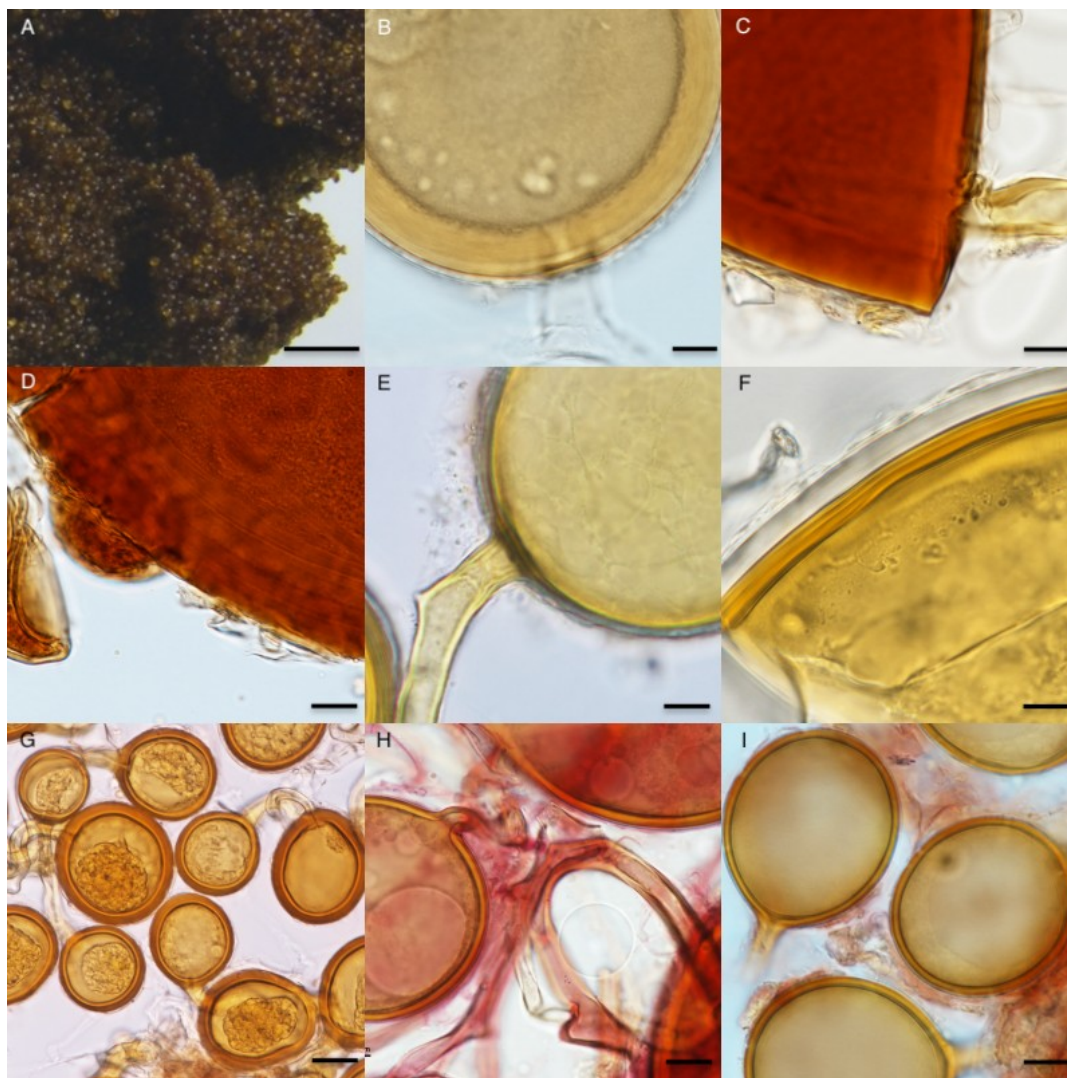
Figure 1. Diversity of glomoid spores representing different species and genera. A-B *Rhizoglomus maiae,* a glomerocarpic species described from Brazil. **C** Detail of glomerospore base of *Glomus melanosporum,* a species molecularly uncharacterized. **D** Glomerospore and aborted spores in *Corymbiglomus globiferum.* **E** Detail of spore base with subtending hypha attached in *Rhizoglomus silesianum*. **F** Spore wall of *Rhizoglomus clarum*. **G** Loose cluster of *Rhizoglomus sp.* obtained from Brazilian soil. **H** Glomerospores in clusters of *Glomus chinense*. **I** Glomerospores in *Dominikia gansuensis* from China. **B, C, D, E, F, G, I** Spores in PVLG. **H** Spores in PVLG+Melzer's reagent. Scale bars: **A** = 250 µm, **B, C, D, E, F, H, I** = 10 µm, **G** = 25 µm.

reproductive system remains largely unknown. Ropars *et al*. (2016) identified putative mating-type locus, suggesting multi-allelic locus and that AMF could be heterothallic and bipolar. However, parasexual processes may also play a role in generating nuclear diversity in *Glomeromycota*. Finally, there are few mycologists in the world dealing with the morphological and molecular characterization of this commercially important group of fungi for crop production and ecological restoration. Nine collections maintain cultures of

Glomeromycota worldwide, in the American continent (**INVAM**, **BGIV**, **GINCO_CAN**, **CICG, Embrapa)**, Europe (**BEG** and **GINCO-BEL**), India (**CMCC**), and Japan (**Naro - Maff - Japan**) among others maintained by labs. However, only three are located in the Global South (BGIV, CICG, and Embrapa). Although the use of the morphological species concept remains the rule, in recent years there have been initiatives to establish species and taxa of other ranks based on environmental sequences (Öpik *et al.* 2010; Lücking *et al.* 2021). The

environmental sequences available in databases suggest the presence of new taxa in *Glomeromycota* of different taxonomic ranks.

Despite being recognized as a basic science, the *Glomeromycota* taxonomy remains a challenge for new generations, attracting a limited number of researchers throughout history. For those uninitiated in the taxonomy of *Glomeromycota*, species identification based on morphological characters could lead to a real nightmare. The main challenge lies in the difficulty of recognizing the characteristics of the spore subcellular structure and components. Although the spores of *Glomeromycota* species are the largest compared to spores of other taxa of the fungi kingdom, they are structurally complex, these structures are difficult to detect, and their characteristics change during spore development (Goto and Maia 2006). Some proceedings have been suggested (or are required) to minimize these difficulties, such as (i) the use of pure (monosporic) cultures as a basis for describing species, ensuring single origin, and quality and reproducibility in analyses, (ii) performing ontogenetic analyses of the glomerospores to reveal the stages of differentiation of the spore subcellular components and structure throughout the development of the fungus; (iii) conducting phylogenetic analyzes to recognize the taxonomic affiliation and the positions within the *Glomeromycota* of the analyzed specimens; and (iv) learning the nature of mycorrhiza and the phenotypic and histochemical features of its structures, i.e., arbuscules, vesicles, and hyphae. All these steps are used as a "gold standardized protocol" to describe AMF species. Technical improvements are required to overcome the need of trap and single-species cultures. Obtaining high-quality complete genome sequences from single spores or nuclei, as achieved by Montoliu-Nerin *et al*. (2021), may revolutionize the description of *Glomeromycota* species.
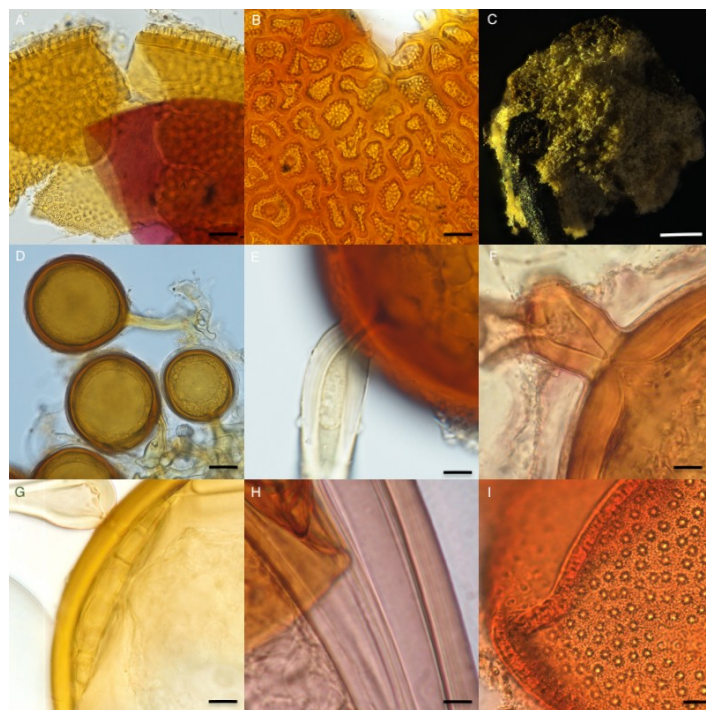

Figure 2. Diversity of glomerospores representing undescribed or described species from Brazilian soils, and species not characterized molecularly. A-B Ornamented *Acaulospora* spp. **C** Glomerocarps of *Sclerocarpum amazonicum*. **D-F** Glomoid glomerospores of new undescribed species. G-H Scutellosporoid glomerospores of undescribed species. **I** Ornamentation in *Racocetra minuta*, a molecularly uncharacterized AMF species. **B, D, E, G, I** Spores in PVLG. **A, F, H** Spores in PVLG+Melzer's reagent. Scale bars: **A, B, E, F, G, H, I** = 10 µm, **C** = 1.0 mm, **D** = 50 µm.

The rate of discovering new AMF taxa over the past two decades has been only 4.6 species per year, taking more than 200 years to describe 1,000 species, as estimated by Mueller and Schmit (2007). Analyses of environmental sequences deposited in public databases, using a 98% identity threshold, suggested a very high diversity (7,247) (Tedersoo *et al*. 2022), with new families and genera still awaiting description. In total, around 360 species have been described (**Fig. 3**) in the *Glomeromycota*, distributed in three classes, six orders, 17 families, and 49 genera (https://biologiademicorrizas.wixsite.com/glomeromycota).This represents only a very small fraction (0.04%) of the estimated richness using environmental sequences, making the task of describing all *Glomeromycota* species a
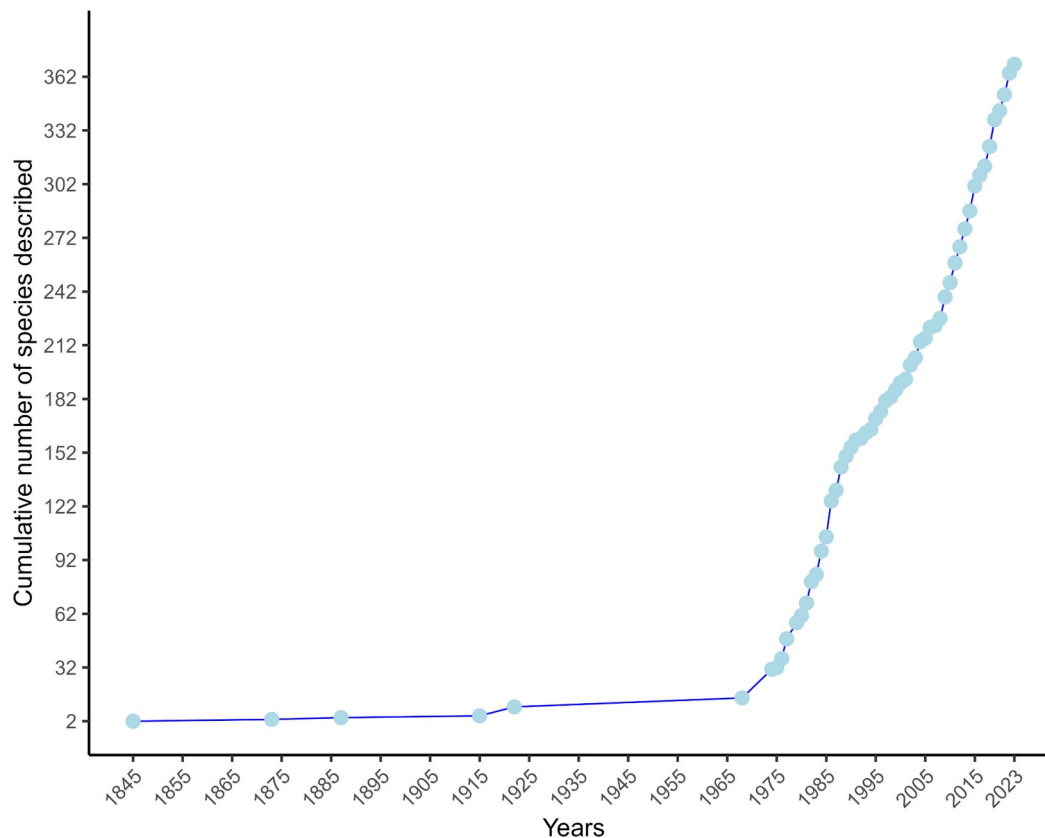
Figure 3. Evolution of glomeromycotan *sp. nov.* description through time.

"mission impossible" (more than 1,500 years), considering the current rate of descriptions (**Fig. 3**). However, in our opinion, a precise estimation is not possible at the moment since it would be largely biased by the criteria used for species boundaries and by the sampling effort.

Hyde *et al*. (2023) gathered 12 researchers linked to the *Glomeromycota* taxonomy in the Global consortium for the classification of fungi and fungus-like taxa, interestingly the majority (8) came from the Global South (Brazil and Peru). Historically, AMF taxonomists are from the Global North (England, United States, Germany, and Poland), but taxonomists from Spain and Switzerland are also involved. Unfortunately, there are no *Glomeromycota* taxonomists working in Africa, and only a few in Asia and Oceania - both hotspots of AMF biodiversity.

Only a few specialists with many years of

experience and knowledge about the morphology of AMF are able to prepare reliable descriptions of these fungi. Despite all the difficulties (financial and time resources, high expertise), this challenge is still worth it! Members of the *Glomeromycota* are wonderful organisms with enormous impacts on nature and the effects of their use by humans, so continuing to learn about AMF is of the utmost importance. This will enable, among others, (i) targeted organization of biological diversity, (ii) deeper understanding of evolution, (iii) promoting the conservation of biodiversity, (iv) more effective application of biotechnology, (v) identifying and communicating, (vi) preserving scientific heritage, and (vii) environmental education. A small group of taxonomists can be responsible for a significant scientific contribution. The authors who signed this short article participated in the description of two classes, two orders, six families, 27 genera, and 100 new species of *Glomeromycota*. This represents 66% of

the classes, 33% of the orders, 35% of the families, 55% of the genera, and 27% of the species described in the phylum. If we want to increase the quality of AMF species descriptions, we need to encourage the training of new taxonomists, develop or strengthen collaborations with taxonomists and phylogeneticists, explore new habitats and environments, and finally, fight for financial resources (as the SPUN initiative - https://www.spun.earth/) and permanent positions, so that these professionals can continue describing species that will be used in future applied work. Considering all the challenges awaiting at the horizon, we are wishing for a "new dawn" of AMF taxonomy, with renewed interest and effort from the members of the scientific community, in order to guarantee a promising future for this beautiful and exciting research field.

References

- Antonelli A, Fry C, Smith, *et al*. 2023. *State of the World's Plants and Fungi 2023*. Royal Botanic Gardens, Kew.
https://doi.org/10.34885/wnwn-6s63
- Bills RB, Morton JB. 2015. A combination of morphology and 28S rRNA gene sequences provide grouping and ranking criteria to merge eight into three Ambispora species (Ambisporaceae. Glomeromycota). *Mycorrhiza* 25: 485-498.
https://doi.org/10.1007/s00572-015-0626-7
- Błaszkowski J, Sanchez-Garcıa M, Niezgoda P, *et al*. 2022. A new order, Entrophosporales, and three new Entrophospora species in Glomeromycota. Front microbiol 13: 962856.
https://doi.org/10.3389/fmicb.2022.962856
- Bhunjun CS, Chen YJ, Phukhamsakda C, et al. 2024. What are the 100 most cited fungal genera? Studies in Mycology (*in press*).
- Chen ECH, Morin E, Beaudet D, *et al*. 2018. High intraspecific genome diversity in the model arbuscular mycorrhizal symbiont Rhizophagus irregularis. *New Phytol* 220: 1161-1171.
https://doi.org/10.1111/nph.14989
-

- Declerck S, Cranenbrouck S, Dalpé Y, *et al*. 2000. *Glomus proliferum* sp. nov.: a description based on morphological, biochemical, molecular and monoxenic cultivation data. *Mycologia* 92: 1178-1187.
http://dx.doi.org/10.2307/3761485
- Gerdemann JW, Trappe JM. 1974. The Endogonaceae in the Pacific Northwest. *Mycologia Memoir* 5: 1-76.
- Goto BT, Maia LC, Oehl F. 2008. *Ambispora brasiliensis*, a new ornamented species in the arbuscular mycorrhiza-forming Glomeromycetes. *Mycotaxon* 105: 11-18.
- Hyde KD, Abdel-Wahab MA, Abdollahzadeh J, *et al*. 2023. Global consortium for the classification of fungi and fungus-like taxa. *Mycosphere* 14: 1960-2012.
https://doi.org/10.5943/mycosphere/14/1/23
- Koide R, Moose B. 2004. A history of research on arbuscular mycorrhiza. *Mycorrhiza* 14: 145-163.
https://doi.org/10.1007/s00572-004-0307-4
- Kokkoris V, Banchini C, Louis Paré L, et al. 2024. *Rhizophagus irregularis*, the model fungus in arbuscular mycorrhiza research, forms dimorphic spores. *New Phytol* 242: 1771-1784.
https://doi.org/10.1111/nph.19121
- Lücking R, Aime MC, Robbertse B, et al. 2021. Fungal taxonomy and sequence-based nomenclature. *Nat Microbiol* 6: 540-548.
https://doi.org/10.1038/s41564-021-00888-x
- Meng Y, Davison J, Clarke JT, *et al*. 2023. Environmental modulation of plant mycorrhizal traits in the global flora. *Ecol Lett* 26: 1862-1876.
https://doi.org/10.1111/ele.14309
- Montoliu-Nerin M, Sánchez-García M, Bergin C, *et al*. 2021. In-depth Phylogenomic Analysis of Arbuscular Mycorrhizal Fungi Based on a Comprehensive Set of *de novo* Genome Assemblies. *Front Fungal Biol* 2: 716385.
https://doi.org/10.3389/ffunb.2021.716385
- Morton JB. 1988. Taxonomy of VA mycorrhizal fungi: classification, nomenclature, and identification. *Mycotaxon* 32: 267-324.
- Morton JB, Benny GL. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporinae,

and two families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. *Mycotaxon* 37: 471-491.

- Morton JB, Redecker D. 2001. Two new families of Glomales, Archaeosporaceae and Paraglomeraceae, with two new genera Archaeospora and Paraglomus, based on concordant molecular and morphological characters. *Mycologia* 93: 181-195. http://dx.doi.org/10.1080/00275514.2001.12063147

- Mueller G, Schmit JP. 2007. Fungal Biodiversity: What do we know? What can we predict? *Biodivers Conserv* 16: 1-5. https://doi.org/10.1007/s10531-006-9117-7

- Oehl F, Silva GA, Goto BT, *et al*. 2011. Glomeromycota: three new genera and glomoid species reorganized. *Mycotaxon* 116: 75-120. http://dx.doi.org/10.5248/116.75

- Öpik M, Vanatoa A, Vanatoa E, *et al.* 2010. The online database *MaarjAM* reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytol* 188: 223-241. https://doi.org/10.1111/j.1469-8137.2010.03334.x

- Ropars J, Toro KS, Noel J, *et al*. 2016. Evidence for the sexual origin of heterokaryosis in arbuscular mycorrhizal fungi. *Nat Microbiol* 1: 1-9. https://doi.org/10.1038/nmicrobiol.2016.33

- Sportes A, Hériché M, Boussageon R, *et al*. 2021. A historical perspective on mycorrhizal mutualism emphasizing arbuscular mycorrhizas and their emerging challenges. *Mycorrhiza* 31: 637-653. https://doi.org/10.1007/s00572-021-01053-2

- Schüßler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota : phylogeny and evolution. *Mycol Res* 105: 1413-1421. https://doi.org/10.1017/S0953756201005196

- Spatafora JW, Chang Y, Benny GL, Lazarus K, *et al*. 2016. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* 108: 1028-1046. https://doi.org/10.3852/16-042

- Tedersoo L, Mikryukov V, Zizka A, *et al*. 2022. Global patterns in endemicity and vulnerability of soil fungi. *Glob Change Biol* 28: 6696-6710. https://doi.org/10.1111/gcb.16398

- Wijayawardene NN, Hyde KD, Dai DQ, *et al*. 2022. Outline of Fungi and fungus-like taxa – 2021. *Mycosphere* 13: 53-453. http://dx.doi.org/10.5943/mycosphere/13/1/2

- Walker C. 1983. Taxonomic concepts in the Endogonaceae: spore wall characteristics in species descriptions. *Mycotaxon* 18: 443-455.