Journal of Applied Botany and Food Quality 99, 43 - 59 (2026), DOI:10.5073/JABFQ.2026.099.006

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Revision of Ambisporaceae, with three new genera, one new species and an identification key for all species currently attributed to this family

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(Submitted: February 4, 2026; Accepted: May 14, 2026)

Summary

The objective of this study was to re-analyse the molecular phylogeny and/or the morphology of all species, which have been attributed to the so-far mono-generic fungal family Ambisporaceae. The genus *Ambispora* has been well-known for its spore bi-morphy described even from single spore clusters. Triple-walled spores are differentiated on sporiferous saccules, while mono-walled spores are formed on simple subtending hyphae. New phylogenetic analyses revealed differences of ≥10% in nrDNA sequences of three phylogenetic clades, suggesting the need to divide *Ambispora* into three genera and perform advanced morphological separations. These advances are primarily based on the composition and phenotypic properties of the spore walls of the acaulo-ambisporoid morph, which are more diverse than those of the mono-walled glomoid morph. While all known species of the triple-walled acaulo-ambisporoid morph have an evanescent to semi-permanent outer spore wall, the middle wall of (i) *Am. fennica*, *Am. brasiliensis*, *Am. gerdemannii* and *Am. nicolsonii* is smooth and permanent (*Am. fennica* clade, A), (ii) *Am. appendicula*, *Am. callosa*, *Am. leptoticha*, and *Am. jimgerdemannii* is alveolate (*Am. appendicula* clade, B), and (iii) *Am. granatensis* is smooth and short-lived, easily degrading with age (*Am. granatensis* clade, C). Consequently, we decided that (i) species of the *Am. fennica* clade represent the genus *Ambispora,* (ii) species of the *Am. appendicula* clade represent the new genus *Appendiculispora*, and (iii) sequences previously ascribed to *Am. granatensis* represents a new clade at the rank of genus, here named *Ephemeriparies*. Two species of an additional morph, with triple-walled spores, but apparently formed on subtending hyphae, and having a diagnostic reticulate, football-like middle wall, are here separated from the revised genus *Ambispora* based solely on morphological analyses, since molecular identification analyses so far failed and remained merely unknown. This later morph and genus is based on the type species *Pelotaspora reticulata* comb. nov, and on *P. austrolatina* sp. nov. Concomitant molecular phylogenetic and morphological analyses are needed to attribute not only *Pelotaspora* species, but also those, for which hitherto only the glomoid-ambisporoid morph has been observed within the family Ambisporaceae. Without molecular analyses, such species with a glomoid morph recognized, but unknown acaulo-ambisporoid morph have to be retained within *Ambispora*.

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Keywords: *Appendicispora*, spore wall composition, pedicel, bi-morphy, phylogenetic clades

Introduction

The introduction of *Appendicispora*/*Ambispora* into the Archaeosporales was one of the most 'lively' coincidences in the history of AM fungal taxonomy (Spain et al., 2006; Walker et al., 2007a, 2007b, 2008). *Appendicispora* and *Ambispora* were described shortly one by one for the same fungal clade, but based on two different type species (*Ap. appendicula* and *Am. fennica*, respectively (Spain et al., 2006; Walker et al., 2007a). Since, based on nomenclatural rules, *Appendicispora* (Spain et al., 2006) was a late homonym of *Appendicospora* (Ascomycota) and therefore illegitimate, *Ambispora* (Walker, 2008) was established as the legitimate genus name, although it was described after *Appendicispora*.

Already the descriptions of the first few species attributed to the genus *Ambispora* were confusing for several reasons. *Glomus gerdemannii*, currently *Ambispora gerdemannii*, was described based on its acaulosporoid (sensu lato) morph, which had erroneously been judged to be a typical glomoid (sensu lato) morph of the genus *Glomus* (Rose et al., 1979). A second species, called *Acaulospora gerdemannii* (Nicolson and Schenck, 1979), was described based on its acaulosporoid morph, which did neither fit with the acaulosporoid morph of *G. gerdemannii*, nor with the acaulosporoid morph of *Ac. appendicula* (= *Am. appendicula*; Schenck et al., 1984; Spain et al., 2006). Finally, besides *Am. appendicula*, for which both acaulosporoid and glomoid morphs were described from single species isolates (Schenck et al., 1984), another species, *G. leptotichum*, was described based on its glomoid morph and diagnostic reticulate ornamentation on its outer spore surface (Schenck and Smith, 1982), which is not existing in the glomoid morph of *Am. appendicula* and *Am. gerdemannii*, currently called *Am. leptoticha*. In the meantime, *Ac. nicolsonii*, currently *Am. nicolsonii*, was described as a 'typical' *Acaulospora*, although it did not have the diagnostic beaded inner wall of other *Acaulospora* species, as in the case with *Ac. gerdemannii* and *Ac. appendicula* (Nicolson and Schenck, 1979; Schenck et al., 1984), but had all the characteristics of the acaulo-ambisporoid morph of *Ambispora* (sensu Walker et al., 1984; Spain et al., 2006).

Phylogenetic and/or morphological analyses led to a separation of Archaeosporaceae and Ambisporaceae from the acaulosporoid

species (sensu stricto) of Acaulosporaceae (Morton and Redecker, 2001; Schüssler et al., 2001; Sieverding and Oehl, 2006; Spain et al., 2006; Walker et al., 2007a; 2007b; Walker, 2008; Bills and Morton, 2015). Both families are currently members of Archaeosporomycetes, a fungal class, which was separated, together with Paraglomeromycetes, from Glomeromycetes (Oehl et al., 2011a; 2011b) and also comprises the old family Geosiphonaceae (Engler and Gilg, 1924) and the recently described Polonosporaceae (Błaszkowski et al., 2021). In the following years, two remarkable species were described in Ambisporaceae, the bi-morphic *Am. granatensis* (Palenzuela et al., 2011) and *Am. reticulata* (Oehl et al., 2012). The latter species produced three-walled spores similar to those of the *Ambispora* acaulo-ambisporoid morph, but neither sporiferous saccules nor mono-walled, glomoid-ambisporoid spores were recognized. Here, it is concluded that typical *Ambispora* species are bi-morphic, as are, e.g., *Am. fennica*, *Am. gerdemannii*, *Am. brasiliensis*, *Am. appendicula*, *Am. jimgerdemannii*, and *Am. granatensis*, which form three-walled acaulo-ambisporoid (sensu lato) spores generally on a pedicel arising from the neck or stalk of sporiferous saccules, and mono-walled glomoid-ambisporoid spores, which typically have a subhyaline to creamy or brownish outer, evanescent layer and a persistent, white to hyaline, structural, laminate second layer. In relation to *Am. callosa*, we (i) re-analysed slides with spores of this species collected in the type location in 1987 (Sieverding, 1988) and (ii) newly examined spores that represented both the glomoid-ambisporoid and acaulo-ambisporoid morphs of this species. For *Am. nicolsonii*, only the acaulo-ambisporoid spore type has been known so far. For other species, such as *Am. fecundispora* only the mono-walled glomoid-ambisporoid spores have been known so far, with the subhyaline to creamy or brownish, evanescent outer layer and a persistent, white to hyaline, structural, laminate second layer, typical and diagnostic for this morph (Spain et al., 2006; Walker et al., 2007a; Goto et al., 2008; Palenzuela et al., 2011). Finally, only triple-walled *Am. reticulata* spores have been described so far, which are apparently not formed on sporiferous saccules, but on subtending hyphae (SH); a mono-walled glomoid-ambisporoid morph is not known for this species.

Since a few years, Glomerales genera are separated, when the molecular dissimilarities in the partial nrDNA gene reach approximately 10% between different clades (e.g. Silva et al., 2023). In other orders, these percentages might be slightly smaller such as in Entrophosporales (Silva et al., 2025) or Gigasporales (Hyde et al., 2024; Oehl et al., 2026). In Archaeosporaceae, recently the newly created genera *Andinospora* and *Antiquispora* were separated from *Archaeospora* (Morton and Redecker, 2001) and *Palaeospora* (Oehl et al., 2015) based on phylogenetic analyses (Esmaeilzadeh-Salestani et al., 2025) and morphology (Oehl et al., 2015). The objectives of the present study were (i) to reconstruct phylogenies of members of Ambisporaceae based on available sequence data, (ii) to determine the relationships between these phylogenies and verified data on the morphology of the included members of this family, and (iii) to introduce changes and novelties to the classification of Ambisporaceae, when the obtained results support such a need.

Materials and methods

Phylogenetic Analyses

To reconstruct the phylogeny, three alignments (datasets), based on partial SSU, ITS region and partial LSU nrDNA (dataset 1 - SSU+ITS+LSU), ITS region of the nrDNA (dataset 2 - ITS), and complete SSU nrDNA (dataset 3 - SSU) were generated with AM fungal sequences from species of Ambisporaceae (Supplementary Material, Spreadsheet S1). *Paraglomus brasilianum*, *Archaeospora trappei*, *Polonospora polonica*, and *Geosiphon pyriformis* were included as outgroup. *Geosiphon pyriformis* was used as outgroup only in datasets 1 and 3, because this species does not have ITS sequences. *Polonospora polonica* was not used as outgroup in dataset 3, because it lacks complete SSU sequences. The sequences available for *A. granatensis* are from ITS and complete SSU. The ITS sequences from *Am. granatensis* were used to compose datasets 1 and 2. The datasets were aligned in MAFFT (Katoh et al., 2019) using default parameters. Prior to phylogenetic analyses, the model of nucleotide substitution was estimated with ModelTest-NG (Darriba et al., 2020). Maximum Likelihood - ML (1000 bootstrap) analyses were performed with Felsenstein Bootstrap Proportion (FBP) and Transfer Bootstrap Expectation (TBE), using RaxML-HPC-SSE3 and RaxML-NG, respectively (Stamatakis, 2014; Kozlov et al., 2019; Edler et al., 2020). Sequences from six of the eleven AMF species currently attributed to Ambisporaceae were available from public data bases, except for *Am. brasiliensis, Am. fecundispora, Am. jimgerdemannii,* and *Am. nicolsonii*. *Ambispora reticulata* has only three fragments from partial SSU sequences (probably from a wrongly attributed fungus), which, as shown by BLASTn analysis, belong to *Endogone* species. We could not obtain sequences from the here newly described species *Pelotaspora austrolatina*. In addition, environmental sequences that, according to BLASTn analysis, were closely related to each of the fungi are discussed here.

Specimen analyses

Specimens of almost all AMF species currently attributed to Ambisporaceae were used in this study. These were the type material of *Am. appendicula* (OSC #41,495), *Am. brasiliensis* (HURM 78879, HURM 78880, OSC# 134501, ZT MYC 159), *Am. callosa* (GOET, OSC), *Am. fennica* (a specimen kindly provided by M. Vestberg), *Am. gerdemannii* (OSC #39,476), *Am. granatensis* (ZT Myc 1626, OSC #134,712), *Am. jimgerdemannii*, (OSC #37,417), *Am. leptoticha* (OSC #40,249), and *Am. reticulata* (ZT Myc 24171-24175). In addition, we analyzed specimens of the species newly described here (ZT Myc 15115-15119) and those collected during the visit of the type location of *Am. gerdemannii* (1 km north of Benham Falls at Fort Benham; Rose et al., 1979) in October 2005, together with Joyce L. Spain and Prof. James M. Trappe. Additionally, we re-analysed about 100 slides from the type location of *Am. callosa*, basionym *Glomus callosum* (Sieverding 1988), prepared by Ewald Sieverding in 1987 and originating from an experimental field of the agricultural school Mushweshwe, near Bukavu in the South-Kivu province in Zaire (since 1997 belonging to the Democratic Republic of Kongo, Central Africa). In several of these slides, we found not only the *Am. callosa* glomoid morph, but also an acaulo-ambisporoid morph of an *Ambispora* (sensu lato) species. In 1987, this acaulo-ambisporoid morph had been misidentified as *Acaulospora appendicula*. Selected slides with the later morph were deposited at Z+ZT (the common mycological herbarium of the University and ETH of Zurich, Switzerland) with the accession number ZT Myc 94416, while newly prepared slides from the type location of *Am. gerdemannii* were deposited under the accession ZT Myc 94417. The specimens included in this study were either directly examined in the mycological herbaria we visited (e.g. OSC), or came from living collections grown by, e.g., INVAM (USA), institutions located in Basel, Zurich (Switzerland), Granada (Spain), and Temuco (Chile), as well as by E. Sieverding and F. Oehl (private cultures).

Spores were extracted from field soils, trap cultures, and single-species cultures by wet-sieving and sucrose centrifugation (Sieverding, 1991). About 100 spores of each species, mounted and crushed in polyvinyl alcohol-lactic acid-glycerol (PVLG; Koske and Tessier, 1983), a mixture of PVLG and Melzer's reagent (Brundrett et al., 1994) and water (Spain, 1990), were examined to determine their mode of formation, subcellular structure, and phenotypic and histochemical properties of components of the structure, using

compound microscopes at 100-1000 × magnifications. Spore wall terminology follows that of Spain (2003), Spain et al. (2006), Goto et al. (2008), and Palenzuela et al. (2011). Specimens mounted in PVLG and the mixture of PVLG and Melzer's reagent were deposited at Z+ZT (Switzerland) and HURM, the mycological herbarium of the UFPE in Recife.

Soil sampling for the newly described species

Soil samples were taken with a shovel in a Southern Chilean temperate grassland and a South Chilean deciduous forest (Castillo et al., 2006), two conventionally managed agricultural soils from the rhizosphere of *Triticum aestivum* (September 2011), and a conventionally managed South Brazilian vineyard from the rhizosphere of *Vitis vinifera* (September 2004). The grassland and the forest sites were located at the Experimental Station San Pablo de Tregua of the University Austral de Chile (Valdivia, Chile, 39º30-39º38'S and 72°02-72°09'W) at an elevation between 550 and 1600 m asl (Castillo et al., 2006). The two wheat fields were located in Curacautín (38º51'S, 71°83'W at 605 m asl) and Gorbea (39º12' S, 72º59' W at 179 m), two municipalities in the Araucanía region of Southern Chile, and the vineyard in Caxias do Sul, Rio Grande do Sul, at about 825 m asl (29° 16' 33"S and 51° 07' 06"W; Silveira, 2006). The soil pH (H_2O) was 5.4 in the grassland and the deciduous forest, 5.9 and 4.8 in the wheat fields, and 6.6 in the vineyard. Organic carbon was 88.7 mg kg^{-1} in the grassland, 144.4 mg kg^{-1} in the deciduous forest, 136.0 mg kg^{-1} and 95.0 mg kg^{-1} in the wheat fields, and 44.0 mg kg^{-1} in the vineyard. Available P was 2.9 and 3.6 mg kg^{-1} in the grassland and the deciduous forest, respectively, 47.8 mg kg^{-1} and 31.9 mg kg^{-1} in the wheat fields, while it was >100.0 mg kg^{-1} in the vineyard. All Chilean soils were Andosols, while the soil type of the vineyard in Southern Brazil was a Leptosol.

Results

Molecular phylogeny

Currently Ambisporaceae is monogeneric with *Ambispora*. According to our phylogenetic analyses Ambisporaceae present three different, well supported clades (Figs. 1, 2, and 3). Clade A is represented by *Am. fennica* and a sequence attributed to *Am. gerdemannii*. Clade B is composed by sequences from *Am. callosa*, *Am. leptoticha*, *Am. appendicula*, and two sequences attributed to *Am. gerdemannii* (isolate AU215) in the NCBI (Fig. 2). Clade C has, so far, sequences from *Am. granatensis*. In our opinion, clades B and C represent two new genera.

According to Maximum Identities (MI) determined in BLASTn analyses with SSU-ITS-LSU sequences of *Am. appendicula*, *Am. callosa*, *Am. leptoticha*, *Am. fennica*, and *Am. gerdemannii* (dataset 1), sequences of clade A with *Am. fennica* and *Am. gerdemannii* are most similar to sequences of *Am. callosa* (isolate MAFF520057, 89% MI), *Am. appendicula* (att1235-2, 88.9% MI), and *Am. leptoticha* (MAFF520090, 88.2% MI). The same analyses with ITS sequences of *Am. granatensis* (clade C with the new genus *Ephemeriparies*) showed that the most closely related species is *Am. fennica* (85% MI). The MI of clade B (new genus *Appendiculispora*) to members of clade A (*Ambispora* sensu stricto) is 89% and no species from clades A and B is close to *Am. granatensis* (clade C).

BLASTn queries using SSU-ITS-LSU sequences revealed three environmental sequences ascribed to *Ambispora* (sensu stricto) with ≥ 95% MI. Two of them were obtained from roots of *Littorella uniflora* (LS479906, LS479907) in Norway (Sudová et al., 2021), and one from roots of *Sequoiadendron giganteum* (HQ895806) in USA (Fahey et al., 2012). In relation to the new genus *Appendiculispora*, placed in clade B, we found six environmental sequences with ≥ 95% MI, of which four were obtained from roots of *Solanum tuberosum* (HF970299, HF970300, HF970301, HF970302) in Peru (Senés-Guerrero et al., 2013), and two from the rhizosphere of *Sinocalycanthus chinensis* (MH469316, MH469376) in China. No environmental ITS sequences related to *Am. granatensis* (clade C with the new genus *Ephemeriparies*) were found.

Morphological analyses

Ambisporaceae species have generally been known to be bi-morphic (see Tab. 1), as spores can be formed i) terminally on the appendix of a branching neck of a sporiferous saccule, which is also formed terminally (acaulo-ambisporoid morph), and ii) terminally on subtending hyphae (SH, i.e. typical glomoid-ambisporoid morph). The phylogenetic analyses requested for advanced morphological separations. These can primarily be based on the phenotypic features of components of the triple-walled acaulo-ambisporoid morph. Although the outer wall is semi-permanent to evanescent in all *Ambispora* spores, the middle wall of (i) *Am. fennica*, *Am. brasiliensis*, *Am. gerdemannii*, and *Am. nicolsonii* is smooth and permanent (*Am. fennica* clade A, Figs. 4-9), (ii) *Am. appendicula*, *A. leptoticha*, *Am. jimgerdemannii*, and *Am. callosa* is alveolate (*Am. appendicula* clade B, Figs. 10-27), and (iii) *Am. granatensis* is smooth, short-lived, easily degrading with age (*Am. granatensis* clade C, Figs. 28-30). Species of the *Am. fennica* clade (clade A) represent the genus *Ambispora* (Tab. 1), based on the type species *Am. fennica*, while species of the *Am. appendicula* clade (clade B) represent the new genus *Appendiculispora* (Tab. 1), and the mono-specific *Am. granatensis* clade (clade C) represents the new genus *Ephemeriparies* (Tab. 1). However, species for which only a glomoid-ambisporoid morph has been known cannot be attributed to any of the three clades due to the lack of clear morphological diagnostic characters. Their membership can only be revealed on the basis of phylogenetic analyses. Currently, these must remain in the type genus *Ambispora* of the revised Ambisporaceae.

One additional morph, with triple-walled spores, but apparently formed on subtending hyphae (SH), has a diagnostic reticulate, football-like middle wall (Figs. 31-43). It is represented by *Am. reticulata* (Figs. 31-34), for which numerous attempts to obtain sequence data from field spores and spores extracted from cultures have failed. A similar morph has been found in Southern Chile and Southern Brazil, the phylogeny of which has also failed to be determined (Figs. 35-43). However, due to the consistent spore morphology of the two fungi, which clearly differs from the spore morphology of *Ambispora* (sensu lato) of clades A-C (Tab. 1), we decided to assign these fungi to a new genus in Ambisporaceae here.

Taxonomy

Ambisporaceae C. Walker, Vestberg & A. Schüssler, Mycol. Res. 111 (2): 143 (2007), emended here by Oehl, G.A. Silva, Palenz. & Sieverd.

MycoBank MB 510208

Emended description: Sporocarp formation unknown; species generally bi-morphic with mycorrhizal associations producing both three-walled acaulosporoid and generally mono-walled glomoid morphs on extra- or intraradical hyphae, but acaulosporoid or glomoid morph is not (yet) known for all species. *Acaulosporoid spores* on short hyphal appendix generally arising laterally from the hyphal neck of a terminal sporiferous saccule; with outer, middle and inner wall; germinating from the innermost wall with a germ tube emerging through the appendix attachment or penetrating the outer walls; a germination structure possibly formed between inner and middle wall. *Glomoid spores* formed singly or in loose clusters on extra- or intraradical hyphae, with a hyaline to subhyaline to creamy

Fig. 1: Phylogenetic tree obtained by analysis from partial SSU, ITS region, and partial LSU nrDNA sequences of Ambisporaceae spp. Sequences are labelled with their database accession numbers. Support values (from top) are from ML (maximum likelihood analysis) using RaxML-HPC-SSE3 with FBP (Felsenstein Bootstrap Proportion) and RaxML-NG with TBE (Transfer Bootstrap Expectation), respectively. Only support values of at least 70% are shown. Thick branches represent clades with more than 95% support in all analyses. The tree was rooted by *Paraglomus brasilianum*, *Archaeopora trappei*, *Polonospora polonica*, and *Geosiphon pyriformis*. Sequences from the ITS region only are indicated by *. The nucleotide substitution model used was GTR+I+G for both analyses.

or brown outer layer and a hyaline to white structural layer beneath, rarely with triple walls as known for the acaulosporoid morph; germinating through the subtending hypha. Intraradical mycorrhizal hyphae, arbuscles, and vesicles stain pale blue with trypan blue.
Type genus: ***Ambispora*** C. Walker, Vestberg & A. Schüssler, Mycol. Res. 111 (2): 147 (2007)
Other genera: *Appendiculispora*, *Ephemeriparies*, *Pelotaspora*

Ambispora C. Walker, Vestberg & A. Schüssler, Mycol. Res. 111 (2): 147 (2007), emended here by Oehl, Sieverd., Palenz. & G.A. Silva, Figs. 4-9
MycoBank MB 510209
Emended description: Sporocarp formation unknown. Acaulosporoid spores formed singly on short hyphal appendix generally arising laterally from the hyphal neck of a terminal sporiferous saccule.

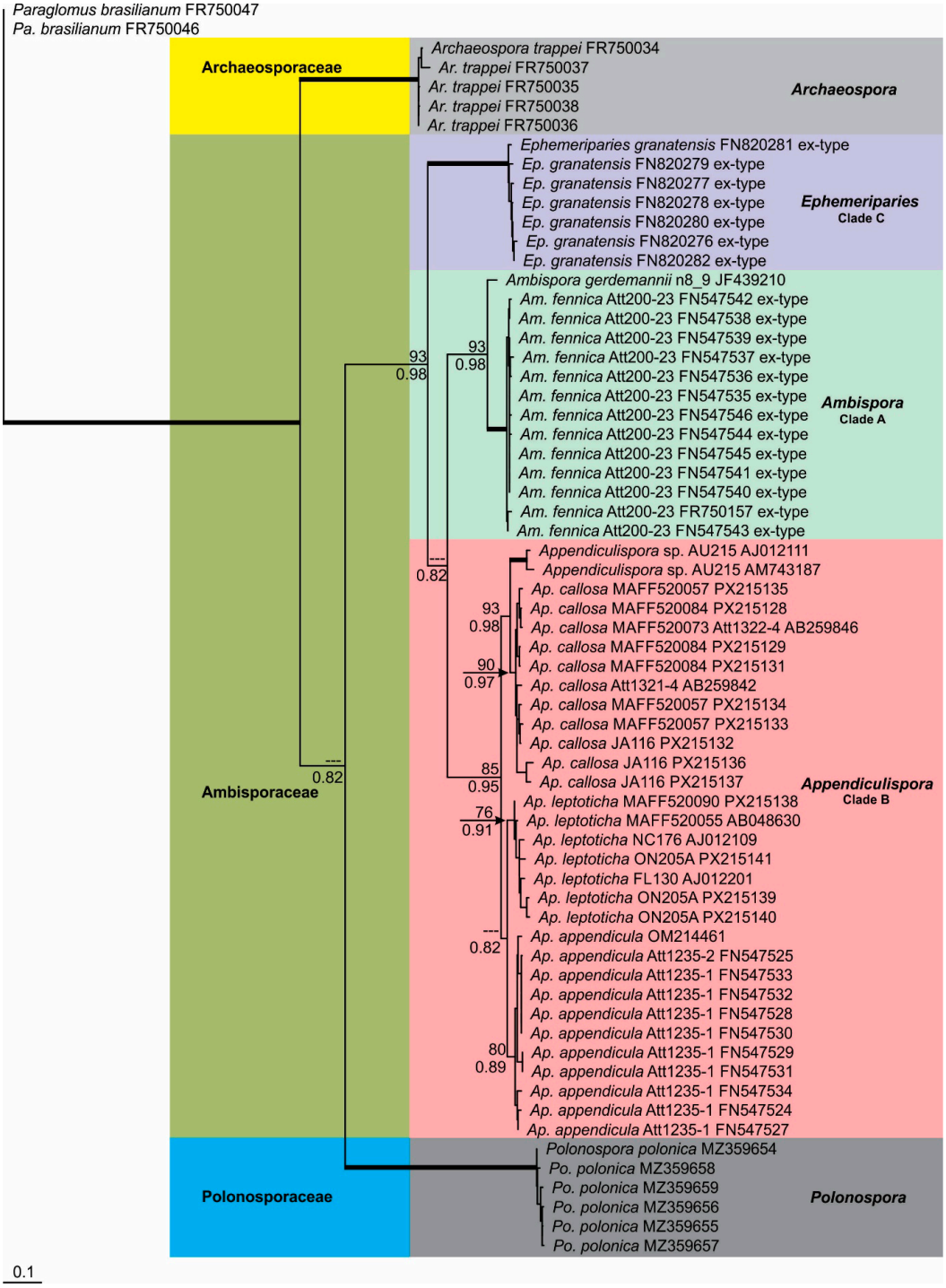


Fig. 2: Phylogenetic tree obtained by analysis from ITS nrDNA sequences of Ambisporaceae spp. Sequences are labelled with their database accession numbers. Support values (from top) are from ML (maximum likelihood analysis) using RaxML-HPC-SSE3 with FBP (Felsenstein Bootstrap Proportion) and RaxML-NG with TBE (Transfer Bootstrap Expectation), respectively. Only support values of at least 70% are shown. Thick branches represent clades with more than 95% support in all analyses. The tree was rooted by *Paraglomus brasilianum*, *Archaeospora trappei*, and *Polonospora polonica*. The nucleotide substitution model used was GTR+G for RaxML-HPC-SSE3 and HKY+G for RaxML-NG analyses.

Glomoid spores formed singly or in loose clusters on extra- or intraradical hyphae. Sporiferous saccules formed terminally on hyphae. The saccule wall is bi- to triple-layered, hyaline to subhyaline. *Acaulosporoid spores* have three walls: outer, middle, inner wall: OW, MW, IW; OW with two to three wall layers (OWL1-3) that may sometimes be lost totally, as they generally are evanescent to semi-permanent. MW is bi-layered (MWL1-2) and permanent. IW is three-layered (IWL1-3). OWL1 is evanescent, only present on young spores; OWL2 is semi-permanent to evanescent, easily seen, often showing a crazed surface with age; OWL3 is semi-permanent to evanescent, thin, tightly pressed to OWL2. In young spores, OW and MWL1 continuous with the appendix wall and mycelial hyphae; MWL1 can produce several septa in the appendix. In mature spores where the appendix is broken off, a septum formed by MWL1 and

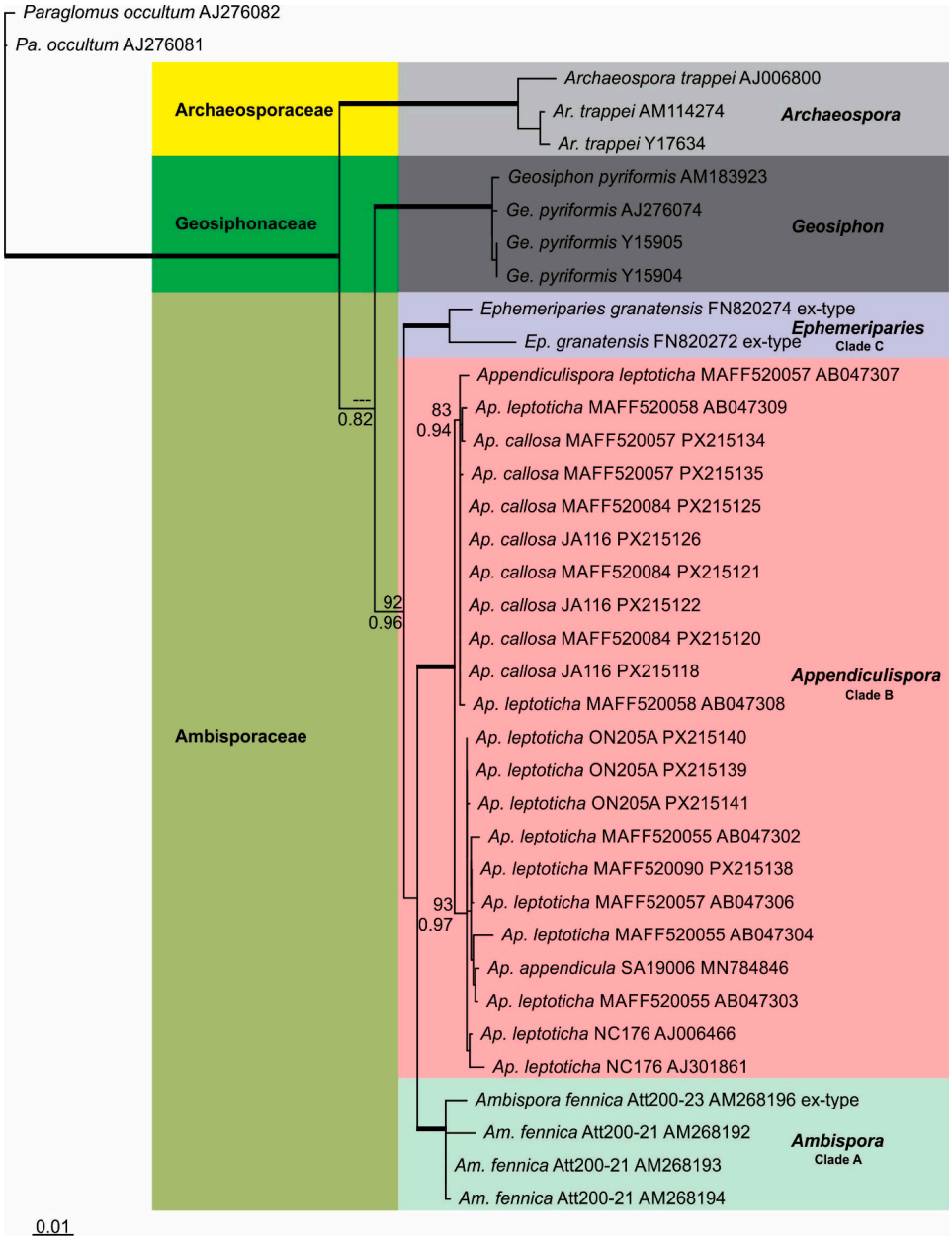


Fig. 3: Phylogenetic tree obtained by analysis from SSU nrDNA sequences of Ambisporaceae spp. Sequences are labelled with their database accession numbers. Support values (from top) are from ML (maximum likelihood analysis) using RaxML-HPC-SSE3 with FBP (Felsenstein Bootstrap Proportion) and RaxML-NG with TBE (Transfer Bootstrap Expectation), respectively. Only support values of at least 70% are shown. Thick branches represent clades with more than 95% support in all analyses. The tree was rooted by *Paraglomus occultum*, *Archaeospora trappei*, and *Geosiphon pyriformis*. The nucleotide substitution model used was GTR+I+G for RaxML-HPC-SSE3 and TIM+I+G for RaxML-NG analyses.

MWL2 closes the pore at the appendix base. MWL1 and MWL2 with smooth surfaces, but often with fracturing character, breaking (i.e. 'plate-like splitting', irregular to rectangular splitting) in crushed spores. IWL2 finely laminate, structural layer, while IWL1 and IWL3 thin, unite, flexible, and rather difficult to detect. Germination of acaulosporoid spores of *Ambispora* spp. has not yet been observed to our knowledge. *Glomoid spores* hyaline to subhyaline, globose to subglobose, with one spore wall (SW), consisting of a mucilaginous, hyaline, creamy to brown outer layer SWL1, about 1.0-2,5 µm thick, frequently with adhering debris on the outer surface; inner wall layer SWL2 several µm thick, laminate, hyaline to white. Subtending hypha cylindric to funnel shaped, 5-30 µm diam at the spore base, bi-layered.

Type species: *Ambispora fennica* C. Walker, Vestberg & A. Schüssler, Mycol. Res. 111 (2): 148 (2007), Figs. 4-8
MycoBank MB 510210
Other species: *Ambispora brasiliensis*, *Ambispora fecundispora, Ambispora gerdemannii*, *Ambispora nicolsonii*

Ambispora brasiliensis B.T. Goto, L.C. Maia & Oehl, Mycotaxon 105: 13 (2008)
MycoBank MB 511612

Ambispora fecundispora (N.C. Schenck & G.S. Sm.) C. Walker, Mycol. Res. 112 (3): 298 (2008)
MycoBank MB 511419

Tab. 1: Major morphological characteristics separating genera for the triple-walled morph of Ambisporaceae

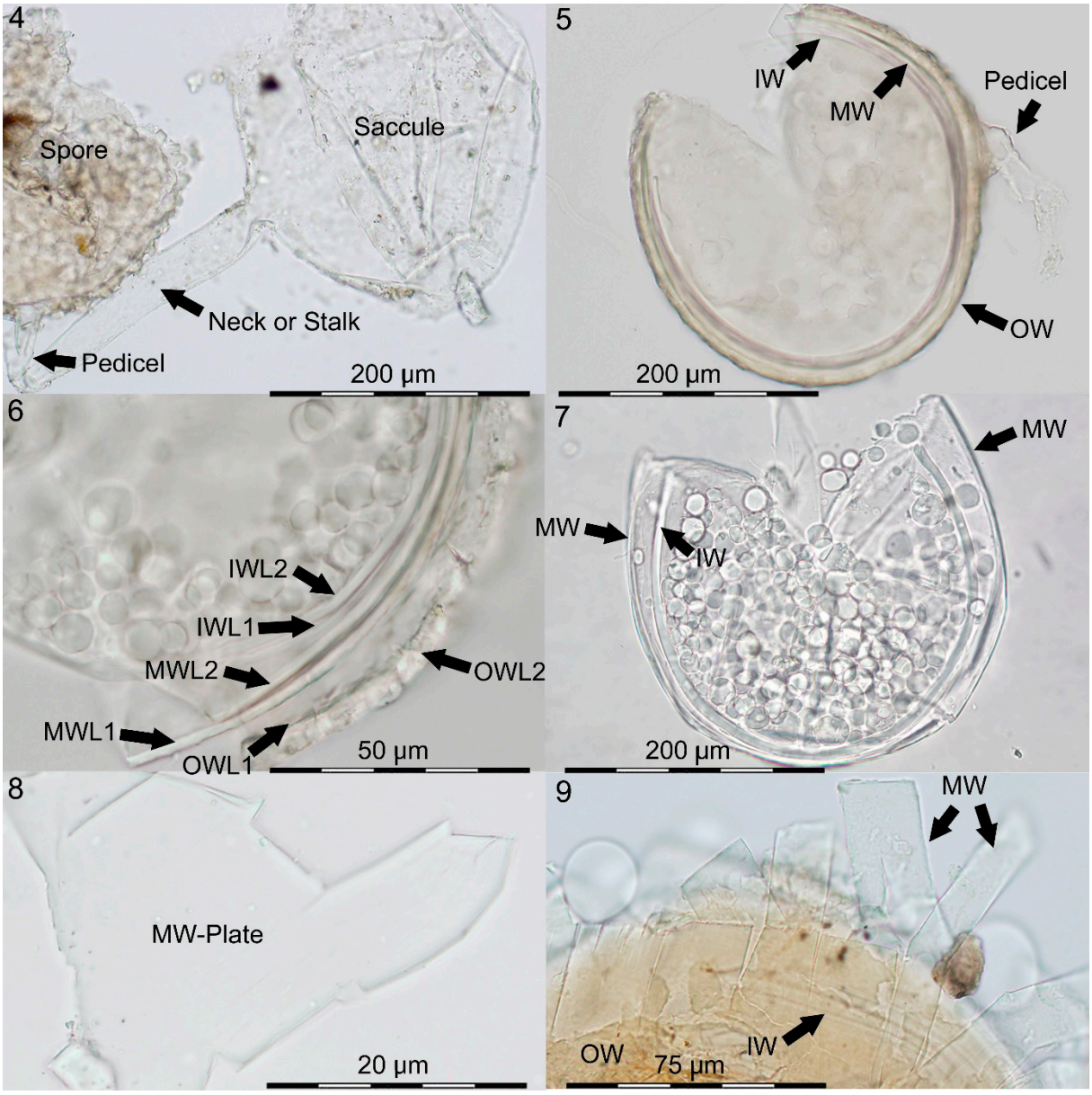
Type species	*Ambispora* *Am. fennica*	*Appendiculispora* *Ap. appendicula*	*Ephemeriparies* *Ep. granatensis*	*Pelotaspora* *Pe. reticulata*
Mycorrhizal symbiosis with flowering plants	Yes	Yes	Yes	Assumed
Staining of mycorrhizal structures	Faintly to pale	Faintly to pale	Faintly to pale	Assumed
Presence of vesicles	Yes	Yes	Not known	Not known
Spore dimorphism	Yes	Yes	Yes	Not known
Spore formation (sensu lato)	Acaulosporoid, Glomoid	Acaulosporoid, Glomoid	Acaulosporoid, Glomoid	Resemble Acaulo-ambisporoid
Number of walls in acaulosporoid (ac) spores	3 (OW, MW, IW)	3 (OW, MW, IW)	3 (OW, MW, IW)	3 (OW, MW, IW)
Number of ac spore walls arising from hyphal and saccule wall	2	2	2	2
Formation characteristics of MW & IW	MW separating from OW; IW forming *de novo* during spore formation	MW separating from OW; IW forming *de novo* during spore formation	MW separating from OW; IW forming *de novo* during spore formation	MW separating from OW; IW forming *de novo* during spore formation
MW characteristics	Permanent, rigid	Permanent, rigid	Evanescent to semi-permanent, fragile	Permanent to semi-permanent, rigid
Pedicel or nipple formation as hyphal branching on saccule neck	Pedicel	Pedicel	Pedicel	Pedicel-like structure; saccule formation unknown
Pore closure in ac spores at spore base by	MWL1	MWL1	MWL1	MWL1
acaulosporoid spore formation (sensu stricto)	Acaulo-Ambisporoid	Acaulo-Appendiculisporoid	Acaulo-Ephemeroid	Pelotasporoid
Known ornamentation types on OW	Pustulae	Cerebriform, (& warty?) projections	Papillae	Unknown
Ornamentation type on MW	None	Alveolate	None	Reticulate
Ornamentation type on IW	None	None	None	None
Species specific staining of OW in Melzer‘s	None to yellow to reddish-brown	None to yellow to reddish-brown	Yellow	Yellow to reddish-brown
Species specific staining of MW in Melzer‘s	None to yellow	None to yellow	None to yellow	None to yellow
Species specific staining of IW in Melzer‘s	None	None	None	None
Germination	From IW through pedicel, forming a specific lobed germination structure on IW surface	From IW through pedicel, forming a specific lobed germination structure on IW surface	From IW through pedicel, forming a specific lobed germination structure on IW surface	Unknown

Basionym: *Glomus fecundisporum* N.C. Schenck & G.S. Sm., Mycologia 74 (1): 81 (1982)
MycoBank MB 110703
Synonym: *Appendicispora fecundispora* (N.C. Schenck & G.S. Sm.) C. Walker, Vestberg & A. Schüssler, Mycol. Res. 111 (3): 254 (2007)
MycoBank MB 510502

Ambispora gerdemannii (S.L. Rose, B.A. Daniels & Trappe) C. Walker, Vestberg & A. Schüssler, Mycol. Res. 111 (2): 148 (2007), Fig. 9
MycoBank MB 510211
Basionym: *Glomus gerdemannii* S.L. Rose, B.A. Daniels & Trappe, Mycotaxon 8(1): 297 (1979)
MycoBank MB 314598
Synonym: *Appendicispora gerdemannii* (S.L. Rose, B.A. Daniels & Trappe) Spain, Oehl & Sieverd., Mycotaxon 97: 174 (2006)
MycoBank MB 510321

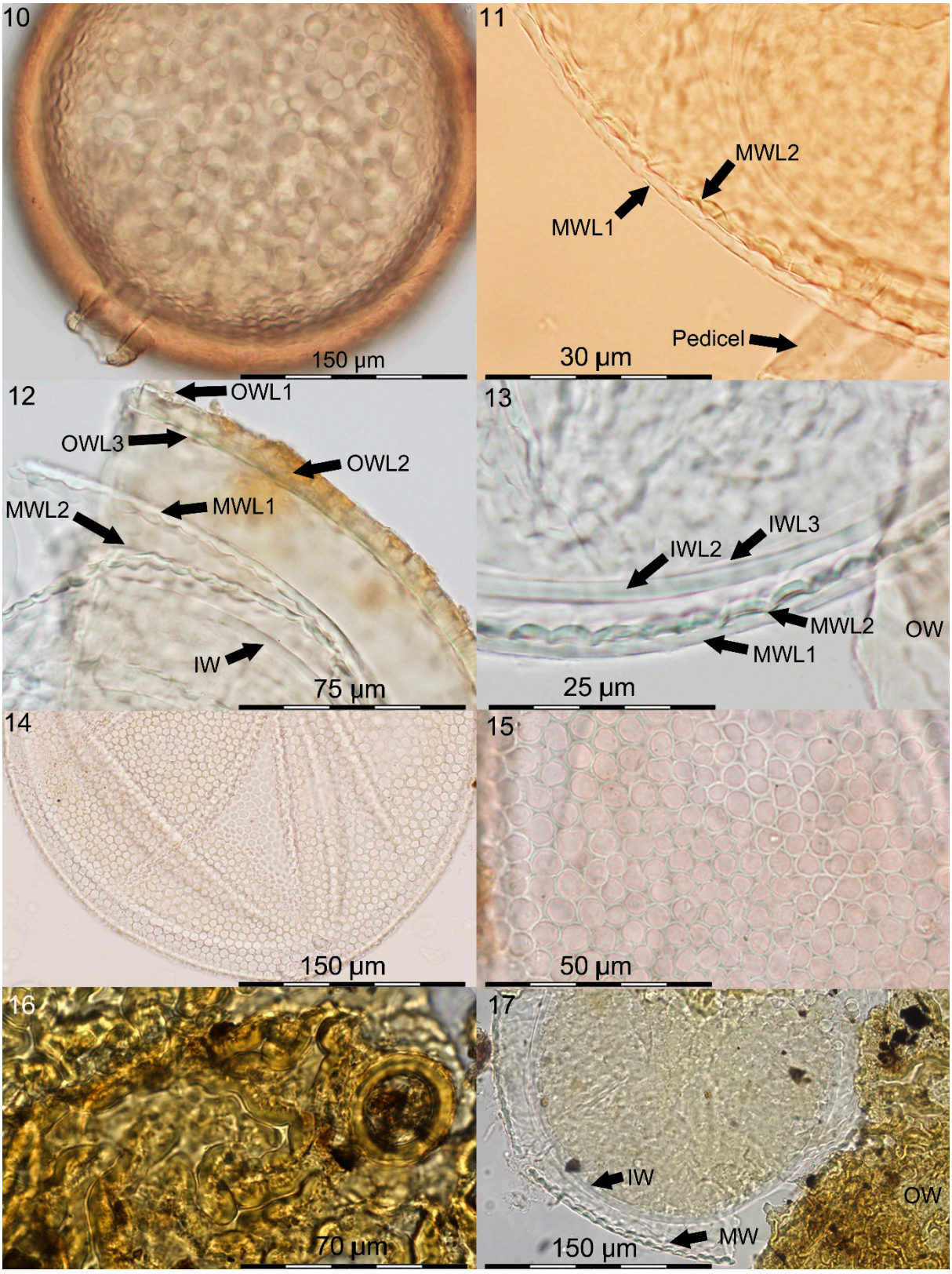
Ambispora nicolsonii (C. Walker, L.E. Reed & F.E. Sanders) Oehl, G.A. Silva, B.T. Goto & Sieverd., Mycotaxon 117: 431 (2011)
MycoBank MB 561663
Basionym: *Acaulospora nicolsonii* C. Walker, L.E. Reed & F.E. Sanders, Trans. Brit. Mycol. Soc. 83(2): 360 (1984)
MycoBank MB 105905

Appendiculispora Sieverd., Oehl, Palenz. & G.A. Silva, gen. nov., Figs. 10-27
MycoBank MB 862213
Description: Sporocarps unknown. Acaulosporoid spores formed singly upon a short appendix that arises laterally on the tapering hyphal neck of a sporiferous saccule, and glomoid spores formed terminally on hyphae, occurring singly or in loose clusters on extra- or intraradical hyphae. Acaulosporoid sporiferous saccules also formed on mycelial, extra- or rarely intraradical hyphae. Both acaulosporoid and glomoid spores may be found on the same mycelium and both

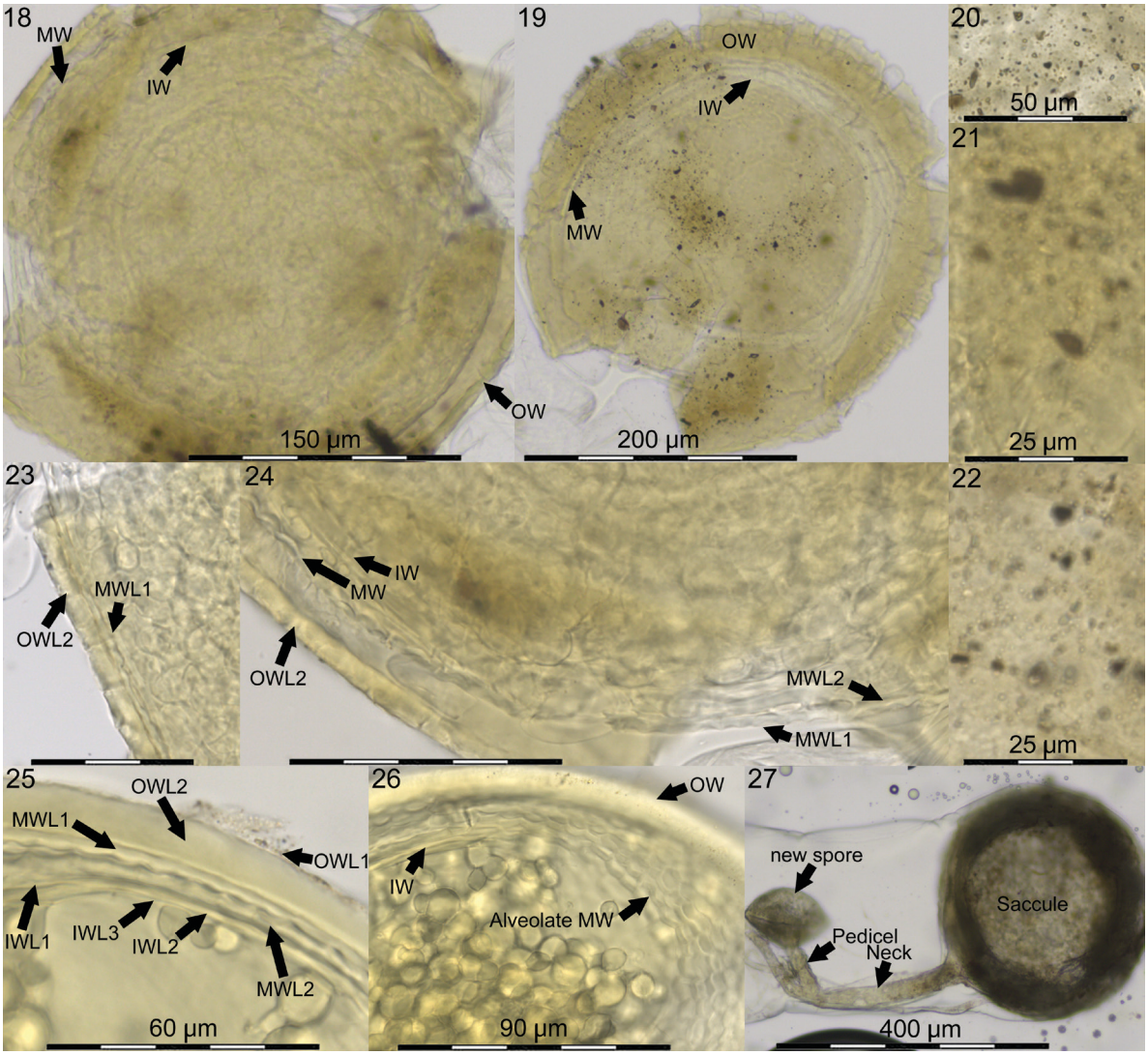


Figs. 4-9: Acaulo-ambisporoid spores and spore characteristics of *Ambispora* spp.: Figs. 4-8: *Ambispora fennica*. Fig. 4. Spore formed on a hyaline pedicel, branching from the neck (stalk) of a sporiferous saccule. Fig. 5. Crushed spore with outer wall (OW), middle wall (MW) & inner wall (IW), showing the pedicel, formed by hyaline MWL1. Fig. 6. Crushed spore with fissured OW, bi-layered MW (MWL1 & MWL2), and bi-to-triple layered IW. Fig. 7. Middle and inner walls (ML, IW); OW completely lost. Fig. 8. A fragment of the middle wall (MW-plate), split in an irregular way, but showing several sharp edges and corners. Fig. 9. *Ambispora gerdemannii*. Middle wall (MW) with rectangular cracks in a crushed spore; OW and IW are indicated.

can function as colonization propagules. Sporiferous saccule wall consists of one wall with 2-3 wall layers; prior to appendix and spore formation, a septum forms in the tapering hypha distal to the saccule where the hyphal appendix is formed. Spores are white-opaque to dull yellow-cream to orange-tan, or creamy to light brown. Three spore walls are present: outer, middle, and inner (OW, MW, IW). OW comprises three layers in young, fully developed spores. OWL1 is hyaline, often completely or partially decomposed during the early stages of spore formation. OWL2 is initially white, becoming yellow to brown, firm and difficult to break on young spores, but with age becoming less rigid, somewhat roughened, with an irregular crazed pattern of fine cracks. OWL3 usually is hard to observe as it is very thin and often tightly adherent to OWL2. When the appendix is broken off, an open pore is formed in the outer wall that is 20-50 µm wide. MW comprises two layers: the outer layer (MWL1) is continuous with the thick, hyaline layer of the appendix and the second layer of the connected hypha and sporiferous saccule; it is hyaline, with a convex, alveolate reticulum. These undulations are about 5-12 µm wide and 2-6 µm deep. MWL2 is hyaline, tightly adherent to MWL1, and thus showing a similar alveolate structure with concave hemispherical depressions on the outer surface that fit into the convex protuberances at the inner surface of MWL1. MWL2 may close the pore of the appendix. MWL1 and MWL2 separate when pressure ruptures the spore. MWL2 may stain yellow in Melzer's reagent. IW is hyaline, smooth, forms only after the full differentiation of the outer and middle walls. A very thin outer layer IWL1 and a thin inner layer IWL3 appear to adhere to the finely laminate middle layer (IWL2) in water mounted specimens, but neither IWL1 nor IWL3 are usually detected in specimens mounted in PVLG. The pedicel (appendix) arises laterally from the hyphal neck of a sporiferous saccule, is often



Figs. 10-17: Acaulo-appendiculisporoid spores and spore characteristics of two *Appendiculispora* spp.: Figs. 10-15: *Appendiculispora appendicula*. Fig. 10. Spore with pigmented OW, alveolate MW, and pedicel with two walls continuous with OW and MW. Fig. 11. Alveolate MW consisting of two layers. MWL1 not staining in Melzer's, while MWL2 stains yellowish. Fig. 12. Three-layered smooth outer wall (OWL1-3), two-layered alveolate middle wall (MWL1, 2), and smooth inner wall (IW). Fig. 13. Alveolate MWL1 & MWL2 and bi- to triple-layered IW. Figs. 14-15. Alveolate MW in planar view. Figs. 16-17: *Appendiculispora jimgerdemannii*. Fig. 16. Labyrinthiforme/cerebriforme surface ornamentation of OW. A round collar of OW visible on the right; pedicel has been detached from the collar. Fig. 17. Ornamented OW, alveolate MW, and smooth IW.



Figs. 18-27: *Appendiculispora callosa*. Figs. 18-19. Crushed spores; Outer wall (OW) subhyaline to light creamy. OWL2 finely laminate, first rigid, but not permanent, as aged (Fig. 19) showing multiple fissures, degraded. Figs. 20-22. Minute (spiny) warts on the OW spore surface in planar view (about 0.5 µm diam). Figs. 23-26. OW with evanescent OWL1 & pigmented OWL2, alveolate MW with MWL1 & MWL2, and triple-layered, smooth IW with IWL1-3. 27. Sporiferous saccule connected to a young, small, developing spore growing on the sporiferous saccule neck and the branching appendix (pedicel) on the neck.

persistent, and resembles a subtending hypha, 25-120 µm long, 20-50 µm wide, cylindric or funnel-shaped, tapering to 10-25 µm at the distal end from the spore. The appendix pore is closed by a septum arising from MWL1 at a short distance (0-10 µm) from the spore base. Acaulosporoid spores germinate with a single or branched germ tube, 6-12 µm diam, that emerges from the inner wall and generally exits through the pore of the appendix. A distinctive germination structure was also observed to form between middle and inner walls in a spore mounted in water from which a germ tube emerged. Single or loose clusters of swollen hyphal tips often form on the germ hyphae at a short distance (100-200 µm) from the base of the acaulosporoid spore. Glomoid spores are hyaline to subhyaline, with one spore wall (SW) consisting of a mucilaginous outer SWL1, regularly about 1.5-2.5 µm thick, frequently with adhering debris on the outer surface, and an about 2-15 µm thick, laminate inner wall layer (SWL2). Subtending hyphae cylindric to slightly funnel shaped, 7-35 µm diam at the spore base tapering to 5-15 µm towards the mycelia hyphae. The pore usually is open, 5-25 µm wide, but sometimes a thin septum deriving from the inner hyphal wall layer is seen at a short distance from the spore base. Glomoid spores germinate through the subtending hypha. Forming vesicular-arbuscular mycorrhiza, with arbuscules, vesicles, and intraradical hyphae staining pale blue with trypan blue.

Etymology: Appendiculi- (appendix) and -spora (spore), referring to the type of spore formation on a pedicel (appendix)

Type species: *Appendiculispora appendicula* (Spain, Sieverd. & N.C. Schenck) Sieverd., G.A. Silva & Oehl, comb. nov., Figs. 10-15

MycoBank MB 862214

Basionym: *Acaulospora appendicula* Spain, Sieverd. & N.C. Schenck, Mycologia 76 (4): 686 (1984)

MycoBank MB 105884

Synonym: *Ambispora appendicula* (Spain, Sieverd. & N.C. Schenck) C. Walker, Mycol. Res. 112 (3): 298 (2008)

MycoBank MB 511420

Synonym: *Appendicispora appendicula* (Spain, Sieverd. & N.C. Schenck) Spain, Oehl & Sieverd., Mycotaxon 97: 170 (2006)

MycoBank MB 510320

Other species: *Appendiculispora callosa*, *Appendiculispora jimgerdemannii*, *Appendiculispora leptoticha*

Appendiculispora callosa (Sieverd.) Sieverd., G.A. Silva & Oehl, comb. nov., Figs. 18-27
MycoBank MB 862215
Basionym: *Glomus callosum* Sieverd., Angew. Bot. 62 (5-6): 374 (1988)
MycoBank MB 134246
Synonym: *Ambispora callosa* (Sieverd.) C. Walker, Vestberg & A. Schüssler, Mycol. Res. 111 (2): 148 (2007)
MycoBank MB 510213
Synonym: *Appendicispora callosa* (Sieverd.) C. Walker, Vestberg & A. Schüssler, Mycol. Res. 111 (3): 254 (2007)
MycoBank MB 510504
Emended description: Sporocarps unknown; spores formed singly upon a short appendix that arises laterally on the tapering hyphal neck of a sporiferous saccule (acaulo-appendiculisporoid), and glomoid spores formed terminally on hyphae, occurring singly or in loose clusters on extra- or intraradical hyphae (glomoid-appendiculisporoid). Sporiferous saccules are 220-320 µm diam, hyaline to subhyaline, with two saccule wall layers, an evanescent hyaline to subhyaline outer layer (1.2-2.0 µm thick), and a permanent hyaline, layer, about 2.5-4.5 µm thick. Acaulo-appendiculisporoid spores 200-280 µm diam, hyaline when young, becoming subhyaline to light creamy with age. OW 4-8 µm thick; OWL1 hyaline, 0.8-1.3 µm thick, evanescent, crowded with minute (spiny-)warts (about 0.5 high and 0.5-0.7 µm broad) on the outer spore surface; OWL2 hyaline to subhyaline, becoming light creamy to creamy with age, 3.5-7.0 µm thick; OWL3 thin, concolorous with and adherent to OWL2. MW hyaline, 4.0-7.0 µm thick in total, comprising two layers: the outer layer MWL1, 2.0-4.0 µm thick, continuous with the thick, hyaline layer of the appendix, the second layer of the connected hypha and sporiferous saccule, and the convex, alveolate reticulum. These undulations are about 5.0-10.0 µm wide and 1.4-2.6 µm deep. MWL2 is hyaline, 2.0-3.5 µm thick, tightly adherent to MWL1 and thus showing a similar alveolate structure with concave hemispherical depressions on the outer surface that fit into the convex protuberances on the inner surface of MWL1. MWL2 may close the pore of the appendix. MWL1 and MWL2 separate when pressure ruptures the spore. IW 3-6 µm thick in total; a very thin outer layer (IWL1) and a thin inner layer (IWL3) appear to adhere to the finely laminate middle layer (IWL2) in water mounted specimens, but neither IWL1 nor IWL3 are usually detected in specimens mounted in PVLG. The pedicel (appendix) arises laterally from the hyphal neck of a sporiferous saccule, is often persistent, and resembles a subtending hypha, 30-100 µm long, 20-40 µm wide, cylindric or funnel-shaped tapering to 10-25 µm at the distal end from the spore. Glomoid spores are hyaline to subhyaline, 220-280 µm, with one spore wall (SW), crowded with minute (spiny-)warts (about 0.5 high and 0.5-0.7 µm broad) on the outer spore surface. SW consisting of a mucilaginous SWL1, about 0.5-1.5 µm thick, frequently with adhering debris on the outer surface and SWL2 that is hyaline, about 2.5-11 µm thick, laminate. Subtending hypha cylindric to slightly funnel shaped, 22-35 diam at the spore base, tapering to 10-12 µm within 10-120 µm distance. The pore usually is open, 8-21 µm wide, but sometimes a thin septum deriving from the inner hyphal wall layer is seen, which can be found even at a distance > 100 µm from the spore base in the transition to the mycelia hyphae.

Appendiculispora jimgerdemannii (Spain, Oehl & Sieverd.) Sieverd., G.A. Silva & Oehl, comb. nov., Figs. 16-17
MycoBank MB 862216
Basionym: *Appendicispora jimgerdemannii* Spain, Oehl & Sieverd., Mycotaxon 97: 176 (2006)
MycoBank MB 510322
Synonym: *Ambispora jimgerdemannii* (Spain, Oehl & Sieverd.) C. Walker, Mycol. Res. 112 (3): 298 (2008)
MycoBank MB 511417

Appendiculispora leptoticha (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl, comb. nov.
MycoBank MB 862217
Basionym: *Glomus leptotichum* N.C. Schenck & G.S. Sm., Mycologia 74 (1): 82 (1982)
MycoBank MB 110705
Synonym: *Ambispora leptoticha* (N.C. Schenck & G.S. Sm.) C. Walker, Vestberg & A. Schüssler, Mycol. Res. 111 (2): 148 (2007)
MycoBank MB 510212
Synonym: *Appendicispora leptoticha* (N.C. Schenck & G.S. Sm.) C. Walker, Vestberg & A. Schüssler, Mycol. Res. 111(3): 255 (2007)
MycoBank MB 510501

Ephemeriparies G.A. Silva, Palenz., Sieverd. & Oehl, gen. nov., Figs. 28-30
MycoBank MB 862218
Description: Sporocarp formation unknown. Species bi-morphic, producing acaulosporoid and glomoid spores, with a spore wall structure typical for Ambisporaceae. Acaulosporoid spores form singly in soils on a short pedicel that branches laterally from the neck of a sporiferous saccule. Glomoid spores are formed singly or in clusters. Sporiferous saccules are hyaline to subhyaline, globose to subglobose, about 100-200 µm diam. The saccule wall is bi-layered, consisting of a subhyaline to light yellow or light creamy, rapidly degrading, evanescent outer layer and a hyaline to subhyaline, semi-persistent inner layer. The hyphal neck of the saccule is ca. 15-30 µm wide at the spore base and tapers to 5-10 µm towards the mycelia hyphae. The sporiferous saccules often detach from mature spores while the pedicel often persists on the spore. Pedicels of acaulosporoid spores branch from the saccule neck about 60-160 µm from the saccule terminus. The acaulosporoid spores are ca. 90-150 µm diam, hyaline to white to white-yellow in water and generally become ochre yellow with age, and when mounted in PVLG. They are white when the outer wall has sloughed off from the spore. The spores have three walls: a three-layered outer wall (OW), a bi-layered middle wall (MW), and a three-layered inner wall (IW). OW is continuous with the undifferentiated outer wall layer of the pedicel and SWL1 of the saccule; it is evanescent, short-lived. Middle wall is bi-layered, hyaline, semi-permanent to regularly evanescent. MWL1 is continuous with the inner layer of the pedicel and with SWL2 of the saccule wall. MWL2 tightly adhered to MWL1, and forms the pore closure of the pedicel at the spore base. Under slight pressure on the cover slide, both layers easily disaggregate into several to many small, irregular pieces, indicating the fragile nature of the middle wall. Inner wall is hyaline, about 2-5 µm thick, and has three wall layers that generally are easily detectable. The IW forms *de novo*, presumably after the spore pore has been closed by the inner layer of the middle wall (MWL2). Second layer IWL2 is finely laminate. IW functions as germinal wall as the germination structure, and subsequently germ tubes emerge from this wall through an initial germ pore (gp). Pedicel is about 10-25 µm long and 10-25 µm wide at the spore base, tapering to 7-15 µm from the spore base. Sometimes two wall layers on the pedicel were observed that are continuous with OW and MWL1 of the spore wall and the saccule wall, but often the OW sloughs. In other cases, the pedicel disappears from the spore base leaving a collar formed by the OW. Sometimes one to two septa arise in the pedicel from MWL1. Germination: the main germ tube generally grows straight through the pedicel and the collar of the OW if they remain present on the germinating spores. A lobed germination structure emerges in germinating spores, which consists of a few (3-6), 5-15 µm long lobes and 1-5 germ tubes arising between the lobes.

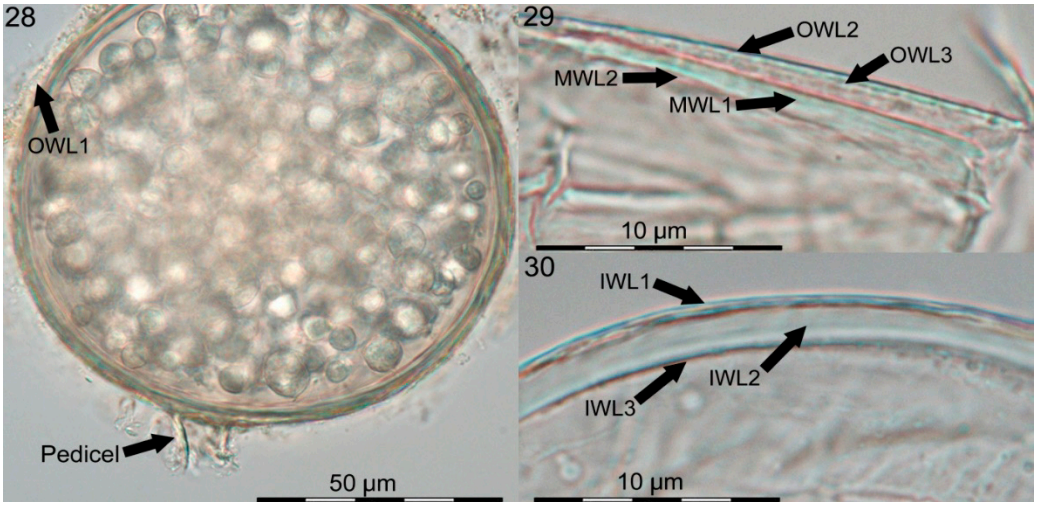
Glomoid spores are hyaline to subhyaline and have two wall layers that are continuous with the two wall layers of the mycelia hyphae. The outer layer (SWL1) is evanescent, hyaline to subhyaline in water. It may become light yellow to light creamy with age and when mounted in PVLG. The inner layer (SWL2) is persistent, hyaline to subhyaline, and finely laminate. The subtending hypha is cylindrical to slightly funnel-shaped; spore pore sometimes is closed by a septum, but generally the spore pore appears to be open. Germinating glomoid spores were not found. Forming vesicular arbuscular mycorrhiza with mycorrhizal structures that stain pale blue in trypan blue.
Etymology: Ephemerus- (evanescent, short-lived), -paries (= wall), as two of the three spore walls are considered to be short-lived, rapidly degrading, not permanent.
Type species: *Ephemeriparies granatensis* (Palenz., N. Ferrol & Oehl) Oehl, Palenz., Sieverd. & G.A. Silva, comb. nov.
MycoBank MB 862219
Basionym: *Ambispora granatensis* Palenz., N. Ferrol & Oehl, Mycologia 103 (2): 334 (2011)
MycoBank MB 513528

Pelotaspora Oehl, G.A. Silva, Palenz. & Sieverd., gen. nov., Figs. 31-43
MycoBank MB 862220
Description: Sporocarp formation unknown; spores resemble acaulo-ambisporoid spores, but apparently are formed singly on short hyphae. The spores are globose, about 100-250 µm diam, hyaline or white to yellow or creamy brown to brown. The spores have three walls: a bi- to triple-layered outer wall (OW), a bi-layered middle wall (MW), and a three-layered inner wall (IW). OW is evanescent to semi-persistent. The structural second layer (OWL2) is 1-6 µm thick and generally stains reddish purple in Melzer's reagent. The pore collar at the spore base is 7-40 µm wide. MW is bi-layered, hyaline, and 2.5-5.0 µm thick. It forms a conspicuous reticulum with 5-7 sided, large pits (15-30 × 12-25 µm diam) that are framed by 3-8 µm broad ridges giving the spore a football-like appearance. MWL1 may stain yellow in Melzer's reagent, at least in the first hours after placing. IW is hyaline and 3-5 μm thick. The IW may function as germinal wall as related to many Glomeromycota species with *de novo* forming IW during spore development.
Etymology: Pelota- (ball) and -spora (spore), referring to the football-like structure of the spores
Type species: *Pelotaspora reticulata* (Oehl & Sieverd.) Oehl, Palenz., G.A. Silva & Sieverd., comb. nov., Figs. 31-34
MycoBank MB 862221
Basionym: *Ambispora reticulata* Oehl & Sieverd., J. Appl. Bot. Food Qual. 85 (2): 130 (2012)
MycoBank MB 800269
Other species: *Pelotaspora austrolatina*

Pelotaspora austrolatina Oehl, C. Castillo, Palenz., I.C. Sánchez & G.A. Silva, sp. nov., Figs. 35-43
MycoBank MB 862222
Type: Holotype 56-5601 (Z+ZT: ZT Myc 15115) isolated from the rhizosphere of *Triticum aestivum* (community Gorbea, Araucanía region, Chile) at about 590 m a.s.l. (39º08' South, 72º38' West). Isotype specimens from the same field samples (56-5602 to 56-5610), deposited at Z+ZT (ZT Myc 15116); isotypes (56-5611 to 56-5613) deposited at HURM (HURM 83558-83560); paratypes from the rhizosphere of *Triticum aestivum* (community Curacautín, Araucanía region, Chile) deposited at Z+ZT (ZT Myc 15117). Collection date: 15.9.2011. Collectors: Claudia Castillo, Victor San Martín, Fritz Oehl.
Etymology austro- (southern), and -latina (latino), referring to the first findings in the South of Brazil and Chile.
Description: Sporocarp formation unknown; spores resemble acaulo-ambisporoid spores but are formed singly on short hyphae. The spores are globose (112-187 µm) to oval to ovoid to rarely irregular, 125-195(-245) × (95-)102-155(-180) µm, hyaline to white to yellow-white, sometimes becoming light ochre-yellow with age or with time when mounted in PVLG. They have three walls: a bi- to triple-layered outer wall (OW), a bi-layered middle wall (MW) and a three-layered inner wall (IW).

Outer wall generally consists of two to three layers. The outer layer (OWL1) is hyaline to white to white yellow, 0.8-2.1 µm thick, and evanescent to semi-persistent. The structural second layer (OWL2) is 1.0-2.5 µm thick. It generally stains reddish purple in Melzer's reagent. The third layer (OWL3) is difficult to detect as it is thin (< 0.6 µm) and tightly adherent to OWL2, and is generally hidden by the outer layer of the middle wall (MWL1).

Middle wall is bi-layered, hyaline and 2.8-4.4 µm thick. It forms a conspicuous reticulum with 5-6 sided, large pits (15-27 × 12-24 µm diam) that are framed by (2.5-)4.5-7.9 µm broad ridges giving the



Figs. 28-30: *Ephemeriparies granatensis*: Acaulo-ephemeroid morph. Fig. 28. Spore with pedicel attached. Fig. 29. Short-lived, thin, evanescent OW with OWL1 & OWL2 and evanescent to semi-permanent MW with MWL1 & MWL2. Fig. 30. Permanent IW with IWL1-3.

spore a football-like appearance. MWL1 is 1.4-2.5 µm thick. MWL2 is 1.0-2.5 µm thick, tightly adherent to MWL1, and regularly stains yellow in Melzer's reagent, at least in the first hours after placing.

Inner wall is hyaline, 3.0-4.5 µm thick, and has three wall layers. The outer layer (IWL1) is 0.5-1.2 µm thick, tightly adherent to the second layer (IWL2). IWL2 is finely laminate, and 2.0-2.4 µm thick. Innermost layer (IWL3) is 0.5-0.9 µm thick, and also tightly adherent to IWL2. IWL3 sometimes separates slightly from IWL2 and often shows several thin folds, especially when pressure is applied to the cover slip.

Pedicel-like subtending hyphae of these pelotasporoid spores at the spore base are 7.5-25(-38) µm wide. Formation of mycorrhizal structures is so far unknown.

Distribution. So far, *Pe. austrolatina* was isolated from two field sites in the Araucanía region of Southern Chile subjected to winter wheat production, and from a viticultural field site in Southern Brazil. It was also found in a grassland and a deciduous forest in the 'de Los Rios' region close to Valdivia, Southern Chile. It co-occurred with other AM fungi, like *Acaulospora punctata*, *Entrophospora claroidea*, *En. infrequens*, *Funneliformis mosseae* and *Septoglomus constrictum* at the San Pablo de Tregua Experimental Station of Valdivia (Castillo et al., 2006; Oehl et al., 2011c), with AM fungi like *Ac. sieverdingii* (Oehl et al., 2011d), *Paraglomus occultum* and *Rhizoglomus invermaium* in Gorbea and Curacautin, and, among others, with several *Acaulospora* species, *S. constrictum*, *Simiglomus hoi*, *Am. gerdemannii*, *Scutellospora calospora* and *Fuscutata heterogama* in Caxias do Sul (RS, Brazil).

Morphological identification key to Ambisporaceae species:
Here, the first morphological identification key is presented for the species attributed to Ambisporaceae. Spore dimensions are generally given in diam, spore wall and wall layer dimensions as thickness.
1 Spores with three walls, generally formed on sporiferous saccules, generally on a lateral branch of the saccule neck (acaulo-ambisporoid),

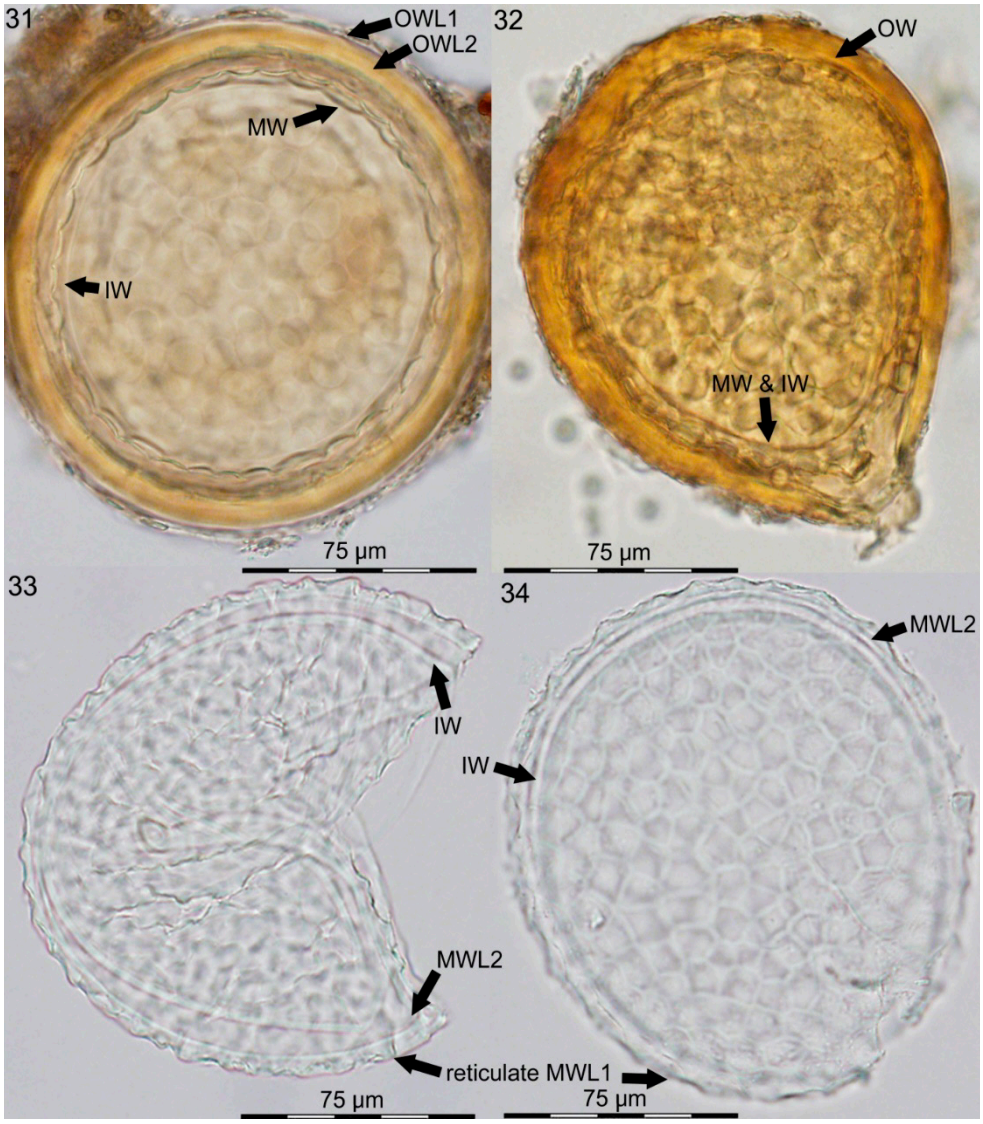
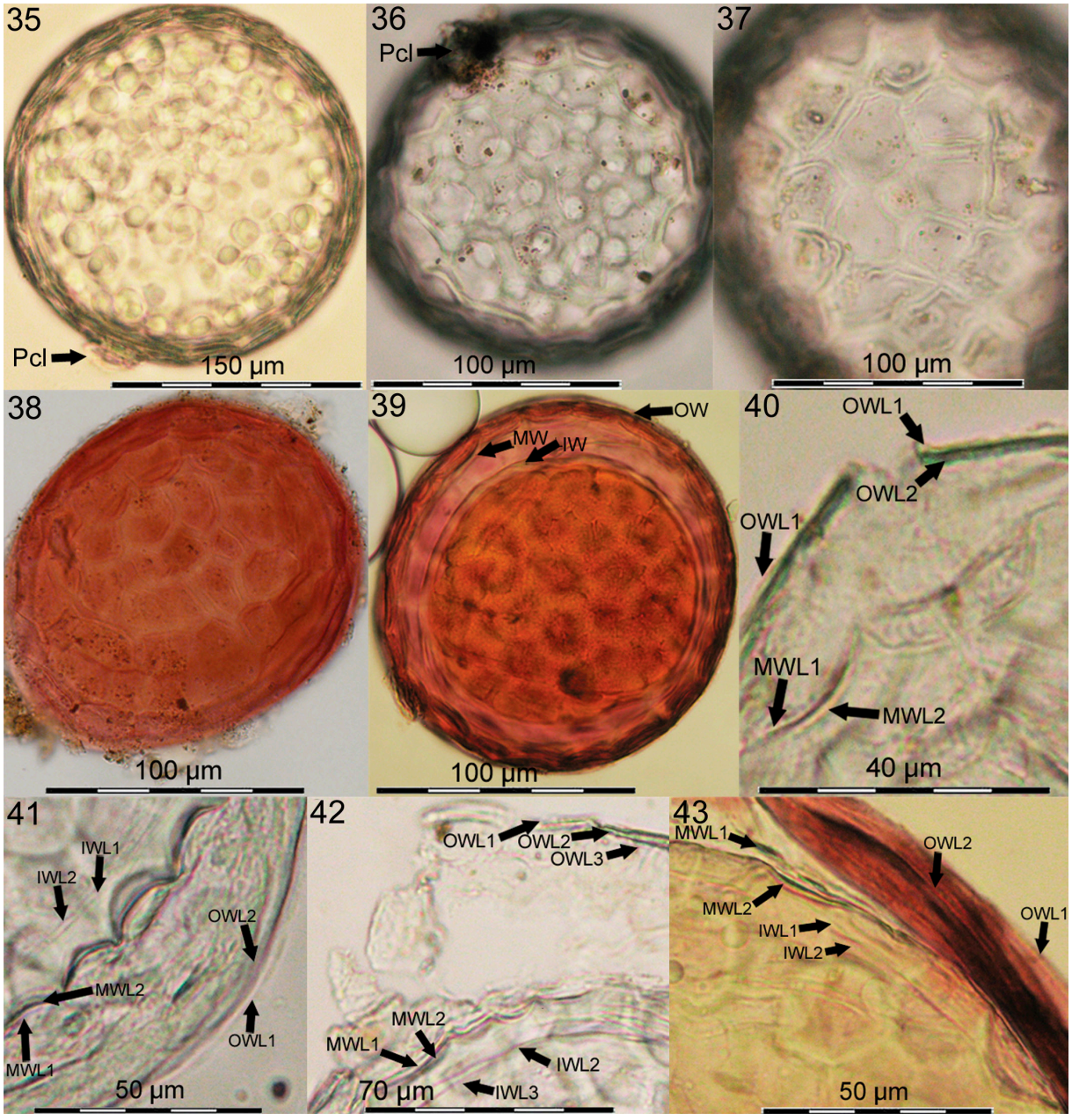


Fig. 31-34: *Pelotaspora reticulata*. Figs. 31-32. Spores with pigmented, smooth OW, with subhyaline OWL1 & brown OWL2, reticulate MW, smooth IW, and a pedicel-like structure at the spore base (Fig. 32). Figs. 33-34. Reticulate MWL1, smooth MWL2, and smooth IW. The reticulum consists of (4-)5-6(-8) sided, large pits (15-30 × 12-25 µm) that are framed by 3-8 µm broad ridges giving the spore the appearance of a football.



Figs. 35-43: *Pelotaspora australolatina*. Figs. 35-37. Spores with pedicel-like (Pcl) subtending hyphae (SH) and reticulate appearance deriving from the reticulum on the MW surface. OW surface is smooth. Figs. 38-39. Spores in Melzer's reagent: OW stained purple; IW yellowish. Fig. 40-42. Crushed spores in PVLG, with smooth, evanescent triple layered OW, reticulate bi-layered MW, and smooth bi- to triple-layered IW. Fig. 43. Crushed spore in PVLG+Melzer's: OWL2 stained purple and MWL2 yellowish, respectively.

rarely on subtending hyphae (triple-walled-glomoid, resembling acaulo-ambisporoid morph) 2
1 Mono-walled spores formed on subtending hyphae (glomoid-ambisporoid morph) 12

2 Spores not formed on sporiferous saccules (triple-walled-glomoid with football like, reticulate ornamentation on the outer MW surface. . . . . (*Pelotaspora*) 3
2 Spores formed on sporiferous saccules, generally on a lateral branch of the saccule neck. . . . . (acaulo-ambisporoid sensu lato) 4

3 Spores hyaline to white to rarely pale yellow, 125-195(-245) × (95-)102-155(-180) µm; the surface of MW with a reticulum consisting of 5-6 sided pits, 15-27 × 12-24 µm framed by (2.5-)4.5-7.9 µm broad ridges. . . . . *Pelotaspora australolatina*
3 Spores yellow-brown to brown, 87-131 × 125-150 µm; the surface of MW with a reticulum consisting of irregular triagonal to octagonal (usually tetra- to hexagonal) pits that are surrounded by ridges. . . . . *Pelotaspora reticulata*

4 Spores with two hyaline, smooth inner walls (MW & IW) below the white to creamy or brown outer wall (OW) 5
4 Spores with alveolate MW and smooth IW below the white to creamy, brown or orange-brown OW. . . . . (*Appendiculispora*) 6

5 OW and MW (semi-)permanent to evanescent, MW often dissolving in liquid mountants, upon pressure on the cover-slide; spores 90-

150 µm, hyaline to white to pale yellow. Outer surface of OW rough and crowded with papillae, which might become difficult to detect within a few hours in lactic acid-based mountings, but are clearly visible in water. The structural OWL2 is only 0.8-1.5 µm thick. . . . . *Ephemeriparies granatensis* (acaulo-ambisporoid morph)
5 MW permanent, fracturing into regular to irregular plate-like pieces under pressure on the cover slide . . . . . (*Ambispora*) 9

6 OW without surface ornamentation 7
6 OW with surface ornamentation 8

7 Middle wall with alveolate ornamentation. Spores (170-)250 (-390) µm, white-opaque when young, becoming dull yellow-cream to yellow to yellowish-brown or brown, rarely orange-tan; main, structural layer (OWL2) firm and difficult to break on young spores, turns orange-red in Melzer's reagent, but with age becoming less rigid and staining less red with Melzer's reagent. . . . . *Appendiculispora appendicula* (acaulo-appendiculisporoid morph)
7 Spores 160-240 µm, cream when young, becoming orange-brown to dark orange-brown, OWL2 staining dark orange-brown to red-brown in Melzer's reagent. . . . . *Appendiculispora leptoticha* (acaulo-appendiculisporoid morph)

8 OW with minute (spiny-)warts on the outer surface (OWL1); spores 200-280 µm, hyaline when young, becoming subhyaline to creamy with age, OW 4-8 µm, MW 4-7.5 µm, IW 3-6 µm thick. . . . . *Appendiculispora callosa* (acaulo-appendiculisporoid morph)
8 OW with labyrinthiform ornamentation on the outer surface of OWL2; spores 200-250 µm, brown at maturity; the main layer (OWL2) is brown in color, becoming brittle with age. It is approximately (8-)10-14 µm thick, showing cerebriform folds over the laminate wall layer that is 1-1.5 µm thick. The cerebriform ridges are up to (6-)10-12 µm high, 4-6 µm broad, and 1-3 µm apart from each other; the outer spore wall does not react with Melzer's reagent. . . . . *Appendiculispora jimgerdemannii* (acaulo-appendiculisporoid morph)

9 Spores < 100 µm, formed on a pedicel branching from the neck of a sporiferous saccule. They are hyaline to pale yellow, globose to subglobose, 62-95 µm, to oval, 59-88 × 69-100(-118) µm, and with three spore walls. Crowded irregular pustules, 2.4-7.0(-10.0) µm high and 4.2-9.8(-15.0) µm wide, are formed on the surface of the outer wall. . . . *Ambispora brasiliensis* (acaulo-appendiculisporoid morph)
9 Spores > 100 µm 10

10 OW not staining purple in Melzer's reagent 11
10 OW staining rust-brown to red or purple in Melzer's reagent; spores hyaline to pale ochraceous, globose to pytiform, 124-201 × 134-201 µm. . . . . *Ambispora fennica* (acaulo-ambisporoid morph)

11 MW regularly cracking into rectangular pieces under pressure on the cover slide; spores 160-310 µm, hyaline to pale yellow-brown. . . . . *Ambispora gerdemannii* (acaulo-ambisporoid morph)
11 MW cracking into irregular pieces under pressure on the cover slide; spores 100-220 µm, hyaline to pale yellow-brown. . . . . *Ambispora nicolsonii* (acaulo-ambisporoid morph)

12 Spores without ornamentation on outer surface 13
12 Spores with surface ornamentation 17

13 Mature spores hyaline, subhyaline to pale yellow 14
13 Mature spores yellow-brown to dark brown, 100-210 × 80-150 µm. . . . *Ambispora fecundispora* (glomoid-ambisporoid morph)

14 Spores regularly < 70 µm 15
14 Spores regularly > 70 µm 16

15 Spores 25-30 µm, hyaline to subhyaline, bi-layered. . . . . *Ambispora brasiliensis* (glomoid-ambisporoid morph)
15 Spores (25)40-70 µm, hyaline to pale yellow, bi-layered. . . . . *Ephemeriparies granatensis* (glomoid-ambisporoid morph)

16 Spores 40-130 µm, hyaline, with a mucilaginous SWL1, about 0.8-1.0 µm thick, frequently with adhering debris on the outer surface, and a permanent, laminate SWL2, 1.5-4.0 µm thick. . . . . *Ambispora fennica* or *Ambispora gerdemannii* (glomoid-ambisporoid morph)
16 Spores 120-240(-280) µm, hyaline to subhyaline, with a mucilaginous SWL1, 1.5-2.5 µm thick, frequently with adhering debris on the outer surface, and a permanent, laminate SWL2, 2-8 (-12) µm thick. . . . . *Appendiculispora appendicula* (glomoid-ambisporoid morph)

17 Spores subhyaline, 220-280 µm, spore surface crowded with minute warts. . . . . *Appendiculispora callosa* (glomoid-ambisporoid morph)
17 Spores hyaline, when young, light yellow when mature, 150-265 × 100-235 µm; spore surface with an alveolate reticulum of shallow ridges, 0.5-1 wide. . . . . *Appendiculispora leptoticha* (glomoid-ambisporoid morph)

Discussion

Our new phylogenetic analyses showed three different clades in Ambisporaceae (A, B, C; *Ambispora*, *Appendiculispora*, and *Ephemeriparies*, respectively). It was already demonstrated for Glomerales that clades that differ by ≥ 10% MI, according to the partial nrDNA gene (Corazon-Guivin et al., 2019; Silva et al., 2023; Silva et al., 2025) can be considered different genera. *Ambispora* has 89% MI to *Appendiculispora*, and the phylogenetic analyses placed *Ambispora* (sensu stricto) in a single, strongly supported clade, well separated from the other two clades. *Ephemeriparies granatensis* has been sequenced only from the SSU and ITS loci. However, BLASTn showed that the sequences do not correspond to any Ambisporaceae sequence. *Ambispora* clustered together with *Appendiculispora*, but there is no support to place these genera in the same group in the SSU tree (Fig. 3). The SSU-ITS-LSU and ITS trees (Figs. 1 and 2) have no support from ML-FBP, and a low support from ML-TBE in the ITS tree (82%) to place these genera together. In the ITS and SSU trees generated by Walker et al. (2007), Ambisporaceae clearly divided into two clades i) with *Am. fennica* and ii) with *Am. gerdemannii*, *Ap. leptoticha*, and *Ap. callosa*. At that time, *Ep. granatensis* (Palenzuela et al., 2011) was not published and there were no sequences from *Ap. appendicula* so far available.
In the tree generated by Esmaeilzadeh-Salestani et al. (2025), *Ambispora* species (*sensu lato*) were also accommodated in two clades (*Ap. appendicula* and *Ap. leptoticha* versus *Am. fennica* and *Am. gerdemannii*). In our ITS tree (Fig. 2), distinct isolates of *Am. gerdemannii* were placed in different clades (together with *Ap. appendicula/leptoticha/callosa* or *Am. fennica*, suggesting that some sequences were wrongly assigned to *Am. gerdemannii* in the NCBI. In the SSU tree (Fig. 3), sequences from the isolate MAFF520057 were attributed to *Ap. callosa* and also to *Ap. leptoticha*. In this case, we believe that the culture contained both species, which were provided with the same isolate number, MAFF520057. The isolate MAFF520058 was attributed to *Ap. leptoticha*, but according to our phylogenetic analyses, this isolate possibly represents *Ap. callosa*. An isolate (n8_9) attributed to *Am. gerdemannii* was sequenced for the SSU+ITS+LSU fragment, while the isolate AU215 has sequences from ITS and LSU. Interestingly, our other analyses showed (data not shown) that LSU sequences obtained from *Am. gerdemannii* derived from the reference culture INVAM MT106 were placed in clade A,

whereas sequences from *Am. gerdemannii* isolate AU215 clustered in clade B. This was probably due to misassignment of the sequences to *Am. gerdemannii* AU215.

The clear results obtained for the three major clades of Ambisporaceae in the phylogenetic analyses requested for advanced morphological separations. Morphologically, four major groups were recognized in Ambisporaceae: i) bi-morphic species with an acaulo-ambisporoid and an glomoid-ambisporoid morph, ii) species, for which so far only an acaulo-ambisporoid morph has been detected, iii) species for which hitherto only a glomoid-ambisporoid morph has been known, and iv) species with triple-walled glomoid-like spores formed on hyphae, whose spores resemble the acaulo-ambisporoid morph, although their formation on sporiferous saccules has not yet been observed so far. All these species were considered before within one genus, *Ambispora*.

These major morphological similarities and differences concern the composition and phenotypic properties of the subcellular components of spores of the acaulo-ambisporoid morph, mainly those of the middle wall: i) *Am. fennica*, *Am. gerdemannii*, *Am. nicolsonii*, and *Am. brasiliensis* have a smooth, permanent middle wall and represent the revised genus *Ambispora*, ii) the middle wall of *Ap. appendicula*, *Ap. callosa*, *Ap. leptoticha*, *and Ap. jimgerdemannii* is permanent, but conspicuously alveolate (new genus *Appendiculispora*), and iii), the middle wall of *Ep. granatensis* is smooth, but short-lived, because it quickly decomposes with time (new genus *Ephemeriparies*). On the other hand, the glomoid-ambisporoid morph alone cannot yet be used to identify a genus, because it has been difficult (so far) to find any feature in it that could reliably distinguish not only genera but also species.

For two additional species, *Pe. reticulata* and *Pe. austrolatina*, the molecular analyses failed so far. Their spores are triple-walled and resemble triple-walled acaulo-ambisporoid spores of *Ambispora*, *Appendiculispora* and *Ephemeriparies*, but they apparently are not formed on sporiferous saccules. The two *Pelotaspora* species further have a diagnostic reticulate middle wall with 4-8 sided, but generally 5-6 sided, large pits that are formed by broad ridges giving the spore a football-like morphological appearance. These two species should no longer be included neither in the newly organized *Ambispora*, nor within one of the two new genera *Appendiculispora* and *Ephemeriparies*, because of their clearly different diagnostic characters. Thus, we here decided to attribute them to the new genus *Pelotaspora*. Further research is needed to understand the phylogeny, spore formation and germination, as well as the features of intra- and extraradical structures of this group of AMF.

Acknowledgments

Daniele Magna Azevedo de Assis and Thays Gabrielle Lins de Oliveira thanks the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for providing fellowship. Gladstone A. Silva has a fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Proc. 312606/2022-2, approval date: 16 February 2023). Samar Velho da Silveira acknowledges the National Council for Scientific and Technological Development (CNPq, Brazil) for financial support through a research fellowship grant (no. 200382/04-7).

Funding information

This study was financially supported by UFPE, FACEPE and CNPq.

Conflict of interest

No potential conflict of interest was reported by the authors.

Supplemental data

GenBank accession numbers for the sequences used in this study are available as csv file at the landing page the DOI indicates.

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GenBank accession numbers for the sequences used in the present study

Species name in NCBI	ID	nrDNA region	Country	GenBank accession numbers (nrDNA)		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547535		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547536		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547537		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547538		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547539		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547540		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547541		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547542		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547543		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547544		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547545		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FN547546		
Ambispora fennica	Att200-23 - ex-type	SSU+ITS+LSU	Finland	FR750157		
Ambispora fennica	Att200-21	SSU	Finland	AM268192		
Ambispora fennica	Att200-21	SSU	Finland	AM268193		
Ambispora fennica	Att200-21	SSU	Finland	AM268194		
Ambispora fennica	Att200-23 - ex-type	SSU	Finland	AM268196		
Ambispora gerdemannii	n8_9	SSU+ITS+LSU	Not available	JF439210		
Ambispora gerdemannii	AU215	ITS	Australia	AJ012111		
Ambispora gerdemannii	AU215	ITS	Australia	AM743187		
Appendiculispora appendicula	Att1235-1	SSU+ITS+LSU	Brazil	FN547524		
Appendiculispora appendicula	Att1235-1	SSU+ITS+LSU	Brazil	FN547525		
Appendiculispora appendicula	Att1235-1	SSU+ITS+LSU	Brazil	FN547527		
Appendiculispora appendicula	Att1235-1	SSU+ITS+LSU	Brazil	FN547528		
Appendiculispora appendicula	Att1235-1	SSU+ITS+LSU	Brazil	FN547529		
Appendiculispora appendicula	Att1235-1	SSU+ITS+LSU	Brazil	FN547530		
Appendiculispora appendicula	Att1235-1	SSU+ITS+LSU	Brazil	FN547531		
Appendiculispora appendicula	Att1235-1	SSU+ITS+LSU	Brazil	FN547532		
Appendiculispora appendicula	Att1235-1	SSU+ITS+LSU	Brazil	FN547533		
Appendiculispora appendicula	Att1235-1	SSU+ITS+LSU	Brazil	FN547534		
Appendiculispora appendicula	MACG1	SSU+ITS+LSU	Peru	OM214461		
Appendiculispora appendicula	SA19006	SSU	Not available	MN784846		
Appendiculispora callosa	JA116	SSU+ITS+LSU	Japan	PX215118		
Appendiculispora callosa	MAFF520084	SSU+ITS+LSU	Japan	PX215120		
Appendiculispora callosa	MAFF520084	SSU+ITS+LSU	Japan	PX215121		
Appendiculispora callosa	JA116	SSU+ITS+LSU	Japan	PX215122		
Appendiculispora callosa	MAFF520084	SSU+ITS+LSU	Japan	PX215125		
Appendiculispora callosa	JA116	SSU+ITS+LSU	Japan	PX215126		
Appendiculispora callosa	MAFF520084	SSU+ITS+LSU	Japan	PX215128		
Appendiculispora callosa	MAFF520084	SSU+ITS+LSU	Japan	PX215129		
Appendiculispora callosa	MAFF520084	SSU+ITS+LSU	Japan	PX215131		
Appendiculispora callosa	JA116	SSU+ITS+LSU	Japan	PX215132		
Appendiculispora callosa	MAFF520057	SSU+ITS+LSU	Japan	PX215133		
Appendiculispora callosa	MAFF520057	SSU+ITS+LSU	Japan	PX215134		
Appendiculispora callosa	MAFF520057	SSU+ITS+LSU	Japan	PX215135		
Appendiculispora callosa	JA116	SSU+ITS+LSU	Japan	PX215136		
Appendiculispora callosa	JA116	SSU+ITS+LSU	Japan	PX215137		
Appendiculispora callosa	Att1321-4	ITS	Japan	AB259842		
Appendiculispora callosa	MAFF520073	ITS	Japan	AB259846		
Appendiculispora leptoticha	MAFF520090	SSU+ITS+LSU	Japan	PX215138		
Appendiculispora leptoticha	ON205A	SSU+ITS+LSU	Canada	PX215139		

Appendiculispora leptoticha	ON205A	SSU+ITS+LSU	Canada	PX215140		
Appendiculispora leptoticha	ON205A	SSU+ITS+LSU	Canada	PX215141		
Appendiculispora leptoticha	MAFF520055	ITS	Japan	AB048630		
Appendiculispora leptoticha	NC176	ITS	USA	AJ012109		
Appendiculispora leptoticha	FL130	ITS	USA	AJ012201		
Appendiculispora leptoticha	MAFF520055	SSU	Japan	AB047302		
Appendiculispora leptoticha	MAFF520055	SSU	Japan	AB047303		
Appendiculispora leptoticha	MAFF520055	SSU	Japan	AB047304		
Appendiculispora leptoticha	MAFF520057	SSU	Japan	AB047306		
Appendiculispora leptoticha	MAFF520057	SSU	Japan	AB047307		
Appendiculispora leptoticha	MAFF520058	SSU	Japan	AB047308		
Appendiculispora leptoticha	MAFF520058	SSU	Japan	AB047309		
Appendiculispora leptoticha	NC176	SSU	USA	AJ006466		
Appendiculispora leptoticha	NC176	SSU	USA	AJ301861		
Archaeospora trappei	Att178-3	SSU+ITS+LSU	UK	FR750034		
Archaeospora trappei	Att178-3	SSU+ITS+LSU	UK	FR750035		
Archaeospora trappei	Att178-3	SSU+ITS+LSU	UK	FR750036		
Archaeospora trappei	Att178-3	SSU+ITS+LSU	UK	FR750037		
Archaeospora trappei	Att178-3	SSU+ITS+LSU	UK	FR750038		
Archaeospora trappei	NB112	SSU	Namibia	AJ006800		
Archaeospora trappei	Att186-1	SSU	Austria	AM114274		
Archaeospora trappei	Att186-1	SSU	Austria	Y17634		
Ephemeriparies granatensis	ex-type	ITS	Spain	FN820276		
Ephemeriparies granatensis	ex-type	ITS	Spain	FN820277		
Ephemeriparies granatensis	ex-type	ITS	Spain	FN820278		
Ephemeriparies granatensis	ex-type	ITS	Spain	FN820280		
Ephemeriparies granatensis	ex-type	ITS	Spain	FN820281		
Ephemeriparies granatensis	ex-type	ITS	Spain	FN820282		
Ephemeriparies granatensis	ex-type	SSU	Spain	FN820272		
Ephemeriparies granatensis	ex-type	SSU	Spain	FN820274		
Geosiphon pyriformis	GEO1	SSU+LSU	Germany	FM876840		
Geosiphon pyriformis	GEO1	SSU+LSU	Germany	FM876841		
Geosiphon pyriformis	GEO1	SSU+LSU	Germany	FM876842		
Geosiphon pyriformis	GEO1	SSU+LSU	Germany	FM876843		
Geosiphon pyriformis	GEO1	SSU+LSU	Germany	FM876844		
Geosiphon pyriformis	GEO1	SSU	Germany	AM183923		
Geosiphon pyriformis	GEO1	SSU	Germany	AJ276074		
Geosiphon pyriformis	GEO1	SSU	Germany	Y15904		
Geosiphon pyriformis	GEO1	SSU	Germany	Y15905		
Paraglomus brasilianum	Att260-8	SSU+ITS+LSU	Brazil	FR750046		
Paraglomus brasilianum	Att260-8	SSU+ITS+LSU	Brazil	FR750047		
Paraglomus occultum	Att677-4	SSU	USA	AJ276081		
Paraglomus occultum	Att677-3	SSU	USA	AJ276082		
Polonospora polonica	ex-type	SSU+ITS+LSU	Poland	MZ359654		
Polonospora polonica	ex-type	SSU+ITS+LSU	Poland	MZ359655		
Polonospora polonica	ex-type	SSU+ITS+LSU	Poland	MZ359656		
Polonospora polonica	ex-type	SSU+ITS+LSU	Poland	MZ359657		
Polonospora polonica	ex-type	SSU+ITS+LSU	Poland	MZ359658		
Polonospora polonica	ex-type	SSU+ITS+LSU	Poland	MZ359659		