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Archaeospora europaea, a new arbuscular mycorrhizal fungus from France, Germany, Italy and Switzerland, with a key to the Archaeosporaceae species

Fritz Oehl[1,*], Iván Sánchez-Castro[2], Viviane Monique Santos[3], Gladstone Alves da Silva[3] & Javier Palenzuela[4]

[1] Agroscope, Competence Division for Plants and Plant Products, Ecotoxicology, Müller-Thurgau-Strasse 29, CH-8820 Wädenswil, Switzerland
[2] Departamento de Microbiología, Campus de Fuentenueva, Universidad de Granada, E-18071 Granada, Spain
[3] Departamento de Micologia, CB, Universidade Federal de Pernambuco, Av. da engenharia s/n, Cidade Universitária, 50740-600, Recife, PE, Brazil
[4] Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, CSIC, Profesor Albareda 1, E-18008 Granada, Spain

* E-mail: fritz.oehl@gmail.com

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Archaeospora europaea, a new fungus in the Archaeosporomycetes (Glomeromycota), was isolated from multiple agricultural production systems in France, Germany, Italy and Switzerland. It was cultivated in trap and monosporic single species cultures on *Trifolium pratense*, *Lolium perenne*, *Plantago lanceolata* and *Hieracium pilosella*. The fungus is bimorphic, but in our cultures it formed predominantly bi-walled acaulo-archaeosporoid spores, globose to subglobose, rarely oblong, ovoid or irregular, and (50)65–87 × (48)64–85 µm in diameter, while the hyaline glomoid spores, 25–38(55) × 20–31 µm, were rarely observed. By morphology, *A. europaea* can be differentiated from all other Archaeosporomycetes species by the small, bi-walled acaulo-archaeosporoid spores formed laterally on the neck of sporiferous saccules without pedicel formation and by a wrinkled second spore wall layer in PVLG-based mountants that stains yellow to pinkish yellow in Melzer's reagent. The glomoid spores resemble glomoid spores of any other Archaeosporaceae species and might, to our opinion, hardly be used for species identification. Phylogenetically, *A. europaea* forms a separate clade closest to *Archaeospora trappei*. The isolation sites of *A. europaea* were various agricultural production systems and soils with variable nutritional and soil cultivation levels. Time to spore formation of the new species was short with only 3–6 months, as observed in several greenhouse cultures.

Keywords: arable lands, biodiversity, Glomeromycota, grasslands, phylogeny. – 1 new species.

Species of Archaeosporaceae J.B. Morton & D. Redecker (Morton & Redecker 2001) generally are bimorphic, have hyaline to subhyaline or white to slightly creamy spores, and the spore wall layers of the outer spore wall of acaulosporoid spores degrade rapidly (Ames & Linderman 1976, Schenck et al. 1986, Sieverding & Toro 1987, Sieverding 1988, Spain 2003), while the inner wall forms de novo and is persistent (Sieverding & Oehl 2006; Oehl et al. 2011b, 2015). Spores of such species are difficult to recognize from field soil samples, and trap or single species culturing is highly recommended for their correct identification (Sieverding & Toro 1987, Spain 2003).

During several biodiversity studies on arbuscular mycorrhizal (AM) fungi in Central Europe, an inconspicuous, small-spored AM fungal species with two spore walls was found. The spores resembled those of *Archaeospora trappei* (Ames & Lindermann 1976, Spain 2003), as they are similar in size, and appeared to have a similar spore wall composition. After the establishment of monosporal cultures, the fungus could thoroughly be analyzed morphologically, and sequences were obtained from the ITS region of the rDNA. The objective of this study is to describe this novel fungal species, based on its spore morphology and molecular phylogeny, and to present the habitats, where the fungus was detected so far in France, Germany, Italy and Switzerland.

Materials and methods

Study sites, soil sampling

During the last fifteen years, multiple natural and agricultural sites were investigated for AM

fungal diversity in France, Germany, Italy and Switzerland (e.g. Oehl et al. 2010, 2011a; Njeru et al. 2015). Soil samples were taken from the top-soils (0–10 cm), as described in Oehl et al. (2005). Soils were air-dried for subsequent AM fungal spore extraction and identification, and for analyses of selected chemical and physical soil parameters. Field moist soils were stored in the fridge (4 °C) until they were used as inoculants for propagation of the natural AM fungal communities in so-called trap cultures.

AM fungal trap and monosporic cultures

AM fungal trap cultures and monosporic cultures were established as described in Tchabi et al. (2010). Trap cultures were established in March 2000 from sites in the three-country corner of France, Germany and Switzerland and maintained for three years until December 2002. Trap cultures established in April 2009 were inoculated with soils from the Kanton Aargau and Bern (Switzerland) and maintained for two years until December 2010. Thereafter, the trap culture substrates were harvested and air-dried. In April 2001 and May 2011, spores of the new fungus were isolated from the substrates and inoculated for the establishment of monosporic cultures. Shortly, single spores were placed in several pipette tips (1 mL) filled with Loess sub-soil and Quartz-sand (1:1; Oehl et al. 2003) in about 5 mm depth. Thereafter, *Hieracium pilosella* L. was seeded on the surface of the pipette tip substrate (Oehl et al. 2003, Tchabi et al. 2010). After five weeks of symbiosis-establishment in the pipette tips, these were cut horizontally in two halves to ease the further root growth, and the upper parts of the tips were immediately transplanted into bigger pots (350 ml) filled with sterile soil substrate. The cultures were maintained for 6, 8 or 14 months before checking for mycorrhizal root colonization and spore formation. In seven of the initiated monosporic cultures, mycorrhizal roots and innumerous single spores were found. They were analyzed applying morphological and molecular methods. The successful culture substrates (original isolate numbers FO-A29, FO-V8, FO-V9, FO-V10 FO107, FO 126, and FO345) were deposited in the Swiss collection of Arbuscular mycorrhizal fungi (SAF) under the accession numbers SAF68, SAF69, SAF113-115 at Agroscope in Zürich (Switzerland), where SAF113-115 have been propagated successfully on *Hieracium pilosella* for several cycles since August 2012 until now.

Morphological analyses

Spores of the new fungus were separated from the soil samples, trap cultures and monosporic culture by a wet sieving process as described by Sieverding (1991). The described morphological spore characteristics and their subcellular structures are based on observations of specimen mounted in polyvinyl alcohol-lactic acid-glycerol (PVLG; Koske & Tessier 1983), in a mixture of PVLG and Melzer's reagent (Brundrett et al. 1994), a mixture of lactic acid and water at 1:1, Melzer's reagent, and in water (Spain 1990). The terminology of the spore structure basically is that presented in Spain et al. (2006), Palenzuela et al. (2011) and Oehl et al. (2012) for species with acaulosporoid spore formation (sensu lato and sensu stricto). Photographs were taken with a digital camera (Leika DFC 295) on a compound microscope (Leitz Laborlux S) using Leica Application Suite Version V 4.1 software. Specimens mounted in PVLG and a (1:1) mixture of PVLG and Melzer's reagent were deposited at Z+ZT (ETH Zurich, Switzerland).

Molecular analyses

All the spores, isolated for the molecular analyses, derived from the first cycle of monosporic pure culture (see above) established on *Hieracium pilosella*. They were surface-sterilized with chloramine-T (2 %), streptomycin (0.02 %) and Tween 20 (2–5 drops; Mosse 1962). Crude extracts were obtained by crushing 5–6 spores with a sterile disposable micropestle in 23 µl milli-Q water, as described by Palenzuela et al. (2013). Direct PCRs of these crude extracts were performed in an automated thermal cycler (Gene Amp PCR System 2400, Perkin-Elmer, Foster City, CA, USA) with a pureTaq Ready-To-Go PCR Bead (Amersham Biosciences Europe GmbH, Germany) following manufacturer's instructions with 0.4 µM concentration of each primer. A two-step PCR was conducted to amplify the ribosomal fragment consisting of partial SSU, ITS1, 5.8S, ITS2 and partial LSU rDNA using the primers SSUmAf/LSUmAr and SSUmCf/LSUmBr consecutively according to Krüger et al. (2009). PCR products from the second round of amplifications (~1500 bp) were separated electrophoretically on 1.2 % agarose gel, stained with Gel Red™ (Biotium Inc., Hayward, CA, USA) and viewed by UV illumination. The band of the expected size was excised with a scalpel and amplified DNA was recovered from the gel with the QIAEX II Gel Extraction kit (QIAGEN, USA) following the manufacturer's protocol. The purified amplicons were cloned into the pCR2.1 vector (Inv-

itrogen, Carlsbad, CA, USA), and transformed into One Shot® TOP10 chemically competent *Escherichia coli* (Invitrogen, Carlsbad, CA, USA). Resulting recombinant colonies were selected by blue/white screening and the presence of inserts detected by PCR amplification with GoTaq® *Green* Master Mix (Promega) using universal forward and reverse M13 vector primers. After isolation from transformed cells, plasmids were sequenced on both strands with M13R/T7 primers using the BigDye Terminator kit 3.1v (Applied Biosystems). The products were analyzed on an automated DNA sequencer (Perkin-Elmer ABI Prism 373). Just the ITS1, 5.8S and ITS2 of the rDNA were sequenced satisfactorily. Sequence data were compared to gene libraries (EMBL and GenBank) using BLAST programs (Altschul et al. 1990). The new sequences were deposited in the EMBL database under the accession numbers MK940274-MK940275.

Phylogenetic analyses

The AM fungal sequences (ITS1, 5.8S and ITS2 of the rDNA) obtained were aligned with other related glomeromycotan sequences from GenBank in ClustalX (Larkin et al. 2007). *Paraglomus brasilianum* (Spain & J. Miranda) J.B. Morton & D. Redecker was included as outgroup. Prior to phylogenetic analysis, the model of nucleotide substitution was estimated using Topali 2.5 (Milne et al. 2004). Bayesian (two runs over 1 x 10^6 generations, with a sample frequency of 100 and a burnin value of 25%) and maximum likelihood (1,000 bootstrap) analyses were performed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), launched from Topali 2.5, using the GTR + G model.

Results

Taxonomy

Archaeospora europaea Oehl, Palenz., Sánchez-Castro, V.M. Santos & G.A. Silva, **sp. nov.**, Figs. 1–8
MycoBank no.: MB 831094

D i a g n o s i s . – Differs from *Archaeospora trappei* by the smooth outermost spore wall layer and by the wrinkling nature and yellow to pinkish yellow staining reaction of the second layer of the outer spore wall in Melzer's reagent.

T y p e . – holotype, deposited at Z+ZT (accession ZT Myc 60147), derived from a monosporic culture established on the host plant *Hieracium pilosella* in the greenhouse of the Swiss Collection for Arbuscular Mycorrhizal Fungi (SAF) at the Institute of Sustainability Sciences, Agroscope, in Zürich, Switzerland. Spores for the culture originated from permanent grassland in Rubigen (Kanton Bern, Switzerland; 46°54'08"N; 7°32'44"E), *leg*. F. Oehl on 16. June 2012. Isotypes deposited at Z+ZT (ZT Myc 60148). The living culture currently maintained at SAF under the accession number SAF115. Paratypes from the three-country corner of France, Germany and Switzerland also deposited at Z+ZT (ZT Myc 60150).

E t y m o l o g y . – *europaea*, referring to the continent, on which this *Archaeospora* species was firstly identified.

D e s c r i p t i o n . – Spore formation generally laterally on the neck of sporiferous saccules (acaulosporoid formation sensu lato; acaulo-archaeosporoid sensu stricto). Glomoid spore formation (glomo-archaeosporoid sensu stricto) rather rarely observed.

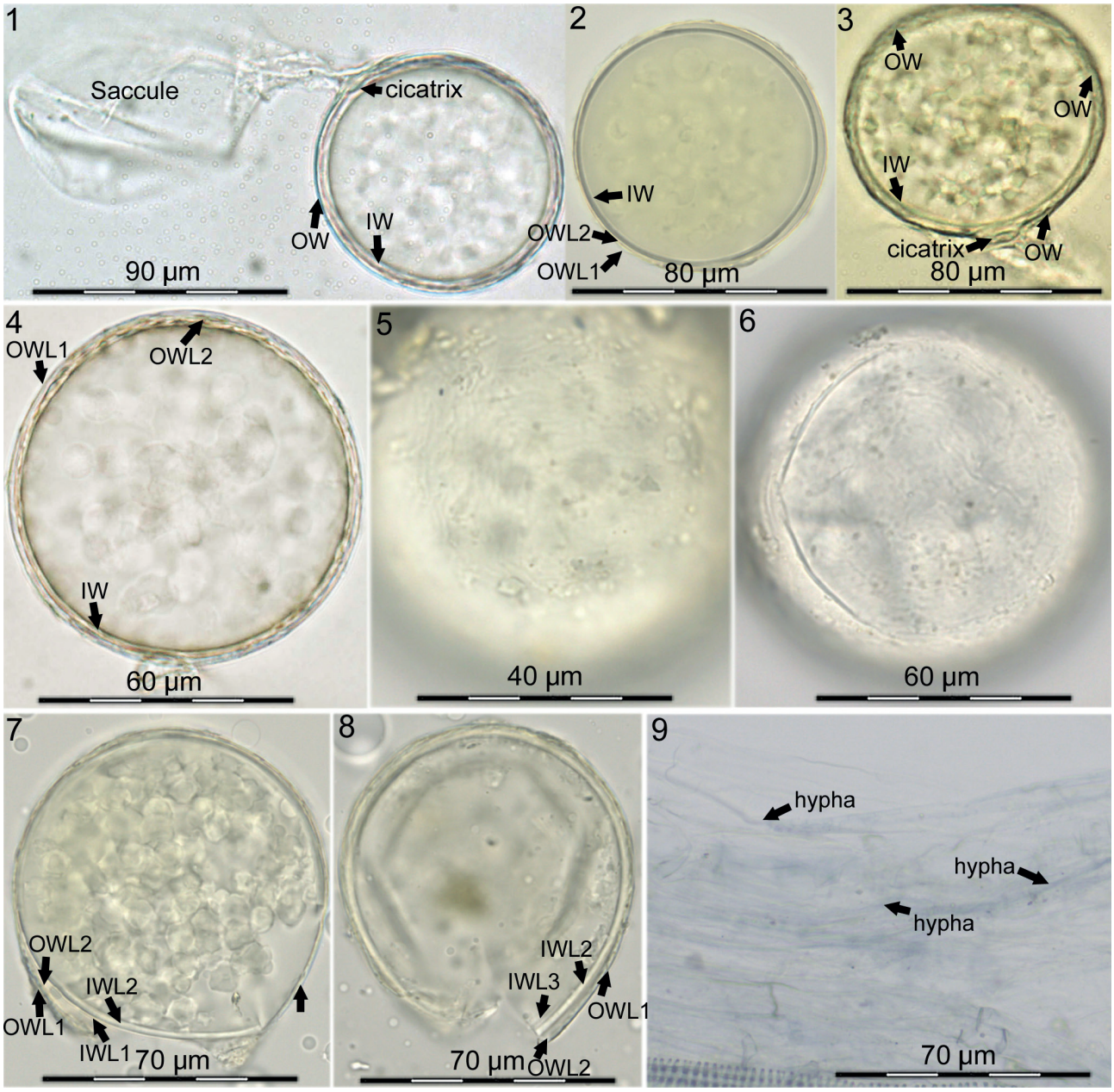
A c a u l o - a r c h a e o s p o r o i d s p o r e f o r m a t i o n . – Sporiferous saccule terminus hyaline to subhyaline, globose to subglobose to oblong, 65–95 × 60–85 µm in diameter, formed terminally on saccule necks that appear as inflated continuations of the mycelia hyphae. Necks 9–14 µm at the base of the saccule terminus, tapering to 4–7 µm in diameter towards the mycelia hyphae. The saccules mono- to bi-layered. Both layers continuous with the two outer layers of the outer spore wall. The saccules regularly collapsing and generally detaching completely from mature spores that are formed directly on the saccule neck in 30–100 µm distance from the saccule terminus.

A c a u l o - a r c h a e o s p o r o i d s p o r e s . – Hyaline to subhyaline, rarely yellowish white in water, becoming creamy to creamy yellow, in degrading stages of the outer wall layers; globose to subglobose, rarely oblong, ovoid or irregular, (50)65–87 × (48)64–85 µm in diameter, formed laterally on saccule necks, with two walls, consisting of a rapidly degrading outer wall and a persistent inner wall.

Outer wall smooth at the outer surface, with two to three layers (OWL1-3), of which the outer layer most rapidly degrading. OWL1 hyaline, evanescent, and 0.7–1.2 µm thick. OWL2 hyaline to sub-hyaline, evanescent to semi-persistent, 0.9–2.2 µm thick, showing innumerous fissures, due to its wrinkling nature. OWL3 hyaline to sub-hyaline, 0.4–0.7 µm thick, rarely observed as tightly adherent to OWL2. OW may degrade completely from the spores in field soils. OWL2 regularly staining yellow to pinkish yellow in Melzer's reagent.

Inner wall bi- to triple-layered, hyaline, and in total 1.9–2.7(–3.4) µm thick. IWL1 0.5–0.7 µm thick and often difficult to observe as tightly adherent to IWL2. IWL2 uniform to finely laminate, 1.0–2.0 µm thick. IWL3 very thin (0.4–0.7 µm), usually difficult to observe. IWL1 usually staining yellow to pinkish yellow in Melzer's reagent.

Spore pore at the spore base 4.0–7.0 µm in diameter and closed by OWL2 and OWL3 as long as the



Figs. 1–9. *Archaeospora europaea*. **1.** Bi-walled (OW, IW) acaulo-archaeosporoid spores; formed laterally on the neck of a sporiferous saccule. Saccule in collapsing stage. Cicatrix formed by OW visible at the spore base. **2–3.** Spores in PVLG+Melzer's reagent staining yellow to pinkish yellow. **4.** Spore in cross view. OW with two layers: OWL1 smooth, OWL2 roughened, wrinkling. **5–6.** Outer spore wall in planar view, showing the roughened (Fig. 5) and wrinkled (Fig. 6) appearance of OWL2. **7–8.** Crushed spores with bi-layered OW (OWL1-3), and triple-layered IW (IWL1-3). **9.** Intraradical hyphae not stained or only faintly staining in trypan blue.

two layers remain intact, as forming an only evanescent to semi-persistent cicatrix-like structure at the spore base.

Glomoid spore formation rarely found in the first and second culturing cycles of the fungus in single species culture on *Hieracium pilosella*, compared to the abundant acaulo-archaeosporoid spore formation.

Glomoid spores hyaline, globose to subglobose, 25–38(55) × 20–31 µm in diameter, forming either singly or in small, loose clusters of about 2–10 spores, terminally or intercalary on mycelia or cy-

lindrical subtending hyphae. Wall bi-layered, 1.1–2.3 µm thick, of which generally only the second wall layer visible. This layer may stain light to bright yellow in Melzer's reagent. Spore pore often closed by a bridging septum arising from the second wall layer. Hyphae 2.4–5.0 µm broad and may not stain in Melzer's. Regularly only the second of two subtending hyphal wall layers detectable (1.1–2.0 µm thick).

Spore germination in acaulo-archaeosporoid spores observed in a few older spores in the region of their spore bases, where single germ trunks penetrate the spore wall at the spore pore region.

Arbuscular mycorrhiza without vesicle formation found in *Hieracium pilosella* roots in monosporic cultures (Fig. 9). Arbuscules, and intraradical and extraradical hyphae not or only faintly blue staining in trypan blue or ink staining procedures (Vierheilig et al. 1998).

Molecular analyses

Phylogenetic analyses of the ITS region of the rDNA reveal that the sequences of the new species group in *Archaeospora* (Archaeosporaceae, Archaeosporales, Archaeosporomycetes) forming a well-separated clade next to *Archaeospora trappei* (Fig. 10). In the BLASTn analysis, the rDNA species sequences with closest match (94–95 % of maximum identity - MI) to the new fungus are from *A. trappei* (FR750034–FR750038). Sequences of *Intraspora schenckii* and *Palaeospora spainiae* showed 89 % of MI with *A. europaea*. Just one environmental sequence, obtained from roots of *Agrostis scabra* (AM942511) in USA (Appoloni et al. 2008), was related to the new species with 97% of MI. Environmental sequences with less than 96 % of MI were not considered.

Distribution

The fungus was found at several sites in Alsace (France), Palatinate and Baden (Germany), Toscana and Umbria (Italy) and Aargau and Basel Land (Switzerland) between 2000 and 2015 (Tab. 1). It was also detected in six out of thirteen sites investigated in the Kanton Bern between 2009 and 2013. These sites comprise permanent or temporary grasslands, or are subjected to low-input organic, to medium to high-input conventional no-tillage and tillage farming systems. The sites had been exposed to very different levels of phosphorus fertilization in the past as suggested by quite diverging levels of soil P availability at sampling time, between 7.9 and 257.0 mg P kg^{-1}. Soil pH ranged from acidic to neutral (5.7–8.0), and organic carbon was between 10.3 and 47.6 mg C kg^{-1}. The fungus was found both in field soil samples and trap cultures and was cultivated in mono-sporal cultures from vineyards and grasslands in Vogtsburg im Kaiserstuhl (Baden), from a permanent grassland in Rubigen (Bern) and from organically managed croplands in Frick (Aargau) and in Uettligen (Bern). Only one environmental sequence might correspond to the new fungus. This sequence, however, does not derive from Central Europe, but from Yellow Stone National Park in USA (Appoloni et al. 2008).

Key to the species in Archaeosporaceae

Archaeosporaceae currently comprise three genera that are all bi-morphic. They are all relatively small-spored (< 100 µm in diameter), hyaline to creamy-hyaline and have a more or less rapidly degrading outer spore wall that might disappear completely from older spores in the nature. Then, morphological spore identification is not anymore possible. If all characters on the spores are more or less intact, the species can readily be identified only by looking at the acaulo-archaeosporoid or entrophosporo-intrasporoid spores, while the glomoid morph might not even serve for genus identification. However, specific identification tools never were elaborated for the glomoid morph. In the following, the first identification key for Archaeosporaceae species is presented that currently comprise six species.

1 Acaulo-archaeosporoid or entrophosporo-intrasporoid spores prevailing, forming two walls, bi-walled spores formed laterally on (acaulo-archaeosporoid) or intrahyphally (entrophosporo-intrasporoid) within the neck of sporiferous saccules; mono-walled glomoid spores rather rarely observed 2

1 Acaulo-palaeosporoid spores prevailing, forming three walls (*Palaeospora*); mono-walled glomoid spores rather rarely observed: ..... ***Palaeospora spainiae*** Oehl, Palenz., Sánchez-Castro & G.A. Silva

Spores white to rarely yellowish white in water, creamy to creamy yellow in lactic acid-based mountants, globose to sub-globose, 52–80 × 51–77 µm. Outer wall smooth to slightly roughened, with three layers (OWL1-3), of which the outer two layers rapidly degrading. OWL2 hyaline to sub-hyaline, becoming creamy in lactic acid based mountants, 0.9–3.2

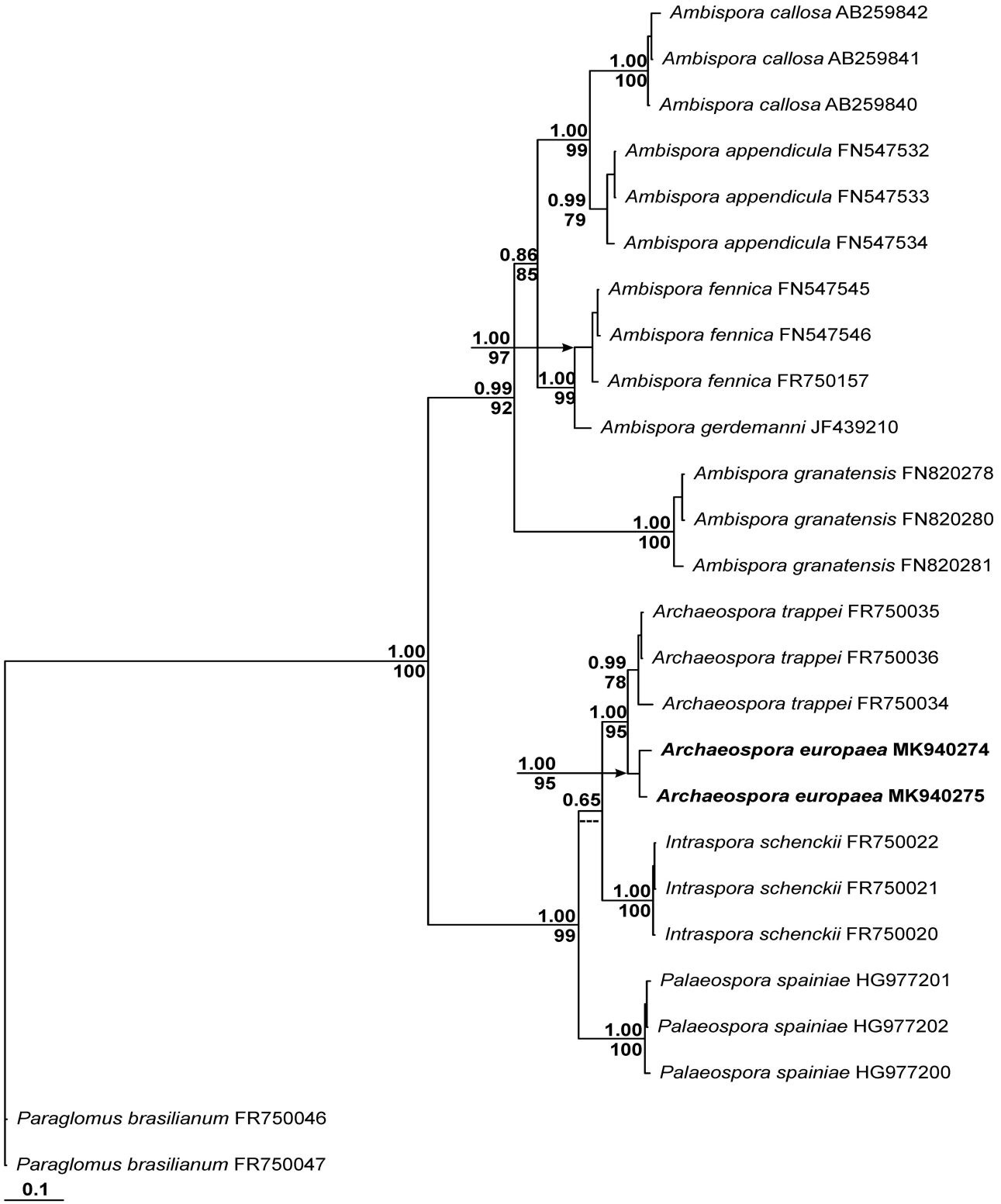


Fig. 10. Phylogenetic tree of Archaeosporomycetes obtained by analysis from sequences of the ITS region of the rDNA. Sequences are labelled with their database accession numbers. Support values (from top) are from Bayesian inference and maximum likelihood analyses, respectively. Only support values of at least 65 % are shown. Sequences obtained in this study are in boldface. The tree was rooted by *Paraglomus brasilianum*.

µm thick, showing innumerous fissures beginning from the outer surface. OWL3 hyaline to sub-hyaline, 0.9–1.5 µm thick, semi-persistent to persistent and often the only OW layer remaining on the spore. OWL2 may stain bright yellow, OWL3 regularly staining bright to dark bright yellow in Melzer's reagent. Middle wall hyaline, flexible, one to bi-layered, in total 0.8–1.9 µm thick. MWL2 staining bright yellow in Melzer's reagent. Inner wall bi- to triple-layered, hyaline, and in total 1.9–3.4 µm thick.

2 Hyaline spores predominantly formed intrahyphally within the stalk of terminal or intercalary sporiferous saccules (entrophosporo-intrasporoid spore formation), or glomoid, terminally or intercalary on hyphae (*Intraspora*): ..... ***Intraspora schenckii*** (Sieverd. & S. Toro) Oehl & Sieverd.

Entrophosporo-intrasporoid spores hyaline, 40–75 µm, with bi-layered outer and triple-layered inner wall; all layers smooth. OWL1 often absent in mature spores, OWL2 closing the two pores, towards the saccule terminus and towards the mycelia hyphae; IWL2 finely laminate, IWL1 and IWL3 each < 0.5 µm thin, flexible and tightly adherent to IWL2. OWL2 staining slightly yellow in Melzer's reagent.

2 Hyaline spores predominantly formed laterally on the neck of sporiferous saccules (*Archaeospora*; acaulo-archaeosporoid) 3

3 Acaulo-archaeosporoid spores with a smooth or roughened outer wall 4

3 Acaulo-archaeosporoid spores with undulate pits on the outer spore wall ..... ***Archaeospora undulata*** (Sieverd.) Sieverd., G.A. Silva, B.T. Goto & Oehl

Acaulo-archaeosporoid spores hyaline, 85–55 µm, rarely oblong, ovoid or irregular, 84–60 × 74–54 µm, with bi-layered outer and triple-layered inner wall. OWL1 smooth, in PVLG separated in young spores from OW2 forming a non-ornamented wall layer that with age attaches and adheres to OWL2 and may become evanescent; OWL2 with depressions on the outer surface, 4–9 µm in diameter and 1–1.5 µm deep. OWL2 staining light yellowish orange in Melzer's. IWL2 finely laminate, IWL1&3 < 0.5 µm thin, flexible and tightly adherent to IWL2. IWL1 minutely roughened in PVLG, staining light yellowish orange in Melzer's reagent.

4 Acaulo-archaeosporoid spores with a smooth outer wall, regularly with a pedicel at the spore base ..... ***Archaeospora myriocarpa*** (Spain, Sieverd. & N.C. Schenck) Oehl, G.A. Silva, B.T. Goto & Sieverd.

Acaulo-archaeosporoid spores formed in sporocarps or singly in soils, or rarely in roots, (22)32–90 µm, rarely oblong, ovoid or irregular, (23)28–95(114) × (21)28–80(96) µm, with bi-layered OW and triple layered IW. OWL2 and IWL1 staining dull yellow in Melzer's reagent.

4 Spores with a roughened or wrinkling outer wall 5

5 Acaulo-archaeosporoid spores with a roughened spore surface formed by thin OWL1, < 0.5 µm thick; OWL2 smooth, finely laminate, 1.5–2.0 µm thick; wall layers non-reactive in Melzer's ..... ***Archaeospora trappei*** (R.N. Ames & Linderman) J.B. Morton & D. Redecker. emend. Spain.

Acaulo-archaeosporoid spores globose to subglobose, 42–90 × 42–72 µm; OW bi-layered, IW triple-layered. Glomoid spores 22–31 µm, bi-layered.

5 Acaulo-archaeosporoid spores with a smooth surface; OWL1 hyaline, evanescent, and 0.7–1.4 µm thick; OWL2 with wrinkled appearance, attributed to foldings of OWL2; OWL2 & IWL1 staining yellow to pinkish yellow. ..... ***Archaeospora europaea*** Oehl, Palenz., Sánchez-Castro & G.A.Silva

Acaulo-archaeosporoid spores globose to subglobose, (49)64–86 µm, rarely oblong, ovoid or irregular, (50)65–87 × (48)64–85 µm, OW bi-layered, IW triple layered (see description above); OWL2 0.9–2.2 µm thick, wrinkling, OWL3 hyaline to sub-hyaline, 0.4–0.7 µm thick, rarely observed as tightly adherent to OWL2. Glomoid spores 25–38(55) × 20–31 µm, bi-layered.

Discussion

Archaeospora europaea can be distinguished from *A. trappei* and all other Archaeosporaceae species by the spore wall structure of its bi-walled acaulo-archaeosporoid spores. *Archaeospora trappei* forms spores of similar size as *A. europaea*, but has a roughened spore surface (OWL1) and a smooth OWL2 (Ames & Schneider 1976, figs. 6–9 in Hafeel 2004), while the spore surface in *A. europaea* is smooth and OWL2 beneath is of wrinkling nature. Also, the staining reaction of the spores in Melzer's reagent separate the two species well (see identification key; Spain 2003). *Archaeospora myriocarpa* forms smaller, smooth spores with a short pedicel-like structure (= nipple) at the spore base (Schenck et al. 1986), which was never observed in *A. euro-*

paea spores, while *A. undulata* has an undulate spore surface consisting of large undulate pits (Sieverding 1988).

Three genera are included in Archaeosporaceae in well supported clades. *Archaeospora* present 91 % of maximum identity (MI) with *Intraspora* and *Palaeospora*. *Intraspora* and *Palaeospora* have 88 % of MI. Phylogenetically, *A. europaea* groups closest to *Archaeospora trappei*. For *A. myriocarpa* and *A. undulata*, molecular data are not yet available. *Intraspora schenckii* and *Palaeospora spainiae,* two other species in the Archaeosporaceae, have either entrophosporoid-intrasporoid spore formation (Sieverding & Oehl 2006), or triple-walled acaulopalaeosporoid spores (Oehl et al. 2015). They are attributed to the two other genera in the Archaeoporaceae, due to their clearly separating phylogenetic position and these major morphological differences. Remarkably, by AM root colonization, AM staining features or their glomoid morphs, these genera cannot be readily distinguished (Oehl et al. 2015).

Acaulospora europaea was found in several countries in Central Europe, and apparently is not negatively affected by intensive agricultural production, as it is regularly found in highly fertilized and intensively cultivated croplands. This observation corresponds well to its fast spore production cycle of 3–6 months in the greenhouses, where in one year it even was an aggressive contaminant of single spore cultures established for other AM fungal species. The new species might have a much larger biogeographical distribution in Europe, especially towards colder and Mediterranean climates, as also recorded from high alpine to nival pioneer sites and from agricultural sites in Central Italy. There are not yet environmental sequences in the data bases either from Europe, or any other place, besides one sequence from Yellowstone National Park in the USA (Appoloni et al. 2008) that might correspond to the new species. This might be related to the fact, that in the past often insensitive primers were used to the basal glomeromycetan clades, to which the Archaeosporaceae belong. The short life cycles, small spores, only fine mycelia hyphae, tiny and non-staining intraradical mycorrhizal structures with only small diameters, and the lack of vesicles in the roots, might be a couple of other reasons, why the new fungus has hitherto remained undetectable by both morphological and molecular approaches. Overall, we assume a much higher diversity of this fungal group than known so far, but this diversity might only be described accurately, when spores with these characteristics are systematically cultured under controlled conditions (Oehl et al. 2015). However, these techniques are time-consuming and not always successful, and thus, they have not been widely used so far.

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