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Acaulospora sieverdingii, an ecologically diverse new fungus in the Glomeromycota, described from lowland temperate Europe and tropical West Africa

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Summary

From a survey of arbuscular mycorrhizal (AM) fungi in agro-ecosystems in Central Europe and West Africa, an undescribed species of *Acaulospora* was recovered and is presented here under the epithet *Acaulospora sieverdingii.* Spores of *A. sieverdingii* are 60-80 µm in diam, hyaline to subhyaline to rarely light yellow and have multiple pitted depressions on the outer spore wall similar to those known for *A. alpina*, *A. cavernata*, *A. paulinae* and *A. scrobiculata.* The pits in *A. sieverdingii* are tiny and often irregular and resemble small dots (0.8-1.8 µm) or lines (0.5-1.2 x 1.8-2.5 µm). Analyses of the ITS1, 5.8S subunit and ITS2 regions of the rDNA resolved each of the five species in a monophyletic well-supported clade and indicate that *A. sieverdingii* is phylogenetically closer to *A. paulinae*, *A. cavernata* and *A. denticulata* than to *A. scrobiculata*. The new species is common in Central Europe only at altitudes below 800 m asl where, to date, it has been detected in crop rotation systems and grasslands in Poland, Germany, France, Switzerland and Italy. Under these conditions it may co-occur with *A. paulinae*, *A. cavernata*, *A. scrobiculata* and several other *Acaulospora* spp. *A. sieverdingii* was also recorded from subtropical and tropical agro-ecosystems and consequently appears to be adapted to ecologically diverse environments.

Introduction

Diversity studies of arbuscular mycorrhizal (AM) fungi in different Central European agro-ecosystems (Oehl et al., 2003, 2004, 2010; Hijri et al., 2006), using morphological and molecular methods, revealed the co-occurrence of two related *Acaulospora* species in the Glomeromycetes. The two species, referred to as ACAU-2 and ACAU-3 in Hijri et al. (2006), can now be attributed to two morphologically similar but distinguishable spore types based on nuclear ribosomal gene sequences. Spores of ACAU-2, showing a pronounced and regular ornamentation on the outer spore wall were found to belong to *Acaulospora paulinae* Błasz. 1988 (Błaszkowski, 1988) whereas spores of ACAU-3, exhibiting inconspicuous and irregular ornamentation, belonged to a hitherto unknown AM fungal species based on the molecular identification (Hijri et al., 2006). This new species, listed as *Acaulospora* sp. BR19 in Oehl et al. (2010), is here described under the epithet *Acaulospora sieverdingii*.

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Materials and methods

Soil sampling and spore isolation

Between March 2000 and April 2009, soil cores of 0-10 cm depth were removed from various agro-ecological systems. These were approximately 300 lowland, mountainous and alpine sites in Germany, France, Italy and Switzerland, and 24 sites in Benin (tropical West Africa). AM fungal spores were separated from the soil samples by a wet sieving process as described by Sieverding (1991). The procedure for spore isolation and mounting on slides in different fixatives followed that of Oehl et al. (2006).

AM fungal trap cultures

The trap cultures for the propagation of the European AM fungal communities were regularly established within a week of sampling using an autoclaved substrate mixture (Terragreen: Loess subsoil = 3:1; Terragreen is American aluminium oxide, Oil Dry US special, type III R, consisting of the granular clay mineral Attapulgite, from Lobbe Umwelttechnik Iserlohn, Germany) according to Oehl et al. (2003, 2006, 2010). The pH-KCl was 6.2; organic carbon 0.3 %; available P (Na-acetate) 2.6 mg kg^{-1}; available K (Na-acetate) 350 mg kg^{-1}. Seeds of each of the three trap plants, *Plantago lanceolata* L., *Lolium perenne* L., *Trifolium pratense* L. were applied in most cases (Oehl et al., 2003, 2010), and in other studies additionally *Hieracium pilosella* L. was sown together with the three other host plants mentioned (Oehl et al., 2006). An automated watering system (Tropf-Blumat, Weninger GmbH, A-6410 Telfs) was installed and the cultures from Europe were kept in a greenhouse under ambient natural light and temperature conditions for two to three years in Basel. The average annual temperature in Basel is about 9.5°C, and the temperature in summer ranges from 20 to 35°C. Spore formation in trap cultures was checked during the vegetation periods (March until beginning of December) at bimonthly intervals as described by Oehl et al. (2003, 2009). The new fungus produced abundant spores in trap cultures inoculated with soils of pH < 7.0, but it was absent in calcareous soils. The cultures initiated with single or multiple spores of the new species failed so far. The trap cultures from West Africa were established and maintained for 8 to 24 months as described in Tchabi et al. (2008, 2009). In the culture systems from African soils, the new fungus was never detected.

Morphological analyses

The described morphological characteristics of spores, including the sporiferous saccules and the subcellular structures are based

on observations of specimens mounted in polyvinyl alcohol-lactic acid-glycerol (PVLG, Omar et al., 1979), in a mixture of PVLG and Melzer's reagent (Brundrett et al., 1994), in a 1:1 (v/v) mixture of lactic acid and water, in solely Melzer's reagent, or in water. The terminology used to describe the spore structures is basically that of Oehl et al. (2006) and Palenzuela et al. (2008, 2011) for species with acaulosporoid spore formation. Photographs were taken using an Olympus digital camera (model DP70-CU) mounted on a Zeiss Axioplan compound microscope. Specimens mounted in PVLG and the mixture of PVLG and Melzer's reagent were deposited at the Z+ZT (Zürich, Switzerland) and the OSC (Corvallis, USA) herbaria. A pure culture, established of the new fungus already in 1989 from Australia (called isolate 'WUM18') and maintained at INVAM (West Virginia, USA) under the accession number AU103, was also analyzed and compared with the type and non-type specimens obtained from Switzerland, France and Germany, and Poland and Benin, respectively. The specimens of the Australian isolate were found to be identical with the specimens from Switzerland. Furthermore, spore analyses on specimens, kindly provided by Hannes Gamper (University of Basel), indicated that sequences obtained from spore populations derived from Swiss agricultural soils do not necessarily belong to *A. paulinae*, as previously assumed, (Gamper and Leuchtmann, 2007), but might rather belong to the fungus described hereafter, or derived from both species.

Molecular analyses

Prior to DNA extraction, spores of the new species were mounted in water on coverless slides and morphologically identified at 200-400x magnifications using a compound microscope. Concomitantly, permanent reference slides were prepared with spores of identical morphology. DNA was extracted from 13 single spores, isolated and identified from a 13-month-old trap culture, originally inoculated with soil from the bio-organically managed plots of a long-term field trial in Therwil, Switzerland (Oehl et al., 2004; Hijri et al., 2006). DNA crude extracts were produced as described by Redecker et al. (1997). The air-dried trap culture substrate had been stored at room temperature for almost four years at the Institute of Botany in Basel prior to the spore isolation and DNA extraction in January 2005. In these samples, no spores of *A. paulinae* were detected. Additionally, DNA was extracted from 11 spores that had been isolated from an air-dried and stored grassland field sample taken in Wintzenheim-La Forge (France). Both *A. paulinae* and the new fungus were present in this sample and sequences of *A. paulinae* were reported previously (Oehl et al., 2006). Single spore DNA extracts were used as templates for a two-step (nested) polymerase chain reaction (PCR) (Redecker et al., 2003) using the primers NS5/ITS4 and ACAU1661/ITS4i consecutively (Redecker, 2000). From the second morphotype, i.e. from the new species, five positive PCR products were obtained, derived from the samples taken at Therwil and La Forge. Three of these PCR products were purified and processed according to Oehl et al. (2006). Sequences were submitted to the EMBL database under the accession numbers AM076375–AM076383.

In addition to the analyses on the new species, two other species with similar spore morphology, *A. cavernata* Błasz. 1989 (Błaszkowski, 1989) and *A. scrobiculata* Trappe 1977 (Trappe, 1977), were analyzed on the same region of the ribosomal gene. No or no reliable sequences exist from these species in the public data bases. The *A. cavernata* isolate had been generated from Great Britain in 1983 at a time when *A. cavernata* had not yet been separated as a phenotypically similar species to *A. scrobiculata* (Błaszkowski, 1989), and was deposited in the European Bank of Glomeromycota under the accession number BEG33. The isolate was re-identified by F. Oehl in 2004. The *A. scrobiculata* isolates were an isolate from Brazil (BR224, identified by J.B. Morton), and an isolate from West Africa (WA-*A. scrob1*), which was established and identified by F. Oehl; see Tchabi et al., 2010). From each of these species, 2-5 spores were used for DNA extraction; PCR, cloning and sequencing was conducted. The new sequences of *A. cavernata* were deposited in the EMBL database under the accession numbers FR692347–FR692348, while the new *A. scrobiculata* sequences were deposited under the numbers FR692349–FR692354.

The sequences were aligned in PAUP*4b10 (Swofford, 2003) in a dataset comprising rDNA ITS1, 5.8S subunit and ITS2 regions of other fungal species of the family Acaulosporaceae and closely related sequences from environmental samples from public databases. In all phylogenetic analyses, the sequence of the AM fungus *Glomus etunicatum* W.N. Becker & Gerd. was used as an 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 burnin value of 2500) and maximum likelihood (1,000 bootstrap) analyses were performed, respectively, in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) and PhyML (Guindon and Gascuel, 2003), launched from Topali 2.5, using the GTR + G model. Neighbor-joining (established with the model cited above) and maximum parsimony analyses were performed using PAUP*4b10 (Swofford, 2003) with 1000 bootstrap replications.

Results

Latin diagnosis

Acaulospora sieverdingii Oehl, Sýkorová, Błaszk. & G.A. Silva sp. nov. (**Figs. 1-6**)

MycoBank MB 518941

Sacculus sporiferus hyalinus aut pallidus luteus, globosus vel subglobosus, 60-75 µm in diametro et sporogenesem praecedens. Sporae singulae lateraliter formatae ad hypham in 40-80 µm distantia ad sacculum terminalem, hyalinae ad albae, globosae 60-80 µm in diametro, vel subglobosae vel ovoideae vel ellipsoideae vel irregulares 60-70 × 65-80(-90) µm. Sporae cum tunicis tribus, tunica exterior, media et interior. Tunica exterior in totum 2.2-3.0 µm crassum, cum stratis tribus: stratum exterior hyalinum, tenue et evanescens; stratum medium unitum vel laminatum, hyalinum ad album, cum depressionibus subtilibus, rotundis (0.8-1.8 µm in diametro) vel ellipsoidibus vel irregularibus (0.5-1.2 × 1.5-2.5µm) in interiorem strati huius insculptis; stratum interior hyalinum, subtile. Tunica media tenuis stratibus duobus et tunica interior stratibus duobus (vel tribus), uterque tunicae hyalinae et flexibiles. Tunica interior 1.4-2.5 µm in totum; solo stratum medium tunicae interioris colorans reagente Melzeri. Typus hic designatus # 42-4201: Z+ZT Myc (1290).

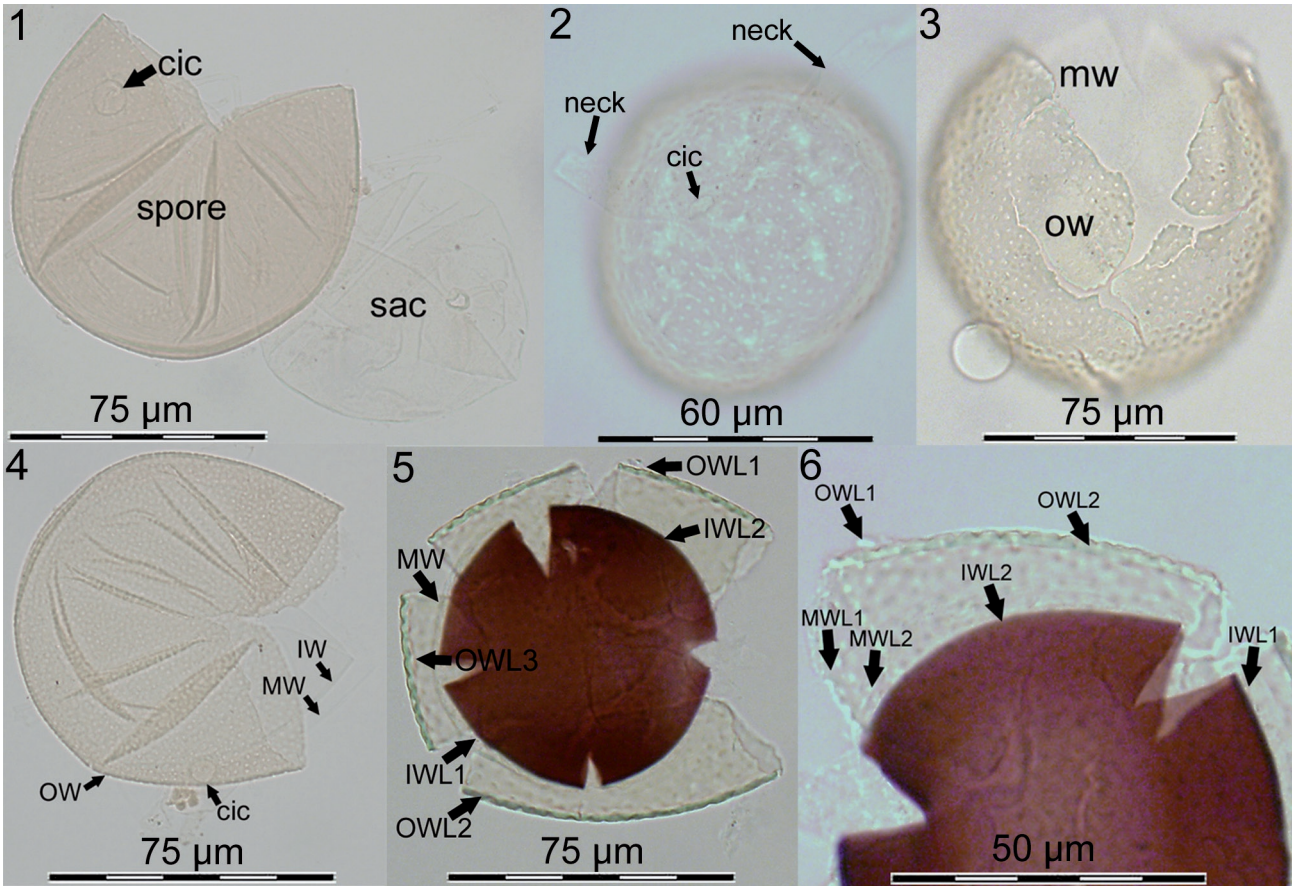
Holotype

Isolated from soil samples in Therwil (Basel Landschaft, Switzerland) from a Luvisol developed on Loess in the bio-organic treatment of the DOK long-term field trial (Mäder et al., 2002). Specimen (42-4201) was deposited at Z+ZT (mycological herbarium of ETHZ & University of Zurich) under the accession number (Z+ZT Myc 1290).

Etymology: in honor of Dr. Ewald Sieverding, University of Hohenheim, Stuttgart, Germany, for his contributions to the knowledge, identification, propagation and use of AM fungi, especially in South American, African and European agricultural ecosystems.

Description

Sporiferous saccules are hyaline, globose (about 60-75 µm in diam) to subglobose (65-75 x 75-85(-92) µm) with one wall layer that is



Figs. 1-6: Spores of *Acaulospora sieverdingii*: 1. Spore with cicatrix (cic) at spore base and sporiferous saccule (sac) attached. 2. Single spore with characteristic minute surface ornamentation, with cicatrix in planar view and lateral saccule neck attached. 3. Broken spore exhibiting the ornamentation of the outer wall and the inner wall complex. 4. Broken spore with outer, middle and inner wall (OW, MW and IW). 5-6. Spore wall structure in PVLG + Melzer's reagent: outer wall layers (OWL1–3), bi-layered middle wall mw (MWL1–2) and bi-layered inner wall (IWL1–2). OWL3 and the beaded ornamentation on the thin outer layer of the inner wall (IWL1) are difficult to see; the second layer of the inner wall (IWL2) stains purple in Melzer's.

1.0-2.1 µm thick (Fig. 1); formed terminally or intercalary in 40-80 µm distance from the location where the spore arises from the saccule neck. The saccule wall layer continues with the second wall layer of the outer spore wall (OWL2). An additional layer was not detected so far on the saccule wall. The saccules usually collapse after the outer spore wall has fully formed, and have often detached completely from mature spores or in the process of spore isolation.

Spores form laterally on the subtending hypha of the sporiferous saccule (Fig. 1). They are hyaline to subhyaline to white to rarely light yellow, 60-80 µm in diam, rarely ovoid to irregular, 60-70 x 65-80(-90) µm (Figs. 2-4).

Outer spore wall consists of three layers (OWL1-3, in total 2.3-3.0 µm thick (Figs. 5-6). The outer layer (OWL1) is hyaline, unite, 0.4-0.8 µm thick, evanescent and, thus, usually absent in mature spores (Figs. 5-6). The second layer (OWL2) is hyaline to white to rarely light yellow, unite or finely laminated, 1.5-2.1 µm thick, including the ornamentation with round to elliptical, rarely oblong pits that are 0.8-1.8 µm in diam when round and 0.5-1.2 x 1.5-2.5 µm when oblong (Figs. 2-4). The pits are concave to conical in shape and 0.5-1.5 µm deep (Figs. 5-6). The distance between pits is 2.5-4.0 µm. The innermost layer (OWL3) is concolorous with OWL2, 0.4-0.7 µm thick, usually tightly adherent to OWL2 and, thus, difficult to detect (Fig. 5).

Middle wall is hyaline, bi-layered and 0.5-1.1 µm thick; both layers (MWL1-2) are semi-flexible and tightly adherent to each other and, thus, difficult to view (Figs. 5-6).

Inner wall is hyaline, with two or three layers (IWL1-3) that are 1.2-2.5 µm thick in total (Figs. 5-6); IWL1 is approximately 0.5 µm thick with a 'beaded' ornamentation that is sometimes difficult to observe; IWL2 is 1.0-2.0 µm thick, and IWL3 is thin and usually difficult to detect due to its close adherence to IWL2; only IWL2 shows a purple to deep purple reaction in Melzer's reagent.

Cicatrix is generally visible after detachment of the sporiferous saccule, 5-8 µm wide (Figs. 1-2). OWL2 which is continuous with the saccule neck wall remains for a short distance of 0.8-1.2 µm on the spore. The pore is closed by laminae of OWL2 and by OWL3.

Specimens examined: Switzerland, Kanton Basel Landschaft: Therwil in a Luvisol developed on Loess in the DOC long-term field trial (MÄDER et al., 2002) (HOLOTYPE: Z+ZT). Germany, Baden-Württemberg: St. Peter (427.7 km East, 5'319.3 km North; 780 m asl) in Cambisols developed on Permian sandstone (pH 5.3-5.6) under grassland; Steinen-Weitenau (408.6 km E, 5'281.9 km N; 420 m asl) in Cambisols under grassland; Steinen-Weitenau (418.3 km E, 5'331.7 km N; 390 m asl) in Cambisols under crop rotation developed on Triassic sandstones (pH 5.6-6.1); Gresgen

(411.7 km E, 5'331.7 km N; 700 m asl) and Sexau-Tennenbach (417.8 km E, 5'330.0 km N; 260 m asl) in Cambisols under grassland; Sexau-Staudenhöfe (418.3 km E, 5'331.7 km N; 250 m asl) in Cambisols under crop rotation; Glottertal in Cambisols developed on dilluvial granite and gneiss sediments under grassland (420.7 km E, 5'322.1 km N; 350 m asl) and under crop rotation (420.5 km E, 5'322.1 km N; 350 m asl); Staufen-Grunern (406.7 km E, 5'301.8 km N; 320 m asl) in a Fluvisol under grassland. France, Alsace. Departement Haut-Rhin: Wintzenheim-La Forge (368.8 km E, 5'324.5 km N; 230 m asl) in a Cambisol developed on Granite under extensive grassland. Italy, Umbria: Assisi in a Cambisol under grassland. Poland: Chałupy (54º46'N, 18º31'E) on the Hel Peninsula, in maritime sand dunes (pH in 1N KCl: 3.8-6.5), under *Rosa canina*. Benin: in the Sudan Savannah (10°07.9 N; 001°51.1 E) in a Ferralsol (pH 5.9) under yam (*Dioscorea cayenensis* L.); in the Northern Guinean forest savannah (09°10.545 N; 002°12.321 E) in a Ferralsol (pH 6.5) and in the Southern Guinean savannah (07°51,537 N; 002°17,246 E) in a Ferralsol (pH 6.6) under peanuts (*Arachis hypogaea* L.).

Commentary*:* In Central Europe, *Acaulospora sieverdingii* was not detected in samples in calcareous soils (pH > 7.0; e.g. Oehl et al., 2010, listed as *Acaulospora* sp. BR19), maize mono-cropping systems or in mountainous or alpine regions above 1.200 m asl. Also in West Africa, the fungus (referred to as *Acaulospora* sp. WAA2 in Tchabi et al., 2008) was only found in soils with pH < 7.0. The fungus was recently detected also in NE Brazil (Mello and Oehl, unpublished) and has been known also from Australia.

Molecular analyses: Sequences of approximately 550 bp length, obtained from DNA extracts of spores, comprised the ITS1, the 5.8S rDNA subunit and ITS2 regions of the ribosomal gene. Phylogenetic analyses firmly placed all sequences of *A. sieverdingii* into the genus *Acaulospora.* The sequences from *A. sieverdingii* comprised a single clade, which was clearly distinct from the other *Acaulospora* spp. and had a strong branch support from Bayesian as well as neighbor joining and parsimony analyses (Fig. 7). Several sequences grouped within this *A. sieverdingii* clade: (i) those obtained from the spores harvested from a field sample in Wintzenheim-La Forge (France), (ii) those obtained from spores of a trap culture originating from the bio-organically managed field plots of the DOK field trial in Therwil (Switzerland), (iii) those obtained from colonized roots of *Triticum aestivum* harvested from the same DOK field plots (Hijri et al., 2006), (iv) a sequence from a colonized *Agathosma betulina* root sample from South Africa (Cloete et al., 2007), and sequences obtained from spores of a pure culture (called hitherto *Acaulospora* sp. WUM18 or AU103, respectively) originating from Australia (Krüger et al., 2009).

The species most closely related to *A. sieverdingii* in our molecular analyses was *A. paulinae.* Its clade comprised sequences obtained from spores originating from a grassland field sample from Wintzenheim-La Forge, France (Oehl et al., 2006), as well as from *T. aestivum* roots harvested in Therwil, Switzerland (Hijri et al., 2006). Also *A. paulinae* formed a monophyletic clade with a strong branch support. Close, but separate from these two clades grouped sequences found in colonized roots of *T. aestivum* in Therwil, Switzerland (presented as sequence type ACAU-1 in Hijri et al., 2006) in addition to one sequence obtained from a *Trifolium* sp. root sample from the Swiss Alps and *Agrostis scabra* roots from USA. Sequences of *A. cavernata* (still called *A. scrobiculata* by Krüger et al. (2009) but re-identified by the authors of the present article, see Materials and methods), and a sequence of *A. denticulata* Sieverd. & S. Toro 1987 (Sieverding and Toro, 1987) clustered together in a closely related clade. Further sequences of *Acaulospora* spp. grouped in other major clade complexes: (i) *A. mellea* Spain & N.C. Schenck 1984, *A. morrowiae* Spain & N.C. Schenck 1984 (Schenck et al., 1984) and a sequence from *Calliandra thirsifolia* roots collected in Costa Rica, (ii) *A. laevis* Gerd. & Trappe 1974 (Gerdemann and Trappe, 1974) and *A. colossica* P.A. Schultz et al. 1999 (Schultz et al., 1999), (iii) *A. alpina* Oehl et al. 2006 (Oehl et al., 2006), (iv) sequences from field collected AM colonized roots from Central European mountainous and alpine areas, (v) *A. lacunosa* J.B. Morton 1986 (Morton, 1986) and other sequences from Costa Rica (Shepherd et al., 2007), and vi) *A. scrobiculata* sequences from Brazil (identified by J.B. Morton, see Materials and methods) and from Benin (Tchabi et al., 2009, 2010).

Discussion

The new AM fungal species *A. sieverdingii* can readily be distinguished from all other species in the genus *Acaulospora* by spore morphology due to its unique combination of spore size, spore color and the characteristic inconspicuous, tiny and irregular pits on the second layer of the outer spore wall. Two other *Acaulospora* species have spores similar in size and color, with a pitted ornamentation on the outer spore wall: *Acaulospora paulinae* and *A. undulata* Sieverd. 1988 (Sieverding, 1988). However, both species have broader and regular shaped pits (Sieverding, 1988; Błaszkowski, 1989; Oehl et al., 2006). Morever, *A. undulata* does not have a spore wall structure characteristic for the Acaulosporaceae, and it has been suggested that this species should belong to the genus *Archaeospora* (Oehl et al., 2006; Sieverding and Oehl, 2006). *Acaulospora taiwanensis* H.T. Hu 1988 (Hu, 1988) and *A. alpina* also form 'pitted' spores and have a similar spore diameter as *A. sieverdingii*. However, these species have yellow to orange-yellow spores. Furthermore, spores of *A. taiwanensis* have densely packed 4- to 5-sided pits giving the appearance of a reticulate, meshed ornamentation, and spores of *A. alpina* have very distinctive, regularly dispersed, circular, truncated cone-shaped pits. Mature spores of *A. scrobiculata*, *A. cavernata* and *A. excavata* Ingleby & C. Walker 1994 (Ingleby et al., 1994) are larger and have thicker walls than those of *A. sieverdingii*. However, the shape of the pits of *A. sieverdingii* is most similar to that of *A. scrobiculata* although smaller in size.

Phylogenetic analyses of the ITS1, 5.8S and ITS2 regions of the nuclear ribosomal gene resolved each of the species analyzed in a monophyletic, well-supported clade, and thus provide confirmation that *A. sieverdingii, A. alpina*, *A. cavernata*, *A. paulinae* and *A. scrobiculata* are separate species. These analyses placed *A. sieverdingii* adjacent to *A. paulinae*. Interestingly, also *A. denticulata*, although morphologically a very different species, is phylogenetically closely related to *A. sieverdingii*, *A. paulinae* and especially to *A. cavernata*. Moreover, a few *Acaulospora* spp., that do not possess outer spore wall ornamentation (*A. morrowiae* and *A. mellea*), also appear to be more closely related to *A. sieverdingii* than to, for example *A. alpina*.

In Central Europe, *A. sieverdingii* was recovered from several lowland grasslands and multiple crop rotation systems in soils of pH 5.0-6.8, but it was not found in calcareous soils or in maize mono-cropping systems (Oehl et al., 2010; Oehl, unpublished). The new species usually shared the habitats with 3-7 other *Acaulospora* spp. Interestingly it was not detected together with *A. alpina,* which was never found below 1300 m asl in the Alps or in the European lowlands (Oehl et al., 2006). While *A. paulinae*, which obviously also occurs in Northern America (e.g. Koske et al., 1997), is frequently recovered in Central Europe from the lowlands and up to high alpine areas of 2800 m asl, *A. sieverdingii* was detected in the European lowland and lower mountainous areas solely below 1.200 m asl. Nevertheless, the

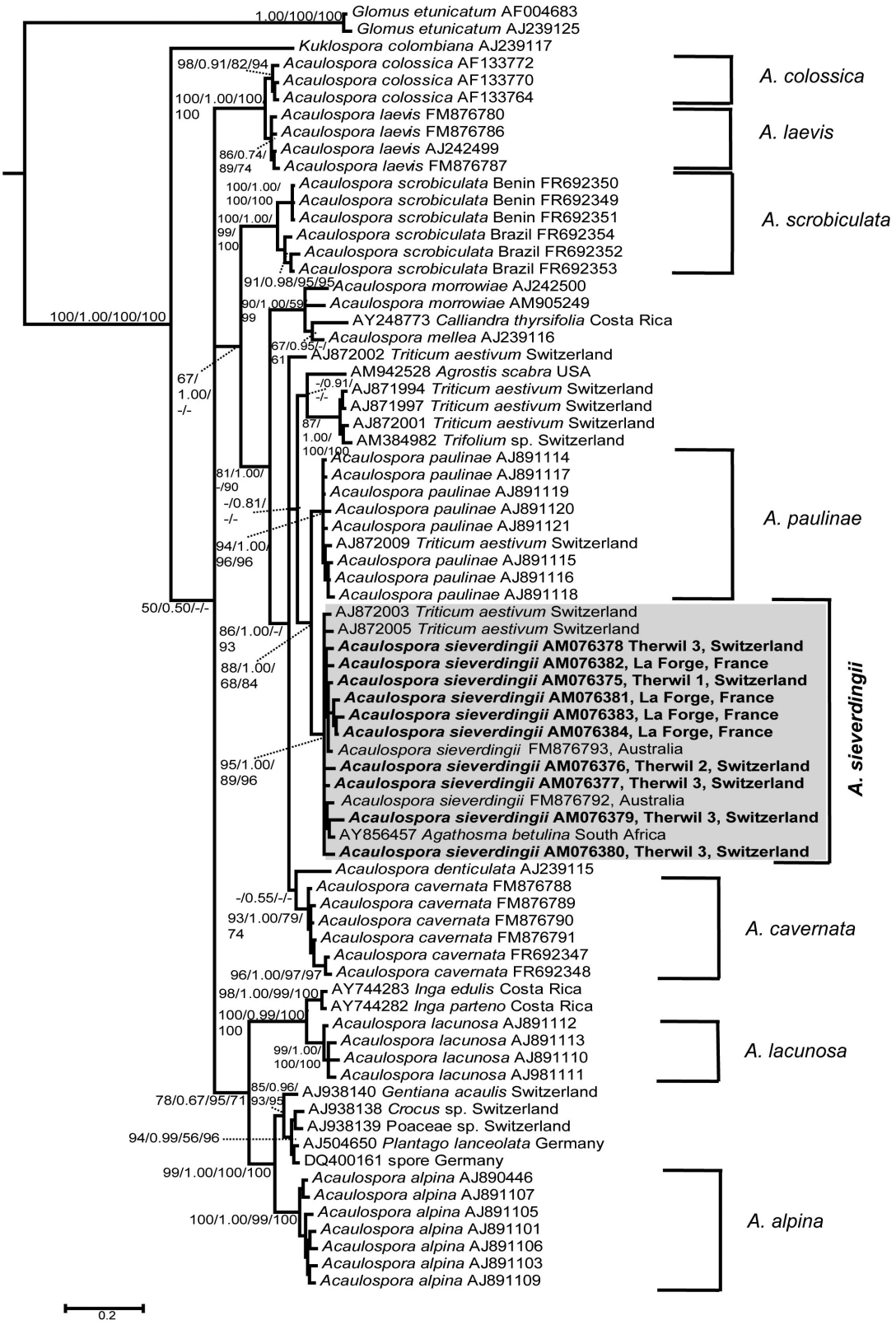


Fig. 7: Phylogenetic tree of the Acaulosporaceae obtained by Bayesian analysis from ITS1, 5.8S rDNA and ITS2 sequences of different *Acaulospora* spp. The numbers above each branch line denotes the support values of maximum likelihood, Bayesian analysis, neighbor joining and maximum parsimony methods, respectively. The tree was rooted by *Glomus etunicatum*. Sequences of *A. sieverdingii* are shown in boldface. They are labeled with the database accession number, name of the field site and country of origin. Multiple spores analyzed from Therwil are numbered 1, 2, 3. GenBank sequences obtained from roots are labeled with the host plant species name, accession number and country of origin. GenBank sequences obtained from spores are labeled with the name of the fungal species and accession number. (Consistency Index = 0.55; Retention Index = 0.85). Note: *Kuklospora colombiana* (sensu Sieverding and Oehl 2006) is the former *Entrophospora colombiana* (SCHENCK et al., 1984). The *A. cavernata* isolate had been deposited in the European Bank of Glomeromycota under the accession number BEG33 as *A. scrobiculata* (see Material and methods).

new species appears to be widely distributed: sequences originating from roots or spores from South Africa (Cloete et al., 2007) and Australia (Krüger et al., 2009) cluster in the *A. sieverdingii* clade. Spores recovered from soils under natural vegetation and in cultivated sites in the Sudan and Guinean Savannahs of Benin (tropical West Africa) are now identified as *A. sieverdingii*. In Benin, *A. paulinae* was not recovered or cultured from any of the samples. Interestingly, it was also observed from lowland Europe samples that *A. sieverdingii* and *A. paulinae* appear to have differing periods of activity during the year. For example, molecular root analyses from field samples and from trap cultures revealed that *A. sieverdingii* is more active earlier in the year, during spring, than *A. paulinae* (Hijri et al., 2006). This was confirmed from trap cultures, with samples taken in May 2001 containing only spores of *A. sieverdingii* but not *A. paulinae* (see above), while abundant spores of *A. paulinae* were recovered from samples taken later in the season (Oehl et al., 2004, 2009). Recently, such differences in temporal activities were also described for two other closely related species occurring in Swiss lowlands: ornamented *Cetraspora helvetica* and smooth *C. pellucida* (Oehl et al., 2010). We suggest that co-occurrence of related species that share different temporal niches in the same soils might be common in the Glomeromycota.

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