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Morphology and phylogeny of *Acaulospora foveata* (Glomeromycetes) from Mexico

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Acaulospora foveata, a glomeromycete fungus with pitted ornamentation on the outer spore surface, was originally isolated from a sugar cane field close to the city Orizaba, Veracruz, Mexico, in 1982. At that time, the concepts of morphological spore descriptions were less evolved, and only two wall layers are mentioned in the protologue, an outer pigmented layer and an inner hyaline layer. In recent years, several *Acaulospora* spp. with pitted ornamented spore surfaces were described. In order to minimize errors on the identification of *A. foveata* and similar fungal species, we studied the holotype, isotype and newly collected epitype material from the type location and morphologically re-described the fungus from these types, and we performed molecular phylogenetic analyses of sequences obtained from the ribosomal gene gained from single spores collected at the type location. *Acaulospora foveata* has - like many other species in the genus - three spore walls, including an inner, 'beaded' wall, and at least eight spore wall layers. Phylogenetically, the fungus clusters in a clade well separated from all other known *Acaulospora* spp., and it is most closely related to *A. lacunosa*. We conclude that with this new information *A. foveata* can now be correctly identified also by molecular analyses.

Keywords: Arbuscular mycorrhizal fungus, Glomeromycota, Acaulosporaceae.

Many arbuscular mycorrhizal (AM) fungal species were described in the 1970's and 1980's, after spores could be isolated by new methods and easier be identified by type of spore formation and spore morphology (e.g. Gerdemann & Trappe 1974, Schenck et al. 1984). The number of species descriptions increased rapidly and a high diversity of AM fungi was virtually found in all terrestrial ecosystems with flowering plants (e.g. Cabello et al. 1994, Oehl & Körner 2014). However, little attention was initially given to exact wall layer descriptions, i.e. outer and inner walls and layers of each wall (e.g. Hall 1977, Nicolson & Schenck 1979). Specific nomenclature for wall layers started with detailed wall and wall layer definitions and elaborated 'murographs' (e.g. Walker 1983, Schenck et al. 1984). Since then specific wall characteristics and definitions were proposed, and new interpretations are still being proposed (e.g. Oehl & Sieverding 2004, Oehl et al. 2008, 2011 a, Lima et al. 2014, Marinho et al. 2014, Sieverding et al. 2014, Błaszkowski et al. 2015). Today it is clear that especially *Acaulospora* spp. have spores with three walls, of which each can have multiply layers (e.g. Schenck et al. 1984, Stürmer & Morton 1999).

Several key species of Acaulosporaceae were already described in the 70th and 80th, e.g. *A. laevis*, *A. elegans*, *A. scrobiculata*, *A. spinosa* and *A. foveata* (Gerdemann & Trappe 1974, Trappe 1977, Walker & Trappe 1981, Janos & Trappe 1982). While some of them have more or less clear diagnostic ornamentations on the outer spore surface, the morphological identification of others has became uncertain, since later other species with similar wall characteristics

have been separated, those usually based on concomitant morphological and molecular phylogenetic analyses in the more recent descriptions. We see the need to isolate again the earlier described *Acaulospora* spp. from the locations of the types, to redescribe the species on the base of new observations, and to generate molecular phylogenetic data for those species. We especially want to more precisely delimit the surface ornamentation structures, and the wall and wall layer composition, as such morphological characteristics are often the first step to identify and organize species in diversity studies.

The objective of this study was therefore to reisolate *A. foveata* Trappe & Janos (Janos & Trappe 1982) from its type location in Orizaba (Veracruz State) of tropical Mexico, to re-examine the spores of the type, to complement the species description, and to extract and amplify DNA from the spores for subsequent molecular phylogenetic analyses on the ribosomal gene.

Material and methods

Study site

The type location was briefly described in the protologue as a sugar cane field 'approximately 5 km Northeast of the city Orizaba' (Janos & Trappe 1982). We took soil samples (about 1 kg) at about the same distance and orientation from the city, and also in a sugar cane field (18° 53' 30" N, 97° 02' 30" W, about 1150 m a.s.l.), in November 5, 2013. The site is located in the central mountainous zone of the Sierra Madre oriental, next to the valley of the volcano Orizaba (5636 m a.s.l.). The soil type is a clayey Andosol with pH of approximately 4.0. The mean annual temperature at study site is about 25°C, and the mean annual rainfall is circa 2700 mm. The natural vegetation in the surroundings of the study site is an evergreen tropical rainforest.

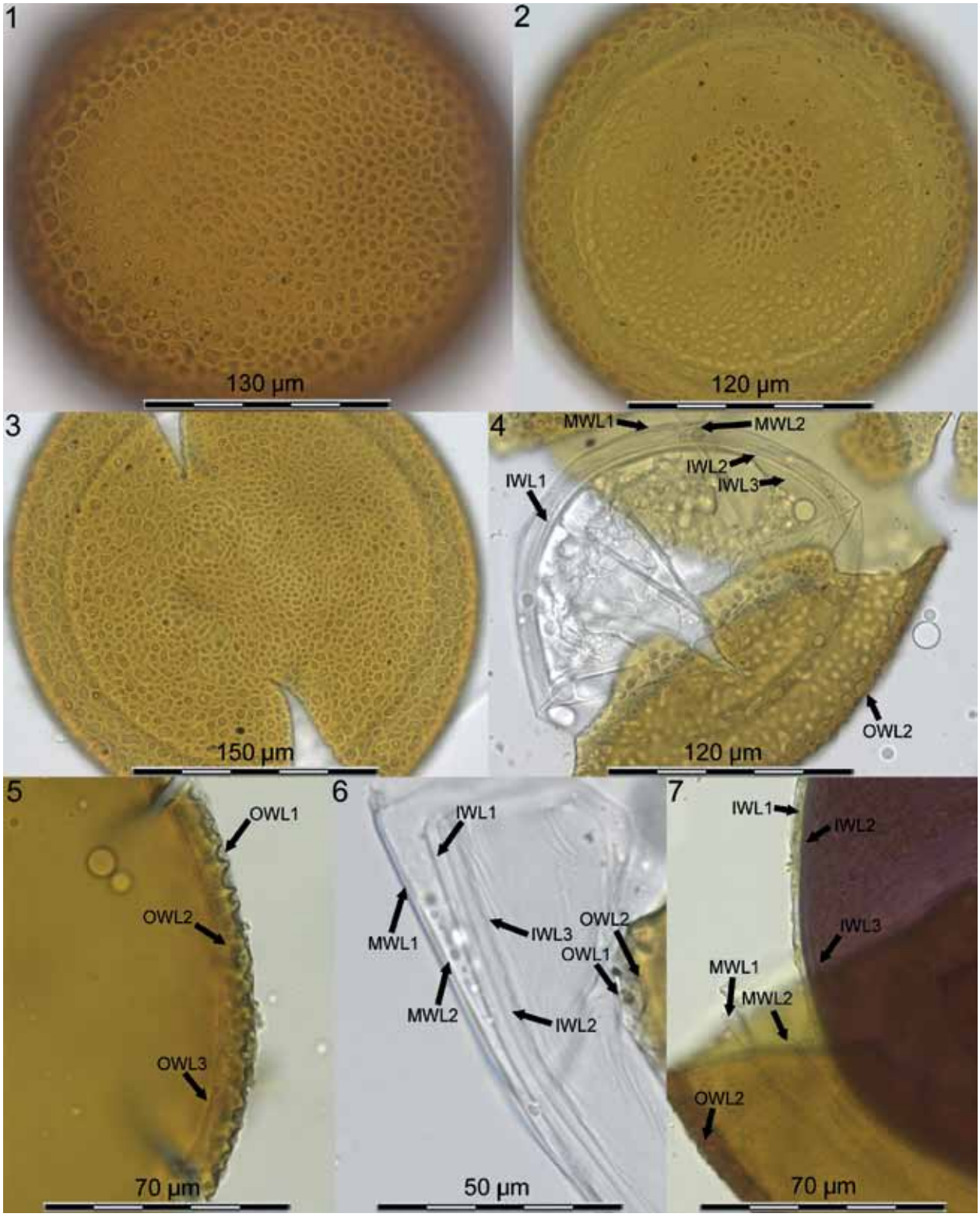
Morphological analyses

Holotypes and isotypes of *A. foveata*, originally deposited by Janos & Trappe at the Mycological Herbarium of the Oregon State University (OSC, in Corvallis, Oregon, USA), at the Escuela Nacional de Ciencias Biológicas (ENCB, in Mexico City, Mexico) and at the Instituto de Ecología (XAL) in Xalapa (Veracruz, Mexico) were re-analyzed as described in preceding publications (e.g. Oehl et al. 2008, 2011 a, 2011 b).

Spores from the newly collected soils, derived from the type area in 'approximately 5 km Northeast of the city Orizaba' (Janos & Trappe 1982), were isolated and analyzed, as described in Sieverding (1991). They were analyzed 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 to water at 1:1, Melzer's reagent, and in water (Spain 1990). The terminology of the spore wall structure basically is that presented in Oehl et al. (2012) for Acaulosporaceae. Newly prepared slides were deposited as epitypes at the Mycological Herbarium of ETH Zurich (Z+ZT), at the Instituto de Ecología (XAL) and at the Universidad Veracruzana (Laboratorio de Organismos Benéficos).

Molecular analyses

Spores were isolated from the soil taken from the type location in November 2013. They were surface-sterilized with chloramine T (2 %) and streptomycin (0.02 %) (Mosse 1962) and crushed singly with a sterile disposable micropestle in 40 µl milli-Q water, as described in Palenzuela et al. (2013). PCRs using the crude extracts as target were performed in an automated thermal cycler (Gene Amp PCR System 2400, Perkin-Elmer, Foster City, California) 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 amplified the SSU end, ITS1, 5.8S, ITS2 and partial LSU rDNA fragment using the SSUmAf/LSUmAr and SSUmCf/LSUmBr primers consecutively (Krüger et al. 2009). PCR products were checked by electrophoresis in 1.2 % agarose gels stained with Gel Red™ (Biotium Inc., Hayward, CA, U.S.A.) and viewed by UV illumination. The amplicons of appropriate size were purified using the GFX PCR DNA kit and Gel Band Purification Illustra, cloned into the PCR 2.1 vector (Invitrogen, Carlsbad, CA, USA), and transformed into One shot® TOP10 chemically competent *Escherichia coli* cells. After plasmid isolation from transformed cells, the cloned DNA fragments were sequenced with universal forward and reverse vector primers (T7 and M13r) on an automated DNA sequencer (Perkin-Elmer ABI Prism 373). Sequence data were compared to public sequence databases using BLAST (Altschul et al. 1990). The percentage of identity among the *Acaulospora* sequences was calculated using the BLASTn analysis. The new sequences were deposited in the EMBL database under the accession numbers LN736022-LN736027.



Figs. 1–7. *Acaulospora foveata*. **1–5.** Intact and crushed spores with three multiple-layered walls: OWL1-3, MWL1-2, and IWL1-3. IWL1 with granular, 'beaded' structure. In dented and crushed spores (Figs. 2-4), the surface pits appear often more irregular than in totally globose spores (Fig. 1). **5.** Spore wall composition; granular structures of IWL1 disappeared through repeated pressure on the cover slide. 6. Crushed spore segment exposed to Melzer's reagent: IWL2 & IWL3 staining purple to dark purple.

Phylogenetic analyses

Some *Acaulospora* spp. have sequences either from LSU rDNA or from ITS regions (e.g. *A. colliculosa*, *A. colossica*, *A. denticulata, A. dilatata, A. herrerae*, *A. longula* and *A. tuberculata*). Thus, the phylogeny was reconstructed by independent analyses of the ITS region and of the partial LSU rDNA. The AM fungal sequences obtained were aligned with other Acaulosporaceae sequences from GenBank in ClustalX (Larkin et al. 2007). *Claroideoglomus etunicatum* (W.N.Becker & Gerd.) C.Walker & A.Schüssler 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 2×10^6 generations with a sample frequency of 200 and a burn-in value of 25 %) and maximum likelihood (1000 bootstrap) analyses were performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), respectively, launched from Topali 2.5, using the GTR + G model. Maximum parsimony analysis was performed using PAUP*4b10 (Swofford 2003) with 1000 bootstrap replications.

Results

Taxonomy

Acaulospora foveata Janos & Trappe – Figs. 1–7
Mycobank MB109576

Epitype. – Mexico, Veracruz, Orizaba (18°53'30"N, 97°02'30"W). Isolated from a sugar cane field. Soil collected by Da. H. Lee Espinoza. Epitype isolated by F. Oehl, L. Capistrán & R. Zulueta, deposited at Z+ZT under the accession ZT Myc 56320, and **here designated.**

Description. – Spores formed laterally on the neck of sporiferous saccules in approximately 100–250 µm distance to the saccule terminus. They are yellowish brown to yellow brown to sometimes reddish brown, becoming brown to black brown with age, globose to ellipsoid, 185–310(410) × 215–350(480) µm in diam., and have three walls (outer, middle, inner wall). The sporiferous saccule termini are of similar size as the spores formed on the saccule necks.

Outer wall with three layers (OWL1-3). OWL1 hyaline to subhyaline, evanescent, 1.0–1.8 µm thick. OWL2 yellowish brown to reddish brown, laminate, 8.5–15 µm thick, with round to oblong, and concave depressions, generally 3.5–8.5(12) × 3.5–12.5(16) µm in diameter, and 1.5-3.5 µm deep. Regularly, some pits were also of smaller sizes (up to 1.3 x 1.3 µm). OWL3 concolorous with OWL2, and regularly tightly adherent to it, persistent and 0.9-1.5 µm thick.

Middle wall with two almost identical, flexible and hyaline layers (MWL1-2), together 1.7–2.7 µm thick.

Inner wall with three layers (IWL1-3). IWL1 hyaline to subhyaline, 1.3–2.4(3.0) µm thick, with granular, 'beaded' structure, that may completely disappear, and become completely hyaline under pressure applied on the cover slide in harshly crushed spores. IWL2 is hyaline, 2.5–5.5 µm thick, expanding up to 15 µm under pressure applied, staining purple to dark purple in Melzer's reagent. IWL3 also hyaline, 0.6–1.2 µm thick, often showing several folds in crushed spores, and also staining purple to dark purple in Melzer's reagent.

Cicatrix at spore base closed by laminae of OWL2 and by adherent OWL3, 18-25 µm in diameter.

Molecular phylogenetic analyses. – In the molecular phylogenetic analyses *A. foveata* and *A. lacunosa* formed together a well supported clade separate from other *Acaulospora* spp. (Figs. 8–9). Only one rDNA sequence of *A. foveata* was found in the NCBI data bank (isolate CR315 from INVAM originating from Costa Rica). The sequences obtained in the present study grouped with the isolate CR315 in the LSU rDNA tree. It was not possible to separate *A. foveata* from *A. lacunosa* in the LSU rDNA tree (Fig. 8). However, the phylogram obtained by the ITS sequences showed *A. foveata* and *A. lacunosa* in different clades with high support values (Fig. 9). The species most closely related to *A. foveata* was *A. lacunosa* with 96 % and 94 % of identity to the LSU rDNA and ITS sequences, respectively.

The environmental LSU rDNA sequences with closest match (97–99 %) to *A. foveata* were found in roots of mangrove plants (HM570008, HQ243214) sampled in Zhuhai Mangrove Area, Guangdong province – China (Wang et al. 2011), roots of *Voyria aphylla* (HQ857180-HQ857181) and spores (JF276256-JF276257) collected in a tropical rainforest of the Guadeloupe Caribbean Island (Courty et al. 2011) and in soil (JN890255) from a tropical rainforest in Guyana (McGuire et al. 2010). For the ITS region, 98 % of identity was found among sequences from *A. foveata* and those from roots of mangrove plants (HM570008, HQ243214) sampled in Zhuhai Mangrove Area, Guangdong province – China (Wang et al. 2011) and roots of *Voyria aphylla* (HQ857180), collected in a tropical rainforest of the Guadeloupe Caribbean Island (Courty et al. 2011).

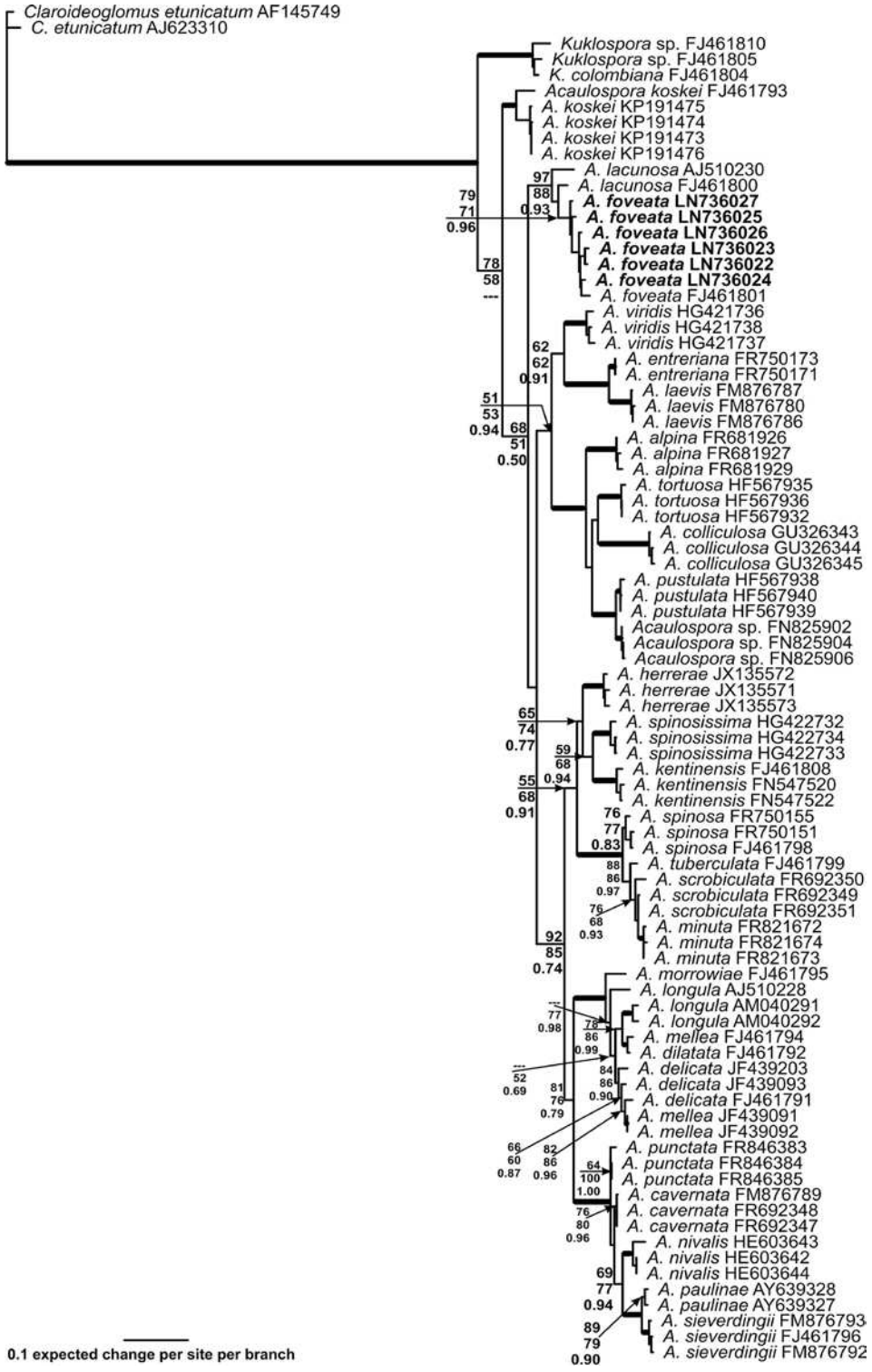


Fig. 8. Phylogenetic tree of the Acaulosporaceae obtained by analysis from partial LSU rDNA sequences of different *Acaulospora* spp. Sequences are labeled with their database accession numbers. Support values (from top) are from maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses, respectively. Topology of the tree is the same presented by the consensus tree of the ML analysis. Sequences obtained in this study are in boldface. Only support values of at least 50 % are shown. Thick branches represent clades with more than 90 % of support in all analyses. The tree was rooted by *Claroideoglomus etunicatum*. (Consistency Index = 0.50; Retention Index = 0.86).

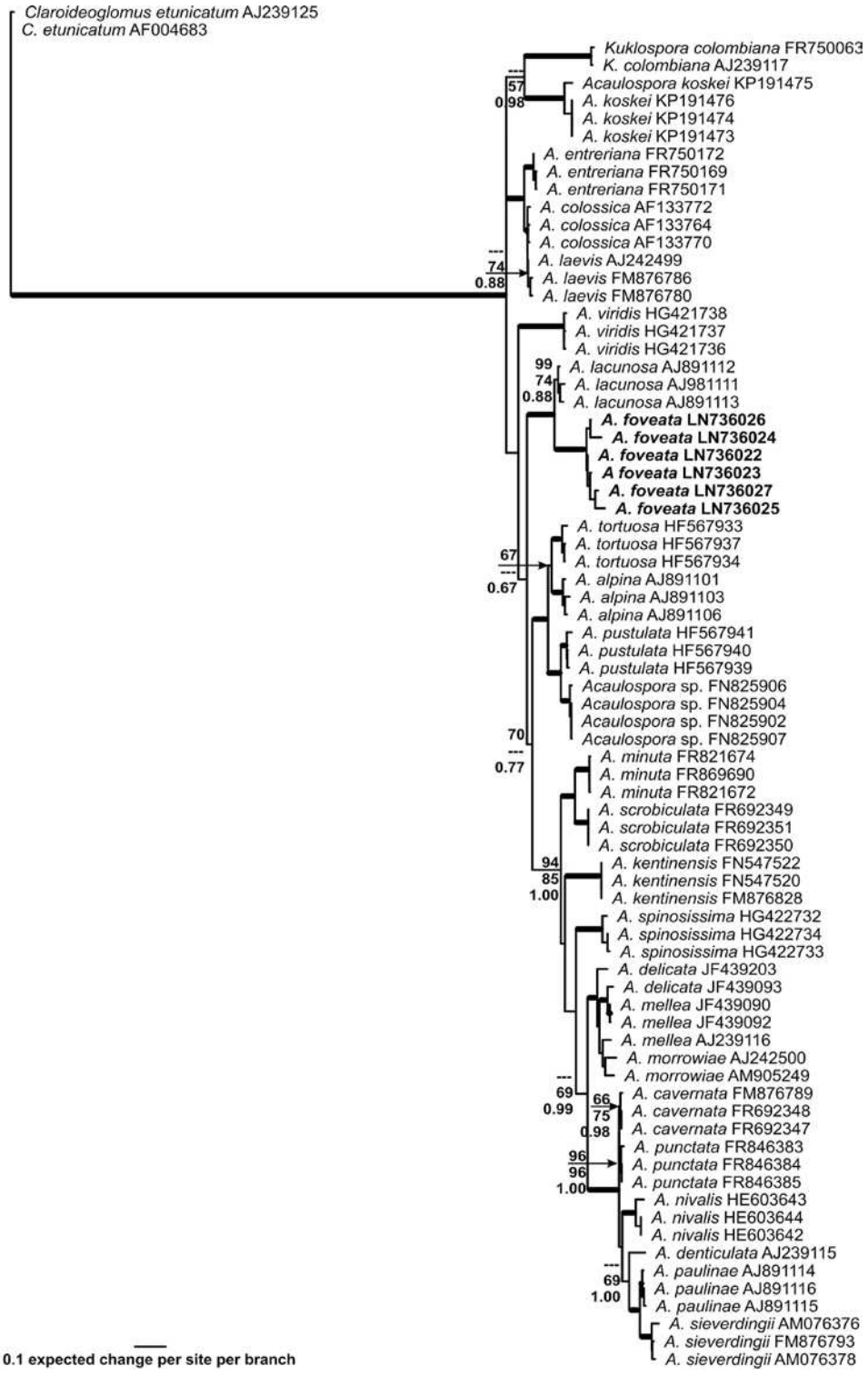


Fig. 9. Phylogenetic tree of the Acaulosporaceae obtained by analysis from ITS1, 5.8S rDNA and ITS2 sequences of different *Acaulospora* spp. Sequences are labeled with their database accession numbers. Support values (from top) are from maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses, respectively. Topology of the tree is the same presented by the consensus tree of the ML analysis. Sequences obtained in this study are in boldface. Only support values of at least 50 % are shown. Thick branches represent clades with more than 90 % of support in all analyses. The tree was rooted by *Claroideoglomus etunicatum*. (Consistency Index = 0.49; Retention Index = 0.84).

Discussion

Acaulospora foveata was originally described with two wall layers (Janos & Trappe 1982). At that time, the species could easily be identified by its acaulosporoid spore formation and the diagnostic surface ornamentation. It was distinct from those of all other *Acaulospora* species with pitted surfaces described until then (*A. srobiculata*, *A. bireticulata* and *A. elegans*; Gerdemann & Trappe 1974; Trappe 1977, Rothwell & Trappe 1979). Five genera with acaulosporoid spore formation (sensu lato), have hitherto been known: *Acaulospora*, *Otospora*, *Ambispora*, *Archaeospora* and *Palaeospora* (Spain et al. 2006, Palenzuela 2008, Oehl et al. 2011 b, 2015). They can clearly be differentiated from each other by spore morphology and especially by spore wall composition (Oehl et al. 2015).

In this study, it was re-inforced that *A. foveata* isolated from the type location in Orizaba (Mexico) belongs to the Acaulosporaceae. It has the characteristic spore wall composition of this family consisting of three spore walls including the 'beaded' structure of the inner wall surface. The large spore size and the characteristic pitted spore surface ornamentation makes it easy to identify *A. foveata* morphologically, and to group it into the genus *Acaulospora* as it was suggested by Janos & Trappe (1982). *Acaulospora foveata* can be differentiated from *A. lacunosa* (Morton 1986) and all other known 'pitted' *Acaulospora* spp. by having substantially bigger spores as summarized in an overview for all *Acaulospora* spp. in recent published identification keys (Błaszkowski 2012, Oehl et al. 2012).

The ITS and partial sequence of the LSU rDNA analyzed also unequivocally confirmed the correctness of the genus and species identification of Janos & Trappe (1982). The fungus is most closely related to *A. lacunosa*. In addition, it was recognized that the *A. foveata* sequences obtained from spores from Costa Rica coincided with our sequences from Orizaba, Mexico. Our sequences obtained from the location of the type can be used in the future to identify this species phylogenetically through molecular analyses, globally and in whatever ecosystem.

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References

Altschul S. F., Gish W., Miller W., Myers E. W., Lipton D. P. (1990) Basic local alignment search tool. *Journal of Molecular Biology* **215**: 403–410.

Błaszkowski J. (2012) *Glomeromycota*. W. Szafer Institute of Botany, Polish Academy of Sciences.

Błaszkowski J., Chwat G., Góralska A., Ryszka P., Kovács G. M. (2015) Two new genera, *Dominikia* and *Kamienska*, and *D. disticha* sp. nov. in Glomeromycota. *Nova Hedwigia* **100**: 225–238.

Brundrett M., Melville L., Peterson L. (1994) *Practical methods in mycorrhizal research*. Mycologue Publications, University of Guelph, Guelph, Ontario, Canada.

Cabello M. N., Gaspar M. L., Pollero R. J. (1994) *Glomus antarcticum* sp. nov., a vesicular-arbuscular mycorrhizal fungus from Antarctica. *Mycotaxon* **51**: 123–128

Courty P. E., Walder F., Boller T., Ineichen K., Wiemken A., Rousteau A., Selosse M. A. (2011) Carbon and nitrogen metabolism in mycorrhizal networks and mycoheterotrophic plants of tropical forests: a stable isotope analysis. *Plant Physiology* **156**: 952–961.

Gerdemann J. W., Trappe J. M. (1974) The Endogonaceae in the Pacific Northwest. *Mycologia Memoir* **5**: 76.

Guindon S., Gascuel O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *System Biology* **52**: 696–704.

Hall I. R. (1977) Species and mycorrhizal infections of New Zealand Endogonaceae. *Transactions of the British Mycological Society* **68**: 341–356.

Janos D. P., Trappe J. M. (1982) Two new *Acaulospora* species from tropical America. *Mycotaxon* **15**: 515–522.

Koske, R.E. Tessier B. (1983) A convenient, permanent slide mounting medium. *Mycological Society of America Newsletter* **34**: 59.

Krüger M., Stockinger H., Schüßler A. (2009) DNA-based species-level detection of arbuscular mycorrhizal fungi: one PCR primer set for all AMF. *New Phytologist* **183**: 212–223.

Larkin M. A., Blackshields G., Brown N. P., Chenna R., McGettigan P. A., McWilliam H., Valentin F., Wallace I. M., Wilm A., Lopez R., Thompson J. D., Gibson T. J., Higgins D. G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**: 2947–2948.

Lima L. L., Kozovits A. R., Assis D. M. A., Silva G. A., Oehl F. (2014) *Cetraspora auronigra*, a new glomeromycete species from Ouro Preto (Minas Gerais, Brazil). *Sydowia* **66**: 299–308.

Marinho F., Silva G. A., Ferreira A. C. A., Veras J. S. N., Sousa N. M. F., Goto B. T., Maia L. C., Oehl F. (2014) *Bulbospora minima*, new genus and new species in the Glomeromycetes from semi-arid Northeast Brazil. *Sydowia* **66**: 313–323.

McGuire K. L., Zak D. R., Edwards I. P., Blackwood C. B., Upchurch, R. (2010) Slowed decomposition is biotically mediated in an ectomycorrhizal, tropical rain forest. *Oecologia* **164**: 785–795.

Milne I., Wright F., Rowe G., Marshal D. F., Husmeier D., McGuire G. (2004) TOPALi: Software for automatic identification of recombinant sequences within DNA multiple alignments. *Bioinformatics* **20**: 1806–1807.

Morton J. B. (1986) Three new species of *Acaulospora* (Endogonaceae) from high aluminium, low pH soils in West Virginia. *Mycologia* **78**: 641–648.

Mosse B. (1962) Establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. *Journal of Genetic Microbiology* **27**: 509–520.

Nicolson T. H., Schenck N. C. (1979) Endogonaceous mycorrhizal endophytes in Florida. *Mycologia* **71**: 178–198.

Oehl F., Körner C. (2014) Multiple mycorrhization at the coldest place known for Angiosperm plant life. *Alpine Botany* **124**: 193–198.

Oehl F., Sieverding E. (2004) *Pacispora*, a new vesicular arbuscular mycorrhizal fungal genus in the Glomeromycetes. *Journal of Applied Botany and Food Quality* **78**: 72–82.

Oehl F., de Souza F. A., Sieverding E. (2008) Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular mycorrhiza-forming *Glomeromycetes*. *Mycotaxon* **106**: 311–360.

Oehl F., Silva G. A., Goto B. T., Sieverding E. (2011 a) Glomeromycota: three new genera, and glomoid species reorganized. *Mycotaxon* **116**: 75–120.

Oehl F., Sieverding E., Palenzuela J., Ineichen K., Silva G. A. (2011 b) Advances in Glomeromycota taxonomy and classification. *IMA Fungus* **2**: 191–199.

Oehl F., Palenzuela J., Sánchez-Castro I., Kuss P., Sieverding E., Silva G. A. (2012) *Acaulospora nivalis*, a new fungus in the Glomeromycetes, characteristic for high alpine and nival altitudes of the Swiss Alps. *Nova Hedwigia* **95**: 105–122.

Oehl F., Sánchez-Castro I., Palenzuela J., Silva G. A. (2015) *Palaeospora spainii*, a new arbuscular mycorrhizal fungus from Swiss agricultural soils. *Nova Hedwigia* **101**: 89–102.

Palenzuela J., Ferrol N., Boller T., Azcón-Aguilar C., Oehl F. (2008) *Otospora bareai*, a new fungal species in the Glomeromycetes from a dolomitic shrub-land in the National Park of Sierra de Baza (Granada, Spain). *Mycologia* **100**: 296–305.

Palenzuela J., Azcón-Aguilar C., Barea J. M., Silva G. A., Oehl F. (2013) *Septoglomus altomontanum*, a new arbuscular mycorrhizal fungus from mountainous and alpine areas in Andalucía (southern Spain). *IMA Fungus* **4**: 243–249.

Ronquist F., Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Rothwell F.M., Trappe JM (1979) *Acaulospora bireticulata* sp. nov. *Mycotaxon* **8**: 471–475.

Schenck N. C., Spain J. L., Sieverding E., Howeler R. H. (1984) Several new and unreported vesicular-arbuscular mycorrhizal fungi (Endogonaceae) from Colombia. *Mycologia* **76**: 685–699.

Sieverding, E. (1991) Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. Deutsche Gesellschaft für Technische Zusammenarbeit Nr. 224. Hartmut Bremer Verlag, Friedland, Germany.

Sieverding E., Silva G. A., Berndt R., Oehl F. (2014) *Rhizoglomus*, a new genus in the Glomeraceae. *Mycotaxon* **129**: 373–386.

Spain J. L. (1990) Arguments for diagnoses based on unaltered wall structures. *Mycotaxon* **38**: 71–76.

Spain J. L., Sieverding E., Oehl F. (2006) Appendicispora, a new genus in the arbuscular mycorrhiza-forming Glomeromycetes, with a discussion of the genus Archaeospora. Mycotaxon **97**: 163–182.

Swofford D.L. (2003) *PAUP*. Phylogenetic analysis using parsimony (*and other methods)*. Sinauer Associates, Sunderland, Massachusetts.

Stürmer S. L., Morton J. B. (1999) Taxonomic reinterpretation of morphological characters in Acaulosporaceae based on developmental patterns. *Mycologia* **91**: 849–857.

Trappe J. M. (1977) Three new Endogonaceae: *Glomus constrictus*, *Sclerocystis clavispora* and *Acaulospora scrobiculata*. *Mycotaxon* **6**: 359–366.

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

Walker C., Trappe J. M. (1981) *Acaulospora spinosa* sp. nov. with a key of *Acaulospora*. *Mycotaxon* **12**: 515–521.

Wang Y., Huang Y., Qiu Q., Xin G., Yang Z., Shi S. (2011) Flooding greatly affects the diversity of arbuscular mycorrhizal fungi communities in the roots of wetland plants. *PLoS ONE* **6**: e24512.

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