ISSN (print) 0093-4666 ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/118.73

Volume 118, pp. 73–81 *October–December 2011*

Diversispora clara (*Glomeromycetes*)— a new species from saline dunes in the Natural Park Cabo de Gata (Spain)

Beatriz Estrada[1], Javier Palenzuela[1], José-Miguel Barea[1], Juan Manuel Ruiz-Lozano[1], Gladstone Alves da Silva[2] & Fritz Oehl[3*]

[1]*Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, CSIC, Profesor Albareda 1, 18008 Granada, Spain*
[2]*Departamento de Micologia, CCB, Universidade Federal de Pernambuco, Av. Prof. Nelson Chaves s/n, Cidade Universitária, 50670-420, Recife, PE, Brazil*
[3]*Federal Research Institute Agroscope Reckenholz-Tänikon ART, Organic Farming Systems, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland*
*Correspondence to: *fritz.oehl@art.admin.ch*

Abstract — A new species of *Diversispora* (*Glomeromycetes*) was found in saline sand dunes of the Natural Park Cabo de Gata (Almería, Andalucía, Southern Spain) in the rhizosphere of *Asteriscus maritimus*, a plant species especially adapted to saline environments. The new fungal species forms brilliant white spores that are 79–130 × 75–125 µm and have one wall consisting of three layers. The subtending hyphae are, as typical for many *Diversispora* spp., thin-walled, hyaline, and cylindrical (or rarely constricted) and flexible and fragile below the septa separating the spore and hyphal contents. The septa form regularly at the spore bases or, less frequently, in subtending hyphae at short distances from the spore base. Phylogenetic analyses of the ITS and partial 28S ribosomal gene confirm that *D. clara* forms a monophyletic, independent clade within *Diversispora*.

Key words — *Glomus*, *Glomeromycota*, Europe, extreme environments, rDNA

Introduction

During recent studies on arbuscular mycorrhiza (AM) fungal diversity in sand dune systems of the Cabo de Gata Natural Park in Almería (Spain), a brilliant-white new glomeromycotean fungus was recovered from the rhizosphere of *Asteriscus maritimus*, a plant species characteristic of saline Mediterranean environments with elevated soil electrical conductivity. The new fungus formed spores in AM fungal bait cultures predominantly in the *Asteriscus maritimus* rhizosphere. The aims of the present study were to analyze this particular fungus applying combined morphological and molecular tools and to describe its characteristics.

Material & methods

Soil and plant sampling

Soil samples were taken in February 2010 from the rhizosphere of ten *Asteriscus maritimus* plants growing in a natural sand dune system of the Natural Park Cabo de Gata in Almería (Andalucía, Spain). The site is located at 36°44′41″N 02°07′26″E. The samples, air-dried in the laboratory, were used to analyze selected chemical soil parameters (pH, soil organic carbon, total nitrogen, soil electrical conductivity) and spore populations. The ten plants and the surrounding rhizosphere soil were also extracted and used as bait cultures for propagation of AM fungal communities indigenous to the natural sand dune system. At the site, the soil was sandy and with pH (H_2O) 8.2, organic carbon 15.3 g kg^{-1}, total nitrogen 1.9 g kg^{-1}, available P 27.0 mg kg^{-1}, and soil electrical conductivity 1.5 µS cm^{-1}.

AM fungal bait cultures

Bait cultures were established and maintained as described in Palenzuela et al. (2008, 2011) by transplanting 10 *Asteriscus maritimus* plants with their rhizosphere soils into 1 L pots and transferring them to the greenhouse at EEZ in Granada immediately after sampling. The pots were irrigated three times per week and fertilized every four weeks with Long-Aston nutrient solution (Hewitt 1966). Pure cultures of the new fungus were initiated in a mixed-culture of three *Allium porrum* L. and three *Hieracium pilosella* L. plantlets in 750 mL pots grown together at three locations in the pots. The plant rhizosphere was inoculated with 20 spores per pot and pot location. A sterile mixture of Terragreen (American aluminum oxide, Oil Dry US special, type III R; Lobbe Umwelttechnik Iserlohn, Germany) and Loess (mixture 3:1; with pH-KCl 6.2; organic carbon 0.3%; available P (Na-acetate) 2.6 mg kg^{-1}; available K (Na-acetate) 350 mg kg^{-1} was chosen as culture substrate (Oehl et al. 2002). So far, pure cultures of the new fungus have not been obtained.

Morphological analyses

AM fungal spores were separated from the soil samples by a wet sieving process (Sieverding 1991). The morphological spore characteristics and their subcellular structures were described from a specimen mounted in: polyvinyl alcohol-lactic acid-glycerol (PVLG; Koske and Tessier 1983); 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 water (Spain 1990). The spore structure terminology follows Oehl et al. (2003, 2005, 2011a) for species with glomoid or diversisporoid spore formation. Photographs (Figs 1–10) were taken with a Leica DFC 290 digital camera on a Leitz Laborlux S compound microscope using Leica Application Suite Version V 2.5.0 R1 software. Specimens mounted in PVLG and the PVLG+Melzer's mixtures were deposited at the herbaria Z+ZT (ETH Zurich, Switzerland), GDA-GDAC (University of Granada, Spain), and URM (Federal University of Pernambuco, Recife).

Molecular analyses

Five spores isolated from the trap cultures were surface-sterilized with chloramine T (2%) and streptomycin (0.02%) (Mosse 1962) and crushed with a sterile disposable micropestle in 40 µL milli-Q water (Ferrol et al. 2004). PCRs of the crude extracts

were obtained 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 partial SSU, ITS1, 5.8S, ITS2 and partial LSU rDNA ribosomal fragment using the SSUmAf/LSUmAr and SSUmCf/LSUmBr primers consecutively (Krüger et al. 2009). The second PCR products were separated electrophoretically on 1.2% agarose gels stained with Gel Red™ (Biotium Inc., Hayward, CA, U.S.A.) and viewed by UV illumination. The band of the expected size was excised with a scalpel and the amplified DNA was isolated from the gel with the QIAEX II Gel Extraction kit (QIAGEN, Valencia, CA, USA), cloned into the PCR2.1 vector (Invitrogen, Carlsbard, CA, USA), and transformed into one shot TOP10 chemically competent *Escherichia coli* cells. After plasmid isolation from transformed cells, cloned DNA fragments were sequenced with vector primers (White et al. 1990) in both directions by Taq polymerase cycle sequencing on an automated DNA sequencer (Perkin-Elmer ABI Prism 373). Sequence data were compared to gene libraries (EMBL and GenBank) using BLAST (Altschul et al. 1990). The new sequences were deposited in the EMBL database under the accession numbers FR873629–FR873633.

Phylogenetic analyses: The AM rDNA ITS1+5.8S +ITS2 fungal sequences were aligned in ClustalX (Larkin et al. 2007) and edited with BioEdit (Hall 1999) to obtain a final alignment with *Acaulospora laevis* Gerd. & Trappe and *A. lacunosa* J.B. Morton as outgroups. Prior to phylogenetic analysis, the model of nucleotide substitution was estimated using Topali 2.5 (Milne et al. 2004). Bayesian (two runs over 1×10^6 generations with a burn in value of 2500) 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. Neighbor-joining (established with the model cited above) and maximum parsimony analyses were performed using PAUP*4b10 (Swofford 2003) with 1000 bootstrap replications.

Results

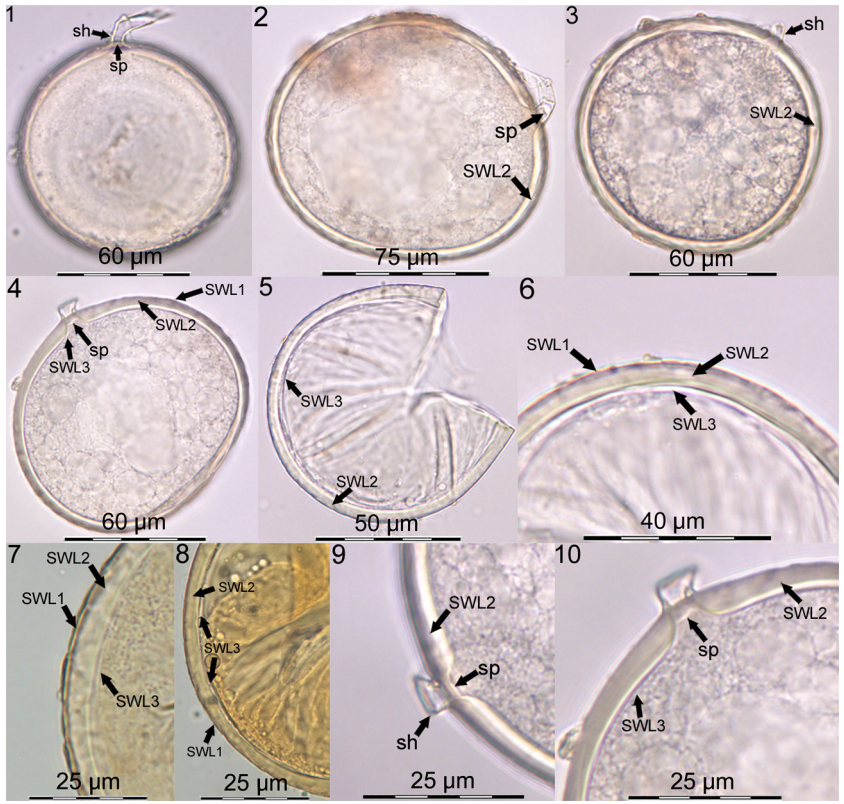
Diversispora clara Oehl, B. Estrada, G.A. Silva & Palenz., **sp. nov.** Figs 1–10

MycoBank MB 561583

Sporae albae, 79–130 × 75–125 μm, tunica tribus stratis, 4.3–8.4 μm. Stratum exterior hyalinum; stratum medium, album ad rarum ochreo-album, 2.8–5.4 μm crassum; stratum interius album. Hypha adhaerenta, 8–13 μm in diametrum. Tunica hyphae 1.0–1.8 μm crassa. Strata medium interiusque septo porum occludentes. Strata exterior mediumque flava colorantes Melzeri.

Type: Spain. Andalucía, Almería, Cabo de Gata Natural Park, sand dune, from the rhizosphere of *Asteriscus maritimus* (L.) Less. (*Asteraceae*), isolation date February 2011, by B. Estrada (**holotype**, ZT Myc 3796 [permanent slide 20-2001]; **isotypes**, ZT Myc 3797 [permanent slides 20-2003 to 20-2012], GDA-GDAC [permanent slides 20-2013 to 20-2018], URM [permanent slides 20-2019, 20-2020]).

Etymology: *clara* (Latin = clear, bright, brilliant, light), referring to the brilliant white spores.



Figs 1–10. *Diversispora clara*: 1–4. Uncrushed spores with a triple-layered spore wall (SWL1-3), a single, cylindrical subtending hypha (sh), and a septum (sp) arising directly or at some distance from the base. Figs 5–6. Spores crushed to show triple layered spore wall (SWL1-3). Figs 7–8. Spore wall structure in PVLG + Melzer's reagent. The SWL1 or SWL2 (or both) stain light to dark yellow in Melzer's, while no such staining reaction is seen on SWL3. However, the yellow stain is usually most noticeable in the spore cell contents. Fig. 9. Septum at spore base formed by SWL2 (SWL3 is not visible in uncrushed spore). Fig. 10. Septum at spore base formed by SWL3.

Spores (Figs 1–4) singly formed in rhizosphere soils, terminally on subtending hyphae, globose (80–110 µm diam.) to subglobose (79–130 × 75–125 µm), one-walled, brilliant to creamy white.

Spore wall 4.3–8.4 µm thick, three-layered (SWL1, SWL2, SWL3; Figs 5–7); outer layer (SWL1) hyaline, 0.8–1.5 µm thick, evanescent and thus often absent in mature spores; second layer (SWL2) bright to (rarely) creamy white, laminated, 2.8–5.4 µm thick (Fig. 7) in uncrushed spores (sometimes ≤ 6.6 µm in crushed spores when pressure is applied on the cover slip); inner layer (SWL3)

brilliant white, 0.7–1.5 µm thick (usually tightly adherent to SWL2 and difficult to observe when < 1.0 µm; see Figs 5–6); both SWL1 and SWL2 generally light to dark yellow in Melzer's reagent (Figs 7–8, with spore cell contents often a more intense yellow than the surrounding cell wall layers (Fig. 8).

Subtending hyphae (sh) generally singly on spores; brilliant white (Figs 1–3), cylindrical or (rarely) slightly constricted at the spore base, 4.0–8.0 µm broad tapering to 3.2–6.1 µm within 100 µm of spore base, although the distance may appear shorter (4.0–10(–25) µm) because the sh walls taper from 1.0–1.8 µm thick to 0.6–1.2 µm within the first 10 µm from the base causing the flexible fragile portion to break from the mature spore (Figs 3, 4, 9) at a point where the septum separates the spore contents from the hyphal contents. Spore pores at the spore bases or in the sh normally closed by a septum (Figs 1–2, 4) arising from SWL2 (Fig. 9), SWL3 (Fig. 10), or both layers (not shown); pores rarely open (Fig. 3).

Distribution: Known only in the natural sand dune system of the Cabo de Gata Natural Park in Andalucía in the rhizosphere of *Asteriscus maritimus*.

Molecular analyses: Phylogenies derived from ITS (Fig. 11) and 28S (data not shown) rDNA analyses cluster the new fungus within the *Diversisporaceae* in a well-separated clade adjacent to several other *Diversispora* species, *Otospora bareae,* and *Tricispora nevadensis* (Oehl et al. 2011b).

Discussion

Our morphological analyses, in particular those of the subtending hyphae and spore bases, clearly support the new fungus in *Diversispora*, with many characters identical to those of other *Diversispora* species (e.g., *D. spurca, D. eburnea;* see Oehl et al. 2011a,b) such as the thin-walled, hyaline, cylindrical (or rarely constricted) subtending hyphae that appear flexible and fragile behind the septum that closes the pore at the spore base or in short distance from the base. In *Diversispora* species with pigmented spores, the subtending hyphae regularly change color conspicuously, becoming hyaline to white behind the septum (Oehl et al. 2011a). This color change, however, was not confirmed for *D. clara*, since the new fungus generally does not form pigmented spores.

Molecular analyses confirm the species as new: in the phylogenetic tree, *D. clara* clusters in a independent, monophyletic clade within a polyphyletic *Diversispora*. These sequence analyses also confirm unequivocally using morphology to identify diversisporoid species within the *Glomeromycota* (Oehl et al. 2011a,c).

Including *D. clara*, there are now 14 *Diversispora* spp. known in the *Glomeromycetes* (Oehl et al. 2011a,b). The 9 species that form significantly pigmented, yellow brown to brown or orange to orange brown spores are *D. arenaria* (Błaszk. et al.) Oehl et al., *D. aurantia* (Błaszk. et al.) C. Walker & A. Schüssler, *D. epigaea* (B.A. Daniels & Trappe) C. Walker & A. Schüssler,

D. insculpta (Błaszk.) Oehl et al., *D. przelewicensis* (Błaszk.) Oehl et al., *D. pustulata* (Koske et al.) Oehl et al., *D. tenera* (P.A. Tandy) Oehl et al., *D. trimurales* (Koske & Halvorson) C. Walker & A. Schüssler, and *D. versiformis* (P. Karst.) Oehl et al. (Oehl et al. 2011a,b). *Diversispora celata* C. Walker et al. forms triple-layered, ochre to ivory to pinkish cream spores (Gamper et al. 2009). Only *D. spurca* (C.M. Pfeiff. et al.) C. Walker & A. Schüssler, *D. eburnea* (L.J. Kenn. et al.) C. Walker & A. Schüssler, and *D. gibbosa* (Błaszk.) Błaszk. & Kovács form hyaline to subhyaline spores that are, however, never brilliant-white as observed for *D. clara*. Moreover, *D. spurca* and *D. eburnea* have bi-layered spore walls (Kennedy et al. 1999), *D. gibbosa* has a five-layered wall (Błaszkowski 1997), and *D. clara* has a three-layered wall.

In the past, large-spored or sporocarpic AM fungi were described from sand dune systems, such as *Gigaspora*, *Scutellospora*, *Pacispora* or *Glomus* species that were generally easy to isolate and recognize from field samples (Koske & Gemma 1995, Gemma et al. 1989, Błaszkowski 1994). Likewise in the current Cabo de Gata Natural Park sand dune system, where *Funneliformis coronatus* (Giovann.) C. Walker & A. Schüssler, *F. mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler, *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders, *Racocetra persica* (Koske & C. Walker) Oehl et al., and *Glomus macrocarpum* Tul & C. Tul. were identified (Estrada, unpublished). When sand dune AM fungal communities were maintained and reproduced in bait cultures, small-spored *Glomus* spp. were also sometimes detected (e.g. Błaszkowski et al. 2009a,b, 2010). It was supposed that species with small, quickly degrading spores were difficult to recover or identify only from field samples. This might also be true for *D. clara*, even though its laminated wall structure is clearly persistent. It will be interesting to see whether future taxonomists will be able to identify the new species directly from field samples, now that the existence and morphology of this unique, brilliant-white, conspicuous but small-spored species is known. Morphological spore and molecular root and spore analyses will hopefully tell us more about the ecology and biogeography of this fungus that is thus far known only from a single *Asteriscus maritimus* rhizosphere in the Natural Park Cabo de Gata of Almería in southern Spain.

Acknowledgments

This study has been supported by the Junta de Andalucía (Spain), project P06-CVI-01876 and by the Swiss National Science Foundation (SNSF; Project 315230_130764/1). We acknowledge the valuable comments on the manuscript and revisions of Dr. Bruno Tomio Goto (Universidade Federal do Rio Grande do Norte, UFRN, Natal, Brazil), Dr. Iván Sánchez-Castro (INRA, Dijon, France) and PD Dr. Ewald Sieverding (University of Hohenheim, Germany) and appreciate the corrections by Shaun Pennycook, Nomenclatural Editor, and suggestions by Lorelei L. Norvell, Editor-in-Chief.

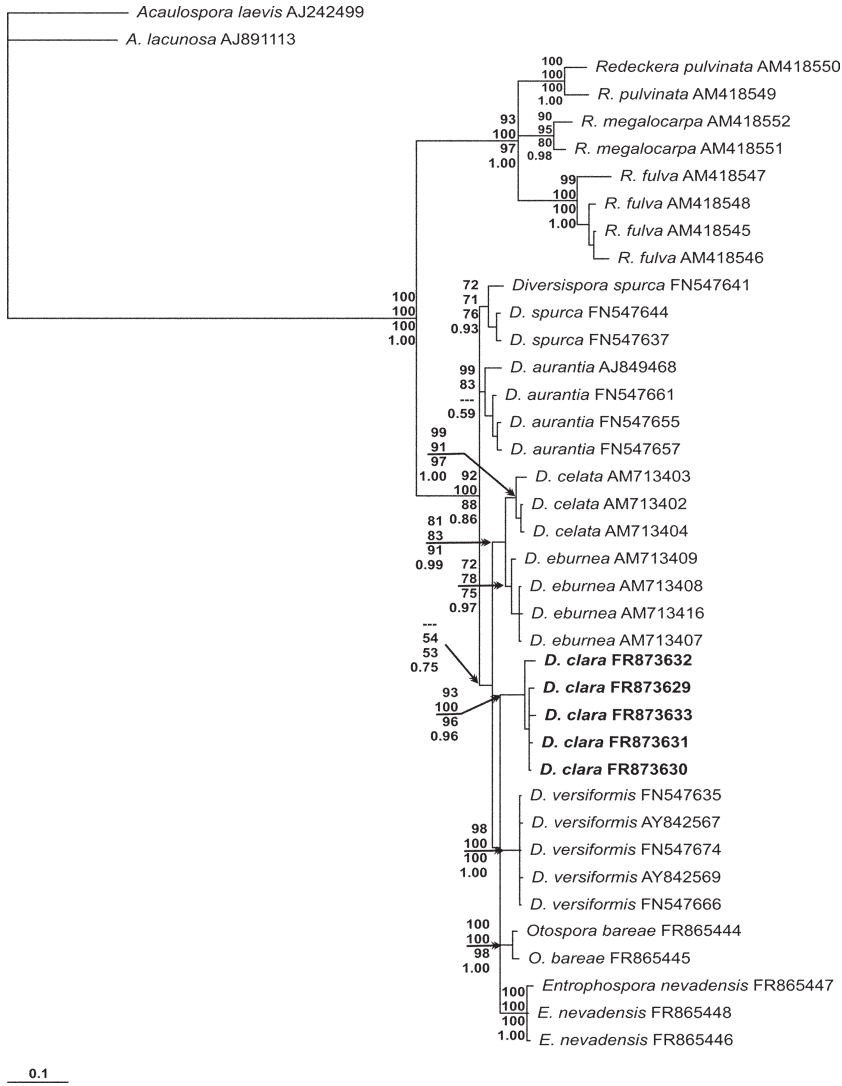


Fig. 11. *Diversisporaceae*. ITS rDNA-based phylogenetic tree rooted by *Acaulospora laevis* and *A. lacunosa*. Sequences are labeled with database accession numbers. Support values are from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and bayesian analyses. *Diversispora clara* sequences are in bold. Only topologies with ≥ 50% bootstrap values are shown. (Consistency Index = 0.72; Retention Index = 0.84).

Literature cited

Altschul SF, Gish W, Miller W, Myers EW, Lipton DJ. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410. http://dx.doi.org/10.1006/jmbi.1990.9999

Błaszkowski J. 1994. Arbuscular fungi and mycorrhizae (*Glomales*) of the Hel Peninsula, Poland. Mycorrhiza 5, 71–88. http://dx.doi.org/10.1007/BF00204022

Błaszkowski J. 1997. *Glomus gibbosum*, a new species from Poland. Mycologia 89(2): 339–345. http://dx.doi.org/10.2307/3761092

Błaszkowski J, Ryszka P, Oehl F, Koegel S, Wiemken A, Kovács GM, Redecker D. 2009a. *Glomus achrum* and *G. bistratum*, two new species of arbuscular mycorrhizal fungi (*Glomeromycota*). Botany 87: 260–271. http://dx.doi.org/10.1139/B08-138

Błaszkowski J, Kovács GM, Balázs TK. 2009b. *Glomus perpusillum*, a new arbuscular mycorrhizal fungus. Mycologia 101(2): 247–255. http://dx.doi.org/10.3852/08-087

Błaszkowski J, Wubet T, Harikumar VS, Ryszka P, Buscot F. 2010. *Glomus indicum*, a new arbuscular mycorrhizal fungus. Botany 88: 132–143. http://dx.doi.org/10.1139/B09-104

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

Ferrol N, Calvente R, Cano C, Barea JM, Azcón-Aguilar C. 2004. Analyzing arbuscular mycorrhizal fungal diversity in shrub-associated resource islands from a desertification-threatened semiarid Mediterranean ecosystem. Appl. Soil Ecol. 25: 123–133. http://dx.doi.org/10.1016/j.apsoil.2003.08.006

Gamper HA, Walker C, Schüßler A. 2009. *Diversispora celata* sp. nov.: molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. New Phytol. 182(2): 495–506. http://dx.doi.org/10.1111/j.1469-8137.2008.02750.x

Gemma JN, Koske RE, Carreiro MM. 1989. Seasonal dynamics of five species of VA fungi in a sand dune. Mycol. Res. 92: 27–32 http://dx.doi.org/10.1016/S0953-7562(89)80072-3

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biol. 52: 696–704. http://dx.doi.org/10.1080/10635150390235520

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41: 95–98.

Hewitt EJ. 1966. Sand and water culture methods used in the study of plant nutrition. Farnham Royal, Farnham, England: Commonwealth Agricultural Bureau. 547 p.

Kennedy LJ, Stutz JC, Morton JB. 1999. *Glomus eburneum* and *G. luteum*, two new species of arbuscular mycorrhizal fungi, with emendation of *G. spurcum*. Mycologia 91(6): 1083–1093. http://dx.doi.org/10.2307/3761638

Koske R., Gemma JN. 1995. *Scutellospora hawaiiensis* (*Gigasporaceae*): a new species of arbuscular mycorrhizal fungi from Hawaii. Mycologia 87: 679–684. http://dx.doi.org/10.2307/3760811

Koske RE, Tessier B. 1983. A convenient, permanent slide mounting medium. Mycol. Soc. Am. Newsl. 34: 59.

Krüger M, Stockinger H, Krüger C, Schüßler A. 2009. DNA-based species-level detection of arbuscular mycorrhizal fungi: one PCR primer set for all AMF. New Phytol. 183(1): 212–223. http://dx.doi.org/10.1111/j.1469-8137.2009.02835.x

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948. http://dx.doi.org/10.1093/bioinformatics/btm404

Milne I, Wright F, Rowe G, Marshal DF, Husmeier D, McGuire G. 2004. TOPALi: Software for Automatic Identification of Recombinant Sequences within DNA Multiple Alignments. Bioinformatics 20: 1806–1807. http://dx.doi.org/10.1093/bioinformatics/bth155

Mosse B. 1962. Establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. J. Gen. Microbiol. 27: 509–520.

Oehl F, Wiemken A, Sieverding E. 2002. *Glomus caesaris*, a new arbuscular mycorrhizal fungus from the Kaiserstuhl in Germany. Mycotaxon 84: 379–385.

Oehl F, Wiemken A, Sieverding E. 2003. *Glomus aureum*, a new sporocarpic species in the *Glomales* from European grasslands. J. Appl. Bot. 77: 111–115.

Oehl F, Redecker D, Sieverding E. 2005. *Glomus badium*, a new sporocarpic arbuscular mycorrhizal fungal species from European grasslands of higher soil pH. J. Appl. Bot. Food Qual. 79: 38–43.

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

Oehl F, Silva GA, Sánchez-Castro I, Goto BT, Maia LC, Vieira HEE, Barea JM, Sieverding E, Palenzuela J. 2011b. Revision of *Glomeromycetes* with entrophosporoid and glomoid spore formation, with three genera nova. Mycotaxon 117: 297–316, http://dx.doi.org/10.5248/117.297

Oehl F, Sieverding E, Palenzuela J, Ineichen K, Silva GA. 2011c. Advances in *Glomeromycota* taxonomy and classification. IMA Fungus 2: 191–199. http://dx.doi.org/10.5598/imafungus.2011.02.02.10

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. http://dx.doi.org/10.3852/mycologia.100.2.296

Palenzuela J, Barea JM, Ferrol N, Oehl F. 2010. *Entrophospora nevadensis*, a new arbuscular mycorrhizal fungus, from Sierra Nevada National Park (southeastern Spain). Mycologia 102: 624–632, http://dx.doi.org/10.3852/09-145

Palenzuela J, Barea JM, Ferrol N, Oehl F. 2011. *Ambispora granatensis*, a new arbuscular mycorrhizal fungus, associated with *Asparagus officinalis* in Andalucía (Spain). Mycologia 103: 333–340. http://dx.doi.org/10.3852/09-146

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Sieverding E. 1991. Vesicular-arbuscular mycorrhizal management in tropical agrosystems. Technical Cooperation (GTZ). Eschborn. Friedland, Bremer, Rossdorf, TZ-Verlagsgesellschaft. Germany.

Sieverding E, Oehl F. 2006. Revision of *Entrophospora* and description of *Kuklospora* and *Intraspora*, two new genera in the arbuscular mycorrhizal *Glomeromycetes*. J. Appl. Bot. Food Qual. 80: 69–81.

Spain JL. 1990. Arguments for diagnoses based on unaltered wall structures. Mycotaxon 38: 71–76.

Swofford DL. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Sinauer Associates, Sunderland, Massachusetts.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR protocols: a guide to methods and applications. Academic Press, San Diego, California.