



The arbuscular mycorrhizal *Paraglomus majewskii* sp. nov. represents a distinct basal lineage in Glomeromycota

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Abstract: *Paraglomus majewskii* sp. nov. (Glomeromycota) is described and illustrated. It forms single spores, which are hyaline through their life cycle, globose to subglobose, (35–)63(–78) μm diam, sometimes egg-shaped, 50–70 \times 65–90 μm , and have an unusually narrow, (3.2–)4.6(–5.9) μm , cylindrical to slightly flared subtending hypha. The spore wall of *P. majewskii* consists of an evanescent, short-lived outermost layer, a laminate middle layer, and a flexible innermost layer, which adheres tightly to the middle layer. None of the spore wall layers stain in Melzer's reagent. In single-species cultures with *Plantago lanceolata* as the host plant *P. majewskii* formed arbuscular mycorrhizae staining violet in trypan blue. *P. majewskii* has been isolated from several, distant geographic regions and from different habitats. In phylogenetic analyses of partial nrDNA SSU and LSU sequences the fungus formed monophyletic group with *Paraglomus* species; however it represents a well separated distinct lineage. Its nrDNA sequences are highly similar to in planta arbuscular mycorrhizal fungal sequences from different habitats in Spain and Ecuador.

Key words: AMF specific PCR primers, ancestral

lineage, arbuscular mycorrhizal fungi, Glomeromycota, mycorrhizae, Paraglomeraceae

INTRODUCTION

The family Paraglomeraceae was erected recently to accommodate the ancestral arbuscular mycorrhizal fungi (AMF) that form small glomoid spores based on rDNA phylogenetic analysis (Morton and Redecker 2001, Schüßler et al. 2001). The species within Paraglomeraceae are the type species *Paraglomus occultum* (C. Walker) J.B. Morton & D. Redecker, *P. brasilianum* (Spain & J. Miranda) J.B. Morton & D. Redecker and *P. lacatum* (Błaszk.) Renker, Błaszk. & Buscot.

The known *Paraglomus* spp. produce glomoid spores (they arise identically to those of *Glomus* spp.) that are colorless or pale and relatively small (mean diameter < 100 μm ; Morton and Redecker 2001, Renker et al. 2007). In addition mycorrhizae of *Paraglomus* spp. stain faintly or not at all in trypan blue and probably lack vesicles (Morton and Redecker 2001). However it is evident that spore morphology and even mode of spore formation of many species of Glomeromycota sometimes correlate weakly or not at all with their molecular phylogenies (Redecker and Raab 2006, Schüßler and Walker 2010). Hence to correctly place newly discovered AMF within Glomeromycota both morphological and molecular phylogenetic analyses are needed.

While *P. occultum* has been recorded from numerous regions of the world (Błaszkowski pers obs, Morton and Redecker 2001), *P. brasilianum* has been found only in Brazil and West Virginia, USA (Morton and Redecker 2001, Spain and de Miranda 1996), and *P. lacatum* has been found only in Denmark, Poland (Błaszkowski 1988, unpubl; Renker et al. 2007) and Great Britain (Chris Walker pers comm).

Examination of pot trap cultures inoculated with rhizosphere soil and root fragments of a variety of plant species collected from several locations in Europe, Africa and Asia produced glomoid spores of an undescribed taxon of Glomeromycota. Molecular phylogenetic analyses showed that these isolates formed a new distinct lineage in a monophyletic group with *Paraglomus* species, herein described as *P. majewskii*.

MATERIALS AND METHODS

Establishment of cultures, extraction of spores and staining of mycorrhizae.—Spores examined in this study were derived

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from both pot trap and single-species cultures. Trap cultures were established to obtain a large number of living spores and to initiate sporulation of species that were present but were not detected in the field samples (Stutz and Morton 1996). The method used to establish trap cultures, growing conditions and the methods of spore extraction and staining of mycorrhizae were those described by Błaszowski et al. (2006). The growing substrate of trap cultures was the field-collected rhizosphere soil and roots of the plant species sampled mixed with an autoclaved coarse-grained sand.

Single-species cultures were established with 10–50 spores isolated from trap cultures and grown as described in Błaszowski et al. (2009a). Attempts of establishing single-species cultures from single spores failed. *Plantago lanceolata* was used as host plant in both trap and single-species cultures.

Microscopy.—Morphological characteristics of spores and wall structures were determined based on examination of at least 100 spores mounted in either lactic acid (spore size) or polyvinyl alcohol/lactic acid/glycerol (PVLG; Omar et al. 1979) and a mixture of PVLG and Melzer's reagent (1:1, v/v). All spores came from single-species cultures. Spores at all developmental stages were crushed to varying degrees by applying pressure to the cover slip and incubated at 65 C for 24 h to clear contents. Spores were examined under a compound microscope equipped with Nomarski differential interference contrast optics. Microphotographs were recorded on a Sony 3CDD color video camera coupled to the microscope.

Spore color was examined under a dissecting microscope on fresh specimens immersed in water. Terminology of spore structure was that suggested by Stürmer and Morton (1997) and Walker (1983). Color names are from Kornerup and Wanscher (1983). Nomenclature of fungi and plants is that of Schüßler and Walker (2010) and Mirek et al. (<http://info.botany.pl/czek/check.htm>) respectively. The authors of the fungal names are those presented at the Index Fungorum Website (<http://www.indexfungorum.org/AuthorsOfFungalNames.htm>). Voucher specimens were mounted in PVLG and a mixture of PVLG and Melzer's reagent (1:1, v/v) on slides and deposited in the Department of Plant Protection (DPP), West Pomeranian University of Technology, Szczecin, Poland, and in the herbarium at Oregon State University (OSC) in Corvallis, Oregon.

DNA extraction, polymerase chain reaction and DNA sequencing.—DNA was extracted from two strains as described by Błaszowski et al. (2009a). Each strain was represented by 3–9 spores isolated from single-species cultures. Strain 1 came from a culture, from which the holotype of *P. majewskii* was selected, and strain 2 came from a culture isolated from maritime sand dunes near Karabucak-Tuzla, Turkey (see *Specimens examined*). The extracts were used as template in polymerase chain reactions (PCR). Partial SSU was amplified as described in Błaszowski et al. (2010) with a nested PCR with GlomerWT0 and Glomer1536 primers (Wubet et al. 2006) and AML1 and AML2 primers (Lee et al. 2008). Partial LSU

region was amplified in a nested PCR reaction first with the primers LR1 (van Tuinen et al. 1998) and FLR2 (Trouvelot et al. 1999) and then the primers 28G1 and 28G2 (da Silva et al. 2006). Subsequent work with amplicons was carried out as described in Błaszowski et al. (2010), and 6–10 clones from each amplicon were sequenced. The sequences were compiled from electrophoregrams with Pregap4 and Gap4 (Staden et al. 2000), and representatives of the different sequences were deposited in GenBank (JN131587–JN131606).

Phylogenetic analyses.—The partial SSU and LSU sequences were analyzed separately. Sequences were aligned with MAFFT (Katoh et al. 2002, Katoh and Toh 2008). The alignments were checked and adjusted manually with ProSeq 2.9 (Filatov 2002). The alignments were deposited in TreeBASE (S11460). The best-fit nucleotide substitution model was selected with the program jModelTest (Posada 2008) considering the selection of Akaike information criterion (AIC). Maximum likelihood (ML) phylogenetic analyses were carried out with the online version of PHYML 3.0 (Guindon and Gascuel 2003). The GTR nucleotide substitution model was used with ML estimation of base frequencies. The proportion of invariable sites was estimated and optimized. Six substitution rate categories were set, and the gamma distribution parameter was estimated and optimized. Bootstrap analysis with 1000 replicates (MLB) was used to test the support of branches. The same substitution model was used in Bayesian analyses performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) using the Computational Biology Service Unit at Cornell University (<http://cbsuapps.tc.cornell.edu/index.aspx>). Four Markov chains were run 5 000 000 generations, sampling every 100 steps, with a burn-in at 7500 sampled trees. The phylogenetic trees were viewed and edited with Tree Explorer of the MEGA4.0 program (Kumar et al. 2004) and a text editor.

TAXONOMY

Paraglomus majewskii Błasz. & Kovács sp. nov.

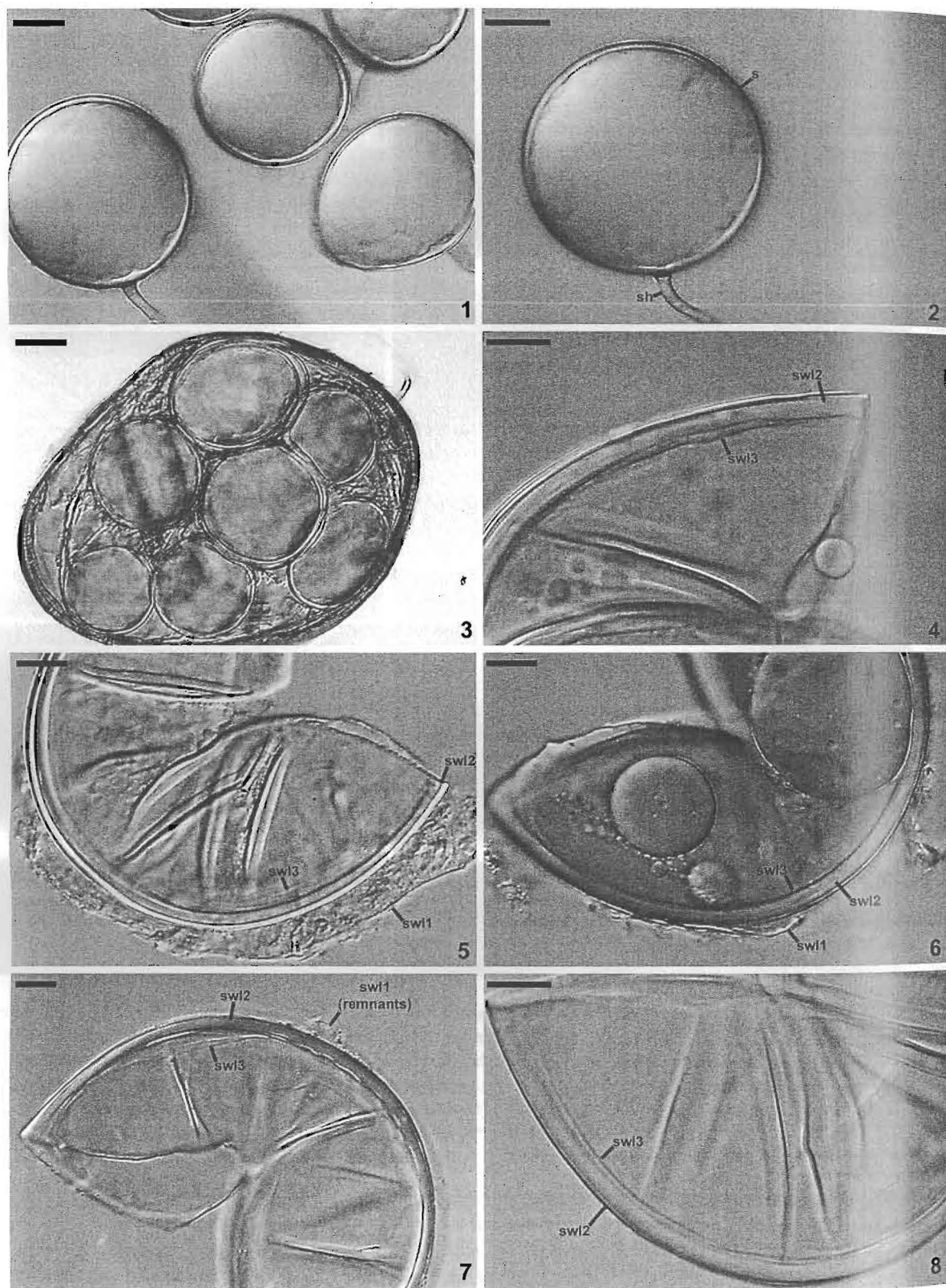
FIGS. 1–16

MycoBank MB561205

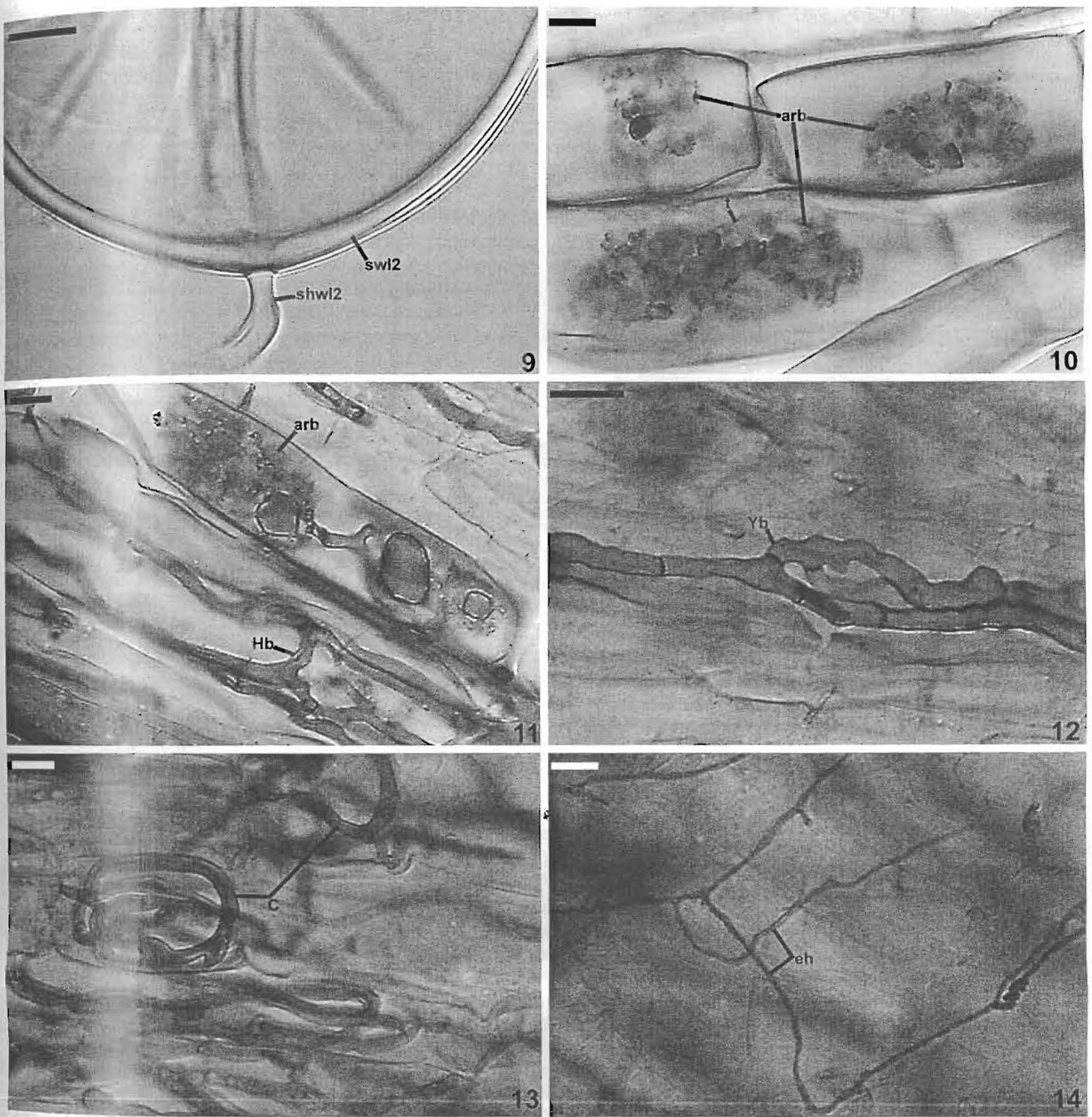
Sporocarpia ignota. Sporae singulatim in solo efformatae. Sporae hyalinae; globosae vel subglobosae; (35–)63(–78) μm diam; raro ovoidae; 50–70 \times 65–90 μm . Tunica sporae e stratis tribus (strati 1–3); stratum 1 evanescens, hyalinum, (0.5–)0.7(–1.0) μm crassum; stratum 2 laminatum, glabrum, hyalinum, (2.5–)3.5(–4.4) μm crassum; stratum 3 flexibile, glabrum, hyalinum, ca. 0.5 μm crassum. Hypha subtenda hyalina; recta vel recurva; cylindrica; (3.2–)4.6(–5.9) μm lata at basim sporae; pariete hyalino, (0.7–)1.4(–2.0) μm crasso, stratis 1 at 2 sporae continuo. Porus (1.2–)1.9(–2.5) diam, aperto. Mycorrhizas arbusculares formans.

Typus. Polonia: Sedinum (Szczecin), infra *P. lanceolata*, 15 Apr 2008, J. Błaszowski, 3064 (Holotypus, DPP).

Sporocarps unknown. Spores formed singly in the soil (FIGS. 1, 2); develop blastically at the tip of



FIGS. 1-8. *Paraglomus majewskii*. 1, 2. Spores (s) with subtending hyphae (sh). 3. Spores inside a spore of *Scutellospora* sp. 4-8. Spore wall layers (swl) 1-3. 1-3. Spores in lactic acid. 4. Spore in PVLG. 5-8. Spores in PVLG + Melzer's reagent. 1-8. Differential interference microscopy. Bars: 1-3 = 20 μ m, 4-8 = 10 μ m.



FIGS. 9-14. *Paraglomus majewskii*. 9. Spore wall layer (swl) 2 and subtending hyphal wall layer (shwl) 2; spore wall layer 1 continuous with subtending hyphal wall layer 1 is completely sloughed in the spore; spore wall layer 3 is invisible, because it tightly adheres to the lower surface of spore wall layer 2. 10-14. Mycorrhizae of *Paraglomus majewskii* in roots of *Plantago lanceolata* stained in 0.1% trypan blue. 10. Arbuscules (arb) with trunk (t). 11. Arbuscule (arb) and H-shaped branch (Hb). 12. Y-shaped branch (Yb). 13. Coil (c). 14. Extraradical hyphae (eh). 9. Spore in PVLG + Melzer's reagent. 10-14. Root fragments in PVLG. 9-11. Differential interference microscopy. Bars: 9, 10, 12-14 = 10 μ m; 11 = 20 μ m.

sporogenous hyphae continuous with mycorrhizal extraradical hyphae; sometimes produced inside empty spores of other AMF (FIG. 3). Spores hyaline; globose to subglobose; (35-)63(-78) μ m diam; sometimes egg-shaped; 50-70 \times 65-90 μ m; with one

subtending hypha (FIGS. 1-3). Spore wall consists of three hyaline layers (1-3). Layer 1, forming the spore surface, evanescent, usually slightly roughened on its upper surface, (0.5-)0.7(-1.0) μ m thick, almost always deteriorated or completely sloughed in mature spores

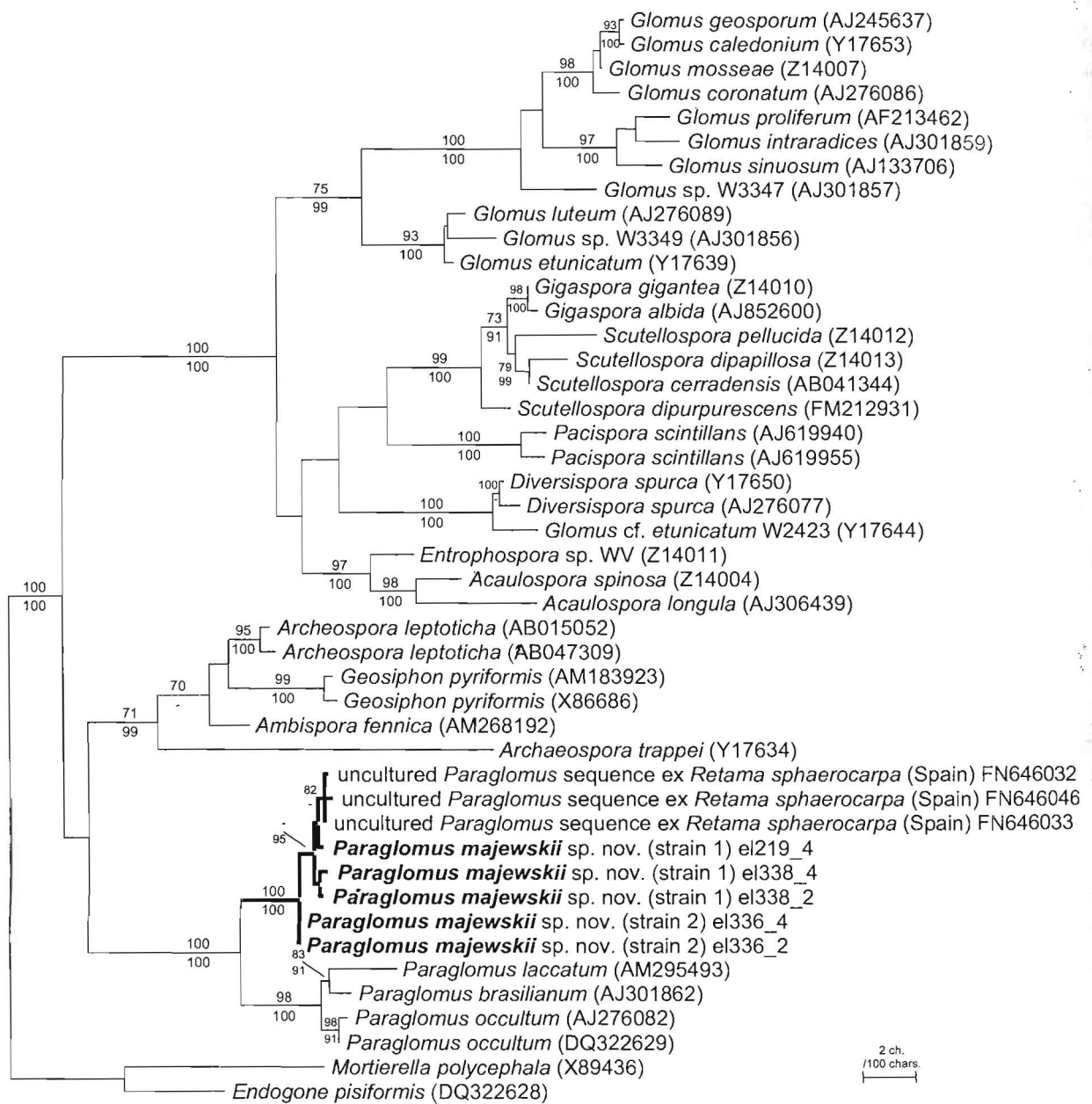


FIG. 15. The maximum likelihood tree inferred from partial SSU rDNA sequences showing the phylogenetic position of *Paraglomus majewskii* in phylum Glomeromycota with *Endogone pisiformis* as outgroup. GenBank accession numbers of the sequences from earlier studies are in parentheses. The value above the branches shows the ML bootstrap, whereas the value below the branches is the Bayesian posterior probabilities (BPP). The values are given as percentages. Values below 70% (bootstrap) and 90% (BPP) are not shown. Bar 2 changes/100 characters.

(FIGS. 4–8). Layer 2 laminate, smooth, (2.5–)3.5(–4.4) μm thick, frequently stratifying into groups of laminae (sublayers) in vigorously crushed spores (FIGS. 4–9). Layer 3 flexible, smooth, ca. 0.5 μm thick, usually tightly adherent to the lower surface of layer 2 in slightly crushed spores, but sometimes separating from layer 2 in vigorously crushed spores (FIGS. 4–8). None of the spore wall layers swell in PVLG or stain in Melzer's reagent. Subtending hypha hyaline; straight

or curved, cylindrical to flared; (3.2–)4.6(–5.9) μm wide at the spore base (FIGS. 1, 2, 9). Wall of subtending hypha hyaline; (0.7–)1.4(–2.0) μm thick at the spore base; composed of two layers continuous with spore wall layers 1 and 2; layer 1 was present in only few intact spores (FIG. 9). Pore (1.2–)1.9(–2.5) μm diam, open (FIG. 9). Germination unknown.

Mycorrhizal associations. In single-species pot cultures with *P. lanceolata* as the host plant, *P. majewskii*

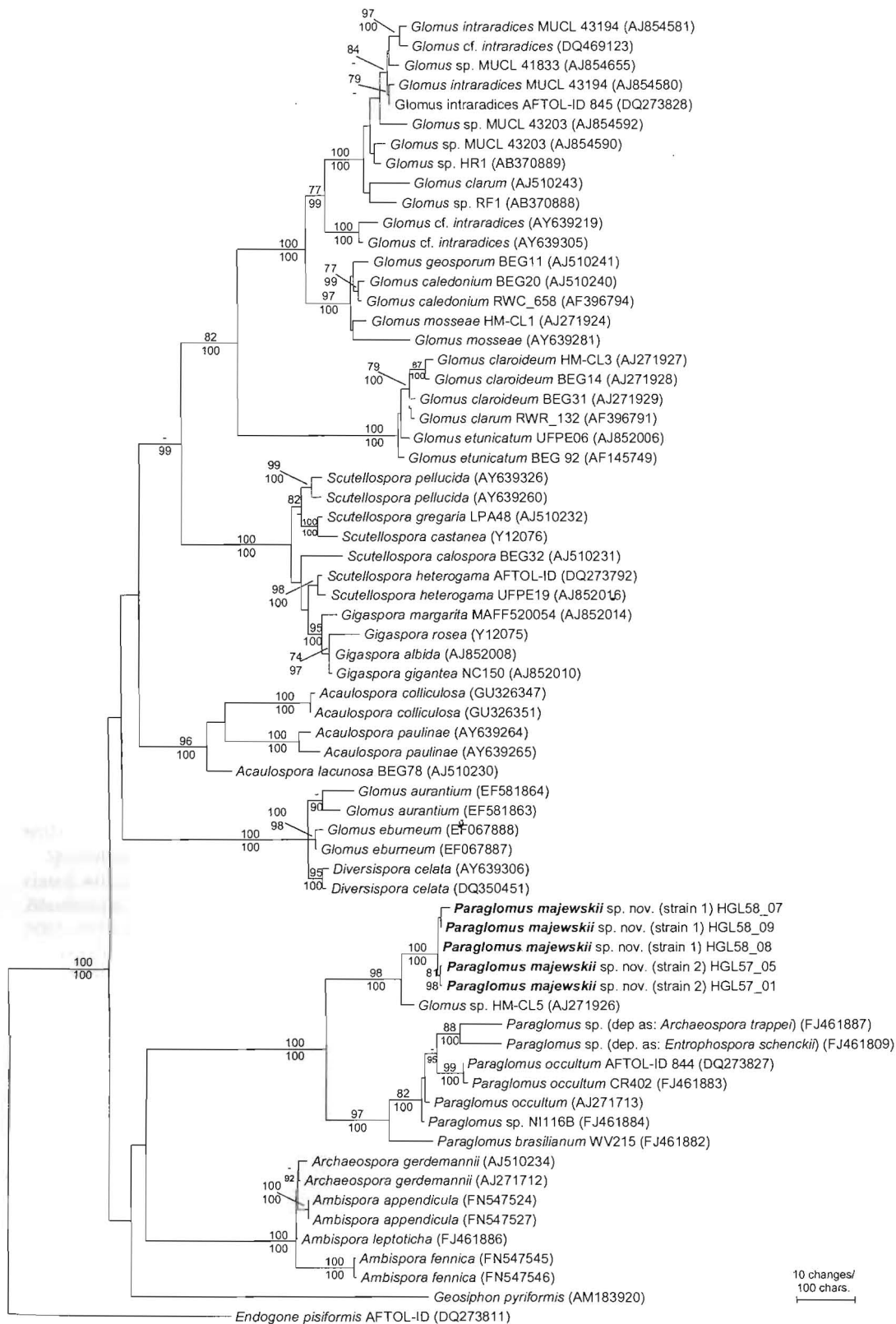


FIG. 16. The maximum likelihood tree inferred from partial LSU rDNA sequences showing the phylogenetic position of *Paraglomus majewskii* in phylum Glomeromycota with *Endogone pisiformis* as outgroup. GenBank accession numbers of the sequences from earlier studies are in parentheses. The values above the branches show the ML bootstrap, whereas the values below the branches are the Bayesian posterior probabilities (BPP). The values are given as percentages. Values below 70% (bootstrap) and 90% (BPP) are not shown. Bar 10 changes/100 characters.

formed mycorrhizae with arbuscules and intra- and extraradical hyphae (FIGS. 10–14). No vesicles were found in any roots (cultures up to 5 y old). Arbuscules were numerous and evenly distributed along the root fragments. They consisted of a short trunk developed from a parent hypha and numerous branches with fine tips (FIGS. 10, 11). Intraradical hyphae grew parallel to the longitudinal root axis, were straight to slightly curved, and (2.2–)4.3(–6.9) μm wide (FIG. 12). They sometimes formed H- or Y-shaped branches and coils (FIGS. 11–13). The coils were primarily ellipsoidal; 7.5–25.0 \times 18.8–87.5 μm ; rarely circular; (12.5–)16.7(–27.5) μm diam when seen in a plan view; and widely dispersed along the root fragments. Extraradical hyphae occurred rarely and were (3.3–)4.8(–5.1) μm wide (FIG. 14). In 0.1% trypan blue arbuscules stained violet-white (16A2) to reddish violet (16C7), intraradical hyphae violet white (16A2) to reddish violet (16B7), coils violet-white (16A2) to lilac (16B5) and extraradical hyphae pale violet (16A3) to grayish violet (16C6).

Phylogenetic position. *Paraglomus majewskii* grouped within the Paraglomeraceae forming a monophyletic group with other *Paraglomus* species based on analyses of both SSU and LSU sequences (FIGS. 15, 16). However the species forms a relatively distant lineage within the genus.

Specimens examined. EUROPE. POLAND, Szczecin, associated with roots of pot-cultured *P. lanceolata*, 15 Apr 2008, *Błaszowski, J.*, 3064 (HOLOTYPE, DPP); *Błaszowski, J.*, 3065–3070 (ISOTYPES, DPP) and two slides at OSC; Brójce (52°18'N, 15°3'E), under *Brassica elongata* Ehrh., 25 Apr 2004, *Błaszowski, J.*, 3080–3084 (DPP); near Bytom (50°20'N, 18°55'E), under *Deschampsia caespitosa* (L.) P. Beauv., 3 Jul 2005, *Błaszowski, J.*, 3100 (DPP); Lubrza (52°18'N, 15°24'E), among roots *Cirsium arvense* (L.) Scop., 13 Aug 2004, *Błaszowski, J.*, 3091 (DPP); Lubrza, in the rhizosphere of *Melandrium album* (Mill.) Garcke, 13 Aug 2004, *Błaszowski, J.*, 3092 (DPP); Łagowiec (52°20'N, 15°41'E), in the root zone of *B. elongata*, 25 Apr 2004, *Błaszowski, J.*, 3085–3088 (DPP); Romanówek (52°17'N, 15°22'E), under *Hypericum perforatum* L., 13 Aug 2004, *Błaszowski, J.*, 3093 (DPP); Trzciel (52°22'N, 15°52'E), among roots of *Asparagus officinalis* L., 25 Apr 2004, *Błaszowski, J.*, 3089–3090 (DPP); Trzciel, under *Jovibarba sobolifera* (Sims) Opiz, 15 Aug 2004, *Błaszowski, J.*, 3094 (DPP); Trzciel, associated with roots of *Juncus effusus* L., 15 Sep 2004, *Błaszowski, J.*, 3097 (DPP); Trzciel, in the root zone of *Plantago arenaria* Waldst. & Kit., 15 Sep 2004, *Błaszowski, J.*, 3098 (DPP); Trzciel, among roots of *A. officinalis*, 22 Jun 2005, *Błaszowski, J.*, 3099 (DPP); Zarzyń (52°23'N, 15°25'E), in the rhizosphere of *Equisetum arvense* L., 13 Sep 2004, *Błaszowski, J.*, 3095 (DPP); Żelechów (52°18'N, 15°21'E), among roots of *P. lanceolata*, 13 Sep 2004, *Błaszowski, J.*, 3096 (DPP). PORTUGAL. Faro (37°1'N, 7°56'W), under *Zea mays* L., 1 Oct 2000, *Błaszowski, J.*, 3071–3073 (DPP). SPAIN. Majorca, near

Cape Salinas (36°19'N, 3°2'E), under *Ammophila arenaria* Link, 22 Sep 2001, *Błaszowski, J.*, 3078–3079 (DPP). AFRICA. ERITREA, near Asmara (15°20'N, 38°55'E), associated with roots of an unknown weed, 20 Oct 2006, *Błaszowski, J.*, 3101–3102 (DPP). ASIA. TURKEY. Near Karabucak-Tuzla (36°43'N, 34°59'E), under *A. arenaria*, 10 Jun 2001, *Błaszowski, J.*, 3074–3077 (DPP).

Etymology. Latin, *majewskii*, in honor of Prof Dr Hab. Tomasz Majewski, Department of Phytopathology, Warsaw University of Life Sciences – SGGW, Warsaw, a long time student of fungi and distinguished mycologist.

Distribution and habitat. Spores of *P. majewskii* first were isolated from a pot trap culture inoculated with rhizosphere soil and roots of *Z. mays* collected near Faro, Portugal, Dec 2000. Later this fungus was isolated from trap cultures containing rhizosphere soils and roots of (i) *A. arenaria* growing in mobile dunes of the Mediterranean Sea adjacent to Cape Salinas, Majorca, Spain, Aug 2001 (one culture), (ii) *A. arenaria* colonizing dunes of the Mediterranean Sea near Karabucak-Tuzla, Turkey, Jun 2001 (one culture), (iii) numerous cultivated and uncultivated plant species growing in different sites of the Lubuskie Province, southwestern Poland, Apr–Aug 2004, 2005 (15 cultures), (iv) an unrecognized weed growing near Asmara, Eritrea, Africa, Nov 2006 (one culture), (v) *A. arenaria* colonizing dunes of the Baltic Sea near Bornholm, Denmark, Oct 2004 (10 cultures) and (vi) *D. caespitosa* growing in soils with high concentrations of heavy metals near Bytom, Upper Silesia, Poland, Jul 2004 (five cultures).

Phylogenetic analyses revealed that several unidentified environmental sequences from public databases were similar to the new species. The SSU sequences of *P. majewskii* showed high similarity and grouped together with AMF sequences originating from the roots of *Retama sphaerocarpa* (L.) Boiss. from semiarid land in Spain (FIG. 15) (Alguacil et al. 2011a). More recently sequences designated as forming group Para-1, some that were identical to the *R. sphaerocarpa* AMF sequences, also were amplified from roots of several plant species growing in heavy metal-polluted soil in southeastern Spain (Alguacil et al. (2011b) (data not shown). Two further environmental sequences similar to *P. majewski* (99% identity) were found in public databases via BLAST queries when we analyzed a ~ 500 bp long fragment at 3' end of our SSU sequences (data not shown). These environmental sequences were obtained from roots of *Setaria* sp. and *Heliocarpus americanus* L. from a molecular diversity study of AMF in Ecuador (EU159183 and EU159184, Haugh et al. 2010). When LSU sequences were analyzed one sequence similar to *P. majewskii* was found (FIG. 16), originating from a spore sample

from southern Poland (Turnau et al. 2001). Thus *P. majewskii* probably is widespread.

Several virtual taxa (VT) are assigned in Paraglomeraceae in the recently opened MaarjAM database (Öpik et al. 2010). *P. majewskii* most probably represents a new VT together with the aforementioned environmental sequences, according to the phylogenetic analyses presented and the BLAST queries.

Notes. The distinctive morphological characters of *P. majewskii* are its small spores produced singly in soil and remaining hyaline throughout their life cycle, their three-layered spore wall structure in which layer 3 is flexible and the unusually narrow subtending hypha (FIGS. 1–9). An interesting property of *P. majewskii* spores also is that they frequently float in water. Morton and Redecker (2001) and Morton (<http://invam.caf.wvu.edu/>) reported that spores of *Archaeospora trappei* (R.N. Ames & Linderman) J.B. Morton & D. Redecker emend. Spain and *P. occultum* also tend to float.

In addition to differences in phylogeny (FIGS. 15, 16) *P. majewskii* also differs morphologically from the other three *Paraglomus* spp. known to date. *P. brasilianum* also produces only hyaline spores with a three-layered spore wall, but spore wall layer 2 of *P. brasilianum* is ornamented (vs. smooth in *P. majewskii*), and layer 3 is laminate (vs. flexible) (Błaszowski pers obs, Morton and Redecker 2001, Spain and de Miranda 1996 SUPPLEMENTARY KEY). Moreover spore wall layers 1 and 3 of *P. brasilianum* are much thicker. Spores of *P. occultum* are smooth and have a three-layered spore wall, but the spores become yellow with age (vs. remain hyaline in *P. majewskii*), spore wall layer 2 is much thinner than that of *P. majewskii* and spore wall layer 3 is laminate and generally much thicker (Błaszowski pers obs, Morton and Redecker 2001, Walker 1982). *Paraglomus laccatum* forms only hyaline spores, which are generally larger than those of *P. majewskii*, have a much wider subtending hypha and have a spore wall composed of only two layers (Błaszowski 1988, Renker et al. 2007, www.zor.zut.edu.pl/Glomeromycota/). In addition the laminate spore wall layer of *P. majewskii* is much thinner than that of *P. laccatum* and consists of thin (< 0.5 µm), tightly adherent sublayers (vs. sublayers 0.5–2.2 µm thick, usually separating from each other even in intact spores). Other morphological differences among *P. majewskii* and the other *Paraglomus* spp. and other described species of AMF forming glomoid only hyaline spores with a three-layered spore wall are provided (SUPPLEMENTARY KEY).

We suggest that *P. majewskii* is a widely distributed fungus, tolerant of a range of biotic and abiotic factors, according to the distribution data. The previous omission of *P. majewskii* from the scientific record probably resulted because its spores are

colorless, small and delicate and thereby might have been overlooked or absent due to their decomposition by soil parasites (Lee and Koske 1994) or possibly because it sporulates seasonally, rarely or not at all in the field (Gemma et al. 1989, Stutz and Morton 1996). Growing successive pot trap cultures frequently stimulates sporulation of AMF and reveals species that produce spores seasonally or not at all in field conditions (Stutz and Morton 1996).

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LITERATURE CITED

- Alguacil MM, Torrecillas E, Caravaca F, Fernández DA, Azcón R, Roldán A. 2011b. The application of an organic amendment modifies the arbuscular mycorrhizal fungal communities colonizing native seedlings grown in a heavy-metal-polluted soil. *Soil Biol Biochem* 43:1498–1508, doi:10.1016/j.soilbio.2011.03.026
- , Torres MP, Torrecillas E, Díaz G, Roldán A. 2011a. Plant type differently promote the arbuscular mycorrhizal fungi biodiversity in the rhizosphere after revegetation of a degraded, semiarid land. *Soil Biol Biochem* 43:167–173, doi:10.1016/j.soilbio.2010.09.029
- Błaszowski J. 1988. Three new vesicular-arbuscular mycorrhizal fungi (Endogonaceae) from Poland. *Bull Pol Acad Sci Biol Sci* 36:271–275.
- , Kovács GM, Balázs KT, Orłowska E, Sadravi M, Wubet T, Buscot F. 2010. *Glomus africanum* and *G. iranicum*, two new species of arbuscular mycorrhizal fungi (Glomeromycota). *Mycologia* 102:1450–1462, doi:10.3852/09-302
- , ———, Balázs T. 2009a. *Glomus perpusillum*, a new arbuscular mycorrhizal fungus. *Mycologia* 101:247–255, doi:10.3852/08-087
- , Ryszka P, Oehl F, Koegel S, Wiemken W, Kovács GM, Redecker D. 2009b. *Glomus achrum* and *G. bistratum*, two new species of arbuscular mycorrhizal fungi (Glomeromycota) found in maritime sand dunes. *Botany* 87:260–271, doi:10.1139/B08-138
- , Renker C, Buscot F. 2006. *Glomus drummondii* and *G. walkeri*, two new species of arbuscular mycorrhizal fungi (Glomeromycota). *Mycol Res* 110:555–566, doi:10.1016/j.mycres.2006.02.006
- da Silva GA, Lumini E, Maia LC, Bonfante P, Bianciotto V. 2006. Phylogenetic analysis of Glomeromycota by partial LSU rDNA sequences. *Mycorrhiza* 16:183–189, doi:10.1007/s00572-005-0030-9

- Filatov DA. 2002. ProSeq: a software for preparation and evolutionary analysis of DNA sequence datasets. *Mol Ecol Notes* 2:621–624, doi:10.1046/j.1471-8286.2002.00313.x
- Gemma JN, Koske RE, Carreiro M. 1989. Seasonal dynamics of selected species of VA mycorrhizal fungi in a sand dune. *Mycol Res* 92:317–321, doi: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. *Syst Biol* 52:696–704, doi:10.1080/10635150390235520
- Haug I, Wubet T, Weiss M, Aguirre N, Weber M, Günter S, Kottke I. 2010. Species-rich but distinct arbuscular mycorrhizal communities in reforestation plots on degraded pastures and in neighboring pristine tropical mountain rain forest. *Tropical Ecology* 51:125–148.
- Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17:754–755, doi:10.1093/bioinformatics/17.8.754
- Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 30:3059–3066, doi:10.1093/nar/gkf436
- , Toh H. 2008. Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BMC Bioinformatics* 9:212, doi:10.1186/1471-2105-9-212
- Kornerup A, Wanscher JH. 1983. *Methuen handbook of colour*. 3rd ed. London: Eyre Methuen. 252 p.
- Kumar S, Tamura K, Nei M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 5:150–163, doi:10.1093/bib/5.2.150
- Lee J, Lee S, Young JP. 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiol Ecol* 65:339–49, doi:10.1111/j.1574-6941.2008.00531.x
- Lee PJ, Koske RE. 1994. *Gigaspora gigantea*: parasitism of spores by fungi and actinomycetes. *Mycol Res* 98:458–466, doi:10.1016/S0953-7562(09)81205-7
- Morton JB, Redecker D. 2001. Two families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. *Mycologia* 93:181–195, doi:10.2307/3761615
- , Walker C. 1984. *Glomus diaphanum*: a new species in the Endogonaceae common to West Virginia. *Mycotaxon* 21:431–440.
- Omar MB, Bolland L, Heather WA. 1979. A permanent mounting medium for fungi. *Bull Br Mycol Soc* 13:31–32, doi:10.1016/S0007-1528(79)80038-3
- Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M. 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytol* 188:223–241, doi:10.1111/j.1469-8137.2010.03334.x
- Posada D. 2008. jModelTest: phylogenetic model averaging. *Mol Biol Evol* 25:1253–1256, doi:10.1093/molbev/msn083
- Redecker D, Raab P. 2006. Phylogeny of the glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia* 98:885–895, doi:10.3852/mycologia.98.6.885
- Renker C, Błaszczowski J, Buscot F. 2007. *Paraglomus laccatum* comb. nov.—a new member of Paraglomeraceae (Glomeromycota). *Nova Hedwig* 84:395–407, doi:10.1127/0029-5035/2007/0084-0395
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574, doi:10.1093/bioinformatics/btg180
- Schüßler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycol Res* 105:1413–1421, doi:10.1017/S0953756201005196
- , Walker C. 2010. *The Glomeromycota. A species list with new families and new genera*. Gloucester, UK: Published in libraries at Royal Botanic Garden Edinburgh, Kew, Botanische Staatssammlung Munich and Oregon State University. 56 p.
- Spain JL, de Miranda JC. 1996. *Glomus brasilianum*: an ornamented species in the Glomaceae. *Mycotaxon* 60:137–142.
- Staden R, Beal KF, Bonfield JK. 2000. The Staden package 1998. *Methods Mol Biol* 132:115–130.
- Stürmer SL, Morton JB. 1997. Developmental patterns defining morphological characters in spores of four species in *Glomus*. *Mycologia* 89:72–81, doi:10.2307/3761174
- Stutz JC, Morton JB. 1996. Successive pot cultures reveal high species richness of arbuscular mycorrhizal fungi in arid ecosystems. *Can J Bot* 74:1883–1889, doi:10.1139/b96-225
- Trouvelot S, van Tuinen D, Hijri M, Gianinazzi-Pearson V. 1999. Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hybridization. *Mycorrhiza* 8:203–206, doi:10.1007/s005720050235
- Turnau K, Ryszka P, Gianinazzi-Pearson V, van Tuinen D. 2001. Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. *Mycorrhiza* 10:169–174, doi:10.1007/s005720000073
- van Tuinen D, Jacquot E, Zhao B, Gollotte A, Gianinazzi-Pearson V. 1998. Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. *Mol Ecol* 7:879–887, doi:10.1046/j.1365-294x.1998.00410.x
- Walker C. 1982. Species in the Endogonaceae: a new species (*Glomus occultum*) and a new combination (*Glomus geosporum*). *Mycotaxon* 15:49–61.
- . 1983. Taxonomic concepts in the Endogonaceae: spore wall characteristics in species descriptions. *Mycotaxon* 18:443–455.
- Wubet T, Weiss M, Kottke I, Teketay D, Oberwinkler F. 2006. Phylogenetic analysis of nuclear small subunit rDNA sequences suggests that the endangered African pencil cedar, *Juniperus procera*, is associated with distinct members of *Glomeraceae*. *Mycol Res* 110:1059–1069, doi:10.1016/j.mycres.2006.04.005