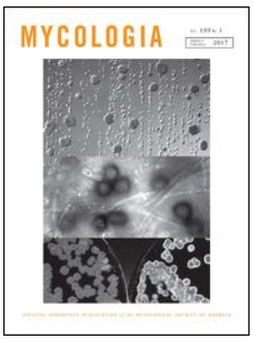

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Septoglomus fuscum and *S. furcatum*, two new species of arbuscular mycorrhizal fungi (Glomeromycota)

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Abstract: Two new arbuscular mycorrhizal fungal species, (Glomeromycota) *Septoglomus fuscum* and *S. furcatum*, are described and illustrated. Spores of *S. fuscum* usually occur in loose hypogeous clusters, rarely singly in soil or inside roots, and *S. furcatum* forms only single spores in soil. Spores of *S. fuscum* are brownish orange to dark brown, globose to subglobose, (20–)47(–90) µm diam, rarely ovoid, 21–50 × 23–60 µm. Their spore wall consists of a semi-persistent, semi-flexible, orange white to golden yellow, rarely hyaline, outer layer, easily separating from a laminate, smooth, brownish orange to dark brown inner layer. Spores of *S. furcatum* are reddish brown to dark brown, globose to subglobose, (106–)138(–167) µm diam, rarely ovoid, 108–127 × 135–160 µm, usually with one subtending hypha that is frequently branched below the spore base, or occasionally with two subtending hyphae located close together. Spore walls consists of a semi-permanent, hyaline to light orange outermost layer, a semipermanent, hyaline to golden yellow middle layer, and a laminate, smooth, reddish brown to dark brown innermost layer. None of the spore-wall layers of *S. fuscum* and *S. furcatum* stain in Melzer's reagent. In the field, *S. fuscum* was associated with roots of *Arctotheca populifolia* colonizing maritime dunes located near Strand in South Africa and *S. furcatum* was associated with *Cordia oncocalyx* growing in a dry forest in the Ceará State, Brazil. In single-species cultures with *Plantago lanceolata* as host plant, *S. fuscum* and *S. furcatum* formed arbuscular mycorrhizae. Phylogenetic analyses of the SSU, ITS and LSU nrDNA sequences placed the two new species in genus *Septoglomus* and both new taxa were separated from described *Septoglomus* species.

Key words: arbuscular fungi, Glomeromycota, molecular phylogeny, mycorrhizae, new species

Introduction

Arbuscular mycorrhizal fungi (AMF; Glomeromycota) have a worldwide distribution and are associated with ca. 70–90% of land plants (Smith and Read 2008, Brundrett 2009). About 62% of known AMF form glomoid spores, that is one-walled spores arising blastically, usually at the tip of sporogenous hyphae, as spores of *Glomus macrocarpum* Tul. & C. Tul., the type species of genus *Glomus* Tul. & C. Tul. (Schüßler and Walker 2010). These AMF are especially difficult to identify based solely on spore morphology because of a limited number of taxonomically significant characters and the changes that can occur among them as spores age.

In the past few years many descriptions of AMF species were based on data obtained from morphological and molecular analyses (e.g. Błaszkowski et al. 2010, Kaonongbua et al. 2010, Palenzuela et al. 2010). This trend resulted in several taxonomic changes in Glomeromycota, including those regarding AMF that form glomoid spores (Schüßler and Walker 2010; Oehl et al. 2011a, b, c; Schüßler et al. 2011).

Based on recent classifications of Glomeromycota, species producing glomoid spores are distributed in 14 genera belonging to six families, four orders and three classes of the phylum (Schüßler and Walker 2010; Oehl et al. 2011a, b, c). Among these taxa the

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genus *Septoglomus* Sieverd. et al. was erected primarily based on phylogenetic analyses of rDNA sequences of four species formerly classified in *Glomus*, *S. africanum* (Błaszk. & Kovács) Sieverd. et al., *S. constrictum* (Trappe) Sieverd. et al., *S. deserticola* (Trappe et al.) G.A. Silva et al. and *S. xanthium* (Błaszk. et al.) G.A. Silva et al. (Oehl et al. 2011b). All these species form dark spores with 2–3-layered spore walls, the innermost layer being laminate. Their spore subtending hypha usually is cylindrical or constricted, and its pore is closed by a septum.

We isolated glomoid spores of two AMF, which seemed to be undescribed species, from pot trap cultures. Subsequent morphological studies of these fungi and molecular phylogenetic analyses of sequences of their small subunit (SSU), internal transcribed spacer (ITS) region, the DNA barcode of the Fungi (Schoch et al. 2012) and large subunit (LSU) of nrDNA genes confirmed our suppositions and indicated their generic position within Glomeromycota. Consequently the AMF are described here as *Septoglomus fuscum* and *S. furcatum* spp. nov.

MATERIALS AND METHODS

Establishment and growth of trap and single-species cultures, extraction of spores and staining of mycorrhizae.—Spores examined in this study were derived from both pot trap and single-species cultures. Trap cultures were established and maintained as described by Błaszkowski et al. (2012) to obtain living spores and to initiate sporulation of species that may not have sporulated in the field collections (Stutz and Morton 1996). The growing substrate for trap cultures was the field-collected rhizosphere soil and roots of the plant species sampled, mixed with autoclaved coarse grained sand.

Single-species cultures also were established and grown as described in Błaszkowski et al. (2012). Briefly, the cultures of *S. fuscum* were established from small clusters of spores (3–5) attached by a common mycelium and those of *S. furcatum* from ca. 10 spores. Attempts to establish single-spore isolates failed. The growing substrate of the cultures was autoclaved, commercially available coarse-grained sand (80.5% grains, 1.0–10.0 mm diam; 17.3% grains, 0.1–1.0 mm diam; 2.2% grains, < 0.1 mm diam) mixed (5:1, v/v) with clinopthilolite (Zeocem, Bystré, Slovakia) grains, 2.5–5 mm. Clinopthilolite is a crystalline hydrated alumosilicate of alkali metals and alkaline earth metals having a high ion exchange capability as well as a reversible hydration and dehydration. pH of the sand-clinopthilolite mixture was 7.3. To prevent contamination with other AMF, cultures were kept in transparent plastic bags, 15 cm wide and 22 cm high as suggested by Walker and Vestberg (1994). The cultures were watered with tap water once or twice a week, and harvested after 5 mo when spores were extracted for study. To reveal mycorrhizal root structures, root fragments located ca. 1–5 cm below the upper level of the growing medium were excised with a scalpel. *Plantago lanceolata* L. was used as a host plant in both trap and single-species cultures.

Microscopy.—Morphological properties of spores and their wall structures were determined after examination of at least 100 spores mounted in water, lactic acid, polyvinyl alcohol/lactic acid/glycerol (PVLG; Omar et al. 1979) and a mixture of PVLG and Melzer's reagent (1:1, v/v). Spores at all developmental stages were crushed to varying degrees by applying pressure to the cover slip, and slides were stored at 65 C for 24 h to clear spore contents from oil droplets. Spores were examined under an Olympus BX 50 compound microscope equipped with Nomarski differential interference contrast optics. Microphotographs were recorded on a Sony 3CDD color video camera attached to the microscope.

Terminology of spore characters is that suggested by Stürmer and Morton (1997) and Walker (1983). Spore color was examined under a dissecting microscope on fresh specimens immersed in water. Color names are from Kornerup and Wanscher (1983). Nomenclature of plants is after Mirek et al. (http://info.botany.pl/czek/check.htm, http://florabase.dec.wa.gov.au/browse/profile.php/7839) and that of fungi and the authors of 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, USA.

DNA extraction, polymerase chain reaction and DNA sequencing.—Crude DNA was isolated from small spore clusters (*S. fuscum*) or 3–8 single spores (*S. furcatum*) crushed with a pipette tip in PCR tubes or a needle in ultraclean water on sterile microscope slides under a dissecting microscope. Amplification, cloning and sequencing were carried out as described in Błaszkowski et al. (2012). Partial nrSSU segment was amplified with the primers AML1 and AML2 (Lee et al. 2008), and partial LSU segment was amplified using the primers LR1 (van Tuinen et al. 1998) and FLR2 (Trouvelot et al. 1999) followed by nested amplification with primers 28G1 and 28G2 (da Silva et al. 2006) as described in Błaszkowski et al. (2012). The ITS region was amplified with GLOM1310 (Redecker 2000) and ITS4 primers following the protocol described in Kovács et al. (2007). In addition, using the methods and primers described above, we obtained partial nrSSU sequences of *S. constrictum* (three sequences) and *S. xanthium* (seven sequences) and partial LSU sequences of *S. africanum* (four sequences) and *S. xanthium* (six sequences) from our single-species culture collection. The *S. constrictum* culture was established from spores obtained from Dr Chris Walker, Royal Botanic Garden, Edinburgh, UK (culture 262-5[6]).

The sequences were deposited in GenBank under accessions numbers JX683146–JX683149 for *S. africanum*, JX683150–JX683151, JX683154–JX683157 and JX683158–JX683163 for *S. furcatum*, JX683152–JX683153, JX683168–JX683170 and JX683165–JX683167 for *S. fuscum* and

JX683171, JX683178–JX683182, JX683172–JX683177 and JX683183 for *S. xanthium*.

Sequence alignment and phylogenetic analyses.—Phylogenetic analyses were performed separately with SSU, ITS and LSU sequences. After pilot analyses of sequences obtained by us with 54 SSU and 53 LSU known sequences representing all recognized genera of Glomeromycota, the final datasets comprised all our sequences of each new species, all our sequences of *S. africanum*, *S. constrictum* and *S. xanthium*, several published sequences of *Septoglomus* spp., except the sequence AF145741 (da Silva et al. 2006) and sequences of other species with glomoid spores mainly deriving from the former *Glomus* group A (Schwarzott et al. 2001). The sequence AF145741 probably represents *Funneliformis coronatus* (Giovann.) C. Walker & A. Schüßler (G.A. da Silva pers comm) whose small-spored isolates closely resemble *S. constrictum* spores (Błaszkowski, unpubl).

The SSU and LSU sequences were aligned with Clustal W (Thompson et al. 1994) with default parameters. The ITS sequence dataset was aligned with MAFFT 6 Q-INS-i (Katoh and Toh 2008). The alignment with LSU sequences was deposited in TreeBASE (S13527).

Maximum likelihood (ML) and Bayesian (BI) analyses were performed with PHYML (Guindon and Gascuel 2003) and MrBayes 3.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) respectively. Before the analyses the best-fit substitution models for the alignments were estimated by the Akaike information criterion (AIC) using either Topali 2.5 (Milne et al. 2004) or jModeltest (Posada 2008). *Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler was outgroup in analyses of SSU and LSU sequences, and *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler in those of ITS sequences. In the ML and BI analyses of both SSU and LSU sequences the models employed were TrN + G and GTR + G respectively. In the ML analysis the transition/transversion ratio for DNA models and the gamma distribution parameter were estimated. Six substitution rate categories were set. Topology and branch lengths and rate parameters were optimized. Support of branches in the ML analysis was estimated in a bootstrap analysis with 1000 replicates.

Because indels can improve the phylogenetic potential of fungal ITS sequences (Nagy et al. 2012), we coded indels in ITS alignment by means of the simple indel-coding algorithm (Simmons and Ochoterena 2001) as implemented in GapCode (Ree 2008), which converts all indels with different starting and/or end positions to a matrix of binary presence/absence characters. Indels showing a complete overlap with a longer indel were coded as unknown characters. Leading and trailing gaps of the alignments were scored as missing data. This binary character set was added to the nucleotide alignment of the ITS dataset. In Bayesian inference nucleotide-only analyses (SSU and LSU) were not partitioned, combined nucleotide plus indel analysis (ITS) was divided into two partitions. Within the nucleotide alignments, gaps were treated as missing data (i.e. not as a fifth character state). We applied the GTR + G model and a two-parameter Markov model (Mk2 Lewis) for nucleotide data and indel matrices respectively. In the BI analyses the Markov chain was run 5 000 000 generations, sampling every 500 steps, with a burn-in at 3000. The details of the analyses are available on request. Phylogenetic trees were visualized and edited in MEGA5 (Tamura et al. 2011). The alignment with LSU sequences was deposited in TreeBASE (S13527).

RESULTS

Molecular analyses.—The analyses of all regions we sequenced (partial SSU, data not shown, ITS and partial LSU) unambiguously placed our two new species in genus *Septoglomus* and separated them from the known species within the genus (FIGS. 1, 2). The analyses of LSU (FIG. 1) and SSU sequences resolved *S. fuscum* as a monophyletic group in a clade together with *S. xanthium* sequences. In the ITS analyses *S. fuscum* sequences formed a monophyletic group with two environmental sequences also in a clade with *S. xanthium* (FIG. 2). In all analyses *S. furcatum* formed a distinct clade within the genus (FIGS. 1, 2). Both the phylogenetic analyses of the SSU and LSU (FIG. 1) sequences indicated that *S. africanum* and *S. constrictum* represent a clade.

TAXONOMY

Septoglomus fuscum Błaszk., Chwat & Kovács, Ryszka, sp. nov. FIGS. 1–10

MycoBank MB801338

Sporocarps unknown. Spores formed in soil in loose clusters, rarely singly, occasionally inside roots (FIGS. 3–8); develop blastically at the tip of, rarely along (intercalary spores) sporogenous hyphae either branched from a parent hypha continuous with a mycorrhizal extraradical hypha (spores in clusters) or directly developed from mycorrhizal extraradical hyphae (single spores). Clusters 89–194 × 141–248 µm with 2–7 spores and usually abundant sterile (without spores) hyphae (FIG. 3). Spores yellowish white (4A2) when juvenile, brownish orange (6C7) to dark brown (7F8) at maturity; globose to subglobose; (20–)47(–90) µm diam; rarely ovoid; 21–50 × 23–60 µm; with one subtending hypha (FIGS. 3–8). Spore wall consists of two layers (FIGS. 5–8). Layer 1, forming the spore surface, semi-persistent, semi-flexible, orange white (5A2) to golden yellow (5B7), rarely hyaline, (1.0–)1.6(–2.5) µm thick, rarely partly deteriorated in mature and older spores, usually easily separating from layer 2 in crushed spores (FIGS. 5–8). Layer 2 laminate, smooth, yellowish white (4A2) in juvenile spores, brownish orange (6C7) to dark brown (7F8) in mature spores, (2.0–)3.8(–7.0) µm thick (FIGS. 5–8). Spore wall layers 1 and 2 do not stain in Melzer's reagent (FIG. 6). Subtending hypha brownish orange (6C7) to dark brown (7F8) in mature

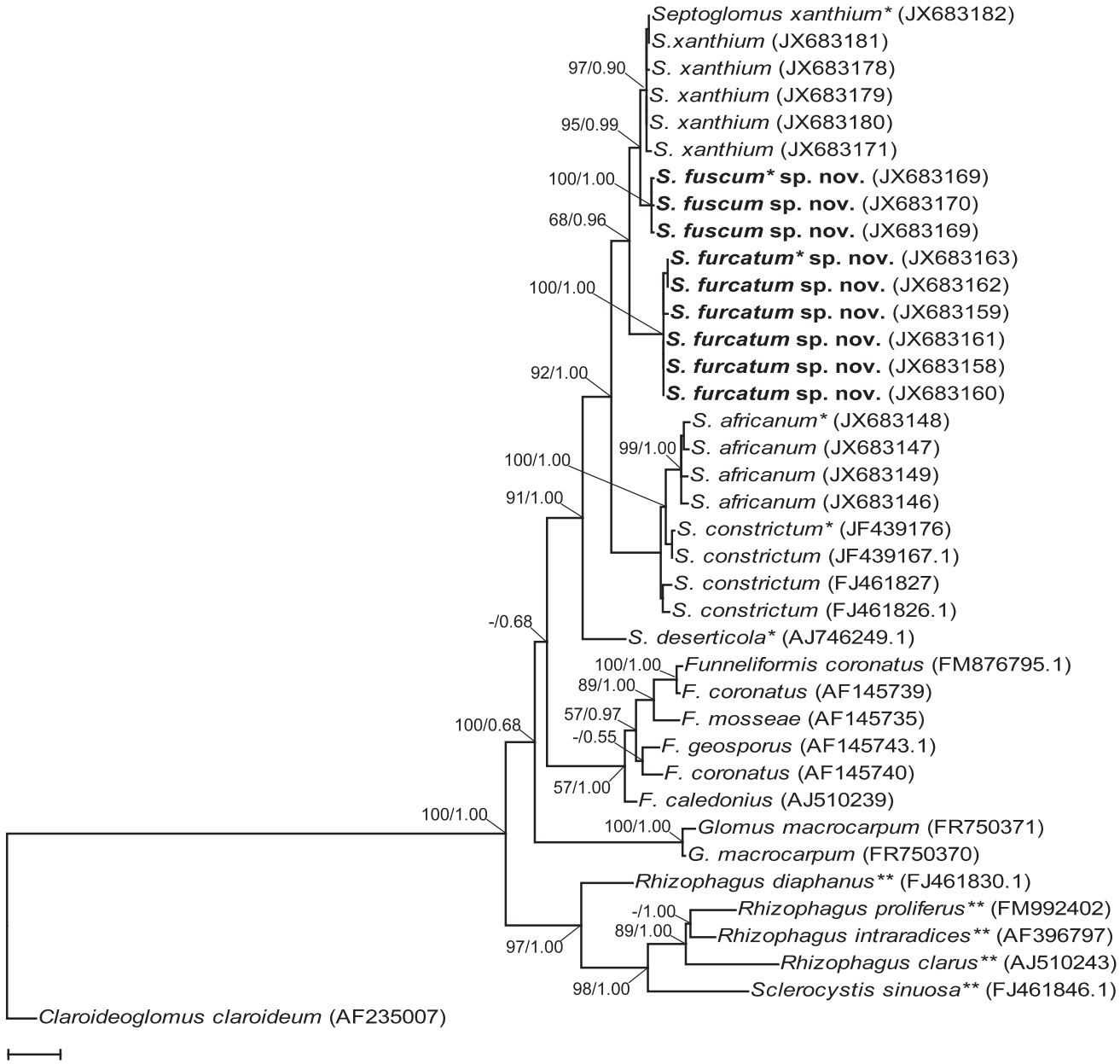


FIG. 1. Maximum likelihood (ML) tree inferred from LSU nrDNA sequences with *Claroideoglomus claroideum* as outgroup. GenBank accession numbers of the sequences are in parentheses. ML bootstrap values ≥ 50% and the Bayesian posterior probabilities ≥ 0.50 are shown near the branches respectively. * = sensu Oehl et al. 2011a, ** = sensu Schüßler and Walker 2010. Bar indicates 0.05 expected change per site per branch.

spores; straight or recurved, cylindrical to funnel-shaped, sometimes slightly constricted at the spore base; (5.5–)7.4(–13.6) µm wide at the spore base (FIGS. 3, 5–7). Wall of subtending hypha brownish orange (6C7) to dark brown (7F8); (1.0–)2.9(–6.2) µm thick at the spore base; continuous with spore wall layers 1 and 2, always extending far below the spore base (FIGS. 7, 8). Pore (1.2–)1.7(–2.4) µm diam, open, with age gradually narrowing due to thickening of wall layer 2 of the subtending hypha toward the center of its lumen (FIGS. 7, 8). Sterile hyphae of spore clusters hyaline to grayish orange (5B3), (2.4–)4.8(–7.3) µm wide, with walls (1.0–)1.6(–2.0) µm thick (FIG. 3). Germination unknown.

Mycorrhizal associations: In the field *S. fuscum* was associated with roots of *Arctotheca populifolia* (P.J. Bergius) Norl. (Asteraceae) in South Africa (see below). In single-species cultures with *P. lanceolata* as host plant, *S. fuscum* formed mycorrhizae with arbuscules, intra- and extraradical hyphae (FIGS. 9,

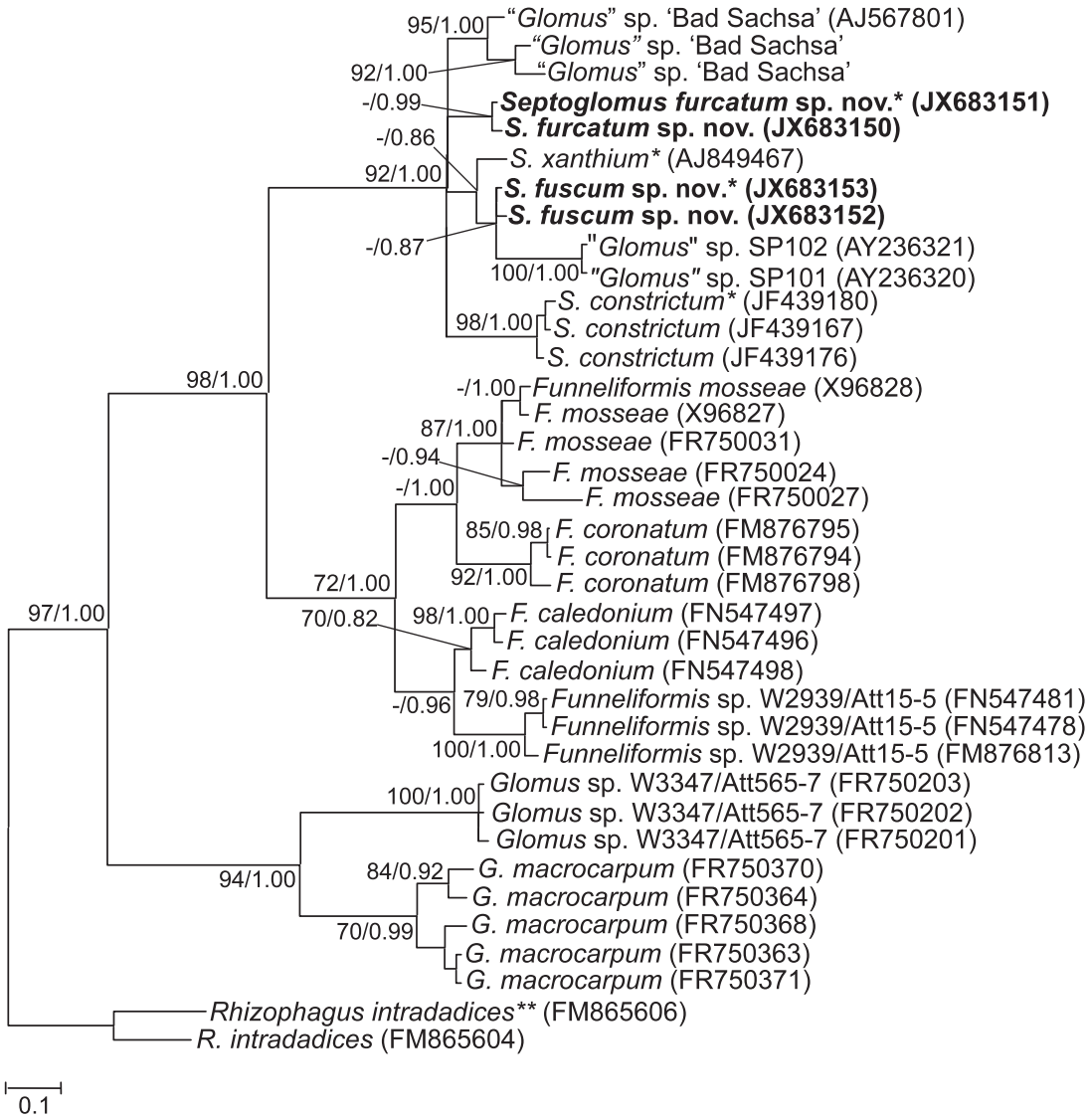


FIG. 2. Fifty percent majority rule consensus phylogram inferred from the Bayesian analysis of ITS dataset (nucleotide + gap coded) showing the phylogenetic position of *Septoglomus fuscum* and *S. furcatum*. The three-genus dataset was analyzed with *Rhizophagus intraradices* as outgroup. GenBank accession numbers of the sequences are in parentheses. ML bootstrap values ≥ 70% and the Bayesian posterior probabilities ≥ 0.90 are near the branches respectively. Bar indicates 0.1 expected change per site per branch.

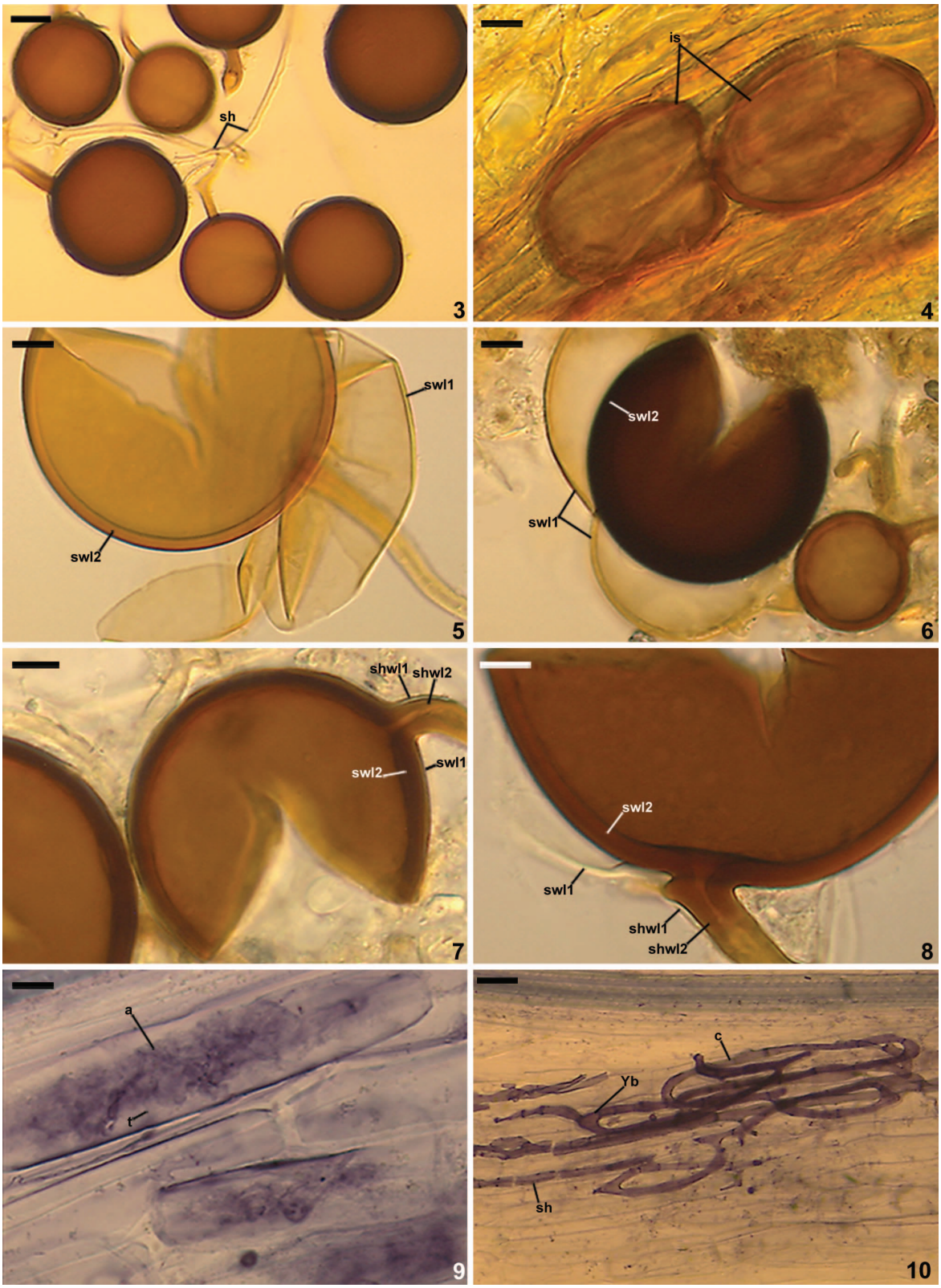
10). No vesicles were found even in roots from 3 y old cultures. Arbuscules were usually widely dispersed along the root fragments examined (FIG. 9). Intraradical hyphae were abundant, (2.0–)4.9(–8.0) µm wide, and frequently formed coils, 10.3–18.0 × 36.5–64.8 µm (FIG. 10). Extraradical hyphae occurred rarely. All the structures stained intensively (violet white, 17A2, to deep violet, 17E8) in 0.1% trypan blue (FIGS. 9, 10).

Specimens examined: POLAND, Szczecin, under pot-cultured *P. lanceolata*, 10 Mar 2012, *Błaszkowski, J., 3281* (HOLOTYPE, DPP); *Błaszkowski, J., 3283–3284* and *3286–3308* (ISTOTYPES, DPP) and two slides at OSC.

Etymology: Latin, *fuscum*, referring to the dark brown spores formed by this species.

Distribution and habitat: Spores of *S. fuscum* were found in one trap culture established from a mixture of the rhizosphere soil and root fragments of *A. populifolia* growing in maritime dunes located near Strand (34°06′S, 18°49′E), ca. 50 km southeast of Cape Town, South Africa. This mixture was sampled on 2 Oct 2005. In the field sample only one other undescribed AMF with glomoid spores was found. *Glomus microcarpum* Tul. & C. Tul. and *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler also occurred in the original trap culture with *S. fuscum*.

Sequences of the partial SSU gene of *S. fuscum* were 99% similar to in planta sequences obtained



FIGS. 3–10. Spores of *Septoglomus fuscum*. 3. Spores in loose cluster with sterile hyphae (sh). 4. Intraradical spores. 5, 6. Spore wall layer (swl) 1 separated from swl2. 7, 8. Subtending hyphal wall layers (shwl) 1 and 2 continuous with spore wall layers (swl) 1 and 2; note the open lumen of the subtending hypha. 9, 10. Mycorrhizae of *S. fuscum* in roots of *Plantago lanceolata* stained in 0.1% trypan blue. 9. Arbuscule (a) with trunk (t). 10. Intraradical straight (sh) and Y-branched (Yb) hyphae and coils (c). 3, 5, 7, 8. spores in PVLG. 4, 6. Spores in PVLG + Melzer's reagent. 9, 10. Mycorrhizae in PVLG. 3–10, differential interference microscopy. Bars: 3 = 20 μm, 4–10 = 10 μm.

from BLAST queries. Among them there were for example sequences from *Juniperus procera* Hochst. ex Endlicher and *Olea europea* L. subsp. *sylvestris* growing in the Afromontane region of Ethiopia, Africa, and in southeastern Spain, Europe respectively (Wubet et al. 2006, Alguacil et al. 2011). No significant conspecific similarity of LSU and ITS sequences of *S. fuscum* was found when they were compared to environmental sequences.

Notes: The most distinctive morphological characters of *S. fuscum* are its loose clusters with small dark spores and abundant sterile hyphae (FIG. 3). The simple, two-layered spore wall with the long-lived, usually colored layer 1 easily separating from the structural laminate layer 2 in crushed spores also distinguishes this species (FIGS. 5–8).

The closest relative of *S. fuscum* is *S. xanthium*, both molecular phylogenetic analyses (FIGS. 1, 2) and study of morphology show. The two species can be separated even under a dissecting microscope. Spores of *S. fuscum* usually occur in loose clusters in soil and rarely inside roots (FIGS. 3, 4). *Septoglomus xanthium* forms only single spores, which usually tightly adhere to roots and frequently develop inside roots (Błaszkowski et al. 2004).

When observed under a compound microscope *S. fuscum* and *S. xanthium* differ clearly. Although spore wall layers 1 and 2 of *S. fuscum* (FIGS. 5–8) are similar phenotypically to spore wall layers 1 and 3 respectively of *S. xanthium* the former species lacks the permanent spore wall layer 2 of the latter fungus (Błaszkowski et al. 2004). In addition the laminate spore wall layer (layer 2) of *S. fuscum* is 2.3–3.2-fold thicker than that (layer 3) of *S. xanthium*.

Glomus antarcticum Cabello, *G. badium* Oehl et al., *G. liquidambaris* (C.G. Wu & Z.C. Chen) Y.J. Yao and *G. fuegianum* (Speg.) Trappe & Gerd. also produce dark, small spores (Wu and Chen 1987, Almeida and Schenck 1990, Cabello et al. 1994, Błaszkowski pers obs, Błaszkowski et al. 1998, Oehl et al. 2005,). However, except for *G. antarcticum*, spores of the other three species arise in compact sporocarps (vs. usually in loose clusters in *S. fuscum*; FIG. 3). Spores of *G. antarcticum* occur in loose clusters, but the spores are much lighter, spore wall layer 1 always is hyaline (vs. usually colored in *S. fuscum*) and their subtending hypha is much wider and has a thicker wall and a wider pore. In addition the spore wall of *G. badium* comprises three layers, and that of *G. liquidambaris* is one-layered (two-layered in *S. fuscum*). Of the species listed above, the molecular phylogenetic position within Glomeromycota is known only for *G. badium* (Oehl et al. 2005, 2011b).

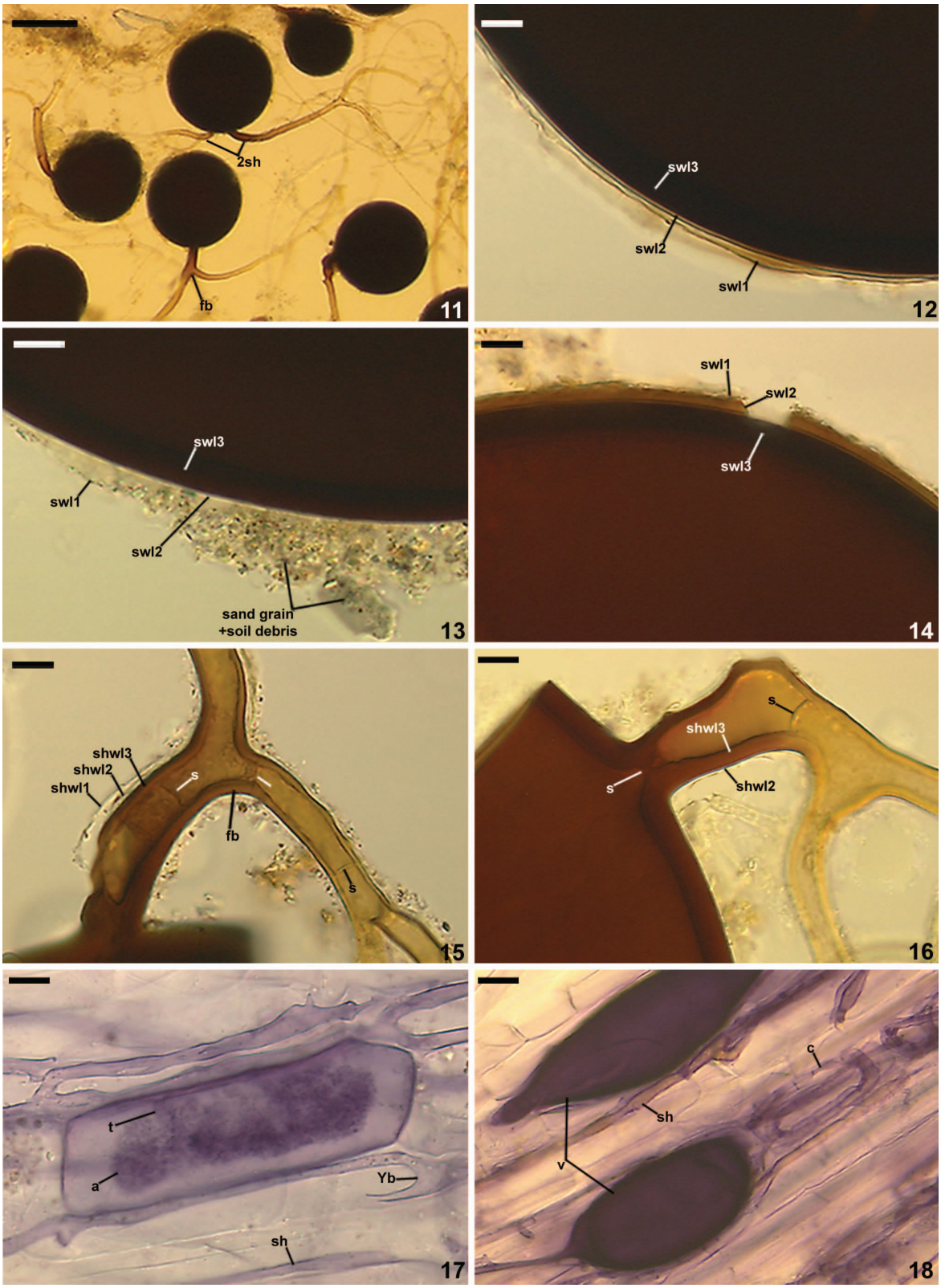
Septoglomus furcatum Błaszk., Chwat & Kovács, Ryszka, sp. nov. FIGS. 1, 2, 11–18

MycoBank MB801339

Sporocarps unknown. Spores formed singly in soil (FIG. 11); develop blastically at the tip of sporogenous hyphae continuous with mycorrhizal extraradical hyphae. Spores reddish brown (9E8) to dark brown (9F8); globose to subglobose; (106–)138(–167) µm diam; rarely ovoid; 108–127 × 135–160 µm; usually with one subtending hypha, sometimes with two subtending hypha, located close together (within a maximum of 28 µm), never on opposite sides of a spore (FIG. 11). Spore wall consists of three layers (FIGS. 12–14). Layer 1, forming the spore surface, semipermanent, hyaline to light orange (5A6), (1.3–)1.6(–2.5) µm thick, smooth when intact in youth, gradually deteriorating with age, rarely completely sloughed, sometimes incorporating sand grains or soil debris (FIGS. 12–14). Layer 2 semipermanent, hyaline to golden yellow (5B7), (1.3–)1.9(–3.3) µm thick, usually intact in mature spores (FIGS. 12–14), occasionally slightly deteriorated in older spores. Layer 3 laminate, smooth, reddish brown (9E8) to dark brown (9F8), (5.3–)7.6(–11.3) µm thick (FIGS. 12–14). None of spore wall layers 1–3 stains in Melzer's reagent (FIGS. 13, 16). Subtending hypha reddish brown (9E8) to dark brown (9F8); straight or recurved, cylindrical to slightly funnel-shaped, sometimes slightly constricted at the spore base; (10.5–)17.9(–30.3) µm wide at the spore base; frequently with a forked branch formed 16–70 µm below the spore base (FIGS. 11, 15, 16). Wall of subtending hypha reddish brown (9E8) to dark brown (9F8); (6.0–)8.2(–12.0) µm thick at the spore base; continuous with spore wall layers 1–3, which extend far below the spore base (FIGS. 15, 16). Pore (3.5–)4.3(–6.5) µm diam, narrowing with spore age due to thickening of wall layer 3 of the subtending hypha toward the center of its lumen, usually closed by a straight or curved septum continuous with some innermost laminae of spore wall layer 2 (FIG. 16); septum

→

FIGS. 11–18. Spores of *Septoglomus furcatum*. 11. Spores with unbranched subtending hyphae, spore with a forked branch (fb) of the subtending hypha and spore with two subtending hyphae (2sh). 12. Intact spore wall layers (swl) 1–3. 13. Highly deteriorated hyaline spore wall layer (swl) 1 with incorporated sand grain and soil debris and intact hyaline swl2 tightly adherent to the colored laminate swl3. 14. Slightly deteriorated spore wall layer (swl) 1 and intact swl2 and 3; note the colored swl1 and 2. 15. Subtending hyphal wall layers (shwl) 1–3; note the septa in the lumen and the forked branch (fb) of the subtending hypha. 16. Subtending hyphal wall layers (shwl) 2 and 3; shwl1 is completely sloughed; note the septa present at



the spore base and in the lumen of the subtending hypha. 17, 18. Mycorrhizae of *S. furcatum* in roots of *Plantago lanceolata* stained in 0.1% trypan blue. 17. Arbuscule (a) with trunk (t) and straight (sh) and Y-branched (Yb) hyphae. 18. Vesicles (v), straight hypha (sh) and coil (c). 11. Spores in lactic acid. 12, 14, 15. Spores in PVLG. 13, 16. Spores in PVLG + Melzer's reagent. 17, 18. Mycorrhizae in PVLG. 11–18, differential interference microscopy. Bars: 11 = 100 µm, 18 = 20 µm, 12–17 = 10 µm.

2.0–3.8 µm thick, always positioned at the level of spore wall layer 3 (not invaginated into the lumen of the subtending hypha); pore rarely open; the subtending hyphal lumen frequently partitioned with numerous transverse or sloping, straight or arched septa, 7.0–11.5 µm wide, 1.0–2.3 µm thick, spaced 12.3–44.5 µm apart (FIGS. 15, 16). Germination unknown.

Mycorrhizal associations: In the field *S. furcatum* was associated with roots of *Cordia oncocalyx* Allemão (Boraginaceae) in Brazil (see below).

In single-species culture with *P. lanceolata* as host plant *S. furcatum* formed mycorrhizae with arbuscules, vesicles, and intra- and extraradical hyphae (FIGS. 17, 18). Arbuscules were numerous and evenly distributed along roots (FIG. 17). Vesicles occurred rarely. They were ellipsoidal to oblong, 42.5–51.0 × 80.0–216.5 µm, when observed in a plan view (FIG. 18). Intraradical hyphae were abundant, evenly distributed along the root fragments examined and measured (2.0–)4.4(–7.8) µm wide (FIGS. 17, 18). They frequently formed Y-shaped branches and coils, 22.5–25.5 × 36.5–65.0 µm (FIGS. 17, 18). Extraradical hyphae were (2.0–)4.6(–8.0) µm wide and occurred not abundantly. All the structures stained intensively, pale violet (17A3) to deep violet (17D8), in 0.1% trypan blue (FIGS. 17, 18).

Specimens examined: POLAND, Szczecin, under pot-cultured *P. lanceolata*, 10 Mar 2012, *Błaszkowski, J., 3309* (HOLOTYPE, DPP); *Błaszkowski, J., 3311, 3313–3327* (ISTOTYPES, DPP) and two slides at OSC.

Etymology: Latin, *furcatum*, referring to the branched subtending hypha frequently formed by the species.

Distribution and habitat: Spores of *S. furcatum* were isolated from one pot trap culture with the rhizosphere soil and root fragments of *Cordia oncocalyx* Allemão (Boraginaceae) growing in a dry forest (5°00′S 40°48′W) in Ceará state, Brazil. The soil and roots were collected by M. Pagano, 4 Apr 2010.

The *S. furcatum* partial SSU gene sequences showed 99% similarity with in planta sequences from environmental samples, suggesting the species occurs in other sites. Closely related sequences were found from *O. europea* subsp. *cuspidata*, *Podocarpus falcatus* (Thunb.) R.Br. ex Mirb. and *Prunus africana* Hook.f. roots growing in the Afromontane region of Ethiopia (Wubet et al. 2009) and from *O. europea* subsp. *sylvestris* growing in southeastern Spain (Alguacil et al. 2011).

Notes: Septoglomus furcatum is distinguished mainly by its dark and relatively large spores with a three-layered spore wall and by a frequently branched subtending hypha located a short distance below the spore base (FIGS. 11–16). Another feature distinguishing *S. furcatum* spores is that they commonly have two subtending hyphae located one near another. This feature is less common or absent in other *Septoglomus* spp.

When observed under a dissecting microscope, older *S. furcatum* spores may be indistinguishable from those of *S. constrictum* (Trappe) Sieverd., G.A. Silva & Oehl. However maturing and freshly mature spores of *S. furcatum* usually are darker due to presence of a higher amount of brown pigments in the structural laminate spore wall layer 3; maturing and freshly mature *S. constrictum* spores usually are brownish orange (6C8; http://www.zor.zut.edu.pl/Glomeromycota/).

The most evident morphological differences readily separating *S. furcatum* from *S. constrictum* regard the number and phenotypic features of their spore wall layers. The structural laminate layer of the spore wall of *S. furcatum* is its third component and it is covered with two long-lived layers, which are hyaline in youth but color with age (FIGS. 12–14). The spore wall of *S. constrictum* consists of only two layers (a semipermanent layer 1 and a laminate layer 2), and layer 1, when not completely sloughed, remains hyaline, even in older spores (http://www.zor.zut.edu.pl/Glomeromycota/).

Spores of other known *Septoglomus* spp. (FIGS. 1, 2) are much lighter and smaller, usually occur in loose clusters (vs. only singly in *S. furcatum*) and do not have branched subtending hypha (Trappe et al. 1984; Błaszkowski et al. 2004, 2010). In addition the spore wall of *S. fuscum* and *S. deserticola* (Trappe, Bloss & J.A. Menge) G.A. Silva, Oehl & Sieverd. comprises only two layers (FIGS. 5–8).

A branched subtending hypha also occurs in *G. brohultii* Sieverd. & R.A. Herrera spores (Błaszkowski unpubl, Herrera-Peraza et al. 2003). However spores of this species are much lighter, have a spore wall with two layers only, and their subtending hypha is clearly lighter than the spore wall (vs. the subtending hypha is the same color as the spore wall in *S. furcatum*; FIGS. 11, 15, 16).

Seven other species also produce glomoid spores of color and size similar to those of *S. furcatum* spores. Of them *G. ambisporum* G.S. Sm. & N.C. Schenck, *G. atrouva* McGee & Pattison, *G. heterosporum* G.S. Sm. & N.C. Schenck, *G. melanosporum* Gerd. & Trappe and *G. warcupii* McGee form spores in compact sporocarps (Gerdemann and Trappe 1974, McGee 1986, Smith and Schenck 1985, McGee and Trappe 2002), and *G. botryoides* F.M. Rothwell & Victor usually in loose clusters (Rothwell and Victor 1984; vs. usually singly in soil in *S. furcatum*; FIG. 11). Moreover, only *G. ambisporum* has a three-layered spore wall, but its layer 3 is uniform, flexible and < 1 µm thick (vs. laminate, semirigid and 5.3–11.3 µm thick in *S.*

furcatum). Although *F. coronatus* forms single spores, they usually are slightly larger than those of *S. furcatum*, have a two-layered spore wall (vs. three-layered; FIGS. 12–14) and their subtending hypha is funnel-shaped (vs. cylindrical to funnel-shaped) and much wider (Giovannetti et al. 1991; http://www.zor.zut.edu.pl/Glomeromycota/). Of the species compared here only *F. coronatus* and *S. furcatum* have known molecular phylogenetic positions within Glomeromycota (Oehl et al. 2011b, Krüger et al. 2012).

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LITERATURE CITED

Alguacil MM, Torrecillas E, Kohler J, Roldán A. 2011. A molecular approach to ascertain the success of in situ AM fungi inoculation in the revegetation of a semi-arid, degraded land. Sci Total Environ 409:2874–2880, doi:10.1016/j.scitotenv.2011.04.029

Almeida RT, Schenck NC. 1990. A revision of the genus *Sclerocystis* (Glomaceae, Glomales). Mycologia 82:703–714, doi:10.2307/3760157

Błaszkowski J, Blanke V, Renker C, Buscot F. 2004. *Glomus aurantium* and *G. xanthium*, new species in Glomeromycota. Mycotaxon 90:447–467.

———, Kovács GM, Balázs T, 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

———, ———, Gáspár BK, Balázs TK, Buscot F, Ryszka P. 2012. The arbuscular mycorrhizal *Paraglomus majewskii* sp. nov. represents a new, distinct basal lineage in Paraglomeraceae (Glomeromycota). Mycologia 104:148–156, doi:10.3852/10-430

———, Madej T, Tadych M. 1998. *Entrophospora baltica* sp. nov. and *Glomus fuegianum*, two species in the Glomales from Poland. Mycotaxon 68:165–184.

Brundrett MC. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77, doi:10.1007/s11104-008-9877-9

Cabello M, Gaspar L, Pollero R. 1994. *Glomus antarcticum* sp. nov., a vesicular-arbuscular mycorrhizal fungus from Argentina. Mycotaxon 60:123–128.

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

Gerdemann JW, Trappe JM. 1974. The Endogonaceae in the Pacific Northwest. Mycol Memoir 5:1–76.

Giovannetti M, Avio L, Salutini L. 1991. Morphological, cytochemical and ontogenetic characteristics of a new species of vesicular-arbuscular mycorrhizal fungus. Can J Bot 69:161–167, doi:10.1139/b91-023

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

Herrera-Peraza RA, Ferrer RL, Sieverding E. 2003. *Glomus brohultii*: a new species in the arbuscular mycorrhiza forming Glomerales. J Appl Bot 77:37–40.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755, doi:10.1093/bioinformatics/17.8.754

Kaonongbua W, Morton JB, Bever JD. 2010. Taxonomic revision transferring species in *Kuklospora* to *Acaulospora* (Glomeromycota) and a description of *Acaulospora colliculosa* sp. nov. from field-collected spores. Mycologia 102:1497–1509, doi:10.3852/10-011

Katoh K, 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 color. 3rd ed. London: Eyre Methuen. 252 p.

Kovács GM, Balázs T, Pénzes Z. 2007. Molecular study of the arbuscular mycorrhizal fungi colonizing the sporophore sporophyte of the eusporangiate rattlesnake fern (*Botrychium virginianum*, Ophioglossaceae). Mycorrhiza 17:597–605, doi:10.1007/s00572-007-0137-2

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A. 2011. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol 193:970–984, doi:10.1111/j.1469-8137.2011.03962.x

Lee J, Lee S, Young PW. 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 65:339–349, doi:10.1111/j.1574-6941.2008.00531.x

McGee PA. 1986. Further sporocarpic species of *Glomus* (Endogonaceae) from south Australia. Trans Br Mycol Soc 87:123–129, doi:10.1016/S0007-1536(86)80011-0

———, Trappe JM. 2002. The Australian zygomycetous mycorrhizal fungi II. Further Australian sporocarpic Glomaceae. Austral Sys Bot 15:115–124, doi:10.1071/SB00038

Milne I, Wright F, Rowe G, Marshal DF, Husmeier McGuire G. 2004. TOPALi: software for automatic identification of recombinant sequences within DNA multiple alignments. Bioinformatics 20:1806–1807, doi:10.1093/bioinformatics/bth155

Nagy GL, Kocsubé S, Csanádi Z, Kovács GM, Petkovics T, Vágvölgyi Cs, Papp T. 2012. Re-Mind the gap! Insertion-deletion data reveal neglected phylogenetic potential of the nuclear ribosomal internal transcribed spacer

(ITS) of fungi. PLoS ONE 7:e49794, doi:10.1371/journal.pone.0049794

Oehl F, da Silva GA, Goto BT, Maia LC, Sieverding E. 2011a. Glomeromycota: two new classes and a new order. Mycotaxon 116:365–379, doi:10.5248/116.365

———, ———, ———, Sieverding E. 2011b. Glomeromycota: three new genera and glomoid species reorganized. Mycotaxon 116:75–120, doi:10.5248/116.75

———, ———, Sánchez-Castro I, Goto BT, Maia LC, Vieira HEE, Barea J-M, Sieverding E, Palenzuela J. 2011c. Revision of Glomeromycetes with entrophosporioid and glomoid spore formation with three new genera. Mycotaxon 117:297–316, doi:10.5248/117.297

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

Omar MB, Bollan 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

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

Posada. 2008. jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256, doi:10.1093/molbev/msn083

Redecker D. 2000. Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. Mycorrhiza 10:73–80, doi:10.1007/s005720000061

Ree R. 2008. Gapcode.py 2.1. Distributed by the author at: http://www.reelab.net/home/node/44

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

Rothwell FM, Victor BJ. 1984. A new species of Endogonaceae: *Glomus botryoides*. Mycotaxon 20:163–167.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci USA (in press). doi: 10.1073/pnas.1117018109.

Schüßler A, Krüger M, Walker C. 2011. Revealing natural relationships among arbuscular mycorrhizal fungi: culture line BEG47 represents *Diversispora epigaea*, not *Glomus versiforme*. PLoS ONE 6:e23333, doi:10.1371/journal.pone.0023333

———, 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, Oregon State University. 56 p.

Schwarzott D, Walker C, Schüßler A. 2001. *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (Glomales) is nonmonophyletic. Mol Phylogenet Evol 21:190–197, doi:10.1006/mpev.2001.1007

Simmons MP, Ochoterena H, Carr TG. 2001. Incorporation, relative homoplasy and effect of gap characters in sequence-based phylogenetic analyses. Syst Biol 50:454–462, doi:10.1080/106351501300318049

Smith GS, Schenck NC. 1985. Two new dimorphic species in the Endogonaceae: *Glomus ambisporum* and *Glomus heterosporum*. Mycologia 77:566–574, doi:10.2307/3793355

Smith SE, Read DJ. 2008. Mycorrhizal symbiosis. 3rd ed. San Diego: Academic Press. 787 p.

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

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol Biol Evol 28:2731–2739, doi:10.1093/molbev/msr121

Thompson JD, Higgins DG, Gibson TJ. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680, doi:10.1093/nar/22.22.4673

Trappe JW, Bloss E, Menge J. 1984. *Glomus deserticola* sp. nov. Mycotaxon 20:123–127.

Trouvelot S, Tuinen D van, 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

van Tuinen D, Zhao B, Gianinazzi-Pearson V. 1998. PCR in studies of AM fungi: from primers to application. In: Varma AK, ed. Mycorrhizal manual. Berlin, Heidelberg, New York: Springer. p 387–399.

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

———, Vestberg M. 1994. A simple and inexpensive method for producing and maintaining closed pot cultures of arbuscular mycorrhizal fungi. Agric Sci Finland 3:233–240.

Wu C-G, Chen Z-C. 1987. The Endogonaceae of Taiwan II: two new species of *Sclerocystis* from Taiwan. Trans Mycol Soc ROC 2:73–83.

Wubet T, Kottke I, Teketay D, Oberwinkler F. 2009. Arbuscular mycorrhizal fungal community structures differ between co-occurring tree species of dry Afromontane tropical forest and their seedlings exhibit potential to trap isolates suited for reforestation. Mycol Prog 8:317–328, doi:10.1007/s11557-009-0602-8

———, 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