



Sieverdingia gen. nov., *S. tortuosa* comb. nov., and *Diversispora peloponnesiaca* sp. nov. in the Diversisporaceae (Glomeromycota)

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Abstract

Phylogenetic analyses of 18S–ITS–28S nuc rDNA sequences indicated that the arbuscular mycorrhizal fungus originally described as *Glomus tortuosum* and later transferred to the genus *Corymbiglomus* represents a separate, previously unrecognized clade at the rank of genus in the family Diversisporaceae (order Diversisporales, phylum Glomeromycota). The analyses located the clade between clades representing the genera *Desertispora* and *Redeckera*. Consequently, a new genus, *Sieverdingia*, was erected, with *S. tortuosa* comb. nov. The unique morphological feature of *S. tortuosa* is the formation of glomoid-like spores with a single-layered spore wall covered with a hyphal mantle. Importantly, the erection of *Sieverdingia* clarified the definition of *Corymbiglomus*, which currently consists of three species producing glomoid-like spores with one, three- to four-layered spore wall. The features of the innermost layer, which is hyaline, laminate, flexible to semi-flexible, indicate that it is a synapomorphy of *Corymbiglomus*. The definitions of *Corymbiglomus* and its species were emended. Moreover, the distribution of *S. tortuosa* and the three species of *Corymbiglomus* was discussed based on own studies, literature data, and molecular sequences deposited in public databases. We concluded that the distribution of *S. tortuosa* and *C. globiferum* known in environmental studies based on their partial 28S nuc rDNA sequences only may be understated because the main molecular characteristics distinguishing these species reside outside the 28S region. Finally, we described a new species in the genus *Diversispora* originating from Mediterranean dunes of the Peloponnese peninsula, Greece. The same phylogenetic analyses mentioned above indicated that the closest relative of the new species, producing dark-coloured spores, is *D. clara*, whose spores are creamy white at most.

Keywords Arbuscular mycorrhizal fungi · Morphology · Molecular phylogeny · 18S–ITS–28S nuc rDNA

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Introduction

The phylum Glomeromycota C. Walker & A. Schüßler, comprising arbuscular mycorrhizal fungi (AMF), is currently represented by ca. 315 species (<http://glomeromycota.wixsite.com/lbmicorrizas/cpia>). About 57% of these species produce glomoid or glomoid-like spores, and the species are distributed in 25 genera belonging to nine families of the Glomeromycota. The glomoid and glomoid-like spores arise blastically at the tip of a cylindrical or funnel-shaped sporogenous hypha, like spores of *Glomus macrocarpum* Tul. & C. Tul., the type species of the genus *Glomus* Tul. & C. Tul. emend. Oehl, G.A. Silva & Sieverd. and the Glomeromycota (Clements and Shear 1931; Oehl et al. 2011), and their subcellular structure contains only one spore wall continuous with the subtending hyphal wall (Błaszowski et al. 2018a, 2018b; Jobim et al. 2019).

The fungus originally described as *G. tortuosum* N.C. Schenck & G.S. Sm. is one of the few species (15, of which probably not all are certain species) of the Glomeromycota, which produces glomoid-like spores singly or in unorganized clusters (with randomly distributed spores), and the spores have a spore wall consisting of only one coloured layer (Schenck and Smith 1982). Based on phylogenetic analyses of sequences of the partial large subunit (28S) nuc rDNA, Błaszowski and Chwat (2013) transferred *G. tortuosum*, along with *G. globiferum* Koske & C. Walker, to *Corymbiglomus* Błasz. & Chwat in the family Diversisporaceae C. Walker & A. Schüßler, order Diversisporales C. Walker & A. Schüßler, and renamed the species as *C. tortuosum* (N.C. Schenck & G.S. Sm.) Błasz. & Chwat and *C. globiferum* (Koske & C. Walker) Błasz. & Chwat. The genus *Corymbiglomus*, with the type species *C. corymbiforme* Błasz., was previously erected following phylogenetic analyses of partial 28S sequences of *G. corymbiforme* Błasz. (Błaszowski 2012). In the presented phylogenetic tree (Fig. 1; Błaszowski and Chwat 2013), each of the species was represented by only one partial 28S sequence and the three sequences clustered in a sister clade to a clade representing the genus *Diversispora* C. Walker & A. Schüßler. Interestingly, the three-species clade consisted of two subclades, one with sequences of *C. corymbiforme* and *C. globiferum*, and the second with a sequence of *C. tortuosum*.

Unfortunately, in the analyses discussed above, no sequence of *Redeckera* spp. was included. However, the ecology and all other morphological features of *Redeckera* spp. and *C. tortuosum* differ fundamentally (Redecker et al. 2007; Schenck and Smith 1982; Oehl et al. 2011). Importantly, our preliminary phylogenetic analyses of available sequences of the partial small subunit (18S), internal transcribed spacer (ITS1–5.8S–ITS2 = ITS)

and partial 28S (= 18S–ITS–28S) nuc rDNA segment or part thereof of representatives of all glomoid-like spore-producing genera of the Diversisporaceae sensu Redecker et al. (2013) suggested that *C. tortuosum* does not belong to *Corymbiglomus*, but it represents a yet undescribed genus in the Diversisporaceae, a hypothesis also postulated by Medina et al. (2014).

In the same paper, Medina et al. (2014) described a new species in *Corymbiglomus*, *C. pacificum* Oehl et al., whose spores were not surrounded by a hyphal mantle, as spores of the three species discussed above. Most importantly, the researches stated that the subcellular structure of the glomoid-like spores of *C. pacificum* consists of two spore walls and suggested that spores of *C. corymbiforme* and *C. globiferum* are also two-walled. Interestingly, *C. corymbiforme* was originally characterized (as *G. corymbiforme*) to produce spores with a single spore wall consisting of three layers (Błaszowski 1995). The original description of *C. globiferum* (as *G. globiferum*) also testifies that its spores have one spore wall (Koske and Walker 1986). Moreover, our observations of the microphotographs of spores of *C. pacificum* published by Medina et al. (2014) suggested that spore wall 2 of *C. pacificum* sensu Medina et al. (2014) is not a separate spore wall and does not arise identically as, for example, spore wall 2 of species of *Pacispora* (Błaszowski 2012).

Literature data and BLAST searches testify that species of *Diversispora* occur in different cultivated and natural habitats and have a worldwide distribution, as well as they suggest that many fungi of this genus remain undescribed (Sridhar and Beena 2001; Schüßler et al. 2011; Błaszowski et al. 2015c; Symanczik et al. 2018). We extracted from a pot trap culture glomoid-like spores, whose morphological features of the subtending hypha and the spore wall at the spore base corresponded to those defined by Oehl et al. (2011) as characteristic for diversisporoid spores of *Diversispora*. In addition, other morphological characters of these spores suggested that they represent an undescribed *Diversispora* sp.

Considering the doubts and suppositions discussed above, the aims of our further studies were (i) to check the phylogenetic position of the four described species of *Corymbiglomus* among other glomoid-like spore-producing taxa of the family Diversisporaceae based on more convincing molecular information; (ii) to definitively define the subcellular structure of spores of these species; (iii) to emend the diagnostic description of the genus *Corymbiglomus*, if necessary; and (iv) to confirm the novelty of the putative undescribed *Diversispora* sp. by growing it in single-species cultures, determining its phylogenetic position among molecularly sequenced glomoid-like spore-producing members of the Diversisporaceae and by characterizing its morphology.

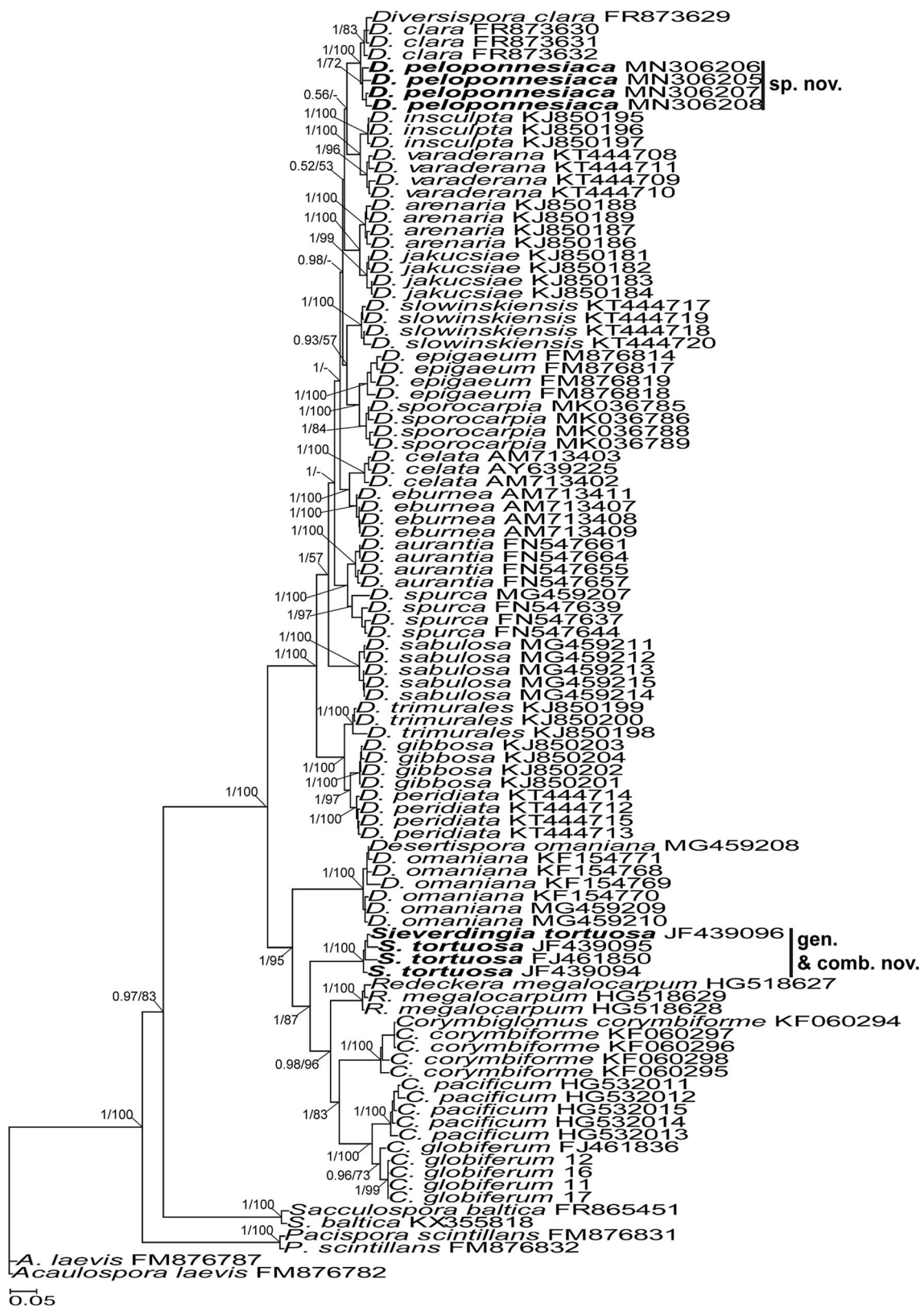


Fig. 1 A 50% majority rule consensus phylogram inferred from a Bayesian analysis of 18S–ITS–28S nuc rDNA sequences of *Diversispora peloponnesiaca* and *Sieverdingia tortuosa*, as well as 24 other species of AMF, including *Acaulospora laevis*, *Pacispora*

scintillans, and *Sacculospora baltica* as outgroup. The Bayesian posterior probabilities ≥ 0.50 and ML bootstrap values $\geq 50\%$ are shown near the branches, respectively. Bar indicates 0.5 expected change per site per branch

Materials and methods

Origin of study material, establishment and growth of the fungi in trap and single-species cultures, extraction of spores, and staining of mycorrhizal structures

We characterized the morphology of the fungus originally described as *G. tortuosum* based on its original description (Schenck and Smith 1982), its features described and illustrated by Dr. J.B. Morton (<http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/tortuosum.html>), spores extracted from a pot trap culture and field-collected rhizosphere soil samples, as well as based on examination of type material (OSC#40251) used to describe the fungus by Schenck and Smith (1982). The trap culture was inoculated with the rhizosphere soil of *Ammophila breviligulata* Fern. (Poaceae Barnh.) that had colonized maritime sand dunes of New Jersey, USA. The soil sample was collected by Dr. M. Tadych in August 2000. Unfortunately, the geographical position of the sampled site was not determined. All attempts at growing the fungus in single-species cultures established from spores extracted from the trap culture failed. The rhizosphere field soils were collected from *Tabebuia roseo-alba* (Ridl.) Sandw. (Bignoniaceae Juss.) and *Guazuma ulmifolia* Lam. [Sterculiaceae (DC.) Bartl.] colonizing sand dunes of the city of Mataraca, Paraíba State, Northeast Brazil (6° 28' 20"–6° 30' 00" S, 34° 55' 50"–34° 57' 10" W; de Souza et al. 2013). The predominant geomorphological formation of the sampled site is clay-sand sedimentary rocks overlaid by fixed dunes, up to 100 m high. The area has a tropical rainy climate (Am type according to Koppen's classifications; Peel et al. 2007), 25 °C annual average temperature and 1700 mm average annual rainfall (Oliveira-Filho and Carvalho 1993).

Spores of *C. corymbiforme* examined in this study were extracted from field-collected rhizosphere soil samples and single-species cultures established from spores collected from trap cultures. The trap cultures were inoculated with the rhizosphere soil samples taken from *Ammophila arenaria* (L.) Link growing in dunes of the Baltic Sea. The dunes are located near the city of Świnoujście (53° 55' 03" N 14° 17' 39" E) in north-western Poland. The climate of Świnoujście is temperate, with an annual sum of rainfall and a mean annual temperature of 550 mm and 8.92 °C, respectively (<https://pl.wikipedia.org/wiki/Świnoujście>). All soil samples were collected by J. Błaszowski in the years 1993–2015.

Corymbiglomus globiferum was characterized here based on its original description (Koske and Walker 1986), the description and pictures presented by Dr. J.B. Morton (<http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/globiferum.html>), and spores extracted from trap cultures. The cultures were inoculated with the rhizosphere soils of *Cyperus crassipes* Vahl

(Cyperaceae Juss.) and *Euploca polyphylla* (Lehm.) J.I.M. Melo & Semir (Boraginaceae Juss.) growing in sand dune soils located near the city of Galinhos and in the Reserva de Desenvolvimento Sustentável Estadual Ponta do Tubarão in the Guamaré Municipality (RDSE Ponta do Tubarão), Rio Grande do Norte State, respectively, Brazil. The *C. crassipes* soil was collected by J.N. de Paiva and K. Jobim in April 2017, and *E. polyphylla* was sampled by K.J.G da Silva and R.C. Theodoro in June and November 2015. The areas of the city of Galinhos and the RDSE Ponta do Tubarão are ca. 342 km² and 1.29 km², respectively, and both are located northwest of the city of Natal (78° 02' S, 94° 37' W and 5° 5' 3.55" S, 36° 28' 19.13" W, respectively). The typical biome of these areas is “restinga”, which consists mainly of plant species from the families Cyperaceae and Poaceae (Oliveira Filho and Carvalho 1993). According to Koppen's classifications (Peel et al. 2007), the region has a tropical humid climate (type Af), with an oscillating temperature of 21 °C and 30 °C (av. 26.1 °C) and an average annual rainfall between 10 and 170 mm.

The morphology of *C. pacificum* was determined in this study based on the original description of the species (Medina et al. 2014) and its holotype and isotype specimens loaned from the herbarium of ETH Zürich (Z+ZT), Switzerland.

Spores of the putative new species of *Diversispora* were originally extracted from a trap culture inoculated with the rhizosphere soil of *A. arenaria*. Subsequently, the spores were used to establish single-species cultures of the fungus. In the field, *A. arenaria* colonized Mediterranean Sea dunes of the beach Voidokolia (36° 57' 46" N 21° 39' 45" E) located on the Peloponnese peninsula, Greece. The climate of the sampled site is warm, with an annual sum of rainfall of 493.8 mm and temperature ranging from 7–14 °C (winter) to 21–34 °C (summer) (<http://www.hnms.gr/emv/el/>; https://www.meteoblue.com/en/weather/forecast/modelclimate/pylos_greece_255293). Dune soils of the site have a pH of 7.64–8.06 and are rich in Mg and Na, whose contents are ca. 74.025 and 72.343 mg/kg, respectively. The soil sample was collected by Dr. Dimitris Arrianas on July 18, 2012.

The methods used to establish trap and single-species cultures of the four species discussed here, the growing conditions of these cultures, and the methods of spore extraction and mycorrhizal staining were as those described previously (Błaszowski et al. 2012).

Microscopy and nomenclature

Except for *C. pacificum*, the morphological features of spores and the phenotypic and histochemical characters of spore wall layers of the other species discussed here were characterized based on at least 50–100 spores of each species 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 of *C. pacificum* were characterized based on the original description of this species (Medina et al. 2014) and specimens permanently mounted on microscope slides loaned from the mycological herbarium of ETH Zurich (Z+ZT), Switzerland. The preparation of spores for study and photography were as those described previously (Błaszowski 2012; Błaszowski et al. 2012). Types of spore wall layers are those defined by Błaszowski (2012), and Walker (1983). Colour names are from Kornerup and Wanscher (1983). Nomenclature of fungi and the authors of fungal names are from the Index Fungorum website <http://www.indexfungorum.org/AuthorsOffungalNames.htm>. The terms glomerospores and glomerocarps were used for spores and fruit bodies, respectively, produced by AMF, as Goto and Maia (2006) and Jobim et al. (2019) proposed.

Voucher specimens of *C. corymbiforme*, *C. globiferum*, the fungus originally described as *G. tortuosum*, and the new species [spores mounted in PVLG and a mixture of PVLG and Melzer's reagent (1:1, v/v) on slides] were deposited at the Herbarium of the Federal University of Rio Grande do Norte [UFRN-Fungos Herbarium; vouchers from type of *G. tortuosum* (UFRN-Fungos 3165) and *C. globiferum* (UFRN-Fungos 3166, 3167)], ETH Zurich, Switzerland (holotype of *D. peloponnesiaca*), and the Department of Ecology, Protection and Shaping of Environment (DEPSE), West Pomeranian University of Technology, Szczecin (isotypes of *D. peloponnesiaca* and specimens of the other three species mentioned above).

Molecular phylogeny, DNA extraction, polymerase chain reaction, cloning, and DNA sequencing

Genomic DNA of *C. globiferum* and the putative *Diversispora* sp. was extracted from eight single spores of each fungus. The origin of spores of both species was presented above. Details of the treatment of the spores prior to polymerase chain reactions (PCRs), the conditions, and primers used in the PCRs to obtain 18S–ITS–28S sequences were as those described in Błaszowski et al. (2015a, 2015b), Krüger et al. (2009), and Symanczik et al. (2014). Cloning and sequencing of PCR products to obtain 18S–ITS–28S sequences of *C. globiferum* and the potentially new *Diversispora* sp. were performed as described by Błaszowski et al. (2015a). The sequences were deposited in GenBank (MN306205–MN306208).

Unfortunately, many attempts to obtain sequences of the largest subunit of RNA polymerase II (*RPB1*) of *C. globiferum* failed, despite nested PCRs being performed in conditions and with primers that allowed obtaining *RPB1* sequences of *C. corymbiforme* (Symanczik et al. 2018).

Sequence alignment and phylogenetic analyses

BLAST queries indicated that our 18S–ITS–28S sequences of the diversisporoid fungus sensu Oehl et al. (2011) represent an undescribed species of *Diversispora*. Subsequently, in order to clarify the doubts regarding the molecular phylogenies of the four so far described species of *Corymbiglomus*, i.e. *C. corymbiforme*, *C. globiferum*, *C. pacificum* and *C. tortuosum*, and to determine the phylogenetic position of the new species within the genus *Diversispora*, an alignment was assembled that consisted of 93 sequences of the 18S–ITS–28S segment and eight partial 28S sequences. These sequences characterized 23 species, including our new *Diversispora* sp., belonging to four described genera of the Diversisporaceae and three species that served as outgroup. Of the eight partial 28S sequences, three characterized *C. globiferum*, *C. tortuosum*, and *Diversispora celata* C. Walker, Gamper & A. Schüßler, and five belonged in *C. pacificum*. The partial 28S sequences of *C. globiferum* and *C. tortuosum* (both treated as members of the genus *Diversispora* C. Walker & A. Schüßler; <http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/tortuosum.html>; <http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/globiferum.html>) were obtained from DNA of spores extracted from cultures grown at INVAM. The outgroup was represented by two 18S–ITS–28S sequences each of single species of the three most closely related families of the Diversisporaceae sensu Redecker et al. (2013), i.e. *Acaulosporaceae* J.B. Morton & Benny (represented by *A. laevis* Gerd. & Trappe), *Pacisporaceae* C. Walker et al. [*P. scintillans* (S.L. Rose & Trappe) Sieverd. et al.], and *Sacculosporaceae* Oehl et al. [*S. baltica* (Błasz., Madej & Tadych) Oehl et al.]. Identity values of the newly obtained 18S–ITS–28S sequences of *C. globiferum* and the new *Diversispora* sp. were calculated using BioEdit (Hall 1999). With the same program, we calculated the percentage divergence of a sequence of each of the four species of *Corymbiglomus* and the *Diversispora* sp. treated separately from a directly neighbouring sequence of the closest relatives of these fungi, as well as the divergence of all analyzed sequences of each species of *Corymbiglomus* and the new *Diversispora* sp. from all sequences clustered in sister species or generic clades of these five species (Fig. 1). All comparisons were performed on sequences of the same length.

The sequence set was aligned with MAFFT v. 7 using the auto option (<http://mafft.cbrc.jp/alignment/server/>). Indels were coded by means of the simple indel coding algorithm (Simmons et al. 2001) as implemented in GapCoder (Young and Healy 2003), and this binary character set was added to the nucleotide alignment, as described and justified in Błaszowski et al. (2014). The phylogenetic position of the four species of *Corymbiglomus* and the new *Diversispora* sp.

within the Diversisporaceae was reconstructed from Bayesian inference (BI) and maximum likelihood (ML) phylogenetic analyses of the alignment. The BI analysis was conducted with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). The nucleotide substitution model used in this analysis was GTR, which was selected by jModelTest (Posada 2008), considering the selection of Akaike criterion. Four Markov chains were run for 5,000,000 generations, sampling every 100 steps, with a burn-in at 7500 sampled trees. The ML analysis was carried out with the raxmlGUI (Silvestro and Michalak 2012) implementation of RAxML (Stamatakis 2014) with the GTRGAMMA algorithm. A rapid bootstrap analysis with 1000 replicates was used to determine the support of branches. In both BI and ML analyses, the alignment [the nucleotide alignment plus the binary (indel) character set] was divided into four partitions, knowing that analyses of partitioned data generally increase the accuracy of phylogenetic reconstruction (Lanfear et al. 2012; Nagy et al. 2012). The generated phylogenetic tree was visualized and edited in MEGA6 (Tamura et al. 2013).

Results

General data and phylogeny

The analyzed sequence alignment had a length of 2063 characters, of which 838 and 755 were variable and parsimony informative, respectively. The identity values of the four 18S–ITS–28S sequences of *C. globiferum* and four 18S–ITS–28S sequences of *D. peloponnesiaca* ranged from 99.6 to 99.9% and from 97.6 to 98.9%, respectively.

Bayesian and ML analyses of the alignment generated trees, in which the position of main clades (five at the rank of genus) to each other and the species composition of sister clades of these generic clades were identical. One of these generic clades contained only sequences of the fungus originally described as *G. tortuosum* (Fig. 1), and this clade was located between the *Desertispora* and *Redeckera* clades. Another generic clade clustered sequences of *C. corymbiforme*, *C. globiferum*, and *C. pacificum*, and this clade was sister to a clade with sequences of *R. megalocarpum*. In the *Corymbiglomus* clade, *C. globiferum* and *C. pacificum* clustered in two separate sister subclades. The generic clade with *G. tortuosum* obtained full supports in both BI and ML analyses. The *Corymbiglomus* clade was fully (BI = 1.0) or strongly (ML = 83%) supported.

The divergences of the neighbouring sequences *C. tortuosum* JF439096 from *Desertispora omaniana* MG459210 and *C. tortuosum* JF439094 from *R. megalocarpum* HG518627 (Fig. 1) were 16.5% and 12.1%, respectively. The sequences *C. corymbiforme* KF060294 and *R. megalocarpum* HG518628 and

C. corymbiforme KF060295 and *C. pacificum* HG532011 were divergent by 12.9% and 12.0%, respectively. The distance between the sequences *C. globiferum* FJ461836 and *C. pacificum* HG532013 was 5.1%.

Both BI and ML analyses of the 18S–ITS–28S sequences of our *Diversispora* confirmed our hypothesis that this fungus is an undescribed species and indicated that its sister relative is *D. clara* (Fig. 1). The clades with the new *Diversispora* sp. and *D. clara* were fully supported in the BI analysis (= 1.0), but moderately in the ML analysis (= 72% and 83%, respectively). The node connecting the two clades obtained full supports in both analyses. The divergences of the four sequences of the putative new *Diversispora* sp. from the four *D. clara* sequences (Fig. 1) ranged from 4.3 to 5.2%.

Thus, based on the data discussed above, we are fully convinced that the fungus originally described as *G. tortuosum* should represent a new genus in the Diversisporaceae and the Greek diversisporoid fungus is a new species in *Diversispora*.

Taxonomy

Erection of a new genus

Sieverdingia Błaszcz., Niezgoda & B.T. Goto, gen. nov.

Mycobank no.: MB 832298

Type species *Sieverdingia tortuosa* (N.C. Schenck & G.S. Sm.) Błaszcz., Niezgoda & B.T. Goto

Basionym *Glomus tortuosum* N.C. Schenck & G.S. Sm. Mycologia 74: 83, 1982.

Specimens examined Brazil: Paraiba state, spores extracted from field-collected rhizosphere soils of *T. roseo-alba* and *G. ulmifolia* colonizing sand dunes of the city of Mataraca, unnumbered slides prepared by de Souza et al. (2013). USA: New Jersey, spores extracted from a trap culture inoculated with the rhizosphere soil of *A. breviligulata* growing at an unknown maritime dune site sampled by Dr. M. Tadych, Błaszczowski J., (slides no.) 3027–3029 (DEPSE); Oregon State University, type material (OSC#40251) used to describe the fungus by Schenck and Smith (1982).

Etymology *Sieverdingia*, in honour of Dr. Ewald Sieverding, Institute for Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, Germany, in recognition of his important contribution to taxonomy and ecology of arbuscular mycorrhizal fungi.

Diagnosis Differs from other genera of the Diversisporaceae in producing glomoid-like spores individually covered with a

hyphal mantle, in having a spore wall consisting of one laminate layer and a cylindrical or slightly flared subtending hypha not inflated slightly below the spore base, as well as in having the specific sequences of the nuc rDNA ITS1: AAAATTTATATAACAAATAT and ITS2: CCTAATATGTTATATATTATGTTTACTTGT, ACTTGTCA TTTAATCGATTTCGTGC regions.

Genus description Producing glomoid-like glomerospores individually covered with a hyphal mantle, having a spore wall consisting of one laminate layer, and a subtending hypha that is cylindrical or slightly flared and is not inflated slightly below the spore base.

***Sieverdingia tortuosa* (N.C. Schenck & G.S. Sm.) Błazsk., Niezgodą & B.T. Goto, comb. nov. (Fig. 2–h)**

Mycobank no.: MB 832299

Basionym *Glomus tortuosum* N.C. Schenck & G.S. Sm. Mycologia 74: 83. 1982.

Synonyms *Corymbi-glomus tortuosum* (N.C. Schenck & G.S. Sm.) Błazsk. & Chwat. Acta Mycol. 48(1):99. 2013.

Etymology Latin, *tortuosum* (= winding), referring to the winding pattern of hyphae in the mantle covering spores of the fungus.

Description Glomerospores formed singly or in loose clusters in soil (Fig. 2, b). *Spores* pale yellow (4A3) to light yellow (4A4), globose to subglobose, (140–)180(–240) μm diam., sometimes ovoid, 140–170 \times 180–240 μm , with one subtending hypha; most mature spores, whether single or arranged in clusters, are surrounded individually by a hyphal mantle (Fig. 2a–h). *Clusters* globose, 280–620 μm diam., to irregular, 140–280 \times 430–640 μm , with 2–6 randomly distributed spores. *Subcellular structure of spores* consists of a single-layered spore wall that is laminate, pale yellow (4A3) to light yellow (4A4), (1.2–)2.2(–2.6) μm thick; its laminae frequently separate from each other in crushed spores (Fig. 2a–h). *Subtending hypha* pale yellow (4A3) to light yellow (4A4), straight or curved, cylindrical to flared, (11.5–)13.5(–15.0) μm wide at the spore base (Fig. 2f), usually covered with the hyphal mantle and difficult to see. *Wall of subtending hypha* pale yellow (4A3) to light yellow (4A4), (1.0–)1.6(–1.9) μm thick at the spore base, composed of one layer continuous with the spore wall layer (Fig. 2f). *Pore* gradually thins with age due to thickening of the subtending hyphal wall layer; no septum was observed in the spores examined (Fig. 2f). *Hyphal mantle* 8–22 μm thick, composed of tightly interwoven, straight and branched, septate, hyaline to yellowish white (4A2), 2.7–7.8 μm wide hyphae, with walls 0.5–

1.0 μm thick (Fig. 2–e, g, h), probably developing from the region of their subtending hypha. Mantle hyphae and spores not staining in Melzer’s reagent. Juvenile and young spores frequently with no hyphal mantle (Fig. 2f). *Germination* through subtending hypha.

Mycorrhizal associations In the field, *S. tortuosa* probably lived in symbiosis with *A. breviligulata*, *Glycine max* (L.) Merr., *Ixeris repens* (L.) A. Gray, *Uniola paniculata* L., as well as with different other plant species (Schenck and Smith 1982; Blaschke 1991; Koske 1987; Cabello 2001; Oehl et al. 2003; Gai et al. 2006; Schalamuk et al. 2006; Wang et al. 2008; Goto et al. 2010; Błazskowski 2012; de Souza et al. 2013; Srinthar and Beena 2012; Yamato et al. 2012; Jobim et al. 2016), although it was confirmed molecularly only with regard to *I. repens* (Yamato et al. 2012). In single-species cultures, *S. tortuosa* formed mycorrhiza without typical arbuscules and vesicles in roots of *Paspalum secundatum* (Walt.) Kuntze (Schenck and Smith 1982).

Distribution and habitat *Sieverdingia tortuosa* was originally identified associated with roots of *G. max* cultivated at the Agricultural Research Center, Live Oak, Florida, USA (Schenck and Smith 1982). Later, spores of this species were found in different dune sites of the USA (Koske 1987; Błazskowski 2012), Brazil (de Souza et al. 2013), India and Japan (Srinthar and Beena 2012), and different cultivated and non-dune uncultivated soils of Argentina (Cabello 2001; Schalamuk et al. 2006), Brazil (Goto et al. 2010; Jobim et al. 2016), Switzerland (Oehl et al. 2003), Germany (Blaschke 1991), and China (Gai et al. 2006; Wang et al. 2008).

BLAST queries did not show any 18S–ITS–28S sequence of identity at the species level ($\geq 97\%$) to the *S. tortuosa* 18S–ITS–28S sequences used in our analyses (Fig. 1). Instead, when the query was the *S. tortuosa* partial 28S sequence FJ461850 used by us, BLAST showed 10 environmental partial 28S sequences, whose identity to the FJ461850 sequence ranged from 97.45 to 98.87%. The sequences represented “uncultured *Diversispora*” associated with *I. repens* growing in saline coastal beach soils in Japan (Yamato et al. 2012).

Emendation of the genus *Corymbi-glomus* Błazsk. & Chwat emend. Błazsk. Niezgodą & B.T. Goto

Mycobank no.: MB 564566

Genus description Forming glomoid-like glomerospores individually covered with a hyphal mantle consisting of non-branched or branched hyphae with or without terminal vesiculate swellings or naked spores (without a hyphal mantle). *Spores* occurring singly or in clusters in soil. *Clusters* with several spores connected by interwoven hyphae of their

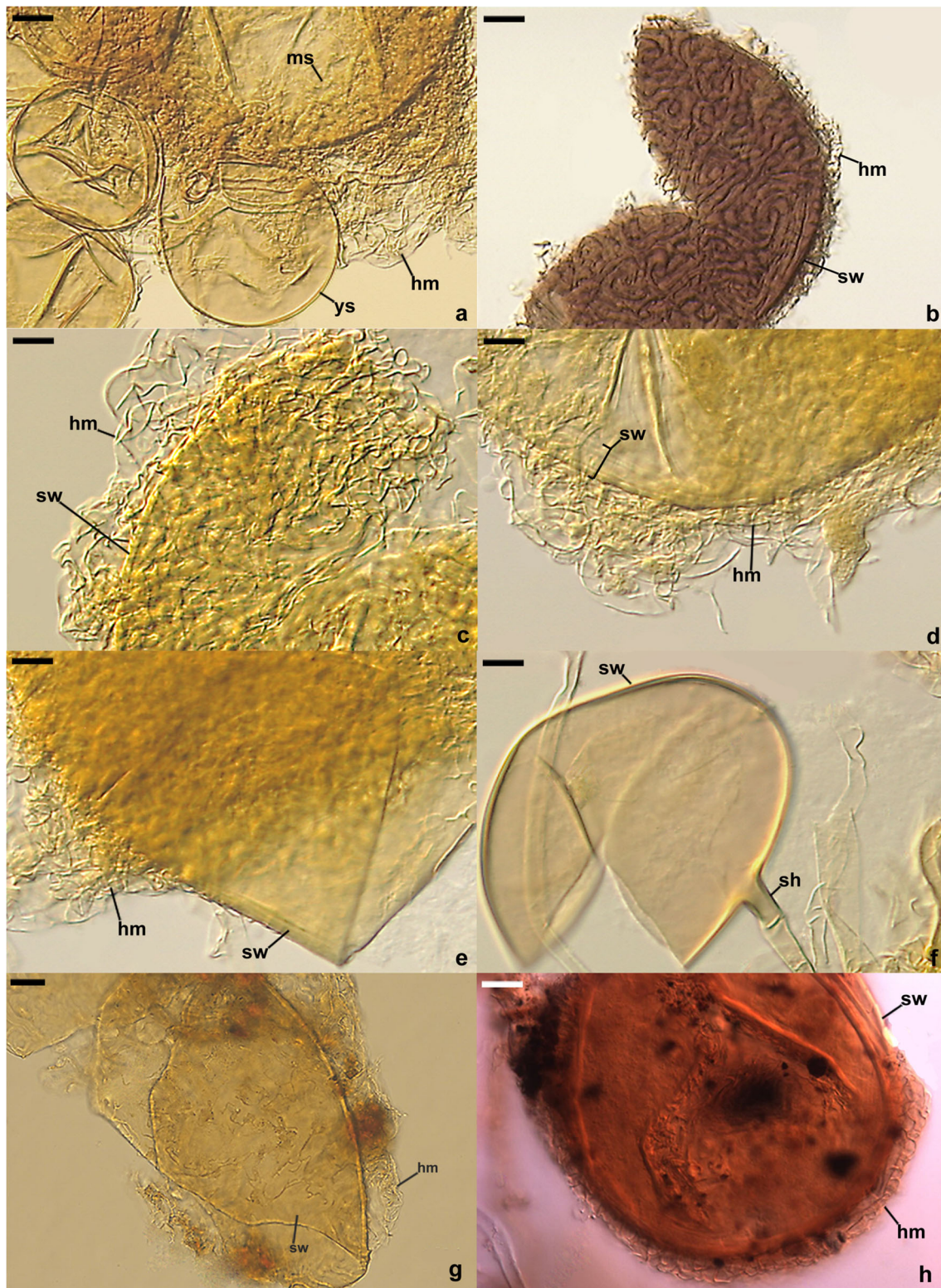


Fig. 2 *Sieverdingia tortuosa*. **a** Cluster with a mature spore (ms) covered with a hyphal mantle (hm) and young, naked (without a mantle) spores. **b–e, g** Spores individually covered with a hyphal mantle (hm); note the single-layered, laminate spore wall (sw), whose laminae clearly separated from each other in **d, f** Spore wall (sw) and subtending hypha (sh) of a young, naked spore. **a–f** Spores from a trap culture inoculated with the

rhizosphere soil of *A. arenaria* growing in USA sand dunes. **g** Spore from sand dunes of Brazil. **h** Spore from type material (OSC #40251). **b, c, g, h** Spores in PVLG. **a, d, e, f** Spores in PVLG+Melzer's reagent. **a–h** Differential interference microscopy. Scale bars: **a, b** = 20 μm , **c–h** = 10 μm

hyphal mantles or with two to < 20 spores arisen at the top of sporogenous hyphae dichotomously branched from a parent hypha continuous with an extraradical mycorrhizal hypha. *Subcellular structure of spores* consists of one spore wall containing three or four layers, of which the innermost layer is laminate, flexible to semi-flexible, hyaline, relatively thick, frequently readily separating from the penultimate spore wall layer above the spore base, but it is associated with the penultimate spore wall layer at the spore base and with the inner surface of the spore subtending hyphal wall at the spore base or much below. *Subtending hypha* straight or recurved, cylindrical to funnel-shaped or constricted. *Subtending hyphal wall* continuous with and coloured similarly to or slightly lighter than the spore wall. *Pore* occluded by (i) ingrowth of the innermost colourless spore wall layer (layer 3 or 4), (ii) a septum continuous with the innermost laminae of the coloured laminate spore wall layer 2, (iii) both the structures, (iv) a septum continuous with the innermost laminae of spore wall layer 2 and adherent spore wall layer 3, and (v) occasionally by thickening of spore wall layer 2; the pore sometimes seems to be open (Medina et al. 2014). *Germination* by a germ tube penetrating through the lumen of the subtending hypha and/or the spore wall (see our Fig. 3h; Medina et al. 2014).

Type species *Corymbiglomus corymbiforme* (Błaszk.) Błaszk. & Chwat. *Glomeromycota* 274. 2012.

Other species *Corymbiglomus globiferum* (Koske & Walker) Błaszk & Chwat.

Basionym *Glomus globiferum* Koske & Walker. *Mycotaxon* 26: 133. 1986.

Corymbiglomus pacificum Oehl, J. Medina, P. Cornejo, Sánchez-Castro, G.A. Silva & Palenz. *Mycotaxon* 127: 176. 2014.

Emendations of *Corymbiglomus corymbiforme*, *C. globiferum*, and *C. pacificum*

Corymbiglomus corymbiforme (Błaszk.) Błaszk. & Chwat emend. Błaszk. Niezgodą & B.T. Goto (Figs. 3–h and 4a, b)

Mycobank no.: MB 564567

Glomeromycota (Kraków): 274. 2012.

Basionym *Glomus corymbiforme* Błaszk. *Mycologia* 87(5): 732. 1995.

Etymology Latin, *corymbiforme*, referring to the clustered, corymbiform organization of spores in clusters of the fungus.

Specimens examined Poland: Glomerospores extracted from: (i) field-collected rhizosphere soils of *A. arenaria*, 6.10.1993,

Błaszkowski J., (slides no.) 2022 (holotype, DEPSE), *Błaszkowski J.*, 2023–2025 (isotypes, DEPSE), 1.10.1991, *Błaszkowski J.*, 2004–2010 (DEPSE), 22.09.1992, *Błaszkowski J.*, 2011–2015 (DEPSE); *Petasites spurius* (Retz.) Rchb., 6.10.1993, *Błaszkowski J.*, 2019 (DEPSE); and *Hieracium umbellatum* L., 6.10.1993, *Błaszkowski J.*, 2020–2021 (DEPSE); and (ii) single-species cultures inoculated with the rhizosphere soil of *A. arenaria*, 5.11.2018, *Błaszkowski J.*, 3660–3667 (DEPSE). All the plant species colonized dunes of the Baltic Sea located near the city of Świnoujście (53° 55' 03" N 14° 17' 39" E) and were sampled by J. Błaszkowski.

Diagnosis Differs from other species of *Corymbiglomus* in the formation of glomerospores mainly in corymbiform clusters (rarely singly), in which spores are individually covered with a hyphal mantle produced by dichotomously branched hyphae grown from spore wall layer 1 and in the nucleotide composition of sequences of the 18S–ITS–28S nuc rDNA region.

Description Glomerospores occurring in corymbiform clusters when formed from branched sporophores (Fig. 3, b), rarely singly in soil when produced from straight sporophores. *Clusters* globose to subglobose, (180–)336(–490) µm diam., sometimes ovoid, 180–350 × 210–500 µm, composed of 2–13 (av. 6) spores enveloped individually by a hyphal mantle (Fig. 3, b, e). *Spores* pastel yellow (3A4) to orange (6A8), globose to subglobose, (50–)142(–220) µm diam., sometimes ovoid or pyriform, 110–125 × 120–200 µm, with one subtending hypha (Fig. 3–h). *Subcellular structure of spores* of one wall with three permanent layers. Layer 1 uniform (not divided into visible sublayers), semi-rigid, smooth or slightly roughened, hyaline to deep yellow (4A8), (0.7–)1.1(–1.7) µm thick, closely attached to layer 2 (Fig. 3c–h). Layer 2 laminate, pastel yellow (3A4) to orange (6A8), (3.9–)7.0(–10.0) µm thick (Fig. 3c–h). Layer 3 laminate, semi-flexible, hyaline, (0.5–)3.4(–5.8) µm thick, usually tightly adherent to layer 2 in young and field-collected, even vigorously crushed, spores, but frequently separating from layer 2 in moderately crushed young and older spores extracted from pot cultures (Fig. 3c–h), usually with a small protrusion invaginated in the lumen of the subtending hypha (Fig. 3d, f, h). *Subtending hypha* cream (4A3) to deep orange (5A8), straight or recurvate, flared, sometimes cylindrical or constricted at the spore base, (9.8–)21.0(–31.1) µm wide at the spore base (Fig. 3e–h). *Wall of subtending hypha* cream (4A3) to deep orange (5A8), (2.2–)6.5(–13.7) µm thick, composed of three layers continuous with spore wall layers 1–3; subtending hyphal wall layer 3 present only at or far below the spore base (Fig. 3d–h). *Pore* occluded by (i) ingrowth of spore wall layer 3; (ii) a septum, (4.5–)9.6(–15.3) µm wide and (1.5–)1.7(–2.0) µm thick, continuous with the innermost lamina of spore wall layer 2; (iii) both the structures; and occasionally by (iv)

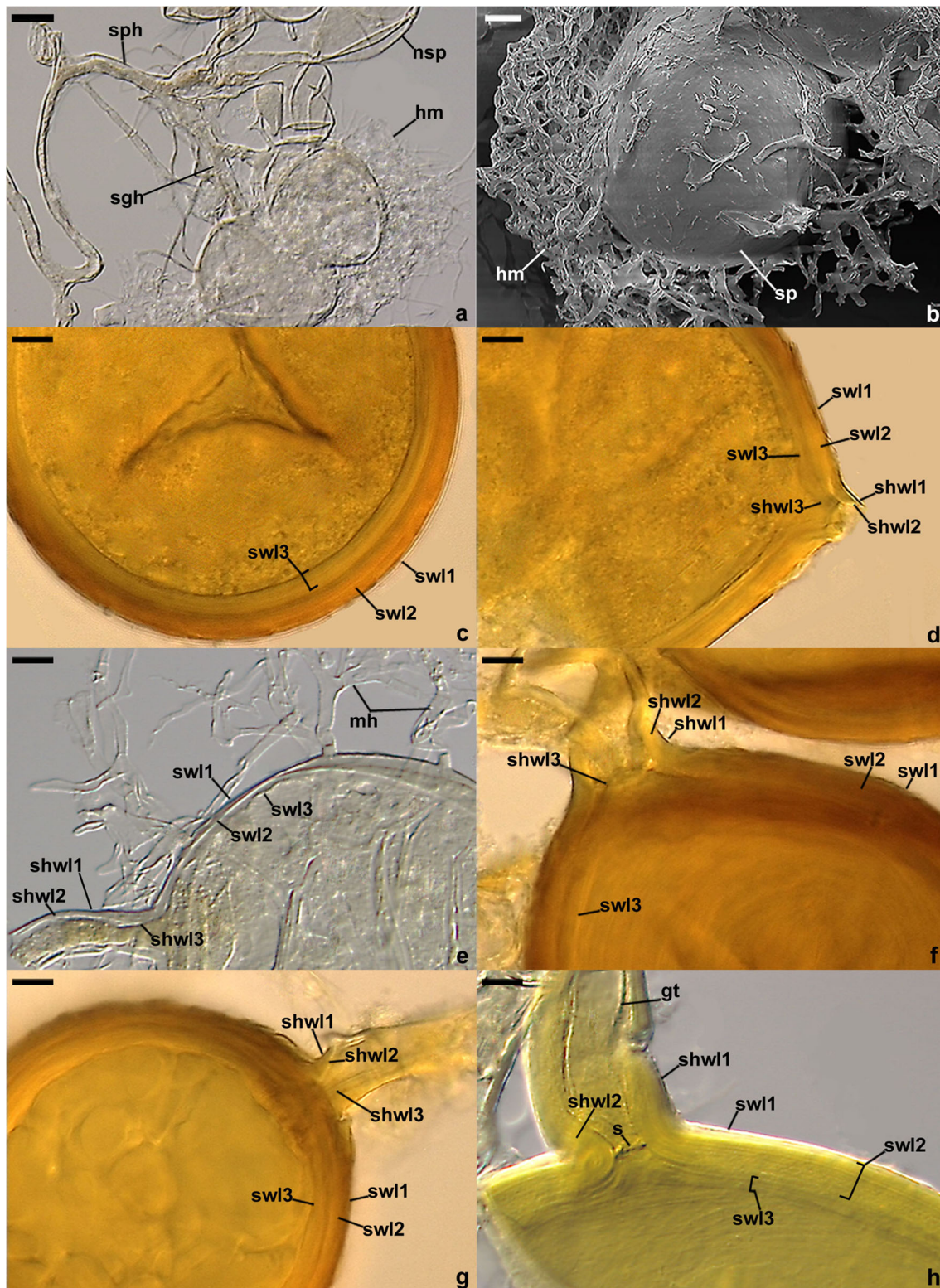


Fig. 3 *Corymbioglomus corymbiforme*. **a** Cluster with young naked spores (nsp) and spores covered with hyphal mantle (hm); spores arisen terminally on sporogenous hyphae (sgh) dichotomously branched from sporophore (sph). **b** Spore (sp) with hyphal mantle (hm). **c** Spore wall layers (swl) 1–3; note the thick, colourless swl3. **d, f–h** Spore wall layers (swl) 1–3 continuous with subtending hyphal wall layers (shwl) 1–3; note the subtending hyphal pore closed by the invaginated swl3 in **d, f** and **h** and both swl3 and a septum (s) connecting the inner surfaces of the laminate

swl2 in **h**; in **g**, the pore is open; a germ tube (gt) in the subtending hyphal lumen is visible in **h**. **e** Subtending hyphal wall layers (shwl) 1–3 continuous with spore wall layers (swl) 1–3 and mantle hyphae (mh) grown from swl1; note shwl3 that started developing far below the spore base. **a, c, e–h** Spores in PVLG. **d** Spore in PVLG+Melzer's reagent. **a, c–h** Differential interference microscopy. **b** Scanning electron microscopy. Scale bars: **a** = 20 μm , **b–h** = 10 μm

thickening of spore wall layer 2 (Fig. 3d, f, h), rarely open (Fig. 3e). *Mantle* (20.0–)47.5(–90.0) μm thick, consisting of a network of hyphae branching dichotomously three to four times at more or less right angles; hyphae thin-walled, hyaline to yellowish white (3A2), septate; length and diameter of branches diminishing with each successive dichotomy; initial hypha (9.8–)17.7(–27.2) μm long, (4.2–)4.9(–6.9) μm wide; developing from spore wall layer 1; final branch (13.5–)19.4(–25.0) μm long, (1.2–)1.8(–2.9) μm wide; distance between septa (14.5–)25.7(–31.4) μm ; mantle frequently absent in mature spores (Fig. 3, b, e). *Sporophore* coenocytic to sparsely septate, hyaline to yellow (5A6), (10.3–)14.7(–17.5) μm wide, with a wall (1.5–)1.6(–1.7) μm thick, usually with two to three main monopodial branches, rarely straight; main branches frequently with one to ten (av. 4) monopodial second branches sometimes branched monopodially one or two times; main, second, and third branches slanted at 30–45° towards their parent hyphae; straight and branched sporophores bearing spores by swelling hyphal tips (Fig. 3). Mantle and spores not reacting in Melzer's reagent. *Germination* by a germ tube penetrating through the lumen of the subtending hypha (Fig. 3 h).

Mycorrhizal associations In the field, *C. corymbiforme* was probably associated with roots of *A. arenaria*, *Artemisia campestris* L., *Corynephorus canescens* (L.) P. Beauv., *Festuca polesica* Zapal., *Galium mollugo* L., *Helichrysum arenarium* (L.) Moench, *Hieracium pilosella* L., *H. umbellatum*, *Petasites spurius*, *Potentilla anserina* L., *Rosa rugosa* Thunb., *Solidago virgaurea* L., and *Viola tricolor* L. (Błaszowski 1995, unpubl. data; Tadych and Błaszowski 2000; Błaszowski et al. 2002a, 2002b; Błaszowski and Czerniawska 2006). However, no molecular analysis was performed on roots of these plant species to confirm this supposition. In single-species cultures, *C. corymbiforme* formed mycorrhiza with arbuscules and intra- and extraradical hyphae staining moderately to intensively [pale violet (17A3) to violet (17B7)] in 0.1% Trypan blue (Fig. 4a, b).

Distribution and habitat Glomerospores of *C. corymbiforme* were originally extracted from maritime dunes located near Świnoujście in north-western Poland (Błaszowski 1995). Other records of this species also originate mainly from dune sites: the Słowiński National Park (Tadych and Błaszowski 2000) and the Mierzeja Wiślana spit (Błaszowski et al. 2002a), both belonging to Poland, dunes of the Mediterranean Sea located near Karabucak-Tuzla (Turkey), Tel-Aviv (Israel), Majorca (Spain; Błaszowski and Czerniawska 2006; Błaszowski, unpubl. data), and inland dunes of the Pustynia Błędowska desert (Poland, Błaszowski et al. 2002b). In addition, *C. corymbiforme* was found in semiarid open sandy grasslands of Hungary (Takacs and Bratek 2006; Błaszowski, unpubl. data).

Comparisons of the 18S–ITS–28S sequences of *C. corymbiforme* with sequences deposited in public databases did not show any record of this species.

***Corymbiglomus globiferum* (Koske & C. Walker) Błasz. & Chwat emend. Błasz. Niezgodna & B.T. Goto (Fig. 4c–f)**

MycoBank no.: MB 622179

Acta Mycol. 48(1): 99. 2013.

Basionym *Glomus globiferum* Koske & Walker. Mycotaxon 26: 133. 1986.

Etymology Latin, *globiferum* (= “sphere bearing”), referring to the spherical or near-spherical vesiculate swellings produced by mantle hyphae on the outside of spores (Koske and Walker 1986).

Specimens examined Brazil: Glomerospores extracted from trap cultures inoculated with the rhizosphere soils of two sand dune plant species: *C. crassipes* growing near the city of Galinhos (UFRN Fungos-3166) and *E. polyphyla* growing in the RDSE Ponta do Tubarão (UFRN-Fungos-3167), both located in the Rio Grande do Norte State.

Diagnosis Differs from other species of *Corymbiglomus* in the formation of single glomerospores individually covered with a hyphal mantle containing vesiculate swellings, whose hyphae grow from spore wall layer 1 and the subtending hyphal wall, as well as in the nucleotide composition of sequences of the 18S–ITS–28S nuc rDNA region.

Description Glomerospores hypogeous, formed singly, occasionally in clusters with two to several spores connected by interwoven hyphae of a mantle individually covering each spore. *Spores* dark orange (5A8) to brownish red (9C8), globose to subglobose, (160–)249(–320) μm diam., with one subtending hypha (Fig. 4c–f). *Subcellular structure of spores* consists of one wall composed of three layers. Layer 1 uniform (not divided into visible sublayers), semi-permanent, semi-rigid, smooth or slightly roughened, hyaline to light orange (5A4), (1.2–)1.7(–2.3) μm thick, closely attached to layer 2, occasionally slightly deteriorated in older spores (Fig. 4d–f). Layer 2 laminate, permanent, smooth, dark orange (5A8) to brownish red (9C8), (10.0–)17.1(–23.8) μm thick (Fig. 4d–f); the thickness is frequently higher in crushed spores due to the plastic properties of this layer. Layer 3 laminate, permanent, semi-flexible, hyaline, (2.0–)2.9(–4.8) μm thick, usually tightly adherent to layer 2, usually with a small protrusion invaginated in the lumen of the subtending hypha (Fig. 4d–f). *Subtending hypha* pale yellow (4A3) to reddish orange (7A6), straight or recurvate, cylindrical or slightly constricted at the spore base, rarely funnel-shaped, 15.0–27.0 μm wide at the spore base

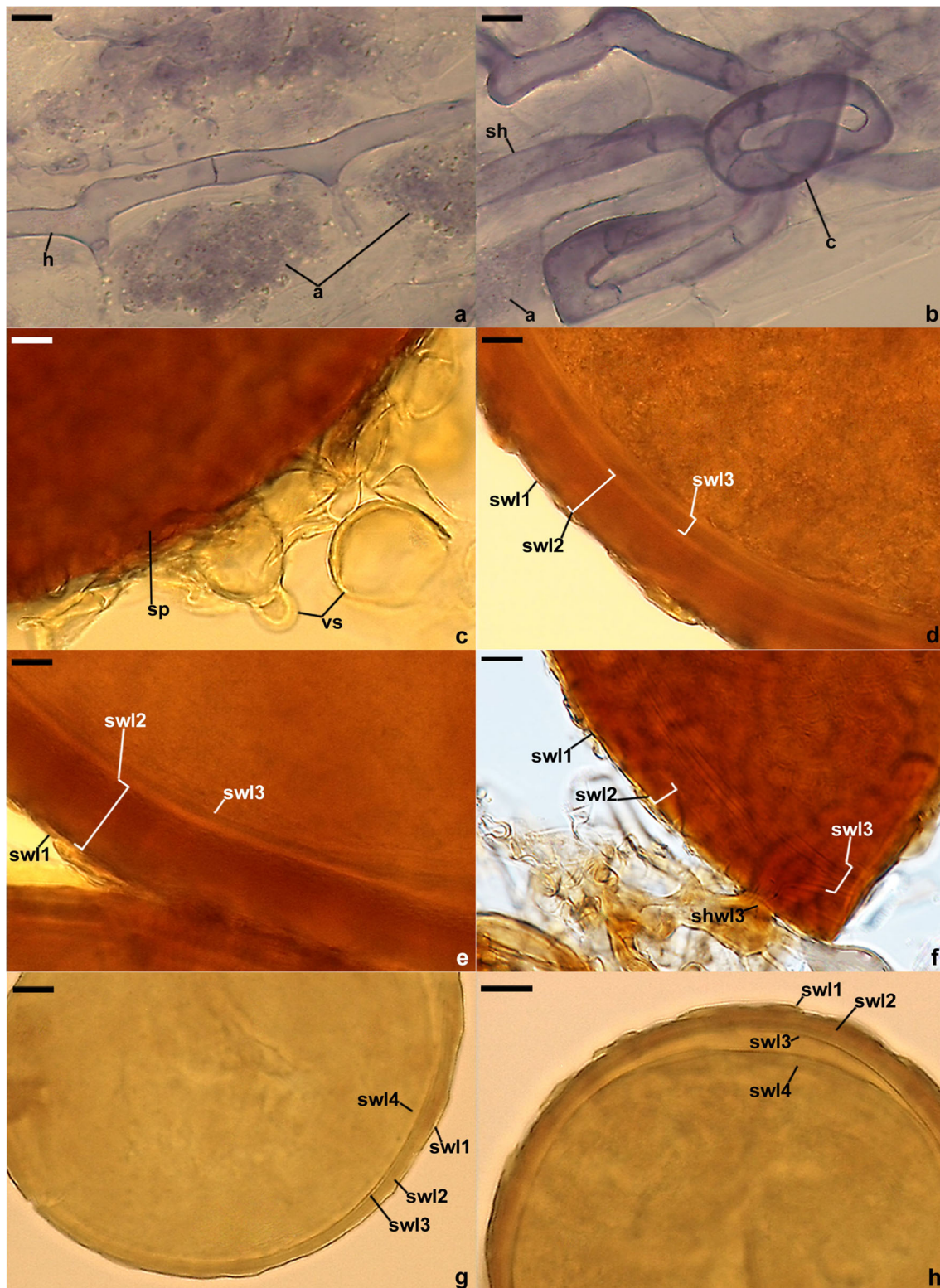


Fig. 4 **a, b** *Corymbioglomus corymbiforme*. **a** Arbuscules (a) and intraradical hypha (h). **b** Arbuscule (a) and straight (sh) and coiled (c) intraradical hyphae. **c–f** *Corymbioglomus globiferum*. **c** Spore (s) covered with a hyphal mantle with vesiculate swellings (vs). **d–f** Spore wall layers

1–3; note the separated sublayers of swl3 in **e** and **f**. **g, h** *Corymbioglomus pacificum*. **g, h** Spore wall layers (swl) 1–4. **a, b, d, f–h** Spores in PVLG. **c, e** Spores in PVLG+Melzer's reagent. **a–h** Differential interference microscopy. Scale bars: **a–h** = 10 μ m

(Fig. 4f). Wall of subtending hypha pale yellow (4A3) to reddish orange (7A6), 2.0–8.0 μ m thick, composed of three layers

continuous with spore wall layers 1–3 (Fig. 4f). Pore occluded by (i) ingrowth of spore wall layer 3, (ii) a septum continuous

with the innermost lamina of spore wall layer 2, and (iii) occasionally by a granular plug. *Germination* by a germ tube penetrating through the lumen of the subtending hypha. *Mantle* 5–47 μm thick, consisting of loosely or tightly interwoven hyphae bearing numerous terminal or intercalary vesiculate swellings (Fig. 4c); hyphae coenocytic or sparsely septate, hyaline to pale yellow to yellow-brown, 5.0–50.0 μm wide, with walls (1.0–)1.5(–1.8) μm thick; swellings hyaline to pale yellow-brown, globose to ovoid, 12–75 μm diam., with a single-layered wall, 0.8–2.0 μm thick. Mantle and spores not reacting in Melzer's reagent.

Mycorrhizal associations In the field, *C. globiferum* probably formed mycorrhiza with *A. breviligulata* and *U. paniculata* (Koske and Walker 1986; Sylvia 1986; Sylvia and Will 1988; Wu and Sylvia 1993), as well as with *C. crassipes* and *E. polyphylla* (pers. observ.), although no molecular analysis was performed on roots of these plant species to confirm this supposition. BLAST searches indicated that, of the *C. globiferum* sequences used in our analyses (Fig. 1), only the partial 28S sequence FJ461836 showed 97.32–97.74% identity to two partial 28S sequences (AB670091, AB670092) obtained from DNA extracted from roots of *I. repens* (Yamato et al. 2012). However, the association of *C. globiferum* with *I. repens* was not confirmed when our 18S–ITS–28S sequences were used in the searches. In single-species cultures, *C. globiferum* formed mycorrhiza with *U. paniculata*, but the level of colonization was low (Sylvia and Burks 1988).

Distribution and habitat *Corymbioglomus globiferum* was originally characterized from glomerospores found in sand dunes at Cape May, New Jersey (Koske and Walker 1986). Sylvia (1986), Sylvia and Will (1988), and Wu and Sylvia (1993) extracted spores of this species from coastal sand dunes in Florida. The specimens of this fungus used in our analyses originated from saline and no saline sand dunes located at the city of Galinhos (78° 02' S, 94° 37' W) and in the RDSE Ponta do Tubarão (5° 5' 3.55" S, 36° 28' 19.13" W), Brazil. In addition, Yamato et al. (2012) concluded from molecular environmental analyses that *C. globiferum* occurred in roots of *I. repens* sampled near the Tottrii sand dunes in Japan.

***Corymbioglomus pacificum* Oehl, J. Medina, P. Cornejo, Sánchez-Castro, G.A. Silva & Palenz. emend. Błaszczak, Niezgodna & B.T. Goto (Figs. 4g, h and 5a–h).**

Mycobank no.: MB 805601
Mycotaxon 127: 176. 2014.

Etymology Latin, *pacificum*, referring to the Pacific Ocean, which is adjacent to the isolation site (Medina et al. 2014).

Specimens examined Slides with glomerospores permanently mounted in PVLG and PVLG+Melzer's reagent loaned from the mycological herbarium of ETH Zurich (Z+ZT, Switzerland): ZT Myc 49005–holotype (two slides), ZT Myc 49006–isotypes (six slides).

Diagnosis Differs from other species of *Corymbioglomus* in the formation of single glomerospores not covered with a hyphal mantle, in having spores with a four-layered spore wall, and in the nucleotide composition of sequences of the 28S nuc rDNA region.

Description Glomerospores hypogeous, formed singly. *Spores* bright yellow to dark yellow to rarely brownish yellow, globose to subglobose to rarely ellipsoid to irregular, (85–)95–130(–135) \times (75–)85–125(–131) μm diam., with one subtending hypha (Figs. 4g, h and 5a–h). *Subcellular structure of spores* consists of one spore wall with four layers. Layer 1 evanescent to semi-permanent, subhyaline to light yellow, 0.6–1.2 μm thick, usually tightly adherent to layer 2 (Figs. 4g, h and 5a–d). Layer 2 permanent, laminate, smooth, bright yellow to dark yellow to brownish dark yellow, 1.8–3.5(–4.5) μm thick (Figs. 4g, h and 5a–d). Layer 3 permanent, uniform (not divided into visible sublayers), hyaline to dull yellow (4B3), 0.4–0.7 μm thick, usually separating from layer 3 only in vigorously crushed spores, and, therefore, difficult to see (Figs. 4g, h and 5a). Layer 4 permanent, laminate, hyaline, (2.3–)3.4(–4.7) μm thick, usually with a small protrusion invaginated in the lumen of the subtending hypha (Fig. 4g, h and 5a–d). In Melzer's reagent, only spore wall layer 4 may stain light yellow to bright dark yellow. *Subtending hypha* usually lighter than spores, light yellow (4A4) to greyish orange (5B5), straight, rarely recurvate, cylindrical, sometimes constricted at the spore base, 7.0–12.0 μm wide at the spore base (Fig. 5c, d). *Wall of subtending hypha* light yellow (4A4) to greyish orange (5B5), 2.4–5.7 μm thick at the spore base, composed of four layers continuous with spore wall layers 1–4 at the spore base, and two layers continuous with spore wall layers 1 and 2 below the spore base (Fig. 5c, d). *Pore* usually occluded by a straight or slightly curved septum, (3.2–)3.7(–4.4) μm wide and ca. 2.0 μm thick, continuous with spore wall layers 2–4 (Fig. 5d), rarely open. *Germination* by a germ tube growing from spore wall layer 4 and penetrating through the other spore wall layers.

Mycorrhizal associations In the field, *C. pacificum* was associated with roots of *A. arenaria* (Medina et al. 2014). Attempts to propagate this species in culture failed. Thus, morphological features of *C. pacificum* mycorrhizal structures remain unknown.

Distribution and habitat So far, *C. pacificum* was physically identified only among roots of *A. arenaria* growing at the mouth of Lake Budi, a saline ecosystem periodically connecting with

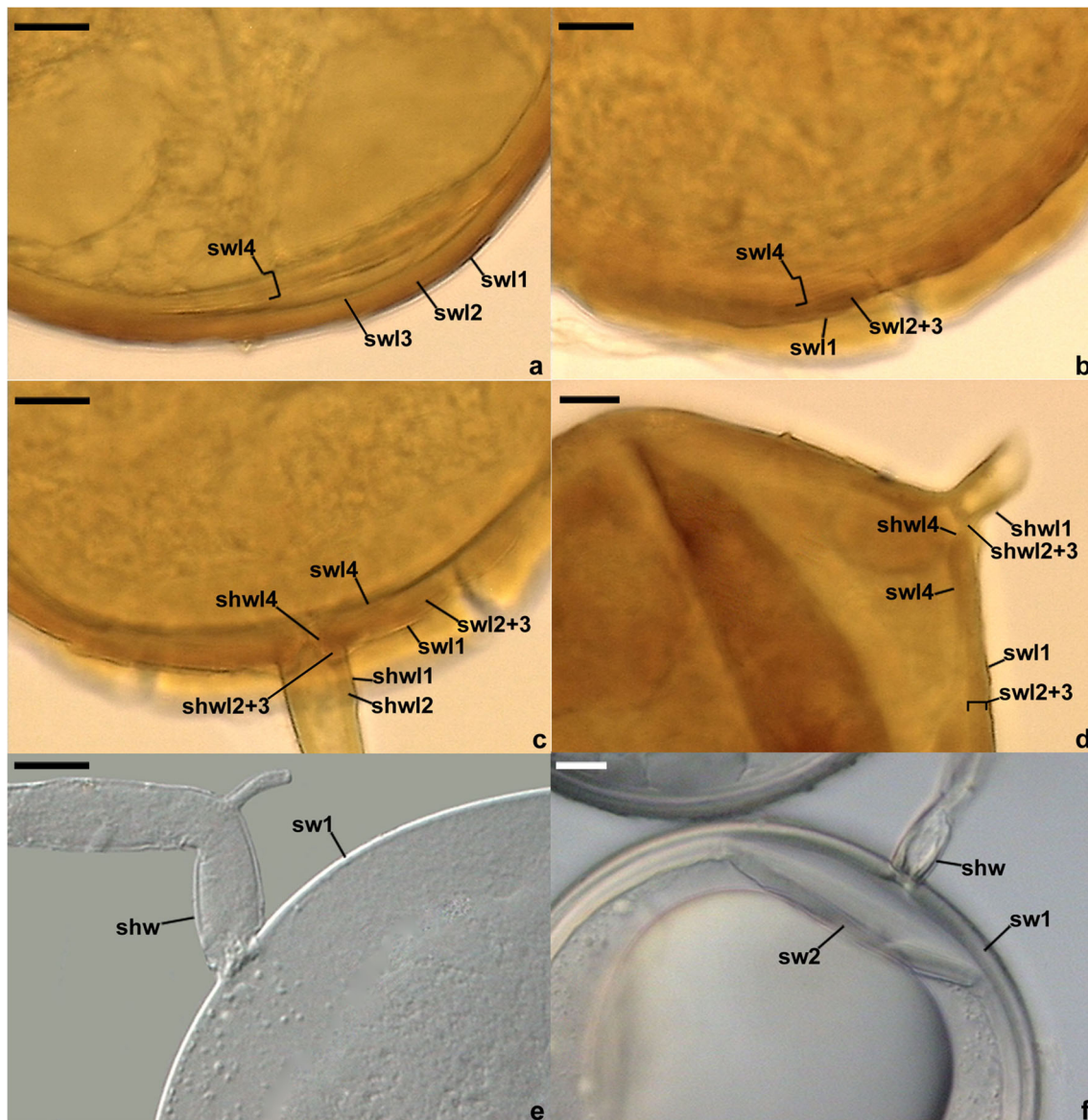


Fig. 5 **a–d** *Corymbioglomus pacificum*. **a, b** Spore wall layers (swl) 1–4; note the laminae of the laminate swl4. **c, d** Spore wall layers (swl) 1–4 continuous with subtending hyphal wall layers (shwl) 1–4; note shwl4 is present only at the spore base; shwl3 probably is present, but is invisible because it is very thin and of colour similar to that of shwl2. **e, f** *Pacispora scintillans*. **e** Subtending hyphal wall (shw) continuous with spore wall 1

(swl) of a juvenile specimen; note the single-walled subcellular structure of the spore. **f** Subtending hyphal wall (shw) continuous with spore wall 1 (sw1) having no physical contact with spore wall 2 (sw2) of a mature specimen. **a–f** Spores in PVLG. **a–f** Differential interference microscopy. Scale bars: **a–f** = 10 μm

the Pacific Ocean, located near the municipality Puerto Saavedra in La Araucanía Region (southern Chile; Medina et al. 2014). There are no available molecular data that would indicate that *C. pacificum* also occurs in other sites of the world.

Description of a new species

Diversispora peloponnesiaca Błaszcz., B.T. Goto, Orfanoudakis & Niezgodna sp. nov. (Fig. 6a–h).

Mycobank no.: MB 832300

Etymology Latin, *peloponnesiaca*, referring to the Peloponnese peninsula, where this species was originally found.

Specimens examined Poland: Glomerospores from a single-species culture established from spores extracted from a trap culture inoculated with the rhizosphere soil of *A. arenaria* that colonized Mediterranean Sea dunes of the beach Voidokoilia (36° 57' 46" N 21° 39' 45" E) located on the Peloponnese peninsula, Greece. The field soil sample was collected by Dr. Dimitris Arrianas on July 18, 2012. Holotype. Slide with spores no. ZT Myc 60277 (Z+ZT), isotypes: slides with spores no. 3650–3659 (DEPSE).

Diagnosis Differs from other *Diversispora* spp. in producing glomerospores that are darkest in colour and in the nucleotide composition of sequences of the 18S–ITS–28S nuc rDNA region.

Description Glomerospores formed singly in soil (Fig. 6a). Spores arise blastically at the tip of sporogenous hyphae. Spores orange (5B8) to reddish brown (8E8) when mature, light yellow (3A5) to reddish yellow (4A7) when young; usually ovoid; 108–142 × 127–171 µm; less often globose to subglobose; (113–)139(–163) µm diam.; with one subtending hypha (Fig. 6a–f). Spore wall composed of three permanent layers. Layer 1, forming the spore surface, semi-flexible, smooth, deep yellow (4A8) to yellowish brown (5D8) in mature spores, hyaline in young spores, (1.3–)1.8(–2.3) µm thick (Fig. 6b–f, h). Layer 2 laminate, semi-flexible, orange (5B8) to reddish brown (8E8) in mature spores, light yellow (3A5) to reddish yellow (4A7) in young spores, (6.3–)8.4(–11.3) µm thick (Fig. 6b–f, h). Layer 3 uniform (not divided into visible sublayers), semi-flexible, hyaline to greyish yellow (4C3), (1.0–)1.1(–1.3) µm thick, usually tightly adherent to the lower surface of layer 2, occasionally separating from this layer in vigorously crushed spores (Fig. 6b–f, h). Layers 1–3 not staining in Melzer’s reagent. Subtending hypha hyaline to greyish yellow (4B3); straight or recurved, cylindrical, rarely slightly constricted at the spore base; (9.0–)11.8(–14.5) µm wide at the spore base (Fig. 6g, h); frequently breaking in crushed spores. Wall of subtending hypha hyaline to greyish yellow (4B3); (1.8–)2.1(–2.3) µm thick at the spore base; consisting of one layer continuous with spore wall layer 1 (Fig. 6g, g). Pore (6.3–)8.9(–10.3) µm wide at the spore base, occluded by either a straight or slightly curved septum, 1.5–2.0 µm thick, connecting the inner surfaces of the subtending hyphal wall at or up to 2.5 µm below the spore base (Fig. 6g), or a curved septum continuous with spore wall layer 3 (Fig. 6h). Germination unknown.

Mycorrhizal associations In the field, *D. peloponnesiaca* probably lived in symbiosis with *A. arenaria*, although no molecular analysis was performed on roots of this plant species to confirm this hypothesis. In single-species cultures with *P. lanceolata* as the host plant, *D. peloponnesiaca* formed mycorrhiza with arbuscules, vesicles, and intraradical and extraradical hyphae that stained violet white (16A2) to dark violet (16F8) in 0.1% Trypan blue.

Distribution and habitat To date, *D. peloponnesiaca* was physically found in only one trap culture representing dunes of the Mediterranean beach Voidokoilia (36° 57' 46" N 21° 39' 45" E) located on the Peloponnese peninsula, Greece. BLAST queries did not show any sequence of ≥ 97% identity to the 18S–ITS–28S sequences of *D. peloponnesiae*. The identity of all listed sequences was < 95.73%.

Discussion

The molecular phylogenetic analyses discussed above fully confirmed our hypothesis that the fungus originally described as *G. tortuosum* (Schenck and Smith 1982) and later accommodated in the genus *Corymbiglomus* (Błaszowski and Chwat 2013) should be transferred to a new genus, here described as *Sieverdingia*, in the family Diversisporaceae. Importantly, the transfer removed inconsistencies between the morphological features of spores of *C. corymbiforme*, *C. globiferum*, and *C. pacificum* versus those of *S. tortuosa* (Schenck and Smith 1982; Koske and Walker 1986; Błaszowski 2012; Medina et al. 2014; <http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/tortuosum.html>; <http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/globiferum.html>). Finally, the analyses confirmed our supposition, resulting from morphological observations, that the diversisporoid spore-producing fungus that we found and grew in cultures is a new species in the genus *Diversispora* (Fig. 1).

The phylogeny of *Sieverdingia* was reconstructed from analyses of 18S–ITS–28S nuc rDNA sequences and partial sequences of the 28S gene of the so far sole species of the new genus, *S. tortuosa* (Fig. 1). The nesting of the FJ461850 partial 28S sequence obtained from spores of *S. tortuosa* grown in the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM; treated as *D. tortuosa*) between three *S. tortuosa* 18S–ITS–28S sequences obtained from spores originating from China proved that the four sequences characterize one species and strongly supported the validity of the erection of *Sieverdingia*. The accuracy and resolution power of phylogenies generally are higher when the number of analyzed molecular sequences and the amount of information contained in these sequences are higher (Redecker et al. 2013; Stockinger et al. 2014).

Currently, the living culture from which type specimens of the fungus originally described as *G. tortuosum* were extracted (Schenck and Smith 1982) does not exist (<http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/tortuosum.html>). However, the morphology of spores of the type specimens permanently mounted on slides (B.T. Goto, pers. observ.; Fig. 2h), as well as that of spores extracted from living cultures grown by Dr. J.B. Morton in INVAM, B.T. Goto (Fig. 2g), and by J. Błaszowski (Fig. 2a–f; Błaszowski 2012; <http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/tortuosum.html>; <http://www.zor.zut.edu.pl/Glomeromycota/>) unambiguously indicates that the spores were produced by one species, *S. tortuosa*. Our *S. tortuosa* morphologically characterized above (see the section “Erection of a new genus”; Fig. 2a–h) slightly differed in only three features from *G. tortuosum* and *D. tortuosa* described and illustrated by Schenck and Smith

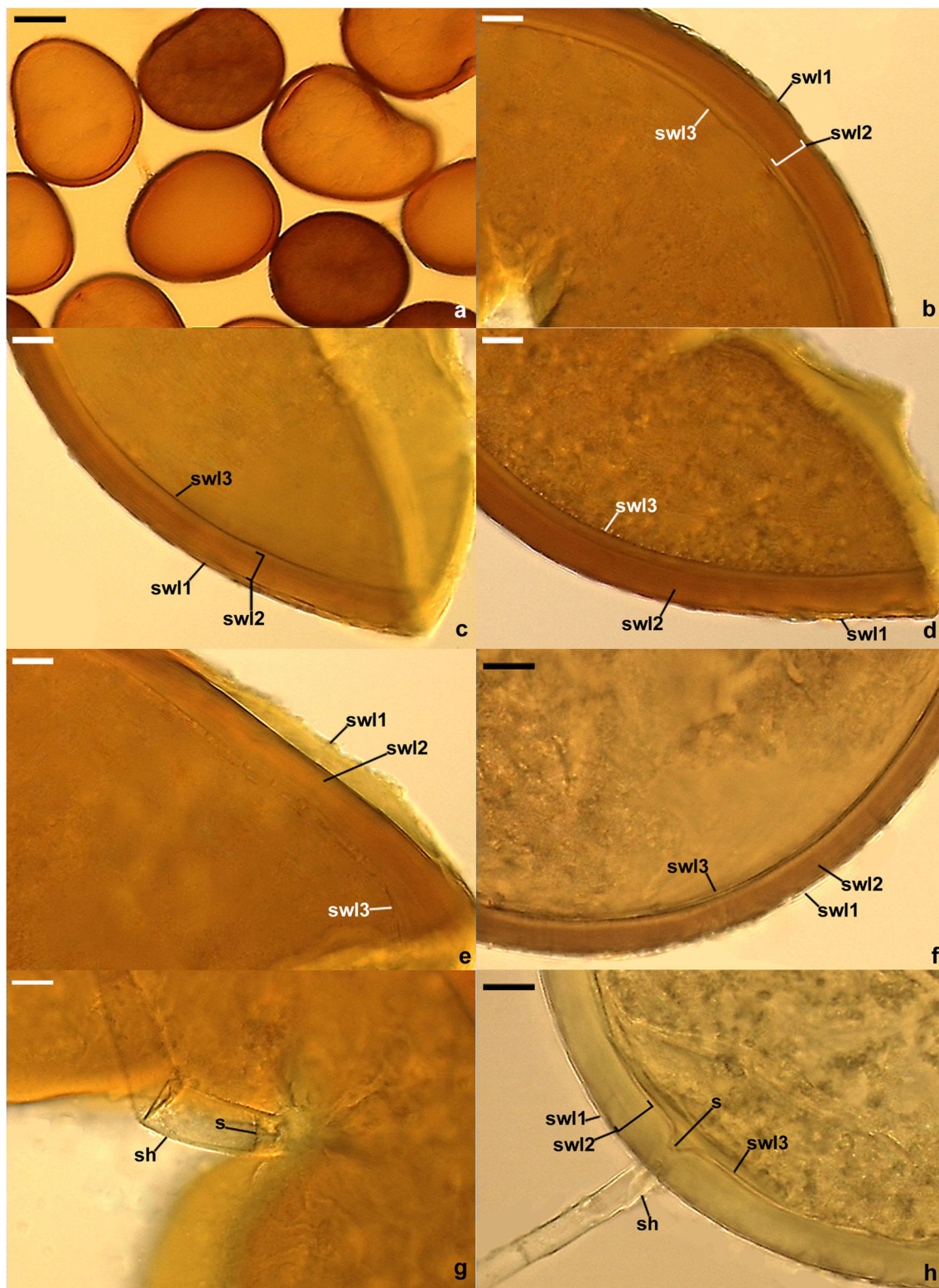


Fig. 6 **a–f** *Diversispora peloponnesiaca*. **a** Intact spores. **b–f** Spore wall layers (swl) 1–3. **g** A straight septum (s) connecting the inner surfaces of the subtending hyphal (sh) wall located slightly below the spore base. **h** Spore wall layers (swl) 1–3, subtending hypha (sh), and a curved septum

(s) continuous with swl3, closing the lumen of sh. **a** Spores in lactic acid. **b, c, e–h** Spores in PVLG+Melzer's reagent. **d** Spore in PVLG. **a–h** Differential interference microscopy. Scale bars: **a** = 50 μ m, **b–h** = 10 μ m

(1982) and Morton (<http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/tortuosum.html>),

respectively. The spores were (i) slightly lighter than those defined by Schenck and Smith (1982; yellow to dull grey-

brown) and Morton (light yellow-brown to orange brown), (ii) their spore wall was slightly thicker than 0.5–2.0 μm (Schenck and Smith 1982; no data in Morton's website), and (iii) their subtending hypha was not as wide as 20–26 μm (Schenck and Smith 1982), but the range of its width was similar to that given by Morton.

Morphologically, *S. tortuosa* is distinguished mainly by its single-layered, coloured spore wall, which is covered with a mantle consisting of straight or sinuous hyphae (Schenck and Smith 1982; Błaszowski 2012; <http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/tortuosum.html>; Fig. 2–h). The mantle hyphae are frequently interwoven with those of a spore or spores arising nearby, and therefore, *S. tortuosa* usually forms clusters with randomly distributed spores. Of the 15 described species of the Glomeromycota that produce glomoid or glomoid-like coloured spores with a single-layered spore wall, which do not arise radially from a central sterile hyphal plexus as spores of *Sclerocystis* spp., only spores of *S. tortuosa* and *G. convolutum* Gerd. & Trappe are individually enclosed in a hyphal mantle (Gerdemann and Trappe 1974; Schenck and Smith 1982; Błaszowski 2012). Both species are easily distinguishable from one another based on their morphology. While spores of *S. tortuosa* arise singly or in rather loose clusters (Fig. 2a–h), those of *G. convolutum* are formed in compact glomerocarps (Gerdemann and Trappe 1974). The laminate spore wall layer of the former species is nonreactive in Melzer's reagent (Fig. 2a, d–f), and in the latter fungus it stains deep orange brown in this reagent. Finally, the oily content of *S. tortuosa* spores is colourless, whereas the oil filling *G. convolutum* spores is deep yellow. Unfortunately, we cannot support the unambiguous morphological separateness of the two species using molecular proofs because *G. convolutum* has no DNA markers sequenced and known molecular phylogeny so far, similarly as the other species of this fungal group, except for *R. fulva* (Berk. & Broome) C. Walker & A. Schüßler, *R. megalocarpa* (D. Redecker) C. Walker & A. Schüßler, and *R. pulvinata* (Henn.) C. Walker & A. Schüßler. Our research revealed that the genus *Redeckera* is a sister of *Sieverdingia* (Fig. 1), whose molecular divergence is high (by 10.5–11.9%).

The morphological analyses discussed here also confirmed our hypothesis that the genus *Corymbiglomus* should contain only species, whose a common feature is the formation of spores with a single spore wall, in which the innermost component is a relatively thick, colourless, laminate layer that frequently separates from the other spore wall layers, but usually adheres to the inner surface of the subtending hyphal wall in crushed spores (Figs. 3c, d, f–h, 4d–h and 5a–d). Such requirements meet only *C. corymbiforme*, *C. globiferum*, and *C. pacificum*. The following data presented below clearly justify our conclusion.

Species of *Pacispora* produce spores with two spore walls, of which the inner wall 2 arises de novo after the full differentiation

of wall 1, forming the spore surface, and wall 2 has no physical contact with wall 1 (Fig. 5e, f; Błaszowski 1988, 2012; Oehl and Sieverding 2004; Walker et al. 2004). Instead, Fig. H illustrating the spore base of *C. corymbiforme* (Błaszowski 2012), pictures of *C. globiferum* (treated as *D. globifera*; <http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/globiferum.html>), and Fig. 8 showing a crushed *C. pacificum* spore (Medina et al. 2014) clearly show that the innermost hyaline spore subcellular component [characterized by Błaszowski (2012) and Morton (<http://fungi.invam.wvu.edu/the-fungi/classification/diversisporaceae/diversispora/globiferum.html>) as spore wall layer 3 in *C. corymbiforme* and *C. globiferum*, respectively, and by Medina et al. (2014) as spore wall 2 in *C. pacificum*] is associated with the spore base. Moreover, detailed examination of the subcellular structure of *C. corymbiforme* spores freshly extracted from single-species cultures, *C. globiferum* spores extracted from trap cultures and field-collected sandy soils (Błaszowski and B.T. Goto, pers. observ.), as well as type specimens of *C. pacificum* proved that the innermost spore subcellular component consists of sublayers (laminae) and adheres to the inner surfaces of both spore wall layer 2 (in *C. corymbiforme* and *C. globiferum*) or layer 3 (in *C. pacificum*) at the spore base and the penultimate subtending hyphal wall layer, and is present either only at or far below the spore base (Figs. 3c, d, f–h, 4d–h and 5a–d), where it likely begins arising. Thus, the location of the innermost spore subcellular component in the spore subcellular structure of the three species of *Corymbiglomus* is identical to that of the innermost spore wall component of, for example, *Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler (layer 4) and *D. clara* (layer 3). Ontogenetic studies of *C. claroideum* spores revealed that the attachment of spore wall layer 4 to the inner surface of the subtending hyphal wall indicates common origin of the subtending hypha and the spore wall, and, consequently, testifies that spore wall layer 4 represents one structure, the spore wall (Stürmer and Morton 1997). Thus, although the ontogeny of the three species of *Corymbiglomus* discussed here is unknown, the attachment of the innermost spore subcellular component to the penultimate layers of the spore and subtending hyphal walls supports our conclusion that all components of the subcellular spore structure of these species also constitute one spore wall. Finally, the position of the innermost spore wall layer in the subcellular spore structure of *Corymbiglomus* spp. and, especially, its phenotypic features (a relatively thick, colourless laminate layer; Figs. 3c, d, f–h, 4d–h and 5a–d) suggest that this layer is a synapomorphy.

Interestingly, our preliminary molecular phylogenetic analyses indicated that the position of *C. corymbiforme* among the analyzed members of the Diversisporaceae (Fig. 1) changed depending on the species composition of the outgroup (data not shown). When the outgroup of the analyzed alignment was represented by two sequences of only *A. laevis* or only *S. baltica*, the position of *C. corymbiforme* was as that

depicted in Fig. 1. However, when the outgroup was two sequences of only *P. scintillans*, *C. corymbiforme* formed a two-species generic clade with *R. megalocarpum*, a species differing fundamentally in morphology, ecology, and highly distant molecularly (by 12.9%) from *C. corymbiforme* (Błaszowski 2012; Redecker et al. 2007). Moreover, our sequence comparisons indicated the *C. corymbiforme* clade is divergent from the neighbouring *C. pacificum* clade by 11.4–13.7%, which is roughly equivalent to intergeneric divergences of other taxa of the Glomeromycota (Błaszowski et al. 2018a, 2018b; Jobim et al. 2019). Instead, the molecular distance between the sister *C. globiferum* and *C. pacificum* clades is at the level of interspecies divergences of AMF, i.e. 4.3–6.0%. Thus, the analyses and comparisons suggest that the relationship of *C. corymbiforme* with *C. globiferum* and *C. pacificum* is not only distant but also weak, and that *C. corymbiforme* could alone occupy a generic clade. This may turn out to be true when at least one other new species will be discovered and its molecular similarity to *C. corymbiforme* will be higher than to *C. pacificum*.

Based on available own and literature data, as well as on comparisons of molecular sequences of *S. tortuosa* and the three species retained here in *Corymbioglomus* with molecular sequences of AMF deposited in public databases, we conclude that these species probably occur rather infrequently on Earth.

However, as indicated in the section “Taxonomy”, the intraradical presence of *S. tortuosa* and *C. globiferum* was revealed in environmental studies only based on the partial 28S FJ461850 and FJ461836 sequences, respectively, obtained from spores of these species. Instead, BLAST searches using 18S–ITS–28S sequences of these two species, as well as *C. corymbiforme*, showed no hits. In order to explain this inconsistency, we compared the *S. tortuosa* and *C. globiferum* 18S–ITS–28S sequences with 18S–ITS–28S sequences of the other AMF contained in the phylogenetic tree showed in Fig. 1. The comparisons indicated that the molecular content particularly strongly distinguishing the three species from the other species of the tree resides outside the 28S segment. Moreover, BLAST queries using the 18S CCGAAAGG TGGCCTTTTT, ITS1 AATAATTTTTTACCCCTCCT TTTGA and ATTATATATAAATTGTATTAATAAATATAC, 5.8S-ITS2 CTAAATATTAATCGTAAAT, and ITS2 ATCTCAGATGGGTTTC sequences present in all analyzed 18S–ITS–28S sequences of *C. globiferum* did not show any other member of the Kingdom Fungi. The molecular content strongly diverging *S. tortuosa* from all other representatives of the Glomeromycota and all members of the Kingdom Fungi also resides in the ITS1 and ITS2 parts of the 18S–ITS–28S sequences of this species. Thus, we conclude that the predominance of the molecular power contained in the 18S–ITS segment of the *S. tortuosa* and *C. globiferum* 18S–ITS–28S sequences is so large that in comparisons it moves these 18S–ITS–28S sequences outside the barcodes of these species

originally determined by the partial 28S FJ461850 and FJ461836 sequences. Therefore, BLAST searches omitted *S. tortuosa* and *C. globiferum* when the queries were their 18S–ITS–28S sequences.

Our phylogenetic analyses showed that the closest natural (molecular) relative of *D. peloponnesiaca* is *D. clara* (Fig. 1), whose morphology is strikingly different than that of the new species (Fig. 6a–h). Compared with all described species of *Diversispora*, mature *D. peloponnesiaca* spores are darkest in colour, whereas those of *D. clara* are colourless to creamy white (Estrada et al. 2011; Błaszowski, pers. observ.). The spore wall of both species consists of three layers, but spore wall layer 1 of *D. peloponnesiaca* is permanent (Fig. 6b–f, e), i.e. it does not deteriorate with age as spore wall layer 1 of *D. clara*, which often is absent (completely sloughed off) in mature spores. Moreover, the adherence of spore wall layers 2 and 3 of *D. peloponnesiaca* is clearly stronger than in *D. clara*. In *D. peloponnesiaca*, these spore wall layers rarely separate from one another, even in vigorously crushed spores (Fig. 6b–f, h). Instead, crushing of *D. clara* spores by applying only moderate pressure usually separates spore wall layers 2 and 3 (Błaszowski, pers. observ.). Finally, spores of *D. peloponnesiaca* are ca. 1.5-fold larger when globose, have a ca. 1.9-fold thicker spore wall, and their subtending hypha at the spore base is 1.8–2.3-fold wider and has a 1.3–1.8-fold thicker wall.

Morphologically, *D. peloponnesiaca* most resembles *D. jakucsiae* Błasz., T.K. Balázs & Kovács because spores of the latter species are almost as darkly coloured [reddish yellow (4A7) to brownish red (8C8)] as mature spores of *D. peloponnesiaca* and the spore wall of both species consists of three permanent layers (Balázs et al. 2015). The only characters separating the two species are the tint and thickness of spore wall layer 1, and the strength of the adherence of spore wall layers 2 and 3. In *D. jakucsiae*, spore wall layer 1 is clearly lighter [hyaline to yellowish white (4A2)] and ca. 1.7-fold thinner than in *D. peloponnesiaca*, and spore wall layer 3 is more loosely associated with the laminate spore wall layer 2 because it usually easily separates from this layer in crushed spores. Most importantly, the two species are highly divergent molecularly from one another: three clades at the species level separate *D. peloponnesiaca* from *D. jakucsiae* in our phylogenetic tree (Fig. 1).

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