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Glomus rugosae, a new arbuscular mycorrhizal species in Glomeraceae (phylum Glomeromycota) from maritime sand dunes of Poland and an ash pond of Czech Republic

JANUSZ BŁASZKOWSKI[1*], SZYMON ZUBEK[2], PAWEŁ MILCZARSKI[3], RYSZARD MALINOWSKI[4], BRUNO TOMIO GOTO[5] & PIOTR NIEZGODA[1]

[1]*Department of Environmental Management, West Pomeranian University of Technology in Szczecin, Słowackiego 17, PL-71434 Szczecin, Poland*
janusz.blaszkowski@zut.edu.pl; https://orcid.org/0000-0003-3688-164X
np12457@zut.edu.pl; https://orcid.org/0000-0002-0560-6154
[2]*Institute of Botany, Faculty of Biology, Jagiellonian University, 30-387, Kraków, Poland*
szymon.zubek@uj.edu.pl
[3]*Department of Genetic, Plant Breeding & Biotechnology, West Pomeranian University of Technology in Szczecin, Słowackiego 17, PL-71434 Szczecin, Poland*
pawel.milczarski@zut.edu.pl; https://orcid.org/0000-0003-2622-8938
[4]*Department of Shaping of Environment, West Pomeranian University of Technology in Szczecin, Słowackiego 17, PL-71434 Szczecin, Poland*
ryszard.malinowski@zut.edu.pl; https://orcid.org/0000-0003-3413-2653
[5]*Departamento de Botânica e Zoologia, Universidade Federal do Rio Grande do Norte, Campus Universitário, 59078-900, Natal, RN, Brazil*
bruno.goto@ufrn.br; https://orcid.org/0000-0001-6157-4954
**Author for correspondence: janusz.blaszkowski@zut.edu.pl*

Abstract

Preliminary morphological analyzes of an isolate producing glomoid spores in culture and comparison of its 45S nuc rDNA sequences (= 18S-ITS-28S) with sequences available in GenBank suggested that this isolate is an undescribed arbuscular mycorrhizal fungus of the genus *Glomus* in the family Glomeraceae (phylum Glomeromycota). This suggestion was confirmed by phylogenetic analyzes with sequences of 45S and the largest subunit of RNA polymerase II (*rpb1*) gene that placed this isolate in an autonomous clade sister to that with *Glomus macrocarpum*, the type species of *Glomus* and Glomeromycota. In the field, this species, here named *G. rugosae sp. nov.*, was associated with roots of *Rosa rugosa*, which inhabited the coastal dunes of the Hel Peninsula in northern Poland. In single-species cultures, *G. rugosae* formed typical vesicular-arbuscular mycorrhiza. Phylogenetic analyzes with 45S sequences of *Glomus* species and environmental sequences with a similarity of >96% to the 45S sequences of *G. rugosae* showed that *G. rugosae* was previously detected in the Czech Republic.

Keywords: arbuscular mycorrhizal fungi, new taxon, morphology, nuc rDNA, phylogenetic taxonomy, *rpb1*

Introduction

A potentially new species of an arbuscular mycorrhizal fungus (AMF) of the phylum Glomeromycota was found in a dune site in Poland and then grown in cultures. This fungus produced glomoid spores sensu Morton & Redecker (2001) with morphological features most corresponding to those of spores of members of the four-generic family Glomeraceae defined by Oehl *et al.* (2011). The main morphological features linking species of the four genera were the continuity and co-coloration of all components of their subtending hyphal wall with all components of the spore wall.

Currently, Glomeraceae consists of 19 genera with 146 species, which were mainly introduced based on phylogenetic analyses of species originally described in the genus *Glomus* and newly discovered fungi with glomoid spores (Wijayawardene *et al.* 2022; da Silva *et al.* 2023; Błaszkowski *et al.* 2023). Recognizing the taxonomic affiliation of most species in these genera and glomoid spore-producing undescribed species is difficult and often impossible. This is mainly due to the simplicity of the spore structure (spores are unicellular), crypticity (no morphological differences

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in phylogenetically different species), homoplasy (the presence of similar features in different taxa, but not present in the last common ancestor of these taxa), high phenotypic and histochemical variability of spore components, and dimorphism (the ability of one species to produce two different morphological forms) (Błaszkowski *et al.* 2021, 2022; da Silva *et al.* 2022). Moreover, such recognition is further complicated by the fact that glomoid spores are also formed by representatives of nine other families of Glomeromycota. Each of these families contains species with colourless spores, in which the typical features of members of Glomeraceae, mentioned above, are difficult to see or are invisible due to the lack of contrast. Finally, the phylogeny of ca. 40% of glomoid spore-producing species of Glomeromycota is still unknown or doubtful. This makes it impossible to reliably determine the position of these species within Glomeromycota and, consequently, to know their role in the evolution of this group of fungi.

To check our initial supposition that the AM fungus found belongs to Glomeraceae, we obtained sequences of its 45S nuc rDNA region (= 18S-ITS-28S) and compared them with sequences available in GenBank, using BLAST. This comparison suggested that this AMF is an undescribed *Glomus* species.

The aims of the further studies described in this paper were (i) to confirm the membership of this fungus in the genus *Glomus* and its novelty, (ii) to determine its phylogenetic position among sequenced *Glomus* species, (iii) to describe the morphology of its spores and mycorrhiza, and (iv) to compare the morphological features of these spores with those of spores of phylogenetically most closely related species and not sequenced species with similar morphology. In introducing the new species, we follow the recommendations for a polyphasic approach (Chethana *et al.* 2021).

Materials and methods

Origin of Study Material

The potentially new species, initially named Isolate 476 (numbers are from an AMF database maintained by J. Błaszkowski), was characterized based on spores extracted from a trap and single-species pot cultures. The single-species cultures were established from spores extracted from a trap culture inoculated with a field mixture of rhizosphere soil and root fragments of *Rosa rugosa* Thunb. The plant colonized maritime sand dunes located near Kuźnica, Hel Peninsula, Poland (54°44′04″N 18°34′54″E). The soil sample was collected by J. Błaszkowski on May 2, 2022. Data about the climate and vegetation of the Hel Peninsula are in Błaszkowski (1994).

The trap and single-species cultures were established and grown, spores were extracted, and mycorrhizal structures were stained as described previously (Błaszkowski *et al.* 2006, 2009). Single-species cultures were established using clusters with five to twenty spores connected by a common parent hypha.

Microscopy and Nomenclature

Morphological features of spore clusters and spores, as well as phenotypic and histochemical characters of spore wall layers of Isolate 476 were characterized based on at least 50–100 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 for study and photography were prepared as described in Błaszkowski (2012) and Błaszkowski *et al.* (2012). The types of spore wall layers were defined by Błaszkowski (2012) and Walker (1983). Color names were from Kornerup & Wanscher (1983). Nomenclature of fungi and the authors of fungal names are from the Index Fungorum website http://www.indexfungorum.org/AuthorsOfFungalNames.htm. The term "glomerospores" was used for spores produced by AMF as proposed by Goto & Maia (2006).

Voucher specimens of the proposed new species [spores permanently mounted in PVLG and a mixture of PVLG and Melzer's reagent (1:1, v/v) on slides] were deposited at ZT Myc (ETH Zurich, Switzerland; holotypes) and in the Laboratory of Plant Protection, Department of Environmental Management (LPPDEM), West Pomeranian University of Technology in Szczecin, Poland (isotypes).

DNA Extraction, PCR, Cloning, and DNA Sequencing

Genomic DNA of Isolate 476 was extracted separately from eight clusters of spores, each with 4–15 spores produced from branches of a parent hypha. The method of processing the spores prior to PCR, conditions and primers used for

PCR, as well as cloning and sequencing of PCR products to obtain 45S sequences of the isolate were as those described by Krüger *et al.* (2009) and Błaszkowski *et al.* (2021). Sequences of the *rpb1* gene were obtained using the RPB1-HS_A1a and RPB1-DR1730rr primers and PCR conditions proposed by Stockinger *et al.* (2014). Both 45S and *rpb1* sequences were deposited in GenBank (PP532762–PP532768, PP556328, PP556329).

Phylogenetic Analyses

As mentioned previously, comparisons of 45S sequences of Isolate 476 with sequences available in GenBank showed that it to be an undescribed species of the *Glomus*. Then, to use an appropriate outgroup in subsequent analyses, ML analysis was performed with 45S sequences of Isolate 476 and selected representatives of all genera of Glomeraceae sequenced from this locus. This analysis showed that the outgroup should be represented by *Complexispora* species (data not shown). Then, to find the position of Isolate 476 among *Glomus* species, three alignments were produced using MAFFT 7 with the E-INS-i option (Katoh *et al.* 2019). In all alignments, the outgroup were sequences (45S, *rpb1*, or 45S+*rpb1*) of *C. multistratosa* and *C. mediterranea*. The ingroup of the 45S alignment contained 27 sequences of the 45S region, which characterized Isolate 476 and six *Glomus* species, i.e., all sequenced members of this genus, and that of the *rpb1* alignment consisted of 16 sequences of Isolate 476 and five *Glomus* species. The 45S+*rpb1* alignment had all sequences of the 45S alignment concatenated with sequences of the *rpb1* alignment.

The percentage sequence divergences of Isolate 476 from sequences of its closest relative were calculated in BioEdit (Hall 1999). All comparisons were performed on sequences of the same length.

The phylogenetic position of Isolate 476 among the analyzed *Glomus* species was reconstructed based on Bayesian inference (BI) and maximum likelihood (ML) phylogenetic analyses of the 45S, *rpb1*, and 45S+*rpb1* alignments, performed via CIPRES Science Gateway 3.1 (Miller *et al.* 2010). The 45S and *rpb1* alignments were divided into five partitions (45S into: 18S, ITS1, 5.8S, ITS2, 28S; *rpb1* into: three exons and two introns). In both BI and ML analyses, GTR+I+G was used as nucleotide substitution model for each nucleotide partition (Abadi *et al.* 2019).

The BI reconstruction was made based on four Markov chains run over one million generations in MrBayes 3.2 (Ronquist *et al.* 2012), sampling every 1,000 generations, with a burn-in at 30% sampled trees. The ML phylogenetic tree inference was performed with RAxML-NG 1.0.1 (Kozlov *et al.* 2019), using a maximum likelihood/1000 bootstrapping run, and ML estimated proportion of invariable sites and base frequencies. The alignments and tree files were deposited as supplementary materials. Clade and node supports were considered strong, moderate, and marginal when BI and ML support values were 0.98–0.99 and 81–99%, 0.96–0.97 and 71–80%, and 0.95 and 70%, respectively.

The phylogenetic trees were visualized and edited in FigTree ver. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and MEGA6 (Tamura *et al.* 2013).

To detect possible other findings of Isolate 476, its 45S sequences were used as queries in BLASTn to retrieve environmental sequences of potentially identical species from GenBank. The sequences were selected according to the percentage of identity >96%. Their likely identity was then verified in BI and ML analyzes of the alignment with 45S+environmental sequences.

Results

General Data and Phylogeny

Of the 45S and *rpb1* sequences analyzed, 7 and 2, respectively, were newly obtained in this study. The numbers of analyzed sequences and species, as well as the numbers of base pairs, variable, and parsimony informative sites of each of the alignments studied are presented in Table 1.

TABLE 1. Characteristics of the sequence alignments with *Glomus rugosae*.

Name of alignment	No. of sequences	No. of fungal species	No. of base pairs	No. of variable sites	No. of parsimony informative sites
45S	33	10	1703	375	319
rpb1	20	8	1175	112	120
45+*rpb1*	33	10	2878	497	439

The topologies of the 45S, *rpb1*, and 45S+*rpb1* trees generated by BI and ML analyses were identical (Fig. 1; Figs. S1, S2). The analyses placed Isolate 476 in a sister position to *G. macrocarpum*. In all analyses, the Isolate 476 clade received full and strong BI and ML supports, respectively. In the 45S+*rpb1* and 45S trees, also the node connecting the Isolate 476 clade with the *G. macrocarpum* clade was fully and strongly supported (Fig. 1, Fig. S3). However, this node did not receive ML support in the *rpb1* tree. In all trees, BI and ML supports of the clade with *G. macrocarpum* sequences were moderate to full.

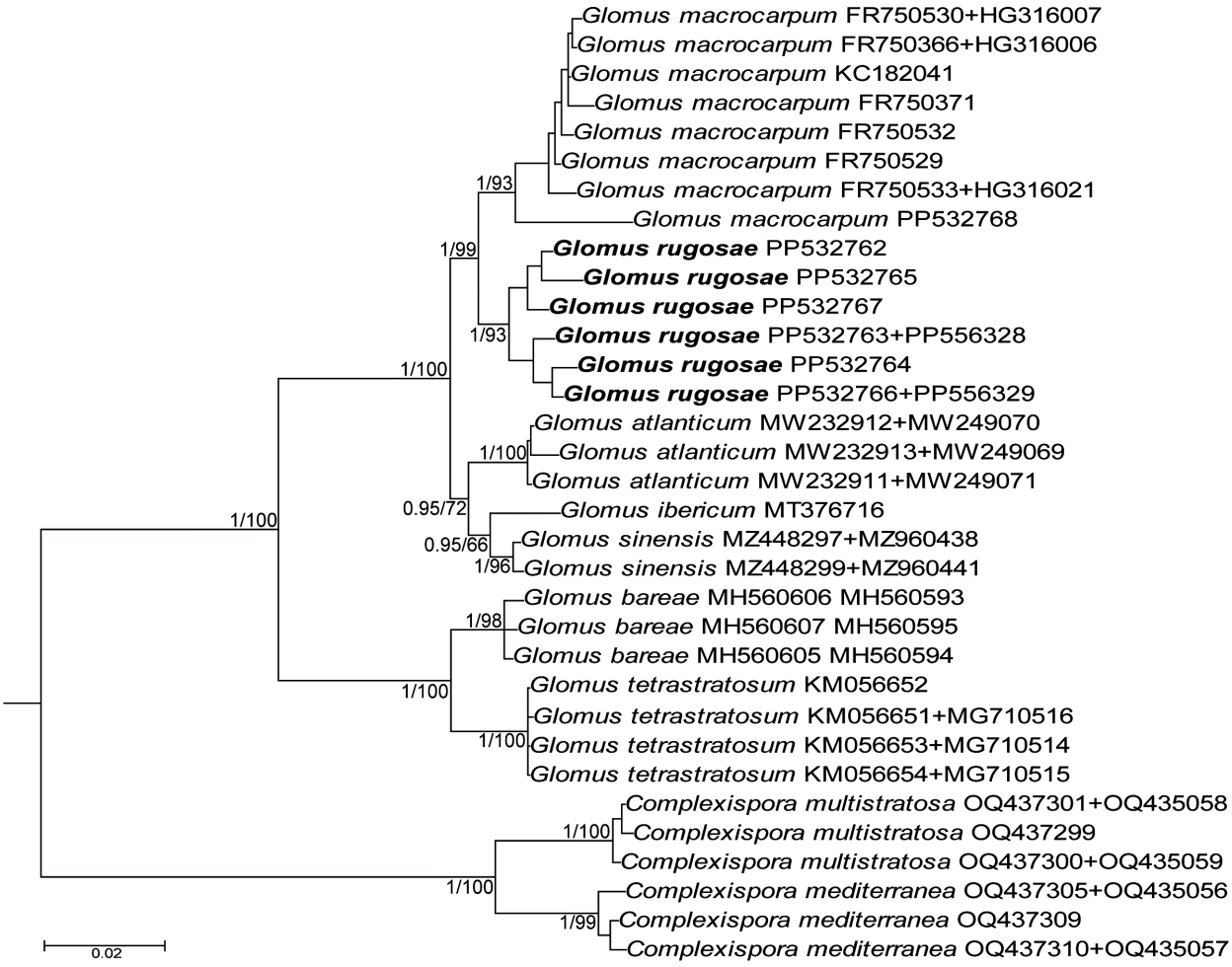

FIGURE 1. 50% majority-rule consensus tree from the Bayesian analysis of sequences of 45S nuc rDNA concatenated with *rpb1* sequences of *Glomus rugosae*, six other *Glomus* species, and two *Complexispora* species serving as outgroup. The new species is in bold font. The Bayesian posterior probabilities ≥0.90 and ML bootstrap values ≥50% are shown near the branches, respectively. Bar indicates 0.02 expected change per site per branch.

The differences between the 45S and *rpb1* sequences of Isolate 476 and *G. macrocarpum* were 4.3–6.9% and 1.4–2.2%, respectively.

Taxonomy

The results of the phylogenetic analyses and comparisons of sequences discussed above confirmed our assumption that Isolate 476 is an unknown species of the genus *Glomus*, here described as *G. rugosae sp. nov*.

Glomus rugosae Błaszk., B.T. Goto et Niezgoda, *sp. nov.* Figures 2A–H.
MycoBank No. MB 853099

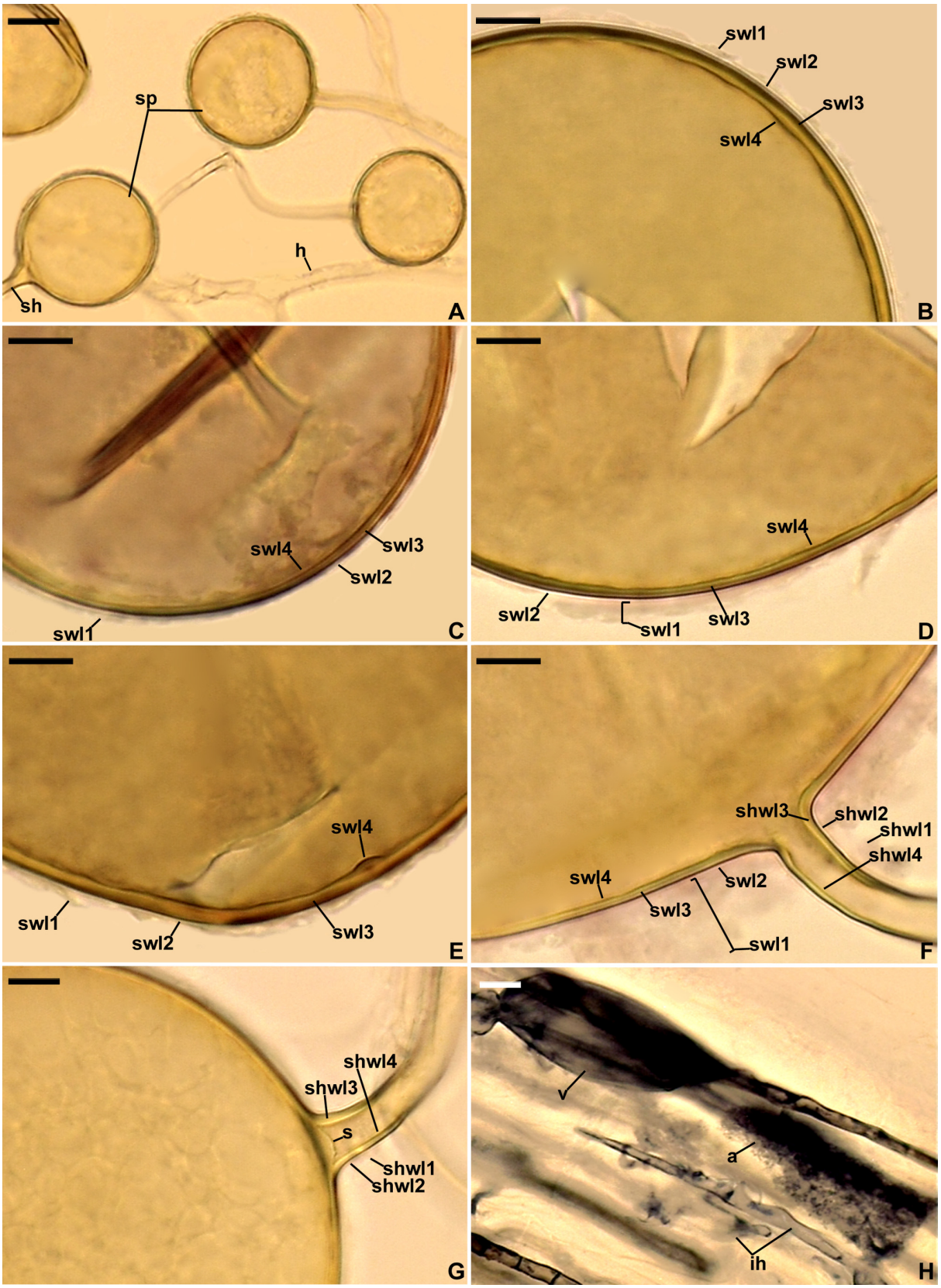

FIGURE 2A–H. *Glomus rugosae*. A. Cluster with sporogenous hyphae (h), spores (sp), and a spore subtending hypha (sh). B–F. Spore wall layers (swl) 1–4. F, G. Subtending hyphal wall layers (shwl) 1–4 continuous with spore wall layers (swl) 1–4. H. Arbuscule (a), intraradical hyphae (ih), and vesicle (v) in *Plantago lanceolata* root stained in 0.1% Trypan blue. A, B, G, H. Spores and mycorrhizal structures in PVLG. C–F. Spores in PVLG+Melzer's reagent. A–H. Differential interference microscopy. Scale bars: A = 20 µm, B–H = 10 µm.

Etymology: Latin, *rugosae*, referring to the plant species, *Rosa rugosa*, under which this species was originally found.

Typification: POLAND. Spores from a single-species culture established from spores extracted from a trap culture inoculated with a field-collected mixture of rhizosphere soil and root fragments of *Rosa rugosa* from a maritime sand dune site located near Kuźnica, Hel Peninsula, Poland (54°44′04″N 18°34′54″E), 2 May 2022, J. Błaszkowski (holotype slide with spores no. ZT Myc 0067314, isotype slides with spores no. 3969–3978, LPPDSE).

Diagnosis: Differs from *G. macrocarpum*, the closest phylogenetic relative (Fig. 1, Figs. S1, S2), in: (i) the spore wall and subtending hyphal wall structure, (ii) morphometric features of spores, the spore and subtending hyphal wall, as well as (iii) nucleotide composition of sequences of the 45S nuc rDNA region and the *rpb1* gene. Differs from *G. spinuliferum*, the only not sequenced glomoid spore-producing species having a four-layered spore wall in (i) spore colour, (ii) morphometric features of spores, subtending hyphae and the spore wall, and (iii) the phenotypic and histochemical properties of spore wall layers 2 and 1, respectively (see Discussion for details).

Description: Glomerospores (= spores) formed in soil, in loose clusters with 2–21 spores or singly, arise blastically at tips of (*i*) sporogenous hyphae branched from a parent hypha continuous with an extraradical mycorrhizal hypha (spores in clusters) or (*ii*) sporogenous hyphae directly continuous with extraradical mycorrhizal hyphae (single spores; Fig. 2A, F, G). *Spores* pale yellow (4A3) to greyish yellow (4B3); globose to subglobose; (25–)62(–100) µm diam; rarely ovoid; 70–98 × 81–110 µm; with one subtending hypha (Fig. 2A–G). *Spore wall* composed of four layers (Fig. 2B–F). Layer 1, forming the spore surface, uniform (not containing visible sublayers), mucilaginous, short-lived, flexible, hyaline, (0.6–)0.7(–0.8) µm thick, often highly swelling/expanding in spores mounted in PVLG and separating from the upper surface of spore wall layer 2 by up to 15 µm, frequently entirely sloughed off in mature spores (Fig. 2C–F). Layer 2 uniform, permanent, smooth, semi-flexible, hyaline, (0.5–)0.8(–1.3) µm thick, tightly adherent to the upper surface of layer 3, not separating from this layer in even vigorously crushed spores (Fig. 2C–F). Layer 3 laminate, permanent, smooth, semi-flexible to semi-rigid, pale yellow (4A3) to greyish yellow (4B3), (0.8–)1.5(–3) µm thick, consisting of very thin, <0.5 µm thick, laminae, tightly adherent to and not separating from each other in even vigorously crushed spores (Fig. 2B–F). Layer 4 uniform, permanent, smooth, semi-flexible to semi-rigid, yellowish white (4A2) to pale yellow (4A3), (0.5–)0.6(–0.8) µm thick, tightly adherent to the inner surface of layer 3 in moderately crushed spores, occasionally separating slightly and locally from this layer in vigorously crushed spores (Fig. 2B–F). In Melzer's reagent, only spore wall layer 1 stains reddish white (7A2) to pale red (8A3) (Fig. 2C–F). *Subtending hypha* pale yellow (4A3) to greyish yellow (4B3); straight or recurved, cylindrical to slightly funnel-shaped, rarely slightly constricted at the spore base; (7.6–)9.8(–13.7) µm wide at the spore base (Fig. 2A, F, G); not braking in crushed spores. *Wall of subtending hypha* pale yellow (4A3) to greyish yellow (4B3); (1.7–)2.7(–3.8) µm thick at the spore base; consisting of four layers continuous with spore wall layers 1–4; subtending hyphal wall layer 1 swelling in PVLG and usually highly deteriorated or, occasionally, entirely sloughed off in mature spores (Fig. 2F, G). *Pore* (1.6–)4.8(–8.6) µm wide at the spore base, open, rarely closed by a curved septum connecting the inner surface of spore wall layer 4 (Fig. 2A, F, G). Spore content of hyaline oily substance. *Germination* unknown.

Ecology and distribution: In the field, *Glomus rugosae* probably lived in arbuscular mycorrhizal symbiosis with roots of *Rosa rugosa*, but no molecular analyses were performed to confirm this assumption. In single-species cultures with *Plantago lanceolata* as the host plant, *G. rugosae* formed mycorrhiza with arbuscules, vesicles, as well as intra- and extraradical hyphae that stained clearly [violet white (18A2) to blackish blue (19F8)] in 0.1% trypan blue (Fig. 2H). Phylogenetic analyzes with the 45S alignment used in this study and environmental sequences with >96% identity to 45S sequences of *G. rugosae*, revealed by BLASTn, indicated that *G. rugosae* was previously recognized in roots of *Acer platanoides* L., which grew in the ash sedimentation pond in Melnik, Cetral Bohemia, Czech Republic (data not shown). The environmental sequences that clustered with 45S sequences of *G. rugosae* were HG425911 and HG425912, with query covers = 100% and identities = 97.54% and 97.29%, respectively.

Discussion

The morphological and phylogenetic analyzes, as well as comparisons of sequences described above confirmed the assumption that the AM fungus associated with roots of *Rosa rugosa* growing in the coastal dunes of the Hel Peninsula, Poland, is a new species of the genus *Glomus*, here described as *G. rugosae*. Phylogenetic analyzes with 45S and *rpb1* sequences showed that its closest relative is *G. macrocarpum* (Fig. 1, Figs. S1, S2).

Glomus rugosae differs substantially from *G. macrocarpum*. The most significant difference resides in the

structure of the spore wall. In *G. rugosae*, the spore wall consists of four layers (Fig. 2B–G), while the spore wall of *G. macrocarpum* contains only two layers (Gerdemann & Trappe 1974; Berch & Fortin 1983, 1984; Błaszkowski 1993, 2012), lacking layers 2 and 4 of the spore wall of the former species. Furthermore, *G. rugosae* spores are 1.6–3.7-fold smaller when globose, the spore wall is 1.6–2.9-fold thinner, and at the spore base the subtending hypha is 1.6–2.3-fold narrower and its wall may be 2.1–2.6-fold thinner. Finally, these two species certainly differ in their ecological requirements. In the literature, there is no convincing evidence that *G. macrocarpum* sporulated in culture. This confirms the conclusion of, e.g., Gerdemann & Trappe (1974) and Błaszkowski *et al.* (2021) that the cultivation of sporocarpic species of Glomeromycota, of which *G. macrocarpum* is a typical representative, usually fails. In contrast, *G. rugosae* produced hundreds of spores both in a trap culture and six single-species cultures, i.e., all that were established.

The only other glomoid spore-producing species with a four-layered spore wall is *G. spinuliferum* (Oehl *et al.* 2003). Although *G. spinuliferum* has not yet been sequenced, its spore wall layer 2, ornamented with spines, readily separates it from *G. rugosae*, in which no spore wall layer is ornamented (Fig. 2A–G). In addition, spores of *G. rugosae* are clearly lighter (vs. dark yellow to brown in *G. spinuliferum*), 1.4–4.4-fold smaller when globose, have a ca. 3-fold thinner spore wall and up to 1.5-fold wider subtending hypha at the spore base, as well as have a spore wall layer 1 that stains in Melzer's reagent (vs. no spore wall layer reacts in this reagent).

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Janusz Błaszkowski, Bruno Tomio Goto, Paweł Milczarski, Piotr Niezgoda, and Szymon Zubek. The first draft of the manuscript was written by Janusz Błaszkowski, and all authors commented on previous versions of the manuscript. Conceptualisation: Janusz Błaszkowski, Bruno Tomio Goto; methodology: Janusz Błaszkowski, Bruno Tomio Goto, Piotr Niezgoda; formal analysis and investigation: Janusz Błaszkowski, Bruno Tomio Goto, Paweł Milczarski, Piotr Niezgoda, and Szymon Zubek; writing original draft preparation: Janusz Błaszkowski and Bruno Tomio Goto; writing—review and editing: Janusz Błaszkowski, Bruno Tomio Goto, Paweł Milczarski, Piotr Niezgoda, and Szymon Zubek; funding acquisition: Bruno Tomio Goto, Piotr Niezgoda, Szymon Zubek; resources: Janusz Błaszkowski, Piotr Niezgoda; Supervision: Janusz Błaszkowski. All authors read and approved the final manuscript.

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Data availability Datasets generated during and/or analyzed during the current study are available from the corresponding author upon request.

Declarations

Ethics approval Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no competing interests.

References

Abadi, S., Azouri, D., Pupko, T. & Mayrose, I. (2019) Model selection may not be a mandatory step for phylogeny reconstruction. *Nature Communications* 10: 934.
https://doi.org/10.1038/s41467-019-08822-w

Berch, S.M. & Fortin, J.A. (1983) Lectotypification of *Glomus macrocarpum* and proposal of new combinations: *Glomus australe*, *Glomus versiforme*, and *Glomus tenebrosum* (Endogonaceae). *Canadian Journal of Botany* 61: 2608–2617. https://doi.org/10.1139/b83-287

Berch, S.M. & Fortin, J.A. (1984) Some sporocarpic *Endogonaceae* from eastern Canada. *Canadian Journal of Botany* 62: 170–180. https://doi.org/10.1139/b84-029

Błaszkowski, J. (1993) Polish *Glomales* XII. *Glomus macrocarpum* Tul. & Tul. and *Glomus microcarpum* Tul. & Tul. *Bulletin Polish Academy Biological Sciences* 41: 29–39.

Błaszkowski, J. (1994) Arbuscular fungi and mycorrhizae (*Glomales*) of the Hel Peninsula, Poland. *Mycorrhiza* 5: 71–88. https://doi.org/10.1007/s005720050043

Błaszkowski, J. (2012) *Glomeromycota*. Kraków: W. Szafer Institute of Botany, Polish Academy of Sciences, 303 pp.

Błaszkowski, J., Jobim, K., Niezgoda, P., Meller, E., Malinowski, M., Milczarski, P., Zubek, Sz., Magurno, F., Casieri, L., Bierza, W., Błaszkowski, T., Crossay, T. & Goto, B.T. (2021) New glomeromycotan taxa, *Dominikia glomerocarpica* sp. nov. and *Epigeocarpum crypticum* gen. nov. et sp. nov. from Brazil, and *Silvaspora* gen. nov. from New Caledonia. *Frontiers in Microbiology* 12: 655910. https://doi.org/10.3389/fmicb.2021.655910

Błaszkowski, J., Kovács, G.M., Gáspár, B.K., Balázs, T.K., 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. https://doi.org/10.3852/10-430

Błaszkowski, J., Kovács, G.M. & Balázs, T. (2009) *Glomus perpusillum*, a new arbuscular mycorrhizal fungus. *Mycologia* 101: 245–253. https://doi.org/10.3852/08-087

Błaszkowski, J., Renker, C. & Buscot, F. (2006) *Glomus drummondii* and *G. walkeri*, two new species of arbuscular mycorrhizal fungi (*Glomeromycota*). *Mycological Research* 110: 555–566. https://doi.org/10.1016/j.mycres.2006.02.006

Błaszkowski, J., Sánchez-García, M., Niezgoda, P., Zubek, Sz., Fernández, F., Vila, A., Al-Yahya'ei, M.N., Symanczik, S., Milczarski, P., Malinowski, R., Cabello, M., Goto, B.T., Casieri, L., Malicka, M., Bierza, W. & Magurno, F. (2022) A new order, *Entrophosporales*, and three new *Entrophospora* species in *Glomeromycota*. *Frontiers in Microbiology* 13: 962856. https://doi.org/10.3389/fmicb.2022.962856

Błaszkowski, J., Yamato, M., Niezgoda, P., Zubek, Sz., Milczarski, P., Malinowski, R., Meller, E., Malicka, M., Goto, B.T., Uszok, S., Casieri, L. & Magurno, F. (2023) A new genus, *Complexispora*, with two new species, *C. multistratosa* and *C. mediterranea*, and *Epigeocarpum japonicum* sp. nov. *Mycological Progress* 22: 34. https://doi.org/10.1007/s11557-023-01882-9

Chethana, T.K.W., Manawasinghe, I.S., Hurdeal, V.G., Bhunjun, C.S., Appadoo, M.A., Gentekaki, E., Raspé, O. & Promputtha, I. (2021) What are fungal species and how to delineate them? *Fungal Diversity* 109: 1–25. https://doi.org/10.1007/s13225-021-00483-9

da Silva, G.A., Corazon-Guivin, M.A., de Assis, D.M.A. & Oehl, F. (2023) *Blaszkowskia*, a new genus in *Glomeraceae. Mycological Progress* 22: 74. https://doi.org/10.1007/s11557-023-01919-z

da Silva, K.J.G., Fernandes, J.A.L., Magurno, F., Leandro, L.B.A., Goto, B.T. & Theodoro, R.C. (2022) Phylogenetic review of *Acaulospora* (*Diversisporales*, *Glomeromycota*) and the homoplasic nature of its ornamentations. *Journal of Fungi* 8*:* 892. https://doi.org/10.3390/jof8090892

Gerdemann, J.W. & Trappe, J.M. (1974) The *Endogonaceae* in the Pacific Northwest. *Mycologia Memoir* 5: 1–76.

Goto, B.T. & Maia, L.C. (2006) Glomerospores: a new denomination for the spore of *Glomeromycota*, a group molecularly distinct from the *Zygomycota*. *Mycotaxon* 96: 129–132.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nuclei Acids Symposium Series* 41: 95–98.

Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166. https://doi.org/10.1093/bib/bbx108

Kornerup, A. & Wanscher, J.H. (1983) *Methuen handbook of colour*, 3rd Edn. Eyre Methuen, London, 252 pp.

Kozlov, A.M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. (2019) RAxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 35: 4453–4455. https://doi.org/10.1093/bioinformatics/btz305

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

https://doi.org/10.1111/j.1469-8137.2009.02835.x

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. *In*: IEEE (Org.) *Proceedings of the Gateway Computing Environments Workshop*. 14 Nov 2010. IEEE, New Orleans, LA, pp. 1–8. https://doi.org/10.1109/GCE.2010.5676129

Morton, J.B. & Redecker, D. (2001) Two families of *Glomales*, *Archaeosporaceae* and *Paraglomaceae*, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. *Mycologia* 93: 181–195. https://doi.org/10.2307/3761615

Oehl, F., da Silva, G.A., Goto, B.T. & Sieverding, E. (2011) *Glomeromycota*: three new genera and glomoid species reorganized. *Mycotaxon* 116: 75–120. https://doi.org/10.5248/116.75

Oehl, F., Wiemken, A. & Sieverding, E. (2003) *Glomus spinuliferum*, a new ornamented species in the *Glomales*. *Mycotaxon* 86: 157–162.

Omar, M.B., Bollan, L. & Heather, W.A. (1979) A permanent mounting medium for fungi. *Bulletin of the British Mycological Society* 13: 31–32. https://doi.org/10.1016/S0007-1528(79)80038-3

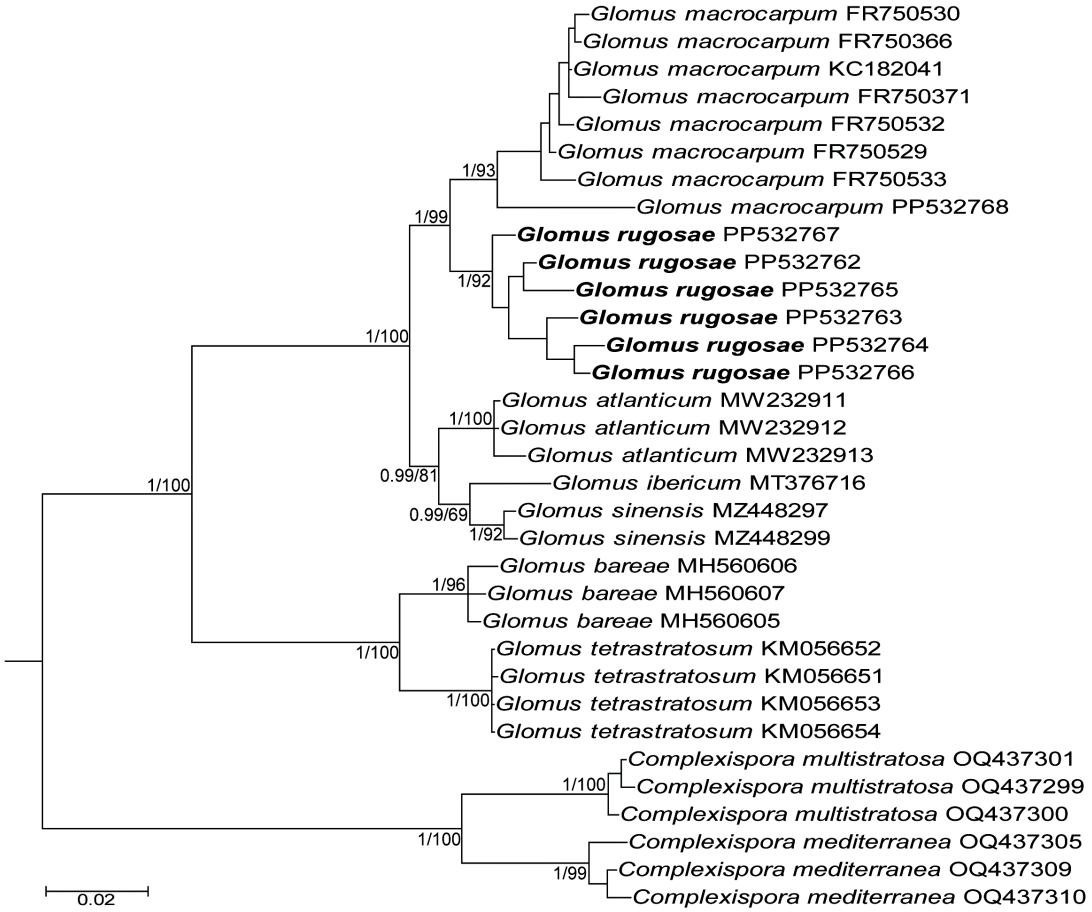
Ronquist, F., Teslenko, M,, van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Stockinger, H., Peyret-Guzzon, M., Koegel, S., Bouffaud, M.-L. & Redecker, D. (2014) The Largest Subunit of RNA Polymerase II as a New Marker Gene to Study Assemblages of Arbuscular Mycorrhizal Fungi in the Field. *PLoS ONE* 9: e107783. https://doi.org/10.1371/journal.pone.0107783

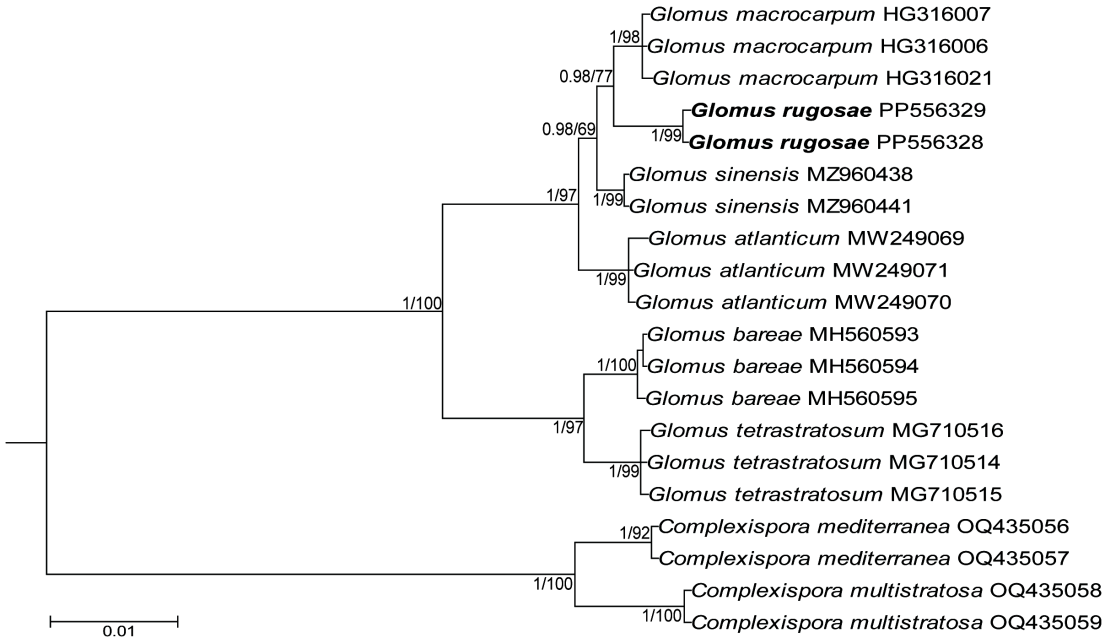
Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

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

Wijayawardene, N.N., Hyde, K.D., Dai, D.Q. & Sánchez-García, M. (2022) Outline of Fungi and fungus-like taxa—2021. *Mycosphere* 13 (1): 53–453. https://doi.org/10.5943/mycosphere/13/1/2



SUPPLEMENTARY FIGURE S1. 50% majority-rule consensus tree from the Bayesian analysis of nuc 45S rDNA sequences of *Glomus rugosae*, six other *Glomus* species, and two *Complexispora* species serving as outgroup. The new species is in bold font. The Bayesian posterior probabilities ≥0.90 and ML bootstrap values ≥50% are shown near the branches, respectively. Bar indicates 0.02 expected change per site per branch.



SUPPLEMENTARY FIGURE S2. 50% majority-rule consensus tree from the Bayesian analysis of *rpb1* sequences of *Glomus rugosae*, five other *Glomus* species, and two *Complexispora* species serving as outgroup. The new species is in bold font. The Bayesian posterior probabilities ≥0.90 and ML bootstrap values ≥50% are shown near the branches, respectively. Bar indicates 0.01 expected change per site per branch.