Glomus Chinense and *Dominikia Gansuensis*, Two New Glomeraceae Species of Arbuscular Mycorrhizal Fungi From High Altitude in The Tibetan Plateau

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Abstract

Two glomoid spore-producing arbuscular mycorrhizal fungi were grown in single-species cultures established from soil-borne spores collected from high altitude (2,800 m a.s.l.) of Tibet Plateau, China. Phylogenetic analyses were performed with sequences of nuclear rDNA (spanning the partial small subunit, whole internal transcribed spacer, and partial large subunit segment = 18S-ITS-28S) and the largest subunit of RNA polymerase II (*RPB1*) gene. Morphological and phylogenetic analyses indicated that the two fungi are undescribed species of the genera *Glomus* and *Dominikia*. The first fungus, described here as *G. chinense* sp. nov., formed spores singly and in loose clusters. The spores are orange-yellow to dark brown, globose, (47–)64(–93) µm diam. *Dominikia gansuensis* sp. nov. produced glomerocarps with pale yellow to yellow-brown, globose, (20–)47(–86) µm diam spores. Spore wall of both species consists of three layers. Both species differ clearly in morphology and phylogeny from their closest phylogenetic relatives, which are *G. atlanticum* and *G. ibericum*, and *D. glomerocarpica*, respectively.

Introduction

Arbuscular mycorrhizal (AM) fungi (phylum Glomeromycota) form mutualistic associations with the roots of most terrestrial plant species (Brundrett and Tedersoo 2018). It is generally accepted that plants supply AM fungi with carbohydrates and fatty acids (Jiang et al. 2017), and in return, the fungi provide their hosts with mineral nutrients, particularly phosphorous (Smith and Smith 2011), as well as enhance tolerance of the hosts to biotic and abiotic stresses (Porter et al. 2020). Furthermore, rich evidence shows that these fungi play key roles in determining diversity of plant communities (van der Heijden et al. 2008), regulating nutrient cycles (Bender et al. 2015), stabilizing soil structure (Daynes et al. 2013) and, consequently, in influencing many terrestrial ecosystem functions (Powell and Rillig 2018). Given the comprehensive benefits of AM fungi on plants and ecosystem functioning, their potential in sustainable agriculture and ecological restoration have been increasingly recognized (Koziol and Bever 2017; Rillig et al. 2019). Nonetheless, effective application of AM fungi is still scarce, which is due partly to the limited availability of AM fungal species/strains (Rillig et al. 2020).

Since the description of the first AM fungal species in 1840s (Koide and Mosse 2004), approximately 342 AM fungal species have been validly described (www.amf-phylogeny.com; accessed on September 20, 2021). This number is much lower (by *ca.* 50%) than the number of AM fungal phylotypes estimated based on published DNA sequences (Kivlin et al. 2011; Öpik et al. 2014), indicating that many undescribed taxa of AM fungi are waiting for discovery (Błaszkowski et al. 2021b; Kolaříková et al. 2021). At least five reasons caused the low recognition of the species diversity of AM fungi. First, isolation, monospecific culture, and identification of AM fungi are difficult and time-consuming due to their obligate symbiotic nature and simple morphology (Pawlowska et al. 1999; Fracchia et al. 2001). Second, many AM fungal species sporulate seasonally or only under certain conditions (Bever et al. 2001), and, therefore, may be omitted during sampling. Third, some AM fungi can be easily overlooked or lost during spore extraction from soils because their spores are small, hyaline, and frequently short-lived

(Błaszkowski et al. 2015). Forth, there is a growing number of potentially new species that cannot be distinguished from described species based on morphology alone due to the lack of convincing evidence or difficulties of detecting them using traditional microscopy (Błaszkowski et al. 2013, 2021b). Finally, the number of experienced mycologists dealing with identification of AM fungi is exceptionally low compared to the number of mycologists working on other fungal groups.

Fortunately, DNA sequencing and phylogenetic analyses have provided fast and accurate methods to discriminate AM fungal species (da Silva et al. 2011; Öpik et al. 2014). Of the molecular loci tested in studies aimed at identifying AM fungi, the most effective appeared to be the nuclear rDNA partial small subunit (18S), internal transcribed spacer region (ITS1-5.8S-ITS2 = ITS), and partial large subunit (28S) segment (18S-ITS-28S), as well as the largest subunit of RNA polymerase II (*RPB1*) gene (Krüger et al. 2009; Stockinger et al. 2010, 2014; Błaszkowski et al. 2021c). With the advancements of molecular identification, in the past few years more than thirty new species of AM fungi have been discovered, mainly from Europe and America (e.g., Błaszkowski et al. 2019; Jobim et al. 2019; Oehl et al. 2019; Schüßler and Walker 2019; Chimal-Sánchez et al. 2020; Corazon-Guivin et al. 2020; Guillén et al. 2020a, b; Błaszkowski et al. 2021a, c).

The Tibetan Plateau, referred to as the “roof of the world”, is the highest and largest plateau on the earth (Royden et al. 2008), where the extreme environments may have favored the evolution of some organisms that differ from those inhabiting regions with moderate climates. In fact, studies of AM fungal communities in this region have detected diverse AM fungal phylotypes, of which only few were related to described species (Liu et al. 2011, 2012, 2015; Jiang et al. 2018). Moreover, many morphotypes of AM fungal spores have been detected in this region at sites with altitude up to 5,500 m a.s.l. (Gai et al. 2009; Pan et al. 2013; Bahadur et al. 2019), but none of them was further investigated.

Our group established many monospecific pot cultures of AM fungi from single spores extracted from soil samples collected from the eastern Tibetan Plateau. Two of these cultures produced spores whose morphological characters were unlike those of described species forming glomoid spores. Therefore, the aims of our further studies were to characterize in detail the morphology of these fungi and to determine their closest phylogenetic relatives among sequenced members of the Glomeromycota.

Materials And Methods

Sampling locations and establishment of single-spore cultures

The two potentially new AM fungal species (initially named Species 1 and Species 2) were isolated from rhizosphere soils (*ca.* 0-20 cm depth) collected in September 2016 nearby the Hezuo city of the Gannan Tibetan Autonomous Prefecture, Gansu province, China. This region is located on the eastern edge of Tibetan Plateau, where the climate is humid-alpine with a mean annual temperature of 1.7°C and a mean annual rainfall of 545 mm. Species 1 was found in a shrubland (35°13'8.86"N, 102°48'30.83"E; 2843 m a.s.l.), where the dominant plant species were *Potentilla fruticosa* Linn. (Rosaceae), *Spiraea alpina* Pall. (Rosaceae) and *Potentilla sischanensis* Bge. (Rosaceae). Species 2 was from a temperate broad-leaved

mixed forest (35°06'27.77"N, 102°51'18.78"E; 2816 m a.s.l.) dominated by *Betula platyphylla* Suk. (Betulaceae), *Euonymus alatus* (Thunb.) Sieb. (Celastraceae), and some understory grasses.

Spores of AM fungi were extracted from field-collected soils using wet sieving and sucrose centrifugation (Brundrett et al. 1994), and the spores of Species 1 and Species 2 were picked out according to their morphology under a dissecting microscope. Monospecific cultures were established by inoculating roots of *Sorghum bicolor* (L.) Moench seedlings with single spores with healthy and shiny appearance, as described by the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (https://invam.wvu.edu/methods/culture-methods/single-species-cultures; accessed in September 2016). Inoculated seedlings were gently planted into 500-mL plastic pots filled with a sterilized mixture of fine sand and clinopthilolite (1:1, v/v). Up to thirty inoculated pots were established for each species. Plants were grown in the greenhouse under controlled temperature of *ca*. 20°C and artificial illumination of *ca*. 170 µmol photons m^{-2} s^{-1} (16 h per day) for four months, during which all pots were watered with distilled water every three or four days and fertilized with Hoagland's low-phosphorus solution every two weeks. All pots were dried for two weeks and then the roots and growth medium were harvested. Single-spore cultures were considered successfully established when their growth substrate contained spores of different developmental stages and stained roots were colonized by mycorrhizal structures. Then, the cultures were further cultured in large pots under the same conditions as described above to obtain more material for study. Extracted spores were used for morphological and molecular identification using the methods described below.

Morphological analysis

Spores were mounted in water, polyvinyl alcohol-lactic acid-glycerol (PVLG), and a mixture of PVLG and Melzer's reagent (1:1, v/v). Morphological characteristics of spores and their subcellular structure were examined and photographed using dissecting and compound microscopes. The terminology of spore characters basically follows Błaszkowski et al. (2021a, b, c) and Oehl et al. (2019), and the colors of spores and spore wall components were described and coded according to the Pantone® refreshed CMYK Guide (Pantone, NJ, USA). Voucher specimens of the two new species were deposited in the herbarium of Lanzhou University, China, and in the UFRN herbarium, Brazil. Types of spore wall layers are those defined by Walker (1983) and Błaszkowski (2012). Nomenclature of fungi and the authors of fungal names are from the Index Fungorum database (www.indexfungorum.org). The terms "glomerospores" and "glomerocarps" were used for spores and fruit bodies (sporocarps) produced by AM fungi, respectively, as Goto and Maia (2006) and Jobim et al. (2019) proposed.

Molecular analysis

Genomic DNA was extracted from single glomerospores/glomerocarps. Briefly, fresh spores were sonicated for 10 min and rinsed three times with sterilized distilled water. Single glomerospores/glomerocarps with healthy and intact appearance were transferred into a PCR tube with 10 µL of ddH_2O and crushed with a sterilized pipette tip under the dissecting microscope. Up to twenty

replications of single glomerospores/glomerocarps were prepared for each species. All tubes were incubated at 80°C for 7 minutes and the supernatant was used as DNA template in the subsequent PCR.

All spore-derived DNA samples were used to amplify 18S-ITS-28S and *RPB1* gene using nested or semi-nested PCR approaches. The rDNA sequences were amplified with the primer pairs SSUmAf–LSUmAr (1st PCR) and SSUmCf–LSUmBr (2nd PCR), producing a *ca*. 1.5-kb fragment (Krüger et al. 2009; Stockinger et al. 2010). The first PCR was carried out in a final volume of 25 µL with 4 µL of DNA template and 0.25 µM of each primer using the PrimeSTAR® HS DNA Polymerase Premix (Takara, Dalian, China) with the following cycling conditions: 94°C for 5 min, 35 × (94°C for 10 s, 55°C for 30 s and 72°C for 2 min) and 72°C for 10 min. One microliter of the first PCR product was used as template for the second PCR under conditions as described above, with the exception of 30 thermal cycles and annealing temperature of 60°C. For the *RPB1* sequences, we used the primer pairs RPB1_HS_A1a–RPB1_5R (1st PCR) and RPB1_HS_A1a–RPB1_DR1210r (2nd PCR) to amplify a *ca*. 2-kb fragment covering the majority of coding region (Stockinger et al. 2014; Błaszkowski et al. 2021c). The PCR conditions were the same as those for rDNA amplification, except that the cycling conditions of both PCRs were as follows: 98°C for 30s, 30 × (98°C for 10 s, 58°C for 10 s and 72°C for 2 min) and 72°C for 10 min.

PCR products were examined on 1% (w/v) agarose gels with GelGreen® staining, and up to ten successful PCR products per species of each target gene were purified using the TIANgel Midi Purification Kit (Tiangen, Beijing, China). After an A-tailing procedure, purified DNA fragments were ligated into pGEM-T vector (Promega, Shanghai, China) and cloned into *Escherichia coli* DH5α according to the manufacturer's instructions. After screening, representative positive clones for each library were sequenced using the vector primers. Sequences were assembled using the ContigExpress module in Vector NTI suite 6.0 (InforMax Inc., MD, USA). All sequences were deposited in GenBank database under the accession numbers MZ448287–MZ448306 (rDNA sequences) and MZ960429-MZ960444 (*RPB1* sequences).

Bioinformatic analysis and phylogenetic analysis

The sequences were blasted (BLASTn; https://blast.ncbi.nlm.nih.gov) versus the GenBank database to assess the putative novelty of the two species isolated. For each species, the percentage of 18S-ITS-28S and *RPB1* sequence divergence was also calculated using BLASTn. With the same program, we calculated the percentage of sequence divergence of these species from sequences of their closest relatives.

An 18S-ITS-28S alignment was obtained using five sequences from both new species obtained in this study and 107 sequences representing 33 species and 17 genera in the Glomeraceae. Nine sequences from three species in the Claroideoglomeraceae (as a sister family of the Glomeraceae in the order Glomerales) were added to the sequence set as outgroup. The alignment was performed with MAFFT 7, using E-INS-i as iterative refinement method (http://mafft.cbrc.jp/alignment/server/). The alignment was divided into five partitions as in Błaszkowski et al. (2021b): 18S, ITS1, 5.8S, ITS2, 28S. An additional

partition was represented by indel strings coded with FastGap 1.2 (Borchsenius 2009) and merged using a customized Python script.

The phylogenetic position of the two undescribed species was reconstructed based on Bayesian inference (BI) and maximum likelihood (ML) phylogenetic analyses of the 18S-ITS-28S alignment, performed via CIPRES Science Gateway 3.3 (Miller et al. 2010). In both analysis GTR+G+I was used as substitution model for the DNA partition as suggested by Abadi et al. (2019), while the F81 model was chosen for the indel partition, according to the MrBayes manual. For the BI analysis, four Markov chains were run over one million generations in MrBayes 3.2 (Ronquist et al. 2012), sampling every 1000 generations, with a burn-in at 3000 sampled trees. The ML analysis was performed with RAxML-NG 1.0.1 (Kozlov et al. 2019), using a maximum likelihood/1000 bootstrapping run, branch length estimation method = linked, and ML estimated proportion of invariable sites and base frequencies.

Similarly, an 18S-ITS-28S+*RPB1* alignment was obtained merging manually the alignment described above with the *RPB1* alignment containing all species of the 18S-ITS-28S alignment that have been provided with *RPB1* sequences as well. In detail, the *RPB1* dataset included five sequences from both new species, 81 sequences representing 27 species and 15 genera in the Glomeraceae, and 5 sequences from 3 species in the Claroideoglomeraceae. The *RPB1* alignment was performed with the same settings used for the 18S-ITS-28S alignment and provided with indel strings.

For the BI and ML analyses of the 18S-ITS-28S+*RPB1* alignment, twelve partitions plus an indel partition were added to the six described for the 18S-ITS-28S portion: a single partition was applied to each intron (4 introns in total) of the *RPB1* gene, while for the exon 3, 4 and 5 two separate partitions were applied for the first two and for the third codon positions; a single partition was applied to the exon 1 and 2 because of the very short length. The same setting described for the 18S-ITS-28S alignment were used for BI and ML analysis.

The phylogenetic trees obtained in the analyses were visualized in Archaeopteryx.js (https://sites.google.com/site/cmzmasek/christian-zmasek/software/archaeopteryx-js).

Results

Molecular phylogeny

The topologies of the 18S-ITS-28S and 18S-ITS-28S + *RPB1* trees generated from BI and ML analyses of the alignments were similar (Fig. S1 and Fig. 1, respectively). Both BI and ML analyses indicated that our fungi, initially named Species 1 and Species 2, are new species in the genera *Glomus* and *Dominikia*, respectively (Fig. 1 and Fig. S1). Consequently, these species are newly described here as *G. chinense* and *D. gansuensis*. The intraspecific similarities of 18S-ITS-28S sequences of *G. chinense* and *D. gansuensis* were 97.2–100% and 97.2–99.5%, respectively; the similarities of *RPB1* sequences of these species were 99.53–100% and 99.54–100%, respectively.

The phylogenetic analyses showed that the closest relatives of *G. chinense* are *G. atlanticum* and *G. ibericum* (Fig. 1 and Fig. S1), whose 18S-ITS-28S and *RPB1* sequences differed by *ca*. 3–4% and 1.3–1.5% (*G. atlanticum* only), respectively, from those of the new species. Instead, the sister relative of *D. gansuensis* is *D. glomerocarpica*, whose 18S-ITS-28S and *RPB1* sequence divergences were *ca*. 7–9% and 5.5–5.8%, respectively. In the analyses, the *G. chinense* clade was fully or strongly supported (18S-ITS-28S tree: BI = 1.0, ML = 99%; 18S-ITS-28S + *RPB1* tree: BI = 1.0, ML = 100%). Also, the node linking this clade with the clades with *G. atlanticum* and *G. ibericum* obtained sufficient supports (18S-ITS-28S tree: BI = 1.0, ML = 84%; 18S-ITS-28S + *RPB1* tree: BI = 1.0, ML = 74%). The clade with *D. gansuensis* was fully supported in both BI and ML analyses, as well as the node linking it with the *D. glomerocarpica* clade obtained full or strong supports (18S-ITS-28S tree: BI = 1.0, ML = 100%; 18S-ITS-28S + *RPB1* tree: BI = 1.0, ML = 99%).

Taxonomy

Glomus chinense F. Yu, B.T. Goto, H. Feng & Y. Liu, sp. nov. Figure 2A-F

MycoBank number: MB841240

Etymology. Latin, *chinense*, referring to the country (China) in which this fungus was originally discovered.

Diagnosis. Differs from *G. atlanticum* and *G. ibericum*, the sister phylogenetic relatives, in the spore wall structure and the phenotypic and biochemical properties of the spore wall layers, as well as in the nucleotide composition of sequences of the 18S-ITS-28S nuclear rDNA region and *RPB1* gene.

Description. Spores formed singly or occasionally in loose clusters in soil; arise blastically at tips of sporogenous hyphae continuous with extraradical mycorrhizal hyphae. *Spores* pale orange-yellow (0-45-100-6) to dark brown (37-76-92-51); globose to subglobose, (47–)64(–93) µm diam, rarely ovoid, 22–43 × 45–74 µm, with one subtending hypha (Fig. 2A). *Spore wall* consists of three layers (layers 1–3; Fig. 2A–E). Layer 1, forming the spore surface, evanescent (short lived), hyaline, slightly roughened in mature spores, (1.7–)2.0(–2.5) µm thick, staining pinkish purple (0-60-0-40) in Melzer's reagent (Fig. 2C–E). Layer 2 laminate, permanent, orange-yellow (0-45-100-6) to dark brown (37–76–92–51), smooth, (1.1–)2.4(–3.8) µm thick, staining yellow brown (0-51-76-36) in Melzer's reagent (Fig. 2C–E). Layer 3 light brown (0-50-75-28) to bright yellow (0-5-85-5), (0.6–)0.9(–1.6) µm thick, sometimes difficult to see, not reacting in Melzer's reagent (Fig. 2C). *Subtending hypha* pale orange-yellow (0-45-100-6) to dark brown (37-76-92-51), straight or curved, cylindrical, usually constricted at the spore base, (4.4–)9.1(–14.7) µm wide at the spore base (Fig. 2A, D). *Pore* of subtending hyphae open. *Wall of subtending hypha* pale orange-yellow (0-45-100-6) to dark brown (37-76-92-51), (2.6–)3.7(–5.8) µm thick at the spore base, composed of three layers continuous with spore wall layers 1–3 (Fig. 2C). *Germination* unknown.

Mycorrhizal associations. In the field, *G. chinense* was found in mixed rhizosphere soils of *Potentilla fruticose, Spiraea alpina*, and *Potentilla sischanensis* in a shrubland located in eastern Tibetan Plateau of China. In single-species cultures with *Sorghum bicolor* as host plant, *G. chinense* formed abundant arbuscules and intraradical hyphae (Fig. 2F).

Specimens examined. Slides with glomerospores permanently mounted in PVLG and a mixture of PLVG and Melzer's reagent. Holotype deposited in the herbarium of Lanzhou University, China (accession number: LZU_AMF_1708). Isotypes deposited in the herbarium of Lanzhou University, China (LZU_AMF_1709 to 1722) and in the UFRN herbarium, Brazil (Fungos 3388).

Distribution and habitat. The shrubland nearby the Hezuo city, Gansu province, China (35°13'8.86"N, 102°48'30.83"E; 2843 m a.s.l.) is the only site in which this fungus was physically found so far. Soil pH at this site was 7.62 ± 0.08 (mean ± SD, $n = 5$) and the concentration of soil available phosphorus was 10.4 ± 3.5 mg kg^{-1}. This type of shrubland, mainly dominated by *Potentilla fruticose*, is very common on the eastern Tibetan Plateau. BLAST searches showed only one environmental sequence (from an alpine meadow on the Tibetan Plateau; GenBank accession number: JX096603) suggesting conspecificity to *G. chinense*. Thus, *G. chinense* may be an endemic species on the Tibetan Plateau.

Dominikia gansuensis F. Yu, B.T. Goto, H. Feng & Y. Liu, sp. nov. Figure 3A-F

MycoBank number: MB841241

Etymology. Latin, *gansuensis*, referring to the Gansu Province of China, where this fungus was originally found.

Diagnosis. Differs from *D. glomerocarpica*, the sister phylogenetic relative, in (*i*) morphology of glomerocarps, (*ii*) the spore wall structure and the histochemical properties of the spore wall layers, as well as in (*iii*) the nucleotide composition of sequences of the 18S-ITS-28S nuclear rDNA region and *RPB1* gene.

Description. Spores usually produced in hypogeous glomerocarps, rarely singly in soil. *Glomerocarps* dull yellow (8-40-83-0) to yellow-brown (0-51-76-36), 99–(198)–354 × 188–(434)–463 µm, with dozens randomly distributed spores (Fig. 3A). *Spores* pale yellow (0-4-29-0) to yellow-brown (0-51-76-36), globose to subglobose, (20–)47(–86) µm diam, rarely ovoid, 18–42 × 30–60 µm, with one subtending hypha (Fig. 3B). *Spore wall* composed of three layers (Fig. 3B–D). Layer 1, forming the spore surface, evanescent, short-lived, hyaline, (0.4–)0.8(–1.4) µm thick. Layer 2 < 0.5 µm thick, usually tightly adherent to layer 3, even in vigorously crushed spores. Layer 3 laminate, dull yellow (8-40-83-0) to yellow-brown (0-51-76-36), (0.3–)0.7(–1.2) µm thick. Only spore wall layer 1 stains royal purple (0-40-0-0) to pinkish purple (0-80-65-0) in Melzer's reagent (Fig. 3C, D). *Subtending hypha* straight or recurved, cylindrical, (4.4–)6.6(–9.9) µm wide at the spore base (Fig. 3B). *Pore* (1.0–)2.5(–4.5) µm wide, occluded by a septum continuous with spore wall layer 3 near the spore base (Fig. 3C). *Germination* by a germ tube

arising from the septum at the spore base and emerging through the lumen of the subtending hypha (Fig. 3E).

Mycorrhizal associations. *Dominikia gansuensis* was originally extracted from rhizosphere soil sampled in a forest dominated by *Betula platyphylla*, *Euonymus alatus*, and some understory grasses. However, the plant species with which *D. gansuensis* formed symbiosis was not determined. In the greenhouse, *D. gansuensis* formed abundant arbuscules in the roots of *Sorghum bicolor* (Fig. 3F).

Specimens examined. Permanent slides with glomerocarps/glomerospores. Holotype deposited in the herbarium of Lanzhou University, China (LZU_AMF_1727); isotypes deposited in the herbarium of Lanzhou University, China (LZU_AMF_1728 to1741) and in the UFRN herbarium, Brazil (Fungos 3389).

Distribution and habitat. So far, *D. gansuensis* was physically found only in a forest site nearby the Hezuo city, Gansu province, China (35°06'27.77"N, 102°51'18.78"E; 2816 m a.s.l.), where the soil pH was 6.44 ± 0.22 (mean ± SD, $n = 5$) and the concentration of soil available phosphorus was 11.0 ± 4.9 mg kg^{-1}. BLAST queries indicated several environmental sequences of an identity of > 97% to *D. gansuensis* 18S-ITS-28S sequences. These sequences were derived from a wetland ecosystem in southern China, a semi-mangrove forest in southern China, a grassland in northern China, and a forest in Czech Republic.

Discussion

The phylogenetic and morphological analyses performed in this study clearly proved that the two AM fungi found at high altitude of Tibetan Plateau are new species of the genera *Glomus* and *Dominikia* (Glomeraceae), here described as *G. chinense* and *D. gansuensis*. Both species are culturable and maintained in the greenhouse with *Sorghum bicolor* as host plants. This is the first report of new AM fungal species isolated from the high altitude of Tibetan Plateau, China.

Glomus chinense is phylogenetically close to *G. ibericum*, *G. atlanticum*, and *G. macrocarpum* (Fig. 1). However, the divergences of the 18S-ITS-28S and *RPB1* sequences of the new species to those of *G. ibericum*, *G. atlanticum*, and *G. macrocarpum* clearly exceed or only reach the thresholds of species conspecificity (97% and 1% for 18S-ITS-28S and *RPB1* sequences, respectively), widely accepted in phylogenetic studies of AM fungi (Krüger et al. 2012; Stockinger et al. 2014). Morphologically, it is easy to distinguish *G. chinense* from both *G. ibericum* and *G. macrocarpum* because the latter two species usually produce spores in glomerocarps and their spore wall is two-layered (Berch and Fortin 1983; Guillén et al. 2020b). Both *G. chinense* and *G. atlanticum* produce spores singly and in loose clusters, as well their spore wall consists of three layers (Fig. 2). But, spore wall layer 2 of *G. chinense* covers a thin spore wall layer 3, laminate, thickest, and is the main structural layer of this wall (Fig. 2). Instead, in *G. atlanticum* the main laminate structural layer of the spore wall is layer 3, which is covered with a thin layer 2 (Błaszkowski et al. 2021c).

The closest phylogenetic relative of *D. gansuensis* is *D. glomerocarpica* (Fig. 1), a recently described species from northeastern Brazil (Błaszkowski et al. 2021a). The 18S-ITS-28S and *RPB1* sequences of

these species differ by 7–9% and 5–6%, respectively. The two species also differ clearly in morphology. Both species form spores in glomerocarps and their spore wall is three-layered, but *D. gansuensis* glomerocarps are hypogeous and much smaller than the epigeous glomerocarps of *D. glomerocarpica* (3.30 × 3.38 mm). Most importantly, spore wall layer 1 of *D. gansuensis* is a short-lived structure and stains in Melzer's reagent (Fig. 3). In *D. glomerocarpica*, all three spore wall layers are permanent and the spore wall component staining in Melzer's reagent is a laminate layer 2. In addition, the spore wall of *D. glomerocarpica* is 3—4.2-fold thicker.

Currently, it is widely recommended to analyze multiple gene loci to improve the reliability of fungal phylogenies (Sung et al. 2007; Chethana et al. 2021). In our study, we analyzed sequences of two unlinked loci, the 18S-ITS-28S rDNA segment and *RPB1* gene, and these analyses unambiguously indicated the phylogenetic positions of our new species within the Glomeraceae, independently whether the loci were considered separately or as concatenated. The information contained in the 18S-ITS-28S segment is generally sufficient to delimit even closely related species (Krüger et al. 2009) and numerous studies of AM fungal taxonomy were based solely on this barcoding region (e.g., Błaszkowski et al. 2015; Oehl et al. 2019; Schüßler and Walker 2019; Chimal-Sánchez et al. 2020; Corazon-Guivin et al. 2020; Guillén et al. 2020a, b). However, the concatenation of 18S-ITS-28S and *RPB1* sequences was shown to increase the robustness of phylogenetic inference of AM fungi and revealed relationships unexposed when the single 18S-ITS-28S locus was analyzed (Błaszkowski et al. 2021c). Unfortunately, so far only *ca*. 25% of described species of AM fungi were provided with *RPB1* sequences (Błaszkowski et al. 2021c). So, obtaining *RPB1* sequences of more Glomeromycotan members is strongly encouraged.

Declarations

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Author contributions

Yongjun Liu and Huyuan Feng contributed to the study conception and design. Jian Wang and Fengxia Yu collected field samples and established monospecific cultures. Fengxia Yu conducted the morphological and molecular analyses with help of Bruno Tomio Goto, Franco Magurno, Wenxia Ma and Yongjun Liu. The first draft of the manuscript was written by Fengxia Yu and Yongjun Liu, and carefully edited by Janusz Błaszkowski. All authors contributed to revisions.

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Data availability

DNA sequence data have been deposited in GenBank database. Datasets generated or analyzed during this study are available from the corresponding author upon request.

Conflict of interest: The authors declare no competing interests.

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Figures

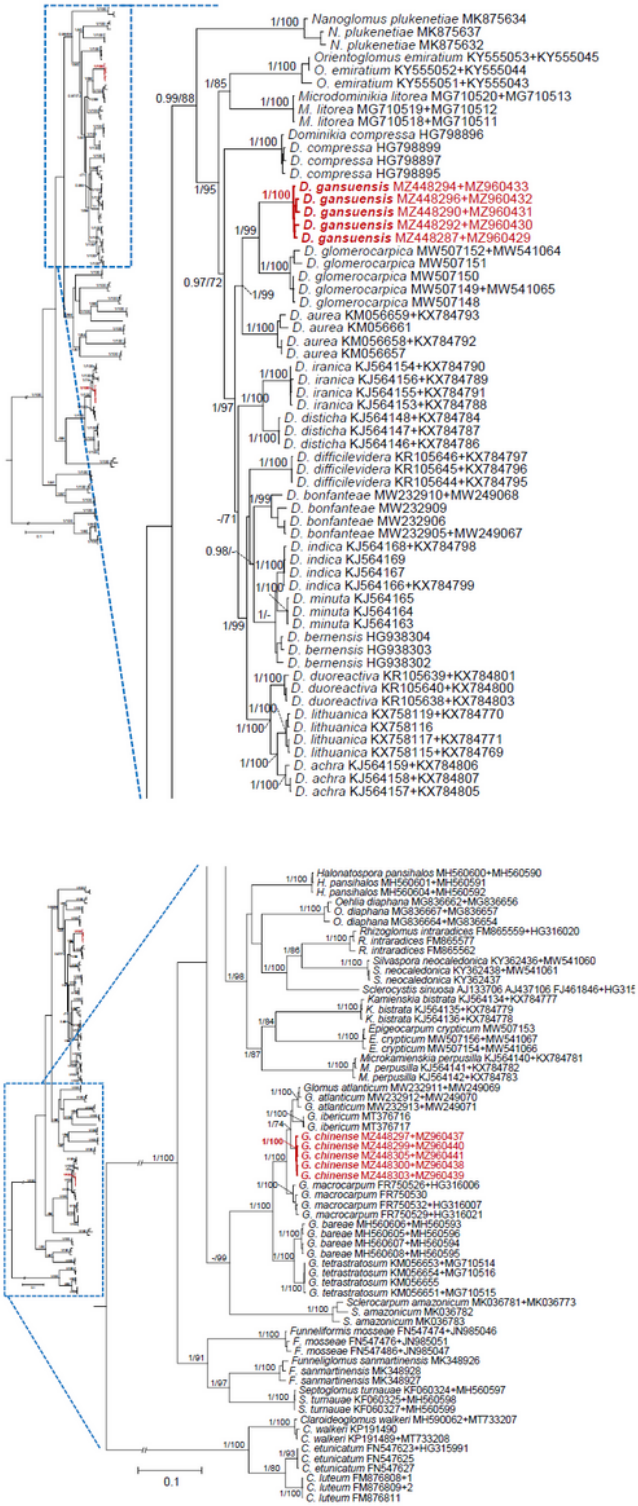

Figure 1

Bayesian phylogenetic tree inferred from the representative 18S-ITS-28S + RPB1 sequences of Glomus chinense and Dominikia gansuensis (in bold and red), 13 described species in Dominikia, 5 described species in Glomus, and representative species of other 15 genera of the Glomeraceae. Three species in the Claroideoglomeraceae serve as outgroup. Sequences are labeled with their GenBank accession numbers. The Bayesian posterior probabilities ≥ 0.95 and ML bootstrap values ≥ 70% are shown near

the branches, respectively. Bar indicates 0.1 expected change per site per branch. Some branches are shortened to 30% in length to improve visibility (indicated by //).

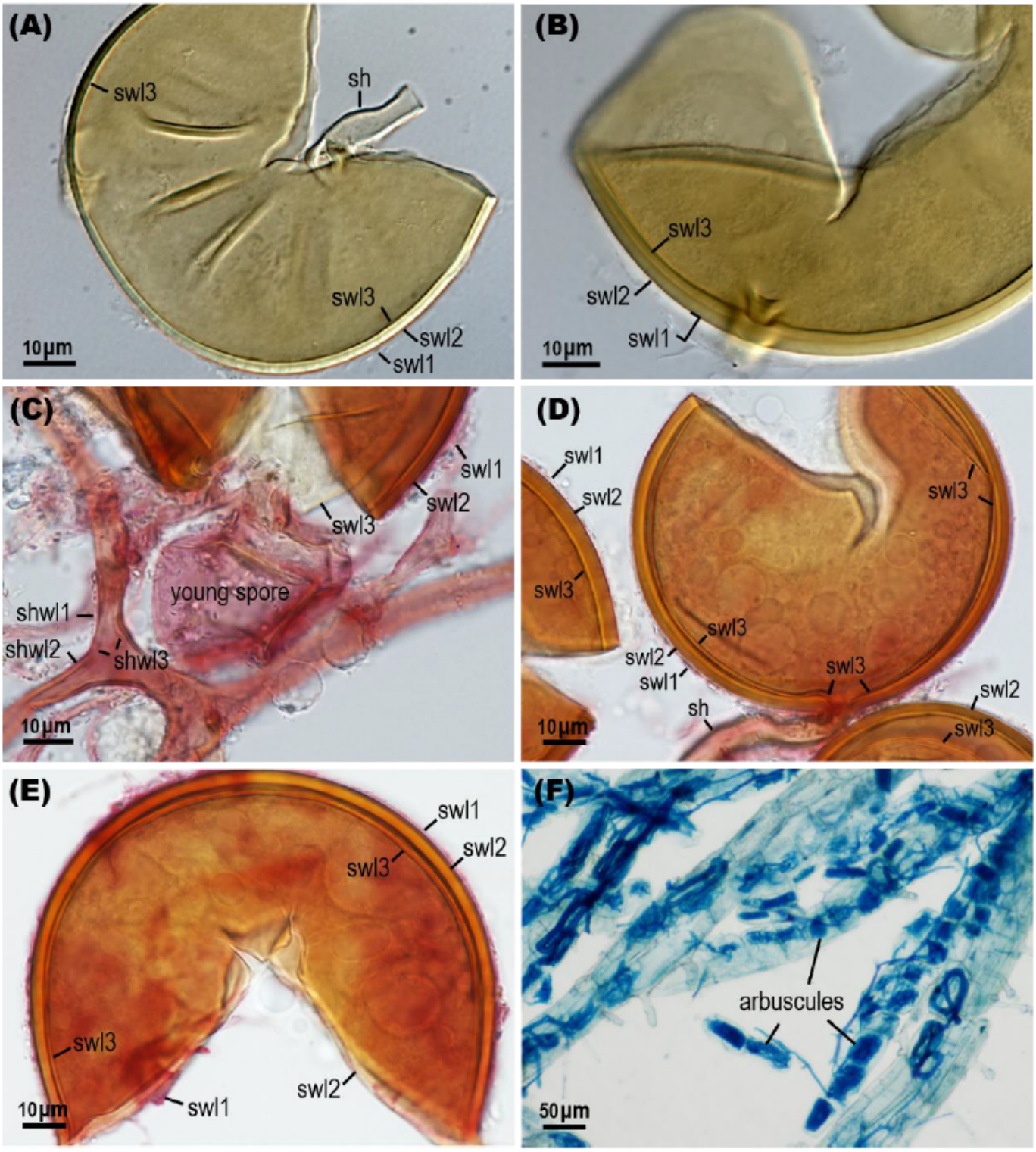

Figure 2

Morphological characteristics of Glomus chinense. (A) Broken spores in PVLG with three layers and subtending hyphae attached. (B) Details of spore wall layers and expansion of swl1 in PVLG mountant. (C) Hypha with three layers producing young and mature spores in the cluster condition. (D-E) Details of

three spore wall layers and Melzer's reactions of swl1 and swl2. (F) Arbuscules colonization in roots of Sorghum bicolor stained in 0.05% Trypan blue. sh, subtending hypha; swl, spore wall layer; shwl, subtending hyphal wall layer.

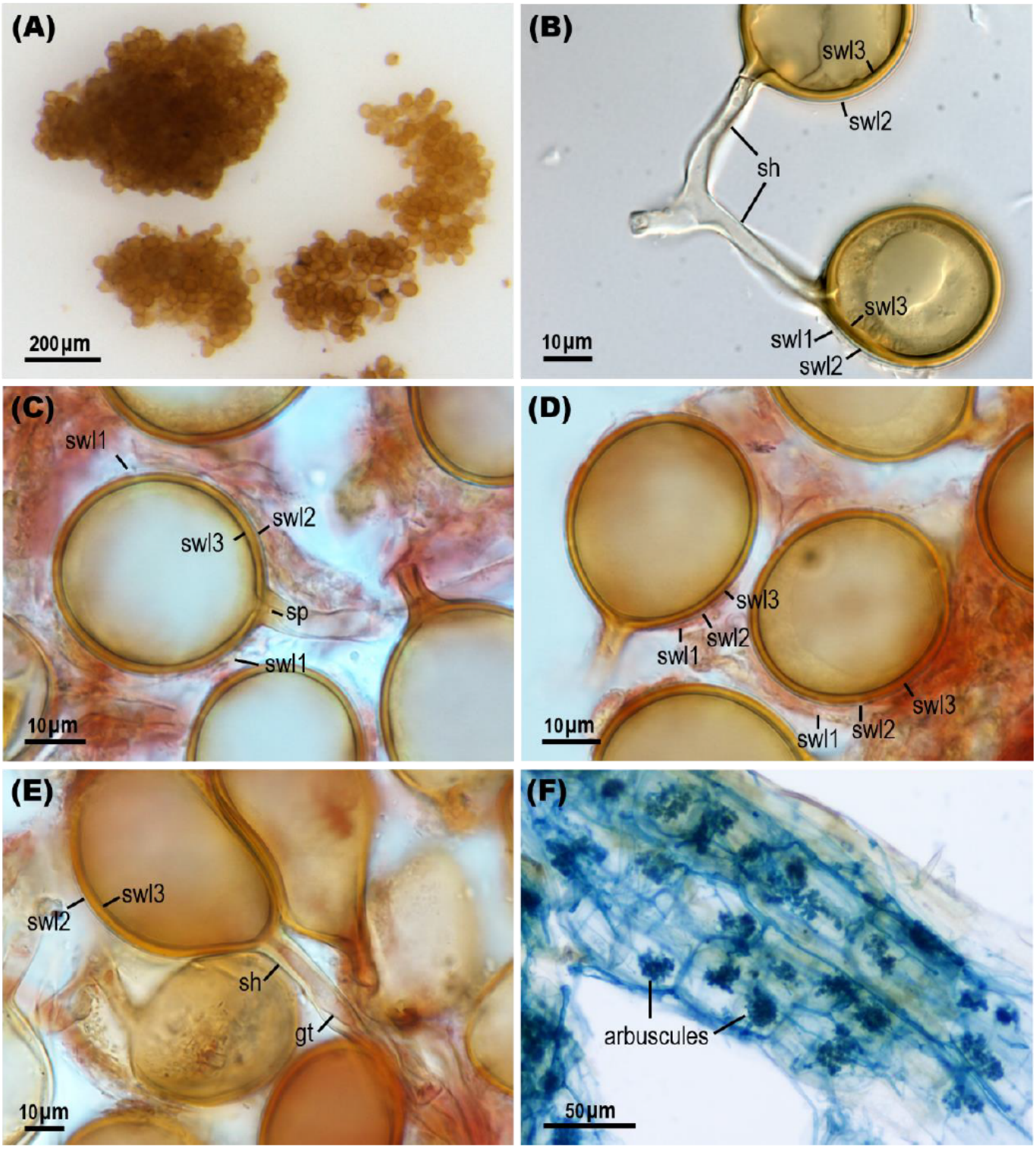

Figure 3

Morphological characteristics of Dominikia gansuensis. (A) General aspects of irregular glomerocarps produced in monospecific pot cultures on filter paper. (B) Detail of spore arrangement removed from

glomerocarps in PVLG. (C) Spore wall layers and detail of pore occluded by septum produced in the base of subtending hyphae. (D) Spores with three spore wall layers inside glomerospores organization and Melzer's reaction of swl1. Hyphae of peridium present strong Melzer's reaction and stain purple. (E) Detail of germinal tube produced at the spore base inside of subtending hyphae. (H) Detail of arbuscules produced in roots of Sorghum bicolor stained in 0.05% Trypan blue. sh, subtending hypha; swl, spore wall layer; sp, septum; gt, germination tube.

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