**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 that were established from soil-borne spores collected from high altitude (2800 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., forms spores singly or in loose clusters. The spores are orange-yellow to dark brown, globose, and (47–64)(–93) μm diam. *Dominikia gansuensis* sp. nov. produces glomerocarps with pale yellow to yellow-brown, globose, (20–47)(–86) μm diam spores. The 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.

**Keywords** Mycorrhiza · Glomeromycota · Qinghai-Tibet · Molecular phylogeny · SSU-ITS-LSU · Two new species

**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 host tolerance 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, 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 has 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 341 AM fungal species have been validly described (www.amfphylogeny.com; accessed on December 5, 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; Říčková et al. 2014), indicating that many undescribed taxa of AM fungi are waiting for discovery (Blaszkowski et al. 2021b; Kolaříková et al. 2021). At least five reasons cause 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 (Blaszkowski 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 (Blaszkowski 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; Říčková et al. 2014). Of the molecular loci used for identifying AM fungi, the partial nuclear rDNA region that covers partial small subunit (18S), the whole internal transcribed spacer (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, have been evidenced to be very effective (Krüger et al. 2009; Stockinger et al. 2010, 2014; Blaszkowski 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., Blaszkowski et al. 2019; Jobim et al. 2019; Oehl et al. 2019; Schüßler and Walker 2019; Chimal-Sánchez et al. 2020; Corazon-Guivín et al. 2020; Guillén et al. 2020a, b; Blaszkowski 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). The extreme environments on this plateau 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 5500 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 sitchensis* 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 platyphylly 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 clinoptilolite (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 4 months, during which all pots were watered...
with distilled water every 3 or 4 days and fertilized with Hoagland’s low-phosphorus solution every 2 weeks. All pots were dried for 2 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 colors of spores and spore wall components were described and coded according to the Pantone® refreshed CMYK Guide (Pantone, NJ, USA). The terminology of spore characters basically follows Oehl et al. (2019) and Błaszkowski et al. (2021a, b, c). 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. Voucher specimens of the two new species were deposited in the herbarium of Lanzhou University, China, and in the UFRN herbarium, Brazil.

**Molecular analysis**

Genomic DNA was extracted from single glomerospores/glomerocarps. Briefly, fresh spores were sonicated for 10 min and rinsed three times with distilled water. Single glomerospores/glomerocarps with healthy and intact appearance were transferred into a PCR tube with 10 μL of ddH2O and crushed with a sterilized pipette tip under the appearance were transferred into a PCR tube with 10 μL of ddH2O. Genomic DNA was extracted from single glomerospores/glomerocarps for each species. Briefly, fresh spores were sonicated for 10 min and rinsed three times with sterilized distilled water. Ten microliters of the first PCR product was used as template for the second PCR using the same cycling 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: 96 °C for 30 s, 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 of each target gene of each species 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**

To assess the putative novelty of the two AM fungal species, their DNA sequences were compared with the GenBank database using BLASTn (https://blast.ncbi.nlm.nih.gov). The percentages of 18S-ITS-28S or RPB1 sequence divergence within each new species or between the new species and their closest relatives were also calculated using BLASTn.

An 18S-ITS-28S alignment was obtained using five representative sequences from each of the 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 Entrophosporaceae (as a sister family of the Glomeraceae in the order Glomerales; please note that this family is synonymous with Claroideoglomeraceae; Wijayawardene et al. 2020; Säle et al. 2021) 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, which were coded with FastGap 1.2.
To determine the phylogenetic positions of the two undescribed species, Bayesian inference (BI) and maximum likelihood (ML) phylogenetic analyses of the 18S-ITS-28S alignment were performed using MrBayes 3.2 (Ronquist et al. 2012) and RAxML-NG 1.0.1 (Kozlov et al. 2019), respectively. In both analysis, GTR+G+I was used as substitution model for the DNA partition (Abadi et al. 2019), while F81 model was chosen for the indel partition according to the MrBayes manual. For the BI analysis, four Markov chains were run for one million generations (sampling frequency = 1000) with a burn-in set of 3000 sampled trees. The ML analysis was performed using a maximum likelihood/1000 bootstrapping run, in which the branch length estimation was performed using a maximum likelihood/1000 bootstrap ratio.

We also created an 18S-ITS-28S+RPB1 alignment by concatenating the 18S-ITS-28S alignment (same as above) and a RPB1 alignment. The latter one contained all species of the 18S-ITS-28S alignment that have been provided with RPB1 sequences. In detail, the RPB1 dataset included five representative sequences from each of the new species, 81 sequences representing 27 species and 15 genera in the Glomeraceae, and 5 sequences from 3 species in the Entrophosporaceae. The RPB1 sequences were aligned using the same program and settings as those for the 18S-ITS-28S dataset, and the indel strings were also provided.

For the phylogenetic analyses of the 18S-ITS-28S+RPB1 alignment, twelve partitions plus an indel partition were added to the six described partitions for the 18S-ITS-28S portion. The twelve partitions of RPB1 gene included the following: 4 partitions to the introns (a single partition was applied to each of the four introns); 6 partitions to the exons 3, 4, and 5 (for each exon, one partition was applied to the first two codon position and one to the third codon position); and 2 partitions to the exons 1 and 2 (a single partition to each exon because of the very short length). The models and parameters used for BI and ML analyses of 18S-ITS-28S+RPB1 alignment were the same as those for the 18S-ITS-28S dataset.

All phylogenetic analyses were performed on the CIPRES Science Gateway v.3.3 server (Miller et al. 2010), and the phylogenetic trees were visualized with 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 Dominikaia, 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, whereas the intraspecific similarities of RPB1 sequences of both species were above 99.5%.

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 differ 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 are 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, and the node linking it with the D. glomerocarpica clade also 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. Fig. 2A-F

Mycobank number: MB841240

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

Diagnosis. This fungus differs from its closest phylogenetic relatives, G. atlanticum and G. ibericum, in the spore wall structure and the phenotypic and biochemical properties of the spore wall layers, as well as in the sequences of the 18S-ITS-28S nuclear rDNA region and RPB1 gene.

Description. Spores arise blastically at the tips of sporogenous hyphae and are formed singly or occasionally in loose clusters in the soil. Spores: pale orange-yellow (0-45-100-6) to dark brown (37-76-92-51); globose to subglobose, (1.7–2.0(-2.5)-μm diam, rarely ovoid, 22-43 × 45–74 μm, with one subtending hypha (Fig. 2A). Spore wall: consisting 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, no reaction 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, consisting 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*,

![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 *Entrophosporaceae* (syn. *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 //).
Spiraea alpina, and *Potentilla sischanensis* in a shrubland on the 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 the mixture of PLVG and Melzer’s reagent were deposited. Holotype was deposited in the herbarium of Lanzhou University, China (accession number: LZU AMF_1708). Isotypes were deposited in the herbarium of Lanzhou University, China (LZU AMF_1709 to 1722) and in the UFRN herbarium, Brazil (Fungos 3388). The living culture of this fungus is currently maintained by the mycorrhizal research group of Lanzhou University.

**Distribution and habitat.** The shrubland nearby 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. Fig. 3A-F

*MycoBank* number: MB841241

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

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

**Description.** Spores are usually produced in hypogeous glomerocarps, rarely single 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 of 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: 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: 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 the rhizosphere soil sampled in a forest that was dominated by Betula platyphylla, Euonymus alatus, and some understory grasses. However, the plant species with which D. gansuensis formed symbiosis were 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 were deposited. Holotype was deposited in the herbarium of Lanzhou University, China (LZU_AMF_1727); isotypes were deposited in the herbarium of Lanzhou University, China (LZU_AMF_1728 to 1741) and in the UFRN herbarium, Brazil (Fungos 3389). The living culture of this fungus is currently maintained by the mycorrhizal research group of Lanzhou University.

Distribution and habitat. So far, *D. gansuensis* was physically found only in a forest site nearby 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 that several environmental sequences showed > 97% identity to the 18S-ITS-28S sequences of *D. gansuensis*. 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

Our analyses clearly proved that the two AM fungi found at high altitude of Tibetan Plateau are new species of the genera Glomus and Dominikia (Glomeraeaceae), namely, 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 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 reach or exceed the thresholds of species conspecificity that were widely accepted in phylogenetic studies of AM fungi (3% for 18S-ITS-28S sequences and 1% for RPB1 sequences; 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, and their spore wall consists of three layers (Fig. 2). But, spore wall layer 2 of G. chinense is laminate and thick, covers a thin spore wall layer 3, 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 two 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 walls are three-layered, but D. gansuensis glomerocarps are hypogeous and much smaller than the epi-geous 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 only the laminate layer 2 stains in Melzer’s reagent. In addition, the spore wall of D. glomerocarpica is 3–4-fold thicker than that of D. gansuensis.

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 Glomeraeaceae, regardless of 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., Blaszkowski 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 can increase the robustness of phylogenetic inference of AM fungi and can also reveal the phylogenetic relationships that are difficultly exposed by single 18S-ITS-28S locus (Blaszkowski et al. 2021c). Unfortunately, so far only ca. 25% of described AM fungal species have been provided with RPB1 sequences (Blaszkowski et al. 2021c). Thus, obtaining RPB1 sequences of more Glomeromycotan members is strongly encouraged.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11557-022-01799-9.

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Author contribution Yongjin 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, Wenzia Ma, and Yongjun Liu. The first draft of the manuscript was written by Fengxia Yu and Yongjun Liu and carefully edited by Janusz Blaszkowski. 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.

Declarations

Conflict of interest The authors declare no competing interests.

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