

RESEARCH ARTICLE

First Report of Arbuscular Mycorrhizal Fungi in Korea: *Acaulospora delicata*, *Dentiscutata colliculosa*, and *Racocetra alborosea*.

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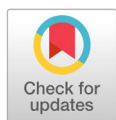
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ABSTRACT

Spores of arbuscular mycorrhizal fungi (phylum Glomeromycota) were isolated from rhizosphere soil collected in Korea. We identified the morphological characteristics of the spores and performed a phylogenetic analysis using the rDNA 18S, 5.8S, and 28S regions. To the best of our knowledge, we confirm the presence of three species of glomeromycotan fungi previously not reported in Korea, namely *Acaulospora delicata*, *Dentiscutata colliculosa*, and *Racocetra alborosea*. We described the morphological characteristics and results of phylogenetic analysis of these species.

Keywords: *Acaulospora delicata*, Arbuscular mycorrhizal fungi, *Dentiscutata colliculosa*, Glomeromycota, *Racocetra alborosea*



OPEN ACCESS

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2020 September, 48(3): 209-215
<https://doi.org/10.4489/KJM.20200022>

Received: August 26, 2020

Revised: September 11, 2020

Accepted: September 21, 2020

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INTRODUCTION

Fungi belonging to the phylum Glomeromycota participate in symbiotic relationships with the roots of terrestrial plants, called arbuscular mycorrhiza [1]. Arbuscular mycorrhizal fungi (AMF) produce asexual spores in the rhizosphere of plants [2], and identification of AMF is generally based on the morphological and molecular characteristics of their spores. Glomeromycota is known as a monophyletic clade [3], and more than 300 species of AMF have been recorded worldwide [4]. In Korea, approximately 100 species have been reported [5,6]; however, many of them were identified using only the morphological characteristics of uncultured spores. For a complete and accurate understanding of the biological characteristics of AMF, it is necessary to identify AMF using cultured, fresh spores. In this study, we report the morphological characteristics and phylogenetic analysis of three AMF species, previously undescribed in Korea, using spores isolated from trap cultures.

MATERIALS AND METHODS

The soil samples used in this study were collected from Hwasungun, and Sinangun in Korea. Soils were collected from rhizospheres at each sampling site. The soil samples were sealed within polyethylene bags containing the host plants and transported to the laboratory within 24 h of collection. For the proliferation of spores, the field-collected soils were mixed with autoclaved sands, and cultivated with a host plant, *Sorghum bicolor*, in a plastic pot for approximately 4 months in a greenhouse [6]. After cultivation, spores were extracted from soils by the wet sieving method [7]. The spores were collected under dissecting microscopes (SZX7, OLYMPUS, Japan) and mounted using polyvinyl alcohol-lactic acid-glycerol (PVLG). Morphological characteristics of AMF spores, such as spore size, color, shape, and surface ornamentation [8, 9], were determined using DIC microscopes (AXIO Imager, A1, Carl Zeiss, Germany).

Genomic DNA was extracted from the three morphologically unique types of the spores, and the 18S rDNA region was amplified by nested PCR. The first PCR was performed using the GeoA1/ART4 primer set with a 59°C annealing temperature. The second PCR utilized the GeoA2/Geo11 primer set with a 63°C annealing temperature [10]. The DNA barcoding region for Glomeromycota, including the small subunit of rDNA (SSU), internal transcribed spacer (ITS) region, and large subunit of rDNA (LSU) was also amplified [11]. Amplification was carried out using nested PCR; the SSUmAf-LSUmAr primer pair was used for the first PCR with an annealing temperature of 60°C [11]. The product of the first PCR was diluted (1/10) and used as the template DNA for the second round of PCR with the SSUmCf-LSUmBr primer pair and an annealing temperature of 63°C [11]. DNA sequencing was performed using the final PCR products (SolGent, Daejeon, Korea). The analyzed nucleotide sequences were identified by BLAST using the National Center for Biological Information (NCBI) database to confirm the best matching species. The neighbor-joining phylogenetic trees were constructed using the MEGA7 program [12]. The DNA sequences were deposited with the NCBI; GenBank accession numbers were obtained.

RESULTS AND DISCUSSION

In this study, we identified three Glomeromycota species which were not previously documented in Korea. The morphological characteristics and phylogenetic analysis were as follows.

***Acaulospora delicata* C. Walker, C.M. Pfeiff. & Bloss, Mytaxon 25 (2): 622 (1986), Fig. 1 A, B, & C**

This species was isolated from the trap cultures of *Lindera obtusiloba* rhizosphere soil. Spores were mostly globose, which developed from spherical sporiferous saccules. The spores (n = 200) were 69–92 (–124) µm in diameter. The spores were mainly pale yellow in color; rarely transparent white spores were also observed. The spore walls consisted of two layers (L1 and L2) of spore wall and two layers of germination wall (GW1 and GW2). The L1 layer had a thickness of approximately 0.5–0.8 µm, and the L2 layer consisted of a distinct laminate layer, approximately 2–3 µm in thickness. GW2 appeared red or dark pink after application of Melzer's reagent. Occasionally, a circular or elliptical cicatrix (approximately 6–9 µm

m in diameter), which indicates a trace of the saccule and the spore, was observed.

Specimen examined. Cheonbo-san mountain, Yangju-si, Gyeonggi-do, Korea, N37°46'02.7" E127°05'12.9", August 30, 2010, *Acaulospora delicata*, isolated from the rhizosphere of *Lindera obtusiloba*, strain KG10295, GenBank no. MN792874.

Notes. *A. delicata* spores are similar to those of *Acaulospora longula* in terms of shape and color; however, *A. longula* spores have a thin wall between the spore wall and germination wall and *A. delicata* spores do not exhibit this feature [13,14]. In this study, the isolated spores exhibited a four-layered spore wall; therefore, they were more consistent with the characteristics of *A. delicata* [13] than the five-layered spore wall of *A. longula* [14]. The DNA sequence of the 18S rDNA region showed 99.05% similarity to that of *A. delicata* AM713431.1, and the SSU-ITS-LSU DNA sequence showed 99.43% similarity to that of *A. delicata* AM713412.1. It was confirmed that the sequences obtained from this study were located within the same clade as *A. delicata* (Fig. 2).

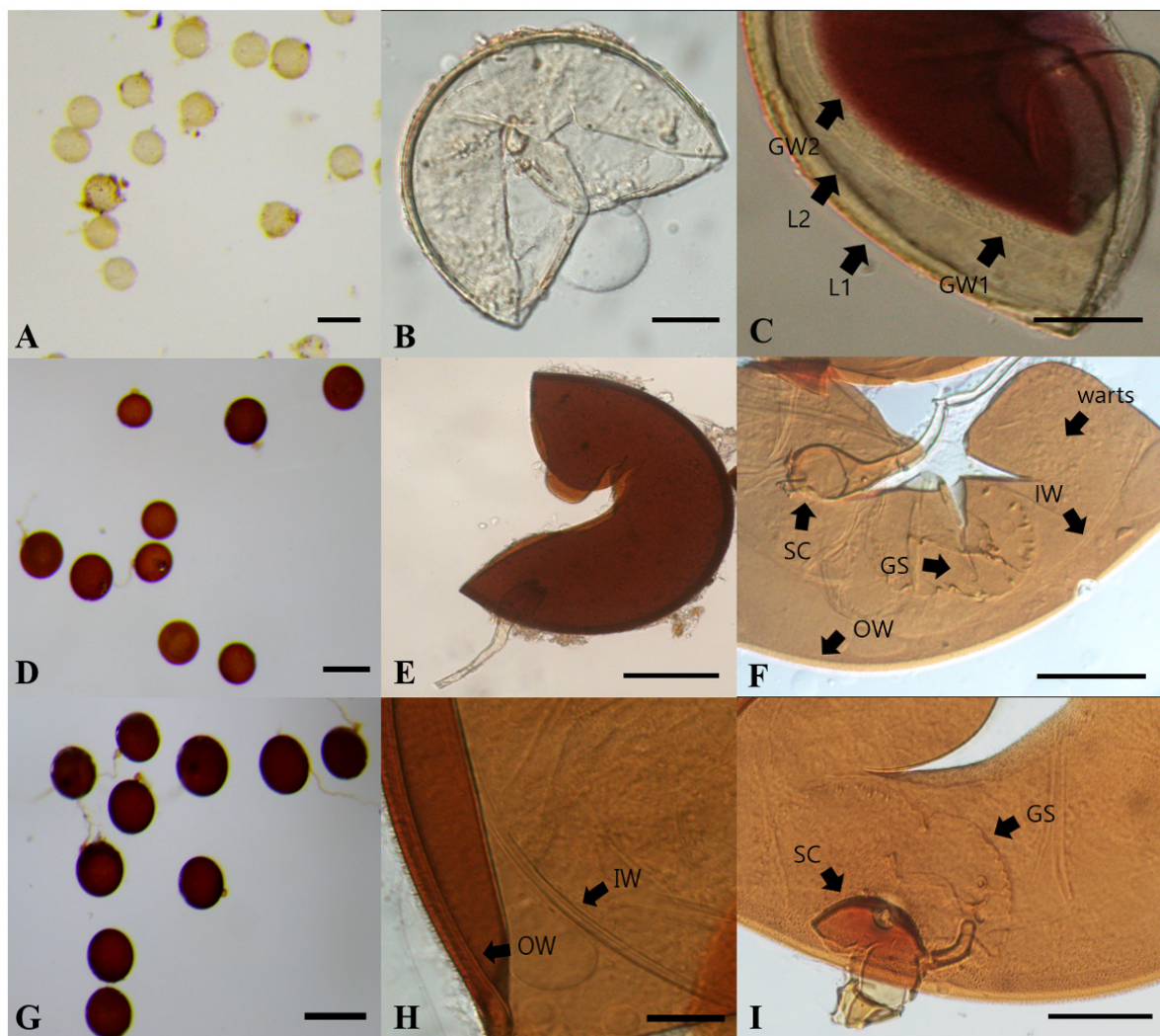


Fig. 1. Spores of *Acaulospora delicata* (A), in PVLG (B), in Melzer's reagent (C); Spores of *Dentiscutata colliculosa* (D), in PVLG (E, F); Spores of *Racocetra alborosea* (G), in PVLG (H, I) (scale bars: A-C = 10 μ m; D, G = 500 μ m; E, F = 100 μ m, H, I = 50 μ m). GW: germination wall, L1 & L2: spore wall layers, IW: inner wall, OW: outer wall, SC: sporogenous cell, GS: germination shield.

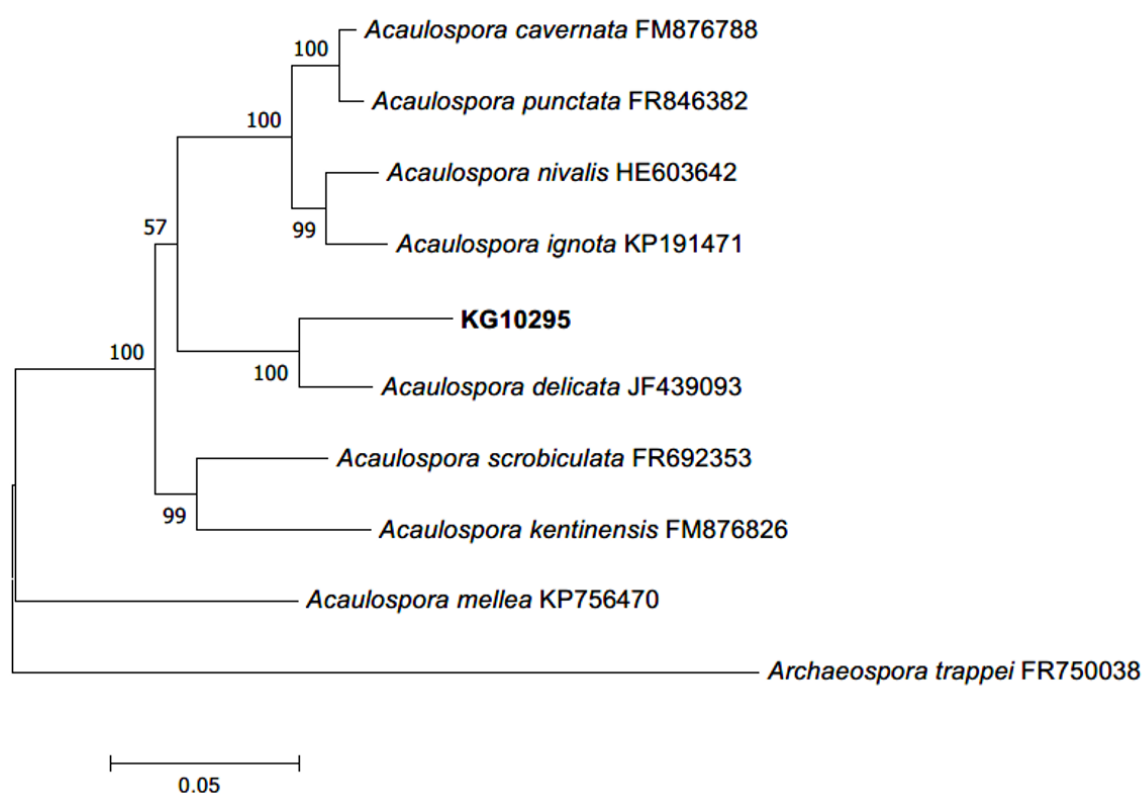


Fig. 2. Neighbor-joining phylogenetic tree based on SSU-ITS-LSU combined DNA sequences. *Archaeospora trappei* was used as an outgroup. Numbers on branches indicate bootstrap values (1,000 replicates). The fungal strain isolated in this study is in bold.

***Dentiscutata colliculosa* B.T. Goto & Oehl, Nova Hedwigia 90(3-4): 385 (2010), Fig. 1 D, E, & F**

This species was isolated from the trap cultures of *Zanthoxylum schinifolium* rhizosphere soil. Spores were formed solely in the soil and mostly exhibited globose characteristics. The spores ($n = 200$) were estimated to be about $(343\text{--}425\text{--}492)$ μm in diameter, and spores were reddish-brown to dark brown in color. The spore wall consisted of a thick outer spore wall (OW) and an inner wall (IW). A transparent, ovoid germination shield was observed in the IW, approximately $30\text{--}40$ μm wide. Sporogenous cells of approximately $50\text{--}60$ μm in size were observed between the basal part of the spore and subtending hyphae.

Specimen examined. Songgong-san mountain, Sinan-gun, Jeollanam-do, Korea, N34°51'06.2" E126°15'19.6", May 24, 2012, *Dentiscutata colliculosa*, isolated from the rhizosphere of *Zanthoxylum schinifolium*, strain JN12191, GenBank no. MT573391.

Notes. *D. colliculosa* was first reported by Goto *et al.* in 2010 [15], including an analysis of the entire 18S rDNA sequence. Although the external characteristics (color and spore size) were similar to those of other species belonging to *Dentiscutata*, *D. colliculosa* was distinguished by the dense distribution of

low-colliculate warts [15] inside the spore wall. However, warts inside the spore wall are often difficult to observe, and warts have also been observed on the spore wall of *D. heterogama* [16,17]. Therefore in this study, *D. colliculosa* was identified by sequence analysis of the entire 18S rDNA region [15]. The sequences from this study showed 99.19% similarity to that of *D. colliculosa* GQ376067.1 [15], and it was confirmed that the sequences were located within the same clade as *A. colliculosa* (Fig. 3).

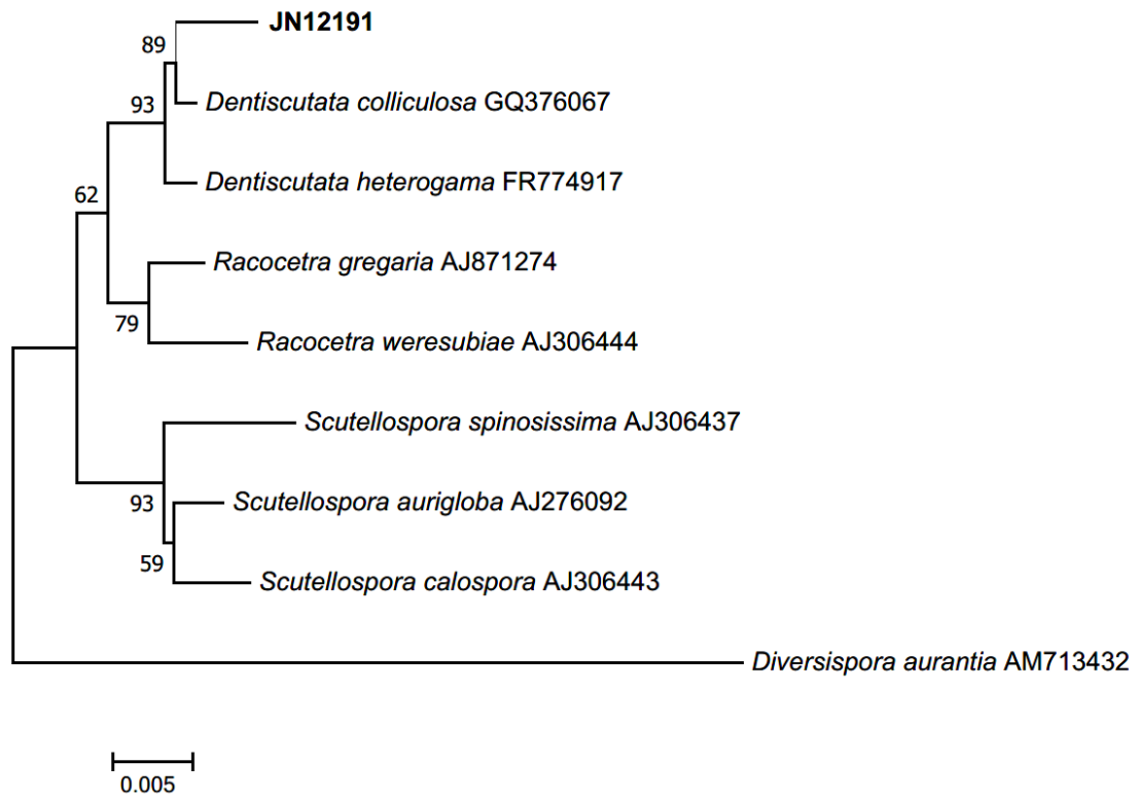


Fig. 3. Neighbor-joining phylogenetic tree based on a 18S rDNA entire sequence. *Diversispora aurantia* was used as an outgroup. Numbers on branches indicate bootstrap values (1,000 replicates). The fungal strain isolated in this study is in bold.

***Racocetra alborosea* (Ferrer & R.A. Herrera) Oehl, F.A. Souza & Sieverd., Mycotaxon 106: 336 (2009), Fig. 1 G, H, & I**

This species was isolated from the trap cultures of *Myrica cerifera* rhizosphere soil. The spores formed solely in the soil and were mostly globose. The spores ($n = 200$) were approximately 343-421 (-516) μm in diameter. The spore color was usually red or reddish-brown. The germination shield was elliptical and had irregular wavy edges. The spore wall comprised a triple-thick, laminate-layered outer wall (OW) and an inner wall (IW) composed of a triple thin hyaline layer. Between OW and IW, there were many small wart-like ornaments. The sporogenous cell was elongated in an obovate manner, and it was approximately 15-20 μm in size.

Specimen examined. Mohu-san mountain, Hwasun-gun, Jeollanam-do, Korea, N35°01'34.8", E127°09'53.4", May 18, 2012, *Racocetra alborosea*, isolated from the rhizosphere of *Myrica cerifera*, strain JN12159, GenBank No. MT573390.

Notes. The morphological characteristic that distinguishes *R. alborosea* from other species belonging to the *Racocetra* taxon includes small warts distributed inside the spore wall [18]. However, it was not easy to identify this species using morphological characteristics. The DNA sequence of the 18S rDNA region showed 99.15% similarity to that of *R. alborosea* JQ080259.1, and it was confirmed that the sequences were located within the same clade as *R. alborosea* (Fig. 4).

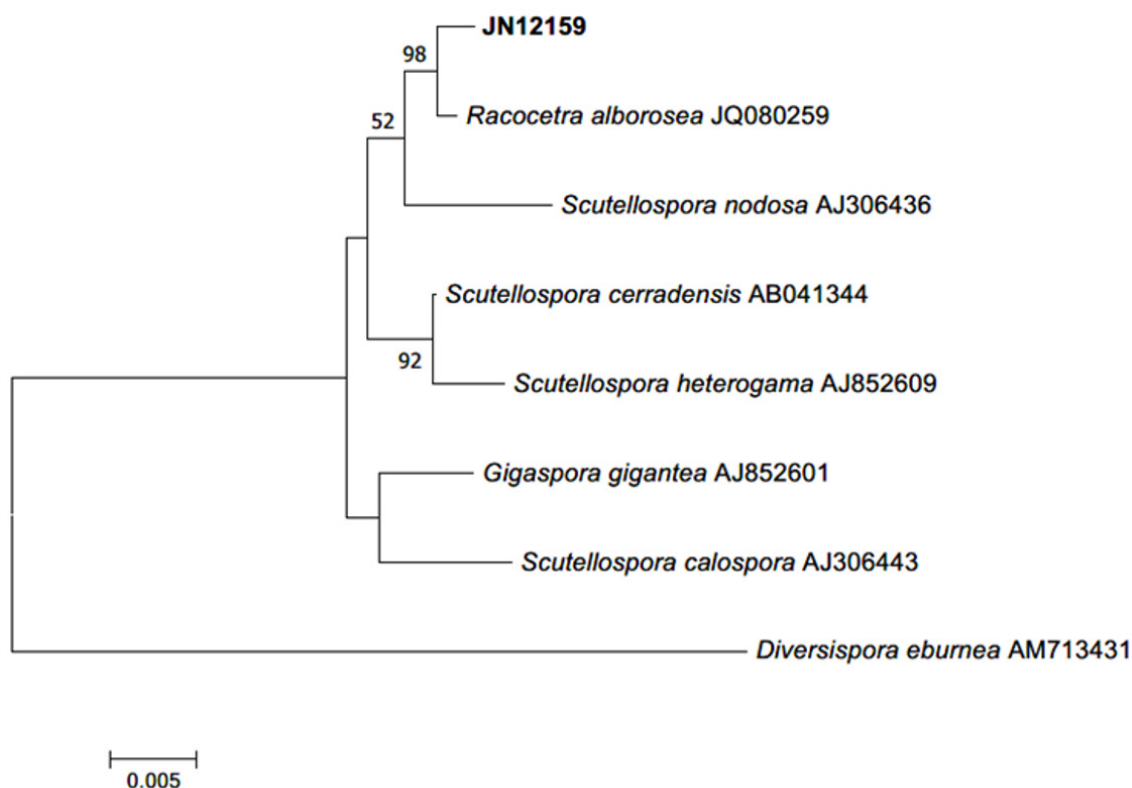


Fig. 4. Neighbor-joining phylogenetic tree based on a 18S rDNA entire sequence. *Diversispora eburnea* was used as an outgroup. Numbers on branches indicate bootstrap values (1,000 replicates). The fungal strain isolated in this study is in bold.

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