Four New Records of Ascomycete Species from Korea

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ABSTRACT
While evaluating fungal diversity in freshwater, grasshopper feces, and soil collected at Dokdo Island in Korea, four fungal strains designated CNUFC-DDS14-1, CNUFC-GHD05-1, CNUFC-DDS47-1, and CNUFC-NDR5-2 were isolated. Based on combination studies using phylogenies and morphological characteristics, the isolates were confirmed as Ascodesmis sphaerospora, Chaetomella raphigera, Gibellulopsis nigrescens, and Myrmecridium schulzeri, respectively. This is the first records of these four species from Korea.

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Ascomycetes; fecal; freshwater; fungal diversity; soil

1. Introduction

Fungi represent an integral part of the biomass of any natural environment including soils. In soils, they act as agents governing soil carbon cycling, plant nutrition, and pathology. Many fungal species also adapt to invade, colonize, and ensure decomposition of keratinous debris of other organisms [1] and mineralization of herbivore feces [2]. The distribution of the fungal community is related to different physiological, ecological, and bio-geographical features, closely linked to the surrounding terrestrial habitat [3–5].

Dokdo Island is characterized by high soil salinity, drought, high winds, low amounts of organic matter, high concentrations of uric acid in the soil, and steep inclines [6]. The weather usually features snow in the winter, with the climate influenced by warm ocean currents. Organisms living there have adapted to the environmental conditions by changing their genetic composition and enzymatic systems, and thus constitute a resource with great biotechnological potential. However, fungi occurring on this island remain poorly studied. Thus it is important to investigate the fungal diversity of this isolated area to understand their natural ecology. Reports have described a variety of bacterial and fungal species on Dokdo Island belonging to the genera Absidia, Alternaria, Aspergillus, Cladosporium, Clonostachys, Fusarium, Diaporthe, Metahizium, Mortierella, Mucor, Paeilomyces, Paraphoma, Penicillium, Plectosphaerella, and Stemphylium [7–11]. However, comparatively few species of fungi have been described [8–10].

Freshwater nourishes diverse habitats for fungi, such as fallen leaves, plant litter, decaying wood, aquatic plants and insects, and soils. Little information is available for fungi belonging to Basidiomycetes and Zygomycetes in comparison to freshwater Ascomycetes, which comprise approximately 622 species and 170 genera; more than 531 Hyphomycetes species (55 genera), and 183 species of Trichomycetes (3 orders) [12]. Various studies related to fungal diversity from diverse habitats have been carried out by Korean mycologists. In comparison to the terrestrial environment, knowledge is scant in Korea regarding fungi belonging to freshwater habitats, particularly Ascomycetes. Freshwater fungi, especially Ascomycetes, are important in freshwater ecosystems as they provide nutrients for other aquatic microorganisms by decomposing complex organic compounds and because of enzymatic activity including that of cellulase, xylanase, and ligninase that degrades wood [13]. Moreover, they are able to produce various compounds that act against pathogenic bacteria, fungi, and nematodes [12].

Fungi in the fecal environment help to biodegrade organic materials and return the nutrients to the environment for reuse [14]. Very little information has been published concerning the diversity of fungi in insect feces in comparison to that in...
Table 1. Taxa, collection numbers, sequences, and GenBank accession numbers used in this study.

| Taxon name         | Collection No. (Isolate No.) | GenBank accession No. |
|--------------------|------------------------------|-----------------------|
| **ITS LSU SSU**    |                              |                       |
| Acremonium antarcticum | CBS 987.87                  | JX158422              | JX158444 |
| A. furcatum        | CBS 122.42                   | NR_145349             | EF543831 |
| A. stromaticum     | CBS 863.73                   | DQ285969              | HQ232143 |
| Ascocereus nigricans | CBS 389.68                  | DQ168335              |          |
| A. nigricans       | CBS 428.91                   | KO12665               |          |
| A. pfaersdorpia    | AFTOL-ID 920                 | FJ176858              | FJ176804 |
| A. pfaersdorpia    | RK 93.55                    | US3372                |          |
| **A. pfaersdorpia**| **CNFUC-DDS14-1**            | **MH542151**          | **MH542149** |
| **A. pfaersdorpia**| **CNFUC-DDS14-2**            | **MH542152**          | **MH542150** |
| Caloscypha fulgens | CBS 218.62                   | KO12668               |          |
| C. tropica         | CBS 133.33                   | KO12669               |          |
| Cephalosporium sordum var. fuscum | CBS 389.68 | DQ168335           |          |
| C. oblonga         | BPI 843552                   | AY487079              | AY487083 |
| C. phragmitis      | CBS 101221                   | EF543848              | EF543840 |
| C. nigricans       | CBS 577.50                   | EF543856              |          |
| C. nigricans       | YIMPH30017                   | KP203818              |          |
| C. nigricans       | DADM226890                   | GU180648              |          |
| C. nigricans       | CNUFC-DDS547-1               | MH540113              | MH542153 |
| C. nigricans       | CNUFC-DDS547-2               | MH540114              | MH542154 |
| C. pisces          | CBS 892.70                   | DQ825985              | EF543835 |
| Gloeobacter biotii | CBS109240                    | DQ825980              | EF543842 |
| Gloeobiella cingulata | ARS788                      | DQ82600               |          |
| G. cirrhata        | HKCC 9036                    | AY03820               |          |
| Hydnophora tubusae | ITB                         | KS-94-005             | DQ168338 |
| Lasiobsolus ciliatus | KS-94-05                   | DQ168338              |          |
| L. cuniculi        | C F-54526                    | DQ167411              |          |
| Mycrocystidum banksiae | CPC 19852 (T)            | JX069871              | JX069855 |
| M. flexuosum       | CBS 398.76 (T)               | EU041768              | EU041825 |
| M. fluvaiae        | CNFUC-YRF61-1 (T)            | KS839678              | KS839677 |
| M. hialae          | CBS 141017 (T)               | KP714695              | KU302612 |
| M. indis           | CPC 25084 (T)                | KR476744              | KR476777 |
| M. obovoidum       | HGUP 0314 (T)                | KC136140              | KC136139 |
| M. phragmitis      | CBS 131311 (T)               | JQ444205              | JQ44434  |
| M. schulzera       | NRRL 62975                   | KM06332               |          |
| M. schulzera       | CBS 100.54                   | EU041769              | EU041826 |
| M. schulzera       | CBS 134.68                   | EU041770              | EU041827 |
| M. schulzera       | CBS 156.63                   | EU041771              | EU041828 |
| M. schulzera       | CBS 175.74                   | EU041775              | EU041832 |
| M. schulzera       | CBS 642.76                   | EU041777              | EU041834 |
| M. schulzera       | CNFUC-NDRS-2                 | MH540115              | MH542155 |
| M. schulzera       | CNFUC-NDRS-3                 | MH540116              | MH542156 |
| M. sportii         | CPC 24953 (T)                | KR611884              | KR611902 |
| M. thallandicum    | CPC 21604 (T)                | KF777169              | KF777222 |
| Oidiodon leporina  | H6000348                     | KF715758              |          |
| Peziza succosa     | TA-134                      | JN588568              |          |
| Plectosphaerella alismatis | CBS113362 | JF780523              | JF780521 |
| P. acerinum        | BPI 843554                   | AY487088              | AY487092 |
| P. aerinum         | BPI 843555                   | AY487091              | AY487089 |
| P. concavum        | BPI 1107275                  | AY487094              | AY487095 |
| P. concavum        | BPI 1107274                  | AY487097              | AY487098 |
| Pyronema domestica | CBS 666.88                   | DQ47813               |          |
| Sarcocoma globosa | KH-07.04                    | FJ499393              |          |
| Seridium banksiae  | CBS 133108                   | JQ444242              | JQ444442 |
| Smardae amethystina | KH-97-132                  | AF335176              |          |
| Sphaeroporella brunnea | KNE 2116                   | U38587                |          |
| Tarzetta catinus   | UME 29731                    | U38587                |          |
| Trichophora hybrida | VL283                      | VL283                 | JT477811 |
| Wilcoxia mikolae   | ML-18                       | ML-18                 | JT477811 |

Bold letters indicate isolates and accession numbers determined in our study. BPI: U.S. National Fungus Collection; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CNFUC: Chonnam National University Fungal Collection, Gwangju, South Korea; CPC: Culture collection of P.W. Crous; DAOM: Canadian Collection of Fungal cultures, at the Ottawa Research and Development Centre Agriculture and Agri-Food, Ottawa, Canada; MAFF: National Institute of Agrobiological Sciences, Ibaraki, Japan; NRRL: ARS Culture Collection, Peoria, Illinois, USA. T: ex-type cultures.
various animal dung substrates [15]. Melanoplus sanguinipes was isolated from grasshopper gut, but no detailed information was provided on the fungal communities [16]. Among the fungal isolates from fecal samples in Korea, five species (two new species and three new records) were reported from rat feces and two (new records) from grasshopper. Absidia stercoraria, Mucor stercorarius, Absidia glauca, Paecilomyces variotii, and Cephalophora trocica were reported from rat feces [10,17–20]. Cunninghamella echinulata and Albicinumibus terrestris were reported from grasshopper feces [19,21]. Korean contributions to the occurrence of coprophilous fungi are still rare.

During an inventory of fungal species belonging to the classes Leotiomycetes, Pezizomycetes, and Sordariomycetes, four new records from freshwater, fecal, and soil samples were identified. The objective of the present study was to perform morphological and molecular analyses to characterize four undescribed species in Korea: Ascodesmis sphaerospora, Chaetomella raphigera, Gibellulopsis nigrescens, and Myrmecridium schulzeri.

2. Materials and methods

2.1. Isolation of fungal strains

Freshwater samples were collected from a branch stream of the Nakdong river located in Gyeongsangbuk-do, Korea. Grasshoppers were collected at the CNU Arboretum located in Chonnam National University, Gwangju, Korea. Soil samples were collected from Dokdo Island in July 2014. In this study, the isolation of fungi from freshwater, grasshoppers, and soil samples was performed as described previously [10,19]. Pure isolates were maintained in potato dextrose agar (PDA) slant tubes and stored in 20% glycerol at −80°C at the Chonnam National University Fungal Collection.
2.2. Morphological studies

To obtain samples for microscopic examination, CNUFC-DDS14-1, CNUFC-DDS47-1, and CNUFC-GHD05-1 were cultured on PDA, corn meal agar (CMA: 20 g cornmeal, 20 g agar, 1 L distilled water), and malt extract agar (MEA: 20 g malt extract, 20 g agar, 1 L distilled water). Plates were incubated at 10, 20, 25, 30, and 35°C in the dark for 7 days. Samples were mounted in lactophenol solution (Junsei Chemical Co. Ltd., Tokyo, Japan) and observed using a BX51 microscope equipped with DIC optics (Olympus, Tokyo, Japan).

2.3. DNA extraction, PCR, and sequencing

Genomic DNA was extracted directly from the mycelia of fungal isolates using the Solg TM Genomic DNA prep kit (Solgent Co. Ltd., Daejeon, South Korea). The rDNA ITS1-5.8S-ITS2 region, small subunit (SSU) 18S, and large subunit (LSU) 28S ribosomal DNA were amplified with the primer pairs ITS1/ITS4 [22], NS1/NS4 [23], and LROR/LR5F [24]. The PCR amplification mixture (total volume, 20 μL) contained 10 ng of fungal DNA template, 5 pmol/μL of each primer, and Accupower PCR Premix (Taq DNA polymerase, dNTPs, buffer, and a tracking dye; Bioneer Corp., Daejeon, Korea). Purification of the PCR products was carried out using the Accuprep PCR Purification Kit (Bioneer Corp.) according to the manufacturer’s instructions. DNA sequencing was performed on an ABI 3700 Automated DNA sequencer (Applied Biosystems Inc., Foster City, CA).

2.4. Phylogenetic analysis

Fungal sequences obtained from the GenBank database (Table 1) were aligned using Clustal_X v.1.83 [25] and edited with Bioedit v.5.0.9.1 [26]. Phylogenetic analyses were performed using MEGA 6 software [27], and maximum likelihood (ML) was constructed by Kimura’s two-parameter correction method. The sequences of Acremonium alcalophilum, Caloscypha fulgens, Glomerella cingulata, Pezzia succosa, Seridium banksiae, and Smardaec amethystina were used as outgroups. The reliability of internal branches was assessed using the p-distance substitution model with 1000 bootstrap replications.
3. Results

3.1. Phylogenetic analysis

The results constructed by ML analyses of the four isolates are shown in Figures 1–4. A Basic Local Alignment Search Tool (BLAST) search of ITS sequences via the NCBI database indicated that the isolates CNUFC-GHD05-1, CNUFC-DDS47-1, and CNUFC-NDR5-2 most closely resembled "Chaetomella raphigera" (GenBank accession no. AY487076), "Gibellulopsis nigrescens" (GenBank accession no. AY487077), "G. nigrescens" (GenBank accession no. GU180648), and "Myrmecridium schulzeri" (GenBank accession no. EU041826) with similarities of 100% (372/372 bp), 99.6% (854/857 bp), 99.8% (849/851 bp), and 99.9% (795/796 bp), respectively. In addition, the BLASTn search results for the CNUFC-DDS14-1 18S rDNA showed 99.8% (1021/1031 bp) homology with "A. sphaerospora" (GenBank accession no. U53372).

3.2. Taxonomy

3.2.1. Taxonomy of CNUFC-DDS14-1

"Ascodesmis sphaerospora" W. Obrist, Canadian Journal of Botany 39:948 (1961) (Table 2; Figure 5).

Description: The strain grew rapidly at 25°C on PDA, filling the petri dish after 4–5 days of incubation. The initial colony color was white and later turned to grayish white. Apothecia were superficial, sessile, and obconical. Asci were clavate, oblong, or ovoid, and measured 40–62 × 18–24 μm. Each ascus contained 4–8

Figure 3. Phylogenetic tree based on ML analysis of internal transcribed rDNA and 28S sequences for "Gibellulopsis nigrescens" CNUFC-DDS47-1 and G. nigrescens CNUFC-DDS47-2. The sequence of "Acremonium alcalophilum" was used as an outgroup. Bootstrap support values of ≥50% are indicated at the nodes. The bar indicates the number of substitutions per position.
Figure 4. Phylogenetic tree based on ML analysis of internal transcribed rDNA and 28S sequences for *Myrmecridium schulzeri* CNUFC-NDR5-2 and *M. schulzeri* CNUFC-NDR5-3. The sequence of *Seiridium banksiae* was used as an outgroup. Bootstrap support values of $\geq 50\%$ are indicated at the nodes. The bar indicates the number of substitutions per position.

Table 2. Morphological characteristics of CNUFC-DDS14-1 compared to those of the reference *Ascodesmis sphaerospora* strain.

| Characteristics      | Present isolate                                      | *Ascodesmis sphaerospora* |
|----------------------|-------------------------------------------------------|---------------------------|
| Colony               | Rapidly-growing, white at first becoming grayish     | NA                        |
|                      | white in age.                                        |                           |
| Apothecia            | Superficial, sessile, obconical                       | Superficial, hemisphaerical to subglobe  |
| Asci                 | Clavate, oblong or ovoid, and measured                | Clavate, oblong or ovoid, and measured |
|                      | 40–62 × 18–24 μm, four to eight ascospores           | 55–80 × 24–33 μm, three to eight ascospores |
| Ascospores           | Subglobose to ellipsoid, and measured                 | Spherical to ellipsoid, 11–15 × 10.5–14 μm |

NA: Not available.

*From the description by Obrist [29].
ascospores. Ascospores were one-celled, subglobose to ellipsoid, hyaline when young, becoming brown at maturity, and covered with dark brown markings in the form of spines, ridges, or reticulations, and measured 10–12 × 9–11 μm.

3.2.2. Taxonomy of CNUFC-GHD05-1

*Chaetomella raphigera* Swift, Mycologia 22:165 (1930) (Table 3; Figure 6).

= *Volutellospora raphigera* (Swift) Thirum. & P.N. Mathur, Sydowia 18 (1-6):38 (1965).

= *Chaetomella terricola* P.Rama Rao, Mycopathologia et Mycologia Applicata 19 (3):255 (1963).

**Description:** Colonies of the strain grew slowly on PDA, reaching 20–22 mm in diameter at 25°C after 7 days of incubation. The initial colony color was white and later turned to cinnamon. Pycnidia were elongated, reniform, pale to dark reddish brown, and measured 72.5–148.5 × 46.5–88.5 μm. Setae were pale to dark brown, mostly 2 septate, and measured 21.3–47.8 × 2.0–3.5 μm. Conidiophores were cylindrical, branched, 26.0–100 × 1.0–2.3 μm. Conidia were ellipsoid, and measured 4.8–7.2 × 1.8–2.6 μm.

Table 3. Morphological characteristics of CNUFC-GHD05-1 compared to those of the reference *Chaetomella raphigera* strain.

| Characteristics | Present isolate | Chaetomella raphigera* |
|-----------------|-----------------|------------------------|
| Colony          | Slowly-growing, white at first, becoming cinnamon in age | Slowly-growing, cinnamon to dark brick |
| Pycnidia        | Elongated, reniform, pale to dark reddish brown, and measured 72.5–148.5 × 46.5–88.5 μm | Elongated, reniform, pale to dark reddish brown, and measured 200–320 × 140–200 μm |
| Setae           | Pale to dark brown, mostly 2 septate, and measured 21.3–47.8 × 2.0–3.5 μm | Pale to dark brown, 0–2 septate, and measured 40.0–90.0 × 2.0–5.0 μm |
| Conidiophores   | Cylindrical, branched, 26.0–100 × 1.0–2.3 μm | Filiform, cylindrical, branched, up to 85 × 1–2 μm ellipsoid, and measured 3.2–7.5 × 2.0–3.0 μm |
| Conidia         | Ellipsoid, and measured 4.8–7.2 × 1.8–2.6 μm | |

*From the description by Rossman et al. [39].

3.2.3. Taxonomy of CNUFC-DDS47-1

*Gibellulopsis nigrescens* R Zare, Gams W, Summerb, Nova Hedwigia 85:477 (2007) (Table 4; Figure 7).

= *Verticillium nigrescens* Pethybr., Transactions of the British Mycological Society 6:117 (1919).

= *Cephalosporium serrae* Maffei, Atti dell'Istituto Botanico della Università e Laboratorio Crittogamico di Pavia 1:196 (1929).
Verticillium amaranti Verona & Ceccar. (1935) = Verticillium amaranthi Verona & Ceccar., Phytopathol. Z.: 379 (1935).

Verticillium dahliae f. zonatum J.F.H. Beyma, Antonie van Leeuwenhoek 6: 42 (1940).

**Description:** Colonies of the strain grew slowly on PDA, reaching 25–27 mm in diameter at 20°C after 10 days of incubation. The color of the colonies on PDA was whitish with cotton-like at the center. Conidiophores arise from vegetative hypha measuring 55–100 × 1.5–2.5 μm. Chlamydospores were formed as single or in short chains, and measured 4–6 × 2.5–5 μm. Conidia were smooth-walled and elongate-ellipsoidal, and measured 4.0–6.0 × 1.5–2.5 μm. Colony on MEA was light brown with 24–30 mm in diameter.

### Table 4. Morphological characteristics of CNUFC-DDS47-1 compared to those of the reference Gibellulopsis nigrescens strain.

| Characteristics                     | Present isolate                                                                 | Gibellulopsis nigrescens*                                                                 |
|-------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| Conidiophores                       | Arising from vegetative hypha, 55–100 μm long and 1.5–2.5 μm wide              | Arising from substratum or aerial hyphae, 50–100 μm long and 1.5–2.5 μm wide in the lower part |
| Chlamydospores                      | Formed as single or in short chains and measured 4–6 × 2.5–5 μm               | Grey-brown, smooth-walled, single or in short chains, 4.5–6(-7.5) × 2.5–4(-5) μm          |
| Conidia                             | Smooth-walled, elongate-ellipsoidal, 4.0–6.0 × 1.5–2.5 μm                      | Hyaline, smooth-walled, elongate-ellipsoidal, 4–5.5 (−7) × 1.0–2(−2.5) μm                 |
| Colonies grown on PDA at 20°C       | Diameter: 25–27 mm; whitish, cotton-like at the centre, finely floccose        | Diameter: 20–30 mm; whitish, finely floccose                                             |
| Colony grown on MEA at 20°C         | Diameter: 24–30 mm; light brown                                                | NA                                                                                       |

NA: Not available.

*From the description by Zare et al. [45] and Wu et al. [48].

3.2.4. Taxonomy of CNUFC-NDR5-2

Myrmecridium schulzeri (Sacc.) Arzanlou, W. Gams & Crous, Studies in Mycology 58:84 (2007) (Table 5; Figure 8).

≡Psilobotrys schulzeri Sacc. (1884).
≡Psilobotrys schulzeri Sacc., Hedwigia 23: 126 (1884).
≡Chloridium schulzeri (Sacc.) Sacc., Sylloge Fungorum 4: 322 (1886).
Rhinocladiella schulzeri (Sacc.) Matsush., Icones Microfungorum a Matsushima lectorum: 124 (1975).
Ramichloridium schulzeri (Sacc.) de Hoog, Studies in Mycology 15: 64 (1977).
¼ Acrotheca acuta Grove, Journal of Botany, British and Foreign 54: 222 (1916).
¼ Rhinotrichum multisporum Doguet, Revue Mycol., Paris: 78 (1952).

**Description:** Colonies of the strain grew slowly on MEA, reaching 29 mm diameter at 25°C after 15 days of incubation. The colony color was pale orange. The colony reverse was also pale orange. Conidiophores were straight, almost unbranched, sometimes branched, reddish brown, septate, 2.5–3.5 μm in width, and variable in length. Conidiogenous cells were cylindrical and forming a rachis with scattered pimple-shaped denticles. Conidia were ellipsoid, obovoid, fusiform, 2.5–3.5 × 5.5–7.5 μm. On OA, the colonies grew more rapidly than on MEA and PDA, but abundant sporulation when grown on MEA.

**Discussion**

Here, we discussed morphological characteristics and the phylogeny of Ascodesmis sphaerospora, Chaetomella raphigera, Gibellulopsis nigrescens, and...
Myrmecridium schulzeri and compared these aspects to the most closely related species.

The genus *Ascodesmis* belonging to the class Pezizomycetes, order Pezizales, family Ascodesmidaceae, was first described by Van Tiegham [28] with *A. nigricans* as the type species. This genus is of operculate discomycetes representing a primitive form of ascomycetes with no excipulum. According to Index Fungorum (www.indexfungorum.org), 13 species were assigned to this genus. There were no *Ascodesmis* species reported from Korea until this study. *A. sphaerospora* was first isolated from dung samples of Brazilian animals [29]. This species is distinguished by its globose or subglobose ascospores with a reticulate ornamentation. *A. sphaerospora* shows a considerable variation in ascospores number, size, and shape growing on dung (spores are spherical) or on artificial agar media (spores tend to be more elliptical). These species are characterized by having relatively long spines [29]. Comparing the morphology of the CNUFC-DDS14-1 isolate with previous descriptions by Obrist [29], the present isolate was most similar to those of *A. sphaerospora*. In our molecular analyses, *A. sphaerospora* CNUFC-DDS14-1 formed a well-supported clade (Figure 1).

There is relatively little literature examining the molecular characteristics of *Ascodesmis* species in comparison to morphological identifications [30,31]. *A. sphaerospora* is reported to be isolated from the dung of jaguar, lion, ocelot, tiger, dog, elk, toad, rabbit, pig, and giraffe [29,32–34]. This is the first isolation of *A. sphaerospora* from a soil sample. *A. sphaerospora* produces antifungal and antibacterial metabolite arugosin F [35].

The genus *Chaetomella* belonging to the class Leotiomycetes, order Helotiales, family Chaetomellaceae, was established by Fuckel in 1869, including *C. atra* and *C. oblonga*, based on the production of pycnidium fruiting body [36]. The genus *Chaetomella* was designated considering its resemblance to genus *Chaetomium*, showing an external appearance of the fruiting bodies with characteristic appendages. Until now, 25 species of *Chaetomella* have been described according to Index Fungorum. *Chaetomella* spp. can be isolated from soil and plants [37]. *C. raphigera* is reported to cause leaf spot disease to *Cuphea* spp., *Rosa chinensis*, blueberry, and pomegranate [38–40]. Phylogenetic analysis based on ITS and LSU sequences showed that our strains CNUFC-GHD05-1 and CNUFC-GHD05-2 grouped together with *C. raphigera* (Figure 2). In addition, *C. raphigera* CNUFC-GHD05-1 fits well with the description provided by
Rossman et al. [39]. *C. raphigera* was reported to produce pectinase, cellulase and xylanase activity, which could play a major virulence role for rot in pomegranates [41]. Yoneda et al. [42] reported that β-glucosidase secreted by *C. raphigera*. *C. raphigera* isolated from a medicinal plant *Terminalia arjuna* and Maia [44], and was further reinvigorated by Plectosphaerellaceae, was established by Batista Sordariomycetes, order Glomerellales, family Gibellulopsis piscis [46]. Until now, three species (Gibellulopsis piscis, *G. nigrescens*, and *G. chrysanthemi*) have been described according to the Index Fungorum. The genus *Gibellulopsis* contains only one valid species, *G. nigrescens* [46]. In 2012, *G. chrysanthemi* was isolated from a garland of Chrysanthemum leaves [47]. *G. nigrescens* is a plant pathogen and can be isolated from soil and the lower parts of the stem of plants. The phylogeny formed by the separate ITS-rDNA and 28S-rDNA of CNUFC-DDS47-1 supports the taxonomic identification as *G. nigrescens* (Figure 3). This species was also isolated from a soil sample and was shown to be the cause of wilt of sugar beets in China [48,49]. Compared with the morphological characters of *G. nigrescens* as described by Zare et al. [45], the isolated strain CNUFC-DDS47-1 displays hyaline conidia that are smooth, ellipsoidal often in chains, and form abundant chlamydospores.

The genus *Myrmecridium* belonging to the class Sordariomycetes, order Glomerellales, family Bartaliniaceae, was described by Arzanlou et al. [50] with the type species being *M. schulzeri*. To date, 13 species of *Myrmecridium* have been described according to the Index Fungorum. The species belonging to this genus are characterized by the production of obovoid or fusiform conidia, tapering towards a narrowly truncate base, hyaline mycelium with pale to unpigmented, pimple-like denticles. They are frequently isolated from freshwater, soil, and plant tissue [15,51–53]. *M. schulzeri* SCGFAP0135 strain was reported to have antibacterial activity [54]. In the phylogenetic tree (Figure 4), CNUFC-NDR5-2 and CNUFC-NDR5-3 strains clustered with a strain putatively named *M. schulzeri*. Comparing the morphology of the CNUFC-NDR5-2 isolate with previous descriptions by Arzanlou et al. [50], the present isolate was generally similar to those of *M. schulzeri*. However, some morphological features differed. The size of conidia described by Arzanlou et al. [50] was larger [3–4 × (6–9)–10(–12) μm] than that (2.5–3.5 × 5.5–7.5 μm) observed in our isolate. *M. schulzeri* isolate presented conidiophores that were sometimes branched, which was not described by Arzanlou et al. [50]. In a previous study, we found a new species, *M. fluviac*, from a freshwater sample in Korea [20]. Our results suggest that freshwater habitats are a good source of *Myrmecridium* species.

This is the first report of Ascodesmis spheraspora, Chaetomella raphigera, Gibellulopsis nigrescens, and Myrmecridium schulzeri in Korea. Future studies should investigate the ability of these species to produce extracellular enzymes as well as secondary metabolites.

**Disclosure statement**

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