

New Recorded Species in Three Genera of the Sordariomycetes in Korea

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Abstract In an ongoing survey of Korean indigenous fungi, three fungal strains belonging to the Sordariomycetes were isolated from soil samples. These strains were designated KNU16-001, KNU16-002, and KNU16-009, and identified as *Ambrosiella grosmanii*, *Acremonium sclerotigenum*, and *Trichocladium asperum*, respectively, based on morphological characterization and phylogenetic analysis using internal transcribed spacer region sequences of ribosomal DNA. This is the first report of these species in Korea.

Keywords *Acremonium*, *Ambrosiella*, Soil fungi, Sordariomycetes, *Trichocladium*

Fungi are one of the most important groups of soil microbes that play diverse and often critical roles in the ecosystem [1, 2]. Fungi perform a range of important ecological functions associated with the cycling of all major elements (e.g., C, N, P, S), and this cycling affects the structure and functions of soil ecosystems [1-3]. In Korea, the composition of soil fungal communities at the phylum level (Ascomycota and Basidiomycota) is probably similar to that reported worldwide, but variations at the species level reflect local environmental conditions, plant and animal communities, and land use. Detailed knowledge of the structure of fungal communities has important implications in various fields such as agriculture, environment, and human health [4-6].

It is well known that the Sordariomycetes is the second largest class of the phylum Ascomycota [7]. Based on literature until end of 2015, the Sordariomycetes included 6 subclasses, 32 orders, 105 families, 1,331 genera, and over

10,000 species [8]. The Sordariomycetes have a cosmopolitan distribution and include mostly terrestrial taxa, although several members can be found in aquatic habitats [7, 9]. Members of this class of fungi are known as pathogens and endophytes of plants, disease-causing agents in arthropods and mammals, as well as mycoparasites and saprobes involved in decomposition and nutrient cycling. Some species of the Sordariomycetes are economically important biocontrol agents [10], producers of bioactive compounds with medicinal applications [11], and extremely useful organisms for biotechnological industries [12].

During screening for unrecorded fungal species in soil samples collected from various fields around Korea, we isolated few dozen morphologically distinct strains. Among them, three strains belonging to the Sordariomycetes, namely, KNU16-001, KNU16-002, and KNU16-009, were identified as *Ambrosiella grosmanii*, *Acremonium sclerotigenum*, and *Trichocladium asperum*, respectively. In this report, we describe the isolation and identification of these strains and discuss potential directions for further study. To the best of our knowledge, these fungi have not been previously reported in Korea.

MATERIALS AND METHODS

Soil sampling and isolation of fungi. Soil samples were collected in 2016 from fields at various locations in Daegu (35°53'45.23" N, 128°35'10.94" E), Nonsan (36°21'43.38" N, 127°22'02.61" E), and Jeonju (35°79'32.46" N, 127°10'86.82" E) in Korea. The samples were taken from a depth of 10–15 cm, air-dried, and stored in plastic bags at 4°C until use. Fungi were isolated using a conventional dilution plating

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technique. For this purpose, 1 g of each soil sample was suspended in 10 mL of sterile distilled water, and the prepared suspension was vortexed, serially diluted, and spread onto potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA) plates. The plates were incubated for 3–7 days at 25°C, until fungal colony growth was observed. Single colonies on these plates were purified by transferring them onto new plates, followed by incubation on PDA at 25°C. The pure cultures were maintained on PDA slants at 4°C for future use.

Morphological characterization. Morphological characteristics of isolates KNU16-001, KNU16-002, and KNU16-009 were observed on PDA (MB Cell, Los Angeles, CA, USA) agar. The strains were inoculated on 9-cm petri dishes and incubated at 25°C in the dark for 14 days. After incubation, colony characteristics such as color, shape, and size were recorded. Conventional slide culture technique was applied to observe the conidia of the isolated fungi

[13]. A PDA block, 5 mm × 5 mm, was cut from a clean PDA plate and placed inside the lid. The sides of the block were inoculated with the culture, and a sterilized cover glass was placed over the block. Then, the lid was placed back on the PDA plate, and the plate was incubated at 25°C in the dark for 20 days. When the fungus had grown onto the cover glass, it was removed from the PDA block, placed on an objective slide, and fixed with 85% lactic acid. The morphology of the isolate was examined under an Olympus BX50 light microscope (Olympus, Tokyo, Japan).

DNA extraction, PCR, sequencing, and phylogenetic analysis. Total genomic DNA was extracted from isolates KNU16-001, KNU16-002, and KNU16-009 by using the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea), per manufacturer's instructions. The internal transcribed spacer (ITS) regions, including the 5.8S rRNA region, were amplified with primers ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-CCTCCGCTTATTGATATGC-3') [14]

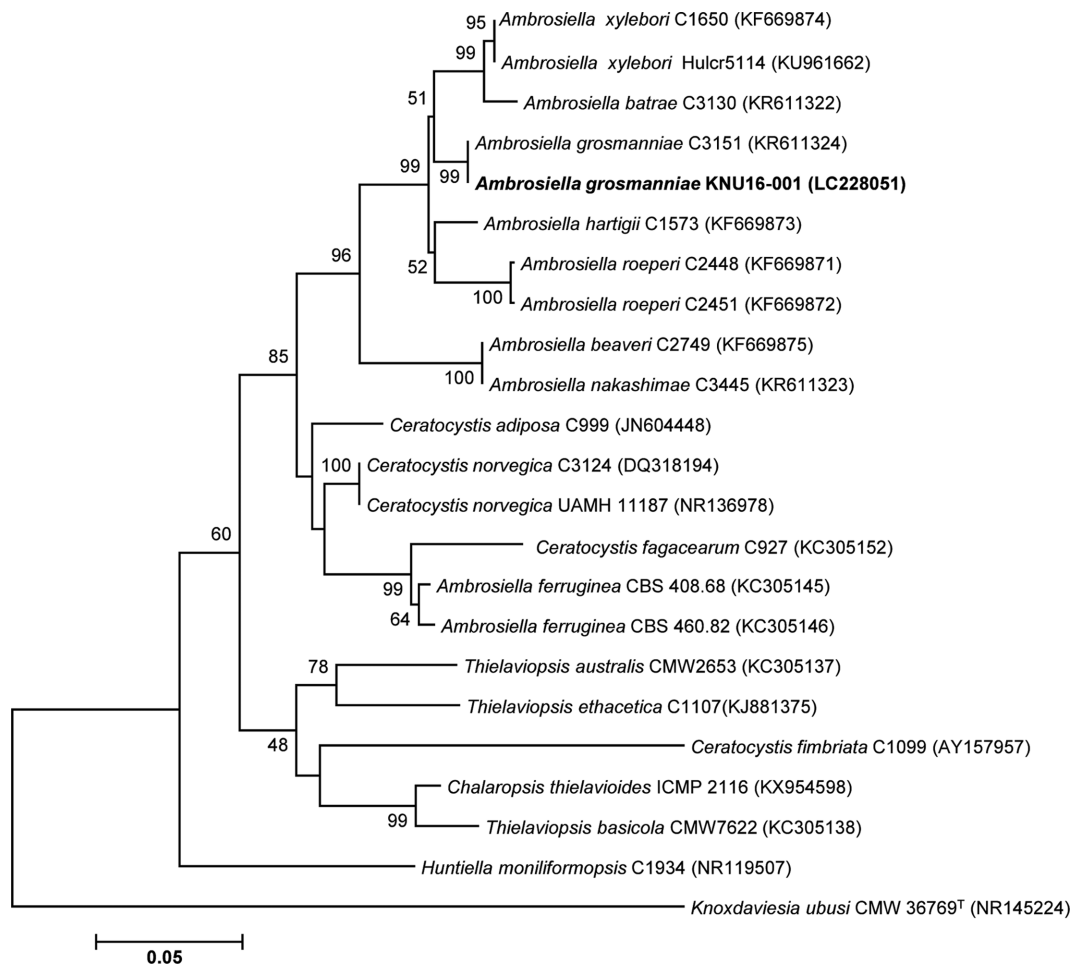


Fig. 1. Neighbor-joining phylogenetic tree, based on internal transcribed spacer region sequences, shows the phylogenetic position of *Ambrosiella grosmanniae* KNU16-001 among members of the genus *Ambrosiella* and other representatives of the Sordariomycetes. The strain isolated in this study is shown in boldface. Bootstrap values (based on 1,000 replications) greater than 50% are shown at the branch points. Accession numbers are shown in parentheses. *Knoxdaviesia ubusi* CMW 36769^T was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

under the following conditions: 95°C for 2 min, followed by 35 cycles consisting of 95°C for 30 sec, 55°C for 45 sec, and 72°C for 1 min, and one final step of extension at 72°C for 7 min. The amplified PCR products were sequenced using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were compared with reference ITS sequences from the GenBank database at the National Center for Biotechnology Information (NCBI), using the basic local alignment search tool (BLAST). Evolutionary distance matrices based on the neighbor-joining algorithm

were calculated using Kimura's two-parameter model [15]. Tree topology was inferred by the neighbor-joining method in the program MEGA7 [16], with bootstrap values based on 1,000 replications.

RESULTS AND DISCUSSION

In a survey of the diversity of microfungi in soils collected from various regions in Korea, several fungal strains were isolated and identified based on phylogenetic analysis and

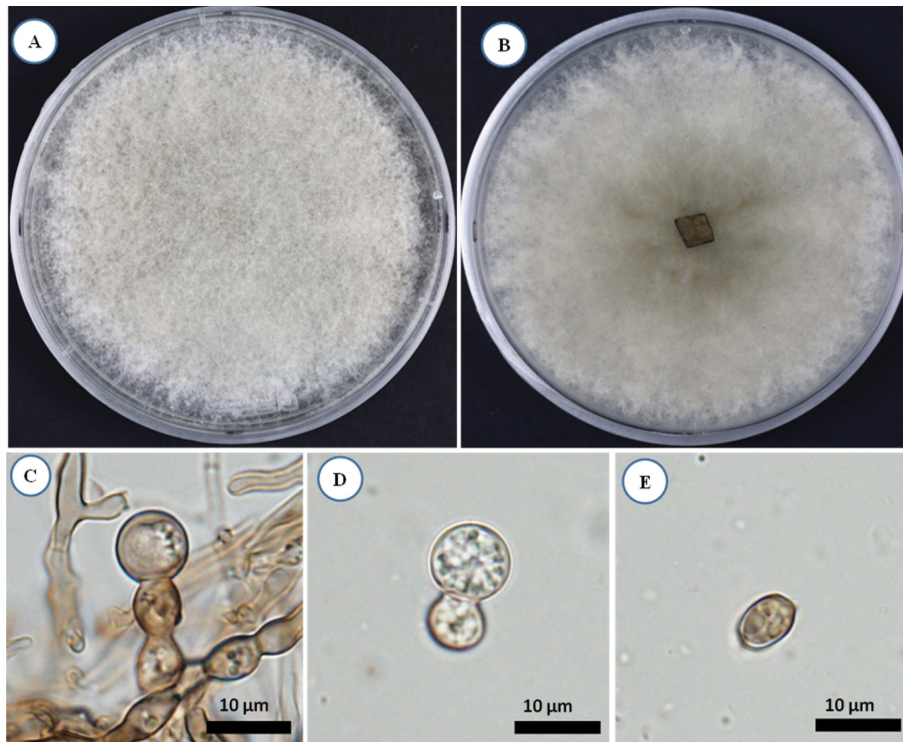


Fig. 2. Morphology of *Ambrosiella grosmaniae* KNU16-001 observed using a light microscope. A, Colony in front; B, Colony in reverse; C, Microscopic image of aleurioconidiophores; D, E, Microscopic images of aleurioconidia (scale bars: C–E = 10 µm).

Table 1. Morphological characteristics of *Ambrosiella grosmaniae* isolated in this study

Characteristics		<i>Ambrosiella grosmaniae</i> isolated in this study	<i>Ambrosiella grosmaniae</i> ^a
Colony	Color	White to light olivaceous, becoming darker with age	White margin, underside olivaceous, becoming darker with age
	Size	8.5–8.7 cm after 14 days on PDA agar	4.5–6.0 cm after 4 days on MYE agar
	Shape	Surface covered with dense, light olivaceous, aerial mycelium	Surface covered with dense, buff to olivaceous, aerial mycelium
Aleurioconidiophores	Size	21–38 µm long	20–60 µm long
	Shape	Hyaline, simple to branched (mostly simple), composed of moniloid cells	Occurring singly or grouped on white to buff sporodochia, hyaline, simple to branched, composed of moniloid cells
Aleurioconidia	Size	6.2–9.0 × 6.5–9.0 µm	7.5–12 × 7.5–12 µm
	Shape	Smooth, hyaline, aseptate, globose to subglobose	Thick-walled, smooth, hyaline, aseptate, globose to subglobose

PDA, potato dextrose agar; MYE, malt yeast extract.

^aSource of description [17].

morphological characterization of the isolates. Currently, sequence-based identification is considered the new “gold standard” for fungal species delimitation and identification, and the nuclear ribosomal DNA operon (nrDNA) has been used extensively for this purpose [18]. ITS1 was found between the 18S rRNA and the 5.8S rRNA genes, and ITS2 between the 5.8S rRNA and 28S rRNA genes. The two spacers, ITS1 and ITS2, and the 5.8S rRNA gene are usually referred to as the ITS region, and this region is the most common target for species-level identification [19]. Three isolates, KNU16-001, KNU16-002, and KNU16-

009, were subjected to molecular identification based on PCR amplification of ITS regions and their molecular identifications were confirmed based on morphological and culture characteristics of the isolates. The nucleotide sequences were deposited in GenBank, with accession numbers LC228051, LC228050, and LC228052 for KNU16-001, KNU16-002, and KNU16-009, respectively.

Ambrosiella grosmaniae. The first isolated strain, designed KNU16-001, was assigned to the genus *Ambrosiella*. A 585-bp ITS region sequence was obtained for the isolate.

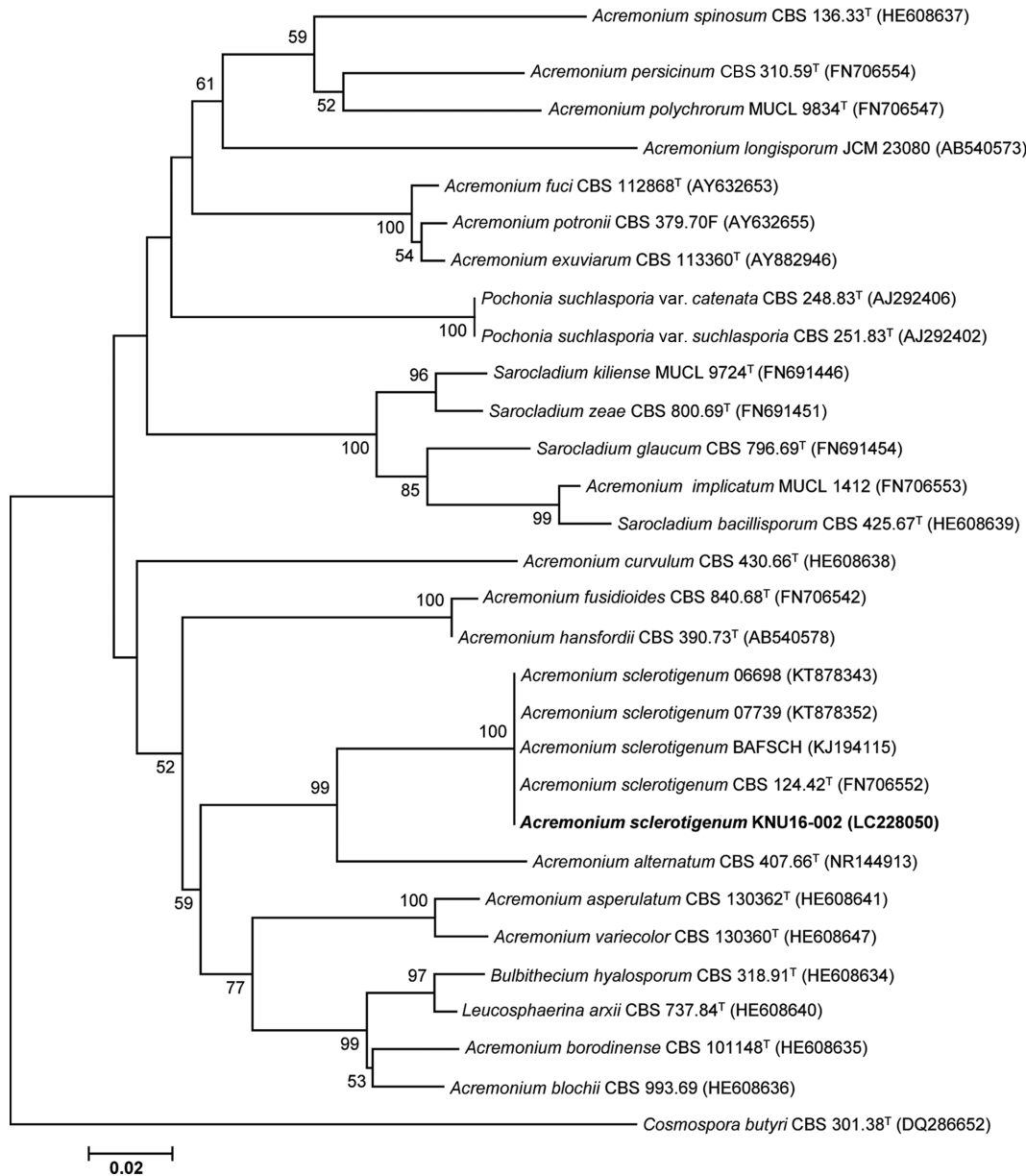


Fig. 3. Neighbor-joining phylogenetic tree, based on internal transcribed spacer region sequences, shows the phylogenetic position of *Acromonium sclerotigenum* KNU16-002 among members of the genus *Acromonium* and other representatives of the Sordariomycetes. The strain isolated in this study is shown in boldface. Bootstrap values (based on 1,000 replications) greater than 50% are shown at the branch points. Accession numbers are shown in parentheses. *Cosmospora butyri* CBS 301.38^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

BLAST searches against the GenBank database showed that the ITS sequence of KNU16-001 had 100% identity with that of *Ambrosiella grosmanii* strain C3151 (KR611324). In the phylogenetic tree based on the neighbor-joining algorithm (Fig. 1), strain KNU16-001 occupied a position within the genus *Ambrosiella* and clustered together with *A. grosmanii*, indicating their closest relationship at the species level.

The genus *Ambrosiella* was first invalidly described by Brader [20] as *A. xylebori*, but no type was designated. Later, Von Arx and Hennebert [21] illustrated and designated a type for the genus and species based on Brader's isolate. The genus *Ambrosiella* accommodates species belonging to the *Ceratocystidaceae* within the class Sordariomycetes those are morphologically characterized as having conidiophores single to aggregated in sporodochia, hyaline, unbranched or sparingly branched, one-celled to septate, and producing terminal aleurioconidia or chains of conidia from phialides [22].

After 14 days of incubation on PDA agar, KNU16-001 colony was 8.5–8.7 cm in diameter (Fig. 2A and 2B). The isolate produced white to olivaceous aerial mycelium and light olivaceous pigment at the bottom of the medium; the color became darker with age. Aleurioconidiophores occurred singly or grouped, composed of moniliod cells (chain of cells), were hyaline and 21–38 μm in size (Fig. 2C). Aleurioconidia were smooth, hyaline, aseptate, globose to subglobose, and 6.2–9.0 \times 6.5–9.0 μm in size (Fig. 2D and 2E). As shown in

Table 1, these morphological characteristics of isolate KNU16-001 were completely consistent with those previously reported for *Ambrosiella grosmanii* [17], thus strongly supporting the results of phylogenetic analysis of KNU16-001 based on the ITS sequence. Besides that, KNU16-001 can be distinguished morphologically from phylogenetically closely related *A. xylebori* by its light olivaceous pigmentation and by absence of a straight, hyphoid aleurioconidiophore with a single attached aleurioconidium that is unique feature of *A. xylebori*. In contrast to KNU16-001, other closely related species *A. batrae* was characterized by the presence of both aleurioconidia borne in chains and terminal aleurioconidia that tear away with conidiophore cells attached [17]. This is the first report of *Ambrosiella grosmanii* isolated from soil in Korea.

***Acremonium sclerotigenum*.** The second isolated strain, designated KNU16-002, was assigned to the genus *Acremonium*. An ITS region sequence of 585 bp was obtained for the isolate. BLAST searches against the GenBank database showed that the ITS sequence of KNU16-002 had 100% identity with several *Acremonium sclerotigenum* strains such as *A. sclerotigenum* CBS 124.42^T (FN706552), *A. sclerotigenum* BAFSCH (KJ194115), *A. sclerotigenum* 06698 (KT878343), and *A. sclerotigenum* 07739 (KT878352). In the neighbor-joining phylogenetic tree, strain KNU16-002 occupied a position within the genus *Acremonium* and clustered together with *A. sclerotigenum* strains, indicating

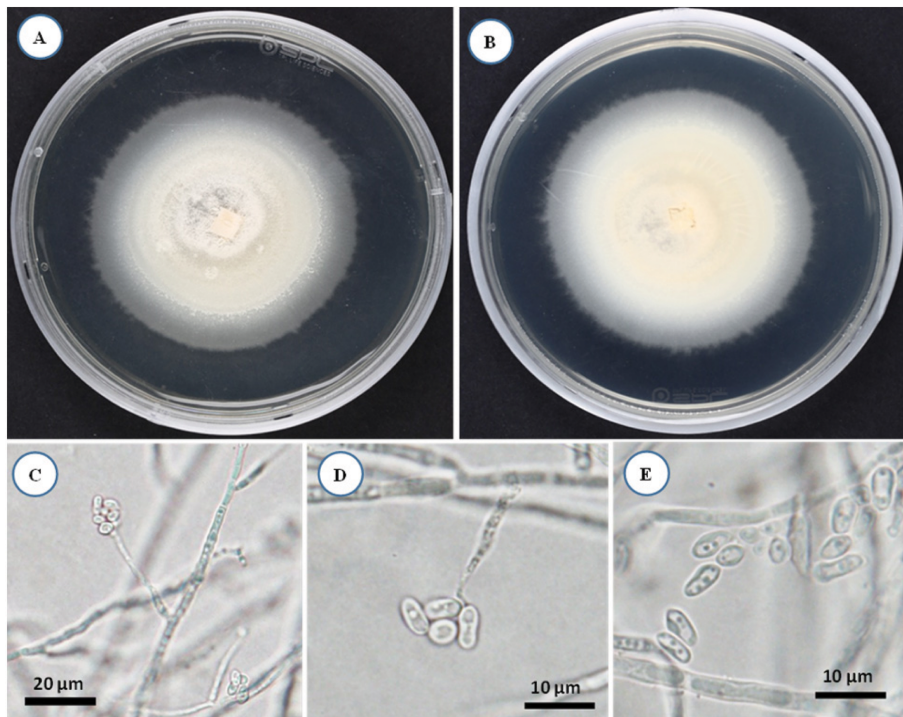


Fig. 4. Morphology of *Acremonium sclerotigenum* KNU16-002 observed using a light microscope. A, Colony in front; B, Colony in reverse; C, D, Microscopic images of phialides; E, Microscopic image of solitary conidia (scale bars: C = 20 μm , D, E = 10 μm).

Table 2. Morphological characteristics of *Acremonium sclerotigenum* isolated in this study

Characteristics		<i>Acremonium sclerotigenum</i> isolated in this study	<i>Acremonium sclerotigenum</i> ^a
Colony	Color	Whitish, flat, slightly cottony	Whitish, with floccose mycelium
	Size	6.5 cm, 14 days on PDA agar at 25°C	2.0 cm, 10 days on oatmeal agar at 20°C
Phialides	Size	15–46 µm long	9.8–47.5 µm long
	Shape	Simple, form from substratum, chromophilic near the base	Simple, erect from the substratum, with a chromophilic septum at the base
Conidia	Size	3.9–5.7 × 0.8–2.1 µm	3.0–5.0 × 0.7–1.8 µm
	Shape	One-celled, hyaline, cylindrical or slightly fusiform, smooth-walled, arranged in slimy heads	Obovoid, cylindrical or slightly fusiform, smooth, hyaline, or pale greenish, in chains or mostly collapsing in heads

PDA, potato dextrose agar.

^aSource of description [27, 28].

their closest relationship at the species level (Fig. 3).

The genus *Acremonium* was erected by Link, with *A. alternatum* Link as the type species, and currently contains more than 100 species, mainly belonging to the Hypocreales within the class Sordariomycetes [23]. *Acremonium* spp. are common soil and plant saprophytes [24], but some species are known as opportunistic human and animal pathogens [25] or as plant pathogens [26]. The species of this genus are morphologically characterized by colonies with slow or moderate growth; thin hyphae; simple or poorly basitonously branched, orthotropic phialides, usually gradually tapering toward the tip; and conidia that are usually small, unicellular, and aggregated in slimy heads, chains, or both [24, 27].

The colony formed by isolate KNU16-002 was whitish and floccose in appearance, and reached a diameter of 6.5 cm after 14 days incubation on PDA agar at 25°C (Fig. 4A and 4B). Phialides were simple without branching, formed from substratum and fasciculated aerial hyphae, and were chromophilic near the base (Fig. 4C and 4D). Conidia were aggregated in heads, cylindrical, or tapering towards the tips and slightly fusiform, smooth-walled, one-celled, and hyaline (Fig. 4E). Morphological characteristics of isolate KNU16-009 were in good agreement with the above-mentioned description of the genus *Acremonium* and most closely matched the characteristics of *A. sclerotigenum* (Table 2) [27, 28]. Similar to other strains of the species [28], *A. sclerotigenum* KNU16-009 distinguished morphologically from other *Acremonium* species by its conidial arrangement with slimy heads. Thus, the results of phylogenetic analysis of KNU16-009 were well supported by its morphological characteristics. Although this is a common fungal species with a cosmopolitan distribution, this is the first report of *A. sclerotigenum* in Korea.

***Trichocladium asperum*.** On the basis of morphological characterization and phylogenetic analysis third isolated strain KNU16-009 was assigned to *Trichocladium* Harz. Isolate KNU16-009 was subjected to molecular identification based on sequence analysis of the ITS regions. A search of

the GenBank database via BLAST revealed that the ITS region sequence of KNU16-009 exhibited high similarity (99–100%) with various *T. asperum* strains. This relationship was also evident from the phylogenetic tree constructed using ITS region sequences. The isolate KNU16-009 clustered together with *T. asperum* strains, thus confirming their closest relationship at the species level (Fig. 5).

The genus *Trichocladium* was introduced by Harz, with *T. asperum* Harz as its type species [29]. Currently, MycoBank lists 41 described species for the genus. Members of the genus *Trichocladium* reproduce asexually and are dematiaceous (dark pigmented) hyphomycetes within the family Chaetomiaceae. Molecular studies supported the affinity of this genus with the order Sordariales within the class Sordariomycetes [30]. Following the principal of “one fungus, one name” and considering that *T. asperum* occurs in the same clade as *Chaetomium* [30], Rėblová et al. [31] proposed that the name *Chaetomium* Kunze 1817 be used rather than *Trichocladium* Harz 1871. However, the authors emphasized that “eventually all generic names proposed for protection will be evaluated by the Nomenclature Committee for Fungi (NCF), and formally accepted or not at the Nomenclature Section meeting of the next International Botanical Congress in 2017.” This recommendation had not been formally accepted yet at the time of writing; therefore, we used the generic name *Trichocladium* in this paper.

The genus *Trichocladium* is characterized by effuse colonies, mycelium partly superficial partly immersed; stroma absent; conidiophores micronematous, semi-, macronematous, mononematous, straight or flexuous, pale brown; conidia solitary, dry, acrogenous or acropleurogenous, simple, clavate, cylindrical, rounded at the apex, obovoid or pyriform, smooth or verrucose, and thick walled with one to many dark brown transverse septa [29, 32].

The mycelium of isolate KNU16-009 was approximately 6.5 cm in diameter after 14 days incubation on PDA at 25°C (Fig. 6A and 6B). Aleurioconidia were oval, straight, dark brown, mostly two-celled, and approximately 26.5 × 9.5 µm in size (Fig. 6C–6E). Morphological characteristics of isolate KNU16-009 were in good agreement with the

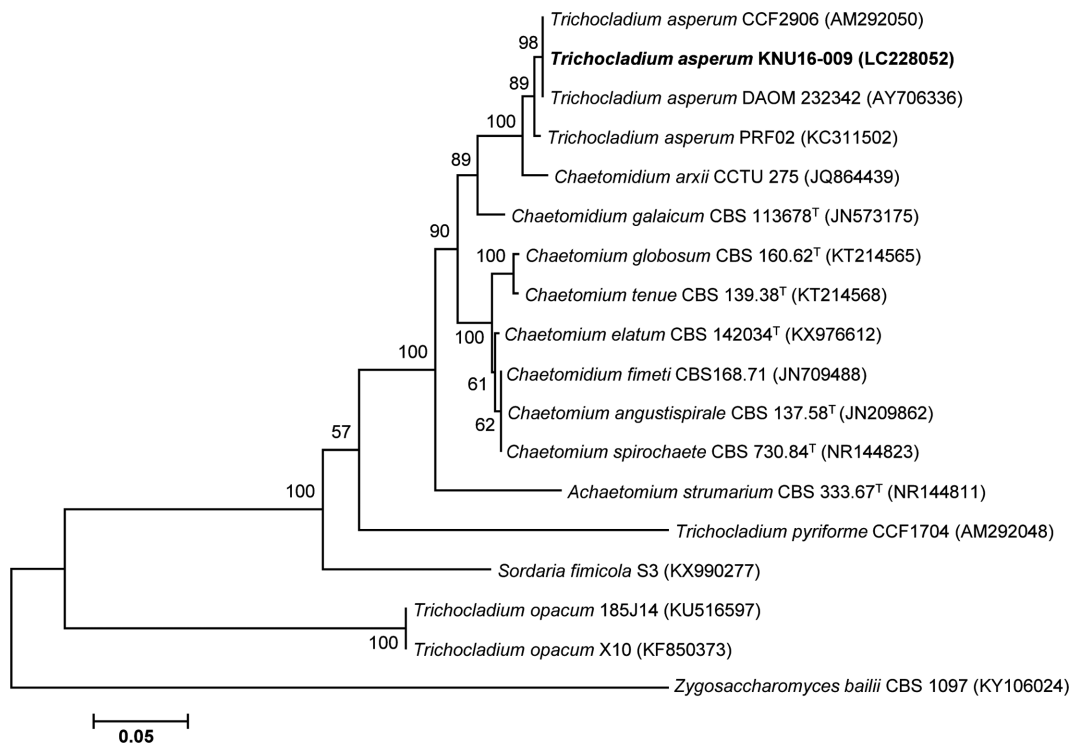


Fig. 5. Neighbor-joining phylogenetic tree, based on internal transcribed spacer region sequences, shows the phylogenetic position of *Trichocladium asperum* KNU16-009 among members of the genus *Trichocladium* and other representatives of the Sordariomycetes. The strain isolated in this study is shown in boldface. Bootstrap values (based on 1,000 replications) greater than 50% are shown at the branch points. Accession numbers are shown in parentheses. *Zygosaccharomyces bailii* CBS 1097 was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

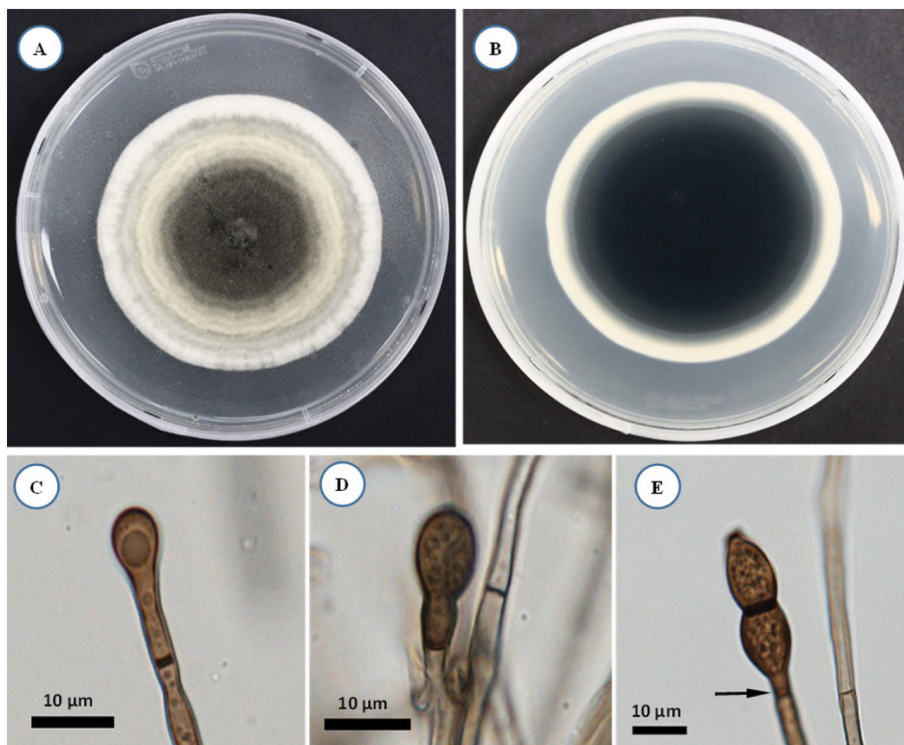


Fig. 6. Morphology of *Trichocladium asperum* KNU16-009 observed using a light microscope. A, Colony in front; B, Colony in reverse; C-E, Microscopic images of aleurioconidia (scale bars: C-E = 10 µm).

Table 3. Morphological characteristics of *Trichocladium asperum* isolated in this study

Characteristics		<i>Trichocladium asperum</i> isolated in this study	<i>Trichocladium asperum</i> ^a
Colony		White, cottony, effuse, mycelium mostly immersed	Effuse, cottony, at first white, later grey, mycelium immersed
Aleurioconidia	Size	14–26 × 7–10 μm	12–28 × 10–15 μm (two-celled)
	Shape	Oval, straight, dark brown, mostly two-celled	Oval, cylindrical-oval, straight, dark brown, coarsely roughened, mostly one-septate

^aSource of description [29, 30, 32].

above-mentioned description of the genus *Trichocladium* and most closely matched the characteristics of *T. asperum* (Table 3) [29, 32]. Moreover, rhexolytic secession is a specific characteristic of *T. asperum* conidia. Sometimes two closely located septa can develop, and secession can occur at this “separating cell” [30]. In Fig. 6E, the arrow indicates the location of the double septa of the eventual site of rhexolytic secession that was also found in the isolate. Morphological characteristics obtained here support the results of phylogenetic analysis of KNU16-009 as belonging to *T. asperum*. The isolate shared unique morphological features with *T. asperum* summarized by Goh and Hyde [32] that differentiated it from other *Trichocladium* species. *T. asperum* has not been previously reported from Korea, and this is the first detailed report of its isolation and identification in Korea.

Among members of the genera *Acremonium* and *Trichocladium* there are producers of bioactive compounds with antifungal activity, such as pyrrocidines A and B [33], and antibacterial activity, such as furanone [34] and trichocladinols A–C, which showed modest cytotoxic effects against the human tumor cell lines HeLa and MCF-7 [35]. In contrast, data regarding similar activities of *Ambrosiella* spp. are practically nonexistent. Thus, further investigation of *Ambrosiella grosmaniae* KNU16-001, *Acremonium sclerotigenum* KNU16-002, and *T. asperum* KNU16-009 would be worthwhile.

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