RESEARCH ARTICLE

A new record of *Trichocladium griseum* in Korea: morphological and molecular characterization

Setu Bazie Tagele[1,†], Thuong T.T. Nguyen[2,†], Sang Woo Kim[1], Mahesh Adhikari[1], Sun Kumar Gurung[1], Hyun Goo Lee[1], Byeong Heon Gwon[1], Han Jun Ju[1], San Kosol[1], Hyang Burm Lee[2,*], and Youn Su Lee[1,*]

[1]Division of Biological Resources Sciences, Kangwon National University, Chuncheon 24341, Korea
[2]Division of Food Technology, Biotechnology & Agrochemistry, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 61186, Korea

*Corresponding author: younslee@kangwon.ac.kr, hblee@jnu.ac.kr
[†]This authors contributed equally to this work.

ABSTRACT

A unrecorded species of *Trichocladium*, *Trichocladium griseum*, was isolated in 2017 during a survey of fungal diversity in Ulsan province, South Korea. This species was identified based on morphological characteristics and phylogenetic analysis of the internal transcribed spacer (ITS) rDNA and β-tubulin gene sequences. *T. griseum* has not yet been reported in South Korea. Thus, we report for the first time a new record of *Trichocladium griseum* in Korea, and we include the descriptions and morphological illustrations of this fungus.

Keywords: β-tubulin, ITS rDNA, Morphology, Taxonomy, *Trichocladium griseum*

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Mahesh Adhikari
https://orcid.org/0000-0001-9646-8633

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Introduction

The genus *Trichocladium*, which belongs to the subphylum Pezizomycotina, class Sordariomycetes, order Sordariales, and family Chaetomiaceae, was first described by Harz in 1871 with *Trichocladium asperum* as the type species [1]. Members of this genus are characterized by the production of darkly pigmented, septate conidia. Species belonging to the genus *Trichocladium* have been frequently isolated from soil, estuarine sediment, leaf, dung, grass, meal, and *Usnea* cf. *aurantio-atra* [2]. Recently, Wang et al. [2] redefined *Humicola* sensu stricto and related genera in the family Chaetomiaceae based on the phylogenetic analyses of multi-gene sequences combined with morphological observations, and they transferred certain species of *Beniowskia, Chaetomium, Chaetomidium, Gilmaniella, Humicola, Monodictys, Thielavia,* and *Monodictys* to the new genus *Trichocladium*. To date, 12 species belonging to this genus are known [2]. Prior to its renaming, *Trichocladium griseum* was classified under the name *Humicola grisea*.

Knowledge of the taxonomy of certain fungi within the genera *Trichocladium* in Korea is limited. A recent study reported the presence of *Trichocladium asperum* in soil [3]. A survey of Korean indigenous

fungal diversity was conducted in 2017 in Ulsan, South Korea. Fungal isolates possessing various morphologies were isolated from soil samples obtained from paddy fields. Consequently, an unrecorded species belonging to the genus *Trichocladium* (*T. griseum*) was discovered. This fungal species has not previously been reported in Korea. In this report, macro-morphological and micro-morphological characteristics of this newly recorded species are presented.

Materials and methods

Isolation of the fungal isolate

Soil samples were collected in 2017 from a paddy field located in Ulsan (35°31′45.36″N, 129°06′23.12″ E), South Korea. Crop debris were removed and soil samples were collected at a depth of 10~15 cm. The soil samples were dried and then stored at 4°C in a sterile polythene bag prior to use. Morphologically different isolates of fungal species were isolated on potato dextrose agar (PDA, Difco Laboratories, Detroit, Michigan, USA) supplemented with 100 μg L^{-1} chloramphenicol using conventional soil dilution techniques [4]. The diluted soil suspensions were streaked onto the petri plates and then incubated for 5 days at 25°C. The developing morphologically different colonies were then aseptically transferred to fresh PDA plates to obtain pure cultures. The pure fungal isolates were finally reserved on PDA slants at 4°C for further use.

Table 1. The ITS and β-tubulin gene sequences obtained from *Trichocladium griseum* used in this study, but also the reference strains and their GenBank accession numbers

Taxon name	Collection no. (strain no.)	GenBank accession numbers	
		ITS	tub2
Trichocladium griseum	CBS 119.14 (NT)	LT993639	LT993720
T. griseum	**EML-KNU17-5**	MK785135	MK782052
T. griseum	CGMCC 3.13888	LT993641	LT993722
T. asperum	CBS 903.85 (eT)	LT993632	LT993713
T. gilmaniellae	CBS 388.75 (T)	LT993638	LT993719
Trichocladium sp.	CBS 351.77	LT993647	LT993728
T. crispatum	CBS 149.58	LT993636	LT993717
T. antarcticum	CBS 123565 (T)	LT993629	LT993710
T. arxii	CBS 104.79 (T)	LT993631	LT993712
T. seminis-citr ulli	CBS 143.58 (T)	LT993645	LT993726
T. amorphum	CBS 127763 (T)	LT993628	LT993709
T. acropullum	CBS 114580 (T)	LT993626	LT993707
Melanocarpus albomyces	CBS 638.94 (T)	KX976679	KX977021
Ovatospora medusarum	CBS 148.67 (T)	KX976684	KX977032
O. senegalensis	CBS 728.84 (T)	KX976687	KX977035
O. mollicella	CBS 583.83 (T)	KX976685	KX977033
Microascus trigonosporus	CBS 218.31 (T)	LM652443	LM652655

Bold letters indicate isolates and accession numbers determined by our study. CBS: Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands); CGMCC: China General Microbiological Culture Collection Center; T, eT, and NT: ex-type, ex-epitype, and neo-type cultures, respectively.

Morphological examination

For detailed morphological studies, the EML-KNU17-5 strain was cultured on malt extract agar (MEA), oatmeal agar (OA; 30 g of oatmeal and 20 g of agar in 1 L of deionized water), and PDA at 25°C for 7 days. The colonies grown on OA were observed microscopically. Fragments of mycelia were removed from the cultures and placed on microscope slides with lactic acid. An Olympus BX51 microscope possessing differential interference contrast optics (Olympus, Tokyo, Japan) was used to obtain digital images.

Genomic DNA extraction, PCR, sequencing, and phylogenetic analysis

The genomic fungal DNA was isolated from mycelia of the fungal isolate using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The β-tubulin (tub2) gene and the ITS region from EML-KNU17-5 isolates were amplified using the primer pairs Bt2a and Bt2b [5] and ITS1 and ITS4 [6], respectively. The PCR products were sequenced at Macrogen (Seoul, Korea) using an ABI PRISM 3730XL Analyzer (Life Technologies, Gaithersburg, MD, USA). The DNA sequences were subjected to BLAST analysis using the GenBank database. The construction of phylogenetic trees was accomplished by neighbor-joining (NJ) method using Kimura's 2-parameter model [7] implemented in the program MEGA version 6 [8] with 1,000 bootstrap replicates. The EML-KNU17-5 isolate used in this study was deposited at the National Institute of Biological Resources (NIBR, Incheon, Korea), as NIBRFG000499999. The sequences of EML-KNU17-5 were deposited into the NCBI database under the accession numbers indicated in Table 1.

Results

Phylogenetic analysis

For the phylogenetic analysis, ITS and β-tubulin gene sequences were used to analyze the phylogenetic relationships between EML-KNU17-5 and related species. BLASTn search of ITS rDNA indicated that the strain EML-KNU17-5 was most closely related to *H. grisea* CGMCC 3.13888 (current name: *T. griseum*) (GenBank accession no. LT993641) with 100% identity. BLASTn analysis of tub2 of EML-KNU17-5 revealed a similarity of 99.6% to *H. grisea* CBS 119.14 (current name: *T. griseum*) (Genbank accession no. LT993720). The phylogenetic relationships among the strain EML-KNU17-5 and related species were analyzed using single genes and the combined datasets for ITS and tub2 (Fig. 1-3). The strain EML-KNU17-5 was grouped within the same clade as *T. griseum* with 90%, 96%, and 99% bootstrap support according to ITS, tub2, and a combination of ITS and tub2 sequences, respectively.

Morphological characterization

Taxonomic descriptions of the morphological structures of the *T. griseum* EML-KNU17-5 are detailed below.

Colony characteristics

***Trichocladium griseum* (Traaen) X. Wei Wang & Houbraken, Studies in Mycology 93: 141 (2018) [MB#824469] (Table 2, Fig. 4).**

Colony characteristics of the fungal isolate EML-KNU17-5 on various agar media are shown in Figure 4. Colonies on PDA exhibited greenish-black centers and grey-white margins, and they were reverse olivaceous black with moderate sporulation and reached 37~40 mm in diameter after 7 days at 25°C. Colonies on MEA were olivaceous grey with a velvety to floccose texture, and they were also reverse greenish black and reached 35~38 mm in diameter after 7 days at 25°C. On OA, colonies were pale olivaceous grey to mouse-grey throughout with greenish-black centers and light grey margins, and they possessed floccose colony texture and reached 40~43 mm in diameter after 7 days at 25°C.

Micromorphology

Conidiophores were hyaline, unbranched, and occasionally exhibited swelling at the tops, and these structures were 3.5~69.0 μm long and 2.5~5.0 μm wide. Conidia were globose or subglobose, brown to dark brown, smooth, and measured 9.5~16.5 × 8.5~15.0 μm. *Acremonium*-like conidia formed in chains that were obovoid to ellipsoidal.

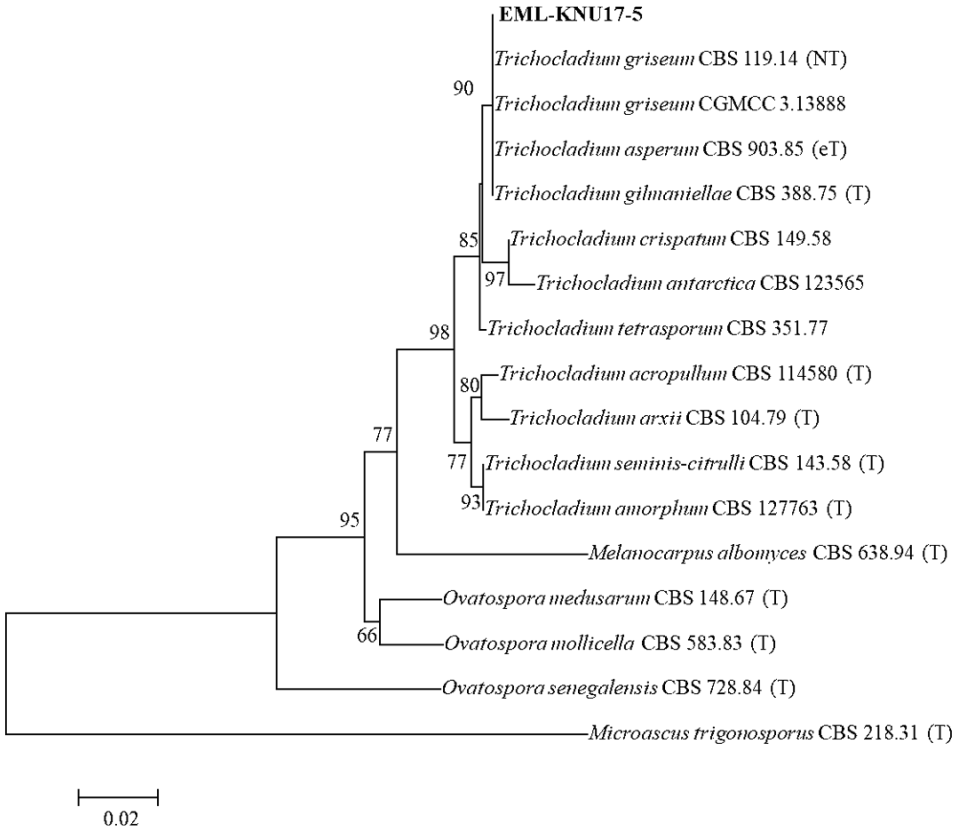

Fig. 1. Phylogenetic tree based on neighbor-joining analysis of the internal transcribed rDNA sequence obtained from EML-KNU17-5. *Microascus trigonosporus* was used as outgroup. Bootstrap scores of > 60% are indicated at the nodes. The scale bar represents the number of substitutions per site. The new isolate described in this study is shown in bold.

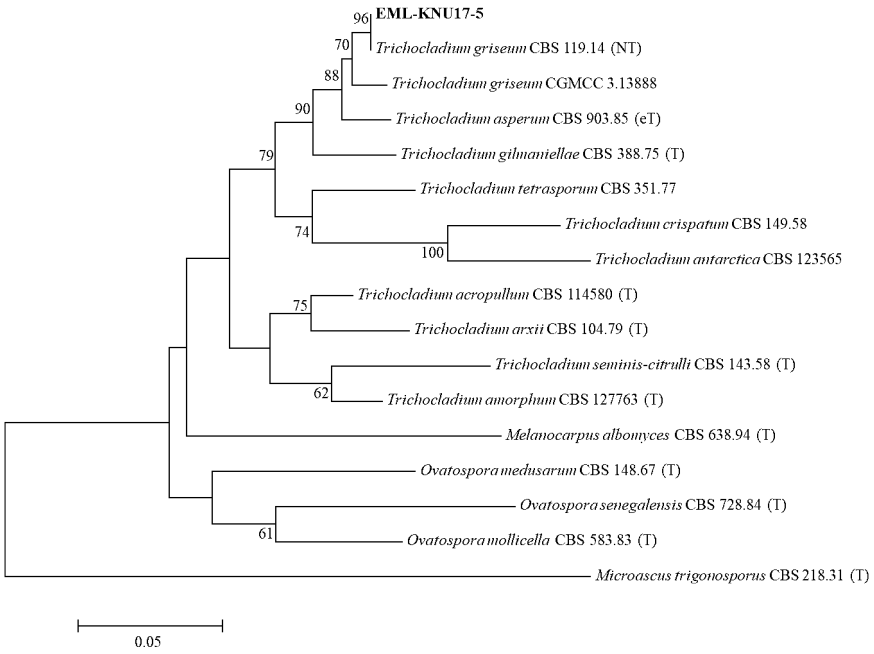

Fig. 2. Phylogenetic tree based on neighbor-joining analysis of β-tubulin gene sequence obtained from EML-KNU17-5. *Microascus trigonosporus* was used as outgroup. Bootstrap scores of > 60% are indicated at the nodes. The scale bar represents the number of substitutions per site. The new isolate described in this study is shown in bold.

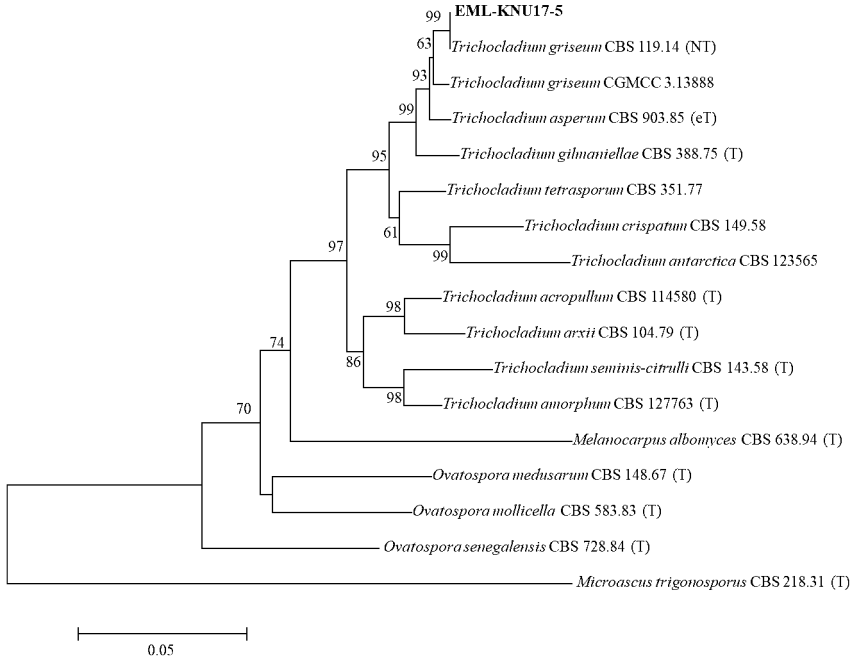

Fig. 3. Phylogenetic tree based on neighbor-joining analysis of the combined datasets for ITS and β-tubulin sequences obtained from EML-KNU17-5. *Microascus trigonosporus* was used as outgroup. Bootstrap scores of ≥ 60% are indicated at the nodes. The scale bar represents the number of substitutions per site. The new isolate described in this study is shown in bold.

Table 2. Morphological characteristics of EML-KNU17-5 and the reference species *Trichocladium griseum* cultured on different agar mediums at 25°C

Character	EML-KNU17-5	*Trichocladium griseum*[a]
Colony	Rapid growth, pale olivaceous grey to mouse grey, floccose colony texture at the center on OA; reverse greenish black at the center and light gray at the margins	Pale olivaceous grey to mouse grey; texture floccose; reverse greenish black
Conidiophores	Hyaline, unbranched, 3.5~69.0 µm long, 2.5~5.0 µm wide	Hyaline, unbranched, 2.5~50 µm long, 2~5 µm wide
Conidia	Globose or subglobose, brown to dark brown, 9.5~16.5 × 8.5~15.0 µm	Single-celled, solitary, sometimes 2(~4) in chains, globose or subglobose, (8~)12~16.5(~18) µm high, (7.5~)12~16(~17,5) µm wide

[a]Based on the description by Wang *et al*. [2].

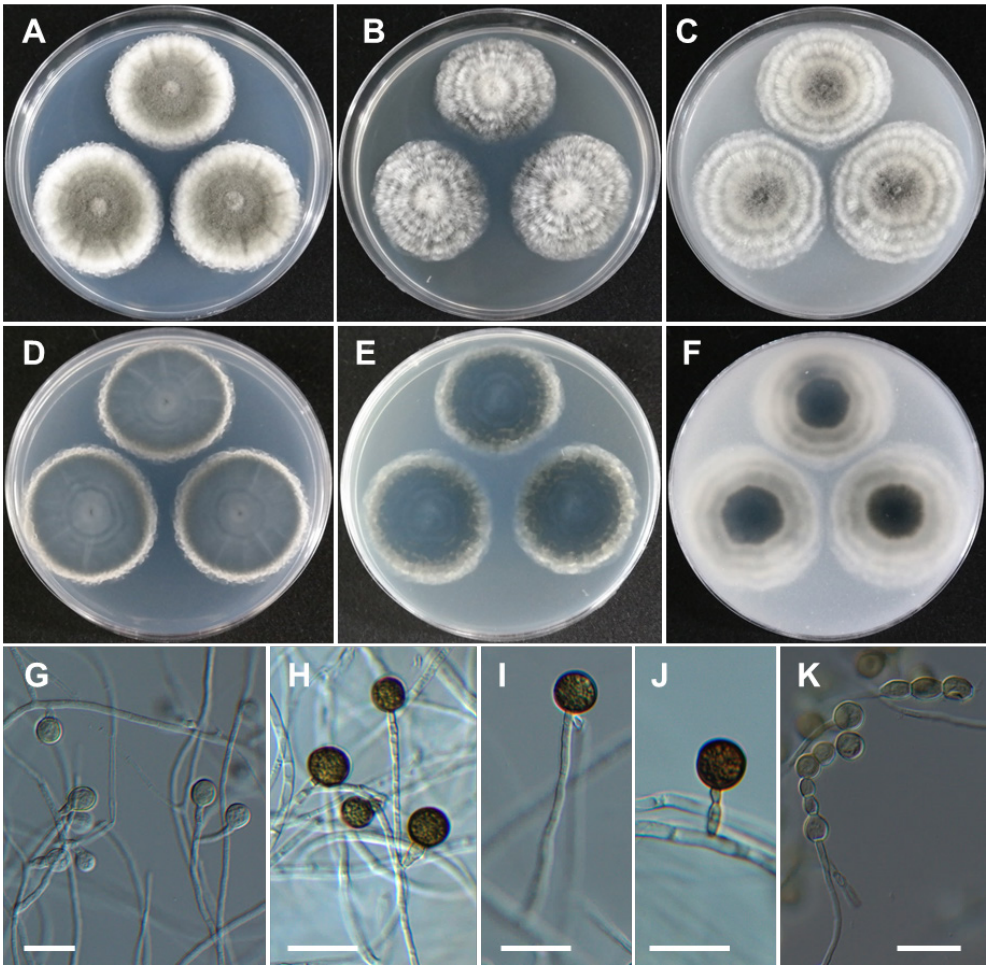

Fig. 4. *Trichocladium griseum* EML-KNU17-5 grown on different media at 25°C for 7 days. A-F, Colonies grown on PDA, MEA, and OA (top=obverse, bottom=reverse). G-J, Conidiophore and aleurioconidia. K, Conidiophores and conidia of the acremonium-like synanamorph (Scale bars: 20 µm).

Discussion

To date, only one species of the genus *Trichocladium*, *T. asperum*, has been reported in Korea. Given this, our findings increase the knowledge of the occurrence and distribution of *T. griseum* in paddy field habitats.

In the β-tubulin phylogenetic tree (Figure 2), our experimental strain (EML-KNU17-5) was clustered with other *T. griseum* species through well-supported branches. However, the ITS tree of EML-KNU17-5 was grouped with certain species that included *T. asperum, T. gilmaniellae,* and *T. griseum* (Figure 1). In the combined datasets for the *tub2* and ITS phylogenetic tree, EML-KNU17-5 was clustered into the same clade with *Trichocladium griseum* CBS 119.14 (Figure 3) through well-supported branches, and this was consistent with a previous study by Wang et al. [2]. There were a few differences observed in the colony diameter of *Trichocladium griseum* when compared to that of previous descriptions. The colony diameter on OA and MEA media was observed to be different from that of previously described *Trichocladium griseum* (OA: 42~48 mm; MEA: 41~47 mm). However, the morphological characteristics of our isolate were largely similar to those previously described by Wang et al. [2]. To the best of our knowledge, this study is the first report of *Trichocladium griseum* in South Korea.

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