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Research Note

A New Record of *Volutella ciliata* Isolated from Crop Field Soil in Korea

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Abstract During a survey of fungal species in South Korea, a species of *Volutella ciliata* was isolated and described based on the analysis of the internal transcribed spacer region of its rDNA and its morphological characteristics. This is the first record of *Volutella ciliata* isolated from crop field soil in Korea.

Keywords Molecular identification, Morphology, Nectriaceae, Setae, Sporodochia, *Volutella*

Volutella is a widespread genus of the Nectriaceae family with significant morphological and ecological diversity among the members. Approximately 120 described species of *Volutella* have been identified from various parts of the world [1]. *Volutella* spp. grow in diverse habitats—in soil as facultative plant pathogens and as saprophytes and decomposers on plant debris. The fungus *Volutella pachysandricola* causes Volutella blight (sometimes called leaf blight and stem canker) on Japanese pachysandra (*Pachysandra terminalis*) [2]. Chilton [3] found *Volutella colletotrichoides* to be a facultative plant pathogen on the stems and leaves of alfalfa, and provided evidence that *Medicago sativa* (alfalfa), *Trifolium pratense* (red clover), and *Lotus corniculatus* (bird's foot trefoil) were susceptible to infection by this organism. Boxwood Volutella stem blight or canker is another very destructive disease caused by the fungus *Volutella buxi* on boxwood (*Buxus* spp.) [4]. It has been proposed that the metabolic detoxification capabilities of *Volutella ciliata* protect plants against potentially toxic compounds in the soil [5]. Osono and Takeda [6] reported that *V. ciliata* plays an important role in plant litter decomposition in forest ecosystems through soil nutrient recycling and accumulation of organic matter in soil. However, the genus *Volutella* is poorly researched and no modern monograph is available despite the common occurrence and broad distribution of these species.

During an investigation of soil fungi in Korea, a *V. ciliata* strain was isolated from the crop field soils of the Gangwon-do province. This fungus, to the best of our knowledge, had not been previously reported in Korea. The objectives of this study were to characterize this fungus morphologically and to determine its phylogenetic placement by sequence analysis of the internal transcribed spacer region of the rRNA gene.

The fungus was isolated from crop field soil, collected (up to 0~15 cm) from Gangwon-do province. The fungus was isolated by the dilution method [7]. The dilution was plated on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA) supplemented with 100 μg chloramphenicol (bacteriostat) per mL. The plates were incubated for 7 days at 24°C until colonies of the culture could be distinguished. Pure culture was maintained in sterile distilled water at 4°C. The fungus genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The internal transcribed spacer (ITS) regions of rDNA were amplified with the ITS1 and ITS4 primers [8]. The amplified PCR product was purified using a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. The PCR product was sequenced using an ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

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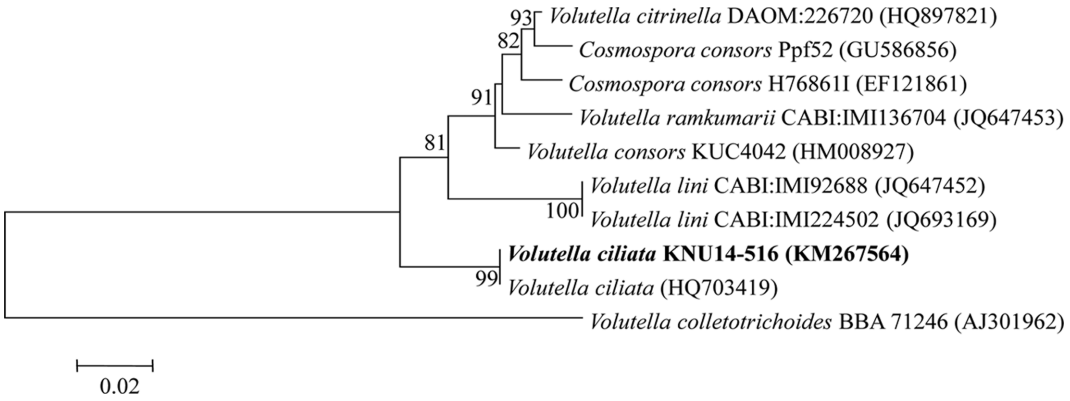

Fig. 1. Neighbor-joining phylogenetic analysis of *Volutella ciliata* KNU14-516 partial 18S-ITS1-5.8S-ITS2-28S rDNA region sequence obtained from crop field soil in Korea. The sequence obtained in the study is shown in boldface. Numerical values (> 50) on branches are the bootstrap values as percentage of bootstrap replication from 1,000 replicate analysis. *Volutella colletotrichoides* (AJ301962) was used as the outgroup.

The sequence was compared with known ITS1~ITS4 rDNA sequences in the GenBank database using BLAST analysis (http://www.ncbi.nlm.nih.gov/Blast). The nucleotide sequence has been deposited in NCBI-GenBank under the accession No. KM267564. The sequence was aligned with closely related strains using the MultAlin program. The evolutionary relationship tree was constructed using the distance based neighbor-joining method with Kimura 2-parameter model using MEGA software [9]. The reliability of the tree was evaluated by 1,000 bootstrap replications (Fig. 1). *Volutella colletotrichoides* (AJ301962) was used as the outgroup.

The fungus KNU14-516 was identified as *V. ciliata*, as the ITS sequence matched that of *V. ciliata* (HQ703419) in GenBank with 99% similarity. In order to determine the phylogenetic relationship among the isolate and its related species, the ITS region was compared. The isolate obtained from the soil and the GenBank isolate *V. ciliata* (isolate E12, accession No. HQ703419) clustered together in a group with a high bootstrap value (99%) (Fig. 1). These results confirmed that the isolate is *V. ciliata*.

The typical features of *Volutella* species are fusiform or biconic, equally or unequally, two-celled ascospores and eight-spored asci, discoid sporodochia or synnemata with setae, simple to verticillate conidiophores, compact and phialidic conidiogenous cells, and one-celled, ovoid to oblong conidia [1, 10-12]. The presence of sporodochial conidiomata with conspicuous hyaline, thick-walled, unbranched, spine-like setae, phialidic conidiogenous cells arising from somewhat penicillately branched conidiophores, and profuse ameroconidia allows *V. ciliata* species to be easily distinguished from the other species of *Volutella*. To confirm the molecular result, the morphology of the isolate KNU14-516 was determined by comparison to previously described *V. ciliata* morphology [2, 13-15]. Photomicrographs were taken with a Kodak14n digital camera (Tokyo, Japan) attached to a compound microscope and scanning electron microscope. Slide material was mounted in water and, in some experiments, stained with aniline blue. For morphological analysis, the strain was grown on PDA by inoculating three points in 9-cm petri plates that were incubated in the

Table 1. Comparison of morphological characteristics of the study isolate with respect to previously reported *Volutella ciliata*

Characteristics	Study isolate *Volutella ciliata* KNU14-516	*Volutella ciliata*[a]
Setae	Setae subglobose, arising from stromatic base and also surrounding the conidiophores, usually with 20~25 setae around, 215~735 μm long, 5~7.5 μm wide at base, tapering to a round apex, septate, spinulose, hyaline, walls 0.5~1.5 μm thick	Conspicuous hyaline, thickwalled, unbranched, spine-like setae or median and psuedoseptate, tapering to a round apex, 510 × 5~5.5 μm
Sporodochia	Sporodochia solitary or gregarious on substrate, hemispheric, usually with over 20 setae around, substipitate with a small and basal stroma, 300~560 μm diam.	Sporodochial conidiomata, hemispheric, substipitate, 130~440 μm diam.
Conidiophore	Conidiophores hyline, branched, bearing conidia apically and 61.5~89.5 μm tall. Phialides determinate, discrete, cylindrical, smooth, hyaline, slightly curved when developed from more or less penicillately branched conidiophores	Conidiophores phialosporous, hyaline, brached, one-celled, cylindrical; phialidic conidiogenous cells arising from more or less penicillately branched conidiophores
Conidia	Conidial masses slimy; conidia ellipsoid, unicellular, aseptate, one celled, distally rounded ends, smooth, 5~7 × 2~2.4 μm (n = 50)	Smooth, elliptical straight, or equilateral, hyaline, 5~5.5 × 1.7~2 μm

[a]Source of description and illustrations [1, 13-15].

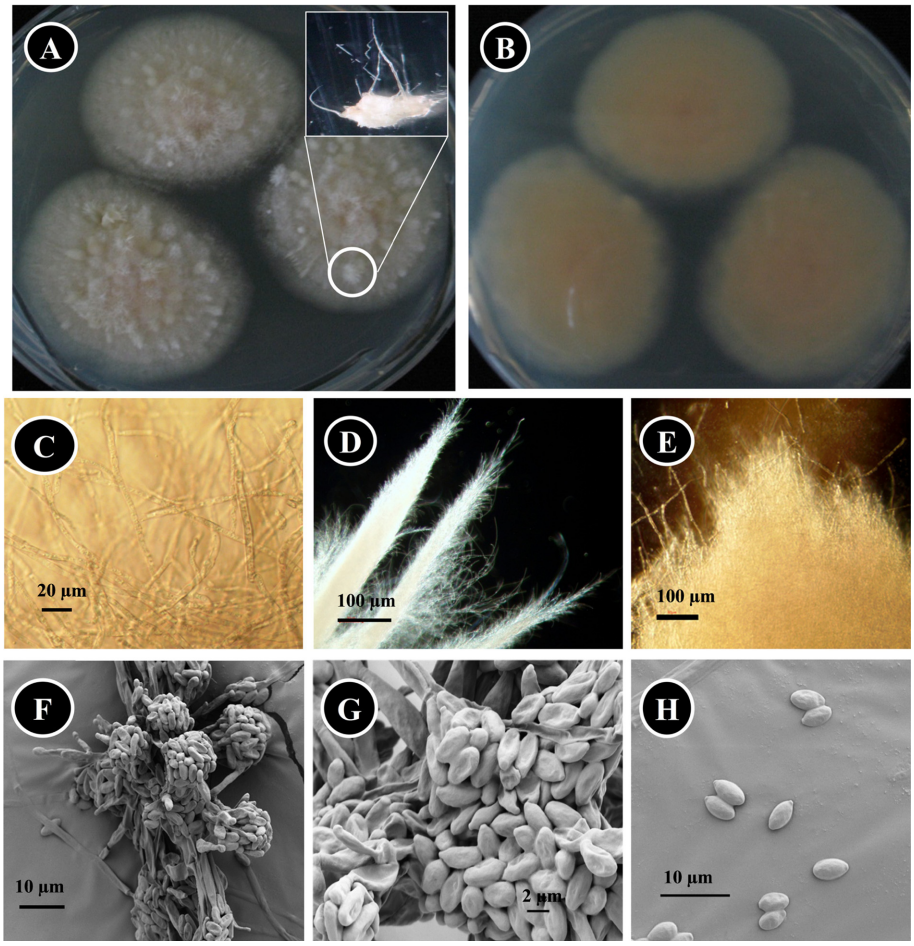

Fig. 2. Morphological characterization of *Volutella ciliata* KNU14-516 observed using a compound microscope and scanning electron microscope (SEM). A, Colony front (insert, sporodochia with setae, 2×); B, Colony reverse; C, Aerial mycelium (compound microscope image); D, Setae (compound microscope image); E, Sporodochia (compound microscope image); F, Conidiophores with phialidic conidiogenous cells and conidia masses (SEM micrograph); G, Conidia mucoid masses (SEM micrograph); H, Conidia (SEM micrograph) (scale bars: C = 20 μm, D, E = 100 μm, F, H = 10 μm, G = 2 μm).

dark at 28°C for 14 days.

The description and illustrations of the morphology of the fungus are shown in Table 1 and Fig. 2, respectively. Colonies on PDA were 25~30 mm in diameter after 14 days incubation at 25°C in the dark, whitish to off-white or pale brown, and light brown on the reverse (Fig. 2A and 2B). Sporodochia were superficial, solitary, whitish, subglobose, associated with stromatic hyphae, surrounded by setae, shortly stipitate basally and 300~560 μm in diameter (Fig. 2D). Setae were hyaline, long septate tapering from base toward acute apex, and up to 735 × 5~7.5 μm (Fig. 2C) Conidiophores were hyaline, one celled and cylindrical (Fig. 2F). Conidia were numerous, hyaline, smooth and, 5~7 × 2~2.4 μm (Fig. 2G and 2H). Based on the phylogenetic analysis and morphological characteristics, strain KNU14-516 was identified as *V. ciliata*. These morphological characteristics of the fungus *V. ciliata* were fully consistent with our phylogenetic analyses based on ITS sequence (Fig. 1). Although *Volutella* species occur commonly and have broad distribution, this genus has received little study [1]. Generally, the genus *Volutella* is typified by the fungus *V. ciliata*, which has been reported from various parts of the world [16]. However, this is the first report of this fungus isolated from crop field soils of Korea.

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REFERENCES

1. Gräfenhan T, Schroers HJ, Nirenberg HI, Seifert KA. An overview of the taxonomy, phylogeny, and typification of

nectriaceous fungi in *Cosmospora*, *Acremonium*, *Fusarium*, *Stilbella*, and *Volutella*. Stud Mycol 2011;68:79-113.
2. Šafránková I. Volutella leaf blight and stem canker on Japanese pachysandra in the Czech Republic. Plant Prot Sci 2007;43:10-2.
3. Chilton JE. *Volutella* species on alfalfa. Mycologia 1954;46: 800-9.
4. Shi F, Hsiang T. *Pseudonectria buxi* causing leaf and stem blight on *Buxus* in Canada. Eur J Plant Pathol 2014;138:763-73.
5. Vaughan D, Jones D, Ord BG. Amelioration by *Volutella ciliata* of the phytotoxicity of vanillic acid towards the growth of *Pisum sativum* L. Soil Biol Biochem 1993;25:11-7.
6. Osono T, Takeda H. Microfungi associated with *Abies* needles and *Betula* leaf litter in a subalpine coniferous forest. Can J Microbiol 2007;53:1-7.
7. Davet P, Rouxel F. Soil fungi: laboratory manuals. Detection and isolation of soil fungi. Enfield: Science Publishers; 2000.
8. Govinda Rajulu MB, Thirunavukkarasu N, Babu AG, Aggarwal A, Suryanarayanan TS, Reddy MS. Endophytic Xylariaceae from the forests of Western Ghats, southern India: distribution and biological activities. Mycology 2013;4:29-37.
9. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 2007;24:1596-9.
10. Rossman AY, Samuels GJ, Lowen R. *Leuconectria clusiae* gen. nov. and its anamorph *Gliocephalotrichium bulbilum* with notes on *Pseudonectria*. Mycologia 1993;85:685-704.
11. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. 4th ed. St. Paul: APS Press; 1998.
12. Samuels GJ, Rossman AY, Chaverri P, Overton BE, Põldmaa K. Hypocreales of the southeastern United States: an identification guide. CBS Biodiversity Series 4. Utrecht: Cebtraalbureau voor Schimmelcultures; 2006.
13. Gilman JC. A manual of soil fungi. 2nd ed. Calcutta: Oxford & IBH Publishing Co.; 1957.
14. Samuels GJ. *Nectria consors* and its *Volutella* conidial state. Mycologia 1977;69:255-62.
15. Saccardo PA. Sylloge fungorum. Vol. 4. Padua: Typis Seminarii; 1886.
16. Iacob V, Ulea E, Bălău AM, Lipşa F. Saprophytic and parasitic fungi on ornamental plants from moldova area (Romania). Agron Ser Sci Res 2012;55:85-7.