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. 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.

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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*<sup>a</sup> |
|-----------------|---------------------------------------------|---------------------------------|
| 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 pseudoseptate, 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, branched, 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 |

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