

RESEARCH ARTICLE

First Record of *Trichophaea scutelloides* in Korea: Morphological and Phylogenetic Characterization

Seong-Eun Kim and Ahn-Heum Eom*

Department of Biology Education, Korea National University of Education, Cheongju 28173, Korea

*Corresponding author: eomah@knue.ac.kr

ABSTRACT

Trichophaea scutelloides (Ellis) Van Vooren, Valade & Khomenko is a globose-spored operculate discomycete recently distinguished from the morphologically similar *T. brunnea*. In this study, *T. scutelloides* was reported for the first time in Korea based on morphological and phylogenetic analyses. Apothecia were found during the cultivation of *Tuber*-inoculated hazelnut (*Corylus* sp.) seedlings, and the morphological characteristics of the apothecia and the pure culture were examined. Phylogenetic analysis based on concatenated internal transcribed spacer (ITS) and large subunit (LSU) sequences placed the Korean isolate within the *T. scutelloides* clade, which is clearly separate from *T. brunnea* and *T. ellipsoideospora*. Ectomycorrhizae that formed on hazelnut roots were also characterized, and their ITS sequences were identical to those obtained from the apothecium and the pure culture. These results confirm the first record of *T. scutelloides* in Korea and provide additional information on its ectomycorrhizal association with *Corylus*.

Keywords: Ectomycorrhiza, Pezizales, *Sphaerosporella*, *Trichophaea*, Truffle

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INTRODUCTION

Globose-spored, operculate discomycetes historically treated under the broad concept of *Sphaerosporella brunnea* have long presented taxonomic difficulties because of their overlapping morphological characteristics. Recent taxonomic studies incorporating morphological, ecological, and molecular data have clarified this complex [1,2]. True *Trichophaea brunnea* is now restricted to pyrophilous collections from burnt substrates, whereas *Trichophaea scutelloides* is recognized as a morphologically similar fungus occurring on unburned substrates [1].

S. brunnea and *T. brunnea* are combinations based on the same basionym, *Peziza brunnea*. Fungi identified as *S. brunnea* have frequently been reported in ectomycorrhizae of *Tuber*-inoculated nursery seedlings and are considered potential competitors of cultivated *Tuber* species [3–5]. Following the recent taxonomic revision of the *T. brunnea* complex, some collections from unburned nursery substrates have been referred to *T. scutelloides* [1]. To date, neither *T. brunnea* nor *T. scutelloides* has been reported in Korea.

During the cultivation of *Tuber*-inoculated hazelnut (*Corylus* sp.) seedlings in Korea, apothecia of a globose-spored discomycete appeared on the potting substrate. In this study, the morphological characteristics of the apothecia and the pure culture were examined, and phylogenetic analyses were conducted based on internal transcribed spacer (ITS) and large subunit (LSU) rDNA sequences to determine the identity of the fungus. Ectomycorrhizae formed on hazelnut roots were characterized and molecularly compared with apothecial and cultured materials. Based on morphological and phylogenetic evidence, *T. scutelloides* was reported for the first time in Korea, providing additional information on its ectomycorrhizal association with *Corylus*.

MATERIALS AND METHODS

Sample collection and fungal isolation

During the cultivation of *Tuber*-inoculated hazelnut (*Corylus* sp.) seedlings in a greenhouse at the Korea National University of Education (KNUE), Cheongju, Korea, apothecia appeared on a potting substrate consisting of vermiculite and perlite mixed at a 1:1 (v/v) ratio. Moreover, ectomycorrhizae morphologically distinct from those of *Tuber* were observed on the seedling roots. The seedlings, from which the apothecia and ectomycorrhizae examined in this study were obtained, had been sown in April 2022. They were initially maintained in a growth room at approximately 25°C, 60–70% relative humidity, and a 16-h light/8-h dark photoperiod, then transferred to a greenhouse. Apothecia and colonized root tips were collected from multiple pots in July 2026. A pure culture was obtained from fresh apothecial material. The resulting isolate was designated KNUE26T001 and maintained at KNUE.

Morphological characterization

Macroscopic characteristics of the apothecia were observed under a stereomicroscope (SZX7; Olympus, Tokyo, Japan). Hand sections and squash mounts were prepared from the hymenium, excipulum, and marginal tissues, and their microscopic characteristics were examined using a light microscope (Axio Imager A2; Carl Zeiss, Oberkochen, Germany). Measurements of ascospores ($n = 50$) and asci ($n = 19$) were taken from mature apothecia and reported as the (minimum–) mean (–maximum). For cultural characterization, the isolate was grown on malt extract agar (MEA; Kisan Bio, Seoul, Korea) and commeal agar (CMA; Kisan Bio, Seoul, Korea) at 25°C in darkness for 7 days. Colony growth, hyphal characteristics, and conidiogenous structures were examined. Ectomycorrhizae formed on *Corylus* roots were also characterized based on color, branching pattern, mantle surface, emanating hyphae, and rhizomorph formation.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted separately from the pure culture, fresh apothecium, and ectomycorrhizal root tips using a HiGene Genomic DNA Prep Kit (BioFact, Daejeon, Korea). The ITS region was amplified from all three materials using the primers ITS1F [6] and ITS4 [7] to confirm that the apothecium, pure culture, and ectomycorrhizae represented the same fungus. The LSU rDNA region was amplified from the pure culture and apothecium using the primers LR0R and LR5 [8]. The PCR products were sequenced by MacroGen (Sejong, Korea), and the resulting sequences were deposited in GenBank.

Phylogenetic analysis

The newly generated ITS and LSU sequences were compared to sequences available in GenBank using BLASTn for preliminary identification. The reference sequences of *T. scutelloides* and closely related taxa were selected primarily from Van Vooren and Valade [1] (Table 1). *Paranthracobia rehmsii* was used as the outgroup. Sequence alignment, concatenation, model selection, and phylogenetic analyses were performed using MEGA 12 [9]. ITS and LSU sequences were aligned separately and subsequently concatenated. The best-fit nucleotide substitution model was selected based on the Bayesian information criterion. Maximum likelihood analysis was performed using the Kimura two-parameter model with invariant sites (K2+I) [10] and 1,000 bootstrap replicates. Bootstrap values $\geq 70\%$ are shown in the phylogenetic tree.

Table 1. Taxa and sequences used in the phylogenetic analysis

Taxon / material	Voucher or isolate	ITS accession	LSU accession
<i>Trichophaea scutelloides</i> (culture)	KNUE26T001_C (this study)	PZ756363	PZ756364
<i>Trichophaea scutelloides</i> (apothecium)	KNUE26T001_A (this study)	PZ736826	PZ736828
<i>Trichophaea scutelloides</i>	RD 25.31.242.98	MW476529	MW546567
<i>Trichophaea scutelloides</i>	LY NV 2022.11.02	OR606626	OR606632
<i>Trichophaea brunnea</i>	LY NV 2021.06.03	OR606625	OR606631
<i>Trichophaea brunnea</i>	Bru_JvP	MF066094	MF066066
<i>Trichophaea ellipsoideospora</i>	PC0714861	MW476527	MW546565
<i>Paranthracobia rehmsii</i>	PC0714858	MW476530	MW476531

ITS: internal transcribed spacer; LSU: large subunit.

RESULTS AND DISCUSSION

Sequence comparison and phylogenetic placement

The ITS sequences obtained directly from the apothecium and ectomycorrhizal root tips were identical to that obtained from the cultured isolate, confirming that all three materials represented the same fungus. In the maximum likelihood analysis of the concatenated ITS+LSU dataset, the Korean isolate clustered with the reference sequences of *T. scutelloides* and was clearly separated from *T. brunnea* and *T. ellipsoideospora* (Fig. 1).

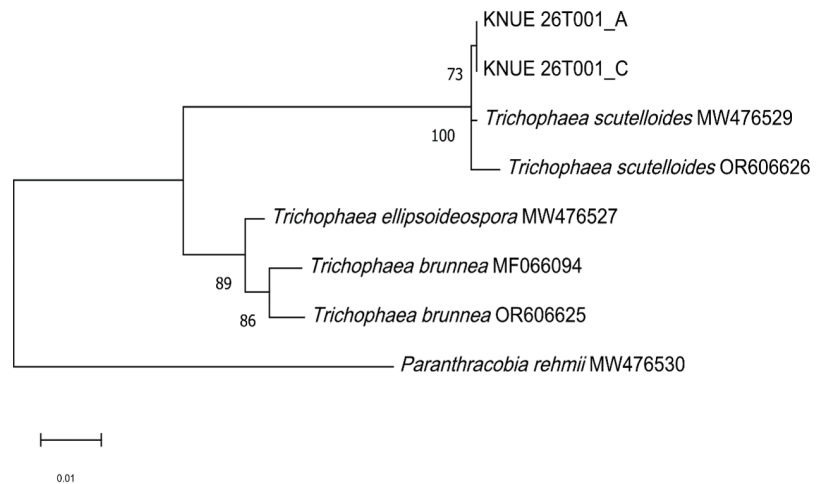


Fig. 1. Maximum likelihood phylogenetic tree based on the concatenated ITS and LSU rDNA dataset showing the placement of *Trichophaea scutelloides* KNUE26T001. The tree was constructed using the Kimura two-parameter model with invariant sites (K2+I). Bootstrap values $\geq 70\%$ from 1,000 replicates are shown at the nodes. *Paranthracobia rehmii* was used as the outgroup. The scale bar represents 0.01 substitutions per site. ITS: internal transcribed spacer; LSU: large subunit.

Taxonomy

Trichophaea scutelloides (Ellis) Van Vooren, Valade & Khomenko, *Ascomycete.org* 15(4): 120 (2023).

Basionym: *Peziza scutelloides* Ellis, Bull. Torrey Bot. Club 9: 18 (1882).

Ascomata gregarious. Apothecia developing on the potting substrate of *Tuber*-inoculated seedlings, sessile, (3.07–)5.14(–10.26) mm in diameter ($n = 8$), discoid, often deformed by mutual pressure, orangish brown to fulvous brown. Hymenium orange-brown, smooth. External surface is orange and smooth. Margins fringed with dense brown hairs (Fig. 2A–E). Asci (87.64–)120.53(–155.87) $\times$ (12.17–)14.11(–17.65) μm ($n = 19$), cylindrical, 8-spored, operculate, inamyloid, arising from croziers. Ascospores (10.62–)12.98(–14.38) μm in diameter ($n = 50$), globose, hyaline, smooth, uniguttulate with a single oil drop, thick-walled. Paraphyses cylindrical, apically widened, $\pm$ clavate to subcapitate, up to 10 μm wide, hyaline to diffusely orange, occasionally with light brown pigment deposited on the outer wall at the apex. Excipulum composed of *textura angularis*. Marginal hairs conical, tapering to acute apices, (63.44–)78.82(–89.56) $\times$ (4.04–)5.31(–7.98) μm . Excipular hairs were long, filamentous, and pale brown in color (Fig. 3A–F). Ectomycorrhizae (0.5–)0.95(–2.79) $\times$ (0.11–)0.18(–0.39) mm, brown, with a smooth mantle surface. Branching simple, mostly unbranched. Tips straight (linear, smooth-sided) and occasionally beaded (Fig. 2F). In culture, the hyphae on MEA were hyaline to pale brown and produced thick-walled brownish monilioid hyphae. Conidiophores and globose to subglobose conidiospores were observed on CMA and MEA (Fig. 4A–E).

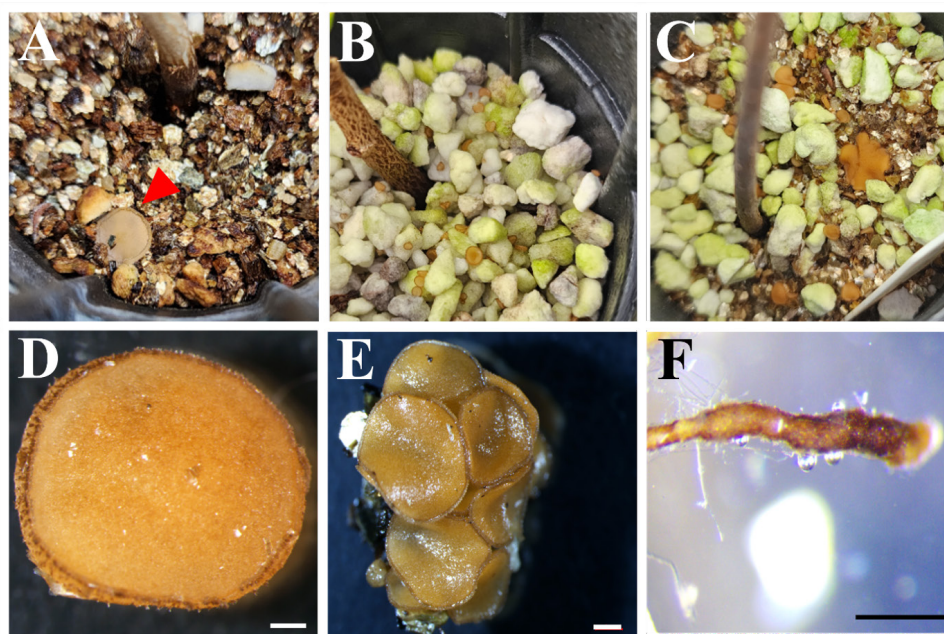


Fig. 2. Apothecia and ectomycorrhizae of *Trichophaea scutelloides* KNUE26T001 from *Tuber*-inoculated hazelnut seedlings in Korea. A–C, apothecia on potting substrate; the red arrowhead in A indicates an apothecium; D, single apothecium; E, gregarious apothecia; F, ectomycorrhizae on *Corylus* roots. Scale bars: D and E, 1 mm; F, 0.2 mm.

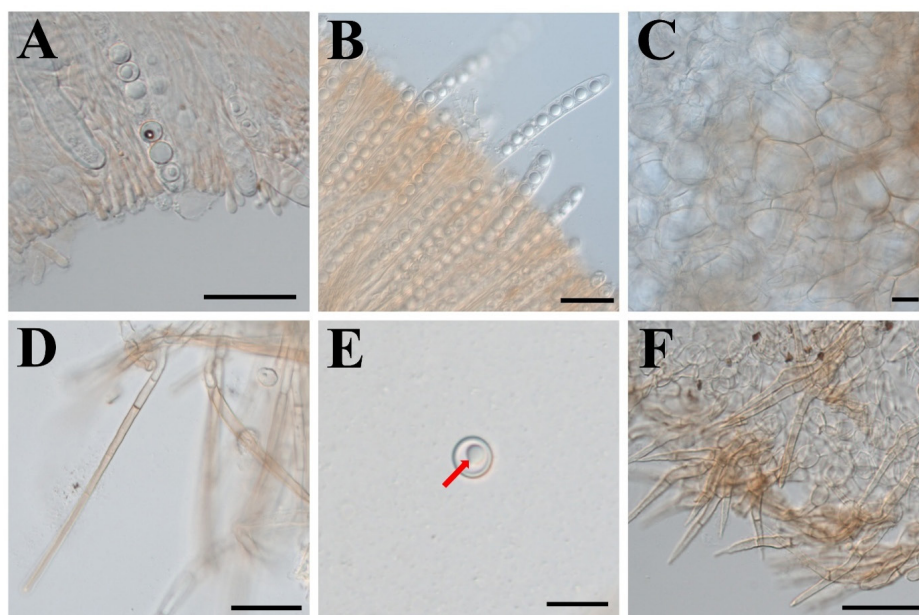


Fig. 3. Microscopic characteristics of the apothecia of *Trichophaea scutelloides* KNUE26T001. A, asci and paraphyses; B, asci; C, excipulum; D, excipular hairs; E, ascospore, with the red arrow indicating the single oil drop (guttule); F, marginal hairs. Scale bars: A, B, D, and F, 50 μ m; C and E, 20 μ m.

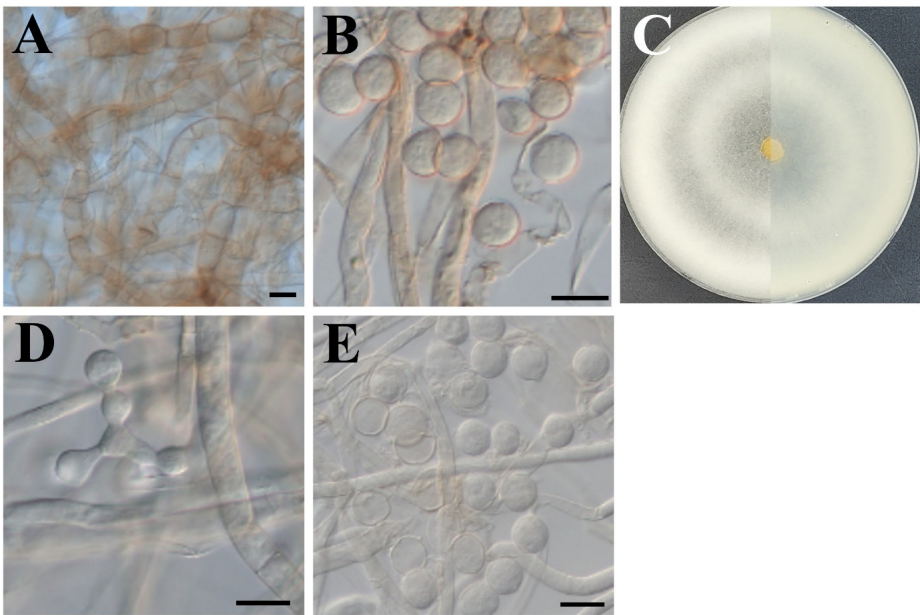


Fig. 4. Cultural characteristics of *Trichophaea scutelloides* KNUE26T001 on malt extract agar (MEA) and cornmeal agar (CMA). A, thick-walled monilioid hyphae on MEA; B, conidiophores and conidia on CMA; C, colony after 7 days on MEA; D, conidiophore with developing conidia on MEA; E, conidia on MEA. Scale bars: A, B, D, and E, 20 μ m.

Note. The isolate examined in this study was identified as *T. scutelloides* based on its morphological characteristics and ITS+LSU phylogenetic analysis. Its occurrence on unburned substrates is consistent with the ecology of *T. scutelloides*. The morphological characteristics overlap considerably with those of *T. brunnea*, making it difficult to distinguish these taxa based on morphology alone (Table 2).

Table 2. Comparison of *Trichophaea brunnea* and *T. scutelloides* with the isolate examined in this study

Character	<i>T. brunnea</i> [1]	<i>T. scutelloides</i> [1]	<i>T. scutelloides</i> (this study)
Substrate ecology	Burnt substrate; pyrophilous	Unburned substrate	Unburned nursery potting substrate
Apothecia	1–5 mm; brown tones	5–11 mm; fulvous to brown or orangish brown	(3.07–)5.14(–10.26) mm; orangish brown to fulvous brown
Ascospores	Globose, smooth, uniguttulate; 12–16.5 μ m	Globose, smooth, uniguttulate; 13–15 μ m	Globose, smooth, uniguttulate; (10.62–)12.98(–14.38) μ m
Asci	150–210 $\times$ 13–22 μ m	(160–)210–270 $\times$ 12–16 μ m	(87.64–)120.53(–155.87) $\times$ (12.17–)14.11(–17.65) μ m
Ectomycorrhizae	Reported as ectomycorrhizal	Reported as ectomycorrhizal / associated with unburned substrates	(0.5–)0.95(–2.79) $\times$ (0.11–)0.18(–0.39) mm, brown, smooth mantle, simple/mostly unbranched, observed on <i>Corylus</i> roots
Molecular placement	<i>T. brunnea</i> clade	<i>T. scutelloides</i> clade	<i>T. scutelloides</i> clade

Specimen examined: KOREA. Cheongju, on potting substrate and roots of *Tuber*-inoculated *Corylus* sp. seedlings, July 2026, KNUE26T001 (NIBRFGC000515587); GenBank accession numbers: (ITS) PZ756363 (culture), PZ736826 (apothecium), PZ736827 (ectomycorrhiza), (LSU) PZ756364 (culture), and PZ736828 (apothecium).

This study provides the first record of *T. scutelloides* in Korea, supported by its morphological characteristics and ITS+LSU phylogenetic analysis. *T. scutelloides* is currently found in North America and Europe, with documented collections from the United States, Canada, France, and Germany [1]. Reference sequences used in the phylogenetic analysis were obtained from collections in France. The isolate from this study clustered with the reference sequences of *T. scutelloides* and was clearly separated from *T. brunnea* and *T. ellipsoideospora*. *T. ellipsoideospora* differs from *T. scutelloides* in having ellipsoid rather than globose ascospores and in its association with burnt substrates. The morphological characteristics of these species overlap considerably, which limits the reliability of morphology alone for species identification within this complex [1]. The shorter asci observed in the Korean material, compared with those reported by Van Vooren and Valade [1], may partly reflect variation in developmental stage. The occurrence of *T. scutelloides* on an unburned substrate in the present study is consistent with its known ecology, whereas true *T. brunnea* is regarded as a pyrophilous species associated with burnt substrates [1,2]. ITS sequences obtained from the apothecium, pure culture, and ectomycorrhizal root tips were identical, demonstrating that these materials represent the same fungus. This provides direct evidence that *T. scutelloides* forms ectomycorrhizae with *Corylus* under the cultivation conditions examined. Globose-spored fungi, previously identified as *S. brunnea*, have frequently been reported as ectomycorrhizal associates of *Tuber*-inoculated seedlings in European truffle nurseries [3–5], and some material from this ecological context is now referred to as *T. scutelloides* under the revised taxonomy [1]. Therefore, the present study extends the known geographic distribution of *T. scutelloides* to Korea and provides additional morphological and molecular documentation of its ectomycorrhizal association with *Corylus*.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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