

## *Triangularia manubriata* sp. Nov.: A Novel Fungal Species Belonging to the Family Podosporaceae Isolated from Soil in Korea

Seong-Keun Lim<sup>a</sup> , Gwang-Jae Lim<sup>a</sup>, Jin-Sil Choi<sup>a</sup>, Seung-Yeol Lee<sup>a,b</sup>  and Hee-Young Jung<sup>a,b</sup> 

<sup>a</sup>Department of Plant Medicine, Kyungpook National University, Daegu, Republic of Korea; <sup>b</sup>Institute of Plant Medicine, Kyungpook National University, Daegu, Republic of Korea

### ABSTRACT

The fungal strain designated as KNUF-21-020, belonging to the genus *Triangularia*, was isolated from a soil sample collected in the Chungnam province, Korea. Phylogenetic analyses based on the concatenated nucleotide sequences of internal transcribed spacer regions and partial sequences of large subunit rRNA, beta-tubulin, and RNA polymerase II subunit genes revealed that the strain was grouped in a clade with *Triangularia* species. However, it occupied a distinct phylogenetic position. We also observed morphological differences between strain KNUF-21-020 and closely related species. Here, we provided detailed descriptions, illustrations, and discussions regarding the morphological and phylogenetic analyses of the closely related species to support the novelty of this isolated species. The phylogenetic analyses and morphological observations indicate that the strain KNUF-21-020 represents a novel species in the genus *Triangularia* (family: Podosporaceae). We have designated this species as *Triangularia manubriata* sp. nov.

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Morphology; phylogeny; Podosporaceae; soil fungi; *Triangularia manubriata*

## 1. Introduction

Soil-inhabiting fungi play a vital ecological role as decomposers, producing a variety of enzymes that break down organic matter and regulate nutrient balance. Additionally, these important ecosystem regulators strongly influence plant diversity and productivity [1]. The family Podosporaceae was introduced by Wang et al. [2]. It includes three redefined genera, namely, *Cladorrhinum*, *Podospora*, and *Triangularia*, all of which were previously classified in the family Lasiosphaeriaceae. Generally, species in the Podosporaceae family are plant endophytes or saprobes found on rotting wood [2]. These three genera produce single-cell pigmented ascospores or double-cell ascospores comprising a pigmented upper cell and a smaller, usually hyaline, lower cell. The genus *Triangularia* is typically found in terrestrial habitats, often growing as saprophytes on the ground among leaf litter or in association with plant roots [3]. Morphologically, it is characterized by the specific shape of its double-celled sexual spores, as described above [2,4]. There are currently 23 species in the genus *Triangularia*, and the type species is *Triangularia bambusae* [3]. Since *Triangularia* species

have been reclassified from other genera, including *Sordaria*, *Apiosordaria*, *Cercospora*, and *Zopfiella*, this genus is phylogenetically confusing [4]. Furthermore, several species produce bioactive compounds, such as anserinone A and B, dethiosecoemestrin, and emestrin [5].

This study aimed to expand our knowledge of fungal species diversity in Korea and the use of fungal resources in various industries. Here, we present the morphological and molecular characteristics of strain KNUF-21-020, belonging to the family Podosporaceae.

## 2. Materials and methods

### 2.1. Collection of soil samples and fungal strain isolation

Fungal strains were isolated from soil samples collected from Unsan-myeon, Seosan-si, Chungnam province, South Korea (36°46'41.0"N, 126°36'15.8"E). Isolation was performed using the plate dilution method as previously described [6]. Strains KNUF-21-020 and KNUF-21-021 were selected from numerous fungal strains for further morphological and molecular phylogenetic analysis.

**CONTACT** Seung-Yeol Lee  [leesy1123@knu.ac.kr](mailto:leesy1123@knu.ac.kr)

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## 2.2. Cultural and morphological characteristics

Strains KNUF-21-020 and KNUF-21-021 were cultured on potato dextrose agar (PDA; Difco, Detroit, MI), malt extract agar (MEA; Difco, Detroit, MI), oatmeal agar (OA; Difco, Detroit, MI), and potato carrot agar (PCA; potato, 20 g; carrot, 20 g; agar, 20 g; distilled H<sub>2</sub>O, 1000 mL) to study its morphology and growth [3]. The cultural characteristics, including color, shape, and size were recorded after seven days. Morphological characteristics were observed under a BX-50 microscope (Olympus, Tokyo, Japan) and the observed characteristics of both strains were identical.

## 2.3. Genomic DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Due to the identical characteristics of both strains, out of both, only KNUF-21-020 was selected for phylogenetic analysis. We extracted genomic DNA from the mycelia of strain KNUF-21-020 cultured on PDA using the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, South Korea) according to the manufacturer's instructions. Molecular identification was conducted by analyzing sequences of the internal transcribed spacer (ITS) regions and large subunit (LSU) rRNA, beta-tubulin (TUB2), and RNA polymerase II subunit (RPB2) genes, which were amplified separately using the primer pairs ITS1F/ITS4, LROR/LR5, T1/Bt2b, and RPB2-5F/RPB2AM-7R, respectively [7–10]. The amplified PCR products were purified with the EXOSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA) and sequenced by SolGent (Daejeon, South Korea). The obtained KNUF-21-020 sequence was deposited in the National Center for Biotechnology Information (NCBI) GenBank database (Table 1).

## 2.4. Phylogenetic analysis

Sequences obtained from the NCBI were used to construct phylogenetic relationships (Table 1). The ITS region sequences and partial sequences of LSU, TUB2, and RPB2 gene were analyzed for identification supported by the construction of phylogenetic trees based on the neighbor-joining method as described by the Kimura model, using MEGA version X with bootstrap values based on 1000 replications [11–14].

## 3. Results

### 3.1. Taxonomy

The morphology of strains KNUF-21-020 and KNUF-21-021 was distinct from the other allied species of *Triangularia*. Therefore, it was described as a new species. However, since the cultural and morphological characteristics of both KNUF-21-020 and KNUF-21-021 were identical, the strain KNUF-21-020 was selected for analysis.

*Triangularia manubriata* S. K. Lim, S.Y. Lee, and H.Y. Jung sp. nov. (Figure 1).

MycoBank: 851263

*Etymology*: The specific epithet is derived from the Latin word *manubrium* (“handle”), which refers to the shape of the lower cell of the ascospore.

*Typus*: The strain was isolated from soil containing plant debris collected from Seosan-si, Chungnam province, South Korea (36°46'41.0"N, 126°36'15.8"E) in 2021. The stock culture was deposited in the National Institute of Biological Resources (NIBR) as a metabolically inactive culture (NIBRFGC000509195).

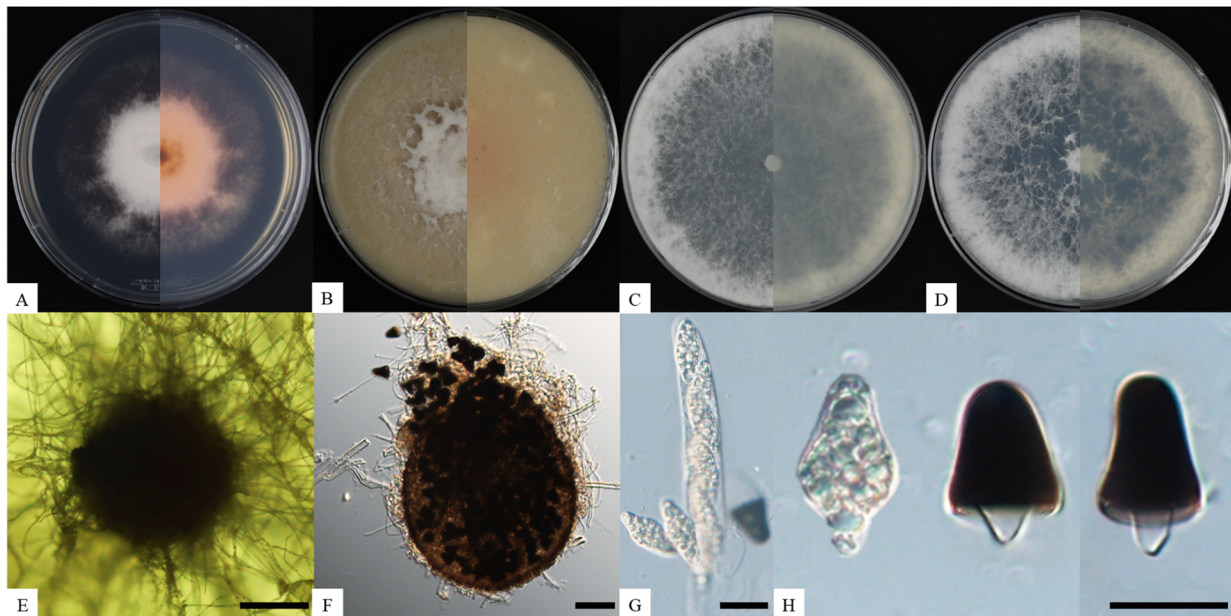
*Habitat*: The novel species proposed in this study, *Triangularia manubriata*, was isolated from soil collected in Korea.

*Description*: Colonies on PDA reached 63–65 mm in diameter after seven days of culture at 25 °C.

**Table 1.** GenBank accession numbers of the sequences used for phylogenetic analyses in this study.

Species	Strain	GenBank accession number			
		ITS	LSU	RPB2	TUB2
<i>Podospora fimicola</i>	CBS 482.64	MK926862	MK926862	MK876824	MK926962
<i>Triangularia allahabadensis</i>	CBS 724.68 <sup>T</sup>	MK926865	MK926865	MK876827	MK926965
<i>Triangularia anserina</i>	CBS 433.50	MK926864	MK926864	MK876826	MK926964
<i>Triangularia bambusae</i>	CBS 352.33 <sup>T</sup>	MK926868	MK926868	MK876830	MK926968
<i>Triangularia backusii</i>	CBS 539.89 <sup>T</sup>	MK926866	MK926866	MK876828	MK926966
<i>Triangularia backusii</i>	CBS 106.77	MK926867	MK926867	MK876829	MK926967
<i>Triangularia longicaudata</i>	FMR 12365	MT784141	KP981448	KP981631	KP981574
<i>Triangularia longicaudata</i>	FMR 12782	MT784142	KP981449	KP981632	KP981575
<b><i>Triangularia manubriata</i></b> <b>sp. nov.</b>	<b>KNUF-21-020<sup>T</sup></b>	<b>OR727458</b>	<b>OR727459</b>	<b>OR729840</b>	<b>OR729841</b>
<i>Triangularia phialophoroides</i>	CBS 301.90 <sup>T</sup>	MK926871	MK926871	MK876833	MK926971
<i>Triangularia setosa</i>	CBS 311.58	MK926872	MK926872	MK876834	MK926972
<i>Triangularia setosa</i>	CBS 369.59	MK926873	MK926873	MK876835	MK926973
<i>Triangularia verruculosa</i>	CBS 148.77	MK926874	MK926874	MK876836	MK926974

ITS: internal transcribed spacer; LSU: large subunit of 28S rRNA; RPB2: RNA polymerase subunit II; TUB2: beta-tubulin. Bold values indicate new strains of the genus *Triangularia*.



**Figure 1.** Cultural and morphological characteristics of *Triangularia manubriata* KNUF-21-020. Cultures were grown at 25°C for seven days. (A–D) Front and reverse view of the colony on PDA, OA, MEA, and PCA, respectively; (E, F) ascomata produced on PDA; (G) asci; (H) premature (hyaline) and matured (melanzated) ascospore. Scale bars: E, F = 50  $\mu\text{m}$ , G, H = 20  $\mu\text{m}$ .

They appeared white, dense, and round with abundant aerial mycelia in the center and a flat edge with pink pigmentation on the media. The reverse side showed shades from pink to dark brown (Figure 1(A)). On OA, the colonies reached up to 80 mm in diameter, and the surface was white, floccose, and circular with entire margins (Figure 1(B)). On MEA, the colonies reached up to 90 mm after six days, and the surface was white, circular, and floccose, with entire margins on the edge of the mycelium and flat in the center (Figure 1(C)). Colonies on PCA were similar in size and appearance to colonies on MEA, but were slightly buff in the center (Figure 1(D)). The ascomata were superficial, dark brown to black under reflected light due to the mass of released ascospores, solitary to aggregated, nonstiolate, and 54–115  $\mu\text{m}$  in diameter ( $n = 10$ ) (Figure 1(E,F)). The asci were cylindrical, containing eight hyaline ascospores that sometimes persisted until the ascospores were mature (Figure 1(G)). The ascospores were double-celled, 12.0–20.2  $\times$  15.3–25.9  $\mu\text{m}$  long (average: 16.5–20.1,  $n = 50$ ), and composed of an upper triangular cell with an obtuse end and a handle-shaped hyaline lower cell. The upper triangular cell was hyaline when it matured, at which point the color changed from dark brown to black (Figure 1(H)). Asexual morphs were absent.

**Notes:** A comparison of strain KNUF-21-020 with phylogenetically related strains *T. bambusae* CBS 325.33<sup>T</sup> and *T. setosa* CBS 311.58 revealed morphological differences. Strain KNUF-21-020 produced double-celled ascospores composed of a triangular

upper cell and a handle-shaped lower cell. *T. bambusae* and *T. setosa* also produced double-celled ascospores, but they consisted of an olivaceous brown to dark upper cell and a hyaline lower cell [2] and were oblong shaped. Additionally, KNUF-21-020 ascospores (12.0–20.2  $\times$  15.3–25.9  $\mu\text{m}$ ) were smaller than those of *T. bambusae* (17.0–20.5  $\times$  10.5–12.0  $\mu\text{m}$ ) and *T. setosa* (15.5–16.0  $\times$  18.5–20.0  $\mu\text{m}$ ). Thus, the strains were morphologically unique in this genus by the shape and size of their ascospores, specifically the lower cells (Table 2). Additionally, its habitat is similar with other *Triangularia* species, that most species from this genus are isolated from soil or rotting wood that support saprotrophic habitat.

### 3.2. Phylogenetic analysis

Amplification of the ITS, LSU, RPB2, and TUB2 genes of strain KNUF-21-020 yielded fragments of 391, 747, 1036, and 699 bp, respectively. The ITS regions of strain KNUF-21-020 showed 99.2% similarity with *T. bambusae* CBS 352.33<sup>T</sup> and 97.9–98.5% similarity with *T. setosa* CBS 311.58, *T. tetraspora* IFO32904, *T. unicaudata* CBS 313.58, and *T. longicaudata* FMR 12365. Based on the partial LSU gene sequence, strain KNUF-21-020 showed 100% similarity with *T. bambusae* CBS 352.33<sup>T</sup>, and 99.2–99.5% similarity with *T. setosa* CBS 311.58, *T. unicaudata* CBS 313.58, and *T. longicaudata* FMR 12367. Using the partial RPB2 gene sequence, the strain showed a maximum similarity

**Table 2.** Morphological characteristics of *Triangularia manubriata* (KNUF-21-020<sup>T</sup>) and comparison with the closest species of *Triangularia*.

Characteristics		<i>T. manubriata</i> KNUF-21-020 <sup>a</sup>	<i>T. setosa</i> CBS 311.58 <sup>b</sup>	<i>T. bambusae</i> CBS 325.33 <sup>Tb</sup>
Colony	Color	OA: white, buff in center and layer on edge by aerial mycelium, absence of exudate and ascomata PCA: white, layer in center and buff on edge by aerial mycelium, absence of exudate and ascomata MEA: white, layer in center and buff on edge by aerial mycelium, absence of exudate and ascomata	OA: vinaceous, without aerial mycelium, no exudate, black dot-like ascomata PCA: uncolored, crenate edge, without aerial mycelium MEA: grey white to vinaceous, buff aerial mycelium, numerous ascomata	OA: white, layer aerial mycelium, salmon to peach-colored exudate, black dot-like ascomata PCA: white, thin layer of aerial mycelium, absence of exudate and ascomata MEA: white, thick layer aerial mycelium, fulvous exudate, distributed black dot-like ascomata
	Diameter	OA: 80 mm in 7 d PCA: up to 90 mm in 6 d MEA: up to 90 mm on 6 d	OA: 24–30 mm in 7 d PCA: 21–27 mm in 7 d MEA: 18–24 mm in 7 d	OA: 70 mm in 7 d PCA: 70 mm in 7 d MEA: 70 mm in 7 d
Ascospores	Shape and color	Triangular upper cell: obtuse end, olivaceous brown; lower cell: handle-shaped, hyaline	Ellipsoidal upper cell: olivaceous brown; lower cell: handle-shaped, hyaline	Triangular upper cell: aciculiform end, olivaceous brown; lower cell: paler vinaceous buff
	Size	12.0–20.2 × 15.3–25.9 μm	17.0–20.5 × 10.5–12.0 μm	15.5–16.0 × 18.5–20.0 μm
Asci	Shape and color	Superficial, dark brown to black, solitary to aggregated, nonostiolate	Superficial, mouse-gray, solitary, ostiolate	Superficial to semi-immersed, setae, ostiolate, obpyriform

d: days; MEA: malt extract agar; OA: oatmeal agar; PCA: potato carrot agar.

<sup>a</sup>Fungal strain of this study.

<sup>b</sup>Source of description [2].

<sup>T</sup>Type strain.

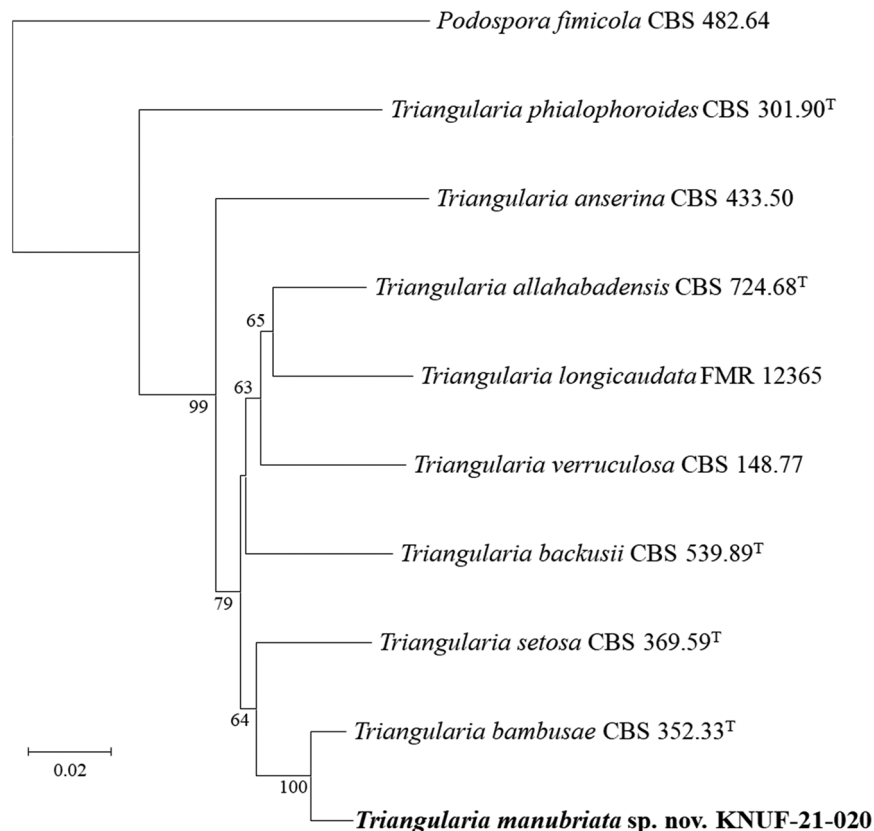
of 96.5% with *T. bambusae* CBS 352.33<sup>T</sup>. Similarly, the partial TUB2 gene sequence showed a maximum similarity of 94.5% with *T. bambusae* CBS 352.33<sup>T</sup>, and other *Triangularia* strains showed 89.3–89.7% similarity with *T. backusii* CBS 539.89, *T. unicaudata* CBS 313.58, *T. longicaudata* FMR 12365, and *T. allahabadensis* CBS 724.68<sup>T</sup>. Using the four aforementioned molecular markers, all results clearly indicated that comparative analysis based on the sequence of any one gene was insufficient for precise identification of the novel fungal strain at the species level. Therefore, multilocus sequence analysis was performed using concatenated ITS regions and partial LSU, RPB2, and TUB2 gene sequences of strain KNUF-21-020 (Table 1). In combination, these four molecular markers were highly effective in species resolution of the genus *Triangularia*. The NJ phylogenetic tree (Figure 2) based on the concatenated sequences demonstrated that strain KNUF-21-020 occupied a distinct position from other *Triangularia* species, and its phylogenetically closest neighbor in the genus *Triangularia* was *T. bambusae*. Accordingly, the novel strain was considered to represent a single, novel, and phylogenetically distinct *Triangularia* species.

#### 4. Discussion

Strains KNUF-21-020 and KNUF-21-021 were collected from soil in Chungnam province, South Korea, and identified as *Triangularia manubriata* sp. nov. To the best of our knowledge, this is the

first report of the genus *Triangularia* in Korea. The genus *Triangularia* was originally classified under the family Lasiosphaeriaceae [2] in the early nineteenth century as a fungal species growing on a decayed substrate that produced ellipsoidal or cylindrical melanized ascospores [15–18]. However, based on molecular analyses, the defined genera belonging to Lasiosphaeriaceae were revealed as polyphyletic [19–21]. Thus, the family Podosporaceae was reclassified from Lasiosphaeriaceae by Wang et al. and established with three genera, namely, *Cladorrhinum*, *Podospora*, and *Triangularia*, based on phylogenetic analysis [2]. At the same time, various species in several genera that previously belonged to Lasiosphaeriaceae, including *Sordaria*, *Apiosordaria*, *Cercospora*, and *Zopfiella* were redefined to the genus *Triangularia*, and several species in the genus *Triangularia* were reclassified to other genera [2–4]. Thus, *Triangularia* is phylogenetically complex, and our report of *T. manubriata* sp. nov. is expected to contribute to the resolution of this phylogeny [4].

Some species in this genus produce different secondary metabolites [5]. For example, *T. anserina* produces larvicidal bioactive compounds called anthraquinones and the antibacterial and antifungal compound 1,4-benzoquinone [22]. Antineoplastic and inflammatory metabolites of polyketide synthase and nonribosomal peptide synthetase hybrids have been reported from *T. effusa* [23]. Additionally, *T. bambusae*, a species closely related to strain KNUF-21-020, was reported to produce the antifungal metabolite tetrahydrofuran



**Figure 2.** Neighbor-joining phylogenetic tree based on a combined dataset of the internal transcribed spacer (ITS) regions and partial large-subunit (LSU), beta-tubulin (TUB2), and RNA polymerase II subunit 2 (RPB2) genes sequences showing the phylogenetic position of the strain KNUF-21-020 among *Triangularia* species. Bootstrap values greater than 60% (percentage of 1000 replications) are shown at branching points. The strain isolated in this study is in bold. The tree was rooted using *Podospora fimicola* CBS 482.64<sup>T</sup> as an out-group. Bar, 0.02 substitutions per nucleotide position.

[24]. Therefore, further research on the secondary metabolites of KNUF-21-020 and KNUF-21-021 is necessary to determine the potential application of these strains.

### Disclosure statement

No potential conflict of interest was reported by the author(s).

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### ORCID

Seong-Keun Lim  <http://orcid.org/0009-0006-7552-0838>  
 Seung-Yeol Lee  <http://orcid.org/0000-0003-1676-0330>  
 Hee-Young Jung  <http://orcid.org/0000-0002-4254-3367>

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