RESEARCH ARTICLE

Morphological and phylogenetic analyses of an unreported species *Diatrype rubi* isolated from soil in Korea

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ABSTRACT

The fungal strain KNUF-21-015 was isolated from soil in Palgongsan, Daegu-si, Gyeongbuk province in Korea, and identified as a previously unreported species within the family *Diatrypaceae*. Observations of conidiogenous cells, pycnidia, and conidia were conducted, and morphological differences between strain KNUF-21-015 and closely related species were compared. As the asexual morph of this species had not been documented previously, phylogenetic analyses were performed using concatenated nucleotide sequences from the internal transcribed spacer (ITS) regions and β-tubulin (TUB) gene to elucidate its identity and evolutionary relationships. The results placed the strain within the genus *Diatrype*, with high sequence similarities of 99.8 and 99.0% to *D. rubi* GMB0429^{T} for the ITS regions and TUB gene, respectively. Phylogenetic and morphological evidence collectively support the identification of the strain KNUF-21-015 as the asexual morph of *D. rubi*. To the best of our knowledge, this is the first report of *D. rubi* and its anamorph in Korea.

Keywords: *Diatrypaceae*, *Diatrype rubi*, Morphology, Phylogenetic analyses, Soil

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INTRODUCTION

The family *Diatrypaceae*, which belongs to the order Xylariales, class Sordariomycetes, and phylum Ascomycota, comprises 28 genera and is widely distributed [1–3]. Members of this family are commonly found on decaying wood and the bark of diverse plant species, contributing to their ubiquitous presence across diverse ecosystems [3]. The genus *Diatrype*, a key member of the *Diatrypaceae* family, has a documented distribution spanning Asia, Europe, North America, Oceania, and South Africa [4]. According to the Mycobank database (https://mycobank.org) approximately 401 species of *Diatrype* are recognized, though only two species, *D. stigma* and *D. disciformis*, have been previously reported in Korea [5–7].

The taxonomic framework of *Diatrypaceae* has historically been unstable, with numerous new genera emerging through the integration of morphological traits and multi-locus phylogenetic analyses [3]. Traditionally, the classification of *Diatrypaceae* species has relied heavily on stromatal characteristics.

However, this approach has led to significant confusion, resulting in polyphyletic genera and frequent species reassignments [3]. The genus *Diatrype* was first established by Fries in 1894 under the family *Diatrypaceae*, with *D. disciformis* as the type species [8]. The asexual morph of this genus is described as libertella-like, characterized by pycnidial conidiomata, while its sexual morph features stromata that are widely effused or verrucose, with eight-spored, long-stalked asci, and hyaline or brownish, allantoid ascospores [2,8,9].

This study aimed to isolate fungi from domestic soil samples in Korea and characterize them based on morphological and phylogenetic traits. The goal is to document potential endemic species, preserve domestic fungal resources, and contribute to a more comprehensive understanding of the fungal biodiversity in Korea.

MATERIALS AND METHODS

Sample collection and fungal isolation

Soil samples for fungal isolation were collected from Mt. Palgongsan, Daegu-si, Gyeongbuk province, Korea (35°59'33.9" N, 128°41'12.7" E). Fungi were isolated using the serial dilution method, as described in a previous study [10]. Single colonies were then transferred to fresh potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates and incubated at 25℃. The fungal strain KNUF-21-015 was selected for molecular, cultural, and morphological analyses. This isolate is preserved at the National Institute of Biological Resources (NIBR) under the accession number NIBRFGC000509191.

Cultural and morphological characterization

The strain KNUF-21-015 was cultured at 25℃ on PDA and potato carrot agar (PCA: potato, 20 g; carrot, 20 g; agar, 20 g; distilled H_2O, 1,000 mL) for morphological and cultural characterization. Cultures were maintained in the dark, and after 7 days, characteristics such as the size, color, and shape of the mycelium, as well as the conidiogenous cells and conidia, were observed. A stereoscopic microscope (Digital Micro Scope-M5; Siwon, Anyang, Korea) and a light microscope (BX-50; Olympus, Tokyo, Japan) were used to examine the morphological and cultural properties.

Genomic DNA extraction, PCR amplification, and sequencing

For phylogenetic analysis, genomic DNA from strain KNUF-21-015 was extracted using the HiGene Genomic DNA Prep Kit (Biofact, Daejeon, Korea) following the manufacturer's instructions. The internal transcribed spacer (ITS) regions and β-tubulin (TUB) gene were amplified using the ITS1F/ITS4 and T1/Bt2b primer pairs, respectively [11–13]. Successful amplification was confirmed through electrophoresis on 1.0% HP Agarose (BIOPURE, Cambridge, USA) gels. The amplified products were purified using ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and submitted to Macrogen (Seoul, Korea)

for sequencing.

Phylogenetic analyses

The sequences of strain KNUF-21-015 were analyzed for similarity using the Basic Local Alignment Search Tool (BLAST) against datasets in the National Center for Biotechnology Information (NCBI) database. Subsequently, several related sequences were retrieved from the database to conduct phylogenetic analyses (Table 1). Phylogenetic trees were constructed using the concatenated sequences of the ITS regions and TUB gene, employing the neighbor-joining (NJ) method in MEGA X [14,15]. Evolutionary distance matrices for the NJ analysis were calculated using Kimura's two-parameter model, with bootstrap values based on 1,000 replications [16].

Table 1. List of species used in phylogenetic analyses along with their GenBank accession numbers

Species name	Strain	GenBank accession number	
		ITS	TUB
Diatrype betulaceicola	FCATAS 2725^{T}	OM040386	OM240966
Diatrype betulaceicola	FCATAS 2726	OM040387	OM240967
Diatrype betulae	CFCC 52416^{T}	MW632943	MW656391
Diatrype bullata	UCDDCh400	DQ006946	DQ007002
Diatrype camelliae-japonicae	GMB0427^{T}	OP935172	OP938734
Diatrype camelliae-japonicae	GMB0428	OP935173	OP938735
Diatrype castaneicola	CFCC55425^{T}	MW632941	MW656389
Diatrype castaneicola	CFCC52426	MW632942	MW656390
Diatrype disciformis	GB 5815	KR605644	KY352434
Diatrype lancangensis	GMB0045^{T}	MW797113	MW814885
Diatrype lancangensis	GMB0046	MW797114	MW814886
Diatrype larissae	FCATAS 2723^{T}	OM040384	OM240964
Diatrype larissae	FCATAS 2724	OM040385	OM240965
Diatrype lijiangensis	MFLU 19-0717^{T}	MK852582	MK852583
Diatrype quercicola	CFCC 52418^{T}	MW632938	MW656386
Diatrype quercicola	CFCC 52419	MW632939	MW656387
Diatrype rubi	GMB0429^{T}	OP935182	OP938740
Diatrype rubi	**KNUF-21-015**	**OR727457**	**OR729842**
Diatrype stigma	DCash200	GQ293947	GQ294003
Diatrype stigma	Kern307	OP038055	OP079884
Xylaria hypoxylon	CBS 122620	KY610407	KX271279

ITS: internal transcribed spacer regions; TUB: β-tubulin gene.
^{T} Type strain. Strain used in this research is highlighted in bold.

RESULTS AND DISCUSSION

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Cultural and morphological characteristics of strain KNUF-21-015

After 7 d of incubation on PDA at 25℃, strain KNUF-21-015 formed colonies with a 90 mm diameter. The obverse side of the colony was white and dense, gradually thinning toward the edge, with a rough margin. The reverse side exhibited a sepia tone extending from the center to the margin, lacked pigmentation on the medium, and appeared filamentous (Fig. 1A). Under the same conditions, the strain grown on PCA formed smaller colonies, measuring 37–38 mm in diameter. The obverse side of the colony was white and filamentous, with a rough margin, while the reverse ranged from yellowish to light brown, also without media pigmentation (Fig. 1B). Orange color pycnidia were observed on PCA media (Fig. 1C). Conidiogenous cells derived from the pycnidia were cylindrical, straight, discrete or integrated, hyaline, and produced conidia at the apex (Fig. 1D and E). The conidia were hyaline, straight to slightly curved, allantoid, and aseptate, measuring 10.8–23.9 × 1.0–2.0 µm (avg. = 19.5 × 1.5 µm, n = 50) (Fig. 1F).

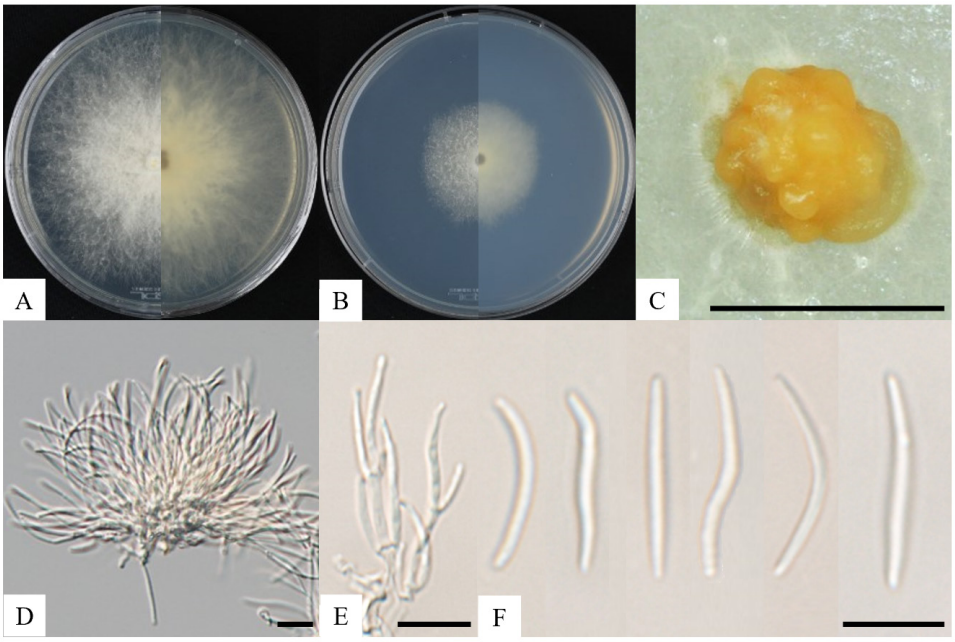

Fig. 1. Cultural and morphological characteristics of *Diatrype rubi* KNUF-21-015. Cultures were grown at 25℃ for 7 days. A and B: Obverse and reverse view of the colony on PDA and PCA; C: Pycnidia on PCA; D: Mass of conidiogenous cell and conidia; E: Conidia with conidiogenous cell; F: Conidia. Scale bars: C = 50 µm, D–F = 10 µm.

Molecular phylogeny of strain KNUF-21-015

Molecular identification of the isolated fungal strain KNUF-21-015 involved amplifying its ITS regions and TUB gene, yielding sequences of 602 and 707 bp, respectively. The ITS regions exhibited 99.8% similarity to *D. rubi* GMB0429^{T}, compared to 99.0% with *D. camelliae-japonicae* GMB0427^{T}. Similarly, the TUB gene showed 99.0% similarity with *D. rubi* GMB0429^{T}, compared to 97.3% with *D. camellia e-japonicae* GMB0427^{T}. The NJ phylogenetic tree constructed from the concatenated sequences of the ITS regions and TUB gene clustered strain KNUF-21-015 with *D. rubi* GMB0429^{T} (Fig. 2). Combined morphological, cultural, and phylogenetic analyses identified the strain as *D. rubi* (Table 2).

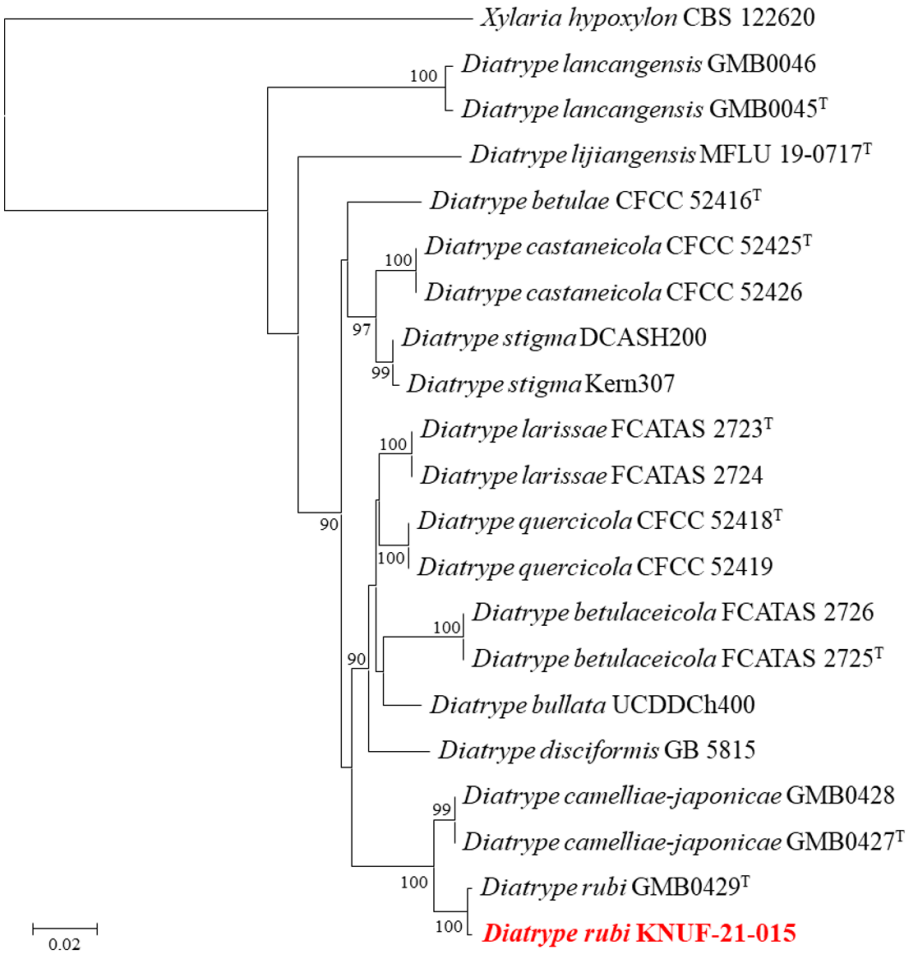

Fig. 2. Neighbor-joining phylogenetic tree based on a combined dataset of partial sequences internal transcribed spacer (ITS) regions and β-tubulin (TUB) gene showing the phylogenetic position of the strain KNUF-22-015 among *Diatrype* species. Bootstrap values greater than 90% (percentage of 1,000 replications) are shown at branching points. The strain isolated in this study is in bold and red. The tree was rooted using *Xylaria hypoxylon* CBS 122620 as an out-group. Bar = 0.02 substitutions per nucleotide position.

Table 2. Morphological characteristics of *Diatrype rubi* KNUF-21-015 compared to closely related *Diatrype species*

Characteristics		*Diatrype rubi* KNUF-21-015[a]	*Diatrype rubi* GMB0429[Tb]	*Diatrype castaneicola* CFCC52425[Tc]
Colony on PDA	Color	White, reverse sepia, no pigmentation on media	White to light yellow, reverse white at the margin, mauve to sepia at the center	White, no pigmentation on media
	Shape	Dense, thinning toward the edge, rough margin, filamentous	Dense, thinning toward the edge, rough margin	Dense, pycnidia distributed irregularly with yellow cream conidial drops
Conidiogenous cell		Cylindrical, straight, discrete or integrated, hyaline, producing conidia at the apex	N/A	Cylindrical, mostly straight, discrete or integrated, hyaline, unicellular, with wide base producing conidia at the apex
Conidia	Size	10.8–23.9 × 1.0–2.0 μm	N/A	4.0–6.0 × 1.0–1.5 μm
	Shape	Hyaline, slightly curved, allantoid, aseptate		Hyaline, elongate-allantoid, slightly curved, smooth, aseptate, multi-guttulate

PDA: potato dextrose agar; N/A: not available in the described paper.

[a] Fungal strain used in this study; [b] Source of description [1]; [c] Source of description[22]; [T] Type strain.

The ambiguity in *Diatrype* taxonomy, largely driven by the reliance on stromatal characteristics, has resulted in polyphyletic genera and inconsistent species classifications. To resolve these issues, we employed multi-locus phylogenetic analyses, focusing on the ITS regions and TUB gene [4]. These analyses provided a more accurate determination of *Diatrype* species and assisted in identifying their plant pathogenicity. Members of the genus *Diatrype* are known to cause grapevine trunk diseases (GTDs) by attacking woody tissues and inducing trunk rots [17]. While several *Diatrype* species are recognized as pathogenic to grapevines [4,18], specific research on the pathogenicity of *D. rubi* is lacking. Notably, this species has also been observed to grow saprophytically on its host, suggesting the need for further study to assess its pathogenic potential. *Diatrype rubi* was first reported in China [1], where it was isolated as a saprobe on the branch surface of its host *Rubus corchorifolius*. The species was first discovered in its sexual morph, while its asexual morph remained uncharacterized. Previous studies indicate that the asexual morphs of many fungi are commonly observed when strains are grown on PCA media [19–22]. Consistent with these findings, strain KNUF-21-015 was cultured on PCA, revealing pycnidia, conidiogenous cells, and conidia. As the anamorph of *D. rubi* had not been reported previously, the observed characteristics were compared to the anamorphs of closely related *Diatrype* species, such as *D. castaneicola* (Table 2). This study presents the first report of the species *D. rubi* in Korea, as well as the first documentation of its anamorph, increasing the number of *Diatrype* species reported in Korea to three: *D. disciformis*, *D. stigma*, and *D. rubi* [5–7]. Considering the high number of reported species worldwide, there is a high likelihood of more species of the genus *Diatrype* to be reported in the Korean peninsula. The identification of the anamorph not only enhances the taxonomic understanding of *Diatrype* species but also serves as a critical step in exploring their ecological roles and pathogenicity. These findings lay a strong foundation for future research into the biology, distribution, and pathogenicity of *D. rubi* and other *Diatrype* species.

CONFLICT OF INTERESTS

No conflict of interests was reported or declared by the authors.

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