MYCOBIOLOGY
2025, VOL. 53, NO. 2, 243–249
https://doi.org/10.1080/12298093.2024.2438463

REVIEW ARTICLE

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A Novel *Phaeoacremonium* Species Isolated from Galls on the Chinese Magnolia-Vine (*Schisandra chinensis*) in Korea

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ABSTRACT

The fungal strain KNUF-24-9L1a, belonging to the genus *Phaeoacremonium*, was isolated from gall-midge (*Lasioptera* sp.; Diptera: Cecidomyiidae) larvae and their galleries on a Chinese magnolia-vine (*Schisandra chinensis*) sample collected in Mungyeong-si, Gyeongbuk province, Korea. Phylogenetic analyses based on concatenated nucleotide sequences of the beta-tubulin and actin genes revealed that the strain clustered with *Phaeoacremonium* species but occupied a distinct phylogenetic position. Morphological differences between strain KNUF-24-9L1a and closely related species were also observed. In this study, we provide detailed descriptions, illustrations, and discussions of the morphological and phylogenetic analyses of closely related species to support the novelty of this isolated species. The phylogenetic and morphological evidence suggests that strain KNUF-24-9L1a represents a novel species within the genus *Phaeoacremonium*, which we have designated this species as *Phaeoacremonium schisandrae* sp. nov.

ARTICLE HISTORY
Received 4 November 2024
Revised 2 December 2024
Accepted 3 December 2024

KEYWORDS
Morphology; phylogeny; *Schisandra chinensis*; gall-midge; *Phaeoacremonium*

1. Introduction

The Chinese magnolia-vine (*Schisandra chinensis*), native to northeastern Asia, is renowned for its distinctive purple-red berries, often referred to as "five-flavor fruits" due to their combination of sweet, bitter, pungent, salty, and sour tastes [1]. *S. chinensis* is highly valued for its adaptogenic properties and is widely used in traditional medicine and phytotherapy, enhancing stress tolerance, energy, and physical performance [2]. Dried fruits and extracts from this plant are known for their benefits in treating neurological, cardiovascular, and gastrointestinal disorders, as well as reducing fatigue and obesity. They also offer antioxidant, anti-inflammatory, antiviral, anticancer, and liver-protective effects [3,4]. The genus *Phaeoacremonium*, belonging to the family Togniniaceae, currently includes 72 described species, with *P. parasiticum* designated as the type species (*MycoBank*; https://www.mycobank.org) [5]. To date, no species from this genus have been reported in Korea. *Phaeoacremonium* species are known pathogens in both plants and humans, with some species isolated from arthropods and soil. Notably, some of *Phaeoacremonium* species are responsible for diseases, such as Petri disease and esca in grapevines [6,7]. The genus is commonly isolated from diseased woody plants, humans with phaeohyphomycosis, arthropods, and soil [8]. Moreover, several species including *P. parasiticum*, *P. scolyti*, and *P. minimum* have been isolated from bark beetle larvae, highlighting the diverse ecology of the genus [9–11].

In this study, we extracted fungi from gall-midge larvae (*Lasioptera* sp.; Diptera: Cecidomyiidae) and their galleries found inside the stem galls of a Chinese magnolia-vine fruit tree (*S. chinensis*) collected in Korea. This work aims to expand our understanding of fungal diversity and to isolate fungi with potential industrial applications. Here, we document and report the morphological and phylogenetic characteristics of the fungal strains isolated.

2. Materials and methods

2.1. Sample collection and fungal strain isolation

Insect gall samples were collected from Mungyeong-si, Gyeongbuk province, Korea (36°42′55.1″N 128°18′53.6″E) in 2024. The samples were placed in individual zip-lock bags and kept cool during transport to the laboratory. The sampled galls were cut longitudinally to examine their contents, and larvae were extracted from the larval

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chambers using sterilized pins. Each larva and tissue of larval chamber was washed once with 70% ethanol and 1% NaClO, then washed three times with sterile distilled water. They were then ground using a hand grinder to isolate the fungi. The samples were suspended in 1.0 mL of sterile distilled water, gently vortexed, and serially diluted. Following this, 100 µL of each sample was spread onto PDA (Difco, Detroit, MI) plates and incubated at 25 °C for 2–3 d. Single colonies were transferred to fresh PDA plates and incubated at 25 °C for 5–7 d. The strains selected for molecular analyses were based on various morphological characteristics. The fungal strains were stored in 20% glycerol at −80 °C for further study. Strains KNUF-24-9L1a, KNUF-24-9L2b, KNUF-24-10L1c, KNUF-24-10L2b, and KNUF-24-15Wb which displayed similar cultural characteristics and were isolated from different larvae, were chosen for further morphological and molecular phylogenetic analyses.

2.2. Cultural and morphological characteristics

Strains KNUF-24-9L1a, KNUF-24-9L2b, KNUF-24-10L1c, KNUF-24-10L2b, and KNUF-24-15Wb were cultured on potato dextrose agar (PDA; Difco, Detroit, MI), malt extract agar (MEA; Difco, Detroit, MI), and oatmeal agar (OA; Difco, Detroit, MI) to study their morphology and growth [12]. Cultural characteristics, such as color, shape, and size, were recorded after 14 d. Morphological features were observed using a BX-50 microscope (Olympus, Tokyo, Japan), and the characteristics of all five strains were found to be identical.

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

Total genomic DNA was extracted from the fungal mycelia of strains KNUF-24-9L1a, KNUF-24-9L2b, KNUF-24-10L1c, KNUF-24-10L2b, and KNUF-24-15Wb, which were cultured on PDA plates, using the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) according to the manufacturer's instructions. Partial beta-tubulin (*TUB*) and actin (*ACT*) genes were amplified using the primer pairs T1 and Bt2b, and ACT-512F and ACT-783R, respectively [13–15]. The internal transcribed spacer (ITS) regions of rDNA were also amplified using the primer pairs ITS1F and ITS4; however, further analysis of these regions was not performed due to insufficient sequence data [16,17]. The PCR products were purified using the ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA) and sequenced by SolGent (Daejeon, Korea). The sequences obtained from the five strains were deposited in the National Center for Biotechnology Information (NCBI) GenBank database (Table 1).

2.4. Phylogenetic analysis

Sequences retrieved from the NCBI were used to establish phylogenetic relationships (Table 1). Partial sequences of the *TUB* and *ACT* genes were analyzed for species identification, supported by the construction of phylogenetic trees using the neighbor-joining method, as described by the Kimura model. The analysis was conducted using MEGA version X, with bootstrap values calculated from 1000 replications [18–20].

3. Results

3.1. Taxonomy

The morphology of strains KNUF-24-9L1a, KNUF-24-9L2b, KNUF-24-10L1c, KNUF-24-10L2b, and KNUF-24-15Wb were distinct from other closely related species of *Phaeoacremonium* such as *P. venezuelense*, *P. aureum*, and *P. alvesii*. As a result, they were classified as a new species. However, since the cultural and morphological characteristics of KNUF-24-9L1a, KNUF-24-9L2b, KNUF-24-10L1c, KNUF-24-10L2b, and KNUF-24-15Wb were identical, only strain KNUF-24-9L1a was selected for further analysis.

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

MycoBank: 856177

Etymology: *schisandrae*: Latin name and specific epithet of the host plant genus (*Schisandra*).

Typus: The strain was isolated from a larva of gall-midge on a Chinese magnolia-vine (*S. chinensis*) collected in Gyeongbuk province, Korea (36°42′55.1″N 128°18′53.6″E) in 2024. The stock culture was deposited in the National Institute of Biological Resources (NIBR) as a metabolically inactive culture (NIBRFGC000512622).

Habitat: Mungyeong-si, Gyeongbuk province, Korea, from a gall-midge larva on the Chinese magnolia-vine (*S. chinensis*).

Description: Colonies on PDA reached 30–31 mm in diameter after 14 d of culture at 25 °C. They appeared circular, beige to grayish orange, dense, with entire margins; reverse whitish orange (Figure 1(D)). On OA, the colonies reached 42–44 mm in diameter after 14 d of culture at 25 °C. They were flat and circular with entire margins, dark brown at the center and white at the edges; reverse whitish yellow (Figure 1(E)). On MEA, the colonies reached 35 mm after

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

Species	Strain	GenBank accession number	
		TUB	ACT
Phaeoacremonium alvesii	CBS 729.97	AY579302	AY579235
Phaeoacremonium alvesii	CBS 110034^T	AY579301	AY579234
Phaeoacremonium amstelodamense	CBS 110627	AY579295	AY579228
Phaeoacremonium aureum	CBS 142691^T	KY906657	KY906656
Phaeoacremonium australiense	CBS 113592	AY579297	AY579230
Phaeoacremonium australiense	CBS 113589^T	AY579296	AY579229
Phaeoacremonium fuscum	CBS 120856^T	EU128098	EU128141
Phaeoacremonium griseorubrum	CBS 111657^T	AY579294	AY579227
Phaeoacremonium griseorubrum	CBS 566.97	AF246801	AY579226
Phaeoacremonium italicum	CBS 137763	KJ534074	KJ534046
Phaeoacremonium alvesii	CBS 729.97	AY579302	AY579235
Phaeoacremonium junior	CBS 142697^T	KY906709	KY906708
Phaeoacremonium parasiticum	CBS 514.52	AY579306	AY579240
Phaeoacremonium parasiticum	CBS 806.73^T	AF246803	AY579253
Phaeoacremonium paululum	CBS 142705	KY906881	KY906880
Phaeoacremonium pravum	CBS 142686	KY084246	KY084248
Phaeoacremonium prolifenatum	CBS 142706^T	KY906903	KY906902
Phaeoacremonium rubrigenum	CBS 112046	AY579305	AY579239
Phaeoacremonium rubrigenum	CBS 498.94^T	AF246802	AY579238
Phaeoacremonium scolyti	CBS 113593	AY579293	AY579225
Phaeoacremonium scolyti	CBS 113597^T	AF246800	AY579224
Phaeoacremonium sphinctrophorum	CBS 694.88	DQ173114	DQ173143
Phaeoacremonium sphinctrophorum	CBS 337.90^T	DQ173113	DQ173142
Phaeoacremonium subulatum	CBS 113587	AY579299	AY579232
Phaeoacremonium subulatum	CBS 113584^T	AY579298	AY579231
Phaeoacremonium venezuelense	CBS 113595	AY579319	AY579255
Phaeoacremonium venezuelense	CBS 651.85^T	AY579320	AY579256
***Phaeoacremonium schisandrae* sp. nov.**	**KNUF-24-9L1a^T**	**PQ276746**	**PQ276742**
***Phaeoacremonium schisandrae* sp. nov.**	**KNUF-24-9L2b**	**PQ276747**	**PQ276743**
***Phaeoacremonium schisandrae* sp. nov.**	**KNUF-24-10L1c**	**PQ276748**	**PQ276744**
***Phaeoacremonium schisandrae* sp. nov.**	**KNUF-24-10L2b**	**PQ276749**	**PQ276745**
***Phaeoacremonium schisandrae* sp. nov.**	**KNUF-24-15Wb**	**PQ540990**	**PQ540991**
Pleurostoma ootheca	CBS 115329^T	MT501325	JX073276

TUB2: beta-tubulin; *ACT*: actin.
^{T}Type strain.
Strains used in this study are indicated in bold.

14 d of culture at 25 °C, with a flat, circular surface and entire margins, displaying a radiating pattern on the mycelium; reverse beige (Figure 1(F)). Conidiophores were hyaline to pale brown, rarely unbranched, arising from aerial or submerged hyphae. They were erect to flexuous, up to 4-septate, and measured 12.5–32.2 × 1.5–2.5 µm (av. = 26.3 × 1.7 µm, $n = 15$). Phialides were terminal or lateral, mostly monophialidic, hyaline to subhyaline; type I phialides were subcylindrical, attenuated at the base, measuring 4.2–14.4 × 1.5–2.0 µm (av. = 9.0 × 1.7 µm, $n = 10$); type II phialides were subcylindrical to ampulliform, tapering toward the apex, measuring 8.6–13.7 × 1.5–2.3 µm (av. = 11.6 × 1.8 µm, $n = 10$); Type III phialides were subcylindrical, measuring 12.1–24.4 × 1.5–2.4 µm (av. = 19.8 × 2.0 µm, $n = 15$) (Figure 1(G–L)). Two types of conidia appear, allantoid or oblong-ellipsoidal, hyaline, 2.3–8.7 × 1.6–3.1 µm (av. = 6.2 × 2.1 µm, $n = 100$) size measured (Figure 1(M)). Sexual morphs and chlamydospores were absent.

Other materials examined: Mungyeong-si, Gyeongbuk province, Korea (36°42′55.1″N 128°18′53.6″E), on gall-midge larva, May 2024, KNUF-24-9L1a, KNUF-24-9L2b, KNUF-24-10L1c, and KNUF-24-10L2b; Mungyeong-si, Gyeongbuk province, Korea (36°42′55.1″N 128°18′53.6″E), larval chamber, May 2024, KNUF-24-15Wb.

Notes: A comparison of strain KNUF-24-9L1a with phylogenetically related strains *P. venezuelense* CBS 651.85^T, *P. aureum* CBS 142691^T, and *P. alvesii* CBS 110034^T revealed distinct morphological differences. Strain KNUF-21-020 produced cream to pale yellow mycelium on MEA, whereas *P. venezuelense* CBS 651.85^T, *P. aureum* CBS 142691^T, and *P. aureum* CBS 142691^T produced beige to orange-brown, dark brick to white, and grayish red to grayish rose color mycelium on MEA, respectively [9,12,21]. Additionally, the conidia of strain KNUF-24-9L1a (2.3–8.7 × 1.6–3.1 µm) were longer than those of *P. venezuelense* CBS 651.85^T (3–4.5 × 1–1.5 µm), *P. aureum* CBS 142691^T (3–5 × 1.5–2 µm), and *P. alvesii* CBS 110034^T (3–6 × 1–2 µm) [9,12,21]. These differences in conidial size and cultural characteristics clearly distinguish strain KNUF-24-9L1a from other species in the genus (Table 2).

3.2. Phylogenetic analysis

The obtained *TUB* and *ACT* gene sequences of KNUF-24-9L1a, KNUF-24-9L2b, KNUF-24-10L1c, KNUF-24-10L2b, and KNUF-24-15Wb were 727 and 233 bp in length, respectively, and the sequences of both genes were identical across all five strains. The partial *TUB* gene sequence of strain KNUF-24-9L1a exhibited maximum similarity (83.7%) with

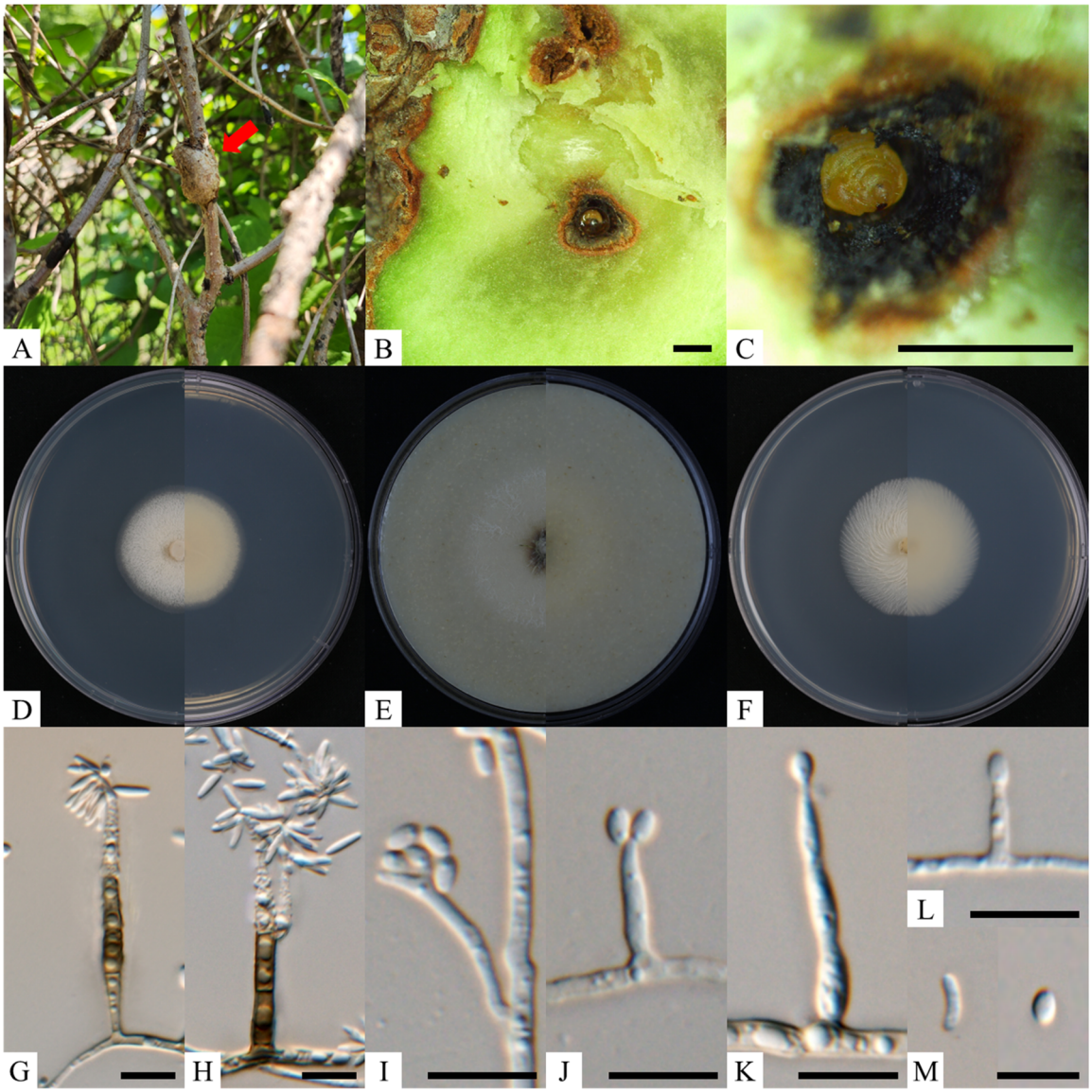

Figure 1. Symptoms observed on *Schisandra chinensis* and morphological characteristics of the *Phaeoacremonium schisandrae* sp. nov. KNUF-24-9L1a^{T}. (A): gall symptom observed on stem (red arrow); (B and C): cross-sections of gall showing larval chamber and larva; (D–F): front and reverse views of colonies on PDA, OA, and MEA, respectively; (G): Conidiophores with phialide and conidia; (H): branched conidiophore with conidia; (J and L): Type I phialide; (I): Type II phialide; (K): Type III phialide; (M): allantoid and oblong-ellipsoidal conidia, respectively. Scale bars: B and C = 1 mm, G–M = 10 µm.

P. venezuelense CBS 113595, while other *Phaeoacremonium* strains showed 78.7%–80.6% similarity with *P. fuscum* CBS 120856^{T}, *P. aureum* CBS 142691^{T}, *P. italicum* CBS 137763^{T}, *P. griseorubrum* CBS 111657^{T}, and *P. alvesii* CBS 729.97. Similarly, the partial *ACT* gene sequence showed a maximum similarity of 82.3% with *P. alvesii* CBS 729.97, while other *Phaeoacremonium* strains, such as *P. venezuelense* CBS 113595, *P. fuscum* CBS 120856^{T}, and *P. aureum* CBS 142691, showed 73.7–82.1% similarity. Using these two molecular markers, the results indicated that analyzing a single gene was insufficient for accurately identifying this novel fungal strain at the species level. Phylogenetic analysis based on *TUB* and *ACT* gene sequences positioned strains KNUF-24-9L1a, KNUF-24-9L2b, KNUF-24-10L1c, KNUF-24-10L2b, and KNUF-24-15Wb in a distinct clade, separate from other *Phaeoacremonium* species in the neighbor-joining tree (Figure 2). Consequently, this novel strain is considered to represent a single, phylogenetically distinct species within the genus *Phaeoacremonium*.

4. Discussion

In this study, the combination of morphological characteristics, cultural traits, and DNA sequence analyses led to the identification of a novel species of *Phaeoacremonium*, isolated from gall-midge larvae and larvae galleries within the stem gall of the Chinese magnolia-vine (*Schisandra chinensis*). This newly discovered species, designated *P. schisandrae*, brings the total number of recognized species within

Table 2. Morphological characteristics of *Phaeoacremonium schisandrae* (KNUF-24-9L1a[T]) and a comparison with the most closely related species of *Phaeoacremonium*.

Characteristics		*P. schisandrae* sp. nov. KNUF-24-9L1aTa	*P. venezuelense* CBS 651.85Tb	*P. aureum* CBS 142691Tc	*P. alvesii* CBS 110034Td
Colony	Shape and color	PDA: flat, felty, beige to grayish orange	PDA: N/A	PDA: flat, felty, dark brick	PDA: flat, wooly textured, and brownish orange
		OA: flat, dark brown to white	OA: N/A	OA: flat, felty, mouse grey to grayish sepia	OA: flat, felty, grayish orange to orange-white
		MEA: flat, velvety, cream to pale yellow	MEA: beige to orange-brown	MEA: flat, felty, dark brick to white	MEA: flat, felty, grayish red to grayish rose
Conidiophore		Hyaline to pale brown, 0–4 septate, 12–32 × 1.5–2.5 µm	Hyaline to pale brown, 1–4 septate, 28–34 × 1–2.5 µm	Hyaline to golden brown, 0–4 septate, 15–38 × 2.5–4 µm	Hyaline to pale brown, 0–2 septate, 14–50 × 1.5–2 µm
Phialide	Type I	Subcylindrical, 4.2–14.4 × 1.5–2.0 µm	Subcylindrical, 5–14 × 1–1.5 µm	Subcylindrical 4–17 × 1–2.5 µm	Cylindrical, 4–12 × 1–1.5 µm
	Type II	Subcylindrical to ampulliform, 8.6–13.7 × 1.5–2.3 µm	Subcylindrical to navicular, 12–14 × 1.5–2 µm	Elongate-ampulliform to subcylindrical, 9–13.5 × 2–3 µm	Subcylindrical to navicular, 10–14 × 1.5–2 µm
	Type III	Subcylindrical 12.1–24.4 × 1.5–2.4 µm	Subcylindrical, 15–23 × 1–2 µm	Subcylindrical 14.5–21.5 × 2–2.5 µm	Subcylindrical 13–22 × 1.5–2.5 µm
Conidia		Oblong-ellipsoidal or allantoid 2.3–8.7 × 1.6–3.1 µm	Oblong-ellipsoidal or fusiform-ellipsoidal, 3–4.5 × 1–1.5 µm	Oblong-ellipsoidal to subcylindrical, 3–5 × 1.5–2 µm	Obovoid or oblong-ellipsoidal, reniform 3–6 × 1–2 µm

[a]Fungal strain of this study, [b]Source of description [9], [c]Source of description [12], [d]Source of description [21], and [T]Type strain.
PDA: potato dextrose agar; OA: oatmeal agar; MEA: malt extract agar.

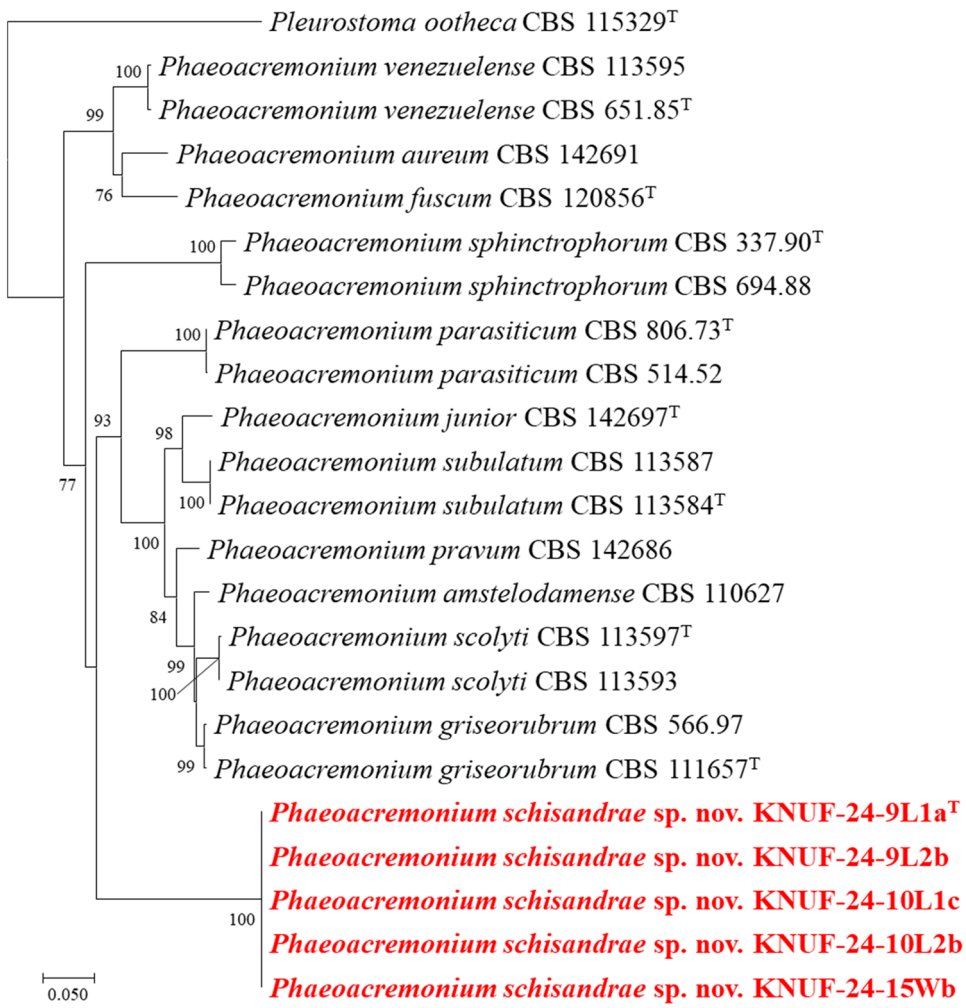

Figure 2. Neighbor-joining phylogenetic tree based on a combined dataset of partial beta-tubulin (*TUB*) and actin (*ACT*) gene sequences, showing the phylogenetic position of five isolated strains in the genus *Phaeoacremonium*. Bootstrap values greater than 70% (from 1000 replications) are indicated at branching points. The novel strain isolated in this study is highlighted in bold and red. The tree is rooted with *Pleurostoma ootheca* CBS 115329[T] as the outgroup. The bar represents 0.050 substitutions per nucleotide position.

the genus *Phaeoacremonium* to 73 and represents the first report of insect-isolated species. Although there are no reports of insect pathogens in the genus *Phaeoacremonium*, however several species including *P. chlamydospora* and *P. parasiticum* have been reported as plant pathogens causing esca disease on grapevine stems and leaves [21,22]. While most known *Phaeoacremonium* isolates have been obtained from plants and humans, some have also been isolated from arthropods, including bark beetles [23]. Numerous studies on plant diseases have shown that arthropods can act as vectors, carrying fungal spores either externally or internally, thereby facilitating their spread to susceptible infection sites [24]. Typically, fungi in this genus are associated with larvae of bark beetles (Scolytidae) and metallic wood-boring beetles (Buprestidae), which have caused significant damage, including dieback in cherry trees [25,26]. Prior to this study, there had been no reports of *Phaeoacremonium* isolated from gall-midge larvae or *S. chinensis*. However, there have been reports of secondary fungal infections, such as those caused by *Alternaria* following gall-midge (*Procontarinia matteiana*) infestations [25]. Additionally, *P. chlamydospora*, a known phytopathogen of grapevines, has been shown to cause dieback in trunks and vine arms, with arthropods acting as vectors [21]. This suggests the possibility that *P. schisandrae* could similarly infect *S. chinensis*. Furthermore, gall-midge species, such as *Lasioptera* and *Asphondylia*, create galls that also serve as habitats for various fungi [26]. The intricate triad of interactions among galls, gall midges, and fungi has been documented in various host plants [27–31]. This emphasizes the ecological significance of the interactions between *P. schisandrae*, its gall-midge vectors, and the host plant, warranting further investigation into their potential roles in plant health.

The results of this study open new avenues for further research to explore the role of gall-midge larvae as vectors in the spread of *Phaeoacremonium* species, including *P. schisandrae*, in *S. chinensis*. It is widely accepted that species within the genus *Phaeoacremonium* have a broad host range and exhibit little host specificity [32]. Given that *P. schisandrae* is the first species in the genus to be reported from gall-midge larvae on *S. chinensis*, additional species within the genus are likely to be discovered. Furthermore, since many *Phaeoacremonium* species are pathogenic, the pathogenicity of *P. schisandrae* warrants further investigation. In addition, various novel and unreported fungal species continue to be reported from various isolation sources, such as insect, plant, and soil collected in Korea [32–36]. The report of *P. schisandrae* sp. nov. isolated from *Schisandra chinensis* and gall-midge larvae in this study expands our understanding of the distribution of fungi in Korea.

Disclosure statement

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

Funding

This work was supported by a grant from the National Institute of Biological Resources (NIBR) – Korea funded by the Ministry of Environment (MOE) of the Republic of Korea [NIBRE202406].

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