RESEARCH ARTICLE

First Report of *Varicosporellopsis shangrilaensis* Isolated from Soil in Korea

You-Jin Kim[1], Seong-Keun Lim[1], Song-Woon Nam[1], Chang-Gi Back[2], Leonid N. Ten[3], Seung-Yeol Lee[1,3], and Hee-Young Jung[1,3*]

[1]Department of Plant Medicine, Kyungpook National University, Daegu, 41566, Korea
[2]Department of Environmental Horticulture and Landscape Architecture, Environmental Horticulture, Dankook University, Cheonan, 31116, Korea
[3]Institute of Plant Medicine, Kyungpook National University, Daegu, 41566, Korea

*Corresponding author: heeyoung@knu.ac.kr

ABSTRACT

The fungal strain designated as KNUF-21-033 was isolated from a soil sample collected in Gunwi-gun, Daegu, Korea. It resembled *Varicosporellopsis shangrilaensis* in morphological characteristics, including colony color and the shapes of macroconidia and microconidia. The colonies formed white and cottony mycelium on potato dextrose agar (PDA). The conidiophores were erect, septate, and either unbranched or branched at the base, measuring 21.5–53.5 × 2.5–5.1 µm. The macroconidia and microconidia measured 13.7–22.6 × 3.8–6.9 µm and 4.0–8.8 × 3.5–7.1 µm, respectively. The obtained sequences of the internal transcribed spacer (ITS) regions and the 28S large subunit (LSU) of the ribosomal RNA, β-tubulin (*TUB*), and actin (*ACT*) genes of isolate KNUF-21-033 exhibited high similarity to strains CGMCC 3.21000 and KLF 01 of *V. shangrilaensis.* The topology of the maximum likelihood phylogenetic tree constructed using the concatenated ITS, LSU, *TUB*, and *ACT* sequences confirmed the affiliation of KNUF-21-033 with *V. shangrilaensis.* Morphological observations and phylogenetic analyses indicated that KNUF-21-033 was indeed a *V. shangrilaensis* strain. To the best of our knowledge, this is the first report of this fungal species in Korea.

Keywords: Multilocus sequence analysis, Nectriaceae, Soil-inhabiting fungi, Unreported species, *Varicosporellopsis shangrilaensis*

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2025 March, 53(1):21-28
https://doi.org/10.4489/kjm.2025.53.1.3

Received: January 22, 2025
Revised: March 15, 2025
Accepted: March 17, 2025

INTRODUCTION

The family Nectriaceae within the phylum Ascomycota occupies a broad range of ecological niches. Members of this family are predominantly soil-borne saprobes or plant pathogens, whereas some species parasitize other fungi or insects. Additionally, several species have been reported to be significant human pathogens, some of which produce mycotoxins [1]. Since 1865, at least 69 genera have been described in the family Nectriaceae, and advancements in molecular biology have continued to expand the family to include new genera and species [2]. The representative genera within this family include *Xenoacremonium*, *Paracremonium*, and *Varicosporellopsis*. The genus *Varicosporellopsis* was first established by Lechat

and Fournier (2016) with the description of *V. aquatilis* isolated from freshwater habitats in southern France [3]. Subsequently, *V. americana*, discovered in the United States, was described by Crous et al. [4]. Another species, *V. shangrilaensis* was reported in China and isolated from the rhizosphere soil of *Astragalus polycladus* [5]. These examples highlight that species of *Varicosporellopsis* have been isolated from diverse environments, including freshwater, waterlogged wood, and rhizosphere soils, classifying them as environmental microorganisms [3–6]. To date, this genus comprises three described species. Morphologically, the type species of *Varicosporellopsis* is characterized by macronematous, mononematous, unbranched, flexuous conidiophores with smooth, hyaline walls. Conidiogenous cells are monophialidic, featuring a slightly flared collarette, and produce narrowly ellipsoidal to subcylindrical, smooth, hyaline conidia. The establishment of the three above-mentioned members of the genus *Varicosporellopsis* was performed by applying phylogenetic analysis based on molecular markers such as internal transcribed spacer (ITS) regions and the 28S large subunit (LSU) of the ribosomal RNA, β-tubulin (*TUB*), and actin (*ACT*) genes [3–6].

The aim of this study was to explore the diversity of indigenous fungal species in Korea. As part of this effort, a fungal strain, designated KNUF-21-033, was isolated from soil in Korea. This study aimed to identify and characterize previously unreported fungal species belonging to the genus *Varicosporellopsis*. Morphological and molecular analyses were conducted to identify the fungal strains, and the findings are presented below.

MATERIALS AND METHODS

Sample collection and fungal isolation

A soil sample was collected from Gunwi-gun, Daegu, Korea (35°59'33.9"N 128°41'12.7"E) and brought to the laboratory. Fungal strains were isolated using serial dilutions. One gram of each soil sample was mixed with 10 mL of sterile distilled water, vortexed, serially diluted, and spread onto potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates. All plates were incubated at 25°C for 7 days. Well-growing colonies were isolated, cultured on fresh PDA plates, and incubated under the same conditions. Several fungal strains were isolated and initially identified using ITS sequences. As a result, isolate KNUF-21-033 was preliminarily identified as a fungal species not previously reported in Korea. Further cultural, morphological, and molecular phylogenetic analyses were conducted to identify the isolates. Isolate KNUF-21-033 was deposited at the National Institute of Biological Resources (NIBR) as a metabolically inactive culture (NIBRFGC000509258).

Cultural and Morphological characterization

For morphological analysis, isolate KNUF-21-033 was incubated at 25°C on PDA for 10 days [5]. After 10 days, the morphological characteristics of the strain, such as color, size, and shape, were

recorded. Observations, measurements, and photographs of the colonies, conidia, conidiogenous cells, and conidiophores were taken using a light microscope (BX-50, Olympus, Tokyo, Japan).

DNA extraction, PCR, and sequencing

Total genomic DNA was extracted using the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) following the manufacturer's instructions. For molecular analysis, ITS regions and LSU, *TUB*, and *ACT* genes were amplified via PCR. PCR amplification was performed using primer pairs ITS1/ITS4 for the ITS regions [7,8], LR0R/LR7 for LSU [9], T1/T22 for *TUB* [10], and ACT512F/ACT1Rd for *ACT* [11,12]. The amplification conditions for each gene have been previously described [5].

Phylogenetic analysis

A phylogenetic tree was constructed using ITS, LSU, *TUB*, and *ACT* sequences for strain KNUF-21-033, along with closely related strains whose sequences were retrieved from the National Center for Biotechnology Information (NCBI) GenBank database. Basic Local Alignment Search Tool (BLAST) was used for sequence comparison. Twelve taxa were included in the phylogenetic analysis (Table 1). The sequences, including those of KNUF-21-033, were aligned to each molecular marker. The aligned datasets for each strain were concatenated in the following order: ITS regions, LSU, *TUB*, and *ACT* genes. The Kimura model was used to estimate evolutionary distances and distance matrices were generated based on these calculations [13]. A phylogenetic tree was constructed using the maximum likelihood (ML) method in MEGA 11.0 software and bootstrapped with 1,000 replicates to ensure statistical robustness [14,15].

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

Species	Strain	GenBank accession numbers			
		ITS	LSU	*TUB*	*ACT*
Corallomycetella elegans	CBS 275.60	KM231828	KM231710	KM232100	KM231237
Microcera coccophila	MAFF 241482	KC291752	KC291787	KC291936	–
Paracremonium contagium	CBS 110348	NR_154313	NG_058130	KM232103	KM231240
Paracremonium inflatum	CBS 485.77	NR_154312	NG_058129	KM232101	KM231238
Varicosporellopsis americana	CBS 148257^{T}	NR_175234	NG_081341	OK651211	OK651131
Varicosporellopsis aquatilis	CBS 143509	MH107922	MH107968	MH108052	MH107987
Varicosporellopsis shangrilaensis	CGMCC 3.21000^{T}	OM956089	OP223500	OQ658529	OQ658531
Varicosporellopsis shangrilaensis	**KNUF-21-033**	**LC858579**	**LC858580**	**LC858581**	**LC858582**
Varicosporellopsis shangrilaensis	KLF 01	OP223502	OP223501	OQ658530	OQ658532
Xenoacremonium falcatus	CBS 400.85	KM231832	HQ232025	KM232104	–
Xenoacremonium recifei	CBS 137.35^{T}	KM231833	HQ232106	KM232105	KM231241
Atractium crassum	CBS 180.31^{T}	KM231790	MH866623	KM232049	HQ897859

ITS: internal transcribed spacer regions; LSU: the 28S large subunit of the ribosomal RNA gene; *TUB*: β-tubulin gene; *ACT*: actin gene.
^{T} Type strain. The strain isolated in this study is indicated in boldface.

RESULTS AND DISCUSSION

Cultural and morphological characteristics

After 10 days of incubation, the colony diameter of strain KNUF-21-033 reached 26.0–28.0 mm and exhibited a white, cottony surface (Fig. 1A and B). After more than two weeks of incubation, the colonies gradually turned pale yellow. The conidiophores of the isolate measured 21.5–53.5 × 2.5–5.1 μm, were straight, segmented, and either unbranched or branched at the base. Its conidiogenous cells measured 2.9–7.7 × 3.1–5.9 μm and featured a diminutive collarette, bearing small conidia at the apex. Strain KNUF-21-033 produced two types of conidia: macroconidia and microconidia. The macroconidia were kidney-shaped or boat-shaped, linear or curved, guttulate, and measured 13.7–22.6 × 3.8–6.9 μm. The microconidia were spherical or droplet-shaped, guttulate, and measured 4.0–8.8 × 3.5–7.1 μm (Fig. 1C–F). The cultural and morphological characteristics of strain KNUF-21-033 closely resembled those of *V. shangrilaensis* [5] (Table 2). However, as shown in Table 2, strain KNUF-21-033 can be distinguished from its close relative *V. americana* by several morphological traits. The conidiophores of *V. americana* are larger (40.0–70.0 × 3.0–4.0 μm), solitary, and branched at the base. Additionally, the conidiogenous cells of *V. americana* are significantly larger (30.0–50.0 × 3.0–4.0 μm), subcylindrical, and feature a flared collarette that produces slimy, hyaline, ellipsoid conidia, with a subobtuse apex and a tapered base. These conidiogenous cells and conidia differed markedly from those of strain KNUF-21-033 in terms of size and shape.

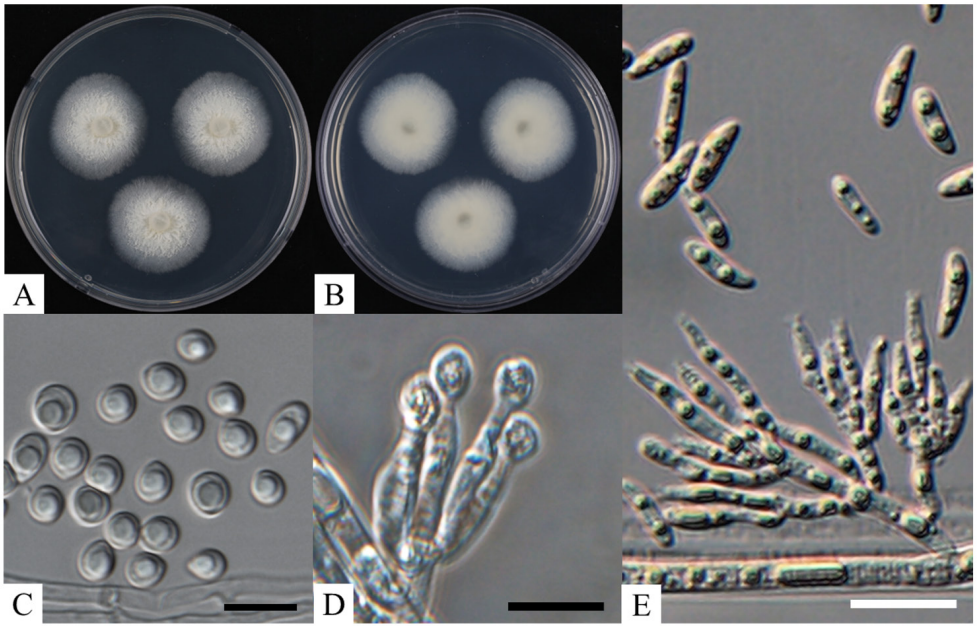

Fig. 1. Culture and morphological characteristics of KNUF-21-033. A: surface of the colony on potato dextrose agar (PDA); B: reverse of the colony on PDA; C: microconidia; D: conidiogenous cell and conidiophore; E: macroconidia, conidiogenous cell, and conidiophore (Scale bar C, D = 10 μm, E = 20 μm).

Table 2. Comparison of the morphological characteristics of strain KNUF-21-033 with those of previously reported *Varicosporellopsis* species

Characteristics		*Varicosporellopsis shangrilaensis*[a] KNUF-21-033	*V. shangrilaensis*[b] CGMCC 3.21000	*V. americana*[c] CBS 143509
Colony	Color	White, gradually turning to pale yellow	Initially white, gradually turning to pale yellow	Saffron on both the surface and reverse
	Size (diam)	26.0–28.0 mm in 10 days at 25℃	30 mm in 10 days at 26℃	30 mm in 14 days at 25℃
	Shape	Cottony	Cottony	Fat, smooth, and lobate margin
Conidiophores	Size (μm)	21.5–53.5 × 2.5–5.1	N/A	40.0–70.0 × 3.0–4.0
	Shape	Straight, segmented, either unbranched or branched at the base	Macronematous, erect, septate, unbranched or branched at the base	Solitary, erect, branched at base, 0–2-septate cells
Conidiogenous cell	Size (μm)	2.9–7.7 × 3.1–5.9	10.0–20.0 × 3.0–5.0	30.0–50.0 × 3.0–4.0
	Shape	Phialidic with a diminutive collarette, bearing small conidia at the apex	Phialidic with a minute collarette	Subcylindrical with slight apical taper, hyaline, smooth, apex phialidic with flared collarette, giving rise to clusters of slimy conidia
Conidia	Size (μm)	Macroconidia: 13.7–22.6 × 3.8–6.9 Microconidia: 4.0–8.8 × 3.5–7.1	Macroconidia: 8.0–20.0 × 2.5–8.5 Microconidia: 3.5–6.5 × 3–6	(10 –)12–16(–24) × (3–)4(–4.5)
	Shape	Macroconidia: kidney-shaped or boat-shaped, linear or curved, guttulate Microconidia: round or droplet-shaped, guttulate	Macroconidia: reniform or cymbiform, straight or slightly curved, guttulate Microconidia: spherical or t eardrop-shaped, guttulate	Solitary, hyaline, smooth, guttulate, ellipsoid, aseptate, straight to curved, apex subobtuse, base tapered to a truncate hilum

PDA: potato dextrose agar; diam: diameter.
[a] Fungal strain used in this study; [b] Source of the description [5]; [c] Source of the description [4].

Phylogenetic relationships of KNUF-21-033

To identify strain KNUF-21-033 at the species level, the nucleotide sequences of the ITS, LSU, *TUB*, and *ACT* loci were obtained (572, 1328, 1332, and 1191 bp, respectively) and compared with those of other strains in the GenBank database using BLAST. The ITS sequence of the isolate exhibited 99.8% similarity with those of two *V. shangrilaensis* strains, CGMCC 3.21000 (OM956089) and KLF 01 (OP223502); 96.0% similarity with *V. americana* CBS 148257 (NR_175234); and a significantly lower similarity of 92.9% with *Paracremonium inflatum* CBS 485.77 (NR_154312). The LSU sequence showed 99.9% similarity with the two *V. shangrilaensis* strains, CGMCC 3.21000 (OP223500) and KLF 01 (OP223501), and 97.7% similarity with several strains of *Fusicolla quarantenae*, including GZUIFR 21.906 (OL897056), GZUIFR 21.907 (OL897057), and CGMCC 3.20777 (OL897054). Unfortunately, BLAST searches for the *TUB* and *ACT* gene sequences of strain KNUF-21-033 did not return results for the aforementioned *Varicosporellopsis* strains. Therefore, similarity values for the *TUB* and *ACT* sequences were calculated via pairwise comparisons. The *TUB* sequence of strain KNUF-21-033 exhibited 99.6% similarity with *V. shangrilaensis* CGMCC 3.21000 (OQ658529) and KLF 01 (OQ658530) and only 92.3% similarity with *V. americana* CBS 148257 (OK651211). The *ACT* sequence showed 96.2% similarity with *V. shangrilaensis* CGMCC 3.21000 (OQ658531), 96.9% similarity with KLF 01 (OQ658532), and 95.5% similarity with *V. americana* CBS 148257 (OQ651131). Based on these comparisons, it was evident that the use of a single molecular marker is insufficient for accurate species-level identification. Therefore, a multilocus sequence analysis (MLSA) was performed. For the establishment of the genus *Varicosporellopsis*, ITS and LSU sequences were initially used [6], while more recently, ITS, LSU, *TUB*, and *ACT* sequences were utilized

to establish *V. shangrilaensis* [5]. Following the latter approach, MLSA was performed using concatenated sequences of the ITS, LSU, *TUB*, and *ACT* loci. The combined sequences were used to construct an ML phylogenetic tree (Fig. 2). The resulting topology clearly indicated that three strains, KNUF-21-033, CGMCC 3.21000, and KLF 01, belonged to *V. shangrilaensis*. The results of both morphological and phylogenetic analyses identified strain KNUF-21-033 as *V. shangrilaensis*. To the best of our knowledge, this is the first report on this fungal species in Korea.

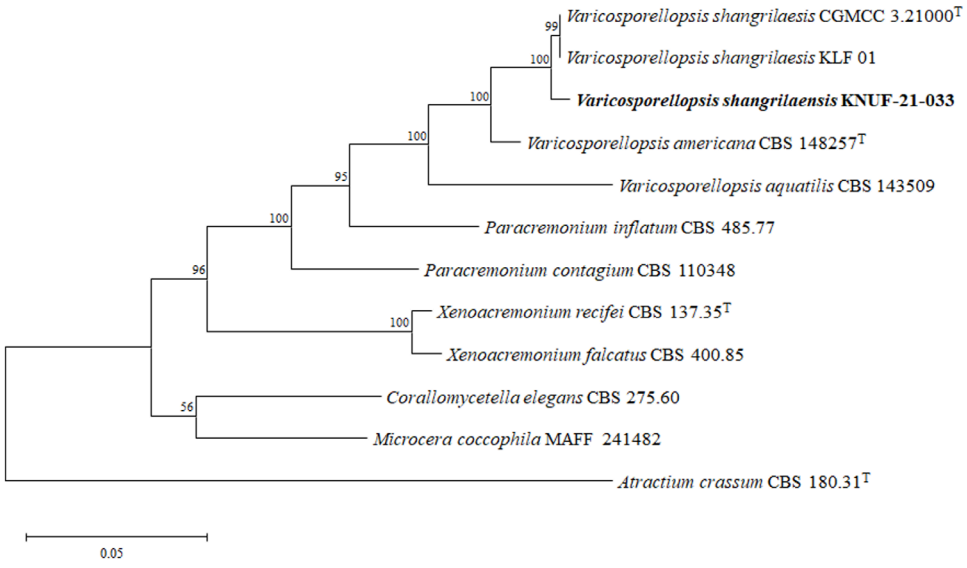

Fig. 2. Maximum likelihood phylogenetic tree based on a combined dataset of particle sequences of internal transcribed spacer (ITS) regions and the 28S large subunit (LSU), β-tubulin (*TUB*) and actin (*ACT*) genes. *Atractium crassum* CBS 180.31^{T} was used as an out-group. The strain isolated in this study is in bold, and the bootstrap values are based on 1,000 replications. Bar, 0.05 substitutions per nucleotide position.

Members of the genus *Varicosporellopsis* have been isolated from submerged wood in freshwater environments and rhizosphere soil and are considered environmental microorganisms [3–6]. Molecular phylogenetic analyses showed that *Varicosporellopsis* is closely related to the genera *Paracremonium*, *Xenoacremonium*, and *Corallomycetella* [2], with *Paracremonium* being the closest relative. Notable species of *Paracremonium* include *P. inflatum* and *P. contagium*, which were isolated from granulomatous and subcutaneous lesions in humans in India and Canada, respectively [1]. Additionally, *P. pembeum* was recovered from an ambrosia beetle (*Euwallacea* sp.), and *P. lepidopterorum* was isolated from a lepidopteran pupa [16,17]. Although *Varicosporellopsis* species are generally regarded as environmental microorganisms, the discovery of a pathogenic *Paracremonium* species isolated from human lesions underscores their potential medical significance of *Varicosporellopsis*. These findings highlight the need for detailed studies on the distribution, biological activities, and potential pathogenicity of *Varicosporellopsis*

spp. Moreover, reports on *Paracremonium* species forming symbiotic relationships with insects suggest that *Varicosporellopsis* may exhibit similar associations. Despite its recent establishment as a genus, *Varicosporellopsis* currently includes only three described species [3–6], and no substantial follow-up studies have been conducted on them. This limited knowledge emphasizes the importance of exploring new members of this genus and studying the ecological, etiological, and biological roles of the known species. Beyond the documented sources of its isolation and its cultural, morphological, and phylogenetic traits, little information is available regarding this genus. Further investigation is essential to achieve a more comprehensive understanding of *Varicosporellopsis* and its species.

This study is the first report of *V. shangrilaensis* infection in Korea. The isolated strain, KNUF-21-033, identified as a member of *V. shangrilaensis*, provides a valuable resource for future research on this species in Korea. Its isolation offers an opportunity to deepen our insight into the ecological, etiological, and biological roles of *V. shangrilaensis*, particularly under the unique environmental conditions of Korea. This study also contributes to enhancing the understanding of the fungal diversity indigenous to Korea, underscoring the need for continued exploration of the country's fungal biodiversity.

CONFLICT OF INTERESTS

The authors declare that they have no potential conflicts of interest.

ACKNOWLEDGEMENTS

This study was supported by the National Institute of Biological Resources funded by the Ministry of Environment of the Republic of Korea (NIBR202102107).

REFERENCES

1. Lombard L, van der Merwe NA, Groenewald JZ, Crous PW. Generic concepts in *Nectriaceae*. Stud Mycol 2015;80:189–245.
2. Li X, Han SL, Zhang YY, Cai L, Zhao P. *Heteroverticillium phytelephatis* gen. et sp. nov. intercepted from nuts of *Phytelephas macrocarpa*, with an updated phylogenetic assessment of *Nectriaceae*. Mycology 2023;14:155–74.
3. Lechat C, Fournier J. *Varicosporellopsis*, a new aquatic genus from southern France. Ascomycete.org 2016;8:96–100.
4. Crous PW, Osieck ER, Jurjević Ž, Boers J, van Iperen AL, Starink-Willemse M, Dima B, Balashov S, Bulgakov TS, Johnston PR, et al. Fungal planet description sheets: 1284–382. Persoonia 2021;47:178–374.
5. Dou ZR, Zhang F, Zhou GJ, Gong GF, Ding JJ, Fan XH, Jiang B, Wang K. *Varicosporellopsis shangrilaensis* sp. nov. (Nectriaceae, Hypocreales), a new terricolous species isolated from the rhizosphere soil of *Astragalus polycladus* in Northwestern Yunnan, China. Phytotaxa 2023;600:230–38.

6. Lechat C, Fournier J. *Varicosporella*, a new aquatic genus in the *Nectriaceae* from France. Ascomycete.org 2015;7:1–8.
7. White TJ, Bruns TD, Lee SB, Taylor JW. Amplification, and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York: Academic Press; 1990. p. 315–22.
8. Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 1993;2:113–8.
9. Bunyard BA, Nicholson MS, Royse DJ. A systematic assessment of *Morchella* using RFLP analysis of the 28S ribosomal RNA gene. Mycologia 1994;86:762–72.
10. Bills GF, Platas G, Overy DP, Collado J, Fillola A, Jiménez MR. Martín J, del Val AG, Vicente F, Tormo JR, et al. Discovery of the parnafungins, antifungal metabolites that inhibit mRNA polyadenylation, from the *Fusarium larvarum* complex and other Hypocrealean fungi. Mycologia 2009;101:449–72.
11. Cabral A, Rego C, Nascimento T, Oliveira H, Groenewald JZ, Crous PW. Multi-gene analysis and morphology reveal novel *Ilyonectria* species associated with black foot disease of grapevines. Fungal Biol 2009;116:62–80.
12. Groenewald JZ, Nakashima C, Nishikawa J, Shin HD, Park JH, Jama AN, Groenewald M, Braun U, Crous PW. Species concepts in *Cercospora*: spotting the weeds among the roses. Stud Mycol 2013;75:115–70.
13. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980;16:111–20.
14. Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 1971;20:406–16.
15. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol 2021;38:3022–7.
16. Lynch SC, Twizeyimana M, Mayorquin JS, Wang DH, Na F, Kayim M, Kasson MT, Thu PQ, Bateman C, Rugman-Jones P, et al. Identification, pathogenicity and abundance of *Paracremonium pembeum* sp. nov. and *Graphium euwallaceae* sp. nov.—two newly discovered mycangial associates of the polyphagous shot hole borer (*Euwallacea* sp.) in California. Mycologia 2016;108:313–29
17. Ming DQ, Luo LY, He XX, Wang MS, Fang WX, Chen SF, Chen WH, Han YF, Liang ZQ. *Paracremonium lepidopterorum*, a new insect-associated fungus. Phytotaxa 2021;524:85–91.