RESEARCH ARTICLE

A New Report of *Biscogniauxia petrensis* Isolated from Mosquitoes in Korea

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ABSTRACT

A fungal strain designated KNU-WDM2A2 was isolated from mosquitoes in Gimcheon, Korea. The pure culture was transferred to potato dextrose agar (PDA) and synthetic nutrient agar (SNA) media and attained a diameter of 90 mm after 10 days of incubation at 25°C. The colonies were whitish to light pink and cottony to wooly, with an abundant production of aerial mycelia. The strain produced hyaline to slightly yellowish conidiophores that were rough-walled and branched, with conidiogenous cells arising terminally or laterally. Conidia were unicellular, hyaline to light brown, smooth, and oval or ovoid to clavate, with a size of 4.1-6.9×2.5-3.3 µm (n=65). A phylogenetic analysis was conducted using the internal transcribed spacer (ITS) regions and 28S rDNA of large subunit (LSU) sequences, to support the cultural and morphological characteristics. The KNU-WDM2A2 strain was identified here as *Biscogniauxia petrensis*, new to Korea.

Keywords: *Biscogniauxia petrensis*, morphology, mosquito, phylogeny

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INTRODUCTION

Sordariomycetes is the second largest class of Ascomycota [1] and includes 1,331 genera distributed across 105 families, 32 orders, and six subclasses [2]. The Xylariaceae family includes 85 genera and more than 1,350 species, which render it one of the largest and most diverse families of Ascomycota [2,3]. *Biscogniauxia* is a genus of the Xylariaceae family that is characterized by stromatic ascomata, unitunicate asci with an amyloid apical apparatus, a brown germ slit of ascospores, and hyphomycetous asexual morphs with holoblastic conidiogenesis [4]. The phylogenetic affinities between *Biscogniauxia* and Hypoxyloideae have been shown, as well as its clustering with *Camillea* on a sister clade to *Annulohypoxylon*, *Daldinia*, and *Hypoxylon* [5]. Recently, a large-scale multilocus phylogenetic analysis of Xylariaceae revealed a well-supported *Biscogniauxia* clade within Hypoxyloideae, which includes *Camillea* and *Obolarina* [3]. However, phylogenetic studies have placed it with the Graphostromataceae family, which is close

to Xylariaceae, while genera such as *Biscogniauxia* and *Camillea* have been described to share similar morphological characters [6,7]. Therefore, the recognition of a separate family called Graphostromataceae was deemed doubtful based on the molecular data available at the time, and Graphostromataceae was synonymized with Xylariaceae [2]. However, the family Graphostromataceae was resurrected and the *Biscogniauxia*, *Camillea*, and *Obolarina* genera were added to it based on additional molecular evidence [8].

During the screening of fungal species in Korea, the KNU-WDM2A2 strain was isolated from mosquitoes and identified as *Biscogniauxia petrensis*, which is a fungal species that had not been reported in Korea. In this study, molecular phylogenetic analyses were used to identify the undescribed fungal species, and its cultural and morphological characteristics were examined.

MATERIALS AND METHODS

Mosquito collection and fungal strain isolation

In 2019, mosquito samples were collected in Gimcheon (36°11'00.6"N, 128°07'05.8" E), Korea, using an insect net. The mosquito samples were placed in a 1.5 mL Eppendorf tube and transported to the laboratory for fungal strain isolation. The captured mosquitoes were identified as *Ades albopictus* based on morphological structures. Each mosquito sample was washed once with 70% ethanol, twice with sterile distilled water, and then grinded using a hand grinder. Subsequently, the samples were suspended in 1.0 mL of sterile distilled water, vortexed gently, and diluted serially, followed by the spreading of 100 μL of each sample onto PDA (Difco, Detroit, MI, USA) plates and incubation at 25°C for 2-3 days. Single colonies were transferred to PDA plates and incubated at 25°C for 5-7 days. The strain was selected for molecular analyses based on various morphological characteristics and the fungal strain was stored for further study in 20% glycerol at -80°C.

Morphological characterization

PDA culture medium and synthetic nutrient agar (SNA: agar 14.0 g/L of agar, 1.0 g/L of KH_2PO_4, 1.0 g/L of KNO_3, 0.25 g/L of $MgSO_4.7H_2O$, 0.5 g/L of KCl, 0.2 g/L of glucose-, and 0.2 g/L of sucrose) [9] were used to study the cultural and morphological characteristics of the KNU-WDM2A2 strain after incubation at 25°C for 14 days [10]. Subsequently, the cultural characteristics, such as colony color, fungal growth, and texture, were recorded, and the morphology of the fungus was observed using a light microscope (BX-50; Olympus, Tokyo, Japan).

Genomic DNA extraction, PCR amplification, and sequencing

The genomic DNA was extracted from fungal mycelia grown on PDA for 5-7 days at 25°C. The HiGene Genomic DNA prep kit (BIOFACT, Daejeon, Korea) was used as per the manufacturer's instructions. The PCR amplification process was carried out on the internal transcribed spacer (ITS) regions using the primer pairs ITS1F/ITS4 [11,12] and the 28S rDNA large subunit (LSU) with LROR/LR5 [13]. Finally, the

amplified PCR products were purified using EXOSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced by Solgent Co., Ltd. (Daejeon, Korea).

Molecular phylogenetic analysis

To perform the phylogenetic analysis, the consensus sequences were retrieved from the GenBank database of the National Center for Biotechnology Information (NCBI) (Table 1). Evolutionary distance matrices were generated based on neighbor-joining algorithm with the Kimura model [14]. The phylogenetic analyses were performed using the MEGA 7.0 software with bootstrap analysis of 1,000 replications [15].

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

Species	Strain Number	GenBank Accession Number	
		ITS	LSU
Biscogniauxia capnodes	YMJ 142	DQ631933	DQ840055
Biscogniauxia nummularia	MUCL 51395	KY610382	KY610427
Biscogniauxia petrensis	LC5751	KU746669	KU746715
Biscogniauxia petrensis	**KNU-WDM2A2**	**LC535367**	**LC535368**
Collariella quadrangulate	CBS 142.58	KX976650	MH869267
Collariella robusta	LZT0008	KY132106	KY247256
Gymnoascus exasperates	LC5640	KU746682	KU746728
Gymnoascus reesii	LCP 60.1696	JQ434569	JQ434633
Humicola olivacea	DTO 319-C7	KX976676	KX976770
Microdochium albescens	CBS 243.83	KP858994	KP858930
Microdochium phragmitis	CBS 423.78	KP859012	MH872924
Phaeosphaeria caricicola	CBS 603.86	KF251182	KF251685

ITS: Internal transcribed spacer regions; LSU: 28S rDNA.
The isolated strain obtained from the present study is indicated in bold.

RESULTS AND DISCUSSION

Morphology of the KNU-WDM2A2 strain

Colonies were whitish to light pink and cottony to wooly, with abundant production of aerial mycelia; moreover, they reached a fungal growth of 90 mm on PDA at 25℃ after 10 days of incubation. The colonies also produced red-droplet secretions after 14 days of incubation and their reverse side was yellowish to red-brown (Fig. 1A). In turn, the colonies were pink-white and cottony to wooly, with a fascicular shape on SNA media, and attained a size of 90 mm after 10 days of incubation at 25℃. The colonies exhibited a white to pink-white color on the reverse side (Fig. 1B). The strain also produced blackish conidiomata-like structures on the agar surface (Fig. 1C). Hyphae were hyaline to brown, branched, thin-walled, septate, and produced a great number of aerial mycelia. Conidiophores were hyaline to slightly yellowish and rough-walled with one or more branches, and produced conidiogenous cells terminally or laterally (Fig. 1D and E). Conidiogenous cells were hyaline to slightly yellowish, top swollen, thin,

sometimes rough-walled, and cylindrical to oblong; they exhibited a size of 6.2-10.5×2.3-3.4 μm with an average diameter of 7.3-2.9 μm (Fig. 1F). Conidia were unicellular, hyaline to light brown, smooth, oval or ovoid to clavate, and measured 4.1-6.9×2.5-3.3 μm (mean, 5.3±0.6×2.9±0.2 μm, n=65) (Fig. 1G). The cultural and morphological characteristics were similar to those described previously for *Biscogniauxia petrensis* (Table 2). Therefore, the KNU-WDM2A2 fungal strain was closely related to *B. petrensis*.

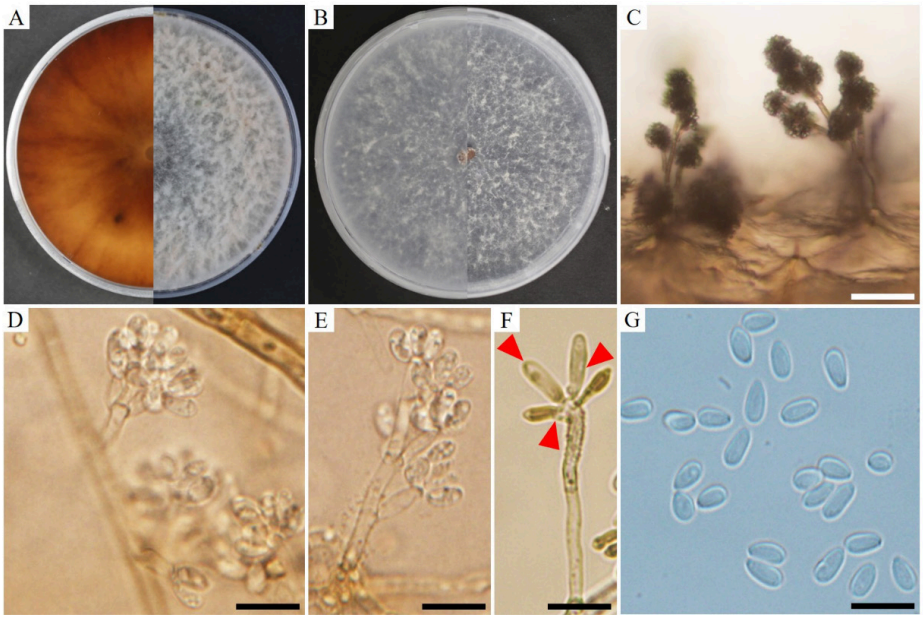

Fig. 1. Cultural and morphological characteristics of *Biscogniauxia petrensis* KNU-WDM2A2. Colonies on potato dextrose agar (A) and synthetic nutrient agar (B) after 14 days of inoculation at 25℃; Conidiomata observed under a stereomicroscope (C); Conidiophores (D-E); Conidiogenous cells (F); Conidia (G). The arrows indicate conidiogenous cells. Scale bars: C=50 μm; D-G=10 μm.

Table 2. Morphological characteristics of the KNU-WDM2A2 strain with reference to *Biscogniauxia petrensis*

Characteristics		*Biscogniauxia petrensis* KNU-WDM2A2[a]	*Biscogniauxia petrensis*[b]
Colony	Color and shape	Initially white, later light pink, aerial mycelium, cottony to wooly, tiny red droplets appeared after 2 weeks; reverse yellow to brown on PDA. Cottony to wooly, pink-white, reverse pink-white on SNA	Whitish to light pink, red-droplet secretions appeared within 2 weeks, abundant cottony to wooly aerial mycelia; reverse yellowish to red-brown on PDA. On SNA, cottony to wooly, pink-white, reverse pink-white
	Size (diam.)	PDA: 90 mm, SNA: 90 mm at 25°C after 10 days	PDA: 85 mm, SNA: 85 mm at 23-25°C after 10 days
Hyphae	Color and shape	Hyaline to brown, septate, thin-walled, branched	Hyaline to brown, septate, branched, abundant thin-walled aerial mycelia
Conidiophores	Shape and color	Hyaline to slightly yellowish, rough-walled, branched, with conidiogenous cells arising terminally or laterally	Hyaline to slightly yellowish, rough-walled, sometimes one or more major branches, with conidiogenous cells arising terminally or laterally
Conidiogenous cells	Shape and size (diam.)	Thin- and rough-walled, cylindrical to oblong, 6.2-10.5×2.3-3.4 μm	Thin- and rough-walled, cylindrical to oblong, 7-13×3-4.5 μm
Conidia	Shape and color	Unicellular, smooth, oval, ovoid to clavate, hyaline to light brown	Unicellular, ovoid to clavate, holoblastic, hyaline, smooth
	Size (diam.)	4.1-6.9×2.5-3.3 μm (mean=5.3±0.6×2.9±0.2 μm). (n=65)	4.5-7.5×2.5-4.5 μm (mean=5.7±0.6×3.3±0.4 μm). (n=35)

Diam.: diameter; PDA: Potato dextrose agar; SNA: synthetic nutrient agar.
[a]Fungal strain studied in this paper.
[b]Sources of the descriptions [10].

Molecular phylogeny of the KNU-WDM2A2 strain

After the analysis of nucleotide sequences, the sequences of 686 and 846 bp were obtained from the ITS regions and 28S rDNA, respectively. The BLAST results of the ITS sequence exhibited a similarity of 100% with different strains of *Biscogniauxia petrensis* (MN844525, MN844517, MN844529, MN844513, and MN844542). The 28S rDNA of the large subunit displayed maximum similarity of 99.88% with the different strains of *B. petrensis* (KU746717, KU746716, KU746715, MK951680). A phylogenetic tree was constructed based on the combined sequences of ITS regions and partial sequences of the 28S rDNA using a neighbor-joining method. The generated phylogenetic tree revealed that the KNU-WDM2A2 strain was clustered with the previously identified *B. petrensis* (Fig. 2). Thus, the KNU-WDM2A2 fungal strain was identified as *B. petrensis* and deposited in the National Institute of Biological Resources (NIBRFG0000507057).

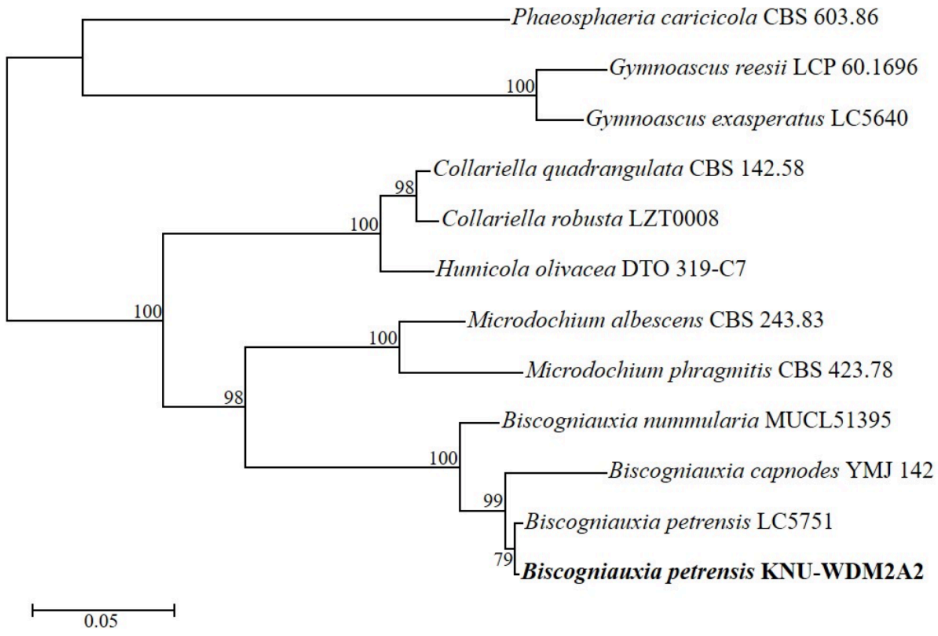

Fig. 2. Neighbor-joining phylogenetic tree based on the combined internal transcribed spacer (ITS) regions and the partial sequence of 28S rDNA genes, showing the relationships of *Biscogniauxia petrensis*. The numbers above the branches represent the bootstrap values obtained for 1,000 replicates. The strain isolated in this study is indicated in bold. Bar, means 0.05 substitutions per nucleotide position.

Some fungal species were reportedly associated with the larvae of several mosquitoes isolated from municipalities of the Brazilian Amazon: *Acremonium kiliense*, *Penicillium oxalicum* from *Mansonia titillans*; *Gliocladium viride* and *Paecilomyces* sp. from *Anopheles darling* [16]. Also several species of *Penicillium* (such as *P. decumbens*, *P. fellutanun*, *P. corylophilum*, *P. waksmanii*, and *P. janthinellum)* were detected in some adults and larvae of mosquitoes (*Aedes* spp., *Anopheles* spp., *Culex* spp., and *Mansonia* spp.) collected in Minas Gerais, Rio de Janeiro, and Rondônia in Brazil [17]. Though there are so many

fungal species isolated from mosquitoes in different countries, but still now, there is no study in Korea. However, there are some fungal species namely *P. brasilianum* and *Blakeslea trispora* isolated from dead insect bodies and from gut of grasshopper in Korea, respectively [18,19]. Moreover, two *Mucor* species, i.e., *Mucor irregularis* and *M. fragilis,* were also isolated from the gut of soldier fly larvae inhabiting bulrush at a pond located in Gwangju, Korea [20]. The member of *Biscogniauxia* called *Biscogniauxia mediterranea* transmitted through ambrosia beetle *Platypus cylindrus* and responsible for the charcoal canker disease of *Quercus suber* [21]. Moreover, *B. mangiferae* was isolated from dead branch of *Mangifera indica* (Anacardiaceae) [22]. *B. rosacearum* was isolated from rosaceous hosts in Italy. And *Biscogniauxia* spp. have been reported as endophytes or secondary invaders that attack only stressed plants [23]. Even, *B. nummularia*, a xylariaceous fungus that causes strip canker and wood decay on European beech (*Fagus sylvatica* L.) [24]. On the other hand, recently, *B. petrensis* strain was isolated from rocks in an unnamed karst cave located in Guizhou province, China [10]. In case of *B. petrensis* was not reported from any other hosts as well as responsible for any diseases yet.

In this study, KNU-WDM2A2 strain was isolated from adult mosquitoes and identified as *Biscogniauxia petrensis* in Korea. Therefore, further research is required to provide in-depth knowledge about this species based on ecology and aspects of the agricultural field in Korea.

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