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Re-Identification of Dollar Spot Pathogen on Creeping Bentgrass and Kentucky Bluegrass in South Korea

Jae Uk Jee [1,2], Ju Hyun Ryu [2], Jeong Ho Lee [3], Seog Won Chang [3,*] and Se Chul Chun [1,*]

[1] Department of Environmental Health Science, Konkuk University, Seoul 05029, Republic of Korea; jee1303@smartscore.kr
[2] E&L Turfgrass Institute, E&L Co., Ltd., Hwaseong 18471, Republic of Korea
[3] Department of Golf Course Management, Korea Golf University of Science & Technology, Hoengseong 25247, Republic of Korea
* Correspondence: changsw802@hanmail.net (S.W.C.); scchun@konkuk.ac.kr (S.C.C.); Tel.: +82-33-810-1066 (S.W.C.); +82-2-450-3727 (S.C.C.)

Abstract: Dollar spot is one of the world's most widely distributed turfgrass diseases. The pathogen of the disease has been re-identified as a fungus belonging to the genus *Clarireedia* in the United States, Japan, and China. Since *Clarireedia* species vary depending on the response to fungicides, hosts, and distribution ranges, it is necessary to re-identify dollar spot in South Korea for effective turfgrass management. In this study, the amplified nucleotide sequences with primer sets of the internal transcribed spacer (ITS) region, Calmodulin (CaM), and Mini-chromosome maintenance complex component 7 (Mcm7) were analyzed to re-identify *Clarireedia* spp. isolated from creeping bentgrass and Kentucky bluegrass on golf courses in South Korea. The ITS and CaM regions were analyzed through multiple sequence alignments. The isolates were identified as *C. paspali*, and *Clarireedia* sp. When cultured on PDA, three groups formed fast growing, cottony colonies with white aerial hyphae that later collapsed and turned tan to brown. Most isolates formed apothecia, but ascospores were not observed. The apothecia formation of *C. paspali* has never been reported. All isolates exhibited pathogenicity on creeping bentgrass and Kentucky bluegrass. These results indicated that the pathogens causing dollar spot on creeping bentgrass and Kentucky bluegrass in South Korea might be *C. paspali* and *Clarireedia* sp. The present study reports the first re-identification of the turfgrass dollar spot pathogen *Sclerotinia homoeocarpa* into the genus *Clarireedia* in South Korea.

Keywords: *Clarireedia*; apothecia; ascospore; *Sclerotinia homoeocarpa*

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1. Introduction

Dollar spot is one of the major diseases affecting both cool season and warm season turfgrasses worldwide [1]. The pathogen of dollar spot has been detected irrespective of disease symptoms and has shown a high detection rate of 83.3% in thatch layers [2]. Dollar spot, characterized by coin-sized bleached spots on turfgrass [3], is one of the most economically significant diseases affecting turfgrass worldwide [4]. It causes severe blighting of the foliage in roughly circular foci 2 to 5 cm in diameter and could cause golf course surfaces to be unplayable for days or weeks at a time [5], requiring considerable effort and expense for management on golf courses, sports fields, and recreational areas [6].

The dollar spot pathogen was first named *Sclerotinia homoeocarpa* by Bennett [7]. Subsequently, based on the observation of apothecia and ascospores, it was proposed that the pathogen be classified under the family *Rutstroemiaceae* [8], but it was not adopted, and

over 70 years of taxonomic debate followed. In 2018, a multi-locus phylogenetic analysis using three DNA markers of the rDNA internal transcribed spacer (ITS) region, Calmodulin (CaM), and DNA replication licensing factor Mcm7 in a total of 1810 bp revealed that *S. homoeocarpa* does not belong to the genus *Sclerotinia* but to a new genus, *Clarireedia*, within the family *Rutstroemiaceae* [9]. Since then, phylogenetic studies of dollar spot pathogens distributed across regions such as Europe, Japan, and China have been actively conducted [10,11]. Six species within the genus *Clarireedia* have been reported, including *C. bennettii*, *C. hainanense*, *C. homoeocarpa*, *C. jacksonii*, *C. monteithiana*, and *C. paspali.* Each species responds differently to fungicides, hosts, and distribution ranges [9–15]. Cultural practices offer only modest suppression of dollar spot, while intensive fungicide usage and misuse have resulted in widespread fungicide resistance among *Clarireedia* spp., increasing financial strain on many golf facilities and heightened concerns about chemical exposure to humans and the environment [16,17]. Four primary fungicide classes are commonly used worldwide for managing dollar spot: dicarboximides (DCFs), demethylase inhibitors (DMIs), succinate dehydrogenase inhibitors (SDHIs), and methyl benzimidazole carbamates (MBCs) [18–21]. However, the repeated use of fungicides has led to selecting *Clarireedia* isolates with reduced sensitivity to fungicides from different modes of action [16,19,21,22]. To prevent such a problem, it is important to re-identify the pathogens causing dollar spot and determine the species of dollar spot pathogens distributed in South Korea. However, despite the global efforts to re-identify the species causing dollar spot on turfgrass, re-identification studies on the pathogen have not been conducted in South Korea.

In this study, the pathogens causing dollar spot on cool season turfgrass in South Korea were isolated from creeping bentgrass (*Agrostis stolonifera*) and Kentucky bluegrass (*Poa pratensis*). The pathogens were re-identified through cultural characteristics and molecular biological methods to guide effective chemical turfgrass management. The present study reports, firstly, the re-identification of the turfgrass dollar spot pathogen *Sclerotinia homoeocarpa* into the genus *Clarireedia* in South Korea.

2. Materials and Methods

2.1. Isolation of the Pathogen from Hosts

The dollar spot pathogens used in the experiment were collected from creeping bentgrass and Kentucky bluegrass samples showing dollar spot symptoms at Blackstone Golf Club in Icheon, Club 72 Golf and Resort in Incheon, and the putting green of the short game course at Korea Golf University of Science and Technology located at Wonju City. Leaves with dollar spot symptoms were surface sterilized in 1% sodium hypochlorite for 1 min and plated on Potato Dextrose Agar (PDA. KisanBio, Seoul, Republic of Korea). The growing hyphae was transferred to a new PDA to isolate the fungi (Table 1).

Table 1. Isolates of dollar spot pathogen used in this study.

Isolate Location	Host	Isolate	Isolated Year
Icheon, Gyeonggi	Kentucky bluegrass	KKBD-02, KKBD-36	2022
Incheon	creeping bentgrass	KCBD-01	2020
Hoengseong, Gangwon	creeping bentgrass	KCBD-02, KCBD-03, KCBD-04	2022
	Kentucky bluegrass	KKBD-01	2022

2.2. Pathogenicity Tests in Pots

The pot experiment for pathogenicity tests was conducted from March 2023 to December 2023 in a plant growth chamber (SUN0022, Neulpureunchae, Gunpo, Republic of Korea) and a greenhouse at Konkuk University in Seoul. Creeping bentgrass and Kentucky

bluegrass were sown (seeding rate: 0.1 g per pot) in pots (70 mm in diameter, 100 mm in height) filled with potting soil (Tobaeki, Sung-Hwa Co., Boseong, Republic of Korea). The isolated pathogens were inoculated onto 12-week-old mature plants [23]. The pathogen inoculation was performed following the method of Rengwalska and Simon [24], where circular mycelial plugs, 10 mm in diameter, were taken from the edge of colonies grown on PDA and inoculated onto the aboveground parts of the turfgrass. The inoculated turfgrass plants were maintained in a greenhouse at a temperature range of 15–30 °C for approximately one month, during which disease development was observed and evaluated. The plots were arranged in a completely randomized design. Mowing was performed using scissors, maintaining a height of approximately 10 mm for creeping bentgrass and 20 mm for Kentucky bluegrass, 2–3 times per week. The plants were irrigated once a week through bottom watering.

2.3. Pathogenicity Tests in Field

The pathogenicity field experiment was conducted from April 2023 to May 2023 on the putting green of the short game course at Korea Golf University of Science & Technology. The isolate used in the experiment was KKBD-36, isolated from the same location. Preparation of the inoculum and the inoculation method followed the protocol described by Chang et al. [25]. Kentucky bluegrass seeds (cultivar: Midnight) were sterilized twice in an autoclave at 121 °C for 40 min. The sterilized seeds were inoculated with the fungal isolate cultured on PDA and incubated at room temperature for one month, shaking once a week. After sufficient drying, the inoculum was evenly spread at 20 g/m^2 on the putting green of the short game course. Mowing was performed daily in the morning using a riding greens mower (Greensmaster 3250-D, Toro, Bloomington, MN, USA) at a height of approximately 5 mm. Each replicate covered an area of 1 m^2, with four replicates. After inoculation, the occurrence of small spots on leaf blades was observed weekly, and the progression of the disease was characterized by brown or reddish-brown margins and light brown, coin-shaped lesions. Disease infection and lesion size were evaluated weekly.

2.4. Koch's Postulates Test

To confirm that all strains used in the pot and field experiments were the causal agents of dollar spot, experiments were conducted according to Koch's postulates. After inoculation, leaves of creeping bentgrass and Kentucky bluegrass showing dollar spot symptoms were collected, surface-sterilized in 1% sodium hypochlorite for 1 min, and cultured on PDA to isolate hyphae. The re-isolated strains were compared to the inoculated strains through phylogenetic classification and identification, using DNA markers to analyze the nucleotide sequences of three regions: internal transcribed spacer (ITS), Calmodulin gene (CaM), and Mini-chromosome Maintenance Complex Component 7 (Mcm7).

2.5. Apothecia Production and Morphological Examinations

The experiment on apothecia formation and morphological characteristics on artificial media was conducted following the method of Salgado-Salazar [9]. The hyphae of the isolated dollar spot pathogen were suspended in 300 µL of sterile water and spread-plated onto PDA containing 2.5 mM ascorbic acid. The plates were incubated at 25 °C under 24 h LED lighting, and observations were made for more than four weeks to observe the formation of apothecia and ascospores. The morphological characteristics of the fungi cultured were examined by inoculating a 10 mm diameter mycelial plug, taken from the edge of the dollar spot pathogen colonies grown on PDA using a cork borer, onto PDA containing 2.5 mM ascorbic acid. The changes in colony color and growth rate were observed for three weeks. All experiments were conducted in four replicates.

2.6. DNA Extractions, PCR Amplification, and Sequencing

Phylogenetic classification and re-identification using three primer pairs were performed: mycelial fragments of dollar spot pathogens cultured on PDA were transferred to PDB (Potato Dextrose Broth) and incubated with shaking at 28 °C for three days. The mycelium was then collected using a benchtop centrifuge, and gDNA was extracted using a gDNA extraction kit (Biofact Co., Daejeon, Republic of Korea) following the manufacturer's instructions. gDNA was extracted from dollar spot hyphae using a silica-based spin column method. Dollar spot hyphae were resuspended in 200 µL of GD1 buffer and mixed with 5 µL of Proteinase K (20 mg/mL). The mixture was vortexed for 1 min and incubated at 65 °C for 10 min. Then, 200 µL of GD2 buffer was added, vortexed for 10 s, and centrifuged at 13,000 rpm for 5 min. The supernatant was transferred to a new 1.5 mL microcentrifuge tube, and 200 µL of GB buffer was added, followed by inverting 10–20 times for complete mixing. For DNA binding, the mixture was transferred to a spin column pre-equilibrated with 200 µL of Help B buffer and centrifuged at 10,000 rpm for 30 s. The passing solution was thrown away with the collection tube. All supernatant was loaded into the spin column and centrifuged at 7000 rpm for 1 min. The flow-through solution was discarded together with the collection tube, and the spin column was placed into a new 2 mL collection tube. The column was washed twice with 500 µL of WB buffer (80% ethanol) by centrifugation at 13,000 rpm for 30 s. A final dry spin was performed at 13,000 rpm for 3 min to remove residual ethanol. DNA was eluted with 50–100 µL of DNA Hydration Solution by incubating at room temperature for 1 min and centrifuging at 13,000 rpm for 2 min. The concentration and purity of the extracted gDNA were measured using a NanoDrop spectrophotometer (Nano-100 Micro-Spectrophotometer, Luscience, Seoul, Republic of Korea). The analysis was performed by Macrogen (Seoul, Republic of Korea). The nucleotide sequence analysis of the strains was conducted for three regions: internal transcribed spacer (ITS), Calmodulin gene (CaM), and Mini-chromosome Maintenance Complex Component 7 (Mcm7). PCR amplification was performed using the primer sets ITS4/ITS5, CAL-228F/CAL-737R, and Mcm7-709for/Mcm7-1348rev, respectively (Table 2). Sequence analysis and alignment were performed using CLC Workbench software v.8.0. A phylogenetic tree was constructed to identify the datasets using the neighbor-joining method with CLC workbench software v.8.0. A complete list of isolates used in the present study is found in Table 3.

Table 2. Primer information for DNA amplification used in this study.

Gene	Primer	Sequence (5′-3′)	Annealing Temperature (°C)	References
Mcm7	Mcm7-709for Mcm7-1348rev	ACIMGIGTITCVGAYGTHAARCC GAYTTDGCIACICCIGGRTCWCCCAT	56	[26]
Cam	CAL-228F CAL-737R	GAGTTCAAGGAGGCCTTCTCCC CATCTTTCTGGCCATCATGG	55	[27]
ITS	ITS4 ITS5	TCCTCCGCTTATTGATATGC GGAAGTAAAAGTCGTAACAAGG	55	[28]

Table 3. Fungal isolates used in this study.

Fungal Species	Isolate ID	Locale	Year	GenBank Number	
				CaM	ITS
Ciboria aestivalis	CBS 119.47	Australia	1947	KF545281	KF545326
Clarireedia bennettii	CBS 309.37	United Kingdom	1937	MF964321	MF964270
Clarireedia bennettii	CBS 311.37	United Kingdom	1937	MF964272	MF964323

Table 3. *Cont.*

Fungal Species	Isolate ID	Locale	Year	GenBank Number	
				CaM	ITS
Clarireedia homoeocarpa	HP-50	NJ, USA	N/A	KF545247	KF545291
Clarireedia homoeocarpa	MB-01	OH, USA	2001	KF545244	KF545290
Clarireedia homoeocarpa	D19	OH, USA	2002	KF545252	KF545298
Clarireedia homoeocarpa	SH44	Canada	2000	KF545251	KF545299
Clarireedia homoeocarpa	RCCPG-1	NC, USA	2003	KF545253	KF545297
Clarireedia homoeocarpa	236-941	NJ, USA	2013	KF545248	KF545296
Clarireedia homoeocarpa	A4	OH, USA	2001	KF545243	KF545295
Clarireedia homoeocarpa	SH80	Canada	2000	KF545245	KF545294
Clarireedia jacksonii	CBS 510.89	Netherlands	1989	KF545261	KF545289
Clarireedia jacksonii	LEF17T-21	Italy	2008	KF545250	KF545293
Clarireedia jacksonii	LWC-10	NC, USA	2003	MF964269	MF964320
Clarireedia jacksonii	MAFF 235854	Japan	1987	KF545242	KF545301
Clarireedia jacksonii	MAFF 235856	Japan	1987	KF545246	KF545302
Clarireedia jacksonii	MAFF 235858	Japan	1988	MF964273	MF964324
Clarireedia monteithiana	TEKP-2	HI, USA	2008	KF545259	KF545304
Clarireedia monteithiana	BC-14	NC, USA	2008	KF545255	KF545307
Clarireedia monteithiana	MAFF 236938	Japan	1991	KF545258	KF545305
Clarireedia paspali	Hawaii2	GA, USA	2021	MZ620646	MZ578438
Clarireedia paspali	Hawaii3	GA, USA	2021	MZ620644	MZ578439
Clarireedia paspali	KCBD-01	Incheon, Republic of Korea	2020	LC867002	LC867009
Clarireedia paspali	KCBD-02	Icheon, Republic of Korea	2022	LC867003	LC867010
Clarireedia paspali	KCBD-03	Hoengseong, Republic of Korea	2022	LC867004	LC867011
Clarireedia paspali	KCBD-04	Hoengseong, Republic of Korea	2022	LC867005	LC867012
Clarireedia paspali	KKBD-01	Hoengseong, Republic of Korea	2022	LC867006	LC867013
Clarireedia paspali	KKBD-02	Hoengseong, Republic of Korea	2022	LC867007	LC867014
Clarireedia sp.	KKBD-36	Icheon, Republic of Korea	2022	LC867008	LC867015
Monilinia vaccinii-corymbosi	SSI-1	NJ, USA	2009	MF964274	MF964325
Sclerotinia matthiolae	CBS 111.17	Switzerland	N/A	MF964263	MF964314
Sclerotinia minor	CBS 112.17	Netherlands	N/A	MF964264	MF964315
Sclerotinia sclerotiorum	SS1	NJ, USA	2009	KF545279	KF545320
Sclerotinia sclerotiorum	SS5	NJ, USA	2009	KF545280	KF545319
Sclerotinia sulcata	CBS 303.31	MD, USA	2017	MF964266	MF964317

2.7. Alignment and Phylogenetic Analysis

The phylogenetic tree was constructed using the CLC genomics workbench (Insilicogen Inc., Yongin, Republic of Korea). The Multiple Sequence Alignment feature was utilized for nucleotide sequence alignment based on the Clustal W algorithm. The gap penalty was set to 10, and the gap extension penalty was set to 0.1. A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method based on the aligned nucleotide sequences. The reliability of the tree was evaluated through bootstrap analysis with 1000 replicates. The phylogenetic tree was visualized using the tree editing tool in the CLC genomics workbench, and the phylogenetic relationships were analyzed based on the visualized tree. Sequencing services, including the generation of the phylogenetic tree, were outsourced to Macrogen Inc. (Seoul, Republic of Korea).

2.8. Statistical Analyses

Statistical analyses were performed using the statistical software SigmaStat (Version 2.03, Systat Software Inc., San Jose, CA, USA). The significance of differences between means was evaluated using Fisher's LSD test ($p = 0.05$).

3. Results

3.1. Isolation of the Pathogen and Pathogenicity Tests and Koch's Postulates Test

In this study, four strains were isolated from creeping bentgrass and three strains from Kentucky bluegrass. All strains were collected from turfgrass showing dollar spot symptoms and were used in the experiments (Table 1). In both pot and field experiments for pathogenicity testing, all isolates caused the characteristic symptoms of dollar spot infection, including reddish-brown margins and light tan discoloration on the leaves of creeping bentgrass and Kentucky bluegrass 7 days after inoculation (Figure 1). Among the strains used in the experiment, KCBD-01 and KCBD-03 exhibited the strongest pathogenicity. In contrast, the pathogenicity of KKBD-01, KKBD-02, KKBD-36, KCBD-02, and KCBD-04 showed no significant differences (Table 4).

Table 4. The pathogenicity of isolates on creeping bentgrass and Kentucky bluegrass in the pot and field.

Isolate	Disease Severity [y]			
	Creeping Bentgrass		Kentucky Bluegrass	
	Pot	Field	Pot	Field
KKBD-01	++	N/A [z]	+	N/A
KKBD-02	++	N/A	+	N/A
KKBD-36	++	++++	+	N/A
KCBD-01	+++	N/A	+	N/A
KCBD-02	++	N/A	+	N/A
KCBD-03	+++	N/A	+	N/A
KCBD-04	++	N/A	+	N/A

[y] Degree of turfgrass mortality at three weeks after inoculation +: 1–20%, ++: 21–40%, +++: 41–60%, ++++: 61–80%. [z] Not applicable (pathogenicity tests were not conducted).

The creeping bentgrass and Kentucky bluegrass inoculated with the pathogens of dollar spots showed the typical symptoms of dollar spot (Figure 1). The pathogens were re-isolated from the symptomatic leaves of creeping bentgrass and Kentucky bluegrass and confirmed to be the same pathogens through the morphological characteristics and PCR.

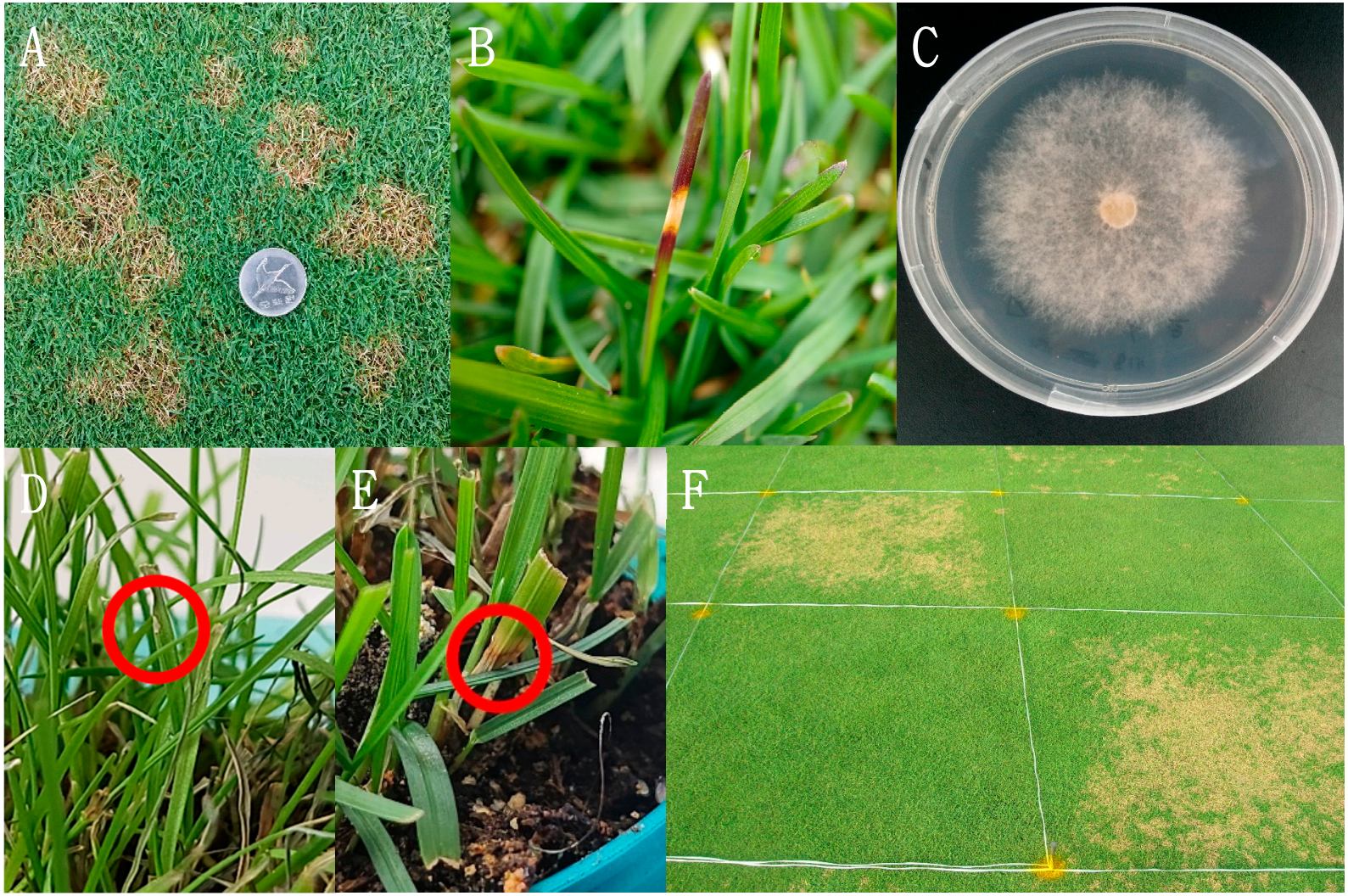


Figure 1. Pathogenicity test of dollar spot isolates. (**A**): Typical dollar spot symptoms of dollar spot on creeping bentgrass putting green. (**B**): Typical symptom of dollar spot on Kentucky bluegrass rough. (**C**): Mycelial colony of dollar spot pathogen on PDA. (**D**): At 7 days after KKBD-02 inoculation, a typical dollar spot symptom (leaf blade bleached in red circle) on creeping bentgrass. (**E**): At 7 days after KCBD-01 inoculation, stroma, a typical dollar spot symptom (red circle) on Kentucky bluegrass leaf blade. (**F**): Typical dollar spot symptoms on creeping bentgrass putting green at 14 days after KKBD-36 inoculation.

3.2. Morphological Examinations and Apothecia Production

The morphological characteristics of the dollar spot pathogens confirmed as causal agents through pathogenicity tests in pots and fields are shown in Figure 2 and Tables 5 and 6. The isolates KKBD-01, KKBD-36, KCBD-01, KCBD-02, and KCBD-04, cultured on PDA containing 2.5 mM ascorbic acid, exhibited rapid growth and formed white aerial hyphae. Over time, the initially white stroma turned dark brown or yellowish-brown. In contrast, the strain KKBD-02 grew at approximately half the rate of the other isolates and formed white aerial hyphae (Tables 5 and 6). The aerial hyphae later collapsed and turned yellowish-brown or gray, with wavy colony margins, and did not form stroma. The strain KCBD-03 exhibited unique growth and morphological characteristics that differed from other isolates (Table 6 and Figure 2). The strain KCBD-03 exhibited rapid growth and formed white aerial hyphae, which later collapsed and turned brown. Unlike other strains, it did not form a dark stroma even after 15 days of incubation. The mycelial growth rates of KKBD-01, KKBD-36, KCBD-01, KCBD-02, KCBD-03, and KCBD-04 on PDA containing 2.5 mM ascorbic acid were approximately twice as fast as those reported for *C. jacksonii*, *C. monteithiana*, and *C. bennettii* by Salgado-Salazar [9]. Similarly, the growth rate of KKBD-02 was about twice as fast as that of *C. homoeocarpa* reported by Salgado-Salazar [9] (Table 5).



Figure 2. Colony morphology of dollar spot isolates after incubation at 25 °C. Colony front (left side petri dish) (**A**) and colony back (right side petri dish) (**B**) on PDA with ascorbic acid at 10 days after incubation, respectively; colony front (**C**) and colony back (**D**) on PDA with ascorbic acid at 3 weeks after incubation, respectively. Apothecium (**E**,**F**) on ascorbic PDA culture and close-up images of an Apothecium (**G**–**I**) (bar size: 10 mm).

Table 5. The mycelial growth of the isolates of *Clarireedia* spp. on potato dextrose agar medium amended with 2.5 mM ascorbic acid.

Salgado-Salazar (2018) [9] [w]		Present Isolate [x]	
Species	**Growth (mm) [y]**	**Isolate**	**Growth (mm) [y]**
C. jacksonii	80 a [z]	KKBD-01	77.9 a
C. homoeocarpa	40 b	KKBD-02	41.7 b
C. monteithiana	80 a	KKBD-36	79.8 a
C. bennettii	80 a	KCBD-01	78.0 a
		KCBD-02	74.2 a
		KCBD-03	80.1 a
		KCBD-04	76.9 a

[w] At 6 days after incubation. [x] At 3 days after incubation. [y] Mycelia diameter grown on PDA amended with 2.5 mM ascorbic acid at 25 °C. [z] Means followed by the same letters are not significantly different within the column at $p = 0.05$ (LSD).

KCBD-01, KCBD-02, KCBD-03, KCBD-04, KKBD-01, and KKBD-36 strains formed apothecia; however, this did not lead to the formation of ascospores (Figure 2). The KKBD-02 strain did not form apothecia and ascospores.

3.3. Molecular Re-Identification of Dollar Spot Pathogen and Phylogenetic Analysis

Molecular classification and re-identification using three primer pairs (ITS, CaM, and Mcm7) revealed that among the seven strains used in this study, KCBD-01, KCBD-02, KCBD-03, KCBD-04, KKBD-01, and KKBD-02 were identified as *C. paspali*. KKBD-36 did not match any known species within the genus *Clarireedia*. Due to the limitations of the primer results, it could not be specifically assigned to any species but was re-identified as belonging to the genus *Clarireedia* (Figure 3).

Table 6. Cultural and morphological characteristics of dollar spot isolates used in this study.

	Clarireedia jacksonii [9]	*C. homoeocarpa* [9]	KKBD-01, KKBD-36, KCBD-01, KCBD-02, and KCBD-04	KKBD-02	KCBD-03
Cultural characteristics	Colonies are fast growing, cottony, front white to off-white with light brown spots, back white to off-white, later collapsing and turning tan to brown. Colony reaches 8 cm radial growth after 6 days at 25 °C under continuous light on PDA + ascorbic acid. Colonies > 15 days old form thick, flat, black stroma on PDA + ascorbic acid. Hyphae septate, hyaline.	Thalli at first aerial, white to off-white, later collapsing and turning brown, tan, olive, or gray, sometimes slightly pink. Colonies on PDA raised, aerial mycelium white to offwhite, collapsing and turning brown, tan, olive, or gray, with undulate margins. The colony reaches 4 cm radial growth after 6 days at 25 °C under continuous light on PDA + ascorbic acid. Colonies > 15 days old do not form a dark stroma on PDA + ascorbic acid. Hyphae septate, hyaline.	Colonies fast growing, cottony, front white to off-white with light brown spots, back white to off-white, later collapsing and turning tan to brown. Colony reaches 8 cm radial growth after 3 days at 25 °C under continuous light on PDA + ascorbic acid. Colonies > 15 days old form thick, flat, black stroma on PDA + ascorbic acid. Hyphae septate, hyaline.	Thalli at first aerial, white to off-white, later collapsing and turning brown, tan, olive, or gray, sometimes slightly pink. Colonies on PDA raised, aerial mycelium white to off-white, collapsing and turning brown, tan, olive, or gray, with undulate margins. Colony reaches 4 cm radial growth after 3 days at 25 °C under continuous light on PDA + ascorbic acid. Colonies > 15 days old do not form a dark stroma on PDA + ascorbic acid.	Colonies are fast growing, cottony, and front white to off-white with light brown spots and back white to off-white, later collapsing and turning brown. The colony reaches 8 cm radial growth after 3 days at 25 °C under continuous light on PDA + ascorbic acid. Colonies > 15 days old do not form a dark stroma on PDA + ascorbic acid.
Apothecia characteristics	Apothecia arising from a substratal stroma, cupulate to discoid, brown, cinnamon, or light orange, receptacle pubescent. Apothecia 2.73 × 1.91 mm arising from dark, substratal stroma. Ascospores and conidia have not been observed.	Hyphae septate, hyaline. Apothecia 0.5–1.5 mm in diameter, arising from a dark substratal stroma, cupulate to discoid, brown, cinnamon, or light orange, receptacle pubescent. Ascus 162.9 × 12.5 µm, on average. Ascospores hyaline, oblong to elliptical, mostly unicellular, occasionally with a medium septum, 20.7 × 8.3 µm. Conidia are not observed. Microconidia spherical, hyaline, 2.0 mm in diameter, formed in cream-colored pustules [7].	Apothecia 0.5–2.0 mm in diameter, arising from a substrate stroma, cupulate to discoid, brown, cinnamon, or light orange, receptacle pubescent.Ascospores and conidia were not observed.	Apothecia, ascospores, and conidia were not observed.	Apothecia 0.5–2.0 mm in diameter, arising from a substrate stroma, cupulate to discoid, brown, cinnamon, or light orange, receptacle pubescent.Ascospores and conidia were not observed.

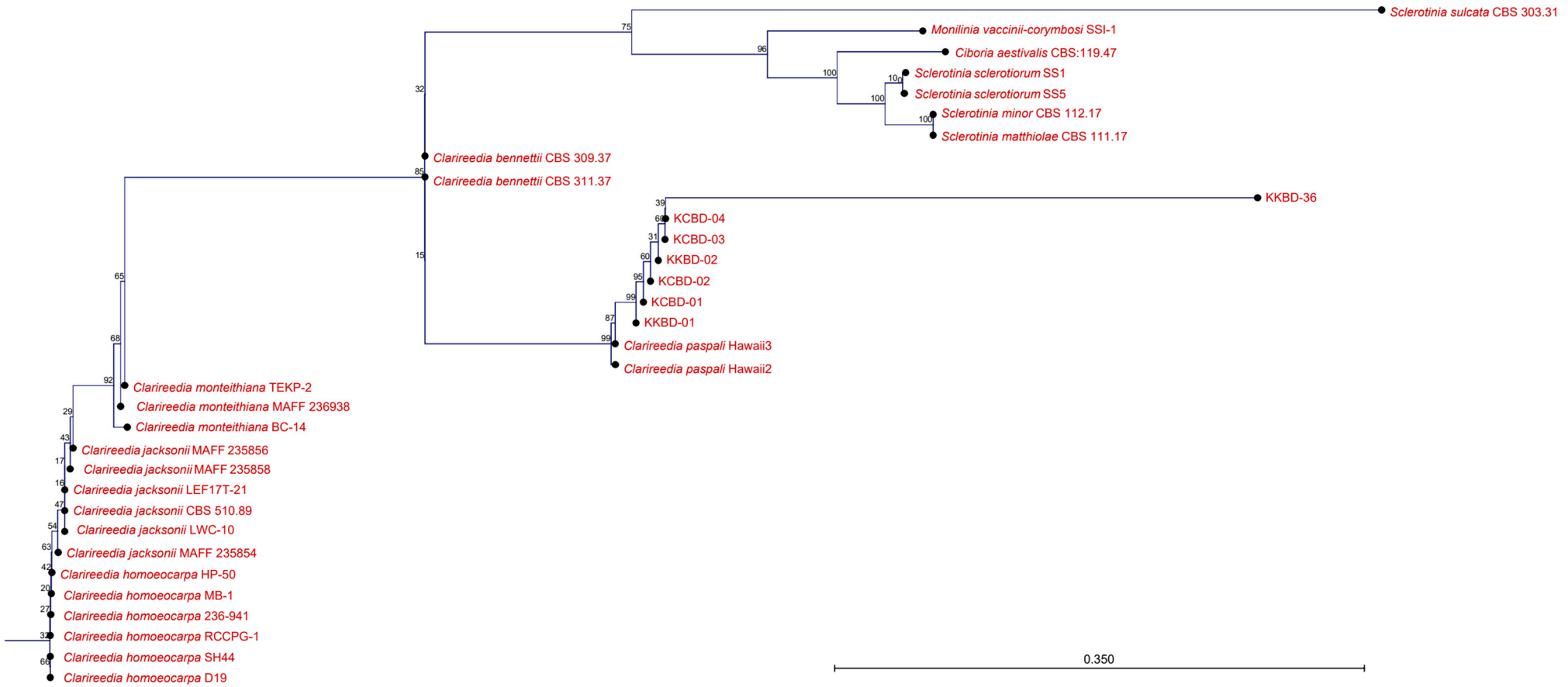


Figure 3. A majority rule Bayesian phylogenetic tree from the combined ITS and CaM analysis showing relationships among fungal isolates in the Sclerotiniaceae and Rutstroemiaceae families. The Mcm7 sequences were excluded due to insufficient reference data in NCBI. Support values (posterior probability (PP)/maximum likelihood (ML) bootstrap) are indicated above the branches. Branch lengths are proportional to levels of sequence divergence.

4. Discussion

The present study reports for the first time in South Korea that dollar spot occurring on creeping bentgrass and Kentucky bluegrass is caused by *C. paspali* and *Clarireedia* sp. The fungi belonging to these three species exhibited pathogenicity on creeping bentgrass and Kentucky bluegrass, forming coin-sized bleached spots (Figure 1). These results were similar to those reported by Smiley [1]. Among them, *C. paspali* KCBD-01 and *C. paspali* KCBD-03 exhibited the strongest pathogenicity on creeping bentgrass, while the other strains showed less pathogenicity. However, all isolates used in the experiment exhibited the same level of pathogenicity on Kentucky bluegrass. Previous studies have reported that *C. jacksonii* exhibits stronger pathogenicity compared to *C. monteithiana* [29]. *Metarhizium anisopliae* exhibited higher conidial hydrophobicity, increased host penetration ability, accelerated growth under hypoxic conditions, and enhanced utilization of diverse carbon sources compared to *Metarhizium acridum*, resulting in higher overall virulence [30]. This is similar to findings from comparative genomics, which reveal that *Fusarium* species, such as *F. oxysporum* and *F. solani*, possess pathogenicity-related chromosomes containing host-specific virulence genes, enabling them to exhibit different pathogenicity depending on the host plant they infect [31]. These results suggest genetic variation may lead to differences in pathogenicity and physiological functions among *Clarireedia* species [12].

KCBD-01, KCBD-02, KCBD-03, KCBD-04, KKBD-01, and KKBD-02 strains exhibited morphological characteristics similar to those of *C. jacksonii* and *C. homoeocarpa* as reported by Salgado-Salazar [9]. However, based on molecular biological analysis, the strains were re-identified as *C. paspali* (Table 6). These strains displayed three distinct cultural characteristics (Figure 2). The KKBD-36 strain was similar to the morphological characteristics of the KCBD-01, KCBD-02, KCBD-04, and KKBD-01 strains, which were re-identified as *C. paspali*, as well as with *C. jacksonii* reported by Salgado-Salazar [9], but it was found to be a different species. These findings did not fully agree with the report by Salgado-Salazar [9], which stated that species within the genus *Clarireedia* exhibit morphological differences depending on the species. Micro-morphological features such as the size and shape of conidia, appressoria, and setae or teleomorph characters overlapped in many species, and these features could change under different growing conditions [32].

This result is similar to the cases where strains morphologically re-identified as *Fusarium graminearum* were re-identified as *F. boothii* based on phylogenetic analyses using primers [33]. Similar to cases where *Botrytis cinerea* and *Botrytis pseudocinerea* are morphologically indistinguishable but identified as different species through molecular methods [34], the classification of species within this species complex based solely on host reference and morphological characteristics appears to have limitations [35]. Consequently, it is difficult to reliably classify species within the genus *Clarireedia* based on morphological characteristics alone, as this may lead to misinterpretations [36]. Molecular re-identification and phylogenetic analysis of the dollar spot pathogen were conducted using three primer pairs (ITS, CaM, and Mcm7). However, due to the insufficient dollar spot sequencing data amplified with all three primer pairs in the National Center for Biotechnology Information (NCBI) database, a phylogenetic tree was constructed using two sequences amplified with ITS and CaM. Therefore, further studies will be necessary if new primers are developed in the future to enhance classification accuracy.

Since the re-identification of *C. paspali* in 2019 [11], there has been no report of successful induction of apothecia and ascospores in the dollar spot pathogen. Similarly, few experiments on the induction of apothecia and ascospores in *Clarireedia* spp. have been reported, apart from Salgado-Salazar [9]. In contrast, successful formation of apothecia and ascospores has been reported for *C. homoeocarpa* strains in the United States and the United Kingdom [7,9], whereas *C. jacksonii* has been observed to form apothecia but not

ascospores [9]. In this study, most strains formed apothecia but did not produce ascospores. Notably, this study is the first to successfully observe apothecia formation in *C. paspali* in artificial media. The differing results observed in this study may be attributed to differences in culture conditions or strain characteristics. In the future, additional experiments under various conditions, such as changes in the concentration of ascorbic acid or light intensity, are considered necessary to induce the formation of ascospores.

Meanwhile, it has been reported that *C. monteithiana*, *C. paspali*, and *C. jacksonii* exhibit different fungicide responses [15]. Specifically, *C. paspali* showed the lowest growth rate on media containing boscalid, while *C. jacksonii* exhibited higher resistance to demethylation inhibitor (DMI) fungicides such as propiconazole and tebuconazole than other species [15]. High fungicide efficiency can lower the optimal application rate, reducing overall costs while maintaining effective disease control [37]. The application of propiconazole plus tebuconazole and azoxystrobin plus epoxiconazole significantly increased the economic yield of spring rapeseed by 0.90 t/ha and 0.77 t/ha, respectively, demonstrating the economic benefits of selecting effective fungicides for disease management [38]. The correct selection of fungicides impacts direct costs and indirect costs, such as the development of fungicide resistance, which may lead to increased future expenses [39]. These findings suggested that accurate species identification within the genus *Clarireedia* might be essential for proper fungicide application.

In the United States, *C. bennettii*, *C. homoeocarpa*, *C. jacksonii*, *C. monteithiana*, and *C. paspali* have been reported [9,12], while in Japan and China, *C. hainanense*, *C. jacksonii*, *C. monteithiana*, and *C. paspali* have been identified [10,11,14]. In South Korea, *C. paspali* and *Clarireedia* sp. were discovered, showing differences in the distribution of *Clarireedia* species compared to neighboring countries. This indicates that the distribution of *Clarireedia* species varies depending on geographical location. For effective dollar spot management, further studies on the distribution of *Clarireedia* species in other regions of South Korea, their host range, degree of pathogenicity, and responses to fungicides are needed.

5. Conclusions

This study is the first re-identification of dollar spot pathogens in South Korea and identifies *Clarireedia paspali* and *Clarireedia* sp. as the causal agents of infections in creeping bentgrass and Kentucky bluegrass. Among the methods used to distinguish species, morphological classification was found insufficient for accurate identification, and molecular approaches using ITS and CaM gene sequences were confirmed to be essential for precise classification. The *Clarireedia* species exhibited differences in pathogenicity as well as fungicide responses, suggesting that species-level identification is essential for effective management of dollar spot. These results in the importance of precise species identification for sustainable and effective turfgrass disease management. Further research is needed to investigate the regional distribution, host range, pathogenicity, and fungicide sensitivity of *Clarireedia* species in South Korea.

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References

1. Smiley, R.W.; Dernoden, P.H.; Clarke, B.B. *Compendium of Turfgrass Diseases*, 2nd ed.; American Phytopathological Society: St. Paul, MN, USA, 1992.
2. Lee, J.H.; Min, G.Y.; Shim, G.Y.; Kim, D.S.; Sang, H.; Jung, G.; Kwak, Y.S. Toothpick-aided detection of *Sclerotinia homoeocarpa* in the turfgrass leaf canopy, thatch, and soil in relation to dollar spot infection centers. *Weed Turfgrass Sci.* **2015**, *4*, 376–382. [CrossRef]
3. Smiley, R.W.; Dernoeden, P.P.; Clarke, B.B. *Compendium of Turfgrass Diseases*, 3rd ed.; American Phytopathological Society: St. Paul, MN, USA, 2005.
4. Goodman, D.M.; Burpee, L.L. Biological control of dollar spot disease of creeping bentgrass. *Phytopathology* **1991**, *81*, 1438–1446. [CrossRef]
5. Tredway, L.P.; Tomaso-Peterson, M.; Kerns, J.P.; Clarke, B.B. *Compendium of Turfgrass Diseases*, 4th ed.; American Phytopathological Society: St. Paul, MN, USA, 2023.
6. Held, D.W.; Potter, D.A. Prospects for managing turfgrass pests with reduced chemical inputs. *Annu. Rev. Entomol.* **2012**, *57*, 329–354. [CrossRef]
7. Bennett, F.T. Dollar spot disease of turf and its causal organism, *Sclerotinia homoeocarpa* n. sp. *Ann. Appl. Biol.* **1937**, *24*, 236–257. [CrossRef]
8. Whetzel, H.H. The cypericolous and juncicolous species of *Sclerotinia*. *Farlowia* **1946**, *2*, 385–437. [CrossRef]
9. Salgado-Salazar, C.; Beirn, L.A.; Ismaiel, A.; Boehm, M.J.; Carbone, I.; Putman, A.I.; Tredway, L.P.; Clarke, B.B.; Crouch, J.A. *Clarireedia*: A new fungal genus comprising four pathogenic species responsible for dollar spot disease of turfgrass. *Fungal Biol.* **2018**, *122*, 761–773. [CrossRef]
10. Takao, T.; Toshihiro, H.; Koya, S. Re-identification of *Clarireedia* spp. causing dollar spot disease of turfgrasses in Japan. *J. Jpn. Soc. Turfgrass Sci.* **2022**, *49*, 71–77.
11. Hu, J.; Zhou, Y.; Geng, J.; Dai, Y.; Ren, H.; Lamour, K. A new dollar spot disease of turfgrass caused by *Clarireedia paspali*. *Mycol. Prog.* **2019**, *18*, 1423–1435. [CrossRef]
12. Bahri, B.A.; Parvathaneni, R.K.; Spratling, W.T.; Saxena, H.; Sapkota, S.; Raymer, P.L.; Martinez-Espinoza, A.D. Whole genome sequencing of *Clarireedia* aff. *paspali* reveals potential pathogenesis factors in *Clarireedia* species, causal agents of dollar spot in turfgrass. *Front. Genet.* **2023**, *13*, 1033437. [CrossRef]
13. Stackhouse, T.; Bass, A.; Waliullah, S.; Ali, E.; Bahri, B.A.; Martinez-Espinoza, A. Probe-based loop-mediated isothermal amplification assay for rapid detection of two *Clarireedia* spp., the causal agent of dollar spot of turfgrass. *Phytopathology* **2024**, *108*, 3115–3122. [CrossRef]
14. Huangwei, Z.; Yinglu, D.; Yuxin, Z.; Jian, H.; Kurt, L.; Zhimin, Y. *Clarireedia hainanense*: A new species is associated with dollar spot of turfgrass in Hainan, China. *Plant Dis.* **2022**, *106*, 996–1002.
15. Hu, J.; Zhang, H.; Dong, Y.; Jiang, S.; Lamour, K.; Liu, J.; Chen, Y.; Yang, Z. Global distributions of *Clarireedia* species and their in vitro sensitivity profiles to fungicides. *Agronomy* **2021**, *11*, 2036. [CrossRef]
16. Sang, H.; Hulvey, J.; Popko, J.T., Jr.; Lopes, J.; Swaminathan, A.; Chang, T.; Jung, G. A pleiotropic drug resistance transporter is involved in reduced sensitivity to multiple fungicide classes in *Sclerotinia homoeocarpa* (F.T. Bennett). *Mol. Plant Pathol.* **2015**, *16*, 251–261. [CrossRef]
17. Sapkota, S.; Catching, K.E.; Raymer, P.L.; Martinez-Espinoza, A.D.; Bahri, B.A. New approaches to an old problem: Dollar spot of turfgrass. *Phytopathology* **2022**, *112*, 469–480. [CrossRef]
18. Ok, C.H.; Popko, J.T., Jr.; Campbell-Nelson, K.; Jung, G. In vitro assessment of *Sclerotinia homoeocarpa* resistance to fungicides and plant growth regulators. *Plant Dis.* **2011**, *95*, 51–56. [CrossRef]
19. Popko, J.T., Jr.; Ok, C.H.; Campbell-Nelson, K.; Jung, G. The association between in vitro propiconazole sensitivity and field efficacy of five New England *Sclerotinia homoeocarpa* populations. *Plant Dis.* **2012**, *96*, 552–561. [CrossRef] [PubMed]
20. Zhang, H.; Jiang, S.; Zhao, Z.; Guan, J.; Dong, Y.; Hu, J.; Lamour, K.; Yin, S.; Yang, Z. Fungicide sensitivity of *Clarireedia* spp. isolates from golf courses in China. *Crop Prot.* **2021**, *149*, 105785. [CrossRef]
21. Ghimire, B.; Aktaruzzaman, M.; Chowdhury, S.R.; Spratling, W.T.; Vermeer, C.B.; Buck, J.W.; Martinez-Espinoza, A.D.; Bahri, B.A. Sensitivity of *Clarireedia* spp. to benzimidazoles and dimethyl inhibitors fungicides and efficacy of biofungicides on dollar spot of warm season turfgrass. *Front. Plant Sci.* **2023**, *14*, 1155670. [CrossRef]

22. Hu, J.; Yang, J.; Li, J.; Ma, Z.; Yao, W.; Ren, H.; Zhang, F.; Yang, G.; Sun, X.; Xiao, Y. Sensitivity of *Sclerotinia homoeocarpa* from turfgrass to thiophanate-methyl, iprodione and propiconazole. *Chin. J. Pestic. Sci.* **2017**, *19*, 694–700.
23. Chang, S.W.; Chang, T.H.; Tredway, L.; Jung, G. Aggressiveness of *Typhula ishikariensis* isolates to cultivars of bentgrass species (*Agrostis* spp.) under controlled environment conditions. *Plant Dis.* **2006**, *90*, 951–956. [CrossRef]
24. Rengwalska, M.; Simon, P.W. Laboratory evaluation of pink root and *Fusarium* basal rot resistance in garlic. *Plant Dis.* **1986**, *70*, 670–672. [CrossRef]
25. Chang, S.W.; Chang, T.H.; Hong, J.K.; Park, J.H.; Jung, S.W. Vegetative compatibility grouping of *Sclerotinia homoeocarpa* isolates infecting turfgrass in South Korea. *Asian J. Turfgrass Sci.* **2012**, *25*, 171–176.
26. Schmitt, I.; Crespo, A.; Divakar, P.K.; Fankhauser, J.D.; Herman-Sackett, E.; Kalb, K.; Nelsen, M.P.; Nelson, N.A.; Rivas Plata, E.; Shimp, A.D.; et al. New primers for promising single-copy genes in fungal phylogenetics and systematics. *Persoonia* **2009**, *23*, 35–40. [CrossRef] [PubMed]
27. Carbone, I.; Kohn, L.M. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **1999**, *91*, 553–556. [CrossRef]
28. White, T.J.; Bruns, T.; Lee, S.; Taylor, J.W. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., Eds.; Academic Press: San Diego, CA, USA, 1990; pp. 315–322.
29. Aynardi, B.A.; Jiménez-Gasco, M.M.; Uddin, W. Effects of isolates of *Clarireedia jacksonii* and *Clarireedia monteithiana* on severity of dollar spot in turfgrasses by host type. *Eur. J. Plant Pathology.* **2019**, *155*, 817–829. [CrossRef]
30. Du, Y.; Li, J.; Chen, S.; Xia, Y.; Jin, K. Pathogenicity analysis and comparative genomics reveal the different infection strategies between the generalist *Metarhizium anisopliae* and the specialist *Metarhizium acridum*. *Pest Manag. Sci.* **2024**, *80*, 820–836. [CrossRef]
31. Rep, M.; Kistler, H.C. The genomic organization of plant pathogenicity in *Fusarium* species. *Curr. Opin. Plant Biol.* **2010**, *13*, 420–426. [CrossRef]
32. Weir, B.S.; Johnston, P.R.; Damm, U. The *Colletotrichum gloeosporioides* species complex. *Stud. Mycol.* **2012**, *73*, 115–180. [CrossRef]
33. Choi, Y.H.; Mageswari, A.; Choi, H.R.; Lee, J.S.; Lee, D.S.; Hong, S.B. Re-identification of *Fusarium sambucinum* species complex strains in Korea and their literature review. *Res. Plant Dis.* **2023**, *29*, 118–129. [CrossRef]
34. Dučkena, L.; Bimšteine, G.; Bankina, B.; Skinderskis, E.; Roga, A.; Frîdmanis, D. Morphological diversity and mycelial compatibility of *Botrytis pseudocinerea* and *Botrytis cinerea* isolated in Latvia. *Proc. Latv. Acad. Sci. Sect. B* **2024**, *78*, 197–205. [CrossRef]
35. Thao, L.D.; Choi, H.; Choi, Y.; Mageswari, A.; Lee, D.; Kim, D.H.; Shin, H.D.; Choi, H.; Ju, H.J.; Hong, S.B. Re-identification of *Colletotrichum gloeosporioides* species complex isolates in Korea and their host plants. *Plant Pathol. J.* **2024**, *40*, 16–29. [CrossRef] [PubMed]
36. Manawasinghe, I.S.; Phillips, A.J.L.; Xu, J.; Balasuriya, A.; Hyde, K.D.; Stępień, Ł.; Harischandra, D.L.; Karunarathna, A.; Yan, J.; Weerasinghe, J.; et al. Defining a species in fungal plant pathology: Beyond the species level. *Fungal Divers.* **2021**, *109*, 267–282. [CrossRef]
37. Dallacorte, L.V.; Bosse, M.A.; Capelin, D.; Paladini, M.V.; Cattani, F.; Remor, M.B.; Lima, J.D.; Perboni, A.T.; Marchese, J.A. Economic versus technical efficiency in using ASM combined with fungicides to elicit wheat varieties with different disease susceptibilities. *Heliyon* **2023**, *9*, e17012. [CrossRef] [PubMed]
38. Devyatkina, T.F.; Chigorin, S.S.; Silaev, A.I.; Bochkarev, D.V. Effectiveness of fungicides in reducing harmfulness of biotrophic pathogens on spring rape. *Agrar. Sci. J.* **2024**, 4–10. [CrossRef]
39. Mikaberidze, A.; Gokhale, C.S.; Bargués-Ribera, M.; Verma, P. Economic analysis of disease and control of multi-field epidemics in agriculture. *bioRxiv* 2025. [CrossRef]

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