MYCOBIOLOGY
2020, VOL. 48, NO. 4, 321–325
https://doi.org/10.1080/12298093.2020.1768626

RESEARCH NOTE

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Colletotrichum cymbidiicola Causing Anthracnose on *Cymbidium* Orchids in Korea

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ABSTRACT

A *Colletotrichum* species was isolated from leaves of *Cymbidium* exhibiting symptoms of anthracnose. In this study, the isolates obtained were identified based on recent taxonomic approaches for the genus *Colletotrichum*. The identity of the causal pathogen was confirmed using morphological data and phylogenetic analysis of combined multi-gene dataset (internal transcribed spacer, glyceraldehyde 3-phosphate dehydrogenase, chitin synthase-1, actin, histone3, beta-tubulin, and calmodulin). Pathogenicity testing revealed that the isolates were pathogenic to *Cymbidium*. Based on these results, the fungal pathogen occurring on *Cymbidium* orchids was identified as *Colletotrichum cymbidiicola,* which is a newly recorded species in Korea.

ARTICLE HISTORY
Received 15 August 2019
Revised 7 October 2019
Accepted 28 October 2019

KEYWORDS
Anthracnose; *Colletotrichum cymbidiicola*; Cymbidium

The genus *Cymbidium*, one of the genera belonging to the family Orchidaceae, is mainly distributed throughout Asian countries including India, Malaysia, China, Japan, and Korea. Cymbidiums are commonly classified into temperate oriental and tropical cymbidiums [1]. *Cymbidium* orchids are popular horticultural plants used for decorative purposes worldwide. Numerous cultivars of *Cymbidium* have been bred to improve horticultural traits, and then made commercially available [2]. In 2016, the area of cultivation for cymbidiums in Korea was estimated to be approximately 52.4 ha (35.9% of the growing areas of all orchids) which is larger than that of other orchid genera such as *Phalaenopsis*, *Dendrobium, Oncidium*, and so on [3].

The genus *Colletotrichum* is one of the most important phytopathogenic fungal genera; it causes anthracnose diseases on a wide range of plants, including crops, globally. The taxonomy of *Colletotrichum* has been advanced based on polyphasic approaches in the last decade. Eleven species complexes present in *Colletotrichum*, i.e., *acutatum* complex [4], *boninense* complex [5], *caudatum* complex [6], *dematium* complex [7], *destructivum* complex [8], *gigasporum* complex [9], *gloeosporioides* complex [10], *graminicola* complex [11], *orbiculare* complex [12], *spaethianum* complex [7], and *truncatum* complex [7], have been intensively studied to delineate species, and 190 species of *Colletotrichum* were accepted within the species complexes as of 2016 [13]. Recent phylogenetic studies have led to the recognition of three species complexes of *Colletotrichum*: *Colletotrichum dracaenophilum*, *Colletotrichum magnum*, and *Colletotrichum orchidearum* as 21 separate species [14].

Anthracnose caused by *Colletotrichum gloeosporioides* on *Cymbidium* species was first reported in the year 1996 in Korea [15]. Later, in 2013, *C. gloeosporioides* was recorded as the causal agent of anthracnose on *Cymbidium kanran,* in Korea [16]. In this study the two new isolates of *Colletotrichum* obtained from cymbidiums in 2013 and 2017 were identified based on morphological characteristics and multigene sequence analysis.

Symptoms typical of anthracnose, which decrease the esthetic value of the plants, have been often found on cymbidiums in the cultivated areas of Korea. Acervuli with conidial masses were formed as concentric rings on dark brown to blackish lesions on the tips or margins of the cymbidium leaves (Figure 1(A,B)). Occasionally, heavily infected leaves turned completely brown with several concentric rings in dead tissues (Figure 1(C)).

Diseased leaf tissues were surface sterilized with 70% ethanol for 3 min and 1% sodium hypochlorite for 1 min, rinsed in sterile distilled water, and placed on potato dextrose agar (PDA) plates. The hyphal tips emerging from the plant tissues were transferred onto new PDA plates to obtain pure cultures. Two fungal isolates were obtained from the diseased

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Figure 1. Symptoms of anthracnose disease occurring on *Cymbidium* species caused by *Colletotrichum cymbidiicola*. (A) Dark brown lesion developed on margin of a leaf. On the lesion, salmon-colored conidial masses formed concentrically; (B) Some lesions that coalesce to form enlarged legion; (C) Heavily Infected leaf turning completely brown and forming several concentric rings; (D) Disease symptoms appearing on young leaves of *Cymbidium* in pathogenicity test.

samples collected from different localities of Korea (Table 1). Morphological features of fungal structures from fresh plant materials and cultures were examined and photographed using a Zeiss AXIO Zoom V16 and AXIO Imager A2 microscopes equipped with AxioCam 506 color (Carl Zeiss, Oberkochen, Germany). Colonies on PDA were light gray with cottony aerial mycelium and reached 80 mm diameter after seven days at 25 °C (Figure 2(H)). Acervuli, sometimes rupturing the epidermis, were arranged in concentric patterns on the lesions and were cushion-shaped with simple, short, septate, and hyaline conidiophores (Figure 2(A–C, F)). Setae were brown, 2–4-septate, verruculous in the upper part, and 62.5–150 µm long (Figure 2(D)). Appressoria, produced using a slide culture technique [17], were brown, lobate, and measuring 5–15 × 5–8.5 µm (Figure 2(E)). Conidia were hyaline, aseptate, and cylindrical, with a prominent scar, and measuring 14–16 × 5–6 µm (Figure 2(G)). The morphological and cultural features of the causal fungus corresponded to those of *Colletotrichum cymbidiicola* described by Damm et al. [5].

Multigene phylogenetic analysis was carried out to confirm the morphological identification. For phylogenetic analysis, sequences of 11 *Colletotrichum* species available from GenBank were used (Table 1). Genomic DNA was extracted from fresh cultures by using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Mycelial mats were scraped using a sterile scalpel from the surface of colonies grown for a week. Polymerase chain reaction (PCR) was performed to amplify seven genes from the genomic DNA templates. DNA amplicons of the internal transcribed spacer (ITS), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), chitin synthase 1 (CHS-1), actin (ACT), histone3 (HIS3), beta-tubulin (TUB2), and calmodulin (CAL) were obtained and sequenced using the primer pairs described by Damm et al. [5]. The resulting seven gene sequences of two isolates (13-040, 17-228) were registered in GenBank (accession numbers; Table 1). A neighbor-joining (NJ) tree based on the combined sequence dataset was constructed in MEGA7 [18]. *C. gloeosporioides* (CBS112999) was used as an outgroup. In the phylogenetic tree, the *Colletotrichum* isolates from cymbidiums were placed in a clade including *C. cymbidiicola* with a bootstrap value of 99% (Figure 3).

The two isolates of *C. cymbidiicola* were tested for pathogenicity on three five-month-old cymbidium plants in a glasshouse. Three leaves wounded with needles were inoculated by spraying with a conidial suspension of 10^6 conidia/mL prepared from each isolate. Plants inoculated with sterile water served as control. The inoculated plants were maintained in a growth chamber at 26 °C for 48 h. Symptoms of anthracnose disease were visible seven days after inoculation (Figure 1(D)). No symptoms were observed in the control plants. The same fungus was re-isolated from the inoculated plants. Pathogenicity test revealed that the two isolates of *C. cymbidiicola* were pathogenic to *Cymbidium*, thus satisfying Koch's postulates.

Five species of *Colletotrichum*: *C. boninense*, *C. cliviae*, *C. cymbidiicola*, *C. gloeosporioides*, *C. orchidearum* and *C. plurivorum*, have been known to occur on *Cymbidium* species worldwide [19]. In Korea, the anthracnose pathogen of cymbidiums has been thought since 1996 to be *C. gloeosporioides*

Table 1. List of *Colletotrichum* species analyzed in this study.

Species	Isolate number	Country (locality)	GenBank accession number						
			ITS	GAPDH	CHS-1	ACT	HIS3	TUB2	CAL
C. cymbidiicola	13-040	Korea (Seosan)	MN258707	MN271661	MN271663	MN271665	MN271667	MN271669	MN271671
	17-228	Korea (Jeonju)	MN258708	MN271662	MN271664	MN271666	MN271668	MN271670	MN271672
	CBS 123757	Japan	JQ005168	JQ005255	JQ005342	JQ005516	JQ005429	JQ005602	JQ005689
	IMI 347923	Australia	JQ005166	JQ005253	JQ005340	JQ005514	JQ005427	JQ005600	JQ005687
	CBS 128543	New Zealand	JQ005167	JQ005254	JQ005341	JQ005515	JQ005428	JQ005601	JQ005688
C. beeveri	CBS 128527	New Zealand	JQ005171	JQ005258	JQ005345	JQ005519	JQ005432	JQ005605	JQ005692
C. boninense	CBS 123755	Japan	JQ005153	JQ005240	JQ005327	JQ005501	JQ005414	JQ005588	JQ005674
C. brasiliense	CBS 128501	Brazil	JQ005235	JQ005322	JQ005409	JQ005583	JQ005496	JQ005669	JQ005756
C. colombiense	CBS 129818	Colombia	JQ005174	JQ005261	JQ005348	JQ005522	JQ005435	JQ005608	JQ005695
C. constrictum	CBS 128504	New Zealand	JQ005238	JQ005325	JQ005412	JQ005586	JQ005499	JQ005672	JQ005759
C. dacrycarpi	CBS 130241	New Zealand	JQ005236	JQ005323	JQ005410	JQ005584	JQ005497	JQ005670	JQ005757
C. gloeosporioides	CBS 112999	Italy	JQ005152	JQ005239	JQ005326	JQ005500	JQ005413	JQ005587	JQ005673
C. hippeastri	CBS 125376	China	JQ005231	JQ005318	JQ005405	JQ005579	JQ005492	JQ005665	JQ005752
C. oncidii	CBS 129828	Germany	JQ005169	JQ005256	JQ005343	JQ005517	JQ005430	JQ005603	JQ005690
C. parsonsiae	CBS 128525	New Zealand	JQ005233	JQ005320	JQ005407	JQ005581	JQ005494	JQ005667	JQ005754

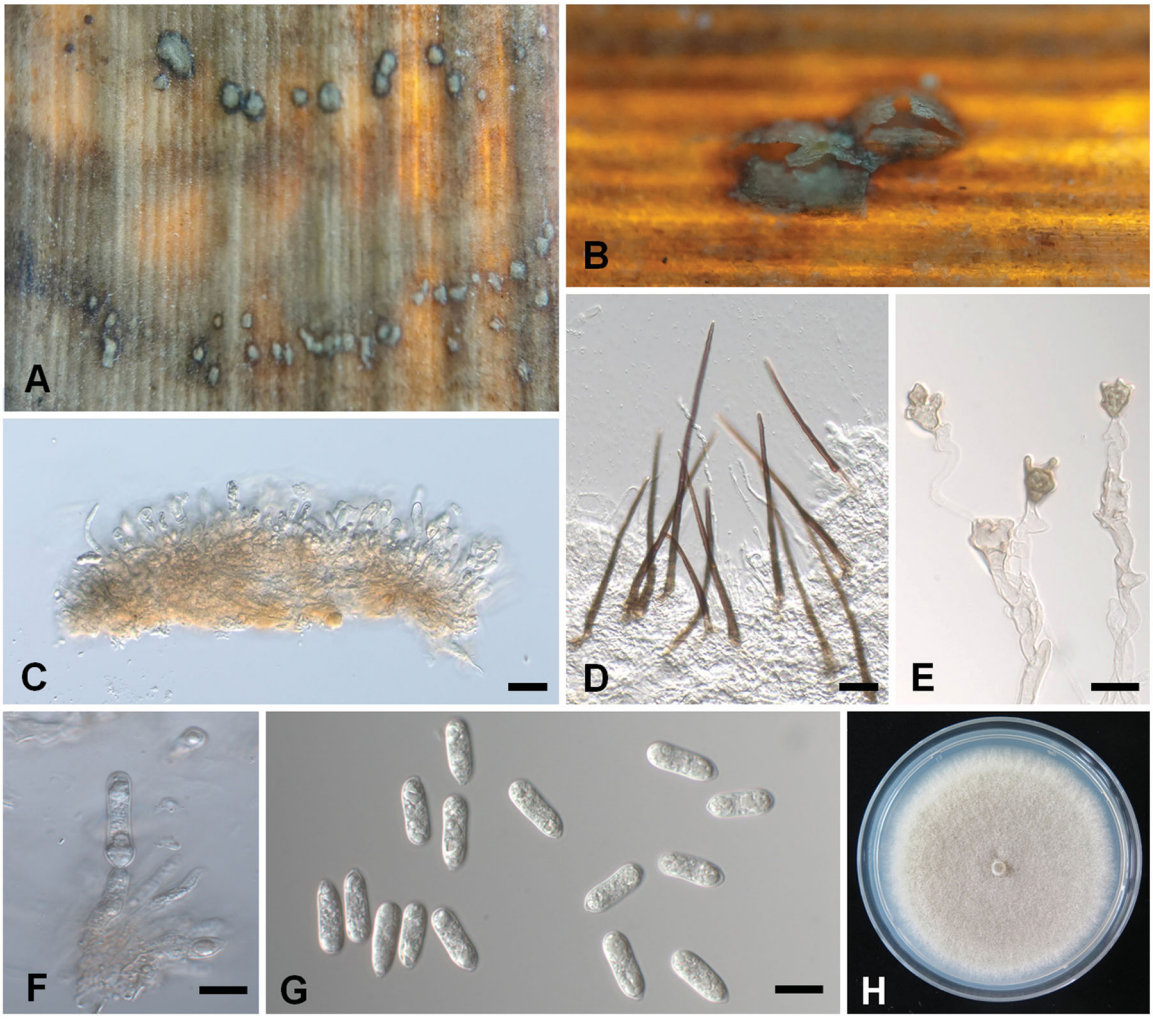

Figure 2. Morphologies of *Colletotrichum cymbidiicola* isolated from *Cymbidium* sp. (A) Close-up view of acervuli formed as concentric rings; (B) Acervuli; (C) Vertical section of acervulus containing conidiophores; (D) Setae; (E) Appressoria; (F) Conidiophore bearing conidium; (G) Conidia; (H) Colonies of *C. cymbidiicola* on potato dextrose agar plate after seven days of incubation at room temperature. Scale bars: Figures Cand D = 20 μm; Figures E, F, and G = 10 μm.

[20]. Our study shows that the Korean isolates newly obtained from cymbidium anthracnose are *C. cymbidiicola*, a newly recorded species on this host plant in Korea. Recent multi-locus analysis has shown that *C. cymbidiicola* is host-specific to *Cymbidium* orchids [5]. The species is phylogenetically placed within the *C. boninense* species complex. *Colletotrichum oncidii*, specific to *Oncidium*, is another *Colletotrichum* species associated with orchid anthracnose. A sister relationship exists between the two *Colletotrichum* species [5]. *C. cymbidiicola* has been recorded as an anthracnose

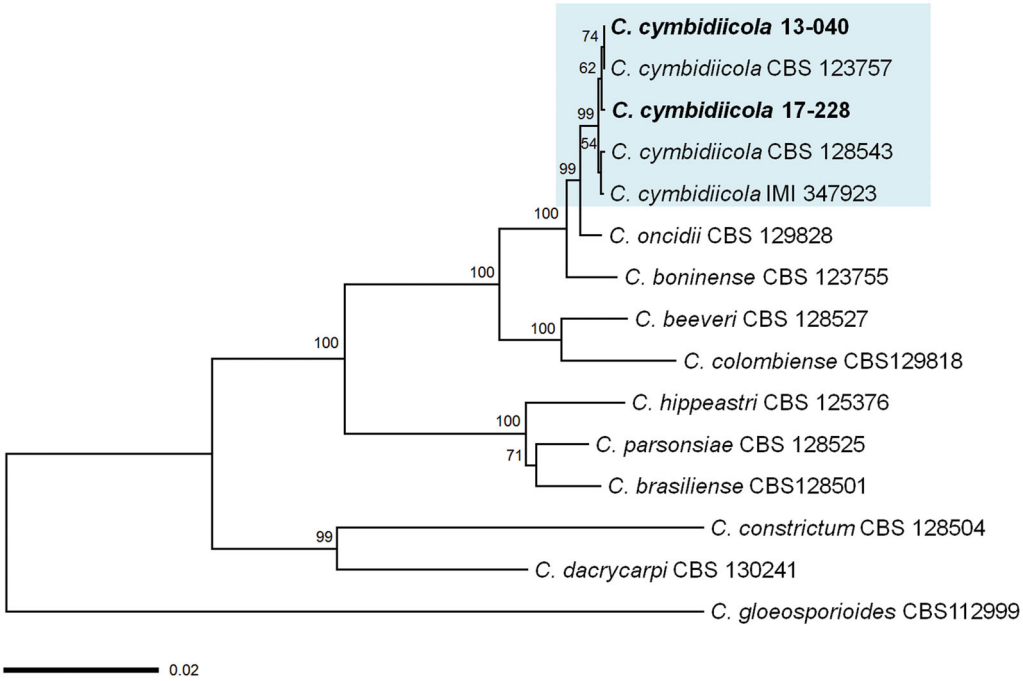

Figure 3. Neighbor-joining tree inferred from the combined ITS, GAPDH, CHS-1, ACT, HIS3, TUB2, and CAL sequence data for *Colletotrichum cymbidiicola* and related species. The numbers above nodes indicate bootstrap values obtained from 1000 replicates. Branch lengths are proportional to number of nucleotide changes. Scale bar = 0.02 substitutions per site.

pathogen in cymbidiums from Australia [5], New Zealand [5], Japan [5], India [21], and China [22]. This is the first report of the occurrence of *C. cymbidiicola* causing anthracnose on *Cymbidium* orchids in Korea.

Disclosure statement

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

Funding

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development [Project No. PJ01136802]", Rural Development Administration, Republic of Korea.

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