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.

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], spathianum 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 magnus, and Colletotrichum orchi deedum 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. gloeo sporoioides 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 leaves.
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.
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

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

| Species          | Isolate number | Country (locality) | GenBank accession number |
|------------------|----------------|--------------------|--------------------------|
| *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 JQ005629 JQ005689 |
|                  | IMI 347923     | Australia          | JQ005166 JQ005253 JQ005340 JQ005514 JQ005427 JQ005600 JQ005687 |
|                  | CBS 128543     | New Zealand        | JQ005167 JQ005254 JQ005341 JQ005513 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 JQ005388 JQ005674 |
| *C. brasiliense* | CBS 128501     | Brazil             | JQ005235 JQ005322 JQ005409 JQ005583 JQ005496 JQ005669 JQ005756 |
| *C. colombiense* | CBS 129818     | Colombia           | JQ005174 JQ005251 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. gloeosporoides* | CBS 112999   | Italy              | JQ005152 JQ005239 JQ005326 JQ005500 JQ005413 JQ005587 JQ005673 |
| *C. hippocastri* | 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 ¼ 20 l m; Figures E, F, and G ¼ 10 l m.
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).

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