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Brief Report

Morphology, Phylogeny, and Pathogenicity of *Colletotrichum* Species Causing Anthracnose in *Camellia japonica* in China

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Abstract: *Camellia japonica* is a renowned flower and an influential plant in Chinese urban landscaping. However, *Colletotrichum*, one of the world's most commercially important phytopathogenic genera that causes anthracnose on a wide range of plant species, have annually caused significant economic losses to *Ca. japonica*. In this study, 115 strains were isolated from *Ca. japonica* leaves with typical symptoms from the provinces of Hunan, Jiangxi, Hainan, Guangxi, Hubei, Chongqing, Guizhou, and Shanxi. They were then subjected to pathogen identification and using method of morphology combined with *ApMat* gene sequence analysis, along with the pathogenicity tests based on Koch's postulates. The 115 strains were identified as *C. gloeosporioides*, *C. fructicola*, *C. siamense*, *C. camelliae* or *C. aeschynomenes*. Pathogenicity tests revealed that all species produced brown lesions on healthy *Ca. japonica* leaves, indicating significant virulence. Furthermore, *C. fructicola* had the broadest distribution and the highest isolation rate., Most importantly, this is the first report in China of *C. aeschynomenes* causing the anthracnose disease in *Ca. japonica*.

Keywords: *Camellia japonica*; anthracnose; *Colletotrichum*; species complex

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1. Introduction

Camellia japonica is one of the ten traditional famous flowers and an important plant in urban landscaping in China. Its cultivation has a long history and it has high ornamental and economic value [1]. *Ca. japonica* contains high concentrations of saponins, tannins, flavonoids, and other active compounds that have health-promoting and disease-prevention properties. [2]. *Ca. japonica* plants have been highlighted as exhibiting antimicrobial (antibacterial, antifungal, antiviral) and antitumoral activity, as well as antioxidant properties and biological activity [3]. Moreover, these plants' flowers are tonic, astringent, hemostatic, antihemorrhagic, and the leaves have a high concentration of anti-inflammatory compounds [2,3]. Thus, the development of the *Ca. japonica* industry is of great significance to the national economy of China.

More than 20 *Ca. japonica* diseases have been recorded, including leaf spot disease, putrefaction disease, and sooty blotch, among others [4]. Anthracnose, caused by Colletotrichum spp., is one of the most serious diseases [5]. Leaves infected by *Colletotrichum* generally cause water-soaked lesions in the early stages of the disease. As the disease progresses, the lesions get larger and necrotic, resulting in significant yield losses [6]. However, relatively little is known about the taxonomy, genetic diversity, and pathogenicity of *Ca. japonica* anthracnose.

Colletotrichum can inhabit plants as a pathogen, endophyte, epiphyte, or saprobe [7–11]. However, until recently, *Colletotrichum* species identification was limited to inconsistent morphological characteristics and host relations. To date, the combination of morphology and molecular systematics has shown to be an effective identification strategy, and knowledge on taxonomy of *Colletotrichum* has improved [8–10]. Nearly all acknowledged species

studied were grouped into 16 *Colletotrichum* species complexes [11,12]. *Colletotrichum* species, considered to be the main causative agents of anthracnose on *Ca. Sinensis,* include six known species (*C. camelliae*, *C. cliviae*, *C. fioriniae*, *C. fructicola*, *C. karstii*, *C. siamense*), three new record species (*C. aenigma*, *C. endophytica*, *C. truncatum*), one novel species (*C. wuxiense*) [9]. In a previous study, seven *Colletotrichum* species caused *Ca. oleifera* anthracnose in China [13,14]. Moreover, *Colletotrichum* species can be identified accurately and rapidly using the *ApMat* gene sequence analysis [15–17]. This study aimed to identify the anthracnose pathogen associated with *Ca. japonica* in China based on both morphological characteristics and molecular phylogeny.

2. Materials and Methods

2.1. Sample Collection and Isolation

In this study, the samples were collected from *Ca. japonica* with irregular brownish-grey lesions on leaves. Samples were collected from the *Ca. japonica* production fields in the Hunan, Jiangxi, Hainan, Guangxi, Hubei, Chongqing, Guizhou and Shanxi provinces in 2021. *Colletotrichum species* were isolated using the protocol described in [17].

2.2. Morphological Characterization

Inoculations of isolates were conducted on potato dextrose agar (PDA) plates at 28 °C for five days. The colony diameter was measured, and the growth rate of mycelium was calculated. As morphological characteristics, we measured the size, growth rate, and color of conidia and appressoria on each isolate. A 10 µL volume of spore suspension (1×10^5 spores/mL) was placed in the center of hydrophobic slides and cultured at 28 °C for 12 h to observe the formation of appressoria [17]. Three replicates were prepared per sample.

2.3. DNA Extraction, PCR Amplification, and Sequencing

To further confirm the identification, genomic DNA was extracted using the CTAB [cetyltrimethylammonium bromide] method [18]. The *Apn2-Mat1-2* intergenic spacer and partial mating type (*Mat1-2*) (*ApMat*) gene region were amplified using the primer pair, Am-F2 (5′-TCATTCTACGTATGCCCG-3′) and Am-R2 (5′-CCAGAAATACACCGAACTTGC-3′) [17], under the conditions described in [18]. PCR products were sequenced by Tsingke Biotechnology Co., Ltd. (Beijing, China).

2.4. Phylogenetic Analyses

All *Colletotrichum* isolates were selected for *ApMat* sequencing and analysis [19]. The GenBank accession numbers for *ApMat* gene sequences of the examined *Colletotrichum* isolates are shown in Table 1. Nineteen reference strains were downloaded with the *ApMat* sequences from GenBank database (Table 1), according to recent publications of the genus. A phylogenetic tree was generated via the Neighbor-Joining method with 1000 bootstrap replications. Evolutionary analyses were conducted in MEGA7 [20].

Table 1. GenBank accession numbers of nucleotide sequences used in this study.

Taxon	Isolate Designation	Host	Geographic Location	*ApMat* GenBank	References
C. camelliae	LF152	*Camellia* sp.	China	KJ954506.1	[21]
	LF790	*Cinamomum zeylanicum*	India	KU239747.1	Direct Submission
	LC1364 *	*Camellia sinensis*	China	KJ954497.1	[21]
	HNCS1	*Camellia japonica*	China	OQ198468	In this study
	HNCS4	*Camellia japonica*	China	OQ198469	In this study
	HNCS10	*Camellia japonica*	China	OQ198470	In this study

Table 1. *Cont.*

Taxon	Isolate Designation	Host	Geographic Location	*ApMat* GenBank	References
	HNCS11	*Camellia japonica*	China	OQ198471	In this study
	JXGZ18-1	*Camellia japonica*	China	OQ198472	In this study
	JXGZ18-2	*Camellia japonica*	China	OQ198473	In this study
	JXGZ25-2	*Camellia japonica*	China	OQ198474	In this study
	JXNC4-1	*Camellia japonica*	China	OQ198475	In this study
	JXNC4-2	*Camellia japonica*	China	OQ198476	In this study
	JXNC14-1	*Camellia japonica*	China	OQ198477	In this study
	JXNC14-2	*Camellia japonica*	China	OQ198478	In this study
	JXNC15-2	*Camellia japonica*	China	OQ198479	In this study
	JXNC17-1	*Camellia japonica*	China	OQ198480	In this study
	JXNC17-2	*Camellia japonica*	China	OQ198481	In this study
	JXNC23-2	*Camellia japonica*	China	OQ198482	In this study
	JXNC24-1	*Camellia japonica*	China	OQ198483	In this study
C. siamense	AGMy0249	*Citrus pennivesiculata*	Bangladesh	KX578769.1	[22]
	LC0034	*Coffee berry*	Thailand	JQ899288.1	Direct Submission
	HJM	*Loropetalum chinense*	China	MG717312.1	Direct Submission
	GL12-3	*Plum*	China	OM816816.1	Direct Submission
	GYL	*Magnolia grandiflora*	China	MG717298.1	Direct Submission
	LF148	*Camellia* sp.	China	KJ954504.1	[21]
	DBST-1	*Cycas debaoensis*	China	MT786728.1	Direct Submission
	EIPP77	*Coffee*	China	MK344209.1	Direct Submission
	ICMP18649 *	*Coffea arabica*	China	JQ899289	[21]
	HNCS3	*Camellia japonica*	China	OQ198484	In this study
	HNCS5	*Camellia japonica*	China	OQ198485	In this study
	HNYY39-1	*Camellia japonica*	China	OQ198486	In this study
	HNHH2-1	*Camellia japonica*	China	OQ198487	In this study
	HNHH2-2	*Camellia japonica*	China	OQ198488	In this study
	HNHH9-2-1	*Camellia japonica*	China	OQ198489	In this study
	HNHH10-1	*Camellia japonica*	China	OQ198490	In this study
	JXXY7-1	*Camellia japonica*	China	OQ198491	In this study
	JXNC15-1	*Camellia japonica*	China	OQ198492	In this study
	GXNN5-1	*Camellia japonica*	China	OQ198493	In this study
	GXNN7-2	*Camellia japonica*	China	OQ198494	In this study
	GXNN9-2	*Camellia japonica*	China	OQ198495	In this study
	GXNN18-1	*Camellia japonica*	China	OQ198496	In this study
	GXNN22-1	*Camellia japonica*	China	OQ198497	In this study
	GXNN22-2	*Camellia japonica*	China	OQ198498	In this study
	GXNN23-1-1	*Camellia japonica*	China	OQ198499	In this study
	GXNN23-1-2	*Camellia japonica*	China	OQ198500	In this study
	GXNN23-2-1	*Camellia japonica*	China	OQ198501	In this study

Table 1. *Cont.*

Taxon	Isolate Designation	Host	Geographic Location	*ApMat* GenBank	References
	GXNN23-2-2	*Camellia japonica*	China	OQ198502	In this study
	CQFJ1-1-1	*Camellia japonica*	China	OQ198503	In this study
	CQFJ6-3	*Camellia japonica*	China	OQ198504	In this study
	CQFJ6-4	*Camellia japonica*	China	OQ198505	In this study
	CQFJ20-1	*Camellia japonica*	China	OQ198506	In this study
	CQFJ22-2	*Camellia japonica*	China	OQ198507	In this study
	CQFJ26-1	*Camellia japonica*	China	OQ198508	In this study
	GZTR2-2	*Camellia japonica*	China	OQ198509	In this study
C. gloeosporioides	LF318	*Ca. sinensis*	China	KJ954541.1	[21]
	ICMP1782 *	*Citrus sinensis*	Italy	JQ807843	[21]
	HNCS7	*Camellia japonica*	China	OQ198510	In this study
	JXNC9-2	*Camellia japonica*	China	OQ198511	In this study
	HBWH9-1	*Camellia japonica*	China	OQ198512	In this study
	HBWH9-2	*Camellia japonica*	China	OQ198513	In this study
	HBWH13-1	*Camellia japonica*	China	OQ198514	In this study
	HBWH13-2	*Camellia japonica*	China	OQ198515	In this study
	GXNN16-1	*Camellia japonica*	China	OQ198516	In this study
	CQGX1-1	*Camellia japonica*	China	OQ198517	In this study
	CQGX1-2	*Camellia japonica*	China	OQ198518	In this study
	CQGX17-1	*Camellia japonica*	China	OQ198519	In this study
	CQGX21-2	*Camellia japonica*	China	OQ198520	In this study
	CQGX24-1	*Camellia japonica*	China	OQ198521	In this study
	CQGX24-2	*Camellia japonica*	China	OQ198522	In this study
C. aeschynomenes	ICMP1767 *	*Aeschynomene viginica*	China	KM360145.1	[21]
	HNTJ20-1	*Camellia oleifera*	China	MZ8321172.1	[17]
	HNCS8	*Camellia japonica*	China	OQ198523	In this study
	HNCS12	*Camellia japonica*	China	OQ198524	In this study
	HNCS13	*Camellia japonica*	China	OQ198525	In this study
	JXPX17-2	*Camellia japonica*	China	OQ198526	In this study
	JXXY18-1-2	*Camellia japonica*	China	OQ198527	In this study
	GXNN4-1	*Camellia japonica*	China	OQ198528	In this study
	GXNN9-1	*Camellia japonica*	China	OQ198529	In this study
	GXNN11-1	*Camellia japonica*	China	OQ198530	In this study
	GXNN12-1-1	*Camellia japonica*	China	OQ198531	In this study
	GXNN12-1-2	*Camellia japonica*	China	OQ198532	In this study
	GXNN12-2	*Camellia japonica*	China	OQ198533	In this study
	GXNN15-1	*Camellia japonica*	China	OQ198534	In this study
	GXNN18-2-1	*Camellia japonica*	China	OQ198535	In this study
	GXNN20-1	*Camellia japonica*	China	OQ198536	In this study
	GXNN20-2	*Camellia japonica*	China	OQ198537	In this study

Table 1. *Cont.*

Taxon	Isolate Designation	Host	Geographic Location	*ApMat* GenBank	References
C. theobromicola	ICMP18649 *	*Theobroma cacao*	Panama	KC790726	[21]
C. fructicola	LF896	*Ca. sinensis*	China	KJ954624.1	[21]
	LC0033	*Coffea arabica*	India	JQ807838.1	Direct Submission
	ICMP18581 *	*Coffea arabica*	Thailand	JQ07838	[21]
	HNCS9	*Camellia japonica*	China	OQ198538	In this study
	HNYY3.24	*Camellia japonica*	China	OQ198539	In this study
	HNYY9-1	*Camellia japonica*	China	OQ198540	In this study
	HNYY23-1	*Camellia japonica*	China	OQ198541	In this study
	HNYY23-2	*Camellia japonica*	China	OQ198542	In this study
	HNYY27-1	*Camellia japonica*	China	OQ198543	In this study
	HNYY27-2	*Camellia japonica*	China	OQ198544	In this study
	HNYY38-1	*Camellia japonica*	China	OQ198545	In this study
	HNYY38-2	*Camellia japonica*	China	OQ198546	In this study
	HNHH4-2	*Camellia japonica*	China	OQ198547	In this study
	HNHH7-1	*Camellia japonica*	China	OQ198548	In this study
	HNHH8-2	*Camellia japonica*	China	OQ198549	In this study
	HNHH11-2	*Camellia japonica*	China	OQ198550	In this study
	HNHH13-2	*Camellia japonica*	China	OQ198551	In this study
	JXPX7-2	*Camellia japonica*	China	OQ198552	In this study
	JXGZ5-2	*Camellia japonica*	China	OQ198553	In this study
	HBMC2-2	*Camellia japonica*	China	OQ198554	In this study
	HBMC11-2-1	*Camellia japonica*	China	OQ198555	In this study
	HBMC11-2-2	*Camellia japonica*	China	OQ198556	In this study
	HBMC25-1	*Camellia japonica*	China	OQ198557	In this study
	HBMC25-2	*Camellia japonica*	China	OQ198558	In this study
	GXNN4-2	*Camellia japonica*	China	OQ198559	In this study
	GXNN7-1	*Camellia japonica*	China	OQ198560	In this study
	CQGX8-3	*Camellia japonica*	China	OQ198561	In this study
	CQFJ1-1-2	*Camellia japonica*	China	OQ198562	In this study
	CQFJ1-2-2	*Camellia japonica*	China	OQ198563	In this study
	CQFJ5-1	*Camellia japonica*	China	OQ198564	In this study
	CQFJ5-2	*Camellia japonica*	China	OQ198565	In this study
	CQFJ10-2	*Camellia japonica*	China	OQ198566	In this study
	CQFJ22-1	*Camellia japonica*	China	OQ198567	In this study
	CQFJ26-2	*Camellia japonica*	China	OQ198568	In this study
	CQFJ27-1	*Camellia japonica*	China	OQ198569	In this study
	CQFJ27-2	*Camellia japonica*	China	OQ198570	In this study
	CQYY4-2	*Camellia japonica*	China	OQ198571	In this study
	CQYY5-2	*Camellia japonica*	China	OQ198572	In this study

Table 1. *Cont.*

Taxon	Isolate Designation	Host	Geographic Location	*ApMat* GenBank	References
	CQYY9-2	*Camellia japonica*	China	OQ198573	In this study
	CQYY12-1	*Camellia japonica*	China	OQ198574	In this study
	HNHK4-4	*Camellia japonica*	China	OQ198575	In this study
	HNHK13-2	*Camellia japonica*	China	OQ198576	In this study
	HNHK49-1	*Camellia japonica*	China	OQ198577	In this study
	GZTR12-1	*Camellia japonica*	China	OQ198578	In this study
	GZTR12-2	*Camellia japonica*	China	OQ198579	In this study
	SXQL2-2	*Camellia japonica*	China	OQ198580	In this study
	SXQL32-2	*Camellia japonica*	China	OQ198581	In this study
	SXQL36-2-1	*Camellia japonica*	China	OQ198582	In this study

Note: the asterisk indicates the ex-type strain.

2.5. Pathogenicity Testing

Young and healthy leaves of *Ca. japonica* were collected from trees growing in the greenhouse. Three isolates of each species were used for a pathogenicity test, which was performed as previously described [8,17]. The pathogen was re-isolated from the symptomatic tissue using the method described in [23].

3. Results

3.1. Phylogenetic Analyses

The sequences of 115 tested *ApMat* genes and other known species of *Colletotrichum* in GenBnak were analyzed, and the Neighbor-Joining tree was constructed. The sequence datasets for the *ApMat* were analyzed in combination to establish interspecific relationships within *Colletotrichum*. The *Colletotrichum* isolates' combined species phylogeny comprising 134 sequences, including the outgroup *C. theobromicola* ICMP18649* (Figure 1).

Phylogenetic analyses using the Neighbor-Joining algorithm showed the 115 isolated strains were clustered into five obvious evolutionary branches with a very high support rate, i.e., *C. gloeosporioides*, *C. fructicola*, *C. siamense*, *C. camelliae* and *C. aeschynomenes*, including forty-five isolates of *C. fructicola*, fifteen isolates of *C. aeschynomenes*, twenty-six isolates of *C. siamense*, and sixteen isolates of *C. camelliae*. *C. fructicola* is the predominant species among *Colletotrichum* of *Ca. japonica* in China, accounting for 39% (n = 115) of the isolates tested. (Figure 2).

3.2. Taxonomy

Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. 1884

Description: *Colonies* on PDA reaching 67–69 mm diam after five days. *Colonies* flat with rose edge, scattered acervuli with orange conidial ooze near center, fuscous black pigment near the edge; reverse honey with fuscous black near the edge. *Chlamydospores* not observed. *Conidia* hyaline, aseptate, smooth-walled, cylindrical, both ends bluntly rounded, 15–16.5 × 4.5–5.5 µm. *Appressoria* medium to dark brown, aseptate, solitary or in groups. Variable in shape, circular, clavate, ellipsoidal or irregular in outline, crenate or slightly lobed at edge, 7.5–9.5 × 5.5–6.5 µm (Figure 3).

Colletotrichum fructicola Priastuti, L. Cai. and K.D. Hyde. 2009.

Description: *Colonies* on PDA reaching 64–66 mm diam after five days. *Colonies* flat with entire edge, aerial mycelium dense, cottony, pale prey to white aerial mycelium and numerous black stroma scattered over the surface, grey in the center, white at the margin;

reverse greyish green. *Conidia* hyaline, aseptate, smooth-walled, cylindrical, both ends obtuse, 14.5–16.5 × 4.9–5.5 µm (Figure 4).

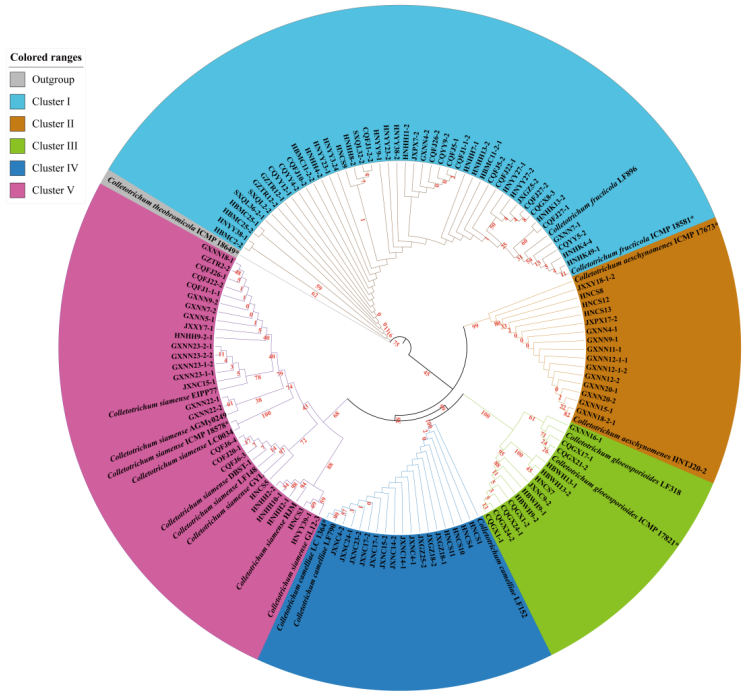

Figure 1. Phylogenetic tree based on *ApMat* gene sequences using the Neighbor-Joining method in MEGA7 with 1000 bootstrap replications. The isolates obtained in this study are marked in bold. The corresponding sequences for the reference species were retrieved from NCBI GenBank. The numbers at nodes represent their bootstrap support. *: type strain.

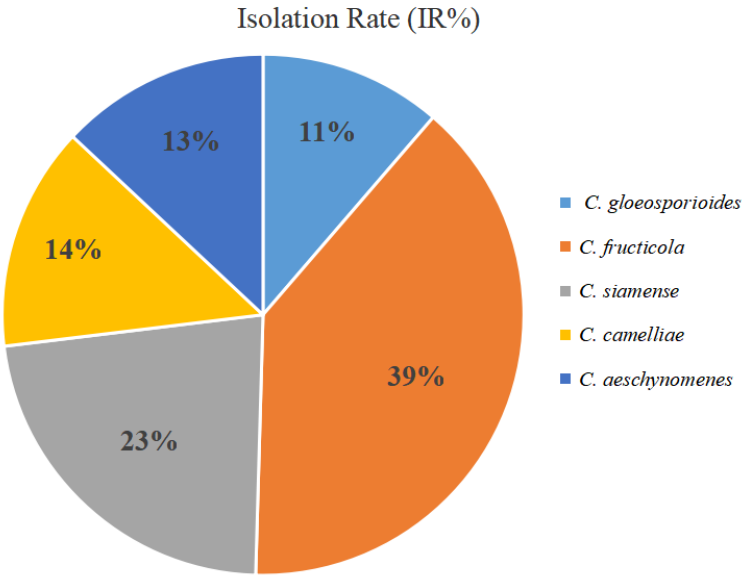

Figure 2. Isolation Rate (IR%) of *Colletotrichum* species isolated from *Ca. japonica* leaves.

Colletotrichum siamense Priastuti, L. Cai. and K.D. Hyde. 2009.

Description: *Colonies* on PDA reaching 58–61 mm diam after five days. *Colonies* pale yellow-white, grey, dense cottony aerial mycelium with orange acervular conidiomata at the center; reverse pale yellowish. *Conidia* hyaline, aseptate, smooth-walled, fusiform to cylindrical, both ends bluntly rounded, 14–15.5 × 4.9–5.2 µm. *Appressoria* dark brown, solitary, circular, entire to crenate margin, 7.5–10 × 5–6.5 µm (Figure 5).

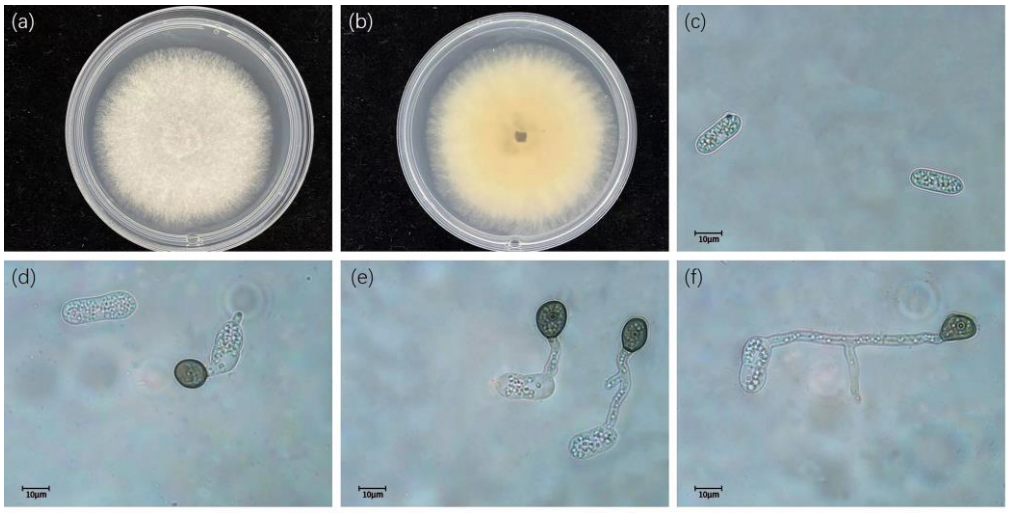

Figure 3. *C. gloeosporioides* (CQGX 24-1). (**a**) Colony on PDA. (**b**) Reverse side of the colony on PDA. (**c**) Conidia. (**d**–**f**) Appressoria. Scale bars = 10 μm.

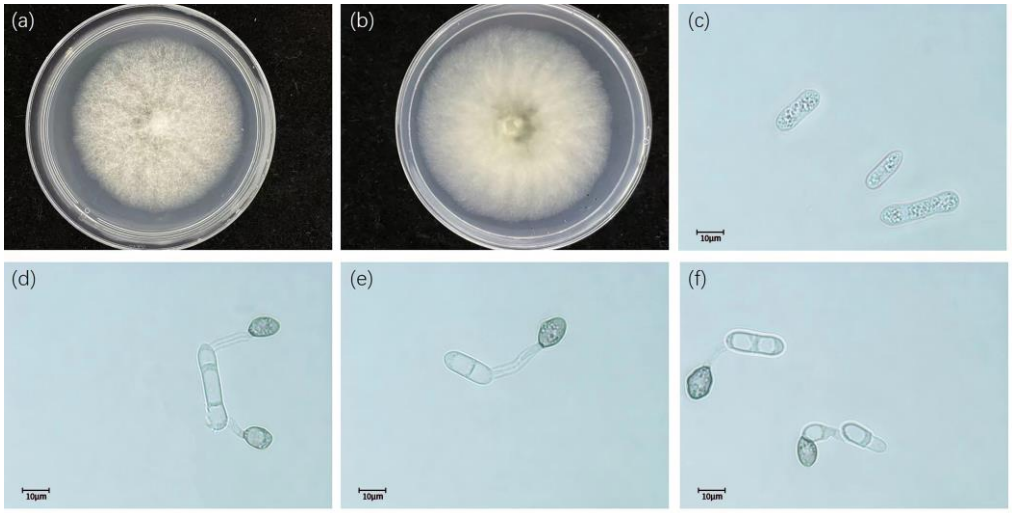

Figure 4. *Colletotrichum fructicola* (HNHH7-1). (**a**) Colony on PDA. (**b**) Reverse side of the colony on PDA. (**c**) Conidia. (**d**–**f**) Appressoria. Scale bars = 10 μm.

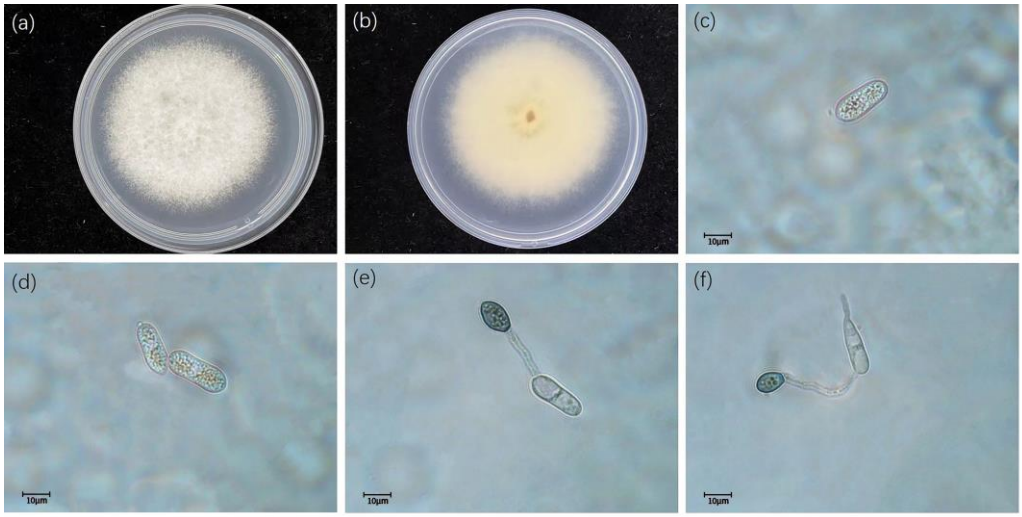

Figure 5. *Colletotrichum siamense* (CQFJ6-3). (**a**) Colony on PDA. (**b**) Reverse side of the colony on PDA. (**c**,**d**) Conidia. (**e**,**f**) Appressoria. Scale bars = 10 μm.

Colletotrichum camelliae Massee. 2012

Description: *Colonies* on PDA reaching 53–56 mm diam after five days. *Colonies* flat with entire edge, aerial mycelium white, cottony, sparse; reverse white at first, then grey to black at the center. *Conidia* hyaline, smooth-walled, guttulate, cylindrical with obtuse ends, sometimes narrowed at the center or towards the base, 14–17 × 4.5–5.5 µm. *Appressoria* irregularly shaped, clavate, crenate, lobed, brown to dark brown, solitary, branched, catenate, with age sometimes complex chlamydospore-like structures develop, 8–11.5 × 5–8.5 µm (Figure 6).

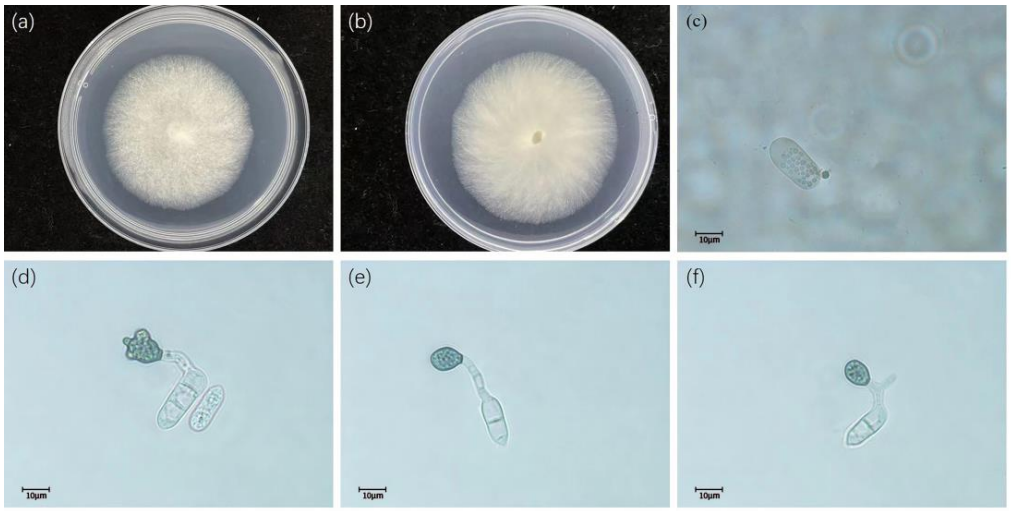

Figure 6. *Colletotrichum camelliae* (JXGZ18-1). (**a**) Colony on PDA. (**b**) Reverse side of the colony on PDA. (**c**) Conidia. (**d**–**f**) Appressoria. Scale bars = 10 µm.

Colletotrichum aeschynomenes B.S. Weir, P.R. Johnston, and U. Damm. 2012

Description: *Colonies* on PDA reaching 63–67 mm diam after five days. *Colonies* pale light grey color with dense cottony aerial mycelium and white to orange conidial masses at the center. *Conidia* hyaline, aseptate, fusiform to cylindrical, both ends bluntly rounded, 7–9 × 17.5–24 µm. *Appressoria* were brown, mostly elliptic to cuboid, deeply lobed, 9–11.5 × 9.5–13 µm (Figure 7).

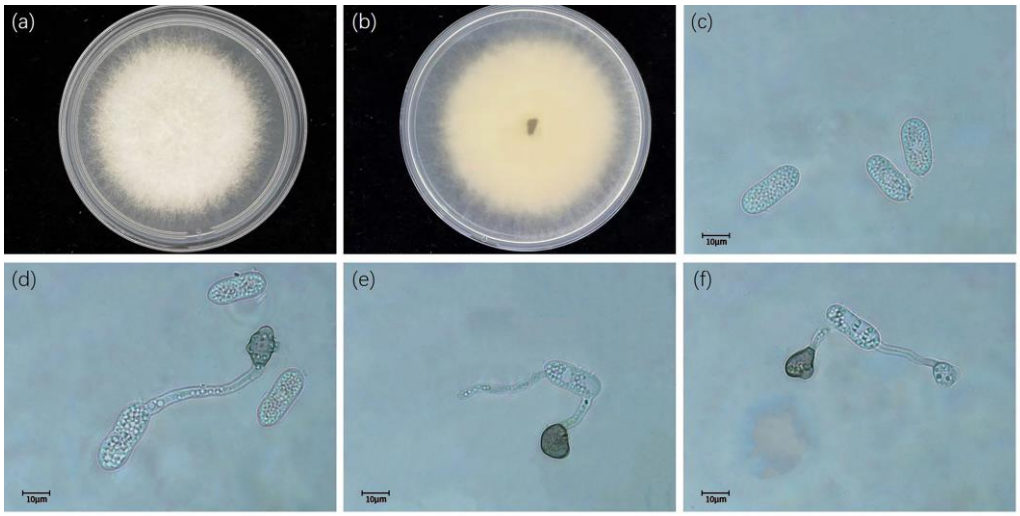

Figure 7. *Colletotrichum aeschynomenes* (GXNN4-1). (**a**) Colony on PDA. (**b**) Reverse side of the colony on PDA. (**c**) Conidia. (**d**–**f**) Appressoria. Scale bars = 10 µm.

3.3. Pathogenicity Assay

In the pathogenicity tests, *C. gloeosporioides*, *C. fructicola*, *C. siamense*, *C. camelliae*, and *C. aeschynomenes* developed brown lesions on wounded leaves after three days, whereas the controls exhibited no symptoms (Figure 8). Koch's postulates were confirmed by reisolating the same fungi and verifying its colony and morphological characteristics.

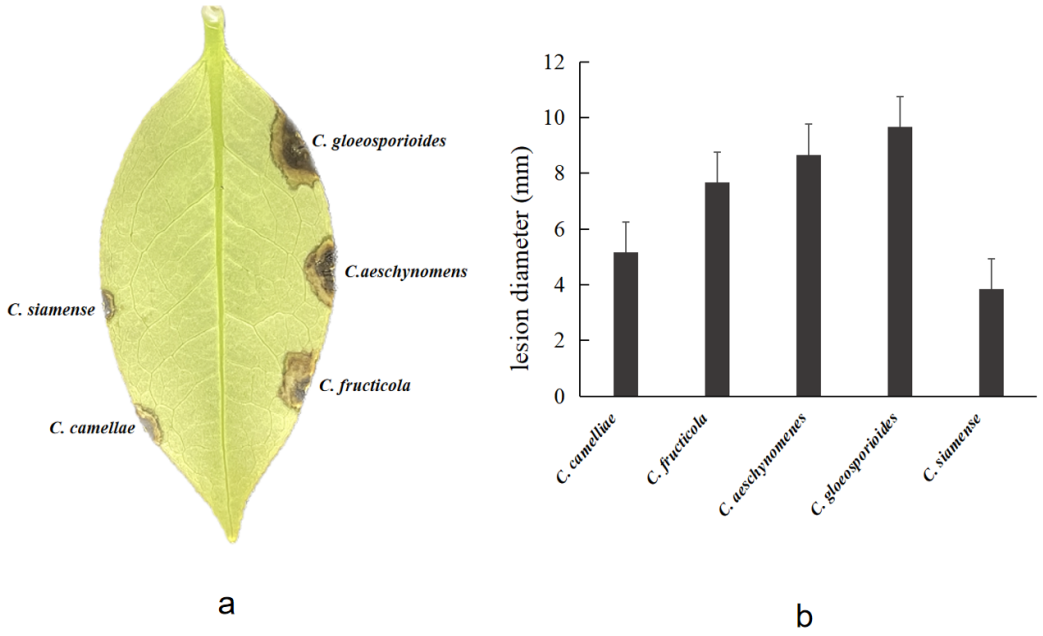

Figure 8. Pathogenicity of five *Colletotrichum* species from *Ca. japonica* leaves. (**a**) Induced symptoms on non-wounded *Ca. japonica* leaves after 3 days. (**b**). The virulence of the isolates was evaluated by measuring the diameters of the necrotic lesions.

4. Discussion

Ca. japonica anthracnose caused by *Colletotrichum* fungi in China was observed as a common disease. Taking this into account, an investigation of *Ca. japonica* diseases in China was carried out. A total of 115 isolates were obtained from eight *Ca. japonica* tree plantations in eight provinces, representing the broad geographical distribution of camellia tree plantations in China. Identification of our collections was processed based on isolates from symptomatic leaves of *Ca. japonica* using *ApMat* gene, as well as morphological characteristics. The results showed the isolates identified as *C. gloeosporioides*, *C. fructicola*, *C. siamense*, *C. camelliae* and *C. aeschynomenes*, as previously described [24–27]. However, some species of *Gloeosporium theae-sinensis*, *C. aenigma*, *C. aracearum* and *C. camelliae-japoncae* were not isolated in this study [28–31]. Further research is needed to find more species of pathogens.

Colletotrichum is one of the most significant plant within the pathogenic fungi genera, with 200 or more species known to cause diseases in plants and crops across the world [7]. Weir et al. [32] characterized the taxonomy of *C. gloeosporioides* species complex based on multi-gene and morphological features, which was a significant taxonomic breakthrough. They incorporated multiple gene regions in their phylogenetic analyses, but heavily relied on *ACT*, *CAL*, *CHS1*, *GAPDH* and *ITS* gene-regions to redefine species boundaries within this species complex [5,6,9,10,22]. The ITS sequences of *Colletotrichum* species complex have been considered to be an insufficient variable to reliably distinguish between the different members [33]. In addition, the *ApMat* marker gene has been used to resolve and improve the systematic classification of *Colletotrichum* species complexes [15–17,19,24]. In this study, phylogenetic analysis was performed on *ApMat* gene sequences and the 115 isolates were clustered into five obvious evolutionary branches with a very high support rate. Among them, *C. fructicola* exhibited the most widespread distribution and the highest isolated rate. This is probably because *C. fructicola* has stronger adaptability to heterogenous habitat.

Pathogenicity experiments of five *Colletorchum* species from *Ca. japonica* revealed that all species were capable of infecting wounded leaves. *C. gloeosporioides*, *C. fructicola* and *C. aeschynomenes* were the most virulent. Interestingly, the major pathogens of *Ca. japonica* anthracnose were found to be distinct in different provinces. This is the first report of *C. aeschynomenes* causing anthracnose disease in *Ca. japonica* in China. Further studies will be needed to understand how the disease can be controlled.

5. Conclusions

Five *Colletotrichum* species on *Ca. japonica* were described and illustrated. This is the first report of *C. aeschynomenes* causing anthracnose in *Ca. japonica* in China. Pathogenicity tests indicated that there were significant differences in virulence among the five *Colletotrichum* species when inoculated on the leaves of *Ca. japonica*. This study provides valuable information for the identification and control of this plant disease.

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