Characterization and Pathogenicity of *Colletotrichum* Species on *Philodendron tatei* cv. Congo in Gansu Province, China

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Abstract

In recent years in China, leaf spot caused by *Colletotrichum* species has been an emerging disease of *Philodendron tatei* cv. Congo. From 2016 to 2019, typical symptoms, appearing as circular or ovoid, sunken, and brown lesions with a yellow halo, were commonly observed on *P. tatei* cv. Congo in and around Lanzhou, Gansu Province, China. Conidiomata were often visible on infected leaf surfaces. Leaf disease incidence was approximately 5 to 20%. A total of 126 single-spored *Colletotrichum* isolates were obtained from leaf lesions. Multilocus phylogenetic relationships were analyzed based on seven genomic loci (ITS, *ACT*, *GAPDH*, *HIS3*, *CAL*, *CHS-1*, and *TUB2*) and the morphological characters of the isolates determined. These isolates were identified as three *Colletotrichum* species in this study. A further 93 isolates, accounting for 74% of all *Colletotrichum* isolates, were described as new species and named as *Colletotrichum philodendricola* sp. nov. after the host plant genus name, *Philodendron*; another two isolates were named as *C. pseudoboninense* sp. nov. based on phylogenetic and morphological relatedness to *C. boninense*; the other 31 isolates, belonging to the *C. orchidearum* species complex, were identified as a known

species—*C. orchidearum*. Both novel species *C. philodendricola* and *C. pseudoboninense* belong to the *C. boninense* species complex. Pathogenicity tests by both spray and point inoculations confirmed that all three species could infect leaves of *P. tatei* cv. Congo. For spray inoculation, the mean infection rate of leaves on the three species was only 4.7% (0 to 12%), and the size on lesions was mostly 1 to 2 mm in length. For point inoculation, 30 days after nonwounding inoculation, the infection rate on leaves was 0 to 35%; in wounding inoculation, the infection rate of leaves was 35 to 65%; wounding in healthy leaves greatly enhanced the pathogenicity of these three species to *P. tatei* cv. Congo; however, the sizes of lesions among the three species were not significantly different. To our knowledge, this is the first report of *Colletotrichum* species associated with anthracnose diseases on *P. tatei* cv. Congo. Results obtained in this study will assist the disease prevention and appropriate management strategies.

Keywords: *Philodendron tatei* cv. Congo, new species of *Colletotrichum*, brown spot, identification

Philodendron tatei cv. Congo (or *Philodendron* ‘Congo’), family Araceae, is a cultivar of *P. tatei* (Osiecki 2000). *P. tatei* is widely used as a pot plant in homes and offices and is famous for its long lifespan in China (Lin et al. 2012; Zhou et al. 2014). It can improve the air quality in buildings because of its capability to absorb various toxic gases, including SO₂, SO₃, NO, etc. (Chen et al. 2012). Broader scale cultivation of *P. tatei* cv. Congo has increased the chance of leaf diseases in *Philodendron* fields in China. For example, a severe outbreak of a soft rot caused by *Dickeya dieffenbachiae* on *P. ‘Congo’* has occurred in three areas of *Philodendron* fields (5 ha) in Guangzhou, China, from 2010 to 2011 (Lin et al. 2012). In October 2013, a leaf spot of *P. tatei* cv. Congo caused by *Alternaria alternata* was observed in a residential district of Luoyang, China (Zhou et al. 2014).

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Anthracnose disease caused by *Colletotrichum* is one of the foremost diseases in crops and ornamental plants (Damm et al. 2012, 2019; Guarnaccia et al. 2016, 2017; Meneses et al. 2019). However, only a few studies of anthracnose on *Philodendron* have been conducted. *Colletotrichum aracearum*, a synonym of *C. orchidearum*, was reported on *P. selloum* in China (Hou et al. 2016). Other *Colletotrichum* species associated with *Philodendron* were *C. gloeosporioides* (French 1989) and *C. philodendri* (Cho and Shin 2004). Previously, *Colletotrichum* species were only identified by using morphological and cultural characteristics (Smith and Black 1990; Tai 1979), whereas many studies demonstrated that morphological characters alone were misleading or unreliable for identification of *Colletotrichum* species because of their variations by different conditions (such as culture medium, temperature, host, strains, etc.) (Cavalcante et al. 2019; Damm et al. 2012). For example, the conidia of strain CBS 125377 (= CSSK4, *C. hippeastri*, China) on PDA were $29.2 \pm 5.5 \times 8.8 \pm 1.0$, L/W = 3.3 (Yang et al. 2009); whenever cultured on Spezieller Nahrstoffarmer agar (SNA), conidia of CBS 125377 were $28.5 \pm 4.1 \times 6.8 \pm 0.6$ μ m, L/W = 4.2, and another strain CBS 241.78 (*C. hippeastri*, Netherlands) has shorter and broader conidia ($24.4 \pm 7.3 \times 7.2 \pm 0.7$ μ m, L/W = 3.4) (Damm et al. 2012). Currently, including multi-gene phylogenetic analyses along with morphological characterization has become a common approach to accurately identify *Colletotrichum* species (Damm et al. 2012, 2019; Wang et al. 2019). Hence, the aims of this paper were to: (i) accurately identify new *Colletotrichum* isolates recovered from *Philodendron* leaf lesions to species level using morphological and phylogenetic studies; and (ii) determine their pathogenicity to *Philodendron* plants using pathogenicity tests.

Materials and Methods

Collection and isolation. In 2016, a leaf spot disease of *P. tatei* cv. Congo was first found by Li Mengyuan in a private residence, and

then often observed in another location in Lanzhou, Gansu Province, China. In order to identify the pathogens causing leaf spot on *P. tatei* cv. Congo, successive samplings were performed in 2016 and 2019. In total, 17 locations (11 private residences and six flower markets) were investigated in Lanzhou city. Symptoms of leaf spot and leaf morbidity were observed and recorded, and symptomatic samples were collected. Pieces (about 1 × 0.5 cm²) of leaf tissues at the border of lesions were cut from these samples. *Colletotrichum* species were isolated from the leaf lesions as described by Xue et al. (2019). According to the procedure provided by Cai et al. (2009), single-spored cultures were obtained for each *Colletotrichum* isolate. Representative isolates were deposited at the Mycological Herbarium of Lanzhou University (MHLZU), China General

Microbiological Culture Collection Center (CGMCC), and Agricultural Culture Collection of China (ACCC), respectively.

Morphological and cultural characterization. Fresh mycelial plugs (6 mm diameter) cut from the colonies were maintained at 20°C for 5 days in the dark and transferred to fresh oatmeal agar (OA) and potato dextrose agar (PDA). The growth rates in various temperatures were measured as described by Xue et al. (2019). After 4 days, growth rates were measured (computing formula: growth rate (mm) = (colony diameter – 6)/2). The colony characteristics on OA or PDA were recorded between 4 and 30 days. Appressoria were observed by using the slide culture technique described by Cai et al. (2009). Colony colors were noted according to Rayner (1970). Morphological characters of cultures incubated under UV light with a

Table 1. Collection details and GenBank accession numbers of *Colletotrichum* species included in this study

<i>Colletotrichum</i> species	Strain ^y	Host	Locality
The <i>Colletotrichum boninense</i> species complex (CBSC)			
<i>C. annellatum</i>	CBS 129826*	<i>Hevea brasiliensis</i>	Colombia
<i>C. beeveri</i>	CBS 128527*	<i>Brachyglottis repanda</i>	New Zealand
<i>C. boninense</i>	CBS 112115	<i>Leucospermum</i> sp.	Australia
	CBS 123755*	<i>Crinum asiaticum</i> var. <i>sinicum</i>	Japan
	CBS 128506	<i>Solanum lycopersicum</i>	New Zealand
	CBS 128549	<i>Solanum betaceum</i>	New Zealand
	CBS 101059*	<i>Brassica oleracea</i> var. <i>gemmifera</i>	New Zealand
<i>C. brassicicola</i>	CBS 134228*	<i>Citrus unchiu</i>	China
<i>C. citricola</i>	CBS 129818*	<i>Passiflora edulis</i>	Colombia
<i>C. colombiense</i>	CBS 128503	<i>Solanum betaceum</i>	New Zealand
<i>C. constrictum</i>	CBS 128504*	<i>Citrus limon</i>	New Zealand
<i>C. cymbidiicola</i>	IMI 347923*	<i>Cymbidium</i> sp.	Australia
	CBS 128543	<i>Cymbidium</i> sp.	New Zealand
<i>C. dacrycarpi</i>	CBS 130241*	<i>Dacrycarpus dacrydioides</i>	New Zealand
<i>C. hippastris</i>	CBS 125376*	<i>Hippeastrum vittatum</i>	China
<i>C. karstii</i>	CBS 110779	<i>Eucalyptus grandis</i>	South Africa
	CBS 126532	<i>Citrus</i> sp.	South Africa
	CBS 128545	<i>Capsicum annuum</i>	New Zealand
<i>C. novae-zelandiae</i>	CBS 128505*	<i>Capsicum annuum</i>	New Zealand
<i>C. oncidii</i>	CBS 129828*	<i>Oncidium</i> sp.	Germany
	CBS 130242	<i>Oncidium</i> sp.	Germany
<i>C. parsonsia</i>	CBS 128525*	<i>Parsonsia capsularis</i>	New Zealand
<i>C. petchii</i>	CBS 125957	<i>Dracaena</i> sp.	Netherlands
<i>C. philodendricola</i> sp. nov.	LZJZ1* = CGMCC 3.19290, MHLZU 0312, ACCC 39680	<i>Philodendron tatei</i> cv. Congo	China
	CGMCC 3.19931 = LZJZ2	<i>P. tatei</i> cv. Congo	China
	LZJZ3	<i>P. tatei</i> cv. Congo	China
	LZJZ4	<i>P. tatei</i> cv. Congo	China
<i>C. phyllanthi</i>	CBS 175.67*	<i>Phyllanthus acidus</i>	India
<i>C. pseudoboninense</i> sp. nov.	LZJZ5* = CGMCC 3.19755, MHLZU 0364, ACCC 39681	<i>Philodendron tatei</i> cv. Congo	China
	CBS 123921	<i>Dendrobium kingianum</i>	Japan
<i>C. torulosum</i>	CBS 128544*	<i>Solanum melongena</i>	New Zealand
<i>C. gloeosporioides</i>	CBS 112999*	<i>Citrus sinensis</i>	Italy
The <i>C. orchidearum</i> species complex (COSC)			
<i>C. cattleyicola</i>	CBS 170.49*	<i>Cattleya</i> sp.	Belgium
<i>C. cliviicola</i>	CBS 125375*	<i>Clivia miniata</i>	China
<i>C. musicola</i>	CBS 132885*	<i>Musa</i> sp.	Mexico
<i>C. orchidearum</i>	CBS 135131*	<i>Dendrobium nobile</i>	Netherlands
	UTFC 265	<i>Philodendron</i> sp.	Iran
Origin of <i>C. aracearum</i>	CGMCC 3.14982*	<i>Monstera deliciosa</i>	China
Origin of <i>C. aracearum</i>	CGMCC 3.14983	<i>P. selloum</i>	China
	LZJZ6 = CGMCC 3.19756, MHLZU 0364, ACCC 39682	<i>P. tatei</i> cv. Congo	China
<i>C. piperis</i>	IMI 71397*	<i>Piper nigrum</i>	Malaysia
<i>C. plurivorum</i>	CBS 125474*	<i>Coffea</i> sp.	Vietnam
<i>C. sojae</i>	ATCC 62257*	<i>Glycine max</i>	U.S.A.
	CBS 128510	<i>Medicago sativa</i>	U.S.A.
<i>C. vittalense</i>	CBS 181.82*	<i>Theobroma cacao</i>	India
<i>C. brevisporum</i>	CBS 129957	<i>Anthurium</i> sp.	Thailand

(Continued on next page)

^y Ex-type cultures are emphasized with an asterisk (*). Strains in this study are shown in bold.

^z ITS: internal transcribed spacers 1 and 2 together with 5.8S nrDNA; *GAPDH*: partial glyceraldehyde-3-phosphate dehydrogenase gene; *CHS1*: partial chitin synthase 1 gene; *HIS3*: histone3; *ACT*: partial actin gene; *TUB2*: partial beta-tubulin gene; *CAL*: partial calmodulin gene.

photoperiod of 12 h light/12 h darkness were observed after 6 to 14 days on both PDA and OA. Microscopic slides were observed with an LCD-Digital NSZ-808 (NOVEL) or a Nikon ECLIPSE Ti microscope. Thirty to 50 measurements were performed for each structure (conidia, appressoria, ascospores, asci, etc.). Duncan's test ($P \leq 0.05$) was used to compare relevant data by IBM SPSS Statistics v.19.

DNA extraction, PCR amplification, and DNA sequencing. DNA extractions and PCR amplifications were performed according to Xue et al. (2019). PCR products were sent to Sangon Biotech Co. Ltd. (Chengdu, China) for sequencing.

Phylogenetic analysis. Sequences used in this study are displayed in Table 1. Single sequences were first aligned using Clustal X

function in MEGA v.6 (Tamura et al. 2013). Multiple loci were then combined with SequenceMatrix 1.8 (Vaidya et al. 2011). MEGA v.6 was used to determine the best DNA model for maximum likelihood (ML) analysis. Aligned sequences were analyzed using ML in MEGA v.6. The bootstrap analyses were based on 1,000 replicates. For Bayesian Inference (BI) analysis, the best-fit nucleotide substitution models of each gene were assessed by MrModeltest 2.3 software (Nylander 2004). Then BI were executed in MrBayes v. 3.2.6 (Ronquist et al. 2012). Analyses of MCMC chains based on the full dataset were run for two million generations and sampled every 100 generations. The first 25% of the generations were discarded as burn-in. Finally, the tree was decorated in Microsoft PowerPoint 2016. The alignments and trees were deposited in TreeBASE (<https://treebase.org/>).

Table 1. (Continued from previous page)

GenBank accessions ^z							References
ITS	GAPDH	CHS-1	HIS3	ACT	TUB2	CAL	
The <i>Colletotrichum boninense</i> species complex (CBSC)							
JQ005222	JQ005309	JQ005396	JQ005483	JQ005570	JQ005656	JQ005743	Damm et al. 2012
JQ005171	JQ005258	JQ005345	JQ005432	JQ005519	JQ005605	JQ005692	Damm et al. 2012
JQ005160	JQ005247	JQ005334	JQ005421	JQ005508	JQ005594	JQ005681	Damm et al. 2012
JQ005153	JQ005240	JQ005327	JQ005414	JQ005501	JQ005588	JQ005674	Damm et al. 2012
JQ005157	JQ005244	JQ005331	JQ005418	JQ005505	JQ005591	JQ005678	Damm et al. 2012
JQ005156	JQ005243	JQ005330	JQ005417	JQ005504	JQ005590	JQ005677	Damm et al. 2012
JQ005172	JQ005259	JQ005346	JQ005433	JQ005520	JQ005606	JQ005693	Damm et al. 2012
KC293576	KC293736	KY856140	KY856311	KC293616	KC293656	KC293696	Guarnaccia et al. 2017
JQ005174	JQ005261	JQ005348	JQ005435	JQ005522	JQ005608	JQ005695	Damm et al. 2012
JQ005237	JQ005324	JQ005411	JQ005498	JQ005585	JQ005671	JQ005758	Damm et al. 2012
JQ005238	JQ005325	JQ005412	JQ005499	JQ0055856	JQ005672	JQ005759	Damm et al. 2012
JQ005166	JQ005253	JQ005340	JQ005427	JQ005514	JQ005600	JQ005687	Damm et al. 2012
JQ005167	JQ005254	JQ005341	JQ005428	JQ005515	JQ005601	JQ005688	Damm et al. 2012
JQ005236	JQ005323	JQ005410	JQ005497	JQ005584	JQ005670	JQ005757	Damm et al. 2012
JQ005231	JQ005318	JQ005405	JQ005492	JQ005579	JQ005665	JQ005752	Damm et al. 2012
JQ005198	JQ005285	JQ005372	JQ005459	JQ005546	JQ005632	JQ005719	Damm et al. 2012
JQ005209	JQ005296	JQ005383	JQ005470	JQ005557	JQ005643	JQ005730	Damm et al. 2012
JQ005207	JQ005294	JQ005381	JQ005468	JQ005555	JQ005641	JQ005728	Damm et al. 2012
JQ005228	JQ005315	JQ005402	JQ005489	JQ005576	JQ005662	JQ005749	Damm et al. 2012
JQ005169	JQ005256	JQ005343	JQ005430	JQ005517	JQ005603	JQ005690	Damm et al. 2012
JQ005170	JQ005257	JQ005344	JQ005431	JQ005518	JQ005604	JQ005691	Damm et al. 2012
JQ005233	JQ005320	JQ005407	JQ005494	JQ005581	JQ005667	JQ005754	Damm et al. 2012
JQ005226	JQ005313	JQ005400	JQ005487	JQ005574	JQ005660	JQ005747	Damm et al. 2012
MH105257	MH105261	MH105265	MH105269	MH105273	MH105277	MH105281	This study
MH105258	MH105262	MH105266	MH105270	MH105274	MH105278	MH105282	This study
MH105259	MH105263	MH105267	MH105271	MH105275	MH105279	MH105283	This study
MH105260	MH105264	MH105268	MH105272	MH105276	MH105280	MH105284	This study
JQ005221	JQ005308	JQ005395	JQ005482	JQ005569	JQ005655	JQ005742	Damm et al. 2012
MK796540	MK796573	...	MK796580	MK796547	MK796554	...	This study
JQ005163	JQ005250	JQ005337	JQ005424	JQ005511	JQ005597	JQ005684	Damm et al. 2012
JQ005164	JQ005251	JQ005338	JQ005425	JQ005512	JQ005598	JQ005685	Damm et al. 2012
JQ005152	JQ005239	JQ005326	JQ005413	JQ005500	JQ005587	JQ005673	Damm et al. 2012
The <i>C. orchidearum</i> species complex (COSC)							
MG600758	MG600819	MG600866	MG600905	MG600963	MG601025	...	Damm et al. 2019
MG600733	MG600795	MG600850	MG600892	MG600939	MG601000	...	Damm et al. 2019
MG600736	MG600798	MG600853	MG600895	MG600942	MG601003	...	Damm et al. 2019
MG600738	MG600800	MG600855	MG600897	MG600944	MG601005	...	Damm et al. 2019
MG600740	MG600802	...	...	MG600946	MG601007	...	Damm et al. 2019
KX853166	KX893585	...	...	KX893577	KX893581	...	Hou et al. 2016
KX853167	KX893586	...	...	KX893578	KX893582	...	Hou et al. 2016
MK796541	MK796574	MK796567	MK796581	MK796548	MK796555	MK796561	This study
MG600760	MG600820	MG600867	MG600906	MG600964	MG601027	...	Damm et al. 2019
MG600718	MG600781	MG600841	MG600887	MG600925	MG600985	...	Damm et al. 2019
MG600749	MG600810	MG600860	MG600899	MG600954	MG601016	...	Damm et al. 2019
MG600751	MG600812	MG600862	MG600901	MG600956	MG601018	...	Damm et al. 2019
MG600734	MG600796	MG600851	MG600893	MG600940	MG601001	...	Damm et al. 2019
MG600762	MG600822	MG600869	MG600908	MG600966	MG601029	...	Damm et al. 2019

Pathogenicity tests. Conidia were obtained by growing single-spored colonies on PDA or OA for 7 to 16 days as described. The conidial suspension was adjusted to a concentration of 1×10^8 conidia/ml in sterile water. Qualitative filter papers (New Star No.102, Hangzhou Special Paper Industry, Hangzhou, China) were divided into 6-mm-diameter papers, and then dipped in conidial suspension, serving as the next pathogenicity tests. Pathogenicity tests were carried out on young and healthy plants (height: 20 to 30 cm) of *P. tatei* cv. Congo.

Spray inoculation. For each isolate, five *P. tatei* cv. Congo plants were spray-inoculated with conidial suspensions (1×10^8) by both wounding and nonwounding methods (Than et al. 2008). Control plants were spray-inoculated with sterile water. All plants were individually covered with transparent polyethylene bags for 10 days to maintain high relative humidity.

Point inoculation. For each isolate, 20 leaves of *P. tatei* cv. Congo plants were inoculated by both wounding and nonwounding

methods. For nonwounding inoculation, 20 filter papers (6 mm diameter) immersed in conidial suspension were placed onto the upper surface of each leaf (the middle of one site per leaf along the longitudinal axis). For wounding inoculation, 20 leaves were first pinpricked, and then filter papers placed on the wound site (Scandiani et al. 2011). Control plants were inoculated with filter papers immersed in sterile water. Thereafter, all plants were spray-inoculated with sterile water, and individually covered with transparent polyethylene bags that had been moistened with sterile distilled water for 10 days (at the onset of symptoms). All plants were incubated in a greenhouse at 18 to 25°C. Pathogenicity trials by both spray and localizing (filter paper) inoculations were performed three times as previously described. 30 days after inoculation, the morbidity of infected leaves was assessed and the length of lesions was measured to evaluate the virulence. Tukey's test ($P \leq 0.05$) was used to analyze the data using IBM SPSS Statistics v.19. All fungal isolates included

Fig. 1. Phylogram tree resulting from a maximum likelihood analysis based on the combined ITS, *GAPDH*, *CHS-1*, *HIS3*, *ACT*, *TUB2*, and *CAL* alignments of the *Colletotrichum boninense* species complex. Bootstrap support values (BS)/Bayesian posterior probability scores (PP) above 70%/0.70 are shown at the nodes. Strains in this study are shown in bold. The tree was rooted to *C. gloeosporioides* (CBS 112999). Ex-epitype isolates are emphasized with an asterisk. TreeBASE: S25515.

Fig. 2. Anthracnose of *Philodendron tatei* cv. Congo caused by *Colletotrichum* species. **a-b**, Leaf spots under natural conditions; **c-e**, symptoms on plants after spray inoculation with LZJZ1 (**c**, nonwounded plants; **d-e**, wounded plants); **f-h**, symptoms inoculated with filter papers on wounded leaves at 30 days (**f**, LZJZ5; **g**, LZJZ1; **h**, LZJZ6).

Fig. 3. Phylogram tree resulting from a maximum likelihood analysis based on the combined ITS, *GAPDH*, *CHS-1*, *HIS3*, *ACT*, and *TUB2* alignments of the *Colletotrichum orchidearum* species complex. Bootstrap support values (BS)/Bayesian posterior probability scores (PP) above 70%/0.70 are shown at the nodes. Strains in this study are shown in bold. The tree was rooted to *C. brevisporum* (CBS 129957). Ex-epitype isolates are emphasized with an asterisk. TreeBASE: S25518.

in the pathogenicity tests were reisolated from infected lesions to confirm their identity using both morphological and molecular approaches.

Genealogical concordance phylogenetic species recognition (GCPSR) analysis. In order to better reveal the novel species in this study, phylogenetically closely related isolates (Fig. 1, clade A and B) were selected for GCPSR analysis on a seven-locus concatenated dataset. A pairwise homoplasy index (PHI, or Φ_w) test was performed in SplitsTree4 (Huson and Bryant 2006) to determine the recombination level. If the PHI results were below a 0.05 level ($\Phi_w < 0.05$), it indicated significant recombination present in the

dataset (Bruen et al. 2006; Quaadvlieg et al. 2014). The splits graphs from the datasets based on both the LogDet transformation and splits decomposition options were used to reveal the relationships between these closely related species groups.

Results

Symptoms. During 2016 and 2019, anthracnose on leaves of *P. tatei* cv. Congo was frequently observed in and around Lanzhou, Gansu Province, China. Leaf disease incidence was approximately 5 to 20%. The disease generally occurred at the tips and the margins

Fig. 4. Cultural traits of *Colletotrichum* species in this study.

of older leaves. Early symptoms appeared as small, grayish white to yellow, circular or irregular, sunken spots (Fig. 2a). Leaf spots gradually expanded, leaving a dark-brown edge surrounded by an obvious yellow halo (Fig. 2b). Eventually, the infected sites of leaves formed a hole in the middle position of lesions. Conidiomata were often visible on the diseased leaf surfaces.

Colletotrichum species collection. In total, 126 single-spored *Colletotrichum* isolates were obtained from 78 symptomatic samples. Based on the high similarity of morphology on PDA as well as ITS, *GAPDH*, and *TUB2* sequences, these cultures were preliminarily classified into three groups (i.e., *Colletotrichum* 1, *Colletotrichum* 2, and *Colletotrichum* 3). *Colletotrichum* 1 included 93 isolates that appeared as the most frequent species, including 74% of all isolates, followed by *Colletotrichum* 3 (31 isolates) and *Colletotrichum* 2 (two isolates). Six representative isolates from these three groups were subsequently selected for further phylogenetic studies (Table 1).

Phylogenetic analyses. Multiple loci (ITS, *ACT*, *CAL*, *GAPDH*, *HIS3*, *CHS-1*, and *TUB2*) of 32 isolates were selected from GenBank, including 26 isolates from the *C. boninense* species complex (CBSC), five representative *Colletotrichum* strains used in this study, and the type strain CBS 112999 (*C. gloeosporioides*), which was used as an outgroup. The dataset contained 2,714 characters including gaps after sequence alignment (546 for ITS; 278 for *ACT*; 442 for *CAL*; 280 for *CHS-1*; 277 for *GAPDH*; 389 for *HIS3*; 502 for *TUB2*). The TN93+G model was selected to reconstruct the ML tree. For BI analysis, the corresponding models were selected by MrModeltest: SYM+I+G for ITS; GTR + I + G for *HIS3*; HKY+G for *ACT* and *GAPDH*; GTR+G for *CAL*; and K80+G for *CHS1*; and HKY+I for *TUB2*. Only the ML tree is shown. The ML bootstrap support values (BS)/Bayesian posterior probability scores (PP) above 70%/0.70 are displayed at the tree nodes. The ML phylogenetic tree (Fig. 1; TreeBASE: S25515) showed that LZJZ1, LZJZ2, LZJZ3, and LZJZ4 isolates from *Colletotrichum* 1 group did not cluster well with any other *Colletotrichum* species, and were identified as *C. philodendricola* in this study; LZJZ5 from *Colletotrichum* 2, clustering with an unnamed isolate (CBS 123921) from the CBSC (Damm et al. 2012), was identified as *C. pseudoboninense* in this study. In order to better distinguish these two new species, the single-locus trees of each gene (ITS, *ACT*, *CAL*, *GAPDH*, *HIS3*, *CHS-1*, and *TUB2*) were also compared by both ML and BI analyses. In the CBSC analysis, the single loci *GAPDH*, *HIS* and *CAL*, separated *C. pseudoboninense* and *C. philodendricola*, respectively. In addition, *C. philodendricola* was separated also by *TUB2* and *ACT*.

With the *C. orchidearum* species complex (COSC), a total of 2,243 characters (539 for ITS; 262 for *ACT*; 293 for *CHS-1*; 242 for *GAPDH*; 390 for *HIS3*; 517 for *TUB2*) including gaps were obtained from 14 *Colletotrichum* isolates (including 12 isolates from COSC, one representative *Colletotrichum* strain isolated from *P. tatei* cv. Congo, and a reference strain *C. brevisporum* (CBS 129957), which was used as an outgroup). For BI analysis, a GTR+I model was selected for ITS, HKY+I for *GAPDH* and *HIS3*, K80+I for *CHS-1*, HKY for *ACT*, and HKY+G for *TUB2*. A T92+G model was selected for ML analysis. The phylogram in Figure 3 (TreeBASE: S25518) showed that LZJZ6 from *Colletotrichum* 3 clustered well with four reported *C. orchidearum* isolates (Damm et al. 2019; Hou et al. 2016).

Morphological and cultural characteristics. Based on both analyses of multiple loci (Table 1) and morphological characterizations (Fig. 4; Table 2), the result of the final identification on these 126 cultures is consistent with those of the preliminary identification (only by morphology on PDA as well as ITS, *GAPDH*, and *TUB2* sequences). *Colletotrichum* 3 isolates on PDA were cottony, dark greenish with white edges on the upper side, and black-green at the center on the reverse side (Fig. 5a and b). Colonies on OA were white to olivaceous on the upper side, reverse gray to gray-brown, and grew 16 to 17.3 mm in 4 days. Conidia on PDA were hyaline, aseptate, cylindrical, rounded at both ends, 14.6 to 21.1 $\times$ 3.7 to 5.8 μ m (mean $\pm$ SD = 17.4 $\pm$ 1.4 $\times$ 4.9 $\pm$ 0.4, n = 50, L/W ratio = 3.6). Appressoria were dark brown, clavate, elliptic to irregular, 6.8 to 12.3 $\times$ 3.7 to 7.1 μ m (mean $\pm$ SD = 8.7 $\pm$ 1.5 $\times$ 6.1 $\pm$ 1.0, n = 14, L/W ratio = 1.4). The sexual morph of *Colletotrichum* 3 was produced on OA

for 6 to 14 days. Ascomata were solitary, subglobose, dark brown to black, 74 to 363 $\times$ 93 to 474 μ m. Asci were fasciculate, 8-spored, brown, clavate, 61.3 to 101.5 $\times$ 5.4 to 13.7 μ m (Fig. 5e). Ascospores were hyaline to yellow-brown, aseptate, allantoid, straight or slightly curved, sometimes with one or two acute ends, 10.1 to 23.7 $\times$ 3.7 to 6.6 μ m (mean $\pm$ SD = 17.7 $\pm$ 3.3 $\times$ 5.5 $\pm$ 0.7, n = 50, L/W ratio = 3.2) (Fig. 5f to l). The morphological features on PDA were generally similar to those of *C. orchidearum* (Ariyawansa et al. 2015; Yang et al. 2011). Phylogenetic analyses of multiple loci along with morphological characteristics confirmed that *Colletotrichum* 3 was *C. orchidearum* – a known species of the *C. orchidearum* species complex. The *Colletotrichum* 1 and *Colletotrichum* 2, both belonging to the *C. boninense* species complex, were named as *C. philodendricola* and *C. pseudoboninense*, respectively, based on their morphological and molecular characteristics in this study. The specific descriptions of the two novel species are as follows:

Colletotrichum philodendricola L. H. Xue & C. J. Li, sp. nov.

Mycobank MB828468; Figure 6.

Etymology: Named after the host *Philodendron tatei* cv. Congo.

Morphological descriptions. Anamorph on PDA. Hyphae hyaline, aseptate, branched, 1.6 to 4.9 μ m diam. Conidiomata acervular, setae and conidiophores formed on pale to medium brown, thick-walled angular cell. Setae pale to dark brown, aseptate, sometimes including geniculate at the middle, 30 to 150 μ m long; base inflated or cylindrical, 3 to 6.5 μ m diam., tip acute. Conidiophores hyaline to gray, branched, to 102 μ m long. Conidia hyaline, aseptate, cylindrical, rounded or acute at ends, 9.3 to 18.1 $\times$ 4.8 to 6.5 μ m (mean $\pm$ SD = 14.0 $\pm$ 1.6 $\times$ 5.6 $\pm$ 0.5, n = 50, L/W ratio = 2.5). Appressoria brown, ellipsoidal to irregular, or navicular, 6.0 to 17.9 $\times$ 4.0 to 8.9 μ m (mean $\pm$ SD = 10.0 $\pm$ 2.1 $\times$ 6.8 $\pm$ 1.3, n = 33, L/W ratio = 1.5).

Anamorph on OA. Hyphae hyaline, aseptate, branched, 1.5 to 6.5 μ m diam. Conidiomata acervular, setae and conidiophores formed from hyphae or on pale to medium brown, thick-walled angular cell 3.5 to 7 μ m diameter. Setae pale to dark brown, aseptate, straight to slightly curved, 43 to 295 μ m long, base inflated or cylindrical, tip acute, 3.2 to 6.3 μ m diameter, and the tip acute. Conidiophores unbranched or branched, hyaline to dark gray, to 85 μ m long. Conidia straight, aseptate, cylindrical to fusiform, 10.3 to 17.4 $\times$ 4.1 to 6.8 μ m (mean $\pm$ SD = 13.8 $\pm$ 1.3 $\times$ 5.6 $\pm$ 0.7, n = 50, L/W ratio = 2.5).

Colonies on PDA buff to honey, covered with orange, dark gray to black conidiomata, and white to gray aerial mycelium; orange conidial masses were visible; reverse pale salmon to honey. Colonies on OA usually white to pale honey-colored, visible with gray to dark conidiomata, and sparse white aerial mycelium; reverse gray, with dark spots because of conidiomata shining through, 19 to 21.3 mm in 7 days (30.5 to 31.5 mm in 10 days).

Specimen examined. Holotype: China. Gansu Province: Lanzhou, isolated from leaf lesions of *P. tatei* cv. Congo, Oct. 2016, Longhai Xue and Yongwen Zhang. Ex-type culture LZJZ1 = CGMCC 3.19290 = MHLZU 0312 = ACCC 39680.

Notes: Phylogenetic analysis of multiple loci showed that *C. philodendricola* is a sister clade to *C. cymbidiicola* and *C. oncidii*, formed a single clade with high BS/PP values (100/1). BLAST analyses of each gene showed that the *ACT*, *CAL*, *GAPDH*, *HIS3*, and *TUB2* sequences of *C. philodendricola* differ from those of the two species (*C. cymbidiicola* and *C. oncidii*) by 4 to 5, 1 to 3, 7 to 9, 1 to 2, and 3 to 5 bp, respectively. Although the features of conidia are similar to those of both *C. cymbidiicola* and *C. oncidii*, *C. philodendricola* is different as setae are aseptate and 30 to 150 μ m shorter than both of the other species (*C. cymbidiicola* and *C. oncidii*) (75 to 180/210 μ m, Table 2), and the growth rate on OA (19 to 21.3 mm in 7 days) is significantly slower than the two species (*C. cymbidiicola*: 25 to 27.5 mm in 7 days; *C. oncidii*: 30 to 31.5 mm in 7 days). In addition, *C. philodendricola* was morphologically different with acute ends of its conidia.

Colletotrichum pseudoboninense L. H. Xue & C. J. Li, sp. nov.

Mycobank MB832454; Figure 7.

Etymology: Named after both the phylogenetic proximity and morphological similarity to *C. boninense*.

Morphological descriptions. Teleomorph on OA (CGMCC 3.19755). Ascomata solitary, globose to ellipsoidal, dark brown to black, 55 to 137 × 107 to 253 µm. Asci fasciculate, 8-spored, pale to dark brown, cylindrical to clavate, 29 to 64 × 4.8 to 12.7 µm (mean ± SD = 45.5 ± 9.4 × 10.2 ± 2.2, *n* = 30). Ascospores yellow brown, aseptate, straight to slightly curved, allantoid, round or acute at ends, 9.7 to 18.3 × 3.8 to 7.3 µm (mean ± SD = 15.7 ± 1.8 × 5.8 ± 0.8, *n* = 50, L/W ratio = 2.7).

Anamorph on PDA (CGMCC 3.19755). Hyphae hyaline, aseptate, branched, 1.4 to 5.6 µm diam. Conidiomata acervular, setae and conidiophores formed on pale brown, roundish to angular cells. Setae rare, pale to dark brown, 0 to 4 septate, straight to curved, 70 to 170 µm long, tip acute. Conidiophores hyaline to brown, branched or unbranched, to 30 µm long. Conidia hyaline, aseptate, cylindrical to clavate, round at both ends, sometimes base with a hilum, 10 to 16.4 × 4.8 to 6.8 µm (mean ± SD = 13.2 ± 1.5 × 5.9 ± 0.4, *n* = 50, L/W = 2.2). Appressoria medium to dark brown, ovoid to irregular in shape, the margin entire to undulate, 6.6 to 11.0 × 4.8 to 8.4 µm (mean ± SD = 8.3 ± 1.3 × 6.1 ± 0.9, *n* = 30, L/W ratio = 1.4).

Colonies on PDA cottony, white to gray-brown, covered with white aerial mycelium, no conidial masses were visible; reverse white with brown on the center. Colonies on OA usually felty white to gray, covered with white aerial mycelium; reverse gray, with small black spots, 25.3 to 27.5 mm in 7 days.

Specimen examined. Holotype: China. Gansu Province: Lanzhou, isolated from leaf lesions of *P. tatei* cv. Congo, Mar. 2019, Longhai Xue. Ex-type culture LZJZ5 = CGMCC 3.19755 = MHLZU 0364 = ACCC 39681. (culture CBS 123921 = MAFF 238642, isolate from *Dendrobium kingianum*. 1996, Kochi Pref.)

Notes: *C. pseudoboninense* was isolated from *P. tatei* cv. Congo in China, and *D. kingianum* in Japan (Damm et al. 2012; Moriwaki et al.

2003). Multilocus phylogenetic analysis revealed that *C. pseudoboninense* is a sister clade to *C. boninense*, formed a single clade with high BS/PP values (90/1). BLAST analyses of each gene showed that the *ACT*, *CAL*, *CHS-1*, *GAPDH*, and *HIS3* sequences of *C. pseudoboninense* were different from those of *C. boninense* by 1 to 5, 8 to 13, 1 to 2, 7 to 8, and 1 to 2 bp, respectively. The morphology of *C. pseudoboninense* is similar to that of *C. boninense*, but the asci of *C. pseudoboninense* are narrower (29 to 64 × 4.8 to 12.7 µm), and the length of setae is longer (70 to 170 µm) than those of *C. boninense* (CBS 123755 and CBS 123756) (asci: 45 to 60 × 12.5 to 17 µm; setae: 20 to 60, or 30 to 70 µm). In addition, the ascospores on OA of *C. pseudoboninense* were aseptate when mature, which was clearly distinguishable from those of *C. boninense* (1 to 3 septate).

Effect of temperature on mycelial growth. For *Colletotrichum* 1 and 2 (i.e., *C. philodendricola* and *C. pseudoboninense*), the optimum growth temperature was 25°C (Table 3), while the optimum temperature for *Colletotrichum* 3 (i.e., *C. orchidearum*) was 30°C (Fig. 4). The three species isolated from *P. tatei* cv. Congo were easily differentiated at 25, 30, and 35°C, respectively (*P* ≤ 0.05); when cultured at these three temperatures, *Colletotrichum* 3 had the maximum growth rate of hyphae, followed by *Colletotrichum* 2 and *Colletotrichum* 1 (except that *Colletotrichum* 1 was higher than *Colletotrichum* 2 at 35°C). No mycelial growth was observed at 0°C for any species. When compared at 10°C, the growth rate of *Colletotrichum* 3 was significantly higher than *Colletotrichum* 1, but had not significant difference with *Colletotrichum* 2; *Colletotrichum* 3 was significantly higher than the other two groups at 15°C; however, *Colletotrichum* 2 had a higher growth rate than *Colletotrichum* 1 and *Colletotrichum* 2 at 20°C. In terms of temperature sensitivity, isolates of *Colletotrichum* 1 were sensitive to low temperatures (10, 15°C), and the growth rate was only 0.7 mm/4 days at 10°C; isolates of

Table 2. Comparison of morphological and cultural characteristics of six close species from the *Colletotrichum boninense* species complex^z

<i>Colletotrichum</i> species	Strains	Colony on oatmeal agar
<i>C. boninense</i>	CBS 123755* (= MAFF 305972), CBS 123756 (= MAFF 306094)	Felty white, covered with rosy buff or pale glaucous gray aerial mycelium, in the center pale luteous aerial mycelium
<i>C. pseudoboninense</i> sp. nov.	CGMCC 3.19755* (= LZJZ5)	Felty white to gray, covered with pale to gray aerial mycelium, reverse with small black spots
Origin of <i>C. boninense</i>	CBS 123921 (= MAFF 238642)	...
<i>C. torulosum</i>	CBS 128544*, CBS 102667	...
<i>C. cymbidiicola</i>	IMI 347923*, CBS 128543, CBS 123757	Buff to straw, covered with white aerial mycelium or black conidiomata
	OORC1	...
<i>C. oncidii</i>	CBS 129828*, CBS 130242	Buff to honey, covered with orange to black conidiomata and lacking aerial mycelium
<i>C. philodendricola</i> sp. nov.	CGMCC 3.19290* (= LZJZ1), LZJZ2, LZJZ3, LZJZ4	White to pale honey-colored, visible with gray to dark conidiomata, and sparse white aerial mycelium

(Continued on next page)

^z Strains in this study are shown in bold. Ellipsis (...) indicates absent. Ex-type cultures are emphasized with an asterisk (*).

Colletotrichum 2 were more susceptible to high temperatures (only 0.8 mm/4 days at 35°C) compared with the other two species; relatively speaking, isolates of *Colletotrichum* 3 were tolerant to high temperatures (11.6 mm/4 days at 35°C).

Pathogenicity. Pathogenicity tested by both spray and point inoculations showed that all tested *Colletotrichum* isolates caused anthracnose lesions on leaves of *P. tatei* cv. Congo inoculated by both wounding and nonwounding methods. No symptoms developed on the controls. Thirty days after inoculation, symptoms appeared as circular or ovoid, sunken, and brown lesions with a yellow halo, which were similar to the symptoms developed under natural conditions (Fig. 2). For spray inoculation, the proportions of leaves infected by the three species were 0 to 12% (mean 4.7%); the lengths of leaf lesions on both nonwounded and wounded leaves were mostly 1 to 2 mm (e.g., Figure 2c, d, e). Because of the low rate and the small length of infected leaves, the differences between the three species were not compared in this study. For point inoculation, the mean infected rate of the three species was 35.8%; when plants were inoculated without wounding, few leaves (0 to 35%) developed lesions; with the wounding inoculation, the infection rate was 30% higher (Table 4). *C. philodendricola*, *C. pseudoboninense*, and *C. orchidearum* were reisolated from the infected leaves, and identified by morphological characters and sequence analyses to fulfill Koch's postulates.

GCPSR analysis. Clade A contains six isolates that had been identified as either belonging to closely related *C. boninense* (ex-type CBS 123755) or *C. pseudoboninense* (ex-type CGMCC 3.19755). Both species are phylogenetically distinct (Fig. 1) but are morphologically indistinguishable (Table 2). When applying the GCPSR concept to these isolates, we detect no significant recombination between these isolates ($\Phi_w = 1$) (Fig. 8A), which is

good support for the proposition that these isolates belong to two distinct taxa.

Clade B contains eight isolates that had been identified as *C. oncidii* and *C. cymbidiicola* (previously), and *C. philodendricola* (in this study) based on both morphology and multilocus DNA dataset. When applying the GCPSR concept to these isolates, we detected no significant recombination between these isolates ($\Phi_w = 1$) (Fig. 8B). The split tree decomposition network of these multiple combinations was clearly detected within three separate groups. These results correspond to the phylogenetic tree (Fig. 1, clade B), which supports the proposition that *C. oncidii* and *C. cymbidiicola*, and *C. philodendricola* represent three different taxa, respectively.

Discussion

This study describes three pathogenic species of the genus *Colletotrichum*, of which *C. orchidearum* was a previously known species of COSC, and *C. pseudoboninense* and *C. philodendricola* were two novel species of CBSC. Species were identified and described using both multilocus phylogenetic analyses and morphological characteristics. To date, 23 species of CBSC have been clearly described (Damm et al. 2012; Guarnaccia et al. 2017; Hou et al. 2016; Ma et al. 2018). None of these species have been previously reported on *Philodendron*.

It is almost impossible to accurately identify *Colletotrichum* species using morphological characteristics alone (Baroncelli et al. 2015; Cannon et al. 2000, 2012; Damm et al. 2009, 2012, 2019; Hou et al. 2016). Additionally, the ITS sequence region is too conserved to distinguish taxa in *Colletotrichum* (Begerow et al. 2010; Damm et al. 2012, 2019; Hou et al. 2016). For instance, *C. boninense*, *C. oncidii*, and *C. cymbidiicola* were not distinguished from each other by BLAST analysis of ITS sequence (Damm et al.

Table 2. (Continued from previous page)

Anamorph		Teleomorph		References
Setae	Conidia	Asci	Ascospores	
Rare, medium brown, 1 to 2 septate, 20 to 60 or 30 to 70 μ m long	Cylindrical to clavate, base round with a prominent hilum, 9 to 16.5 $\times$ 4 to 6.5 μ m, av. = 13.2 $\times$ 5.8 μ m, L/W = 2.3	Fascicle, cylindrical to clavate, 8-spored, 45 to 60 $\times$ 12.5 to 17 μ m	1 to 3-septate, allantoid, 12.5 to 18 $\times$ 4 to 6.5 μ m, av. = 15.6 $\times$ 5.4 μ m, L/W = 2.9	Damm et al. 2012
Rare, pale to dark brown, 0 to 4 septate, 70 to 170 μm long	Cylindrical to clavate, round at both ends, sometimes base with a hilum, 10 to 16.4 $\times$ 4.8 to 6.8 μm, av. = 13.2 $\times$ 5.9 μm, L/W = 2.2	Fascicle, cylindrical to clavate, 8-spored, 29 to 64 $\times$ 4.8 to 12.7 μm	Aseptate, allantoid, 9.7 to 18.3 $\times$ 3.8 to 7.3 μm, av. = 15.7 $\times$ 5.8 μm, L/W = 2.7	This study
...	Cylindrical, obtuse at both ends, 11.5 to 17 $\times$ 4 to 7 μ m	...	...	Moriwaki et al. 2003
Medium brown, 0 to 2 septate, 20 to 60 μ m long	Cylindrical, round at both ends, with a prominent scar, 13.5 to 17.5 $\times$ 5 to 6.5 μ m, av. = 15.5 $\times$ 5.8 μ m, L/W = 2.7 (CBS 102667: 12 to 18 $\times$ 5 to 6.5 μ m, av. = 13.4 $\times$ 5.7 μ m, L/W = 2.7)	...	...	Damm et al. 2012
Medium brown, 1 to 4 septate, 75 to 180 μ m long	Cylindrical, round at both ends, with a prominent scar, 11.5 to 16.5 $\times$ 5 to 6.5 μ m, av. = 14.6 $\times$ 5.7 μ m, L/W = 2.6	Cylindrical, 8-spored, 55 to 77 $\times$ 11.5 to 13.5 μ m	Aseptate, cylindrical to fusiform, 15 to 31 $\times$ 5 to 7 μ m, av. = 21.2 $\times$ 5.5 μ m, L/W = 3.9	Damm et al. 2012
...	Cylindrical, round at both ends, with a prominent scar, 13.9 to 15.8 $\times$ 5.6 to 5.9 μ m, av. = 14.7 $\times$ 5.8 μ m, L/W = 2.53	...	...	Chowdappa et al. 2014
Medium brown, 2 to 5 septate, 75 to 210 μ m long	Cylindrical, round at both ends, with a prominent scar, 14 to 17.5 $\times$ 5 to 6 μ m, av. = 16.0 $\times$ 5.4 μ m, L/W = 3.0	...	...	Damm et al. 2012
Pale to dark brown, aseptate, 30 to 150 μm long	Cylindrical, round or acute at ends, 9.3 to 18.1 $\times$ 4.8 to 6.5 μm, av. = 14.0 $\times$ 5.6 μm, L/W = 2.5	...	...	This study

2012). In this study also, the ITS tree alone in both ML and BI analyses could not differentiate *C. pseudoboninense*, *C. philodendricola*, *C. boninense*, *C. oncidii*, and *C. cymbidiicola*. Multigene analyses had been indispensable for the species-level identification of the genus *Colletotrichum* in recent decades (Cai et al. 2009, 2011; Damm et al. 2012, 2019; Hou et al. 2016).

C. pseudoboninense was isolated from *P. tatei* cv. Congo in China and *Dendrobium kingianum* (CBS 123921) in Japan. The isolate CBS 123921 (= MAFF 238642) was originally identified as *C. boninense* based on its conidial characters and ITS sequence (Moriwaki et al. 2003). Later, it (CBS 123921) was recognized as an unnamed taxon by forming a single clade on the phylogenetic tree of multiple loci (Damm et al. 2012). In this study, multilocus analysis of seven genes (Fig. 1) showed that isolate CGMCC 3.19755 from *P. tatei* cv. Congo and CBS 123921 from *D. kingianum* formed a single clade with high BS/PP values (90/1), and a sister group to *C. boninense*. The single-locus tree showed that this species was clearly differentiated from *C. boninense* in *GAPDH* and *CAL* phylogenetic trees. The morphology of conidia (CGMCC 3.19755) was similar to that of CBS 123921 (Table 2). Therefore, these two isolates (CGMCC 3.19755 and CBS 123921) grouped together with *C. pseudoboninense*, which is distinct from *C. boninense*, but belongs to the same species complex. Although the morphology of this species was similar to those of *C. boninense* (described by Damm et al. 2012), the ascospores on OA were aseptate when mature, different from *C. boninense*. This new species was named *C. pseudoboninense* sp. nov.

As for *C. philodendricola*, phylogenetic analyses of multiple loci showed that this species was closely related to five species of the CBSC (i.e., *C. boninense*, *C. cymbidiicola*, *C. oncidii*, *C. torulosum*, and *C. pseudoboninense*). Referring to the features described by Damm et al. (2012), the growth rate of *C. philodendricola* on OA was slower than that of five other species (Table 2); *C. philodendricola* developed the anamorph only on OA; on the other hand, *C.*

boninense (CBS 123756) produced the teleomorph. The setae of *C. philodendricola* were aseptate while those of the other five species were 0 to 5 septate. Another species, *C. philodendri*, was first isolated from *Philodendron* in Germany (Hennings 1904). *C. philodendri* on *Philodendron* was thereafter recorded in the U.S.S.R. (Bondarzewa-Monteverde et al. 1936), Florida (Alfieri et al. 1984), and Korea (Cho and Shin 2004); most recently, *C. philodendri* was mentioned as the causal agent of leaf spot on *Philodendron* in New Jersey (Horst 2013). Kobayashi (1958) thought that *C. philodendri* should be a synonym of *C. gloeosporioides*; however, this hypothesis was based only on cultural and morphological characters because of the lack of molecular techniques. Presently, no strains of *C. philodendri* reported in the original publication are available; moreover, it was impossible to refer to the manuscript by Hennings (1904) in which the author gives the complete morphological descriptions of *C. philodendri* (MB168038). It is, therefore, not possible to compare *C. philodendricola* and *C. philodendri* for which there is neither a type strain nor a precise and complete description. So, according to morphological and molecular characters, the species (ex-type strain CGMCC 3.19290) on *Philodendron* was named as *C. philodendriicola* sp. nov.

Recently, *C. orchidearum* Allescher was isolated from *Cattleya* sp., *Cymbidium hookerianum*, and *Oncidium flexuosum* in China, and identified using morphological and phylogenetic studies (Yang et al. 2011). Then Damm et al. (2019) stated that both *C. aracearum* (Hou et al. 2016) and *C. hymenocallidicola* (Ariyawansa et al. 2015) were synonyms of *C. orchidearum*. *C. orchidearum* has been reported on *P. selloum* in China (Hou et al. 2016) and *Philodendron* sp. in Iran (Damm et al. 2019). To our knowledge, this is the first formal report of *C. orchidearum* causing anthracnose on *P. tatei* cv. Congo in China.

The colony growth rate is an important feature to distinguish *Colletotrichum* species, and the maximum growth temperature is often applied to characterize species (Damm et al. 2012; Weir et al.

Fig. 5. Morphological features of *Colletotrichum orchidearum* (strain LZJZ6/CGMCC 3.19756). **a, b, m-p**, from potato dextrose agar (PDA); **c-l**, from oatmeal agar (OA). **a-b**, The colony on PDA at 20°C for 8 days in the dark; **c-d**, the colony on OA at 20°C for 8 days in the dark; **e**, asci; **f-l**, ascospores; **m**, conidia; **n-p**, appressoria.

2012). For example, the slower growth rate of *C. scovillei* separated it from *C. gloeosporioides*, *C. siamense*, *C. fruticola*, *C. truncatum*, *C. plurivorum* (= *C. sichuanensis*), and *C. brevisporum* (Liu et al. 2016). Previous studies have suggested that growth rates were valuable for distinguishing between *C. truncatum*, *C. gloeosporioides*, and *C. acutatum* on chilli (Than et al. 2008), *C. acutatum* and *C. gloeosporioides* on coffee (Nguyen et al. 2010; Kenny et al. 2012), *C. cymbidiicola* and *C. cliviae* on orchids (Chowdappa et al. 2014), and *C. gloeosporioides* and *C. karstii* on citrus (Daoud et al. 2019). In this study, the growth rate of *C. philodendricola* on OA differentiated it from *C. cymbidiicola* and *C. oncidii* described by Damm et al. (2012). Data on growth rates (Table 3) also showed that these three species were distinguishable

at 25 or 30°C (maximum growth temperature). Considering the importance of temperature for the development of plant diseases, these data would be useful to implement strategies to model and control this disease.

In the present study, the data on pathogenicity indicated that all tested species could infect leaves of *P. tatei* cv. Congo by both spray and point inoculations on both wounded and nonwounded plants. On one hand, the pathogenicity was higher for point inoculation than for spray inoculation as assessed by percent infected leaves (35.8% versus 4.7%). This is the opposite of what was found in a previous study where Fusarium head blight of wheat was more readily caused by spray inoculation (Miedaner et al. 2003). The reasons for the difference in infection efficiency is unknown but may

Fig. 6. Morphological features of *Colletotrichum philodendricola* (ex-holotype strain LZJ1/CGMCC 3.19290). **a-m**, From potato dextrose agar. **n-w**, From oatmeal agar. **a**, Culture at 20°C for 8 days; **b, n, o**, cultures at 20°C for 20 days (**o**, reverse); **c, p**, conidiomata and seta; **d, q**, conidia; **e, f, s-v**, seta; **g-i, r, w**, conidiophores; **j-m**, appressoria.

depend on the different fungi (i.e., *Colletotrichum* versus *Fusarium*), differences in the type of infected organ (i.e., leaf and spike), and differing environmental conditions. On the other hand, wounding in healthy leaves under point inoculation conditions greatly enhanced (more than 30%) virulence of *Colletotrichum* in accordance with previous findings on *C. capsici* (= *C. truncatum*, Pring et al. 1995), although the sizes of lesions between these three species were not significantly different (Table 4). Chen et al. (2017) also confirmed that wounding of the leaves infected with *C. acutatum* and *C. gloeosporioides* could significantly increase the number and sizes of lesions on tea leaves. *C. cymbidiicola*, a sister clade of *C. philodendricola*, could infect the detached leaves of a *Cymbidium* hybrid by wounding/drop inoculation (Chowdappa et al. 2014); Liu et al. (2018) showed that conidial suspensions of this species were also pathogenic to nonwounding leaves of *Cymbidium ensifolium* in China. Damm et al. (2019) showed that *C. orchidearum*, an ex-epitype culture CBS 135131, was isolated from leaf lesions of *Dendrobium nobile* in the Netherlands but showed a lack of pathogenicity. In other pathogenicity tests, Yang et al. (2011) found that *C. orchidearum*, CORCX6, developed lesions on three fruits by wounding, but no symptom on nonwounding fruits. However, Xu et al. (2016) reported that isolate SAUCC 1407 could infect nonwounding leaves of *Arctium lappa*.

GCPSR is a pragmatic tool for the assessment of species limits (Quaedvlieg et al. 2014) and has been widely applied to describe

species in the fungal kingdom (Geiser et al. 1998; Koufopanou et al. 1997; Laurence et al. 2014; Peterson 2008). In this study, the results of GCPSR analyses correspond to the phylogenetic tree of the seven-loci data (Fig. 1). Therefore, it will be more reasonable for the identification of fungal species (especially for novel species) by adding GCPSR analysis of phylogenetically related species on the basis of morphological and molecular characters.

This is the first study of the diversity of *Colletotrichum* species associated with anthracnose on *P. tatei* cv. Congo in China. Two novel species (*C. pseudoboninense* sp. nov. and *C. philodendricola* sp. nov.) were separated phylogenetically from known species of the *C. boninense* complex, and both showed limited morphological differences from the closely related species. *C. orchidearum*, as a previously described species of COSC, was reported for the first time on *P. tatei* cv. Congo. All three species showed pathogenicity with minimal variability in virulence as expressed in terms of lesion size. The most prevalent species of anthracnose on *P. tatei* cv. Congo was *C. philodendricola*, followed by *C. orchidearum* and *C. pseudoboninense*. This study provides an insight for the identification and pathogenicity evaluation of *Colletotrichum* species associated with anthracnose on *P. tatei* cv. Congo and is useful for implementation of accurate and appropriate management strategies.

Fig. 7. Morphological features of *Colletotrichum pseudoboninense* (ex-holotype strain LZJZ5/CGMCC 3.19755). a-l, From oatmeal agar. m-t, From potato dextrose agar. a, b, Culture at 20°C for 8 days; c, d, cultures at 20°C for 20 days (d, reverse); e, ascomata; f-h, asci; i-l, ascospores; m, conidia; n-p, appressoria; q, r, seta; s, t, conidiophores.

Table 3. Growth rate (mm/4 days) of *Colletotrichum* isolates in this study cultured on potato dextrose agar (PDA) at different temperatures^z

Species (group)	Strains	5°C	10°C	15°C	20°C	25°C	30°C	35°C
<i>Colletotrichum</i> 1	LZJZ1, LZJZ2, LZJZ3, LZJZ4	0 a	0.7 ± 0.7 b	7.2 ± 0.8 b	13.2 ± 0.9 b	15.0 ± 1.1 c	6.0 ± 0.9 c	1.9 ± 0.3 b
<i>Colletotrichum</i> 2	LZJZ5	0 a	2.1 ± 0.1 a	9.0 ± 0.2 b	18.2 ± 0.5 a	19.5 ± 0.2 b	8.6 ± 0.3 b	0.8 ± 0.3 c
<i>Colletotrichum</i> 3	LZJZ6	0 a	2.3 ± 1.5 a	11.3 ± 0.4 a	13.1 ± 0.4 b	21.9 ± 0.8 a	30.3 ± 0.4 a	11.6 ± 0.3 a

^z Columns with the same letter do not differ significantly according with Duncan's test ($P \leq 0.05$).

Table 4. Pathogenicity of *Colletotrichum* isolates on leaves of *Philodendron tatei* cv. Congo plants, 30 days after wounding and nonwounding inoculation^x

Species (group)	Strains	Infected leaves (%)		Lesion diameter (mm)	
		Nonwounded ^y	Wounded ^z	Nonwounded ^y	Wounded ^z
Check		...	...	...	...
<i>Colletotrichum</i> 1	LZJZ1, LZJZ2, LZJZ3, LZJZ4,	23.3 ± 10.4	53.3 ± 10.4	1.9 ± 0.7 × 1.4 ± 0.4	3.0 ± 1.9 × 2.2 ± 1.2
<i>Colletotrichum</i> 2	LZJZ5	10.0 ± 10.0	43.3 ± 10.4	2.2 ± 0.6 × 1.6 ± 0.4	2.7 ± 0.9 × 2.1 ± 0.7
<i>Colletotrichum</i> 3	LZJZ6	16.7 ± 2.9	53.3 ± 5.8	2.1 ± 0.8 × 1.5 ± 0.6	3.0 ± 2.1 × 2.1 ± 1.1

^x Table shows incidence of disease by percent and lesion diameter following inoculation of wounded and nonwounded leaves of *P. tatei* cv. Congo. Data represent the mean of three replications, each with 20 leaves on *P. tatei* cv. Congo plants. Check = leaves inoculated with filter papers with sterile water (6 mm diameter), as controls. Ellipsis (...) indicates no symptoms developed on leaves of *P. tatei* cv. Congo.

^y Nonwounded, inoculated with a 6-mm conidial paper.

^z Wounded by sterile pinprick, inoculated with a 6-mm conidial paper.

Fig. 8. The results of the pairwise homoplasy index (PHI) test of closely related species using both LogDet transformation and splits decomposition. **A**, clade A; **B**, clade B.

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