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RESEARCH ARTICLE

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Colletotrichum species causing anthracnose disease of chili in China

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Key words

DNA phylogeny
multi-gene analysis
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systematics

Abstract Anthracnose caused by *Colletotrichum* species is a serious disease of more than 30 plant genera. Several *Colletotrichum* species have been reported to infect chili in different countries. Although China is the largest chili-producing country, little is known about the species that have been infecting chili locally. Therefore, we collected samples of diseased chili from 29 provinces of China, from which 1285 strains were isolated. The morphological characters of all strains were observed and compared, and multi-locus phylogenetic analyses (ITS, *ACT*, *CAL*, *CHS-1*, *GAPDH*, *TUB2*, and *HIS3*) were performed on selected representative strains. Fifteen *Colletotrichum* species were identified, with *C. fioriniae*, *C. fructicola*, *C. gloeosporioides*, *C. scovillei*, and *C. truncatum* being prevalent. Three new species, *C. conoides*, *C. grossum*, and *C. liaoningense*, were recognised and described in this paper. *Colletotrichum aenigma*, *C. cliviae*, *C. endophytica*, *C. hymenocallidis*, *C. incanum*, *C. karstii*, and *C. viniferum* were reported for the first time from chili. Pathogenicity of all species isolated from chili was confirmed, except for *C. endophytica*. The current study improves the understanding of species causing anthracnose on chili and provides useful information for the effective control of the disease in China.

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INTRODUCTION

Chili (*Capsicum* spp.) is an important vegetable crop worldwide. China maintains the largest planted area of chili, producing more than 28 M tons per year for domestic consumption and export (Li et al. 2009). One of the most destructive diseases restricting chili production is anthracnose, caused by *Colletotrichum* spp. (Bailey & Jeger 1992, Poonpolgul & Kumphai 2007, Than et al. 2008), resulting in up to 40 % yield loss in China (Lin et al. 2004).

Colletotrichum species can infect more than 30 plant genera (Perfect et al. 1999, Dean et al. 2012, Farr & Rossman 2016). More than 10 *Colletotrichum* species have been reported from chili, with different distributions among countries (Than et al. 2008, Liao et al. 2012, Kanto et al. 2014, Sharma et al. 2014, Diao et al. 2015). For example, anthracnose on chili is caused by *C. coccodes*, *C. fructicola*, *C. siamense*, and *C. truncatum* in India (Sharma & Shenoy 2014); by *C. acutatum*, *C. coccodes*, and *C. gloeosporioides* in the USA; by *C. acutatum*, *C. dematium*, *C. gloeosporioides*, and *C. truncatum* in Australia; by *C. acutatum*, *C. coccodes*, *C. dematium*, *C. gloeosporioides*, and *C. panacicola* in Korea (Than et al. 2008); and by *C. acutatum*, *C. gloeosporioides*, *C. truncatum*, and *C. coccodes* in China (Shin et al. 1999, Liao et al. 2012). Most of these reports, however, were based on morphology and ITS sequences or a combination of ITS and *TUB2* sequences, which have been shown to be insufficient in distinguishing closely related taxa in several species complexes. In addition, these records were mostly based on a small sampling from restricted areas, and, thus, may underestimate the species diversity.

The current study aimed to investigate the *Colletotrichum* species causing anthracnose on chili in China, by employing large-scale sampling and isolation, and via morphological characterisation and multi-locus phylogeny of the obtained strains.

MATERIALS AND METHODS

Sample collection and isolation

From 2008 to 2014, fruits and leaves of chili (*Capsicum* spp.) with anthracnose symptoms were collected from 50 locations in 29 provinces of China (Fig. 1). In each location, a hierarchical sampling method was used as previously described (Kohli et al. 1995). Five fields were chosen at each sampling location, and 25 chili fruits and also leaves in some cases were collected from each field along a diagonal transect. *Colletotrichum* species were isolated as described by Cai et al. (2009). All isolates were grown at 28 °C for further study. Type specimens of new species from this study were deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS), and ex-type living cultures were deposited in the China General Microbiological Culture Collection Centre (CGMCC), Beijing, China.

Morphological characterisation

Mycelial plugs (5 mm) were transferred from the edge of actively growing cultures to fresh potato dextrose agar (PDA, 1.5 %, Difco) plates. Cultures were kept at 28 °C with a 12/12 h fluorescent light/dark cycle. The morphological characters for all isolates, including colony and conidial characteristics, were observed. Microscopic characters were examined with the Carl Zeiss Imager A2 microscope after 1 mo of cultivation. Among the 1 285 obtained isolates (Table 1), 121 representative isolates were selected for further multilocus phylogenetic analyses based on geographical location, morphology (colony shape and colour and characteristics of aerial mycelia and conidia), and

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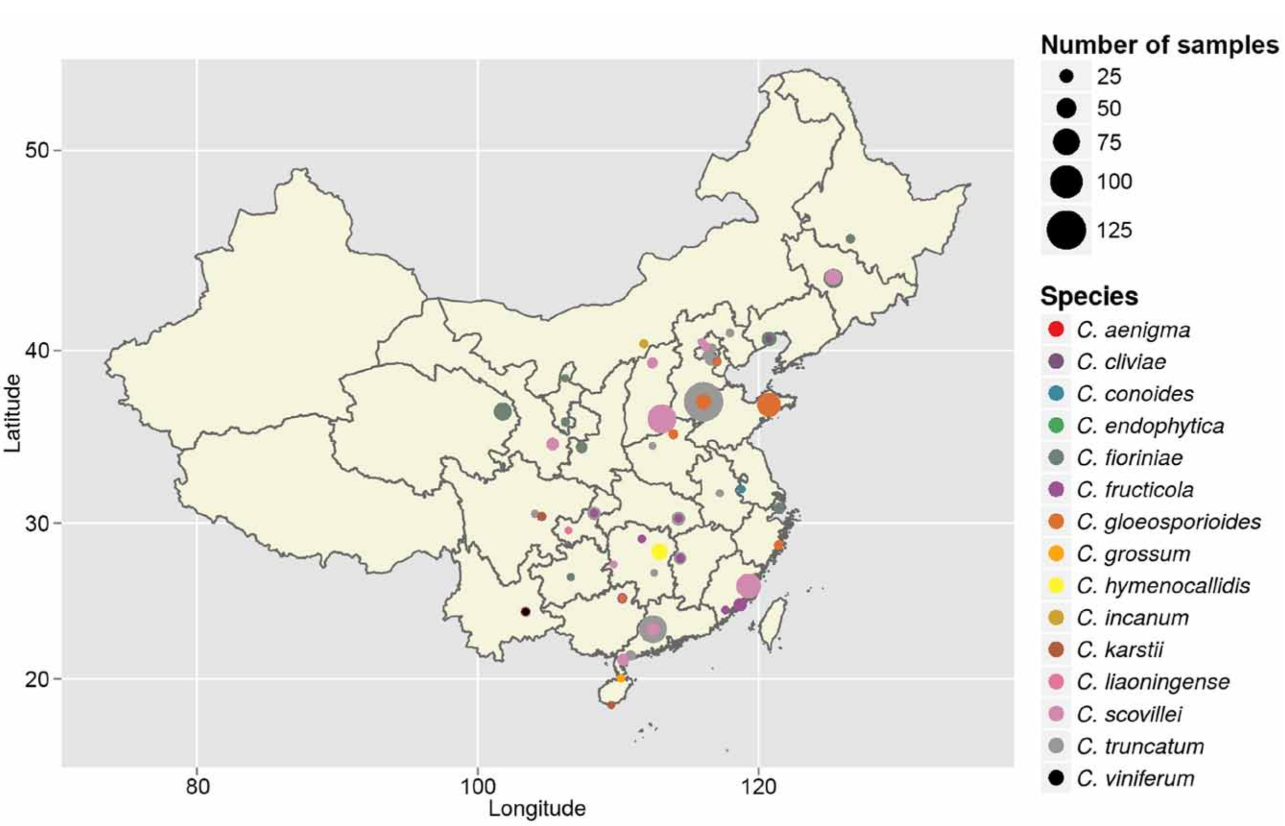


Fig. 1 Map showing locations in China where chili was sampled for *Colletotrichum* species. Each coloured circle represents one species by preliminary identification, and the size of the circle indicates the number of isolates collected from that location.

ITS sequences. Different morphological types were selected from each location, and the number of representative isolates selected depended on the number of isolates with different morphologies. Furthermore, if the number of isolates with high morphological and ITS sequence similarities was less than 10 in one location, then one isolate was randomly selected as a representative. If the number was more than 10, on the other hand, then an additional isolate (one from each of the 10 isolates) was selected for multi-locus phylogenetic analyses. The length and width of 30 conidia for each isolate were measured in lactic acid, and mean values calculated. The formation of appressoria was induced as described by Cai et al. (2009).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from 121 representative isolates as previously described (Murray & Thompson 1980, Diao et al. 2015). The following loci were amplified with the indicated primers: the internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) with primers ITS4/ITS5 (White et al. 1990); partial sequences of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) with primers GDF1/GDR1 (Templeton et al. 1992); actin gene (*ACT*) with primers ACT512F/ACT783R (Carbone & Kohn 1999); beta-tubulin (*TUB2*) with primers T1/Bt2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997); calmodulin (*CAL*) with primers CL1/CL2A (O'Donnell et al. 2000); chitin synthase 1 (*CHS-1*) with primers CHS-79F/CHS-345R (Carbone & Kohn 1999); and histone3 (*HIS3*) with primers CYLH3F/CYLH3R (Crous et al. 2004b). PCR reactions were performed as described by Damm et al. (2009). DNA sequencing was conducted by Sunbiotech, Beijing, China with a 3730 DNA Analyzer (Applied Biosystems, USA). The sequences obtained from forward and reverse primers were used to obtain consensus sequences with DNAMAN v. 6.0 (Lynnon Biosoft, USA). Sequences were aligned using MAFFT v. 6 (Katoh & Toh 2010).

Phylogenetic analysis

All sequences of the 121 representative isolates were blasted in GenBank. Sequences with high similarities were selected and included in the analyses (Table 2a–c). Concatenated analyses of ITS, *GAPDH*, *CHS-1*, *HIS3*, *ACT*, and *TUB2* were conducted for the *C. acutatum* species complex and *Colletotrichum* species with curved conidia, while ITS, *GAPDH*, *CAL*, *ACT*, *CHS-1*, and *TUB2* were concatenated for the analysis of the *C. gloeosporioides* species complex and other species. Maximum parsimony (MP) analyses based on the combined datasets were conducted using PAUP v. 4.0b10 (Swofford 2002). Phylogenetic trees were generated using the heuristic search option with TBR branch swapping and 1 000 random sequence additions. Maxtrees were unlimited, with branches of zero length collapsed, and all multiple parsimonious trees were saved. Clade stability was assessed using a bootstrap analysis with 1 000 replicates. Afterward, tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated. Bayesian inference (BI) was used to reconstruct the phylogenetic tree using MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003). Best-fit models of nucleotide substitution were selected using MrModelTest v. 2.3 (Nylander 2004). Two analyses of four MCMC chains were run from random trees for 1 000 000 generations, and trees were sampled every 100 generations resulting in 20 000 total trees. The first 25 % of the trees were discarded as the burn-in phase of each analysis, and the remaining trees were used to calculate posterior probabilities (Cai et al. 2006, Liu et al. 2012, 2013). An additional Maximum likelihood (ML) analysis was implemented in the *C. gloeosporioides* species complex using the CIPRES Science Gateway v. 3.3 (www.phylo.org), and the RAxML-HPC BlackBox was selected with default parameters. Sequences derived in this study were deposited in GenBank (Table 2), and the concatenated alignments were deposited in TreeBASE (http://treebase.org/treebase-web/home.html; study

Table 1 A list of all *Colletotrichum* isolates collected from chili in China based on preliminary identification.

Species	Location	Host tissue	Year	Number of isolates	Longitude	Latitude
C. aenigma	Yangliuqing, Tianjin	Fruit	2012	1	39.4	117.01
C. cliviae	Xingcheng, Liaoning	Fruit	2012	1	40.63	120.74
C. conoides	Nanjing, Jiangsu	Fruit	2010	3	32.06	118.79
C. endophytica	Mile, Yunnan	Fruit	2011	1	24.41	103.41
C. fioriniae	Fengxiang, Shanxi	Fruit	2011	17	34.55	107.4
	Changchun	Fruit	2011	47	43.81	125.32
	Xining, Qinghai	Fruit	2011	42	36.61	101.78
	Sanya, Hainan	Fruit	2012	2	18.25	109.51
	Guiyang, Guizhou	Fruit	2012	3	26.64	106.63
	Xingcheng, Liaoning	Fruit	2012	29	40.63	120.74
	Yinchuan, Ningxia	Fruit	2012	4	38.48	106.23
	Guyuan, Ningxia	Fruit	2012	8	36.01	106.24
	Fengxian, Shanghai	Fruit	2012	16	30.91	121.47
	Harbin, Heilongjiang	Fruit	2012	9	45.8	126.53
C. fructicola	Fuzhou, Fujian	Fruit	2011	2	26.07	119.29
	Guilin, Guangxi	Fruit	2011	10	25.27	110.29
	Hengyang, Hunan	Fruit	2012	4	29.03	111.69
	Changsha, Hunan	Fruit	2012	20	28.23	112.94
	Laiyang, Shandong	Fruit	2011	32	36.99	120.74
	Wucheng, Shandong	Fruit	2012	15	37.16	116.08
	Zhangzhou, Fujian	Fruit	2008	5	24.51	117.64
	Quanzhou, Fujian	Fruit	2009	23	24.87	118.67
	Fengxiang, Shanxi	Fruit	2011	5	34.55	107.4
	Xinxiang, Henan	Fruit	2011	5	35.3	113.93
	Yichun, Jiangxi	Fruit	2011	6	27.81	114.41
	Jianyang, Sichuan	Fruit	2011	8	30.41	104.55
	Mile, Yunnan	Fruit	2011	10	24.41	103.41
	Yangliuqing, Tianjin	Fruit	2012	4	39.4	117.01
	Wuhan, Hubei	Fruit	2012	4	30.28	114.29
	Xingcheng, Liaoning	Fruit	2012	4	40.63	120.74
	Shizhu, Chongqing	Fruit	2013	4	30.6	108.29
C. gloeosporioides	Guilin, Guangxi	Fruit	2011	1	25.27	110.29
	Qingyuan, Guangdong	Fruit	2013	21	23.28	112.48
	Qingyuan, Guangdong	Fruit	2014	1	23.28	112.48
	Laiyang, Shandong	Fruit	2011	64	36.99	120.74
	Wucheng, Shandong	Fruit	2011	30	37.16	116.08
	Fengxiang, Shanxi	Fruit	2011	10	34.55	107.4
	Xinxiang, Henan	Fruit	2011	10	35.3	113.93
	Taizhou, Zhejiang	Fruit	2011	8	28.65	121.42
	Changsha, Hunan	Fruit	2012	20	28.23	112.94
	Mile, Yunnan	Fruit	2011	5	24.41	103.41
	Jianyang, Sichuan	Fruit	2011	4	30.41	104.55
	Guyuan, Ningxia	Fruit	2012	2	36.01	106.24
	Wuqing, Tianjin	Fruit	2012	1	39.38	117.04
	Xingcheng, Liaoning	Fruit	2012	4	40.63	120.74
C. grossum	Haikou, Hainan	Fruit	2011	3	20.04	110.19
C. hymenocallidis	Changsha, Hunan	Fruit	2012	35	28.23	112.94
C. incanum	Helingeer, Inner Mongolia	Fruit	2012	6	40.37	111.82
C. karstii	Sanya, Hainan	Fruit	2012	3	18.25	109.51
	Jianyang, Sichuan	Fruit	2011	5	30.41	104.55
	Mile, Yunnan	Fruit	2011	1	24.41	103.41
C. liaoningense	Xingcheng, Liaoning	Fruit	2012	11	40.63	120.74
	Shapingba, Chongqing	Fruit	2012	1	29.54	106.46
C. scovillei	Yanqing, Beijing	Fruit	2011	4	40.45	115.97
	Changping, Beijing	Fruit	2013	8	40.22	116.23
	Fuzhou, Fujian	Fruit	2011	68	26.07	119.29
	Gangu, Gansu	Fruit	2012	21	34.73	105.33
	Jida, Jilin	Fruit	2013	33	43.88	125.31
	Changzhi, Shanxi	Fruit	2011	84	36.19	113.11
	Shuozhou, Shanxi	Fruit	2012	14	39.33	112.43
	Sanya, Hainan	Fruit	2012	1	18.25	109.51
	Zhijiang, Hunan	Fruit	2011	1	27.44	109.68
	Zhanjiang, Guangdong	Fruit	2011	18	21.27	110.35
	Qingyuan, Guangdong	Fruit	2012	20	23.28	112.48
C. truncatum	Qingyuan, Guangdong	Fruit	2013	80	23.28	112.48
	Qingyuan, Guangdong	Fruit	2014	10	23.28	112.48
	Maoming, Guangdong	Fruit	2013	13	21.55	110.88
	Yichun, Jiangxi	Leave	2011	20	27.81	114.41
	Shizhu, Chongqing	Fruit	2013	23	30.6	108.29
	Wuhan, Hubei	Fruit	2013	25	30.28	114.29
	Fengxiang, Shanxi	Fruit	2011	12	34.55	107.4
	Wucheng, Shandong	Fruit	2011	125	37.16	116.08
	Laiyang, Shandong	Fruit	2011	10	36.99	120.74
	Yangliuqing, Tianjin	Fruit	2012	11	39.4	117.01

Table 1 (cont.)

Species	Location	Host tissue	Year	Number of isolates	Longitude	Latitude
C. truncatum (cont.)	Langfang, Hebei	Fruit	2011	20	39.52	116.61
	Chengde, Hebei	Fruit	2013	5	40.95	117.96
	Daxing, Beijing	Fruit	2011	9	39.73	116.34
	Shunyi, Beijing	Fruit	2011	10	40.13	116.65
	Xingcheng, Liaoning	Fruit	2012	16	40.63	120.74
	Changchun, Jilin	Fruit	2012	7	43.71	125.54
	Chengdu, Sichuan	Fruit	2011	3	30.57	104.07
	Hefei, Anhui	Fruit	2011	2	31.82	117.23
	Fuzhou, Fujian	Fruit	2011	2	26.07	119.29
	Luoyang, Henan	Fruit	2011	1	34.62	112.45
	Xinxiang, Henan	Fruit	2011	2	35.3	113.93
	Changsha, Hunan	Fruit	2012	1	28.23	112.94
	Hengyang, Hunan	Fruit	2012	1	26.89	112.57
	Changde, Hunan	Fruit	2012	3	29.03	111.69
	Mile, Yunnan	Fruit	2011	4	24.41	103.41
	Zhanjiang, Guangdong	Fruit	2011	5	21.27	110.36
	Xining, Qinghai	Fruit	2011	1	36.61	101.78
	Shuozhou, Shanxi	Fruit	2012	1	39.33	112.43
C. viniferum	Mile, Yunnan	Fruit	2011	1	24.41	103.41
Total				1285		

S17061), and the descriptions and nomenclature in MycoBank (Crous et al. 2004a).

Genealogical concordance phylogenetic species recognition analysis

New species and their most closely related neighbours were analysed using the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) model with a pairwise homoplasy index (PHI) test as described by Quaedvlieg et al. (2014). The PHI tests were performed in SplitsTree4 (Huson 1998, Huson & Bryant 2006) to determine the recombination level within phylogenetically closely related species using a six-locus concatenated dataset (*ACT*, *CAL*, *CHS*, *GAPDH*, ITS, and *TUB2*) for *C. conoides*, *C. grossum*, and *C. liaoningense* and their respective related species. A pairwise homoplasy index below a 0.05 threshold ($\Phi w < 0.05$) indicated the presence of significant recombination in the dataset. The relationship between closely related species was visualised by constructing a split graph.

Pathogenicity assay

Seven chili cultivars, *Capsicum annuum* cv. Chaotianjiao, Denglongjiao, Sanyingjiao, Zidantou, *C. frutescens* cv. Shuangla, Xiaomila and *C. chinense* cv. Huangdijiao, were inoculated with representative strains of 15 *Colletotrichum* species respectively (Table 3, 4). Pathogenicity tests were conducted on chili following the methods described by Montri et al. (2009) and Mongkolporn et al. (2010). Healthy, ripe red and green chili fruits were surface sterilised in 1 % NaClO for 5 min separately, washed twice with sterile-distilled water, and air dried on sterile filter paper. Each fruit was inoculated with 1 µL of a conidial suspension (1×10^6 conidial/mL), which was injected onto the non-wounded fruit surface using a microsyringe (Shanghai, China). Control fruits were treated with 1 µL of distilled water. Each isolate was inoculated to five replicate fruits. The inoculated fruits were incubated in a moist chamber at 28 °C and were examined for symptoms daily for 9 d. The virulence and pathotypes were evaluated as described by Montri et al. (2009). The experiment was conducted twice.

RESULTS

Disease survey and strain isolation

Symptoms of anthracnose were circular or angular sunken lesions on chili fruits and irregularly shaped brown spots with dark brown edges on leaves. A total of 1 285 isolates of *Colletotrichum* spp. were obtained from 29 provinces in China (Fig. 1, Table 1). Attempts were made to collect samples from multiple locations in Tibet and Xinjiang provinces for several years but failed to locate symptomatic plants. All strains were isolated from fruits except those from Jiangxi province, where serious damage was found on chili leaves rather than on fruits.

Group assessment

Based on megablast searches in GenBank using ITS sequences and the colony morphologies on PDA, all strains were assigned to four groups, i.e., those that produce cylindrical conidia with round ends were assigned to the *C. gloeosporioides* species complex; those that produce acute ends or ± cylindrical conidia with only one acute end were assigned to the *C. acutatum* species complex; those that produce dark setae and curved conidia were assigned to the *Colletotrichum* species with curved conidia; and the remaining strains were assigned to a fourth group. Among the 121 isolates, 31 belonged to the *C. gloeosporioides* complex; 48 belonged to the *C. acutatum* complex; 34 belonged to the *Colletotrichum* species with curved conidia, and eight belonged to the fourth group (Damm et al. 2012a, b, 2013, 2014, Weir et al. 2012, Crouch 2014).

Multi-locus phylogenetic analyses

The 121 representative isolates from chili were subjected to multilocus phylogenetic analyses (Table 2a–c). The trees generated from the Bayesian and RaxML analyses were essentially similar to that from the MP analysis (Fig. 2) and are therefore not shown. In Fig. 2, the 31 isolates in the *C. gloeosporioides* complex clustered in eight clades, eight with *C. fructicola*, 13 with *C. gloeosporioides*, and four with *C. aenigma*, *C. endophytica*, *C. hymenocallidis*, and *C. viniferum*, respectively. In addition, two distinct lineages, which clustered distantly from any known species in the complex, were recognised as new species and herein described as *C. conoides* and *C. grossum* (Fig. 2). In Fig. 3, the isolates of the *C. acutatum* complex clustered in two clades, 31 with *C. scovillei* and 17 with *C. fioriniae*. In the *Colletotrichum* species with curved conidia, 33 isolates

Table 2a Strains used for the phylogenetic analysis of the *Colletotrichum gloeosporioides* species complex and other species with details about host, location, and GenBank accession numbers.

Species	Isolate	Host	Location	GenBank accessions					
				ITS	*GAPDH*	*CAL*	*ACT*	*CHS-1*	*TUB2*
C. aenigma	ICMP 18686	*Pyrus pyrifolia*	Japan	JX010243	JX009913	JX009684	JX009519	JX009789	JX010390
	ICMP 18608*	*Persea americana*	Israel	JX010244	JX010044	JX009683	JX009443	JX009774	JX010389
C. aeschynomenes	ICMP 17673*	*Aeschynomene virginica*	USA	JX010176	JX009930	JX009721	JX009483	JX009799	JX010392
C. alatae	CBS 304.67*	*Dioscorea alata*	India	JX010190	JX009990	JX009738	JX009471	JX009837	JX010383
	ICMP 18122	*Dioscorea alata*	Nigeria	JX010191	JX010011	JX009739	JX009470	JX009846	JX010449
C. alienum	ICMP 12071*	*Malus domestica*	New Zealand	JX010251	JX010028	JX009654	JX009572	JX009882	JX010411
	ICMP 18621	*Persea americana*	New Zealand	JX010246	JX009959	JX009657	JX009552	JX009755	JX010386
C. aotearoa	ICMP 18537*	*Coprosma* sp.	New Zealand	JX010205	JX010005	JX009611	JX009564	JX009853	JX010420
C. asianum	ICMP 18580*	*Coffea arabica*	Thailand	FJ972612	JX010053	FJ917506	JX009584	JX009867	JX010406
	ICMP 18696	*Mangifera indica*	Australia	JX010192	JX009915	JX009723	JX009576	JX009753	JX010384
C. boninense	MAFF 305972*	*Crinum asiaticum* var. *sinicum*	Japan	JX010292	JX009905	JQ005674	JX009583	JX009827	JQ005588
	CBS 128547	*Camellia* sp.	New Zealand	JQ005159	JQ005246	JQ005680	JQ005507	JQ005333	JQ005593
C. brevisporum	BCC 38876*	*Neoregalia* sp.	Thailand	JN050238	JN050238	JN050222	JN050216	KF687760	JN050244
	MFLUCC100182	*Pandanus pygmaeus*	Thailand	JN050239	JN050228		JN050217		JN050245
C. clidemiae	ICMP 18706	*Clidemia hirta*	USA	JX010274	JX009909	JX009639	JX009476	JX009777	JX010439
C. cliviae	CBS 125375*	*Clivia miniata*	China	JX519223	JX546611		JX519240	JX519232	JX519249
	CSSS1	*Clivia miniata*	China	GU109479	GU085867		GU085861		GU085869
	CSSS2	*Clivia miniata*	China	GU109480	GU085868		GU085862		GU085870
	CAUOS6	***Capsicum annuum***	**China**			**KP890123**	**KP890100**	**KP890131**	**KP890115**
C. conoides	**CAUG17***	***Capsicum annuum***	**China**	**KP890168**	**KP890162**	**KP890150**	**KP890144**	**KP890156**	**KP890174**
	CAUG33	***Capsicum annuum***	**China**	**KP890169**	**KP890163**	**KP890151**	**KP890145**	**KP890157**	**KP890175**
	CAUG34	***Capsicum annuum***	**China**	**KP890170**	**KP890164**	**KP890152**	**KP890146**	**KP890158**	**KP890176**
C. cordylinicola	ICMP 18579	*Cordyline fruticosa*	Thailand	JX010226	JX009975	HM470238	HM470235	JX009864	JX010440
C. dracaenophilum	CBS 118199*	*Dracaena* sp.	China	JX519222	JX546707		JX519238	JX519230	JX519247
C. endophytica	**CAUG28**	***Capsicum annuum***	**China**	**KP145441**	**KP145413**	**KP145357**	**KP145329**	**KP145385**	**KP145469**
	LC0324*	*Pennisetu purpureum*	Thailand	KC633854	KC832854	KC810018	KF306258		
	MFLUCC 100676	*Pennisetu purpureum*	Thailand	KF242123	KF242181	KF254846	KF157827		
C. fructicola	**CAUG1**	***Capsicum* sp.**	**China**	**KP145416**	**KP145388**	**KP145332**	**KP145304**	**KP145360**	**KP145444**
	CAUG5	***Capsicum* sp.**	**China**	**KP145420**	**KP145392**	**KP145336**	**KP145308**	**KP145364**	**KP145448**
	CAUG6	***Capsicum* sp.**	**China**	**KP145421**	**KP145393**	**KP145337**	**KP145309**	**KP145365**	**KP145449**
	CAUG8	***Capsicum* sp.**	**China**	**KP145422**	**KP145394**	**KP145338**	**KP145310**	**KP145366**	**KP145450**
	CAUG10	***Capsicum* sp.**	**China**	**KP145424**	**KP145396**	**KP145340**	**KP145312**	**KP145368**	**KP145452**
	CAUG11	***Capsicum* sp.**	**China**	**KP145425**	**KP145397**	**KP145341**	**KP145313**	**KP145369**	**KP145453**
	CAUG16	***Capsicum* sp.**	**China**	**KP145430**	**KP145402**	**KP145346**	**KP145318**	**KP145374**	**KP145458**
	CAUG18	***Capsicum* sp.**	**China**	**KP145431**	**KP145403**	**KP145347**	**KP145319**	**KP145375**	**KP145459**
	CBS 125395	*Theobroma cacao*	Panama	JX010172	JX009992	JX009666	JX009543	JX009873	JX010408
	ICMP 17789	*Malus domestica*	USA	JX010178	JX009914	JX009665	JX009451	JX009809	
	ICMP 12568	*Persea americana*	Australia	JX010166	JX009946	JX009680	JX009529	JX009762	
	ICMP 18581*	*Coffea arabica*	Thailand	JX010165	JX010033	FJ917508	FJ907426	JX009866	JX010405
	ICMP 18613	*Limonium sinuatum*	Israel	JX010167	JX009998	JX009675	JX009491	JX009772	JX010388
	ICMP 18727	*Fragaria ananassa*	USA	JX010179	JX010035	JX009682	JX009565	JX009812	JX010394
	CBS 238.49*	*Ficus edulis*	Germany	JX010181	JX009923	JX009671	JX009495	JX009839	JX010400
	CBS 125397*	*Tetragastris panamensis*	Panama	JX010173	JX010032	JX009674	JX009581	JX009874	JX010409
C. gloeosporiodes	**CAUG2**	***Capsicum* sp.**	**China**	**KP145417**	**KP145389**	**KP145333**	**KP145305**	**KP145361**	**KP145445**
	CAUG3	***Capsicum* sp.**	**China**	**KP145418**	**KP145390**	**KP145334**	**KP145306**	**KP145362**	**KP145446**
	CAUG12	***Capsicum* sp.**	**China**	**KP145426**	**KP145398**	**KP145342**	**KP145314**	**KP145370**	**KP145454**
	CAUG13	***Capsicum* sp.**	**China**	**KP145427**	**KP145399**	**KP145343**	**KP145315**	**KP145371**	**KP145455**
	CAUG14	***Capsicum* sp.**	**China**	**KP145428**	**KP145400**	**KP145344**	**KP145316**	**KP145372**	**KP145456**
	CAUG15	***Capsicum* sp.**	**China**	**KP145429**	**KP145401**	**KP145345**	**KP145317**	**KP145373**	**KP145457**
	CAUG19	***Capsicum* sp.**	**China**	**KP145432**	**KP145404**	**KP145348**	**KP145320**	**KP145376**	**KP145460**
	CAUG20	***Capsicum* sp.**	**China**	**KP145433**	**KP145405**	**KP145349**	**KP145321**	**KP145377**	**KP145461**

C. gloeosporiodes (cont.)	**CAUG22**	***Capsicum* sp.**	**China**	**KP145435**	**KP145407**	**KP145351**	**KP145323**	**KP145379**	**KP145463**
	CAUG23	***Capsicum* sp.**	**China**	**KP145436**	**KP145408**	**KP145352**	**KP145324**	**KP145380**	**KP145464**
	CAUG24	***Capsicum* sp.**	**China**	**KP145437**	**KP145409**	**KP145353**	**KP145325**	**KP145381**	**KP145465**
	CAUG25	***Capsicum* sp.**	**China**	**KP145438**	**KP145410**	**KP145354**	**KP145326**	**KP145382**	**KP145466**
	CAUG26	***Capsicum* sp.**	**China**	**KP145439**	**KP145411**	**KP145355**	**KP145327**	**KP145383**	**KP145467**
	CAUG29	***Capsicum* sp.**	**China**	**KP145442**	**KP145414**	**KP145358**	**KP145330**	**KP145386**	**KP145470**
	IMI 356878*	*Citrus sinensis*	Italy	JX010152	JX010056	JX009731	JX009531	JX009818	JX010445
	CORCG4	*Vanda* sp.	Thailand	HM034808	HM034806	HM034802	HM034800		
	CORCG5	*Vanda* sp.	Thailand	HM034809	HM034807	HM034803	HM034801	HM034805	HM034811
C. grevillea	CBS 132879*	*Grevillea* sp.	Italy	KC297078	KC297010	KC296963	KC296941	KC296987	KC297102
C. grossum	**CAUG7***	***Capsicum* sp.**	**China**	**KP890165**	**KP890159**	**KP890147**	**KP890141**	**KP890153**	**KP890171**
	CAU31	***Capsicum* sp.**	**China**	**KP890166**	**KP890160**	**KP890148**	**KP890142**	**KP890154**	**KP890172**
	CAUG32	***Capsicum* sp.**	**China**	**KP890167**	**KP890161**	**KP890149**	**KP890143**	**KP890155**	**KP890173**
C. hebeiense	JZB330024	*Vitis vinifera* cv. Cabernet Sauvignon	China	KF156873	KF377505		KF377542		
	JZB330028*	*Vitis vinifera* cv. Cabernet Sauvignon	China	KF156863	KF377495		KF377532	KF289008	KF288975
C. hymenocallidis	**CAUG9**	***Capsicum* sp.**	**China**	**KP145423**	**KP145395**	**KP145339**	**KP145311**	**KP145367**	**KP145451**
	ICMP 18642*	*Hymenocallis americana*	China	JX010278	JX010019	JX009709	GQ856775	GQ856730	JX010410
C. kahawae subsp. *ciggaro*	ICMP 12952	*Persea americana*	New Zealand	JX010214	JX009971	JX009648	JX009431	JX009757	JX010426
	ICMP 18539*	*Olea europaea*	Australia	JX010230	JX009966	JX009635	JX009523	JX009800	JX010434
C. karstii	CGMCC 3.14194*	*Vanda* sp.	China	HM585409	HM585391	HM581995	HM585428		
	CAUOS1	***Capsicum* sp.**	**China**	**KP890103**	**KP890134**	**KP890118**	**KP890096**	**KP890126**	**KP890110**
	CAUOS7	***Capsicum* sp.**	**China**	**KP890108**	**KP890139**	**KP890124**	**KP890101**	**KP890132**	**KP890116**
	CAUOS8	***Capsicum* sp.**	**China**	**KP890109**	**KP890140**	**KP890125**	**KP890102**	**KP890133**	**KP890117**
C. liaoningense	**CAUOS2***	***Capsicum* sp.**	**China**	**KP890104**	**KP890135**	**KP890119**	**KP890097**	**KP890127**	**KP890111**
	CAUOS3	***Capsicum* sp.**	**China**	**KP890105**	**KP890136**	**KP890120**	**KP890098**	**KP890128**	**KP890112**
	CAUOS4	***Capsicum* sp.**	**China**	**KP890106**	**KP890137**	**KP890121**	**KP890099**	**KP890129**	**KP890113**
	CAUOS5	***Capsicum* sp.**	**China**	**KP890107**	**KP890138**	**KP890122**		**KP890130**	**KP890114**
C. musae	ICMP 19119*	*Musa* sp.	USA	JX010146	JX010050	JX009742	JX009433	JX009896	HQ596280
	IMI 52264	*Musa sapientum*	Kenya	JX010142	JX010015	JX009689	JX009432	JX009815	JX010395
C. nupharicola	ICMP 17938	*Nuphar lutea* subsp. *polysepala*	USA	JX010189	JX009936	JX009661	JX009486	JX009834	JX010397
	ICMP 17940	*Nymphaea ordorata*	USA	JX010188	JX010031	JX009662	JX009582	JX009836	JX010399
	ICMP 18187*	*Nuphar lutea* subsp. *polysepala*	USA	JX010187	JX009972	JX009663	JX009437	JX009835	JX010398
C. psidii	ICMP 19120*	*Psidium* sp.	Italy	JX010219	JX009967	JX009743	JX009515	JX009901	JX010443
C. queenslandicum	ICMP 1778*	*Carica papaya*	Australia	JX010276	JX009934	JX009691	JX009447	JX009899	JX010414
C. salsolae	ICMP 19051	*Salsola tragus*	Hungary	JX010242	JX009916	JX009696	JX009562	JX009863	JX010403
C. siamense	CBS 130420	*Jasminum sambac*	Vietnam	HM131511	HM131497	JX009713	HM131507	JX009895	JX010415
C. thailandicum	BCC 38879*	*Hibiscus rosa-sinensis*	Thailand	JN050242	JN050231		JN050220		JN050248
	MFLUCC10092	*Alocasia* sp.	Thailand	JN050243	JN050232		JN050221		JN050249
C. theobromicola	CBS 124945*	*Theobroma cacao*	Panama	JX010294	JX010006	JX009591	JX009444	JX009869	JX010447
	ICMP 17814	*Fragaria vesca*	USA	JX010288	JX010003	JX009589	JX009448	JX009819	JX010379
	ICMP 17957	*Stylosanthes viscosa*	Australia	JX010289	JX009962	JX009597	JX009575	JX009821	JX010380
C. ti	ICMP 4832*	*Cordyline* sp.	New Zealand	JX010269	JX009952	JX009649	JX009520	JX009898	JX010442
	ICMP 5285	*Cordyline australis*	New Zealand	JX010267	JX009910	JX009650	JX009553	JX009897	JX010441
C. tropicale	CBS 124949*	*Theobroma cacao*	Panama	JX010264	JX010007	JX009719	JX009489	JX009870	JX010407
	ICMP 18672	*Litchi chinensis*	Japan	JX010275	JX010020	JX009722	JX009480	JX009826	JX010396
C. tropicicola	BCC 38877*	*Citrus maxima*	Thailand	JN050240	JN050229		JN050218		JN050246
	MFLUCC100167	*Paphiopedilum bellatolum*	Thailand	JN050241	JN050230		JN050219		JN050247
C. viniferum	**CAUG27**	***Capsicum* sp.**	**China**	**KP145440**	**KP145412**	**KP145356**	**KP145328**	**KP145384**	**KP145468**
	GZAAS 5.08601*	*Vitis vinifera*, cv. 'Shuijing'	China	JN412804	JN412798	JQ309639	JN412795		JN412813
	GZSSS 5.08608	*Vitis vinifera*, cv. 'Hongti'	China	JN412802	JN412800	JQ412782	JN412793		JN412811
C. xanthorrhoeae	ICMP 17903*	*Xanthorrhoea preissii*	Australia	JX010261	JX009927	JX009653	JX009478	JX009823	JX010448
C. yunnanense	CBS 132135*	*Buxus* sp.	China	JX546804	JX519248	JX546706	JX519239	JX519231	
Glomella cingulata 'f. sp. *camelliae*'	ICMP 10643	*Camellia williamsii*	UK	JX010224	JX009908	JX009630	JX009540	JX009891	JX010436
Monilochaetes infuscans	CBS 869.96*	*Ipomoea batatas*	South Africa	JQ005780	JX546612		JQ005843	JQ005801	JQ005864

* = Ex-type culture. Strains studied in this paper are in **bold** font.

Table 2b Strains used for the phylogenetic analysis of the *Colletotrichum acutatum* species complex with details about host, location, and GenBank accession numbers.

Species	Isolate	Host	Location	GenBank accessions					
				ITS	*GAPDH*	*CHS-1*	*HIS3*	*ACT*	*TUB2*
C. acutatum	CBS 112996*	*Carica papaya*	Australia	JQ005776	JQ948677	JQ005797	JQ005818	JQ005839	JQ005860
	CBS 144.29	*Capsicum annuum*	Sri Lanka	JQ948401	JQ948732	JQ949062	JQ949392	JQ949722	JQ950052
C. chrysanthemi	CBS 126518	*Carthamus* sp.	Netherlands	JQ948271	JQ948601	JQ948932	JQ949262	JQ949592	JQ949922
	IMI 364540	*Chrysanthemum coronarium*	China	JQ948272	JQ948602	JQ948933	JQ949263	JQ949593	JQ949923
C. fioriniae	**CAUA18**	***Capsicum annuum***	**China**	**KP145016**	**KP145096**	**KP145048**	**KP145064**	**KP145032**	**KP145080**
	CAUA20	***Capsicum annuum***	**China**	**KP145226**	**KP145162**	**KP145290**	**KP145194**	**KP145130**	**KP145258**
	CAUA24	***Capsicum annuum***	**China**	**KP145017**	**KP145097**	**KP145049**	**KP145065**	**KP145033**	**KP145081**
	CAUA25	***Capsicum annuum***	**China**	**KP145018**	**KP145098**	**KP145050**	**KP145066**	**KP145034**	**KP145082**
	CAUA26	***Capsicum annuum***	**China**	**KP145019**	**KP145099**	**KP145051**	**KP145067**	**KP145035**	**KP145083**
	CAUA27	***Capsicum annuum***	**China**	**KP145020**	**KP145100**	**KP145052**	**KP145068**	**KP145036**	**KP145084**
	CAUA28	***Capsicum annuum***	**China**	**KP145021**	**KP145101**	**KP145053**	**KP145069**	**KP145037**	**KP145085**
	CAUA29	***Capsicum annuum***	**China**	**KP145022**	**KP145102**	**KP145054**	**KP145070**	**KP145038**	**KP145086**
	CAUA30	***Capsicum annuum***	**China**	**KP145023**	**KP145103**	**KP145055**	**KP145071**	**KP145039**	**KP145087**
	CAUA31	***Capsicum annuum***	**China**	**KP145024**	**KP145104**	**KP145056**	**KP145072**	**KP145040**	**KP145088**
	CAUA32	***Capsicum annuum***	**China**	**KP145025**	**KP145105**	**KP145057**	**KP145073**	**KP145041**	**KP145089**
	CAUA37	***Capsicum annuum***	**China**	**KP145026**	**KP145106**	**KP145058**	**KP145074**	**KP145042**	**KP145090**
	CAUA38	***Capsicum annuum***	**China**	**KP145027**	**KP145107**	**KP145059**	**KP145075**	**KP145043**	**KP145091**
	CAUA39	***Capsicum annuum***	**China**	**KP145028**	**KP145108**	**KP145060**	**KP145076**	**KP145044**	**KP145092**
	CAUA40	***Capsicum annuum***	**China**	**KP145029**	**KP145109**	**KP145061**	**KP145077**	**KP145045**	**KP145093**
	CAUA41	***Capsicum annuum***	**China**	**KP145030**	**KP145110**	**KP145062**	**KP145078**	**KP145046**	**KP145094**
	CAUA48	***Capsicum annuum***	**China**	**KP145031**	**KP145111**	**KP145063**	**KP145079**	**KP145047**	**KP145095**
	CBS 125396	*Malus domestica*	USA	JQ948299	JQ948629	JQ948960	JQ949290	JQ949620	JQ949950
	CBS 127537	*Vaccinium* sp.	USA	JQ948317	JQ948647	JQ948978	JQ949308	JQ949638	JQ949968
	CBS 128517*	*Fiorinia* sp.	USA	JQ948292	JQ948622	JQ948953	JQ949283	JQ949613	JQ949943
	CBS 129916	*Vaccinium* sp.	USA	JQ948317	JQ948647	JQ948978	JQ949308	JQ949638	JQ949968
	CBS 293.67	*Persea* sp.	Australia	JQ948310	JQ948640	JQ948971	JQ949301	JQ949631	JQ949961
	CBS 127601	*Mangifera* sp.	Australia	JQ948311	JQ948641	JQ948972	JQ949302	JQ949632	JQ949962
	CBS 129947	*Vitis* sp.	Portugal	JQ948343	JQ948673	JQ949004	JQ949334	JQ949664	JQ949994
	CBS 200.35	*Rubus* sp.	USA	JQ948293	JQ948623	JQ948954	JQ949284	JQ949614	JQ949944
C. gloeosporioides	IMI 356878*	*Citrus sinensi*	Italy	JX010152	JX010056	JX009818		JX009531	JX010445
C. godetiae	CBS 133.44*	*Godetia* sp.	Denmark	JQ948402	JQ948733	JQ949063	JQ949393	JQ949723	JQ950053
C. guajavae	IMI 350839*	*Psidium* sp.	India	JQ948270	JQ948600	JQ948931	JQ949261	JQ949591	JQ949921
C. laticiphilum	CBS 112989*	*Hevea* sp.	India	JQ948289	JQ948619	JQ948950	JQ949280	JQ949610	JQ949940
C. nymphaeae	CBS 515.78*	*Nymphaea* sp.	Netherlands	JQ948197	JQ948527	JQ948858	JQ949188	JQ949518	JQ949848
C. orchidophilum	CBS 632.80*	*Dendrobium* sp.	USA	JQ948151	JQ948481	JQ948812	JQ949142	JQ949472	JQ949802
C. salicis	CBS 607.94*	*Salix* sp.	Netherlands	JQ948460	JQ948791	JQ949121	JQ949451	JQ949781	JQ950111
C. scovillei	**CAUA1**	***Capsicum annuum***	**China**	**KP145208**	**KP145144**	**KP145272**	**KP145176**	**KP145112**	**KP145240**
	CAUA2	***Capsicum annuum***	**China**	**KP145209**	**KP145145**	**KP145273**	**KP145177**	**KP145113**	**KP145241**
	CAUA3	***Capsicum annuum***	**China**	**KP145210**	**KP145146**	**KP145274**	**KP145178**	**KP145114**	**KP145242**
	CAUA4	***Capsicum annuum***	**China**	**KP145211**	**KP145147**	**KP145275**	**KP145179**	**KP145115**	**KP145243**
	CAUA5	***Capsicum annuum***	**China**	**KP145212**	**KP145148**	**KP145276**	**KP145180**	**KP145116**	**KP145244**
	CAUA6	***Capsicum annuum***	**China**	**KP145213**	**KP145149**	**KP145277**	**KP145181**	**KP145117**	**KP145245**
	CAUA7	***Capsicum annuum***	**China**	**KP145214**	**KP145150**	**KP145278**	**KP145182**	**KP145118**	**KP145246**
	CAUA8	***Capsicum annuum***	**China**	**KP145215**	**KP145151**	**KP145279**	**KP145183**	**KP145119**	**KP145247**
	CAUA9	***Capsicum annuum***	**China**	**KP145216**	**KP145152**	**KP145280**	**KP145184**	**KP145120**	**KP145248**
	CAUA10	***Capsicum annuum***	**China**	**KP145217**	**KP145153**	**KP145281**	**KP145185**	**KP145121**	**KP145249**
	CAUA11	***Capsicum annuum***	**China**	**KP145218**	**KP145154**	**KP145282**	**KP145186**	**KP145122**	**KP145250**

	CAUA12	***Capsicum annuum***	**China**	**KP145219**	**KP145155**	**KP145283**	**KP145187**	**KP145123**	**KP145251**
	CAUA13	***Capsicum annuum***	**China**	**KP145220**	**KP145156**	**KP145284**	**KP145188**	**KP145124**	**KP145252**
	CAUA14	***Capsicum annuum***	**China**	**KP145221**	**KP145157**	**KP145285**	**KP145189**	**KP145125**	**KP145253**
	CAUA15	***Capsicum annuum***	**China**	**KP145222**	**KP145158**	**KP145286**	**KP145190**	**KP145126**	**KP145254**
	CAUA16	***Capsicum annuum***	**China**	**KP145223**	**KP145159**	**KP145287**	**KP145191**	**KP145127**	**KP145255**
	CAUA17	***Capsicum annuum***	**China**	**KP145224**	**KP145160**	**KP145288**	**KP145192**	**KP145128**	**KP145256**
	CAUA19	***Capsicum annuum***	**China**	**KP145225**	**KP145161**	**KP145289**	**KP145193**	**KP145129**	**KP145257**
	CAUA21	***Capsicum annuum***	**China**	**KP145227**	**KP145163**	**KP145291**	**KP145195**	**KP145131**	**KP145259**
	CAUA22	***Capsicum annuum***	**China**	**KP145228**	**KP145164**	**KP145292**	**KP145196**	**KP145132**	**KP145260**
	CAUA23	***Capsicum annuum***	**China**	**KP145229**	**KP145165**	**KP145293**	**KP145197**	**KP145133**	**KP145261**
	CAUA33	***Capsicum annuum***	**China**	**KP145230**	**KP145166**	**KP145294**	**KP145198**	**KP145134**	**KP145262**
	CAUA34	***Capsicum annuum***	**China**	**KP145231**	**KP145167**	**KP145295**	**KP145199**	**KP145135**	**KP145263**
	CAUA35	***Capsicum annuum***	**China**	**KP145232**	**KP145168**	**KP145296**	**KP145200**	**KP145136**	**KP145264**
	CAUA36	***Capsicum* sp.**	**China**	**KP145233**	**KP145169**	**KP145297**	**KP145201**	**KP145137**	**KP145265**
	CAUA42	***Capsicum* sp.**	**China**	**KP145234**	**KP145170**	**KP145298**	**KP145202**	**KP145138**	**KP145266**
	CAUA43	***Capsicum* sp.**	**China**	**KP145235**	**KP145171**	**KP145299**	**KP145203**	**KP145139**	**KP145267**
	CAUA44	***Capsicum* sp.**	**China**	**KP145236**	**KP145172**	**KP145300**	**KP145204**	**KP145140**	**KP145268**
	CAUA45	***Capsicum* sp.**	**China**	**KP145237**	**KP145173**	**KP145301**	**KP145205**	**KP145141**	**KP145269**
	CAUA46	***Capsicum* sp.**	**China**	**KP145238**	**KP145174**	**KP145302**	**KP145206**	**KP145142**	**KP145270**
	CAUA47	***Capsicum* sp.**	**China**	**KP145239**	**KP145175**	**KP145303**	**KP145207**	**KP145143**	**KP145271**
	CBS 120708	*Capsicum* sp.	Thailand	JQ948269	JQ948599	JQ948930	JQ949260	JQ949590	JQ949920
	CBS 126529*	*Capsicum* sp.	Indonesia	JQ948267	JQ948597	JQ948928	JQ949258	JQ949588	JQ949918
	CBS 126530	*Capsicum* sp.	Indonesia	JQ948268	JQ948598	JQ948929	JQ949259	JQ949589	JQ949919
C. simmondsii	CBS 122122*	*Carica* sp.	Australia	JQ948276	JQ948606	JQ948937	JQ949267	JQ949597	JQ949927

* = Ex-type culture. Strains studied in this paper are in **bold** font.

clustered with *C. truncatum*, and one clustered with *C. incanum* (Fig. 4). The remaining isolates were assigned to *C. cliviae* and *C. karstii*. A new lineage belonging to the fourth group, distinct from all known species, is herein described as a new species, *C. liaoningense* (Fig. 6).

Pathogenicity

All tested isolates except that of *C. endophytica* were pathogenic to most of the detached ripe red chili fruits (Table 3). All *Capsicum annuum* and *Ca. frutescens* cultivars were susceptible to all tested *Colletotrichum* species (except *C. endophytica*), with disease scores from 5 to 9. *Capsicum chinense* was susceptible to most of the *Colletotrichum* species, except *C. cliviae* CAUOS5, *C. endophytica* CAUG28, and *C. hymenocallidis* CAUG9. *Capsicum annuum* and *Ca. frutescens* were the most susceptible, with average scores of 7. Three pathotypes (PC1-R, PC2-R, and PC3-R) were identified (Table 3) based on three differential reactions of tested strains with *Capsicum chinense* cv. Huangdijiao, *Ca. annuum* and *Ca. frutescens*. Host reactions of the mature green fruit were similar to those of the ripe fruit (Table 4). Similar to the ripe fruit, all three cultivars of the mature green fruit of *Capsicum annuum*, Zidantou, Denglongjiao, and Sanyingjiao were susceptible to all tested *Colletotrichum* species, except *C. endophytica*, with average scores from 5 to 6. Most of the *Capsicum frutescens* cultivars and *Ca. chinense* cv. Huangdijiao at the green fruit stage were susceptible to most isolates, except *C. aenigma* CAUG26, *C. conoides* CAUG17, *C. gloeosporioides* CAUG2, *C. grossum* CAUG7, *C. fructicola* CAUG1 and *C. karstii* CAUOS1. Five pathotypes were identified based on the differential reactions with Xiaomila, Shuangla, and Huangdijiao (Table 4). All of the pathogenic isolates formed sunken, brown to dark lesions on the fruits. No symptoms developed on the negative controls.

Prevalence of Colletotrichum species

To determine the prevalence of the *Colletotrichum* species associated with chili in China, the sample locations and the number of isolates were assessed for each species. Isolates with highly similar morphology and ITS sequences to those of the ex-type of *C. truncatum* appear to be most common (N = 422), representing 33 % of all isolates, and presenting in 56 % of all sampling locations (Fig. 5). All 34 isolates chosen from this group for multi-locus phylogenetic analysis were confirmed to be *C. truncatum* (Fig. 4). It therefore appears that *C. truncatum* is the most prevalent species of *Colletotrichum* on chili in China. The next most prevalent species included *C. scovillei*, *C. gloeosporioides*, *C. fioriniae*, and *C. fructicola*, which accounted for 21, 14, 14, and 13 % of all the isolates, respectively. The remaining species were detected in less than 3 % of the sampling locations.

TAXONOMY

Based on the morphology and the multi-locus phylogeny, the 121 isolates were assigned to 15 species. Seven species (*C. aenigma*, *C. cliviae*, *C. endophytica*, *C. hymenocallidis*, *C. incanum*, *C. karstii*, and *C. viniferum*) were reported from chili for the first time. Three other species (*C. fioriniae*, *C. fructicola*, and *C. scovillei*) were reported for the first time in China, and a further three species newly described.

Colletotrichum conoides Y.Z. Diao, C. Zhang, L. Cai & X.L. Liu, *sp. nov*. — MycoBank MB812003; Fig. 7

Etymology. Referring to the host variety (*Capsicum annuum* var. *conoides*) from which the fungus was first collected.

Colonies on PDA attaining 53–55 mm diam in 4 d at 28 °C; aerial mycelia greyish white; reverse light grey to medium grey

Table 2c Strains used for the phylogenetic analysis of *Colletotrichum* species with curved conidia with details on host, location, and GenBank accession numbers.

Species	Isolate	Host	Location	GenBank accession numbers					
				ITS	*ACT*	*TUB2*	*CHS-1*	*GAPDH*	*HIS3*
C. anthrisci	CBS 125335	*Anthriscus sylvestris*	Netherlands	GU227846	GU227944	GU228140	GU228336	GU228238	GU228042
C. chlorophyti	IMI 103806*	*Chlorophytum* sp.	India	GU227894	GU227992	GU228188	GU228384	GU228286	GU228090
	CBS 142.79	*Stylosanthes hamata*	Australia	GU227895	GU227993	GU228189	GU228385	GU228287	GU228091
C. circinans	CBS 111.21	*Allium cepa*	USA	GU227854	GU227952	GU228148	GU228344	GU228246	GU228050
	CBS 221.81*	*Allium cepa*	Serbia	GU227855	GU227953	GU228149	GU228345	GU228247	GU228051
C. dematium	CBS 125.25*	*Eryngium campestre*	France	GU227819	GU227917	GU228113	GU228309	GU228211	GU228015
	CBS 125340	*Apiaceae*	Czech Republic	GU227820	GU227918	GU228114	GU228310	GU228212	GU228016
C. fructi	CBS 346.37*	*Malus sylvestris*	USA	GU227844	GU227942	GU228138	GU228334	GU228236	GU228040
C. incanum	ATCC 64682*	*Glycine max*	USA	KC110789	KC110825	KC110816		KC110807	KC110798
	IL6A	*Glycine max*	USA	KC110787	KC110823	KC110814		KC110805	KC110796
	IL9A	*Glycine max*	USA	KC110788	KC110824	KC110815		KC110806	KC110797
	CAUCT34	***Capsicum* sp.**	**China**	**KP145641**	**KP145505**	**KP145675**	**KP145539**	**KP145573**	**KP145607**
C. lilii	CBS 109214	*Lilium* sp.	Japan	GU227810	GU227908	GU228104	GU228300	GU228202	GU228006
C. lindemuthianum	CBS 151.28	*Lilium* sp.	Netherlands	GU227800	GU227898	GU228094	GU228290	GU228192	GU227996
C. lineola	CBS 125337*	*Apiaceae* sp.	Czech Republic	GU227829	GU227927	GU228123	GU228319	GU228221	GU228025
	CBS 125339	*Apiaceae* sp.	Czech Republic	GU227830	GU227928	GU228124	GU228320	GU228222	GU228026
C. liriopes	CBS 119444*	*Lirope muscari*	Mexico	GU227804	GU227902	GU228098	GU228294	GU228196	GU228000
	CBS 122747	*Lirope muscari*	Mexico	GU227805	GU227903	GU228099	GU228295	GU228197	GU228001
C. phaseolorum 1	CBS 157.36	*Phaseolus radiatus var. aureus*	Japan	GU227896	GU227994	GU228190	GU228386	GU228288	GU228092
C. phaseolorum 2	CBS 158.36	*Vigna sinensis*	Japan	GU227897	GU227995	GU228191	GU228387	GU228289	GU228093
C. rusci	CBS 119206*	*Ruscus* sp.	Italy	GU227818	GU227916	GU228112	GU228308	GU228210	GU228014
C. spaethianum	CBS 167.49*	*Hosta sieboldiana*	Germany	GU227807	GU227905	GU228101	GU228297	GU228199	GU228003
	CBS 100063	*Lilium* sp.	South Korea	GU227808	GU227906	GU228102	GU228298	GU228200	GU228004
	CBS 101631	*Hemerocallis* sp.	New Zealand	GU227809	GU227907	GU228103	GU228299	GU228201	GU228005
C. spinaciae	CBS 128.57	*Spinacia oleracea*	Netherlands	GU227847	GU227945	GU228141	GU228337	GU228239	GU228043
	IMI 104607	*Spinacia* sp.	Italy	GU227850	GU227948	GU228144	GU228340	GU228242	GU228046
C. tofieldiae	CBS 168.49	*Lupinus polyphyllus*	Germany	GU227802	GU227900	GU228096	GU228292	GU228194	GU227998
	CBS 495.85	*Tofieldia calyculata*	Switzerland	GU227801	GU227899	GU228095	GU228291	GU228193	GU227997
C. trichellum	CBS 118198	*Hedera* sp.	Guatemala	GU227813	GU227911	GU228107	GU228303	GU228205	GU228009
	CBS 217.64	*Hedera helix*	UK	GU227812	GU227910	GU228106	GU228302	GU228204	GU228008
C. truncatum	CBS 182.52	*Glycine max*	USA	GU227866	GU227964	GU228160	GU228356	GU228258	GU228062
	CBS195.32	*Glycine max*	USA	GU227865	GU227963	GU228159	GU228355	GU228257	GU228061
	CBS 345.70	*Glycine max*	Denmark	GU227867	GU227965	GU228161	GU228357	GU228259	GU228063
	CBS 151.35*	*Phaseolus lunatus*	USA	GU227862	GU227960	GU228156	GU228352	GU228254	GU228058
	CAUCT1	***Capsicum annuum***	**China**	**KP145608**	**KP145472**	**KP145642**	**KP145506**	**KP145540**	**KP145574**
	CAUCT2	***Capsicum annuum***	**China**	**KP145609**	**KP145473**	**KP145643**	**KP145507**	**KP145541**	**KP145575**
	CAUCT3	***Capsicum annuum***	**China**	**KP145610**	**KP145474**	**KP145644**	**KP145508**	**KP145542**	**KP145576**
	CAUCT4	***Capsicum annuum***	**China**	**KP145611**	**KP145475**	**KP145645**	**KP145509**	**KP145543**	**KP145577**
	CAUCT5	***Capsicum annuum***	**China**	**KP145612**	**KP145476**	**KP145646**	**KP145510**	**KP145544**	**KP145578**
	CAUCT6	***Capsicum annuum***	**China**	**KP145613**	**KP145477**	**KP145647**	**KP145511**	**KP145545**	**KP145579**
	CAUCT7	***Capsicum* sp.**	**China**	**KP145614**	**KP145478**	**KP145648**	**KP145512**	**KP145546**	**KP145580**
	CAUCT8	***Capsicum* sp.**	**China**	**KP145615**	**KP145479**	**KP145649**	**KP145513**	**KP145547**	**KP145581**
	CAUCT9	***Capsicum* sp.**	**China**	**KP145616**	**KP145480**	**KP145650**	**KP145514**	**KP145548**	**KP145582**
	CAUCT10	***Capsicum* sp.**	**China**	**KP145617**	**KP145481**	**KP145651**	**KP145515**	**KP145549**	**KP145583**
	CAUCT11	***Capsicum* sp.**	**China**	**KP145618**	**KP145482**	**KP145652**	**KP145516**	**KP145550**	**KP145584**

C. truncatum (cont.)	**CAUCT12**	***Capsicum* sp.**	**China**	**KP145619**	**KP145483**	**KP145653**	**KP145517**	**KP145551**	**KP145585**
	CAUCT13	***Capsicum* sp.**	**China**	**KP145620**	**KP145484**	**KP145654**	**KP145518**	**KP145552**	**KP145586**
	CAUCT14	***Capsicum* sp.**	**China**	**KP145621**	**KP145485**	**KP145655**	**KP145519**	**KP145553**	**KP145587**
	CAUCT15	***Capsicum* sp.**	**China**	**KP145622**	**KP145486**	**KP145656**	**KP145520**	**KP145554**	**KP145588**
	CAUCT16	***Capsicum* sp.**	**China**	**KP145623**	**KP145487**	**KP145657**	**KP145521**	**KP145555**	**KP145589**
	CAUCT17	***Capsicum* sp.**	**China**	**KP145624**	**KP145488**	**KP145658**	**KP145522**	**KP145556**	**KP145590**
	CAUCT18	***Capsicum* sp.**	**China**	**KP145625**	**KP145489**	**KP145659**	**KP145523**	**KP145557**	**KP145591**
	CAUCT19	***Capsicum* sp.**	**China**	**KP145626**	**KP145490**	**KP145660**	**KP145524**	**KP145558**	**KP145592**
	CAUCT20	***Capsicum* sp.**	**China**	**KP145627**	**KP145491**	**KP145661**	**KP145525**	**KP145559**	**KP145593**
	CAUCT21	***Capsicum* sp.**	**China**	**KP145628**	**KP145492**	**KP145662**	**KP145526**	**KP145560**	**KP145594**
	CAUCT22	***Capsicum* sp.**	**China**	**KP145629**	**KP145493**	**KP145663**	**KP145527**	**KP145561**	**KP145595**
	CAUCT23	***Capsicum* sp.**	**China**	**KP145630**	**KP145494**	**KP145664**	**KP145528**	**KP145562**	**KP145596**
	CAUCT24	***Capsicum* sp.**	**China**	**KP145631**	**KP145495**	**KP145665**	**KP145529**	**KP145563**	**KP145597**
	CAUCT25	***Capsicum* sp.**	**China**	**KP145632**	**KP145496**	**KP145666**	**KP145530**	**KP145564**	**KP145598**
	CAUCT26	***Capsicum* sp.**	**China**	**KP145633**	**KP145497**	**KP145667**	**KP145531**	**KP145565**	**KP145599**
	CAUCT27	***Capsicum* sp.**	**China**	**KP145634**	**KP145498**	**KP145668**	**KP145532**	**KP145566**	**KP145600**
	CAUCT28	***Capsicum* sp.**	**China**	**KP145635**	**KP145499**	**KP145669**	**KP145533**	**KP145567**	**KP145601**
	CAUCT29	***Capsicum* sp.**	**China**	**KP145636**	**KP145500**	**KP145670**	**KP145534**	**KP145568**	**KP145602**
	CAUCT30	***Capsicum* sp.**	**China**	**KP145637**	**KP145501**	**KP145671**	**KP145535**	**KP145569**	**KP145603**
	CAUCT31	***Capsicum* sp.**	**China**	**KP145638**	**KP145502**	**KP145672**	**KP145536**	**KP145570**	**KP145604**
	CAUCT32	***Capsicum* sp.**	**China**	**KP145639**	**KP145503**	**KP145673**	**KP145537**	**KP145571**	**KP145605**
	CAUCT33	***Capsicum* sp.**	**China**	**KP145640**	**KP145504**	**KP145674**	**KP145538**	**KP145572**	**KP145606**
	CAUCT33	***Capsicum* sp.**	**China**	**KP145640**	**KP145504**	**KP145674**	**KP145538**	**KP145572**	**KP145606**

* = Ex-type culture. Strains studied in this paper are in bold

with white margin. *Chlamydospores* not observed. *Vegetative hyphae* hyaline, smooth-walled, septate, branched. *Conidiomata* and *setae* not observed. *Conidiophores* formed directly on aerial mycelium, hyaline, aseptate. *Conidiogenous cells* hyaline, cylindrical to clavate, 22–30 × 3.5–5 µm, opening 2.5–3 µm. *Conidia* hyaline, aseptate, smooth-walled, cylindrical to clavate, both ends obtusely rounded, contents granular and mostly equally distributed, 13–17.5 × 5–6.5 µm (av. = 15.9 × 5.9 µm), L/W ratio = 2.7. *Appressoria* single or in small groups, medium to dark brown, aseptate, mostly ellipsoidal to irregular in outline, and crenate or deeply lobed at margin, 4–11.5 × 6–10.5 µm (av. = 8.35 × 7.1 µm), L/W ratio = 1.2. Sexual morph not observed after 8 wk.

Specimen examined. China, Jiangsu Province, Nanjing City, on fruits of *Capsicum annuum* var. *conoides*, Sept. 2010, *Y.Z. Diao* (holotype HMAS 246481, ex-type living culture CGMCC 3.17615 = CAUG17 = LC6226); ibid., NJ26, living culture CAUG33; ibid., NJ27, living culture CAUG34.

Notes — *Colletotrichum conoides* is phylogenetically most closely related to *C. hebeiense* (Fig. 2). Sequence data from ITS and *CHS-1* could not separate the two species, but they can be distinguished by *GAPDH* (12 bp), *ACT* (4 bp), or *TUB* (3 bp). The two species also differ in the following characteristics: the granules are uniformly distributed in the conidia of *C. conoides* but mostly present at the polar ends in the conidia of *C. hebeiense*; most appressoria of *C. conoides* are ovoid ellipsoidal with crenate or deeply lobed margin, while those of *C. hebeiense* are clavate to subglobose; conidia of *C. conoides* are slightly larger than those of *C. hebeiense* (13–17.5 × 5–6.5 µm vs 11.6–15.3 × 4.47–6.88 µm). In addition, *C. conoides* was described from *Capsicum annuum* var. *conoides*, while *C. hebeiense* was described from *Vitis vinifera* (Yan et al. 2015). A PHI test revealed no significant recombination event between *C. conoides* and *C. hebeiense* (Fig. 8).

Colletotrichum grossum Y.Z. Diao, C. Zhang, L. Cai & X.L. Liu, *sp. nov*. — MycoBank MB812006; Fig. 9

Etymology. Referring to the host variety (*Capsicum annuum* var. *grossum*) from which the fungus was first collected.

Colonies on PDA attaining 49–52 mm diam in 4 d at 28 °C; aerial mycelia white, reverse light grey with white margin. *Chlamydospores* not observed. *Vegetative hyphae* hyaline, smooth-walled, septate, branched. *Conidiomata* and *setae* not observed. *Conidiophores* formed directly on aerial mycelium, hyaline, aseptate. *Conidiogenous cells* hyaline, cylindrical to clavate, 22–32 × 3–3.5 µm, opening 2–2.5 µm. *Conidia* hyaline, aseptate, smooth-walled, cylindrical to clavate, both ends rounded or one end acute, contents granular and mostly present at the polar ends, 14.5–20.5 × 5–7.5 µm (av = 16.8 × 6.3 µm), L/W ratio = 2.7. *Appressoria* single, medium brown, aseptate, mostly ovoid or ellipsoidal to irregular in outline, and crenate in margin. 5.5–11.5 × 4–10.5 µm (av = 8.65 × 6.1 µm), L/W ratio = 1.4. Sexual morph not observed after 8 wk.

Specimen examined. China, Hainan Province, Haikou city, on chili fruits (*Capsicum annuum* var. *grossum*), Oct. 2010, *Y.Z Diao* (holotype HMAS 246480, ex-type living culture CGMCC3.17614 = CAUG7 = LC6227); ibid., HN2, living culture CAUG31; ibid., HN3, living culture CAUG32.

Notes — *Colletotrichum grossum* is phylogenetically most closely related to *C. theobromicola* (Fig. 2). The sequence data of ITS and *CAL* do not separate the two species, but they can be distinguished by *GAPDH* (3 bp), *ACT* (5 bp), and *TUB* (8 bp). In morphology, *C. grossum* differs from *C. theobromicola* by having wider conidia (14.5–20.5 × 5–7.5 µm vs 14.5–18.7 × 4.5–5.5 µm) and colonies that are flat white rather than black as in *C. theobromicola* (Rojas et al. 2010). A PHI test revealed no significant recombination event between *C. grossum* and *C. theobromicola* (Fig. 8).

Fig. 2 Maximum parsimony tree of isolates in the *Colletotrichum gloeosporioides* species complex obtained from a heuristic search of combined *ACT*, *CAL*, *CHS-1*, *GAPDH*, ITS, and *TUB2* gene sequences. *Colletotrichum boninense* was used as the outgroup. Bootstrap support values ≥ 50 %, Bayesian posterior probability values ≥ 0.95 and RAxML bootstrap support values (ML ≥ 50 %) are shown at the nodes. Tree length = 1665, CI = 0.672, RI = 0.889, RC = 0.597, HI = 0.328. Ex-type strains are emphasised in **bold**.

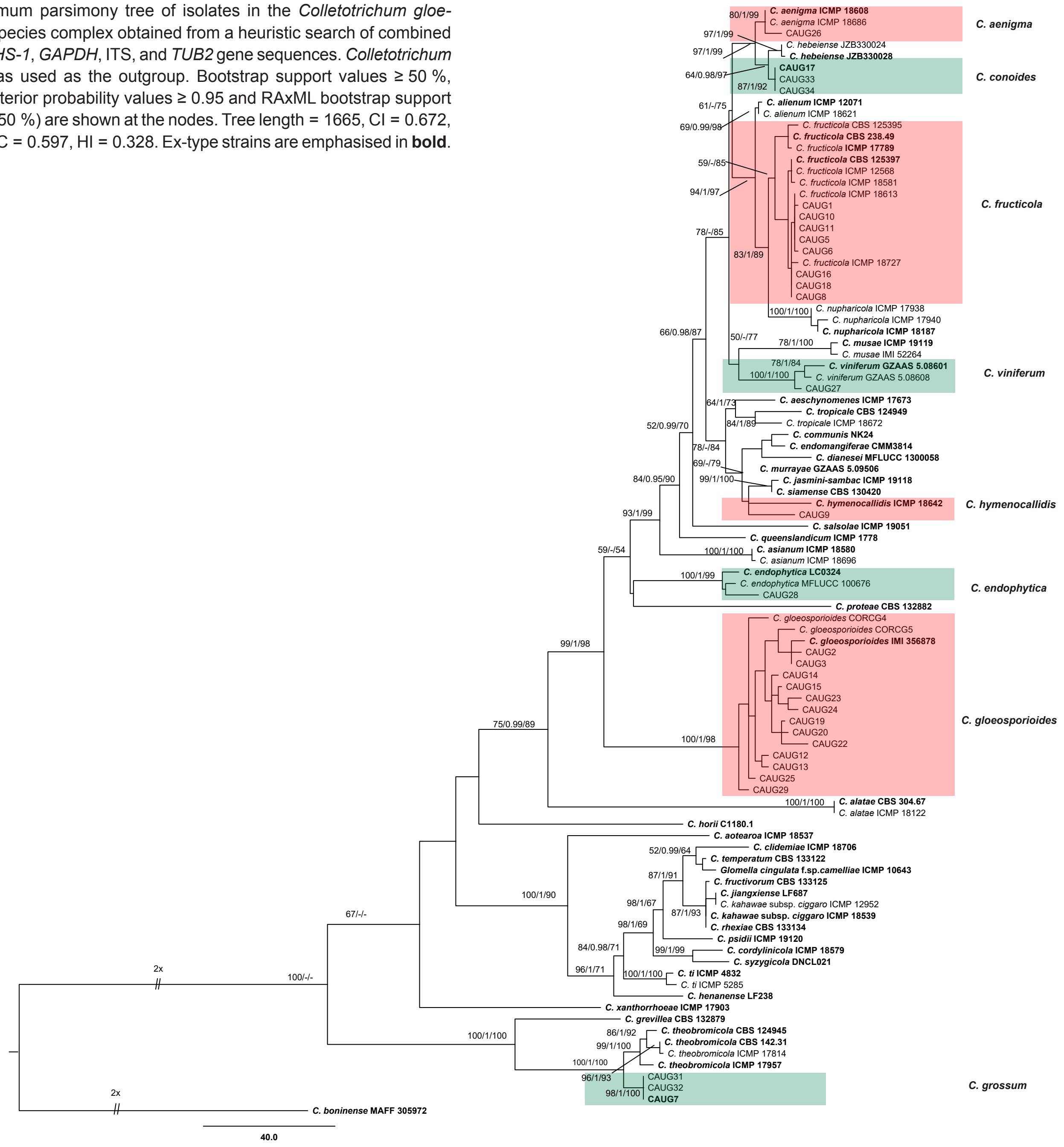


Table 3 Anthracnose severity scores on a 0–9 scale and pathotypes of 15 *Colletotrichum* species isolates at the ripe red fruit stage of seven chili cultivars.

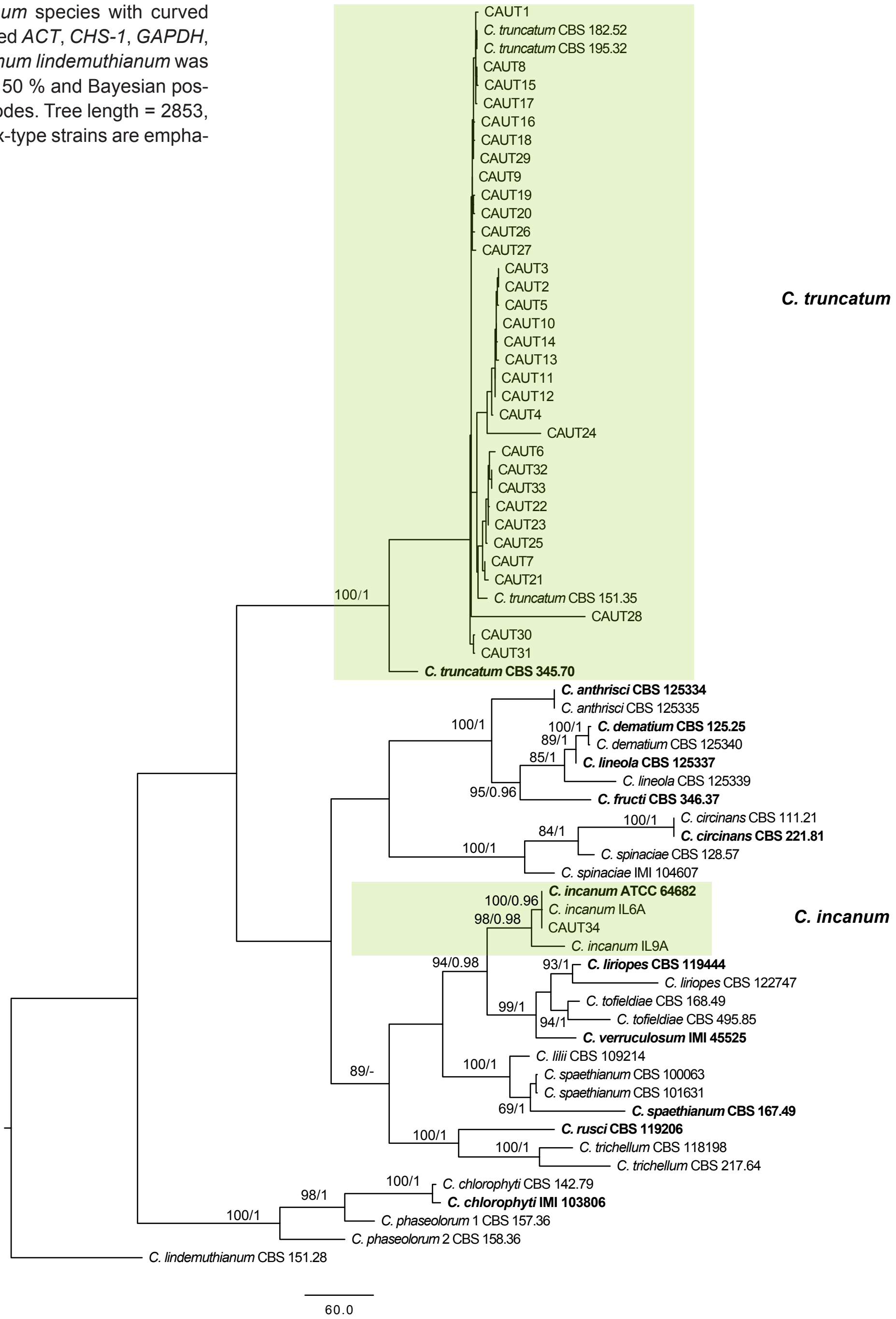
Isolate	*Capsicum annuum*				*C. frutescens*		*C. chinense*	Mean	Pathotype
	Chaotianjiao	Denglongjiao	Sanyingjiao	Zidantou	Shuanla	Xiaomila	Huangdijiao		
C. aenigma CAUG26	5	5	7	5	5	7	5	6	PC1-R
C. cliviae CAUOS5	7	7	5	7	7	5	0	5	PC2-R
C. conoides CAUG17	7	7	7	7	7	5	5	6	PC1-R
C. endophytica CAUG28	0	0	0	0	0	0	0	0	PC3-R
C. fioriniae CAUT34	7	7	9	9	9	9	7	8	PC1-R
C. fructicola CAUG1	7	7	9	9	9	9	7	8	PC1-R
C. gloeosporioides CAUG2	7	7	9	9	7	7	5	7	PC1-R
C. grossum CAUG7	7	7	7	7	5	5	7	6	PC1-R
C. hymenocallidis CAUG9	5	7	9	9	7	7	0	6	PC2-R
C. incanum CAUT34	5	7	7	5	7	5	5	6	PC1-R
C. karstii CAUOS1	7	5	7	7	9	9	7	7	PC1-R
C. liaoningense CAUOS2	9	7	9	9	5	9	5	8	PC1-R
C. scovillei CAUA1	7	9	9	9	9	7	9	8	PC1-R
C. truncatum CAUT1	7	7	9	7	9	9	7	8	PC1-R
C. viniferum CAUG27	5	7	9	9	9	9	5	8	PC1-R
Mean	6	6	7	7	7	7	5	6	–

Fig. 3 Maximum parsimony tree of isolates in the *Colletotrichum acutatum* species complex obtained from a heuristic search of combined *ACT*, *CHS-1*, *GAPDH*, *HIS3*, ITS, and *TUB2* gene sequences. *Colletotrichum gloeosporioides* was used as the outgroup. Bootstrap support values ≥ 50 % and Bayesian posterior probability values ≥ 0.95 are shown at the nodes. Tree length = 943, CI = 0.757, RI = 0.912, RC = 0.691, HI = 0.243. Ex-type strains are emphasised in **bold**.

Table 4 Anthracnose severity scores on a 0–9 scale and pathotypes of 15 *Colletotrichum* species isolates at the mature green fruit stage of seven chili cultivars.

Isolate	*Capsicum annuum*				*C. frutescens*		*C. chinense*	Mean	Pathotype
	Chaotianjiao	Denglongjiao	Sanyingjiao	Zidantou	Shuanla	Xiaomila	Huangdijiao		
C. aenigma CAUG26	5	5	5	5	0	0	0	3	PC1-G
C. cliviae CAUOS5	7	9	5	7	7	5	7	7	PC2-G
C. conoides CAUG17	7	5	0	5	7	5	0	4	PC3-G
C. endophytica CAUG28	0	0	0	0	0	0	0	0	PC4-G
C. fioriniae CAUT34	9	9	7	7	9	9	7	8	PC2-G
C. fructicola CAUG1	7	9	9	5	0	7	7	6	PC5-G
C. gloeosporioides CAUG2	5	9	7	5	0	7	7	6	PC5-G
C. grossum CAUG7	3	3	0	5	5	5	0	3	PC3-G
C. hymenocallidis CAUG9	5	5	5	5	7	7	7	6	PC2-G
C. incanum CAUT34	5	5	5	3	7	5	5	5	PC2-G
C. karstii CAUOS1	5	7	7	7	0	0	0	4	PC1-G
C. liaoningense CAUOS2	5	5	9	7	5	3	7	6	PC2-G
C. scovillei CAUA1	7	7	7	7	9	9	7	7	PC2-G
C. truncatum CAUT1	7	5	5	7	7	7	5	6	PC2-G
C. viniferum CAUG27	5	5	5	7	9	9	7	7	PC2-G
Mean	5	6	5	5	5	5	4	5	–

Fig. 4 Maximum parsimony tree of *Colletotrichum* species with curved conidia obtained from a heuristic search of combined *ACT*, *CHS-1*, *GAPDH*, *HIS3*, ITS, and *TUB2* gene sequences. *Colletotrichum lindemuthianum* was used as the outgroup. Bootstrap support values ≥ 50 % and Bayesian posterior probability values ≥ 0.95 are shown at the nodes. Tree length = 2853, CI = 0.467, RI = 0.859, RC = 0.401, HI = 0.533. Ex-type strains are emphasised in **bold**.



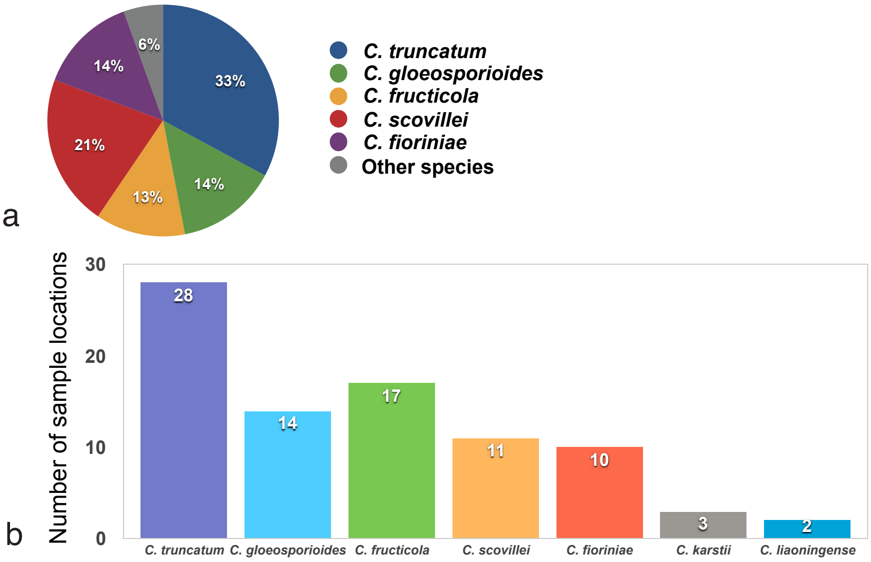


Fig. 5 Prevalence of *Colletotrichum* species on chili in China based on preliminary identifications. a. The percentage of isolates represented by the indicated *Colletotrichum* species on chili; b. number of sampling locations where the seven most prevalent species were isolated.

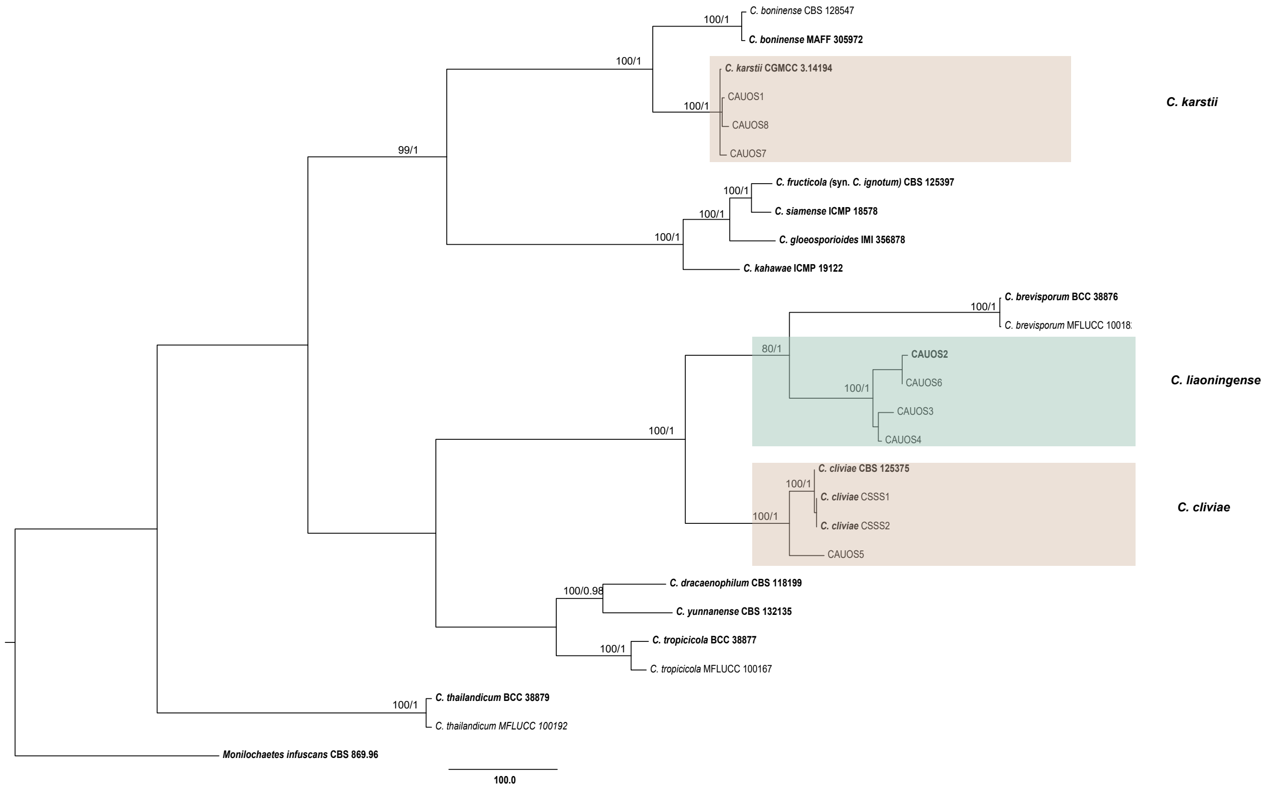


Fig. 6 Maximum parsimony tree of isolates of *Colletotrichum* species in the fourth group obtained from a heuristic search of combined *ACT*, *CAL*, *CHS-1*, *GAPDH*, ITS, and *TUB2* gene sequences. *Monilochaetes infuscans* was used as the outgroup. Bootstrap support values ≥ 50 % and Bayesian posterior probability values ≥ 0.95 are shown at the nodes. Tree length = 2913, CI = 0.717, RI = 0.870, RC = 0.624, HI = 0.283. Ex-type strains are emphasised in **bold**.

Fig. 7 *Colletotrichum conoides* (CAUG17). a–b. Colonies on PDA above and below; c. conidiophores; d–e, g–h: appressoria; f. conidia. — Scale bars: c–h = 10 µm.

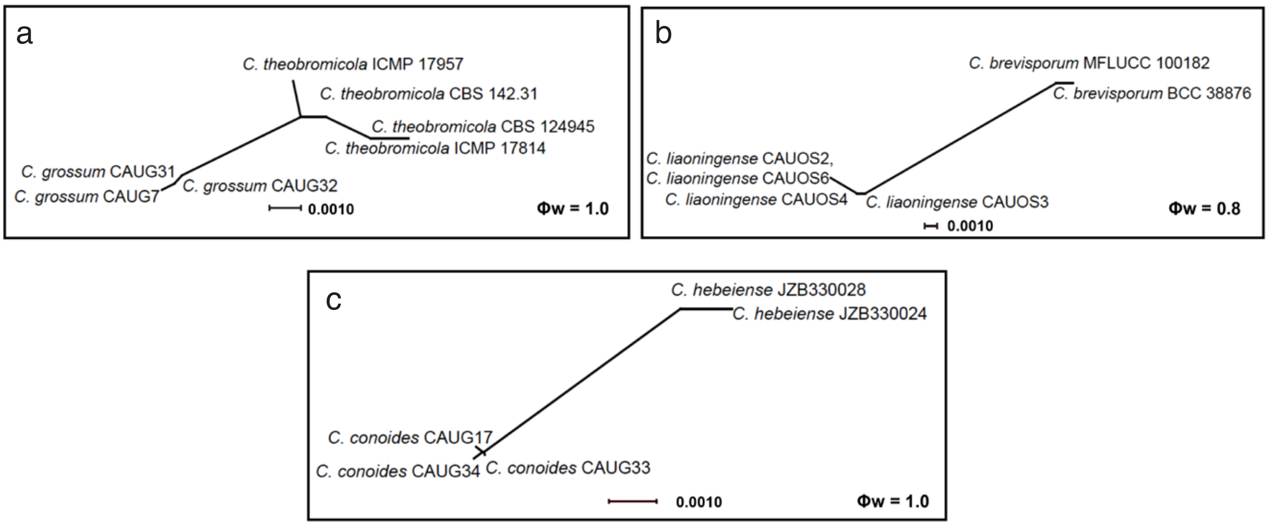


Fig. 8 The results of the pairwise homoplasy index (PHI) test of closely related species using both LogDet transformation and splits decomposition. PHI test results (Φw) < 0.05 indicate significant recombination within the dataset.

Colletotrichum liaoningense Y.Z. Diao, C. Zhang, L. Cai & X.L. Liu, *sp. nov*. — MycoBank MB812007; Fig. 10

Etymology. Referring to the province in China where the fungus was first collected.

Colonies on PDA attaining 48–51 mm diam in 4 d at 28 °C; aerial mycelia light grey, reverse medium to dark brown with white margin. *Chlamydospores* not observed. *Vegetative hyphae* hyaline, smooth-walled, septate, branched. *Conidiomata* acervular. *Setae* medium grey, smooth-walled to verruculose, 3–6-septate, 46–68 μm long, base cylindrical, conical, or slightly inflated, 4.5–6.5 μm diam at the widest part, tip rounded. *Conidiophores* formed directly on aerial mycelium, hyaline, aseptate. *Conidiogenous cells* hyaline, cylindrical to clavate, 27–30 × 3.5–4.5 μm, opening 2–4 μm. *Conidia* cylindrical to clavate, both ends rounded or one end acute, contents granular and mostly present at the polar ends, hyaline, aseptate, smooth-walled, 14–18.5 μm × 5–7.5 μm (av. = 16.3 × 6.1 μm), L/W ratio = 2.7. *Appressoria* single, medium to dark brown, aseptate, mostly ellipsoidal to irregular in outline, and crenate at margin, 3.5–5 × 2.5–4.5 μm (av. = 4.1 × 2.9 μm), L/W ratio = 1.4. Sexual morph not observed after 8 wk.

Specimen examined. CHINA, Xingcheng city, Liaoning Province on chili fruits (*Capsicum annuum* var. *conoides*), Oct. 2012, *Y.Z. Diao* (holotype HMAS 246479, ex-type living culture CGMCC3.17616 = CAUOS2 = LC6228); ibid., LN3, living culture CAUOS3; ibid., LN4, living culture CAUOS4; ibid., LN6, living culture CAUOS6.

Notes — *Colletotrichum liaoningense* is phylogenetically most closely related to *C. brevisporum* (Fig. 6). The sequence data from ITS and *ACT* could not separate the two species; however, they can be distinguished from each other via *GAPDH* (10 bp) or *TUB* (12 bp). The granules are equally distributed in the conidia of *C. liaoningense* but mostly present at the polar ends in conidia of *C. brevisporum*. The appressoria of *C. liaoningense* are smaller than those of *C. brevisporum* (3.5–5 × 2.5–4.5 μm vs 10–13 × 8–11 μm) (Noireung et al. 2012). A PHI test revealed no significant recombination event between *C. liaoningense* and *C. brevisporum* (Fig. 8).

DISCUSSION

Colletotrichum truncatum, the most frequently isolated species in this study, has been reported from more than 460 plant species (Farr & Rossman 2016). This taxon has also been shown to cause serious damage to chili production in Australia, China, India, Thailand, and other countries (Poonpolgul & Kumphai 2007, Than et al. 2008, Sharma et al. 2014, Diao et al. 2015). In China, *C. truncatum* has been reported from tomato, dragon fruit, pumpkin, and other crops (Chai et al. 2014, Cheng et al. 2014, Diao et al. 2014, Guo et al. 2014). Geographic populations of *C. truncatum* in China exhibit significant genetic differentiation and recombination abilities, which can probably be attributed to the prevalence of this species (Diao et al. 2015).

Colletotrichum gloeosporioides has been reported to infect chili in Australia, China, India, Korea, Thailand, the USA, and other countries (Shin et al. 1999, Kim et al. 2008, Than et al. 2008). However, a recent study revealed this taxon to be a species complex comprising many morphologically similar taxa (Weir et al. 2012). Therefore, this new classification system necessitates a re-investigation of species in the *C. gloeosporioides* species complex on chili, as species in this complex exhibit biological and physiological differences. In the current study, *C. gloeosporioides* s.str. and *C. fructicola* were revealed to be most prevalent in this complex, representing 47 % and 42 % of the isolates, respectively (Fig. 2). *Colletotrichum fructicola* was originally isolated from coffee berries (Prihastuti et al. 2009), and has since been found on a wide range of host plants (Weir et al. 2012). However, this is the first report of *C. fructicola* infecting chili. In previous studies, *C. gloeosporioides* s.str. was shown to be an uncommon pathogen on chili and other fruits in the tropics (Phoulivong et al. 2010). Additionally, we failed to isolate *C. gloeosporioides* s.str. from chili in the tropical regions of China, e.g. Hainan, south of Guangdong, and Yunnan provinces (Table 1), which suggested a significant effect of climate on the distribution of these pathogens. Pathogenicity of all obtained species from chili in this study was confirmed by inoculation tests, except for that of *C. endophytica*. *Colletotrichum endophytica*, which was originally reported as an endophytic fungus in tropical grasses (Manamgoda et al. 2013), did not show pathogenicity to any chili cultivars in our test, further underlining the possible endophytic nature of this species.

Colletotrichum acutatum is a commonly reported species, and causes anthracnose on numerous plants worldwide (Damm et al. 2012a). It was originally described from *Carica papaya*, *Capsicum frutescens*, and *Delphinium ajacis* in Australia (Simmonds 1965), but has subsequently been reported to infect chili in almost all pepper-growing countries, such as Australia, China, India, Korea, New Zealand, Thailand, and the USA (Than et

Fig. 9 *Colletotrichum grossum* (CAUG7). a–b. Colonies on PDA above and below; c–e: conidiophores; f. conidia; g–k: appressoria. — Scale bars: c–f, j–k = 10 µm (j applied to g–j).

al. 2008). Like *C. gloeosporioides*, *C. acutatum* has also been shown to represent a species complex (Damm et al. 2012a). Interestingly, *C. acutatum* s.str. was not found on chili in China (Fig. 3). Only *C. scovillei* and *C. fioriniae* were identified from this complex (Fig. 3).

No *Colletotrichum* species were detected on chili in Tibet and Xinjiang, despite the fact that several field trips have been made to these provinces, and attempts have been made for to isolate these fungi. The failure to detect *Colletotrichum* species from these regions might be explained by the high latitude, small growing area, dry climate, and high day/night variation in temperature. *Colletotrichum fructicola* and *C. truncatum* were isolated from leaves in the Jiangxi province, and were also found from fruits in other sampling regions. In previous studies, these two species were primarily isolated from fruits from various plants (Poonpolgul & Kumphai 2007, Than et al. 2008, Alaniz et al. 2015, Diao et al. 2015).

In summary, the current study represents the hitherto most intensive investigation of *Colletotrichum* species on chili in China, which revealed 15 species, with the dominant species

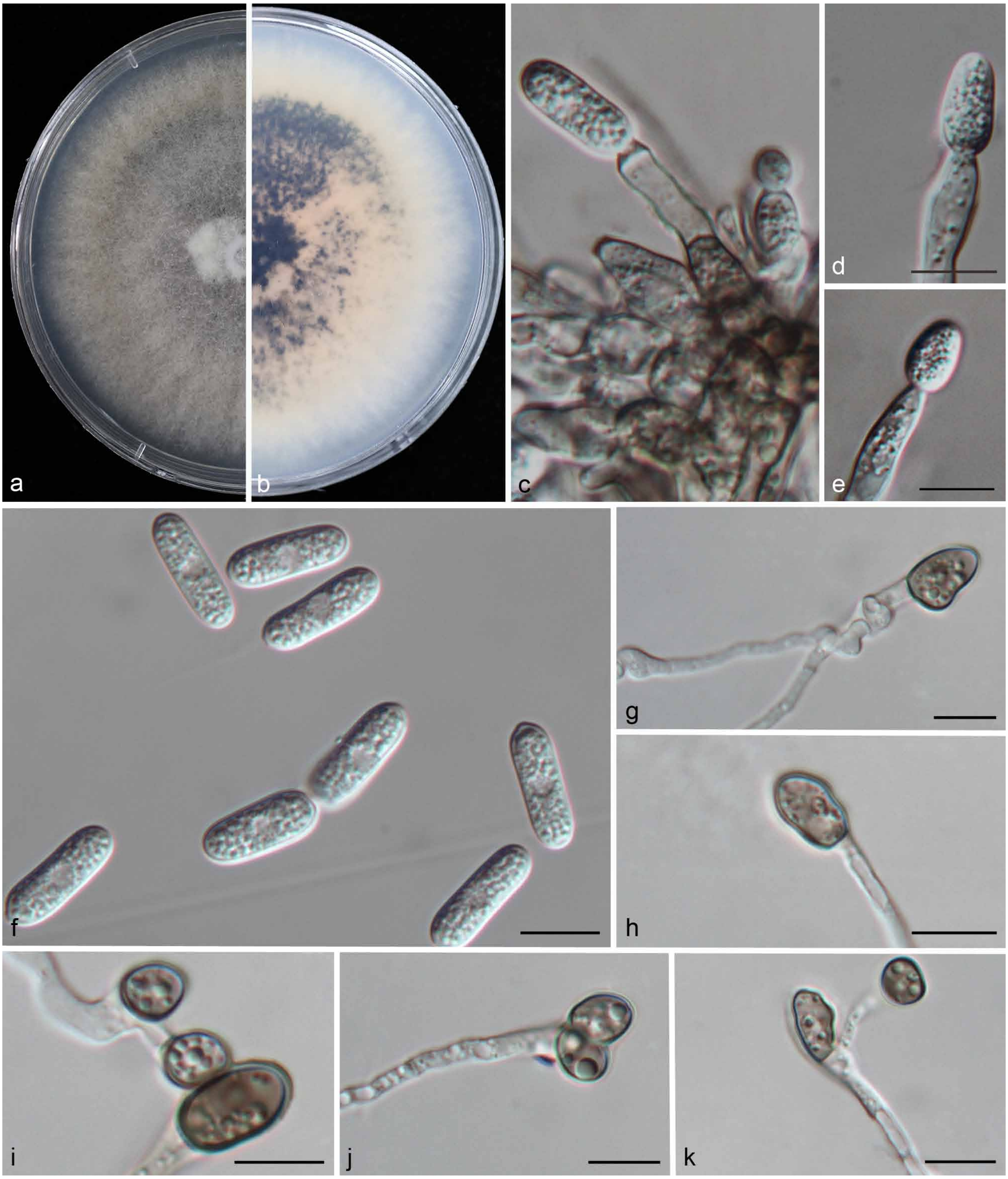


Fig. 10 *Colletotrichum liaoningense* (CAUOS2). a–b. Colonies on PDA above and below; c–e: conidiophores; f. conidia; g–k: appressoria. — Scale bars: d–k = 10 µm (d applies to c–d).

being *C. fioriniae*, *C. fructicola*, *C. gloeosporioides*, *C. scovillei*, and *C. truncatum*. The information provided here could prove useful for the control of anthracnose on chili, as well as for the screening of new chili cultivars against anthracnose.

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