



# *Colletotrichum* species causing anthracnose disease of chili in China

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## Key words

DNA phylogeny  
multi-gene analysis  
plant pathogen  
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

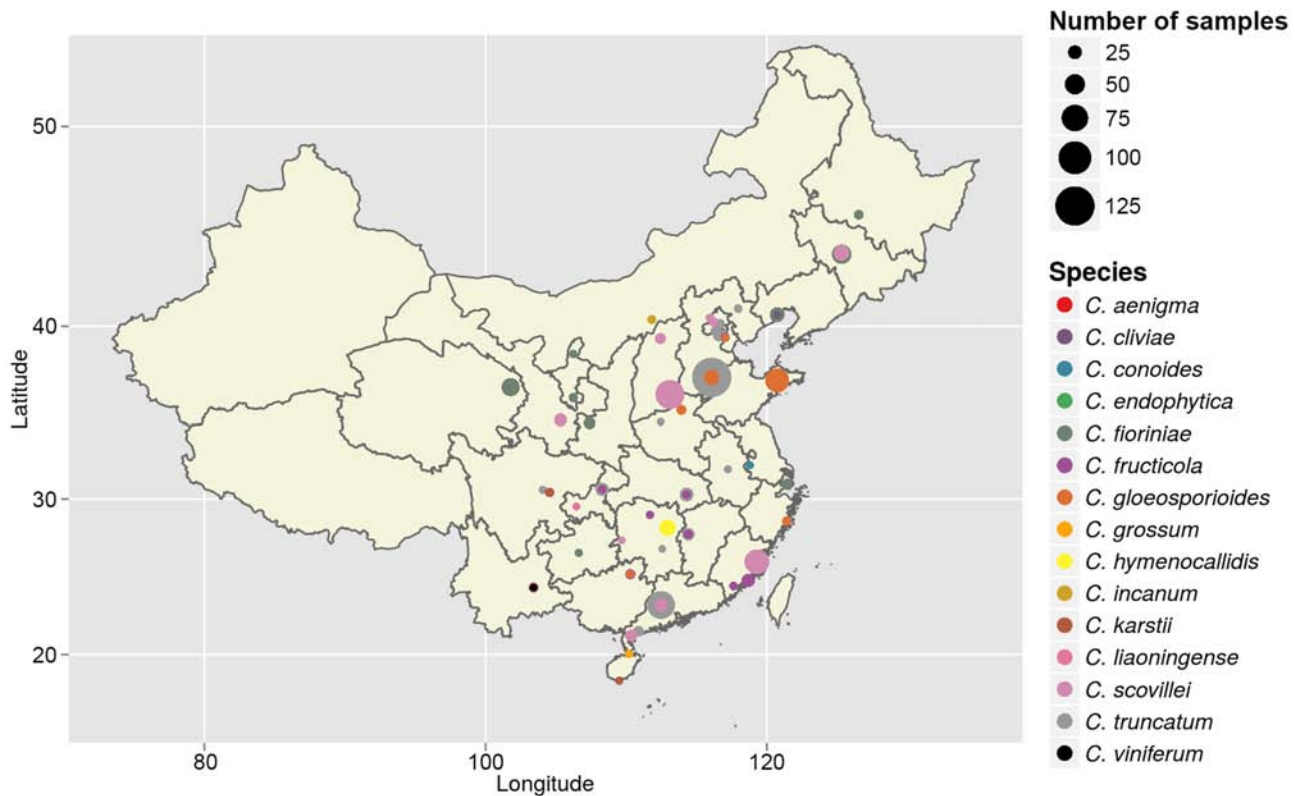
From 2008 to 2014, fruits and leaves of chili (*Capsicum* spp.) with anthracnose symptoms were collected from 50 locations in 29 provinces of China (Fig. 1). In each location, a hierarchical sampling method was used as previously described (Kohli et al. 1995). Five fields were chosen at each sampling 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 Sunbitech, 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](http://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
<i>C. aenigma</i>	Yangliuqing, Tianjin	Fruit	2012	1	39.4	117.01
<i>C. cliviae</i>	Xingcheng, Liaoning	Fruit	2012	1	40.63	120.74
<i>C. conoides</i>	Nanjing, Jiangsu	Fruit	2010	3	32.06	118.79
<i>C. endophytica</i>	Mile, Yunnan	Fruit	2011	1	24.41	103.41
<i>C. fioriniae</i>	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
<i>C. fruticola</i>	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
	Jiayang, 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	
<i>C. gloeosporioides</i>	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
	Jiayang, 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
<i>C. grossum</i>	Haikou, Hainan	Fruit	2011	3	20.04	110.19
<i>C. hymenocallidis</i>	Changsha, Hunan	Fruit	2012	35	28.23	112.94
<i>C. incanum</i>	Helingeer, Inner Mongolia	Fruit	2012	6	40.37	111.82
<i>C. karstii</i>	Sanya, Hainan	Fruit	2012	3	18.25	109.51
	Jiayang, Sichuan	Fruit	2011	5	30.41	104.55
	Mile, Yunnan	Fruit	2011	1	24.41	103.41
<i>C. liaoningense</i>	Xingcheng, Liaoning	Fruit	2012	11	40.63	120.74
	Shapingba, Chongqing	Fruit	2012	1	29.54	106.46
<i>C. scovillei</i>	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
	<i>C. truncatum</i>	Qingyuan, Guangdong	Fruit	2013	80	23.28
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
<i>C. truncatum</i> (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
	<i>C. viniferum</i>	Mile, Yunnan	Fruit	2011	1	24.41
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  $\mu$ L of a conidial suspension ( $1 \times 10^6$  conidial/mL), which was injected onto the non-wounded fruit surface using a microsyringe (Shanghai, China). Control fruits were treated with 1  $\mu$ L 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  $\pm$  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 multi-locus 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. fruticola*, 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 *Colletrichium 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	
<i>C. aenigma</i>	ICMP 18686	<i>Pyrus pyrifolia</i>	Japan	JX010243	JX009913	JX009684	JX009519	JX009789	JX010390	
	ICMP 18608*	<i>Persea americana</i>	Israel	JX010244	JX010044	JX009683	JX009443	JX009774	JX010389	
<i>C. aeschynomenes</i>	ICMP 17673*	<i>Aeschynomene virginica</i>	USA	JX010176	JX009930	JX009721	JX009483	JX009799	JX010392	
	CBS 304.67*	<i>Dioscorea alata</i>	India	JX010190	JX009990	JX009738	JX009471	JX009837	JX010383	
<i>C. alatae</i>	ICMP 18122	<i>Dioscorea alata</i>	Nigeria	JX010191	JX010011	JX009739	JX009470	JX009846	JX010449	
	ICMP 12071*	<i>Malus domestica</i>	New Zealand	JX010251	JX010028	JX009654	JX009572	JX009882	JX010411	
<i>C. aotearoa</i>	ICMP 18621	<i>Persea americana</i>	New Zealand	JX010246	JX009959	JX009657	JX009552	JX009755	JX010386	
	ICMP 18537*	<i>Coprosma</i> sp.	New Zealand	FJ972612	JX010005	JX009611	JX009564	JX009853	JX010420	
<i>C. asianum</i>	ICMP 18580*	<i>Coffea arabica</i>	Thailand	JX010205	JX010063	FJ917506	JX009584	JX009867	JX010406	
	ICMP 18696	<i>Mangifera indica</i>	Australia	JX010192	JX009915	JX009723	JX009576	JX009753	JX010384	
<i>C. boninense</i>	MAFF 305972*	<i>Crinum asiaticum</i> var. <i>sinicum</i>	Japan	JX010292	JX009905	JQ005674	JX009583	JX009827	JQ005588	
	CBS 128547	<i>Camelia</i> sp.	New Zealand	JQ005159	JQ005246	JQ005680	JQ005507	JQ005333	JQ005593	
<i>C. brevisporum</i>	BCC 38876*	<i>Neoregalia</i> sp.	Thailand	JN050238	JN050228	JN050222	JN050216	KF687760	JN050244	
	MFLUCC100182	<i>Pandanus pygmaeus</i>	Thailand	JN050239	JN050228	JN050222	JN050217	JN050245	JN050245	
<i>C. clidemiae</i>	ICMP 18706	<i>Clidemia hirta</i>	USA	JX010274	JX009909	JX009639	JX009476	JX009777	JX010439	
	CBS 125375*	<i>Givia miniata</i>	China	JX519223	JX546611	JX519240	JX519240	JX519232	JX519249	
<i>C. clivae</i>	CSSS1	<i>Givia miniata</i>	China	GU109479	GU085867	GU085861	GU085861	GU085869	GU085869	
	CSSS2	<i>Givia miniata</i>	China	GU109480	GU085868	GU085862	GU085862	GU085870	GU085870	
<b><i>C. conoides</i></b>	<b>CAUO6</b>	<b><i>Capsicum annuum</i></b>	<b>China</b>	<b>KP890168</b>	<b>KP890162</b>	<b>KP890123</b>	<b>KP890100</b>	<b>KP890131</b>	<b>KP890115</b>	
	<b>CAUG17*</b>	<b><i>Capsicum annuum</i></b>	<b>China</b>	<b>KP890169</b>	<b>KP890163</b>	<b>KP890150</b>	<b>KP890144</b>	<b>KP890156</b>	<b>KP890174</b>	
<i>C. cordylimicola</i>	<b>CAUG3</b>	<b><i>Capsicum annuum</i></b>	<b>China</b>	<b>KP890170</b>	<b>KP890164</b>	<b>KP890152</b>	<b>KP890146</b>	<b>KP890157</b>	<b>KP890175</b>	
	<b>CAUG34</b>	<b><i>Capsicum annuum</i></b>	<b>China</b>	<b>KP890170</b>	<b>KP890164</b>	<b>KP890152</b>	<b>KP890146</b>	<b>KP890158</b>	<b>KP890176</b>	
<i>C. dracaenophilum</i>	ICMP 18579	<i>Cordylone fruticosa</i>	Thailand	JX010226	JX009975	HM470238	HM470235	JX009864	JX010440	
	CBS 118199*	<i>Dracaena</i> sp.	China	JX519222	JX546707	JX519238	JX519238	JX519230	JX519247	
<b><i>C. endophytica</i></b>	<b>CAUG28</b>	<b><i>Dracaena</i> sp.</b>	<b>China</b>	<b>KP145441</b>	<b>KP145413</b>	<b>KP145357</b>	<b>KP145329</b>	<b>KP145385</b>	<b>KP145469</b>	
	LC0324*	<b><i>Pennisetum purpureum</i></b>	<b>Thailand</b>	<b>KC633854</b>	<b>KC832854</b>	<b>KC810018</b>	<b>KF306258</b>			
<i>C. fructicola</i>	MFLUCC 100676	<b><i>Pennisetum purpureum</i></b>	<b>Thailand</b>	<b>KF242123</b>	<b>KF254846</b>	<b>KF157827</b>				
	<b>CAUG1</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145416</b>	<b>KP145388</b>	<b>KP145332</b>	<b>KP145304</b>	<b>KP145360</b>	<b>KP145444</b>	
<i>C. gloeosporioides</i>	<b>CAUG5</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145420</b>	<b>KP145392</b>	<b>KP145336</b>	<b>KP145308</b>	<b>KP145364</b>	<b>KP145448</b>	
	<b>CAUG6</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145421</b>	<b>KP145393</b>	<b>KP145337</b>	<b>KP145309</b>	<b>KP145365</b>	<b>KP145449</b>	
<i>C. gloeosporioides</i>	<b>CAUG8</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145422</b>	<b>KP145394</b>	<b>KP145338</b>	<b>KP145310</b>	<b>KP145366</b>	<b>KP145450</b>	
	<b>CAUG10</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145424</b>	<b>KP145396</b>	<b>KP145340</b>	<b>KP145312</b>	<b>KP145368</b>	<b>KP145452</b>	
<i>C. gloeosporioides</i>	<b>CAUG11</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145425</b>	<b>KP145397</b>	<b>KP145341</b>	<b>KP145313</b>	<b>KP145369</b>	<b>KP145453</b>	
	<b>CAUG16</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145430</b>	<b>KP145402</b>	<b>KP145346</b>	<b>KP145318</b>	<b>KP145374</b>	<b>KP145458</b>	
<i>C. gloeosporioides</i>	<b>CAUG18</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145431</b>	<b>KP145403</b>	<b>KP145347</b>	<b>KP145319</b>	<b>KP145375</b>	<b>KP145459</b>	
	CBS 125395	<i>Theobroma cacao</i>	Panama	JX010172	JX009992	JX009666	JX009543	JX009873	JX010408	
<i>C. gloeosporioides</i>	ICMP 17789	<i>Malus domestica</i>	USA	JX010178	JX009914	JX009665	JX009451	JX009809		
	ICMP 12568	<i>Persea americana</i>	Australia	JX010166	JX009946	JX009680	JX009529	JX009762	JX010405	
<i>C. gloeosporioides</i>	ICMP 18581*	<i>Coffea arabica</i>	Thailand	JX010165	JX010033	FJ917508	FJ907426	JX009866	JX010388	
	ICMP 18613	<i>Limonium sinuatum</i>	Israel	JX010167	JX009998	JX009675	JX009491	JX009772	JX010394	
<i>C. gloeosporioides</i>	ICMP 18727	<i>Fragaria ananassa</i>	USA	JX010179	JX010035	JX009682	JX009565	JX009812	JX010394	
	CBS 238.49*	<i>Ficus edulis</i>	Germany	JX010181	JX009923	JX009671	JX009495	JX009839	JX010400	
<i>C. gloeosporioides</i>	CBS 125397*	<i>Tetragastris panamensis</i>	Panama	JX010173	JX010032	JX009674	JX009581	JX009874	JX010409	
	<b>CAUG2</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145417</b>	<b>KP145389</b>	<b>KP145333</b>	<b>KP145305</b>	<b>KP145361</b>	<b>KP145445</b>	
<i>C. gloeosporioides</i>	<b>CAUG3</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145418</b>	<b>KP145390</b>	<b>KP145334</b>	<b>KP145306</b>	<b>KP145362</b>	<b>KP145446</b>	
	<b>CAUG12</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145426</b>	<b>KP145398</b>	<b>KP145342</b>	<b>KP145314</b>	<b>KP145370</b>	<b>KP145454</b>	
<i>C. gloeosporioides</i>	<b>CAUG13</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145427</b>	<b>KP145399</b>	<b>KP145343</b>	<b>KP145315</b>	<b>KP145371</b>	<b>KP145455</b>	
	<b>CAUG14</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145428</b>	<b>KP145400</b>	<b>KP145344</b>	<b>KP145316</b>	<b>KP145372</b>	<b>KP145456</b>	
<i>C. gloeosporioides</i>	<b>CAUG15</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145429</b>	<b>KP145401</b>	<b>KP145345</b>	<b>KP145317</b>	<b>KP145373</b>	<b>KP145457</b>	
	<b>CAUG19</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145432</b>	<b>KP145404</b>	<b>KP145348</b>	<b>KP145320</b>	<b>KP145376</b>	<b>KP145460</b>	
<i>C. gloeosporioides</i>	<b>CAUG20</b>	<b><i>Capsicum</i> sp.</b>	<b>China</b>	<b>KP145433</b>	<b>KP145405</b>	<b>KP145349</b>	<b>KP145321</b>	<b>KP145377</b>	<b>KP145461</b>	

<b>C. gloeosporioides</b> (cont.)	CAUG22	<i>Capsicum</i> sp.	China	KP145435	KP145407	KP145351	KP145323	KP145379	KP145463
	CAUG23	<i>Capsicum</i> sp.	China	KP145436	KP145408	KP145352	KP145324	KP145380	KP145464
	CAUG24	<i>Capsicum</i> sp.	China	KP145437	KP145409	KP145353	KP145325	KP145381	KP145465
	CAUG25	<i>Capsicum</i> sp.	China	KP145438	KP145410	KP145354	KP145326	KP145382	KP145466
	CAUG26	<i>Capsicum</i> sp.	China	KP145439	KP145411	KP145355	KP145327	KP145383	KP145467
	CAUG29	<i>Capsicum</i> sp.	China	KP145442	KP145414	KP145358	KP145330	KP145386	KP145470
	IMI 356878*	<i>Citrus sinensis</i>	Italy	JX010152	JX010056	JX009731	JX009531	JX009818	JX010445
	CORCG4	<i>Vanda</i> sp.	Thailand	HM034808	HM034806	HM034802	HM034800		
	CORCG5	<i>Vanda</i> sp.	Thailand	HM034809	HM034807	HM034803	HM034801	HM034805	HM034811
<i>C. grevillea</i>	CBS 132879*	<i>Grevillea</i> sp.	Italy	KC297078	KC297010	KC296963	KC296941	KC296987	KC297102
<b>C. grossum</b>	CAUG7*	<i>Capsicum</i> sp.	China	KP890165	KP890159	KP890147	KP890141	KP890153	KP890171
	CAU31	<i>Capsicum</i> sp.	China	KP890166	KP890160	KP890148	KP890142	KP890154	KP890172
	CAUG32	<i>Capsicum</i> sp.	China	KP890167	KP890161	KP890149	KP890143	KP890155	KP890173
<i>C. hebelense</i>	JZB330024	<i>Vitis vinifera</i> cv. Cabernet Sauvignon	China	KF156873	KF377505		KF377532	KF289008	KF289875
	JZB330028*	<i>Vitis vinifera</i> cv. Cabernet Sauvignon	China	KF156863	KF377495		KF377532	KP145367	KP145451
<b>C. hymenocallidis</b>	CAUG9	<i>Capsicum</i> sp.	China	KP145423	KP145395	KP145339	KP145311	GQ856730	JX010410
	ICMP 18642*	<i>Hymenocallis americana</i>	China	JX010278	JX010019	JX009709	GQ856775	JX009757	JX010426
	ICMP 12952	<i>Persea americana</i>	China	JX010214	JX009971	JX009648	JX009431	JX009757	JX010426
<i>C. kahawae</i> subsp. <i>ciggaro</i>	ICMP 18539*	<i>Olea europaea</i>	New Zealand	JX010230	JX009966	JX009635	JX009523	JX009800	JX010434
<i>C. karstii</i>	CGMCC 3.14194*	<i>Vanda</i> sp.	Australia	HM585409	HM585391	HM581995	HM585428		
	CAUOS1	<i>Capsicum</i> sp.	China	KP890103	KP890134	KP890118	KP890096	KP890126	KP890110
	CAUOS7	<i>Capsicum</i> sp.	China	KP890108	KP890139	KP890124	KP890102	KP890132	KP890116
	CAUOS8	<i>Capsicum</i> sp.	China	KP890109	KP890140	KP890125	KP890102	KP890133	KP890117
	CAUOS2*	<i>Capsicum</i> sp.	China	KP890104	KP890135	KP890119	KP890097	KP890127	KP890111
	CAUOS3	<i>Capsicum</i> sp.	China	KP890105	KP890136	KP890120	KP890098	KP890128	KP890112
	CAUOS4	<i>Capsicum</i> sp.	China	KP890106	KP890137	KP890121	KP890099	KP890129	KP890113
	CAUOS5	<i>Capsicum</i> sp.	China	KP890107	KP890138	KP890122	KP890114	KP890130	KP890114
<i>C. musae</i>	ICMP 19119*	<i>Musa</i> sp.	USA	JX010146	JX010050	JX009742	JX009433	JX009896	HQ596280
	IMI 52264	<i>Musa sapientum</i>	USA	JX010142	JX010015	JX009689	JX009432	JX009815	JX010395
<i>C. nupharicola</i>	ICMP 17938	<i>Nuphar lutea</i> subsp. <i>polysepala</i>	Kenya	JX010189	JX009936	JX009661	JX009486	JX009834	JX010397
	ICMP 17940	<i>Nymphaea odorata</i>	USA	JX010188	JX010031	JX009662	JX009582	JX009836	JX010399
	ICMP 18187*	<i>Nuphar lutea</i> subsp. <i>polysepala</i>	USA	JX010187	JX009972	JX009663	JX009437	JX009835	JX010398
	ICMP 19120*	<i>Psidium</i> sp.	Italy	JX010219	JX009967	JX009743	JX009515	JX009901	JX010443
<i>C. queenslandicum</i>	ICMP 1778*	<i>Carica papaya</i>	Australia	JX010276	JX009934	JX009691	JX009447	JX009899	JX010414
<i>C. saesolae</i>	ICMP 19051	<i>Salsola tragus</i>	Hungary	JX010242	JX009916	JX009696	JX009562	JX009863	JX010403
<i>C. siamense</i>	CBS 130420	<i>Jasminum sambac</i>	Vietnam	HM131511	HM131497		HM131507	JX009895	JX010415
<i>C. thailandicum</i>	BCC 38879*	<i>Hibiscus rosa-sinensis</i>	Thailand	JN050242	JN050231	JX009713	JN050220	JN050248	JN050248
	MFLUCC10092	<i>Alocasia</i> sp.	Thailand	JN050243	JN050232		JN050221	JN050249	JN050249
<i>C. theobromicola</i>	CBS 124945*	<i>Theobroma cacao</i>	Panama	JX010294	JX010006	JX009591	JX009444	JX009869	JX010447
	ICMP 17814	<i>Fragaria vesca</i>	USA	JX010288	JX010003	JX009589	JX009448	JX009819	JX010379
	ICMP 17957	<i>Stylosanthes viscosa</i>	Australia	JX010289	JX009962	JX009597	JX009575	JX009821	JX010380
<i>C. ti</i>	ICMP 4832*	<i>Cordylone</i> sp.	New Zealand	JX010269	JX009952	JX009649	JX009520	JX009898	JX010442
	ICMP 5285	<i>Cordylone australis</i>	New Zealand	JX010267	JX009910	JX009650	JX009553	JX009897	JX010441
<i>C. tropicale</i>	CBS 124949*	<i>Theobroma cacao</i>	Panama	JX010264	JX010007	JX009719	JX009489	JX009870	JX010407
	ICMP 18672	<i>Litchi chinensis</i>	Japan	JX010275	JX010020	JX009722	JX009480	JX009826	JX010396
<i>C. tropicicola</i>	BCC 38877*	<i>Citrus maxima</i>	Thailand	JN050240	JN050229		JN050218	JN050246	JN050246
	MFLUCC100167	<i>Paphiopedilum bellatolum</i>	Thailand	JN050241	JN050230		JN050219	JN050247	JN050247
<b>C. viniferum</b>	CAUG27	<i>Capsicum</i> sp.	China	KP145440	KP145412	KP145356	KP145328	KP145384	KP145468
	GZAAS 5.08601*	<i>Vitis vinifera</i> , cv. 'Shuijing'	China	JN412804	JN412798	JQ309639	JN412795		JN412813
	GZSSS 5.08608	<i>Vitis vinifera</i> , cv. 'Hongji'	China	JN412802	JN412800	JQ412782	JN412793		JN412811
<i>C. xanthorrhoeae</i>	ICMP 17903*	<i>Xanthorrhoea preissii</i>	Australia	JX010261	JX009927	JX009653	JX009478	JX009823	JX010448
<i>C. yunnanense</i>	CBS 132135*	<i>Buxus</i> sp.	China	JX546804	JX519248	JX546706	JX519239	JX519231	
<i>Glomella circinata</i>	ICMP 10643	<i>Camellia williamsii</i>	UK	JX010224	JX009908	JX009630	JX009540	JX009891	JX010436
<i>Monilochaetes infuscans</i>	CBS 869.96*	<i>Ipomoea batatas</i>	South Africa	JQ005780	JX546612	JQ005843	JQ005801	JQ005801	JQ005864

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

Table 2b Strains used for the phylogenetic analysis of the *Colletothrichum 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	
<i>C. acutatum</i>	CBS 112996*	<i>Carica papaya</i>	Australia	JQ005776	JQ948677	JQ005797	JQ005818	JQ005839	JQ005860	
	CBS 144.29	<i>Capsicum annuum</i>	Sri Lanka	JQ948401	JQ948732	JQ949062	JQ949392	JQ949722	JQ950052	
	CBS 126518	<i>Carthamus</i> sp.	Netherlands	JQ948271	JQ948601	JQ948932	JQ949262	JQ949592	JQ949922	
<i>C. chrysanthemi</i>	IMI 364540	<i>Chrysanthemum coronarium</i>	China	JQ948272	JQ948602	JQ948933	JQ949263	JQ949593	JQ949923	
	CAUA18	<i>Capsicum annuum</i>	China	KP145016	KP145096	KP145048	KP145064	KP145032	KP145080	
<i>C. fioriniae</i>	CAUA20	<i>Capsicum annuum</i>	China	KP145226	KP145162	KP145290	KP145194	KP145130	KP145258	
	CAUA24	<i>Capsicum annuum</i>	China	KP145017	KP145097	KP145049	KP145065	KP145033	KP145081	
	CAUA25	<i>Capsicum annuum</i>	China	KP145018	KP145098	KP145050	KP145066	KP145034	KP145082	
	CAUA26	<i>Capsicum annuum</i>	China	KP145019	KP145099	KP145051	KP145067	KP145035	KP145083	
	CAUA27	<i>Capsicum annuum</i>	China	KP145020	KP145100	KP145052	KP145068	KP145036	KP145084	
	CAUA28	<i>Capsicum annuum</i>	China	KP145021	KP145101	KP145053	KP145069	KP145037	KP145085	
	CAUA29	<i>Capsicum annuum</i>	China	KP145022	KP145102	KP145054	KP145070	KP145038	KP145086	
	CAUA30	<i>Capsicum annuum</i>	China	KP145023	KP145103	KP145055	KP145071	KP145039	KP145087	
	CAUA31	<i>Capsicum annuum</i>	China	KP145024	KP145104	KP145056	KP145072	KP145040	KP145088	
	CAUA32	<i>Capsicum annuum</i>	China	KP145025	KP145105	KP145057	KP145073	KP145041	KP145089	
	CAUA37	<i>Capsicum annuum</i>	China	KP145026	KP145106	KP145058	KP145074	KP145042	KP145090	
	CAUA38	<i>Capsicum annuum</i>	China	KP145027	KP145107	KP145059	KP145075	KP145043	KP145091	
CAUA39	<i>Capsicum annuum</i>	China	KP145028	KP145108	KP145060	KP145076	KP145044	KP145092		
CAUA40	<i>Capsicum annuum</i>	China	KP145029	KP145109	KP145061	KP145077	KP145045	KP145093		
CAUA41	<i>Capsicum annuum</i>	China	KP145030	KP145110	KP145062	KP145078	KP145046	KP145094		
CAUA48	<i>Capsicum annuum</i>	China	KP145031	KP145111	KP145063	KP145079	KP145047	KP145095		
<i>C. gloeosporioides</i>	CBS 125396	<i>Malus domestica</i>	USA	JQ948299	JQ948629	JQ948960	JQ949290	JQ949620	JQ949950	
	CBS 127537	<i>Vaccinium</i> sp.	USA	JQ948317	JQ948647	JQ948978	JQ949308	JQ949638	JQ949968	
	CBS 128517*	<i>Florinia</i> sp.	USA	JQ948292	JQ948622	JQ948953	JQ949283	JQ949613	JQ949943	
	CBS 129916	<i>Vaccinium</i> sp.	USA	JQ948317	JQ948647	JQ948978	JQ949308	JQ949638	JQ949968	
	CBS 293.67	<i>Persea</i> sp.	Australia	JQ948310	JQ948640	JQ948971	JQ949301	JQ949631	JQ949961	
	CBS 127601	<i>Mangifera</i> sp.	Australia	JQ948311	JQ948641	JQ948972	JQ949302	JQ949632	JQ949962	
	CBS 129947	<i>Vitis</i> sp.	Portugal	JQ948343	JQ948673	JQ949004	JQ949334	JQ949664	JQ949994	
	CBS 200.35	<i>Rubus</i> sp.	USA	JQ948293	JQ948623	JQ948954	JQ949284	JQ949614	JQ949944	
	IMI 356878*	<i>Citrus sinensis</i>	Italy	JX010152	JX010056	JX009818		JX009531	JX010445	
	CBS 133.44*	<i>Godetia</i> sp.	Denmark	JQ948402	JQ948733	JQ949063	JQ949393	JQ949723	JQ950053	
	IMI 350839*	<i>Psidium</i> sp.	India	JQ948270	JQ948600	JQ948931	JQ949261	JQ949591	JQ949921	
	CBS 112989*	<i>Hevea</i> sp.	India	JQ948289	JQ948619	JQ948950	JQ949280	JQ949610	JQ949940	
CBS 515.78*	<i>Nymphaea</i> sp.	Netherlands	JQ948197	JQ948527	JQ948858	JQ949188	JQ949518	JQ949848		
CBS 632.80*	<i>Dendrobium</i> sp.	USA	JQ948151	JQ948481	JQ948812	JQ949142	JQ949472	JQ949802		
CBS 607.94*	<i>Salix</i> sp.	Netherlands	JQ948460	JQ948791	JQ949121	JQ949451	JQ949781	JQ950111		
<i>C. scovillei</i>	CAUA1	<i>Capsicum annuum</i>	China	KP145208	KP145144	KP145272	KP145176	KP145112	KP145240	
	CAUA2	<i>Capsicum annuum</i>	China	KP145209	KP145145	KP145273	KP145177	KP145113	KP145241	
	CAUA3	<i>Capsicum annuum</i>	China	KP145210	KP145146	KP145274	KP145178	KP145114	KP145242	
	CAUA4	<i>Capsicum annuum</i>	China	KP145211	KP145147	KP145275	KP145179	KP145115	KP145243	
	CAUA5	<i>Capsicum annuum</i>	China	KP145212	KP145148	KP145276	KP145180	KP145116	KP145244	
	CAUA6	<i>Capsicum annuum</i>	China	KP145213	KP145149	KP145277	KP145181	KP145117	KP145245	
	CAUA7	<i>Capsicum annuum</i>	China	KP145214	KP145150	KP145278	KP145182	KP145118	KP145246	
	CAUA8	<i>Capsicum annuum</i>	China	KP145215	KP145151	KP145279	KP145183	KP145119	KP145247	
	CAUA9	<i>Capsicum annuum</i>	China	KP145216	KP145152	KP145280	KP145184	KP145120	KP145248	
	CAUA10	<i>Capsicum annuum</i>	China	KP145217	KP145153	KP145281	KP145185	KP145121	KP145249	
	CAUA11	<i>Capsicum annuum</i>	China	KP145218	KP145154	KP145282	KP145186	KP145122	KP145250	

CAUA12	<i>Capsicum annuum</i>	China	KP145219	KP145155	KP145283	KP145187	KP145123	KP145251
CAUA13	<i>Capsicum annuum</i>	China	KP145220	KP145156	KP145284	KP145188	KP145124	KP145252
CAUA14	<i>Capsicum annuum</i>	China	KP145221	KP145157	KP145285	KP145189	KP145125	KP145253
CAUA15	<i>Capsicum annuum</i>	China	KP145222	KP145158	KP145286	KP145190	KP145126	KP145254
CAUA16	<i>Capsicum annuum</i>	China	KP145223	KP145159	KP145287	KP145191	KP145127	KP145255
CAUA17	<i>Capsicum annuum</i>	China	KP145224	KP145160	KP145288	KP145192	KP145128	KP145256
CAUA19	<i>Capsicum annuum</i>	China	KP145225	KP145161	KP145289	KP145193	KP145129	KP145257
CAUA21	<i>Capsicum annuum</i>	China	KP145227	KP145163	KP145291	KP145195	KP145131	KP145259
CAUA22	<i>Capsicum annuum</i>	China	KP145228	KP145164	KP145292	KP145196	KP145132	KP145260
CAUA23	<i>Capsicum annuum</i>	China	KP145229	KP145165	KP145293	KP145197	KP145133	KP145261
CAUA33	<i>Capsicum annuum</i>	China	KP145230	KP145166	KP145294	KP145198	KP145134	KP145262
CAUA34	<i>Capsicum annuum</i>	China	KP145231	KP145167	KP145295	KP145199	KP145135	KP145263
CAUA35	<i>Capsicum annuum</i>	China	KP145232	KP145168	KP145296	KP145200	KP145136	KP145264
CAUA36	<i>Capsicum</i> sp.	China	KP145233	KP145169	KP145297	KP145201	KP145137	KP145265
CAUA42	<i>Capsicum</i> sp.	China	KP145234	KP145170	KP145298	KP145202	KP145138	KP145266
CAUA43	<i>Capsicum</i> sp.	China	KP145235	KP145171	KP145299	KP145203	KP145139	KP145267
CAUA44	<i>Capsicum</i> sp.	China	KP145236	KP145172	KP145300	KP145204	KP145140	KP145268
CAUA45	<i>Capsicum</i> sp.	China	KP145237	KP145173	KP145301	KP145205	KP145141	KP145269
CAUA46	<i>Capsicum</i> sp.	China	KP145238	KP145174	KP145302	KP145206	KP145142	KP145270
CAUA47	<i>Capsicum</i> sp.	China	KP145239	KP145175	KP145303	KP145207	KP145143	KP145271
CBS 120708	<i>Capsicum</i> sp.	Thailand	JQ948269	JQ948599	JQ948930	JQ949260	JQ949590	JQ949920
CBS 126529*	<i>Capsicum</i> sp.	Indonesia	JQ948267	JQ948597	JQ948928	JQ949258	JQ949588	JQ949918
CBS 126530	<i>Capsicum</i> sp.	Indonesia	JQ948268	JQ948598	JQ948929	JQ949259	JQ949589	JQ949919
CBS 122122*	<i>Carica</i> 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	
<i>C. anthrisci</i>	CBS 125335	<i>Anthriscus sylvestris</i>	Netherlands	GU227846	GU227944	GU228140	GU228336	GU228238	GU228042	
<i>C. chlorophyti</i>	IMI 103806*	<i>Chlorophytum</i> sp.	India	GU227894	GU227992	GU228188	GU228384	GU228286	GU228090	
	CBS 142.79	<i>Stylosanthes hamata</i>	Australia	GU227895	GU227993	GU228189	GU228385	GU228287	GU228091	
<i>C. circinans</i>	CBS 111.21	<i>Allium cepa</i>	USA	GU227854	GU227952	GU228148	GU228344	GU228246	GU228050	
	CBS 221.81*	<i>Allium cepa</i>	Serbia	GU227855	GU227953	GU228149	GU228345	GU228247	GU228051	
<i>C. dematium</i>	CBS 125.25*	<i>Eryngium campestre</i>	France	GU227819	GU227917	GU228113	GU228309	GU228211	GU228015	
	CBS 125340	Apiaceae	Czech Republic	GU227820	GU227918	GU228114	GU228310	GU228212	GU228016	
<i>C. fructi</i>	CBS 346.37*	<i>Malus sylvestris</i>	USA	GU227844	GU227942	GU228138	GU228334	GU228236	GU228040	
<i>C. incanum</i>	ATCC 64682*	<i>Glycine max</i>	USA	KC110789	KC110825	KC110816		KC110807	KC110798	
	IL6A	<i>Glycine max</i>	USA	KC110787	KC110823	KC110814		KC110805	KC110796	
	IL9A	<i>Glycine max</i>	USA	KC110788	KC110824	KC110815		KC110806	KC110797	
	<b>CAUCT34</b>	<b>Capsicum</b> sp.	<b>China</b>	<b>KP145641</b>	<b>KP145505</b>	<b>KP145675</b>	<b>KP145539</b>	<b>KP145573</b>	<b>KP145607</b>	
<i>C. lilii</i>	CBS 109214	<i>Lilium</i> sp.	Japan	GU227810	GU227908	GU228104	GU228300	GU228202	GU228006	
<i>C. lindemuthianum</i>	CBS 151.28	<i>Lilium</i> sp.	Netherlands	GU227800	GU227898	GU228094	GU228290	GU228192	GU227996	
<i>C. lineola</i>	CBS 125337*	Apiaceae sp.	Czech Republic	GU227829	GU227927	GU228123	GU228319	GU228221	GU228025	
	CBS 125339	Apiaceae sp.	Czech Republic	GU227830	GU227928	GU228124	GU228320	GU228222	GU228026	
<i>C. liriopes</i>	CBS 119444*	<i>Liriope muscari</i>	Mexico	GU227804	GU227902	GU228098	GU228294	GU228196	GU228000	
	CBS 122747	<i>Liriope muscari</i>	Mexico	GU227805	GU227903	GU228099	GU228295	GU228197	GU228001	
<i>C. phaseolorum</i> 1	CBS 157.36	<i>Phaseolus radiatus</i> var. <i>aureus</i>	Japan	GU227896	GU227994	GU228190	GU228386	GU228288	GU228092	
<i>C. phaseolorum</i> 2	CBS 158.36	<i>Vigna sinensis</i>	Japan	GU227897	GU227995	GU228191	GU228387	GU228289	GU228093	
<i>C. rusci</i>	CBS 119206*	<i>Ruscus</i> sp.	Italy	GU227818	GU227916	GU228112	GU228308	GU228210	GU228014	
	CBS 167.49*	<i>Hosta sieboldiana</i>	Germany	GU227807	GU227905	GU228101	GU228297	GU228199	GU228003	
<i>C. spaethianum</i>	CBS 100063	<i>Lilium</i> sp.	South Korea	GU227808	GU227906	GU228102	GU228298	GU228200	GU228004	
	CBS 101631	<i>Hemerocallis</i> sp.	New Zealand	GU227809	GU227907	GU228103	GU228299	GU228201	GU228005	
<i>C. sphinaciae</i>	CBS 128.57	<i>Sphinacia oleracea</i>	Netherlands	GU227847	GU227945	GU228141	GU228337	GU228239	GU228043	
	IMI 104607	<i>Sphinacia</i> sp.	Italy	GU227850	GU227948	GU228144	GU228340	GU228242	GU228046	
<i>C. tofieldiae</i>	CBS 168.49	<i>Lupinus polyphyllus</i>	Germany	GU227802	GU227900	GU228096	GU228292	GU228194	GU227998	
	CBS 495.85	<i>Tofieldia calyculata</i>	Switzerland	GU227801	GU227899	GU228095	GU228291	GU228193	GU227997	
<i>C. trichellum</i>	CBS 118198	<i>Hedera</i> sp.	Guatemala	GU227813	GU227911	GU228107	GU228303	GU228205	GU228009	
	CBS 217.64	<i>Hedera helix</i>	UK	GU227812	GU227910	GU228106	GU228302	GU228204	GU228008	
<i>C. truncatum</i>	CBS 182.52	<i>Glycine max</i>	USA	GU227866	GU227964	GU228160	GU228356	GU228258	GU228062	
	CBS195.32	<i>Glycine max</i>	USA	GU227865	GU227963	GU228159	GU228355	GU228257	GU228061	
	CBS 345.70	<i>Glycine max</i>	Denmark	GU227867	GU227965	GU228161	GU228357	GU228259	GU228063	
	CBS 151.35*	<i>Phaseolus lunatus</i>	USA	GU227862	GU227960	GU228156	GU228352	GU228254	GU228058	
	<b>CAUCT1</b>	<b>Capsicum annuum</b>	<b>China</b>	<b>KP145608</b>	<b>KP145472</b>	<b>KP145642</b>	<b>KP145506</b>	<b>KP145540</b>	<b>KP145574</b>	
	<b>CAUCT2</b>	<b>Capsicum annuum</b>	<b>China</b>	<b>KP145609</b>	<b>KP145473</b>	<b>KP145643</b>	<b>KP145507</b>	<b>KP145541</b>	<b>KP145575</b>	
	<b>CAUCT3</b>	<b>Capsicum annuum</b>	<b>China</b>	<b>KP145610</b>	<b>KP145474</b>	<b>KP145644</b>	<b>KP145508</b>	<b>KP145542</b>	<b>KP145576</b>	
	<b>CAUCT4</b>	<b>Capsicum annuum</b>	<b>China</b>	<b>KP145611</b>	<b>KP145475</b>	<b>KP145645</b>	<b>KP145509</b>	<b>KP145543</b>	<b>KP145577</b>	
	<b>CAUCT5</b>	<b>Capsicum annuum</b>	<b>China</b>	<b>KP145612</b>	<b>KP145476</b>	<b>KP145646</b>	<b>KP145510</b>	<b>KP145544</b>	<b>KP145578</b>	
	<b>CAUCT6</b>	<b>Capsicum annuum</b>	<b>China</b>	<b>KP145613</b>	<b>KP145477</b>	<b>KP145647</b>	<b>KP145511</b>	<b>KP145545</b>	<b>KP145579</b>	
	<b>CAUCT7</b>	<b>Capsicum</b> sp.	<b>China</b>	<b>KP145614</b>	<b>KP145478</b>	<b>KP145648</b>	<b>KP145512</b>	<b>KP145546</b>	<b>KP145580</b>	
	<b>CAUCT8</b>	<b>Capsicum</b> sp.	<b>China</b>	<b>KP145615</b>	<b>KP145479</b>	<b>KP145649</b>	<b>KP145513</b>	<b>KP145547</b>	<b>KP145581</b>	
	<b>CAUCT9</b>	<b>Capsicum</b> sp.	<b>China</b>	<b>KP145616</b>	<b>KP145480</b>	<b>KP145650</b>	<b>KP145514</b>	<b>KP145548</b>	<b>KP145582</b>	
	<b>CAUCT10</b>	<b>Capsicum</b> sp.	<b>China</b>	<b>KP145617</b>	<b>KP145481</b>	<b>KP145651</b>	<b>KP145515</b>	<b>KP145549</b>	<b>KP145583</b>	
	<b>CAUCT11</b>	<b>Capsicum</b> sp.	<b>China</b>	<b>KP145618</b>	<b>KP145482</b>	<b>KP145652</b>	<b>KP145516</b>	<b>KP145550</b>	<b>KP145584</b>	

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

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

with white margin. *Chlamydo*spores 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

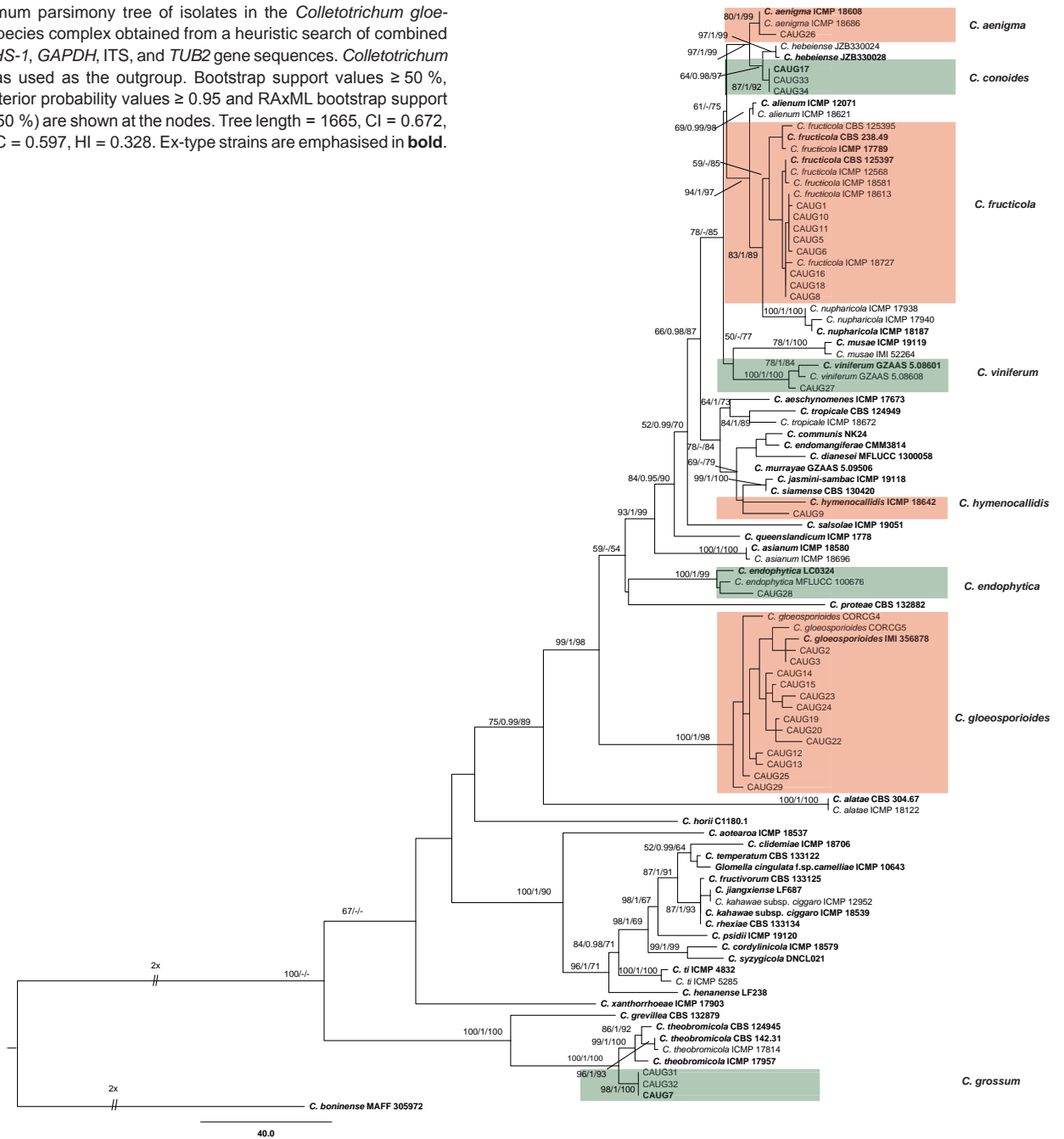
*Etymology.* Referring to the host variety (*Capsicum annuum* var. *grossum*) from which the fungus was first collected.

*Colonies* on PDA attaining 49–52 mm diam in 4 d at 28 °C; aerial mycelia white, reverse light grey with white margin. *Chlamydo*spores 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  $\geq 50\%$ , Bayesian posterior probability values  $\geq 0.95$  and RAxML bootstrap support values (ML  $\geq 50\%$ ) 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	<i>Capsicum annuum</i>				<i>C. frutescens</i>		<i>C. chinense</i>	Mean	Pathotype
	Chaotianjiao	Denglongjiao	Sanyingjiao	Zidantou	Shuanla	Xiaomila	Huangdijiao		
<i>C. aenigma</i> CAUG26	5	5	7	5	5	7	5	6	PC1-R
<i>C. cliviae</i> CAUOS5	7	7	5	7	7	5	0	5	PC2-R
<i>C. conoides</i> CAUG17	7	7	7	7	7	5	5	6	PC1-R
<i>C. endophytica</i> CAUG28	0	0	0	0	0	0	0	0	PC3-R
<i>C. fiorinae</i> CAUT34	7	7	9	9	9	9	7	8	PC1-R
<i>C. fructicola</i> CAUG1	7	7	9	9	9	9	7	8	PC1-R
<i>C. gloeosporioides</i> CAUG2	7	7	9	9	7	7	5	7	PC1-R
<i>C. grossum</i> CAUG7	7	7	7	7	5	5	7	6	PC1-R
<i>C. hymenocallidis</i> CAUG9	5	7	9	9	7	7	0	6	PC2-R
<i>C. incanum</i> CAUT34	5	7	7	5	7	5	5	6	PC1-R
<i>C. karstii</i> CAUOS1	7	5	7	7	9	9	7	7	PC1-R
<i>C. liaoningense</i> CAUOS2	9	7	9	9	5	9	5	8	PC1-R
<i>C. scovillei</i> CAUA1	7	9	9	9	9	7	9	8	PC1-R
<i>C. truncatum</i> CAUT1	7	7	9	7	9	9	7	8	PC1-R
<i>C. viniferum</i> 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  $\geq 50\%$  and Bayesian posterior probability values  $\geq 0.95$  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**.

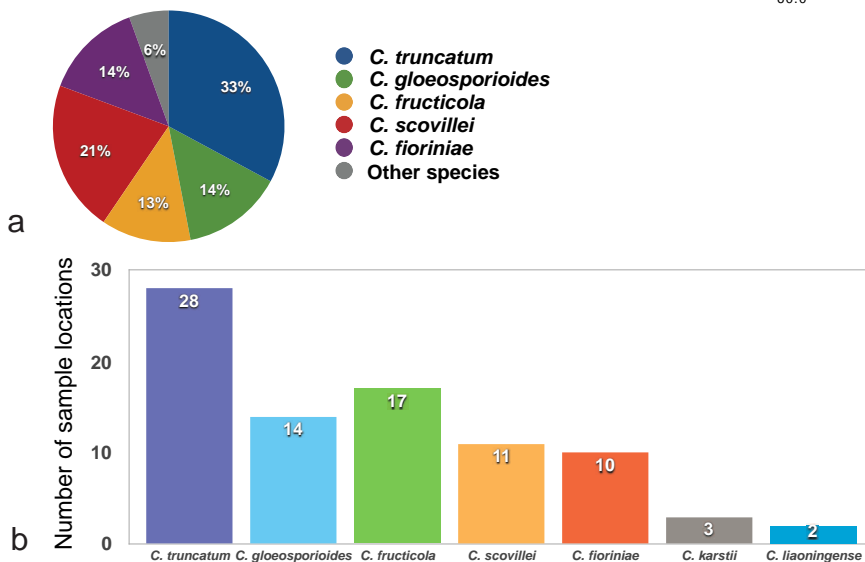
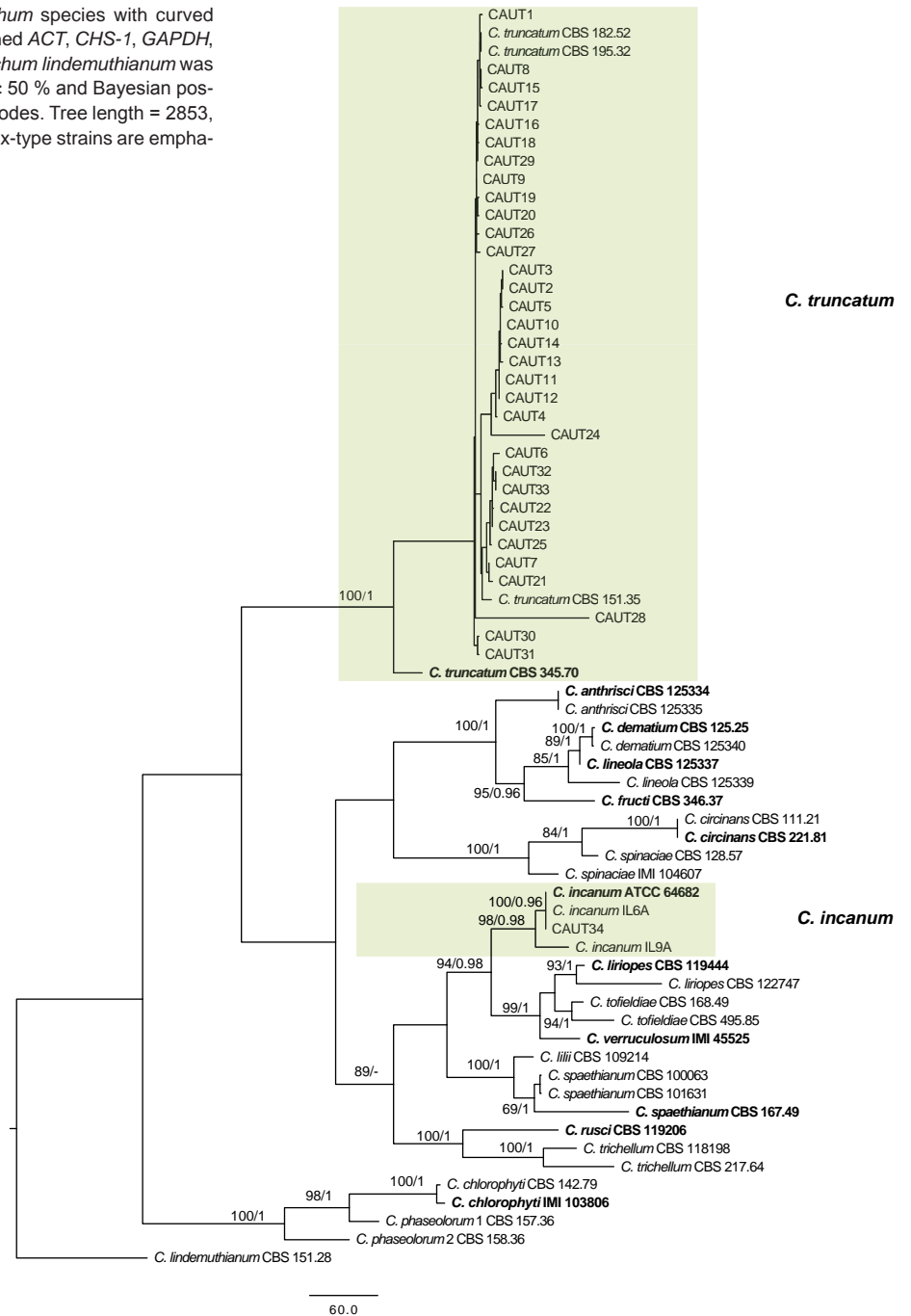


**Table 4** Anthracnose severity scores on a 0–9 scale and pathotypes of 15 *Colletotrichum* species isolates at the mature green fruit stage of seven chili cultivars.

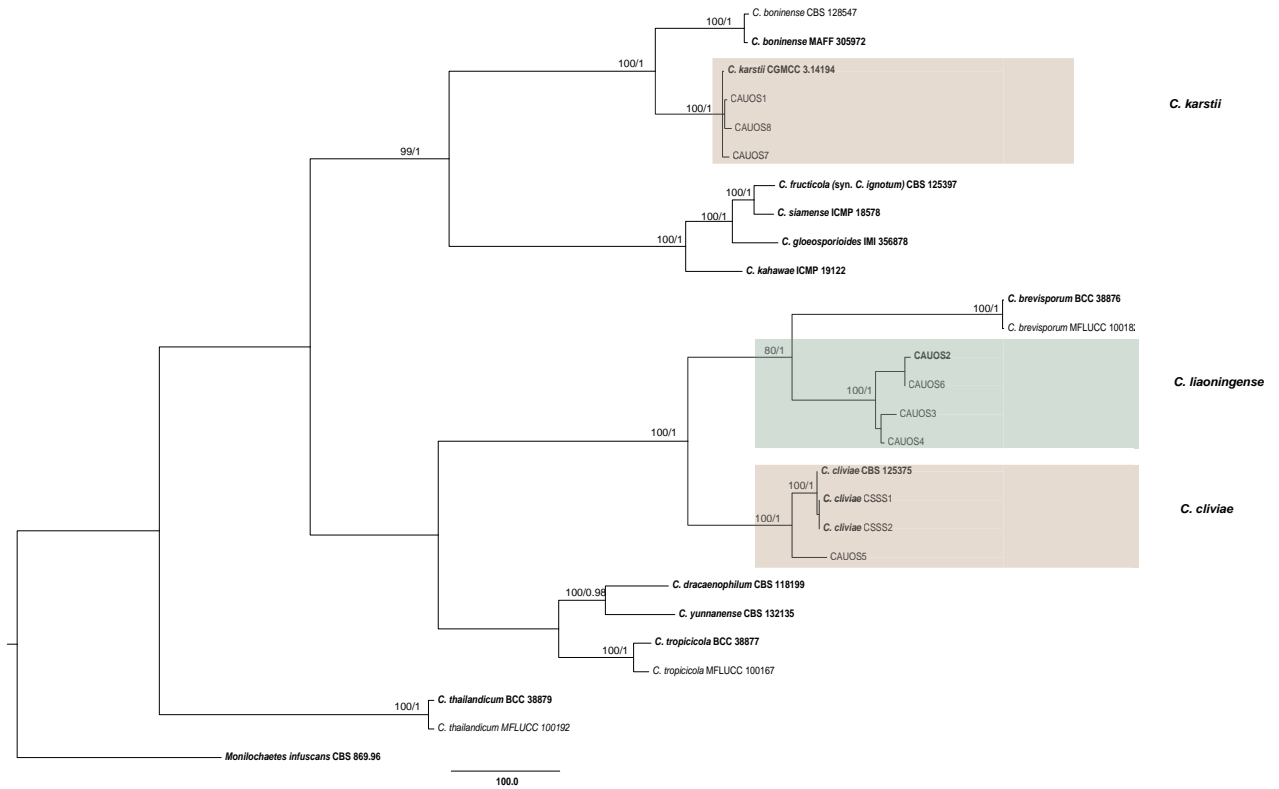
Isolate	<i>Capsicum annuum</i>				<i>C. frutescens</i>		<i>C. chinense</i>	Mean	Pathotype
	Chaotianjiao	Denglongjiao	Sanyingjiao	Zidantou	Shuanla	Xiaomila	Huangdijiao		
<i>C. aenigma</i> CAUG26	5	5	5	5	0	0	0	3	PC1-G
<i>C. cliviae</i> CAUOS5	7	9	5	7	7	5	7	7	PC2-G
<i>C. conoides</i> CAUG17	7	5	0	5	7	5	0	4	PC3-G
<i>C. endophytica</i> CAUG28	0	0	0	0	0	0	0	0	PC4-G
<i>C. fioriniae</i> CAUT34	9	9	7	7	9	9	7	8	PC2-G
<i>C. fruticola</i> CAUG1	7	9	9	5	0	7	7	6	PC5-G
<i>C. gloeosporioides</i> CAUG2	5	9	7	5	0	7	7	6	PC5-G
<i>C. grossum</i> CAUG7	3	3	0	5	5	5	0	3	PC3-G
<i>C. hymenocallidis</i> CAUG9	5	5	5	5	7	7	7	6	PC2-G
<i>C. incanum</i> CAUT34	5	5	5	3	7	5	5	5	PC2-G
<i>C. karstii</i> CAUOS1	5	7	7	7	0	0	0	4	PC1-G
<i>C. liaoningense</i> CAUOS2	5	5	9	7	5	3	7	6	PC2-G
<i>C. scovillei</i> CAUA1	7	7	7	7	9	9	7	7	PC2-G
<i>C. truncatum</i> CAUT1	7	5	5	7	7	7	5	6	PC2-G
<i>C. viniferum</i> 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  $\geq 50\%$  and Bayesian posterior probability values  $\geq 0.95$  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**.



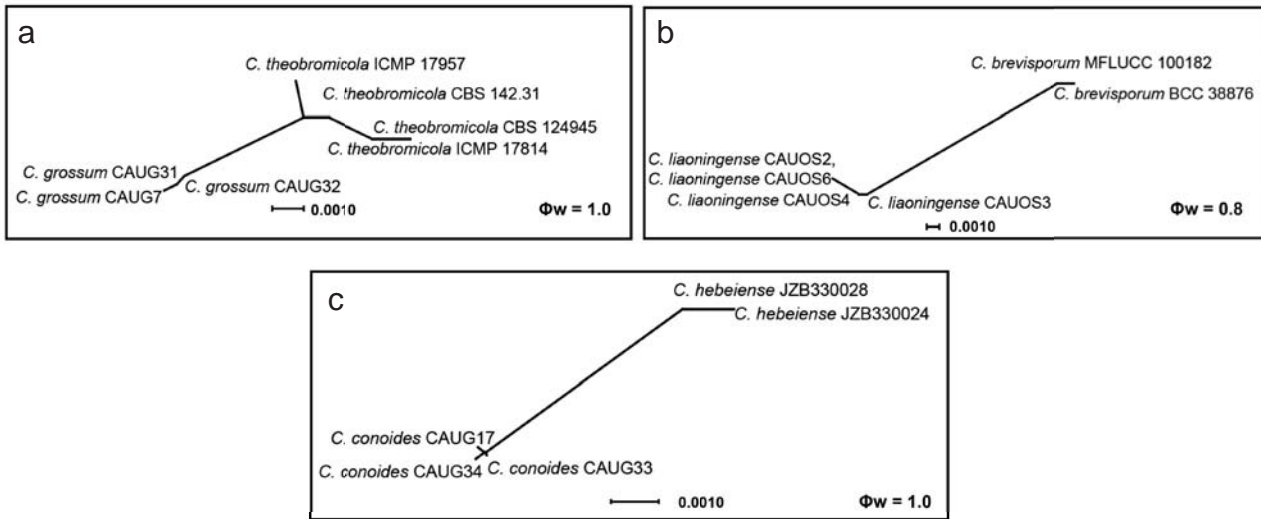
**Fig. 5** Prevalence of *Colletotrichum* species on chili in China based on preliminary identifications. a. The percentage of isolates represented by the indicated *Colletotrichum* species on chili; b. number of sampling locations where the seven most prevalent species were isolated.



**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  $\geq 50\%$  and Bayesian posterior probability values  $\geq 0.95$  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 homoplasies index (PHI) test of closely related species using both LogDet transformation and splits decomposition. PHI test results ( $\Phi_w$ ) < 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. fiorinae*, *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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