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Colletotrichum species associated with anthracnose of *Pyrus* spp. in China

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Key words

Colletotrichum
multi-gene phylogeny
pathogenicity
Pyrus

Abstract *Colletotrichum* species are plant pathogens, saprobes, and endophytes on a range of economically important hosts. However, the species occurring on pear remain largely unresolved. To determine the morphology, phylogeny and biology of *Colletotrichum* species associated with *Pyrus* plants, a total of 295 samples were collected from cultivated pear species (including *P. pyrifolia*, *P. bretschneideri*, and *P. communis*) from seven major pear-cultivation provinces in China. The pear leaves and fruits affected by anthracnose were sampled and subjected to fungus isolation, resulting in a total of 488 *Colletotrichum* isolates. Phylogenetic analyses based on six loci (*ACT*, *TUB2*, *CAL*, *CHS-1*, *GAPDH*, and ITS) coupled with morphology of 90 representative isolates revealed that they belong to 10 known *Colletotrichum* species, including *C. aenigma*, *C. citricola*, *C. conoides*, *C. fioriniae*, *C. fructicola*, *C. gloeosporioides*, *C. karstii*, *C. plurivorum*, *C. siamense*, *C. wuxiense*, and two novel species, described here as *C. jinshuiense* and *C. pyrifoliae*. Of these, *C. fructicola* was the most dominant, occurring on *P. pyrifolia* and *P. bretschneideri* in all surveyed provinces except in Shandong, where *C. siamense* was dominant. In contrast, only *C. siamense* and *C. fioriniae* were isolated from *P. communis*, with the former being dominant. In order to prove Koch's postulates, pathogenicity tests on pear leaves and fruits revealed a broad diversity in pathogenicity and aggressiveness among the species and isolates, of which *C. citricola*, *C. jinshuiense*, *C. pyrifoliae*, and *C. conoides* appeared to be organ-specific on either leaves or fruits. This study also represents the first reports of *C. citricola*, *C. conoides*, *C. karstii*, *C. plurivorum*, *C. siamense*, and *C. wuxiense* causing anthracnose on pear.

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INTRODUCTION

Colletotrichum species are important plant pathogens, saprobes, and endophytes, and can infect numerous plant hosts (Cannon et al. 2012, Dean et al. 2012, Diao et al. 2017, Guarnaccia et al. 2017). In recent years, the *Colletotrichum* species isolated from many host plants, e.g., *Camellia sinensis* (*Theaceae*), *Capsicum annuum* (*Solanaceae*), *Citrus reticulata* (*Rutaceae*), *Mangifera indica* (*Anacardiaceae*), and *Vitis vinifera* (*Vitaceae*), have been studied at a broad geographical level, which contributed to a better understanding of the genus (Huang et al. 2013, Lima et al. 2013, Vieira et al. 2014, Liu et al. 2015, Yan et al. 2015, Diao et al. 2017, Guarnaccia et al. 2017). Although *Pyrus* is an important host genus for *Colletotrichum* spp., the *Colletotrichum* spp. associated with pear anthracnose remained largely unresolved, with only six individual species identified including *C. acutatum*, *C. aenigma*, *C. fioriniae*, *C. fructicola*, *C. pyricola*, and *C. salicis* (Damm et al. 2012b, Weir et al. 2012). Moreover, previous reports chiefly investigated morphology and ITS sequence data (Wu et al. 2010, Liu et al. 2013b), which is insufficient for distinguishing closely related taxa in several species complexes (Liu et al. 2016a). Additionally, most of the species reported from pear were based on small sample sizes from restricted areas, thus underestimating the species diversity on this host (Damm et al. 2012b, Weir et al. 2012).

In the genus *Pyrus*, *P. bretschneideri*, *P. communis*, *P. pyrifolia*, *P. sinkiangensis*, and *P. ussuriensis* are commercially cultivated (Wu et al. 2013). Of these, *P. bretschneideri*, *P. communis*, and *P. pyrifolia* represent the major cultivated species in China (Zhao et al. 2016). Pear is the third most widespread temperate fruit crop after apple and grape, with the largest production in China (Wu et al. 2013). The pear industry is also one of the most important fruit industries worldwide. Statistical data for 2016 indicated that pear-cultivation area was 1 121 675 ha, yielding 19.5 MT fruit in China, accounting for 70 % of the global pear fruit yield (FAO 2016). Furthermore, *Pyrus* also originated from the tertiary period (about 65 to 55 M yr ago) in western China, which represents one of the two subcentres for genetic diversity of this genus (Rubtsov 1944, Vavilov 1951, Zeven & Zhukovsky 1975, Wu et al. 2013, Silva et al. 2014).

Characterisation of the *Colletotrichum* spp. associated with *Pyrus* plants is expected to provide a better insight into the biology of this important genus. Moreover, pear anthracnose caused by *Colletotrichum* spp. is an important disease in major pear-cultivation areas of China, occurring in the growth and fruit maturation periods of pear, mainly damaging leaves and fruits. Pear anthracnose has led to substantial economic losses due to excessive fruit rot, or the severe suppression of tree growth. However, a detailed study and knowledge of the *Colletotrichum*

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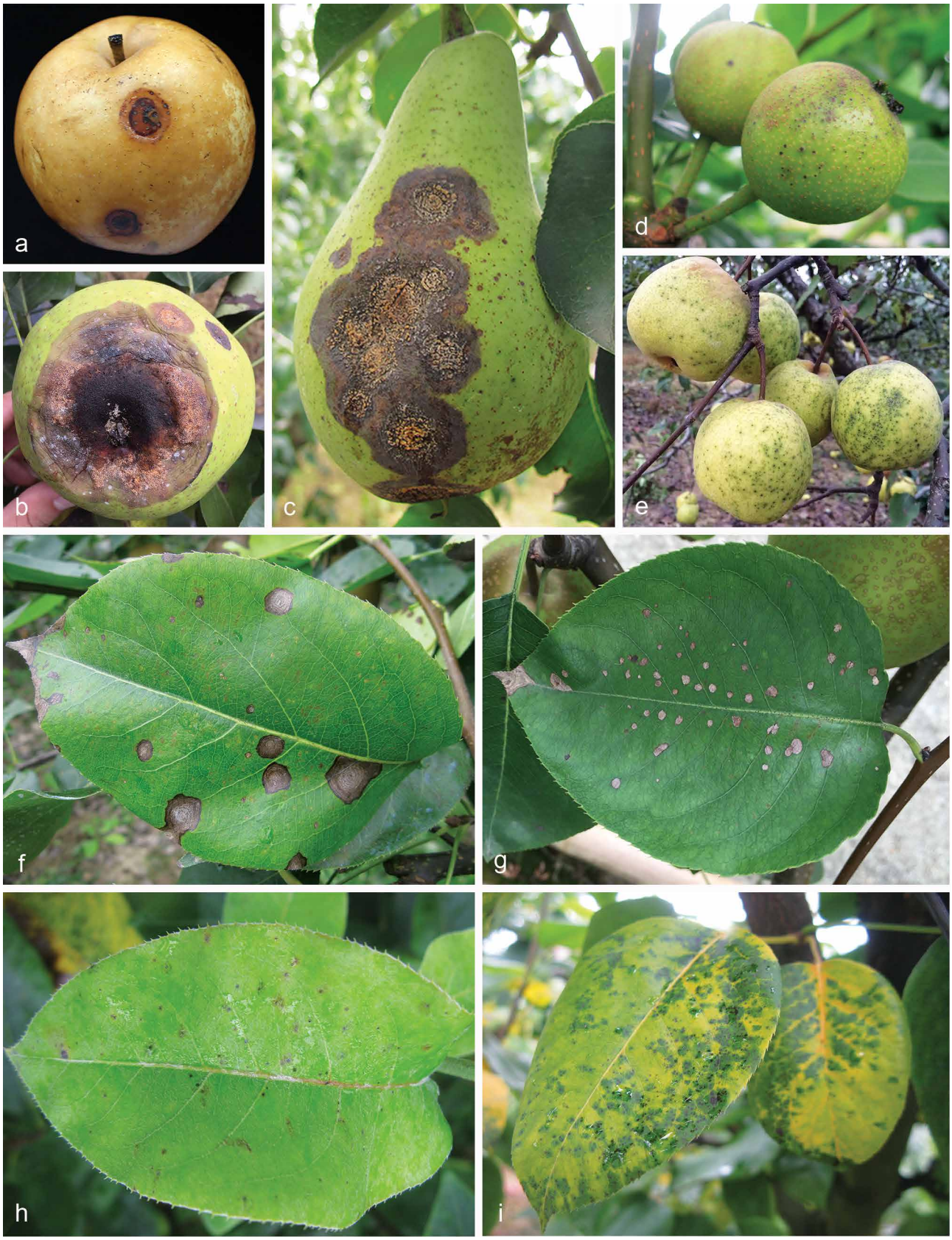

Fig. 1 Representative symptoms of pear anthracnose on fruits and leaves in the field. a–c. Symptoms of big sunken rot lesions (BrL; 10–35 mm diam) on fruits of *P. pyrifolia* (a, b) and *P. communis* cultivar (cv.) Gyuiot (c); d, e. symptoms of tiny black spots (TS; < 1 mm diam) on young pear fruits of *P. pyrifolia* cv. Cuiguan and mature pear fruit of *P. bretschneideri* cv. Huangguan, respectively; f. symptoms of big necrotic lesions (BnL; 5–10 mm diam) on leaves of *P. pyrifolia* cv. Xiangnan; g. symptoms of small round spots (SS; 3–4 mm diam) on leaves of *P. pyrifolia* cv. Jinshui No.1; h, i. initial and latter symptoms of TS on *P. pyrifolia* cv. Cuiguan.

spp. affecting pear production has been lacking in China and is also poorly documented worldwide.

The taxonomy of the genus *Colletotrichum* has in the past mainly relied on host range and morphological characters (Von Arx 1957, Sutton 1980), which is limited in species resolution (Cai et al. 2009, Hyde et al. 2009, Cannon et al. 2012). Recently, multi-locus phylogenetic analyses together with morphological characteristics have significantly influenced the classification and species concepts in *Colletotrichum* (Cai et al. 2009, Cannon et al. 2012, Damm et al. 2012a, b, 2013, 2014, 2019, Weir et al. 2012, Liu et al. 2013a, 2014, Vieira et al. 2014, Yan et al. 2015, Guarnaccia et al. 2017). Phylogenetic analyses based on multi-locus DNA sequence data and the application of Genealogical Concordance Phylogenetic Species Recognition (GCPSR) represent an enhanced ability for species resolution (Quaedvlieg et al. 2014, Liu et al. 2016a, Diao et al. 2017), e.g., *C. siamense* was previously assumed to be a species complex composed of several taxa (Yang et al. 2009, Wikee et al. 2011, Lima et al. 2013, Vieira et al. 2014, Sharma et al. 2015), but was shown to represent a single variable species in the *C. gloeosporioides* species complex (Weir et al. 2012, Liu et al. 2016a). Based on recent progress, 14 *Colletotrichum* species complexes and 15 singleton species have been identified (Marin-Felix et al. 2017, Damm et al. 2019).

The aims of the present study were as follows:

i. identify the prevalence of *Colletotrichum* spp. associated with *Pyrus* anthracnose in the major production provinces in China;
ii. validate the taxonomy of the *Colletotrichum* spp. through morphology, DNA phylogenetic analysis; and
iii. evaluate their pathogenicity by proving Koch's postulates.

MATERIALS AND METHODS

Sampling and isolation

A survey was conducted in 15 commercial pear orchards and four nurseries (Aug. 2013 to Oct. 2016) in the seven major pear-cultivation provinces (Anhui, Fujian, Hubei, Jiangsu, Jiangxi, Shandong, and Zhejiang) of China. Two kinds of symptoms were observed on fruit, namely 1) bitter rot showing big sunken rot lesions (BrL), 10–35 mm diam, with embedded concentric acervuli, secreting an orange conidial mass under humid conditions (Fig. 1a–c); and 2) tiny black spots (TS) less than 1 mm diam, gradually increasing in number instead of in size during the season (Fig. 1d, e). Three symptom types were observed on leaves, namely 1) big necrotic lesions (BnL); 2) small round spots (SS); and 3) TS. The BnL symptoms were characterised by sunken necrotic lesions 5–10 mm diam, brown in the centre but black along the margin, with black acervuli on the surface, secreting orange conidial tendrils under humid conditions (Fig. 1f). The SS symptoms were characterised by grey-white spots, 3–4 mm diam, circular to subcircular, grey-white in the centre, with a dark-brown margin (Fig. 1g). The TS symptoms were characterised by tiny black spots of less than 1 mm diam, which increased in number instead of in the size, accompanied by chlorosis, yellowing, and 'green island regions', resulting in defoliation (Fig. 1h, i).

Fruits and leaves showing the symptoms explained above were collected from pear trees of *P. pyrifolia* cultivars (cvs.) Cuiguan, Guanyangxueli, Hohsui, Huanghua, Huali No.1, Imamuraaki, Jinshui No. 1, Jinshui No. 2, and Xiangnan, *P. bretschneideri* cvs. Chili, Dangshansuli, Huangguan, Huangxianchangba, and Yali, and *P. communis* cv. Gyuiot in the surveyed orchards.

Fungi were isolated and linked to symptom types. Diseased tissues (neighbouring the asymptomatic regions) without sporulation were cut into small pieces (4–5 mm^2) after surface sterilisation (1 % NaOCl for 45 s, 75 % ethanol for 45 s, washed three times in sterile water and dried on sterilised filter paper; Photita et al. 2005). Excised tissues were placed onto potato dextrose agar (PDA, 20 % diced potato, 2 % glucose, and 1.5 % agar, and distilled water) plates and incubated at 28 °C. For diseased tissues with sporulation, conidia were collected, suspended in sterilised water, diluted to a concentration of 1×10^4 conidia per mL, and spread onto the surface of water agar (WA, 2 % agar, and distilled water) to generate discrete colonies (Choi et al. 1999). Six single colonies of each isolate were picked up with a sterilised needle (insect pin, 0.5 mm diam) and transferred onto PDA plates. Pure cultures were stored in 25 % glycerol at -80 °C until use. 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.

DNA extraction, PCR amplification and sequencing

Mycelial discs were transferred to PDA plates covered with sterile cellophane and incubated at 28 °C in the dark for 5–7 d. Fungal genomic DNA was extracted with cetyltrimethylammonium bromide (CTAB) buffer (2 % w/v CTAB, 1.42 M NaCl, 20 mM EDTA, 100 mM Tris·HCl, pH 8.0, 0.2 % (w/v) β-mercaptoethanol) as previously described (Freeman et al. 1996). Six loci including the 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS), a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and partial actin (*ACT*), beta-tubulin (*TUB2*), chitin synthase (*CHS-1*), and calmodulin (*CAL*) genes were amplified using the primer pairs ITS4/ITS5 (White et al. 1990), GDF1/GDR1 (Guerber et al. 2003), ACT-512F/ACT-783R (Carbone & Kohn 1999), T1/Bt2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997), CHS-79F/CHS-345R (Carbone & Kohn 1999), and CL1C/CL2C (Weir et al. 2012), respectively.

PCR amplification was conducted as described by Weir et al. (2012) but modified by using an annealing temperature of 56 °C for ITS, 59 °C for *ACT* and *GAPDH*, 58 °C for *TUB2* and *CHS-1*, and 57 °C for *CAL*. PCR amplicons were purified and sequenced at the Sangon Biotech (Shanghai, China) Company, Ltd. Forward and reverse sequences were assembled to obtain a consensus sequence with DNAMAN (v. 9.0; Lynnon Biosoft). Sequences generated in this study were deposited in GenBank (Table 1).

Phylogenetic analyses

Multiple sequences of concatenated *ACT*, *TUB2*, *CAL*, *CHS-1*, *GAPDH* and ITS sequences were aligned using MAFFT v. 7 (Katoh & Standley 2013) with default settings, and if necessary, manually adjusted in MEGA v. 7.0.1 (Kumar et al. 2016). Bayesian inference (BI) was used to construct phylogenies using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). MrModeltest v. 2.3 (Nylander 2004) was used to carry out statistical selection of best-fit models of nucleotide substitution using the corrected Akaike information criterion (AIC) (Table 2). Two analyses of four Markov Chain Monte Carlo (MCMC) chains were conducted from random trees with 1×10^7 generations for the *C. gloeosporioides* species complex, 3×10^6 for the *C. dematium* species complex and the related reference species involved in the same phylogenetic tree, and 2×10^6 generations for *C. acutatum* and *C. boninense* species complexes. The analyses were sampled every 1 000 generations, which were stopped once the average standard deviation of split frequencies was below 0.01. Convergence of all parameters was checked using the internal diagnostics of the standard deviation of split frequencies and performance scale reduction factors (PSRF),

Table 1 List of 90 representative isolates of 12 *Colletotrichum* spp. collected from pear in China, with details about host, symptoms, origins, and GenBank accession numbers.

Species	Isolate No.	Host	Symptoms	Origin	GenBank accession number					
					ITS	*GAPDH*	*CAL*	*ACT*	*CHS-1*	*TUB2*
C. aenigma	PAFQ1	*P. pyrifolia* cv. Xiangnan, leaf	BnL	Zhongxiang, Hubei	MG747997	MG747915	MG747769	MG747687	MG747833	MG748079
	PAFQ5	*P. pyrifolia* cv. Huali No.1, leaf	BnL	Zhongxiang, Hubei	MG747998	MG747916	MG747770	MG747688	MG747834	MG748080
	PAFQ21	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG747999	MG747917	MG747771	MG747689	MG747835	MG748081
	PAFQ23	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG748000	MG747918	MG747772	MG747690	MG747836	MG748082
	PAFQ24	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG748001	MG747919	MG747773	MG747691	MG747837	MG748083
	PAFQ45	*P. bretschneideri* cv. Yali, leaf	BnL	Yancheng, Jiangsu	MG748002	MG747920	MG747774	MG747692	MG747838	MG748084
	PAFQ47	*P. bretschneideri* cv. Chili, fruit	BrL	Yancheng, Jiangsu	MG748003	MG747921	MG747775	MG747693	MG747839	MG748085
	PAFQ64	*P. bretschneideri* cv. Huangguan, leaf	BnL	Dangshan, Anhui	MG748004	MG747922	MG747776	MG747694	MG747840	MG748086
	PAFQ66	*P. bretschneideri* cv. Huangguan, fruit	BrL	Dangshan, Anhui	MG748005	MG747923	MG747777	MG747695	MG747841	MG748087
	PAFQ81	*P. pyrifolia* cv. Guanyangxueli, leaf	SS	Hangzhou, Zhejiang	MG748006	MG747924	MG747778	MG747696	MG747842	MG748088
	PAFQ83	*P. pyrifolia* cv. Guanyangxueli, leaf	SS	Hangzhou, Zhejiang	MG748007	MG747925	MG747779	MG747697	MG747843	MG748089
C. citricola	PAFQ13	*P. pyrifolia*, leaf	BnL	Wuhan, Hubei	MG748062	MG747980	MG747819	MG747752	MG747898	MG748142
C. conoides	PAFQ6	*P. pyrifolia*, fruit	BrL	Wuhan, Hubei	MG748008	MG747926	MG747780	MG747698	MG747844	MG748090
C. fioriniae	PAFQ8	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG748047	MG747965	–	MG747737	MG747883	MG748128
	PAFQ9	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG748048	MG747966	–	MG747738	MG747884	–
	PAFQ10	*P. pyrifolia* cv. Jinshui No.2, leaf	SS	Wuhan, Hubei	MG748049	MG747967	–	MG747739	MG747885	MG748129
	PAFQ11	*P. pyrifolia* cv. Jinshui No.2, leaf	SS	Wuhan, Hubei	MG748050	MG747968	–	MG747740	MG747886	MG748130
	PAFQ12	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG748051	MG747969	–	MG747741	MG747887	MG748131
	PAFQ17	*P. pyrifolia*, fruit	BrL	Wuhan, Hubei	MG748052	MG747970	–	MG747742	MG747888	MG748132
	PAFQ18	*P. pyrifolia*, fruit	BrL	Wuhan, Hubei	MG748053	MG747971	–	MG747743	MG747889	MG748133
	PAFQ19	*P. pyrifolia*, fruit	BrL	Wuhan, Hubei	MG748054	MG747972	–	MG747744	MG747890	MG748134
	PAFQ34	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jianning, Fujian	MG748055	MG747973	–	MG747745	MG747891	MG748135
	PAFQ35	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jianning, Fujian	MG748056	MG747974	–	MG747746	MG747892	MG748136
	PAFQ36	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jianning, Fujian	MG748057	MG747975	–	MG747747	MG747893	MG748137
	PAFQ49	*P. pyrifolia*, fruit	BrL	Nanjing, Jiangsu	MG748060	MG747978	–	MG747750	MG747896	MG748140
	PAFQ50	*P. pyrifolia*, fruit	BrL	Nanjing, Jiangsu	MG748061	MG747979	–	MG747751	MG747897	MG748141
	PAFQ55	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jinxi, Jiangxi	MG748058	MG747976	–	MG747748	MG747894	MG748138
	PAFQ75	*P. communis* cv. Gyuiot, fruit	BrL	Yantai, Shandong	MG748059	MG747977	–	MG747749	MG747895	MG748139
C. fructicola	PAFQ20	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG748011	MG747929	MG747783	MG747701	MG747847	MG748093
	PAFQ25	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG748012	MG747930	MG747784	MG747702	MG747848	MG748094
	PAFQ31	*P. pyrifolia* cv. Cuiguan, leaf	TS	Jianning, Fujian	MG748013	MG747931	MG747785	MG747703	MG747849	MG748095
	PAFQ32	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jianning, Fujian	MG748014	MG747932	MG747786	MG747704	MG747850	MG748096
	PAFQ33	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jianning, Fujian	MG748015	MG747933	MG747787	MG747705	MG747851	MG748097
	PAFQ46	*P. bretschneideri* cv. Yali, leaf	BnL	Yancheng, Jiangsu	MG748016	MG747934	MG747788	MG747706	MG747852	MG748098
	PAFQ48	*P. bretschneideri* cv. Dangshanshuli, fruit	TS	Yancheng, Jiangsu	MG748017	MG747935	MG747789	MG747707	MG747853	MG748099
	PAFQ51	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jiangxi	MG748018	MG747936	MG747790	MG747708	MG747854	MG748100
	PAFQ57	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jinxi, Jiangxi	MG748019	MG747937	MG747791	MG747709	MG747855	MG748101
	PAFQ62	*P. bretschneideri* cv. Huangguan, leaf	BnL	Dangshan, Anhui	MG748020	MG747938	MG747792	MG747710	MG747856	MG748102
	PAFQ63	*P. bretschneideri* cv. Huangguan, leaf	BnL	Dangshan, Anhui	MG748021	MG747939	MG747793	MG747711	MG747857	MG748103
	PAFQ77	*P. pyrifolia* cv. Guangyangxueli, leaf	BnL	Hangzhou, Zhejiang	MG748023	MG747941	MG747795	MG747713	MG747859	MG748105
	PAFQ79	*P. pyrifolia* cv. Guanyangxueli, leaf	BnL	Hangzhou, Zhejiang	MG748024	MG747942	MG747796	MG747714	MG747860	MG748106
	PAFQ84	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Tonglu, Zhejiang	MG748022	MG747940	MG747794	MG747712	MG747858	MG748104
C. gloeosporioides	PAFQ7	*P. bretschneideri* cv. Huangxianchangba, leaf	BnL	Wuhan, Hubei	MG748025	MG747943	MG747797	MG747715	MG747861	MG748107
	PAFQ27	*P. pyrifolia* cv. Hohsui, leaf	SS	Wuhan, Hubei	MG748026	MG747944	MG747798	MG747716	MG747862	MG748108
	PAFQ29	*P. pyrifolia* cv. Hohsui, leaf	SS	Wuhan, Hubei	MG748027	MG747945	MG747799	MG747717	MG747863	MG748109
	PAFQ44	*P. bretschneideri* cv. Yali, leaf	SS	Yancheng, Jiangsu	MG748028	MG747946	MG747800	MG747718	MG747864	MG748110

Table 1 (cont.)

Species	Isolate No.	Host	Symptoms	Origin	GenBank accession number					
					ITS	*GAPDH*	*CAL*	*ACT*	*CHS-1*	*TUB2*
C. gloeosporioides (cont.)	PAFQ56	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jinxi, Jiangxi	MG748029	MG747947	MG747801	MG747719	MG747865	MG748111
	PAFQ58	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jinxi, Jiangxi	MG748030	MG747948	MG747802	MG747720	MG747866	MG748112
	PAFQ59	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jinxi, Jiangxi	MG748031	MG747949	MG747803	MG747721	MG747867	MG748113
	PAFQ60	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jinxi, Jiangxi	MG748032	MG747950	MG747804	MG747722	MG747868	MG748114
	PAFQ61	*P. pyrifolia* cv. Huanghua, fruit	BrL	Jinxi, Jiangxi	MG748033	MG747951	MG747805	MG747723	MG747869	MG748115
	PAFQ80	*P. pyrifolia* cv. Guangyangxueli, leaf	SS	Hangzhou, Zhejiang	MG748035	MG747953	MG747807	MG747725	MG747871	MG748117
	PAFQ86	*P. pyrifolia*, leaf	BnL	Hangzhou, Zhejiang	MG748034	MG747952	MG747806	MG747724	MG747870	MG748116
C. jinshuiense	PAFQ26, CGMCC 3.18903*	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG748077	MG747995	–	MG747767	MG747913	MG748157
	PAFQ26a	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG874830	MG874822	–	MG874807	MG874814	MG874838
	PAFQ26b	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG874831	MG874823	–	MG874808	MG874815	MG874839
	PAFQ26c	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG874832	MG874824	–	–	MG874816	MG874840
	PAFQ26d	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG874833	MG874825	–	MG874809	MG874817	MG874841
C. karstii	PAFQ14	*P. pyrifolia*, leaf	BnL	Wuhan, Hubei	MG748063	MG747981	MG747820	MG747753	MG747899	MG748143
	PAFQ15	*P. pyrifolia*, leaf	BnL	Wuhan, Hubei	MG748064	MG747982	MG747821	MG747754	MG747900	MG748144
	PAFQ16	*P. pyrifolia*, leaf	BnL	Wuhan, Hubei	MG748065	MG747983	MG747822	MG747755	MG747901	MG748145
	PAFQ28	*P. pyrifolia* cv. Hohsui, leaf	BnL	Wuhan, Hubei	MG748066	MG747984	MG747823	MG747756	MG747902	MG748146
	PAFQ37	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jianning, Fujian	MG748067	MG747985	MG747824	MG747757	MG747903	MG748147
	PAFQ38	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jianning, Fujian	MG748068	MG747986	MG747825	MG747758	MG747904	MG748148
	PAFQ39	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jianning, Fujian	MG748069	MG747987	MG747826	MG747759	MG747905	MG748149
	PAFQ40	*P. pyrifolia* cv. Huanghua, leaf	BnL	Jianning, Fujian	MG748070	MG747988	MG747827	MG747760	MG747906	MG748150
	PAFQ41	*P. pyrifolia* cv. Huanghua, leaf	BnL	Jianning, Fujian	MG748071	MG747989	MG747828	MG747761	MG747907	MG748151
	PAFQ42	*P. pyrifolia* cv. Huanghua, leaf	BnL	Jianning, Fujian	MG748072	MG747990	MG747829	MG747762	MG747908	MG748152
	PAFQ43	*P. pyrifolia* cv. Huanghua, leaf	BnL	Jianning, Fujian	MG748073	MG747991	MG747830	MG747763	MG747909	MG748153
	PAFQ52	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jinxi, Jiangxi	MG748074	MG747992	MG747831	MG747764	MG747910	MG748154
	PAFQ82	*P. pyrifolia* cv. Guanyangxueli, leaf	BnL	Hangzhou, Zhejiang	MG748075	MG747993	MG747832	MG747765	MG747911	MG748155
C. plurivorum	PAFQ65	*P. bretschneideri* cv. Huangguan, leaf	BnL	Dangshan, Anhui	MG748076	MG747994	–	MG747766	MG747912	MG748156
C. pyrifolia	PAFQ22, CGMCC 3.18902*	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG748078	MG747996	–	MG747768	MG747914	MG748158
	PAFQ22a	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG874834	MG874826	–	MG874810	MG874818	MG874842
	PAFQ22b	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG874835	MG874827	–	MG874811	MG874819	MG874843
	PAFQ22c	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG874836	MG874828	–	MG874812	MG874820	MG874844
	PAFQ22d	*P. pyrifolia* cv. Jinshui No.1, leaf	SS	Wuhan, Hubei	MG874837	MG874829	–	MG874813	MG874821	MG874845
C. siamense	PAFQ67	*P. communis* cv. Gyuiot, fruit	BrL	Yantai, Shandong	MG748036	MG747954	MG747808	MG747726	MG747872	MG748118
	PAFQ68	*P. communis* cv. Gyuiot, fruit	BrL	Yantai, Shandong	MG748037	MG747955	MG747809	MG747727	MG747873	MG748119
	PAFQ69	*P. communis* cv. Gyuiot, fruit	BrL	Yantai, Shandong	MG748038	MG747956	MG747810	MG747728	MG747874	MG748120
	PAFQ70	*P. communis* cv. Gyuiot, fruit	BrL	Yantai, Shandong	MG748039	MG747957	MG747811	MG747729	MG747875	MG748121
	PAFQ71	*P. communis* cv. Gyuiot, fruit	BrL	Yantai, Shandong	MG748040	MG747958	MG747812	MG747730	MG747876	MG748122
	PAFQ72	*P. communis* cv. Gyuiot, fruit	BrL	Yantai, Shandong	MG748041	MG747959	MG747813	MG747731	MG747877	MG748123
	PAFQ73	*P. communis* cv. Gyuiot, fruit	BrL	Yantai, Shandong	MG748042	MG747960	MG747814	MG747732	MG747878	MG748124
	PAFQ74	*P. communis* cv. Gyuiot, fruit	BrL	Yantai, Shandong	MG748043	MG747961	MG747815	MG747733	MG747879	MG748125
	PAFQ76	*P. communis* cv. Gyuiot, fruit	BrL	Yantai, Shandong	MG748044	MG747962	MG747816	MG747734	MG747880	–
	PAFQ78	*P. pyrifolia* cv. Guanyangxueli, leaf	BnL	Hangzhou, Zhejiang	MG748046	MG747964	MG747818	MG747736	MG747882	MG748127
	PAFQ85	*P. pyrifolia*, leaf	BnL	Hangzhou, Zhejiang	MG748045	MG747963	MG747817	MG747735	MG747881	MG748126
C. wuxiense	PAFQ53	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jinxi, Jiangxi	MG748009	MG747927	MG747781	MG747699	MG747845	MG748091
	PAFQ54	*P. pyrifolia* cv. Cuiguan, leaf	BnL	Jinxi, Jiangxi	MG748010	MG747928	MG747782	MG747700	MG747846	MG748092

* = Ex-type culture.
BrL: big sunken rot lesions; BnL: big necrotic lesions; SS: small round spots; TS: tiny black spots.

Table 2 Nucleotide substitution models used in the phylogenetic analyses.

Gene	Gloeosporioides clade	Acutatum clade	Boninense clade	Dematium clade and other taxa
ITS	GTR+I+G	GTR+I	SYM+I+G	GTR+I+G
ACT	GTR+G	HKY+G	HKY+G	HKY+I+G
GAPDH	HKY+G	GTR+G	HKY+I	HKY+I+G
TUB2	SYM+G	GTR+G	HKY+I	HKY+I+G
CHS-1	K80+I	SYM+G	GTR+I	GTR+I+G
CAL	GTR+I+G		HKY+I	

and then externally with Tracer v. 1.6 (Rambaut et al. 2013). The first 25 % of trees were discarded as the burn-in phase of each analysis and posterior probabilities determined from the remaining trees. Additionally, maximum parsimony analyses (MP) were performed on the multi-locus alignment using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). Phylogenetic trees were generated using the heuristic search option with Tree Bisection Reconnection (TBR) branch swapping and 1 000 random sequence additions. Maxtrees were set up to 5 000, branches of zero length collapsed, and all multiple parsimonious trees were saved. Clade stability was assessed using a bootstrap analysis with 1 000 replicates. Afterwards, tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated. Furthermore, maximum likelihood (ML) analyses were implemented on the multi-locus alignments using the RaxmlGUI v. 1.3.1 (Silvestro & Michalak 2012). Clade stability was assessed using bootstrap analyses with 1 000 replicates. A general time reversible model (GTR) was applied with an invgamma-distributed rate variation. Phylogenetic trees were visualised in FigTree v. 1.4.2 (Rambaut 2014). The alignments and phylogenetic trees were deposited in TreeBASE (study 22264).

For the phylogenetically close but not clearly delimited species, sequences were analysed using the GCPSR model by performing a pairwise homoplasy index (PHI) test as described by Quaedvlieg et al. (2014). The PHI test was performed in SplitsTree 4 (Huson 1998, Huson & Kloepper 2005, Huson & Bryant 2006) to determine the recombination level within phylogenetically closely related species using a six-locus concatenated dataset (*ACT*, *TUB2*, *CAL*, *CHS-1*, *GAPDH*, and ITS). If the resulting pairwise homoplasy index was below a 0.05 threshold ($\Phi w < 0.05$), it was indicative of significant recombination in the dataset. The relationship between closely related species was visualised by constructing a splits graph.

Morphological analysis

Morphological and cultural features were characterised according to Yan et al. (2015). Briefly, mycelial discs (5 mm diam) were taken from the growing edge of 5-d-old cultures in triplicate, transferred on PDA, oatmeal agar (OA; Crous et al. 2009) and synthetic nutrient-poor agar medium (SNA; Nirenberg 1976), and incubated in the dark at 28 °C. Colony diameters were measured daily for 5 d to calculate their mycelial growth rates (mm/d). The shape, colour and density of colonies were recorded after 6 d. Moreover, the shape, colour and size of sporocarps, conidia, conidiophores, asci and ascospores were observed using light microscopy (Nikon Eclipse 90i or Olympus BX63, Japan), and 50 conidia or ascospores were measured to determine their sizes unless no or less spores were produced. Conidial appressoria were induced by dropping a conidial suspension (10^6 conidia/mL; 50 µL) on a concavity slide, placed inside plates containing moistened filter papers with distilled sterile water, and then incubated at 25 °C in the dark. After incubating for 24 to 48 h, the sizes of 30 conidial appressoria formed at the ends of germ tubes were measured (Yang et al. 2009).

Prevalence

To determine the prevalence of *Colletotrichum* species in sampled provinces, the *Pyrus* spp. and pear organ (leaf or fruit) involved were established. The Isolation Rate (R^I) was calculated for each species with the formula, $R^I\ \% = (N^S / N^I) \times 100$, where N^S was the number of isolates from the same species, and N^I was the total number of isolates from each sample-collected province, *Pyrus* sp. or pear organ (Vieira et al. 2014, Wang et al. 2016). The overall R^I was calculated using the N^I value equal to the total number of isolates obtained from pear plants.

Pathogenicity tests

Representative *Colletotrichum* isolates were selected for pathogenicity tests with a spore suspension on detached leaves (approx. 4-wk-old) of *P. pyriforia* cv. Cuiguan in eight replicates as previously described (Cai et al. 2009). Briefly, tender healthy-looking leaves were collected, washed three times with sterile water, and air-dried on sterilised filter paper. The leaves are inoculated using the wound/drop and non-wound/drop inoculation methods (Lin et al. 2002, Kanchana-udomkan et al. 2004, Than et al. 2008). For the wound/drop method, an aliquot of 6 µL of spore suspension (1.0×10^6 conidia or ascospores per mL) was dropped on the left side of a leaf after wounding once by pin-pricking with a sterilised needle (insect pin, 0.5 mm diam), and sterile water on the right side of the same leaf in parallel as control. For non-wound/drop method, the spore suspension was dropped on the left side of a leaf without being unwounded, and sterile water on the right side of the same leaf in parallel as control. The infection rates were calculated using the formula (infection rate = the number of infected leaves or fruits/the number of inoculated leaves or fruits) at 14 d post inoculation (dpi) (Huang et al. 2013).

Additionally, pathogenicity was also determined on detached mature pear fruits of *P. bretschneideri* cv. Huangguan in triplicate as previously described (Cai et al. 2009). Briefly, healthy fruits were surface-sterilised with 1 % sodium hypochlorite for 5 min, washed three times with sterile water, and air-dried. Wound/drop and non-wound/drop inoculation methods were also used (Lin et al. 2002, Kanchana-udomkan et al. 2004, Than et al. 2008). For the wound/drop method, an aliquot of 6 µL of spore suspension (1×10^6 conidia or ascospores per mL) was dropped on the fruits after wounding three times by pin-pricking with a sterilised needle (5 mm deep). For the non-wound/drop method, the same spore suspension was also directly dropped on the surface of unwounded pear fruits. Sterile water was dropped on the fruit in parallel as control. Symptom development under wounded conditions was evaluated by determining the mean lesion lengths at 10 dpi. Symptom development on fruits was studied by determining the infection rates at 30 dpi using the aforementioned formula.

After inoculation, the detached leaves and fruits were put on plastic trays, covered with plastic wrap to maintain a 99 % relative humidity, and incubated at 25 °C with a 12/12 h light/dark photoperiod. Pathogens were re-isolated from the resulting

Table 3 List of isolates of the *Colletotrichum* species used in this study, with details about host/substrate, country, and GenBank accession numbers.

Species	Culture[x]	Host/Substrate	Country	GenBank accession number					
				ITS	*GAPDH*	*CAL*	*ACT*	*CHS-1*	*TUB2*
C. abscissum	COAD 1877*	*Citrus sinensis* cv. Pera	Brazil	KP843126	KP843129	–	KP843141	KP843132	KP843135
C. acerbum	CBS 128530*	*Malus domestica*	New Zealand	JQ948459	JQ948790	–	JQ949780	JQ949120	JQ950110
C. acutatum	CBS 112996*	*Carica papaya*	Australia	JQ005776	JQ948677	–	JQ005839	JQ005797	JQ005860
C. aenigma	ICMP 18608*	*Persea americana*	Israel	JX010244	JX010044	JX009683	JX009443	JX009774	JX010389
	ICMP 18686	*Pyrus pyrifolia*	Japan	JX010243	JX009913	JX009684	JX009519	JX009789	JX010390
C. aeschynomenes	ICMP 17673*	*Aeschynomene virginica*	USA	JX010176	JX009930	JX009721	JX009483	JX009799	JX010392
C. agaves	CBS 118190	*Agave striate*	Mexico	DQ286221	–	–	–	–	–
C. alatae	CBS 304.67*	*Dioscorea alata*	India	JX010190	JX009990	JX009738	JX009471	JX009837	JX010383
C. alienum	ICMP 12071*	*Malus domestica*	New Zealand	JX010251	JX010028	JX009654	JX009572	JX009882	JX010411
C. annellatum	CBS 129826*	*Hevea brasiliensis*, leaf	Colombia	JQ005222	JQ005309	JQ005743	JQ005570	JQ005396	JQ005656
C. anthrisci	CBS 125334*	*Anthriscus sylvestris*,dead stem	Netherlands	GU227845	GU228237	–	GU227943	GU228335	GU228139
	CBS 125335	*Anthriscus sylvestris*,dead stem	Netherlands	GU227846	GU228238	–	GU227944	GU228336	GU228140
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
C. australe	CBS 116478*	*Trachycarpus fortunei*	South Africa	JQ948455	JQ948786	–	JQ949776	JQ949116	JQ950106
C. beeveri	CBS 128527*	*Brachyglottis repanda*	New Zealand	JQ005171	JQ005258	JQ005692	JQ005519	JQ005345	JQ005605
C. boninense	CBS 123755*	*Crinum asiaticum* var. *sinicum*	Japan	JQ005153	JQ005240	JQ005674	JQ005501	JQ005327	JQ005588
	CBS 128506	*Solanum lycopersicum*, fruit rot	New Zealand	JQ005157	JQ005244	JQ005678	JQ005505	JQ005331	JQ005591
C. brasiliense	CBS 128501*	*Passiflora edulis*, fruit anthracnose	Brazil	JQ005235	JQ005322	JQ005756	JQ005583	JQ005409	JQ005669
C. brassicicola	CBS 101059*	*Brassica oleracea*, leaf spot	New Zealand	JQ005172	JQ005259	JQ005693	JQ005520	JQ005346	JQ005606
C. brevisporum	BCC 38876*	*Neoregalia* sp.	Thailand	JN050238	JN050238	–	JN050216	KF687760	JN050244
C. brisbanense	CBS 292.67*	*Capsicum annuum*	Australia	JQ948291	JQ948621	–	JQ949612	JQ948952	JQ949942
C. cairnsense	BRIP 63642*	*Capsicum annuum*	Australia	KU923672	KU923704	–	KU923716	KU923710	KU923688
C. camelliae-japonicae	CGMCC 3.18118*	*Camellia japonica*	Japan	KX853165	KX893584	–	KX893576	–	KX893580
	CGMCC 3.18117	*Camellia japonica*	Japan	KX853164	KX893583	–	KX893575	–	KX893579
C. carthami	SAPA100011*	*Carthamus tinctorium*	Japan	AB696998	–	–	–	–	AB696992
C. cattleyicola	CBS 170.49*	*Cattleya* sp.	Belgium	MG600758	MG600819	–	MG600963	MG600866	MG601025
C. chlorophyti	IMI 103806*	*Chlorophytum* sp.	India	GU227894	GU228286	–	GU227992	GU228384	GU228188
C. chrysanthemi	IMI 364540	*Chrysanthemum coronarium*, leaf spot	China	JQ948273	JQ948603	–	JQ949594	JQ948934	JQ949924
C. circinans	CBS 221.81*	*Allium cepa*	Serbia	GU227855	GU228247	–	GU227953	GU228345	GU228149
C. citricola	CBS 134228*	*Citrus unshiu*	China	KC293576	KC293736	KC293696	KC293616	KC293696	KC293656
	CBS 134229	*Citrus unshiu*	China	KC293577	KC293737	KC293697	KC293617	KC293793	KC293657
	CBS 134230	*Citrus unshiu*	China	KC293578	KC293738	KC293698	KC293618	KC293794	KC293658
C. clidemiae	ICMP 18658*	*Clidemia hirta*	USA, Hawaii	JX010265	JX009989	JX009645	JX009537	JX009877	JX010438
C. cliviicola	CBS 125375*	*Clivia miniata*	China	JX519223	JX546611	–	JX519240	JX519232	JX519249
	CSSS1	*Clivia miniata*	China	GU109479	GU085867	–	GU085861	GU085865	GU085869
	CSSS2	*Clivia miniata*	China	GU109480	GU085868	–	GU085862	GU085866	GU085870
C. colombiense	CBS 129818*	*Passiflora edulis*, leaf	Colombia	JQ005174	JQ005261	JQ005695	JQ005522	JQ005348	JQ005608
C. conoides	CGMCC 3.17615*	*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. constrictum	CBS 128504*	*Citrus limon*, fruit rot	New Zealand	JQ005238	JQ005325	JQ005759	JQ005586	JQ005412	JQ005672
C. cordylinicola	ICMP 18579*	*Cordyline fruticosa*	Thailand	JX010226	JX009975	HM470238	HM470235	JX009864	JX010440
C. cosmi	CBS 853.73*	*Cosmos* sp., seed	Netherlands	JQ948274	JQ948604	–	JQ949595	JQ948935	JQ949925
C. costaricense	CBS 330.75*	*Coffea arabica*, cv. Typica, berry	Costa Rica	JQ948180	JQ948510	–	JQ949501	JQ948841	JQ949831
C. curcumae	IMI 288937*	*Curcuma longa*	India	GU227893	GU228285	–	GU227991	GU228383	GU228187

Table 3 (cont.)

Species	Culture[x]	Host/Substrate	Country	GenBank accession number					
				ITS	*GAPDH*	*CAL*	*ACT*	*CHS-1*	*TUB2*
C. cuscutae	IMI 304802*	*Cuscuta* sp.	Dominica	JQ948195	JQ948525	–	JQ949516	JQ948856	JQ949846
C. cymbidiicola	IMI 347923*	*Cymbidium* sp., leaf lesion	Australia	JQ005166	JQ005253	JQ005687	JQ005514	JQ005340	JQ005600
C. dacrycarpi	CBS 130241*	*Dacrycarpus dacrydioides*, leaf endophyte	New Zealand	JQ005236	JQ005323	JQ005757	JQ005584	JQ005410	JQ005670
C. dematium	CBS 125.25*	*Eryngium campestre*,dead leaf	France	GU227819	GU228211	–	GU227917	GU228309	GU228113
	CBS 123728	*Genista tinctoria*, leaf spot	Czech Republic	GU227822	GU228214	–	GU227920	GU228312	GU228116
C. dracaenophilum	CBS 118199*	*Dracaena* sp.	China	JX519222	JX546707	–	JX519238	JX519230	JX519247
C. euphorbiae	CBS 134725*	*Euphorbia* sp.	South Africa	KF777146	KF777131	–	KF777125	KF777128	KF777247
C. fioriniae	CBS 125396	*Malus domestica*, fruit lesion	USA	JQ948299	JQ948629	–	JQ949620	JQ948960	JQ949950
	IMI 324996	*Malus pumila*	USA	JQ948301	JQ948631	–	JQ949622	JQ948962	JQ949952
	CBS 126526	*Primula* sp., leaf spots	Netherlands	JQ948323	JQ948653	–	JQ949644	JQ948984	JQ949974
	CBS 124958	*Pyrus* sp., fruit rot	USA	JQ948306	JQ948636	–	JQ949627	JQ948967	JQ949957
	IMI 504882	*Fragaria* × *ananassa*	New Zealand	KT153562	KT153552	–	KT153542	KT153547	KT153567
	CBS 129938	*Malus domestica*	USA	JQ948296	JQ948626	–	JQ949617	JQ948957	JQ949947
	CBS 119292	*Vaccinium* sp., fruit	New Zealand	JQ948313	JQ948643	–	JQ949634	JQ948974	JQ949964
	CBS 129930	*Malus domestica*	New Zealand	JQ948304	JQ948634	–	JQ949625	JQ948965	JQ949955
	ATCC 28992	*Malus domestica*	USA	JQ948297	JQ948627	–	JQ949618	JQ948958	JQ949948
C. fructi	CBS 346.37*	*Malus sylvestris*, fruit	USA	GU227844	GU228236	–	GU227942	GU228334	GU228138
C. fructicola	ICMP 18581*	*Coffea arabica*	Thailand	JX010165	JX010033	FJ917508	FJ907426	JX009866	JX010405
	ICMP 18613	*Limonium sinuatum*	Israel	JX010167	JX009998	JX009675	JX009491	JX009772	JX010388
	ICMP 18645	*Theobroma cacao*	Panama	JX010172	JX009992	JX009666	JX009543	JX009873	JX010408
	ICMP 18727	*Fragaria* × *ananassa*	USA	JX010179	JX010035	JX009682	JX009565	JX009812	JX010394
	ICMP 18120	*Dioscorea alata*	Nigeria	JX010182	JX010041	JX009670	JX009436	JX009844	JX010401
C. fructicola (syn. *C. ignotum*)	ICMP 18646*	*Tetragastris panamensis*	Panama	JX010173	JX010032	JX009674	JX009581	JX009874	JX010409
C. fructicola (syn. *Glomerella cingulata* var. *minor*)	ICMP 17921*	*Ficus edulis*	Germany	JX010181	JX009923	JX009671	JX009495	JX009839	JX010400
C. fructivorum	CBS 133125*	*Vaccinium macrocarpon*	USA	JX145145	–	–	–	–	JX145196
	CBS 133135	*Rhexia virginica*	USA	JX145133	–	–	–	–	JX145184
C. gloeosporioides	IMI 356878*	*Citrus sinensis*	Italy	JX010152	JX010056	JX009731	JX009531	JX009818	JX010445
	ICMP 12939	*Citrus* sp.	New Zealand	JX010149	JX009931	JX009728	JX009462	JX009747	–
	ICMP 18695	*Citrus* sp.	USA	JX010153	JX009979	JX009735	JX009494	JX009779	–
	ICMP 18694	*Mangifera indica*	South Africa	JX010155	JX009980	JX009729	JX009481	JX009796	–
C. gloeosporioides (syn. *Gloeosporium pedemontanum*)	ICMP 19121*	*Citrus limon*	Italy	JX010148	JX010054	JX009745	JX009558	JX009903	–
C. godetiae	CBS 133.44*	*Clarkia hybrida*	Denmark	JQ948402	JQ948733	–	JQ949723	JQ949063	JQ950053
C. hebeiense	JZB330024	*Vitis vinifera* cv. Cabernet Sauvignon	China	KF156873	KF377505	–	KF377542	–	–
	CGMCC 3.17464*	*Vitis vinifera* cv. Cabernet Sauvignon	China	KF156863	KF377495	–	KF377532	KF289008	KF288975
C. hemerocallidis	CDLG5*	*Hemerocallis fulva* var. *kwanso*	China	JQ400005	JQ400012	–	JQ399991	JQ399998	JQ400019
C. hippeastri	CBS 125376*	*Hippeastrum vittatum*, leaf	China	JQ005231	JQ005318	JQ005752	JQ005579	JQ005405	JQ005665
C. horii	ICMP 10492*	*Diospyros kaki*	Japan	GQ329690	GQ329681	JX009604	JX009438	JX009752	JX010450
C. insertae	MFLU 15-1895*	*Parthenocissus inserta*	Russia	KX618686	KX618684	–	KX618682	KX618683	KX618685
C. jasminigenum	MFLUCC 10-0273	*Jasminum sambac*	Vietnam	HM131513	HM131499	–	HM131508	–	HM153770
C. jiangxiense	CGMCC 3.17362	*Camellia sinensis*, endophyte	China	KJ955198	KJ954899	KJ954749	KJ954469	–	KJ955345
	CGMCC 3.17363*	*Camellia sinensis*, pathogen	China	KJ955201	KJ954902	KJ954752	KJ954471	–	KJ955348
C. johnstonii	CBS 128532*	*Solanum lycopersicum*, fruit rot	New Zealand	JQ948444	JQ948775	–	JQ949765	JQ949105	JQ950095
C. kahawae subsp. *ciggaro*	ICMP 18539*	*Olea europaea*	Australia	JX010230	JX009966	JX009635	JX009523	JX009800	JX010434
	ICMP 18534	*Kunzea ericoides*	New Zealand	JX010227	JX009904	JX009634	JX009473	JX009765	JX010427
	ICMP 12952	*Persea americana*	New Zealand	JX010214	JX009971	JX009648	JX009431	JX009757	JX010426
C. kahawae subsp. *kahawae*	IMI 319418*	*Coffea arabica*	Kenya	JX010231	JX010012	JX009642	JX009452	JX009813	JX010444

Table 3 (cont.)

Species	Culture[x]	Host/Substrate	Country	GenBank accession number					
				ITS	*GAPDH*	*CAL*	*ACT*	*CHS-1*	*TUB2*
C. kahawae subsp. *kahawae* (cont.)	ICMP 17905	*Coffea arabica*	Cameroon	JX010232	JX010046	JX009644	JX009561	JX009816	JX010431
	ICMP 17915	*Coffea arabica*	Angola	JX010234	JX010040	JX009638	JX009474	JX009829	JX010435
C. karstii	CBS 113087	*Malus* sp.	USA	JQ005181	JQ005268	JQ005702	JQ005529	JQ005355	JQ005615
	CBS 128524	*Citrullus lanatus*, rotten fruit	New Zealand	JQ005195	JQ005282	JQ005716	JQ005543	JQ005369	JQ005629
	CBS 128551	*Citrus* sp.	New Zealand	JQ005208	JQ005295	JQ005729	JQ005556	JQ005382	JQ005642
	CBS 129832	*Musa* sp.	Mexico	JQ005177	JQ005264	JQ005698	JQ005525	JQ005351	JQ005611
	CBS 129824	*Musa* AAA, fruit	Colombia	JQ005215	JQ005302	JQ005736	JQ005563	JQ005389	JQ005649
	CBS 128552	*Synsepalum dulcificum*, leaves	Taiwan	JQ005188	JQ005275	JQ005709	JQ005536	JQ005362	JQ005622
C. kinghornii	CBS 198.35*	*Phormium* sp.	UK	JQ948454	JQ948785	–	JQ949775	JQ949115	JQ950105
C. laticiphilum	CBS 112989*	*Hevea brasiliensis*	India	JQ948289	JQ948619	–	JQ949610	JQ948950	JQ949940
C. ledebouriae	CBS 141284*	*Ledebouria floridunda*	South Africa	KX228254	–	–	KX228357	–	–
C. liaoningense	CGMCC 3.17616*	*Capsicum* sp.	China	KP890104	KP890135	–	KP890097	KP890127	KP890111
C. lindemuthianum	CBS 144.31*	*Phaseolus vulgaris*	Germany	JQ005779	JX546712	–	JQ005842	JQ005800	JQ005863
C. lineola	CBS 125337*	*Apiaceae*, dead stem	Czech Republic	GU227829	GU228221	–	GU227927	GU228319	GU228123
	CBS 124.25	*Trillium* sp., leaf spot	Czech Republic	GU227836	GU228228	–	GU227934	GU228326	GU228130
C. lupini	CBS 109225*	*Lupinus albus*	Ulkraine	JQ948155	JQ948485	–	JQ949476	JQ948816	JQ949806
C. magnum	CBS 519.97*	*Citrullus lanatus*	USA	MG600769	MG600829	–	MG600973	MG600875	MG601036
C. menispermi	MFLU 14-0625*	*Menispermum dauricum*	Russia	KU242357	KU242356	–	KU242353	KU242355	KU242354
C. musae	CBS 116870*	*Musa* sp.	USA	JX010146	JX010050	JX009742	JX009433	JX009896	HQ596280
C. musicola	CBS 132885*	*Musa* sp.	Mexico	MG600736	MG600798	–	MG600942	MG600853	MG601003
C. neosansevieriae	CBS 139918*	*Sansevieria trifasciata*	South Africa	KR476747	KR476791	–	KR476790	–	KR476797
C. novae-zelandiae	CBS 128505*	*Capsicum annuum*, fruit rot	New Zealand	JQ005228	JQ005315	JQ005749	JQ005576	JQ005402	JQ005662
C. nupharicola	CBS 470.96*	*Nuphar lutea* subsp. *Polysepala*	USA	JX010187	JX009972	JX009663	JX009437	JX009835	JX010398
C. nymphaeae	CBS 515.78*	*Nymphaea alba*	Netherlands	JQ948197	JQ948527	–	JQ949518	JQ948858	JQ949848
C. oncidii	CBS 129828*	*Oncidium* sp., leaf	Germany	JQ005169	JQ005256	JQ005690	JQ005517	JQ005343	JQ005603
C. orbiculare	CBS 514.97	*Cucumis sativus*	Japan	JQ005778	KF178491	–	JQ005841	JQ005799	JQ005862
C. orchidearum	CBS 135131*	*Dendrobium nobile*	Netherlands	MG600738	MG600800	–	MG600944	MG600855	MG601005
C. orchidophilum	CBS 632.80*	*Dendrobium* sp.	USA	JQ948151	JQ948481	–	JQ949472	JQ948812	JQ949802
C. paranaense	CBS 134729*	*Malus domestica*	Brazil, Parana	KC204992	KC205026	–	KC205077	KC205043	KC205060
C. parsonsiae	CBS 128525	*Parsonsia capsularis*, leaf endophyte	New Zealand	JQ005233	JQ005320	JQ005754	JQ005581	JQ005407	JQ005667
C. paxtonii	IMI 165753*	*Musa* sp.	Saint Lucia	JQ948285	JQ948615	–	JQ949606	JQ948946	JQ949936
C. petchii	CBS 378.94*	*Dracaena marginata*, spotted leaves	Italy	JQ005223	JQ005310	JQ005744	JQ005571	JQ005397	JQ005657
C. phormii	CBS 118194*	*Phormium* sp.	Germany	JQ948446	JQ948777	–	JQ949767	JQ949107	JQ950097
C. phyllanthi	CBS 175.67*	*Phyllanthus acidus*, anthracnose	India	JQ005221	JQ005308	JQ005742	JQ005569	JQ005395	JQ005655
C. piperis	IMI 71397*	*Piper nigrum*	Malaysia	MG600760	MG600820	–	MG600964	MG600867	MG601027
C. plurivorum	CBS 125474*	*Coffea* sp.	Vietnam	MG600718	MG600781	–	MG600925	MG600841	MG600985
	CBS 125473	*Coffea* sp.	Vietnam	MG600717	MG600780	–	MG600924	MG600840	MG600984
	CGMCC 3.17358	*Camellia sinensis*, endophyte	China	KJ955215	KJ954916	–	KJ954483	–	KJ955361
	CMM 3742	*Mangifera indica*	Brazil	KC702980	KC702941	–	KC702908	KC598100	KC992327
	LJTJ30	*Capsicum annuum*	China	KP748221	KP823800	–	KP823741	–	KP823853
	MAFF 243073	*Amorphophallus rivieri*	Japan	MG600730	MG600793	–	MG600936	MG600847	MG600997
	MAFF 305790	*Musa* sp.	Japan	MG600726	MG600789	–	MG600932	MG600845	MG600993
C. psidii	CBS 145.29*	*Psidium* sp.	Italy	JX010219	JX009967	JX009743	JX009515	JX009901	JX010443
C. pyricola	CBS 128531*	*Pyrus communis*, fruit rot	New Zealand	JQ948445	JQ948776	–	JQ949766	JQ949106	JQ950096
C. queenslandicum	ICMP 1778*	*Carica papaya*	Australia	JX010276	JX009934	JX009691	JX009447	JX009899	JX010414
C. quinquefoliae	MFLU 14-0626*	*Parthenocissus quinquefolia*	Russia	KU236391	KU236390	–	KU236389	–	KU236392
C. rhexiae	CBS 133134*	*Rhexia virginica*	USA	JX145128	–	–	–	–	JX145179

Table 3 (cont.)

Species	Culture[x]	Host/Substrate	Country	GenBank accession number					
				ITS	*GAPDH*	*CAL*	*ACT*	*CHS-1*	*TUB2*
C. rhexiae (cont.)	CBS 133132	*Vaccinium macrocarpon*	USA	JX145157	–	–	–	–	JX145209
C. rhombiforme	CBS 129953*	*Olea europaea*	Portugal	JQ948457	JQ948788	–	JQ949778	JQ949118	JQ950108
C. salicis	CBS 607.94*	*Salix* sp., leaf, spot	Netherlands	JQ948460	JQ948791	JQ949781	JQ949121	JQ950111	–
C. salsolae	ICMP 19051*	*Salsola tragus*	Hungary	JX010242	JX009916	JX009696	JX009562	JX009863	JX010403
C. sansevieriae	MAFF 239721*	*Sansevieria trifasciata*	Japan	AB212991	–	–	–	–	–
C. sedi	MFLUCC 14-1002*	*Sedum* sp.	Russia	KM974758	KM974755	–	KM974756	KM974754	KM974757
C. siamense	ICMP 18578*	*Coffea arabica*	Thailand	JX010171	JX009924	FJ917505	FJ907423	JX009865	JX010404
	ICMP 12567	*Persea americana*	Australia	JX010250	JX009940	JX009697	JX009541	JX009761	JX010387
	ICMP 18574	*Pistacia vera*	Australia	JX010270	JX010002	JX009707	JX009535	JX009798	JX010391
	ICMP 18121	*Dioscorea rotundata*	Nigeria	JX010245	JX009942	JX009715	JX009460	JX009845	JX010402
	ICMP 17795	*Malus domestica*	USA	JX010162	JX010051	JX009703	JX009506	JX009805	JX010393
C. siamense (syn. *C. hymenocallidis*)	ICMP 18642*	*Hymenocallis americana*	China	JX010278	JX010019	JX009709	GQ856775	GQ856730	JX010410
C. siamense (syn. *C. jasmini-sambac*)	ICMP 19118*	*Jasminum sambac*	Vietnam	HM131511	HM131497	JX009713	HM131507	JX009895	JX010415
C. simmondsii	CBS 122122*	*Carica papaya*	Australia	JQ948276	JQ948606	–	JQ949597	JQ948937	JQ949927
C. sloanei	IMI 364297*	*Theobroma cacao*, leaf	Malaysia	JQ948287	JQ948617	–	JQ949608	JQ948948	JQ949938
C. sojae	ATCC 62257*	*Glycine max*	USA	MG600749	MG600810	–	MG600954	MG600860	MG601016
	CGMCC 3.15171	*Bletilla ochracea*	China	HM751813	KC843501	–	KC843550	–	KC244161
C. sonchicola	JZB330117	*Sonchus* sp.	Italy	KY962756	KY962753	–	KY962747	KY962750	–
	MFLUCC 17-1300	*Sonchus* sp.	Italy	KY962758	KY962755	–	KY962749	KY962752	–
C. spinaciae	CBS 128.57	*Spinacia oleracea*	Netherlands	GU227847	GU228239	–	GU227945	GU228337	GU228141
C. sydowii	CBS 135819	*Sambucus* sp.	China, Taiwan	KY263783	KY263785	–	KY263791	KY263787	KY263793
C. tamarilloi	CBS 129814*	*Solanum betaceum*, fruit, anthracnose	Colombia	JQ948184	JQ948514	–	JQ949505	JQ948845	JQ949835
C. temperatum	CBS 133122*	*Vaccinium macrocarpon*	USA	JX145159	–	–	–	–	JX145211
	CBS 133120	*Vaccinium macrocarpon*	USA	JX145135	–	–	–	–	JX145186
C. theobromicola	CBS 124945*	*Theobroma cacao*	Panama	JX010294	JX010006	JX009591	JX009444	JX009869	JX010447
C. ti	ICMP 4832*	*Cordyline* sp.	New Zealand	JX010269	JX009952	JX009649	JX009520	JX009898	JX010442
C. torulosum	CBS 128544*	*Solanum melongena*	New Zealand	JQ005164	JQ005251	JQ005685	JQ005512	JQ005338	JQ005598
C. tropicale	CBS 124949*	*Theobroma cacao*	Panama	JX010264	JX010007	JX009719	JX009489	JX009870	JX010407
C. tropicicola	BCC 38877*	*Citrus maxima*	Thailand	JN050240	JN050229	–	JN050218	–	JN050246
	MFLUCC100167	*Paphiopedilum bellatolum*	Thailand	JN050241	JN050230	–	JN050219	–	JN050247
C. truncatum	CBS 151.35*	*Phaseolus lunatus*	USA	GU227862	GU228254	–	GU227960	GU228352	GU228156
C. viniferum	GZAAS 5.08601*	*Vitis vinifera* cv. Shuijing	China	JN412804	JN412798	JQ309639	JN412795	–	JN412813
C. vittalense	CBS 181.82*	*Theobroma cacao*	India	MG600734	MG600796	–	MG600940	MG600851	MG601001
C. walleri	CBS 125472*	*Coffea* sp., leaf tissue	Vietnam	JQ948275	JQ948605	–	JQ949596	JQ948936	JQ949926
C. wuxiense	CGMCC 3.17894*	*Camellia sinensis*	China	KU251591	KU252045	KU251833	KU251672	KU251939	KU252200
	JS1A44	*Camellia sinensis*	China	KU251592	KU252046	KU251834	KU251673	KU251940	KU252201
C. xanthorrhoeae	ICMP 17903*	*Xanthorrhoea preissii*	Australia	JX010261	JX009927	JX009653	JX009478	JX009823	JX010448
C. yunnanense	CBS 132135*	*Buxus* sp.	China	JX546804	JX546706	–	JX519239	JX519231	JX519248
Colletotrichum sp.	CGMCC 3.15172	*Bletilla ochracea*	China	HM751816	KC843522	–	KC843547	–	KC244162
	Q026	*Rubus glaucus*	Colombia	JN715839	KC860013	–	KC859970	KC859995	KC860039
Glomerella 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

[x] ATCC: American Type Culture Collection; BCC: BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), Khlong Luang, Pathumthani, Thailand; BRIP: Plant Pathology Herbarium, Department of Employment, Economic, Development and Innovation, Queensland, Australia; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection; CMM: Culture Collection of Phytopathogenic Fungi Prof. Maria Menezes, Federal Rural University of Pernambuco, Brazil; COAD: Coleção Octávio Almeida Drummond, Viçosa, Brazil; GZAAS: Guizhou Academy of Agricultural Sciences Herbarium, China; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; MAFF: MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MFLU: Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

* = ex-type culture.

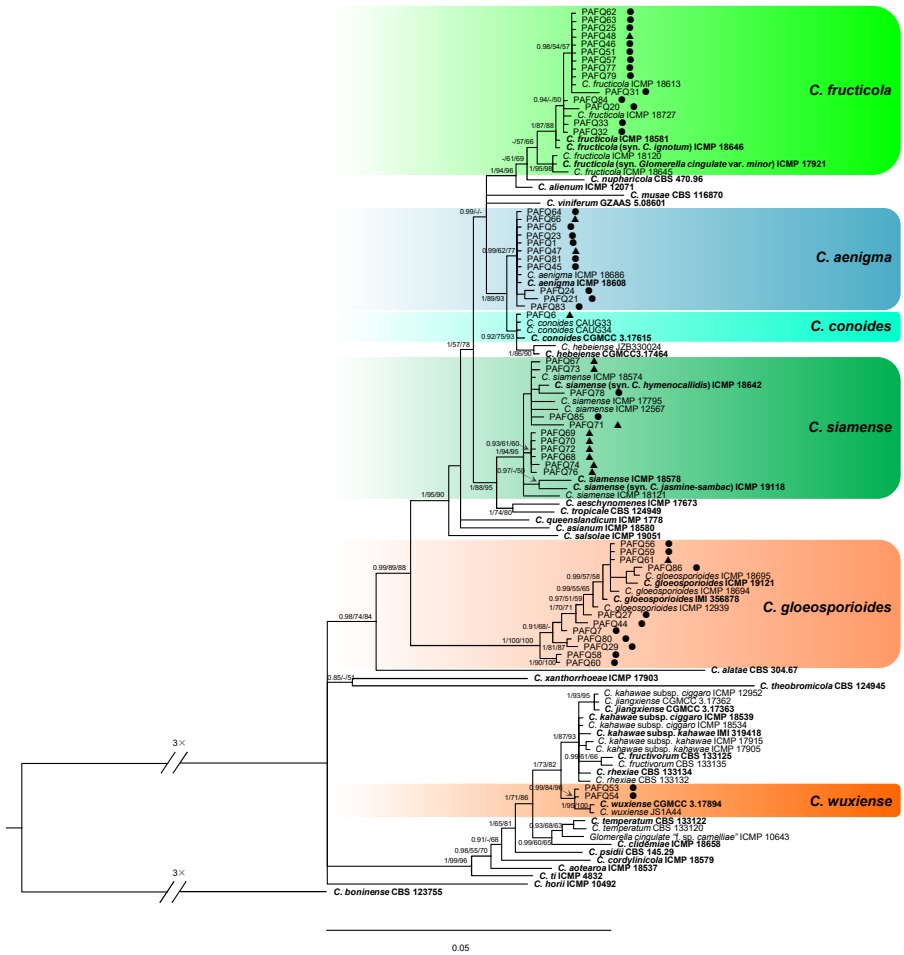

Fig. 2 A Bayesian inference phylogenetic tree of 111 isolates in the *C. gloeosporioides* species complex. The species *C. boninense* (CBS 123755) was selected as an outgroup. The tree was built using concatenated sequences of the *ACT*, *TUB2*, *CAL*, *CHS-1*, *GAPDH*, and ITS genes. Bayesian posterior probability (PP ≥ 0.90), MP bootstrap support values (ML ≥ 50 %), and RAxML bootstrap support values (ML ≥ 50 %) were shown at the nodes (PP/MP/ML). Ex-type isolates are in **bold**. Coloured blocks indicate clades containing isolates from *Pyrus* spp. in this study; circles indicate isolates isolated from leaves, triangles indicate isolates isolated from fruits. The scale bar indicates 0.05 expected changes per site.

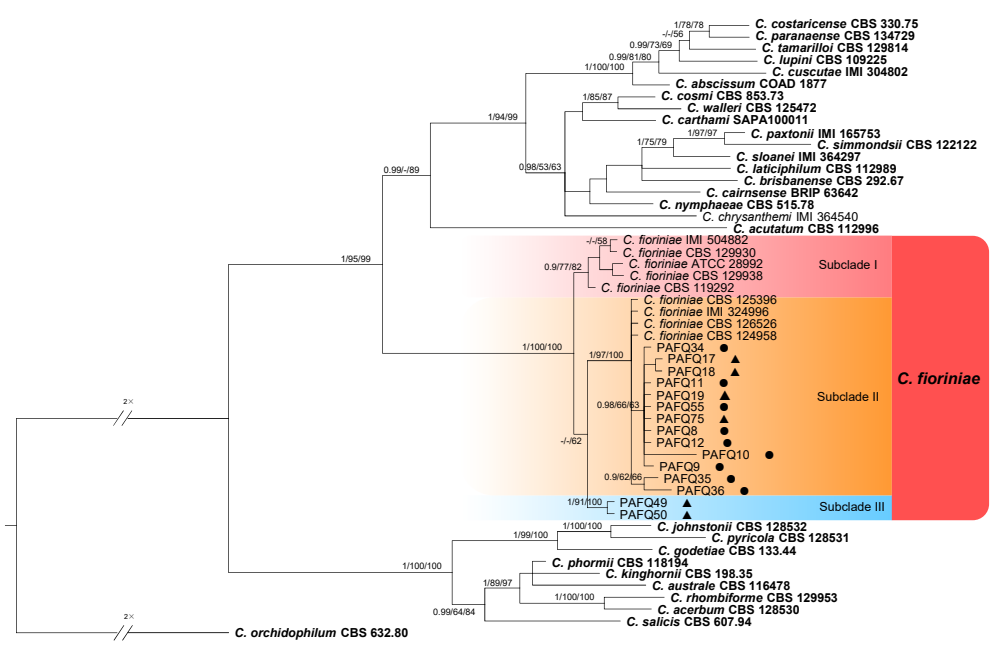

Fig. 3 A Bayesian inference phylogenetic tree of 51 isolates in the *C. acutatum* species complex. The species *C. orchidophilum* (CBS 632.80) was selected as an outgroup. The tree was built using concatenated sequences of the *ACT*, *TUB2*, *CHS-1*, *GAPDH*, and ITS genes. Bayesian posterior probability (PP ≥ 0.90), MP bootstrap support values (ML ≥ 50 %), and RAxML bootstrap support values (ML ≥ 50 %) were shown at the nodes (PP/MP/ML). Ex-type isolates are in **bold**. Coloured blocks indicate clades containing isolates from *Pyrus* spp. in this study; circles indicate isolates isolated from leaves, triangles indicate isolates isolated from fruits. The scale bar indicates 0.02 expected changes per site.

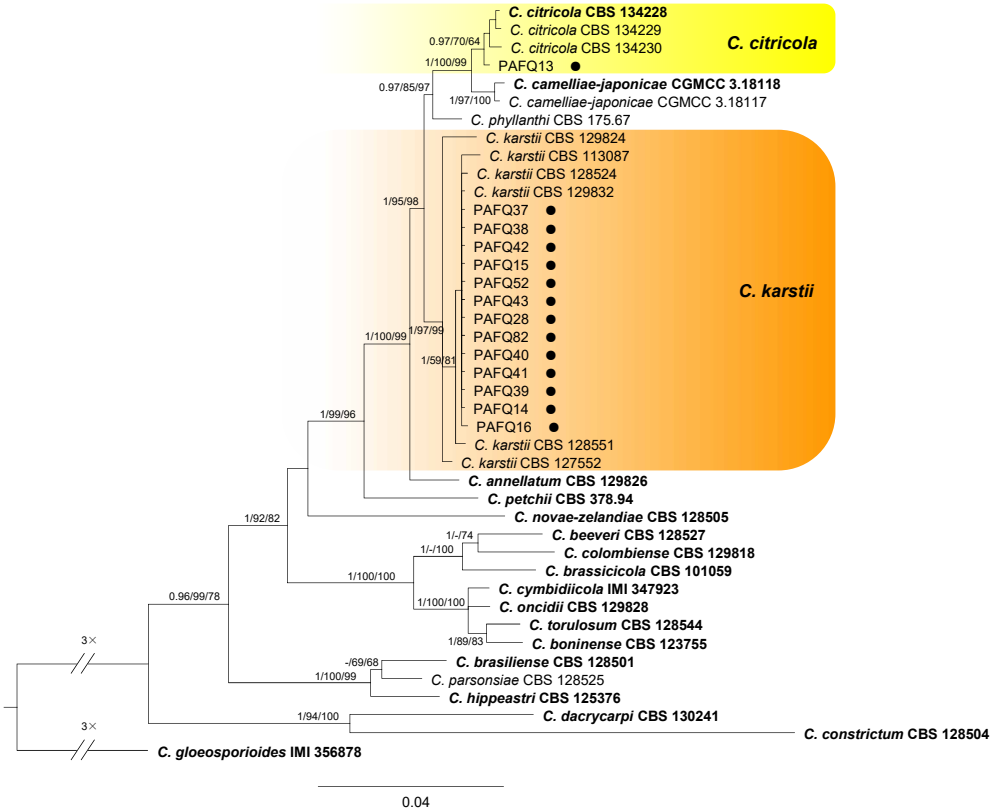

Fig. 4 A Bayesian inference phylogenetic tree of 41 isolates in the *C. boninense* species complex. The species *C. gloeosporioides* (IMI 356878) was selected as an outgroup. The tree was built using concatenated sequences of the *ACT*, *TUB2*, *CAL*, *CHS-1*, *GAPDH*, and ITS genes. Bayesian posterior probability (PP ≥ 0.90), MP bootstrap support values (ML ≥ 50 %), and RAxML bootstrap support values (ML ≥ 50 %) were shown at the nodes (PP/MP/ML). Ex-type isolates are in **bold**. Coloured blocks indicate clades containing isolates from *Pyrus* spp. in this study; circles indicate isolates isolated from leaves. The scale bar indicates 0.04 expected changes per site.

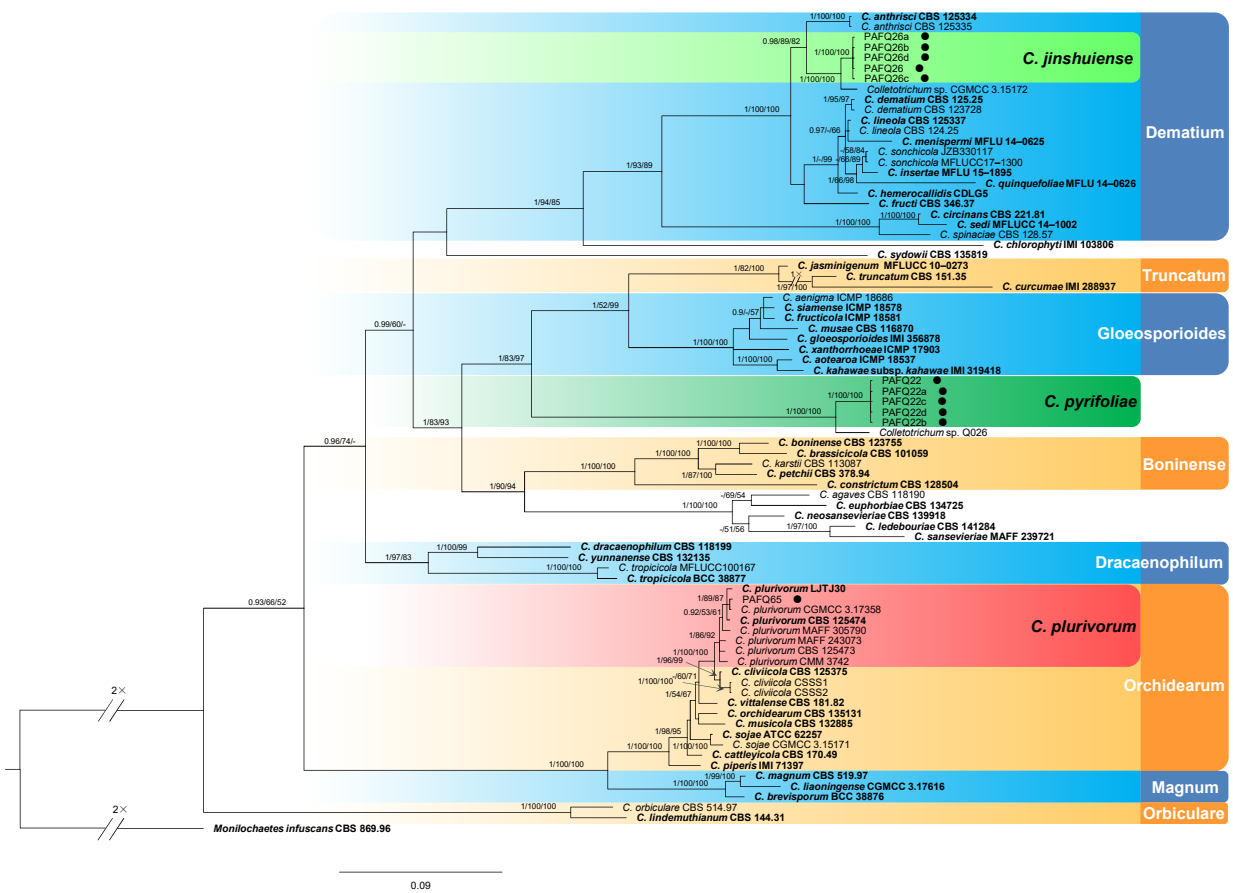

Fig. 5 Phylogenetic tree generated by Bayesian inference based on concatenated sequences of the *ACT*, *CHS-1*, *GAPDH*, ITS, and *TUB* genes. *Monilochaetes infuscans* (CBS 869.96) was selected as an outgroup. Bayesian posterior probability (PP ≥ 0.90), MP bootstrap support values (ML ≥ 50 %), and RAxML bootstrap support values (ML ≥ 50 %) were shown at the nodes (PP/MP/ML). Ex-type isolates are in **bold**. Coloured blocks are used to indicate clades containing isolates from *Pyrus* spp. in this study; circles indicate isolates isolated from leaves. The scale bar indicates 0.09 expected changes per site.

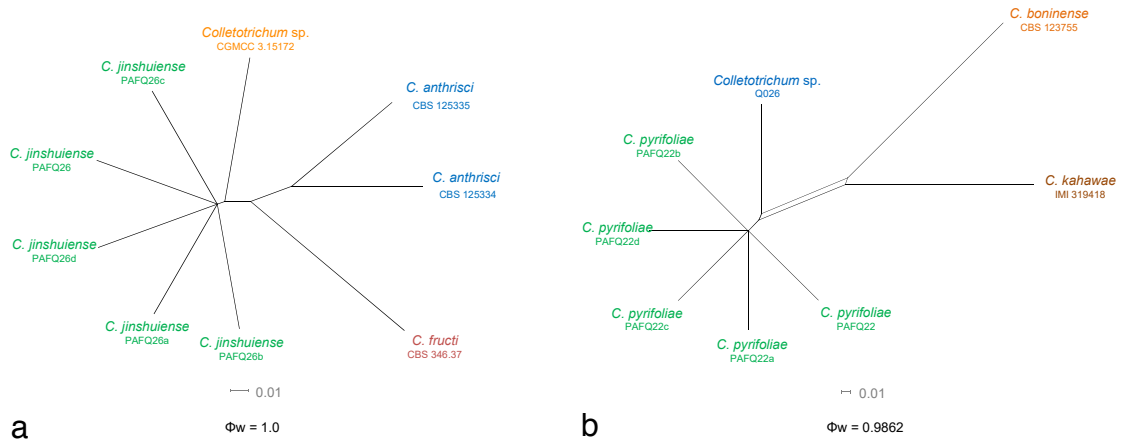

Fig. 6 The result of the pairwise homoplasy index (PHI) tests of closely related species using both LogDet transformation and splits decomposition. a, b. The PHI of *C. jinshuiense* (a) or *C. pyrifoliae* (b) and their phylogenetically related isolates or species, respectively. PHI test value (Φw) < 0.05 indicate significant recombination within the dataset.

lesions and identified as described above. The pathogenicity tests were repeated once.

RESULTS

Colletotrichum isolates associated with pear anthracnose

A total of 295 pear samples (249 leaves and 46 fruits) affected by pear anthracnose, including BrL and TS on fruits, and BnL, SS, and TS on leaves were collected for fungal isolation, resulting in a total of 488 *Colletotrichum* isolates identified based on morphology and ITS sequence data. A total of 90 representative isolates were chosen for further analyses based on their morphology (colony shape, colour, and conidial morphology), ITS sequence data, symptom type, origin, and host cultivar involved (Table 1).

Multi-locus phylogenetic analyses

The 90 representative isolates (Table 1) together with 181 reference isolates from previously described species (Table 3) were subjected to multi-locus phylogenetic analyses with concatenated *ACT*, *TUB2*, *CAL*, *CHS-1*, *GAPDH*, and ITS sequences for those belonging to the *C. gloeosporioides* and *C. boninense* species complexes, or with concatenated *ACT*, *TUB2*, *CHS-1*, *GAPDH*, and ITS sequences for other species of which no *CAL* sequences are available. The results showed that isolates clustered together with 12 species in five *Colletotrichum* species complexes, including gloeosporioides (50 isolates), acutatum (15), boninense (14), dematium (5), and orchidearum (1), and one singleton species (5) (Fig. 2–5).

In the phylogenetic tree constructed for the isolates in the *C. gloeosporioides* species complex, 50 isolates clustered in six clades corresponding to *C. fructicola* (14 isolates), *C. aenigma* (11), *C. siamense* (11), *C. gloeosporioides* (11), *C. wuxiense* (2), and *C. conoides* (1) (Fig. 2). For the isolates in the *C. acutatum* species complex, 13 isolates grouped in subclade II of *C. fioriniae* (Bayesian posterior probabilities value 1/PAUP bootstrap support value 97/RAxML bootstrap support value 100) as defined in a previous study (Damm et al. 2012b), while two isolates (PAFQ49 and PAFQ50) formed a further subclade, which is designated as subclade III (Fig. 3). For isolates in the *C. boninense* species complex, 13 isolates clustered with *C. karstii*, and one with *C. citricola* (Fig. 4). For the remaining 11 isolates, PAFQ65 clustered with *C. plurivorum* (1/86/92), while five isolates formed a distinct clade (1/100/100) as sister to *Colletotrichum* sp. isolate CGMCC 3.15172 in the *C. dematium* species complex. In addition, the remaining five isolates, which formed a distinct clade (1/100/100), clustered distantly from any known *Colletotrichum* species complex (Fig. 5).

To exclude the possibility that species delimitation might be interfered by recombination among the genes used for phylogenetic analyses, the multi-locus (*ACT*, *TUB2*, *CHS-1*, *GAPDH*, and ITS) concatenated datasets were subjected to two PHI tests (Fig. 6) to determine the recombination level within phylogenetically closely related species. The results showed that no significant recombination events were observed between *C. jinshuiense* and phylogenetically related isolates or species (*Colletotrichum* sp. isolate CGMCC 3.15172, *C. anthrisci* and *C. fructi*) (Fig. 6a), and between *C. pyrifoliae* and phylogenetically related isolates or species (*Colletotrichum* sp. isolate Q026, *C. boninense* and *C. kahawae*) (Fig. 6b).

Taxonomy

Based on morphology and multi-locus sequence data, the 90 isolates were assigned to 12 *Colletotrichum* spp. Of these, two species proved to represent new taxa that are described below. Six species are reported from pear for the first time. Eight species formed sexual morphs *in vitro*.

Colletotrichum aenigma B.S. Weir & P.R. Johnst., Stud. Mycol. 73: 135. 2012. — Fig. 7

Description & Illustration — Weir et al. (2012), Wang et al. (2016).

Materials examined. China, Hubei Province, Zhongxiang City, on leaves of *P. pyrifolia* cv. Xiangnan, 1 Sept. 2015, *M. Fu* (culture PAFQ1); ibid., on leaves of *P. pyrifolia* cv. Huanghua, 1 Sept. 2015, *M. Fu* (PAFQ3); ibid., on leaves of *P. pyrifolia* cv. Huali No.1, 1 Sept. 2015, *M. Fu* (PAFQ5); Jiangsu Province, Yancheng City, on fruits of *P. bretschneideri* cv. Renli, 1 Sept. 2015, *M. Fu* (PAFQ47); ibid., on leaves of *P. bretschneideri* cv. Yali, 1 Sept. 2015, *M. Fu* (PAFQ45); Zhejiang Province, Hangzhou City, on leaves of *P. pyrifolia* cv. Guanyangxueli, 18 Aug. 2016, *M. Fu* (PAFQ81); Anhui Province, Dangshan County, on fruits of *P. bretschneideri* cv. Huangguan, 4 Aug. 2016, *M. Fu* (PAFQ66).

Notes — A total of 40 isolates were collected. *Colletotrichum aenigma* has been reported to cause anthracnose diseases of *P. pyrifolia* from Japan (Weir et al. 2012), and *P. communis* from Italy (Schena et al. 2014). This is the first report of *C. aenigma* causing anthracnose on *P. bretschneideri* and on *Pyrus* in China.

Colletotrichum citricola F. Huang et al., Fung. Diversity 61: 67. 2013. — Fig. 8

Description & Illustration — Huang et al. (2013).

Materials examined. China, Hubei Province, Wuhan City, on leaves of *P. pyrifolia*, 1 Sept. 2015, *P.F. Zhang* (culture PAFQ13).

Notes — *Colletotrichum citricola* was first reported as a saprobe from *Citrus unshiu* in China (Huang et al. 2013). Isolate PAFQ13 was isolated from pear leaves, and clustered together with the ex-type culture of *C. citricola* (CBS 134228) in the multi-locus phylogenetic tree (Fig. 4). This is the first report of *C. citricola* causing anthracnose on *P. pyrifolia*.

Ascospores of the isolate PAFQ13 (13.5–20 × 5–8 µm, mean ± SD = 17.4 ± 1.4 × 7.1 ± 0.7 µm) are slightly larger than those of the ex-type isolate CBS 134228 (12.8–18.4 × 5.3–6.7 µm, mean = 15.8 × 6.1 µm) of *C. citricola*. Setae were observed in the acervuli formed on pear leaves, being brown, smooth-walled, 2-septate, 41–84 µm long, base rounded, 6 µm diam, tip more or less acute.

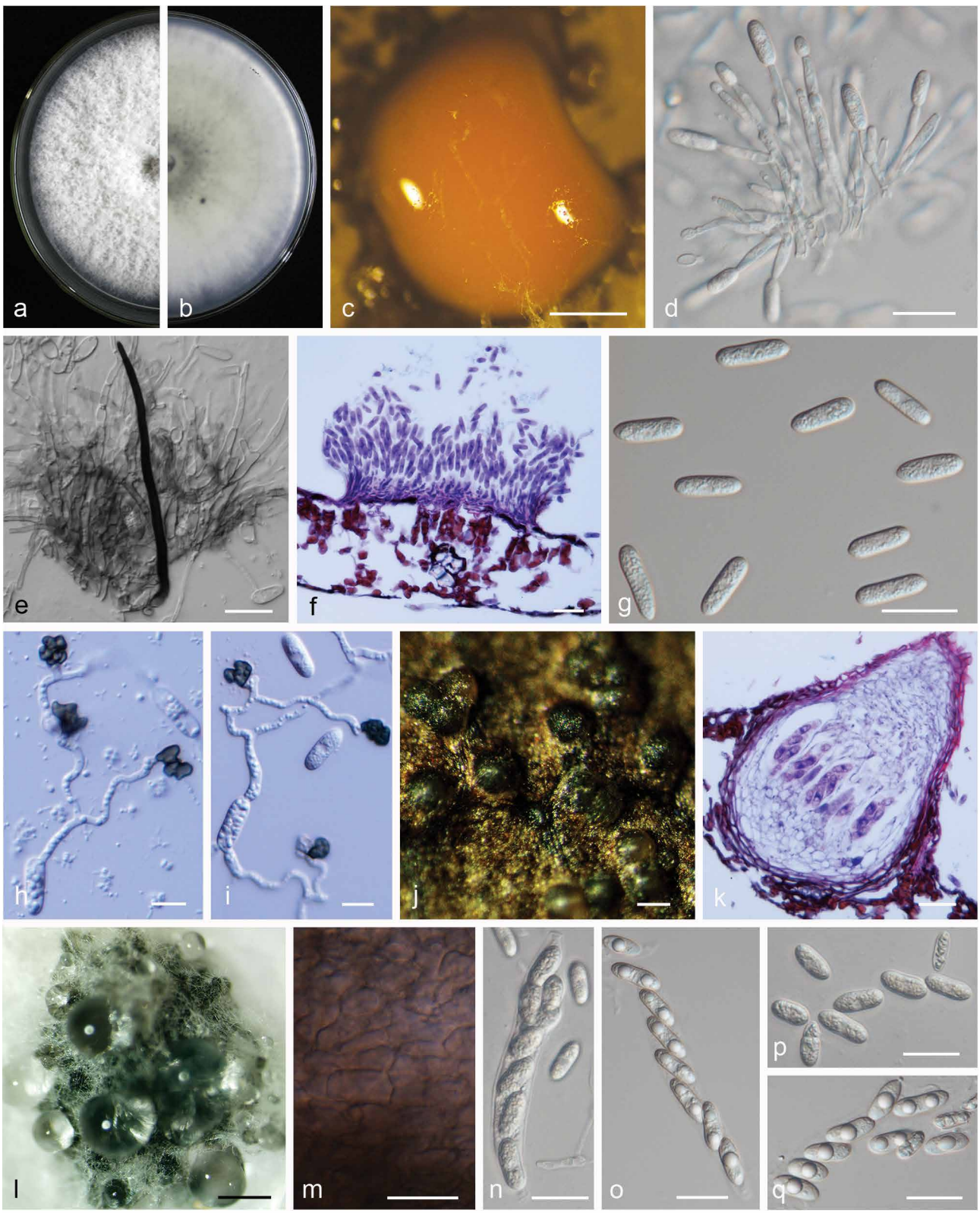

Fig. 7 *Colletotrichum aenigma*. a, b. Front and back view, respectively, of 6-d-old PDA culture; c. conidiomata; d. conidiophores; e. seta; f. section view of acervulus produced on pear leaf (*P. pyrifolia* cv. Cuiguan); g. conidia; h, i. appressoria; j. ascomata produced on pear leaf (*P. bretschneideri* cv. Dangshansuli); k. section view of ascoma produced on pear leaf (*P. pyrifolia* cv. Cuiguan); l. ascomata; m. outer surface of peridium; n, o. asci; p, q. ascospores (a–c, i–m. isolate PAFQ1; d–h. isolate PAFQ47; n, p. isolate PAFQ3; o, q. isolate PAFQ2; a–e, g, l–q produced on PDA agar medium). — Scale bars: c, l = 500 µm; d–g, k, m–q = 20 µm; h, i =10 µm; j = 100 µm.

Colletotrichum conoides Y.Z. Diao et al., Persoonia 38: 27. 2017. — Fig. 9

Sexual morph developed on PDA. *Ascomata* ovoid to obpyriform, light to dark brown, 77–180 × 69–159 µm, ostiolate. *Asci* cylindrical to clavate, 59.5–99 × 13.5–18.5 µm, 8-spored. *Ascospores* hyaline, smooth-walled, aseptate, cylindrical, sometimes slightly curved, both sides rounded, contents granular, 12.5–21 × 5.5–7.5 µm, mean ± SD = 15.9 ± 1.3 × 6.8 ± 0.5 µm, L/W ratio = 2.3.

Asexual morph developed on PDA. *Conidiophores* hyaline, smooth-walled, septate, branched. *Conidiogenous cells* hyaline, cylindrical to clavate, 18–34.5 × 2–3 µm. *Conidia* hyaline, aseptate, smooth-walled, cylindrical, both ends round or one end slightly acute, usually broader towards one side, contents granular, 16–20 × 4.5–6 µm, mean ± SD = 18.4 ± 0.8 × 5.6 ± 0.3 µm, L/W ratio = 3.3. *Appressoria* dark brown, irregular, but often square to ellipsoid in outline, the margin lobate, 7–12.5 × 5–8.5 µm, mean ± SD = 9.7 ± 1.3 × 6.9 ± 1.1 µm, L/W ratio = 1.4.

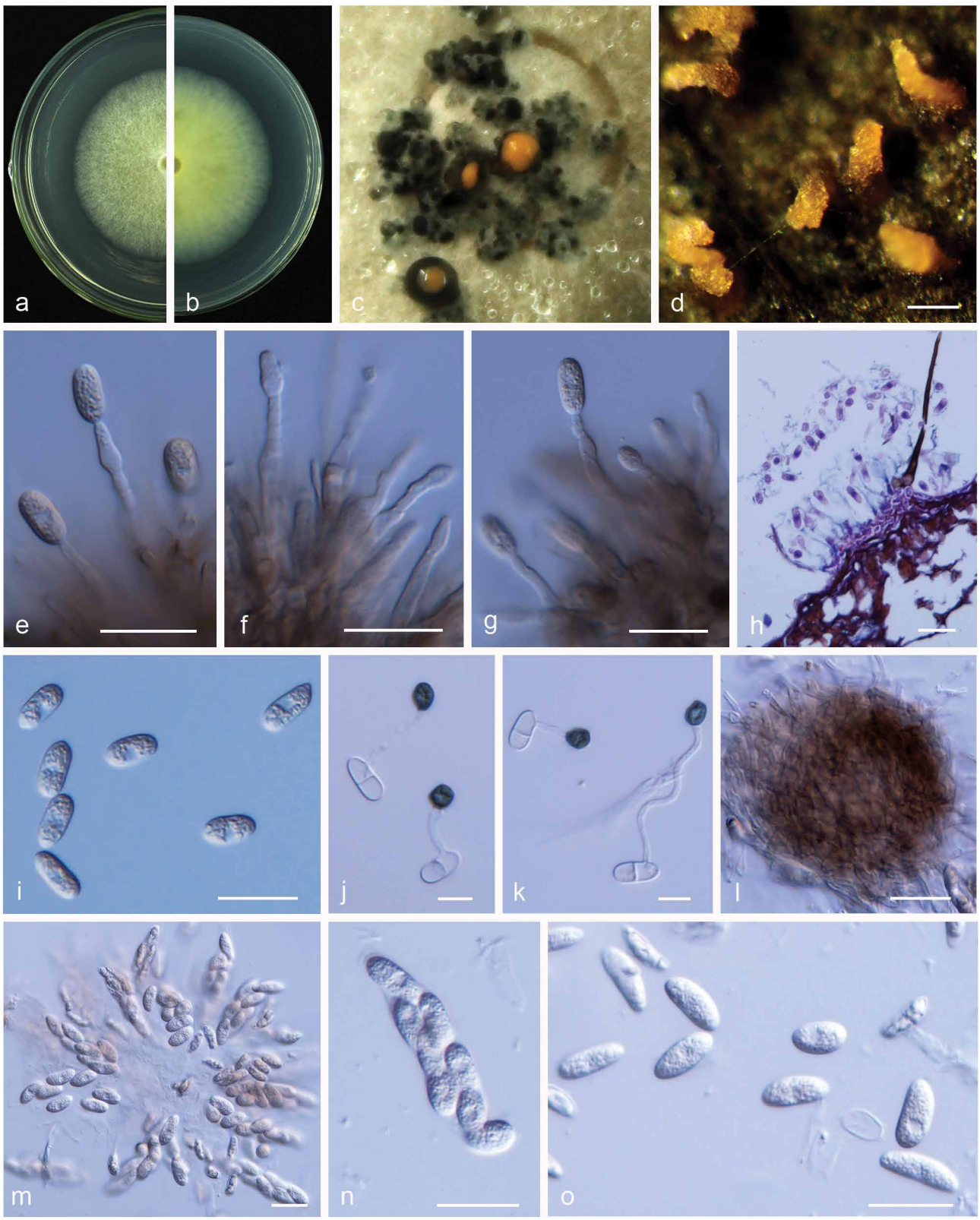

Fig. 8 *Colletotrichum citricola*. a, b. Front and back view, respectively of 6-d-old PDA culture; c, d. conidiomata; e–g. conidiophores; h. section view of acervulus produced on pear leaf (*P. pyrifolia* cv. Cuiguan); i. conidia; j, k. appressoria; l. ascoma; m, n. asci; o. ascospores (a–o. isolate PAFQ13; a–c, e–g, i, l–o. produced on PDA agar medium, d. produced on pear leaf (*P. bretschneideri* cv. Dangshansuli)). — Scale bars: d = 100 µm; e–i, l–o = 20 µm; j, k = 10 µm.

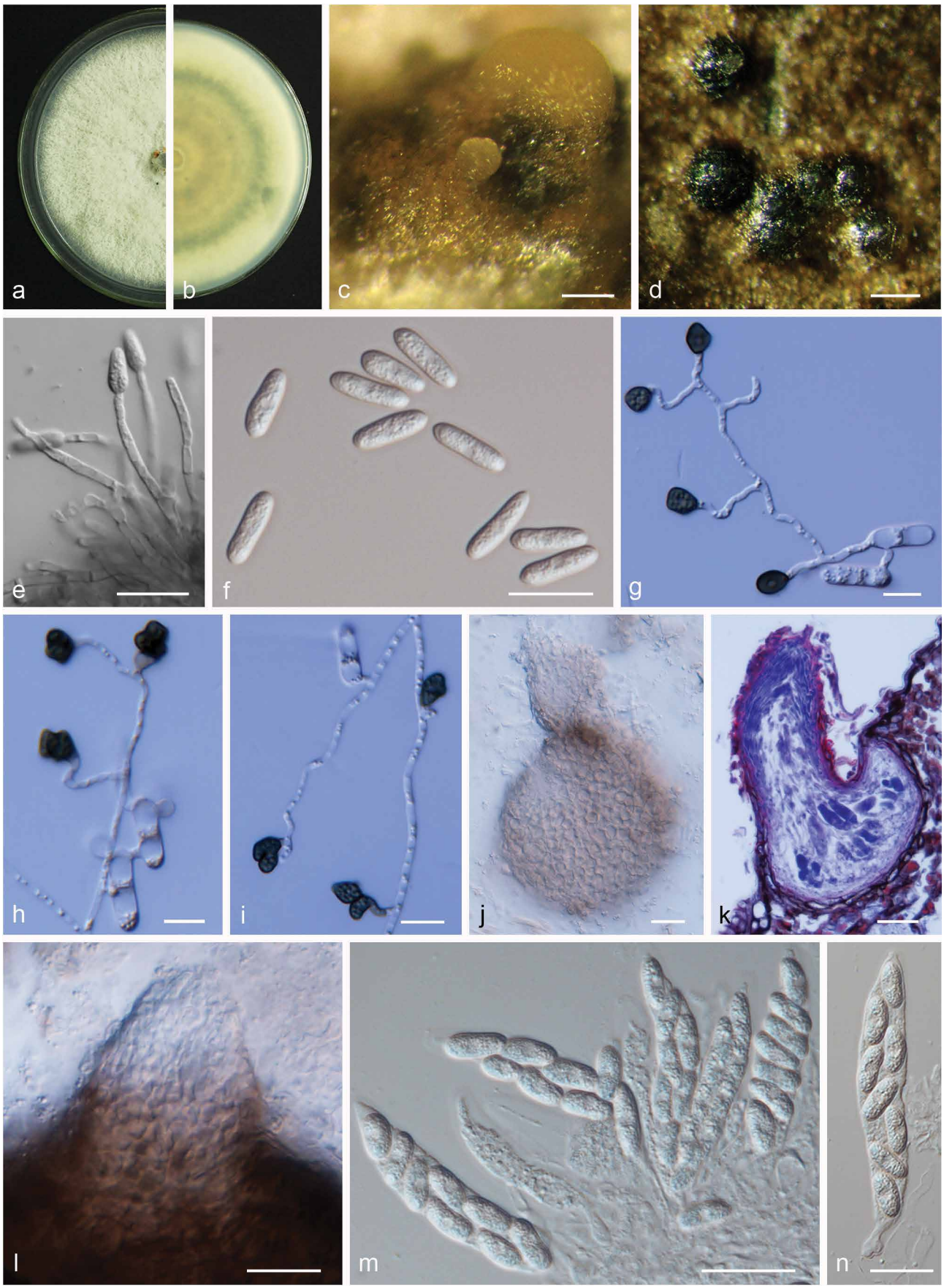

Fig. 9 *Colletotrichum conoides.* a, b. Front and back view, respectively, of 6-d-old PDA culture; c. conidiomata; d. ascomata produced on pear leaf (*P. bretschneideri* cv. Dangshansuli); e. conidiophores; f. conidia; g–i. appressoria; j. ascoma; k. section view of ascoma produced on pear leaf (*P. pyrifolia* cv. Cuiguan); l. neck of ascoma; m, n. asci (a–n. isolate PAFQ6; a–c, e, f, j, l–n. produced on PDA agar medium). — Scale bars: c, d = 100 µm; e, f, j–n = 20 µm; g–i =10 µm.

Culture characteristics — Colonies on PDA flat with entire margin, aerial mycelium white, cottony, dense; reverse light grey in the centre and pale white margin, olivaceous coloured pigments formed in the shape of a concentric ring pattern; colony diam 77–78 mm in 5 d. *Conidia in mass* orange.

Materials examined. China, Hubei Province, Wuhan City, on fruits of *P. pyrifolia*, 1 Sept. 2015, *M. Fu* (culture PAFQ6).

Notes — *Colletotrichum conoides* was first reported on *Capsicum annuum* (chili) from China (Diao et al. 2017). In the present study, one isolate (PAFQ6) from pear fruit clustered together with the ex-type culture of *C. conoides* (CGMCC 3.17615) in the multi-locus phylogenetic tree (Fig. 2). This is the first report of *C. conoides* to cause anthracnose on *P. pyrifolia* and the first description of its sexual morph.

Conidia of the isolate PAFQ6 (16–20 × 4.5–6 µm, mean ± SD = 18.4 ± 0.8 × 5.6 ± 0.3 µm) are longer than those of the ex-type isolate CGMCC 3.17615 (13–17.5 × 5–6.5 µm, mean = 15.9 × 5.9 µm) of *C. conoides*.

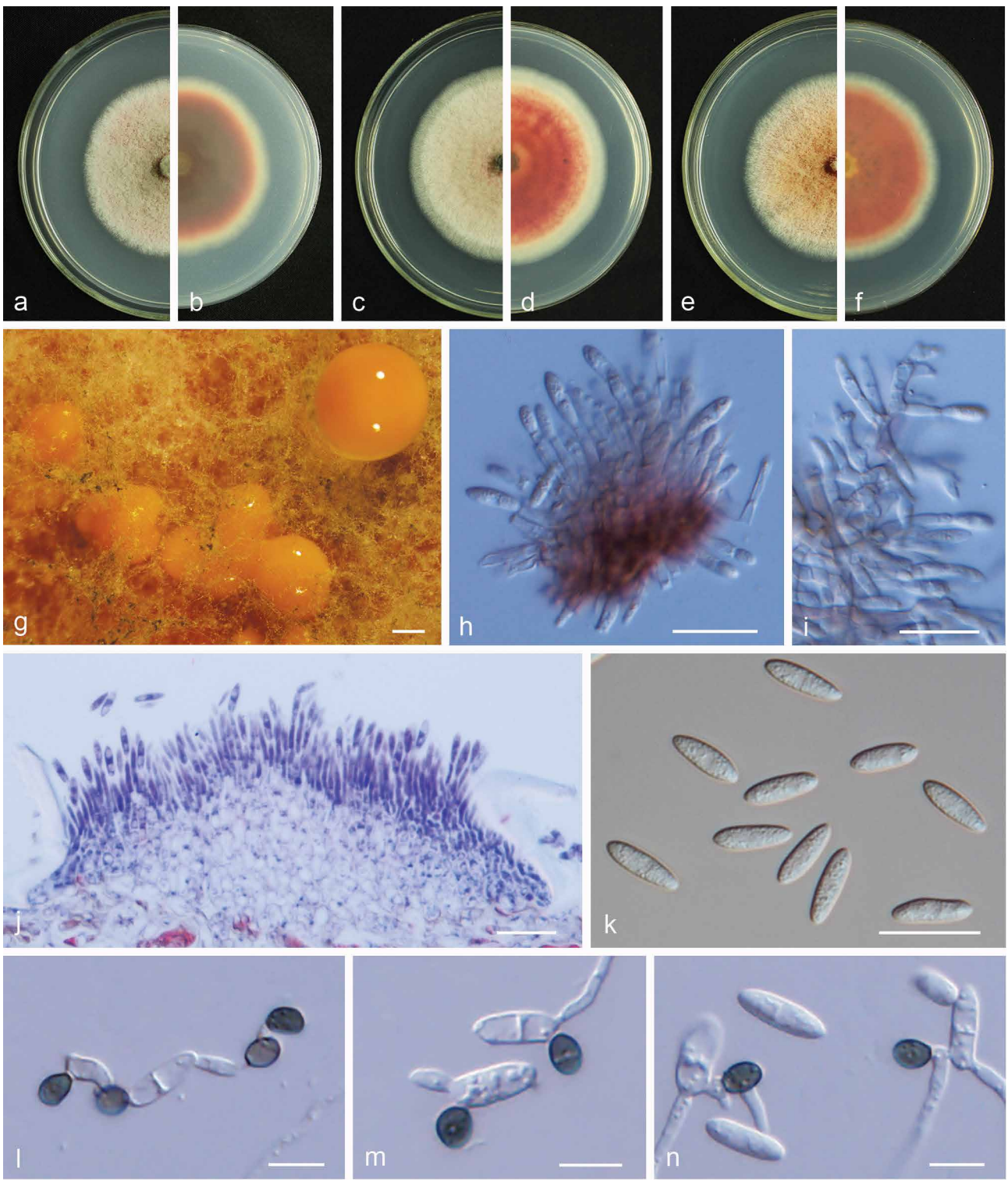

Fig. 10 *Colletotrichum fioriniae.* a, c, e. Front views of 6-d-old PDA culture; b, d, f. back views of 6-d-old PDA culture; g. conidiomata; h, i. conidiophores; j. section view of acervulus produced on pear fruit (*P. bretschneideri* cv. Huangguan); k. conidia; l–n. appressoria (a, b, g–l. isolate PAFQ8, c, d, m. isolate PAFQ36, e, f, n. isolate PAFQ49; a–i, k produced on PDA agar medium). — Scale bars: g = 400 µm; h–k = 20 µm; l–n = 10 µm.

Colletotrichum fioriniae (Marcelino & Gouli) Pennycook, Mycotaxon 132: 150. 2017. — Fig. 10

Description & Illustration — Damm et al. (2012b).

Materials examined. China, Hubei Province, Wuhan City, on leaves of *P. pyrifolia* cv. Jinshui No. 1, 1 Sept. 2015, *M. Fu* (cultures PAFQ8 and PAFQ9); ibid., on fruits of *P. pyrifolia*, 1 Aug. 2016, *M. Fu* (PAFQ17); Fujian Province, Jianning County, on leaves of *P. pyrifolia* cv. Cuiguan, 1 Apr. 2016, *M. Fu* (PAFQ35, PAFQ36); Jiangxi Province, Jinxi County, on leaves of *P. pyrifolia* cv. Cuiguan, 23 July 2016, *M. Fu* (PAFQ55); Shandong Province, Yantai City, on fruits of *P. communis* cv. Gyuiot, 27 Aug. 2016, *M. Fu* (PAFQ75); Jiangsu Province, Nanjing City, on leaves of *P. pyrifolia*, 20 Aug. 2016, *M. Fu* (PAFQ49).

Notes — *Colletotrichum fioriniae* was first reported on *Persea americana* and *Acacia acuminata* from Australia (Shivas & Tan 2009) and also caused fruit rot on *Pyrus* sp. in the USA (Damm et al. 2012b). In the study of Damm et al. (2012b), iso-

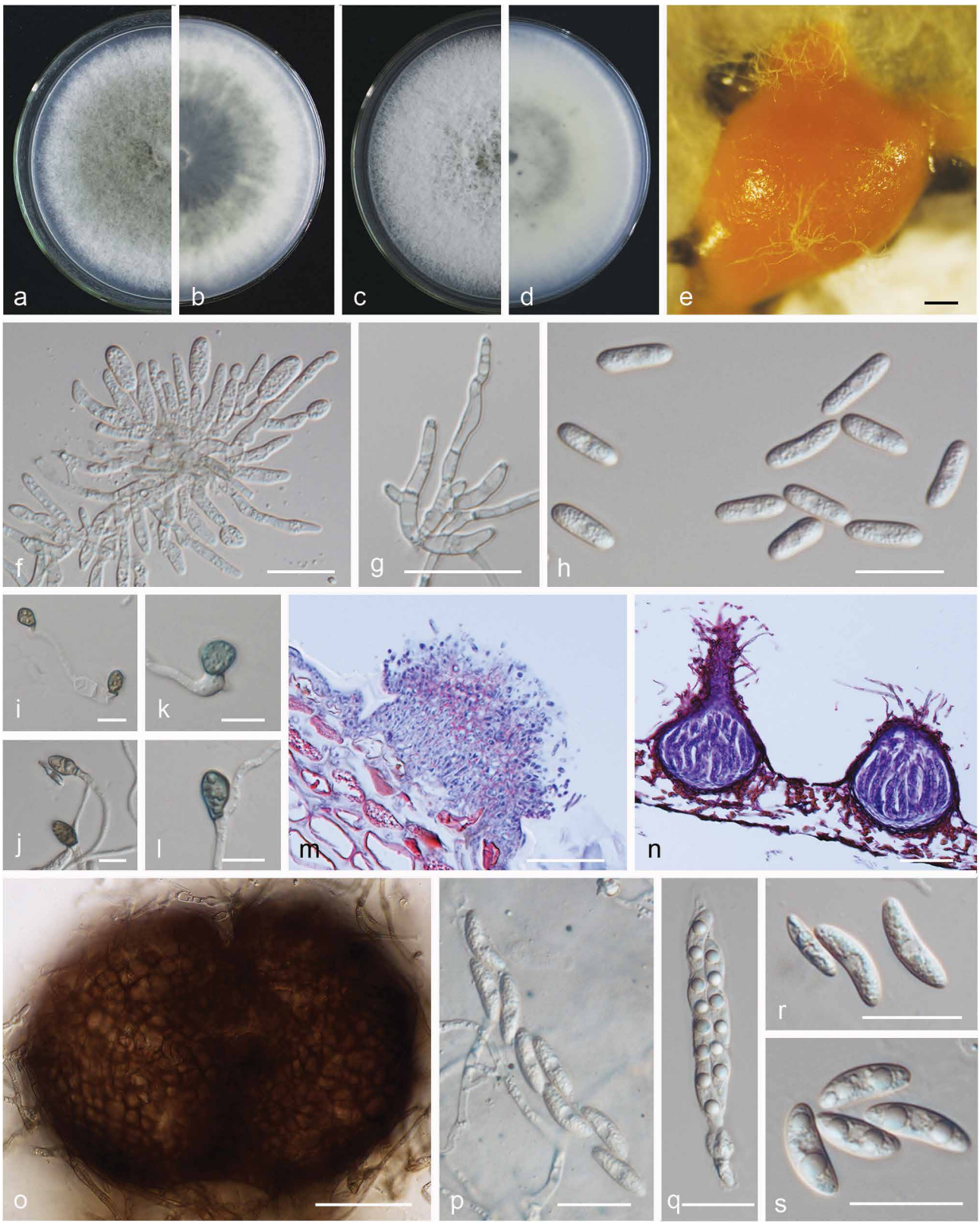

Fig. 11 *Colletotrichum fructicola*. a, c. Front views of 6-d-old PDA culture; b, d. back views of 6-d-old PDA culture; e. conidiomata; f, g. conidiophores; h. conidia; i–l. appressoria; m. section view of acervulus produced on pear fruit (*P. bretschneideri* cv. Huangguan); n. section view of ascomata produced on pear leaf (*P. pyrifolia* cv. Cuiguan); o. ascomata; p, q. asci; r, s. ascospores (a, b, h–l, o, q, r. isolate PAFQ31, c–e, m, n. isolate PAFQ32, p, s. isolate PAFQ48, f, g. isolate PAFQ30; a–h, o–s produced on PDA agar medium). — Scale bars: e = 500 µm; f–h, p–s = 20 µm; i–l = 10 µm; m–o = 50 µm.

lates clustered in two subclades, here designated as I and II. In the current study, an additional subclade (III) was detected (Fig. 3), which differs from subclade I in 2–3 bp in *ACT*, 1 bp in *CHS*, 1 bp in *GAPDH*, and 1 bp in *TUB2*, and subclade II in 3 bp in *CHS*, 4 bp in *GAPDH*, and 2 bp in *TUB2*.

Colletotrichum fructicola Prihast. et al., Fung. Diversity 39: 96. 2009. — Fig. 11

Description & Illustration — Prihastuti et al. (2009).

Materials examined. China, Fujian Province, Jianning County, on leaves of *P. pyrifolia* cv. Cuiguan, Apr. 2014, *P.F. Zhang* (cultures PAFQ30 and PAFQ31); ibid., 1 Sept. 2015, *M. Fu* (PAFQ32, PAFQ33); Jiangxi Province, Jinxi County, on leaves of *P. pyrifolia* cv. Cuiguan, 23 July 2016, *M. Fu* (PAFQ88); Hubei Province, Wuhan City, on leaves of *P. pyrifolia* cv. Jingshui, 1 Aug. 2016, *M. Fu* (PAFQ20, PAFQ25); Zhejiang Province, Hangzhou City, on leaves of *P. pyrifolia* cv. Guanyangxueli, 18 Aug. 2016, *M. Fu* (PAFQ79); ibid., Tonglu County, on leaves of *P. pyrifolia* cv. Cuiguan, 18 Aug. 2016, *M. Fu* (PAFQ84); Jiangsu Province, Yancheng City, on fruits of *P. bretschneideri* cv. Dangshanshuli, 1 Sept. 2015, *M. Fu* (PAFQ48); ibid., on leaves of *P. bretschneideri* cv. Yali, 1 Sept. 2015, *M. Fu* (PAFQ46); Anhui Province, Dangshan County, on leaves of *P. bretschneideri* cv. Huangguan, 4 Aug. 2016, *M. Fu* (PAFQ62); ibid., on fruits of *P. bretschneideri* cv. Huangguan, 4 Aug. 2016, *M. Fu* (PAFQ90).

Notes — *Colletotrichum fructicola* was first reported on *Coffea arabica* in Thailand (Prihastuti et al. 2009), and subsequent-

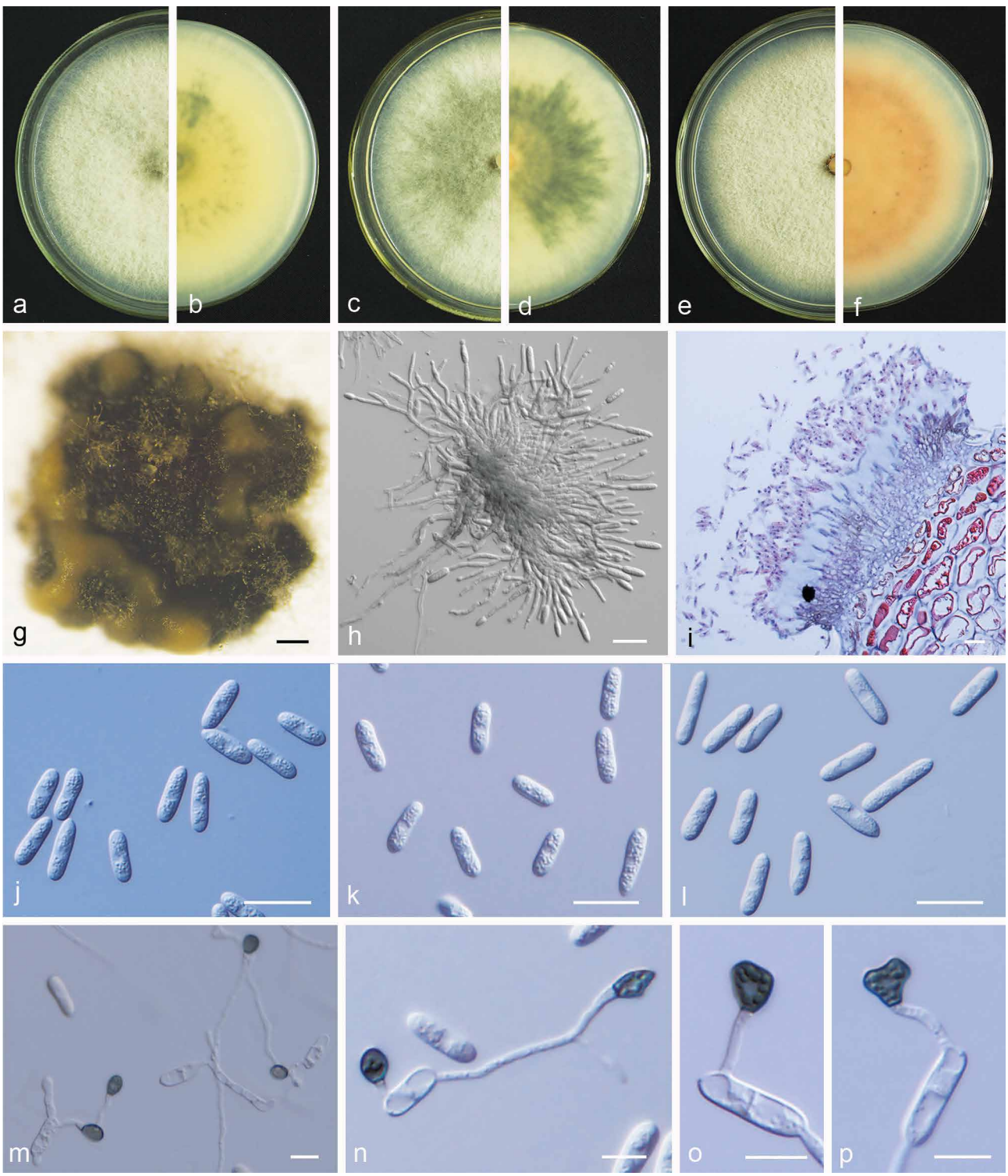

Fig. 12 *Colletotrichum gloeosporioides*. a, c, e. Front views of 6-d-old PDA culture; b, d, f. back views of 6-d-old PDA culture; g. conidiomata; h. conidiophores; i. section view of acervulus produced on pear fruit (*P. bretschneideri* cv. Huangguan); j–l. conidia; m–p. appressoria (a, b, j, m. isolate PAFQ80, c, d, k, n. isolate PAFQ7, e–i, l, o, p. isolate PAFQ56; a–h, j–l produced on PDA agar medium). — Scale bars: g = 200 µm; h–l = 20 µm; m–p = 10 µm.

ly reported on *Pyrus pyrifolia* in Japan (Weir et al. 2012), *Citrus reticulata* in China (Huang et al. 2013), *Pyrus bretschneideri* in China (Li et al. 2013), and other plants (e.g., Lima et al. 2013, Liu et al. 2015, Diao et al. 2017). The species was identified as responsible for pear anthracnose, causing TS symptoms on *P. pyrifolia* leaves (Zhang et al. 2015) and *P. bretschneideri* fruits in China (Jiang et al. 2014).

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., Atti Reale Ist. Veneto Sci. Lett. Arti., ser. 6, 2: 670. 1884. — Fig. 12

Description & Illustration — Cannon et al. (2008), Liu et al. (2015).

Materials examined. China, Jiangxi Province, Jinxi County, on leaves of *P. pyrifolia* cv. Cuiguan, 23 July 2016, *M. Fu* (culture PAFQ56); ibid., on fruits of *P. pyrifolia* cv. Huanghua, 23 July 2016, *M. Fu* (PAFQ61); Hubei Province, Wuhan City, on leaves of *P. pyrifolia* cv. Hohsui, 1 Aug. 2016, *M. Fu* (PAFQ27); ibid., on leaves of *P. bretschneideri* cv. Huangxianchangba,

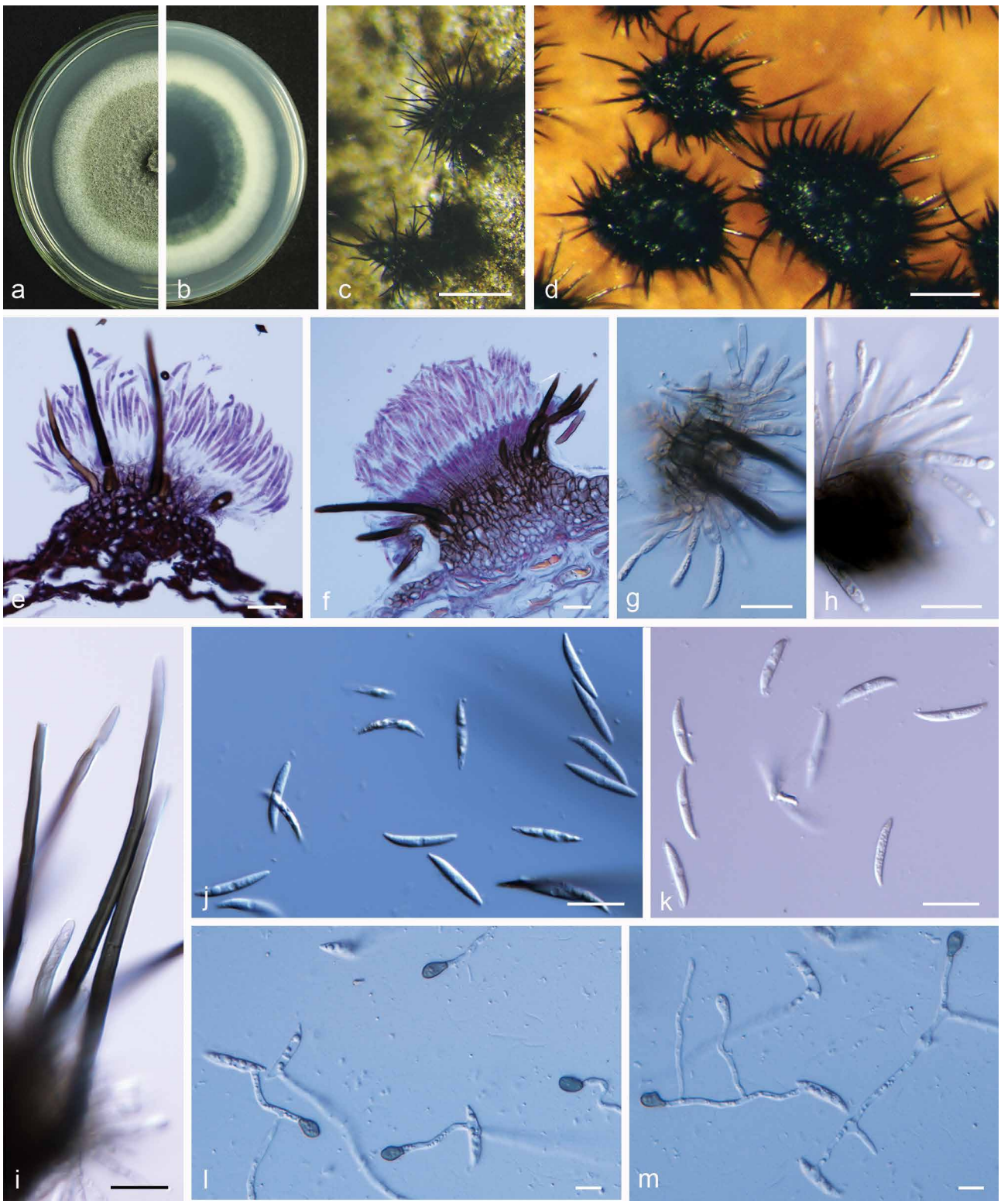

Fig. 13 *Colletotrichum jinshuiense*. a, b. Front and back view, respectively, of 6-d-old PDA culture; c. acervuli produced on pear leaf (*P. bretschneideri* cv. Dangshansuli); d. acervuli produced on pear fruit; e, f. section view of acervulus produced on pear leaf and fruit, respectively; g, h. conidiophores; i. setae; j, k. conidia; l, m. appressoria (a–m. isolate PAFQ26; a, b. produced on PDA agar medium; c, e, j, l. from pear leaf (*P. pyrifoliae* cv. Cuiguan), d, f–i, k–m. from pear fruit (*P. bretschneideri* cv. Huangguan)). — Scale bars: c = 200 µm; d = 100 µm; e–k = 20 µm; l, m = 10 µm.

1 Sept. 2016, *M. Fu* (PAFQ7); Jiangsu Province, Yancheng City, on leaves of *P. bretschneideri* cv. Yali, 1 Sept. 2015, *M. Fu* (PAFQ44); Zhejiang Province, Hangzhou City, on leaves of *P. pyrifolia* cv. Guanyangxueli, 18 Aug. 2016, *M. Fu* (PAFQ80); ibid., on leaves of *Pyrus* sp., 18 Sept. 2016, *M. Fu* (PAFQ86).

Notes — Although *C. gloeosporioides* has been identified as responsible for pear anthracnose in China, these identifications were chiefly based on morphology and/or ITS sequence data (Wu et al. 2010, Liu et al. 2013b). In this study, 20 isolates of *C. gloeosporioides* isolated from fruits and leaves of pear were identified as *C. gloeosporioides* based on multi-loci phylogenetic analyses and confirmed as responsible for pear anthracnose following Koch's postulates.

Colletotrichum jinshuiense M. Fu & G.P. Wang, *sp. nov.* — MycoBank MB824216; Fig. 13

Etymology. Referring to the host variety (*P. pyrofolia* cv. Jinshui) from which the fungus was isolated.

Sexual morph not observed. *Asexual morph* on pear leaves and fruit. *Conidiomata* acervular, conidiophores and setae formed from a brown stroma. Setae dark brown to black, opaque, tip acute, base cylindrical, 1–4-septate, 59–363 (on leaf surface) and 70–272 µm long (on fruit surface). *Conidiophores* pale brown to hyaline, simple to 2-septate, unbranched. *Conidiogenous cells* (on fruit surface) hyaline, smooth-walled, cylindrical, 12.5–27 × 3.5–4.5 µm, opening 1–2 µm. *Conidia*, hyaline, smooth-walled, aseptate, curved, base subtruncate, apex acute, contents with 1–2 guttules, on leaf surface: 25–29.5 × 3.5–4.5

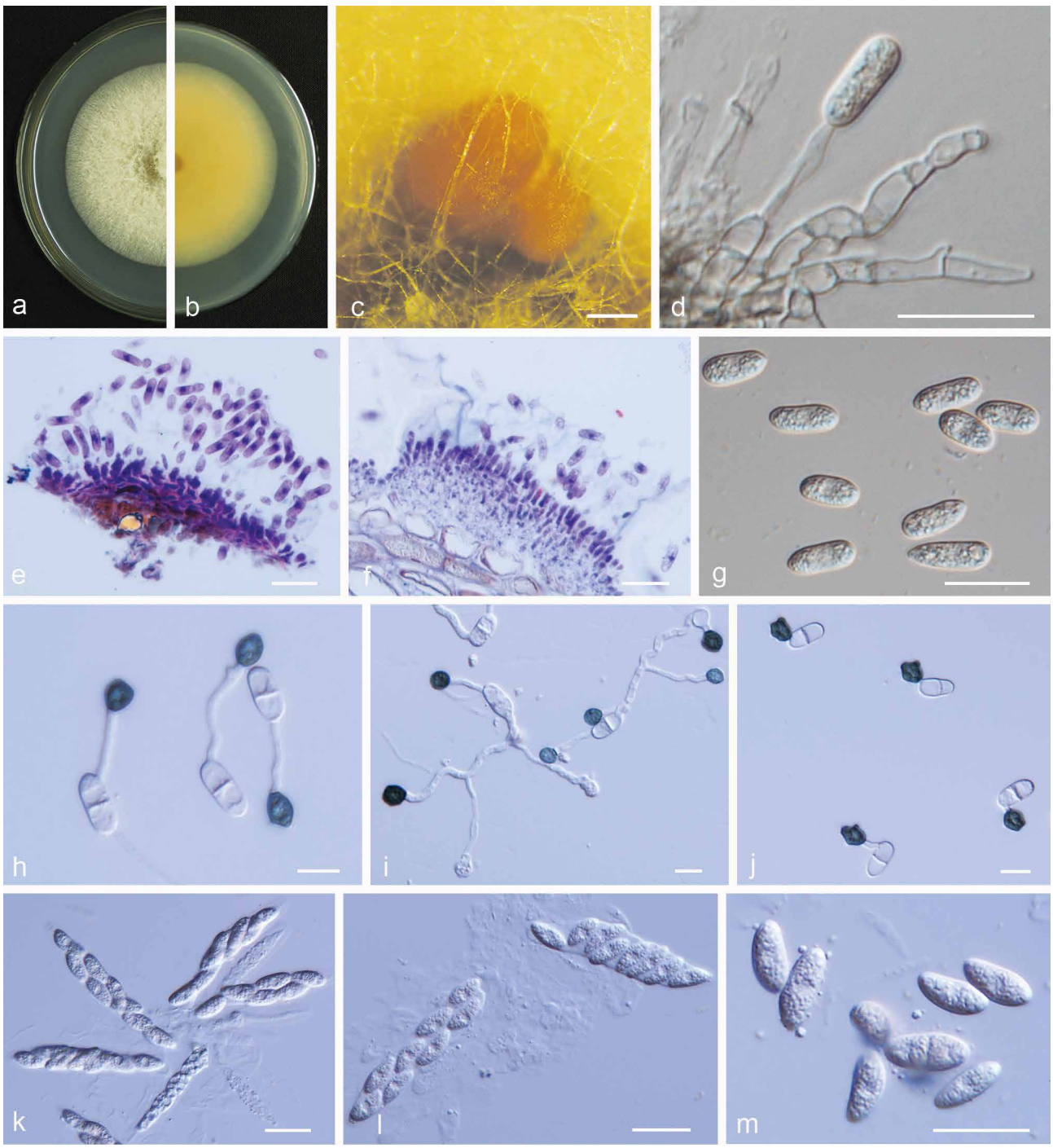

Fig. 14 *Colletotrichum karstii.* a, b. Front and back view, respectively, of 6-d-old PDA culture; c. conidiomata; d. conidiophores; e, f. section view of acervulus produced on pear leaf (*P. pyrifolia* cv. Cuiguan) and fruit (*P. bretschneideri* cv. Huangguan), respectively; g. conidia; h–j. appressoria; k, l. asci; m. ascospores (a–h. isolate PAFQ14, i, k–m. isolate PAFQ40, j isolate PAFQ52; a–d, g, k–m produced on PDA agar medium). — Scale bars: c = 200 µm; d–g, k–m = 20 µm; h–j = 10 µm.

µm, mean ± SD = 27.1 ± 1.7 × 4.0 ± 0.3 µm, L/W ratio = 6.8; on fruit surface: 21–30.5 × 3–4.5 µm, mean ± SD = 24.4 ± 2.1 × 4.0 ± 0.3 µm, L/W ratio = 6.2. *Appressoria* pale brown, smooth-walled, ellipsoidal to clavate, 8–17 × 5–7.5 µm, mean ± SD = 10.7 ± 1.7 × 6.0 ± 0.5 µm, L/W ratio = 1.8.

Culture characteristics — Colonies on PDA flat with entire margin, aerial mycelium sparse, cottony, surface pale grey-black with white margin; reverse black to dark grey-green in centre with white margin. Colony diam 56–57 mm in 5 d. *Conidia in mass* not observed on PDA or SNA.

Materials examined. China, Hubei Province, Wuhan City, on leaves of *P. pyrifolia* cv. Jinshui, 1 Aug. 2016, *M. Fu* (holotype HMAS 247824, culture ex-type CGMCC 3.18903 = PAFQ26); ibid., culture PAFQ26a, PAFQ26b, PAFQ26c, and PAFQ26d.

Notes — Isolates of *C. jinshuiense* are phylogenetically closely related to *Colletotrichum* sp. isolate CGMCC 3.15172 (Fig. 5), which was reported as an endophytic *Colletotrichum* species from *Bletilla ochracea* (*Orchidaceae*) in China (Tao et al. 2013), whereas they are different in *GAPDH* (94.98 %), and *TUB2* (98.12 %). Furthermore, the PHI test (Φw = 1) did not detect recombination between these isolates and *Colletotrichum* sp. isolate CGMCC 3.15172 (Fig. 6a). In this study, *C. jinshuiense* clustered in the *C. dematium* species complex, which is often associated with herbaceous plants (Damm et al. 2009). The asexual and sexual morphs of *C. jinshuiense* were not observed on PDA or SNA, while they easily developed on pear fruit and leaves, indicating that pear tissue plays an important part in the epidemiology and life cycle of *C. jinshuiense*.

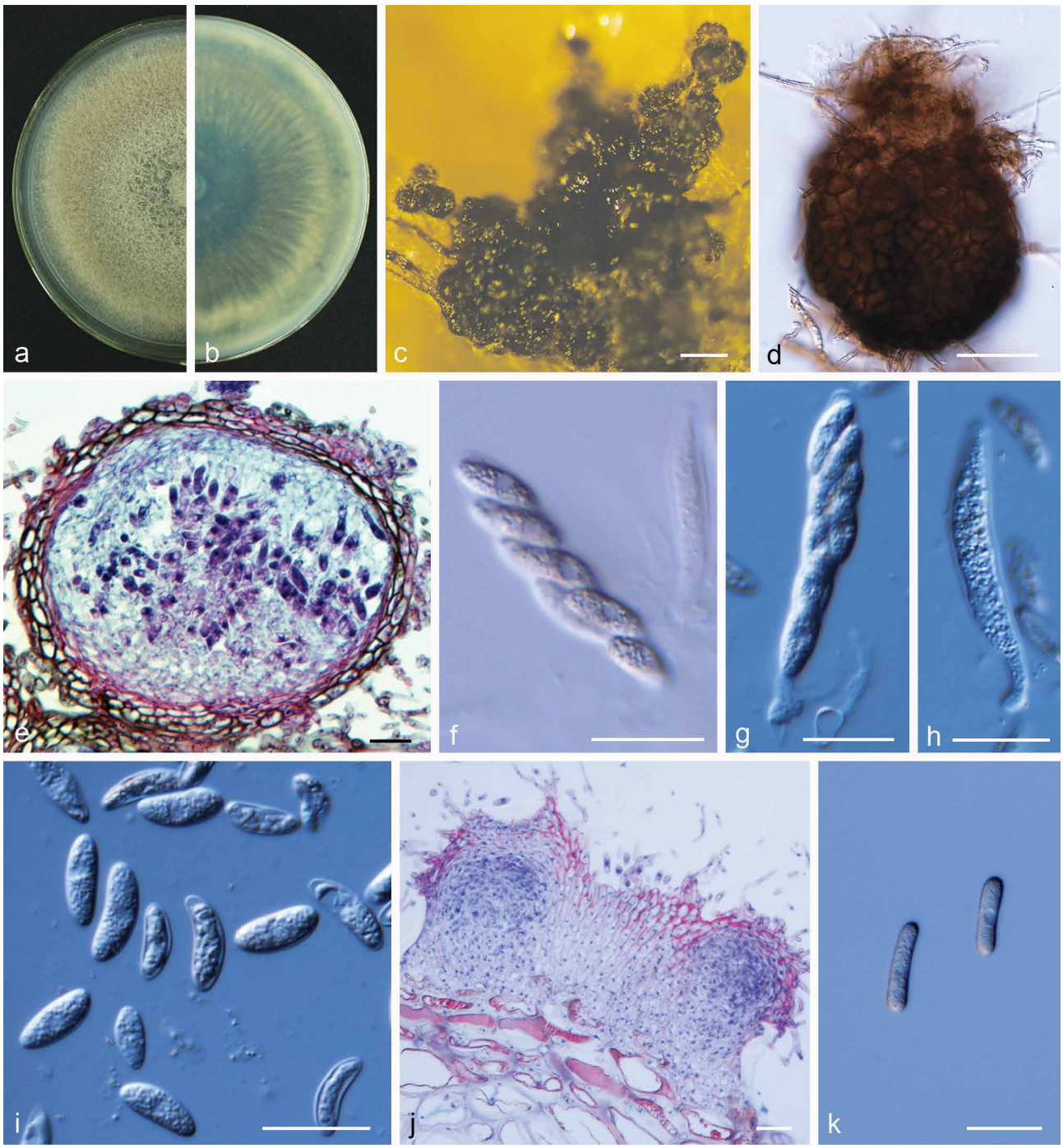

Fig. 15 *Colletotrichum plurivorum*. a, b. Front and back view, respectively, of 6-d-old PDA culture; c, d. ascomata; e. section of ascoma; f, g. asci; h. immature ascus; i. ascospores; j. section view of acervulus produced on pear fruit (*P. bretschneideri* cv. Huangguan); k. conidia (a–k. isolate PAFQ65; a–i. produced on PDA agar medium, j, k. from pear fruits). — Scale bars: c = 200 µm; d = 50 µm; e–k = 20 µm.

Colletotrichum karstii Yan L. Yang et al., Cryptog. Mycol. 32: 241. 2011. — Fig. 14

Description & Illustration — Yang et al. (2011).

Materials examined. China, Hubei Province, Wuhan City, on leaves of *P. pyrifolia*, 1 Sept. 2015, *P.F. Zhang* (culture PAFQ14); ibid., on leaves of *P. pyrifolia* cv. Hohsui, 1 Aug. 2016, *M. Fu* (PAFQ28); Fujian Province, Jianning County, on leaves of *P. pyrifolia* cv. Cuiguan, 20 Oct. 2016, *M. Fu* (PAFQ40); Zhejiang Province, Hangzhou City, on leaves of *P. pyrifolia* cv. Guanyangxueli, 18 Aug. 2016, *M. Fu* (PAFQ82); Jiangxi Province, Jinxi County, on leaves of *P. pyrifolia* cv. Cuiguan, 23 July 2016, *M. Fu* (PAFQ52).

Notes — *Colletotrichum karstii* was first reported on *Vanda* sp. in China (Yang et al. 2011) and is diverse in its geographical distribution and host range (Damm et al. 2012a). In this study, 19 isolates of *Colletotrichum* were identified as belonging to this species, and this is the first report of *C. karstii* causing anthracnose of *P. pyrifolia*.

Conidia of the ex-type (GZAAS 090006, 12–19.5 × (5–)6–7.5 µm, mean ± SD = 15.4 ±1.3 × 6.5 ± 0.5 µm) of *C. karstii* are slightly smaller than that of isolate PAFQ82 (12.5–21 × 5–8 µm, mean ± SD = 16.8 ± 1.6 × 7.2 ± 0.6 µm), but larger than that of isolate PAFQ40 (12.5–16 × 5.5–7.5 µm, mean ± SD = 13.6 ± 0.8 × 6.5 ± 0.4 µm) and isolate PAFQ52 (11.5–16 × 5.5–7.5 µm, mean ± SD = 13.9 ± 1.0 × 6.8 ± 0.3 µm).

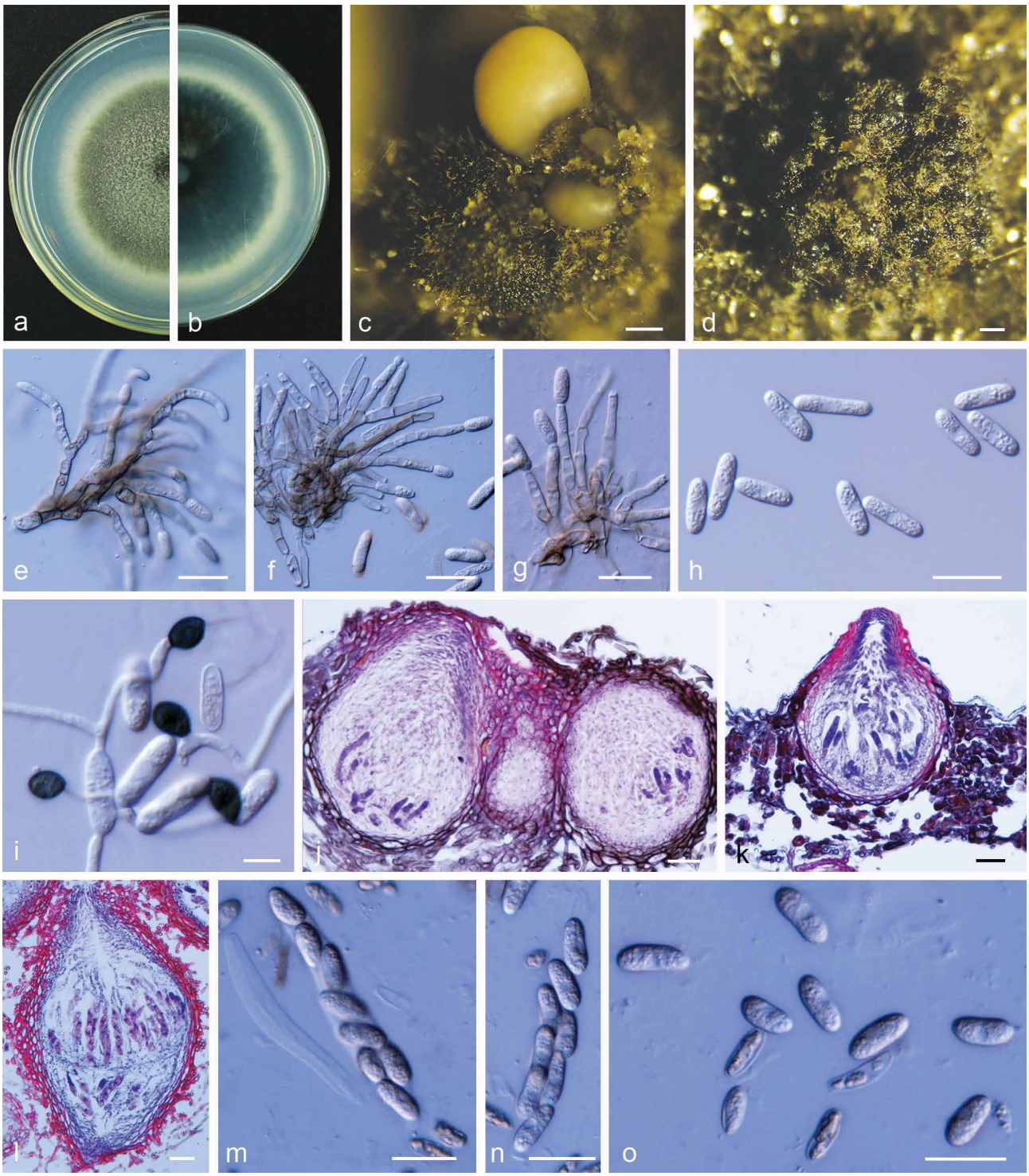

Fig. 16 *Colletotrichum pyrifoliae*. a, b. Front and back view, respectively, of 6-d-old PDA culture; c. conidiomata; d. ascomata; e–g. conidiophores; h. conidia; i. appressoria; j, k. section view of ascomata produced on pear fruit (*P. bretschneideri* cv. Huangguan) and leaf (*P. pyrifolia* cv. Cuiguan), respectively; l. section view of ascoma; m, n. asci; o. ascospores (a–o. isolate PAFQ22; a–e, h, l–o. produced on PDA, f. produced on OA, g. produced on SNA). — Scale bars: c, d = 200 µm; e–h, j–o = 20 µm; i = 10 µm.

Colletotrichum plurivorum Damm et al., Stud. Mycol. 92: 31. 2019. — Fig. 15

Description & Illustration — Damm et al. (2019).

Materials examined. China, Anhui Province, Dangshan County, on leaves of *P. bretschneideri* cv. Huangguan, 4 Aug. 2016, *M. Fu* (culture PAFQ65).

Notes — *Colletotrichum plurivorum* was first reported as *C. sichuanensis* from fruits of *Capsicum annuum* in China (Liu et al. 2016b), further regarded as a synonym of *C. cliviicola* (as *C. cliviae*) (Douanla-Meli et al. 2018), but later distinguished from the latter by Damm et al. (2019). In this study, isolate PAFQ65 was isolated from pear leaves and clustered together

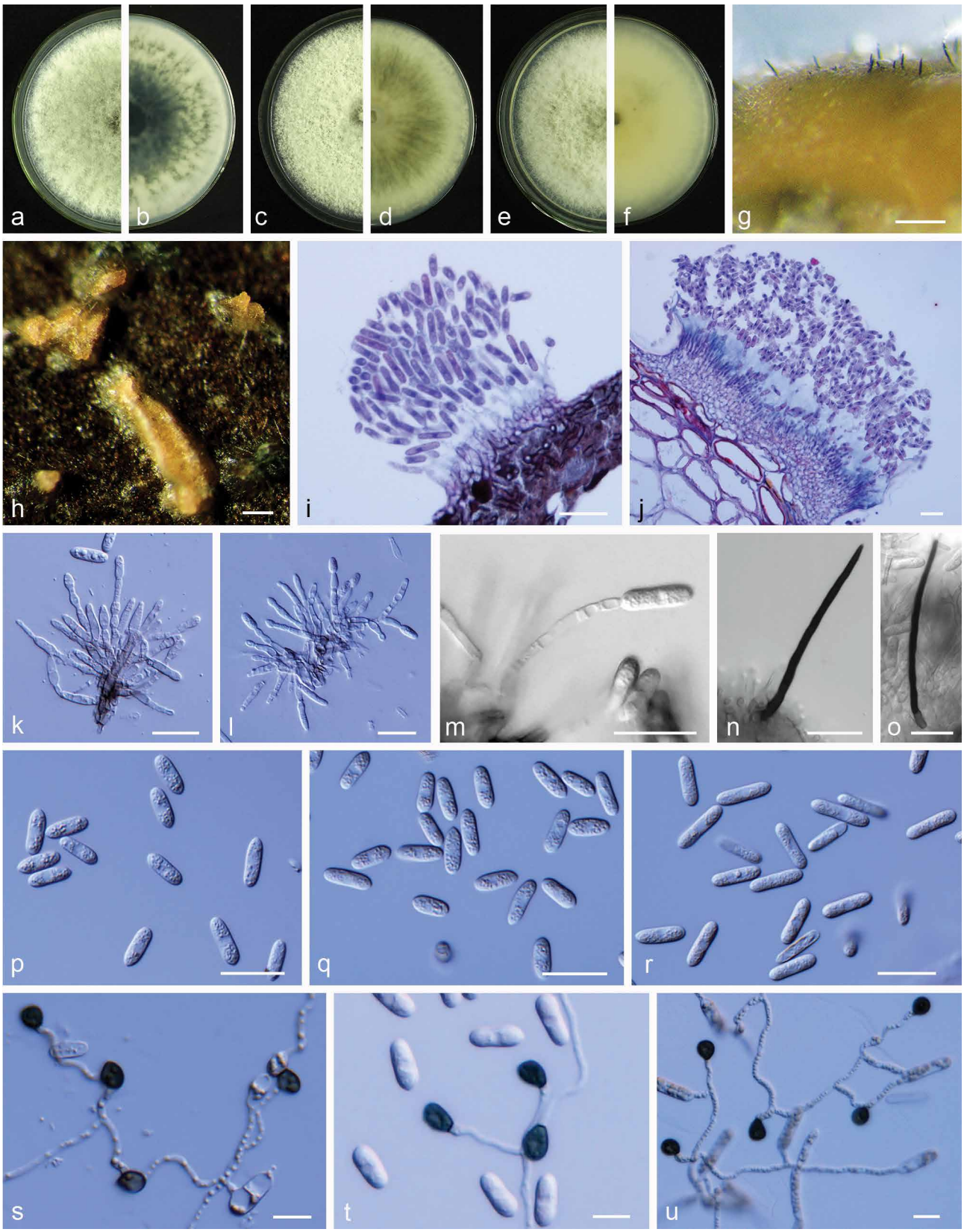

Fig. 17 *Colletotrichum siamense*. a, c, e. Front views of 6-d-old PDA culture; b, d, f. back views of 6-d-old PDA culture; g, h. conidiomata; i, j. section view of acervulus produced on pear leaf (*P. pyrifolia* cv. Cuiguan) and fruit (*P. bretschneideri* cv. Huangguan), respectively; k–m. conidiophores; n, o. setae; p–r. conidia; s–u. appressoria (a, b, k, p, s. from PAFQ67, c, d, g, h, j, l, n, q, t. from PAFQ74, e, f, i, m, o, r, u. from PAFQ78; a–g, k–r. produced on PDA, h. produced on pear leaf (*P. bretschneideri* cv. Dangshansuli)). — Scale bars: g, h = 100 µm; i–r = 20 µm; s–u = 10 µm.

with the ex-type culture of *C. plurivorum* (CBS 125474) in the multi-locus phylogenetic tree. This is the first report of *C. plurivorum* associated with anthracnose in *P. bretschneideri*. Notably, isolate PAFQ65 rapidly developed the sexual morph on PDA, but the asexual morph was not observed on PDA.

Colletotrichum pyrifoliae M. Fu & G.P. Wang, *sp. nov.* — MycoBank MB824217; Fig. 16

Etymology. Referring to the host species and host organ from which the fungus was isolated.

Sexual morph developed on PDA. *Ascomata* formed on PDA after 20–22 d, semi-immersed in the agar medium, pyriform to subglobose, dark brown, 78–212 × 75–160 µm, ostiolate. *Asci* fasciculate, clavate, 66–92 × 11–20 µm, 8-spored. *Ascospores* hyaline, smooth-walled, aseptate, cylindrical with rounded ends, straight, rarely slightly curved, contents granular, 11.5–20.5 × 4.5–7 µm, mean ± SD = 16.8 ± 1.6 × 6.4 ± 0.5 µm, L/W ratio = 2.6.

Asexual morph developed on PDA. Vegetative hyphae 2–6.5 µm diam, hyaline, smooth-walled, septate, branched. *Setae* not observed. *Conidiophores* hyaline to pale brown, smooth-walled,

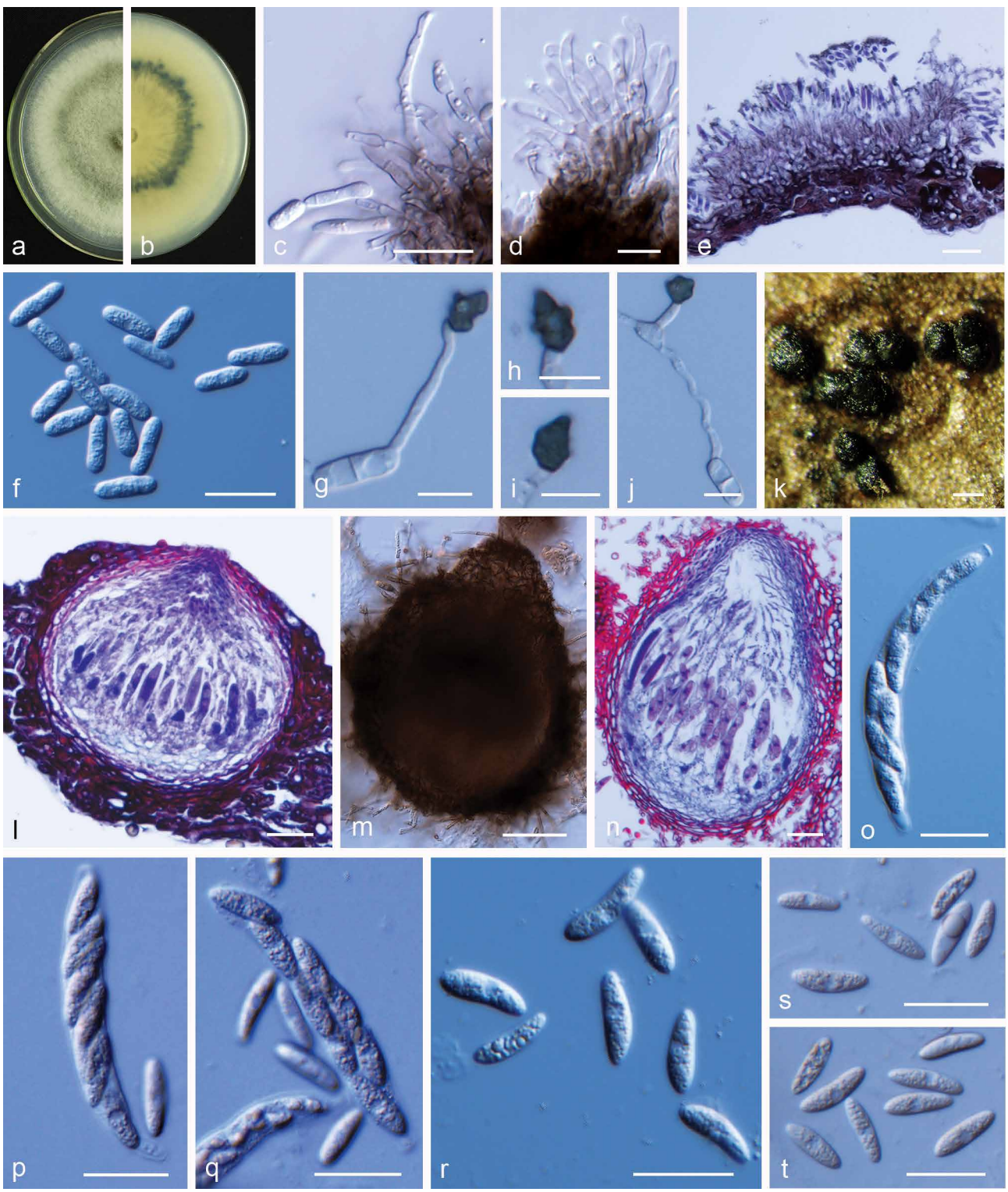

Fig. 18 *Colletotrichum wuxiense.* a, b. Front and back view, respectively, of 6-d-old PDA culture; c, d. conidiophores; e. section view of acervulus produced on pear leaf; f. conidia; g–j. appressoria; k. ascomata; l. section view of ascoma produced on pear fruit; m. ascoma produced on PDA; n. section view of ascoma; o–q. asci; r–t. ascospores (a–l, n, o, q–s. isolate PAFQ53, m, p, t. isolate PAFQ54; a–f, m–t. produced on PDA agar medium, m, n, p, q, s, t. produced on SNA agar medium). — Scale bars: c–f, l, n–t = 20 µm; g–j = 10 µm; k = 100 µm; m = 50 µm.

Table 4 The sizes of conidia, appresoria and ascospores of the representative isolates of *Colletotrichum* spp. obtained in this study.

Species and strain number	Conidia			Appresoria			Ascospores)			Growth rate (mm/d)
	Length (μm)[x]	Width (μm)[y]	Means ± SD of conidia size[z]	Length (μm)[x]	Width (μm)[y]	Means ± SD of appresoria size[z]	Length (μm)[x]	Width (μm)[y]	Means ± SD of ascospores size[z]	
C. aenigma										
PAFQ1	15.5–20	5–6.5	17.2 ± 1.0 × 5.6 ± 0.3	7.5–15.5	6–11	10.5 ± 1.8 × 8.0 ± 1.2	13.5–22	6–8	18.0 ± 1.7 × 6.9 ± 0.5	8.2
PAFQ3	14.5–20	5.5–7.5	17.1 ± 1.1 × 6.6 ± 0.4	/	/	/	14.5–20.5	5–8	17.5 ± 1.6 × 6.5 ± 0.6	3.7
PAFQ5	16–21.5	5.5–7.5	18.5 ± 1.1 × 6.7 ± 0.5	7.5–11	5–9.5	9.2 ± 1.1 × 7.1 ± 1.1	14.5–19	4–8	16.7 ± 1.1 × 6.1 ± 0.8	6.9
PAFQ47	15–19	5.5–7	16.9 ± 0.9 × 6.3 ± 0.3	8–11.5	5.5–9	9.4 ± 1.0 × 7.3 ± 0.9	12.5–19.5	5–8	15.7 ± 1.6 × 6.6 ± 0.8	7.9
PAFQ66	14.5–18	5.5–6.5	16.0 ± 0.7 × 5.8 ± 0.3	6–11.5	6–11.5	9.0 ± 1.3 × 7.6 ± 1.1	15–20	5.5–8.5	17.1 ± 1.1 × 6.5 ± 0.6	7.5
PAFQ81	15–19	5–6	17.1 ± 0.9 × 5.8 ± 0.3	5.5–11	5.5–8	8.8 ± 1.2 × 6.7 ± 0.8	14.5–21	5.5–8	18.0 ± 1.6 × 6.7 ± 0.5	7.5
C. citricola										
PAFQ13	12.5–17	6–8	14.4 ± 1.0 × 7.1 ± 0.4	7–9.5	5.5–7.5	8.2 ± 0.6 × 6.7 ± 0.5	13.5–20	5–8	17.4 ± 1.4 × 7.1 ± 0.7	4.4
C. conoides										
PAFQ6	16–20	4.5–6	18.4 ± 0.8 × 5.6 ± 0.3	7–12.5	5–8.5	9.7 ± 1.3 × 6.9 ± 1.1	12.5–21	5.5–7.5	15.9 ± 1.3 × 6.8 ± 0.5	7.8
C. fioriniae										
PAFQ8	13.5–16	4.5–5.5	15.8 ± 1.0 × 5.6 ± 0.3	5.5–9	3.5–6	7.1 ± 0.9 × 4.9 ± 0.5	/	/	/	3.5
PAFQ17	13–15	4–5	15.2 ± 1.2 × 5.1 ± 0.5	5.5–8.5	3.5–6	7.1 ± 0.6 × 5.2 ± 0.5	/	/	/	4.3
PAFQ36	11.5–14	4.5–5	14.2 ± 1.2 × 5.3 ± 0.4	5.5–8.5	4.5–6	7.2 ± 0.7 × 5.3 ± 0.4	/	/	/	4.7
PAFQ49	13–16	4.5–5.5	16.1 ± 1.3 × 5.7 ± 0.4	6.5–10	4.5–6.5	7.7 ± 0.7 × 5.4 ± 0.5	/	/	/	4.6
PAFQ55	12.5–16.5	4–5	16.3 ± 1.4 × 5.0 ± 0.4	6–9	4.5–6.5	7.3 ± 0.7 × 5.3 ± 0.5	/	/	/	4.6
PAFQ75	13–15.5	4.5–5.5	15.4 ± 1.3 × 5.4 ± 0.3	6.5–10.5	4–7	7.8 ± 1.0 × 5.2 ± 0.6	/	/	/	4.4
C. fructicola										
PAFQ30	14.5–19	5–7.5	17.1 ± 1.1 × 6.4 ± 0.6	6.5–13	5–8.5	8.5 ± 1.7 × 6.7 ± 0.9	15.5–24	4–6	18.8 ± 1.9 × 5.4 ± 0.5	7
PAFQ31	14.5–20	5–7.5	17.1 ± 1.5 × 6.1 ± 0.6	8–12.5	6–9.5	9.9 ± 1.2 × 7.2 ± 0.9	14–22	3.5–6	17.1 ± 1.9 × 4.6 ± 0.6	7.6
PAFQ32	13–17.5	5.5–7	15.1 ± 1.0 × 6.5 ± 0.4	8–14.5	6–9.5	10.9 ± 1.5 × 7.5 ± 0.9	12.5–22.5	4–6	17.1 ± 1.9 × 4.9 ± 0.5	7.3
PAFQ48	13.5–16.5	4–6	15.0 ± 0.7 × 5.1 ± 0.4	7–10	5.5–8	8.2 ± 0.8 × 6.7 ± 0.7	14.5–25.5	4.5–7	18.3 ± 1.9 × 5.4 ± 0.5	7.8
PAFQ77	13.5–19.5	4–6	16.2 ± 1.5 × 5.3 ± 0.4	6.5–13	5–7	9.5 ± 1.5 × 6.0 ± 0.5	12.5–18.5	3.5–6	15.5 ± 1.5 × 4.9 ± 0.7	6.6
PAFQ84	14–19	4.5–6	16.1 ± 1.1 × 5.4 ± 0.4	6.5–14	5–7	7.8 ± 1.4 × 6.0 ± 0.5	/	/	/	7.9
C. gloeosporioides										
PAFQ7	16–22.5	5–7.5	18.0 ± 1.4 × 6.1 ± 0.6	7–10.5	5–7	8.4 ± 0.8 × 6.1 ± 0.5	/	/	/	7.9
PAFQ44	11.5–21	4–6	16.6 ± 1.7 × 5.5 ± 0.4	7.5–12.5	5.5–8.5	9.0 ± 1.2 × 7.0 ± 0.7	/	/	/	8.3
PAFQ56	16–32	4.5–6.5	21.5 ± 4.1 × 5.5 ± 0.4	6–10.5	5–9	8.3 ± 1.0 × 6.6 ± 0.8	/	/	/	7
PAFQ61	15.5–22.5	5–6.5	17.7 ± 1.6 × 5.6 ± 0.3	6.5–10	4.5–7.5	8.2 ± 0.8 × 6.3 ± 0.7	/	/	/	7.4
PAFQ80	15–21	5–6.5	16.9 ± 1.1 × 5.9 ± 0.3	6.5–11	5–6.5	7.8 ± 0.9 × 5.9 ± 0.4	/	/	/	7.4
PAFQ86	14–18	5–6.5	16.1 ± 0.9 × 5.8 ± 0.3	7–11.5	5–7.5	9.0 ± 1.3 × 6.4 ± 0.6	/	/	/	7.1
C. jinshuiense										
PAFQ26	21–30.5[α]	3–4.5[α]	24.4 ± 2.1 × 4.0 ± 0.3[α]	8–17	5–7.5	10.7 ± 1.7 × 6.0 ± 0.5	/	/	/	5.6
C. karstii										
PAFQ14	12.5–18	5.5–8	15.8 ± 1.0 × 7.2 ± 0.5	6.5–10	5.5–7.5	8.3 ± 0.8 × 6.4 ± 0.5	/	/	/	4.3
PAFQ28	12.5–18.5	6–8	15.5 ± 1.4 × 6.8 ± 0.5	6.5–10	5–8.5	8.4 ± 0.7 × 6.9 ± 0.7	/	/	/	5.2
PAFQ40	12.5–16	5.5–7.5	13.6 ± 0.8 × 6.5 ± 0.4	6.5–9.5	6–8.5	8.0 ± 0.7 × 7.3 ± 0.6	14–19	5–8	16.4 ± 1.1 × 6.8 ± 0.7	5.3
PAFQ52	11.5–16	5.5–7.5	13.9 ± 1.0 × 6.8 ± 0.3	7–10.5	5–8	8.8 ± 0.7 × 6.8 ± 0.8	/	/	/	5.3
PAFQ82	12.5–21	5–8	16.8 ± 1.6 × 7.2 ± 0.6	8–14	5–9.5	10.5 ± 1.4 × 7.2 ± 1.0	/	/	/	4.4
C. plurivorum										
PAFQ65	14–24[α]	4.5–7[α]	18.1 ± 2.1 × 5.6 ± 0.7[α]	/	/	/	15–20.5	4.5–6	18.2 ± 1.6 × 5.4 ± 0.4	7.2
C. pyrifoliae										
PAFQ22	14–23	5.5–7	18.1 ± 1.8 × 6.4 ± 0.4	7–12	6–8	8.8 ± 1.0 × 6.9 ± 0.5	11.5–20.5	4.5–7	16.8 ± 1.6 × 6.4 ± 0.5	4.9

Table 4 (cont.)

Species and strain number	Conidia			Appressoria			Ascospores			Growth rate (mm/d)
	Length (μm)[x]	Width (μm)[y]	Means ± SD of conidia size[z]	Length (μm)[x]	Width (μm)[y]	Means ± SD of appresoria size[z]	Length (μm)[x]	Width (μm)[y]	Means ± SD of ascospores size[z]	
C. siamense										
PAFQ67	12–18	5–6.5	15.5 ± 1.0 × 5.6 ± 0.3	6–10.5	4.5–8.5	8.1 ± 1.3 × 6.2 ± 0.7	/	/	/	7.9
PAFQ68	12.5–17.5	5.5–7	14.7 ± 1.0 × 5.8 ± 0.4	5.5–10.5	5.5–7.5	8.0 ± 1.1 × 6.3 ± 0.6	/	/	/	8.2
PAFQ71	13–19	4.5–6.5	15.8 ± 1.1 × 5.3 ± 0.4	5.5–9.5	5–6.5	7.7 ± 1.0 × 5.8 ± 0.4	/	/	/	7.7
PAFQ73	13.5–19	4–6	16.0 ± 1.2 × 5.6 ± 0.4	6.5–8.5	4.5–6.5	7.4 ± 1.0 × 5.7 ± 0.4	/	/	/	/
PAFQ74	13–17.5	4.5–6.5	15.1 ± 0.9 × 5.7 ± 0.3	6–9	4.5–6.5	7.8 ± 0.6 × 5.7 ± 0.5	/	/	/	7.8
PAFQ78	15–21	4–6	17.4 ± 1.1 × 5.4 ± 0.5	6.5–12	5.5–9	9.0 ± 1.2 × 6.7 ± 0.8	/	/	/	7.6
PAFQ85	14–20	4.5–5.9	15.9 ± 1.1 × 5.4 ± 0.3	5.5–10	4.5–6.5	7.8 ± 1.0 × 5.8 ± 0.5	/	/	/	8.3
PAFQ91	12–17.5	5–7	15.0 ± 1.1 × 5.9 ± 0.4	6.5–10	4–7	7.8 ± 1.2 × 5.9 ± 0.5	/	/	/	/
C. wuxiense										
PAFQ53	11.5–17	4.5–6.5	14.9 ± 1.3 × 5.3 ± 0.3	6.5–12	5.5–11	9.4 ± 1.1 × 7.1 ± 1.4	14–20[β]	4–6.5[β]	17.2 ± 1.3 × 5.0 ± 0.5[β]	7.1
PAFQ54	13–18	4.5–6	15.0 ± 1.3 × 5.1 ± 0.4	/	/	/	13–21[β]	4.5–6[β]	17.7 ± 1.5 × 5.2 ± 0.4[β]	7

[x] Numbers indicate minimum and maximum sizes for length of 50 conidia, ascospores and 30 appresoria recorded from the representative strains of *Colletotrichum* spp. obtained in this study. Significance at $P = 0.05$ level.
[y] Numbers indicate minimum and maximum sizes for width of 50 conidia, ascospores and 30 appresoria recorded from the representative strains of *Colletotrichum* spp. obtained in this study. Significance at $P = 0.05$ level.
[z] Numbers indicate mean conidia, appresoria, ascospores sizes of each representative strain calculated by the statistical analysis. Data were analyzed with SPSS Statistics 21.0 (WinWrap® Basic; http://www.winwrap.com) by one-way ANOVA, and means were compared using Duncan's test at a significance level of $P = 0.05$. SD: standard deviation.
/ Appresoria, ascospores or data of growth rate were absent.
[α] Conidia induced on fruit.
[β] Ascospores induced on SNA medium.

septate and branched. *Conidiogenous cells* hyaline to pale brown, cylindrical to clavate, 15–32 × 3–5 μm, opening 1.5–2.5 μm. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, both ends rounded, contents granular, 14–23 × 5.5–7 μm, mean ± SD = 18.1 ± 1.8 × 6.4 ± 0.4 μm, L/W ratio = 2.9. *Appressoria* dark-brown, elliptical, 7–12 × 6–8 μm, mean ± SD = 8.8 ± 1.0 × 6.9 ± 0.5 μm, L/W ratio = 1.3.

Asexual morph developed on OA. *Setae* not observed. *Conidiophores* hyaline to pale brown, smooth-walled, septate and branched. *Conidiogenous cells* hyaline to pale brown, cylindrical to clavate, 8–23 × 4–5 μm. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, both ends rounded, contents granular, 15.5–21.5 × 5–6.5 μm, mean ± SD = 17.8 ± 1.3 × 5.7 ± 0.4 μm, L/W ratio = 3.1.

Asexual morph developed on SNA. *Setae* not observed. *Conidiophores* hyaline to pale brown, smooth-walled, septate and branched. *Conidiogenous cells* hyaline to pale brown, cylindrical to clavate, 12–24.5 × 4–6 μm. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, both ends rounded, contents granular, 16–22 × 5–6.5 μm, mean ± SD = 18.5 ± 1.3 × 5.6 ± 0.3 μm, L/W ratio = 3.3.

Culture characteristics — Colonies on PDA flat with entire margin, aerial mycelium sparse, cottony in the centre, surface grey-green with white margin; reverse dark grey-green with white margin; colony diam 48–50 mm in 5 d. *Conidia in mass* pale yellow.

Materials examined. China, Hubei Province, Wuhan City, on leaves of *P. pyrifolia* cv. Jinshui, 1 Aug. 2016, *M. Fu* (holotype HMAS 247825, culture ex-type CGMCC 3.18902 = PAFQ22); ibid., PAFQ22a, PAFQ22b, PAFQ22c, and PAFQ22d.

Notes — *Colletotrichum pyrifoliae* is phylogenetically closely related to *Colletotrichum* sp. isolate Q026 (Fig. 5), which was reported to be associated with anthracnose of *Rubus glaucus* in Colombia (Afanador-Kafuri et al. 2014). However, *C. pyrifoliae* differs from the latter in *ACT* (with 95.62 % sequence identity), *CHS-1* (96.47 %), *GAPDH* (93.01 %), ITS (99.25 %), and *TUB2* (96.41 %) sequences. Moreover, isolates of *C. pyrifoliae* have larger conidia (PAFQ22, 14–23 × 5.5–7 μm, mean ± SD = 18.1 ± 1.8 × 6.4 ± 0.4 μm) than those of *Colletotrichum* sp. isolate Q026 (mean = 10.4 × 2.9 μm). The PHI test (Φw = 0.9862) detected no significant recombination between the isolates and *Colletotrichum* sp. isolate Q026 (Fig. 6b). *Colletotrichum pyrifoliae* is a singleton species, which grouped neither with the *C. gloeosporioides* nor the *C. boninense* species complexes (Fig. 5).

Colletotrichum siamense Prihast. et al., Fung. Diversity 39: 98. 2009. — Fig. 17

Description & Illustration — Prihastuti et al. (2009).

Materials examined. China, Shandong Province, Yantai City, on fruits of *P. communis* cv. Gyuiot, 27 Aug. 2016, *M. Fu* (cultures PAFQ67, PAFQ68, PAFQ71, PAFQ73, PAFQ74); Zhejiang Province, Hangzhou City, on leaves of *P. pyrifolia* cv. Guanyangxueli, 18 Aug. 2016, *M. Fu* (PAFQ78); ibid., on leaves of *P. pyrifolia* cv. Cuiguan, 18 Aug. 2016, *M. Fu* (PAFQ85).

Notes — *Colletotrichum siamense* was first reported on *Coffea arabica* in Thailand (Prihastuti et al. 2009) and subsequently reported on a wide range of hosts (e.g., Yang et al. 2009, Wikee et al. 2011, Weir et al. 2012, Wang et al. 2016, Liu et al. 2016b). Notably, this is the first report and characterisation of *C. siamense* causing anthracnose on *P. pyrifolia* and *P. communis*.

The isolates of *C. siamense* were divided into three groups (I–III) in this study according to morphology. Group I colonies (13 isolates, representative isolate PAFQ67) flat, grey-green with white margin; reverse dark green to black in the centre and pale white margin, sporadic pigment at the margin. Group

II colonies (25 isolates, representative isolate PAFQ74) flat, surface white; reverse pale yellow in the centre and pale white margin, sometimes grey radial pigment produced. Group III colonies (1 isolate, representative isolate PAFQ78) convex, surface pale white in the centre and white margin; reverse pale yellow in the centre and pale white margin, sometimes grey pigment produced. Moreover, these isolates have similar appressorial sizes but different conidium sizes among the three colony types. Of these, conidium sizes of the type III isolates (PAFQ78, 15–21 µm, mean lengths ± SD = 17.4 ± 1.1 µm) were longer than those of type I (12–19 µm, mean lengths from 15.5 ± 1.0 to 16.0 ± 1.2 µm) and II (12–17.5 µm, mean lengths from 14.7 ± 1.0 to 15.1 ± 0.9 µm) isolates (Table 4 and Fig. 17p–r). Setae were observed in isolates PAFQ78 and PAFQ74 on PDA, and setae were dark brown to black, opaque, tip acute, base cylindrical, 3-septate, 67–95 µm long.

Colletotrichum wuxiense Y.C. Wang et al., Sci. Rep. 6: 8. 2016. — Fig. 18

Sexual morph on SNA. *Ascomata* developed on SNA after 18–22 d, immersed or semi-immersed in the agar medium, subglobose to pyriform, dark brown, 88–249 × 88–224 µm, ostiolate. *Asci* clavate, 43–91 × 9–13 µm, 8-spored. *Ascospores* hyaline, smooth-walled, aseptate, fusiform, slightly curved, rarely straight, rounded ends, contents granular, sometimes with 1–3 guttules, 14–20 × 4–6.5 µm, mean ± SD = 17.2 ± 1.3 × 5.0 ± 0.5 µm, L/W ratio = 3.4.
Sexual morph developed on PDA. *Ascomata* pyriform to subglobose, dark brown, 74–139 × 64–127 µm, ostiolate. *Asci* clavate, 57–96 × 12–16 µm, 8-spored. *Ascospores* hyaline, smooth-walled, aseptate, fusoid, slightly curved, straight with round ends, contents granular, 15.5–22 × 5–6.5 µm, mean ± SD = 18.37 ± 1.39 × 5.80 ± 0.44 µm, L/W ratio = 3.2.
Asexual morph developed on PDA. Vegetative hyphae 1.5–4.5 µm diam, hyaline, smooth-walled, septate, branched. *Setae* not observed. *Conidiophores* hyaline to pale brown, smooth-walled, septate and branched. *Conidiogenous cells* hyaline to pale brown, cylindrical, 8.5–28 × 2.5–4 µm. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, both ends rounded or one end slightly acute, contents granular or guttulate, 11.5–17 × 4.5–6.5 µm, mean ± SD = 14.9 ± 1.3 × 5.3 ± 0.3 µm, L/W ratio = 2.8. *Appressoria* dark-brown, irregular in shape or bullet-shaped with an acute tip, lobed, 6.5–12 × 5.5–11 µm, mean ± SD = 9.4 ± 1.1 × 7.1 ± 1.4 µm, L/W ratio = 1.3.

Culture characteristics — Colonies on PDA convex with entire margin, aerial mycelium dense, surface greenish in the centre, with white margin; reverse pale yellow with white margin, and a dark green concentric ring in the middle of the colony. Colony diam 70–71 mm in 5 d. *Conidia in mass* orange.

Materials examined. China, Jiangxi Province, Jinxi County, on leaves of *P. pyrifolia* cv. Cuiguan, 23 July 2016, *M. Fu* (cultures PAFQ53 and PAFQ54).

Notes — According to the results obtained in the multi-locus phylogenetic analyses (Fig. 2), two isolates (PAFQ53, PAFQ54) from pear leaves clustered together with the ex-type culture of *C. wuxiense* (CGMCC 3.17894), which was initially reported on *Camellia sinensis* in China (Wang et al. 2016). Notably, the conidium sizes of *C. wuxiense* isolates in this study (PAFQ53: 11.5–17 × 4.5–6.5 µm, mean ± SD = 14.9 ± 1.3 × 5.3 ± 0.3 µm; PAFQ54: 13–18 × 4.5–6 µm, mean ± SD = 15.0 ± 1.3 × 5.1 ± 0.4 µm) were smaller than those of the ex-type culture of *C. wuxiense* (CGMCC 3.17894: 16.5–23 × 4.5–6.5 µm, mean ± SE = 19.0 ± 1.4 × 5.6 ± 0.5 µm). This is the first report of *C. wuxiense* to cause anthracnose on *P. pyrifolia* and the first description of its sexual morph.

Prevalence of Colletotrichum species

Analyses of the prevalence of 12 *Colletotrichum* species revealed that *C. fructicola* isolates (298 isolates, 61.1 % of the total isolates) were predominantly isolated from six provinces (Anhui, Fujian, Hubei, Jiangsu, Jiangxi, and Zhejiang), followed by *C. fioriniae* (52 isolates, 10.7 %, isolated from Anhui, Fujian, Hubei, Jiangsu, Jiangxi, and Shandong), *C. siamense* (43 isolates, 8.8 %, isolated from Shandong and Zhejiang), *C. aenigma* (40 isolates, 8.2 %, isolated from Anhui, Hubei, Jiangsu, and Zhejiang), *C. gloeosporioides* (20 isolates, 4.1 %, isolated from Hubei, Jiangsu, Jiangxi, and Zhejiang), and *C. karstii* (19 isolates, 3.9 %, isolated from Fujian, Hubei, Jiangxi, and Zhejiang) (Fig. 19a, b). The remaining six species account for 3.2 % of the isolates (Fig. 19a, b). These results revealed that *C. fructicola* is the most dominant species on pear in China; *C. aenigma*, *C. fioriniae*, *C. gloeosporioides*, *C. karstii*, and *C. siamense* were less dominant and *C. citricola*, *C. conoides*, *C. jinshuiense*, *C. plurivorum*, *C. pyrifoliae*, and *C. wuxiense* the least dominant species. Moreover, *C. fructicola* isolates causing black spot symptoms were mainly detected in the Yangtze valley regions in the Fujian, Hubei, Jiangsu, Jiangxi, and Zhejiang provinces.

Analyses of the isolation rate of these *Colletotrichum* species in each of the sampled provinces revealed that *C. fructicola* was dominantly isolated in Fujian, Jiangxi, Jiangsu, Anhui, and Zhejiang provinces, accounting for 85.2 %, 83.8 %, 80.4 %, 78 %, and 71.4 % of the obtained isolates, respectively. Isolates of each other species accounted for less than 15 % (Fig. 19b). However, in the Shandong province, *C. siamense* isolates were dominantly isolated, accounting for 95 % of the total isolates from this province; in the Hubei province, *C. fructicola*, *C. fioriniae*, and *C. aenigma* isolates were commonly isolated, accounting for 27.5 %, 26.7 %, and 25.0 %, respectively, of the total isolates from this province (Fig. 19b).

Analyses of the isolation rate of these *Colletotrichum* species from each of the sampled pear species revealed that *C. fructicola* isolates were dominant on *P. pyrifolia* and *P. bretschneideri*, accounting for 64.5 % and 79.7 % of the total isolates, respectively, followed by *C. fioriniae* (11.8 %), *C. aenigma* (9.3 %), *C. karstii* (4.9 %), and *C. gloeosporioides* (4.6 %) from *P. pyrifolia*, and *C. fioriniae* (6.8 %), *C. aenigma* (6.8 %), *C. plurivorum* (3.4 %), and *C. gloeosporioides* (3.4 %) from *P. bretschneideri*. The remaining species (*C. citricola*, *C. conoides*, *C. jinshuiense*, *C. pyrifoliae*, *C. siamense*, and *C. wuxiense*) were isolated in a low incidence of less than 5.0 % from *P. pyrifolia*. Only *C. siamense* and *C. fioriniae* were isolated from *P. communis*, with the former accounting for an incidence of 95 % and the latter for 5 % (Fig. 19c). Analyses of the incidence of these *Colletotrichum* species from the leaves and fruits revealed that *C. aenigma*, *C. fructicola*, *C. gloeosporioides*, *C. fioriniae*, and *C. siamense* were isolated from both leaves and fruits, while *C. citricola*, *C. jinshuiense*, *C. karstii*, *C. plurivorum*, and *C. pyrifoliae* were isolated only from leaves, and *C. conoides* only from fruits (Fig. 19d).

Pathogenicity

Thirteen representative *Colletotrichum* isolates (one from each species except two from *C. fructicola* related to two different symptom types) were selected to prove Koch's postulates with a spore suspension on detached leaves of *P. pyriforia* cv. Cuiguan. Under unwounded conditions, only *C. fructicola* (isolate PAFQ31) and *C. siamense* (isolate PAFQ78) were pathogenic to leaves by inducing lesions on the leaf tissues (Fig. 20). Of these, isolate PAFQ31 caused TS symptoms at 8 dpi (Fig. 20b2) and isolate PAFQ78 caused extended BnL symptoms at 14 dpi (Fig. 20b5). Under wounded conditions inoculated at

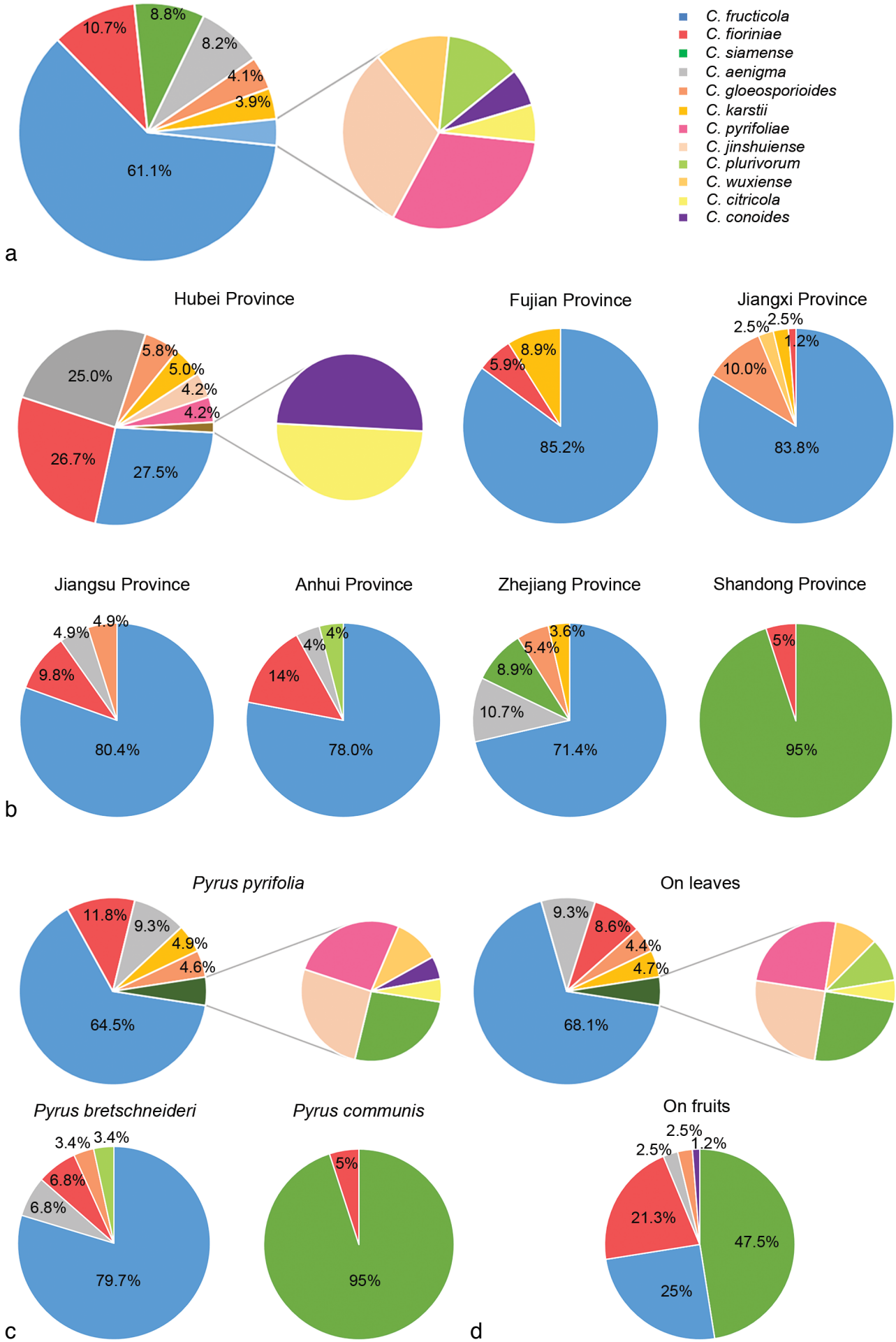

Fig. 19 The prevalence of *Colletotrichum* species isolated from pear. a. Overall isolation rate (%) of *Colletotrichum* species; b–d. isolation rate (%) of *Colletotrichum* species from each sampled province (b), *Pyrus* spp. (c), and pear organs (d), respectively.

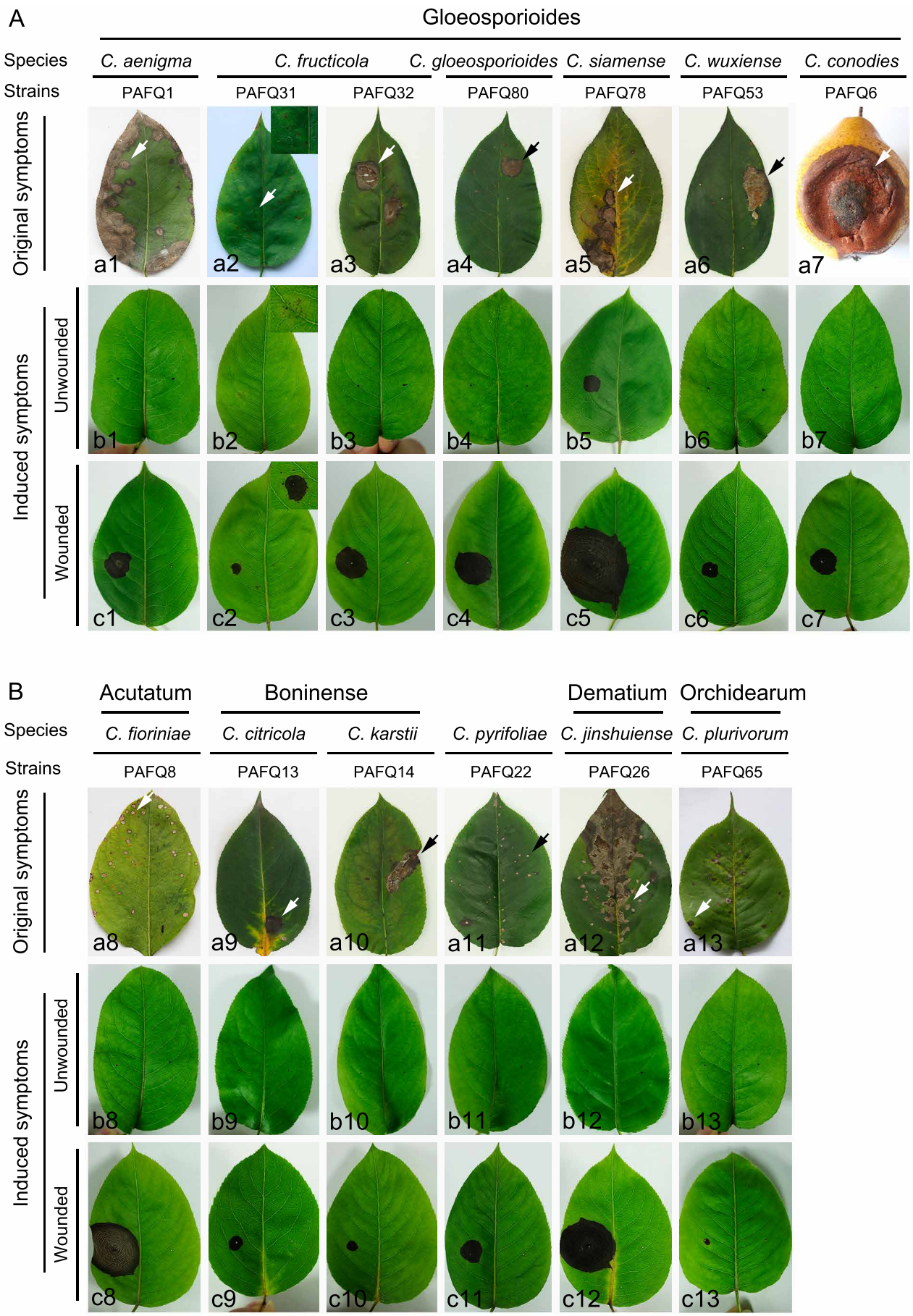

Fig. 20 Representative symptoms of pear leaves (*P. pyrifolia* cv. Cuiguan) induced by inoculation of spore suspensions of 12 *Colletotrichum* spp. under unwounded and wounded conditions. The symptoms caused by these species were photographed at 14 dpi (except for b2, c2, c3 at 8 dpi). A, B. The symptoms induced by the isolates/species belonging to the *C. gloeosporioides* complex (A) and other complexes or singleton species (B), respectively. The inoculation was conducted by dropping 1 × 10^6 spores (conidia or ascospores) per mL on detached about four-weeks-old leaves of *P. pyrifolia* cv. Cuiguan in eight replicates after wounded by pin-pricking each leaf for one time with a sterilized needle (wounded) or kept unwounded (unwounded). Under unwounded conditions, inoculated positions are indicated with blue spots.

Table 5 Infection rates of *Colletotrichum* spp. inoculated on leaves of *P. pyrifolia* cv. Cuiguan.

Species	Strain	Origin	Infection rate
C. aenigma	PAFQ1	Leaf	14/16
C. citricola	PAFQ13	Leaf	7/16
C. conoides	PAFQ6	Fruit	6/16
C. fioriniae	PAFQ8	Leaf	15/16
C. fructicola	PAFQ31	Leaf	16/16
	PAFQ32	Leaf	10/16
C. gloeosporioides	PAFQ80	Leaf	9/16
C. jinshuiense	PAFQ26	Leaf	9/16
C. karstii	PAFQ14	Leaf	7/16
C. plurivorum	PAFQ65	Leaf	2/16
C. pyrifoliae	PAFQ22	Leaf	10/16
C. siamense	PAFQ78	Leaf	12/16
C. wuxiense	PAFQ53	Leaf	7/16
control	H_2O		0

Table 6 Infection rates of *Colletotrichum* spp. inoculated on the fruits of *P. bretschneideri* cv. Huangguan.

Species	Strain	Origin	Infection rate
C. aenigma	PAFQ66	Fruit	4/6
C. citricola	PAFQ13	Leaf	0/6
C. conoides	PAFQ6	Fruit	3/6
C. fioriniae	PAFQ19	Fruit	2/6
C. fructicola	PAFQ31	Leaf	6/6
	PAFQ32	Leaf	5/6
C. gloeosporioides	PAFQ61	Fruit	5/6
C. jinshuiense	PAFQ26	Leaf	0/6
C. karstii	PAFQ14	Leaf	0/6
C. plurivorum	PAFQ65	Leaf	0/6
C. pyrifoliae	PAFQ22	Leaf	0/6
C. siamense	PAFQ74	Fruit	4/6
C. wuxiense	PAFQ53	Leaf	0/6
control	H_2O		0

14 dpi, all the species were pathogenic to leaves, but with obviously varied infection rates depending on the species/isolates (Table 5), with the least 2/16 infection rates for *C. plurivorum* (isolate PAFQ65) to 16/16 for *C. fructicola* (isolate PAFQ31). In the case of successful infection, all species started to induce small dark-brown to black necrotic lesions at 6 dpi but 10 dpi for *C. citricola* (isolate PAFQ13). The small lesions quickly expanded into large dark-brown to black lesions, with the lesion lengths varying among the species (Fig. 20c1–c13) and formed concentric rings of acervuli on the leaf tissues and exuded an orange conidia mass (6–10 dpi) at 25 °C under 99 % relative humidity. It is worth to mention that *C. fructicola* isolate PAFQ31 isolated from a leaf showing TS symptoms in the field induced similar symptoms around the BnL on inoculated leaves (Fig. 20c2), while another *C. fructicola* isolate PAFQ32 from a leaf showing BnL symptoms induced big black lesions only (Fig. 20c3). Moreover, *C. conoides* isolate PAFQ6, which was only isolated from pear fruits, also caused BnL symptoms on pear leaves (Fig. 20c7). No lesions were induced in the control fruits inoculated with sterile water.

Pathogenicity was also accessed on detached pear fruits of *P. bretschneideri* cv. Huangguan. Under unwounded conditions, all the isolates isolated from the fruits were pathogenic to the fruits at 30 dpi, with infection rates ranging from 2/6 for *C. fioriniae* (PAFQ19) to 5/6 for *C. gloeosporioides* (PAFQ61) (Table 6). These isolates started to induce small brown or dark brown lesions at different time points post inoculation, i.e., at 28–30 dpi for *C. aenigma*, *C. conoides*, and *C. fioriniae*, 18–22 dpi for *C. gloeosporioides*, and 6–8 dpi for *C. siamense*. The small lesions expanded to large brown or dark brown lesions over time and formed concentric rings of acervuli at 4–6 dpi, which exuded an orange conidium mass (Fig. 21b1, b4–b6, b8). For the isolates isolated from pear leaves, only *C. fructicola* isolates (PAFQ31 and PAFQ32) were pathogenic to the inoculated fruits, with infection rates of 6/6 for isolate PAFQ31 and 5/6 for isolate PAFQ32 (Table 6). It is worth to note that *C. fructicola* isolates PAFQ31 and PAFQ32 induced black spots (Fig. 21b2) and fruit rot symptoms (Fig. 21b3) at 30 dpi, respectively, similar to those in sizes on the leaves observed in the field. The remaining six species isolated from pear leaves induced no visual fruit symptoms (Fig. 21b7, b9–b13). Under wounded conditions, all species were pathogenic to pear fruits at 10 dpi, but with obviously varying aggressiveness among species (Fig. 21c1–c13 and Fig. 22). Of these, the isolates of the *C. gloeosporioides* species complex induced significantly longer lesions (40–62.5 mm) than those induced by *C. fioriniae* (20–22 mm), *C. citricola* (3 mm), *C. karstii* (31–32 mm), *C. pyrifoliae* (20.5 mm), and *C. jinshuiense* (24.5 mm) (Fig. 22). No lesions were induced in the control fruits inoculated with sterile water.

From the diseased leaf and fruit tissues, fungi were further isolated from the lesions neighbouring the asymptomatic regions. These results showed that the obtained colonies matched the original ones used for inoculation regarding their morphology and ITS sequence data.

DISCUSSION

In this study we employed morphological and multi-locus phylogenetic analyses to identify the species associated with pear anthracnose, and pathogenicity tests to confirm Koch's postulates. We revealed 12 species belonging to five *Colletotrichum* species complexes, including gloeosporioides (*C. aenigma*, *C. conoides*, *C. fructicola*, *C. gloeosporioides*, *C. siamense*, and *C. wuxiense*), acutatum (*C. fioriniae*), boninense (*C. citricola* and *C. karstii*), dematium (*C. jinshuiense*), orchidearum (*C. plurivorum*), and one singleton species (*C. pyrifoliae*). Of these, *C. conoides*, *C. siamense*, *C. wuxiense*, *C. citricola*, *C. karstii*, and *C. plurivorum* were confirmed to be responsible for pear anthracnose for the first time. More importantly, this study differentiated two new species responsible for pear anthracnose, namely *C. jinshuiense* and *C. pyrifoliae*.

Corresponding to the taxonomic classification determined by multi-locus phylogenetic analyses, most *Colletotrichum* species also exhibited characteristic morphological characters, including their colony colours, the density of aerial mycelium, and shapes and sizes of conidia, ascospores, appressoria and setae (Fig. 7–18). Most of these features have been used to delimit species in previous studies (Damm et al. 2012a, b, 2014, Liu et al. 2013a, 2015, Hou et al. 2016, Guarnaccia et al. 2017). It is worth to note that the *Colletotrichum* species associated with pear anthracnose secreted pigments that differed in colour among species and isolates. Moreover, these species also differed in their ability to form a sexual morph in culture. For example, *C. gloeosporioide*s, *C. siamense*, *C. fioriniae*, and *C. jinshuiense* produced no ascospores under the culture conditions employed. Additionally, *C. citricola* and *C. jinshuiense* produced setae on the host tissues, but *C. aenigma* and *C. siamense* did so on PDA. Importantly, the macro- and micro-morphologies of the *Colletotrichum* species isolated

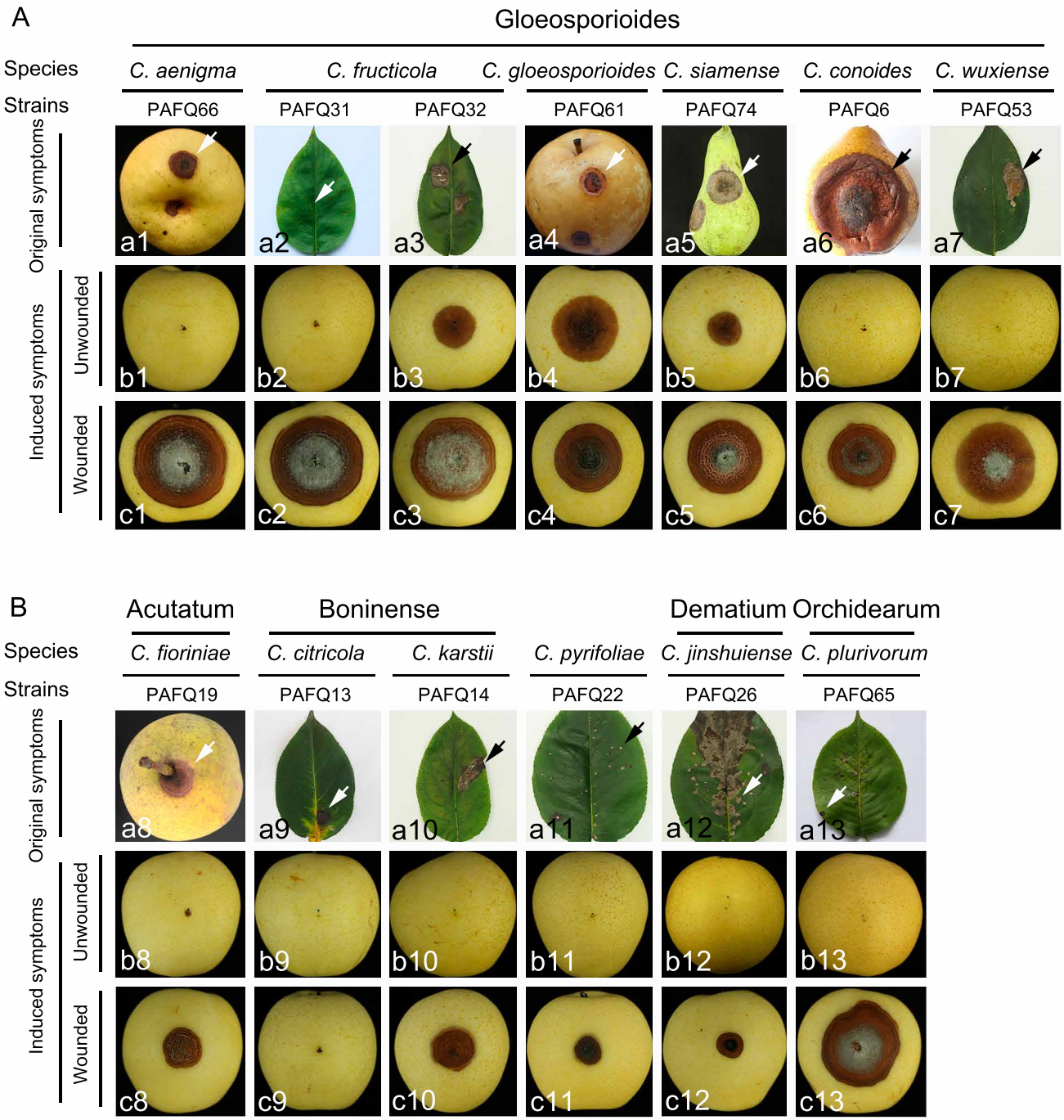

Fig. 21 Representative symptoms of pear fruits (*P. bretschneideri* cv. Huangguan) induced by inoculation with spore suspensions of 12 *Colletotrichum* spp. under unwounded and wounded conditions. The symptoms under unwounded conditions were photographed at 30 dpi, whereas these under the wounded at 10 dpi. A, B. The symptoms induced by the isolates/species belonging to the *C. gloeosporioides* complex (A) and other complexes or singleton species (B), respectively. The inoculation was conducted by dropping 1×10^6 spores (conidia or ascospores) per mL on detached fruits in triplicate after wounded by pin-pricking each position for three times with a sterilized needle (wounded) or kept unwounded (unwounded). Under unwounded conditions, inoculated positions are indicated with blue spots.

from pear showed differences compared with those from other plants. For example, most of the *C. gloeosporioides* isolates (e.g., PAFQ56, PAFQ61, and PAFQ7; 15.5–32 µm) from pear had longer conidia than those from tea (11–15.5 µm) (Liu et al. 2015) and citrus (11.3–14.7 µm) (Huang et al. 2013); and most of *C. fructicola* isolates (PAFQ30, PAFQ31, and PAFQ84; 14.0–20 × 4.5–7.5 µm) from pear had larger conidia than those from coffee (9.7–14 × 3–4.3 µm) (Prihastuti et al. 2009).

The prevalence of a *Colletotrichum* species associated with pear anthracnose is closely related to the sampling area, *Pyrus* sp. and plant organ. For example, *C. fructicola* is the most prevalent species in most pear-growing regions in China studied, and most frequently isolated from *P. pyrifolia* and *P. bretschneideri* in all the sampled areas except for the Shandong province, where *C. siamense* was most frequently isolated and prevalent on *P. communis*. Geographical preference was also found for *C. aenigma* and *C. fioriniae*, which were mainly isolated in the Hubei province. However, *C. jinshuiense*, *C. pyrifoliae*, *C. wuxiense*, *C. plurivorum*, *C. conoides*, and *C. citricola* showed low prevalence and restricted distribution. Moreover, a high species diversity was observed in the Hubei province as compared to the Fujian and Shandong provinces. It is worth to note that *C. acutatum*, *C. pyricola*, and *C. salicis* were not detected in this study although they were linked to pear anthracnose in New Zealand (Damm et al. 2012b).

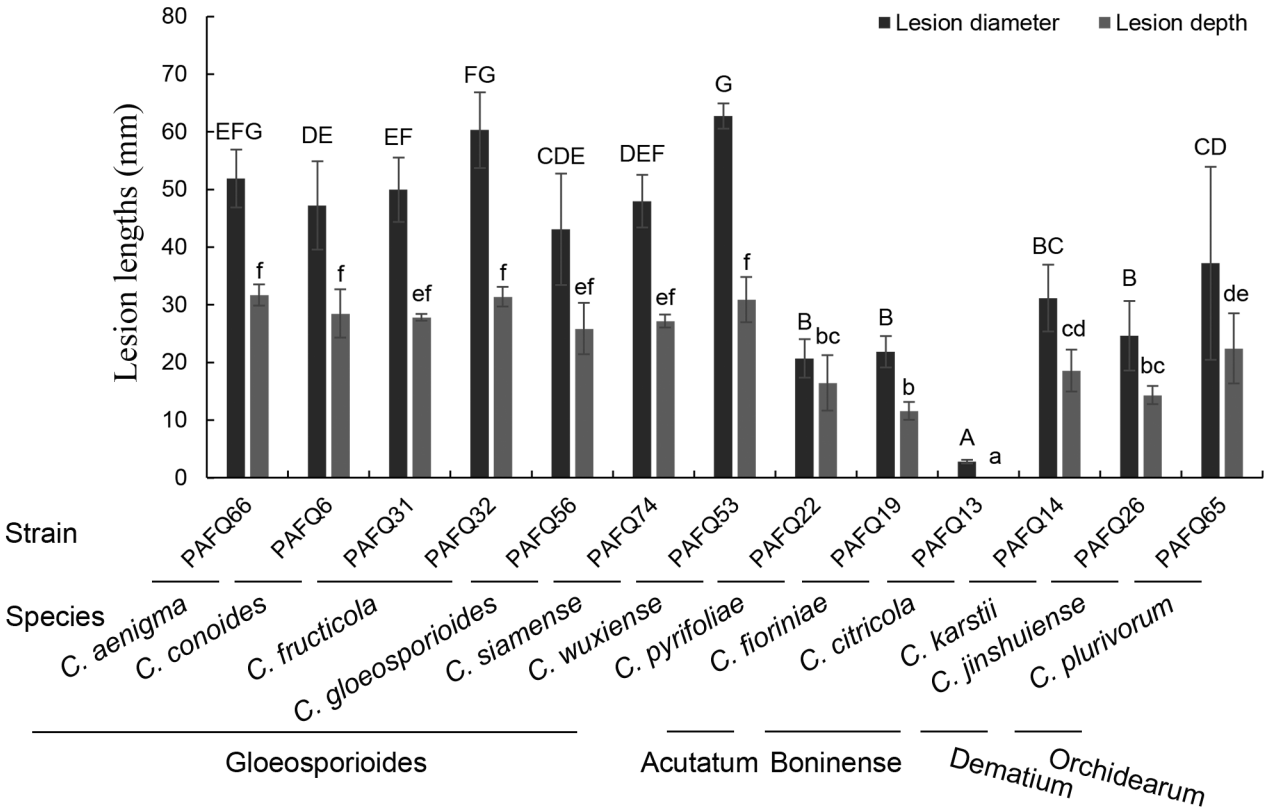

Fig. 22 Lesion lengths and depths on wounded pear fruits (*P. bretschneideri* cv. Huangguan) at 10 dpi induced by conidial suspensions of 13 representative isolates of 12 *Colletotrichum* spp. The involved isolates and their belonging are indicated at the bottom of the bars. Data were analysed with SPSS Statistics 21.0 (WinWrap Basic; http://www.winwrap.com) by one-way analysis of variance, and means were compared using Duncan's test at a significance level of $P = 0.05$. Letters over the error bars indicate the significant difference at the $P = 0.05$ level.

In previous reports the pathogenicity of most of the identified *Colletotrichum* species associated with pear anthracnose, including *C. aenigma*, *C. fructicola*, *C. acutatum*, *C. fioriniae*, *C. pyricola*, and *C. salicis* (Damm et al. 2012b, Weir et al. 2012, Jiang et al. 2014, Schena et al. 2014, Zhang et al. 2015), remained unresolved. Here, pathogenicity tests were conducted in order to confirm Koch's postulates for all the isolated species to clarify their pathogenicity. From these data it was revealed that the *Colletotrichum* species/isolates showed broad diversities in their pathogenicity and aggressiveness. Notably, *C. fructicola* caused TS symptoms on leaves and fruits under unwounded conditions, while it caused rot symptoms on fruits or necrosis lesions on leaves under wounded conditions; the BnL symptoms on leaves could also be induced by *C. fructicola* isolates, if these isolates were isolated from leaves showing BnL symptoms, indicating *C. fructicola* to have two pathogenic types. Other species including *C. aenigma*, *C. citricola*, *C. wuxiense*, *C. gloeosporioides*, *C. karstii*, and *C. siamense* are also related to the leaf BnL symptoms; *C. fioriniae*, *C. fructicola*, *C. aenigma*, *C. gloeosporioides*, *C. pyrifoliae*, and *C. jinshuiense* are related to leaf SS symptoms; and *C. aenigma*, *C. fioriniae*, *C. gloeosporioides*, *C. siamense*, and *C. conoides* are related to fruit BrL symptoms. Notably, many isolates caused obvious lesions on fruits (or leaves) under wounded conditions but not under unwounded conditions. This phenomenon is related to the quiescent infection of these species, which is an important feature of *Colletotrichum* spp. and always occurs at the immature fruit stage, progressively developing to rot as the fruits ripen (Peres et al. 2005, Alkan et al. 2015, De Silva et al. 2017). Previous results indicated that wounding can break the quiescent infection and enhance the infectivity of *C. fructicola*, leading to more rapid rot of young and mature fruits (Jiang et al. 2014). It is worth to note that although the 12 species obtained in this study can infect pear fruits under wounded conditions, those isolated from pear leaves (*C. citricola*, *C. jinshuiense*, *C. karstii*, *C. plurivorum*, *C. pyrifoliae*, and *C. wuxiense*) showed no pathogenicity to pear fruits (*P. bretschneideri* cv. Huangguan) under unwounded conditions up to 30 dpi. These results revealed a clear organ specificity for the pathogenicity of some *Colletotrichum* isolates. Some studies also provide clues that some isolates of *Glomerella cingulata*, *C. gloeosporioides* and *C. acutatum*, are host organ specific; they mainly infected the leaves instead of causing bitter rot on apple and pear fruit (Yano et al. 2004, González et al. 2006, Tashiro et al. 2012). Additionally, most of the isolates belonging to the *C. gloeosporioides* species complex showed higher aggressiveness than those of *C. fioriniae*, *C. citricola*, and *C. pyrifoliae* (Fig. 22).

Previous studies revealed that *C. fructicola* caused anthracnose on many plants, e.g., *Citrus reticulata* (Huang et al. 2013), *Capsicum* sp. (Diao et al. 2017), *Camellia sinensis* (Liu et al. 2015), *Mangifera indica* (Lima et al. 2013), and *Malus* sp. (Munir et al. 2016), resulting in lesions rather than TS symptoms. Therefore, it is interesting that *C. fructicola* causes TS symptoms on pear. *Colletotrichum aenigma* was reported on *P. pyrifolia* in Japan (Weir et al. 2012) and *P. communis* in Italy (Schena et al. 2014) without mention about the infected organs and induced symptoms. This is the first report of *C. aenigma* to induce pear anthracnose of *P. bretschneideri* (on fruits and leaves) and *P. pyrifoliae* (on leaves) in China (Fig. 19c, d), with a dominant incidence on the latter. *Colletotrichum fioriniae* was reported causing leaf spots on *Cinnamomum subavenium* and *Juglans regia* in China (Sun et al. 2012, Zhu et al. 2015), *Salvia leucantha* in Italy (Garibaldi et al. 2016), and bitter rot on *Pyrus* sp. in the USA and Croatia (Damm et al. 2012b, Ivic et al. 2013) and *P. communis* in France (Da Lio et al. 2017). This is the first report of *C. fioriniae* in China, which caused pear bitter rot and was associated with pear leaf spot on *P. pyrifolia*, *P. bretschneideri*, and *P. communis*. *Colletotrichum citricola* was

first reported on *Citrus unchiu* in China, where it was a saprobe on leaves (Huang et al. 2013), but this is the first report of *C. citricola* on *P. pyrifolia*, where it was found to cause anthracnose on pear leaves.

This study provides the first systematic investigation, morphological, molecular and biological characterisation of *Colletotrichum* spp. associated with *Pyrus* plants, and represents the first reports of *C. citricola*, *C. conoides*, *C. karstii*, *C. plurivorum*, *C. siamense*, and *C. wuxiense*, together with the novel species, causing anthracnose on pear. This study also reveals taxonomic, morphological and biological diversity of *Colletotrichum* spp. associated with different *Pyrus* spp. in China in respect to tissue type, geographical location and climate, contributing useful information to help understand the ecology of the *Colletotrichum* spp. involved in pear anthracnose.

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