



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

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Key words

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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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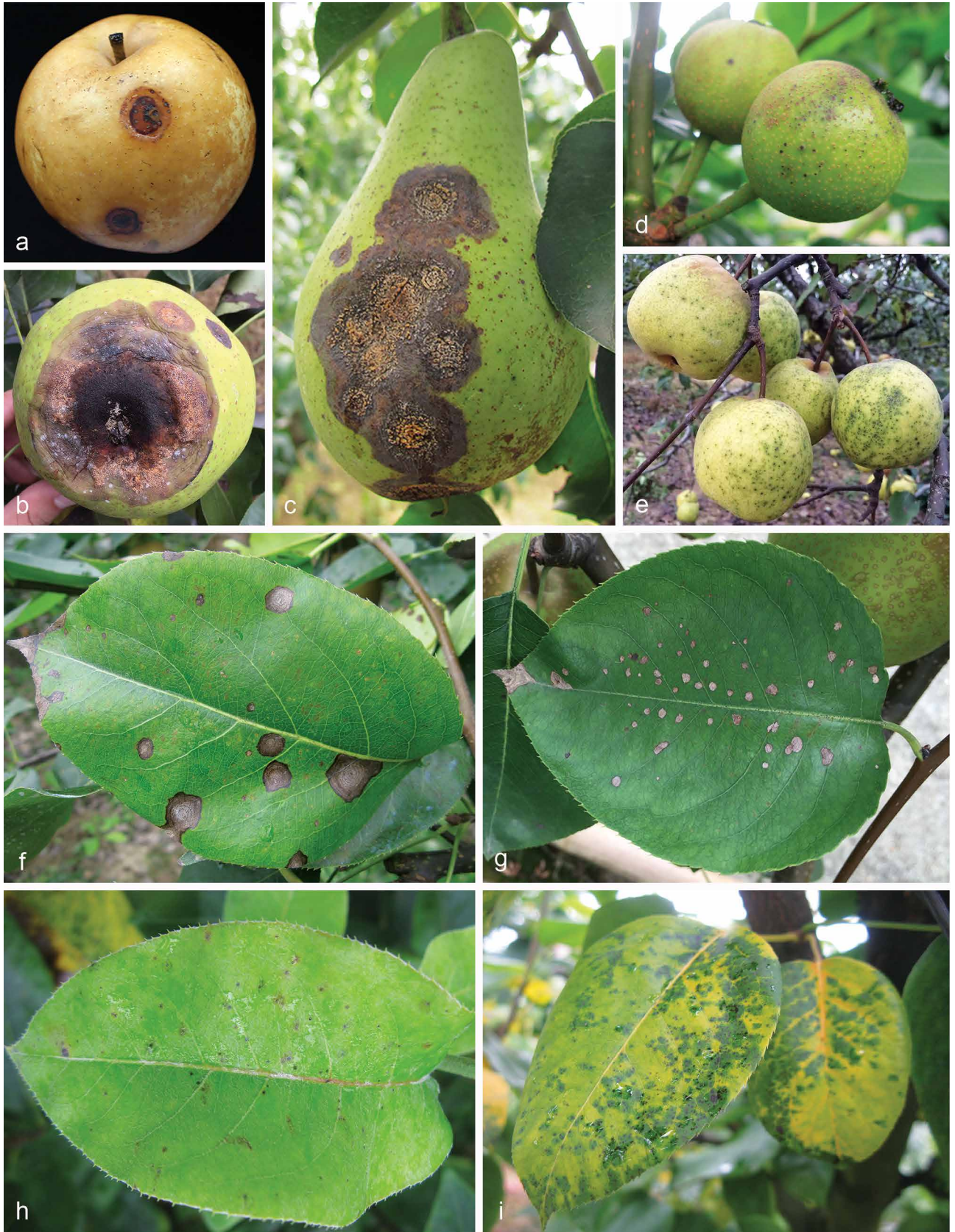


Fig. 1 Representative symptoms of pear anthracnose on fruits and leaves in the field. a–c. Symptoms of big sunken rot lesions (BRL; 10–35 mm diam) on fruits of *P. pyrifolia* (a, b) and *P. communis* cultivar (cv.) Gyuiot (c); d, e. symptoms of tiny black spots (TS; < 1 mm diam) on young pear fruits 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 mm²) 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 1000 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 (cont.)

| Species | Isolate No. | Host | Symptoms | Origin | GenBank accession number | | | | | TUB2 |
|-----------------------------------|---|---|--------------------|--------------------|--------------------------|----------|----------|----------|----------|----------|
| | | | | | ITS | GAPDH | CAL | ACT | CHS-1 | |
| <i>C. gloeosporioides</i> (cont.) | PAFQ56 | <i>P. pyrifolia</i> cv. Cuiguan, leaf | BnL | Jinxi, Jiangxi | MG748029 | MG747947 | MG747801 | MG747719 | MG747865 | MG748111 |
| | PAFQ58 | <i>P. pyrifolia</i> cv. Cuiguan, leaf | BnL | Jinxi, Jiangxi | MG748030 | MG747948 | MG747802 | MG747720 | MG747866 | MG748112 |
| | PAFQ59 | <i>P. pyrifolia</i> cv. Cuiguan, leaf | BnL | Jinxi, Jiangxi | MG748031 | MG747949 | MG747803 | MG747721 | MG747867 | MG748113 |
| | PAFQ60 | <i>P. pyrifolia</i> cv. Cuiguan, leaf | BnL | Jinxi, Jiangxi | MG748032 | MG747950 | MG747804 | MG747722 | MG747868 | MG748114 |
| | PAFQ61 | <i>P. pyrifolia</i> cv. Huanghua, fruit | BnL | Jinxi, Jiangxi | MG748033 | MG747951 | MG747805 | MG747723 | MG747869 | MG748115 |
| | PAFQ80 | <i>P. pyrifolia</i> cv. Guanyangxueli, leaf | SS | Hangzhou, Zhejiang | MG748035 | MG747953 | MG747807 | MG747725 | MG747871 | MG748117 |
| | PAFQ86 | <i>P. pyrifolia</i> , leaf | BnL | Hangzhou, Zhejiang | MG748034 | MG747952 | MG747806 | MG747724 | MG747870 | MG748116 |
| | PAFQ26, CGMCC 3, 18903* | <i>P. pyrifolia</i> cv. Jinshui No.1, leaf | SS | Wuhan, Hubei | MG748077 | MG747995 | – | MG747767 | MG747913 | MG748157 |
| | PAFQ28a | <i>P. pyrifolia</i> cv. Jinshui No.1, leaf | SS | Wuhan, Hubei | MG874830 | MG874822 | – | MG874807 | MG874814 | MG874838 |
| | PAFQ28b | <i>P. pyrifolia</i> cv. Jinshui No.1, leaf | SS | Wuhan, Hubei | MG874831 | MG874823 | – | MG874808 | MG874815 | MG874839 |
| PAFQ28c | <i>P. pyrifolia</i> cv. Jinshui No.1, leaf | SS | Wuhan, Hubei | MG874832 | MG874824 | – | – | MG874816 | MG874840 | |
| PAFQ28d | <i>P. pyrifolia</i> cv. Jinshui No.1, leaf | SS | Wuhan, Hubei | MG874833 | MG874825 | – | MG874809 | MG874817 | MG874841 | |
| <i>C. karstii</i> | PAFQ14 | <i>P. pyrifolia</i> , leaf | BnL | Wuhan, Hubei | MG748063 | MG747981 | MG747820 | MG747753 | MG747899 | MG748143 |
| | PAFQ15 | <i>P. pyrifolia</i> , leaf | BnL | Wuhan, Hubei | MG748064 | MG747982 | MG747821 | MG747754 | MG747900 | MG748144 |
| | PAFQ16 | <i>P. pyrifolia</i> , leaf | BnL | Wuhan, Hubei | MG748065 | MG747983 | MG747822 | MG747755 | MG747901 | MG748145 |
| | PAFQ28 | <i>P. pyrifolia</i> cv. Hohsui, leaf | BnL | Wuhan, Hubei | MG748066 | MG747984 | MG747823 | MG747756 | MG747902 | MG748146 |
| | PAFQ37 | <i>P. pyrifolia</i> cv. Cuiguan, leaf | BnL | Wuhan, Hubei | MG748067 | MG747985 | MG747824 | MG747757 | MG747903 | MG748147 |
| | PAFQ38 | <i>P. pyrifolia</i> cv. Cuiguan, leaf | BnL | Jianning, Fujian | MG748068 | MG747986 | MG747825 | MG747758 | MG747904 | MG748148 |
| | PAFQ39 | <i>P. pyrifolia</i> cv. Cuiguan, leaf | BnL | Jianning, Fujian | MG748069 | MG747987 | MG747826 | MG747759 | MG747905 | MG748149 |
| | PAFQ40 | <i>P. pyrifolia</i> cv. Huanghua, leaf | BnL | Jianning, Fujian | MG748070 | MG747988 | MG747827 | MG747760 | MG747906 | MG748150 |
| | PAFQ41 | <i>P. pyrifolia</i> cv. Huanghua, leaf | BnL | Jianning, Fujian | MG748071 | MG747989 | MG747828 | MG747761 | MG747907 | MG748151 |
| | PAFQ42 | <i>P. pyrifolia</i> cv. Huanghua, leaf | BnL | Jianning, Fujian | MG748072 | MG747990 | MG747829 | MG747762 | MG747908 | MG748152 |
| PAFQ43 | <i>P. pyrifolia</i> cv. Huanghua, leaf | BnL | Jianning, Fujian | MG748073 | MG747991 | MG747830 | MG747763 | MG747909 | MG748153 | |
| PAFQ52 | <i>P. pyrifolia</i> cv. Cuiguan, leaf | BnL | Jinxi, Jiangxi | MG748074 | MG747992 | MG747831 | MG747764 | MG747910 | MG748154 | |
| PAFQ82 | <i>P. pyrifolia</i> cv. Guanyangxueli, leaf | BnL | Hangzhou, Zhejiang | MG748075 | MG747993 | MG747832 | MG747765 | MG747911 | MG748155 | |
| PAFQ65 | <i>P. breitschneideri</i> cv. Huangguan, leaf | BnL | Dangshan, Anhui | MG748076 | MG747994 | – | MG747766 | MG747912 | MG748156 | |
| <i>C. pyrifolia</i> | PAFQ22, CGMCC 3, 18902* | <i>P. pyrifolia</i> cv. Jinshui No.1, leaf | SS | Wuhan, Hubei | MG748078 | MG747996 | – | MG747768 | MG747914 | MG748158 |
| | PAFQ22a | <i>P. pyrifolia</i> cv. Jinshui No.1, leaf | SS | Wuhan, Hubei | MG874834 | MG874826 | – | MG874810 | MG874818 | MG874842 |
| | PAFQ22b | <i>P. pyrifolia</i> cv. Jinshui No.1, leaf | SS | Wuhan, Hubei | MG874835 | MG874827 | – | MG874811 | MG874819 | MG874843 |
| | PAFQ22c | <i>P. pyrifolia</i> cv. Jinshui No.1, leaf | SS | Wuhan, Hubei | MG874836 | MG874828 | – | MG874812 | MG874820 | MG874844 |
| | PAFQ22d | <i>P. pyrifolia</i> cv. Jinshui No.1, leaf | SS | Wuhan, Hubei | MG874837 | MG874829 | – | MG874813 | MG874821 | MG874845 |
| | PAFQ67 | <i>P. communis</i> cv. Gyuot, fruit | BnL | Yantai, Shandong | MG748036 | MG747954 | MG747808 | MG747726 | MG747872 | MG748118 |
| | PAFQ68 | <i>P. communis</i> cv. Gyuot, fruit | BnL | Yantai, Shandong | MG748037 | MG747955 | MG747809 | MG747727 | MG747873 | MG748119 |
| PAFQ69 | <i>P. communis</i> cv. Gyuot, fruit | BnL | Yantai, Shandong | MG748038 | MG747956 | MG747810 | MG747728 | MG747874 | MG748120 | |
| PAFQ70 | <i>P. communis</i> cv. Gyuot, fruit | BnL | Yantai, Shandong | MG748039 | MG747957 | MG747811 | MG747729 | MG747875 | MG748121 | |
| PAFQ71 | <i>P. communis</i> cv. Gyuot, fruit | BnL | Yantai, Shandong | MG748040 | MG747958 | MG747812 | MG747730 | MG747876 | MG748122 | |
| PAFQ72 | <i>P. communis</i> cv. Gyuot, fruit | BnL | Yantai, Shandong | MG748041 | MG747959 | MG747813 | MG747731 | MG747877 | MG748123 | |
| PAFQ73 | <i>P. communis</i> cv. Gyuot, fruit | BnL | Yantai, Shandong | MG748042 | MG747960 | MG747814 | MG747732 | MG747878 | MG748124 | |
| PAFQ74 | <i>P. communis</i> cv. Gyuot, fruit | BnL | Yantai, Shandong | MG748043 | MG747961 | MG747815 | MG747733 | MG747879 | MG748125 | |
| PAFQ76 | <i>P. communis</i> cv. Gyuot, fruit | BnL | Yantai, Shandong | MG748044 | MG747962 | MG747816 | MG747734 | MG747880 | – | |
| PAFQ78 | <i>P. pyrifolia</i> cv. Guanyangxueli, leaf | BnL | Hangzhou, Zhejiang | MG748046 | MG747964 | MG747818 | MG747736 | MG747882 | MG748127 | |
| PAFQ85 | <i>P. pyrifolia</i> , leaf | BnL | Hangzhou, Zhejiang | MG748045 | MG747963 | MG747817 | MG747735 | MG747881 | MG748126 | |
| <i>C. wuxiense</i> | PAFQ53 | <i>P. pyrifolia</i> cv. Cuiguan, leaf | BnL | Jinxi, Jiangxi | MG748009 | MG747927 | MG747781 | MG747699 | MG747845 | MG748091 |
| | PAFQ54 | <i>P. pyrifolia</i> cv. Cuiguan, leaf | BnL | Jinxi, Jiangxi | MG748010 | MG747928 | MG747782 | MG747700 | MG747846 | MG748092 |

* = Ex-type culture.

BnL: 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 1000 random sequence additions. Maxtrees were set up to 5000, branches of zero length collapsed, and all multiple parsimonious trees were saved. Clade stability was assessed using a bootstrap analysis with 1000 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 1000 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 Quaerndt et al. (2014). The PHI test was performed in SplitsTree 4 (Huson 1998, Huson & Klopper 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 μ L) 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 (Rⁱ) was calculated for each species with the formula, $R^i \% = (N^s / N^t) \times 100$, where N^s was the number of isolates from the same species, and N^t 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 Rⁱ was calculated using the N^t 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. pyrifolia* 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 μ L 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 μ L 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* | Host/Substrate | Country | GenBank accession number | | | | | | | |
|-------------------------------|----------------|---|--------------|--------------------------|----------|----------|----------|----------|----------|----------|--|
| | | | | ITS | GAPDH | CAL | ACT | CHS-1 | TUB2 | | |
| <i>C. abscissum</i> | COAD 1877* | <i>Citrus sinensis</i> cv. Pera | Brazil | KP843126 | KP843129 | – | KP843141 | KP843132 | – | KP843135 | |
| <i>C. acerbum</i> | CBS 128530* | <i>Malus domestica</i> | New Zealand | JQ948459 | JQ948790 | – | JQ949180 | JQ949127 | – | JQ950110 | |
| <i>C. acutatum</i> | CBS 112996* | <i>Carica papaya</i> | Australia | JQ005776 | JQ948677 | – | JQ005839 | JQ005797 | – | JQ005860 | |
| <i>C. aenigma</i> | ICMP 18608* | <i>Persea americana</i> | Israel | JX010244 | JX010044 | JX009683 | JX009443 | JX009774 | JX009774 | JX010369 | |
| | ICMP 18686 | <i>Pyrus pyrifolia</i> | Japan | JX010243 | JX009913 | JX009684 | JX009519 | JX009789 | JX009789 | JX010390 | |
| <i>C. aeshchynomenes</i> | ICMP 17673* | <i>Aeschynomene virginica</i> | USA | JX010176 | JX009930 | JX009721 | JX009483 | JX009799 | JX009799 | JX010392 | |
| <i>C. agaves</i> | CBS 118190 | <i>Agave striate</i> | Mexico | DQ286221 | – | – | – | – | – | – | |
| <i>C. alatae</i> | CBS 304.67* | <i>Dioscorea alata</i> | India | JX010190 | JX009990 | JX009738 | JX009471 | JX009837 | JX009837 | JX010383 | |
| <i>C. allenium</i> | ICMP 12071* | <i>Malus domestica</i> | New Zealand | JX010251 | JX010028 | JX009654 | JX009572 | JX009882 | JX009882 | JX010411 | |
| <i>C. annellatum</i> | CBS 129826* | <i>Hevea brasiliensis</i> , leaf | Colombia | JQ005222 | JQ005309 | JQ005743 | JQ005570 | JQ005396 | JQ005396 | JQ005656 | |
| <i>C. antrisci</i> | CBS 125334* | <i>Anthriscus sylvestris</i> , dead stem | Netherlands | GU227845 | GU228237 | – | GU227943 | GU228335 | GU228335 | GU228139 | |
| | CBS 125335 | <i>Anthriscus sylvestris</i> , dead stem | Netherlands | GU227846 | GU228238 | – | GU227944 | GU228336 | GU228336 | GU228140 | |
| <i>C. aotearoa</i> | ICMP 18537* | <i>Coprosma</i> sp. | New Zealand | JX010205 | JX010005 | JX009611 | JX009564 | JX009853 | JX009853 | JX010420 | |
| <i>C. asianum</i> | ICMP 18580* | <i>Coffea arabica</i> | Thailand | FJ972612 | JX010053 | FJ917506 | JX009584 | JX009867 | JX009867 | JX010406 | |
| <i>C. australe</i> | CBS 116478* | <i>Trachycarpus fortunei</i> | South Africa | JQ948455 | JQ948786 | – | JQ949776 | JQ949116 | JQ949116 | JQ950106 | |
| <i>C. beeveri</i> | CBS 128527* | <i>Brachyglottis repanda</i> | New Zealand | JQ005171 | JQ005258 | JQ005692 | JQ005519 | JQ005345 | JQ005345 | JQ005605 | |
| <i>C. boninense</i> | CBS 123755* | <i>Crinium asiaticum</i> var. <i>sinicum</i> | Japan | JQ005153 | JQ005240 | JQ005674 | JQ005501 | JQ005327 | JQ005327 | JQ005588 | |
| | CBS 128506 | <i>Solanum lycopersicum</i> , fruit rot | New Zealand | JQ005157 | JQ005244 | JQ005678 | JQ005505 | JQ005331 | JQ005331 | JQ005591 | |
| | CBS 128501* | <i>Passiflora edulis</i> , fruit anthracnose | Brazil | JQ005235 | JQ005322 | JQ005756 | JQ005583 | JQ005409 | JQ005409 | JQ005669 | |
| <i>C. brassilense</i> | CBS 101059* | <i>Brassica oleracea</i> , leaf spot | New Zealand | JQ005172 | JQ005259 | JQ005693 | JQ005520 | JQ005346 | JQ005346 | JQ005606 | |
| <i>C. brassicicola</i> | BCC 38876* | <i>Neoregalia</i> sp. | Thailand | JN050238 | JN050238 | – | JN050216 | KF687760 | KF687760 | JN050244 | |
| <i>C. brisporum</i> | CBS 292.67* | <i>Capsicum annuum</i> | Australia | JQ948291 | JQ948621 | – | JQ949612 | JQ948952 | JQ948952 | JQ949942 | |
| <i>C. brisbanense</i> | BRIP 63642* | <i>Capsicum annuum</i> | Australia | KU923672 | KU923704 | – | KU923716 | KU923710 | KU923710 | KU923688 | |
| <i>C. cairnsense</i> | CGMCC 3.18118* | <i>Camellia japonica</i> | Japan | KX853165 | KX893584 | – | KX893576 | – | – | KX893580 | |
| <i>C. camelliae-japonicae</i> | CGMCC 3.18117 | <i>Camellia japonica</i> | Japan | KX853164 | KX893583 | – | KX893575 | – | – | KX893579 | |
| | SAPA100011* | <i>Carthamus tinctorium</i> | Japan | AB696998 | – | – | – | – | – | AB696992 | |
| <i>C. carthami</i> | CBS 170.49* | <i>Cattleya</i> sp. | Belgium | MG600758 | MG600819 | – | MG600963 | MG600866 | MG600866 | MG601025 | |
| <i>C. cattleyicola</i> | IMI 103806* | <i>Chlorophytum</i> sp. | India | GU227894 | GU228286 | – | GU227992 | GU228384 | GU228384 | GU228188 | |
| <i>C. chlorophyti</i> | IMI 364540 | <i>Chrysanthemum coronarium</i> , leaf spot | China | JQ948273 | JQ948603 | – | JQ949594 | JQ948934 | JQ948934 | JQ949924 | |
| <i>C. chrysanthemii</i> | CBS 221.81* | <i>Allium cepa</i> | Serbia | GU227855 | GU228247 | – | GU227953 | GU228345 | GU228345 | GU228149 | |
| <i>C. circinans</i> | CBS 134228* | <i>Citrus unshiu</i> | China | KC293576 | KC293736 | KC293696 | KC293616 | KC293696 | KC293696 | KC293656 | |
| <i>C. citricola</i> | CBS 134229 | <i>Citrus unshiu</i> | China | KC293577 | KC293737 | KC293697 | KC293617 | KC293793 | KC293793 | KC293657 | |
| | CBS 134230 | <i>Citrus unshiu</i> | China | KC293578 | KC293738 | KC293698 | KC293618 | KC293794 | KC293794 | KC293658 | |
| <i>C. clidmiae</i> | ICMP 18658* | <i>Clidemia hirta</i> | USA, Hawaii | JX010265 | JX009989 | JX009645 | JX009537 | JX009877 | JX009877 | JX010438 | |
| <i>C. clivicola</i> | CBS 125375* | <i>Clivia miniata</i> | China | JX519223 | JX546611 | – | JX519240 | JX519232 | JX519232 | JX519249 | |
| | CS51 | <i>Clivia miniata</i> | China | GU109479 | GU085867 | – | GU085861 | GU085865 | GU085865 | GU085869 | |
| | CS52 | <i>Clivia miniata</i> | China | GU109480 | GU085868 | – | GU085862 | GU085866 | GU085866 | GU085870 | |
| <i>C. colombiense</i> | CBS 129818* | <i>Passiflora edulis</i> , leaf | Colombia | JQ005174 | JQ005261 | JQ005695 | JQ005522 | JQ005348 | JQ005348 | JQ005608 | |
| <i>C. conoides</i> | CGMCC 3.17615* | <i>Capsicum annuum</i> | China | KP890168 | KP890162 | KP890150 | KP890144 | KP890156 | KP890156 | KP890174 | |
| | CAUG33 | <i>Capsicum annuum</i> | China | KP890169 | KP890163 | KP890151 | KP890145 | KP890157 | KP890157 | KP890175 | |
| | CAUG34 | <i>Capsicum annuum</i> | China | KP890170 | KP890164 | KP890152 | KP890146 | KP890158 | KP890158 | KP890176 | |
| <i>C. constrictum</i> | CBS 128504* | <i>Citrus limon</i> , fruit rot | New Zealand | JQ005238 | JQ005325 | JQ005759 | JQ005586 | JQ005412 | JQ005412 | JQ005672 | |
| <i>C. cordylinicola</i> | ICMP 18579* | <i>Cordyline fruticosa</i> | Thailand | JX010226 | JX009975 | HM470238 | HM470235 | JX009864 | JX009864 | JX010440 | |
| <i>C. cosmi</i> | CBS 853.73* | <i>Cosmos</i> sp., seed | Netherlands | JQ948274 | JQ948604 | – | JQ949595 | JQ948935 | JQ948935 | JQ949925 | |
| <i>C. costaricense</i> | CBS 330.75* | <i>Coffea arabica</i> , cv. <i>Typica</i> , berry | Costa Rica | JQ948180 | JQ948510 | – | JQ949501 | JQ948841 | JQ948841 | JQ949831 | |
| <i>C. curcurnae</i> | IMI 288937* | <i>Curcuma longa</i> | India | GU227893 | GU228285 | – | GU227991 | GU228383 | GU228383 | GU228187 | |

Table 3 (cont.)

| Species | Culture* | Host/Substrate | Country | GenBank accession number | | | | | | |
|--|----------------|---|----------------|--------------------------|----------|----------|----------|----------|----------|--|
| | | | | ITS | GAPDH | CAL | ACT | CHS-1 | TUB2 | |
| <i>C. cuscuteae</i> | IMI 304802* | <i>Cuscuta</i> sp. | Dominica | JQ948195 | JQ948525 | – | JQ949516 | JQ948856 | JQ949846 | |
| <i>C. cymbidicola</i> | IMI 347923* | <i>Cymbidium</i> sp., leaf lesion | Australia | JQ005166 | JQ005253 | JQ005687 | JQ005514 | JQ005340 | JQ005600 | |
| <i>C. dactylocarp</i> | CBS 130241* | <i>Dactylocarpus dactyloides</i> , leaf endophyte | New Zealand | JQ005236 | JQ005323 | JQ005757 | JQ005584 | JQ005410 | JQ005670 | |
| <i>C. dematium</i> | CBS 125.25* | <i>Eryngium campestre</i> , dead leaf | France | GU227819 | GU228211 | – | GU227917 | GU228309 | GU228113 | |
| | CBS 123728 | <i>Genista tinctoria</i> , leaf spot | Czech Republic | GU227822 | GU228214 | – | GU227920 | GU228312 | GU228116 | |
| <i>C. dracaenophilum</i> | CBS 118199* | <i>Dracaena</i> sp. | China | JX519222 | JX546707 | – | JX519238 | JX519230 | JX519247 | |
| <i>C. euphorbiae</i> | CBS 134725* | <i>Euphorbia</i> sp. | South Africa | KF777146 | KF777131 | – | KF777125 | KF777128 | KF777247 | |
| <i>C. floriniae</i> | CBS 125396 | <i>Malus domestica</i> , fruit lesion | USA | JQ948299 | JQ948629 | – | JQ949620 | JQ948960 | JQ949950 | |
| | IMI 324996 | <i>Malus pumila</i> | USA | JQ948301 | JQ948631 | – | JQ949622 | JQ948962 | JQ949952 | |
| | CBS 126526 | <i>Primula</i> sp., leaf spots | Netherlands | JQ948323 | JQ948653 | – | JQ949644 | JQ948984 | JQ949974 | |
| | CBS 124958 | <i>Pyrus</i> sp., fruit rot | USA | JQ948306 | JQ948636 | – | JQ949627 | JQ948967 | JQ949957 | |
| | IMI 504882 | <i>Fragaria × ananassa</i> | New Zealand | KT153562 | KT153552 | – | KT153542 | KT153547 | KT153567 | |
| | CBS 129838 | <i>Malus domestica</i> | USA | JQ948296 | JQ948626 | – | JQ949617 | JQ948957 | JQ949947 | |
| | CBS 119292 | <i>Vaccinium</i> sp., fruit | New Zealand | JQ948313 | JQ948643 | – | JQ949634 | JQ948974 | JQ949964 | |
| | CBS 129930 | <i>Malus domestica</i> | New Zealand | JQ948304 | JQ948634 | – | JQ949625 | JQ948965 | JQ949955 | |
| | ATCC 28992 | <i>Malus domestica</i> | USA | JQ948297 | JQ948627 | – | JQ949618 | JQ948958 | JQ949948 | |
| <i>C. fructi</i> | CBS 346.37* | <i>Malus sylvestris</i> , fruit | USA | GU227844 | GU228236 | – | FJ917508 | GU228334 | GU228138 | |
| <i>C. fructicola</i> | ICMP 18581* | <i>Coffea arabica</i> | Thailand | JX010165 | JX010033 | FJ917508 | FJ907426 | JX009866 | JX010405 | |
| | ICMP 18613 | <i>Limonium sinuatum</i> | Israel | JX010167 | JX009998 | JX009675 | JX009491 | JX009772 | JX010388 | |
| | ICMP 18645 | <i>Theobroma cacao</i> | Panama | JX010172 | JX009992 | JX009666 | JX009543 | JX009873 | JX010408 | |
| | ICMP 18727 | <i>Fragaria × ananassa</i> | USA | JX010179 | JX010035 | JX009682 | JX009565 | JX009812 | JX010394 | |
| | ICMP 18120 | <i>Dioscorea alata</i> | Nigeria | JX010182 | JX010041 | JX009670 | JX009436 | JX009844 | JX010401 | |
| <i>C. fructicola</i> (syn. <i>C. ignotum</i>) | ICMP 18646* | <i>Tetragastris panamensis</i> | Panama | JX010173 | JX010032 | JX009674 | JX009581 | JX009874 | JX010409 | |
| <i>C. fructicola</i> (syn. <i>Glomerella cingulata</i> var. <i>minor</i>) | ICMP 17921* | <i>Ficus edulis</i> | Germany | JX010181 | JX009923 | JX009671 | JX009495 | JX009839 | JX010400 | |
| <i>C. fructivorum</i> | CBS 133125* | <i>Vaccinium macrocarpon</i> | USA | JX145145 | – | – | – | – | JX145196 | |
| | CBS 133135 | <i>Rhexia virginica</i> | USA | JX145133 | – | – | – | – | JX145184 | |
| <i>C. gloeosporioides</i> | IMI 356878* | <i>Citrus sinensis</i> | Italy | JX010152 | JX010056 | JX009731 | JX009531 | JX009818 | JX010445 | |
| | ICMP 12939 | <i>Citrus</i> sp. | New Zealand | JX010149 | JX009931 | JX009728 | JX009462 | JX009747 | – | |
| | ICMP 18695 | <i>Citrus</i> sp. | USA | JX010153 | JX009979 | JX009735 | JX009494 | JX009779 | – | |
| | ICMP 18694 | <i>Mangifera indica</i> | South Africa | JX010155 | JX009980 | JX009729 | JX009481 | JX009796 | – | |
| <i>C. gloeosporioides</i> (syn. <i>Gloeosporium pedermontanum</i>) | ICMP 19121* | <i>Citrus limon</i> | Italy | JX010148 | JX010054 | JX009745 | JX009558 | JX009903 | – | |
| <i>C. godetiae</i> | CBS 133.44* | <i>Clarkia hybrida</i> | Denmark | JQ948402 | JQ948733 | – | JQ949723 | JQ949063 | JQ950053 | |
| <i>C. hebeense</i> | JZB330024 | <i>Vitis vinifera</i> cv. Cabernet Sauvignon | China | KF156873 | KF377505 | – | KF377542 | – | – | |
| | CGMCC 3.17464* | <i>Vitis vinifera</i> cv. Cabernet Sauvignon | China | KF156863 | KF377495 | – | KF377532 | KF289008 | KF288975 | |
| | CDLG5* | <i>Hemerocallis fulva</i> var. <i>kwanso</i> | China | JQ400005 | JQ400012 | – | JQ399991 | JQ399998 | JQ400019 | |
| <i>C. hemerocallidis</i> | CBS 125376* | <i>Hippeastrum vittatum</i> , leaf | China | JQ005231 | JQ005318 | JQ005752 | JQ005579 | JQ005405 | JQ005665 | |
| <i>C. hippeastr</i> | ICMP 10492* | <i>Diospyros kaki</i> | Japan | GO323690 | GO323681 | JX009604 | JX009438 | JX009752 | JX010450 | |
| <i>C. horii</i> | MFLU 15-1895* | <i>Parthenocissus inserta</i> | Russia | KX618686 | KX618684 | – | KX618682 | KX618683 | KX618685 | |
| <i>C. insertae</i> | MFLUCC 10-0273 | <i>Jasminum sambac</i> | Vietnam | HM131513 | HM131499 | – | HM131508 | – | HM153770 | |
| <i>C. jasmiginenum</i> | CGMCC 3.17362 | <i>Camellia sinensis</i> , endophyte | China | KJ955198 | KJ954899 | KJ954749 | KJ954469 | – | KJ955345 | |
| <i>C. jiangxiense</i> | CGMCC 3.17363* | <i>Camellia sinensis</i> , pathogen | China | KJ955201 | KJ954902 | KJ954752 | KJ954471 | – | KJ955348 | |
| | CBS 128532* | <i>Solanum lycopersicum</i> , fruit rot | New Zealand | JQ948444 | JQ948775 | – | JQ949765 | JQ949105 | JQ950095 | |
| <i>C. johnstonii</i> | ICMP 18539* | <i>Olea europaea</i> | Australia | JX010230 | JX009966 | JX009635 | JX009523 | JX009800 | JX010434 | |
| <i>C. kahawae</i> subsp. <i>ciggaro</i> | ICMP 18534 | <i>Kunzea ericoides</i> | New Zealand | JX010227 | JX009904 | JX009634 | JX009473 | JX009765 | JX010427 | |
| | ICMP 12952 | <i>Persea americana</i> | New Zealand | JX010214 | JX009971 | JX009648 | JX009431 | JX009757 | JX010426 | |
| <i>C. kahawae</i> subsp. <i>kahawae</i> | IMI 319418* | <i>Coffea arabica</i> | Kenya | JX010231 | JX010012 | JX009642 | JX009452 | JX009813 | JX010444 | |

Table 3 (cont.)

| Species | Culture* | Host/Substrate | Country | ITS | GAPDH | CAL | ACT | CHS-1 | TUB2 |
|---|----------------|--|----------------|----------|----------|----------|----------|----------|----------|
| <i>C. kahawae</i> subsp. <i>kahawae</i> (cont.) | ICMP 17905 | <i>Coffea arabica</i> | Cameroon | JX010232 | JX010046 | JX009644 | JX009561 | JX009816 | JX010431 |
| | ICMP 17915 | <i>Coffea arabica</i> | Angola | JX010234 | JX010040 | JX009638 | JX009474 | JX009829 | JX010435 |
| <i>C. karstii</i> | CBS 113087 | <i>Malus</i> sp. | USA | JQ005181 | JQ005268 | JQ005702 | JQ005529 | JQ005355 | JQ005615 |
| | CBS 128524 | <i>Citrus</i> sp. | New Zealand | JQ005195 | JQ005282 | JQ005716 | JQ005543 | JQ005369 | JQ005629 |
| | CBS 128551 | <i>Citrus</i> sp. | New Zealand | JQ005208 | JQ005295 | JQ005729 | JQ005556 | JQ005382 | JQ005642 |
| | CBS 129832 | <i>Musa</i> sp. | Mexico | JQ005177 | JQ005264 | JQ005698 | JQ005525 | JQ005351 | JQ005611 |
| | CBS 129824 | <i>Musa</i> AAA, fruit | Colombia | JQ005215 | JQ005302 | JQ005736 | JQ005563 | JQ005389 | JQ005649 |
| | CBS 128552 | <i>Synsepalum dulcificum</i> , leaves | Taiwan | JQ005188 | JQ005275 | JQ005709 | JQ005536 | JQ005362 | JQ005622 |
| <i>C. kinghornii</i> | CBS 198.35* | <i>Phormium</i> sp. | UK | JQ948454 | JQ948785 | - | JQ949775 | JQ949115 | JQ950105 |
| <i>C. laticipitulum</i> | CBS 112989* | <i>Hevea brasiliensis</i> | India | JQ948289 | JQ948619 | - | JQ949610 | JQ948950 | JQ949940 |
| <i>C. ledebouriae</i> | CBS 141284* | <i>Ledebouria floridunda</i> | South Africa | KX228254 | - | - | KX228357 | - | - |
| <i>C. liaoningense</i> | CGMCC 3.17616* | <i>Capsicum</i> sp. | China | KP890104 | KP890135 | - | KP890097 | KP890127 | KP890111 |
| <i>C. lindemuthianum</i> | CBS 144.31* | <i>Phaseolus vulgaris</i> | Germany | JQ005779 | JX546712 | - | JQ005842 | JQ005800 | JQ005863 |
| <i>C. lineola</i> | CBS 125337* | <i>Apiaceae</i> , dead stem | Czech Republic | GU227829 | GU228221 | - | GU227927 | GU228319 | GU228123 |
| | CBS 124.25 | <i>Trillium</i> sp., leaf spot | Czech Republic | GU227836 | GU228228 | - | GU227934 | GU228326 | GU228130 |
| <i>C. lupini</i> | CBS 109225* | <i>Lupinus albus</i> | Ukraine | JQ948155 | JQ948485 | - | JQ949476 | JQ948816 | JQ949806 |
| <i>C. magnum</i> | CBS 519.97* | <i>Citrus</i> sp. | USA | MG600769 | MG600829 | - | MG600973 | MG600875 | MG601036 |
| <i>C. menispermii</i> | MFLU 14-0625* | <i>Menispermum dauricum</i> | Russia | KU242357 | KU242356 | - | KU242353 | KU242355 | KU242354 |
| <i>C. musae</i> | CBS 116870* | <i>Musa</i> sp. | USA | JX010146 | JX010050 | JX009742 | JX009433 | JX009896 | HQ596280 |
| <i>C. musicola</i> | CBS 132885* | <i>Musa</i> sp. | Mexico | MG600736 | MG600798 | - | MG600942 | MG600853 | MG601003 |
| <i>C. neosansevieriae</i> | CBS 139918* | <i>Sansevieria trifasciata</i> | South Africa | KR476747 | KR476791 | - | KR476790 | - | KR476797 |
| <i>C. novae-zelandiae</i> | CBS 128505* | <i>Capsicum annuum</i> , fruit rot | New Zealand | JQ005228 | JQ005315 | JQ005749 | JQ005576 | JQ005402 | JQ005662 |
| <i>C. nupharicola</i> | CBS 470.96* | <i>Nuphar lutea</i> subsp. <i>Polysepala</i> | USA | JX010187 | JQ009972 | JX009663 | JQ009437 | JQ009835 | JX010398 |
| <i>C. nymphphaeae</i> | CBS 515.78* | <i>Nymphaea alba</i> | Netherlands | JQ948197 | JQ948527 | - | JQ949518 | JQ948858 | JQ949848 |
| <i>C. oncidii</i> | CBS 129828* | <i>Oncidium</i> sp., leaf | Germany | JQ005169 | JQ005256 | JQ005690 | JQ005517 | JQ005603 | JQ005603 |
| <i>C. orbiculare</i> | CBS 514.97 | <i>Cucumis sativus</i> | Japan | JQ005778 | KF178491 | - | JQ005841 | JQ005799 | JQ005862 |
| <i>C. orchidearum</i> | CBS 135131* | <i>Dendrobium nobile</i> | Netherlands | MG600738 | MG600800 | - | MG600944 | MG600855 | MG601005 |
| <i>C. orchidophilum</i> | CBS 632.80* | <i>Dendrobium</i> sp. | USA | JQ948151 | JQ948481 | - | JQ949472 | JQ948812 | JQ949802 |
| <i>C. paranaense</i> | CBS 134729* | <i>Malus domestica</i> | Brazil, Parana | KC204992 | KC205026 | - | KC205077 | KC205043 | KC205060 |
| <i>C. parsonsiae</i> | CBS 128525 | <i>Parsonsia capsularis</i> , leaf endophyte | New Zealand | JQ005233 | JQ005320 | JQ005754 | JQ005581 | JQ005407 | JQ005667 |
| <i>C. paxtonii</i> | IMI 165753* | <i>Musa</i> sp. | Saint Lucia | JQ948285 | JQ948615 | - | JQ949606 | JQ948946 | JQ949936 |
| <i>C. petchii</i> | CBS 378.94* | <i>Dracaena marginata</i> , spotted leaves | Italy | JQ005223 | JQ005310 | JQ005744 | JQ005571 | JQ005397 | JQ005657 |
| <i>C. phormii</i> | CBS 118194* | <i>Phormium</i> sp. | Germany | JQ948446 | JQ948777 | - | JQ949767 | JQ949107 | JQ950097 |
| <i>C. phyllanthi</i> | CBS 175.67* | <i>Phyllanthus acidus</i> , anthracnose | India | JQ005221 | JQ005308 | JQ005742 | JQ005569 | JQ005395 | JQ005655 |
| <i>C. piperis</i> | CBS 125474* | <i>Piper nigrum</i> | Malaysia | MG600760 | MG600820 | - | MG600964 | MG600867 | MG601027 |
| <i>C. plumvorum</i> | CBS 125473 | <i>Coffea</i> sp. | Vietnam | MG600718 | MG600781 | - | MG600925 | MG600841 | MG600985 |
| | CGMCC 3.17358 | <i>Coffea</i> sp. | Vietnam | MG600717 | MG600780 | - | MG600924 | MG600840 | MG600984 |
| | GMM 3742 | <i>Camellia sinensis</i> , endophyte | China | KJ955215 | KJ954916 | - | KJ954483 | - | KJ955361 |
| | LJTJ30 | <i>Mangifera indica</i> | Brazil | KC702980 | KC702941 | - | KC702908 | KC598100 | KC992327 |
| | MAFF 243073 | <i>Capsicum annuum</i> | China | KP748221 | KP823800 | - | KP823741 | - | KP823853 |
| | MAFF 305790 | <i>Amorphophallus rivieri</i> | Japan | MG600730 | MG600793 | - | MG600936 | MG600847 | MG600997 |
| | CBS 145.29* | <i>Musa</i> sp. | Japan | MG600726 | MG600789 | - | MG600932 | MG600845 | MG600993 |
| <i>C. psidii</i> | CBS 128531* | <i>Psidium</i> sp. | Italy | JX010219 | JX009967 | JX009743 | JX009515 | JX009901 | JX010443 |
| <i>C. pyricola</i> | ICMP 1778* | <i>Pyrus communis</i> , fruit rot | New Zealand | JQ948445 | JQ948776 | - | JQ949766 | JQ949106 | JQ950096 |
| <i>C. queenslandicum</i> | MFLU 14-0626* | <i>Carica papaya</i> | Australia | JX010276 | JX009934 | JX009691 | JX009447 | JX009899 | JX010414 |
| <i>C. quinquefoliae</i> | CBS 133134* | <i>Parthenocissus quinquefolia</i> | Russia | KU236391 | KU236390 | - | KU236389 | - | KU236392 |
| <i>C. rhexiae</i> | | <i>Rhexia virginica</i> | USA | JX145128 | - | - | - | - | JX145179 |

Table 3 (cont.)

| Species | Culture* | Host/Substrate | Country | ITS | GAPDH | CAL | ACT | CHS-1 | TUB2 |
|---|-----------------|--|---------------|----------|----------|----------|----------|----------|----------|
| <i>C. rhexiae</i> (cont.) | CBS 133132 | <i>Vaccinium macrocarpon</i> | USA | JX145157 | – | – | – | – | JX145209 |
| <i>C. rhombiforme</i> | CBS 129953* | <i>Olea europaea</i> | Portugal | JQ948457 | JQ948788 | – | JQ949778 | JQ949118 | JQ950108 |
| <i>C. salicis</i> | CBS 607.94* | <i>Salix</i> sp., leaf, spot | Netherlands | JQ948460 | JQ948791 | JQ949781 | JQ949121 | JQ950111 | – |
| <i>C. salsolae</i> | ICMP 19051* | <i>Salsola tragus</i> | Hungary | JX010242 | JX009916 | JX009696 | JX009562 | JX009863 | JX010403 |
| <i>C. sansevieriae</i> | MAFF 239721* | <i>Sansevieria trifasciata</i> | Japan | AB212991 | – | – | – | – | – |
| <i>C. sedi</i> | MFLUCC 14-1002* | <i>Sedum</i> sp. | Russia | KM974758 | KM974755 | – | KM974756 | KM974754 | KM974757 |
| <i>C. siamense</i> | ICMP 18578* | <i>Coffea arabica</i> | Thailand | JX010171 | JX009924 | FJ917505 | FJ907423 | JX009865 | JX010404 |
| | ICMP 12567 | <i>Persea americana</i> | Australia | JX010250 | JX009940 | JX009697 | JX009541 | JX009761 | JX010387 |
| | ICMP 18574 | <i>Pistacia vera</i> | Australia | JX010270 | JX009942 | JX009715 | JX009460 | JX009845 | JX010402 |
| | ICMP 18121 | <i>Dioscorea rotundata</i> | Nigeria | JX010245 | JX009942 | JX009715 | JX009460 | JX009845 | JX010402 |
| | ICMP 17795 | <i>Malus domestica</i> | USA | JX010162 | JX010051 | JX009703 | JX009506 | JX009805 | JX010393 |
| <i>C. siamense</i> (syn. <i>C. hymenocallidis</i>) | ICMP 18642* | <i>Hymenocallis americana</i> | China | JX010278 | JX010019 | JX009709 | GQ856775 | GQ856730 | JX010410 |
| <i>C. siamense</i> (syn. <i>C. jasmini-sambac</i>) | ICMP 19118* | <i>Jasminum sambac</i> | Vietnam | HM131511 | HM131497 | JX009713 | HM131507 | JX009895 | JX010415 |
| <i>C. simmondsii</i> | CBS 122122* | <i>Carica papaya</i> | Australia | JQ948276 | JQ948606 | – | JQ949597 | JQ948937 | JQ949927 |
| <i>C. sloanei</i> | IMI 364297* | <i>Theobroma cacao</i> , leaf | Malaysia | JQ948287 | JQ948617 | – | JQ949608 | JQ948948 | JQ949938 |
| <i>C. sojae</i> | ATCC 62257* | <i>Glycine max</i> | USA | MG600749 | MG600810 | – | MG600954 | MG600860 | MG601016 |
| | CGMCC 3.15171 | <i>Bletilla ochracea</i> | China | HM751813 | KC843501 | – | KC843550 | KC244161 | – |
| <i>C. sonchicola</i> | JZB330117 | <i>Sonchus</i> sp. | Italy | KY962756 | KY962753 | – | KY962747 | KY962750 | – |
| | MFLUCC 17-1300 | <i>Sonchus</i> sp. | Italy | KY962758 | KY962755 | – | KY962749 | KY962752 | – |
| <i>C. spinaciae</i> | CBS 128.57 | <i>Spinacia oleracea</i> | Netherlands | GU227847 | GU228239 | – | GU227945 | GU228337 | GU228141 |
| <i>C. sydowii</i> | CBS 135819 | <i>Sambucus</i> sp. | China, Taiwan | KY263783 | KY263785 | – | KY263791 | KY263787 | KY263793 |
| <i>C. tamarilloi</i> | CBS 129814* | <i>Solanum betaceum</i> , fruit, anthracnose | Colombia | JQ948184 | JQ948514 | – | JQ949505 | JQ948845 | JQ949835 |
| <i>C. temperatum</i> | CBS 133122* | <i>Vaccinium macrocarpon</i> | USA | JX145159 | – | – | – | – | JX145211 |
| | CBS 133120 | <i>Vaccinium macrocarpon</i> | USA | JX145135 | – | – | – | – | JX145186 |
| <i>C. theobromicola</i> | CBS 124945* | <i>Theobroma cacao</i> | Panama | JX010294 | JX010006 | JX009591 | JX009444 | JX009869 | JX010447 |
| <i>C. ti</i> | ICMP 4832* | <i>Cordylone</i> sp. | New Zealand | JX010269 | JX009952 | JX009649 | JX009520 | JX009898 | JX010442 |
| <i>C. torulosum</i> | CBS 128544* | <i>Solanum melongena</i> | New Zealand | JQ005164 | JQ005251 | JQ005685 | JQ005512 | JQ005338 | JQ005598 |
| <i>C. tropicale</i> | CBS 124949* | <i>Theobroma cacao</i> | Panama | JX010264 | JX010007 | JX009719 | JX009489 | JX009870 | JX010407 |
| <i>C. tropicicola</i> | BCC 38877* | <i>Citrus maxima</i> | Thailand | JN050240 | JN050229 | – | JN050218 | – | JN050246 |
| | MFLUCC100167 | <i>Paphiopedilum bellatolum</i> | Thailand | JN050241 | JN050230 | – | JN050219 | – | JN050247 |
| <i>C. truncatum</i> | CBS 151.35* | <i>Phaseolus lunatus</i> | USA | GU227862 | GU228254 | – | GU227960 | GU228352 | GU228156 |
| <i>C. viniferum</i> | GZAAS 5.08601* | <i>Vitis vinifera</i> cv. Shuijing | China | JN412804 | JN412798 | JQ309639 | JN412795 | – | JN412813 |
| <i>C. vitalense</i> | CBS 181.82* | <i>Theobroma cacao</i> | India | MG600734 | MG600796 | – | MG600940 | MG600851 | MG601001 |
| <i>C. walleri</i> | CBS 125472* | <i>Coffea</i> sp., leaf tissue | Vietnam | JQ948275 | JQ948605 | – | JQ949596 | JQ948936 | JQ949926 |
| <i>C. wuxiense</i> | CGMCC 3.17894* | <i>Camellia sinensis</i> | China | KU251591 | KU252045 | KU251833 | KU251672 | KU251939 | KU252200 |
| | JST1A44 | <i>Camellia sinensis</i> | China | KU251592 | KU252046 | KU251834 | KU251673 | KU251940 | KU252201 |
| <i>C. xanthorrhoeae</i> | ICMP 17903* | <i>Xanthorrhoea preissii</i> | Australia | JX010261 | JX009927 | JX009653 | JX009478 | JX009823 | JX010448 |
| <i>C. yunnanense</i> | CBS 132135* | <i>Buxus</i> sp. | China | JX546804 | JX546706 | – | JX519239 | JX519231 | JX519248 |
| <i>Colletotrichum</i> sp. | CGMCC 3.15172 | <i>Bletilla ochracea</i> | China | HM751816 | KC843522 | – | KC843547 | – | KC244162 |
| | Q026 | <i>Rubus glaucus</i> | Colombia | JN715839 | KC860013 | – | KC859970 | KC859995 | KC860039 |
| <i>Glomerella cingulata</i> f. sp. <i>camelliae</i> * | ICMP 10643 | <i>Camellia</i> × <i>williamsii</i> | UK | JX010224 | JX009908 | JX009630 | JX009540 | JX009891 | JX010436 |
| <i>Monilochaetes infuscans</i> | CBS 869.96* | <i>Ipomoea batatas</i> | South Africa | JQ005780 | JX546612 | – | JQ005843 | JQ005801 | JQ005864 |

* 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 Oclávio Almeida Drummond, Vitória, Brazil; GZAAS: Guizhou Academy of Agricultural Sciences Herbarium, China; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; IMI: Culture collection of CAB International, 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.

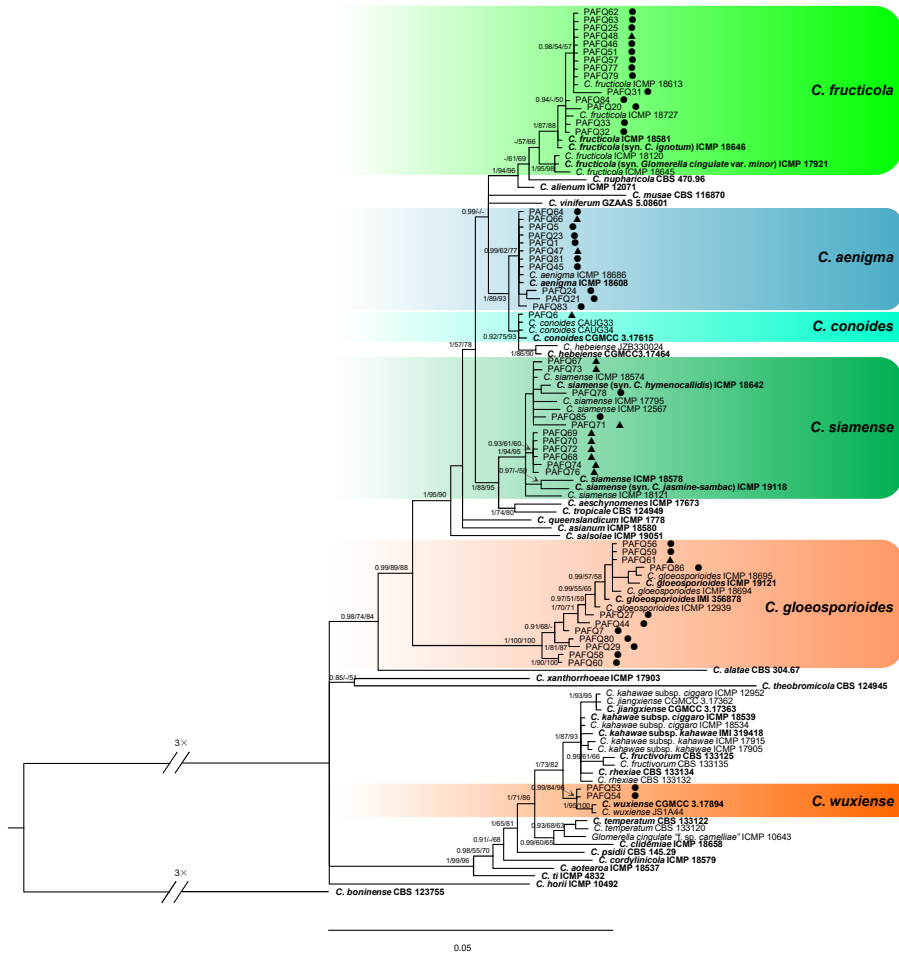


Fig. 2 A Bayesian inference phylogenetic tree of 111 isolates in the *C. gloeosporioides* species complex. The species *C. boninense* (CBS 123755) was selected as an outgroup. The tree was built using concatenated sequences of the *ACT*, *TUB2*, *CAL*, *CHS-1*, *GAPDH*, and ITS genes. Bayesian posterior 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.

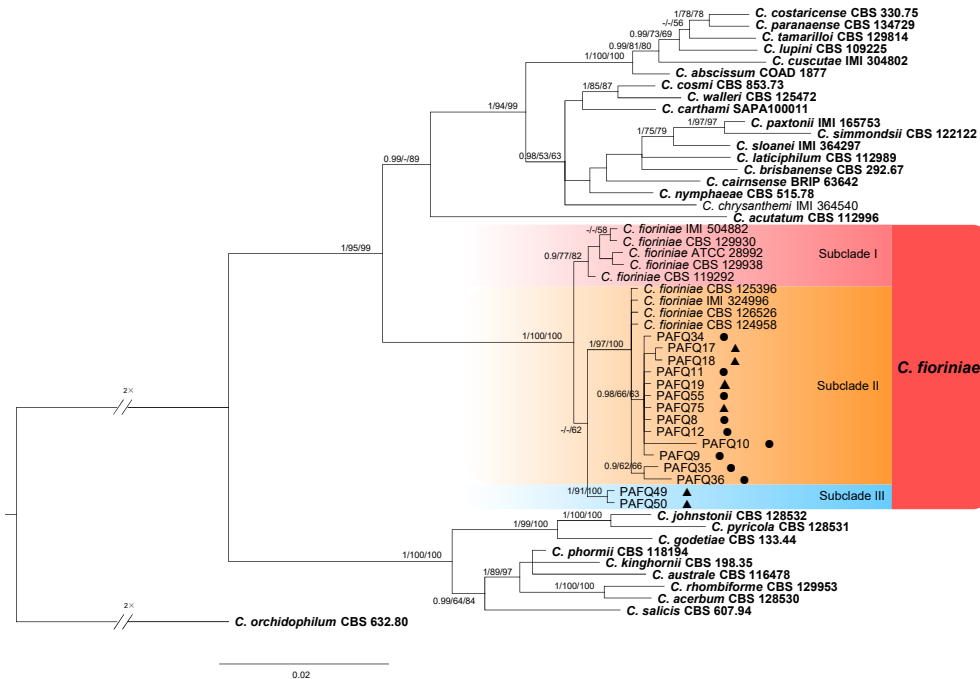


Fig. 3 A Bayesian inference phylogenetic tree of 51 isolates in the *C. acutatum* species complex. The species *C. orchidophilum* (CBS 632.80) was selected as an outgroup. The tree was built using concatenated sequences of the *ACT*, *TUB2*, *CHS-1*, *GAPDH*, and ITS genes. Bayesian posterior probability (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.

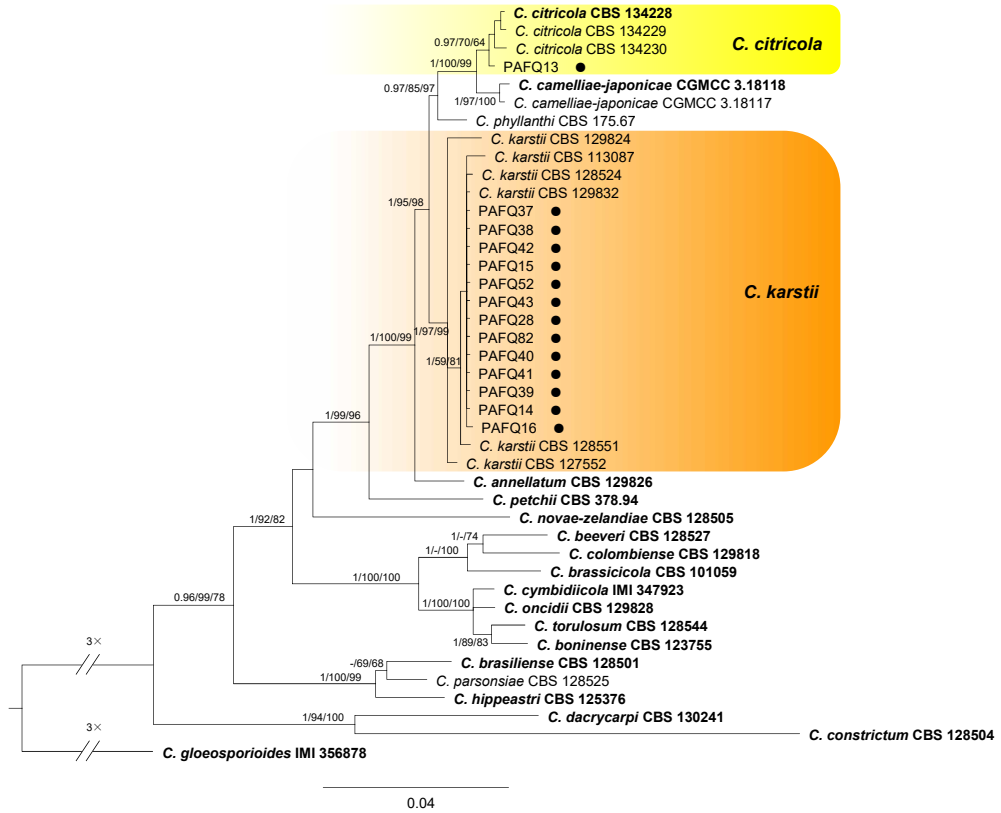


Fig. 4 A Bayesian inference phylogenetic tree of 41 isolates in the *C. boninense* species complex. The species *C. gloeosporioides* (IMI 356878) was selected as an outgroup. The tree was built using concatenated sequences of the *ACT*, *TUB2*, *CAL*, *CHS-1*, *GAPDH*, and ITS genes. Bayesian posterior 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.

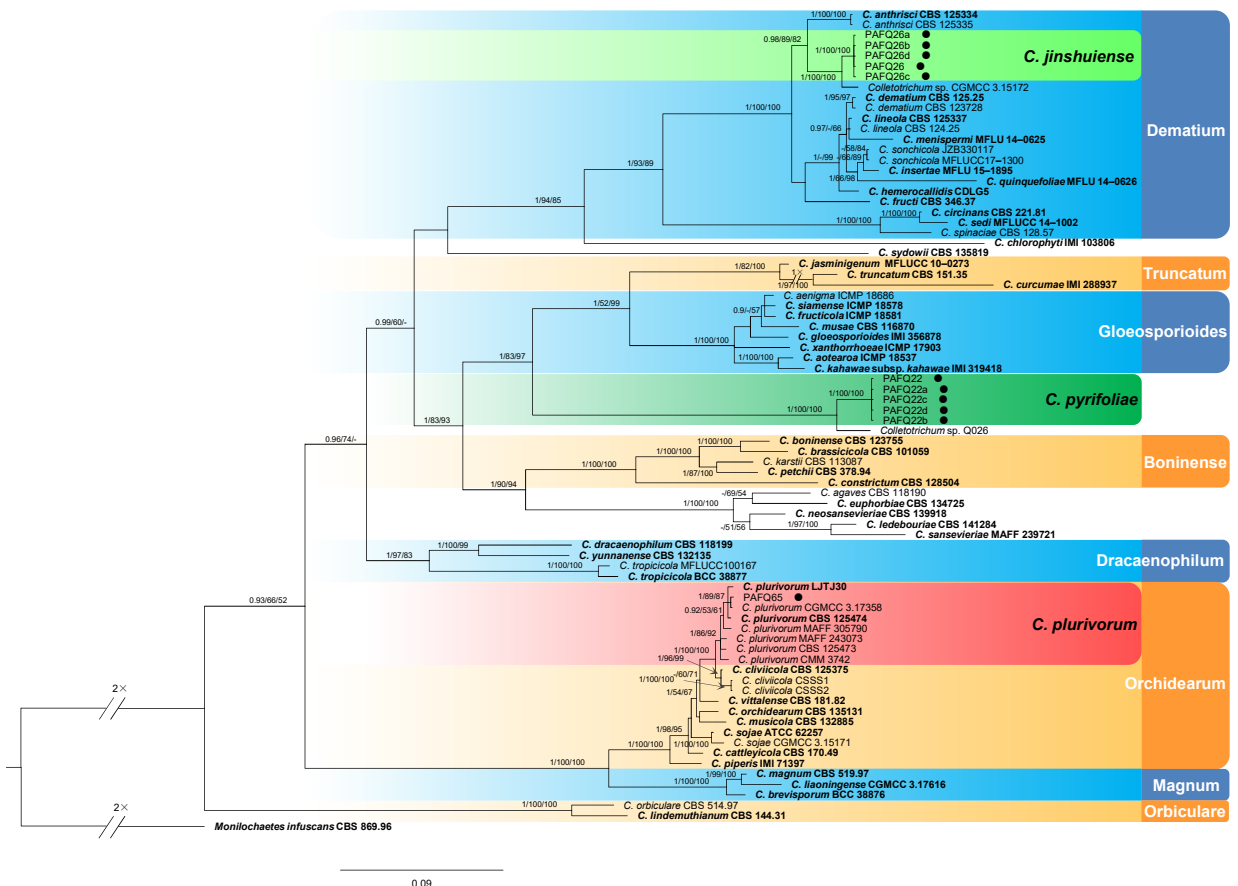


Fig. 5 Phylogenetic tree generated by Bayesian inference based on concatenated sequences of the *ACT*, *CHS-1*, *GAPDH*, ITS, and *TUB* genes. *Monilochaetes infuscans* (CBS 869.96) was selected as an outgroup. Bayesian posterior probability (PP ≥ 0.90), MP bootstrap support values (ML ≥ 50 %), and RAxML 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.

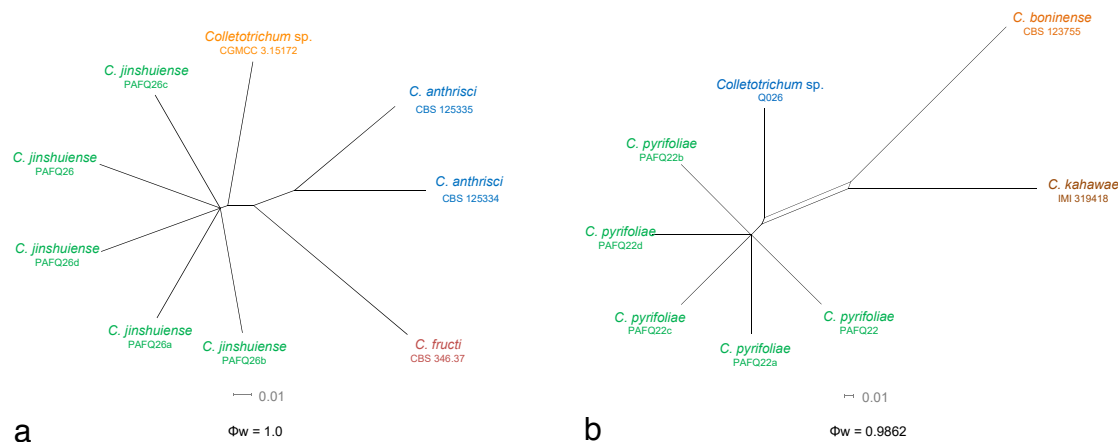


Fig. 6 The result of the pairwise homoplasly index (PHI) tests of closely related species using both LogDet transformation and splits decomposition. a, b. The PHI of *C. jinshuiense* (a) or *C. pyriformae* (b) and their phylogenetically related isolates or species, respectively. PHI test value (Φ_w) < 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. pyriformae* 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. pyriformae* cv. Xiangnan, 1 Sept. 2015, *M. Fu* (culture PAFQ1); *ibid.*, on leaves of *P. pyriformae* cv. Huanghua, 1 Sept. 2015, *M. Fu* (PAFQ3); *ibid.*, on leaves of *P. pyriformae* 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. pyriformae* 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. pyriformae* 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. pyriformae*, 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 ($13.5\text{--}20 \times 5\text{--}8 \mu\text{m}$, mean \pm SD = $17.4 \pm 1.4 \times 7.1 \pm 0.7 \mu\text{m}$) are slightly larger than those of the ex-type isolate CBS 134228 ($12.8\text{--}18.4 \times 5.3\text{--}6.7 \mu\text{m}$, mean = $15.8 \times 6.1 \mu\text{m}$) of *C. citricola*. Setae were observed in the acervuli formed on pear leaves, being brown, smooth-walled, 2-septate, $41\text{--}84 \mu\text{m}$ long, base rounded, $6 \mu\text{m}$ diam, tip more or less acute.

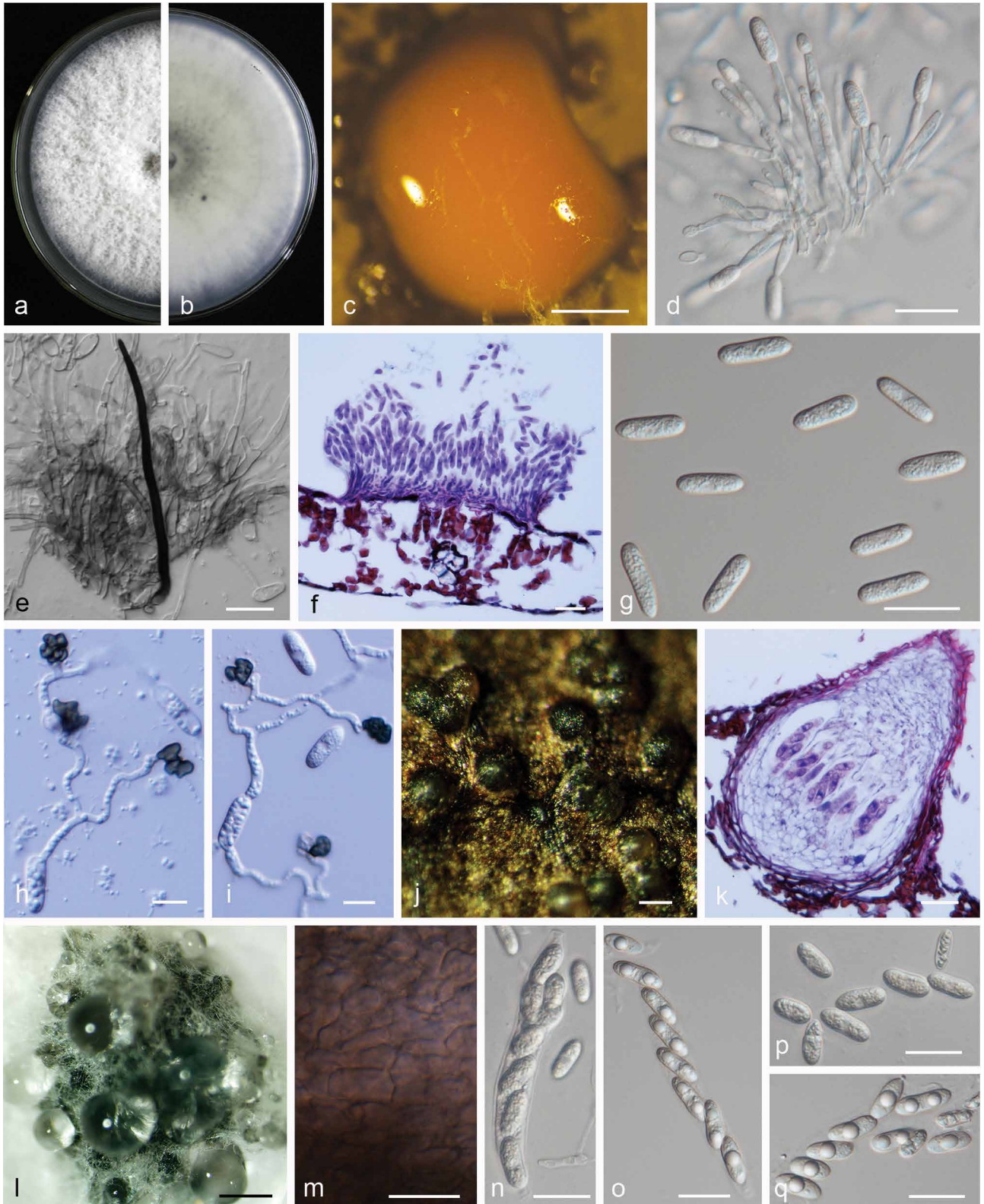


Fig. 7 *Colletotrichum aenigma*. a, b. Front and back view, respectively, of 6-d-old PDA culture; c. conidiomata; d. conidiophores; e. seta; f. section view of acervulus produced on pear leaf (*P. pyrifolia* cv. Cuiguan); g. conidia; h, i. appressoria; j. ascomata produced on pear leaf (*P. bretschneideri* 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.

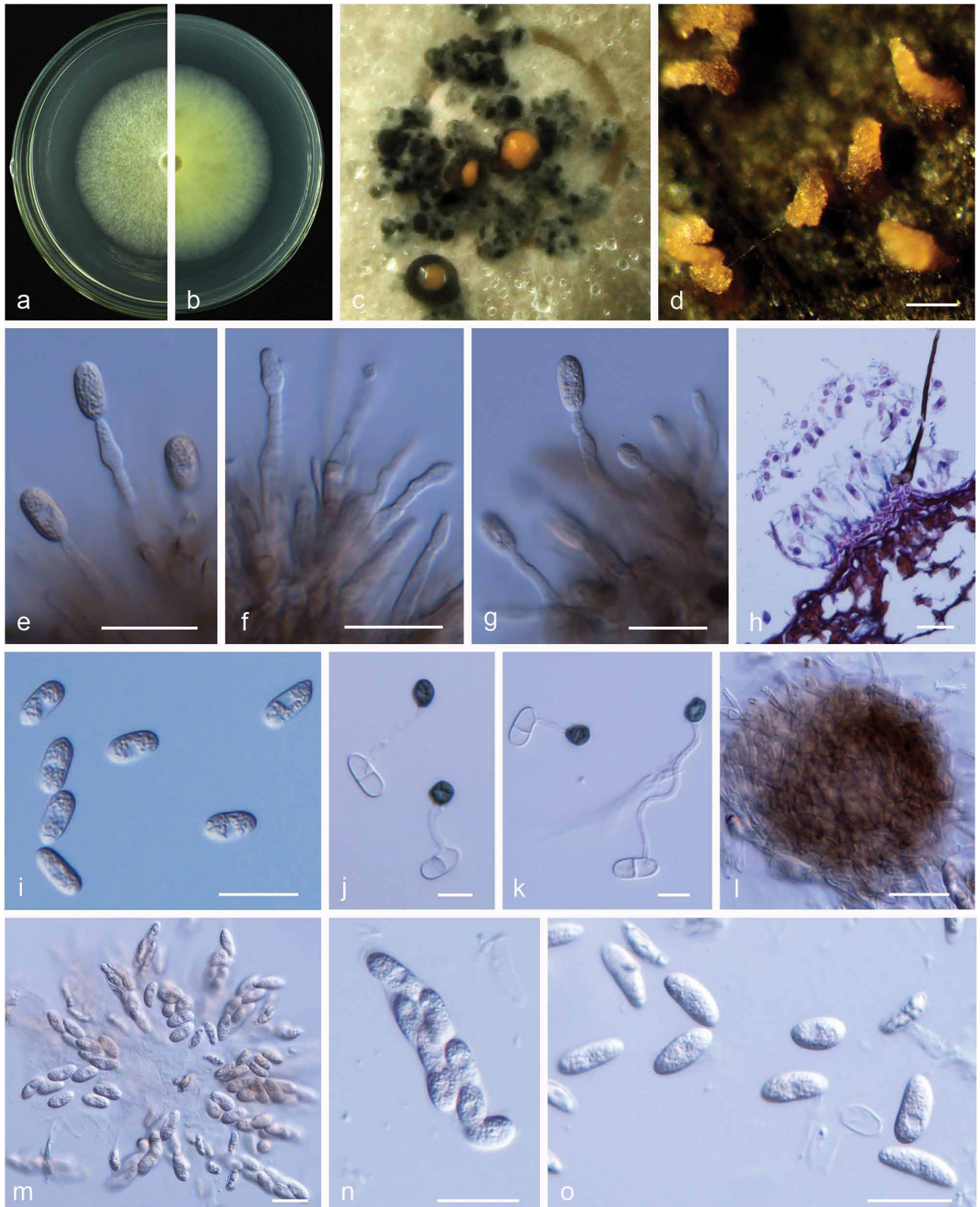


Fig. 8 *Colletotrichum citricola*. a, b. Front and back view, respectively of 6-d-old PDA culture; c, d. conidiomata; e–g. conidiophores; h. section view of acerulus produced on pear leaf (*P. pyrifolia* cv. Cuiguan); i. conidia; j, k. appressoria; l. ascoma; m, n. asci; o. ascospores (a–o. isolate PAFQ13; 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.

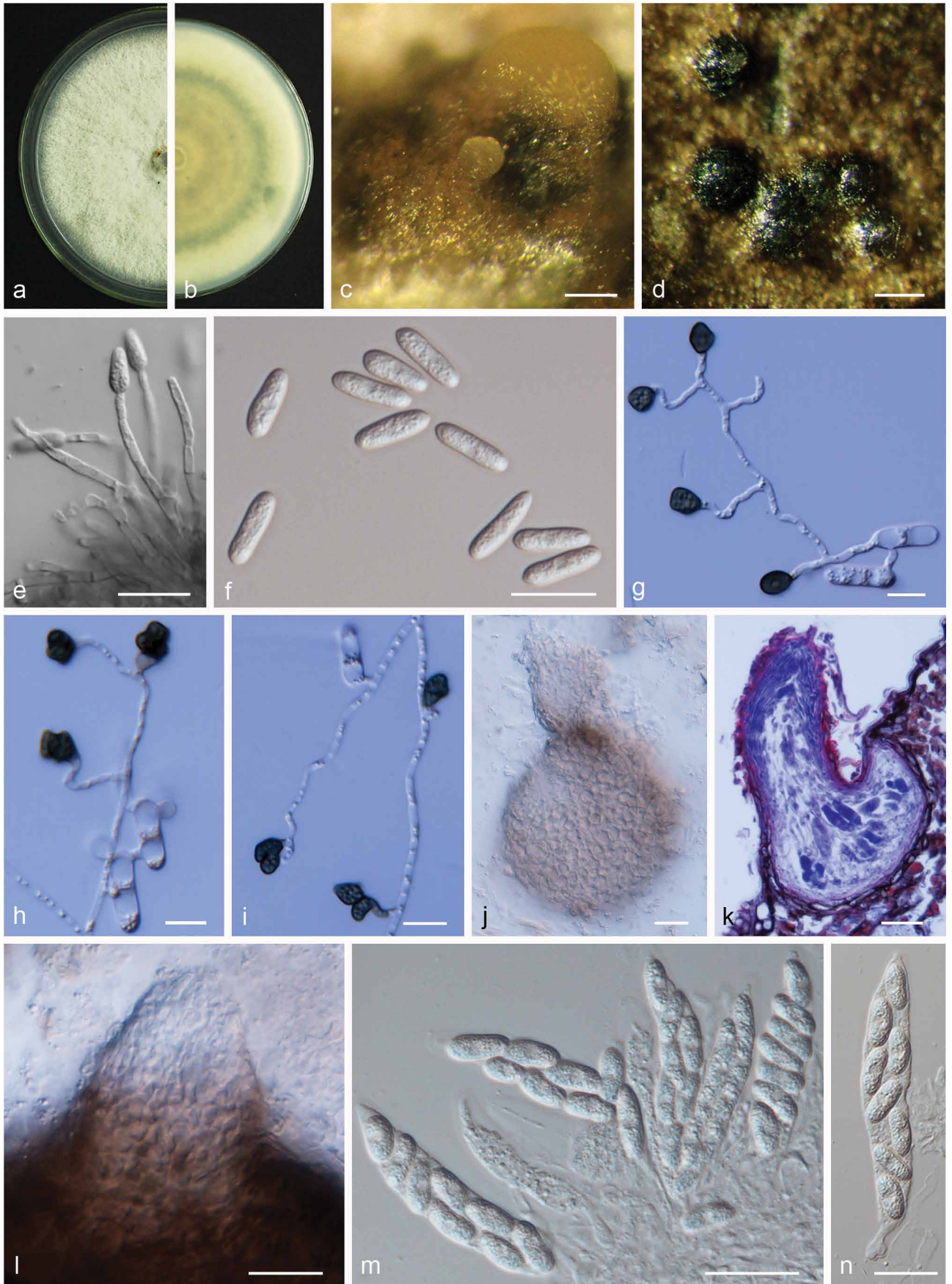


Fig. 9 *Colletotrichum conoides*. a, b. Front and back view, respectively, of 6-d-old PDA culture; c. conidiomata; d. ascomata produced on pear leaf (*P. bretschneideri* cv. Dangshansuli); e. conidiophores; f. conidia; g–i. appressoria; j. ascoma; k. section view of ascoma produced on pear leaf (*P. 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 *Cap-sicum 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 ($16\text{--}20 \times 4.5\text{--}6 \mu\text{m}$, mean \pm SD = $18.4 \pm 0.8 \times 5.6 \pm 0.3 \mu\text{m}$) are longer than those of the ex-type isolate CGMCC 3.17615 ($13\text{--}17.5 \times 5\text{--}6.5 \mu\text{m}$, mean = $15.9 \times 5.9 \mu\text{m}$) of *C. conoides*.

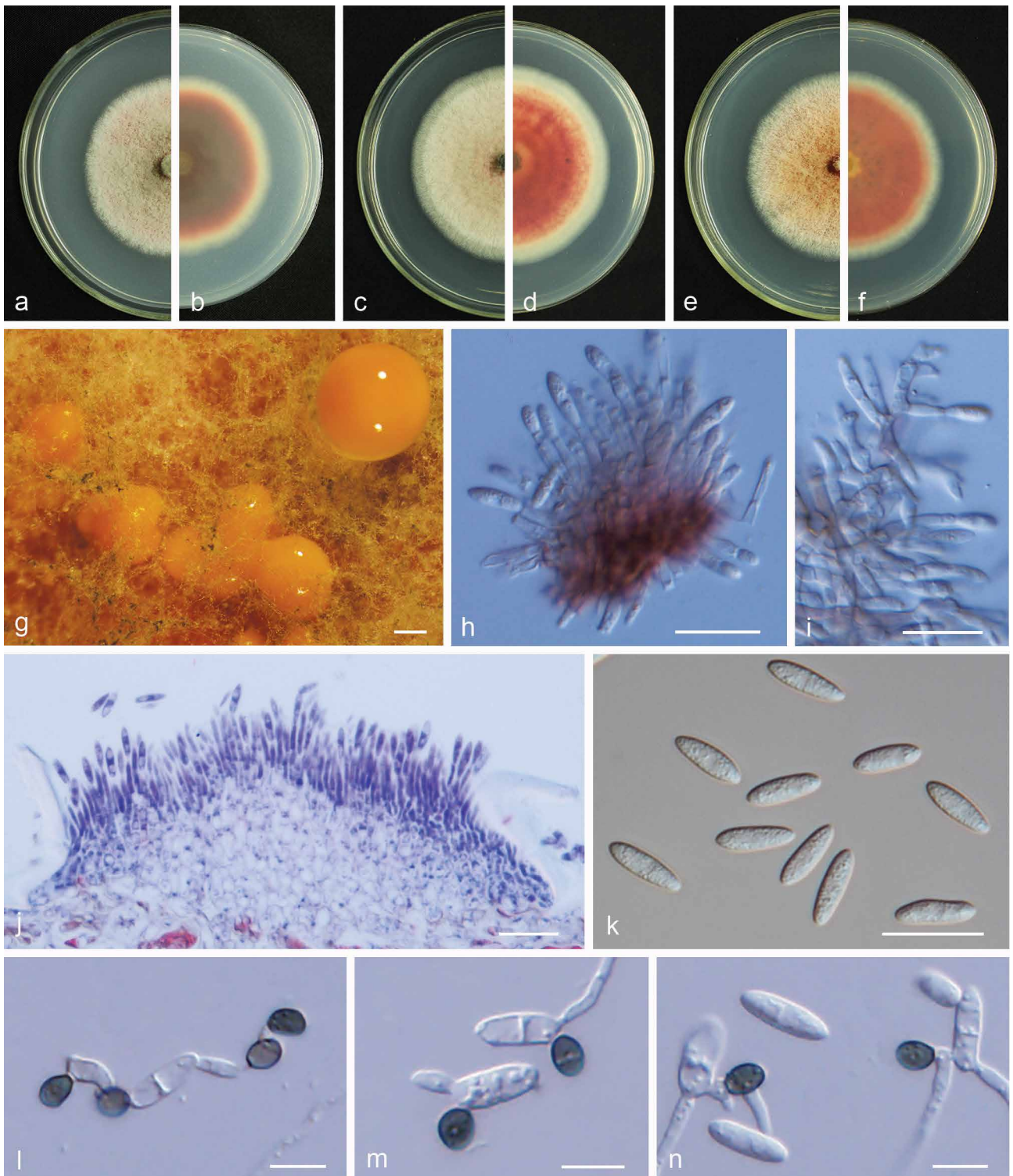


Fig. 10 *Colletotrichum fioriniae*. a, c, e. Front views of 6-d-old PDA culture; b, d, f. back views of 6-d-old PDA culture; g. conidiomata; h, i. conidiophores; j. section view of acervulus produced on pear fruit (*P. bretschneideri* cv. Huangguan); k. conidia; l–n. appressoria (a, b, g–l. isolate PAFQ8, 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. Guyiot, 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-

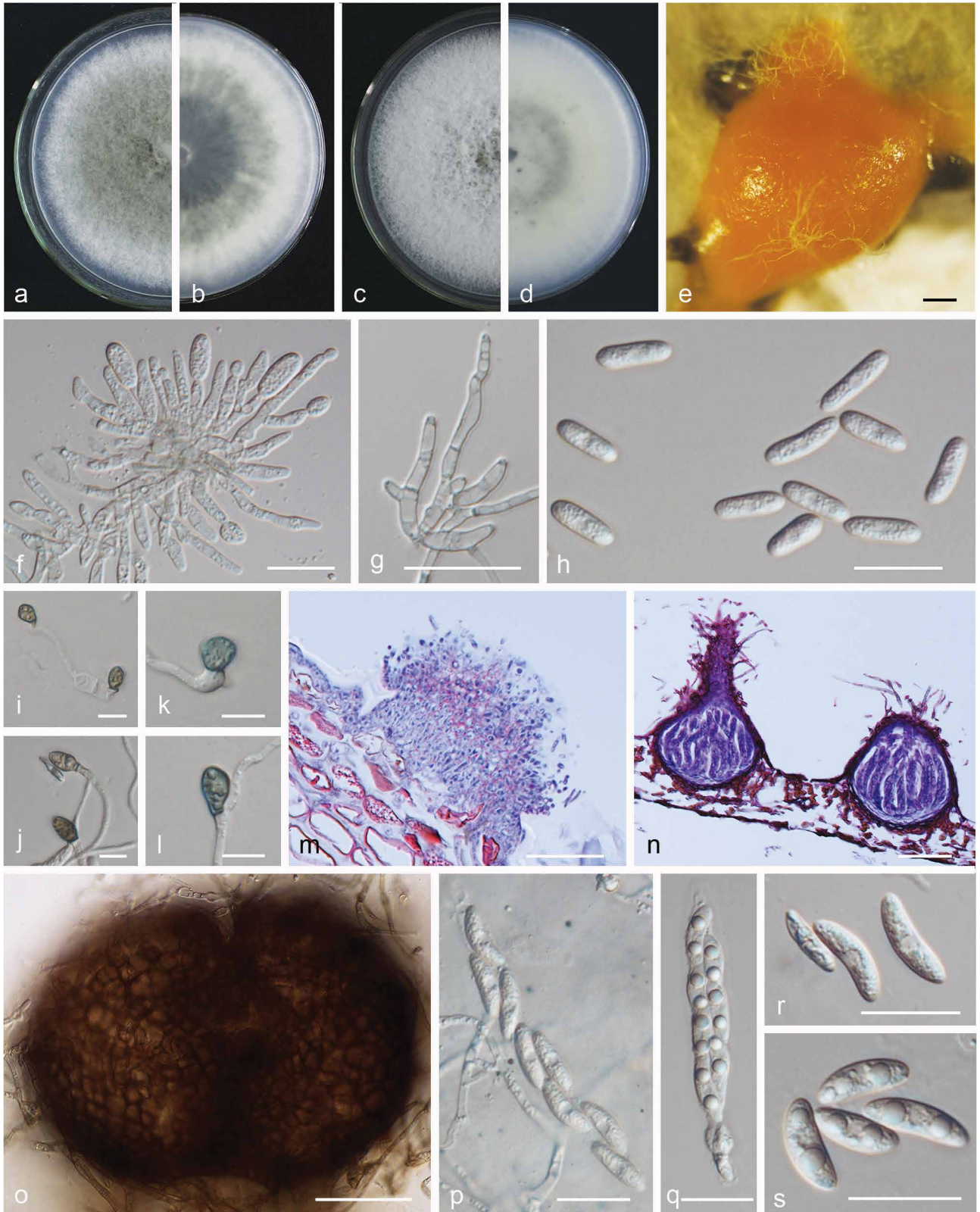


Fig. 11 *Colletotrichum fructicola*. a, c. Front views of 6-d-old PDA culture; b, d. back views of 6-d-old PDA culture; e. conidiomata; f, g. conidiophores; h. conidia; i–l. appressoria; m. section view of acervulus produced on pear fruit (*P. bretschneideri* cv. Huangguan); n. section view of ascomata 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 μ m; f–h, p–s = 20 μ m; i–l = 10 μ m; m–o = 50 μ m.

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-

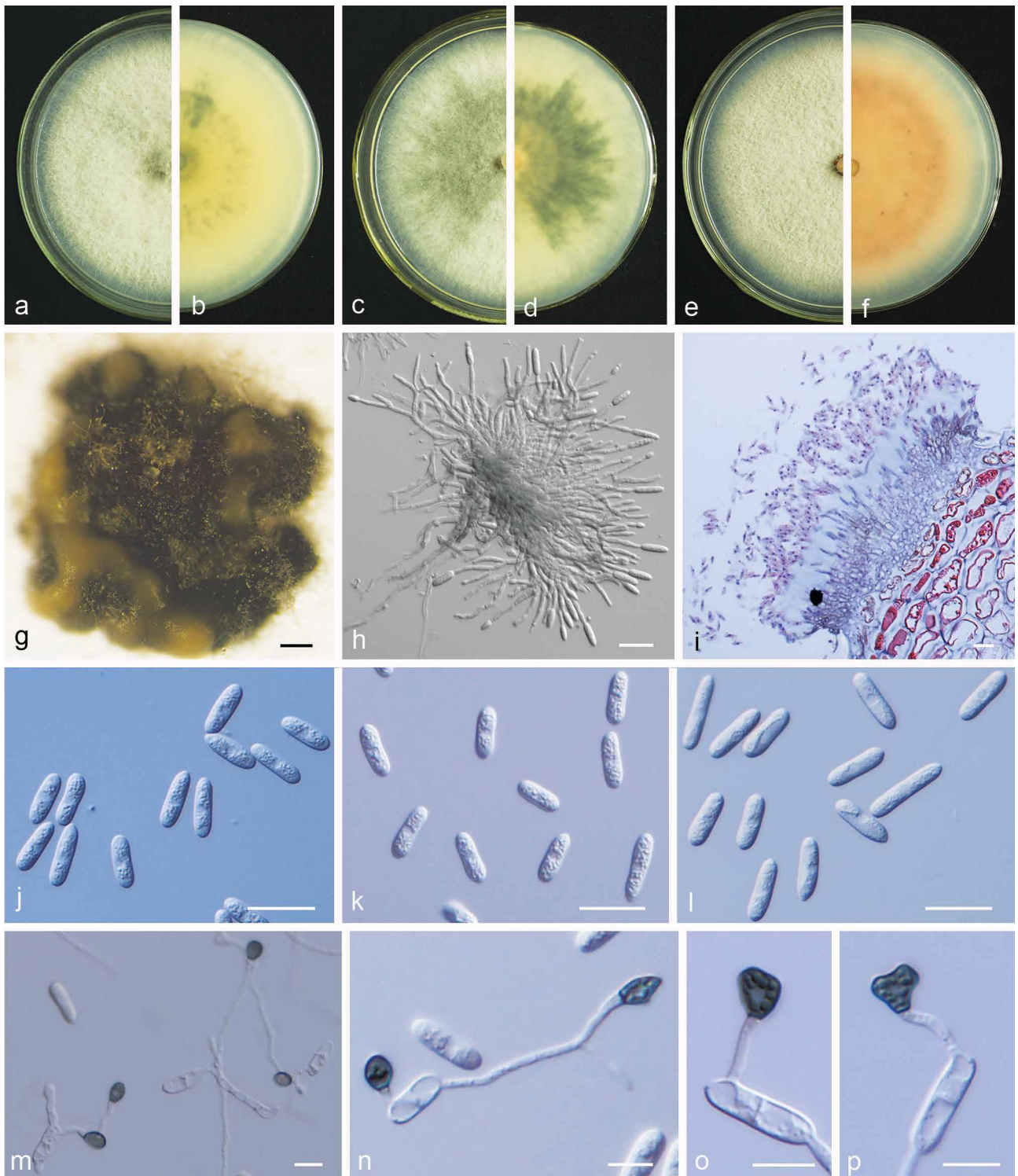


Fig. 12 *Colletotrichum gloeosporioides*. a, c, e. Front views of 6-d-old PDA culture; b, d, f. back views of 6-d-old PDA culture; g. conidiomata; h. conidiophores; i. section view of acervulus produced on pear fruit (*P. bretschneideri* cv. Huangguan); j–l. conidia; m–p. appressoria (a, b, j, m. isolate 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 μ m; h–l = 20 μ m; m–p = 10 μ m.

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,

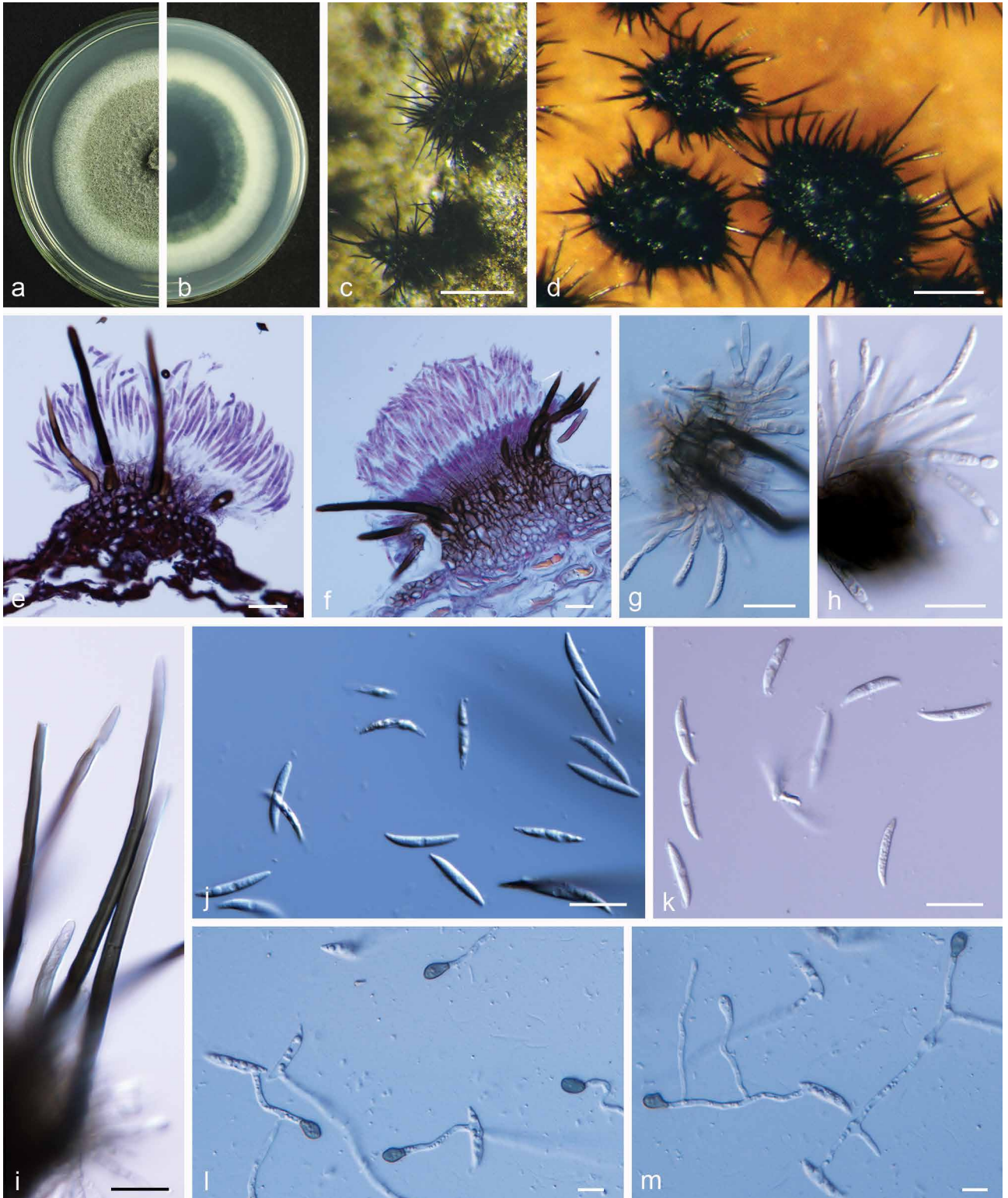


Fig. 13 *Colletotrichum jinshuiense*. a, b. Front and back view, respectively, of 6-d-old PDA culture; c. acervuli produced on pear leaf (*P. bretschneideri* cv. Dangshansuli); d. acervuli produced on pear fruit; e, f. section view of acervulus produced on pear leaf and fruit, respectively; g, h. conidiophores;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

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. pyrifolia* 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. **Conidigenous cells** (on fruit surface) hyaline, smooth-walled, cylindrical, 12.5–27 \times 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 \times 3.5–4.5

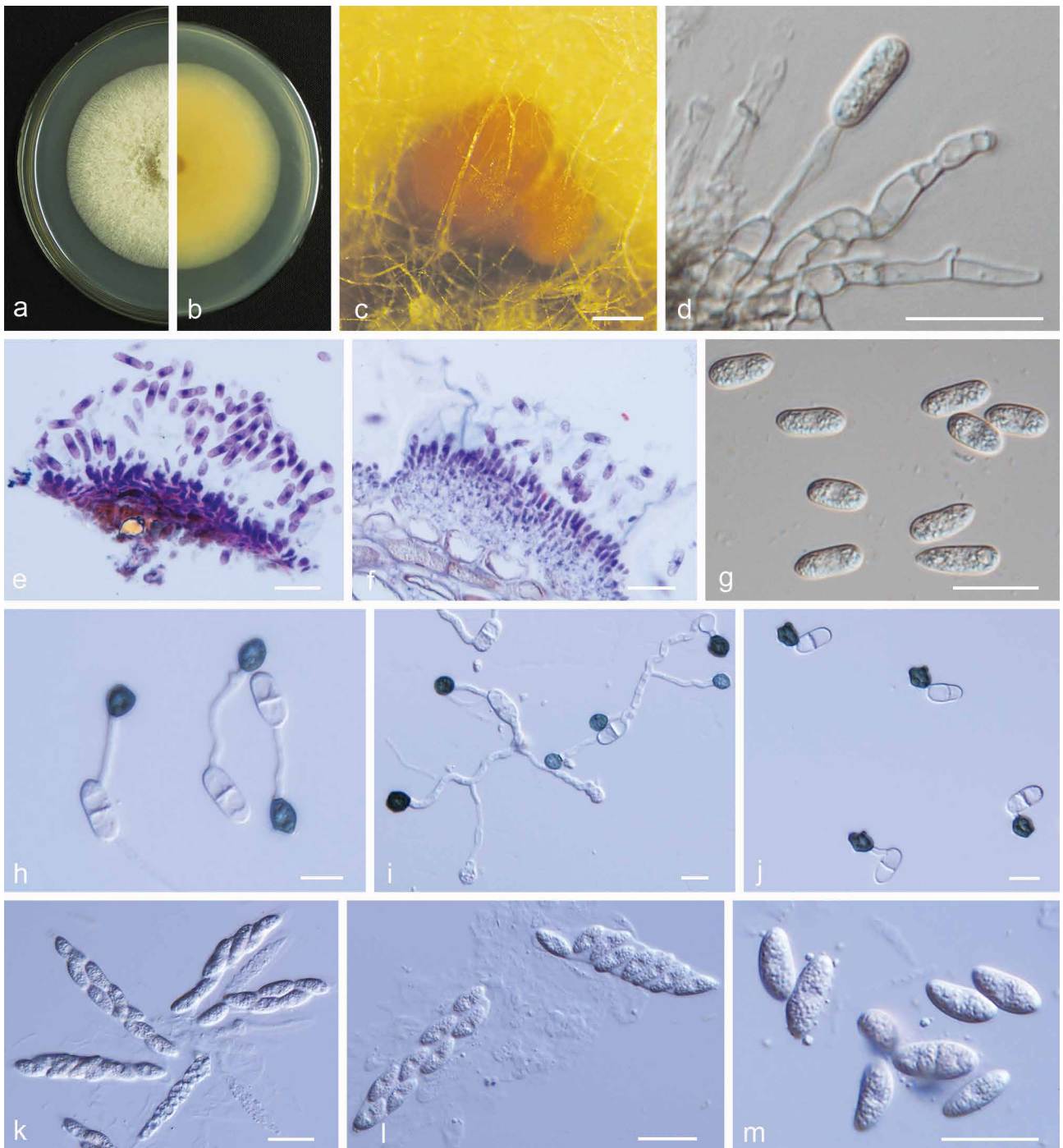


Fig. 14 *Colletotrichum karstii*. a, b. Front and back view, respectively, of 6-d-old PDA culture; c. conidiomata; d. conidiophores; e, f. section view of acervulus produced on pear leaf (*P. pyrifolia* cv. Cuiguan) and fruit (*P. bretschneideri* cv. Huangguan), respectively; g. conidia; h–j. appressoria; 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 \pm SD = $27.1 \pm 1.7 \times 4.0 \pm 0.3 \mu\text{m}$, L/W ratio = 6.8; on fruit surface: $21\text{--}30.5 \times 3\text{--}4.5 \mu\text{m}$, mean \pm SD = $24.4 \pm 2.1 \times 4.0 \pm 0.3 \mu\text{m}$, L/W ratio = 6.2. *Appressoria* pale brown, smooth-walled, ellipsoidal to clavate, $8\text{--}17 \times 5\text{--}7.5 \mu\text{m}$, mean \pm SD = $10.7 \pm 1.7 \times 6.0 \pm 0.5 \mu\text{m}$, 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 ($\Phi_w = 1$) 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*.

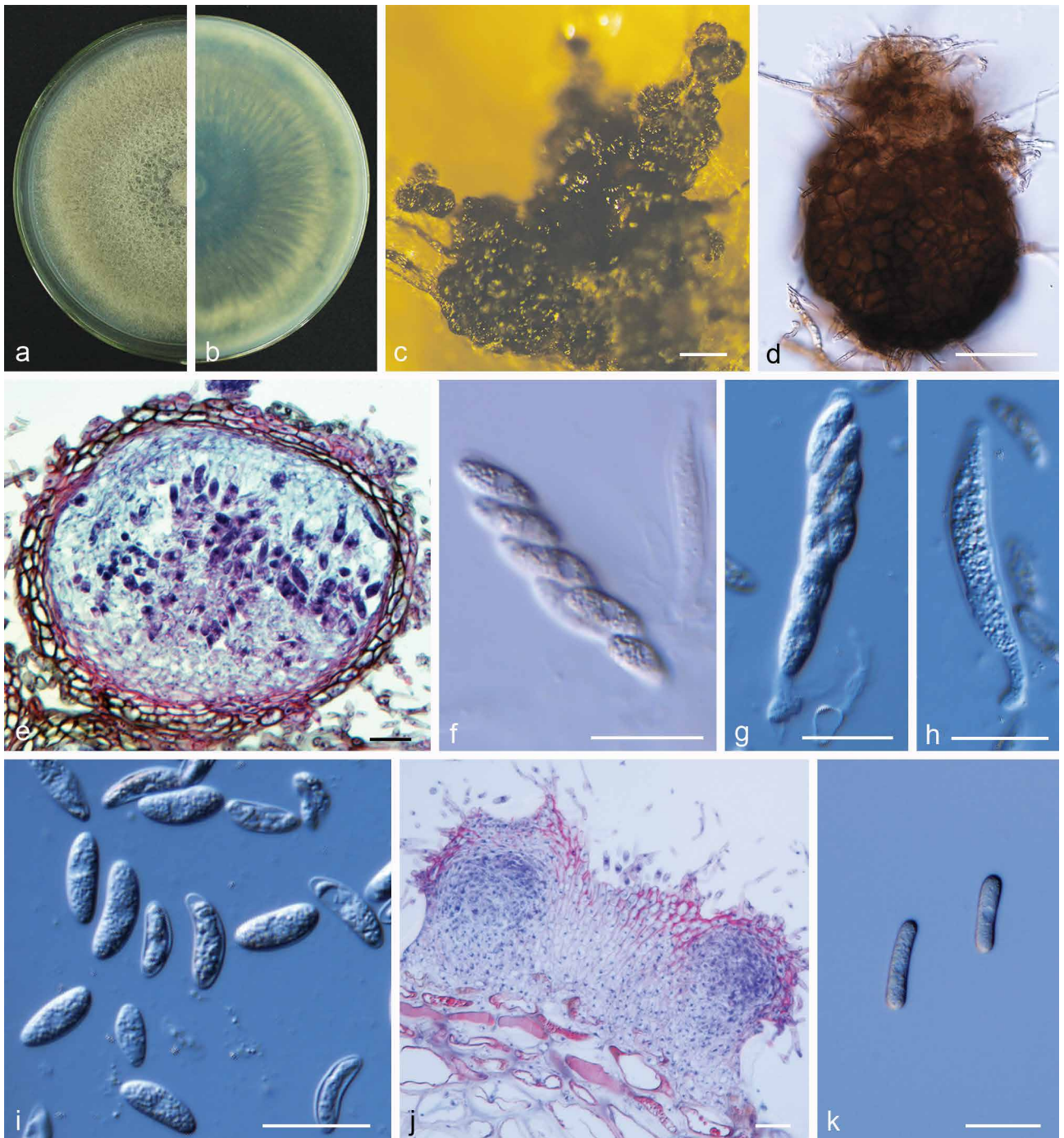


Fig. 15 *Colletotrichum plurivorum*. a, b. Front and back view, respectively, of 6-d-old PDA culture; c, d. ascomata; e. section of ascoma; f, g. asci; h. immature ascus; i. ascospores; j. section view of acervulus produced on pear fruit (*P. bretschneideri* cv. Huangguan); k. conidia (a–k. isolate PAFQ65;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

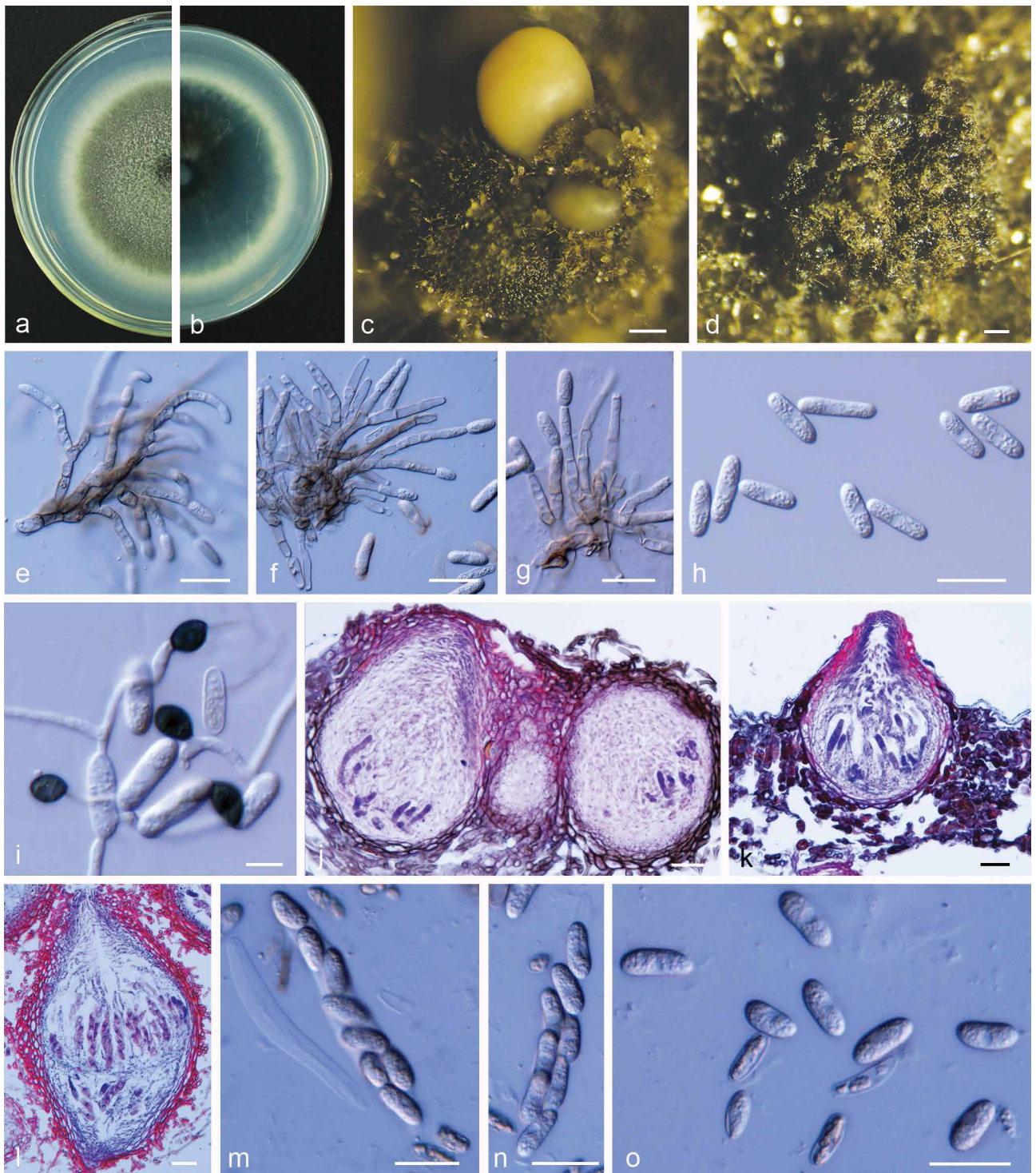


Fig. 16 *Colletotrichum pyrifoliae*. a, b. Front and back view, respectively, of 6-d-old PDA culture; c. conidiomata; d. ascomata; e–g. conidiophores; h. conidia; i. appressoria; j, k. section view of ascomata produced on pear fruit (*P. bretschneideri* cv. Huangguan) and leaf (*P. pyrifolia* cv. Cuiguan), 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. clivicola* (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

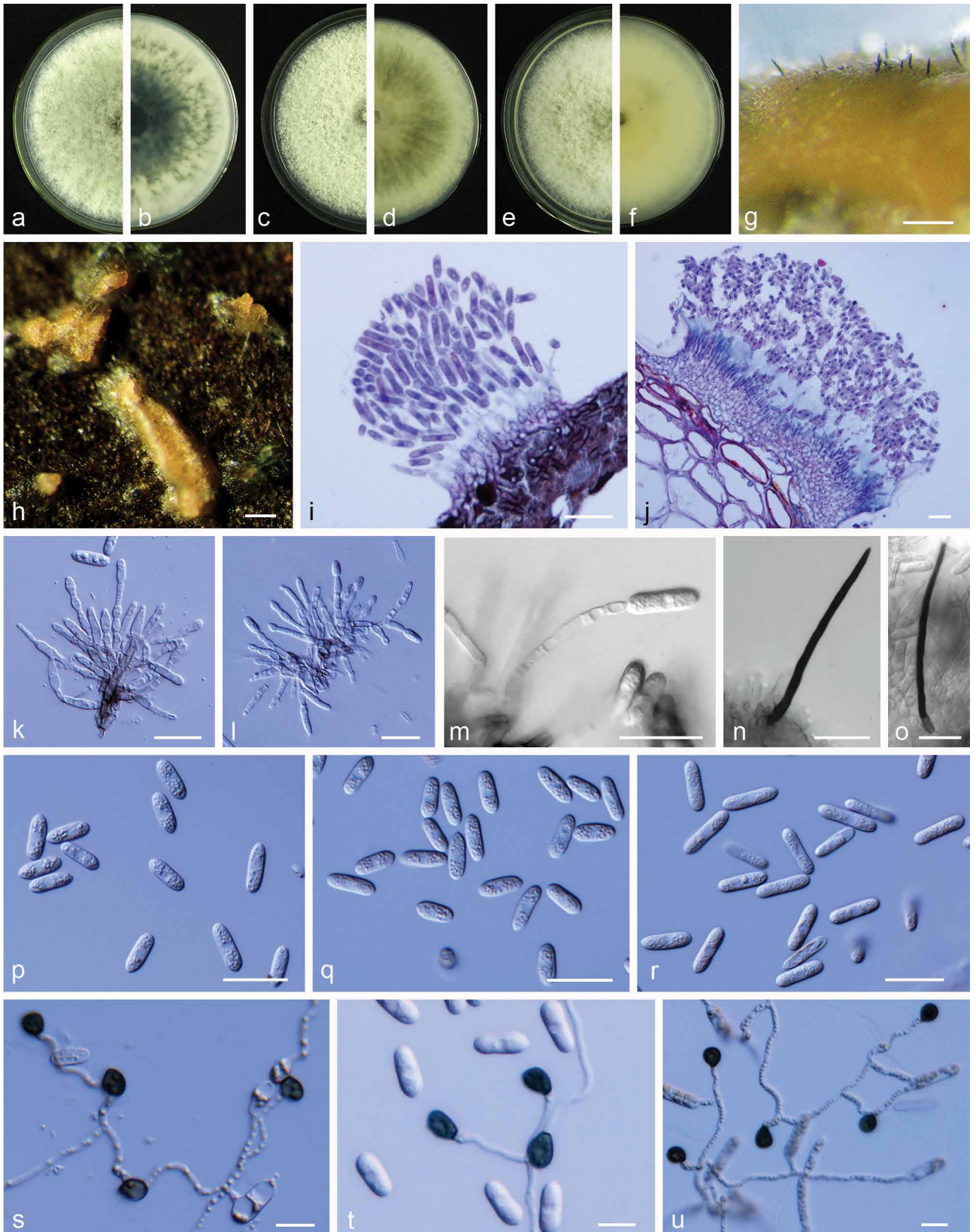


Fig. 17 *Colletotrichum siamense*. a, c, e. Front views of 6-d-old PDA culture; b, d, f. back views of 6-d-old PDA culture; g, h. conidiomata; i, j. section view of acervulus produced on pear leaf (*P. pyrifolia* cv. Cuiguan) and fruit (*P. bretschneideri* cv. Huangguan), respectively; k–m. conidiophores; 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 μ m; i–r = 20 μ m; s–u = 10 μ m.

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,

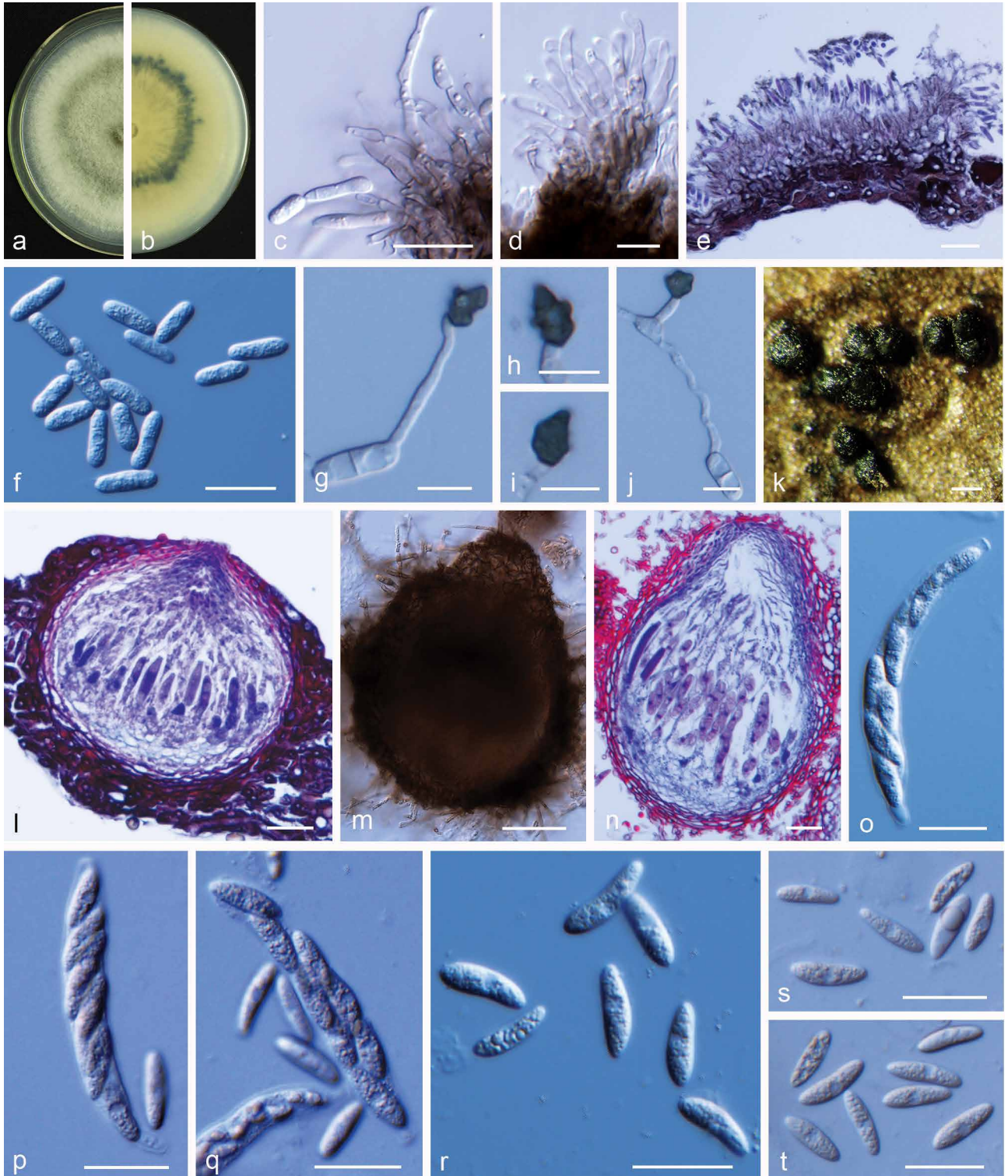


Fig. 18 *Colletotrichum wuxiense*. a, b. Front and back view, respectively, of 6-d-old PDA culture; c, d. conidiophores; e. section view of acervulus produced on pear leaf; f. conidia; g–j. appressoria; k. ascomata; l. section view of ascoma produced on pear fruit; m. ascoma produced on PDA; n. section 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, appressoria and ascospores of *Colletotrichum* spp. obtained in this study.

| 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 appressoria size ^z | Length (µm) ^x | Width (µm) ^y | Means ± SD of ascospores size ^z | |
| <i>C. aenigma</i> | | | | | | | | | | |
| 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 |
| <i>C. citricola</i> | | | | | | | | | | |
| 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 |
| <i>C. conoides</i> | | | | | | | | | | |
| 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 |
| <i>C. floriniae</i> | | | | | | | | | | |
| 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 |
| <i>C. fructicola</i> | | | | | | | | | | |
| 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 |
| <i>C. gloeosporioides</i> | | | | | | | | | | |
| 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 |
| <i>C. jinshulense</i> | | | | | | | | | | |
| PAFQ26 | 21–30.5 ^a | 3–4.5 ^a | 24.4 ± 2.1 × 4.0 ± 0.3 ^a | 8–17 | 5–7.5 | 10.7 ± 1.7 × 6.0 ± 0.5 | / | / | / | 5.6 |
| <i>C. karstii</i> | | | | | | | | | | |
| 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 |
| <i>C. plurivorum</i> | | | | | | | | | | |
| PAFQ65 | 14–24 ^a | 4.5–7 ^a | 18.1 ± 2.1 × 5.6 ± 0.7 ^a | / | / | / | 15–20.5 | 4.5–6 | 18.2 ± 1.6 × 5.4 ± 0.4 | 7.2 |
| <i>C. pyriformae</i> | | | | | | | | | | |
| 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 appressoria 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 |
| PAFQ64 | 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 appressoria 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 appressoria recorded from the representative strains of *Colletotrichum* spp. obtained in this study. Significance at $P = 0.05$ level.

^z Numbers indicate mean conidia, appressoria, 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.

/ Appressoria, ascospores or data of growth rate were absent.

° 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 ($\Phi_w = 0.9862$) 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 \pm SD = 17.4 \pm 1.1 μm) were longer than those of type I (12–19 μm , mean lengths from 15.5 \pm 1.0 to 16.0 \pm 1.2 μm) and II (12–17.5 μm , mean lengths from 14.7 \pm 1.0 to 15.1 \pm 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 \times 88–224 μm , ostiolate. *Asci* clavate, 43–91 \times 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 \times 4–6.5 μm , mean \pm SD = 17.2 \pm 1.3 \times 5.0 \pm 0.5 μm , L/W ratio = 3.4.

Sexual morph developed on PDA. *Ascomata* pyriform to subglobose, dark brown, 74–139 \times 64–127 μm , ostiolate. *Asci* clavate, 57–96 \times 12–16 μm , 8-spored. *Ascospores* hyaline, smooth-walled, aseptate, fusoid, slightly curved, straight with round ends, contents granular, 15.5–22 \times 5–6.5 μm , mean \pm SD = 18.37 \pm 1.39 \times 5.80 \pm 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 \times 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 \times 4.5–6.5 μm , mean \pm SD = 14.9 \pm 1.3 \times 5.3 \pm 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 \times 5.5–11 μm , mean \pm SD = 9.4 \pm 1.1 \times 7.1 \pm 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. Cuiquan, 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 \times 4.5–6.5 μm , mean \pm SD = 14.9 \pm 1.3 \times 5.3 \pm 0.3 μm ; PAFQ54: 13–18 \times 4.5–6 μm , mean \pm SD = 15.0 \pm 1.3 \times 5.1 \pm 0.4 μm) were smaller than those of the ex-type culture of *C. wuxiense* (CGMCC 3.17894: 16.5–23 \times 4.5–6.5 μm , mean \pm SE = 19.0 \pm 1.4 \times 5.6 \pm 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. pyrifolia* cv. Cuiquan. 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

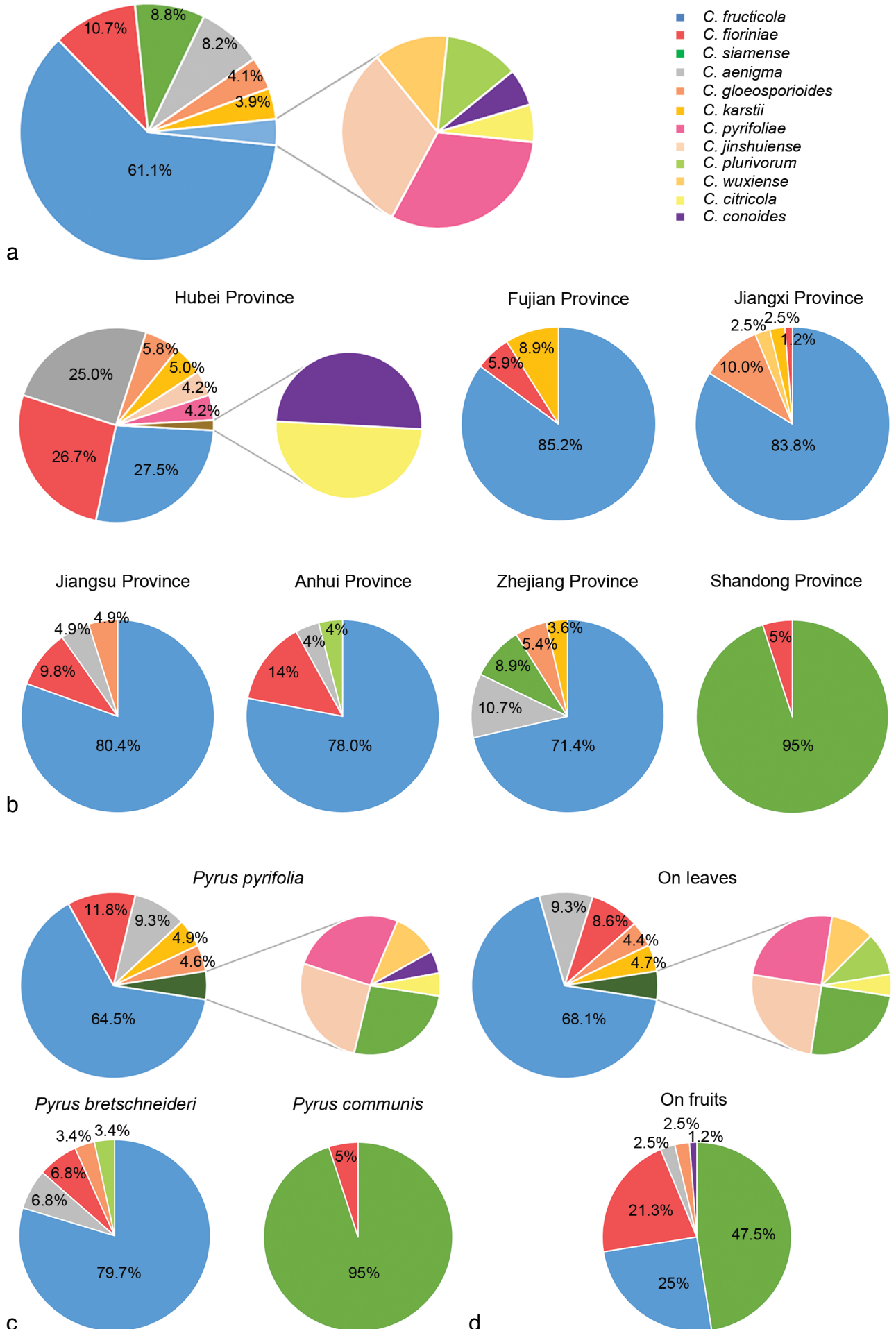


Fig. 19 The prevalence of *Colletotrichum* species isolated from pear. a. Overall isolation rate (%) of *Colletotrichum* species; b–d. isolation rate (%) of *Colletotrichum* species from each sampled province (b), *Pyrus* spp. (c), and pear organs (d), respectively.

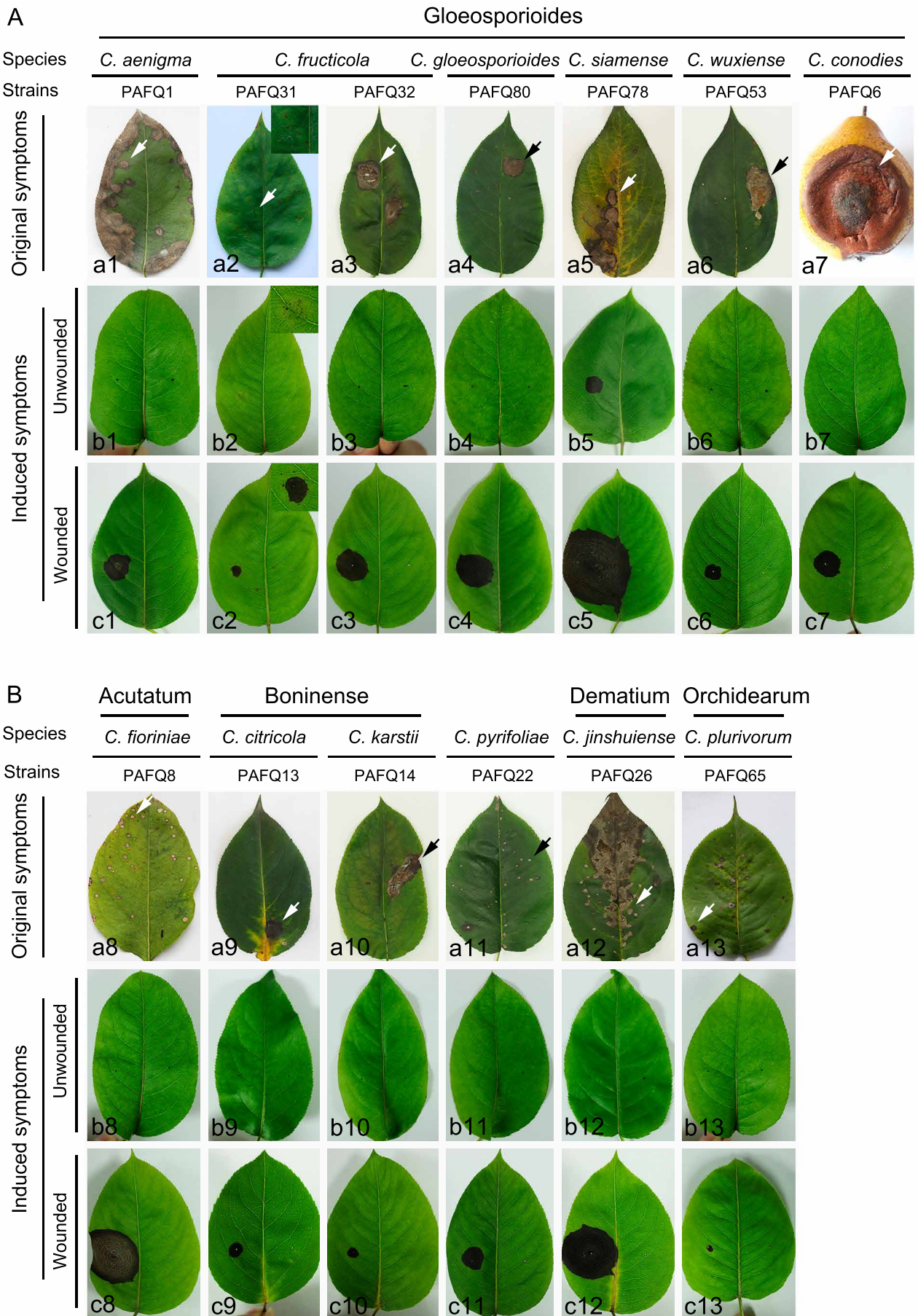


Fig. 20 Representative symptoms of pear leaves (*P. pyriformis* cv. Cuiguan) induced by inoculation of spore suspensions of 12 *Colletotrichum* spp. under unwounded and wounded conditions. The symptoms caused by these species were photographed at 14 dpi (except for b2, c2, c3 at 8 dpi). A, B. The symptoms 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. pyriformis* 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 |
|---------------------------|------------------|--------|----------------|
| <i>C. aenigma</i> | PAFQ1 | Leaf | 14/16 |
| <i>C. citricola</i> | PAFQ13 | Leaf | 7/16 |
| <i>C. conoides</i> | PAFQ6 | Fruit | 6/16 |
| <i>C. fioriniae</i> | PAFQ8 | Leaf | 15/16 |
| <i>C. fructicola</i> | PAFQ31 | Leaf | 16/16 |
| | PAFQ32 | Leaf | 10/16 |
| <i>C. gloeosporioides</i> | PAFQ80 | Leaf | 9/16 |
| <i>C. jinshuiense</i> | PAFQ26 | Leaf | 9/16 |
| <i>C. karstii</i> | PAFQ14 | Leaf | 7/16 |
| <i>C. plurivorum</i> | PAFQ65 | Leaf | 2/16 |
| <i>C. pyrifoliae</i> | PAFQ22 | Leaf | 10/16 |
| <i>C. siamense</i> | PAFQ78 | Leaf | 12/16 |
| <i>C. wuxiense</i> | PAFQ53 | Leaf | 7/16 |
| control | H ₂ O | | 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

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

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

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 *Colletotrichum 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. gloeosporioides*, *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

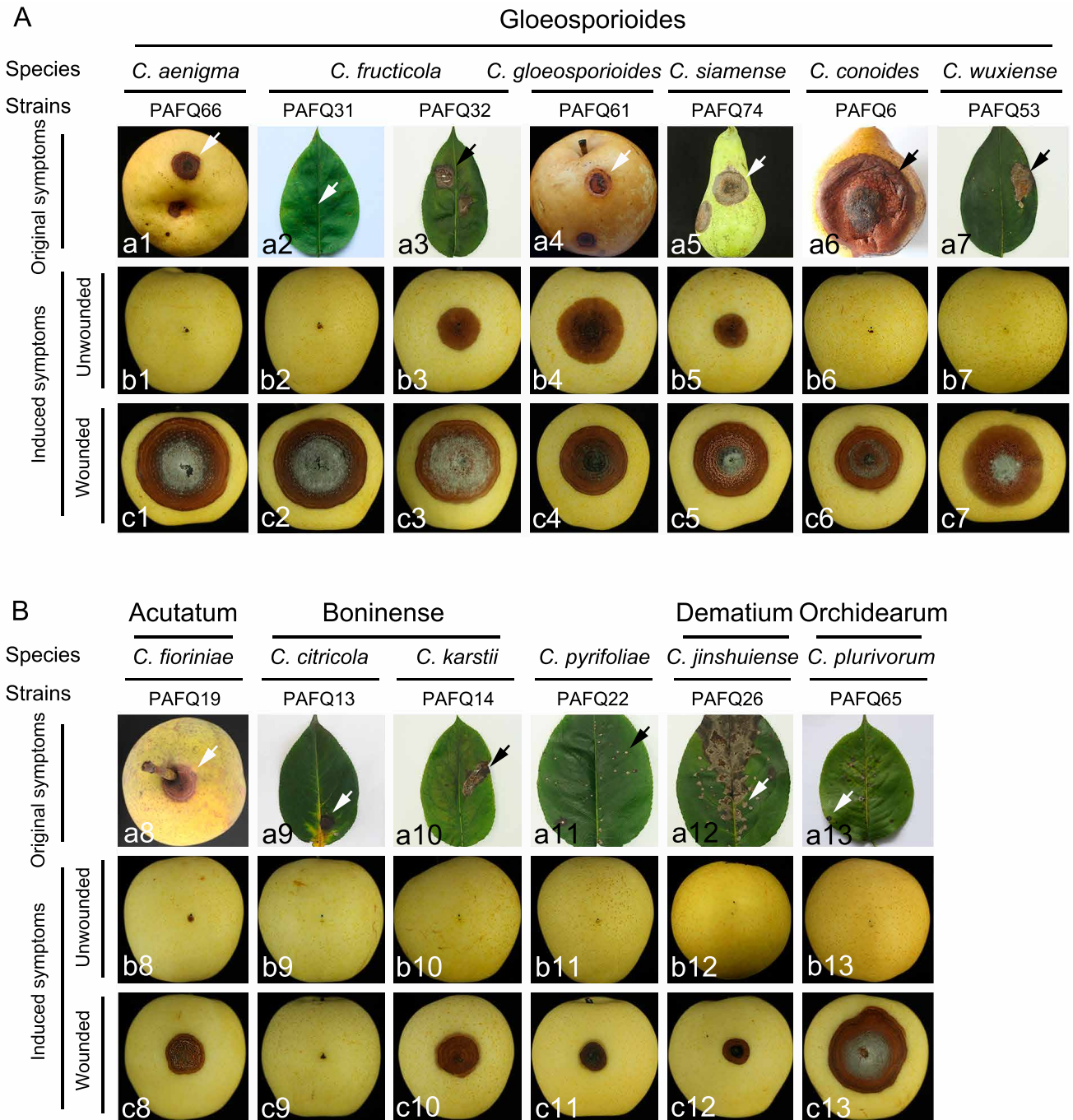


Fig. 21 Representative symptoms of pear fruits (*P. bretschneideri* cv. Huangguan) induced by inoculation with spore suspensions of 12 *Colletotrichum* spp. under unwounded and wounded conditions. The symptoms under unwounded conditions were photographed at 30 dpi, whereas these under the wounded at 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 \times 4.5–7.5 μm) from pear had larger conidia than those from coffee (9.7–14 \times 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. pyriformae* 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. pyriformae*, *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).

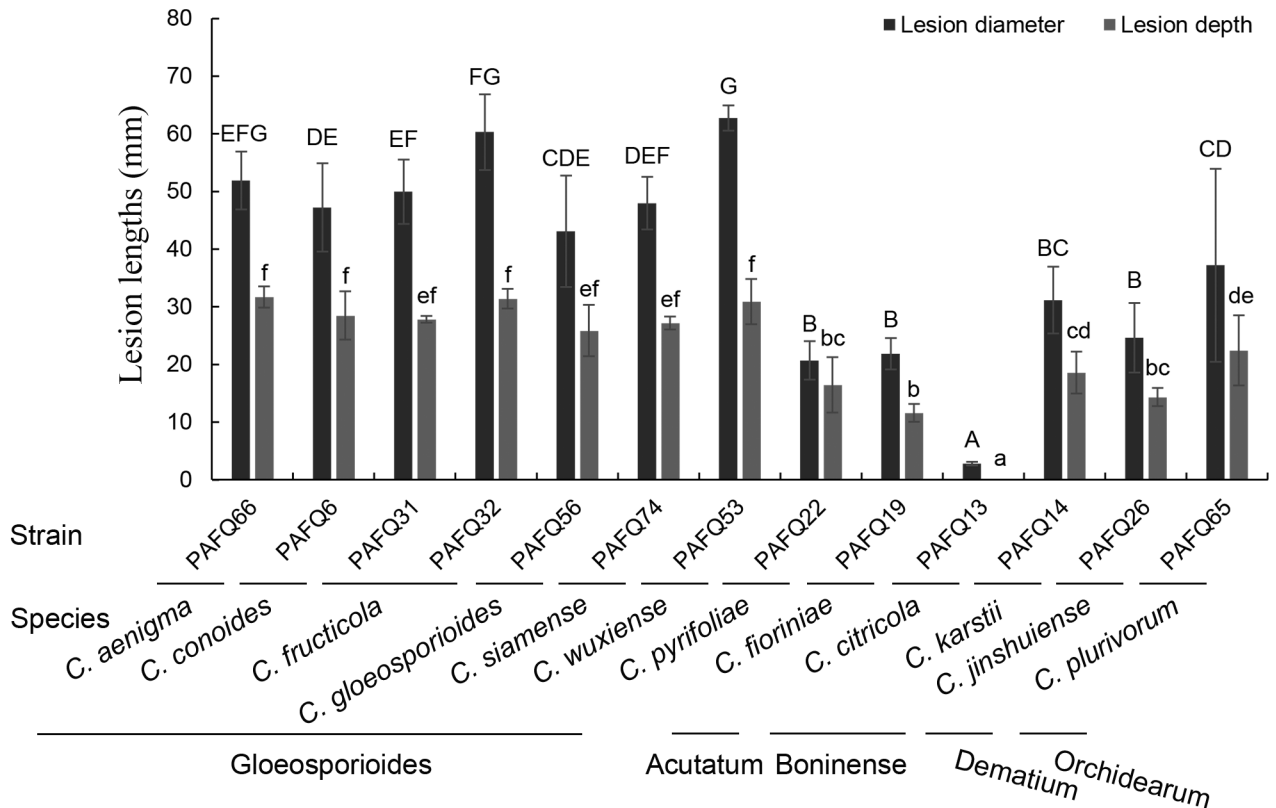


Fig. 22 Lesion lengths and depths on wounded pear fruits (*P. bretschneideri* cv. Huangguan) at 10 dpi induced by conidial suspensions of 13 representative isolates of 12 *Colletotrichum* spp. The involved isolates and their belonging are indicated at the bottom of the bars. Data were analysed with SPSS 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. fiorinae*, *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. fiorinae*, *C. fructicola*, *C. aenigma*, *C. gloeosporioides*, *C. pyriformiae*, and *C. jinshuiense* are related to leaf SS symptoms; and *C. aenigma*, *C. fiorinae*, *C. gloeosporioides*, *C. siamense*, and *C. conoides* are related to fruit BR 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. pyriformiae*, 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. fiorinae*, *C. citricola*, and *C. pyriformiae* (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. pyriformia* 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. pyriformiae* (on leaves) in China (Fig. 19c, d), with a dominant incidence on the latter. *Colletotrichum fiorinae* 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. fiorinae* in China, which caused pear bitter rot and was associated with pear leaf spot on *P. pyriformia*, *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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