Mycosphere 10(1): 490–530 (2019) www.mycosphere.org ISSN 2077 7019
Article
Doi 10.5943/mycosphere/10/1/8

Molecular characterization and pathogenicity of fungal taxa associated with cherry leaf spot disease

Chethana KWT[1,2], Jayawardene RS[2], Zhang W[1], Zhou YY[1], Liu M[1], Hyde KD[2], Li XH[1], Wang J[3], Zhang KC[3] and Yan JY[1]

[1]*Beijing Key Laboratory of Environment Friendly Management on Fruit Diseases and Pests in North China, Institute of Plant and Environment Protection, Beijing Academy of agriculture and forestry sciences, Beijing, China*
[2]*Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand*
[3]*Academy of Forestry and Pomology Sciences, Beijing, China*

Chethana KWT, Jayawardene RS, Zhang W, Zhou YY, Liu M, Hyde KD, Li XH, Wang J, Zhang KC, Yan JY 2019 – Molecular characterization and pathogenicity of fungal taxa associated with cherry leaf spot disease. Mycosphere 10(1), 490–530, Doi 10.5943/mycosphere/10/1/8

Abstract

Cherry leaf spot is one of the most common and devastating diseases of cherries worldwide. The disease causes considerable yield losses in many cherry growing regions. We surveyed cherry leaf spot disease in Beijing City and collected 67 fungal isolates from approximately 60 diseased leaves. Multigene phylogenetic analyses coupled with morphological observations facilitated the identification of species isolated from the diseased tissues. Pathogenicity assays were conducted for six isolates representing all the identified species and Koch's postulates were confirmed on three cultivars of *Prunus avium* under greenhouse conditions. These results confirmed their pathogenicity on cherry leaves as symptoms were reproduced. Based on these results, a novel taxon *Alternaria prunicola* sp. nov. is reported as the main pathogen of Cherry leaf spot in Beijing City. In addition, *Alternaria alternata*, *Alternaria pseudoeichhorniae* sp. nov., *Colletotrichum aenigma*, *Colletotrichum pseudotheobromicola* sp. nov., *Epicoccum pseudokeratinophilum* sp. nov., *Nothophoma pruni* sp. nov., *Nothophoma quercina* and *Stagonosporopsis citrulli* were also isolated from Cherry leaf spots. Significant variations in the virulence were observed among fungal species on different cherry cultivars.

Key words – *Alternaria* – *Colletotrichum* – disease management – *Epicoccum* – *Nothophoma* – pathogenicity – *Prunus* – *Stagonosporopsis* – 5 new species

Introduction

Cherry (*Prunus avium* L., Rosaceae) is an important stone fruit of temperate regions with many beneficial properties. Different cultivars of cherries including sweet cherry, sour cherry, flowering ornamental cherries and wild cherries are cultivated worldwide for their economic value (Holb 2009, Faust & Surányi 2011, Joshua 2012, USDA 2017). According to the statistical data from FAO (2018), world cherry cultivation and production have reached 0.44 million hectares (Mha) and 2.3 million tonnes in 2016. Due to extreme weather conditions, world cherry production forecasted to drop by 3% by 2018 (USDA 2017). Driven by ongoing consumer demand and high market value, cherry cultivation has expanded in China from top cherry producing provinces such as Shandong and Liaoning to inland provinces including Gansu, Henan, Shaanxi, Shanxi and

Submitted 14 December 2018, Accepted 27 April 2019, Published 8 May 2019
Corresponding Author: Jiye Yan – e-mail – jiyeyan@vip.163.com

Yunnan (Scott et al. 2014). In the year 2016, cherry cultivation in China has increased to 9.034 kha resulting an increased production of 0.037 Million tonnes (FAO 2018). Currently, many cherry cultivars are grown in China, most of which are introduced from other countries. Among them 'Brooks' or 'Red Lantern' is known as the most dominant cultivar (Scott et al. 2014). Even with ideal conditions, cherries are susceptible to many diseases including bacterial, fungal, parasitic and viral diseases (Uyemoto et al. 2018). Among these, Cherry leaf spot disease is one of the most common and widely spread diseases in many cherry growing regions in the world with humid conditions (Garcia & Jones 1993, Ellis 2008, Farr & Rossman 2011, Choi et al. 2014) and affect leaves, leaf stems, fruits, and fruit stem causing defoliation and eventual death of the tree (Jones & Sutton 1996, Díaz et al. 2007, Farr & Rossman 2011).

Cherry leaf spot disease was first observed in the USA and then reported from other countries, mainly in mainland Europe, Turkey, China, Chile, Japan and Russia (Ogawa & English 1991, Holb 2009). Several names including cherry leaf blight, cherry anthracnose (Holb 2009) and yellow leaf and shot hole disease have been used in literature to address the same disease (Ogawa & English 1991). *Blumeriella jaapii* (Rehm) Arx. (asexual morph: *Phloeosporella padi* (Lib.) Arx.; *Cylindrosporium padi* P. Karst.) was identified as the main pathogen of Cherry leaf spot disease in many countries (Sjulin et al. 1989, Heald 1993, Joshua & Mmbaga 2013). Over the years, this pathogen has been synonymized under different names (Index Fungorum 2018). In addition to *Blumeriella jaapii*, several other pathogens have been also identified as causative agents of Cherry leaf spot disease. *Pseudocercospora pruni-persicicola* (J.M. Yen) J.M. Yen has been isolated from cherry leaf spots in Taiwan and Korea (Farr & Rossman 2013, Choi et al. 2014). In addition, *Alternaria cerasi* Potebnia. and *Alternaria alternata* (Fr.) Keissl. have been reported from cherry leaf spots in China and in Greece respectively (Zhu & Chang 2004, Thomidis & Tsipouridis 2006). Most of the studies conducted on the disease have focused on *B. jaapii* infection (Schuster & Tobutt 2004, Díaz et al. 2007, Joshua & Mmbaga 2013). Previous studies have characterized the cherry leaf spot symptoms common to all the pathogens, which initiate by forming pinpoint lesions on the leaf surface. These rapidly enlarging lesions change their colour to brown and coalesced together to form large patches of necrotic tissues (Ellis 2008, Holb 2009, Khan et al. 2014). According to Holb (2009) and Higgins (1914), an absiss layer forms around the leaf spot causing rapid cell enlargement which results in the formation of a shot hole. Shot holes with round and evenly curved smooth margins are characteristic for cherry leaf spot disease (Higgins 1914). Heavily infected leaves fall to the ground leading to premature defoliation, a major symptom of cherry leaf spot, and facilitate the fungus to overwinter among the leaf debris by switching its lifestyle (Ellis 2008, Holb 2009). In response to warm and humid weather conditions, conidia discharge and establish new infections (Eisensmith & Jones 1981, Holb 2009). Therefore, timely execution of control strategies can be effective against these pathogens.

There are few reports on Cherry leaf spot disease in China from provinces including Henan and Shandong (Zhu & Chang 2004, Shu-gui 2007, Li et al. 2011, Zhao & Liu 2012). *Alternaria alternata* (Zhao & Liu 2012) and *Alternaria cerasi* Potebnia. (Zhu & Chang 2004) have been reported as the causative agents of cherry leaf spots in China. However, detailed morphological studies combined with phylogenetic analyses based on DNA sequence data and pathogenicity assays have not been conducted. We have observed severe leaf spot conditions in cherry orchards in Beijing during 2016 and 2018. Surprisingly, no studies have been conducted in Beijing yet to identify the causal agent of the disease. Therefore, in the current study, we studied phytopathogenic fungal species associated with cherry leaf spot symptoms in Beijing, China; identified them based on morphology and DNA sequence data, investigate their phylogeny and assess their pathogenicity. Our main objective is to generate information needed to guide species-specific disease management of cherry leaf spot disease and to evaluate the source of the primary inoculum.

Materials & Methods

Sample collection, fungal isolation, and morphological characterization

Cherry leaves with leaf spot symptoms were collected from orchards at Beijing Academy of Forestry and Pomology Sciences, Beijing, China during the springs of 2016 and 2018. Symptomatic leaves were brought to the laboratory in separate plastic bags. Samples were surface sterilized by washing with 1% sodium hypochlorite for 1 min, 70% ethanol for 1 min, rinsed three times in sterilized water, blotted dry and placed on Potato Dextrose Agar (PDA) and on Potato Carrot Agar (PCA). Cultures were maintained at 25 °C for 2–5 days. Fungal structures were observed and photographed using an Axio Imager Z2 photographic microscope (Carl Zeiss Microscopy, Oberkochen, Germany) and measurements were taken using ZEN PRO 2012 software (Carl Zeiss Microscopy, Germany). Fourty conidial measurements were made per isolate. Growth rates and cultural characteristics were recorded after 5 days. The growth rate was calculated as the mean of two perpendicular measurements. Herbarium specimens were deposited in the Mae Fah Luang University (MFLU) herbarium, Thailand, and the Herbarium of Cryptograms (HKAS), Kunming Institute of Botany, China. Cultures were deposited in the culture collections at Mae Fah Luang University, Thailand (MFLUCC), the culture collection at Kunming Institute of Botany (KUMCC), China, and the Beijing Academy of Agricultural and Forestry Sciences (JZB) culture collection, China. Facesoffungi and MycoBank numbers were acquired as in Jayasiri et al. (2015) and (Myco Bank 2018).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from 67 representative isolates using the modified protocol described in Chethana et al. (2017). The following loci were amplified using the primers given in Table 1. For *Alternaria* species: ITS, $GAPDH_{ALT}$, RPB2, TEF 1-α and Alt-a 1; For *Colletotrichum* species: ITS, $GAPDH_{C}$, CHS-1, ACT and TUB2; For Didymellaceae species: LSU, ITS, RPB2 and TUB2. Polymerase chain reactions (PCR) were conducted in an Applied Biosystems Vetri Thermal Cycler with the following PCR conditions for ITS, RPB2, and TEF 1-α regions (White et al. 1990): initial denaturation for 3 min at 95 °C, followed by 34 cycles of denaturation for 30 s at 95 °C and 30 s of annealing and 1 min elongation at 72 °C, and a final extension for 10 min at 72 °C. The annealing temperatures were as follows: 52 °C for LSU, 54 °C for $GADPH_{C}$ and TEF 1-α, 56 °C for ACT, RPB2 and TUB2, and 59 °C for CHS-1 and ITS gene regions. Slightly deviated PCR conditions were provided for the other two genes: initial denaturation for 2 min at 94 °C, followed by 35 cycles of denaturation for 1 min at 96 °C, 1 min of annealing at 58 °C and 1 min elongation at 72 °C and a final extension for 10 min at 72 °C for the $GAPDH_{ALT}$; initial denaturation for 2 min at 94 °C, followed by 35 cycles of denaturation for 1 min at 94 °C, 30 s of annealing at 57 °C and 45 s elongation at 72 °C, and a final extension for 10 min at 72 °C for Alt-a 1. The PCR solution mixture was composed of 0.3 ml of TaKaRa Ex-Taq DNA polymerase (TaKaRa, China), 2.5 ml of 10x Ex-Taq buffer (TaKaRa, China), 3.0 ml of dNTPs (TaKaRa, China), 1 ml of genomic DNA, 1 ml of each primer, and 16.2 ml of ddH2O. The PCR products were visualized on 1% agarose gel under UV light using a GelDoc XR+Molecular Imager (BIO-RAD, USA) after ethidium bromide staining. Sequencing of PCR products was carried at the Sunbiotech Company, Beijing, China. The sequences from forward and reverse primers were used to obtain consensus sequences with DNAMAN 6.0 (Lynnon Biosoft, USA). Those sequences were deposited in the GenBank and their accession numbers are given in Supplementary Tables 2–4.

Phylogenetic analysis

Based on the analyses conducted using the National Center for Biotechnology Information (NCBI) search engine GenBank BLASTn, fungal members similar to the new taxa were included in the analyses. Reference sequences were obtained from GenBank (Weir et al. 2012, Woudenberg et al. 2013, 2015, Chen et al. 2015, 2017, Abdel-Wahab et al. 2017, Crous et al. 2017, Jayasiri et al. 2017, Thambugala et al. 2017, Hyde et al. 2017, 2018, Tibpromma et al. 2018, Valenzuela-Lopez et al. 2018, Wanasinghe et al. 2018) are listed in Tables 2–4. Individual datasets for the genes were aligned using the default settings of MAFFT 7 (Katoh & Toh 2008, http://mafft.cbrc.jp/alignment/server) and improved manually where necessary using BIOEDIT

(Hall 1999). Aligned gene regions were concatenated for the analyses in the following order: ITS, $GAPDH_{ALT}$, RPB2, TEF 1-α and Alt-a 1 for *Alternaria* species; ITS, $GAPDH_C$, CHS-1, ACT and TUB2 for *Colletotrichum* species; LSU, ITS, RPB2, and TUB2 for Didymellaceae species.

Maximum parsimony (MP) analysis based on the combined dataset was conducted in PAUP (phylogenetic analysis using parsimony) 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. Ambiguous regions in the alignment were excluded, and gaps were treated as missing data. Clade stability was assessed using a bootstrap analysis with 1000 replications (Hillis & Bull 1993). Maxtrees were set up to 1000, branches of zero length were collapsed, and all multiple parsimonious trees were saved. Descriptive statistics such as tree length (TL), consistency index (CI), retention index (RI), relative consistency index (RC), and homoplasy index (HI) were calculated for trees inferred under different optimality criteria. Their significance was evaluated using Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989).

Maximum likelihood (ML) analysis was conducted in RAXMLGUI 0.9b2 (Silvestro & Michalak 2010) for 1000 nonparametric bootstrapping iterations, using the general time reversible model (GTR) with a discrete gamma distribution. Trees with the final likelihood values of -6586.660252, -8409.454513, -16 769.656375 were selected as the best-scoring trees for *Alternaria* species, *Colletotrichum* species and Didymellaceae species respectively and the replicates were plotted relative to it.

Furthermore, Bayesian reconstructions were performed using MRBAYES 3.0b4 (Ronquist & Huelsenbeck 2003). The evolutionary models for phylogenetic analyses were determined independently for each locus by MRMODELTEST 2.3 (Nylander 2004). The best model selected for each locus was given in Table 1. Four simultaneous Markov chains were run for 1,000,000 generations with increments of additional generations when needed until the standard deviation of split frequencies reach 0.01. From the 10 000 trees obtained, first 2000 representing the burn-in phase of the analyses were discarded while the remaining 8,000 trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic set to 0.01) (Crous et al. 2006). All the phylogenetic trees were drawn using TREEVIEW 1.6.6 (Page 1996). Sequences derived in this study were deposited in GenBank; alignments and trees were deposited in TreeBase for *Alternaria* species, *Colletotrichum* species and Didymellaceae species (S23535).

Pathogenicity assay

Pathogenicity tests were conducted on young, healthy detached leaves of three *Prunus avium* cultivars namely, 'Tieton', 'Summit' and 'Sunburst' from Tongzhou Experimental Station for Cherries, Beijing Academy of Forestry and Pomology Sciences, Beijing, China. Pathogenicity of three isolates of *Alternaria* species (MFLUCC 18–1597, MFLUCC 18–1599, and MFLUCC 18–1587), three Didymellaceae species (MFLUCC 18–1593, MFLUCC 18–1595 and MFLUCC 18–1600) and three isolates *Colletotrichum* species (MFLUCC 18–1604, MFLUCC 18–1603 and MFLUCC 18–1602) were randomly tested. Leaf surfaces were sterilized by washing in 75% ethanol for 1 min, then in 10% sodium hypochlorite for 1 min, followed by washing with distilled water three times. The experiment was conducted using six leaves per isolate (including one control), inoculated by non-wound and wound inoculation approaches and repeated three times to get stable results. These wound and non-wound inoculations were performed on symmetrical halves of each leaf. For the wound inoculation, the upper epidermal layer of the leaf was injured with a sterile needle. One hundred µl of the inoculum consisting of 10^6 spores/ml conidial suspension were inoculated on both the wound and non-wound sites of each of the leaves. Sterile water was used as the control. Each inoculated leaf was placed in a 12 cm diameter petri dish and incubated in a moist chamber at 25 °C with an 80% relative humidity until symptoms appeared. Lesion lengths were recorded three days after the inoculation. Koch's postulates were confirmed by

re-isolating the inoculated fungus. The re-isolated fungus was identified based on cultural and morphological characters.

Table 1 Primers used in the study, with sequences, their sources, sizes and evolutionary models used in the study

Gene	Product name	Primer	Sequence (5'–3')	Product size	Nucleotide substitution model	Reference
ACT	Actin	ACT-512F ACT-783R	ATG TGC AAG GCC GGT TTC GC TAC GAG TCC TTC TGG CCC AT	256 bp	HKY + G	Carbone & Kohn (1999)
Alt-a 1	*Alternaria* major allergen 1	Alt-F Alt-R	ATG CAG TTC ACC ACC ATC GC ACG AGG GTG AYG TAG GCG TC	457 bp	TrNef+G	Hong et al. (2005)
CHS-1	Chitin synthase	CHS-79F CHS-345R	TGG GGC AAG GAT GCT TGG AAG AAG TGG AAG AAC CAT CTG TGA GAG TTG	282 bp	TrNef+G	Carbone & Kohn (1999)
GAPDH$_{ALT}$	glyceraldehyde-3-phosphate dehydrogenase	gpd1 gpd2	CAA CGG CTT CGG TCG CAT TG GCC AAG CAG TTG GTT GTG C	535 bp	TrN+G	Berbee et al. (1999)
GAPDH$_{C}$	glyceraldehyde-3-phosphate dehydrogenase	GDF GDR	GCC GTC AAC GAC CCC TTC ATT GA GGG TGG AGT CGT ACT TGA GCA TGT	250 bp	HKY+G	Templeton et al. (1992)
ITS	Internal transcribed spacer	ITS 1 ITS 4	TCC GTA GGT GAA CCT GCG G TCC TCC GCT TAT TGA TAT GC	*Alternaria*: 539 bp *Colletotrichum*: 533 bp Didymellaceae: 486 bp	*Alternaria*: SYM+I+G *Colletotrichum*: TrNef+G Didymellaceae: TrNef+I+G	White et al. (1990)
LSU	28S large subunit of nuclear ribosomal RNA	LR5 LROR	TCC TGA GGG AAA CTT CG ACC CGC TGA ACT TAA GC	877 bp	TrNef+I	Vilgalys & Hester (1990) Rehner & Samuels (1994)
RPB$_2$	RNA polymerase II second largest subunit	RPB2–5F RPB2–7cR	GGG GWG AYC AGA AGA AGG C CCC ATR GCT TGY TTR CCC AT	*Alternaria*: 881 bp Didymellaceae: 905 bp	*Alternaria*: TrNef+G Didymellaceae: TIM2ef+I+G	Liu et al. (1999) Sung et al. (2007)

Table 1 Continued

Gene	Product name	Primer	Sequence (5'–3')	*Product size*	Nucleotide substitution model	Reference
TEF 1-α	Partial translation elongation factor 1-α	TEF1-728F TEF1-986R	CAT CGA GAA GTT CGA GAA GG TAC TTG AAG GAA CCC TTA CC	*Alternaria*: 223 bp	TIM1ef+G	Carbone & Kohn (1999)
TUB2	β-Tubulin	BT-2F BT-4R	AAC ATG CGT GAG ATT GTA AGT TAG TGA CCC TTG GCC CAG TTG	*Colletotrichum*: 479 bp Didymellaceae: 338 bp	*Colletotrichum*: TrNef+G Didymellaceae: TPM2uf+I+G	O'Donnell & Cigelnik (1997)

Table 2 GenBank accession numbers of the nucleotide sequences of *Alternaria* species used in this study

Species	Culture Collection number[1]	GenBank Accession Numbers[3]				
		ITS	GAPDH	RPB2	TEF1-α	Alt-a 1
Alternaria alstroemeriae	CBS 118808	KP124296	KP124153	KP124764	KP125071	KP123845
Alternaria alstroemeriae	CBS 118809	KP124297	KP124154	KP124765	KP125072	-
Alternaria alternantherae	CBS 124392	KC584179	KC584096	KC584374	KC584633	KP123846
Alternaria alternata	CBS 174.52	KC584228	KC584152	DQ677964	KC584704	KP123856
Alternaria alternata	CBS 175.52	KC584227	KC584151	KC584445	KC584703	KP123857
Alternaria alternata	CBS 916.96 [T]	AF347031	AY278808	KC584375	KC584634	AY563301
Alternaria alternata	CBS 102595	FJ266476	AY562411	KC584408	KC584666	AY563306
Alternaria alternata	CBS 118812	KC584193	KC584112	KC584393	KC584652	KP123905
Alternaria alternata	**JZB3180002**	**MH827031**	**MH853645**	**MH853718**	**MH853703**	**MH853692**
Alternaria alternata	**MFLUCC 18–1587**	**MH827038**	**MH853652**	**MH853725**	**MH853710**	**MH853699**
Alternaria alternata	**JZB3180011**	**MH827040**	**MH853654**	**MH853727**	**MH853712**	**MH853701**
Alternaria alternata	**JZB3180012**	**MH827041**	**MH853655**	**MH853728**	**MH853713**	**-**
Alternaria alternata	**JZB3180014**	**MH827043**	**MH853657**	**MH853730**	**MH853715**	**-**
Alternaria alternata	**MFLUCC 18–1586**	**MH827044**	**MH853658**	**MH853731**	**MH853716**	**-**
Alternaria anigozanthi	CBS 121920 [T]	KC584180	KC584097	KC584376	KC584635	-
Alternaria arborescens	CBS 102605 [T]	AF347033	AY278810	KC584377	KC584636	AY563303
Alternaria arborescens	CBS 101.13	KP124392	KP124244	KP124862	KP125170	KP123940

Table 2 Continued.

Species	Culture Collection number[1]	GenBank Accession Numbers[3]				
		ITS	GAPDH	RPB2	TEF1-α	Alt-a 1
Alternaria arborescens	CBS 112633	KP124400	KP124252	KP124870	KP125178	KP123947
Alternaria aspera	CBS 115269 T	KC584242	KC584166	KC584474	KC584734	KF533899
Alternaria betae-kenyensis	CBS 118810 T	KP124419	KP124270	KP124888	KP125197	KP123966
Alternaria brassicicola	CBS 118699	JX499031	KC584103	KC584383	KC584642	-
Alternaria burnsii	CBS 108.27	KC584236	KC584162	KC584468	KC584727	KP123850
Alternaria burnsii	CBS 107.38 T	KP124420	JC646305	KP124889	KP125198	KP123967
Alternaria carotiincultae	CBS 109381 T	KC584188	KC584106	KC584386	KC584645	-
Alternaria cheiranthi	CBS 109384	AF229457	KC584107	KC584387	KC584646	JQ905106
Alternaria cinerariae	CBS 116495	KC584190	KC584109	KC584389	KC584648	-
Alternaria cucurbitae	CBS 483.81	FJ266483	AY562418	KC584483	KC584743	-
Alternaria dauci	CBS 117097	KC584192	KC584111	KC584392	KC584651	KJ718678
Alternaria dianthicola	CBS 116491	KC584194	KC584113	KC584394	KC584653	-
Alternaria eichhorniae	CBS 489.92 T	KC146356	KP124276	KP124895	KP125204	KP123973
Alternaria eichhorniae	CBS 119778	KP124426	KP124277	KP124896	KP125205	-
Alternaria gaisen	CBS 632.93	KC584197	KC584116	KC584399	KC584658	KP123974
Alternaria gaisen	CBS 118488	KP124427	KP124278	KP125206	KP124897	KP123975
Alternaria gossypina	CBS 100.23	KP124429	KP124280	KP124899	KP125208	KP123977
Alternaria gossypina	CBS 104.32 T	KP124430	JQ646312	KP124900	KP125209	JQ646395
Alternaria gypsophilae	CBS 107.41 T	KC584199	KC584118	KC584401	KC584660	JQ646304
Alternaria iridiaustralis	CBS 118486 T	KP124435	KP124284	KP124905	KP125214	KP123981
Alternaria iridiaustralis	CBS 118404	KP124434	KP124283	KP124904	KP125213	KP123980
Alternaria jacinthicola	CBS 133751 T	KP124438	KP124287	KP124908	KP125217	KP123984
Alternaria jacinthicola	CPC 25267	KP124439	KP124288	KP124909	KP125218	KP123985
Alternaria japonica	CBS 118390	KC584201	KC584121	KC584405	KC584663	-
Alternaria juxtiseptata	CBS 119673 T	KC584202	KC584122	KC584406	KC584664	-
Alternaria leucanthemi	CBS 421.65 T	KC584240	KC584164	KC584472	KC584732	-
Alternaria longipes	CBS 540.94	AY278835	AY278811	KC584409	KC584667	AY563304
Alternaria longipes	CBS 917.96	KP124442	KP124291	KP124912	KP125226	KP123988
Alternaria longipes	CBS 121333	KP124444	KP124293	KP124914	KP125223	KP123990
Alternaria macrospora	CBS 117228 T	KC584204	KC584124	KC584410	KC584668	KJ718702

Table 2 Continued.

Species	Culture Collection number[1]	GenBank Accession Numbers[3]				
		ITS	GAPDH	RPB2	TEF1-α	Alt-a 1
Alternaria nepalensis	CBS 118700 T	KC584207	KC584126	KC584414	KC584672	-
Alternaria nobilis	CBS 116490	KC584208	KC584127	KC584415	KC584673	JQ646385
Alternaria obovoidea	CBS 101229	FJ266487	FJ266498	KC584485	KC584745	FJ266513
Alternaria panax	CBS 482.81	KC584209	KC584128	KC584417	KC584675	-
Alternaria perpunctulata	CBS 115267 T	KC584210	KC584129	KC584418	KC584676	JQ905111
Alternaria photistica	CBS 212.86 T	KC584212	KC584131	KC584420	KC584678	-
Alternaria porri	CBS 116698	DQ323700	KC584132	KC584421	KC584679	KJ718726
Alternaria prunicola	**MFLUCC 18–1598**	**MH827032**	**MH853646**	**MH853719**	**MH853704**	**MH853693**
Alternaria prunicola	**MFLUCC 18–1596**	**MH827033**	**MH853647**	**MH853720**	**MH853705**	**MH853694**
Alternaria prunicola	**JZB3180005**	**MH827034**	**MH853648**	**MH853721**	**MH853706**	**MH853695**
Alternaria prunicola	**JZB3180006**	**MH827035**	**MH853649**	**MH853722**	**MH853707**	**MH853696**
Alternaria prunicola	**MFLUCC 18–1597 T**	**MH827036**	**MH853650**	**MH853723**	**MH853708**	**MH853697**
Alternaria prunicola	**MFLUCC 18–1599**	**MH827037**	**MH853651**	**MH853724**	**MH853709**	**MH853698**
Alternaria prunicola	**JZB3180013**	**MH827042**	**MH853656**	**MH853729**	**MH853714**	**-**
Alternaria pseudoeichhorniae	**MFLUCC 18–1589 T**	**MH827030**	**MH853644**	**MH853717**	**MH853702**	**-**
Alternaria pseudorostrata	CBS 119411 T	JN383483	AY562406	KC584422	KC584680	-
Alternaria radicina	CBS 245.67 T	KC584213	KC584133	KC584423	KC584681	FN689405
Alternaria saponariae	CBS 116492	KC584215	KC584135	KC584425	KC584683	-
Alternaria septospora	CBS 109.38	FJ266489	FJ266500	KC584487	KC584747	FJ266515
Alternaria simsimi	CBS 115265 T	JF780937	KC584137	KC584428	KC584686	JQ905110
Alternaria solani	CBS 116651	KC584217	KC584139	KC584430	KC584688	-
Alternaria sonchi	CBS 119675	KC584220	KC584142	KC584433	KC584691	-
Alternaria sp.	CBS 115.44	KC584214	KC584134	KC584424	KC584682	-
Alternaria tagetica	CBS 479.81	KC584221	KC584143	KC584434	KC584692	KJ718761
Alternaria tenuissima	CBS 918.96	AF347032	AY278809	KC584435	KC584693	AY563302
Alternaria terricola	CBS 202.67 T	FJ266490	KC584177	KC584490	KC584750	FJ266516
Alternaria tomato	CBS 103.30	KP124445	KP124294	KP124915	KP125224	KP123991
Alternaria tomato	CBS 114.35	KP124446	KP124295	KP124916	KP125225	KP123992
Alternaria vaccariicola	CBS 118714 T	KC584224	KC584147	KC584439	KC584697	-

[1]CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Personal collection of P.W. Crous, Utrecht, The Netherlands; JZB: Beijing Academy of Agriculture and Forestry Sciences culture collection, Beijing, China; MFLUCC: Mae Fah Luang University Culture Collection, Thailand;
Ex-type, neo-type and epi-type cultures are marked with superscript [T] and newly generated sequences are shown in bold face.
ITS: internal transcribed spacer regions 1 & 2 including 5.8S nrDNA gene; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; RPB2: RNA polymerase II second subunit; TEF 1-α: Partial translation elongation factor 1-α; Alt-a 1: *Alternaria* major allergen 1.

Table 3 GenBank accession numbers of the nucleotide sequences of *Colletotrichum* species used in this study.

Species	**Culture Collection number[1]**	**GenBank Accession Numbers[3]**				
		ITS	**GAPDH**	**CHS-1**	**ACT**	**TUB**
Colletotrichum aenigma	ICMP 18608 [T]	JX010244	JX010044	JX009774	JX009443	JX010389
Colletotrichum aenigma	ICMP 18686	JX010243	JX009913	JX009789	JX009519	JX010390
Colletotrichum aenigma	**MFLUCC 18–1604**	**MH817393**	**MH853673**	**MH853676**	**MH853679**	**MH853682**
Colletotrichum aenigma	**MFLUCC 18–1603**	**MH817394**	**MH853674**	**MH853677**	**MH853680**	**MH853683**
Colletotrichum aeschynomenes	ICMP 17673 [T]	JX010176	JX009930	JX009799	JX009483	JX010392
Colletotrichum alatae	ICMP 17919 [T]	JX010190	JX009990	JX009837	JX009471	JX010383
Colletotrichum alatae	ICMP 18122	JX010191	JX010011	JX009846	JX009470	JX010449
Colletotrichum alienum	ICMP 12071 [T]	JX010251	JX010028	JX009882	JX009572	JX010411
Colletotrichum alienum	ICMP 18691	JX010217	JX010018	JX009754	JX009580	JX010385
Colletotrichum aotearoa	ICMP 18537 [T]	JX010205	JX010005	JX009853	JX009564	JX010420
Colletotrichum aotearoa	ICMP 18577	JX010203	JX009978	JX009851	JX009567	JX010417
Colletotrichum asianum	ICMP 18580 [T]	FJ972612	JX010053	JX009867	JX009584	JX010406
Colletotrichum asianum	ICMP 18696	JX010195	JX019915	JX009753	JX009576	JX010384
Colletotrichum boninense	CBS 123755 [T]	JQ005153	JQ005240	JQ005327	JQ005501	JQ005588
Colletotrichum camelliae	CGMCC 3.14925[T]	KJ955081	KJ954782	-	KJ954363	KJ955230
Colletotrichum chengpingense	MFLUCC 15–0022[T]	KP683152	KP852469	KP852449	KP683093	KP852490
Colletotrichum clidemiae	ICMP 18658 [T]	JX010265	JX009989	JX009877	JX009537	JX010438
Colletotrichum conoides	CAUG17 [T]	KP890168	KP890162	KP890156	KP890144	KP890174
Colletotrichum cordylinicola	ICMP 18579 [T]	JX010226	JX009975	JX009864	HM470235	JX010440
Colletotrichum endophytica	LC0324	KC633854	KC832854	-	KF306258	-
Colletotrichum fructicola	ICMP 18581 [T]	JX010165	JX010033	JX009873	FJ907426	JX010405
Colletotrichum fructicola	ICMP 18645	JX010172	JX009992	JX009866	JX009543	JX010408
Colletotrichum fructivorum	Coll1414 [T]	JX145145	-	-	-	JX145196

Table 3 Continued.

Species	Culture Collection number[1]	GenBank Accession Numbers[3]				
		ITS	GAPDH	CHS-1	ACT	TUB
Colletotrichum gloeosporioides	CBS 112999 [T]	JQ005152	JQ005239	JQ005326	JQ005500	JQ005587
Colletotrichum gloeosporioides	ICMP 18697	JX010154	JX009987	JX009780	JX009557	-
Colletotrichum gloeosporioides	ICMP 19121	JX010148	JX010054	JX009903	JX009558	-
Colletotrichum grevilleae	CBS 132879 [T]	KC297078	KC297010	KC296987	KC296941	KC297102
Colletotrichum grossum	CAUG7 [T]	KP890165	KP890159	KP890153	KP890153	KP890171
Colletotrichum hebeiense	MFLUCC13–0726 [T]	KF156863	KF377495	KF289008	KF377532	KF288975
Colletotrichum hebeiense	MFLUCC14–1213 [T]	KF156873	KF377505	-	KF377542	-
Colletotrichum henanense	CGMCC 3.17354[T]	KJ955109	KJ954810	-	KM023257	KJ955257
Colletotrichum horii	ICMP 10492 [T]	GQ329690	GQ329681	JX009752	JX009438	JX010450
Colletotrichum horii	ICMP 17968	JX010212	GQ329682	JX009811	JX009547	JX010378
Colletotrichum jiangxiense	CGMCC 3.17363[T]	KJ955201	KJ954902	-	KJ954471	KJ955348
Colletotrichum kahawae	ICMP 17816 [T]	JX010231	JX010046	JX009813	JX009452	JX010444
Colletotrichum kahawae	ICMP 17905	JX010232	JX010012	JX009816	JX009561	JX010431
Colletotrichum musae	ICMP 19119 [T]	JX010146	JX010050	JX009896	JX009433	HQ596280
Colletotrichum musae	ICMP 17817	JX010142	JX010015	JX009815	JX009432	JX010395
Colletotrichum nupharicola	ICMP 18187 [T]	JX010187	JX009972	JX009835	JX009437	JX010398
Colletotrichum nupharicola	ICMP 17938	JX010189	JX009936	JX009834	JX009486	JX010397
Colletotrichum pandanicola	MFLUCC 17–0571 [T]	MG646967	MG646934	MG646931	MG646938	MG646926
Colletotrichum proteae	CBS 132882 [T]	KC297079	KC297009	KC296986	KC296940	KC297101
Colletotrichum psidii	ICMP 19120 [T]	JX010219	JX009967	JX009901	JX009515	JX010443
Colletotrichum queenslandicum	ICMP 1778 [T]	JX010276	JX009934	JX009899	JX009447	JX010414
Colletotrichum rhexiae	Coll 1026	JX145128	-	-	-	JX145179
Colletotrichum salsolae	ICMP 19051	JX010242	JX009916	JX009863	JX009562	JX010403
Colletotrichum siamense	ICMP 18578	JX010171	JX009924	JX009865	FJ907423	JX010404
Colletotrichum siamense	ICMP 18574	JX010270	JX010002	JX009798	JX009535	JX010391
Colletotrichum syzygicola	MFLUCC 10– 0624 [T]	KF242094	KF242156	-	KF157801	KF254880
Colletotrichum temperatum	Coll883 T	JX145159	-	-	-	JX145211
Colletotrichum theobromicola	ICMP 18649 [T]	JX010294	JX010006	JX009869	JX009444	JX010447
Colletotrichum theobromicola	ICMP 17895	JX010284	JX010057	JX009828	JX009568	JX010382
Colletotrichum theobromicola	ICMP 17958	JX010291	JX009948	JX009822	JX009498	JX010381

Table 3 Continued.

Species	Culture Collection number[1]	GenBank Accession Numbers[3]				
		ITS	GAPDH	CHS-1	ACT	TUB
Colletotrichum theobromicola	ICMP 17927	JX010286	JX010024	JX009830	JX009516	JX010373
Colletotrichum theobromicola	ICMP 17957	JX010289	JX009962	JX009821	JX009575	JX010380
Colletotrichum pseudotheobromicola	**MFLUCC 18–1602 T**	**MH817395**	**MH853675**	**MH853678**	**MH853681**	**MH853684**
Colletotrichum ti	ICMP 4832 T	JX010269	JX009952	JX009898	JX009520	JX010442
Colletotrichum tropicale	CBS 124949 T	JX010264	JX010007	JX009870	JX009489	JX010407
Colletotrichum viniferum	GZAAS5.08601 T	JN412804	JN412798	-	JN412795	JN412813
Colletotrichum viniferum	GZAAS5.08608	JN412802	JN412800	-	JN412793	JN412811
Colletotrichum viniferum	GZAAS5.08614	JN412807	JN412799	-	JN412790	JN412809
Colletotrichum wuxiense	CGMCC 3.17894T	KU251591	KU252045	KU251939	KU251672	KU252200
Colletotrichum xanthorrhoeae	ICMP 17903 T	JX010261	JX009927	JX009823	-	JX010448

[1]CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, China; GZAAS: Guizhou Academy of Agricultural Sciences Herbarium, China; JZB: Beijing Academy of Agriculture and Forestry Sciences culture collection, Beijing, China; ICMP: International Collection of Microorganisms for Plants, Landcare Research, New Zealand; LC: Laboratory of Cryptogamy, National Museum of Natural History, Paris, France; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

[2]Ex-type, neo-type and epi-type cultures are marked with superscript T and newly generated sequences are shown in bold face.

[3] ITS: internal transcribed spacer regions 1 & 2 including 5.8S nrDNA gene; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; TUB: ß-tubulin; CHS-1: Chitin synthase; ACT: Actin.

Table 4 GenBank accession numbers of the nucleotide sequences of Didymellaceae species used in this study.

Species [1]	Culture Collection number[2]	GenBank Accession Numbers [3]			
		LSU	ITS	TUB	RPB2
Epicoccum brasiliense	CBS 120105 T	GU238049	GU237760	GU237588	KT389627
Epicoccum camelliae	CGMCC 3.18343 T	KY742245	KY742091	KY742333	KY742170
Epicoccum camelliae	UTHSC:DI16-201	LN907344	LT592902	LT592971	LT593040
Epicoccum camelliae	UTHSC:DI16-202	LN907345	LT592903	LT592972	LT593041
Epicoccum camelliae	UTHSC:DI16-206	LN907349	LT592906	LT592975	LT593044
Epicoccum camelliae	UTHSC:DI16-280	LN907423	LT592937	LT593006	LT593076

Table 4 Continued.

Species [1]	Culture Collection number[2]	GenBank Accession Numbers [3]			
		LSU	ITS	TUB	RPB2
Epicoccum camelliae	UTHSC:DI16-338	LN907481	LT592959	LT593028	LT593098
Epicoccum camelliae	UTHSC:DI16-345	LN907488	LT592961	LT593030	LT593100
Epicoccum camelliae	LC 4862	KY742246	KY742092	KY742334	KY742171
Epicoccum catenisporum	CBS 181.80 [T]	LT623213	FJ427069	FJ427175	LT623253
Epicoccum cedri	MFLUCC 17–1058 [T]	KY711172	KY711170	KY711168	-
Epicoccum dendrobii	CGMCC 3.18359 [T]	KY742247	KY742093	KY742335	-
Epicoccum dendrobii	LC 8146	KY742248	KY74209	KY742336	-
Epicoccum draconis	CBS 186.83	GU238070	GU237795	GU237607	KT389628
Epicoccum duchesneae	LC 8147	KY742250	KY742096	KY742338	-
Epicoccum duchesneae	CGMCC 3.18345 [T]	KY742249	KY742095	KY742337	-
Epicoccum henningsii	CBS 104.80	GU238081	GU237731	GU237612	KT389629
Epicoccum hordei	CGMCC 3.18360 [T]	KY742251	KY742097	KY742339	-
Epicoccum hordei	LC 8149	KY742252	KY742098	KY742340	-
Epicoccum huancayense	CBS 105.80 [T]	GU238084	GU237732	GU237615	KT389630
Epicoccum italicum	CGMCC 3.18361 [T]	KY742253	KY742099	KY742341	KY742172
Epicoccum italicum	LC 8151	KY74225	KY742100	KY742342	KY742173
Epicoccum keratinophilum	UTHSC:DI16-244	LN907387	LT592924	LT592993	LT593062
Epicoccum keratinophilum	UTHSC:DI16-258	LN907401	LT592928	LT592997	LT593066
Epicoccum keratinophilum	UTHSC:DI16-271 [T]	LN907414	LT592930	LT592999	LT593068
Epicoccum keratinophilum	UTHSC:DI16-272	LN907415	LT592931	LT593000	LT593069
Epicoccum keratinophilum	UTHSC:DI16-299	LN907442	LT592947	LT593016	LT593086
Epicoccum latusicollum	UTHSC:DI16-197	LT907340	LT592898	LT592967	LT593036
Epicoccum latusicollum	CGMCC 3.18346 [T]	KY742255	KY742101	KY742343	KY742174
Epicoccum latusicollum	LC 4859	KY742256	KY742102	KY742344	KY742175
Epicoccum layuense	CGMCC 3.18362 [T]	KY742261	KY742107	KY742349	-
Epicoccum layuense	LC 8156	KY742262	KY742108	KY742350	-
Epicoccum mackenziei	MFLUCC 16–0335 [T]	KX698028	KX698039	KX698032	KX698035
Epicoccum nigrum	CBS 125.82	GU237974	FJ426995	FJ427106	KT389631
Epicoccum nigrum	CBS 173.73 [T]	GU237975	FJ426996	FJ427107	KT389632
Epicoccum ovisporum	CBS 180.80 [T]	LT623212	FJ427068	FJ427174	LT623252

Table 4 Continued.

Species [1]	Culture Collection number[2]	GenBank Accession Numbers [3]			
		LSU	ITS	TUB	RPB2
Epicoccum pimprinum	PD 77/1028	GU237977	FJ427050	FJ427160	KT389633
Epicoccum plurivorum	CBS 558.81 [T]	GU238132	GU237888	GU237647	KT389634
Epicoccum pneumoniae	UTHSC:DI16-257 [T]	LN907400	LT592927	LT592996	LT593065
Epicoccum poaceicola	MFLUCC 15–0448 [T]	KX954396	KX965727	KY197980	-
Epicoccum poae	LC 8161	KY742268	KY742114	KY742356	KY742183
Epicoccum poae	CGMCC 3.18363 [T]	KY742267	KY742113	KY742355	KY742182
Epicoccum poae	LC 8162	KY742269	KY742115	KY742357	KY742184
Epicoccum proteae	CBS 114179 [T]	JQ044452	JQ044433	LT623230	LT623251
Epicoccum pruni	MFLUCC 17–1059	KY711171	KY711169	KY711167	KY711173
Epicoccum pseudokeratinophilum	**MFLUCC 18–1593 [T]**	**-**	**MH827002**	**MH853666**	**MH853659**
Epicoccum rosae	MFLUCC 15–3639	MG829009	MG828899	-	-
Epicoccum sorghinum	CBS 179.80	GU237978	FJ427067	FJ427173	KT389635
Epicoccum sorghinum	CBS 627.68	GU237979	FJ427072	FJ427178	KT389636
Epicoccum sorghinum	UTHSC:DI16-288	LN907431	LT592940	LT593009	LT593079
Epicoccum sorghinum	UTHSC:DI16-301	LN907444	LT592948	LT593017	LT593087
Epicoccum thailandicum	MFLUCC 16–0892 [T]	KY703620	KY703619	-	-
Epicoccum tritici	MFLUCC 16–0277	KX954391	KX926426	KY197979	-
Epicoccum viticis	BRIP 29294	KY742271	KY742117	KY742359	-
Epicoccum viticis	CGMCC 3.18344 [T]	KY742272	KY742118	KY742360	KY742186
Neocucurbitaria aquatica	CBS 297.74 [T]	EU754177	LT623221	LT623238	LT623278
Nothophoma anigozanthi	CBS 381.91 [T]	GU238039	GU237852	GU237580	KT389655
Nothophoma arachidis-hypogaeae	CBS 125.93	GU238043	GU237771	GU237583	KT389656
Nothophoma gossypiicola	CBS 377.67	GU238079	GU237845	GU237611	KT389658
Nothophoma gossypiicola	UTHSC:DI16-294	LN907437	LT592943	LT593012	LT593082
Nothophoma infossa	CBS 123395 [T]	GU238089	FJ427025	FJ427135	KT389659
Nothophoma macrospora	CBS 140674 [T]	LN880537	LN880536	LN880539	LT593073
Nothophoma multilocularis	AUMC-12003 [T]	KY996744	-	-	-
Nothophoma pruni	**JZB380015**	**MH827025**	**MH827004**	**MH853668**	**MH853661**
Nothophoma pruni	**MFLUCC 18–1601**	**MH827026**	**MH827005**	**MH853669**	**MH853662**
Nothophoma pruni	**JZB380017**	**MH827027**	**MH827006**	**MH853670**	**MH853663**

Table 4 Continued.

Species [1]	Culture Collection number[2]	GenBank Accession Numbers [3]			
		LSU	ITS	TUB	RPB2
Nothophoma pruni	**MFLUCC 18–1600 [T]**	**MH827028**	**MH827007**	**MH853671**	**MH853664**
Nothophoma quercina	CBS 633.92	EU754127	GU237900	GU237609	KT389657
Nothophoma quercina	UTHSC:DI16-270	LN907413	LT592929	LT592998	LT593067
Nothophoma quercina	**MFLUCC 18–1588**	**MH827029**	**MH827008**	**MH853672**	**MH853665**
Nothophoma raii	MCC 1082 [T]	-	MF664467	MF664468	-
Nothophoma variabilis	UTHSC:DI16-285 [T]	LN907428	LT592939	LT593008	LT593078
Stagonosporopsis actaeae	CBS 106.96 [T]	GU238166	GU237734	KT389672	GU237671
Stagonosporopsis actaeae	CBS 114303	KT389760	KT389544	-	KT389847
Stagonosporopsis ajacis	CBS 177.93 [T]	GU238168	GU237791	KT389673	GU237673
Stagonosporopsis alianthicola	MFLUCC16–1439 [T]	-	KY100872	KY100878	KY100876
Stagonosporopsis andigena	CBS 101.80	GU238169	GU237714	-	GU237674
Stagonosporopsis andigena	CBS 269.80	GU238170	GU237817	-	GU237675
Stagonosporopsis artemisiicola	CBS 102636	GU238171	GU237728	KT389674	GU237676
Stagonosporopsis astragali	CBS 178.25	GU238172	GU237792	-	GU237677
Stagonosporopsis bomiensis	LC 8167 [T]	KY742277	KY742123	KY742189	KY742365
Stagonosporopsis bomiensis	LC 8168	KY742278	KY742124	KY742190	KY742366
Stagonosporopsis caricae	CBS 248.90	GU238175	GU237807	-	GU237680
Stagonosporopsis caricae	CBS 282.76	GU238177	GU237821	-	GU237682
Stagonosporopsis centaureae	MFLUCC 16–0787	KX611238	KX611240	-	-
Stagonosporopsis citrulli	ATCC TSD-2 [T]	-	KJ855546	KJ855602	-
Stagonosporopsis citrulli	**MFLUCC 18–1595**	**MH827024**	**MH827003**	**MH853667**	**MH853660**
Stagonosporopsis crystalliniformis	CBS 713.85 [T]	GU238178	GU237903	KT389675	GU237683
Stagonosporopsis cucurbitacearum	CBS 133.96	GU238181	GU237780	KT389676	GU237686
Stagonosporopsis dennisii	CBS 631.68 [T]	GU238182	GU237899	KT389677	GU237687
Stagonosporopsis dorenboschii	CBS 426.90 [T]	GU238185	GU237862	KT389678	GU237690
Stagonosporopsis helianthi	CBS 200.87 [T]	KT389761	KT389545	KT389683	KT389848
Stagonosporopsis heliopsidis	CBS 109182	GU238186	GU237747	KT389679	GU237691
Stagonosporopsis hortensis	CBS 104.42	GU238198	GU237730	KT389680	GU237703
Stagonosporopsis hortensis	CBS 572.85	GU238199	GU237893	KT389681	GU237704
Stagonosporopsis ligulicola var. ligulicola	CBS 500.63	GU238190	GU237871	-	GU237695

Table 4 Continued.

Species [1]	Culture Collection number[2]	GenBank Accession Numbers [3]			
		LSU	ITS	TUB	RPB2
Stagonosporopsis ligulicola var. ligulicola	CBS 137.96	GU238191	GU237783	-	GU237696
Stagonosporopsis ligulicola var. inoxydabilis	CBS 425.90 [T]	GU238188	GU237861	KT389682	GU237693
Stagonosporopsis ligulicola var. inoxydabilis	PD 85/259	GU238189	GU237920	GU237694	-
Stagonosporopsis loticola	CBS 562.81 [T]	GU238192	GU237890	KT389684	GU237697
Stagonosporopsis lupini	CBS 101494 [T]	GU238194	GU237724	KT389685	GU237699
Stagonosporopsis oculo-hominis	CBS 634.92 [T]	GU238196	GU237901	KT389686	GU237701
Stagonosporopsis papillata	LC 8169 [T]	KY742279	KY742125	KY742191	KY742367
Stagonosporopsis papillata	LC 8170	KY742280	KY742126	KY742192	KY742368
Stagonosporopsis papillata	LC 8171	KY742281	KY742127	KY742193	KY742369
Stagonosporopsis rudbeckiae	CBS 109180	GU238197	GU237745	-	GU237702
Stagonosporopsis tanaceti	CBS 131484 [T]	JQ897461	NR_111724	-	JQ897496
Stagonosporopsis trachelii	CBS 379.91	GU238173	GU237850	KT389687	GU237678
Stagonosporopsis trachelii	CBS 384.68	GU238174	GU237856	-	GU237679
Stagonosporopsis valerianellae	CBS 273.92	GU238200	GU237819	-	GU237705
Stagonosporopsis valerianellae	CBS 329.67 [T]	GU238201	GU237832	-	GU237706

[1]ATCC: American Type Culture Collection, USA; BRIP: Plant Pathology Herbarium, Department of Employment, Economic, Development and Innovation, Queensland, Australia; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection, Beijing, China; JZB: Beijing Academy of Agriculture and Forestry Sciences culture collection, Beijing, China; LC: Lei Cai's personal collection deposited in laboratory, housed at CAS, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; PD: Plant Protection Service, Wageningen, the Netherlands; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, Texas, USA.

[2]Ex-type, neo-type and epi-type cultures are marked with superscript [T] and newly generated sequences are shown in bold face.

[3]ITS: internal transcribed spacer regions 1 & 2 including 5.8S nrDNA gene; LSU: 28S large subunit of the nrRNA gene; RPB2: RNA polymerase II second subunit; TUB: ß-tubulin.

Results

Disease symptoms identified in the field

During this study, symptomatic cherry leaves were observed in the fields at Academy of Forestry and Pomology Sciences, Beijing (Fig. 1). Initially, small, dark red or purple pinpoint lesions appear on the top leaf surface of infected leaves in early summer. With time these lesions enlarge to

form red-brown, 1–3 mm circular lesions with evenly curved and smooth margins. In some leaves, these enlarged pinpoint spots combined together to form larger dead patches. Following 7–10 days of initial symptoms, necrotic tissues of the leaf spots drop out causing shot holes. Heavily infected leaves turn light green and yellow areas resulted from leaf chlorosis form around the spots. One month after the initial infection, leaves die off and fall from the tree leading to premature tree defoliation. Disease severity of the plants in the field is very high. Almost all of the trees were infected and most of the trees show heavy infection.

Fungal isolation

A total of 67 isolates were obtained from leaf spot tissues of approximately 60 diseased leaves collected from *Prunus avium* at Beijing Academy of Forestry and Pomology Sciences, Beijing, China. Among these, the majority were *Alternaria* species (58 isolates) and the rest divided among *Colletotrichum* (3 isolates) and didymellaceous taxa (6 isolates).

Multi-locus phylogenetic analyses

Phylogenetic analyses were conducted separately for *Alternaria* species, *Colletotrichum* species and for the species in Didymellaceae. The first phylogenetic tree focusses on the *Alternaria* section *Alternaria*, the second one was for *Colletotrichum* species and the last one was produced to estimate the phylogenies of Didymellaceae species. As described in Jeewon & Hyde (2016), recommendations for base pair differences among the species were followed when introducing new species.

Figure 1 – Disease symptoms of Cherry leaf spot on *Prunus avium* in the field. a Development of numerous specks on leaves. b Small, dark red or purple pinpoint lesions on severely infected leaves. c Coalescing and formation of irregular necrotic patches. d Formation of chlorotic areas. e Formation of shot holes. f Curling of chlorotic and a-chlorotic leaves. g Premature defoliation. h infected leaves on the ground. i Heavily infected tree.

The phylogeny of genus *Alternaria* was defined by 76 strains of *Alternaria* species. In the phylogenetic analyses, 14 internal clades (herein called sections) occur consistently in the individual and combined phylogenies. These include sections *Alternaria*, *Alternantherae*, *Brassicicola*, *Cheiranthus*, *Dianthicola*, *Eureka*, *Gypsophilae*, *Japonicae*, *Panax*, *Pseudoulocladium*, *Porri*, *Radicina*, *Sonchi*, *Teretispora* and *Ulocladioides*. Several gene combinations were tested to obtain the best resolution for the identification of *Alternaria* pathogens. Among these combinations, ITS, $GAPDH_{ALT}$, RPB2, TEF 1-α and Alt-a 1 proved to be the best combination. All the isolates from the current study clustered in a subclade within section *Alternaria* (Supplementary Fig. 1). Therefore, separate phylogenetic analyses were conducted for *Alternaria* section *Alternaria*. *Alternaria* section *Alternaria* combined dataset consists of 42 sequences representing 15 taxa with *Alternaria alternantherae* (CBS 124392) of section *Alternantherae* as the outgroup. Multi-gene phylogenetic trees with similar topologies were generated from MP, ML and Bayesian analyses. The parsimony analysis comprised 2681 total characters including gaps. The concatenated alignment contained 179 parsimony informative characters, 184 variable and parsimony-uninformative characters, and 2318 constant characters. The first of 1000 equally parsimonious trees is shown in Fig. 2, which enabled the identification of the isolates to species level, with a better resolution than the single-gene analyses (TL=477, CI=0.830, RI=0.877, RC=0.728, HI=0.170). Maximum likelihood matrix had 309 distinct alignment patterns, with 10.48 % of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.239957, C = 0.281729, G = 0.243707, T = 0.234608; substitution rates AC = 1.097417, AG = 2.879682, AT = 0.925667, CG = 0.528440, CT = 5.893520, GT = 1.000000; gamma distribution shape parameter α = 0.107840. All our isolates divided among three species. Combined phylogenetic analyses provided good evidence that most of the isolates in the current study belong to a new species, which we introduce in this study as *Alternaria prunicola*, with high bootstrap values and high Bayesian posterior probabilities (MP: 81%, ML: 85%, BPP: 1.00). Many of the isolates of the current study were clustered together with *Alternaria prunicola*. Hence, it was considered as the main pathogen of Cherry leaf spot disease in Beijing, China. In addition, another new species *Alternaria pseudoeichhorniae* was identified with high bootstrap values and high Bayesian posterior probabilities (MP: 100%, ML: 95%, BPP: 1.00). Several *Alternaria* isolates were clustered with the ex-type of previously identified pathogen *Alternaria alternata* (CBS 916.96). In the phylogenetic analyses, *Alternaria alternata* isolates clustered into two sub clades. This is similar to the study conducted by Woudenberg et al. (2015) where they clustered into many sub clades. During this study, 35 morphospecies were synonymized under *A. alternata* due to their inability to be reliably distinguished in the multi-gene phylogeny. In the current study, we have selected several *A. alternata* isolates representing these clades. Similarly, we have also observed several sub clades in our analyses for the synonymized *A. alternata* isolates.

For the identification of *Colletotrichum* isolates, a phylogenetic tree was constructed using 63 representative *Colletotrichum* isolates including the isolates from the current study. Based on NCBI GenBank BLASTn search, all *Colletotrichum* isolates of the current study belong to *C. gloeosporioides* species complex. Concatenated analyses of ITS, GAPDH, CHS-1, ACT and TUB2 were performed for the *C. gloeosporioides* species complex. The dataset consists of 62 sequences representing 42 taxa with *Colletotrichum boninense* (MAFF 305972) representing the outgroup. The trees generated from the Bayesian and ML analyses share a similar topology from that of the MP analysis (Fig. 3). The parsimony analysis comprised 1941 total characters including gaps. The concatenated alignment consists of 361 parsimony informative characters, 250 variable and parsimony-uninformative characters and 1330 constant characters. The first of 1000 equally parsimonious trees is shown in Fig. 3, which enabled the identification of the isolates to the species level, with a better resolution than the single-gene analyses (TL=1077, CI=0.703, RI=0.852, RC=0.599, HI=0.297). Maximum Likelihood alignment matrix had 694 distinct alignment patterns, with 13.50 % of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.229422, C = 0.297559, G = 0.244522, T = 0.228496; substitution rates AC = 1.040111, AG = 2.595057, AT = 0.828774, CG = 0.691054, CT = 4.320904, GT = 1.000000; gamma distribution

shape parameter α = 0.388664. In Fig. 3, two of our *C. aenigma* isolates (MFLUCC 18–1603 and MFLUCC 18–1604) clade together strongly with the ex-type strain of *C. aenigma* (ICMP 18608) and another *C. pseudotheobromicola* isolate (MFLUCC 18–1602) clade together with the ex-type of *C. theobromicola* (ICMP 18649).

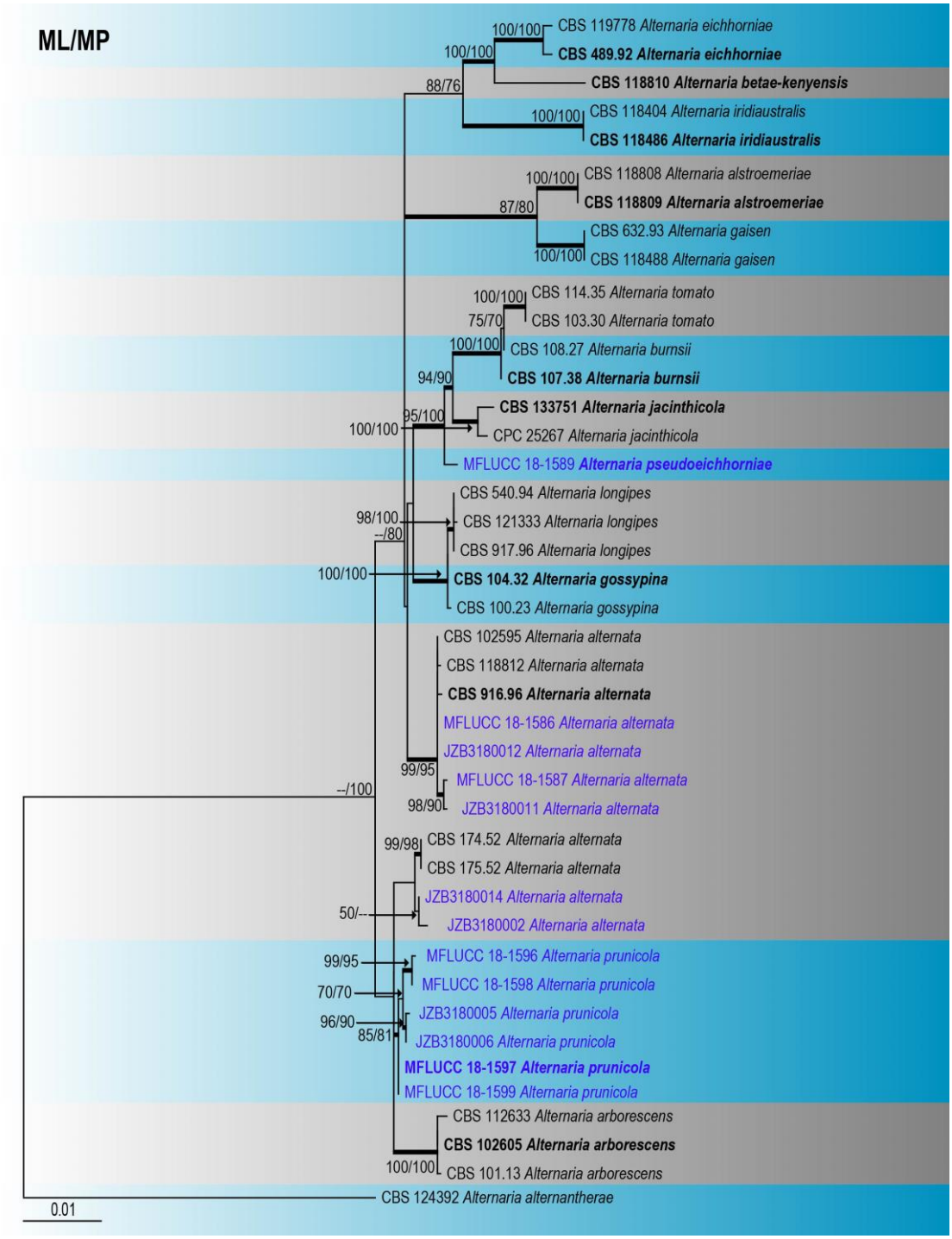


Figure 2 – Phylogenetic tree generated by maximum likelihood analysis of combined ITS, GADPH, RPB2, TEF 1-α and Alt-a 1 sequence data of species belonging to *Alternaria alternata* section *Alternaria*. The tree was rooted with *Alternaria alternantherae* (CBS 124392). Maximum parsimony and RAxML bootstrap support values ≥ 50% (BT) are shown respectively near the nodes. Bayesian posterior probabilities ≥ 0.95 (PP) indicated as thickened black branches. The scale bar indicates 0.01 changes. The ex-type strains are in bold and isolates from the current study are in blue.

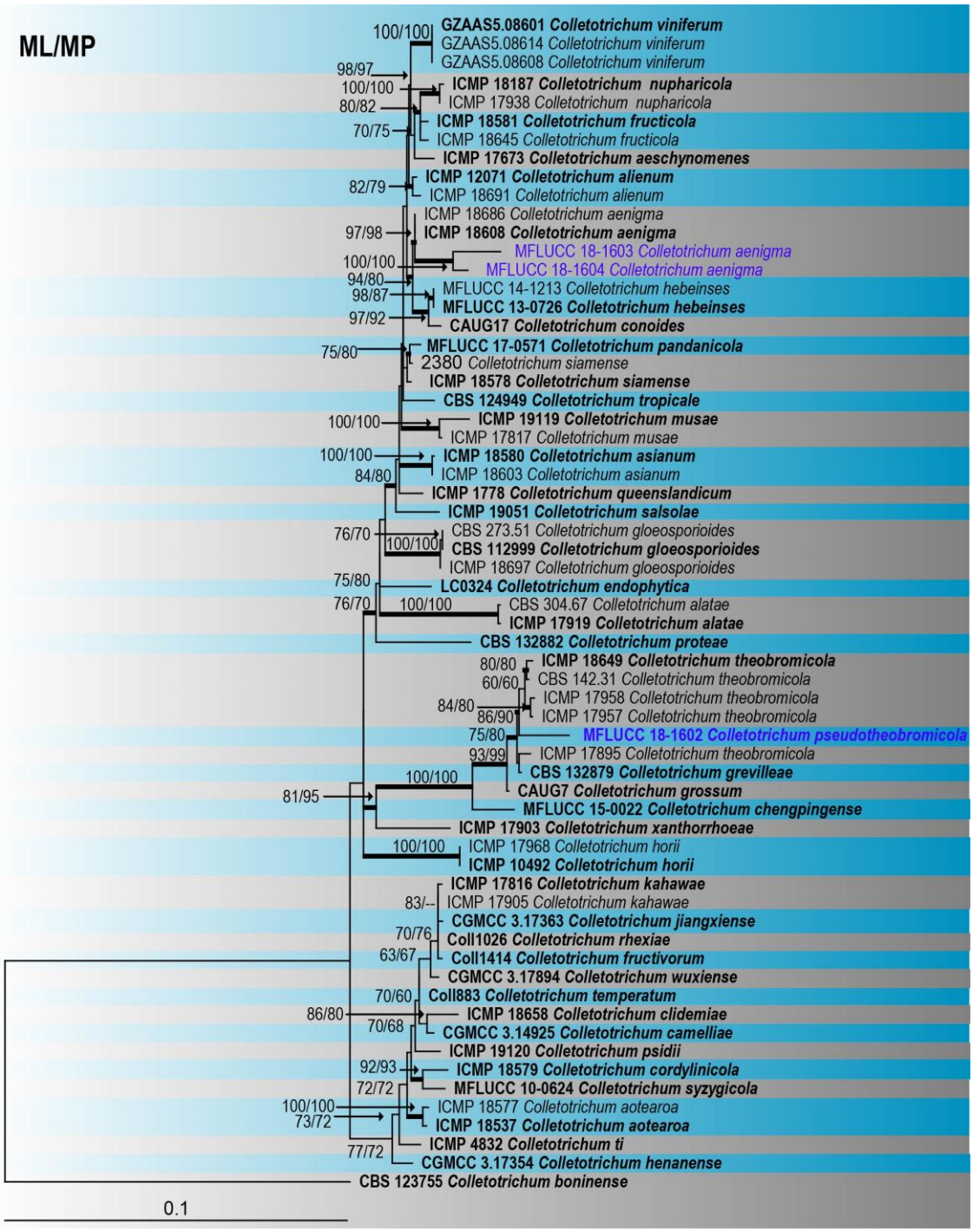
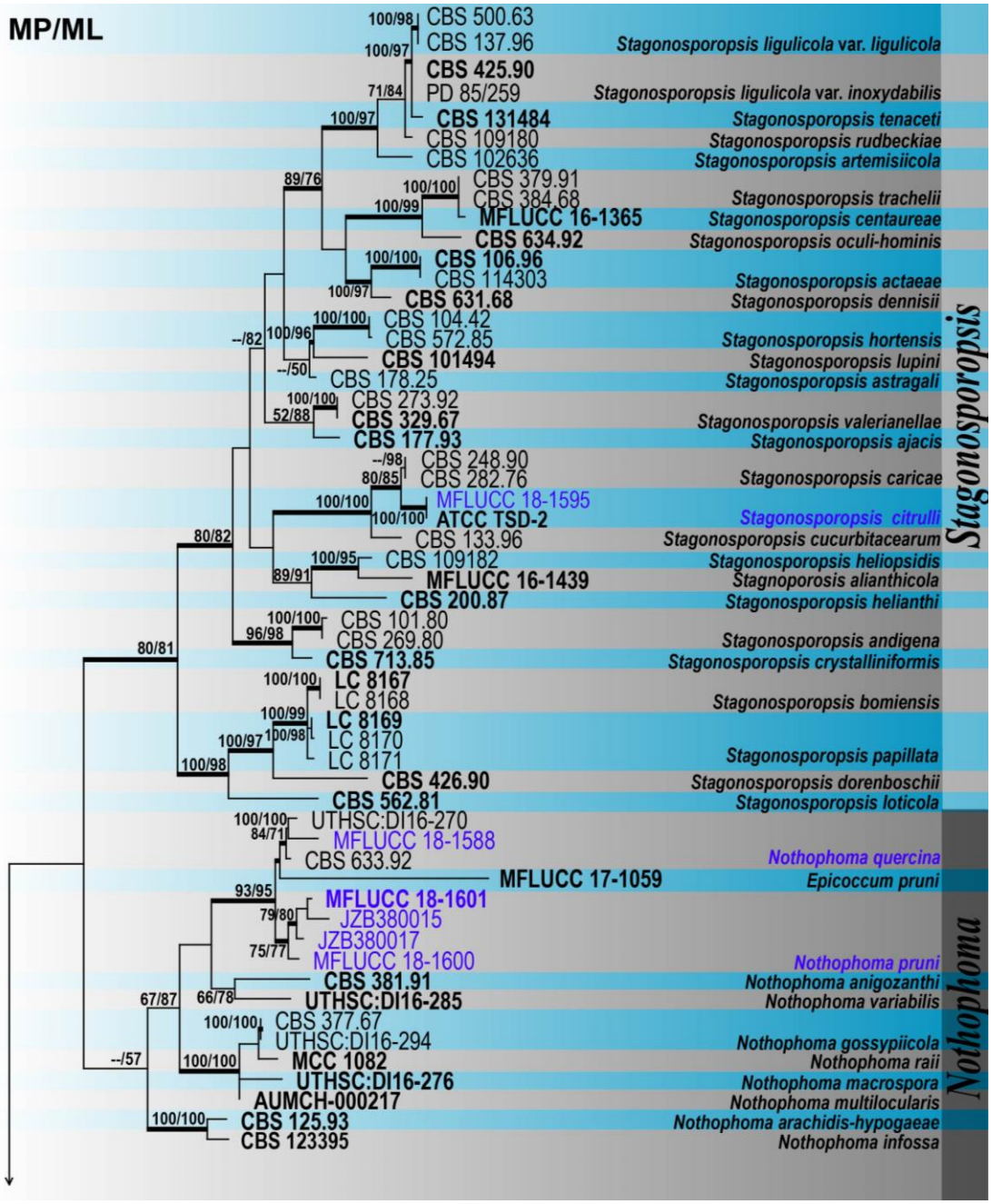


Figure 3 – Phylogenetic tree generated by maximum parsimony analysis of combined ITS, GAPDH, CHS-1, ACT and TUB2 sequence data of *Colletotrichum gloeosporioides* species complex. The tree was rooted with *Colletotrichum boninense* (CBS 123755). Maximum parsimony and RAxML bootstrap support values ≥ 50% (BT) are shown respectively near the nodes. Bayesian posterior probabilities ≥ 0.95 (PP) indicated as thickened black branches. The scale bar indicates 10 changes. The ex-type strains are in bold and new isolates in blue.

Identification of Didymellaceae species was conducted using a concatenated multigene phylogenetic analysis of LSU, ITS, RPB2, and TUB2 gene regions. The Didymellaceae alignment included 113 strains, representing three genera with *Neocucubitaria aquatica* (CBS 297.74) as the outgroup, and consisted of 2328 characters forming 703 unique alignment patterns. Similar topology multigene phylogenetic trees were generated from the Bayesian, ML and MP analyses (Fig. 4). The parsimony analysis comprised 2328 total characters including gaps. The concatenated

alignment consists of 452 parsimony informative characters, 274 variable and parsimony-uninformative characters, and 1602 constant characters. The first of 1000 equally parsimonious trees is shown in Fig. 4, which enabled the identification of the isolates to species level (TL= 2601, CI= 0.392, RI=0.800, RC=0.314, HI=0.608). Maximum likelihood alignment consisted of 9.08% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.238604, C = 0.244685, G = 0.274276, T = 0.242435; substitution rates AC = 1.272245, AG = 3.581243, AT = 1.481142, CG = 0.853475, CT = 8.586284, GT = 1.000000; gamma distribution shape parameter α = 0.175954. In the current tree (Fig. 4), one of our *Nothophoma quercina* isolate (MFLUCC 18–1588) clustered together with the reference strain of *N. quercina* (CBS 633.92) and another four isolates, which were introduced as new species, *N. pruni* (JZB380015, MFLUCC 18–1601, JZB380017 and MFLUCC 18–1600) phylogenetically distinct from *N. quercina* reference strain (CBS 633.92). *Stagonosporopsis citrulli* isolate (MFLUCC 18–1595) clustered with the ex-type isolates of *S. citrulli* (ATCC TSD-2) and an *Epicoccum pseudokeratinophilum* isolate (MFLUCC 18–1593) clustered with the ex-type isolate of *E. keratinophilum* (UTHSCDI 16-271) as new species.



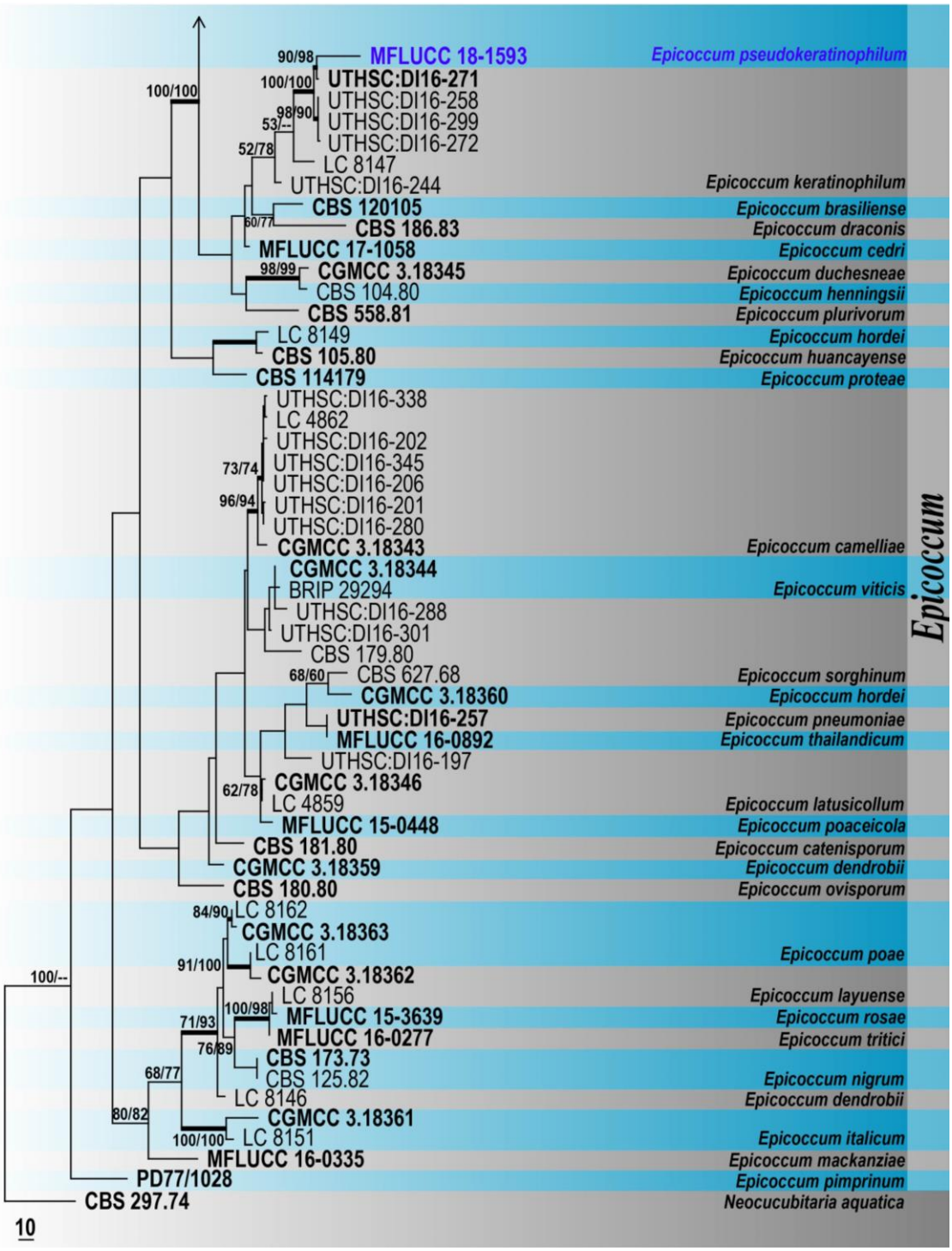


Figure 4 – Phylogenetic tree generated by maximum likelihood analysis of combined LSU, ITS, RPB2 and TUB2 sequence data of Didymellaceae species. The tree was rooted with *Neocucubitaria aquatica* (CBS 297.74). RAxML and Maximum parsimony bootstrap support values ≥ 50% (BT) are shown respectively near the nodes. Bayesian posterior probabilities ≥ 0.95 (PP) indicated as thickened black branches. The scale bar indicates 0.1 changes. The ex-type strains are in bold and new isolates in blue.

Pathogenicity assay

An isolate of *Alternaria alternata* (MFLUCC 18–1587), two isolates of *A. prunicola* (MFLUCC 18–1597, MFLUCC 18–1599), two isolates of *Colletotrichum aenigma* (MFLUCC 18–1603, MFLUCC 18–1604), an isolate of *C. pseudotheobromicola* (MFLUCC 18–1602), an isolate of *Epicoccum pseudokeratinophilum* (MFLUCC 18–1593), an isolate of *Stagonosporopsis citrulli*

(MFLUCC 18–1595) and an isolate of *Nothophoma pruni* (MFLUCC 18–1600) were subjected to detached leaf inoculation assay on three cultivars of *Prunus avium*.

Initially, pinpoint necrotic spots were formed near the inoculated area on leaves. Three days after inoculation, evenly round lesions surrounded by achlorotic margin were recorded on wounded inoculated leaves. These symptoms were similar to the characteristic lesions of cherry leaf spot that were observed in the field. After five days, lesions expanded and coalesced together to form larger necrotic areas. *Colletotrichum* species, being the highly pathogenic taxa, formed larger, dark brown necrotic areas. The major pathogen, *A. prunicola* formed identical necrotic areas for all of its isolates. Compared to these two genera, Didymellaceae species formed insignificant or no necrotic areas on the wounded leaves. No symptoms were observed on non-wounded inoculated leaves or on wounded and non-wounded leaves maintained as controls. Re-isolation from lesions confirmed the inoculated fungus based on cultural and morphological characters such as colony characters and conidial characters.

According to one-way ANOVA analysis, significantly different lesion areas resulted from different isolates (F8= 6.57, p=0.000). Mean difference in the lesion areas were significantly highest between *Epicoccum pseudokeratinophilum* (lowest lesion area) and *Colletotrichum pseudotheobromae* (highest lesion area). Furthermore, significantly different lesion areas resulted in different cherry cultivars (F8= 6.42, p=0.002). Mean lesion area of *Prunus avium* cv. 'Summit' was significantly different from mean lesion areas of *Prunus avium* cv. 'Tieton' and 'Sunburst'. Lesion areas formed by all of the isolates on *Prunus avium* cv. 'Summit' were larger, while smallest lesion areas were formed on *Prunus avium* cv. 'Sunburst'. Therefore, based on the analysis *Prunus avium* cv. 'Summit' was highly susceptible to cherry leaf spot disease whereas *Prunus avium* cv. 'Sunburst' showed the highest resistance to the disease. Two-way ANOVA analysis for lesion area showed that there was a significant interaction between the pathogen and cherry cultivar for forming lesion areas (Table 5) and the interaction plot for lesion area demonstrated these relationships (Figs 5, 6).

Table 5 Two-way analysis of variance for lesion area *vs*. isolate and variety

Source	**DF[1]**	**SS[1]**	**MS[1]**	**F[1]**	**P[1]**
Isolate	8	18.2010	2.27513	8.81	0.000
Variety	2	5.4798	2.73988	10.61	0.000
Interaction (isolate × interaction)	16	10.2596	0.64123	2.48	0.003
Error	108	27.8824	0.25817		
Total	134	61.8229			

[1]DF: Degrees of Freedom; SS: Sums of Squares; MS: Mean Squares; F: F-value; P: P-value

Taxonomy

Pleosporaceae Nitschke, Verh. naturh. Ver. preuss. Rheinl. 26: 74 (1869)
Alternaria Nees, Syst. Pilze (Würzburg): 72. 1816 [1816–1817]

Alternaria, introduced by Nees von Esenbeck (1816), is an ubiquitous genus treated under Pleosporaceae, Pleosporales, Dothideomycetes. The genus includes saprobes, endophytes and plant pathogens associated with a wide variety of substrates (Woudenberg et al. 2013). The genus has been subjected to several major revisions during the last few years (Woudenberg et al. 2013, Ariyawansa et al. 2015).

Alternaria alternata (Fr.) Keissl., Beih. Bot. Centralbl., Abt. 2 29: 434 (1912) Fig. 7

Basionym: *Torula alternata* Fr., Syst. Mycol. (Lundae) 3: 500 (1832) (nom. Sanct.).
≡ *Alternaria tenuis* Nees, Syst. Pilze (Würzburg): 72 (1816).
For additional synonyms refer to Index Fungorum.

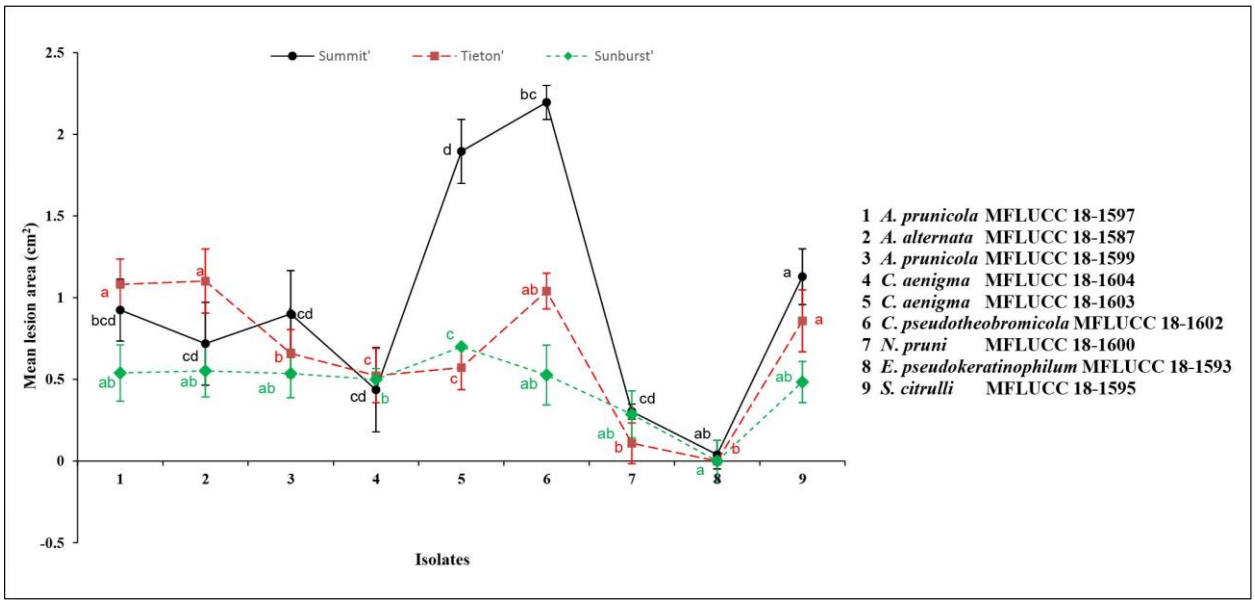


Figure 5 – Variations in the virulence of lesion areas of pathogenic isolates on cherry leaves resulting from the pathogenicity test three days post inoculation. Virulence of isolates depends on both the isolates as well as on the cherry cultivar. Characters indicate the significant differences for lesion areas. Isolates that do not share the same letter are significantly different.

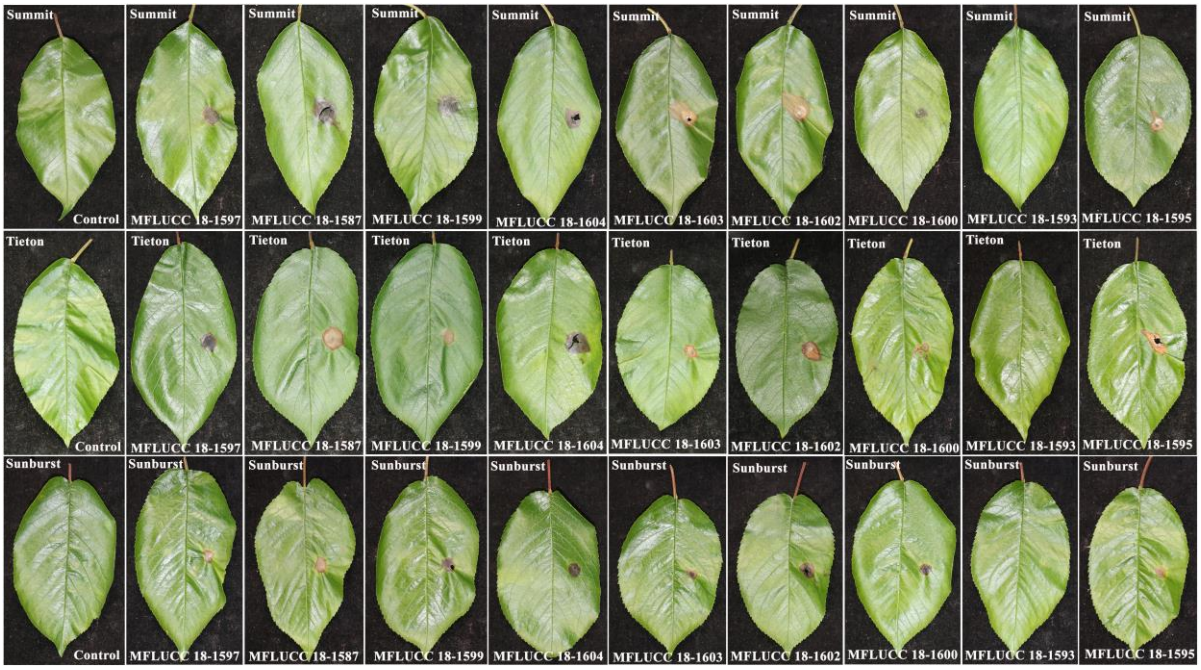


Figure 6 – Detached leaf pathogenicity assay for the fungal isolates on *P. avium* cvs. 'Summit', 'Tieton' and 'Sunburst' three days post inoculation. Symptoms on leaves symmetrically inoculated with a conidial suspension of the isolated pathogen, right: non-wounded inoculation; left: wounded inoculation. Top right corner of each figure indicate the *P. avium* cultivar name and the bottom left indicate the inoculated fungal isolate number. The species names are as follows: MFLUCC 18–1587 = *Alternaria alternata*, MFLUCC 18–1597, MFLUCC 18–1599 = *Alternaria prunicola*, MFLUCC 18–1603, MFLUCC 18–1604 = *Colletotrichum aenigma*, MFLUCC 18–1602 = *Colletotrichum pseudotheobromicola*, MFLUCC 18–1593 = *Epicoccum pseudokeratinophilum*, MFLUCC 18–1595 = *Stagonosporopsis citrulli* and MFLUCC 18–1600 = *Nothophoma pruni*. Sterilized water was used as the control. The highest level of virulence was exhibited by *Colletotrichum* species. All Didymellaceae species induced less or no lesions on the cherry leaves.

Pathogenic on diseased leaves of *Prunus avium*. Sexual morph: Undetermined. Asexual morph: *Hyphae* superficial or submerged, subhyaline, branched, smooth to verruculose, septate, 2–3 μm wide. *Conidiophores* 23–40 × 3–5 μm ($\overline{x}$ = 27 × 3.5 μm, n = 20), solitary, simple or branched, brown, multi-septate, with a single terminal conidiogenous loci. *Conidia* 20–40 × 9–15 μm ($\overline{x}$ = 29 × 10.2 μm, n = 40), solitary or in branched chains of 20 or more, first 1–2 conidia in each chain longer than others, straight, ellipsoidal or ovoid, pale to dark brown to olivaceous green, with smooth outer wall, some muriform, usually with 1–6 transverse septa and 0–3 longitudinal septa, rounded apex. *Conidial beaks* pale brown to subhyaline, not branched, 2–6 × 3–4 μm.

Culture characteristics – Colonies on PCA attaining 80 mm diam. after 5 days at 25 °C, circular, entire-edged, flat, floccose to woolly, surface pale olivaceous grey near the margin changing to dull green in the centre and reverse olivaceous black in the centre and pale olivaceous grey near the margin.

Material examined – CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (MFLU 18–2659) – living culture, MFLUCC 18–1586, KUMCC 18–0394; *ibid*. (MFLU 18–2660) – living culture, MFLUCC 18–1587, KUMCC 18–0395.

Additional material examined – CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (JZB-H 3180012) – living culture, JZB3180012; *ibid*. (JZB-H 3180011) – living culture, JZB3180011; *ibid*. (JZB-H 3180014) – living culture, JZB3180014; *ibid*. (JZB-H 3180002) – living culture, JZB3180002.

Notes – *Alternaria alternata* is a common pathogen of many hosts and mostly found as saprobes (Thomidis & Tsipouridis 2006, Hyde et al. 2009, Jayawardena et al. 2016). There is a report on *A. alternata* causing leaf spot on cherry in Greece (Thomidis & Tsipouridis 2006). Based on our phylogenetic analysis of combined ITS, GAPDH, RPB_2, TEF 1-α and Alt-a sequence data of *Alternaria* species (Fig. 2), some of our isolates (MFLUCC 18–1586, JZB3180012,) clustered together with the ex-type strain of *A. alternata* (CBS 916.96) with high bootstrap and Bayesian probabilities (99% MP, 95% ML and 1.00 PP), while others (JZB3180011, JZB3180014, JZB3180002 and MFLUCC 18–1587) are phylogenetically distant. As discussed under multi-gene phylogenies, this phylogenetically distant group is due to the inclusion of different morphospecies used in the study which are currently synonymized under *A. alternata*. Comparisons of base pair differences for all the genes between our strain (MFLUCC 18–1586) and ex-type strain of *A. alternata* (CBS 916.96) reveal identical or less than 1% base pair differences. When our strain was compared with the type specimen of *A. alternata* (CBS 916.96), it showed similar morphology (Ariyawansa et al. 2015, Woudenberg et al. 2015).

Alternaria prunicola Chethana, Yan, Li & K.D. Hyde, sp. nov. Fig. 8

MycoBank number: MB828515; Facesoffungi number: FoF04913

Etymology – The specific epithet *prunicola* was given after the host genus.

Pathogenic on diseased leaves of *Prunus avium.* Sexual morph: not observed. Asexual morph: *Hyphae* subhyaline to pale olivaceous, branched, smooth, septate, 3–4 μm wide. *Conidiophores* 13–40 × 2.5–5 μm ($\overline{x}$ = 26.5 × 3.8 μm, n = 20), solitary, simple, straight or flexuous, dark brown, multi-septate, with a single or two terminal conidiogenous loci. *Conidia* 18–37.1 × 6–15 μm ($\overline{x}$ = 25.5 × 8.8 μm, n = 40), solitary or in branched chains of 4 or more, straight, clavate to elongated clavate, olivaceous to light brown, with smooth outer wall, some muriform, usually with 3–4 transverse septa and 0–1 longitudinal septa, rarely have oblique septa which divide the septate cells into cuboid portions, often constricted at the primary septa, rounded apex, stalked or stalkless.

Culture characteristics – Colonies on PCA attaining 80 mm diam. after 5 days at 25 °C, circular, entire-edged, effuse, floccose to woolly, surface pale olivaceous grey near the margin changing to dull green in the centre and reverse olivaceous black in the centre and pale olivaceous grey near the margin.

Material examined – CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (MFLU 18–2661, holotype), ex-type culture, MFLUCC 18–

1597; *ibid*. (KUMCC 18–0405, isotype); *ibid.* (MFLU 18–2662) – living culture, MFLUCC 18–1596, KUMCC 18–0404; CHINA. *ibid*. (MFLU 18–2663) – living culture, MFLUCC 18–1599, KUMCC 18–0407; *ibid*. (MFLU 18–2664) – living culture, MFLUCC 18–1598, KUMCC 18–0406.

Additional material examined – CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (JZB-H 3180005) – living culture, JZB3180005; *ibid*. (JZB-H 3180006) – living culture, JZB3180006.

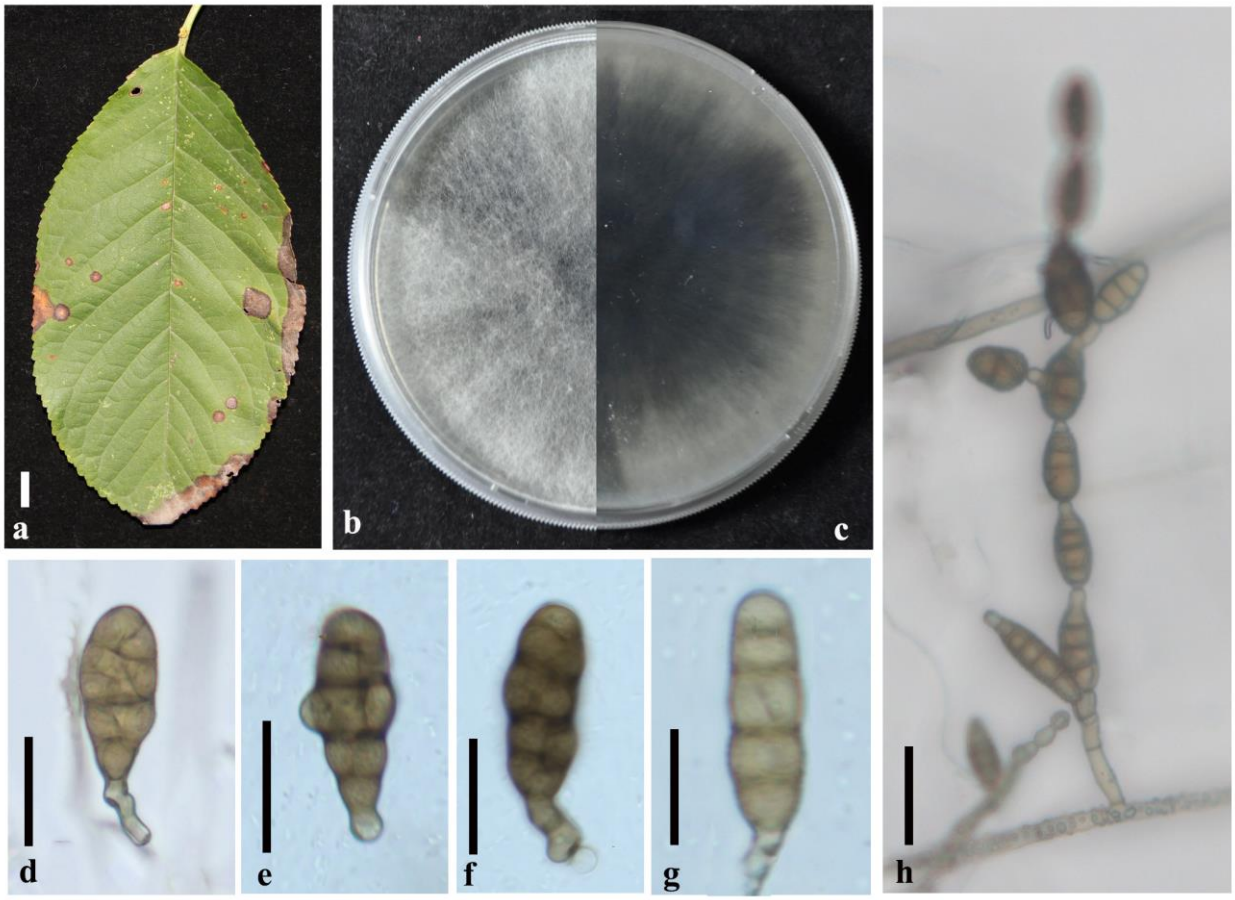


Figure 7 – *Alternaria alternata* (MFLUCC 18–1586) a Host surface from which the pathogen was isolated. b, c Upper-view (b) and the reverse view (c) of the colony on PCA. d–g Beaked or beakless different conidial morphologies. h Sporulation pattern of *A. alternata*. Scale bars: a = 1 cm, d–g = 20 μm, h = 50 μm.

Note – In our phylogenetic analysis of combined ITS, GAPDH, RPB_2, TEF 1-α and Alt-a sequence data of *Alternaria* species (Fig. 2), all of the isolates belonging to *Alternaria prunicola* formed a subclade within section *Alternaria*. *Alternaria prunicola* was well-separated from other *Alternaria* species with a strong 82% ML, 81% MP bootstrap values and 1.00 posterior probability; its sister taxa *A. alternata* (CBS 919.96) and *A. longipes* (CBS 540.94) clustered separately from *A. prunicola* with 100% MP, 100% ML and 1.00 posterior probabilities. A comparison of the 509 nucleotides across the ITS (+5.8S) gene region between *A. prunicola* (MFLUCC 18–1597) and its sister taxa *A. alternata* (CBS 919.96) and *A. longipes* (CBS 540.94) reveal 22.09% and 22.28% base pair differences respectively. In addition, we compared our new taxon with *A. alternata* (CBS 919.96) and *A. longipes* (CBS 540.94) for base pair differences in the protein coding genes and there are 2.86% and 3.43% base pair differences respectively across 524 nucleotides in GAPDH gene region; 1.66% and 8.73% base pair differences respectively across 1070 nucleotides in RPB_2 gene region; 8.33% and 10.13% base pair differences respectively across 202 nucleotides in TEF 1-

α gene region. Another *Alternaria* species, *Alternaria pruni* McAlpine had been isolated from Apricot leaves. However, it is morphologically different from our collection in having larger (52–64 × 13–18 μm), 6–8 septate spores (McAlpine 1902). Due to the unavailability of DNA sequences, this was not included in the phylogenetic analysis. Our collection is distinct from *A. alternata*, another reported cherry leaf spot pathogen, in having clavate to elongated clavate, 3–4 transverse septate and 0–1 longitudinal septate, smaller conidia (18–37.1 × 6–15 μm), in contrast to obclavate, obpyriform, ovoid or ellipsoidal, pale to mid golden brown, 8 transverse and usually several longitudinal or oblique septate, larger (20–63 × 9–18 μm) conidia of *A. alternata* (Ellis 1971).

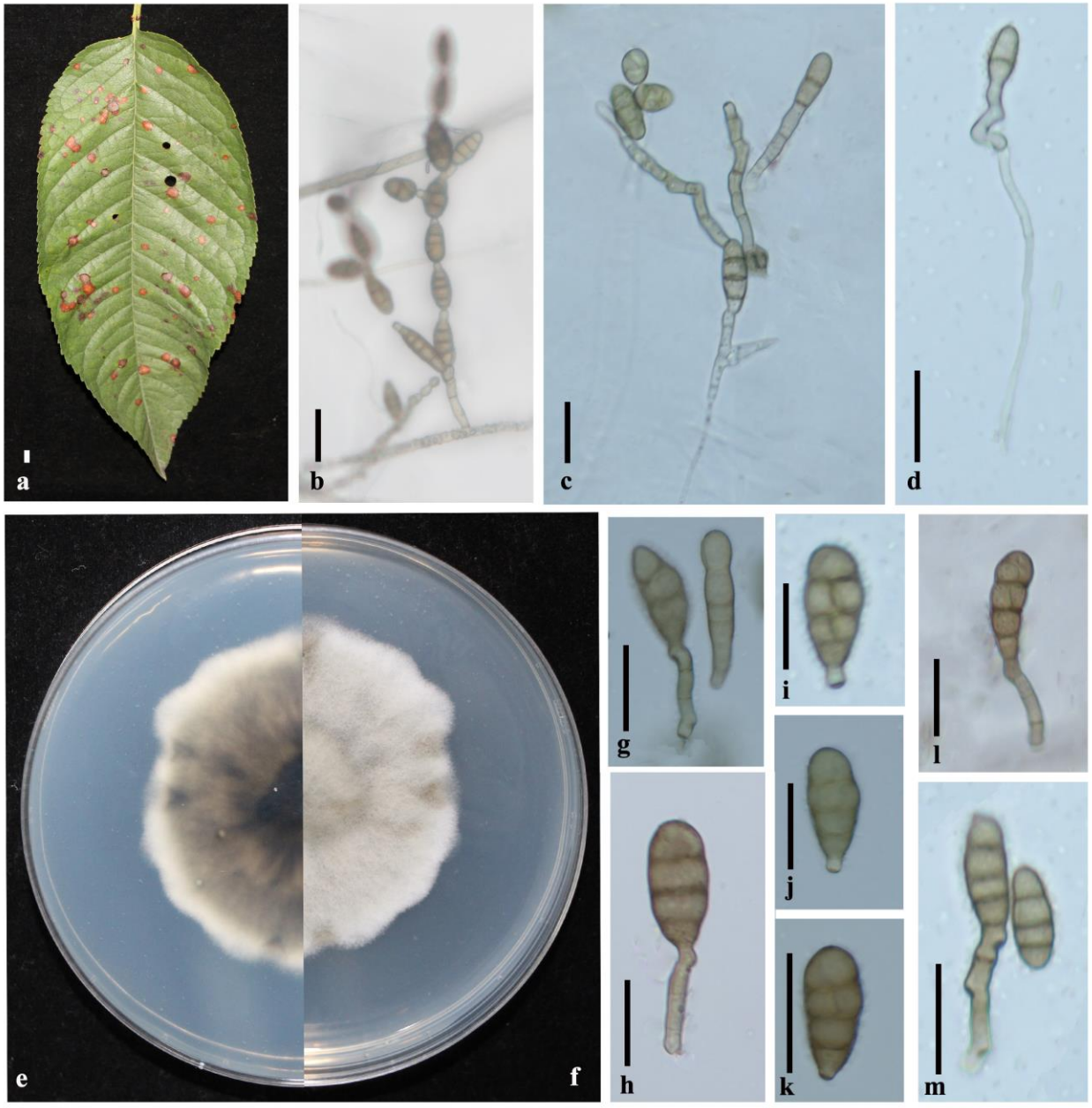


Figure 8 – *Alternaria prunicola* (MFLUCC 18–1597, holotype) a Host surface from which the pathogen was isolated. b Sporulation pattern of *A. prunicola*. c Conidiophores connected to conidia. d Germinating conidia. e, f Upper-view (e) and the reverse view (f) of the colony on PCA. g–m Stalked or stalkless different conidial morphologies. Scale bars: a = 3 mm, b–d, g–m = 20 μm.

Alternaria pseudoeichhorniae Chethana, Yan, Li & K.D. Hyde, sp. nov. Fig. 9
MycoBank number: MB828516; Facesoffungi number*:* FoF04912

Etymology – The specific epithet *pseudoeichhorniae* was given after its close resemblance to *Alternaria pseudoeichhorniae*.

Pathogenic on diseased leaves of *Prunus avium*. Sexual morph: not observed. Asexual morph: *Hyphae* subhyaline to hyaline, branched, smooth, septate. *Conidiophores* 18–48.5 × 2.5–6 µm ($\overline{x}$ = 29.1 × 4.3 µm, n = 10), solitary, simple, straight or flexuous, dark brown, multi-septate, with a single or up to three terminal conidiogenous loci. *Conidia* 16–30.2 × 5–13 µm ($\overline{x}$ = 22.6 × 9.8 µm, n = 40), solitary or in a chain of 2–4 or more, straight, obpyriform to obclavate, light brown, with smooth outer wall, usually with an indistinct basal pore, muriform, with 2–3 transverse and 0–1 longitudinal septa, often constricted at the primary septa. *Conidial beak* absent or present as a short conical, narrowly tapered or almost cylindrical beak.

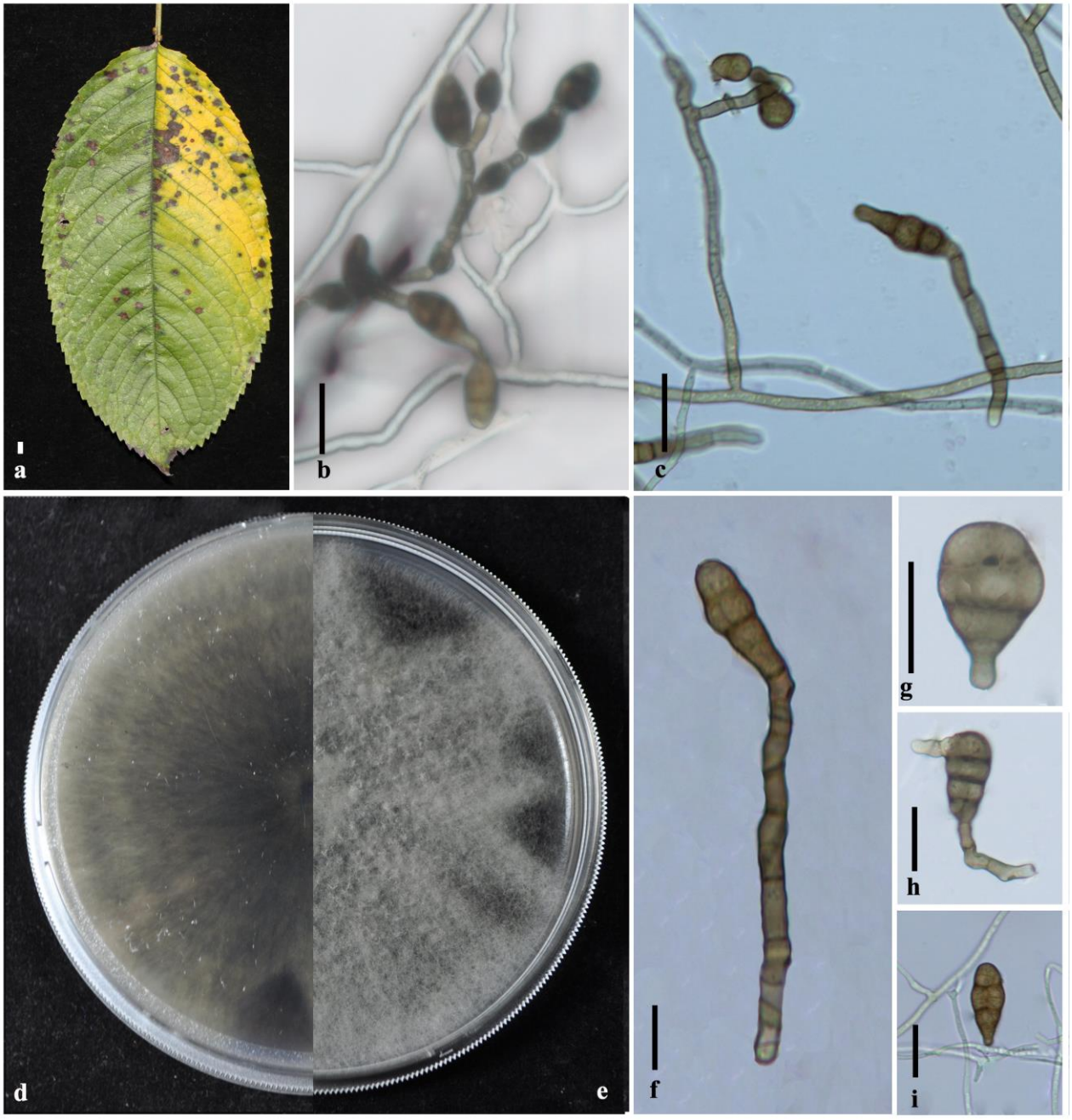


Figure 9 – *Alternaria pseudoeichhorniae* (MFLUCC 18–1589, holotype) a Host surface from which the pathogen was isolated. b Sporulation pattern of *A. eichhorniae*. c Conidiophore. d, e Upper-view (e) and the reverse view (d) of the colony on PCA. f Beaked conidium. g–i Germinating beakless conidia. Scale bars: a = 3 mm, b, c, f–i = 20 µm.

Culture characteristics – Colonies on PCA fast growing, circular, with velvety to cotton abundant greyish aerial mycelium, effuse at the edges, occasionally forming black patches towards the margin of the colonies, with conspicuous concentric zonations of growth, attaining a diameter of 8.5 cm in 7 days at 25 °C.

Material examined – CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (MFLU 18–2665, holotype) – ex-type culture, MFLUCC 18–1589; *ibid*. (KUMCC 18–0397, isotype).

Notes – Based on our phylogenetic analyses of combined ITS, GAPDH, RPB_2, TEF 1-α and Alt-a sequence data of *Alternaria* species (Fig. 2), our strain (MFLUCC 18–1589) clustered in a clade together with the isolates of *Alternaria tomato* (CBS 103.30 and CBS 114.35), *A. burnsii* (CBS 108.27 and CBS 107.38) and *A. jacinthicola* (CBS 133751 and CPC 25267). Our novel taxon was separated from these taxa with high bootstrap values and strong baysean posterior probabilities (100% MP, 95% ML, and 1.00 PP). A comparison of the protein coding regions GADPH, RPB2 and TEF 1-α between our species (MFLUCC 18–1589) and closely associated *A. jacinthicola* (CPC 25267) revealed 3.92%, 4.5% and 5.4% base pair differences respectively. Morphological comparison between them revealed different conidial characters. Compared to our strain, *A. jacinthicola* have larger conidiophores (70 × 2–4 µm) and larger (28–32 × 12–15 µm), very short beaked, 3–7 transverse and 1–2 longitudinal septate conidia (Dagno et al. 2011). As mentioned in the etymology section, our strain show a high resemblance to *A. eichhorniae*. When comparing our strain with the type specimen of *A. eichhorniae* (CBS 489.92), they are similar in morphology except for conidia and conidiomata. Our strain have slightly smaller conidia, and smaller conidiophores as compared to the type strain (Nag Raj & Ponnappa 1970).

Glomerellaceae Locq. Wx Seifert & W. Gams, Zhang et al., Mycologia 98(6): 1083 (2007)
Colletotrichum Corda, Sturm, Deutschl. Fl., 3 Abt. (Pilze Deutschl.) 3(12): 41. (1831)

Colletotrichum was introduced by Corda (1831) for *Colletotrichum lineola* Corda. The genus includes endophytes, saprobes and many plant pathogens (Hyde et al. 2009, Jayawardena et al. 2016). *Colletotrichum* was placed in Glomerellaceae, Sordariomycetes by Kirk et al. (2001) and this was confirmed by other studies (Maharachchikumbura et al. 2015, 2016).

Colletotrichum aenigma B.S. Weir & P.R. Johnst., in Weir, Johnston & Damm, Stud. Mycol. 73: 135 (2012) Fig. 10

Facesoffungi number: FoF04914

Pathogenic on diseased leaves of *Prunus avium*. Sexual morph: Undetermined. Asexual morph: *Pycnidia* on the PDA 0.66–5 mm diam. ($\overline{x}$ = 2.3 mm, n = 10), black, aggregated, verrucose, sometimes reduced to hyaline conidial masses. *Vegetative hyphae* hyaline, smooth-walled, septate, branched. *Conidiophores* not observed. *Conidiogenous cells* poorly differentiated, arise from hyphae without any organization. *Conidia* 14–31.2 × 4–8 µm ($\overline{x}$ = 18.9 × 6.2 µm, n = 40), hyaline, smooth-walled, aseptate, guttulate, straight, cylindrical with broadly rounded ends. *Appressoria* 6–10 µm diam., dark brown or black, sub-globose or with few broad lobes. *Chlamydospores* and *setae* not observed.

Culture characteristics – Colonies on PDA slow growing, attaining a diameter of 5.0 cm in 4 days at 25 °C, circular, with cotton, dense, white aerial mycelium, reverse centre pale olivaceous grey and olivaceous grey towards the margin, becoming black with age.

Material examined – CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (MFLU 18–2658) – living culture, MFLUCC 18–1603, KUMCC 18–0411; CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (MFLU 18–2657) – living culture, MFLUCC 18–1604, KUMCC 18–0412.

Notes – *Colletotrichum aenigma* has been reported from causing diseases on a variety of hosts including *Capsicum* sp., *Citrus sinensis* (L.) Osbeck, *Fragaria x ananassa* Duchesne, *Malus domestica* Borkh., *Olea europaea* L., *Persea americana* Mill., *Pyrus* sp., *Sedum kamtschaticum* Fisch. & C.A. Mey and *Vitis vinifera* L. from Asian and European regions (Schena et al. 2014, Yan

et al. 2015, Han et al. 2016, Choi et al. 2017, Diao et al. 2017). Based on our phylogenetic analysis of combined ITS, GAPDH, CHS, ACT and TUB2 sequence data of *Colletotrichum* species (Fig. 3), our strain (MFLUCC 18–1603) clustered together with the ex-type strain of *C. aenigma* (ICMP 18608) with high bootstrap and Bayesian probabilities (100% MP, 100% ML and 1.00 PP). Comparisons of base pair differences for all the genes between our strain (MFLUCC 18–1603) and ex-type strain of *C. aenigma* (ICMP 18608) reveal identical or less than 1% base pair differences. When comparing our strain with the type specimen of *C. aenigma* (ICMP 18608), it showed similar morphology (Weir et al. 2012).

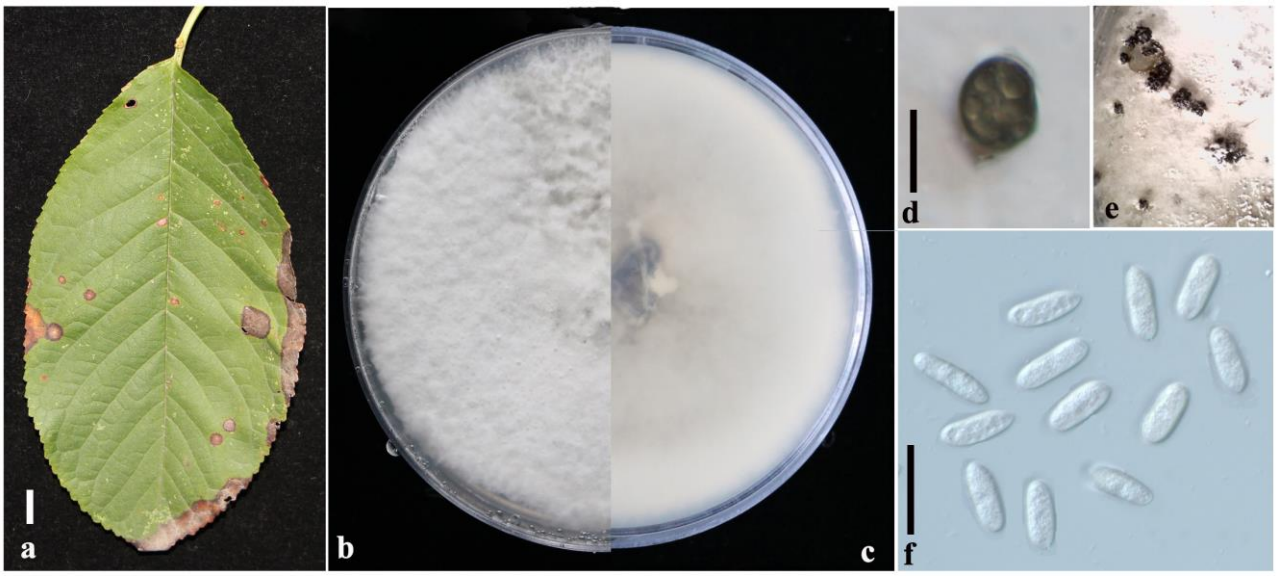


Figure 10 – *Colletotrichum aenigma* (MFLUCC 18–1603) a Host surface from which the pathogen was isolated. b, c Upper-view (b) and the reverse view (c) of the colony on PDA. d Appressoria. e Pycnidia on the medium. f Conidia. Scale bars: a = 1 cm, d = 10 μm, f = 20 μm.

Colletotrichum pseudotheobromicola Chethana, Yan, Li & K.D. Hyde, sp. nov. Fig. 11

MycoBank number: MB828517; Facesoffungi number: FoF04915

Etymology – The specific epithet *pseudotheobromicola* was given after its resemblance to *Colletotrichum theobromicola*.

Pathogenic on diseased leaves of *Prunus avium*. Sexual morph: Undetermined. Asexual morph: *Pycnidia* on the PDA 0.4–0.97 mm diam. ($\bar{x}$ = 0.6 mm, n = 10), solitary, submerged in PDA, globose, black, but mostly rudimentary, reduced to orange conidial masses, forming abundantly. *Vegetative hyphae* hyaline to light brown, smooth-walled, septate, branched. *Conidiogenous cells* 8.6–14.5 × 1–4 μm ($\bar{x}$ = 10.9 × 3.4 μm, n = 20, n = 20), hyaline to pale brown, cylindrical, tapering uniformly from base to tip, arising from highly septate, swollen hyphae on PDA. Phialides 14.5–20 × 3–4 μm produced from short-cell hyphae, cylindrical, tapered toward the tip and tips marked by periclinal thickening. *Conidia* 13–19.7 × 4–6 μm ($\bar{x}$ = 16.6 × 5.0 μm, n = 40), L/W ratio 3.3, hyaline, smooth-walled, aseptate, straight, sub-cylindrical to clavate, often with broadly rounded ends. *Appressoria* 6–10 × 5–8 μm ($\bar{x}$ = 9.8 × 6.9 μm, n = 10), irregular, light brown. *Chlamydospores* and *setae* not observed.

Culture characteristics – Colonies on PDA slow growing, attaining a diameter of 5.0 cm in 4 days at 25 °C, circular, with velvety to cotton, dense, greyish aerial mycelium, initially light grey, with hyaline immersed hyphae, forming dark, grey, concentric rings, becoming black with age.

Material examined – CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (MFLU 18–2656, holotype) – ex-type culture, MFLUCC 18–1602; *ibid*. (KUMCC 18–0410, isotype).

Notes – Based on the phylogenetic analysis of the current study of combined ITS, GAPDH, CHS, ACT and TUB2 sequence data of *Colletotrichum* species (Fig. 3), our taxon *C. pseudotheobromicola* (MFLUCC 18–1602) is phylogenetically distant from the ex-type strain of *C. theobromicola* (CBS 124945; 86% MP 90% ML and 0.98 PP). A comparison of the 521 nucleotides across the ITS (+5.8S) gene region between *C. pseudotheobromicola* (MFLUCC 18–1602) and *C. theobromicola* (CBS 124945) reveal 3.15% base pair difference. Furthermore, comparison of our new taxon with *C. theobromicola* (CBS 124945) for base pair differences in the protein coding genes showed, 4.74% base pair difference across 250 nucleotides in GAPDH gene region; 10% base pair difference across 282 nucleotides in CHS gene region; 3.46% base pair difference across 491 nucleotides in TUB2 gene region; and 10% base pair difference across 256 nucleotides in ACT gene region. When comparing our strain with the type specimen of *C. theobromicola* (CBS 489.92), they are similar in morphology (Rojas et al. 2010) except for spore and appressoria characters. Our taxon *C. pseudotheobromicola* (MFLUCC 18–1602) differs from *C. theobromicola* (CBS 489.92) in having larger spores (13–19.7 × 4–6 µm) and larger appressoria (6–10 × 5–8 µm) compared to smaller spores (14–18.7 × 4–5 µm) and smaller appressoria (6–10 × 5–6 µm) of *C. theobromicola* (CBS 489.92).

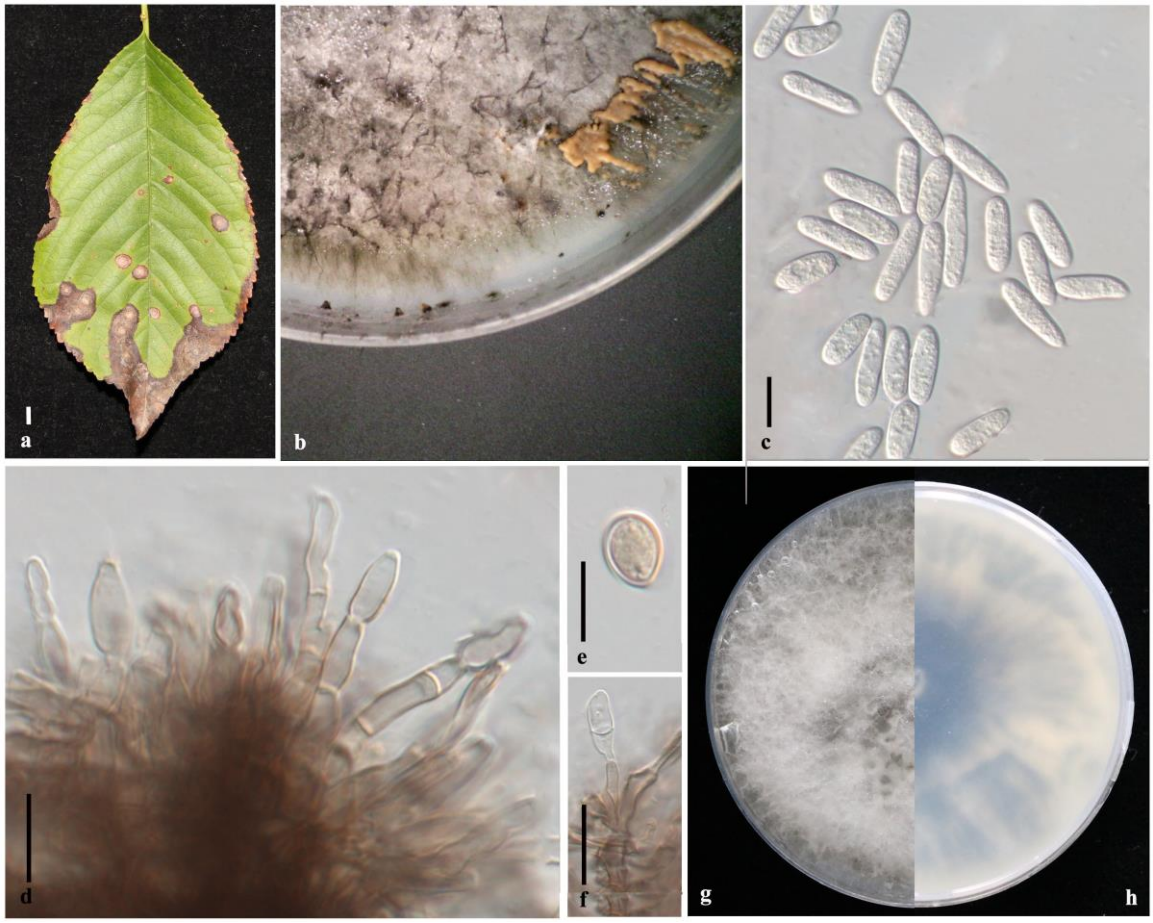


Figure 11 – *Colletotrichum pseudotheobromicola* (MFLUCC 18–1602, holotype) a Host surface from which the pathogen was isolated. b Pycnidia and orange colour spore mass on the PDA. c Conidia. d, f Phialides developed from septate swollen hyphae. e Appressoria. g, h Upper-view (g) and the reverse view (h) of the colony on PDA. d Conidia. Scale bars: a = 7 mm, c–f = 20 µm.

Didymellaceae Gruyter, Aveskamp & Verkley, Mycol. Res. 113(4): 516 (2009)
Nothophoma Qian Chen & L. Cai, Stud. Mycol. 82: 212 (2015)

Nothophoma was introduced by Chen et al. (2015) with *Nothophoma infossa* (Ellis & Everh.) Q. Chen & L. Cai. to accommodate *Nothophoma anigozanthi* (Tassi) Q. Chen & L. Cai.,

Nothophoma arachidis-hypogaeae (V.G. Rao) Q. Chen & L. Cai., *Nothophoma quercina* (Syd.) Q. Chen & L. Cai. and *Nothophoma gossypiicola* (Gruyter) Q. Chen & L. Cai. This ubiquitous, species-rich genus includes many important plant pathogens (Chen et al. 2015).

Nothophoma pruni Chethana, Yan, Li & K.D. Hyde, sp. nov. Fig. 12

MycoBank number: MB828518; Facesoffungi number: FoF04917

Etymology – The specific epithet *pruni* was given after the host genus.

Saprobic on diseased leaves of *Prunus avium.* Sexual morph: Undetermined. Asexual morph: *Pycnidia* on the PDA surface, 0.22–0.43 mm ($\bar{x}$ = 0.28 mm, n = 10) diam., solitary, scattered, globose to irregularly shaped, black, ostiolate. *Conidiogenous cells* phialidic, hyaline, simple, doliiform to ampulliform, variable in size. *Conidia* 4.8–8.5 × 2.7–3.9 µm ($\bar{x}$ = 6 × 3.3 µm, n = 40), cylindrical to obovoid or oblong, hyaline, aseptate, smooth-walled. Conidial exudates hyaline to buff.

Culture characteristics – Colonies on PDA reach 80 mm diam. after 7 days at 25 °C, with regular margin, dull white aerial mycelium surface floccose, with reverse pale vinaceous.

Material examined – CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (MFLU 18–2668, holotype) – ex-type culture, MFLUCC 18–1601; *ibid*. (KUMCC 18–0409, isotype); CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (MFLU 18–2667) – living culture, MFLUCC 18–1600, KUMCC 18–0408.

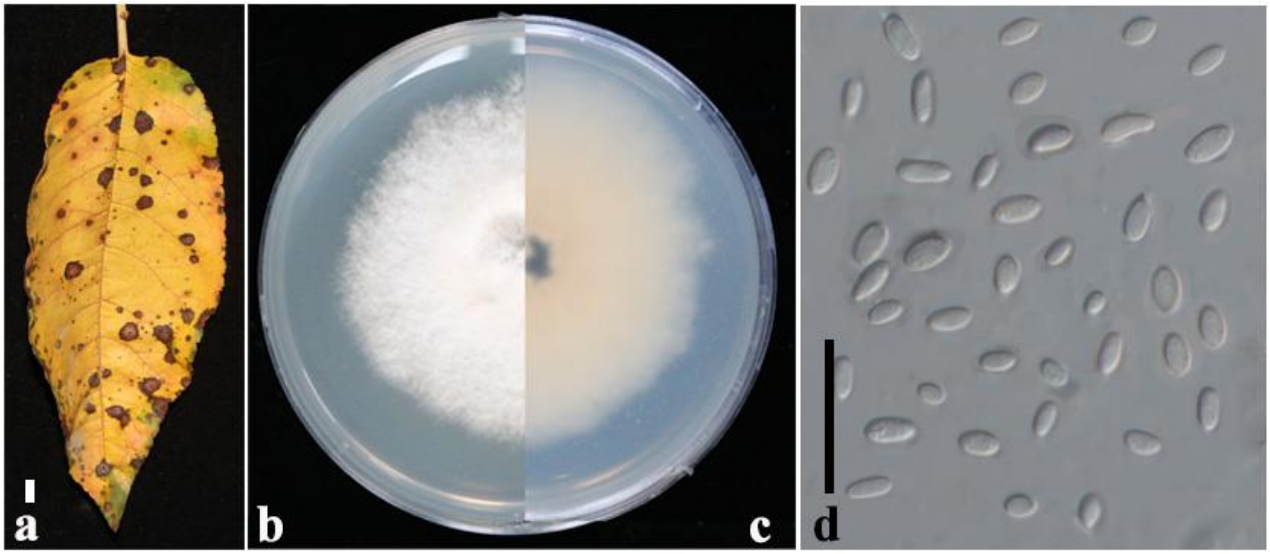


Figure 12 – *Nothophoma pruni* (MFLUCC 18–1601, holotype) a Host surface from which the pathogen was isolated. b, c Upper-view (b) and the reverse view (c) of the colony on PDA. d Conidia. Scale bars: a = 1 cm, d = 20 µm.

Note – According to the phylogenetic analysis of combined LSU, ITS, RPB_2 and TUB2 sequence data of Didymellaceae species (Fig. 4), our collection of *Nothophoma pruni* formed a subclade within genus *Nothophoma*. The *Nothophoma pruni* collection was well-separated from its sister taxa, *N. quercina* (CBS 633.92) with a relatively high bootstrap and Bayesian probabilities (95% ML, 93% MP, 1.00 PP). A comparison of the 486 nucleotides across the ITS (+5.8S) gene region between *N. pruni* (MFLUCC 18–1601) and *N. quercina* (CBS 633.92) reveal 2.46% base pair difference. Furthermore, comparison of our new taxon with *N. quercina* (CBS 633.92) for base pair differences in the protein coding genes confirmed its novelty. There are 3.19% base pair difference across 909 nucleotides in RPB_2 gene region and 4.17% base pair difference across 335 nucleotides in TUB2 gene region. Our collection differs from ex-type of *N. quercina* in having cylindrical to obovoid or oblong, hyaline, slightly smaller (4.8–8.5 × 2.7–3.9 µm) conidia in

contrast to subglobose to oval to obtuse, brown, larger (5.5–7.5 × 3–4.5 µm) conidia of *N. quercina* (Aveskamp et al. 2010).

Nothophoma quercina (Syd. & P. Syd.) Qian Chen & L. Cai, Stud. Mycol. 82: 213 (2015) Fig. 13

Facesoffungi number: FoF04918

Basionym: *Cicinobolus quercinus* Syd., Ann. Mycol. 13: 42 (1915)

≡ *Ampelomyces quercinus* (Syd.) Rudakov, Mikol. Fitopatol. 13: 109 (1979)

≡ *Phoma fungicola* Aveskamp et al., Stud. Mycol. 65: 26 (2010)

Saprobic on diseased leaves of *Prunus avium*. Sexual morph: Undetermined. Asexual morph: *Pycnidia* on the PDA surface, 0.22–0.43 mm ($\bar{x}$ = 0.28 mm, n = 10) diam., solitary, scattered, globose to irregularly shaped, black, ostiolate. *Pycnidial wall* multi-layered, composed of pale brown, pseudoparenchymatous cells, thicker outer layer and thinner inner layer. *Conidiogenous cells* phialidic, hyaline, simple, doliiform to ampulliform, variable in size. *Conidia* 2–5.5 × 1–4 µm ($\bar{x}$ = 3.5 × 2.5 µm, n = 40), variable in size and shape, subglobose to oval or obtuse, initially hyaline, but brown at maturity, aseptate, smooth-walled. Conidial exudates hyaline to buff.

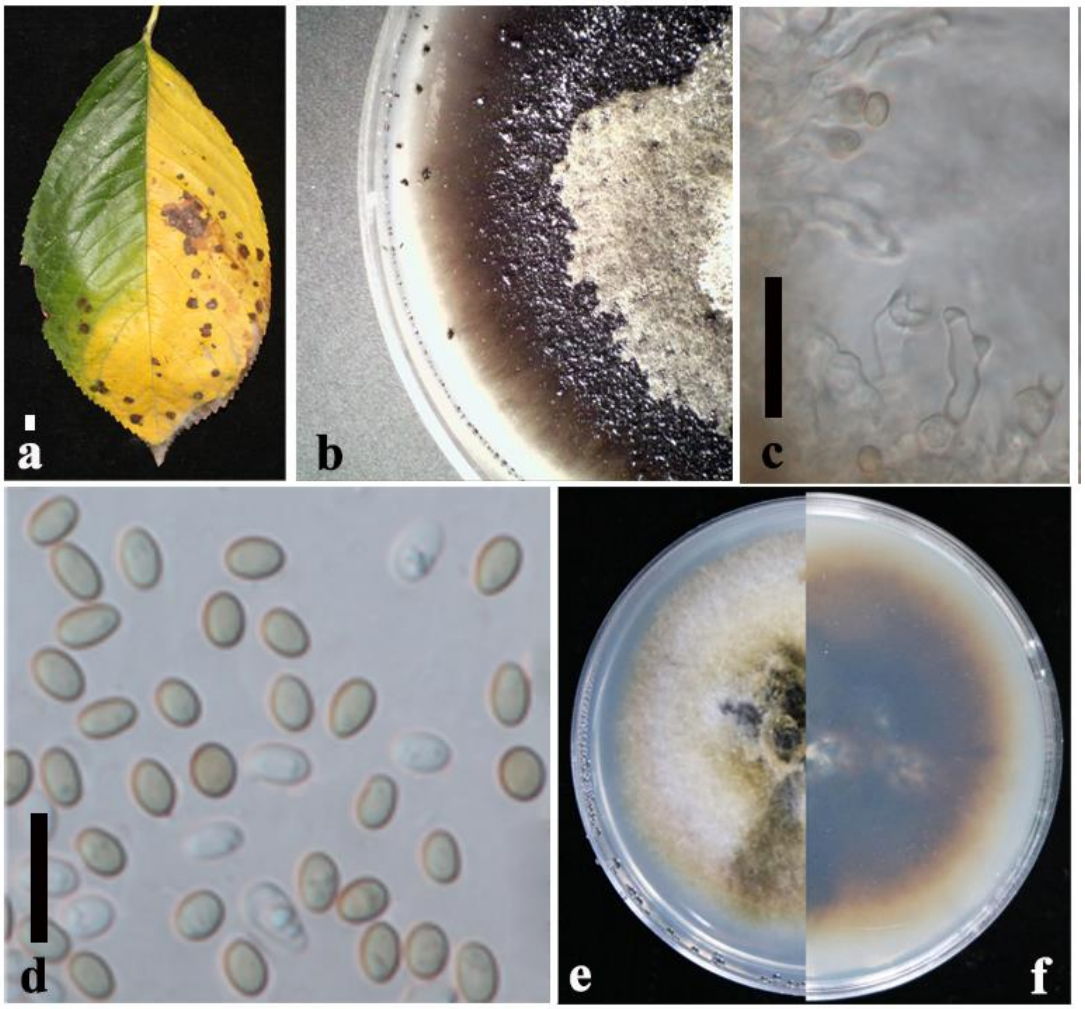


Figure 13 – *Nothophoma quercina* (MFLUCC 18–1588) a Host surface from which the pathogen was isolated. b Pycnidia on agar. c Conidiogenous cells. d Conidia. e, f Upper-view (e) and the reverse view (f) of the colony on PDA. Scale bars: a = 3 mm, c, d = 20 µm.

Culture characteristics – Colonies on PDA reach 80 mm diam. after 7 days at 25 °C, with regular margin, dull white aerial mycelium surface floccose to wooly, with greenish olivaceous to olivaceous near the centre and reverse dark ochreous in the centre and white in the margin.

Material examined – CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (MFLU 18–2666) – living culture, MFLUCC 18–1588, KUMCC 18–0396.

Note – *Nothophoma quercina* has been reported as a saprobe from *Erysiphe alphitoides* Griffon & Maubl., *Quercus* sp., *Ulmus* sp. and *Ziziphus jujuba* Mill. (Aveskamp et al. 2010, Chen et al. 2015, Jianyu et al. 2016) and as a pathogen from *Chaenomeles sinensis* (Thouin) Koehne, *Malus micromalus* Makino, *Phellodendron amurense* Rupr. and *Pistacia vera* L. (Yun et al. 2016, Jiao et al. 2017, Liu et al. 2018, Moral et al. 2018). This is the first record of *N. quercina* reported on *Prunus avium*. When comparing our species with the type specimen of *N. quercina* (CBS 633.92), they are similar in morphology but differ in their host. The conidiomata are larger in size while conidia are smaller in size compared to the type specimen. Based on our phylogenetic analysis of combined ITS, LSU, TUB2 and RPB2 sequence data of Didymellaceae species (Fig. 4), our strain (MFLUCC 18–1588) clustered together with the ex-type strain of *N. quercina* (CBS 633.92) with relatively high bootstrap and Bayesian probabilities (100% ML, 100% MP, 1.00 PP). Base pair comparisons for all the genes between our strain (MFLUCC 18–1588) and ex-type strain of *N. quercina* (CBS 633.92) reveal identical or less than 1% base pair differences.

Stagonosporopsis Died., Annls. Mycol. 10(2): 142 (1912)

Stagonosporopsis Died. was introduced by Diedicke (1912) to separate taxa which occasionally form multi-septate taxa from *Ascochyta*. As no type material was specified, *S. actaeae*, the first species described by Diedicke was considered as the generic type (Boerema et al. 1997, 2004). Aveskamp et al. (2010) recombined *Stagonosporopsis* synanamorphs and proposed an emended description for *Stagonosporopsis.*

Stagonosporopsis citrulli M.T. Brewer & J.E. Stewart, in Stewart, Turner & Brewer, Fungal Biol. 119: 377 (2015) Fig. 14

Facesoffungi number: FoF 04919

Saprobic on diseased leaves of *Prunus avium.* Sexual morph: Undetermined. Asexual morph: *Pycnidia* on the PDA surface, solitary, globose to subglobose, black, immersed or on the surface. *Pycnidial wall* multi-layered, composed of pale brown, pseudoparenchymatous cells, thicker outer layer, and a thinner inner layer. *Conidiogenous cells* phialidic, hyaline, simple, doliiform to ampulliform, variable in size. *Conidia* 4–7.6 × 1.6–3.5 µm ($\bar{x}$ = 6.0 × 2.5 *µm*, n = 40), cylindrical to ellipsoidal, hyaline, aseptate, straight to slightly curved, thin and smooth-walled. Conidial exudates buff.

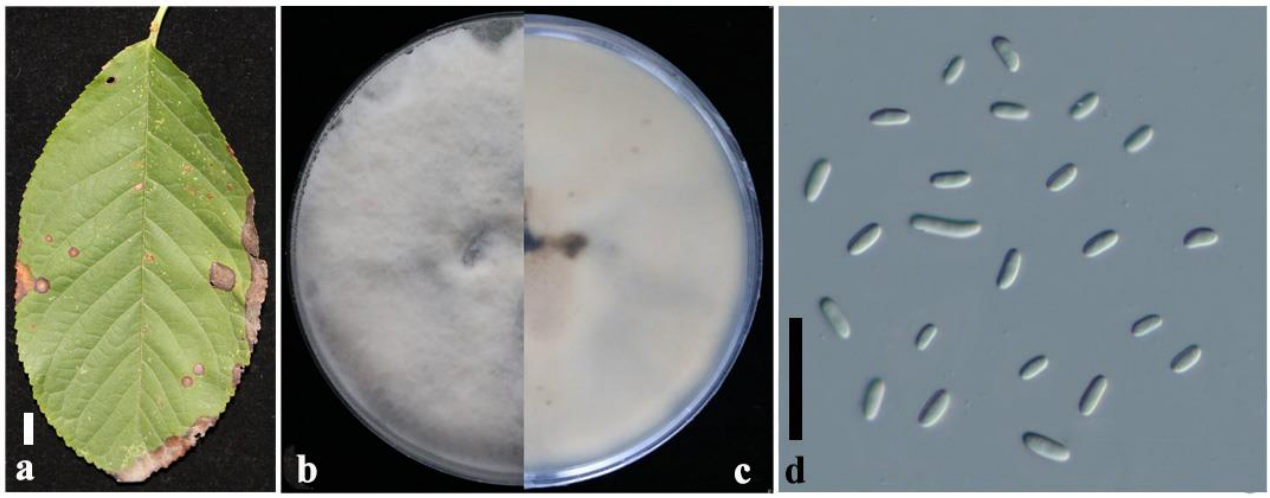


Figure 14 – *Stagonosporopsis citrulli* (MFLUCC 18–1595) a Host surface from which the pathogen was isolated. b, c Upper-view (b) and the reverse view (c) of the colony on PDA. d Conidia. Scale bars: a = 1 cm, d = 20 µm.

Culture characteristics – Colonies on PDA reach 60 mm diam. after 7 days at 25 °C, with regular margin, floccose, white aerial mycelium, with pale olivaceous grey reverse.

Material examined – CHINA, Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (MFLU 18–2669) – living culture, MFLUCC 18–1595, KUMCC 18–0403.

Note – In the current study, a new host record was identified for *S. citrulli* by the phylogenetic analysis of combined ITS, LSU, TUB2 and RPB2 sequence data of Didymellaceae species (Fig. 4). *Stagonosporopsis citrulli* (MFLUCC 18–1595) clusters together with the ex-type of *S. citrulli* (ATCC TSD-2) with relatively high bootstrap and Bayesian probabilities (100% ML, 100% MP, 1.00 PP). A comparison of the 499 nucleotides across the ITS (+5.8S) gene region between *S. citrulli* (JZB380014) and *S. citrulli* (ATCC TSD-2) reveal l.4% base pair difference. Furthermore, comparison of our new taxon with *S. citrulli* (ATCC TSD-2) for base pair differences in the protein coding genes confirmed that it is the same species. There are no base pair difference across 324 nucleotides in TUB2 gene region. When we compared the morphology of our isolate with *S. citrulli* (ATCC TSD-2), both exhibited similar morphological characters (Boerema et al. 2004).

Epicoccum Link, Mag. Gesell. Naturf. Freunde, Belin 7: 32 (1816)

The genus *Epicoccum* was emended by Chen et al. (2015) to incorporate several *Phoma* species with epicoccoid, subcylindrical conidia, and irregular pycnidial conidiomata.

Epicoccum pseudokeratinophilum Chethana, Yan, Li & K.D. Hyde, sp. nov. Fig. 15

MycoBank number: MB828519; Facesoffungi number: FoF04916

Etymology – The specific epithet *pseudokeratinophilum* was given after its resemblance to *Epicoccum keratinophilum*.

Saprobic on diseased leaves of *Prunus avium.* Sexual morph: Undetermined. Asexual morph: *Pycnidia* on the PDA surface, 1.3–2.7 mm ($\bar{x}$ = 1.8 mm, n = 10) diam., solitary, aggregated, glabrous, subglobose, brown, superficial or immersed in the media. *Pycnidial wall* multi-layered, composed of brown to dark brown cells of *textura angularis*. *Conidiogenous cells* phialidic, hyaline, simple, ampulliform to globose. *Conidia* 4.8–6.9 × 1.3–3 µm ($\bar{x}$ = 5.9 × 2.2 µm, n = 40), cylindrical to ellipsoidal, hyaline, aseptate, straight to slightly curved, thin and smooth-walled, mostly with 2 polar guttules and sometimes 3-guttules. Chlamydospores 3.5–5.2 × 2.9–3.6 µm ($\bar{x}$ = 4.4 × 3.2 µm, n = 10), unicellular, pale brown, smooth-walled, disposed singly, globose to subglobose.

Culture characteristics – Colonies on PDA reach 60 mm diam. after 7 days at 25 °C, with regular margin, flattened, dark grey olivaceous surface towards the margin and white in the centre, dark olivaceous black reverse with grey olivaceous margin. *Hyphae* pale brown, smooth- and thin-walled, septate, 2.5–5 µm wide.

Material examined – CHINA. Beijing, on leaf spots of *Prunus avium* L. (Rosaceae), 28 September 2017, K.W.T. Chethana (MFLU 18–2670, holotype) – ex-type culture, MFLUCC 18–1593; *ibid*. (KUMCC 18–0401, isotype).

Note – In our study, we identified an *Epicoccum* species with morphological characters similar to *Epicoccum keratinophilum*. When comparing our species with the type specimen of *E. keratinophilum* (UTHSC: DI16-271), they are similar in morphology. However, conidia are slightly larger in size as compared to the type specimen (4–6 × 1.5–2 µm) and chlamydospores were present in our culture. Based on our phylogenetic analysis of combined ITS, LSU, TUB2 and RPB2 sequence data of *Epicoccum* species (Fig. 4), our strain of *E. pseudokeratinophilum* (MFLUCC 18–1593) clustered basal to the ex-type strain of *Epicoccum keratinophilum* (UTHSC:DI16-271) with relatively high bootstrap and Bayesian probabilities (90% ML, 98 % MP, 0.98 PP). A comparison of the 486 nucleotides across the ITS (+5.8S) gene region between *E. pseudokeratinophilum* (MFLUCC 18–1593) and *Epicoccum keratinophilum* (UTHSC: DI16-271) reveal 2.45% base pair difference. Furthermore, comparison of our new taxon with *Epicoccum keratinophilum* (UTHSC:

DI16-271) for base pair differences in the protein coding genes confirmed its novelty. There are 2.18% base pair difference across 596 nucleotides in RPB_2 gene region and 5.11% base pair difference across 327 nucleotides in TUB2 gene region.

Discussion

With varying severity in different geographical and climatic regions, cherry leaf spot thrives throughout the world with moderately wet conditions and with temperatures above 16 °C (Ogawa & English 1991, Holb 2009, Farr & Rossman 2011, Faust & Surányi 2011, Joshua 2012, Choi et al. 2014). The optimal temperature range for the spread of most of the pathogens is between 16–20 °C (Wilcox 1993, Pederson et al. 2012). Cherry leaf spot is identified as a common disease in Chinese orchards. In China, the fruiting period of cherry falls within the summer rainy season, facilitating the disease spread among the Chinese orchards. During the early summer, initial symptoms appear on the upper surface of leaves and with frequent rains in May and June, fungi spread extremely quickly similar to the observations by Ellis (2008). Disease severity in the orchards differ according to different environmental conditions and sanitary conditions inside the orchards.

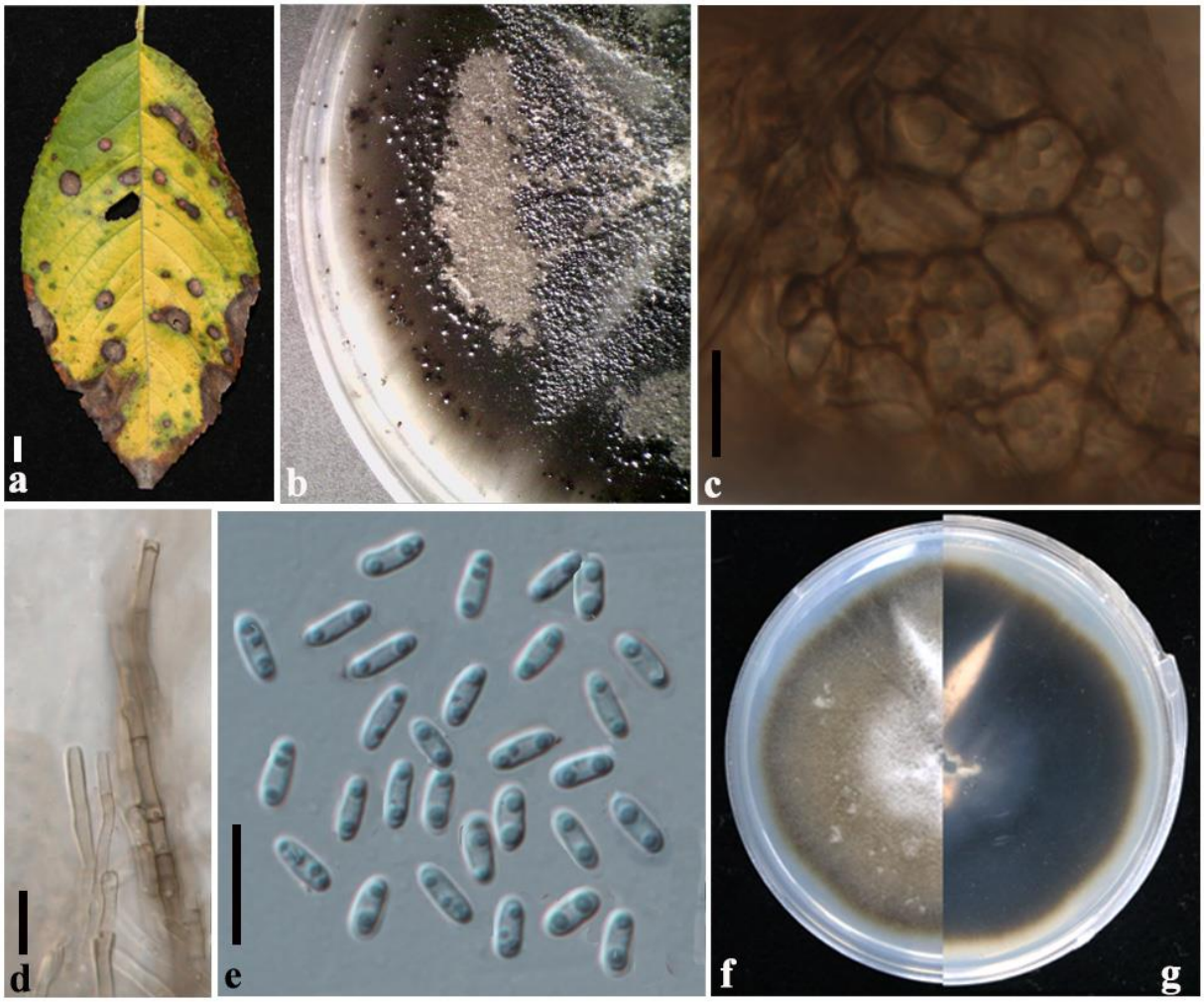


Figure 15 – *Epicoccum pseudokeratinophilum* (MFLUCC 18–1593, holotype) a Host surface from which the pathogen was isolated. b Pycnidia on the PDA. c Pycnidial wall. d Septate hyphae. e Conidia. f, g Upper-view (f) and the reverse view (g) of the colony on PDA. Scale bars: a = 5 mm, c–e = 10 μm.

In the current study, we have obtained 67 isolates from the diseased cherry leaves. The majority belonged to *Alternaria prunicola* (50 isolates) and during the pathogenicity assay, these isolates reproduced identical disease symptoms on detached leaves. Thus, we concluded *A.*

prunicola as the causative agent of the cherry leaf spot in cherry orchards in Beijing. In addition, six isolates of *A. alternata* were isolated from diseased leaves. *Alternaria alternata* has previously been identified as one of the pathogens of Cherry leaf spot in Greece (Thomidis & Tsipouridis 2006) and as the pathogen of black spot disease on cherry fruits (Zhao & Liu 2012). Even though *A. prunicola* is the main pathogen, *Colletotrichum aenigma* and *C. pseudotheobromicola* exhibited a higher level of virulence as compared to *A. prunicola* in the pathogenicity assay. This can be justified as these *Colletotrichum* species are considered to be pathogenic on most of their hosts (Jayawardena et al. 2016). Furthermore, identification of several Didymellaceae species, which are proven to be weakly pathogenic or non-pathogenic during our pathogenicity assay, confirms that these species may cause secondary infections on diseased leaves with cherry leaf spots. In our pathogenicity assay, the disease severity of the cherry leaf spot significantly changed among the cultivars. *Prunus avium* cv. 'Sunburst' exhibited significantly higher resistance as compared to the other two cultivars. Therefore, cultivation of resistant cultivars such as 'Sunburst' can slightly reduce the severity of the disease in the orchards.

For most of the plant diseases, primary inoculum mostly comes from within the orchard through leaf debris, infected plant tissues and from fallen mummified berries. Similarly, for cherry leaf spot, we believe that the primary inoculum came from within the orchard. During our field survey, we observed diseased leaves among the leaf debris on the ground. Furthermore, we were able to isolate *A. prunicola* from the leaf debris, the same pathogen that we isolated from diseased leaves, confirming our observations. Thus, *A. prunicola* can be considered as a true pathogen. It not only relies on the host (*P. avium* leaves) for its growth, but also capable of surviving by overwintering in the leaf debris and contribute significantly in forming the primary inoculum for the disease for the next season. Therefore, if the orchard had been infected previously by the leaf spot pathogen, there is a higher probability for the disease to occur in the new season if proper control measures are not followed. Information on the primary inoculum source, the timing of infection, proper agricultural practices as well as the application of fungicides can help the growers in designing effective disease management strategies. Since the main form of survival for the pathogen is in the leaf litter, removing and destroying the leaf litter can significantly decrease the primary inoculum. Furthermore, pruning is another method practiced by growers to reduce humidity and increase air and light circulation in the orchards. In addition to these agricultural practices, growers and researchers are experimenting the efficacy of different fungicides against cherry leaf spot pathogens (Hamilton et al. 1956, Eisensmith & Jones 1981, Green et al. 2006).

To our knowledge, this is the first study that has identified and characterised the cherry leaf spot pathogen, *A. prunicola* in Beijing, China by morpho-molecular studies and pathogenicity assays. Identification of this new pathogen is important as it is the most critical step in the early detection and monitoring stages of any disease management program (Riley et al. 2002). Correct identification of the pathogen, *A. prunicola*, becomes important as there are many *Alternaria* species occurring on Cherries with varied virulence and varied lifestyles such as pathogens and saprobes (Zhu & Chang 2004, Thomidis & Tsipouridis 2006, Farr & Rossman 2013, Choi et al. 2014). Identification of a new taxon indicates that a new pathogen has been evolved under the existing chemical and traditional control methods. Therefore, a new control strategy must be designed by combining chemical and agricultural approaches. Studies must be designed to confirm the efficacy of traditional fungicides such as Captan, Chlorothalonil, Tebuconazole, and Trifloxystrobin (Joshua 2012) against leaf spot pathogen *A. prunicola*. Furthermore, representative isolates used in our pathogenicity study (JZB3180002, MFLUCC 18–1598, MFLUCC 18–1596 and MFLUCC 18–1597, MFLUCC 18–1599) could be utilized in screening effective fungicides against our new pathogenic taxon. Another successful preventive strategy is to cultivate cherry leaf spot resistant cultivars (Riley et al. 2002). However, according to the previous research conducted against cherry leaf spot caused by *Blumeriella jaapii*, all the sweet cherry cultivars were susceptible to the disease, whereas several sour cherry cultivars such as 'Morina', 'Köröser Gierstädt', 'Hartai' and 'Karneol' showed some resistance (Schuster & Tobutt 2004). In addition to integrating chemical control with proper agricultural practices, a diversified leaf spot disease management

strategy can be implemented in cherry orchards in Beijing. In the current study, we only investigated cherry growing areas in Beijing. In future studies, the disease sample collection area should be further expanded to all the provinces in China. If possible, *Alternaria cerasi* cultures previously identified in China based only on morphological characters should be re-investigated. Research can now focus on species population dynamics and disease epidemiology to design more effective disease management strategies against these pathogens.

Acknowledgements

The project was funded by Beijing Talent Program for Dr. Jiye Yan and JNKYT201605. We would like to thank cherry cultivators for providing disease samples, R. S. Jayawardena and Ms. Wang Jing for their assistance in sample collection and providing isolates for the study. K.D. Hyde thanks Kunming Institute of Botany, Chinese Academy of Sciences (project 2013T2S0030, for the Visiting Professorship for Senior International Scientists.

References

Abdel-Wahab FA, Bahkali AHA, El-Gorban AM, Hodhod MS. 2017 – Natural products of *Nothophoma multilocularis* sp. nov. an endophyte of the medicinal plant Rhazya stricta. Mycosphere 8, 1185–1200.

Ariyawansa HA, Thambugala KM, Manamgoda DS, Jayawardena R et al. 2015 – Towards a natural classification and backbone tree for Pleosporaceae. Fungal Diversity 71, 85–139.

Aveskamp MM, de Gruyter J, Woudenberg JHC, Verkley GJM, Crous PW. 2010 – Highlights of the Didymellaceae: a polyphasic approach to characterize Phoma and related pleosporalean genera. Studies in Mycology 65, 1–60.

Berbee ML, Pirseyedi M, Hubbard S. 1999 – *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 91, 964–977.

Boerema GH, de Gruyter J, Noordeloos ME. 1997 – Contributions towards a monograph of *Phoma* (Coelomycetes) — IV. Section *Heterospora*: Taxa with large sized conidial dimorphs, in vivo sometimes as Stagonosporopsis synanamorphs. Persoonia 16, 335–371.

Boerema GH, De Gruyter J, Noordeloos ME, Hamers MEC. 2004 – Phoma identification manual: differentiation of specific and intraspecific taxa in culture. CABI Publishing, Wallingford.

Carbone I, Kohn LM. 1999 – A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91, 553–556.

Chen Q, Jiang JR, Zhang GZ, Cai L, Crous PW. 2015 – Resolving the *Phoma* enigma. Studies in Mycology 82, 137–217.

Chen Q, Hou LW, Duan WJ, Crous PW, Cai L. 2017 – Didymellaceae revisited. Studies in Mycology 87, 105–159.

Chethana KWT, Zhou Y, Zhang W, Liu M, Xing QK et al. 2017. *Coniella vitis* sp. nov. is the common pathogen of white rot in Chinese vineyards. Plant Disease 101, 2123–2136.

Choi HW, Lee YK, Hong SK. 2017 – First report of *Colletotrichum aenigma* causing anthracnose on Sedum kamtschaticum in Korea. Plant Disease 101, 2150.

Choi IY, Braun U, Park JH, Shin HD. 2014. First report of leaf spot caused by *Pseudocercospora pruni-persicicola* on Sweet Cherry in Korea. Plant Disease 98, 693.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO et al. 2006 – Phylogenetic lineages in the Botryosphaeriaceae. Studies in Mycology 55, 235–253.

Crous PW, Wingfield MJ, Burgess TI, Carnegie AJ, Hardy GEStJ et al. 2017 – Fungal Planet description sheets: 625–715. Persoonia 39, 270–467.

Dagno K, Crovadore J, Lefort F, Lahlali R, Lassois L et al. 2011 – *Alternaria jacinthicola*, a new fungal species causing blight leaf disease on water hyacinth [*Eichhornia crassipes* (Martius) Solms-Laubach]. Journal of Yeast Fungal Research 2, 99–105.

Diao YZ, Zhang C, Liu F, Wang WZ, Liu L et al. 2017 – *Colletotrichum* species causing anthracnose disease of chili in China. Persoonia 38, 20–37.
Díaz R, Zas R, Fernández-lópez, J. 2007 – Genetic variation of *Prunus avium* in susceptibility to cherry leaf spot (*Blumeriella jaapii*) in spatially heterogeneous infected seed orchards. Annals of Forest Science 64, 21–30.
Diedicke H. 1912 – Die Abteilung Hyalodidymae der Sphaerioideen. Annals of Mycology 10, 135–152.
Eisensmith SP, Jones AL. 1981 – A model for detecting infection periods of *Coccomyces hiemalis* on sour cherry. Phytopathology 71, 728–732.
Ellis MB. 1971 – Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, UK.
Ellis AM. 2008 – Cherry leaf spot. Fact Sheet Agriculture and Natural Resources. Ohio State University Extension, Columbus OH, USA. Accessed on June 2, 2018, at URL: https://ohioline.osu.edu.
Farr DF, Rossman AY. 2013 – Fungal Databases. Systematic Mycology and Microbiology Laboratory, Online publication, ARS, USDA, Accessed on August 24, 2018.
Faust M, Surányi D. 2011 – Origin and Dissemination of Cherry. In: Janick, J. 2011 – Origin and Dissemination of *Prunus* Crops: Peach, Cherry, Apricot, Plum, Almond. International Society for Horticultural Science, Belgium.
FAO. 2018 – FAOSTAT database collections. Food and Agriculture Organization of the United Nations. Rome. Accessed on August 24, 2018, at URL: http://faostat.fao.org.
Garcia SM, Jones AL. 1993 – Influence of temperature on apothecial development and ascospore discharge by *Blumeriella jaapii*. Plant Disease 77, 776–779.
Green H, Bengtsson M, Duval X, Pedersen HL, Hockenhull J et al. 2006 – Influence of urea on the cherry leaf spot pathogen, *Blumeriella jaapii*, and on microorganisms in decomposing cherry leaves. Soil Biology & Biochemistry 38, 2731–2742.
Hall TA. 1999 – BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.
Hamilton JM, Szkolnik M, Sondheimer E. 1956 – Systemic control of cherry leaf-spot fungus by foliar sprays of Actidione derivatives. Science 123, 1175–1176.
Han YC, Zeng XG, Xiang FY, Ren L, Chen FY et al. 2016 – Distribution and characteristics of *Colletotrichum* spp. associated with anthracnose of strawberry in Hubei, China. Plant Disease 100, 996–1006.
Heald FD. 1993 – Manual of Plant Diseases, McGraw-Hill, New York.
Higgins BB. 1914 – Contribution to the life history and physiology of *Cylindrosporium* on stone fruit. American Journal of Botany 1, 145–166.
Hillis DM, Bull JJ. 1993 – An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42, 182.
Holb IJ. 2009 – Some biological features of cherry leaf spot (*Blumeriella jappii*) with special reference to cultivar susceptibility. International Journal of Horticultural Sciences 15, 91–93.
Hong SG, Cramer RA, Lawrence CB, Pryor BM. 2005 – Alt-a 1 allergen homologs from *Alternaria* and related taxa: analysis of phylogenetic content and secondary structure. Fungal Genetics & Biology 42, 119–129.
Hyde KD, Cai L, Canon PE, Crouch JA, Crous PW et al. 2009 – *Colletotrichum*: a catalogue of confusion. Fungal Diversity 39, 1–17.
Hyde KD, Norphanphoun C, Abreu VP, Bazzicalupo A, Chethana KWT et al. 2017 – Fungal diversity notes 603–708: Taxonomic and phylogenetic notes on genera and species. Fungal Diversity 87, 1–235.
Hyde KD, Chaiwan N, Norphanphoun C, Boonmee S, Camporesi E et al. 2018 – Mycosphere notes 169–224. Mycosphere 9, 271–430.
Index Fungorum 2018 – Accessed on August 22, 2018, at URL: http://www.indexfungorum.org/.

Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat DJ, Buyck B et al. 2015 – The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74, 3–18.
Jayasiri SC, Hyde KD, Jones EBG, Jeewon R. 2017 – Taxonomy and multigene phylogenetic evaluation of novel species in *Boeremia* and *Epicoccum* with new records of *Ascochyta* and *Didymella* (Didymellaceae). Mycosphere 8, 1080–1101.
Jayawardena RS, Hyde KD, Damm U, Cai L, Liu M et al. 2016 – Notes on currently accepted species of *Colletotrichum*. Mycosphere 7, 1192–1260.
Jeewon R, Hyde KD. 2016 – Establishing species boundaries and new taxa among fungi: recommendations to resolve taxonomic ambiguities. Mycosphere 7, 1669–1677.
Jianyu B, Xiaoming W, Yanjiang S, Canxing D. 2016 – Occurrence and identification of *Nothophoma quercina* causing brown spot of jujube in China. Canadian Journal of Plant Pathology 38, 527–532.
Jiao WL, Zhou RJ, Fu JF, Xu HJ, Hao N. 2017 – First report of *Nothophoma quercina* causing leaf spot disease of *Phellodendron amurense* in China. Plant Disease 101, 1820–1821.
Jones AL, Sutton TB. 1996 – Diseases of tree fruits in the east. Northcentral Regional Bulletin 45, Michigan State University.
Joshua J. 2012 – Studies on the Cherry Leaf Spot Disease in Flowering Cherries (*Prunus* species). PhD Thesis, Tennessee State University, Tennessee, United States.
Joshua J, Mmbaga MT. 2013 – Perpetuation of Cherry Leaf Spot Disease in Ornamental Cherry. Journal of Phytopathology 163, 194–201.
Katoh K, Toh H. 2008 – Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9, 276–285.
Khan NA, Bhat MA, Ahmad K, Qazi NA. 2014 – First record of Cherry Leaf Spot caused By *Cylindrosporium padi* (P. Karst.) from India. Applied Biological Research 16, 122–123.
Kirk PM, Cannon PF, David JC, Stalpers JA. 2001 – Ainsworth and Bisby's dictionary of the fungi, 8th edn. CABI Publishing, London.
Kishino H, Hasegawa M. 1989 – Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data. Journal of Molecular Evolution 29, 170–179.
Li M, Qi W, Cai QX, Guang DX, Yan Z et al. 2011 – Symptoms and treatment of leaf diseases of Cherry (in Chinese). Moderate Rural Science Technology 9, 35.
Liu YL, Whelen S, Hall BD. 1999 – Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology & Evolution 16, 1799–1808.
Liu M, Zhang W, Manawasinghe IS, Zhou Y, Xing QK et al. 2018 – First report of *Nothophoma quercina* causing trunk canker on crab apple (*Malus micromalus*) in China. Plant Disease 102, 1462.
Maharachchikumbura SN, Hyde KD, Jones EBG, McKenzie EHC, Huang SK et al. 2015 – Towards a natural classification and backbone tree for Sordariomycetes. Fungal Diversity 72, 199–301.
Maharachchikumbura SN, Hyde KD, Jones EBG, McKenzie EHC, Jayarama B et al. 2016 – Families of Sordariomycetes. Fungal Diversity 79, 1–317.
McAlpine D. 1902 – Fungus Diseases of stone-fruit trees in Australia and their treatment. Department of Agriculture, Victoria, Australia.
Moral J, Lichtemberg PSF, Papagelis A, Sherman J, Michailides TJ. 2018 – *Didymella glomerata* causing leaf blight on pistachio. European Journal of Plant Pathology 151, 1095–1099.
Nag Raj TR, Ponnappa KM. 1970 – Blight of water-hyacinth caused by *Alternaria eichhorniae* sp. nov. Transactions of the British Mycological Society 55, 123–130.
Nees von Esenbeck CG. 1816 – Das system der pilze und schwämme. Wurzburg, Germany.
Nylander JAA. 2004 – MrModeltest 2.0. Program distributed author. Evolutionary Biology Centre, Uppsala University.

O'Donnell K, Cigelnik E. 1997 – Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7, 103–116.
Ogawa JM, English H. 1991 – Diseases of temperate zone tree fruit and nut crops. Division of Agriculture and Natural Resources, University of California, Oakland, USA.
Page RDM. 1996 – TreeView: an application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12, 357–358.
Pedersen HL, Jensen B, Munk L, Bengtsson MV, Trapman M. 2012 – Reduction in the use of fungicides in apple and sour cherry production by preventative methods and warning systems. Pesticides Research 139, 14–71.
Rehner SA, Samuels GJ. 1994 – Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98, 625–634.
Riley MB, Williamson MR, Maloy O. 2012 – Plant disease diagnosis. The Plant Health Instructor, DOI: 10.1094/PHI-I-2002-1021-01.
Rojas EI, Rehner SA, Samuels GJ, Van Bael SA, Herre EA et al. 2010 – *Colletotrichum gloeosporioides* associated with *Theobroma cacao* and other plants in Panama: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. Mycologia 102, 1318–1338.
Ronquist F, Huelsenbeck JP. 2003 – MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572.
Schena L, Mosca S, Cacciola SO, Faedda R, Sanzani SM et al. 2014 – Species of the *Colletotrichum gloeosporioides* and *C. boninense* complexes associated with olive anthracnose. Plant Pathology 63, 437–446.
Schuster M, Tobutt KR. 2004 – Screening of cherries for resistance to leaf spot, *Blumeriella jaapii*. Acta horticulture 663, 239–244.
Scott RR, Bugang W, Zhen X, Lesny V. 2014 – Annual Report on Stone Fruit in People's Republic of China. Foreign Agricultural Service, United States Department of Agriculture. USA.
Shu gui X. 2007 – Occurrence and Control of Cherry leaf spot. Proceedings of China Meeting, 2007.
Silvestro D, Michalak I. 2010 – RaxmlGUI: a graphical front-end for RAxML. Accessed on 29 September 2015, at URL http://sourceforge.net/projects/raxmlgui/
Sivanesan A. 1990 – CMI description sheets: Set 99. Mycopathlogia 109, 41–62.
Sjulin TM, Jones AL, Andersen RL. 1989 – Expression of partial resistance to cherry leaf-spot in cultivars of sweet, sour, duke, and European ground cherry. Plant Disease 73, 56–61.
Stewart JE, Turner AN, Brewer MT. 2015 – Evolutionary history and variation in host range of three *Stagonosporopsis* species causing gummy stem blight of cucurbits. Fungal Biology 119, 370–382.
Sung GH, Sung JM, Hywel-Jones N, Spatafora JW. 2007 – A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap approach. Molecular Phylogenetics and Evolution 44, 1204–1223.
Swofford DL. 2002 – PAUP* 4.0: phylogenetic analysis using parsimony (* and other methods). Sinauer Associates, Sunderland.
Templeton MD, Rikkerink EHA, Solon SL, Crowhurst RN. 1992 – Cloning and molecular characterization of the glyceraldehyde-3-phosphate dehydrogenase encoding gene and cDNA from the plant pathogenic fungus *Glomerella cingulata*. Gene 122, 225–230.
Thambugala KM, Wanasinghe DN, Phillips AJL, Camporesi E, Bulgakov T et al. 2017 – Mycosphere notes 1–50: Grass (Poaceae) inhabiting Dothideomycetes. Mycosphere 8, 697–796.
Thomidis T, Tsipouridis C. 2006 – First Report of *Alternaria* Leaf Spot on Cherry Trees in Greece. Plant Disease 90, 680.

Tibpromma S, Hyde KD, Jeewon R, Maharachchikumbura SSN, Liu JK et al. 2017 – Fungal diversity notes 491–602: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 83, 1–261.
Tibpromma S, Hyde KD, Bhat JD, Mortimer PE, Xu J et al. 2018 – Identification of endophytic fungi from leaves of Pandanaceae based on their morphotypes and DNA sequence data from southern Thailand. MycoKeys 33, 25–67.
USDA 2017 – USDA Global Analysis - Fresh Peaches and Cherries: World Markets and Trade. Foreign Agricultural Service, United States Department of Agriculture. USA.
Uyemoto JK, Ogawa JM, Jaffee BA. 2018 – Common Names of Plant Diseases: Diseases of Sweet Cherry (*Prunus avium* L.) and Sour Cherry (*P. cerasus* L.). The American Phytopathological Society. USA.
Valenzuela-Lopez N, Cano-Lira JF, Guarro J, Sutton DA, Wiederhold N et al. 2018 – Coelomycetous Dothideomycetes with emphasis on the families Cucurbitariaceae and Didymellaceae. Studies in Mycology 90, 1–69.
Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172, 4238–4246.
Wanasinghe DN, Phukhamsakda C, Hyde KD, Jeewon R, Lee HB. 2018 – Fungal diversity notes 709–839: taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on Rosaceae. Fungal Diversity 89, 1–236.
Weir BS, Johnston PR, Damm U. 2012 – The *Colletotrichum gloeosporioides* species complex. Studies in Mycology 73, 115–180.
White TJ, Bruns T, Lee S, Taylor JW. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, (Eds.) PCR protocols: A guide to methods and applications. Academic, New York, pp. 315–322.
Wilcox WF. 1993 – Cherry Leaf Spot. Integrated Pest Management, Cornell University. Retrieved on February 21, 2019 from www.nysipm.cornell.edu/factsheets/treefruit/diseases/cls/cls.pdf
Woudenberg JHC, Groenewald JZ, Binder M, Crous PW. 2013 – *Alternaria* redefined. Studies in Mycology 75, 171–212.
Woudenberg JHC, Sedi MF, Groenewald JZ, Vries Mde, Stielow JB et al. 2015 – *Alternaria* section *Alternaria*: Species, formae speciales or pathotypes?. Studies in Mycology 82, 1–21.
Yan JY, Jayawardena MMRS, Goonasekara ID, Wang Y, Zhang W et al. 2015 – Diverse species of *Colletotrichum* associated with grapevine anthracnose in China. Fungal Diversity 71, 233–246.
Yun YH, Oh MH. 2016 – First report of *Nothophoma quercina* causing shoot canker on *Chaenomeles sinensis* in Korea. Plant Disease 100, 2533–2534.
Zhao YZ, Liu ZH. 2012 – First report of black spot disease caused by *Alternaria alternata* on cherry fruits in China. Plant Disease 96, 1580.
Zhu J, Chang Y. 2004 – Cherry black ring spot pathogen identification and biological study (in Chinese). China Fruits 3, 9–12.