Mycosphere 7(8) 1147-1163(2016) www.mycosphere.org ISSN 2077 7019
Article
Doi 10.5943/mycosphere/si/2c/6

An account of *Colletotrichum* species associated with strawberry anthracnose in China based on morphology and molecular data

Jayawardena RS[a, b, d, *], **Huang JK**[a, *], **Jin BC**[a], **Yan JY**[b], **Li XH**[b], **Hyde KD**[c], **Bahkali AH**[e], **Yin SL**[a], **Zhang GZ**[a, #]

[a]*College of Plant Protection, China Agricultural University, Beijing 100193, People's Republic of China*
[b]*Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, People's Republic of China*
[c]*Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, People's Republic of China*
[d]*Centre of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[e]*Botany and Microbiology Department, College of Science, King Saud University, Riyadh, KSA 11442, Saudi Arabia*

*****These authors contributed equally to the work.

Jayawardena RS, Huang JK, Jin BC, Yan JY, Li XH, Hyde KD, Bahkali AH, Yin SL, Zhang GZ 2016 – An account of *Colletotrichum* species associated with strawberry anthracnose in China based on morphology and molecular data. Mycosphere 7(8) 1147–1163, Doi 10.5943/mycosphere/si/2c/6

Abstract

Strawberry anthracnose is an important disease in China that results in significant economic losses. A number of *Colletotrichum* species are known to be pathogens of strawberry. A survey of strawberry fields in eight provinces of China was carried out to identify the causal agents of strawberry anthracnose. The disease mainly causes crown rot, leading to plant wilt and death in the nursery stage, after transplanting in green house, which makes anthracnose a major threat to strawberry production and quality. Multi-locus sequence analysis coupled with morphological assessment revealed that the disease-associated taxa belong to two *Colletotrichum* species complexes: *Colletotrichum nymphaeae* (acutatum species complex), *C. fructicola* and *C. changpingense sp. nov.* (gloeosporioides species complex). The novel species is introduced in this paper and illustrated. The new species is closely related to *C. theobromicola* and pathogenicity tests proved that it is pathogenic to the strawberry crown, fruits and leaves.

Keywords – *C. nymphaeae* – crown rot – molecular phylogeny – morphology – pathogen

Introduction

Strawberry (*Fragaria*× *ananassa*) is a major crop cultivated in China (Xie et al. 2010). Strawberry production has expanded rapidly in recent years and China has become one of the world's largest strawberry producers and exporters. Strawberry cultivation occupies 100.5 × 10^3 ha with a yield of 2.8 million tonnes in 2013 (The Ministry of Agriculture of the People's Republic of China 2014). With the increase of production, many fungal diseases have become a major problem in strawberry production, reducing fruit yield and quality (Mass 1998, Smith 2008). One of the most important diseases of strawberry is anthracnose, which is caused by species of *Colletotrichum.*

Submitted 31 October 2016, Accepted 28 November 2016, Published online 6 December 2016
Corresponding Author: GZ Zhang – e-mail – zhanggzh@cau.edu.cn

Colletotrichum is one of the most important plant pathogenic genera worldwide (Cannon et al. 2012, Hyde et al. 2014, Nilsson et al. 2014, Yan et al. 2015), occurring predominantly in tropical and subtropical regions on a wide range of crops (Waller 1993, Hyde et al. 2009 a, b, 2014). A summary of names (Hyde et al. 2009a) and a review of the confusion with names in the genus (Hyde et al. 2009b), helped to kick start a series of studies that used molecular phylogeny and morphology, that have helped to define species in the genus (Cannon et al. 2012). Anthracnose caused by *Colletotrichum* species is a major disease affecting strawberry production across the world (Mass 1998). *Colletotrichum* species infect all plant parts causing serious crown rot, irregular leaf spots, necrotic lesions on the petioles and runners, as well as black spot on the fruits (Mass 1998). Anthracnose can cause up to 80% of plant death in nurseries and over 50% of yield losses in strawberry fields (Sreenivasaprasad & Talhinhas 2005). For successful implementation of any resistance breeding and disease management programme, accurate pathogen identification is important (Freeman et al. 1998).

Strawberry anthracnose was first reported in China as late as 1990 (Hu 1990). Shao (1992) reported strawberry anthracnose in Shanghai for the first time and identified the pathogen as *C. acutatum* (Dai et al. 2006). Zhang et al. (2007) contributed to these findings by identifying *C. gloeosporioides* and *C. fragariae* (syn *C. theobromicola*) as the causal agents based on morphological characters. Ren et al. (2008, 2011) concluded that these three species are the main pathogens causing anthracnose of strawberry in China. Crown rot is the most severe disease problem in most strawberry producing areas in China (Ren et al. 2008). In the Zhejiang region, nearly 50% of seedling deaths in nurseries and over 40% of yield losses in strawberry fields has been recorded (Xie et al. 2010). The yield of greenhouse cultivated strawberries was reduced by over 10% due to crown rot in Donggang city in Laoning Province in 2009 (Wang 2013). The average incidence of crown rot in green houses of Hubei Province was 41.7% in 2011 (Xiang et al. 2012). However, the majority of studies conducted in China regarding this disease were mainly based on morphology and single gene (mainly ITS) analysis (Dai et al. 2006, Ren et al. 2008, 2011). Due to the overlapping characters, species delimitation based on morphology alone is difficult in this genus. Use of multi-gene analysis combined with morphology can provide a better resolution (Cai et al. 2009, Hyde et al. 2014). Therefore the objective of this study was to identify the pathogens causing strawberry anthracnose in China using a morphological and multi-gene approach.

Materials and Methods

Sample collection, Isolation and Identification of fungi

Disease samples showing anthracnose symptoms were collected from different locations (Anhui, Hainan, Hebei, Hubei, Liaoning, Shandong Provinces and Beijing, Shanghai cities) in China during 2011–2014. To promote fungal development and facilitate isolation, samples were incubated in a clean polythene bag with sterilized tissue dipped in distilled water. Samples were surface sterilized in 3.5% Sodium hypochlorite for 1–2 minutes and then rinsed three times in sterilized water before culturing on Potato Dextrose Agar (PDA) medium at 28 °C. Single germinating conidia were transferred to fresh PDA medium and incubated at 28 °C to obtain pure colonies of the fungi (Chomnunti et al. 2014). The pure isolates were maintained on PDA medium with sterilized filter paper and incubated for 7–10 days at 28 °C. Cultures on the filter papers were dried and stored at -20 °C for further study. Fungal mycelia and spores were observed and photographed using a ZEISS Imager M1 microscope and measurements were made on 40 conidia. All microscopic measurements were made with the Axio vision v 4.8 and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, The United States). Facesoffungi and Index Fungorum numbers are provided (Jayasiri et al. 2015, Index Fungorum 2016).

DNA extraction, PCR amplification and DNA sequencing

Total genomic DNA was extracted by the modified protocol of Damm et al. (2008). Total genomic DNA was extracted from fresh mycelium (500 mg), scraped from the margin of a colony on a PDA plate incubated at 28 °C for 7–10 days. The 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS), a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), partial sequences of the chitin synthase 1 (CHS-1), actin (ACT) and β-tubulin genes were amplified using primer pairs ITS1/ITS4 (White et al. 1990), GDF/GDR (Templeton et al. 1992), CHS79F/CHS345R (Carbone & Kohn 1999), ACT512F/ACT783R (Carbone & Kohn 1999) and BT1/BT2 (O'Donnell & Cigelnik 1997) respectively. PCR was performed in a BIORAD 1000 Thermal Cycler in a total volume of 25 μl. PCR mixtures contained TaKaRa Ex-Taq DNA polymerase 0.3 μl, 12.5 μl of 2 × PCR buffer with 2.5 μl of dNTPs, 1μl of each primer, 9.2 μl of double-distilled water and 100–500 ng of DNA template. The thermal cycling program followed that reported in Weir et al. (2012). The PCR products were visualised by staining with Ethidium bromide on 1.2% agarose electrophoresis gels and purified according to the manufacturer's instructions of a Qiagen purification kit (Qiagen, USA). DNA sequencing of the genes was conducted by Sunbiotech Company Ltd., Beijing, China. The sequence data from this study are placed in GenBank.

Phylogenetic Analysis

Consensus sequences from the sequences generated from forward and reverse primers were obtained using DNAStar v.5.1 and SeqMan v.5.00 (Burland 2000). A combined dataset of the five gene regions was prepared using Clustal X1.81 (Thompson et al. 1997). Further alignment of sequences was achieved using default settings in MAFFT v.7 (Katoh & Toh 2008, http://mafft.cbrc.jp/alignment/server/) and manual adjustment was conducted using BioEditv.7.0.9.0 (Hall 1999) where necessary. Two separate phylogenetic trees were constructed for the acutatum species complex and for the gloeosporioides species complex. Maximum Parsimony analysis (MP) was performed using PAUP v. 4.0b10 (Swofford 2002) to obtain the most parsimonious trees. Gaps were treated as missing data and ambiguously aligned regions were excluded. Trees were inferred using the heuristic search option with Tree Bisection Reconnection (TBR) branch swapping and 1000 random sequence additions. Maxtrees were set up to 5,000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Rescaled Consistency index [RC], and Homoplasy index [HI]) were calculated for trees generated under different optimality criteria. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications resulting from maximum parsimony analysis (Hillis & Bull 1993). Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989) were performed in order to determine whether trees were significantly different.

Bayesian inference (BI) was used in addition to construct the phylogenies using Mr. Bayes v.3.1.2 (Ronquist et al. 2003). MrModeltest v. 2.3 (Nylander 2004) was used to carry out statistical selection of best-fit model of nucleotide substitution. HYK+I model was selected for ITS, a HKY+G model for GAPDH and β-tubulin, a K80+I+G model for CHS-1, a GTR+G model for ACT were incorporated into the analysis. Six simultaneous Markov chains were run for 1000000 generations and trees were sampled every 100th generation. The 2000 trees representing the burn-in phase of the analyses were discarded and the remaining 8000 trees used for calculating posterior probabilities (PP) in the majority rule consensus tree. The alignments and trees are deposited in TreeBASE (Sanderson et al. 1994) under accession numbers S20238 respectively. The fungal strains used for the phylogenetic analysis in this study are listed in Table 1.

Genealogical concordance phylogenetic species recognition (GCPSR) analysis

New species and their most closely related species were analysed using the GCPSR model. A pairwise homoplasy index (PHI) (Philippe & Bryant 2006) test was performed in SplitsTree4 (Huson 1998, Huson & Bryant 2006) as described by Quaedvlieg et al. (2014), in order to determine the recombination level within phylogenetically closely related species using a five-locus

concatenated dataset for *C. changpingense* and its related species. If the pairwise homoplasy index is below a 0.05 threshold (Φw < 0.05), it indicated that there is a significant recombination present in the dataset. The relationships between closely related species were visualised by constructing a split graph, using both the LogDet transformation and splits decomposition options (Fig. 6).

Pathogenicity test

In order to test the pathogenicity of the new species, detached fruits and leaves inoculations were conducted. To determine whether this new species can infect the rhizome (crown), seedling inoculations were carried out.

Detached fruits and leaves inoculation

Healthy strawberry fruits, leaves and seedlings were used for the pathogenicity test. Fruits and leaves were surface sterilized with 75% ethanol and washed three times with distilled water. Fifteen leaves and 15 fruits per isolate were wounded by means of sterilized insect needle, while 15 leaves and 15 fruits remained non-wounded. Conidial suspension (10^5/mL, emended by a haemocytometer) was applied on the wounded and non-wounded fruits and uniformly sprayed onto the leaves. Control fruits and leaves were inoculated with sterilized water. The inoculated fruits and leaves with the controls were put into a plastic box covered with plastic film and incubated at 25 °C.

Seedling/ Rhizome inoculation

In order to check the pathogenicity towards the strawberry rhizome, 30 strawberry seedlings planted in plastic pots were inoculated with spore suspension. Each seedling was planted in one pot and a 100mL spore suspension was added. The control seedlings were inoculated with sterilized water. The inoculated seedlings and the controls were kept in a growth chamber at 25 °C with a 12 h day/ night.

Results

Isolation of fungi

Colletotrichum species were isolated from strawberry fruits, petioles, rhizomes and stolons showing typical anthracnose symptoms (Fig. 1). One hundred and twenty-one isolates were obtained from the disease samples and are deposited in the China General Microbiological Culture Collection (CGMCC) / Mae Fah Luang University Culture Collection, Thailand (MFLUCC) and the China Agricultural University Beijing (SA), China.

Phylogenetic analysis

Twenty-one representative strains were used in the analysis. Phylogenies were reconstructed using combined ACT, GAPDH, CHS, ITS and β-tubulin sequence data for our isolates of *Colletotrichum* with those in recent publications on *Colletotrichum* (Damm et al. 2012, Weir et al. 2012, Sharma et al. 2013, Yan et al. 2015, Diao et al. 2017). A single phylogenetic tree was constructed for all *Colletotrichum* strains including our sequence data using five combined gene alignments. The tree was compared with the backbone tree in Hyde et al. (2014) and species narrowed down to the acutatum and gloeosporioides species complexes. Two separate phylogenetic trees were therefore constructed for the complexes. Maximum- parsimony and Bayesian inference produced nearly identical topologies (Bayesian trees are not shown).

The combined gene alignment for the acutatum complex comprised 37 taxa and 1712 characters including gaps with *C. orchidophilum* (CBS 632.80) as the outgroup taxon. Parsimony analysis indicated that 1249 characters were constant, 229 variable characters uninformative and 234 characters parsimony-informative. The parsimony analysis of the data matrix yielded a single most parsimonious tree (TL = 689, CI = 0.729, RI = 0.820, RC = 0.597, HI = 0.271) which is presented in Fig 2. Five isolates (SA0017, SA0024, SA0041, SA0069 and SA0070) clustered together with *C. nymphaeae* with strong support.

Fig. 1 – Symptoms of strawberry anthracnose a. Wilted seedling plants at early disease stage in greenhouse, b. Wilted and dead plants in the field, c. Dead plants with crown rot symptoms, d. Reddish brown tissue of a strawberry crown infected by *Colletotrichum* sp.

The combined gene alignment for the gloeosporioides complex comprised 52 taxa and 1932 characters including gaps with *C. boninense* (CBS 123755) as the outgroup taxon. Parsimony analysis indicated that 1350 characters were constant, 318 variable characters parsimony-uninformative and 264 characters parsimony-informative. Parsimony analysis resulted in 100 most parsimonious trees, one of them (TL = 1040, CI = 0.696, RI = 0.779, RC = 0.542, HI = 0.304) is shown in Fig. 3 where 15 isolates obtained in this study clustered together with *C. fructicola* (CBS 130416) with a high support, while isolates SA0016 and SA0050 are sister to the clade that contains *C. grevilleae*, *C. grossum* and *C. theobromicola* also with high support (100/1.00).

Pathogenicity studies

Detached fruits and leaves inoculation

All of the inoculated leaves and fruits showed symptoms after 72 h inoculation. Symptoms on the wounded leaves and fruits appeared 24 h earlier than the non-wounded tissues. Orange spore masses appeared on the lesion under high humidity condition.

Seedling inoculation

The inoculated strawberry seedlings began wilting after 10 d inoculation. The wilting seedlings were stunt compared with control plants. Necrosis was observed in the roots of the wilted plants. The longitudinal section of the crown showed red brown, hard lesions in rot. Twenty-four inoculated seedlings (80%) died after 20 d inoculation. Control seedlings remained healthy.

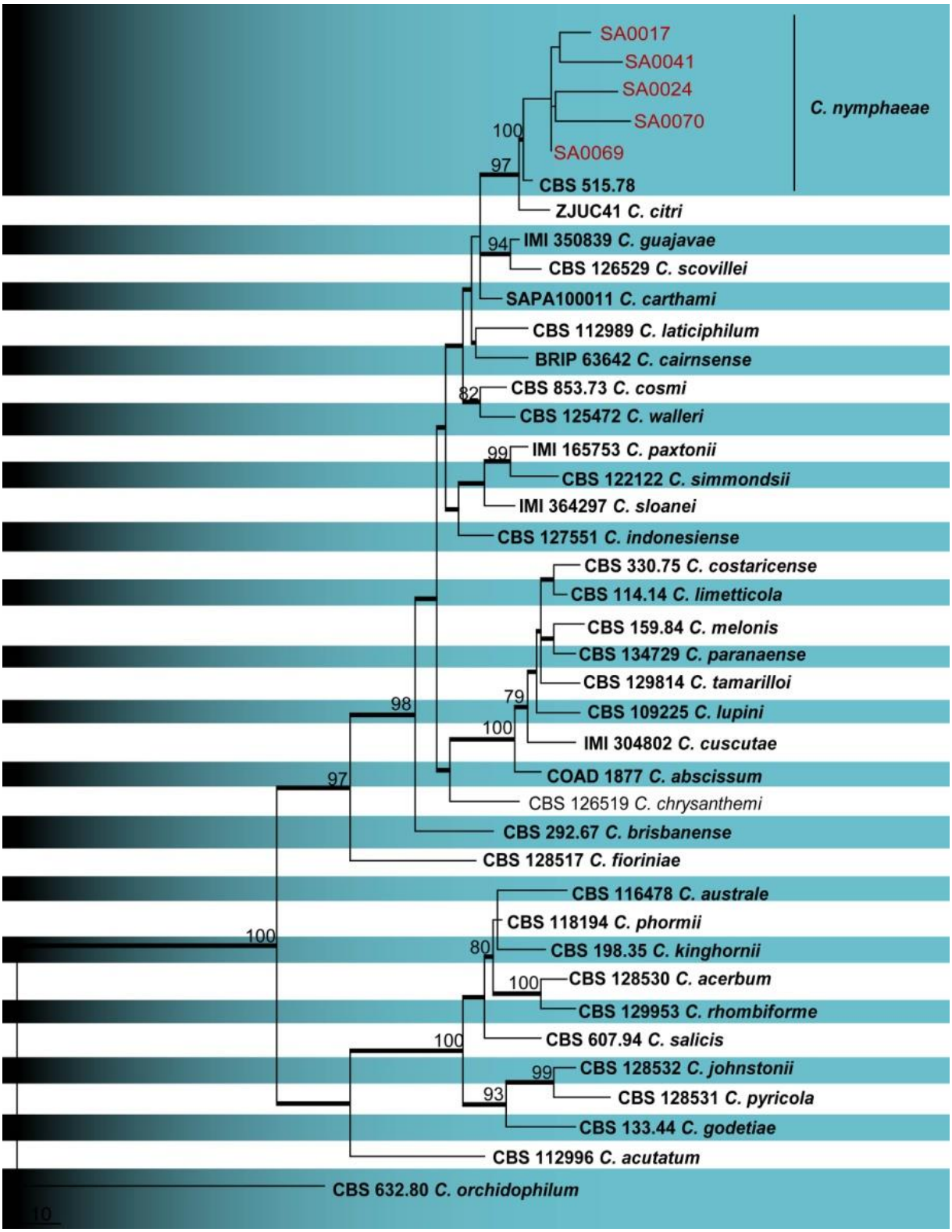

Fig. 2 – One of the 100 most parsimonious trees obtained from a heuristic search of combined ACT, GAPDH, CHS, ITS and β-tubulin sequenced data of the acutatum species complex. Parsimony bootstrap support values greater than 70% are indicated above the nodes and branches with Bayesian posterior probabilities above 0.90 are given in bold. The ex-type strains are in bold; isolates of this study are in red. The scale bar indicates ten changes. The tree is rooted with *C. orchidophilum* CBS 632.80.

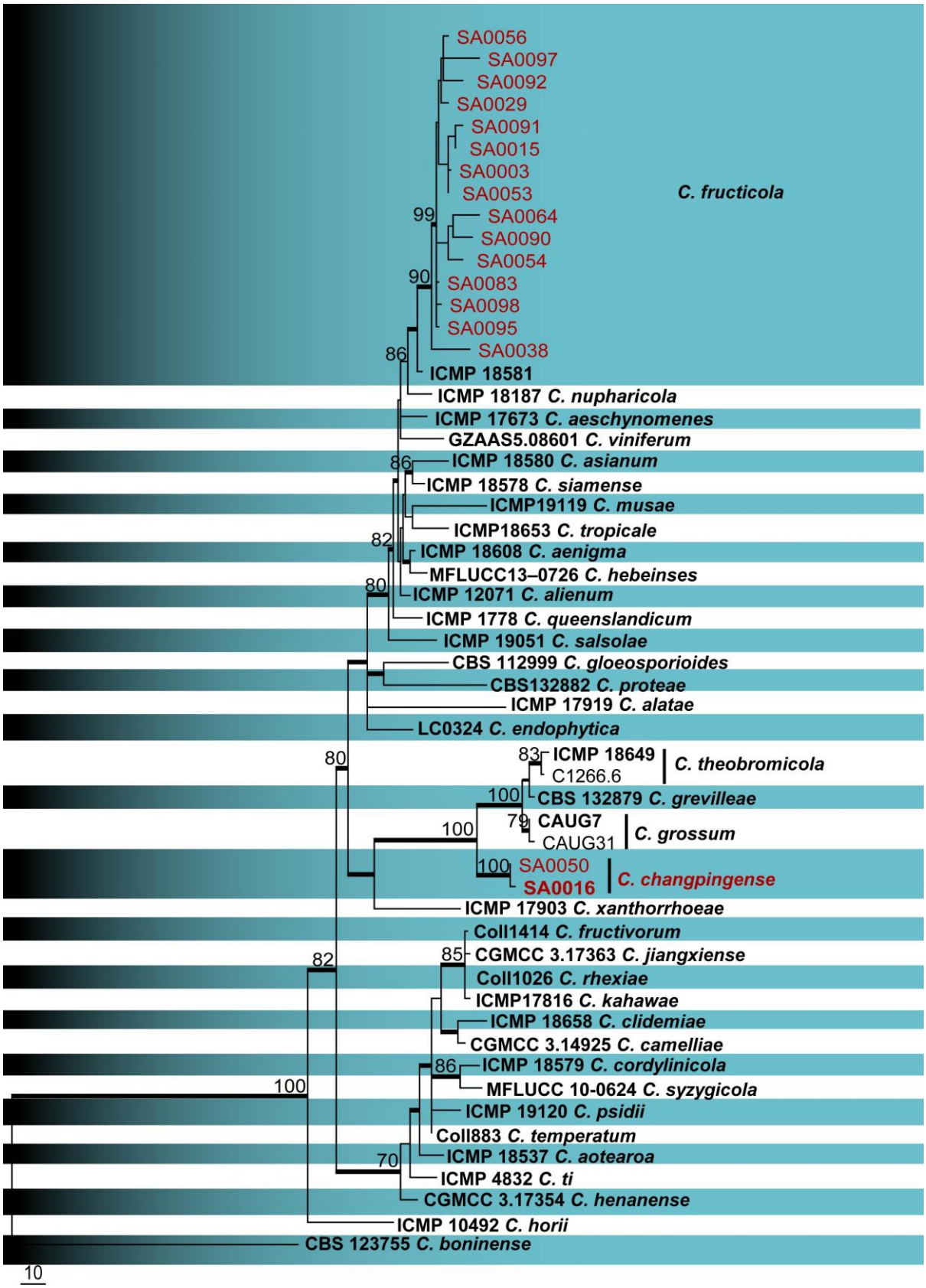

Fig. 3–One of the 100 most parsimonious trees obtained from a heuristic search of combined ACT, GAPDH, CHS, ITS and β-tubulin sequenced data of the gloeosporioides species complex. Parsimony bootstrap support values greater than 70% are indicated above the nodes and branches with Bayesian posterior probabilities above 0.90 are given in bold. The ex-type strains are in bold; isolates of this study are in red. The scale bar indicates ten changes. The tree is rooted with *C. boninense* CBS 123755.

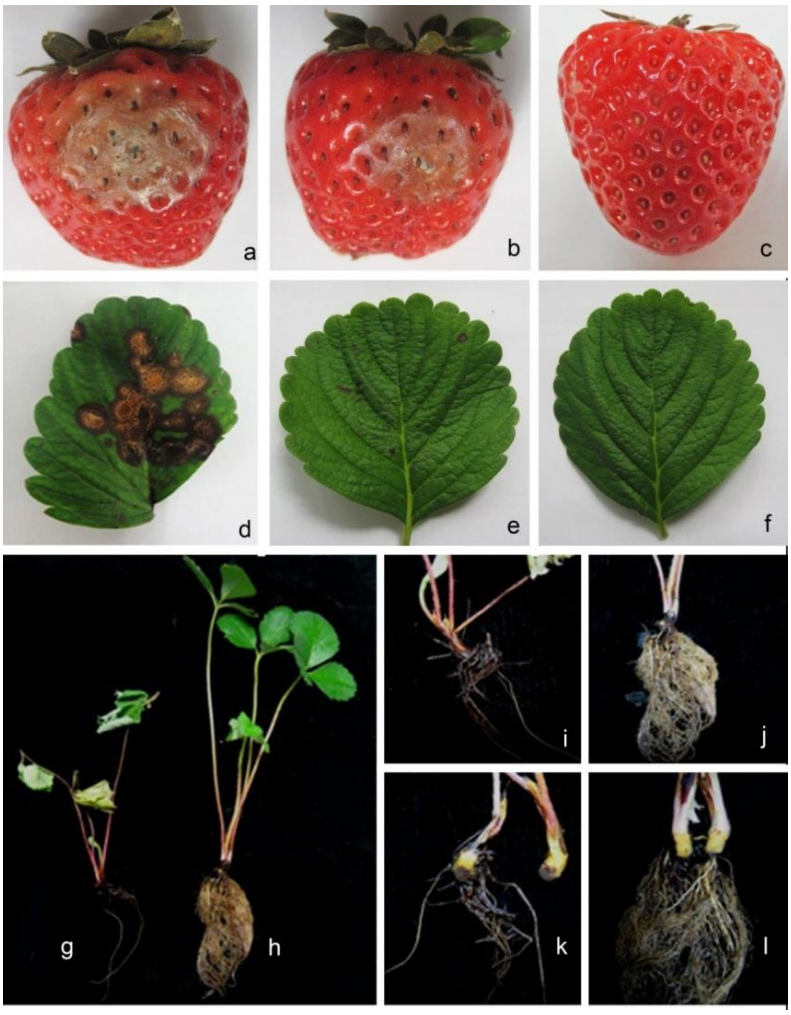

Fig. 4 – Pathogenicity test results of *C. changpingense* (SA0016). a–f. Symptoms on leaves and fruits after 80 h of inoculation. a, d. Wound inoculation. b–c. Non-wound inoculation. g–l. Symptoms of the seedlings and on root after 14days of inoculation. g. Seedling with reddish-brown rhizome and wilted leaves. i, k. Reddish-brown rhizome with retarded root growth. c, f, j, l. Control fruit, leaf and rhizome.

Taxonomy

Colletotrichum fructicola Prihast., L. Cai & K.D. Hyde

Colletotrichum fructicola was originally reported from coffee berries in Thailand (Prihastuti et al. 2009) and has a wide host range (Weir et al. 2012). *Colletotrichum fructicola* belongs to the gloeosporioides species complex and has been reported from Canada and the USA causing strawberry anthracnose (Weir et al. 2012). Fifteen strains of this species were isolated from strawberry in this study; one strain from a fruit, two strains from petioles, 11 strains from rhizomes and one strain from a stolon. In the phylogram, our strains clustered with *C. fructicola* (ICMP 18581) with 99% bootstrap support and posterior probability values of 1.00 (Fig. 3).

Colletotrichum nymphaeae (Pass.) Aa.

A detailed description of *C. nymphaeae* was provided by Damm et al. (2012). This species has a wide host range and belongs to the acutatum species complex. *Colletotrichum nymphaeae* has been recorded from Bulgaria, Canada, France, Israel, Italy, Kenya, Netherlands, South Africa, Spain, Switzerland, the UK and the USA (Damm et al. 2012), where it causes strawberry anthracnose. Five strains of this species were isolated from strawberry; two from fruits, two from petioles and one from a stolon. In the phylogram, our strains clustered with *C. nymphaeae* (CBS515.78) with 100% bootstrap support and posterior probability values of 1.00 (Fig. 2).

Colletotrichum changpingense G. Zhang**,** Jayawardena & KD Hyde, ***sp. nov.***
Index Fungorum No: IF552575; *Facesoffungi* number: FoF: 00644, Fig. 4
Etymology-This species is named after the locality, where this species was found.
Holotype: MFLU 15-0212

Pathogen on strawberry rhizome. *Colonies* growing from single conidia on PDA white, reverse black in centre, pale yellow grey towards the edge, reaching a maximum of 78 mm diam. in 7 days at 28 ºC, growth rate 2–7.8 mm/day. Aerial mycelium, white, dense, cottony. Vegetative hyphae 1–2.1 µm hyaline, smooth-walled, septate, branched. *Asexual morph* developed on PDA. *Conidiomata* 208–425µm diam., abundant, black, an acervulus, oval, solitary to aggregated, with orange spore masses. *Setae* absent. *Conidiophores* 26 µm long, hyaline to light brown, cylindrical or clavate, smooth-walled, simple, wide at the base, occuring in densly arranged clusters. *Conidiogenous cells* 7–26×1.5–3 ($\bar{x}$ = 18.5×1.9, n = 10), enteroblastic, hyaline, smooth–walled, cyllindrical, tapering from base to apex, opening with a 0.5–1.5 µm diam. collarette,< 0.5 µm long, periclinal thickening conspicuous. *Conidia* 9–15×2.5–6 µm ($\bar{x}$ = 12.3×4.6, n = 40) hyaline, smooth-walled or minutely verruculose, aseptate, ovoid to cylindrical or clavate with rounded apices, contents granular and mostly present at the polar ends leaving anopaque region in the centre. *Appressoria* notobserved. *Sexual morph* not observed.

Material examined – CHINA, Beijing City, Changping, Xingshou Town, from rhizome of *Fragaria* × *ananassa*, November 2011, *Zhang Guozhen* (MFLU 15-0212, holotype), ex-type living culture, SA0016 (MFLUCC 15-0022, CGMCC3.17582); CHINA, Guangzhou, from rhizome of *Fragaria* × *ananassa*, April 2012, *Zhang Guozhen* living culture, SA0050.

Fig. 5 – *Colletotrichum changpingense* (MFLUCC 15-0022, ex-type culture). a. Conidioma on PDA b. Orange coloured spore mass. c–d. Conidiogenous cells. e–f**.** Conidia. g. Upper view of colony (7 d old). h. reverse view of colony (7 d old). Scale bars=5µm.

Notes – Based on multi-locus sequence data (ACT, CHS, GAPDH, ITS and β-tubulin), *C. changpingense* is phylogenetically closely related to *C. grevilleae*, *C. grossum* and *C. theobromicola*. Sequence data derived from the ITS region does not separate *C. changpingense* from *C. grevilleae*, *C. theobromicola* and *C. grossum*. The BLASTn search with the ITS sequence of this strain showed 100% similarity to JX573319 *C. gloeosporioides* isolate. The closest match in a BLASTn search in GenBank with the GAPDH sequence was JX009957 *C. theobromicola* strain with 99% similarity. The closest matches in a BLASTn search in GenBank with the CHS sequence was KF772060 *C. siamense* strain with 97% similarity, with the ACT sequence was KT936435 *C. siamense* strain with 99% similarity. The BLASTn search with the β-tubulin is identical to *C. grossum* (CAUG7, KP890171) and *C. theobromicola* (C1273, JX010382). *Colletotrichum changpingense* differs from the type strain of *C. grevilleae* by 17bp changes in ACT, 11bp changes in CHS, 6bp changes in GAPDH and 8bp changes in ITS. *Colletotrichum changpingense* differs from the type strain of *C. theobromicola* by 17bp changes in ACT, 12bp changes in CHS, 2bp changes in GAPDH, 9bp changes in ITS and 8bp changes in β-tubulin. *Colletotrichum changpingense* differs from the type strain of *C. grossum* by 18bp changes in ACT, 9bp changes in CHS and 5bp changes in GAPDH. Therefore *C. changpingense* provides sufficient data to be accommodated as a new species. A PHI test revealed no significant recombination event between *C. changpingense* and its closely related taxa (Fig. 6).

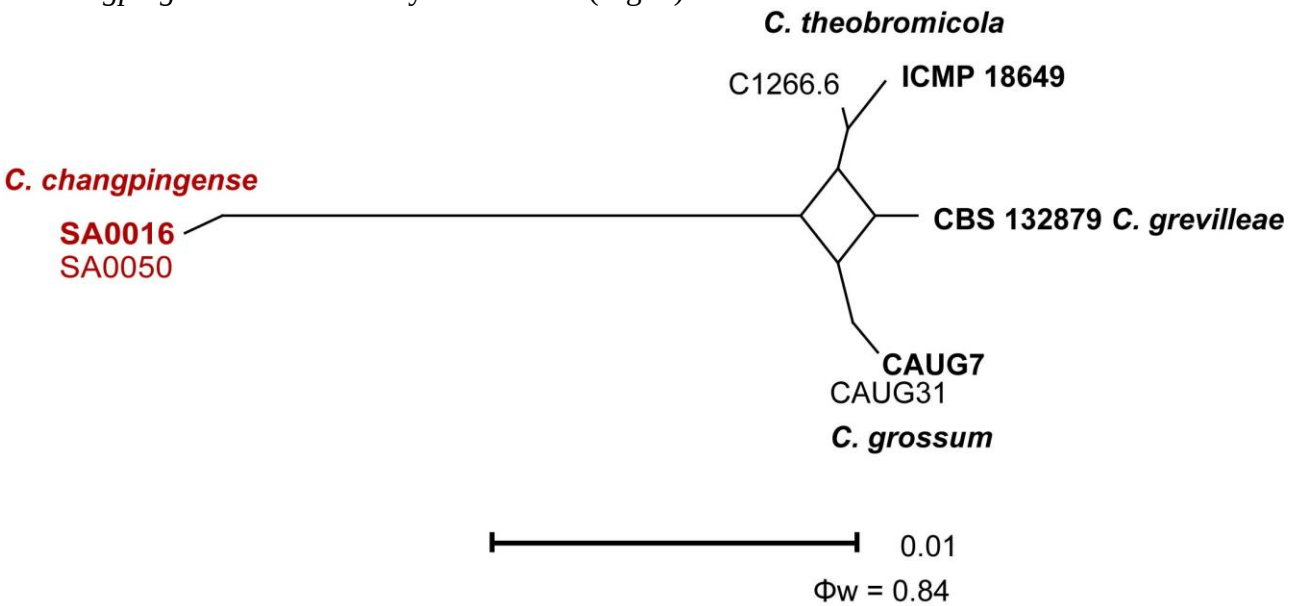

Fig. 6 – The results of the pairwise homoplasy index (PHI) test of closely related species using both LogDet transformation and splits decomposition. PHI test results (Φw) < 0.05 indicate significant recombination within the dataset.

Discussion

Previous studies on *Colletotrichum* species causing strawberry anthracnose were mainly based on morphological characters and single gene based identifications, which would have been identified to species complexes rather than individual species (Ye et al. 2009). Due to the high variability in morphological characters (Sutton1992, Johnston & Jones 1997), differentiation between two main strawberry pathogens, *C. gloeosporioides* and *C. theobromicola* (*fragariae*) became problematic (Ye et al. 1997, Xie et al. 2010), which led to the use of molecular methods (Cai et al. 2009) as they provide useful data in clarifying the systematics of *Colletotrichum* (Hyde et al. 2014, Yan et al. 2015). Xie et al. (2010) identified the *Colletotrichum* species causing anthracnose disease of Strawberry cv. Toyonoka in Zhejiang and Shanghai, China to be *C. acutatum*, *C. gloeosporioides* and *C. theobromicola* using morphology and ITS gene region alone. As ITS gene alone does not resolve *Colletotrichum* species well (Martinez-Culebras 2002, Jelev et al. 2008, Xie et al. 2010), multigene analysis has been adopted to resolve *Colletotrichum* species satisfactorily (Cai et al. 2009, Hyde et al. 2014), and the present study has followed this protocol.

Table 1 List of GenBank accession numbers for the ITS, ACT, CHS-1, GAPDH and β-tubulin gene sequences of the ex-type isolates belonging to the gloeosporioides species complex, acutatum species complex and the strains used in this study with information on taxa, host and geographic location. Strains sequenced in this study are bolded.

Species name	Isolate number	Host	Location	GenBank Accession Numbers				
				ITS	ACT	CHS-1	GAPDH	β -tubulin
C. acerbum	ICMP 12921*	*Malus domestica*	New Zealand	JQ948459	JQ949780	JQ949120	JQ948790	JQ950110
C. acutatum	CBS 112996*	*Carica papaya*	Australia	JQ005776	JQ005839	JQ005797	JQ948677	JQ005860
C. aenigma	ICMP 18608*	*Persea americana*	Israel	JX010244	JX009443	JX009774	JX010044	JX010389
C. aeschynomenes	ICMP 17673*	*Aeschynomene virginica*	USA	JX010176	JX009483	JX009799	JX009930	JX010392
C. alatae	CBS 304.67*	*Dioscorea alata*	India	JX010190	JX009471	JX009837	JX009990	JX010383
C. alienum	ICMP 12071*	*Malus domestica*	New Zealand	JX010251	JX009572	JX009882	JX010028	JX010411
C. aotearoa	ICMP 18537*	*Coprosma* sp.	New Zealand	JX010205	JX009564	JX009853	JX010005	JX010420
C. asianum	ICMP 18580*	*Coffea arabica*	Thailand	FJ972612	JX009584	JX009867	JX010053	JX010406
C. australe	CBS 116478*	*Trachycarpus fortunei*	South Africa	JQ948455	JQ949776	JQ949116	JQ948786	JQ950106
C. brisbanense	CBS 292.67*	*Capsicum annuum*	Australia	JQ948291	JQ949612	JQ948952	JQ948621	JQ949942
C. camelliae	CGMCC 3.14925*	*Camellia sinensis*	China	KJ955081	KJ954782	N.S	KJ954363	KJ955230
C. cairnsense	BRIP 63642*	*Capsicum annuum*	Australia	KU923672	KU923716	KU923710	KU923704	KU923688
C. carthami	SAPA 100011*	*Carthamus tinctorium*	Japan	AB696998	N.S	N.S	N.S	AB696992
C. changpingense	**SA0016 (MFLUCC 15-0022***)	Rhizome of *Fragaria ×ananassa*	China, Beijing (Changping)	KP683152	KP683093	KP852449	KP852469	KP852490
C. changpingense	**SA0050**	Rhizome of *Fragaria × ananassa*	China, Guangzhou	KY214473	KY214470	KY214471	KY214472	KY214474
C. chrysanthemi	IMI 364540*	*Chrysanthemum coronarium*	China	JQ948273	JQ949594	JQ948934	JQ948603	JQ949924
C. citri	CBS 134233*	*Citrus aurantifolia*	China	KC293581	KC293621	N.S	KC293741	KC293661
C. communis	MTCC 11599*	*Mangifera* sp.	India	JQ894681	JQ894546	JQ894617	JQ894632	JQ894602
C. cordylinicola	ICMP 18579*	*Cordyline fruticosa*	Thailand	JX010226	HM470235	JX009864	JX009975	JX010440
C. cosmi	CBS 853.73*	*Cosmos* sp.	Netherlands	JQ948274	JQ949595	JQ948935	JQ948604	JQ949925
C. costaricense	CBS 330.75*	*Coffea arabica*	Costa Rica	JQ948180	JQ949501	JQ948841	JQ948510	JQ949831
C. cuscutae	IMI 304802*	*Cuscuta* sp.	Dominica	JQ948195	JQ949516	JQ948856	JQ948525	JQ949846
C. endophytica	MFLUCC 13-0418*	*Pennisetum purpureum*	Thailand	KC633854	KF306258	N.S	KC832854	N.S
C. fioriniae	CBS 128517*	*Fiorinia externa*	USA	JQ948292	JQ949613	JQ948953	JQ948622	JQ949943
C. fructicola	ICMP 18581*	*Coffea arabica*	Thailand	JX010165	FJ907426	JX009866	JX010033	JX010405
C. fructicola	**SA0003**	Rhizome of *Fragaria ×ananassa*	China, Beijing (Changping)	KP683149	KP683090	KP852446	KP852466	KP852487
C. fructicola	**SA0015**	Rhizome of *Fragaria ×ananassa*	China, Beijing (Changping)	KP683148	KP683089	KP852445	KP852465	KP852486
C. fructicola	**SA0029**	Rhizome of *Fragaria ×ananassa*	China, Beijing (Changping)	KP683153	KP683094	-	KP852470	KP852491
C. fructicola	**SA0038**	Stolon of *Fragaria ×ananassa*	China, Beijing (Changping)	KP683151	KP683092	KP852448	KP852468	KP852489
C. fructicola	**SA0053**	Petiole of *Fragaria ×ananassa*	China, Liaoning (Donggang)	KP683150	KP683091	KP852447	KP852467	KP852488
C. fructicola	**SA0054**	Rhizome of *Fragaria ×ananassa*	China, Liaoning (Donggang)	KP683147	KP683088	KP852444	KP852464	KP852485
C. fructicola	**SA0056**	Rhizome of *Fragaria ×ananassa*	China, Shanghai (Shanghai)	KP683138	KP683079	KP852435	KP852455	KP852476

Species name	Isolate number	Host	Location	GenBank Accession Numbers				
				ITS	ACT	CHS-1	GAPDH	β -tubulin
C. fructicola	**SA0064**	Rhizome of *Fragaria ×ananassa*	China, Shandong (Qingdao)	KP683139	KP683080	KP852436	KP852456	KP852477
C. fructicola	**SA0083**	Rhizome of *Fragaria ×ananassa*	China, Beijing (Fangshan)	KP683143	KP683084	KP852440	KP852460	KP852481
C. fructicola	**SA0090**	Rhizome of *Fragaria ×ananassa*	China, (Changfeng), Anhui	KP683142	KP683083	KP852439	KP852459	KP852480
C. fructicola	**SA0091**	Rhizome of *Fragaria ×ananassa*	China, (Changfeng), Anhui	KP683145	KP683086	KP852442	KP852462	KP852483
C. fructicola	**SA0092**	Rhizome of *Fragaria ×ananassa*	China, Beijing (Tongzhou)	KP683140	KP683081	KP852437	KP852457	KP852478
C. fructicola	**SA0095**	Rhizome of *Fragaria ×ananassa*	China, Hubei (Wuhan)	KP683144	KP683085	KP852441	KP852461	KP852482
C. fructicola	**SA0097**	Petiole of *Fragaria ×ananassa*	China, Beijing (Fangshan)	KP683141	KP683082	KP852438	KP852458	KP852479
C. fructicola	**SA0098**	Fruit of *Fragaria ×ananassa*	China, Hainan (Haikou)	KP683146	KP683087	KP852443	KP852463	KP852484
C. fructivorum	CBS 133125*	*Vaccinium macrocarpo*	USA	JX145145	N.S	N.S	N.S	JX145196
C. gloeosporioides	IMI 356878*	*Citrus sinensis*	Italy	JX010152	JX009531	JX009818	JX010056	JX010445
C. grevilleae	CBS 132879*	*Grevillea* sp.	Italy	KC297078	KC296941	KC296987	KC297010	KC297102
C. grossum	CAUG7*	*Capsicum* sp.	China	KP890165	KP890141	KP890153	KP89015	KP890171
C. godetiae	CBS 133.44*	*Clarkia hybrida*	Denmark	JQ948402	JQ949723	JQ949063	JQ948733	JQ950053
C. guajavae	IMI 350839*	*Psidium guajava*	India	JQ948270	JQ949591	JQ948931	JQ948600	JQ949921
C. hebeiense	MFLUCC 13-0726*	*Vitis vinifera*	China	KF156863	KF377532	KF289008	KF377495	KF288975
C. henanense	CGMCC 3.17354*	*Camilla sinensis*	China	KJ955109	KM023257	N.S	KJ954810	KJ955257
C. horii	ICMP 10492*	*Diospyros kaki*	Japan	GQ329690	JX009438	JX009752	GQ329681	JX010450
C. indonesiense	CBS 127551*	*Eucalyptus* sp.	Indonesia	JQ948288	JQ949609	JQ948949	JQ948618	JQ949939
C. jiangxiense	CGMCC 3.17363*	*Camilla sinensis*	China	KJ955201	KJ954471	N.S	KJ954902	KJ955348
C. johnstonii	CBS 128532*	*Solanum lycopersicum*	New Zealand	JQ948444	JQ949765	JQ949105	JQ948775	JQ950095
C. kahawae	ICMP 17816*	*Coffea arabica*	Kenya	JX010231	JX009452	JX009813	JX010012	JX010444
C. kinghornii	CBS 198.35*	*Phormium* sp.	UK	JQ948454	JQ949775	JQ949115	JQ948785	JQ950105
C. laticiphilum	CBS 112989*	*Hevea brasiliensis*	India	JQ948289	JQ949610	JQ948950	JQ948619	JQ949940
C. limetticola	CBS 114.14*	*Citrus aurantifolia*	USA	JQ948193	JQ949514	JQ948854	JQ948523	JQ949844
C. lupine	CBS 109225*	*Lupinus albus*	Ukraine	JQ948155	JQ949476	JQ948816	JQ948485	JQ949806
C. melonis	CBS 159.84*	*Cucumis melo*	Brazil	JQ948194	JQ949515	JQ948855	JQ948524	JQ949845
C. musae	CBS 116870*	*Musa* sp.	USA	JX010146	JX009433	JX009896	JX010050	HQ596280
C. nupharicola	CBS 470.96*	*Nuphar lutea*	USA	JX010187	JX009437	JX009835	JX009972	JX010398
C. nymphaeae	CBS 515.78*	*Nymphaea alba*	Netherlands	JQ948197	JQ949518	JQ948858	JQ948527	JQ949848
C. nymphaeae	**SA0017**	Petiole of *Fragaria× ananassa*	China, Beijing (Daxing)	KP683133	KP683074	KP852430	KP852450	KP852471
C. nymphaeae	**SA0024**	Petiole of *Fragaria× ananassa*	China, Beijing (Xiaotangshan)	KP683135	KP683076	KP852432	KP852452	KP852473
C. nymphaeae	**SA0041**	Fruit of *Fragaria× ananassa*	China, Hubei (Baoding)	KP683134	KP683075	KP852431	KP852451	KP852472
C. nymphaeae	**SA0069**	Fruit of *Fragaria× ananassa*	China, Beijing (Changping)	KP683137	KP683078	KP852434	KP852454	KP852475
C. nymphaeae	**SA0070**	Stolon of *Fragaria× ananassa*	China, Beijing (Changping)	KP683136	KP683077	KP852433	KP852453	KP852474
C. orchidophilum	CBS 632.80*	*Dendrobium* sp.	USA	JQ948151	JQ949472	JQ948812	JQ948481	JQ949802
C. paranaense	CBS 134729*	*Malus domestica*	Brazil	KC204992	KC205077	KC205043	KC205026	KC205060

Species name	Isolate number	Host	Location	GenBank Accession Numbers				
				ITS	ACT	CHS-1	GAPDH	β -tubulin
C. paxtonii	IMI 165753*	*Musa* sp.	Saint Lucia	JQ948285	JQ949606	JQ948946	JQ948615	JQ949936
C. phormii	CBS 118194*	*Phormium* sp.	Germany	JQ948446	JQ949767	JQ949107	JQ948777	JQ950097
C. proteae	CBS 132882*	*Protea* sp.	South Africa	KC297079	KC296940	KC296986	KC297009	KC297101
C. psidii	CBS 145.29*	*Psidium* sp.	Italy	JX010219	JX009515	JX009901	JX009967	JX010443
C. pyricola	CBS 128531*	*Pyrus communis*	New Zealand	JQ948445	JQ949766	JQ949106	JQ948776	JQ950096
C. queenslandicum	ICMP 1778*	*Carica papaya*	Australia	JX010276	JX009447	JX009899	JX009934	JX010414
C. rhexiae	CBS 133134*	*Rhexia virginica*	USA	JX145128	N.S	N.S	N.S	JX145179
C. rhombiforme	CBS 129953*	*Olea europaea*	Portugal	JQ948457	JQ949778	JQ949118	JQ948788	JQ950108
C. salicis	CBS 607.94*	*Salix* sp.	Netherlands	JQ948460	JQ949781	JQ949121	JQ948791	JQ950111
C. salsolae	ICMP 19051*	*Salsola tragus*	Hungary	JX010242	JX009562	JX009863	JX009916	JX010403
C. scovillei	CBS 126529*	*Capsicum* sp.	Indonesia	JQ948267	JQ949588	JQ948928	JQ948597	JQ949918
C. siamense	ICMP 18578*	*Coffea arabica*	Thailand	JX010171	FJ907423	JX009865	JX009924	JX010404
C. simmondsii	CBS 122122*	*Carica papaya*	Australia	JQ948276	JQ949597	JQ948937	JQ948606	JQ949927
C. sloanei	IMI 364297*	*Theobroma cacao*	Malaysia	JQ948287	JQ949608	JQ948948	JQ948617	JQ949938
C. syzygicola	MFLUCC* 10–0624	*Syzygium samarangense*	Thailand	KF242094	KF157801	N.S	KF242156	KF254880
C. temperatum	CBS 133122*	*Vaccinium macrocarpon*	USA	JX145159	N.S	N.S	N.S	JX145211
C. tamarilloi	CBS 129814*	*Solanum betaceum*	Colombia	JQ948184	JQ949505	JQ948845	JQ948514	JQ949835
C. theobromicola	CBS 124945*	*Theobroma cacao*	Panama	JX010294	JX009444	JX009869	JX010006	JX010447
C. ti	ICMP 4832*	*Cordyline* sp.	New Zealand	JX010269	JX009520	JX009898	JX009952	JX010442
C. tropicale	CBS 124943*	*Theobroma cacao*	Panama	JX010264	JX009489	JX009870	JX010007	JX010407
C. truncatum	CBS 151.35*	*Phaseolus lunatus*	USA	GU227862	GU227960	GU228352	GU228254	N.S
C. viniferum	GZAAS5.08601*	*Vitis vinifera*	China	JN412804	JN412795	N.S	JN412798	JN412813
C. walleri	CBS 125472*	*Coffea* sp.	Vietnam	JQ948275	JQ949596	JQ948936	JQ948605	JQ949926
C. xanthorrhoeae	ICMP 17903*	*Xanthorrhoea preissii*	Australia	JX010261	JX009478	JX009823	JX009927	JX010448

[*Abbreviation: CBS Culture Collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection, China; GZAAS Guizhou Academy of Agricultural Sciences herbarium, China; ICMP International Collection ofMicroorganisms from Plants, Landcare Research, Auckland, New Zealand; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; MFLUCC: Mae Fah Luang University Culture Collection, Thailand; SA Culture collection of China Agriculture University, Beijing, China; N.S. not sequenced

The main taxa reported to cause strawberry anthracnose worldwide are *C. acutatum*, *C. gloeosporioides* and *C. fragariae* (Mass 1998), the latter which has been synonimized under *C. theobromicola* (Weir et al. 2012). Besides *C. acutatum*, *C. gloeosporioides* and *C. theobromicola*, *C. coccodes* (Buddie et al. 1999; Cannon et al. 2012), *C. cuscutae* (Damm et al. 2012), *C. fructicola* (Weir et al. 2012), *C. fioriniae* (MacKenzie et al. 2009; Damm et al. 2012), *C.godetiae* (Damm et al. 2012), *C. nymphaeae* (Damm et al. 2012), *C. salicis* (Damm et al. 2012) and *C. simmondsii* (Whitelaw-Weckertet al. 2007, Damm et al. 2012) have been reported to be associated with strawberry anthracnose; most of these taxa belong to *C. acutatum* species complex.

In this study, we have determined the species of *Colletotrichum* that are associated with strawberry anthracnose in eight strawberry growing provinces of China. A new *Colletotrichum* species that causes strawberry anthracnose in China is described as *C. changpingense*, based on morphological features, multi-gene phylogenetic analysis and pathogenicity assays. The five gene multi-loci analysis revealed that this isolate from rhizomes, clustered separately from other *Colletotrichum* species. Pathogenicity testing showed that the newly introduced species is pathogenic not only on the strawberry crowns, but also on fruits and leaves. This species is capable of causing crown rot disease which is characterized by reddish brown rhizomes with less roots and wilted leaves. Anthracnose lesions on ripe strawberry fruits are firm, slightly sunken and covered with pink masses. This species is also capable of causing leaf spots on strawberry characterized by brown to black necrotic spots along the veins and inter-vein regions. Fifteen strains of *C. fructicola* were recorded from a fruit, petioles, rhizomes and a stolon and five strains of *C. nymphaeae* were also recorded from fruits, petioles and a stolon. As the latter two species are well-known pathogens causing strawberry anthracnose in different countries (Damm et al. 2012, Weir et al. 2012), this paper further contributes that these two species are also associated with strawberry anthracnose in China. In this study we did not find *C. theobromicola.* However, there were 15 more isolates in the single phylogenetic tree which consisted of all the *Colletotrichum* species that were not well-resolved. Further clarifications of those isolates are needed. This study contributes in updating the *Colletotrichum* species associated with strawberry of China. Species with similar morphology may have a considerable physiological and pathogenic variation. It is important to know the host distribution of a particular *Colletotrichum* species which will be important in implementing bio-security measures in quarantine (Jayawardena et al. 2016).

Acknowledgments

The research was funded by special fund for Agro-scientific research in the public interest (201003064). Kevin D. Hyde thanks the Chinese Academy of Sciences, project number 2013T2S0030, for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany. The authors extend their sincere appreciations to the Deanship of Scientific Research at King Saud University for funding this Prolific Research group (PRG-1436-09).

References

Buddie AG, Martínez-Culebras P, Bridge PD, García MD, Querol A, Cannon PF, Monte E. – 1999 Molecular characterisation of *Colletotrichum* strains derived from strawberry. Mycological Research 103, 385–394.

Burland TG. – 2000 DNASTAR's Lasergene sequence analysis software. Methods in Molecular Biology 132, 71–91.

Cai L, Hyde KD, Taylor PWJ, Weir BS, Waller J, Abang MM, Zhang JZ, Yang YL, Phoulivong S, Liu ZY, Prihastuti H, Shivas RG, McKenzie EHC, Johnston PR. 2009 – A polyphasic approach for studying *Colletotrichum*. Fungal Diversity 39, 183–204.

Cannon PF, Damm U, Johnston PR, Weir BS. 2012– *Colletotrichum*-current status and future directions. Studies in Mycology 73, 181–213.

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

Chomnunti P, Hongsanan S, Aguirre-Hudson B, Tian Q, Peršoh D, Dhami MK, Alias AS, Xu J-C, Liu X-Z, Stadler M, Hyde KD. 2014– The sooty moulds. Fungal Diversity 66, 1–36.

Dai FM, Ren XJ, Lu JP. 2006– First report of anthracnose fruit rot of strawberry caused by *Colletotrichum acutatum* in China. Plant Disease 90, p1460.

Damm U, Mostert L, Crous PW, Fourie PH. 2008 – Novel *Phaeoacremonium* species associated with necrotic wood of Prunus trees. Persoonia 20, 87–102.

Damm U, Cannon PF, Woundenberg JHC, Crous PW. 2012 – The *Colletotrichum acutatum* species complex. Studies in Mycology 73, 37–113.

Diao YZ, Zhang C, Liu F, Wang WZ, Cai L, Liu XL. 2017 – *Colletotrichum* species causing anthracnose disease of chili in China. Persoonia 38, 20–37.

Freeman S, Katan T, Shabi E. 1998 – Characterization of *Colletotrichum* species responsible for anthracnose disease of various fruits. Plant Disease 82, 596–605.

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.

Hillis DM, Bull JJ. 1993 – An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42, p182.

Hu S.1990 – The preliminary investigation of strawberry diseases. Collected abstracts for Fifth Meeting of East China, Chinese Society for Plant Pathology, Jiangsu 58–59.

Huson DH, Bryant D. 2006 – Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23, 254–267.

Huson DH. 1998 – Splits Tree: analyzing and visualizing evolutionary data. Bioinformatics 14, 68–73.

Hyde KD, Cai L, Cannon PF, Crouch JA, Crous PW, Damm U, Goodwin PH, Chen H, Johnston PR, Jones EBG, Liu ZY, McKenzie EHC, Moriwaki J, Noireung P, Pennycook SR, Pfenning LH, Prihastuti H, Sato T, Shivas RG, Tan YP, Taylor PWJ, Weir BS, Yang YL, Zhang JZ. 2009a – *Colletotrichum* names in current use. Fungal Diversity 39, 147–182.

Hyde KD, Cai L, McKenzie EHC, Yang YL, Zhang JZ, Prihastuti H. 2009b – *Colletotrichum*: a catalogue of confusion. Fungal Diversity 39, 1–17.

Hyde KD, Nilsson RH, Alias SA, Ariyawansa HA, Blair JE, Cai L, de Cock AWAM, Dissanayake AJ, Glockling SL, Goonasekara ID, Gorczak M, Hahn M, Jayawardena RS, van Kan JAL, Laurence MH, Lévesque CA, Li XH, Liu JK, Maharachchikumbura SSN, Manamgoda DS, Martin FN, McKenzie EHC, McTaggart AR, Mortimer PE, Nair PVR, Pawłowska J, Rintoul TL, Shivas RG, Spies CFJ, Summerell BA, Taylor PWJ, Terhem RB, Udayanga D, Vaghefi N, Walther G, Wilk M, Wrzosek M, Xu JC, Yan JY, Zhou N. 2014 – One stop shop: backbones trees for important pytopathogenic genera: I Fungal Diversity 67, 21–125.

Index Fungorum, 2016. http://www.indexfungorum.org/names/names.asp

Jayasiri SC, Hyde KD, Abd-Elsalam KA, Abdel-Wahab MA,Ariyawansa HA, Bhat J, Buyck B, Dai YC, Ertz D, Hidayat I, Jeewon R, Jones EBG, Karunarathna SC, Kirk P, Lei C, Liu JK, Maharachchikumbura SSN, McKenzie E, Ghobad-Nejhad M, Nilsson H, Pang KL, Phookamsak R, Rollins AW, Romero AI,Stephenson S, Suetrong S, Tsui CKM, Vizzini A, Wen TC, De Silva NI, Promputtha I, Kang JC. 2015 – The faces of fungi database:fungal names linked with morphology, molecular and human attributes. Fungal Diversity 74, 18–357.

Jayawardena RS, Hyde KD, Jeewon R, Liu XH, Liu M, Yan JY. 2016 – Why it is important to correctly name *Colletotrichum* species? Mycosphere (This issue- http://doi 10.5943/mycosphere/si/1c/1).

Jelev ZJ, Bobev SG, Minz D, Maymon M, Freeman S. 2008 – First report of anthracnose fruit rot caused by *Colletotrichum acutatum* on pepper and tomato in Bulgaria. Plant Disease 92, pp172.

Johnston RP, Jones D. 1997 – Relationships among *Colletotrichum* isolates from fruit-rots assessed using rDNA sequences. Mycologia 89, 420–430.

Katoh K, Toh H.2008 – Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9, 276–285.

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.

Mass JL. 1998 – Compendium of strawberry diseases 2nd U.S. Department of Agriculture Beltsville, Maryland. APS Press.

Mackenzie SJ, Mertely JC, Seijo TE, Peres NA. 2009 – *Colletotrichum fragariae*is a pathogen on hosts other than strawberry. Plant Disease 92, 1432–1438.

Martinez-Culebras PV, Barrio E, Suarez- Fernandes MB, Gracia Lopez MD, Querol A. 2002 – RAPD analysis of *Colletotrichum* species isolated from strawberry and the design of specific primers for the identification of *C. fragariae.* Journal of Phytopathology 150, 680–686.

Nilsson RH, Hyde KD, Pawlowska J, Ryberg M, Tedersoo L, Aas AB, Alias SA, Alves A, Anderson CL, Antonelli A, Arnold AE, Bahnmann B, Bahram M, Bengtsson-Palme J, Berlin A, Branco S, Chomnunti P, Dissanayake A, Drenkhan R, Friberg H, Frøslev TG, Halwachs B, Hartmann M, Henricot B, Jayawardena R, Jumpponen A, Kauserud H, Koskela S, Kulik T, Liimatainen K, Lindahl BD, Lindner D, Liu JK, Maharachchikumbura S, Manamgoda D, Martinsson S, Neves MA, Niskane T, Pereira OL, Pinho DB, Porter TM, Queloz V, Riit T, Sánchez-García M, de Sousa F, Stefańczyk E, Tadych M, Takamatsu S, Tian Q, Udayanga D, Unterseher M, Wang Z, Wikee S, Yan J, Larsson E, Larsson KH. Kõljalg U, Abarenkov K. 2014 – Improving ITS sequence data for identification of plant pathogenic fungi. Fungal Diversity67, 11–19.

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 Phylogeny and Evolution 7, 103–116.

Philippe H, Bryant D. 2006 – A simple and robust statistical test for detecting the presence of recombination. Genetics 172, 2665–2681.

Quaedvlieg W, Binder M, Groenewald JZ, Summerell BA, Carnegie AJ, Burgess TI, Crous PW. 2014 – Introducing the consolidated species concept to resolve species in the *Teratosphaeriaceae*. Persoonia 33, 1–40.

Ren HY, Jiang GH, Fang L, Zhang YC, Yang XF, Miao LX, Wang HR. 2011 – Identification and biological characteristics of pathogens of strawberry anthracnose in Jiangsu province. Acta Agriculturae Zhejiangensis 23, 937–941.

Ren XJ, Liang Y, Lu JP, Yang BR, Xu JY, Dai FM. 2008 – Identification of *Colletotrichum* species from strawberry in Shanghai. Acta Phytopathologica Sinica 38, 325–328.

Ronquist F, Huelsenbeck JP. 2003 – MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, p1572.

Sanderson MJ, Donoghue MJ, Piel W, Eriksson T. 1994 – TreeBase: A prototype database of phylogenetic analyses and an interactive tool for browsing the phylogeny of life. The Journal of the Botany American 81: article 6.

Shao XH. 1992 – New disease of strawberry: Strawberry anthracnose. Acta Agriculturae Shanghai 8, 86–87.

Sharma G, Pinnaka AK, Shenoy BD. 2013 – Resolving the *Colletotrichum siamense* species complex using *ApMat* marker. Fungal Diversity 61, 117–138.

Smith BJ. 2008 – Epidemiology and pathology strawberry anthracnose: A North American perspective. HortScience 43, 69–73.

Sreenivasaprasad S, Talhinhas P. 2005 – Genotypic and phenotypic diversity in *Colletotrichum acutatum*, a cosmopolitan pathogen causing anthracnose on a wide range of hosts. Molecular Plant Pathology 6, 361–378.

Sutton BC. 1992 – The genus *Glomerella* and its anamorph *Colletotrichum*. In: *Colletotrichum* Biology, Pathology and Control (Bailey JA, Jeger MJ, eds). CAB International, Wallingford: 1– 26.

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.

The Ministry of Agriculture of the People's Republic of China, 2013 – China Agriculture Statistical Report [M] ISBN978-7-109-19711-4, Beijing: China Agriculture Press, 2014, pp3.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997 – The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24, 4876–4882.

Waller JM, Bridge PD, Black R, Hakiza G. 1993 – Characterisation of the coffee berry disease pathogen, *Colletotrichum kahawae* sp. nov. Mycological Research 97, 989–994.

Wang CH. 2013 – Occurrence and control of strawberry anthracnose in Donggangarea. Northen Fruit. 2, p38.

Weir B, 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.

Whitelaw-Weckert MA, Curtin SJ, Huang R, Steel CC, Blanchard CL, Roffey PE. 2007 – Phylogenetic relationships and pathogenicity of *Colletotrichum acutatum* isolates from grape in subtropical Australia. Plant Pathology 56, 448–463.

Xiang FY, Han YC, Zeng XG, Zhang P, Wu RL, Feng XM, Gu YC. 2012 – A survey on the strawberry anthracnose disease in Hubei Province . Journal of Hubei Agricultural Sciences 51, 5650–5653.

Xie L, Zhang J-Z Wan Y, Hu D-W. 2010 – Identification of *Colletotrichum* spp. isolated from strawberry in Zhejiang province and Shanghai city, China. Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 11, 61–70.

Yan JY, Jayawardena MMRS, Goonasekara I, Wang Y, Zhang W, Liu M, Yan H, Huang JB, Wang ZY, Shang JJ, Peng YL, Bahkali A, Hyde KD, Li XH. 2015 – Diverse species of *Colletotrichum* associated with grapevine anthracnose in China. Fungal Diversity 71, 233–246.

Ye ZW, Zheng HQ, Tong YM. 1997 – First report on species of strawberry anthracnose pathogen in Shanghai suburbs and resistance of some strawberry cultivars to anthracnose. Acta agriculturae Shanghai 13, 75–80.

Zhang HY, Zhang MH, Liu ZH, Yu HB, Wang DL. 2007 – Identification and biological characteristics of *Colletotrichum gloeosporioides* on strawberry. Journal of Shenyang Agricultural University 38, 317–321.