

Re-identification of *Colletotrichum acutatum* Species Complex in Korea and Their Host Plants

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***Colletotrichum acutatum* species complex** is one of the most important groups in the genus *Colletotrichum* with a high species diversity and a wide range of host plants. *C. acutatum* and related species have been collected from different plants and locations in Korea and deposited into the Korean Agricultural Culture Collection (KACC), National Institute of Agricultural Sciences since the 1990s. These fungal isolates were previously identified based mainly on morphological characteristics, and a limitation of molecular data was provided. To confirm the identification of species, 64 *C. acutatum* species complex isolates in KACC were used in this study for DNA sequence analyses of six loci: nuclear ribosomal internal transcribed spacers (ITS), beta-tubulin 2 (TUB2), histone-3 (HIS3), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), chitin synthase 1 (CHS-1), and actin (ACT). The molecular analysis revealed that they were identified in six different species of *C. fioriniae* (24 isolates), *C. nymphaeae* (21 isolates), *C. scovillei* (12 isolates), *C. chrysanthemi* (three isolates), *C. lupini* (two isolates), and *C. godetiae* (one isolate), and a novel species candidate. We compared the hosts of KACC isolates with “The List of Plant Diseases in Korea”, previous reports in Korea and global reports and

found that 23 combinations between hosts and pathogens could be newly reported in Korea after pathogenicity tests, and 12 of these have not been recorded in the world.

Keywords : *Colletotrichum acutatum* species complex, host plant, identification

The fungal family *Glomerellaceae* contains only one genus *Colletotrichum* which consists of many phytopathogenic species with a wide range of hosts. *Colletotrichum* species were commonly reported as causal agents of anthracnose diseases and infected economically important crops such as apple, strawberry, pepper, citrus, peach, mango, avocado, banana, coffee and cereals (Cannon et al., 2012; Crouch and Beirn, 2009; González et al., 2006; Kim et al., 2008; Lenné, 2002; Nguyen et al., 2010; Oo et al., 2018; Peres et al., 2008; Sanders and Korsten, 2003; Tan et al., 2022).

The identification of *Colletotrichum* species was primarily based on morphological characteristics with 11 species recognized by von Arx (1957), 22 species by Sutton (1980), and 39 species by Sutton (1992). Later, 66 species were accepted by Hyde et al. (2009) based on the morphology and/or molecular analysis. However, morphological characteristics and the lack of molecular data cannot be used to identify *Colletotrichum* species accurately (Cai et al., 2011; Sato and Moriwaki, 2013). Therefore, Jayawardena et al. (2016) divided the genus *Colletotrichum* (189 species) into 11 species complexes and 23 single species, then Liu et al. (2022) updated to 280 accepted species with 16 species complexes and 15 singleton species, based on multi-locus phylogeny. The nuclear ribosomal internal transcribed spacer (ITS) region was used to determine *Colletotrichum* species complexes (Cannon et al., 2012).

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While, most members of the *C. acutatum*, *C. dematium*, *C. destructivum*, *C. orchidearum*, *C. spaethianum*, *C. dra- caenophilum*, *C. magnum*, and *C. truncatum* species complexes were identified at species level by the combination of six loci: ITS, beta-tubulin 2 (TUB2), histone-3 (HIS3), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), chitin synthase 1 (CHS-1), and actin (ACT) (Damm et al., 2009, 2012, 2014, 2019; Jayawardena et al., 2016; Liu et al., 2022).

Species in the *C. acutatum* species complex are known as destructive plant pathogens worldwide, including Korea (Baroncelli et al., 2015; Cho et al., 2021; Garrido et al., 2009; Kim et al., 2022; Kwon and Kim, 2011; Lee et al., 2007; Oh, 1995; Peres et al., 2008; Talhinas et al., 2011). To date, this complex was one of the biggest groups in the genus *Colletotrichum* with 43 accepted species and contained species with a wide or narrow range of host plants (Jayawardena et al., 2021; Liu et al., 2022). The variation in the conidial shape of many species in the complex often led to incorrectly identify based on morphology. For example, 39 *Colletotrichum* isolates, morphologically identified as *C. gloeosporioides* or *Glomerella cingulata*, were re-identified as 14 species in or closely related to the *C. acutatum* species complex based on the multi-locus analysis (Damm et al., 2012).

Isolates of *C. acutatum* and related species have been deposited into the Korean Agricultural Culture Collection (KACC), National Institute of Agricultural Sciences since the 1990s. These species were originally identified mainly based on morphological characteristics. In earlier studies, several KACC isolates in the *C. acutatum* species complex

were re-identified using a single locus or the multi-locus combination. Nevertheless, some of these were ambiguous species because of insufficient molecular data (Han et al., 2014; Kim and Kim, 2020; Kim et al., 2006, 2008, 2020; Noh et al., 2014; Park et al., 2020; Xu et al., 2018). Accurate species identification within *C. acutatum* species complex plays an important role to understand species diversity and the host plants of this complex in Korea. Hence, this study aims to: (1) re-identify the isolates of *C. acutatum* species complex in KACC using the combined analysis of six loci (ITS, TUB2, HIS3, GAPDH, CHS-1, and ACT); (2) rearrange combination between host plants and re-identified species under *C. acutatum* species complex in Korea.

Materials and Methods

Fungal isolates. Sixty-four cultures of *Colletotrichum* from many host plants and different locations in Korea have been deposited and preserved in liquid nitrogen at the KACC. Details of these isolates such as sources, locations, collected date and previous re-identifications were documented. Fresh cultures were recovered on potato dextrose agar (PDA) and used in this study.

DNA extraction, polymerase chain reaction amplification, and sequencing. Fungal mycelia were scraped from 7-day-old cultures on PDA plates. Around 50 mg of fresh mycelia was used for DNA extraction using the DNeasy plant mini kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. DNA templates were checked

Table 1. Primer pairs used for PCR amplification and sequencing in this study

Locus	Primer	Direction	Sequence (5'-3')	Reference
ITS	ITS1	Forward	TCCGTAGGTGAAACCTGCGG	White et al. (1990)
	ITS4	Reverse	TCCTCCGCTTATTGATATGC	
TUB2	T1	Forward	AACATGCGTGAGATTGTAAGT	O'Donnell and Cigelnik (1997)
	Bt2b	Reverse	ACCCTCAGTGTAGTGACCCTTGGC	
GAPDH	GDF1	Forward	GCCGTCAACGACCCCTTCATTGA	Guerber et al. (2003)
	GDR1	Reverse	GGGTGGAGTCGTACTTGAGCATGT	
HIS3	CYLH3F	Forward	AGGTCCACTGGTGGCAAG	Crous et al. (2004)
	CYLH3R	Reverse	AGCTGGATGTCCTTGGACTG	
CHS-1	CHS-79F	Forward	TGGGGCAAGGATGCTTGGAAAGAAG	Carbone and Kohn (1999)
	CHS-345R	Reverse	TGGAAGAACCATCTGTGAGAGTTG	
ACT	ACT-512F	Forward	ATGTGCAAGGCCGGTTCGC	Carbone and Kohn (1999)
	ACT-783R	Reverse	TACGAGTCCTCTGGCCCAT	

PCR, polymerase chain reaction; ITS, internal transcribed spacer; TUB2, β-tubulin 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HIS3, histone3; CHS-1, chitin synthase; ACT, actin.

by a NanoDrop Spectrophotometer (Thermo Scientific, Waltham, MA, USA).

The primer pairs, including ITS1/ITS4 (White et al., 1990), T1/BT2b (Glass and Donaldson, 1995; O'Donnell and Cigelnik, 1997), GDF1/GDR1 (Guerber et al., 2003), CYLH3F/CYLH3R (Crous et al., 2004), CHS-79F/CHS-345R (Carbone and Kohn, 1999), and ACT-512F/ACT-783R (Carbone and Kohn, 1999), were used for the amplification of ITS, TUB2, GAPDH, HIS3, CHS-1, and ACT, respectively (Table 1). Each polymerase chain reaction (PCR) volume (25 µl) consisted of 12.5 µl MyTaq HS Mix, 1 µl (4.5 pMol) of each primer, 8.5 µl nuclease-free water and 2 µl DNA template (100 ng/µl). PCR reactions were performed in a MJ Research PTC-200 Thermal Cycler (MJ Research, Ramsey, MN, USA) with an initial denaturation step at 94°C for 5 min, followed by 30 cycles: denaturation at 94°C for 30 s; annealing at 58°C (ITS), 61°C (TUB2 and GAPDH) and 61.5°C (HIS3, CHS-1, and ACT) for 30 s, extension at 72°C for 1 min and final extension at 72°C for 10 min. PCR products were checked by gel electrophoresis before sending to the Macrogen (Seoul, Korea) for sequencing with the amplifying primer pairs.

Phylogenetic analysis. Raw sequences obtained in this study were assembled by MEGA 11 (Tamura et al., 2021) and deposited to RDA-GeneBank (<http://genebank.rda.go.kr>) with accession numbers in Table 2. The sequence datasets contained sequences of 64 KACC isolates, 43 reference species (Supplementary Table 1) in the *C. acutatum* species complex, and *C. orchidophilum* (outgroup) (Liu et al., 2022). The multiple sequence alignment of each locus was separately performed using the ClustalW program in MEGA 11 and concatenated afterward. A maximum likelihood (ML) phylogenetic tree of six loci was inferred using IQ-TREE with the best-fit model “TIM2+F+I+G4”, and 1,000 ultrafast bootstrap replicates. The phylogenetic tree was viewed in MEGA11 and depicted in Adobe Illustrator.

Results

Multi-locus phylogeny. The ITS, TUB2, GAPDH, HIS3, CHS-1, and ACT alignments contained 545, 497, 247, 378, 251, and 234 characters including gaps, respectively. Concatenated alignment of six loci included the members in the *C. acutatum* species complex in this study and references (43 accepted species, and *C. orchidophilum* as an outgroup). A combined phylogenetic tree (Fig. 1) showed that 64 KACC isolates were composed of six different species and a novel species candidate. Of these, 24 KACC

isolates were grouped with the ex-type strain of *C. fioriniae* (CBS 128517), supported by a 100% ML bootstrap value. Twenty-one KACC isolates were clustered together with the ex-type strain of *C. nymphaeae* (CBS 515.78) with a 100% ML bootstrap value. Twelve KACC isolates and the ex-type strain of *C. scovillei* (CBS 126529) formed a single clade with a 99% ML bootstrap value. Three KACC isolates (KACC 40700, KACC 40801, and KACC 47036) were segregated into a separate group with *C. chrysanthemi* (IMI 364540, authentic strain), well supported by a 100% ML bootstrap value. KACC 47255 and KACC 47254 were in a group with the ex-type strain of *C. lupini* (CBS 109225) with a 97% ML bootstrap value. A single clade was generated by KACC 42911 and the ex-type strain of *C. goetiae* (CBS 133.44), well supported with a bootstrap value of 98%. The isolate KACC 40014 formed a single clade and had a distant genetic relationship with others. The GAPDH sequence of KACC 40014 had highest similarities with *C. sloanei* (IMI 364297, ex-type strain, 97.37%), *C. paxtonii* (IMI 165753, ex-type strain, 96.93%) and had 93.86% similarity with *C. simmondsii* (CBS 122122, ex-type strain). The loci of KACC 40014 were lower than 99% similarity with the closely related species such as ACT (98.7% with *C. simmondsii* and *C. paxtonii*), HIS3 (98.66% with *C. simmondsii* and *C. sloanei*), TUB2 (98.98% with *C. paxtonii* and 98.57% with *C. sloanei*), and ITS (98.9% with *C. simmondsii*). The data suggested that *Colletotrichum* sp. (KACC 40014) could be considered as a novel species candidate of the genus *Colletotrichum*.

Host plants of *C. acutatum* species complex in this study. *C. fioriniae* (24 KACC isolates) were collected from 15 different plant species belonging to 15 genera and 11 families with an abundance of Rosaceae (six isolates) and Solanaceae (six isolates). Ten host species (*Actinidia chinensis*, *Capsicum annuum*, *Camellia sinensis*, *Cayratia japonica*, *Chaenomeles sinensis*, *Machilus thunbergii*, *Pinus koraiensis*, *Schizonepeta tenuifolia* var. *japonica*, *Spiraea prunifolia* var. *simpliciflora*, and *Xylosma congesta*) have not been reported in Korea and six of which, including *C. japonica*, *C. sinensis*, *P. koraiensis*, *S. tenuifolia* var. *japonica*, *S. prunifolia* var. *simpliciflora* and *X. congesta*, have not been reported in the world. Five species (*Cucurbita* sp., *Lycium chinense*, *Malus domestica*, *Paeonia lactiflora*, and *Solanum melongena*) were previously reported in Korea. *C. nymphaeae* (21 KACC isolates) were isolated from 10 different host species, from nine genera and seven families. Most of them are from the family Rosaceae (10 isolates) and Solanaceae (four isolates). Eight host species (*Abies holophylla*, *Capsicum annuum*, *Humulus japonicas*,

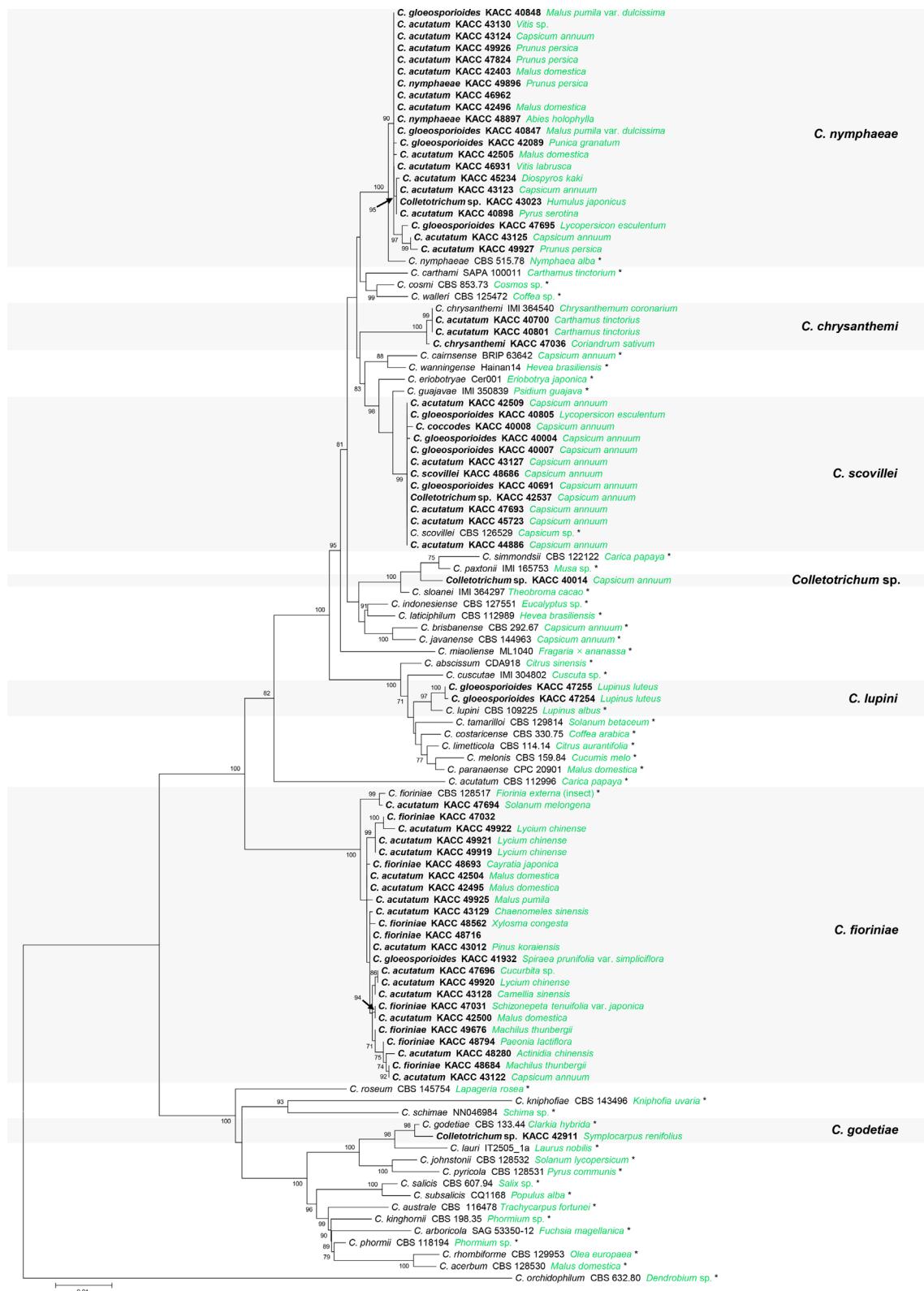


Fig. 1. A maximum likelihood tree was generated based on the analysis of multi-locus sequences of internal transcribed spacers (ITS), beta-tubulin 2 (TUB2), histone-3 (HIS3), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), chitin synthase 1 (CHS-1), and actin (ACT). Species names are followed by isolate numbers and their hosts (green). Isolates in this study are in bold (original species names and isolate numbers). Ex-type strains are noted by *. *Colletotrichum orchidophilum* was used as the outgroup.

Table 2. KACC isolates under *Colletotrichum acutatum* species complex used in this study

Re-identified species	Isolate no.	Location	Collected date	Host	Species name by depositor	Previous identification	RDA-GeneBank accession no.					
							ITS	TUB2	GAPDH			
<i>C. chrysanthemi</i>	KACC 40700	Jecheon	1998 Jun	<i>Carthamus tinctorius</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061920	RDA0062143	RDA0062355	RDA0065775	RDA0065725	RDA0065685
	KACC 40801	Uiseong	1998 Jun	<i>Carthamus tinctorius</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061919	RDA0062144	RDA0062356	RDA0065780	RDA0065720	RDA0065684
	KACC 47036	Eunseong	2012 Sep	<i>Coriandrum sativum</i>	<i>C. chrysanthemi</i>	<i>C. chrysanthemi</i>	RDA0061949	RDA0062247	RDA0062452	RDA0065759	RDA0065741	RDA0065631
<i>C. fioriniae</i>	KACC 47032	Cheongyang	2012 Sep	-	<i>C. fioriniae</i>	<i>C. fioriniae</i>	RDA0061948	RDA0062243	RDA0062448	RDA0065763	RDA0065737	RDA0065635
	KACC 48716	Gangneung	2013 Nov	-	<i>C. fioriniae</i>	<i>C. fioriniae</i>	RDA0061944	RDA0062307	RDA0062501	RDA0065766	RDA0065734	RDA0065638
	KACC 48280	Jeju	2015 Sep	<i>Actinidia chinensis</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061958	RDA0062281	RDA0062476	RDA0065799	RDA0065700	RDA0065665
	KACC 43128	Boseong	2006 Aug	<i>Camellia sinensis</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061938 -	RDA0062418	RDA0065812	RDA0065687	RDA0065677	RDA0065677
	KACC 43122	Jeungpyeong	2004 Jan	<i>Capsicum annuum</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061932	RDA0062207	RDA0062412	RDA0065811	RDA0065688	RDA0065676
	KACC 48693	Jeju	2018 Oct	<i>Cyrtosia japonica</i>	<i>C. fioriniae</i>	<i>C. fioriniae</i>	RDA0061963	RDA0062299	RDA0062494	RDA0065768	RDA0065732	RDA0065639
	KACC 43129	Cheongju	2007 Mar	<i>Chenomeles sinensis</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061939	RDA0062214	RDA0062419	RDA0065777	RDA0065723	RDA0065646
	KACC 47696	Gangneung	2013 Nov	<i>Chaerbita</i> sp.	<i>C. acutatum</i>	<i>C. fioriniae</i>	RDA0061955	RDA0062268	RDA0062462	RDA0065785	RDA0065715	RDA0065651
						(Kim and Kim, 2020)						
	KACC 49919	Cheongyang	-	<i>Lycium chinense</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061969	RDA0062321	RDA0062517	RDA0065808	RDA0065691	RDA0065673
	KACC 49920	Cheongyang	-	<i>Lycium chinense</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061970	RDA0062322	RDA0062518	RDA0065805	RDA0065694	RDA0065670
	KACC 49921	Cheongyang	-	<i>Lycium chinense</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061971	RDA0062323	RDA0062519	RDA0065794	RDA0065705	RDA0065660
	KACC 49922	Cheongyang	-	<i>Lycium chinense</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061972	RDA0062324	RDA0062520	RDA0065752	RDA0065748	RDA0065667
	KACC 48684	Busan	2017 Aug	<i>Machilus thunbergii</i>	<i>C. fioriniae</i>	<i>C. fioriniae</i>	RDA0061961	RDA0062296	RDA0062490	RDA0065804	RDA0065695	RDA0065637
	KACC 42495	Bonghwa	2006 Sep	<i>Malus domestica</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061910	RDA0062184	RDA0062389	RDA0065781	RDA0065719	RDA0065648
	KACC 42500	Yongin	2006 Aug	<i>Malus domestica</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061908	RDA0062189	RDA0062394	RDA0065788	RDA0065712	RDA0065654
	KACC 42504	Jangsu	2006 Aug	<i>Malus domestica</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061907	RDA0062193	RDA0062398	RDA0065776	RDA0065724	RDA0065645
	KACC 49925	Yeongdon	-	<i>Malus pumila</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061973	RDA0062327	RDA0062523	RDA0065797	RDA0065702	RDA0065663
	KACC 48794	Gangjin	2019 Jul	<i>Paeonia lactiflora</i>	<i>C. fioriniae</i>	<i>C. fioriniae</i>	RDA0061965	RDA0062308	RDA0062503	RDA0065798	RDA0065701	RDA0065664
	KACC 43012	Daejeon	2006 May	<i>Pinus koraiensis</i>	<i>C. acutatum</i>	<i>C. acutatum</i>	RDA0061931	RDA0062201	RDA0062407	RDA0065813	RDA0065710	RDA0065655
	KACC 47031	Eunseong	2012 Jul	<i>Schizonepeta tenuifolia</i> var. <i>japonica</i>	<i>C. fioriniae</i>	<i>C. fioriniae</i>	RDA0061947	RDA0062242	RDA0062447	RDA0065770	RDA0065730	RDA0065641
	KACC 47694	Gangneung	2011 Nov	<i>Solanum melongena</i>	<i>C. acutatum</i>	<i>C. fioriniae</i>	RDA0061953	RDA0062266	RDA0062460	RDA0065795	RDA0065704	RDA0065661
						(Xu et al., 2018)						

(Continued)

Table 2. Continued

Re-identified species	Isolate no.	Location	Collected date	Host	Species name by depositor	Previous identification	ITS	TUB2	GAPDH	HIS3	CHS-1	ACT	RDA-GeneBank accession no.
<i>C. fioriniae</i>	KACC 41932	Geoje	2002 May	<i>Spiraea prunifolia</i> var. <i>simpliciflora</i>	<i>C. gloeo sporioides</i> <i>C. acutatum</i>	RDA0061913	RDA0062171	RDA0062375	RDA0065772	RDA0065728	RDA0065643		
	KACC 48562	Seogwipo	2018 Apr	<i>Xylosma congesta</i>	<i>C. fioriniae</i>	RDA0061960	RDA0062287	RDA0062481	RDA0065790	RDA0065709	RDA0065656		
	KACC 49676	Jeju	-	<i>Machilus thunbergii</i>	<i>C. fioriniae</i>	RDA0061967	RDA0065814 -		RDA0065796	RDA0065703	RDA0065662		
<i>C. godetiae</i>	KACC 42911	Hongcheon	2007 Jun	<i>Symplocarpus renifolius</i>	<i>Colletotrichum</i> sp.	RDA0061930	RDA0062200	RDA0062406	RDA0065787	RDA0065713	RDA0065653		
<i>C. lupini</i>	KACC 47254	Seoul	2009 May	<i>Lupinus luteus</i>	<i>C. gloeo sporioides</i> <i>C. lupini</i> (Han RDA0061950 et al., 2014)	RDA0061950	RDA0062249	RDA0065816	RDA0065782	RDA0065718	RDA0065649		
	KACC 47255	Yongin	2013 Apr	<i>Lupinus luteus</i>	<i>C. gloeo sporioides</i>	RDA0061951	RDA0062250	RDA0062454	RDA0065760	RDA0065740	RDA0065632		
<i>C. nymphaea</i>	KACC 46962	Mungyeong	2012 Sep	-	<i>C. acutatum</i>	RDA0061946	RDA0062237	RDA0062442	RDA0065771	RDA0065729	RDA0065642		
	KACC 47824	Cheongdo	2014 Aug	<i>Prunus persica</i>	<i>C. acutatum</i>	RDA0061956	RDA0062270	RDA0062464	RDA0065784	RDA0065716	RDA0065650		
	KACC 48897	Tongyong	-	<i>Abies holophylla</i>	<i>C. nymphaea</i>	RDA0061966	RDA0062310	RDA0062505	RDA0065806	RDA0065693	RDA0065693		
	KACC 43123	Chungbuk	2002 Jan	<i>Capsicum annuum</i>	<i>C. acutatum</i>	RDA0061933	RDA0062208	RDA0062413	RDA0065778	RDA0065722	RDA0065647		
	KACC 43124	Boeun	2004 Jan	<i>Capsicum annuum</i>	<i>C. acutatum</i>	RDA0061934	RDA0062209	RDA0062414	RDA0065758	RDA0065742	RDA0065629		
	KACC 43125	Cheongju	2004 Jan	<i>Capsicum annuum</i>	<i>C. acutatum</i>	RDA0061935	RDA0062210	RDA0062415	RDA0065761	RDA0065739	RDA0065633		
	KACC 45234	Changwon	2008 Oct	<i>Diospyros kaki</i>	<i>C. acutatum</i>	RDA0061943	RDA0062226	RDA0062433	RDA0065765	RDA0065735	RDA0065636		
	KACC 47695	Gangneung	2013 Jun	<i>Lycopersicon esculentum</i>	<i>C. gloeo sporioides</i>	RDA0061954	RDA0062267	RDA0062461	RDA0065773	RDA0065727	RDA0065644		
	KACC 42403	Andong	2006 Sep	<i>Malus domestica</i>	<i>C. acutatum</i>	<i>C. nymphaea</i> RDA0061911	RDA0062178	RDA0062383	RDA0065792	RDA0065707	RDA0065658	(Kim et al., 2020)	
	KACC 42496	Gunwi	2006 Sep	<i>Malus domestica</i>	<i>C. acutatum</i>	RDA0061909	RDA0062185	RDA0062390	RDA0065810	RDA0065689	RDA0065675		
	KACC 42505	Gunwi	2004 Aug	<i>Malus domestica</i>	<i>C. acutatum</i>	RDA0061927	RDA0062194	RDA0062399	RDA0065786	RDA0065714	RDA0065652		
	KACC 40847	Andong	1999 Oct	<i>Malus pumila</i> var. <i>dulcissima</i>	<i>C. gloeo sporioides</i> <i>C. acutatum</i>	RDA0061916	RDA0062147	RDA0062365	RDA0065756	RDA0065744	RDA0065627	(Kim et al., 2006)	
	KACC 40848	Yeongju	1999 Oct	<i>Malus pumila</i> var. <i>dulcissima</i>	<i>C. acutatum</i>	<i>C. nymphaea</i> RDA0061915	RDA0062148	RDA0062366	RDA0065753	RDA0065747	RDA0065624	(Kim et al., 2020)	
	KACC 49896	Jeonju	2021 Oct	<i>Prunus persica</i>	<i>C. nymphaea</i>	RDA0061968	RDA0062320	RDA0062316	RDA0065762	RDA0065738	RDA0065634		
	KACC 49926	Sejong	-	<i>Prunus persica</i>	<i>C. acutatum</i>	RDA0061974	RDA0062328	RDA0062524	RDA0065802	RDA0065697	RDA0065668		

(Continued)

Table 2. Continued

Re-identified species	Isolate no.	Location	Collected date	Host	Species name by depositor	Previous identification	RDA-GeneBank accession no.					
							ITS	TUB2	GAPDH	HIS3	CHS-1	ACT
<i>C. nymphaeae</i> KACC 42089 Hapcheon											RDA0065623	
KACC 40898 Naju	2000 Oct	<i>Pyrus serotina</i>		<i>C. acutatum</i>		RDA0061912 RDA0062376 RDA0065751 RDA0065750 RDA0065674						
KACC 43130 Cheongju	2006 Jun	<i>Vitis</i> sp.		<i>C. acutatum</i>		RDA0061940 RDA0062215 RDA0062420 RDA0065807 RDA0065692 RDA0065672						
C. <i>nymphaeae</i> KACC 46931 Yeongdong	2011 Nov	<i>Vitis labrusca</i>		<i>C. acutatum</i>		<i>C. acutatum</i>	RDA0061945 RDA0062235 RDA0065817 RDA0065793 RDA0065706 RDA0065659					
C. <i>nymphaeae</i>	KAC C 43023 Hongcheon	2007 Jul	<i>Humulus japonicus</i>	<i>Colletotrichum</i> sp.		RDA0061979 RDA0062203 RDA0062409 RDA0065764 RDA0065622						
							RDA0061975 -	RDA0065815 -	RDA0065749 -	RDA0065749 -	RDA0065749 -	
C. <i>scovillei</i>	KACC 49927 Sejong	-	<i>Prunus persica</i>	<i>C. acutatum</i>		RDA0060929 RDA0062124 RDA0062331 RDA0065800 RDA0065699 RDA0065686						
							(Kim et al., 2006)	(Noh et al., 2014)	(RDA0061979 RDA0062203 RDA0062409 RDA0065764 RDA0065622)	(RDA0061975 -)	(RDA0065815 -)	
KACC 40007 Daejeon	-		<i>Capsicum annuum</i>	<i>C. gloeosporioides</i> C. <i>acutatum</i>		RDA0060924 RDA0062126 RDA0062334 RDA0065755 RDA0065745 RDA0065626						
							(Kim et al., 2006)	(RDA0060925 RDA0062127 RDA0062335 RDA0065791 RDA0065708 RDA0065657)				
KACC 40008 Daejeon	-		<i>Capsicum annuum</i>	<i>C. coccodes</i>		RDA0060937 RDA0062140 RDA0062348 RDA0065757 RDA0065743 RDA0065628						
							(Kim et al., 2006)	(RDA0060937 RDA0062140 RDA0062348 RDA0065757 RDA0065743 RDA0065628)				
KACC 40691 Hwangseong	1998 Aug	<i>Capsicum annuum</i>	<i>C. gloeosporioides</i> C. <i>acutatum</i>			RDA0061929 RDA00622198 RDA0062403 RDA0065767 RDA0065733 RDA0065682						
							(Kim et al., 2006)	(RDA0061929 RDA00622198 RDA0062403 RDA0065767 RDA0065733 RDA0065682)				
KACC 42509 Gunwi	2005 Jul	<i>Capsicum annuum</i>	<i>C. acutatum</i>			RDA0061937 RDA0062212 RDA0062417 RDA0065779 RDA0065721 RDA0065680						
							(Kim et al., 2006)	(RDA0061937 RDA0062212 RDA0062417 RDA0065779 RDA0065721 RDA0065680)				
KACC 43127 Jeonbuk	2002 Jan	<i>Capsicum annuum</i>	<i>C. acutatum</i>			RDA0061941 RDA0062224 RDA0062431 RDA0065754 RDA0065746 RDA0065679						
							(Kim et al., 2006)	(RDA0061941 RDA0062224 RDA0062431 RDA0065754 RDA0065746 RDA0065679)				
KACC 44886 Daejeon	2008 May	<i>Capsicum annuum</i>	<i>C. acutatum</i>			RDA0061944 RDA0062229 RDA0062436 RDA0065769 RDA0065731 RDA0065640						
							(Kim et al., 2006)	(RDA0061944 RDA0062229 RDA0062436 RDA0065769 RDA0065731 RDA0065640)				
KACC 45723 Hwaseong	2008 Feb	<i>Capsicum annuum</i>	<i>C. acutatum</i>			RDA0061952 RDA0062265 RDA0062459 RDA0065783 RDA0065717 RDA0065678						
							(Kim et al., 2006)	(RDA0061952 RDA0062265 RDA0062459 RDA0065783 RDA0065717 RDA0065678)				
KACC 47693 Pyeongchang	2012 Sep	<i>Capsicum annuum</i>	<i>C. acutatum</i>			RDA0061962 RDA0062297 RDA0062492 RDA0065803 RDA0065696 RDA0065669						
							(Kim et al., 2006)	(RDA0061962 RDA0062297 RDA0062492 RDA0065803 RDA0065696 RDA0065669)				
KACC 48686 Gimje	2018 Sep	<i>Capsicum annuum</i>	<i>C. scovillei</i>			RDA006197 RDA0062146 RDA0065818 RDA0065774 RDA0065726 RDA0065683						
							(Kim et al., 2006)	(RDA006197 RDA0062146 RDA0065818 RDA0065774 RDA0065726 RDA0065683)				
KACC 40805 Yangpyeong	1996 Sep	<i>Lycopersicon esculentum</i>	<i>C. gloeosporioides</i> C. <i>acutatum</i>			RDA0061978 RDA0062199 RDA0062404 RDA0065789 RDA0065711 RDA0065681						
							(Kim et al., 2006)	(RDA0061978 RDA0062199 RDA0062404 RDA0065789 RDA0065711 RDA0065681)				
Colletotrichum sp.	KACC 42537 Chuncheon	2006 Sep	<i>Capsicum annuum</i>	<i>Colletotrichum</i> sp.		RDA0061976 RDA0062132 RDA0062340 RDA0065801 RDA0065698 RDA0065666						
							(Kim et al., 2006)	(RDA0061976 RDA0062132 RDA0062340 RDA0065801 RDA0065698 RDA0065666)				

ITS, internal transcribed spacer; TUB2, β -tubulin 2; GAPDH, glyceraldehyde-3-phosphate hydrogenase; HIS3, histone3; CHS-1, chitin synthase; ACT, actin; RDA, Rural Development Administration; K/ACC, Korean Agricultural Culture Collection.
 ITS, internal transcribed spacer; TUB2, β -tubulin 2; GAPDH, glyceraldehyde-3-phosphate hydrogenase; HIS3, histone3; CHS-1, chitin synthase; ACT, actin; RDA, Rural Development Administration; K/ACC, Korean Agricultural Culture Collection.

Table 3. Comparison of host plants between KACC isolates and previous reports

Species	KACC	Host			
		The List of Plant Diseases	Previous reports in Korea	Global reports	References
<i>C. chrysanthemi</i>	<i>Carthamus tinctorius</i>	-	-	<i>Carthamus tinctorius</i>	Baroncelli et al. (2015)
	<i>Coriandrum sativum</i> *	-	-	-	-
	-	<i>Chrysanthemum coronarium</i>	<i>Chrysanthemum coronarium</i>	<i>Chrysanthemum coronarium</i>	Sato and Moriwaki (2013)
				(Continued)	
<i>C. fioriniae</i>	<i>Actinidia chinensis</i>	-	-	<i>Actinidia chinensis</i>	Shivas and Tan (2009)
	<i>Capsicum annuum</i>	-	-	<i>Capsicum annuum</i>	Noor and Zakaria (2018)
	<i>Camellia sinensis</i>	-	-	<i>Camellia sinensis</i>	Wang et al. (2016)
	<i>Cayratia japonica</i> *	-	-	-	-
	<i>Chaenomeles sinensis</i> *	-	-	-	-
	<i>Cucurbita</i> sp.	<i>Cucurbita moschata</i>	<i>Cucurbita moschata</i>	<i>Cucurbita moschata</i>	Kim and Kim (2020)
	<i>Lycium chinense</i>	<i>Lycium chinense</i>	<i>Lycium chinense</i>	<i>Lycium chinense</i>	Oo et al. (2016)
	<i>Machilus thunbergii</i>	-	-	<i>Machilus thunbergii</i>	Sato et al. (2013)
	<i>Malus domestica</i>	-	<i>Malus domestica</i>	<i>Malus domestica</i>	Oo et al. (2018)
	<i>Paeonia lactiflora</i>	<i>Paeonia lactiflora</i>	<i>Paeonia lactiflora</i>	<i>Paeonia lactiflora</i>	Park et al. (2020)
	<i>Pinus koraiensis</i> *	-	-	-	-
	<i>Schizonepeta tenuifolia</i> var. <i>japonica</i> *	-	-	-	-
	<i>Solanum melongena</i>	<i>Solanum melongena</i>	<i>Solanum melongena</i>	<i>Solanum melongena</i>	Xu et al. (2018)
	<i>Spiraea prunifolia</i> var. <i>simpliciflora</i> *	-	-	-	-
	<i>Xylosma congesta</i> *	-	-	-	-
<i>C. godetiae</i>	<i>Symplocarpus renifolius</i> *	-	-	-	-
				(Continued)	
<i>C. lupini</i>	<i>Lupinus luteus</i>	<i>Lupinus luteus</i>	<i>Lupinus luteus</i>	-	Han et al. (2014)
				(Continued)	
<i>C. nymphaeae</i>	<i>Abies holophylla</i> *	-	-	-	-
	<i>Capsicum annuum</i>	-	-	<i>Capsicum annuum</i>	Nasehi et al. (2016)
	<i>Diospyros kaki</i>	<i>Diospyros kaki</i>	<i>Diospyros kaki</i>	<i>Diospyros kaki</i>	Hassan et al. (2019a)
	<i>Humulus japonicus</i> *	-	-	-	-
	<i>Lycopersicon esculentum</i> (syn. <i>Solanum lycopersicum</i>)	-	-	<i>Solanum lycopersicum</i>	Santos et al. (2018)
	<i>Malus domestica</i> (syn. <i>Malus pumila</i>)	-	<i>Malus domestica</i>	<i>Malus domestica</i>	Oo et al. (2018)

(Continued)

Table 3. Continued

Species	KACC	Host			
		The List of Plant Diseases	Previous reports in Korea	Global reports	References
<i>C. nymphaeae</i>	<i>Prunus persica</i>	-	-	<i>Prunus persica</i>	Tan et al. (2022)
	<i>Punica granatum</i>	-	-	<i>Punica granatum</i>	Xavier et al. (2019)
	<i>Pyrus serotina</i> (syn. <i>P. pyrifolia</i>)	-	-	<i>Pyrus pyrifolia</i>	Moreira et al. (2019)
	<i>Vitis labrusca</i>	-	-	<i>Vitis labrusca</i>	Chechi et al. (2019)
	-	<i>Actinidia argute</i>	<i>Actinidia argute</i>	<i>Actinidia argute</i>	Kim et al. (2018)
	-	<i>Prunus salicina</i>	<i>Prunus salicina</i>	<i>Prunus salicina</i>	Chang et al. (2018)
	-	-	<i>Vaccinium</i> sect. <i>Cyanococcus</i>	<i>Vaccinium</i> sect. <i>Cyanococcus</i>	Cho et al. (2021)
	-	-	<i>Ziziphus jujube</i>	<i>Ziziphus jujube</i>	Kang et al. (2023)
(Continued)					
<i>C. scovillei</i>	<i>Capsicum annuum</i>	<i>Capsicum annuum</i>	<i>Capsicum annuum</i>	<i>Capsicum annuum</i>	Oo et al. (2017)
	<i>Lycopersicon esculentum</i>*	-	-	-	-
(Continued)					
<i>Colletotrichum</i> sp. (novel species candidate)	<i>Capsicum annuum</i>*	-	-	-	-

Hosts in “bold” and followed by “*” are unreported in Korea and in the world, respectively.

Lycopersicon esculentum, *Prunus persica*, *Punica granatum*, *Pyrus serotina*, and *Vitis labrusca*) have not been reported in Korea, of which two species (*A. holophylla* and *H. japonicus*) have not been recorded in the world. Two other species (*Diospyros kaki* and *Malus domestica*) were previously reported in Korea. *C. scovillei* (12 KACC isolates) were obtained from only the family Solanaceae, including 11 isolates from *Capsicum annuum* (reported in Korea) and one isolate from *Lycopersicon esculentum* (unreported in the world). *C. chrysanthemi* (three KACC isolates) was isolated from *Coriandrum sativum* (unreported in the world) and *Carthamus tinctorius* (unreported in Korea). *C. lupini* (two KACC isolates) was found on *Lupinus luteus* (reported in Korea). *C. godetiae* (one KACC isolate) was from *Symplocarpus renifolius* (unreported in the world). A novel species candidate (KACC 40014) was collected from *Capsicum annuum* (Tables 2 and 3).

Discussion

Sixty-four Korean isolates in *C. acutatum* species complex were accurately identified into six different species (*C. fioriniae*, *C. nymphaeae*, *C. scovillei*, *C. chrysanthemi*, *C. lupini*, and *C. godetiae*) and a novel species candidate,

based on the combination of multi-locus sequences of ITS, TUB2, HIS3, GAPDH, CHS-1, and ACT. Forty-eight isolates changed their species names from the original names given by depositors. The present results also demonstrated that the identifications of the species in the *C. acutatum* species complex using a single ITS region and/or TUB2 gene in the previous publications (Han et al., 2014; Kim et al., 2006, 2008; Noh et al., 2014) were insufficient.

C. fioriniae has been reported as an entomopathogenic, endophytic and phytopathogenic fungus (Damm et al., 2012; Marcelino et al., 2008). This species has been reported as the causal agent of anthracnose diseases on *Cucurbita moschata*, *Lycium chinense*, *Malus domestica*, *Paeonia lactiflora*, *Solana melongena*, *Ilex integra*, *Prunus persica*, *Prunus salicina*, *Schisandra chinensis*, and *Vaccinium* sect. *Cyanococcus* in Korea. Six of them (*M. domestica*, *P. salicina*, *S. chinensis*, and *V. sect. Cyanococcus*) were not listed in The List of Plant Diseases in Korea (<http://genbank.rda.go.kr/english/plntDissInfo.do>), and five species (*I. integra*, *P. persica*, *P. salicina*, *S. chinensis*, and *V. sect. Cyanococcus*) were not found in this work. Meanwhile, ten host species of *C. fioriniae* in this study have not been recorded in Korea or in the world. *C. nymphaeae* was associated with serious anthracnose diseases in a wide range

of host plants, especially strawberries (*Fragaria × ananassa*) (Damm et al., 2012; Jayawardena et al., 2016). In the previous research, six host species of *C. nymphaeae* (*Diospyros kaki*, *Malus domestica*, *Actinidia argute*, *Prunus salicina*, *Vaccinium* sect. *Cyanococcus*, and *Ziziphus jujube*) were reported in Korea, three of that plant (*M. domestica*, *V. sect. Cyanococcus*, and *Z. jujube*) have not been updated in The List of Plant Diseases in Korea, and four species (*A. argute*, *P. salicina*, *V. sect. Cyanococcus*, and *Z. jujube*) were not recorded in this study. However, eight host species in the present study were not introduced in relationship with this fungal species before in Korea or in the world. *C. scovillei* has a narrow host range and was commonly reported as one of the highly aggressive diseases of *Capsicum* spp. in many countries such as Brazil (Giacomin et al., 2021), Korea (Oo et al., 2017) and Asia (de Silva et al., 2019). *C. scovillei* also infected *Clausena lansium* (Lin et al., 2020), *Mangifera indica* (Qin et al., 2019), *Musa* sp. (Zhou et al., 2017), and *Pseudodraconium lacourii* (Liu et al., 2022). In our findings, *C. scovillei* was isolated from *Lycopersicon esculentum* and this has not been reported in the world.

C. chrysanthemi was only reported in the family Asteraceae, including *Carthamus tinctorius* in Italy (Baroncelli et al., 2015), *Glebionis carinata* (vascular discoloration) in the Netherlands, *Glebionis coronaria* (leaf spot) in China (Damm et al., 2012), *Chrysanthemum coronarium* in Korea and *Calendula officinalis* in Japan (Sato and Moriwaki, 2013). In this study, *C. chrysanthemi* was not only collected from the Asteraceae (*Carthamus tinctorius*) but also obtained from an unrecorded family Apiaceae (*Coriandrum sativum*).

C. lupini was first reported to cause anthracnose on yellow lupin (*Lupinus luteus*) in Korea and Asia in 2013 (Han et al., 2014). This species has a narrow range of host plants, but it showed high virulence and globally widespread disease on some host species of the genus *Lupinus* (Alkemade et al., 2021). This fungal species was also found on *Camellia* sp., *Cinnamomum verum*, *Manihot utilissima*, and *Olea europaea* (Alkemade et al., 2021; Damm et al., 2012). *C. godetiae* was infected economically important crops and had a wide host range and global distribution (Alizadeh et al., 2015; Liu et al., 2022; Shivas et al., 2016; Tan et al., 2022; Tóth et al., 2017). However, this species was not commonly associated with valuable crops in Korea. In our study, this fungal species could be a new finding on Asian skunk cabbage (*Symplocarpus renifolius*).

On the other hand, considering species under *C. acutatum* species complex according to economic host plants in

this study, *C. scovillei* ($n = 11$) was dominant on *Capsicum annuum* (pepper) and *C. nymphaeae* ($n = 3$), *C. fioriniae* ($n = 1$) followed. Five *C. nymphaeae* and four *C. fioriniae* isolates were identified from *Malus* spp. including apple. Only four and two isolates of *C. nymphaeae* were isolated from peach (*Prunus persica*) and grape (*Vitis labrusca*), respectively.

The most important finding in this study is that 23 new combinations could be suggested in Korea and 12 of these have not been reported in the world. Of which, *C. fioriniae* on pepper (*Capsicum annuum*) and kiwi (*Actinidia chinensis*), *C. nymphaeae* on peach (*Prunus persica*), pear (*Pyrus serotina*) and grape (*Vitis labrusca*), and *C. scovillei* on tomato (*Lycopersicon esculentum*), are meaningful information in the agricultural field of Korea. However, the pathogenicity of KACC isolates on hosts is not clear. KACC did not confirm the pathogenicity of deposited *Colletotrichum* isolates on host plants, but depended on only the depositor's information. Therefore, new combinations suggested in this study need to be clarified via the pathogenicity tests in further studies.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (<http://www.pppjonline.org/>).

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