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Molecular Re-identification of Strains of the *Colletotrichum acutatum* Species Complex Deposited in the NIAS Genebank and Morphological Characteristics of its Member Species

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Abstract

Recently, nine phylogenetic groups were proposed for a plurivorous fungus, *Colletotrichum acutatum* by some researchers. Some of them were named as *C. simmondsii*, *C. fioriniae*, *C. acutatum sensu stricto* and *C. carthami* based on morphology and phylogenetic analyses of β-tubulin-2 gene partial sequences. We re-identified 170 strains deposited as *C. acutatum* in the NIAS Genebank, Japan based on phylogenetic analysis with the β-tubulin-2 gene sequences. Eighty strains with reddish colony colors and relatively uniform conidia belonged to *C. fioriniae*, 37 with a grayish to yellowish color and short conidia to *C. simmondsii*, 25 with a blackish to cream color and short conidia to *C. carthami*, 12 with a cream to pale orange color and narrow conidia to '*C. acutatum* group A2-P' newly designated in this paper, 5 with a brownish to orange color and larger conidia to *C. acutatum* group A4, and 11 to other species of *Colletotrichum*. No strain of *C. acutatum s. str.* was found. A dichotomous key to the member species and groups was designated based on reverse colony color and conidial morphology. Because pathogenic strains of 18 host plants were re-identified, updating of pathogen names was proposed. Ten of them should be changed to *C. fioriniae*, and the pathogens of each five host plants such as strawberry and apple should be replaced with 2 to 3 species or the group. Sweet pepper pathogen was tentatively classified into *C. acutatum* group A2-P.

Discipline: Plant disease
Additional key words: β-tubulin-2 gene, colony color, conidial morphology, dichotomous key, phylogenetic analysis

Introduction

Colletotrichum acutatum J. H. Simmonds was known as a plurivorous and cosmopolitan anthracnose pathogen, since it was first described as having fusiform conidia and reddish colonies in Australia[27]. Its morphology, e.g. colony color and conidial shape, has been reported to vary among strains[1,23]. Because some pathogenic strains of species causing anthracnose resembled *Colletotrichum gloeosporioides* (Penzig) Penzig & Saccardo, they were often misidentified as the latter species, especially in Japan[21]. A few morphologically distinct groups, e.g. 'larger spored form'[27], appeared to be present in *C. acutatum*[23]. Recently, several researchers applied DNA sequences to define genetic groups within the species[10,14,39]. Groups A1 to A8 were recognized based on sequences of ribosomal DNA internal transcribed spacer (rDNA-ITS) region[28] and A9 was added based on the result of a randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) and β-tubulin-2 gene partial sequences[40]. Some researchers proposed dividing the groups into distinct species[5,28,39]. Shivas and Tan[26] separated two species, *Colletotrichum fioriniae* (Marcelino & Gouli) R. G. Shivas & Y. P. Tan (=group A3)

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and *Colletotrichum simmondsii* R. G. Shivas & Y. P. Tan (=group A2, A9), from the *C. acutatum* species complex and also defined *C. acutatum sensu stricto* (=group A5) based on their morphological characteristics in culture and molecular phylogenetic analyses with the rDNA-ITS region and the β-tubulin-2 gene sequences. Quite recently, *Colletotrichum carthami* (Fukui) S. Uematsu, Kageyama, Moriwaki & Toy. Sato was revived as a member of the species complex, which had distinct β-tubulin-2 gene sequences and specific pathogenicity to three asteraceous plants such as safflower. Moreover, phylogenetic analysis with the β-tubulin-2 gene was confirmed as more effective than with the rDNA-ITS region in identifying the member species of the complex[37]. Although each of the three species except for *C. carthami* reportedly had a characteristic colony color[26,37], differences in the microscopic characters of the four species remained unclear. More than 150 strains of the *C. acutatum* species complex are preserved in the Genebank, National Institute of Agrobiological Sciences (NIAS), Japan. We performed molecular re-identification and subsequent microscopic re-examination of the strains to clarify the morphological differences among the member species of the complex, which enabled everyone to identify them quickly.

The strains included many pathogens of various plants reported in Japan. Re-identification of the strains according to recent taxonomy[26,37] is necessary to determine the host range of each member species of the complex. Moreover, discrimination of the member species helps us evaluate pathogenicity to their host plants appropriately, as shown in the case where *C. simmondsii* was much more virulent to celery than *C. fioriniae*[3].

Materials and Methods

1. Molecular Re-identification

One hundred and seventy strains of "*C. acutatum*" preserved in the NIAS Genebank (Table 1) were re-identified based on phylogenetic analyses with the β-tubulin-2 gene partial sequences and genomic DNA was extracted according to the procedure by Moriwaki et al.[16]. The extracted DNA was used as a template DNA for the subsequent polymerase chain reaction (PCR) analysis. The cycling conditions were 94°C for 3 min, followed by 35 cycles of 94°C for 30 sec - 60°C for 1 min - 72°C for 1 min, and 72°C for 5 min, respectively in a GeneAmp 9700 (Applied Biosystems Japan, Tokyo, Japan). Part of the β-tubulin-2 gene (exons 2–6) was amplified with the *Taq* polymerase (TaKaRa, Otsu, Japan) and T1[18] and Bt2b[4] primers. PCR products were purified using a QIAquick PCR Purification kit (Qiagen, Chatsworth, CA, USA) and sequenced directly with a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Primers T1, Bt2b, TB5[33], and TBCA[34] for the β-tubulin-2 gene were used for bidirectional sequencing. Sequencing reactions were conducted according to the manufacturer's instructions. Extension products were analyzed on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems) according to the manufacturer's instructions. All the sequences determined were published from the web pages, "Detailed information of microorganism genetic resources of Microorganism Search System", NIAS Genebank (http://www.gene.affrc.go.jp/databases-micro_search_en.php) or DDBJ/EMBL/GenBank databases.

For phylogenetic analysis, the sequence data of the β-tubulin-2 gene sequences of *Colletotrichum acutatum s. str.*, *C. acutatum* group A4[28] (larger spored form), *C. simmondsii* and *C. fioriniae*, which were downloaded from DDBJ/EMBL/GenBank databases, were also included as references[26]. Sequences of *C. gloeosporioides* were used as an outgroup. A multiple sequence alignment of β-tubulin-2 was initially performed using the alignment subroutines of Clustal X version 2.0[15,35] whereupon the alignment of all sequences was further optimized manually. Phylogenetic relationships were analyzed by the distance methods. The distance matrix for the aligned sequences was calculated using Kimura's two-parameter model[13] and analyzed with the neighbor-joining (NJ) method[19] using Clustal X version 2.0, excluding positions with gaps. The reliability of the inferred tree was estimated by bootstrap analysis[2].

2. Morphological studies

Two kinds of media, potato dextrose agar (PDA, Difco laboratories, Detroit, MI, USA) and modified Weitzman-Silva-Hunter Agar[37] (WSH: 10 g crushed oat meal, 1 g KH_2PO_4, 1 g $MgSO_4 \cdot 7H_2O$, 1 g $NaNO_3$, 20 g agar, 1,000 mL distilled water), were used because strains of the *C. acutatum* species complex often showed more distinct colors on WSH than on PDA, as described in the results. Mycelial discs (6 mm diameter) of strains with bold numbers of MAFF accessions listed on Table 1 were cultured on PDA and WSH plates (55 mm in diam.) at 25°C in the dark for 14–21 days to observe the reverse colony color and conidial morphology. The length and width of each fifty conidia of the strains were measured with a phase contrast microscope (Nikon Eclipse 80i with an image analyzer, Nikon Digital Sight; Nikon, Tokyo, Japan). Standard deviations of the conidial length of the member species were calculated by the STDV function of Excel 2008 for Mac (Microsoft, Redmond, WA, USA).

Results

1. Molecular Re-identification

An NJ tree was obtained from phylogenetic analysis with the sequences of 170 strains uploaded to the NIAS

Table 1. Re-identification of the *Colletotrichum acutatum* species complex strains deposited in the NIAS Genebank (MAFF[a])

Isolation sources (Host plant)[b]	Re-identified species	MAFF accession[c]	Reference[d]
Actinidia deliciosa var. ***deliciosa***	*C. fioriniae*[e]	237215–237218	**38**
Akebia sp.	*C. fioriniae*	**238947**	
Anemone coronaria	*C. simmondsii*[f]	306487, 306488, 306507–306509	**21**
	C. acutatum A4[g]	**306506**	
Annona squamosa	*C. simmondsii*	**306172**	20
Apium graveolens	*C. simmondsii*	242590	**3**
	C. fioriniae	**242591**	**3**
Bischofia javanica	*C. simmondsii*	**237894**	24
Calendula officinalis	*C. fioriniae*	**237240**	24
	C. carthami[h]	**239355**, 239356–239361, **242919**[k]	**37**
Capsicum annuum var. ***annuum***	*C. acutatum* A2-P[i]	**242420**–**242428**, 242592, **242692**, 242693	**12, 36**
	C. gloeosporioides[j]	306096	25
Carthamus tinctorius	*C. carthami*	**239370**–**239374**, **243248**[l]	**37**
Castanopsis sieboldii	*C. fioriniae*	**238654, 238655**	**32**
Chrysanthemum coronarium var. *spatiosum*	*C. carthami*	239362–239369, **239368**, **239369**	**37**
Citrus unshiu	*C. fioriniae*	239142	
Corchorus olitorius	*C. fioriniae*	**306551**	**7**
Cosmos bipinnatus	*C. fioriniae*	**237758**, 306550	**41**
Cotinus coggygria	*C. fioriniae*	**712312**	**29**
Cydonia oblonga	*C. acutatum* A4	241296	
Diospyros kaki	*C. fioriniae*	**237130**	
Eriobotrya japonica	*C. fioriniae*	**241801**, 305596, 306405, 306408–306410	15, 20, **22**
	C. simmondsii	**306406**, 306407	**22**
Eustoma grandiflorum	*C. fioriniae*	238652, 238653, **306247**, 306249–306252, 306502, 306254	15, 20, **22**, 23
Fagus crenata	*C. fioriniae*	239399, **410888**	
Fragaria* × *ananassa	*C. simmondsii*	239773, **306647**, 306682, 731068, 731069	24
	C. fioriniae	**241293**, **242430**, 306282, **306283**	**9**, 15
	C. carthami	238555, 744062, 744063	
Gentiana scabra	*C. fioriniae*	**241878**, 241879	**17**
var. ***buergeri***	*C. simmondsii*	712289	
Glochidion obovatum	*C. fioriniae*	**238519**	
Hovenia dulcis	*C. fioriniae*	**240052**	
Hyacinthus orientalis	*C. fioriniae*	306542	23
Hydrangea sp.	*C. gloeosporioides*	306735	
Hydrangea sp.	*Colletotrichum* sp.	306736	
Kadsura japonica	*C. simmondsii*	241261	
Machilus thunbergii	*C. fioriniae*	240389	
Mahonia fortunei	*C. gloeosporioides*	242619, 242620	
Malus pumila var. ***domestica***	*C. acutatum* A4	**241297**	
	C. fioriniae	305145, 306543, 306544, **306549**, 306630	15, 8, **23**
	C. simmondsii	306546–306548	**23**
Matthiola incana	*C. simmondsii*	**712311**	**29**
Morus bombycis	*C. fioriniae*	840072, 840073	**44**
Nyssa sinensis	*C. fioriniae*	**239279**	
Origanum vulgare	*C. fioriniae*	240192	
Petroselinum crispum	*C. simmondsii*	242413–242419	

Table 1. (Continued)

Isolation sources (Host plant)[b)]	Re-identified species	MAFF accession[c)]	Reference[d)]
Prunus × *yedoensis*	*Colletotrichum* sp.	240237	
	C. fioriniae	**240249**, **240427**	
Prunus armeniaca	*C. fioriniae*	**306528**, **306529**	16
Prunus domestica	*C. acutatum* A4	**241295**	**6**
	C. fioriniae	306489, **306504**, 306651	**15**, **21**
	C. simmondsii	**241294**, 306503, **306505**	**6**, **21**
Prunus mume	*C. fioriniae*	306526, **306527**	16
Prunus persica	*C. simmondsii*	306430, 306522–306524	16 23
Prunus salicina	*C. fioriniae*	306677	
Punica granatum	*C. fioriniae*	237168	24
Pyrus communis var. *sativa*	*C. fioriniae*	**306520**, **306521**	16
Rhododendron tashiroi	*Colletotrichum* sp.	240193	
	C. fioriniae	240250, 240251	
Rohdea japonica	*C. fioriniae*	**238639**	
Rosa rugosa	*C. fioriniae*	712210, 712211	
Rumex japonicus	*C. fioriniae*	**306490**, 306545	20
Sanguisorba officinalis	*C. acutatum* A4	**240289**	**30**
Stewartia pseudo-camellia	*Colletotrichum* sp.	237922, 306725, 306726, 410809	**11**, 23
Sutera sp.	*C. fioriniae*	**240194**	
Trichosanthes kirilowii var. *japonica*	*C. fioriniae*	238651	20
Vaccinium corymbosum	*C. fioriniae*	**240425**, 240426, 306610, 306611, 306650	**43**
Vigna radiata	*C. simmondsii*	242581	
Vitis vinifera	*C. fioriniae*	238647, 665009	**42**
Woody plant	*C. simmondsii*	239736	

[a)] Acronym of microbe strains in the Genebank, National Institute of Agrobiological Sciences, Japan.
[b)] Bold names were reported as host plants of diseases caused by *Colletotrichum acutatum* in Japan.
[c)] Bold strains were used in the morphological examination, detailed data and DNA sequences of each strain appearing in the website, http://www.gene.affrc.go.jp/databases-micro_search_en.php
[d)] Bold references are initial reports on diseases caused by *Colletotrichum acutatum* in Japan, in which the strains listed were described.
[e)] *Colletotrichum fioriniae* (Marcelino & Gouli) R. G. Shivas & Y. P. Tan [26]
[f)] *Colletotrichum simmondsii* R.G. Shivas & Y. P. Tan [26]
[g)] *Colletotrichum acutatum* J. H. Simmonds group A4 = 'larger spored form' [27,30,39]
[h)] *Colletotrichum carthami* (Fukui) S. Uematsu, Kageyama, Moriwaki & Toy. Sato[37]
[i)] Newly designated in this paper
[j)] *Colletotrichum gloeosporioides* (Penzig) Penzig & Saccardo
[k)] DDBJ/EMBL/GenBank accsession: AB696989
[l)] DDBJ/EMBL/GenBank accsession: AB696990

Genebank website mentioned above and those downloaded from DDBJ/EMBL/GenBank databases (Fig. 1). The phylogenetic tree revealed that 80, 37 and 25 strains belonged to *C. fioriniae, C. simmondsii* and *C. carthami*, respectively. The β-tubulin-2 gene sequences of the 80 strains of *C. fioriniae* in Japan were relatively similar and clustered in a single clade with the holotype. No strains of *C. simmondsii* collected in Japan formed a clade with the holotype of the species. However, 37 strains composed a few sub-clades with other reference strains identified as *C. simmondsii* by Shivas & Tan[26]. Twelve strains, MAFF242420-242428, MAFF24592, MAFF24692 and MAFF242693, all of which isolated from sweet pepper[12,36], constituted a distinct clade without the reference strains of any members of the *C. acutatum* species complex. Thus, the strains could not be identified as the member species. Because the bootstrap value among the three sister clades of the sweet pepper strains and *C. carthami* was less than 22, whereas monophyly of each

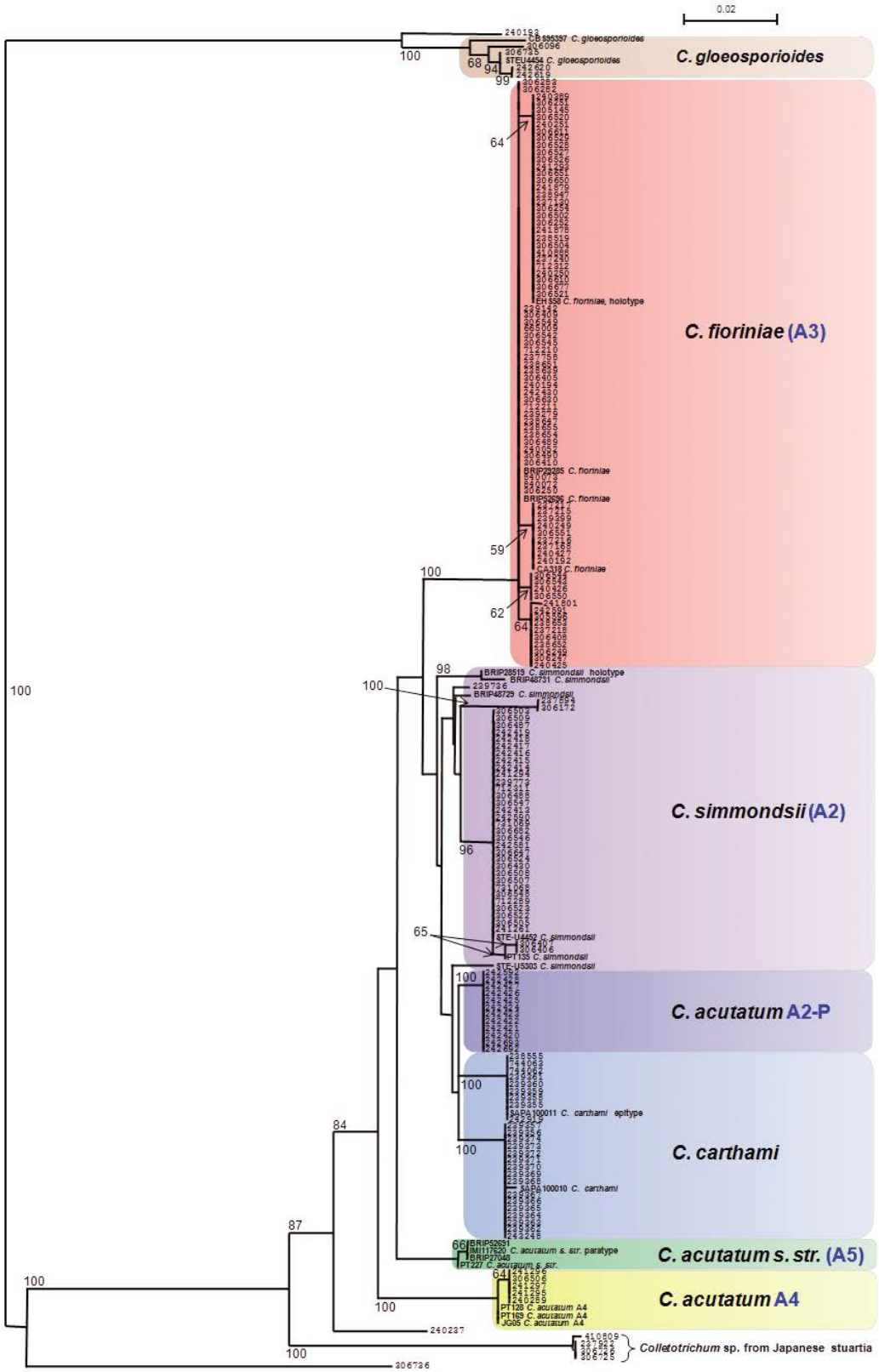

Fig. 1. Neighbor-joining phylogram of 170 strains deposited in the NIAS Genebank as the *Colletotrichum acutatum* species complex based on β-tubulin-2 gene sequences

The bar indicates a distance of two base changes per 100 nucleotide positions. Numbers on branches represent the percentages of congruent clusters in 1,000 bootstrap trials when the values exceeded 50%. Bold types mean reference strains downloaded from DDBJ/EMBL/GenBank databases. A2–A5 attached to scientific names mean phylogenetic groups of *C. acutatum*[26,28] except for A2-P newly designated in this paper.

clade was supported by the highest bootstrap values, it was hard to consider the sweet pepper strains as *C. carthami*. The β-tubulin-2 sequences of the representative strain MAFF242420 had 98.6% (721/738 sites) homology with GU183289 of *C. simmondsii* (BRIP28519 [holotype]), which belongs to *C. acutatum* group A2[26,28]. The twelve strains were regarded as members of the group A2, because the homology was the highest among those of the other groups of *C. acutatum*. They were therefore tentatively classified as '*C. acutatum* (group) A2-P' newly designated in this paper. Twenty five strains of *C. carthami* were located on two sub-clades adjacent to the clade of *C. acutatum* group A2-P. *Colletotrichum acutatum s. str.* and *C. acutatum* group A4 strains were placed with each branch distant from the three other species. No strain was grouped into the *C. acutatum s. str.* clade. Five and eleven belonged to the *C. acutatum* group A4 and *C. gloeosporioides* or unidentified *Colletotrichum* spp., respectively (Table 1).

2. Morphology

Some tendencies in reverse colony color were observed among the three species and two groups. The color of *C. fioriniae* strains tended to be reddish on PDA and/or WSH, whereas that of *C. simmondsii* and *C. carthami* strains varied from dark brown or olive to pale orange or yellow (Fig. 2). Darker and lighter colonies on PDA were more often observed in *C. carthami* and *C. acutatum* A2-P strains than in *C. simmondsii*, respectively. Colonies of the three species on PDA were darker than those on WSH in general. The *C. acutatum* A4 strains showed dark brown to cream color, though the color on WSH was darker than on PDA.

Conidia of *C. fioriniae* strains were uniformly fusiform, sometimes oblong or cylindrical with pointed ends, 6.4–17.5 (–20) × 1.7–4.9 (–6.5) µm (average: 12.7 × 3.9 µm) in size, length/breadth (L/B) ratio 2.19–4.14 (–4.73) (average: 3.26) and standard deviation (SD) of conidial length 1.79 on PDA. Those of *C. simmondsii* strains were fusiform, cylindrical with pointed base or ends, clubate, ellipsoid, boat- or sausage-shaped, oblong, 5.5–16.7 × 1.9–4.6 (–5.7) µm (average: 10.4 × 3.3 µm) in size, L/B ratio 2.85–3.80 (average: 3.15) and SD of conidial length 1.68 on PDA. Those of *C. carthami* strains were thick and short fusiform with slightly rounded ends, ellipsoid, clubate, (4.9–) 5.7–18.6 × 2.1–6.2 µm, (average: 10.9 × 4.1 µm) in size, L/B ratio 2.13–2.97 (–3.63) (average: 2.66) and SD of conidial length 2.19 on PDA. Those of *C. acutatum* A2-P strains were subcylindrical, fusiform, ellipsoid, oblong, attenuated and blunt pointed ends, 7.2–17.8 × 3.0–5.0 µm

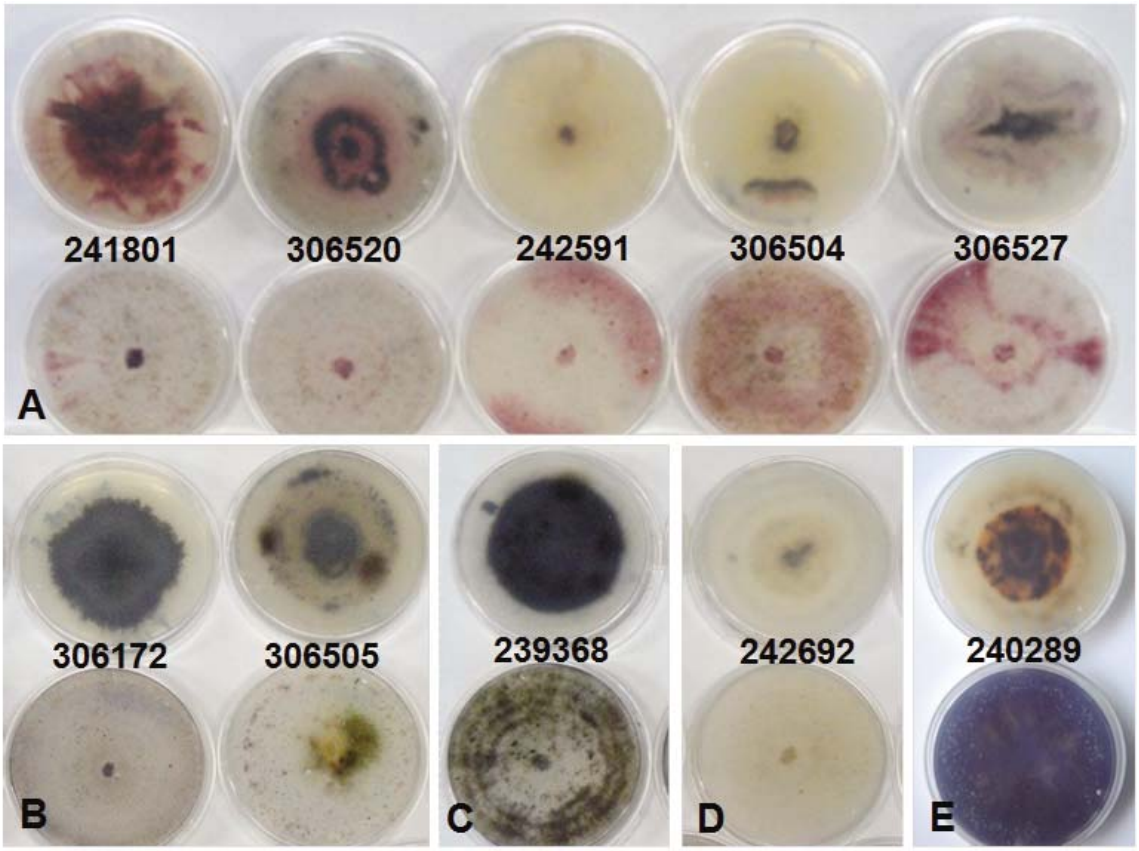

Fig. 2. Reverse colonies of strains of *Colletotrichum fioriniae* (A), *C. simmondsii* (B), *C. carthami* (C), *C. acutatum* group A2-P (D) and *C. acutatum* group A4 (E) on PDA (upper) and WSH (lower) at 25°C 30 days after transplant
Six digit numbers mean MAFF accessions of the NIAS Genebank, Japan.

(average: 11.3 × 3.0 μm) in size, L/B ratio 3.25–4.06 (average: 3.73) and SD of conidial length 2.80 on PDA. Those of *C. acutatum* group A4 strains were cylindrical with pointed ends, fusiform, clubate, long cylindrical with rounded ends, 9.9–30.0 × 2.9–5.3 μm (average: 14.7 × 3.8 μm) in size, L/B ratio 3.37–3.83 (–4.73) (average: 3.82) and SD of conidial length 3.45 on PDA. The conidial morphology on PDA varied more widely than those on WSH (Table 2). Detailed observation and measurements of the conidia clarified the characteristics of the three species and the two groups. Conidia of *C. carthami* and *C. acutatum* group A4 were unique among them because of the small L/B ratio, especially on PDA and large to huge in size, respectively. The average of the conidial length of *C. fioriniae* significantly exceeded that of *C. simmondsii* while the *C. acutatum* group A2-P was characterized by the narrowest conidia, both on PDA and WSH (Fig. 3).

Discussion

1. Phylogenetic analysis

Approximately 50% of 170 strains deposited as *C. acutatum* were re-identified as *C. fioriniae*, which reflects the fact that the member species was easily identified as *C. acutatum*[27] with a reddish colony and fusiform conidia[7,9,21,22,32,38,41,42,43,44]. The strains of *C. simmondsii* were divided into a few sub-clades, suggesting the polyphyletic nature of the species. The species, which consisted of both groups A2 and A9 from the beginning[26], as well as the *C. acutatum* group A2-P, should be examined with other DNA regions or genes to obtain more satisfactory phylogenetic analyses and taxonomy. Three strains isolated from strawberry, MAFF238555, MAFF744062 and MAFF744063, belonged to one of the *C. carthami* sub-clades. The latter two strains showed pathogenicity to the strawberry by inoculation (M. Koitabashi, personal communication). The strawberry therefore seems a new host of *C. carthami*, although the fungus reportedly has specific pathogenicity to asteraceous plants[37]. The *Colletotrichum acutatum* group A4 strains placed distant from four other species should be separated as distinct species. No strain *C. acutatum s. str.* and a few strains of the group A4 suggested absence and limited distribution in Japan, respectively. Seven strains corresponding to all except the member species, the group A4 and *C. gloeosporioides* were regarded as misidentified before being accepted in the NIAS Genebank or as those of nameless member species of the complex. They include pathogenic strains of the Japanese stuartia (*Stewartia pseudo-camellia*)[11] (Fig. 1), conidia and appressoria of which resembled those of *C. acutatum* described previously[20] to some extent.

Table 2. Conidial morphology of *Colletotrichum fioriniae*, *C. simmondsii*, *C. carthami*, *C. acutatum* group A2-P and *C. acutatum* group A4 strains deposited in the NIAS Genebank

Species or group	Shape	Medium	Length			Breadth (μm)		Length/breadth (L/B) ratio	
			Range (μm)	Av[a)](μm)	SD[b)]	Range	Av[a)]	Range	Av[a)]
C. fioriniae[26]	Fusiform, sometimes oblong, cylindrical with pointed ends	PDA[c)]	6.4–17.5 (–20)	12.7	1.79	1.7–4.9 (–6.5)	3.9	2.19–4.14 (–4.73)	3.26
		WSH[d)]	(6.5–) 9.5–17.7	12.9	1.14	(2.1–) 3.0–4.9	3.7	3.04–4.32	3.53
C. simmondsii[26]	Fusiform, cylindrical with pointed base or ends, clubate, ellipsoid, boat- or sausage-shaped, oblong	PDA	5.5–16.7	10.4	1.68	1.9–4.6 (–5.7)	3.3	2.85–3.80	3.15
		WSH	(7.4–) 9.4–16.3	12.3	1.47	2.6–4.5	3.6	2.95–3.74	3.42
C. carthami[37]	Thick and short fusiform with slightly rounded ends, ellipsoid, clubate, short cylindrical	PDA	(4.9–) 5.7–18.6	10.9	2.19	2.1–6.2	4.1	2.13—2.97 (–3.63)	2.66
		WSH	8.0–15.3	11.8	1.30	2.8–4.6	3.5	2.22–3.56	3.33
C. acutatum group A2-P[e)]	Subcylindrical, fusiform, ellipsoid, oblong, attenuated and blunt pointed ends	PDA	7.2–17.8	11.3	2.80	3.0–5.0	3.0	3.25–4.06	3.73
		WSH	9–14.8	12.2	2.01	2.6–4.1	3.3	3.25–4.21	3.72
C. acutatum group A4[f)]	Cylindrical with pointed ends, fusiform, clubate, long cylindrical with rounded ends	PDA	9.9–30.0	14.7	3.45	2.9–5.3	3.8	3.37–3.83 (–4.73)	3.82
		WSH	10.6–19.2 (–22.5)	14.9	2.09	(2.8–) 3.1–5.4	3.9	(3.29–) 3.8–4.17	3.82

[a)] Average

[b)] Standard deviation

[c)] Potato Dextrose Agar

[d)] Modified Weitzman-Silva-Hunter Agar (see Materials and Methods)

[e)] Newly designated in this paper, isolated from sweet pepper [12,36]

[f)] 'Larger spored form' of the *C. acutatum* [26,29,37]

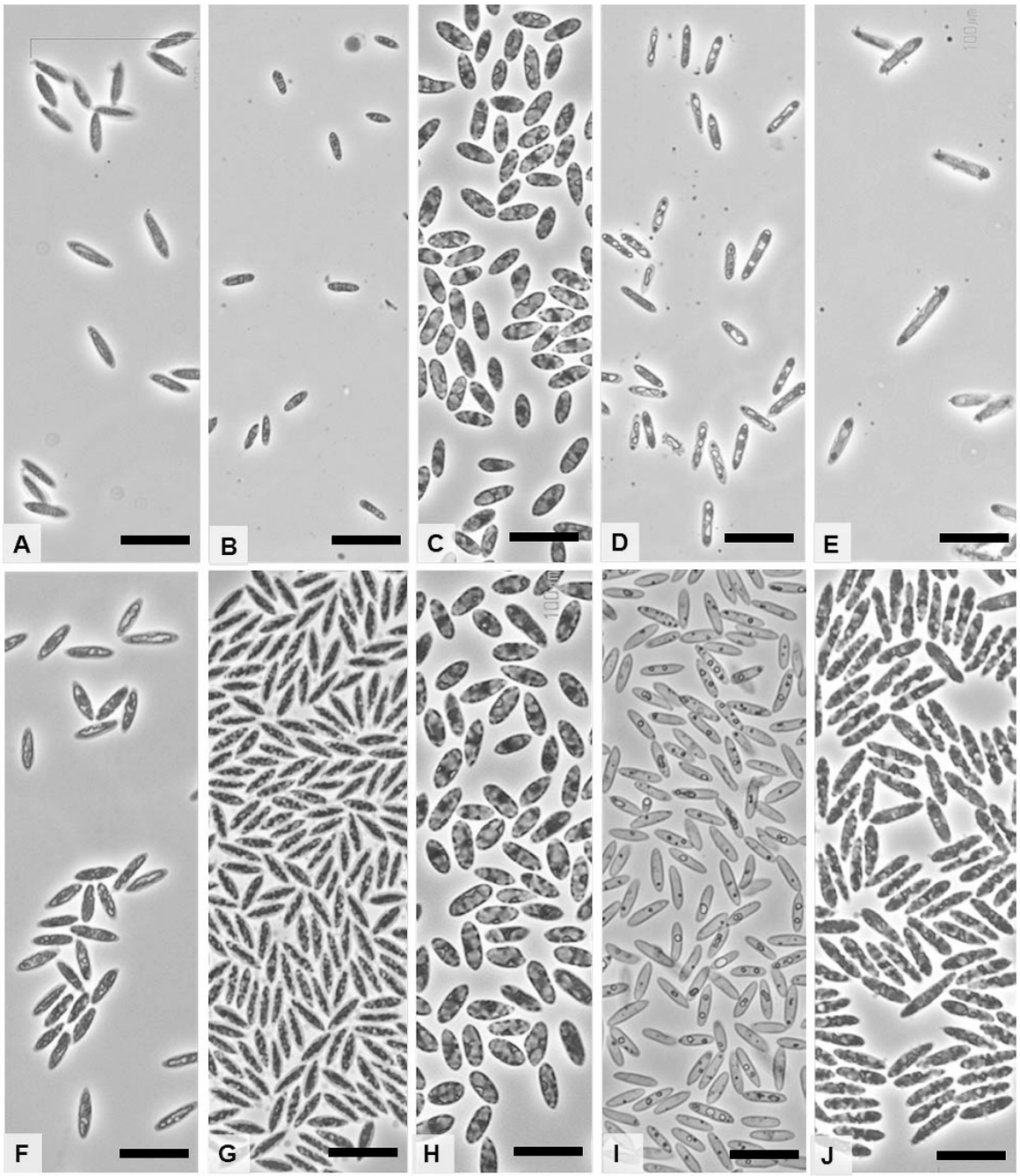

Fig. 3. Conidia of strains of *Colletotrichum fioriniae* (A, F; MAFF306520), *C. simmondsii* (B, G; MAFF306172), *C. carthami* (C, H; MAFF243248), *C. acutatum* group A2-P (D, I; MAFF242420) and *C. acutatum* group A4 (E, J; MAFF241297) produced on PDA (A–E) and WSH (F–J) at 20-25°C, 21 days after transplant (bar : 20 µm, phase contrast optics)

The strains should be re-examined in detail to determine an appropriate name.

2. Morphology

The reverse colony color of some strains of *C. fioriniae* and *C. simmondsii* on PDA resembled those reported by Shivas and Tan[26], but many were deeper and some strains of the former such as MAFF241801 showed vinaceous red color like as *C. acutatum s. str.*[26]. It is noteworthy that some strains of *C. fioriniae* such as MAFF242591 without a reddish color on PDA showed conspicuous pink on WSH (Fig. 2). They can be distinguished from other species more easily on WSH. Almost all strains of *C. carthami* were found to have darker colonies than those of the *C. simmondsii* and *C. acutatum* group A2-P, especially on PDA, though color variation was continuous among them (Fig. 2).

Appressorial morphology on potato carrot agar has been regarded as one of the criteria for taxonomy and iden-

tification of *Colletotrichum* spp., since Sutton[31] showed morphological variation among the species. That of some representative strains of the three member species, groups A2-P and A4 of the species complex, has already been reported[12,21,22,23,30,36,37]. Its shape appeared to show few differences among the species and groups. Appressorial sizes of each of the species and groups were unified as follows: *C. fioriniae*: 5.8–14 (–15) × 4–8 (–9.8) µm[21,22,23], *C. simmondsii*: 5.4–14 × 4–8 (–9.8) µm[23,37], *C. carthami*: 3.7–9.2 (–12.8) × 4.6–6 (–7.4) µm[37], *C. acutatum* A2-P: 4–17 × 3.2–8 µm[12,36] and *C. acutatum* A4: 6.9–15.8 × 4.6–7.8 µm[30]. That of *C. carthami* inclined to small sides in comparison with those of the others, bearing appressoria of similar size. However, the size variation of *C. acutatum* A2-P nearly covered those of others, meaning the appressoria of the members of the *C. acutatum* species complex were considered to have indistinct morphological variation.

A detailed investigation of the conidial size and shape clarified the characteristics of the three species of the *C. acutatum* species complex in this study, although few differences in conidial morphology among them were reported in previous taxonomic studies[26,37]. The three species and two groups were distinguished by their morphological characteristics as follows:

1. Reverse colony color reddish on PDA or WSH ····· *C. fioriniae*
1. Reverse colony color not reddish on PDA or WSH ····· 2
  2. Some conidia more than 18 µm in length ····· *C. acutatum* group A4
  2. Conidial length less than 18 µm ····· 3
    3. Average conidial length less than 11 µm on PDA ····· 4
    3. Average conidial length more than 11 µm on PDA ····· 5
      4. Most conidial L/B ratio less than 3 on PDA ····· *C. carthami*
      4. Most conidial L/B ratio more than 3 on PDA ····· *C. simmondsii*
        5. Average conidial width less than 3.5 µm ··· *C. acutatum* group A2-P
        5. Average conidial width more than 3.5 µm ··· *C. fioriniae*

Colletotrichum acutatum s. str. is absent from this dichotomous key because of the current lack of any strain of this species in the NIAS Genebank. The species was described as forming colonies with a reddish reverse color like certain strains of *C. fioriniae* examined here. Some host plants such as avocado and mango[26] in Japan possibly bear *C. acutatum s. str*., which appears to be easily misidentified as *C. fioriniae*. Therefore, molecular identification with β-tubulin-2 gene sequences is recommended when *C. fioriniae*–like strains will be isolated.

3. Update of pathogen name

A number of pathogenic strains of anthracnose first found in Japan are contained in the materials examined here. All the pathogens were identified as "*C. acutatum*" except for those of celery[3], sweet pepper[12,36] and three asteraceous plants[37] in Japan to date. We propose updates of the other pathogens' names in case the re-identified strains are cited in initial reports on the diseases. New pathogen names and their host plants are as follows:

C. fioriniae ····· blueberry, *Castanopsis sieboldii*, cosmos, gentian, grape, Jew's mallow, kiwifruit, mulberry, Russell prairie gentian, venetian sumac
C. simmondsii ····· stock
C. acutatum group A2-P ····· sweet pepper
C. acutatum group A4 ····· burnet
C. fioriniae and *C. simmondsii* ····· apple, loquat
C. simmondsii and *C. acutatum* group A4 ····· anemone
C. fioriniae, *C. simmondsii* and *C. carthami* ····· strawberry
C. fioriniae, *C. simmondsii* and *C. acutatum* group A4 ····· prune

The provisional host ranges of the member species and group, which also include several plants other than the crops listed above, were clarified in this study (Table 1), though they will expand gradually whenever strains and isolates of the *C. acutatum* species complex from other host plants are re-identified. The narrowed host ranges will facilitate searches for infection sources and efforts to control diseases caused by member species more than before.

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