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Molecular phylogenetic analyses and morphological re-examination of strains belonging to three rare *Colletotrichum* species in Japan

Toyozo Sato[1)]*, Jouji Moriwaki[2)], Shihomi Uzuhashi[3)], Yousuke Degawa[4)], Tsuyoshi Ono[5)] and Kazuko Nishimura[6)]

[1)]Genetic Resources Center, National Institute of Agrobiological Sciences (NIAS)
2-1-2, Kannondai, Tsukuba, Ibaraki 305-8602, Japan
[2)]Toyama Prefectural Agricultural, Forestry & Fisheries Research Center Horticultural Research Institute
288, Goromaru, Tonami, Toyama 939-1327, Japan
[3)]Department of Agricultural, Food and Nutritional Science, University of Alberta
Edmonton, Alberta. T6G 2P5, Canada
[4)]Sugadaira Montane Research Center, University of Tsukuba
1278-294 Sugadaira-kogen, Ueda, Nagano 386-2204 Japan
[5)]Agro-Environment Division, Tokyo Metropolitan Agriculture and Forestry Research Center
3-8-1 Fujimicho, Tachikawa, Tokyo 190-0013, Japan
[6)]First Laboratories, Co., Ltd., 1313, Kamihirama, Nakahara-ku, Kawasaki, Kanagawa 211-0013, Japan

Phylogenetic relationships of strains belonging to three rare *Colletotrichum* species in Japan were clarified based on sequences of the rDNA-ITS region and some other genes. Morphological re-examination of the strains was also carried out. *Colletotrichum hsienjenchang* on a bamboo, *Phyllostachys bambusoides,* collected in Kanagawa Prefecture, Japan in 2011 was found to produce tufted conidia on the top of polyphialides on PDA medium and large appressoria with a few short projections. Its strain was placed on a branch with *C. spaethianum* in an rDNA-ITS phylogram, but it was separated on a branch near *C. tofieldiae* and other closely related species with falcate conidia in phylograms based on actin, chitin synthase 1 or histone 3 partial sequences. Based on the present results, *Gnomonia hsienjenchang,* a teleomorph of *C. hsienjenchang*, was transferred to the emended *Glomerella*. *Colletotrichum metake* found on another bamboo, *Pleioblastus simonii*, in Ibaraki Pref., Japan in 2009 was found to form small cylindrical conidia and lemon-shaped appressoria. Although the species has been regarded as a synonym of *C. falcatum*, the strains were placed on a branch distant from *C. falcatum* in the rDNA-ITS phylogram. *Colletotrichum taiwanense* was isolated from a chrysanthemum (*Dendranthema grandiflorum*) in Okinawa Pref. in 2001, subsequently from the human cornea in Kumamoto, and from a vanilla leaf and a lemon branch in the Bonin Islands, Japan. The species was found to produce arrowhead-shaped appressoria from mycelia, and exceptionally long ascospores with 1-3 septa and large conidia. Strains of the species clustered into a single clade distant from other species of the genus in the phylogram. The three species were demonstrated to be distinct within the genus *Colletotrichum* by both molecular and morphological evidence in this study.

Key words: *Colletotrichum hsienjenchang*, *Colletotrichum metake*, *Colletotrichum taiwanense*, *Glomerella hsienjenchang*, *Glomerella septospora*

INTRODUCTION

The genus *Colletotrichum* belonging to the Glomerellaceae consists of species causing plant anthracnose (Arx, 1987; Sato & Moriwaki, 2009a). Sutton (1992) tentatively recognized thirty eight species, one variety and seven formae speciales in the genus. Moriwaki *et al.* (2002) classified *Colletotrichum* spp. in Japan into 20 groups based on a phylogenetic analysis with ribosomal DNA internal transcribed spacer (rDNA-ITS) region. After that, numbers of new or revived names and new combinations have been proposed as the results of molecular phylogenetic studies and morphological re-examination of various strains in the world (Crouch *et al.*,

*Corresponding author
E-mail: s1043@affrc.go.jp
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2009; Damm *et al*., 2009; Farr *et al*., 2006; Moriwaki *et al*., 2003; Moriwaki & Tsukiboshi, 2009; Sato & Moriwaki, 2009b; Shivas & Tan, 2009; Weir & Johnston 2010). The genus is in the process of revealing many cryptic species based on multi-gene phylogenetic analyses (Damm *et al*., 2012; Rojas *et al*., 2010; Uematsu *et al*., 2012), even after Hyde *et al*. (2009) listed 68 *Colletotrichum* names in current use. Two rare bamboo parasites, *Colletotrichum hsienjenchang* I. Hino & Hidaka (1934) and *Colletotrichum metake* Saccardo (Saccardo, 1908) do not appear in the *Colletotrichum* names in current use (Hyde *et al*., 2009) and *Colletotrichum taiwanense* Sivanesan & W.H. Hsieh (1993) new to Japan was annotated to require confirming its taxonomic identity (Hyde *et al*., 2009), because their phylogenetic relationships based on DNA sequences had not been clarified yet. We carried out molecular phylogenetic analyses of the species and also their morphological re-examination to clarify their phylogenetic relationships in the genus *Colletotrichum* and morphological characteristics not only on natural host but culture media, by using several strains with the three species names in the Genebank, National Institute of Agrobiological Sciences (NIAS).

MATERIALS AND METHODS

Molecular phylogenetic analyses

Seventy-four strains of thirty *Colletotrichum* species including the three rare species preserved in the NIAS Genebank (Table 1) were used for phylogenetic analysis with their 5.8S nuclear ribosomal gene with the two internal transcribed spacers (rDNA-ITS) on both sides sequences. Ten strains of eight species and one of *C. hsienjenchang* in the NIAS Genebank were also examined with partial sequences of actin (ACT), chitin synthetase-1 (CHS-1) and/or histone 3 (HIS3) genes. Genomic DNA was extracted according to the procedure by Moriwaki *et al*. (2002). The extracted DNA was used as a template DNA for the following polymerase chain reaction (PCR) analysis. The rDNA-ITS, ACT, CHS-1 and HIS3 genes were amplified and sequenced using the primer pairs ITS5 & ITS4 (White *et al*, 1990), ACT-512F & ACT-783R (Carbone & Kohn, 1999), CHS-354R & CHS-79F (Carbone & Kohn, 1999) and CYLH3F & CYLH3R (Crous *et al*., 2004), respectively. Every gene region was amplified with the *Taq* polymerase (TaKaRa, Otsu, Japan) in a GeneAmp 9700 (Applied Biosystems, Foster City, CA, USA). Cycling conditions for amplification of the rDNA-ITS were 94℃ for 3 min, followed by 35 cycles of 94℃ for 30 sec, 55℃ for 1 min, 72℃ for 1 min, and a final step 72℃ for 5 min. Those of ACT, CHS-1 and HIS3 genes were 94℃ for 5 min, followed by 40 cycles of 94℃ for 30 sec, 52℃ for 30 sec, 72℃ for 30 sec, and a final step 72℃ for 7 min. PCR products were purified using a QIAquick PCR Purification kit (Qiagen, Chatsworth, CA, USA) and were sequenced directly with a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Sequencing reactions were conducted according to the manufacturer's instructions. Extension products were analyzed on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. All the sequences determined were uploaded to 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). Sequences of the three species were also deposited to DDBJ/EMBL/GenBank databases (Table 1).

For phylogenetic analysis, sequence data of the rDNA-ITS region, ACT, CHS-1 and/or HIS3 gene sequences of 101, 34, 33 and 31 strains respectively of three species discussed in this paper as well as strains of additional forty-one species of *Colletotricum* downloaded from DDBJ/EMBL/GenBank databases were also included as references (Table 1). Two rDNA-ITS sequences of *Magnaporthe oryzae* B.C. Couch deposited in the databases were also used as an outgroup. A multiple sequence alignment of the region and the genes initially was carried out using the alignment subroutines of Clustal X version 2.0 (Larkin *et al*., 2007; Thompson *et al*., 1997). The alignment of all sequences was further optimized manually. Phylogenetic relationships were analyzed by distance methods. The distance matrix for the aligned sequences was calculated using Kimura's two-parameter model (Kimura, 1980) and was analyzed with the neighbor-joining (NJ) method (Saitou & Nei, 1987) using Clustal X version 2.0, excluding positions with gaps. The reliability of the inferred tree was estimated by bootstrap analysis (Felsenstein, 1985).

Morphological studies

Anamorphs of the three species on the host plants, isolation sources of them, were observed and sectioned by a razor blade under a binocular micro-

Table 1 Strains and DNA sequences used in this study

Species[a]	Strain[b]	DNA sequence accsession[c] of DDBJ/EMBL/GenBank or NIAS Genebank			
		rDNA-ITS	Actin	Chitin synthetase 1	Histon3
C. acutatum (A4)	TNOS5	AJ300557			
C. acutatum s.lato	MAFF240613	240613_ITS			
C. acutatum s.str.	**IMI117620**	FJ788417			
C. anthrisci	**CBS125334**	GU227845	GU227943	GU228335	GU228041
C. boninense	MAFF238336	238336_ITS			
	MAFF238641	238641_ITS			
	MAFF238656	238656_ITS			
	MAFF239120	239120_ITS			
	MAFF240190	240190_ITS			
	MAFF305972	305972_ITS			
C. caudatum	MAFF305700	305700_ITS			
C. chlorophyti	**IMI103806**	GU227894	GU227992	GU228384	GU228090
	MAFF425349	425349_ITS	425349_Act	425349_CHS1	425349_His3
C. circinans	**CBS111.21**	GU227855			
	CBS221.81		GU227953	GU228345	GU228051
	MAFF238640	238640_ITS	238640_Act		
C. coccodes	MAFF237459	237459_ITS			
	MAFF305072	305072_ITS			
C. curcumae	**IMI288937**	GU227893	GU227991	GU228383	GU228089
C. dematium	CBS115524		GU227924	GU228316	GU228022
	CBS125.25	GU227819	GU227917	GU228309	GU228015
C. destructivum	MAFF238340	238340_ITS			
	MAFF238453	238453_ITS			
	MAFF238560	238560_ITS			
	MAFF238562	238562_ITS			
	MAFF240106	240106_ITS			
	MAFF240195	240195_ITS			
	MAFF511453	511453_ITS			
C. echinochloae	MAFF305404	305404_ITS			
	MAFF305439	305439_ITS			
	MAFF305460	305460_ITS			
C. falcatum	MAFF306170	306170_ITS			
C. fioriniae	**EHS58**	EF464594			
	MAFF239398	239398_ITS			
	MAFF242727	242727_ITS			
C. fragariae	MAFF241263	241263_ITS			
C. fructi	**CBS346.37**	GU227844	GU227942	GU228334	GU228040
C. gloeosporioides	MAFF237219	237219_ITS			
	MAFF238321	238321_ITS			
	MAFF238494	238494_ITS			
	MAFF238659	238659_ITS			
	MAFF238779	238779_ITS			
	MAFF239160	239160_ITS			
	MAFF239282	239282_ITS			
	MAFF239524	239524_ITS			
	MAFF239563	239563_ITS			
	MAFF240490	240490_ITS			
	MAFF241226	241226_ITS			
	MAFF241260	241260_ITS			
	MAFF241580	241580_ITS			
	MAFF242738	242738_ITS			
	MAFF242913	242913_ITS			
	MAFF305974	305974_ITS			
C. graminicola	MAFF305384	305384_ITS			
	MAFF305427	305427_ITS			
	MAFF305436	305436_ITS			
C. hsienjenchang	MAFF243051	**AB738855**[d]	**AB738845**	**AB738846**	**AB738847**
C. lilii	CBS109214	GU227810	GU227908	GU228300	GU228006
C. lindemuthianum	MAFF305390	305390_ITS			

Table 1 Continued

Species[a]	Strain[b]	DNA sequence accsession[c] of DDBJ/EMBL/GenBank or NIAS Genebank			
		rDNA-ITS	Actin	Chitin synthetase 1	Histon3
C. lineola	CBS124959		GU227940	GU228332	GU228038
	CBS125337	GU227829	GU227927	GU228319	GU228025
	MAFF240431	240431_ITS	240431_Act	240431_CHS1	
C. liriopes	**CBS119444**	GU227804	GU227902	GU228294	GU228000
	MAFF238703	238703_ITS	238703_Act	238703_CHS1	238703_His3
C. metake	MAFF241800	**AB738856**			
	MAFF241845	**AB738857**			
	MAFF241876	**AB738858**			
	NBRC 8974[e]	**AB738859**			
C. musae	MAFF239086	239086_ITS			
C. orbiculare	MAFF240422	240422_ITS			
	MAFF306518	306518_ITS			
C. paspali	**MAFF305403**	305403_ITS			
C. phaseolorum	CBS157.36	GU227896	GU227994	GU228386	GU228092
	CBS158.36	GU227897	GU227995	GU228387	GU228093
C. rusci	**CBS119206**	GU227818	GU227916	GU228308	GU228014
C. sansevieriae	MAFF239175	239175_ITS			
	MAFF239721	239721_ITS			
C. simmondsii	**BRIP28519**	GU183331			
	MAFF240037	240037_ITS			
C. spaethianum	**CBS167.49**	GU227807	GU227905	GU228003	GU228003
	MAFF238024		238024_Act	238024_CHS1	238024_His3
	MAFF238702		238702_Act	238702_CHS1	238702_His3
	MAFF239500	239500_ITS	239500_Act	239500_CHS1	
C. spinaciae	CBS128.57	GU227847	GU227945	GU228337	GU228043
C. sublineolum	MAFF305360	305360_ITS			
	MAFF305361	305361_ITS			
C. taiwanense	MAFF238783	**AB738848**			
	MAFF242697	**AB738849**			
	MAFF243031	**AB738851**			
	MAFF243173	**AB738852**			
	MAFF243176	**AB738853**			
	MAFF243177	**AB738854**			
C. tofieldiae	CBS495.85		GU227899	GU228291	GU227997
	IMI288810	GU227803	GU227901	GU228293	GU227999
	MAFF712333	712333_ITS	712333_Act	712333_CHS1	712333_His3
C. trichellum	CBS217.64	GU227812	GU227910	GU228302	GU228008
	MAFF237918	237918_ITS			
	MAFF410046	410046_ITS			
C. trifolii	MAFF305079	305079_ITS			
	MAFF305080	305080_ITS			
C. truncatum	**CBS151.35**	GU227862	GU227960	GU228352	GU228058
	CBS345.70		GU227965	GU228357	GU228063
	MAFF240453		240453_Act	240453_CHS1	240453_His3
	MAFF726762	726762_ITS	726762_Act	726762_CHS1	726762_His3
C. verruculosum	**IMI45525**	GU227806	GU227904	GU228296	GU228002
G. lindemuthiana	CBS151.28		GU227898	GU228290	GU227996
G. septospora	IFM 59082	**AB738850**			
(*C. taiwanense*)	(MAFF242912)				
	C04098	GU935907			
M. oryzae	DH08037	GU073121			
	K44-111P	FN555119			

[a]*C.=Colletotrichum, G.=Glomerella, M.=Magnaporthe*

[b]Bold strains are type or ex-type

[c]Accsessions with _ITS, _Act, _CHS1 and _His3 are available the website of NIAS Genebank, http://www.gene.affrc.go.jp/databases-micro_search_en.php

[d]Bold accsessions were deposited to DDBJ/EMBL/GenBank in this study

[e]Labelled as *Colletotrichum sasaecola* in NBRC Catalogue of Biological Resources 2nd ed. 2010

scope (Leica MZ125, Lica Microsystems, Wetzlar, Germany), and then examined in detail and photographed using microscope with Nomarski differential interference contrast and phase contrast optics (Nikon Eclipse 80i; Nikon, Tokyo, Japan). One strain of *C. hsienjenchang*, four of *C. metake* and seven of *C. taiwanense* listed on Table 1 were cultured on Potato Dextrose Agar (PDA, Difco laboratories, Detroit, MI, USA) at 25℃ under the black light (Toshiba FL20SBLB, peak emission 352 nm) for 14–30 days to observe their colonies of anamorphs and/or teleomorphs. Appressoria of representative strains, MAFF243051 (*C. hsienjenchang*), MAFF241876 (*C. metake*) and MAFF242912 (*C. taiwanense*), produced after 7–14 days in slide culture with Potato Carrot Agar (PCA, chopped 20 g potato and 20 g carrot, boile10 min. in 1 *l* tap water, filled up 1 *l*, plus 20 g agar) at 25℃ in the dark were studied as follows. Each fifty conidia on the medium and the appressoria of the strains were measured and photographed using the microscope with an image analyzer (Nikon Digital Sight; Nikon, Tokyo, Japan).

RESULTS AND DISCUSSION

Colletotrichum hsienjenchang

Colletotrichum hsienjenchang has been collected on some bamboos such as *Phyllostachys bambusoides*, *P. nigra* var. *henonis* and *P. edulis* f. *pubescens* in Japan and China, since it was first described in 1934 (Degawa *et al.*, 2004). The strain examined here, MAFF243051, was derived from the conidia produced on a diseased culm of *P. bambusoides* collected in Odawara, Kanagawa Prefecture, Japan in 2011. It is the first living strain of the species. The phylogenetic analysis based on the rDNA-ITS region revealed that the strain was on a branch with *Colletotrichum spaethianum* (Allesch.) Damm, P.F. Cannon & Crous (Fig. 1), showing that *C. hsienjenchang* was distantly related to the member species of the *Colletotrichum graminicola* species complex parasitic to the Gramineae (Crouch *et al.*, 2009), even though the bamboo hosts belong to the Gramineae. Three NJ trees were obtained from the analysis each with the ACT, CHS-1 or HIS3 gene sequences of *C. hsienjenchang*, the 10 strains uploaded to the website of NIAS Genebank and 23 downloaded from DDBJ/EMBL/GenBank databases (Fig. 2–4). In every phylogram *Colletotrichum hsienjenchang* was placed on a branch adjacent to clades consisting of *Colletotrichum lilii* Plakidas ex Boerema & Hamers, *Colletotrichum liriopes* Damm, P.F. Cannon & Crous, *C. spaethianum*, *Colletotrichum tofieldiae* (Pat.) Damm, P.F. Cannon & Crous and/or *Colletotrichum verruculosum* Damm, P.F. Cannon & Crous, which all are parasitic to herbaceous except gramineous plants and have falcate conidia (Damm *et al*., 2009).

Acervuli of *C. hsienjenchang* on *P. bambusoides*, were sub-epidermal, ellipsoid to irregular, up to 530 μm in length, with white conidial masses and surrounded by many dark setae (Fig. 5a). The surface side of a colony of the strain MAFF243051 on PDA was pale orange, with a poor aerial mycelium, orange conidial mass on its center, and the reverse side was pale orange with grayish inner area and a few darker sectors at peripheral side (Fig. 5b). The strain produced conidia not only on monophialides but also on apical collarets of polyphialides on PDA, which were often dichotomous and with clavate branches on the top of conidiophores, looking like a broom or a mop (Fig. 5c, d). The polyphialidic conidiogenesis seemed new to the genus *Colletotrichum*. The conidia were aseptate, hyaline, slightly falcate to cylindrical with curved base and both ends pointed, 16－68 (－86)×2.8－5.3 (－6.4) μm in size (Fig. 5e). Appressoria on hyphae in PCA slide culture were grayish brown to brown hemispherical, ellipsoid or irregular often with a few short projections and single large pores, 12－36×8－27 μm in size (Fig. 5f). This study proved that *C. hsienjenchang* is morphologically and phylogenetically distinct from other species with falcate conidia.

Gnomonia hsienjenchang I. Hino & Katumoto described as a teleomorph of *C. hsienjenchang* was known to have 3-septate fusiform ascospores (Hino & Katumoto, 1958a). But the teleomorph was described to have no perithecial beak typical of *Gnomonia* and the anamorph is neither *Asteroma*, *Discula*, *Marssoniella* nor *Zythia* (Arx, 1987). Although it was not observed in this study, the teleomorph should be transferred to *Glomerella*, because the genus was emended to have aseptate to multiseptate ascospores in the original description of *G. septospora*, a teleomorph of *C. taiwanense* (Sivanesan & Hsieh, 1993). This transfer is also supported by the result of the phylogenetic analyses, in which the anamorphic strain was placed among other typical species of *Colletotrichum*.

Glomerella hsienjenchang (I. Hino & Katumoto) Toy. Sato & Y. Degawa, comb. nov.

Basionym: *Gnomonia hsienjenchang* I. Hino &

Fig. 1 Neighbor joining phylogram based on rDNA-ITS sequences of 68 strains of twenty three *Colletotrichum* species including the three rare species preserved in NIAS Genebank and 28 strains of 22 species downloaded from DDBJ/EMBL/GenBank databases. The bar indicates a distance of one base changes per 100 nucleotide positions. Numbers on branches represent the percentage of congruent clusters in 1,000 bootstrap trials when the values were greater than 50%.

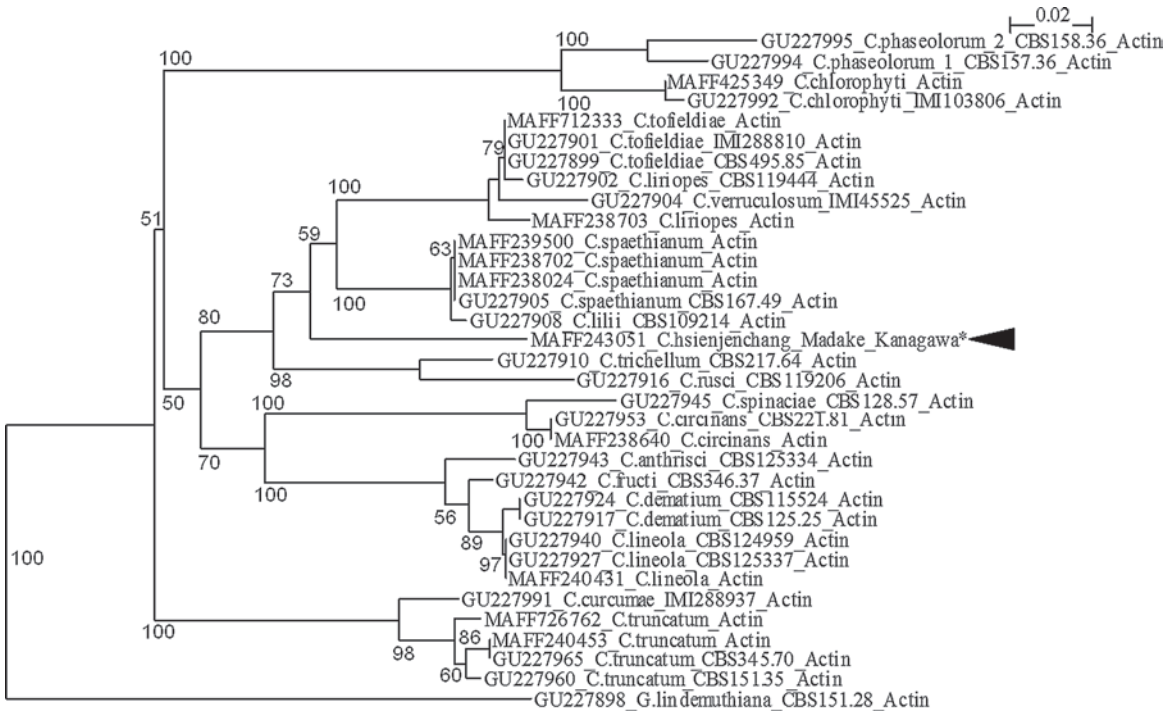

Fig. 2 Neighbor joining phylogram based on actin gene (ACT) partial sequences of a *Colletotrichum hsienjenchang* strain, MAFF243051, ten strains of seven *Colletotrichum* species preserved in NIAS Genebank and 23 strains of 18 species downloaded from DDBJ/EMBL/GenBank databases. The bar indicates a distance of one base changes per 100 nucleotide positions. Numbers on branches represent the percentage of congruent clusters in 1,000 bootstrap trials when the values were greater than 50%. An arrowhead indicates a branch of the *C. hsienjenchang* strain.

Katum., Hino, I. & Katumoto, K. 1958. Bull. Faculty of Agriculture, Yamaguchi University 9: 896
Anamorph: *Colletotrichum hsienjenchang* I. Hino & Hidaka, I. Hino & Hidaka 1934. Bull. Miyazaki College of Agriculture and Forestry 6: 97

Colletotrichum metake

Colletotrichum metake described in Italy in 1908 (Saccardo, 1908) was found on another bamboo, *Pleioblastus simonii*, in Tsukuba, Ibaraki Prefecture, Japan in 2009. The species was listed as a fungus parasitic to bamboo in Japan, but its description and collection locality of Japanese materials were not known (Hino & Katumoto, 1958a, 1958b). The Phytopathological Society of Japan required to re-examine its taxonomic identity (Anonymous, 2012). Morphology of the materials collected in Tsukuba was as follows. Acervuli on the host plant were subcuticular, small cushion-like, average 100 μm in diam., with narrow setae surrounded by conidiophores (Fig. 5g). The species produced conidia on monophialides branched from the short conidiophore on host cuticula (Fig. 5h). The surface side of a colony of a representative strain MAFF241876 on PDA had radiate branch pattern of submerged mycelia in pale orange on the inner side and blackish at its periphery, and its reverse side was similar to that of the surface side (Fig. 5i). Conidia on PDA were aseptate, hyaline, narrowly cylindrical with pointed base and rounded tip, (14.4−)19−27×3.2−5.5 μm in size (Fig. 5j). Appressoria on hyphae in PCA slide culture were dark to light brown sub-spherical, lemon- or fist-shaped, sometimes with a few lobes, 8−17×5.4−15 μm in size (Fig. 5k). The strains were identified as *C. metake* based on the morphology and the host plant.

The species was treated as a synonym of *Glomerella tucumanensis* (Speg.) Arx & E. Müller (Anamorph: *Colletotrichum falcatum* Went) based on the examination of the original specimen (Arx, 1957). But *C. metake* was first described to have "oblong conidia" (Saccardo, 1908), which is obviously distinct from falcate ones of *C. falcatum*. The strains of the former were clustered into a clade distant from the

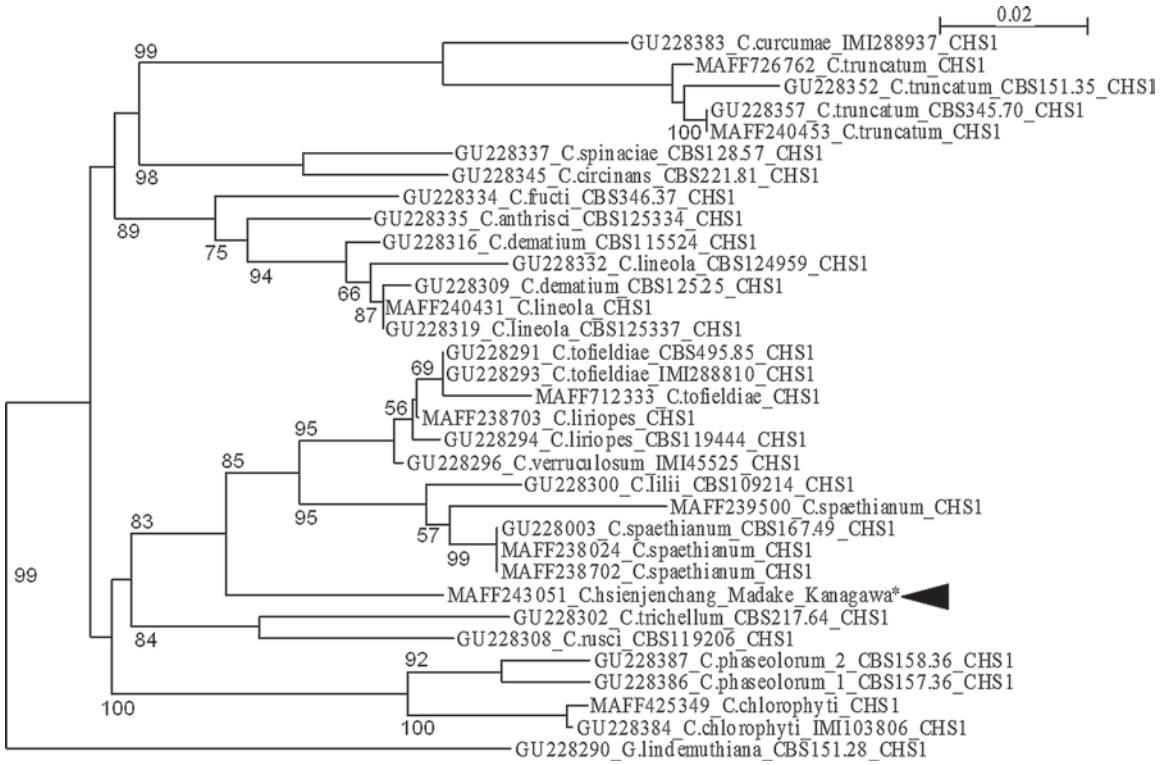

Fig. 3 Neighbor joining phylogram based on chitin synthase-1 (CSH-1) gene partial sequences of a *Colletotrichum hsienjenchang* strain, MAFF243051, nine strains of six *Colletotrichum* species preserved in NIAS Genebank and 23 strains of 18 species downloaded from DDBJ/EMBL/GenBank databases. The bar indicates a distance of one base changes per 100 nucleotide positions. Numbers on branches represent the percentage of congruent clusters in 1,000 bootstrap trials when the values were greater than 50%. An arrowhead indicates a branch of the *C. hsienjenchang* strain.

strain of the latter in the rDNA-ITS phylogram (Fig. 1). The result also indicates that *C. metake* is not a synonym of *C. falcatum*, and that it is distantly relative to other graminicolous species, although the host plant is a member of the Gramineae. The strain NBRC 8974 (=MAFF306419) is labelled as *Colletotrichum sasaecola* I. Hino & Katumoto in the strain catalogue of NITE Biological Resource Centre. Its species epithet was corrected as "*sasicola*" (Index of Fungi vol. 2, page 505), though it was "*sasaecolum*" (Hino & Katumoto, 1958a) originally. The strain was found to have the same rDNA-ITS sequence as that of *C. metake* from Tsukuba. *Colletotrichum sasicola* (*sasaecola*) appears to be distinct from *C. metake*, because the former was described to form clavate to fusiform conidia on *Sasa kurilensis* (Hino & Katumoto, 1958a). The strain produced no conidia in colonies on PDA and also on subsequent colonies covered with autoclaved bamboo leaves. It should be re-examined with inoculations into both *S. kurilensis* and *P. simonii* as well as phylogenetic studies based on other genes.

Colletotrichum taiwanense

Colletotrichum taiwanense reported as endophytic species from Taiwan in 1993 (Sivanesan & Hsieh, 1993) were found on 6 hosts in south-western Japan since 2001. A strain of the species, MAFF242912, was isolated from a human eye with keratomycosis caused by an accidental blow with a plant branch. It is also known as a pathogen of chilli pepper (*Capsicum annuum*) anthracnose in Korea (S.K. Hong, personal communication). The rDNA-ITS phylogram revealed that *C. taiwanense* strains with three kinds of sequences formed two clades distant from other species. One consisted of three strains, MAFF238783 from chrysanthemum in Okinawa, MAFF242697 from persimmon in Shimane Prefecture, and MAFF243173 from vanilla in Chichijima. The other contained five strains,

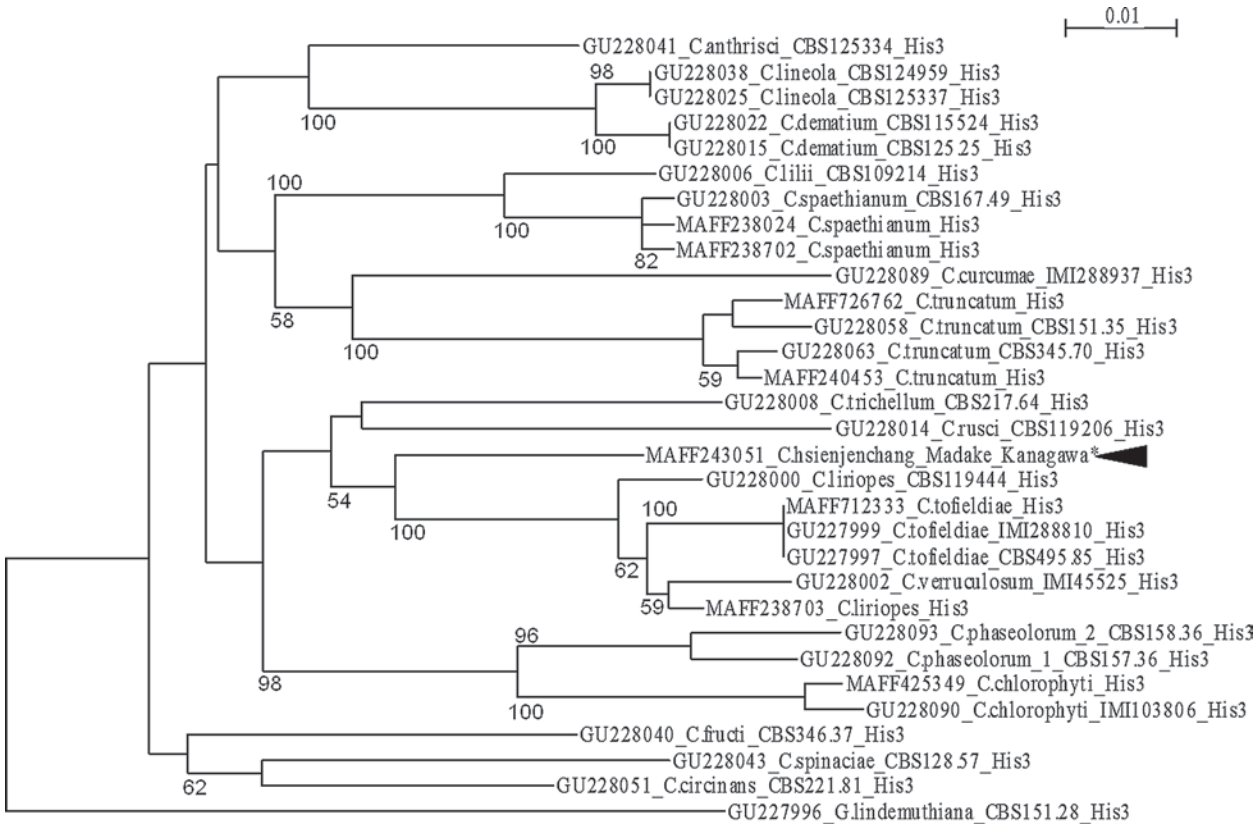

Fig. 4 Neighbor joining phylogram based on histone3 (HIS3) gene partial sequences of a *Colletotrichum hsienjenchang* strain, MAFF243051, eight strains of 5 *Colletotrichum* species preserved in NIAS Genebank and 23 strains of 18 species downloaded from DDBJ/EMBL/GenBank databases. The bar indicates a distance of one base changes per 100 nucleotide positions. Numbers on branches represent the percentage of congruent clusters in 1,000 bootstrap trials when the values were greater than 50%. An arrowhead indicates a branch of the *C. hsienjenchang* strain.

MAFF242912 from human cornea, MAFF243031 from English ivy, C04098 from chilli pepper in Korea and two strains from lemon in Hahajima (Fig. 1). The two clades appeared in the rDNA-ITS phylogram suggest genetic differentiation within the species, although few differences were detected in anamorphic morphology among the strains. It is necessary for its further taxonomy to analyze their phylogeny based on other genes, cross-test with the strains and examine teleomorphic morphology in detail.

Acervuli of *C. taiwanense* on *Vanilla planifolia* were sub-epidermal, ellipsoid along by longitudinal axis of the host leaves, with black base, narrow dark setae and orange conidial masses, 100 to 250 μm in diam (Fig. 6a). The surface side of a colony of a representative strain MAFF242912 on a PDA plate was covered with white to grayish aerial mycelia, and the reverse side was grayish or dark brown to black (Fig. 6b). In most strains of the species, numbers of black nailhead-shaped stromata often developed between PDA slants and inner wall of test tubes (Fig. 6c). Conidia on PDA were aseptate, hyaline, thick cylindrical with rounded ends, $14-28\times7-10.4$ μm in size (Fig. 6d). Appressoria on hyphae in PCA slide culture were grayish brown often arrowhead-shaped or ellipsoid to sub-spherical, sometimes lobed and binary, $12-19\ (-24)\times7.5-11.8$ μm in size (Fig. 6e).

The teleomorph was observed only in PDA culture of MAFF242912 isolated from the human cornea. It formed abundant perithecia on the surface of the PDA plate after 20–30 days' incubation (Fig. 6f). Honey-like droplets containing ascospores were produced on their top (Fig. 6g). The perithecium was flask-shaped, with a short hyaline beak and a dark brown body (Fig. 6h). Asci were unitunicate, broadly

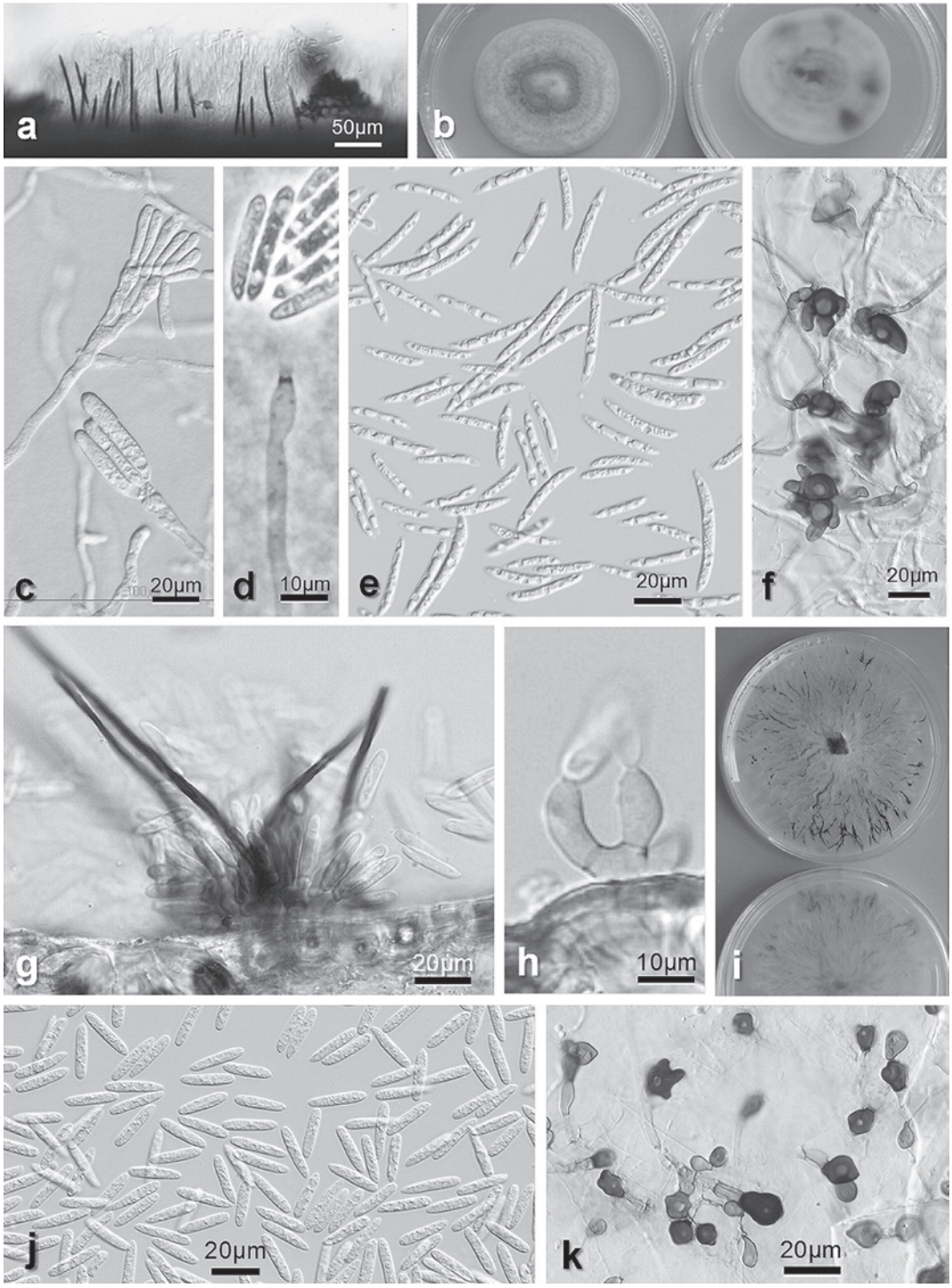

Fig. 5 **a–f: Morphology of *Colletotrichum hsienjenchang*, a:** an acervulus with setae on *Phyllostachys bambusoides*, **b–f:** MAFF243051 on PDA **(b–e)** and on PCA **(f)** incubated at 25℃, **b:** colonies 15 days after culturing (left: surface side, right: reverse side), **c:** polyphialides bearing immature conidia on apical collarets, **d:** a monophialide, **e:** conidia, **f:** appressoria developed from hyphae.
g–k: *Colletotrichum metake*, g: an acervulus with setae on *Pleioblastus simonii*, **h:** monophialides bearing immature conidia, **i–k:** MAFF241876 on PDA **(i, j)** and on PCA **(k)** incubated at 25℃, **i:** colonies 10 days after culturing (upper: surface side, lower: reverse side), **j:** conidia, **k:** appressoria developed from hyphae **(a, c, d, f, g, h, j, k:** Nomarski differential interference contrast optics, **e:** phase contrast optics).

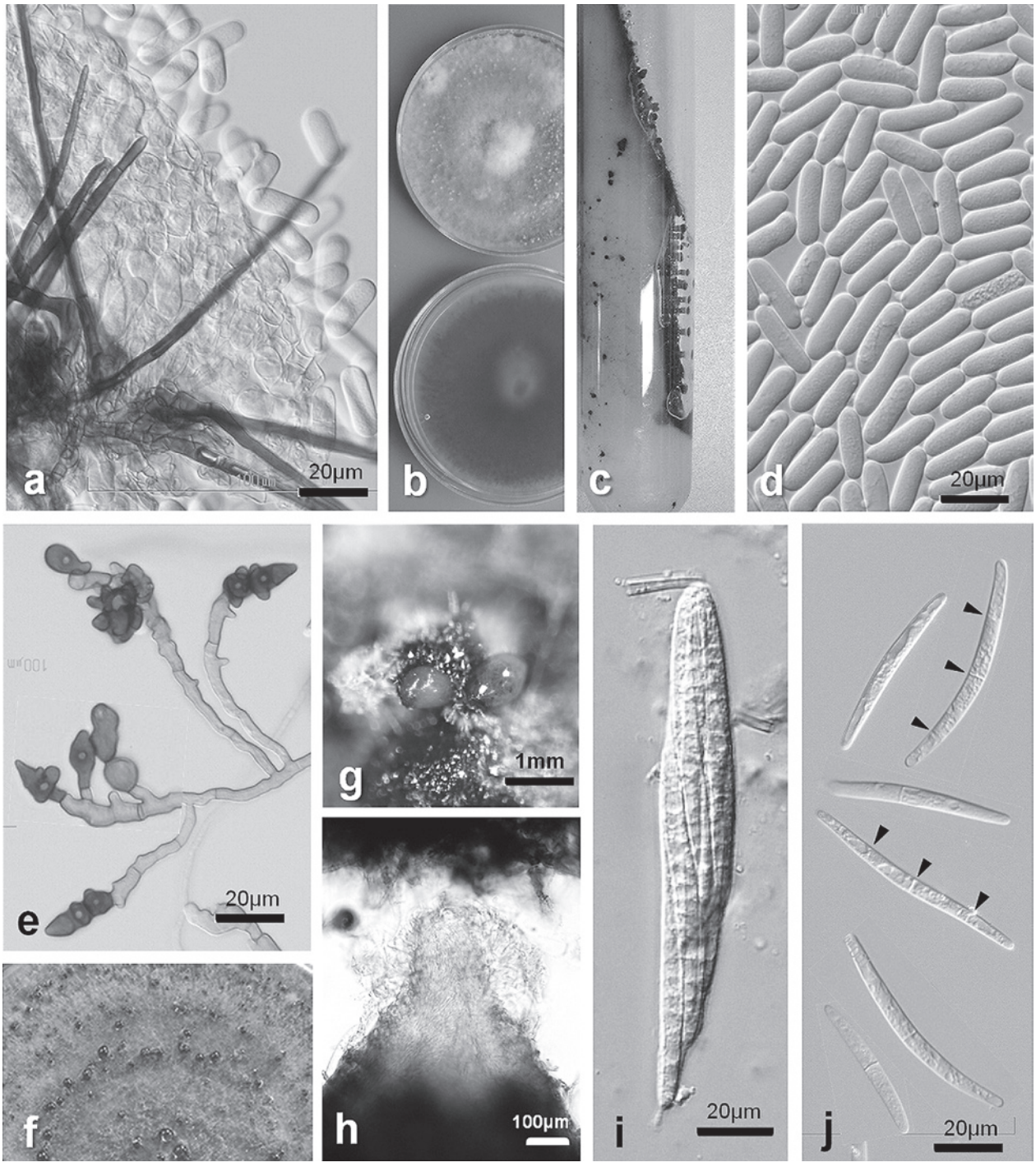

Fig. 6 a–e: Morphology of *Colletotrichum taiwanense*, a: an acervulus with setae on *Vanilla planifolia*, **b–d:** MAFF242912 on PDA **(b–e)** and on PCA **(e)** incubated at 25℃, **b:** colonies 15 days after culturing (upper: surface side, lower: reverse side), **c:** nailhead-shaped structures between PDA slants and inner wall of a tube, **d:** conidia, **e:** appressoria developed from hyphae.
f–j: *Glomerella septospora*, f: black perithecia on PDA culture at 25℃ for 21 days, **g:** honey-like droplets containing ascospores on top of perithecia, **h:** an apical part of a perithecium, **i:** an ascus, **j:** ascospores (arrowheads indicate septa of 3-septate spores) **(a, d, e, i, j:** Nomarski differential interference contrast optics).

cylindrical to clavate or sub-fusoid, thin walled, 8-spored up to 130 μm long and to 23 μm wide (Fig. 6i). Ascospores were cylindrical to narrowly fusiform, often slightly curved, with rounded ends, 3-septate when matured, with single median septa when young, hyaline, smooth, variously arranged in the ascus, 49−86×5−6.7 μm in size (Fig. 6j). Because the extremely long ascospores with 1- or 3-septa were similar to those of *Calonectria* (Arx, 1987), the species appears to belong to a genus other than *Glomerella*. But the rDNA-ITS phylogram demonstrates that the teleomorph belongs to the genus *Colletotrichum* (=*Glomerella*) and thus the emendation of the genus by Sivanesan & Hsieh (1993) was appropriate. Appressoria developed from hyphae of the species were much more complex than those formed on the end of conidial germ tubes (Sivanesan & Hsieh, 1993). The former seems useful for identification of the species, as the importance of hyphal appressoria to *Colletotrichum* taxonomy was already shown (Sutton, 1992). The unique morphology and its phylogenetic position of the species isolated from others except for *Colletotrichum sansevieriae* Miho Nakamura & Ohzono suggests that it has been isolated from other species for a long time among the genus.

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日本産 *Colletotrichum* 属希少 3 種菌株の分子系統解析および形態学的再評価

佐藤豊三[1)]*，森脇丈治[2)]，埋橋志穂美[3)]，出川洋介[4)]，小野　剛[5)]，西村和子[6)]

[1)]農業生物資源研究所遺伝資源センター，[2)]富山県農林水産総合技術センター園芸研究所，
[3)]アルバータ大学農学部食品栄養科学部，[4)]筑波大学菅平高原実験センター，
[5)]東京都農林総合研究センター生産環境科，[6)]株式会社ファーストラボラトリーズ

日本産 *Colletotrichum* 属の希少 3 種菌株について形態的再検討を行うとともに，初めて分子系統関係を明らかにした．2011 年神奈川県のマダケ上で採集された *C. hsienjenchang* は，PDA 培地上で棍棒形のポリフィアライド先端から細長い鎌形分生子を房状に形成し，大型で指状突起のある付着器を持つことが明らかになった．rDNA-ITS 領域の塩基配列に基づく分子系統解析（ITS 系統解析）の結果，本菌は *C. spaethianum* とクレードを形成したが，Actin など 3 遺伝子に基づく分子系統樹では，*C. tofieldiae* 等に隣接する枝に位置付けられた．2009 年つくば市のメダケ上で再発見された *C. metake* は，小型のレモン形付着器を持つことが明らかになった．本菌は *C. falcatum* の異名とされているが，ITS 系統解析の結果，同菌とは全く別の単独クレードを形成した．*C. taiwanense* は，2001 年国内で初めて沖縄県のキクから分離され，その後，熊本県でヒトの角膜，小笠原諸島のバニラやレモンから分離された．本菌は本属菌としては例外的に細長く隔壁のある子のう胞子と大型の分生子および矢尻形の付着器を形成し，また，ITS 系統解析では単独のクレードを形成した．以上より上記 3 種は分子系統的にも形態的にも *Colletotrichum* 属の独立種であると考えられた．

（担当編集委員：田中尚人）