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SHORT COMMUNICATION

Ceratocystis ficicola sp. nov., a causal fungus of fig canker in Japan

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Abstract The causal fungus of *Ceratocystis* canker of fig in Japan is described as *Ceratocystis ficicola* sp. nov. This species is characterized by galeated ascospores and is similar to *Ceratocystis fimbriata* sensu lato. However, the perithecia of the new species are much larger than those of *C. fimbriata*. The fungus grows more rapidly than *C. fimbriata* sensu stricto at 25°C but more slowly at 30°C. Molecular analysis of the nucleotide sequences of rDNA ITS regions showed that *C. ficicola* is phylogenetically placed in the clade of *C. fimbriata* s. l. but is easily distinguishable from other species of *C. fimbriata* s. l.

Keywords *Ficus carica* · Microascales · Ophiostomatoid fungi

The genus *Ceratocystis* contains causal fungi of serious plant diseases such as black rot of sweet potato and wilt diseases of sycamore and oak. In this genus, *Ceratocystis fimbriata* Ellis & Halst. sensu lato (s. l.) is economically important, as it is associated with serious diseases in a large number of woody and herbaceous plants (CAB International 2001). *Ceratocystis fimbriata* s. l. is characterized by galeated ascospores and is a taxonomically problematic species because it contains a complex of many cryptic species. Numerous species have been described as separate taxa from *C. fimbriata* sensu stricto (s. str.) in *C. fimbriata* s. l. based on molecular phylogeny, host specificity, and morphology (e.g., Engelbrecht and Harrington 2005; van Wyk et al. 2010).

In the 1980s, a new canker disease of the fig cultivar Masui-Dofin (*Ficus carica* L. cv. Masui-Dofin) occurred in part of a fig cultivation area in Aichi, Japan (Kato and Miyagawa 1980; Kato et al. 1981). Later, a similar disease was observed on cv. Horaishi in Fukuoka (Kajitani et al. 1992). Kato et al. (1981) reported that the canker disease of fig was incited by *C. fimbriata*, based on morphological observations. According to Kato et al. (1981, 1982), the perithecia of the causal fungus were slightly larger than those of *C. fimbriata* on sweet potato. However, they did not conduct a critical comparison between the fungus and *C. fimbriata* s. str. at that time. Subsequently, Kajitani and Kudo (1993) noted that the fungus differed from *C. fimbriata* on sweet potato in morphology and physiology and proposed a new forma specialis, i.e., *Ceratocystis fimbriata* f. sp. *caricae* Kajitani et Kudo. However, Kajitani and Kanematsu (1997) considered the fungus as a distinct species from *C. fimbriata* s. str.

Ceratocystis fimbriata has been reported on *Ficus* in other countries. The cause of high mortality of *F. carica* was ascribed to *C. fimbriata* in San Paulo, Brazil (Valarini and Tokeshi 1980). Johnson et al. (2005) reported that the Brazilian isolates were placed together with *C. fimbriata* s. str. from sweet potato in the Latin American clade and that Japanese isolates identified as *C. fimbriata* from fig were clearly placed in the Asian clade within *C. fimbriata* s. l. in the phenogram based on allozymes. Also, analysis of genetic relationship within isolates belonging to *C. fimbriata* s. str. and related isolates revealed that the Brazilian isolates were positioned in the same clade with other isolates of *C. fimbriata* s. str. (Ferreira et al. 2010). These results imply that Japanese isolates of *C. fimbriata* from the

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fig are not related to *C. fimbriata* s. str. as is the case with Brazilian isolates. However, the critical taxonomic position of the Japanese isolates remains uncertain. The aim of this study was to characterize the causal fungus of the canker disease of fig found in Japan using morphology, growth rate, and molecular analysis and to provide an appropriate name for the fungus.

The isolates used in this study are listed in Table 1. We conducted morphological observations and growth experiments, as well as molecular analysis, using four isolates (FFCF 9001, FFCF 9101, IFCF 9001, and WFCF 9101) obtained from diseased fig trees. FFCF9001 was deposited as MAFF 625119 into the NIAS Genebank, National Institute of Agrobiological Science, Tsukuba, Japan. IFO 30501 isolated from *Ipomoea* was included to reveal the growth–temperature relationship. The isolates were pregrown on potato dextrose agar (PDA; Difco, Detroit, MI, USA) at 25°C for 2 weeks until use. All isolates are maintained in the culture collection of the NIAS Genebank or the private collection of the first author.

Ascocarps produced on PDA were used for morphological observations. They were mounted in lactophenol and observed with a Nomarsky interference microscopy (Olympus, New Vanox, model AHBS). The sizes of perithecia, ascospores, conidia, and chlamydospores were measured, and their averages were calculated.

Three isolates, i.e., MAFF 625119, FFCF 9101, and IFO 30501, were used to study growth–temperature relationships at 5, 10, 15, 20, 25, 30, and 35°C. A 6-mm plug was taken from the edge of actively growing colonies and placed in the center of 90-mm-diameter Petri dishes containing PDA. Three replicate plates for each isolate were examined 10 days after incubation to determine colony diameter. Average colony diameters were measured, and the linear growth rates of each isolate were calculated.

Molecular analysis utilized a partial ribosomal DNA sequence to clarify the phylogenetic position of the causal fungus of fig canker in Japan. The total DNA was extracted using the method of Lee and Taylor (1990). An Applied Biosystems GeneAmp 9700 thermal cycler was used to amplify the rDNA internal transcribed spacer (ITS) region using primer pairs ITS1 and ITS4 (White et al. 1990). PCR conditions and sequence analysis followed the method of Kanematsu et al. (2000). Sequence data were aligned with other *Ceratocystis* species using the program FSA version 1.15.2 (Bradley et al. 2009). A maximum likelihood tree was obtained in PhyML under the HKY model (Guindon and Gascuel 2003). Bootstrap analysis was also conducted in PhyML.

The isolate obtained from figs in Japan (MAFF 625119) was characterized by galeated ascospores, resembling those of other species of *C. fimbriata* s. l. In particular, the sizes of ascospores, conidia, and chlamydospores coincided with those of *C. fimbriata* s. str.; however, the perithecial size and neck length of the fig fungus were larger than those of all other species belonging to *C. fimbriata* s. l. According to the original descriptions, other species of *C. fimbriata* s. l. have perithecia that range from 100 to 300 μm in diameter and perithecial necks ranging from 300 to 1,200 μm in length. Perithecial sizes of *C. fimbriata* s. str. isolates (NFCF 9010 and MFCF 9210) also fitted well to the range in the descriptions. However, the Japanese isolates from fig had large perithecia, 280–640 μm in diameter, and long necks, 890–2,460 μm in length (Fig. 1). These morphological characters were confirmed on other isolates of the causal fungus of fig canker in Japan (FFCF 9101, IFCF 9001, and WFCF 9101).

The colony diameter of each fungus from fig canker was about 80 mm 10 days after incubation at 25°C and that of *C. fimbriata* s. str. was about 50 mm. Linear growth rates at 25°C on PDA of the fig fungus and *C. fimbriata* were 8 and 4.8 mm/day, respectively. The optimal temperature for growth of the fungus from fig and *C. fimbriata* s. str. was 20°–25°C. No growth was observed at 5°C or 35°C.

We obtained 635 bp of rDNA-ITS partial sequence data from the fig canker fungus (MAFF 625119, FFCF 9101, and IFCF 9001). The accession numbers of the sequence data for other *Ceratocystis* species are shown in Fig. 2. The alignment data set is deposited in TreeBASE (http://www.treebase.org/) under the accession number S10821. The resulting phylogenetic tree showed that the fig fungus belonged to a well-defined taxonomic group with high bootstrap support (Fig. 2).

Table 1 Isolates of *Ceratocystis* used in this study

Species	Isolate no.	Host	Locality	Year
Ceratocystis fimbriata	NFCF9010	*Ipomoea batatas*	Nagasaki	1968
C. fimbriata	MFCF9210	*I. batatas*	Miyazaki	1992
C. fimbriata	IFO30501	*I. batatas*	Nagasaki	–
Ceratocystis ficicola	MAFF625119 (FFCF9001)	*Ficus carica*	Fukuoka	1990
C. ficicola	FFCF9101	*F. carica*	Fukuoka	1991
C. ficicola	IFCF9001	*F. carica*	Aichi	1990
C. ficicola	WFCF9101	*F. carica*	Wakayama	1991
C. ficicola	SFCF9401	*F. carica*	Shizuoka	1994

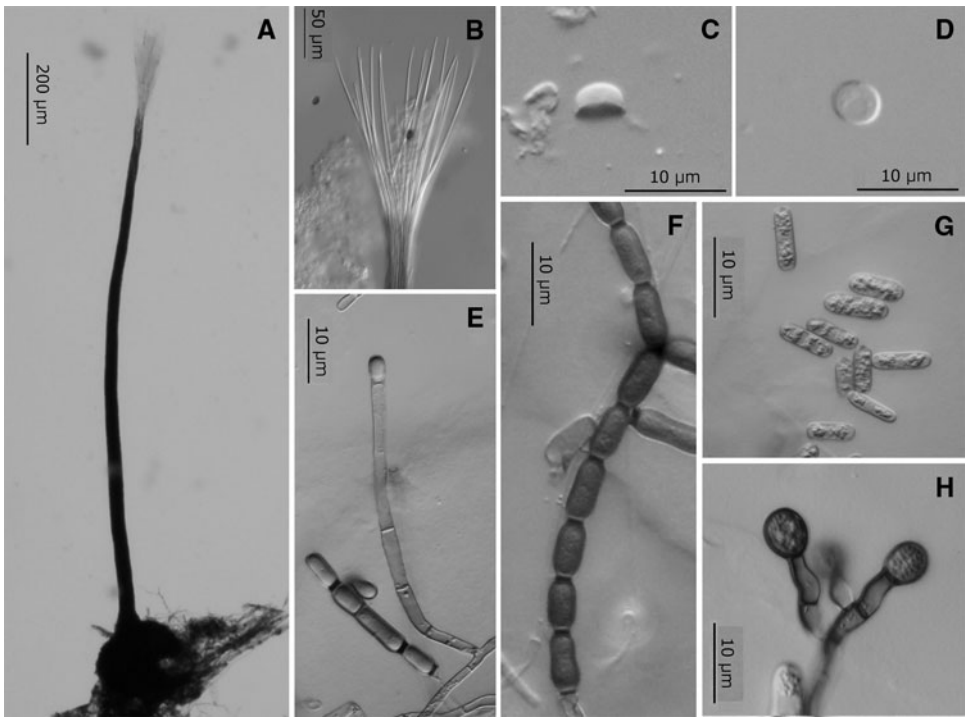

Fig. 1 *Ceratocystis ficicola*. **a** Perithecium. **b** Ostiolar hyphae. **c** Ascospore, *side view*. **d** Ascospore, *top view*. **e** Conidiophores. **f** Conidia in chain. **g** Conidia. **h** Aleurioconidia. *Bars* **a** 200 µm; **b** 50 µm; **c**–**h** 10 µm

Based on morphology, physiology, and molecular data, the causal fungus of fig canker in Japan represents a well-defined taxon and is clearly distinguishable from *C. fimbriata* s. str. and other *Ceratocystis* species. Here we describe the fig *Ceratocystis* canker fungus as a new species within the genus *Ceratocystis*.

Ceratocystis ficicola Kajitani et Masuya, sp. nov. Fig. 1
MycoBank no.: MB518749

≡ *Ceratocystis fimbriata* f. sp. *caricae* Kajitani et Kudo, Ann. Phytopathol. Soc. Jpn 59: 290, 1993

Etymology: Latin, *Ficus* (fig) and -cola (inhabiting) in reference to the habitat of the fungus.

Anamorph: *Thielaviopsis* sp.

Coloniae viridifuscae; mycelium aerium. Hyphae laeves, ad septa non constrictae. Bases ascomatum atrobrunneae vel nigrae, globosae vel subglobosae, 280–640 µm latae. Colla ascomatum atrobrunnea vel nigra, apicem versus pallentia, 890–2,460 µm longa, basi 65–110 µm, apice 27–43 µm lata, basi discoidea. Hyphae ostiolares divergentes, hyalinae 140–300 µm longae. Asci non visi. Ascosporae hyalinae, galeiformes, non septatae, 6.5–8 × 4–5.5 µm, in massis fulvo-flavescentibus mucosis formata ad apicibus collorum ascomatum.

Anamorpha *Thielaviopsis*: Endoconidiophorae in mycelio singulae, 40–133 µm longae, basi 2–4 µm. Phialides hyalinae, 20–42 µm longae, basi 2–4 µm. Endoconidia hyalina vel brunnea, cylindrica, non septata 5–9.5 × 4.5–8 µm. Aleurioconidia hyalina, subglobosa 7.5–16 × 7–12.5 µm.

Holotypus: on twigs of *Ficus carica* L., Japan. Fukuoka Prefecture, Yukihashi, November 1990, leg. Y. Kajitani (NIAES 20600; ex-type culture, MAFF 625119 and FFCF 9001).

Colonies greenish brown in color. Mycelium aerial. Hyphae smooth, not constricted at the septum. Ascomatal bases pale brown to black, globose to subglobose, 280–640 µm wide, ascomatal necks blackish brown to black becoming paler toward the apices, 890–2,460 µm long, 65–110 µm wide at the base, 27–43 µm wide at the apex (see Fig. 1a). Ostiolar hyphae divergent, hyaline, 140–300 µm long (see Fig. 1b). Asci not observed. Ascospores hyaline, galeate, aseptate, 6.5–8 µm long × 4–5.5 µm wide in top view, × 3–4.5 µm high in side view (see Fig. 1c,d), accumulating in buff-yellow mucilaginous masses at the apices of the ascomatal necks.

Thielaviopsis anamorph: Endoconidiophores occurring singly on mycelium, hyaline to pale brown, tapering toward the apices, 40–133 µm long, 2–4 µm wide at bases (see Fig. 1e). Phialides cylindrical or slightly lageniform, 20–42 µm long, 2–4 µm wide at bases. Endoconidia hyaline to brown, aseptate, cylindrical, 5–9.5 × 4.5–8 µm (see Fig. 1g), sometimes remaining in chains (see Fig. 1f). Aleurioconidia brown, aseptate, subglobose, 7.5–16 × 7–12.5 µm (see Fig. 1h).

Host: *Ficus carica* L.

Strains examined: Single ascospore cultures isolated from perithecium on twig of *F. carica*, Japan, Fukuoka, November 1990, leg. Y. Kajitani (MAFF 625119, ex-type culture of NIAES 20600); Fukuoka, September 1991, leg.

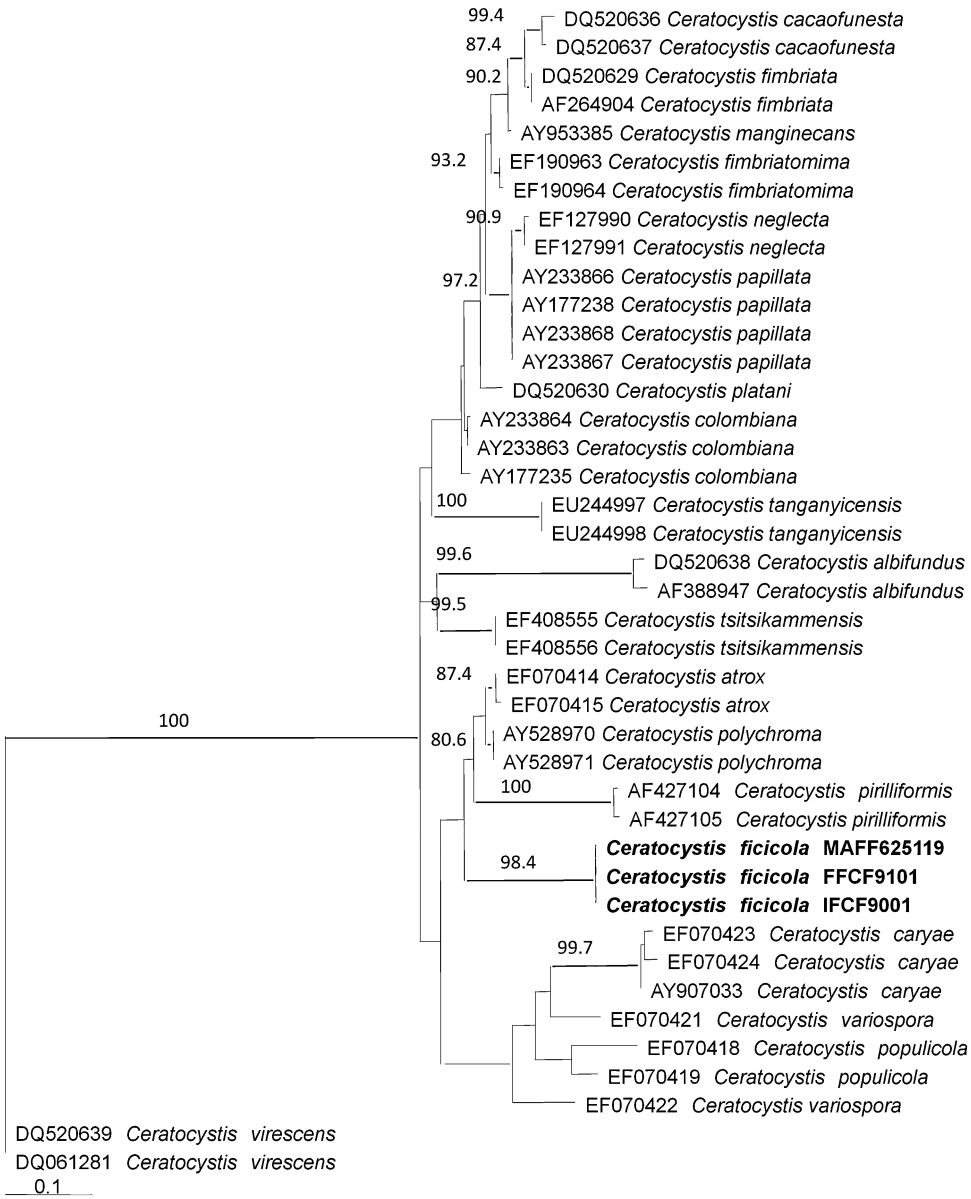

Fig. 2 Maximum likelihood tree of *Ceratocystis fimbriata* s. l. based on rDNA internal transcribed spacer (ITS) regions. Bootstrap support values (1,000 replications) greater than 50% are indicated at the nodes. Alignment data set was deposited on TreeBASE. *C. ficicola* isolates are in bold

Y. Kajitani (FFCF 9101, culture of NIAES 20601); Aichi, September 1990, leg. Y. Kajitani (IFCF 9001); Wakayama, November 1991, leg. Y. Kajitani (WFCF 9101).

Ceratocystis ficicola is characterized by extensively large perithecia. Other species in the *C. fimbriata* s. l. have similar morphology and are difficult to distinguish from each other. However, *C. ficicola* is easily distinguished from other species in this complex by its perithecial size. The anamorphic characteristics of *C. ficicola* are almost the same as those of other species of *C. fimbriata* s. l., but *C. ficicola* do not have apparent doliiform conidia, which are often seen on many other species of *C. fimbriata* s. l., excepting *C. fimbriata* s. str. This characteristic, together with the size of perithecia, helps with identification of *C. ficicola* by morphology.

Molecular data indicate that *C. ficicola* is clearly placed in a well-defined clade and is phylogenetically distinguishable from other species of *C. fimbriata* s. l., supporting the conclusion that *C. ficicola* is a separate taxon. Johnson et al. (2005) reported a phenogram of the *C. fimbriata* complex including Japanese isolates of *C. fimbriata* based on allozyme electromorphs of 44 electrophoretic phenotypes. They showed that Japanese isolates from sweet potato were clearly placed in the *C. fimbriata* s. str. clade, but isolates from fig constituted an independent clade. Although closely allied taxa in the phylogenetic tree include *Ceratocystis polychroma* M. van Wyk, M.J. Wingf. & E.C.Y. Liew from *Syzygium aromaticum* in Sulawesi, Indonesia (van Wyk et al. 2004), and *Ceratocystis atrox* M. van Wyk & M.J. Wingf. (van Wyk et al. 2007) and

Ceratocystis pirilliformis I. Barnes & M.J. Wingf. (Barnes et al. 2003) from *Eucalyptus* in Australia; they differ from *C. ficicola* in terms of morphology, host plant, and distribution.

In this study, we found that *C. ficicola* is not phylogenetically related to the *Ceratocystis* species parasitic on *Ficus* in Brazil. However, Brazilian isolates on *Ficus* are closely related to *C. fimbriata* s. str. (Ferreira et al. 2010). Ferreira et al. (2010) showed that Brazilian fig isolates were genetically associated with other isolates from *Ipomoea*, *Eucalyptus*, *Theobroma*, *Mangifera*, and *Platanus* species. This observation implies that some species belonging to *C. fimbriata* s. l. cannot always be distinguished by host and still require reappraisal. The causal agent of fig canker may contain species of *C. fimbriata* s. l. other than *C. ficicola*, and the identity of the fig isolates other than *C. ficicola* should also be reappraised.

The dispersal biology of *C. ficicola* has not been fully clarified. The ambrosia beetle *Euwallacea interjectus* was suggested as a vector in Fukuoka, southern Japan (Kajitani 1996). This association, which appeared to be found in several parts of Japan (Nitta et al. 2005), should be confirmed through a critical survey over all Japan. Other possibilities include spread by human activity, root systems, or soil-borne inocula. The origin of *C. ficicola* is unknown at present.

Ceratocystis ficicola is strongly virulent on fig trees in Japan and has caused serious losses in several fig plantation areas. The recent emergence of the serious disease in Japan may suggest that *C. ficicola* is an invasive tree pathogen from other localities. Alternatively, the fungus may be native to Japan and could easily cause damage the introduced host plant. To confirm these hypotheses, a phylogeographic study on this fungus, sampling over a wide area around the world, is required, together with pathogenicity experiments.

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