

## Characterisation and neotypification of *Gloeosporium kaki* Hori as *Colletotrichum horii* nom. nov.

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**Abstract** — A neotype is designated for the persimmon anthracnose pathogen *Gloeosporium kaki* Hori and the fungus is transferred to *Colletotrichum* as *Colletotrichum horii* nom. nov. Molecular and morphological analyses place this species as a distinct group within the *Colletotrichum gloeosporioides* sensu lato species complex. The fungus is associated with dieback and canker of twigs and young branches of persimmon, as well as spots on unripe fruit. The disease occurs on persimmon in China, Japan, and Korea, and is reported for the first time from New Zealand.

**Keywords** — *Glomerella*, phylogenetic, taxonomy, *Diospyros kaki*

### Introduction

The common persimmon (*Diospyros kaki* L.f.) has been cultivated in China, Japan, and Korea since prehistoric times, although it is probably of Chinese origin (Yonemori et al. 2008).

An anthracnose disease of persimmon is one of the most serious diseases of persimmon in Japan (Kitagawa & Glucina 1984), China (Kaiqi et al. 1988), and Korea (Lee et al. 2004). The fungus causing this disease was described in a Japanese language article by Shotaro Hori (1910a,b). He received specimens of the disease in July 1909 from an orchard planted in 1902 in the Mie prefecture and named the organism he isolated from the diseased fruits as *Gloeosporium kaki* on the basis of morphological traits similar to *Glomerella rufomaculans* (Berk.) Spauld. & H. Schrenk and *Gloeosporium fructigenum* Berk. (Hori 1910b).

The next year Seiya Ito (1911), probably unaware of the earlier work, published an article (in English) on the same persimmon fruit anthracnose pathogen, which he also named *Gloeosporium kaki*, although based on different specimens. In some works the authority for *G. kaki* has been given as Ito (e.g. Trotter 1931, von Arx 1957), probably due to the obscure place of publication

of Hori's name, "Engei no Tomo" (Friends of Horticulture). However, *G. kaki* Hori was validly published according to the rules of the botanical code of nomenclature at that time. The name is still in common use (e.g. Hu et al. 2006).

Maffei (1921) described a leaf spot pathogen of persimmon from a specimen collected in Italy. He speculated that his new species, *Colletotrichum kaki*, could be the same fungus as that described by Ito (1911) as *Gloeosporium kaki*. Von Arx (1970) agreed, stating that these two names referred to the same fungus. The seminal work of von Arx (1957) synonymised 750 *Gloeosporium* and *Colletotrichum* species to just 11 *Colletotrichum* species, and as part of this work *G. kaki* was placed in synonymy with *Colletotrichum gloeosporioides*, the conidial state of *Glomerella cingulata*.

In this paper we consider that Hori (1910b) and Ito (1911) described a fungus different from that of Maffei (1921). We neotypify *Gloeosporium kaki* Hori in order to stabilise and modernise the taxonomy of this pathogen, and transfer the taxon to *Colletotrichum*. We consider the persimmon pathogen a distinct taxon within the *Colletotrichum gloeosporioides* group species and report it from New Zealand for the first time.

## Materials and methods

### Isolates

New Zealand isolates were recovered from anthracnose lesions on persimmon fruit and twigs. Isolates from Japan were obtained from international culture collections; isolates from China were obtained from JZ Zhang (Zhang et al. 2005). Living cultures have been deposited in the ICMP culture collection (Landcare Research, Auckland), and dried herbarium specimens in PDD and TNS.

### Culture and morphology

Single conidial isolates were prepared and grown on Difco PDA at 18°C under a mixture of white and near UV light with a 12 h photoperiod. Colony morphology was noted after 12 days. Colour names follow Kornerup & Wanscher (1963). Conidia taken from actively growing, 7–10 day old cultures were examined for size and shape in lactic acid. Setae and appressoria were examined in lactic acid. Appressoria were producing using a slide culture. A small square of agar was inoculated on one side with conidia and immediately covered with a sterile cover slip. After 14 days the cover slip was removed and placed in a drop of lactic acid on a glass slide.

### Phylogenetic analysis

DNA was extracted from pure cultures using a Corbett X-tractor Gene robot. PCR amplifications were done in an Applied Biosystems Veriti Thermal Cycler in 25 µL reactions with Roche FastStart Taq DNA Polymerase. The primers used were: Internal transcribed spacer — ITS: ITS1F (CTT GGT CAT TTA GAG GAA GTA A) (Gardes & Bruns 1993), ITS4 (TCC TCC GCT TAT TGA TAT GC) (White et al. 1990); Glycerol-3-phosphate-dehydrogenase – GPDH: GDF1 (GCC GTC AAC GAC CCC TTC ATT

GA), GDRI (GGG TGG AGT CGT ACT TGA GCA TGT) (Templeton et al. 1992); and translation elongation factor – EF1 $\alpha$ : EF728 (CAT YGA GAA GTT CGA GAA GG) (Carbone & Kohn 1999), EF2 (GGA RGT ACC AGT SAT CAT GTT) (O'Donnell et al. 1998). The PCR conditions for GPDH were: 4 min at 95°C, then 35 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 45 s, and then 7 min at 72°C. ITS and EF1 $\alpha$  conditions were identical with the exception of annealing temperatures of 52°C and 50°C, respectively.

DNA sequences were obtained in both directions on an Applied Biosystems 3130xl Avant Genetic analyzer using BigDye 3.1 chemistry, electropherograms were analysed and assembled in Sequencher 4.8 (Gene Codes Corp.). Multiple sequence alignments were made with PRANKSTER (Loytynoja & Goldman 2005). MrModelTest 2.3 (Nylander 2004) was used to determine the optimal analysis method. Bayesian inference trees were constructed with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) for 10 million generations with no prior assumptions. The first 25% of generations before convergence were discarded. A phylogenetic tree was constructed for each gene individually.

### Typification and taxonomy

*Colletotrichum horii* B. Weir & P.R. Johnst., nom. nov.

FIG. 1

MYCOBANK MB 514062

= *Gloeosporium kaki* Hori, Engei no Tomo 6(2): 21, 1910 [in Japanese], non *Colletotrichum kaki* Maffei 1921

= *Gloeosporium kaki* S. Ito, The Botanical Magazine, Tokyo 25: 201, 1911, nom. illegit. [later homonym]

TIPIFICATION: Japan, on *Diospyros kaki*, N. Nishihara A71, 1959, (TNS-F-26102 – dried culture here designated as neotype of *Gloeosporium kaki* Hori; isoneotype PDD 98210; living cultures derived from neotype – IFO 7478 = NBRC 7478 = ICMP 10492).

ETYMOLOGY: *horii*, after Prof. Shotaro Hori, Japanese researcher who first isolated and named *Gloeosporium kaki*.

Colonies on Difco PDA variable. Isolates from Japan 55–60 mm diam. after 12 days, colonies uniform in appearance, aerial mycelium low, pale grey (1B1), cottony, conidia develop across whole colony, forming slimy, pale orange (5A2) conidial masses mostly close to agar surface but also amongst the aerial mycelium, sometimes associated with dark-based conidiomata. In reverse, brown (6E6) pigments towards centre of colony, greenish grey (30E2) near margin, overlaid with narrow, darker concentric bands. Margin of colony regular. Conidiomata comprise groups of closely packed hyphae with short-cylindric to more or less globose cells 3.5–5  $\mu$ m diam. with walls dark and slightly thick. Setae scattered, 50–80(–140)  $\mu$ m long, 6–8  $\mu$ m diam. at swollen basal cell, then tapering gradually to small, rounded apex, wall thick and dark. Conidiogenous cells held on the dark-walled cells, cylindric, 8–15  $\times$  3.5–5  $\mu$ m, wall thickened at the single apical conidiogenous locus. Conidia (13–)15–21(–23)  $\times$  4–5.5  $\mu$ m (mean 17.6  $\times$  4.8  $\mu$ m, n = 76), straight, ends broadly rounded, mostly cylindric, a few tapering towards the base. Isolates from New Zealand and China 68–73

mm diam. after 12 days, colonies with aerial mycelium sparse, or dense and cottony, pale grey (1B1) to grey (1E1), sometimes with small clumps of dark grey (1F1) mycelium on surface, conidial ooze orange (6B7), restricted to tops of the dark, more or less round conidiomata. In reverse, dark conidiomata and orange spore masses show through from colony surface, sometimes overlaid with greenish grey (26F2) pigment within the agar. Margin of colony either regular or irregularly scalloped. Conidiogenous cells and setae the same as Japanese isolates. Conidia 16–29.5(–35) × (4–)4.5–6(–7) µm (mean 21.8 × 5.0 µm, n = 182), straight, ends broadly rounded, some cylindrical but most taper gradually toward the basal end. Appressoria short-cylindric, usually uniform in outline, a few irregularly lobed, 9.5–13.5 × 6–8.5 µm.

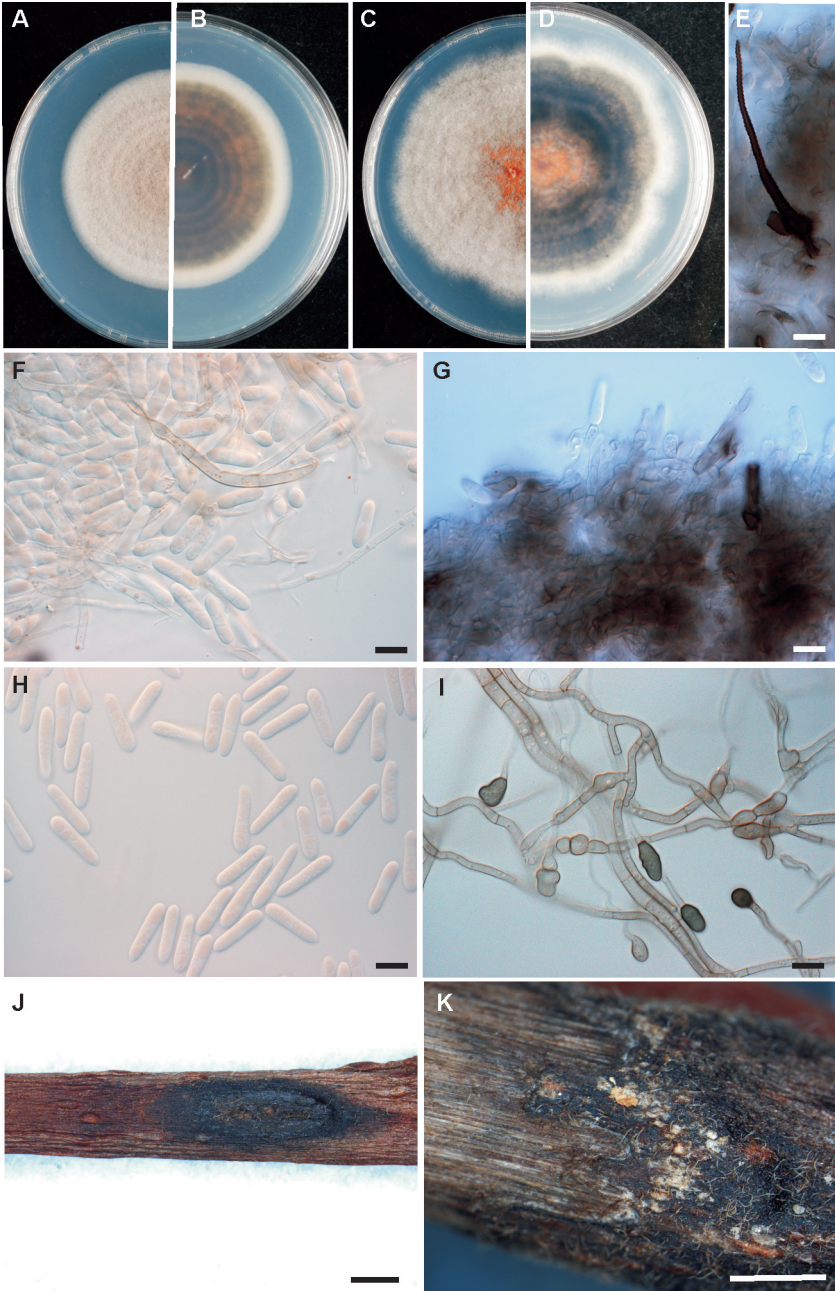
**HABITAT** — Associated with lesions on unripe fruit, young stems, and twigs. On unripe fruit, lesions slightly sunken, more or less round, very dark to black, acervuli erumpent covered with orange conidial masses, present near centre of lesions. Twigs and young stems with tip dieback and lesions, dark grey to black, elliptic, extending along one side of stem, erumpent acervuli and pale conidial masses near margins of lesions, bark often lost from stem in central part of lesion.

**OTHER COLLECTIONS EXAMINED** — China: on *Diospyros kaki* rot of unripe fruit, Jingze Zhang, May 2002 (TSG001 = ICMP 17968 and TSG002 = ICMP 17969 – living cultures). Japan: Fukuoka, on *D. kaki* young shoot, Y. Kajitani, May 1993 (MAFF 306429 = ICMP 17970 – living cultures). New Zealand: Northland, Ohaewai, Hall-Wright orchard, on *D. kaki* rot of unripe fruit, A. Clarke, 1990 (PDD 62825 – dried herbarium specimen, ICMP 12951 – living culture derived from herbarium specimen). Bay of Plenty, Te Puke, on *D. kaki* lesions on living stems and unripe fruit, S. Parkes, P. Glucina, Jan. 1989 (PDD 57148 – dried herbarium specimen, ICMP 12942 – living culture derived from herbarium specimen). Bay of Plenty, Te Puke, on *D. kaki* rot of unripe fruit, M.A. Manning MM150, June 2002 (ICMP 14918 – living culture). Bay of Plenty, Katikati, on *D. kaki* ripe fruit rot, M.A. Manning, 15 June 1989 (PDD 55534 – dried herbarium specimen, ICMP 18126 – living culture derived from herbarium specimen).

**NOTES** — The specimen cited by Hori (1910b; Honshu, Mie Prefecture, on *Diospyros kaki* fruit, S. Hori, 31 July 1909) is not present in the TNS herbarium (Tsuyoshi Hosoya, pers. comm.) and is assumed to have been destroyed or not originally preserved; it is unlikely to be present in other herbaria in Japan.

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FIG. 1. *Colletotrichum horii*. A, top; and B, reverse, of 12 day old culture on PDA, initiated from a single conidium (ICMP 10492, PDA subculture, ex neotype, Japan). C, top; and D, reverse, of 12 day old culture on PDA, initiated from a single conidium (ICMP 12942, PDA subculture, New Zealand). E, seta. F, H, conidia in lactic acid. G, acervulus, squash mount. I, appressoria in culture. J, young lesion on living twig. K, margin of older lesion on twig, sporulating acervuli erumpent through bark at margin of lesion, bark lost from central part of lesion. A–B, E–G, ICMP 10492; C–D, H, ICMP 12942; I, ICMP 17970; J–K, PDD 57148. Scale bars E–I = 10 µm, J–K = 2 mm.



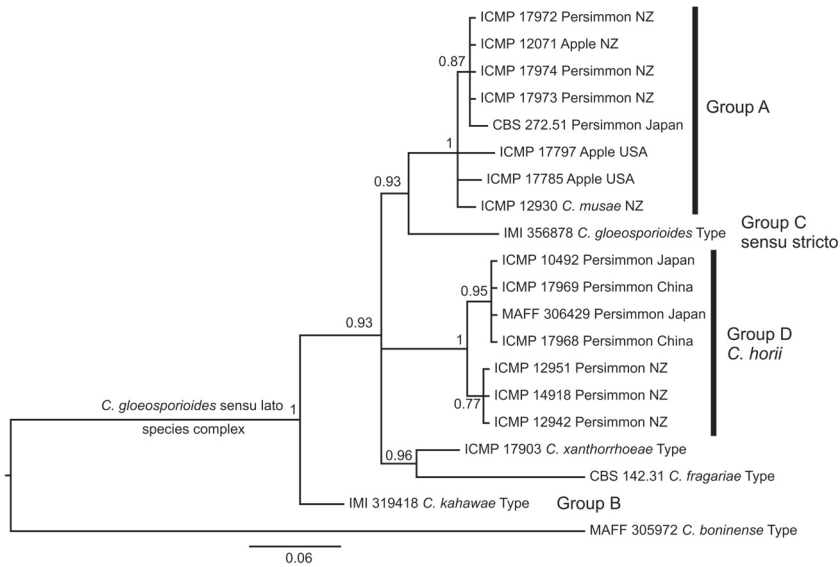


FIG. 2. 50% majority-rule consensus Bayesian inference phylogenetic tree of intron 2 of the GPDH gene. The tree shows the relationship of *Colletotrichum horii* isolates to other described species and relevant isolates within the *C. gloeosporioides sensu lato* species complex, and is rooted with *C. boninense*. Clades labelled as Groups A–D are sensu Johnston & Jones (1997). Clade posterior probabilities are indicated at nodes. Scale bar shows number of inferred changes per site.

The neotype we selected matches the original description of this fungus (Hori 1910a,b, Ito 1911) both morphologically and biologically. Although not from the site where the holotype was collected, it is from the same country. Because species level taxonomy of *Colletotrichum* is increasingly becoming based on molecular phylogenetic analyses, we have selected a type specimen that is available as a living culture, allowing practical and repeated DNA isolation for future genetic studies.

Appearance of the isolates of *C. horii* in culture is variable and this variation appears to reflect geographic origin of the isolates. Compared with the fresh isolates from New Zealand and the Chinese isolates, the Japanese isolates have a slower growth rate, conidia are not always associated with dark-based acervuli, the acervuli that are present are smaller in size, and the conidia are smaller. The New Zealand isolates from Northland differ from those from the Bay of Plenty in lacking dark grey pigment within the agar and in having a uniform rather than irregularly scalloped colony margin. The Chinese isolates are similar to those from New Zealand in having conidial production more or less confined to scattered, dark-based acervuli. Because of the genetic and biological similarity between the isolates from all of these localities, the variation

in cultural appearance is considered as within-species variation. In addition, the storage history of the Japanese isolates is not known, and the differences may relate to changes in cultural appearance following repeated subculturing, a common occurrence in *Colletotrichum*.

### Phylogenetic analyses

All gene trees were congruent, and generally resolved the same major clades as shown in the GPDH tree (FIG. 2). Clades are labelled Group A–D in the sense of Johnston & Jones (1997), with Group A = Clade 1 and Group B = Clade 2 of Johnston et al. (2008). The GPDH tree had the most variation and best resolution of the genes sequenced for the species within the *C. gloeosporioides* complex. The posterior probabilities of the clades in the GPDH tree were very high between groups ranging from 0.93–1.00 indicating strong support for this topology. The posterior probabilities were generally lower (0.77, 0.87, and 0.95) within groups. The ITS gene tree had poor resolution of clades within the *C. gloeosporioides* sensu lato complex. Sequences of *C. horii* genes have been deposited in GenBank as ITS (GQ329687 – GQ329690), GPDH (GQ329680 – GQ329686), EF1 $\alpha$  (GQ329691 – GQ329697). Sequences of other all isolates, including types have been deposited as (GU174542 – GU174577). Trees and alignments are available at TreeBASE # S2470.

## Discussion

### Phylogenetics

Prior to the 1950s the taxonomy of *Colletotrichum* was complicated with many species names based solely on host and without reference to other material. Von Arx (1957) synonymised 750 names to just 11 *Colletotrichum* species, based on morphological similarities. However, this led to the creation of large “group-species” such as *Colletotrichum gloeosporioides* that are genetically and biologically highly diverse. Johnston & Jones (1997) found five morphologically and genetically distinct groups within *C. gloeosporioides* sensu lato isolates from New Zealand (Groups A–D, and *C. musae*); this work was later supported with a multigene phylogeny with a geographically expanded range of isolates (Johnston et al. 2008). The recent typification of *C. gloeosporioides* sensu stricto (Cannon et al. 2008) has clarified the taxonomy of this species, and its relationship to other members of the *C. gloeosporioides* species complex, including *C. musae*, *C. kahawae*, *C. fragariae*, and *C. xanthorrhoeae* (FIG. 2).

Johnston et al. (2008) recognised a distinct persimmon-specialised clade within the *C. gloeosporioides* complex on the basis of a multigene phylogeny. Here we formally name that clade *Colletotrichum horii*. The tree we present (FIG. 2) is based on the intron 2 sequence of the GPDH, as tree topologies from this gene closely replicated those of the multigene topology. Sequences of the

translation elongation factor (EF1 $\alpha$ ) and the internal transcribed spacer (ITS) were identical across all *C. horii* isolates sequenced. The sequences of GPDH intron 2 varied by 7 bp between Asian and New Zealand isolates, as a result of a 3 bp indel and four single nucleotide transitions. It is unlikely that this variation has evolved in New Zealand. Although persimmon has been grown in New Zealand for more than 100 years, the disease associated with *C. horii* has been noticed only recently and it may even have been accidentally introduced in very recent times (see discussion below). More extensive studies of *C. horii* throughout Asia are likely to reveal greater levels of genetic diversity within the species.

The neotypification of *Colletotrichum horii* in this publication will stabilise and modernise the taxonomy of this species. This will in turn allow the application of molecular diagnostic tools, allowing more accurate and specific detection of persimmon pathogens than have previously been possible. For example, the method of Lee et al. (2004) fails to distinguish several different *Colletotrichum* species, any of which could potentially occur on persimmon, but with only *C. horii* likely to be of practical concern on this host.

#### **Synonyms of *Colletotrichum horii***

The name *Gloeosporium kaki* has been proposed twice, one year and 400 km apart on Honshu, Japan (Hori 1910a,b, Ito 1911). Due to the similarity in biology, disease symptoms, and morphology we assume that both authors described the same organism.

*Colletotrichum kaki* was described in 1921 by Maffei as a leaf disease affecting specimens of *Diospyros kaki* cv. Kiombo in the Pavia botanic gardens in Italy. The disease, possibly associated with previous physical damage, started with hazel spots on the leaf margin or apex of mature leaves, and expanded in concentric rings until the leaves dried and fell. Although Maffei (1921) speculated that his organism could be the same as that of Ito (1911), the different biology of the disease would suggest otherwise. Isolates of *C. horii* are consistently associated with lesions on young twigs and shoots and as spots of unripe fruit. Morphologically they differ in that Maffei (1921) described a fungus with numerous setae, whereas *C. horii* produces few or no setae on host tissue, and sparsely in culture. There is no evidence that *Gloeosporium kaki* and *Colletotrichum kaki* are synonyms.

Other reports of *Colletotrichum gloeosporioides* infecting persimmon fruit are impossible to assess accurately with respect to the species being discussed, without extant cultures and genetic data (e.g. Maffei 1921, Moreau 1945). However, the descriptions of associated symptoms reported by da Silva (1940), where he described black, sunken lesions on unripe fruit, suggest that *C. horii* may also occur in Brazil.



### **Host specificity of *Colletotrichum horii***

From the earliest literature, there have been suggestions that *C. horii* may occur on other hosts. For example, Hori (1910b) noted that his isolates were pathogenic to pear. Ito (1911) inoculated a ripe apple and observed disease symptoms identical to those on persimmon, although *Colletotrichum* isolates from bitter rot symptoms of apple were not pathogenic to persimmon. Ikata (1936) inoculated isolates identified as *Gloeosporium kaki* on to ripe *Capsicum annuum* fruit. Such an apparent lack of specificity is not surprising. Johnston (2000) discussed a similar situation with *Glomerella miyabeana*, a pathogen of *Salix* found incidentally on a range of other plants.

### ***Colletotrichum horii* in New Zealand**

Persimmon plants were first imported into New Zealand in 1873 with the intention of growing persimmons as a crop (Kitagawa & Glucina 1984). However, following their initial introduction they were grown largely as garden plants, until the early 1980s when commercial interest was revived with the importation from Japan of a wide range of cultivars for testing under New Zealand conditions (Kitagawa & Glucina 1984). It is likely that the diseased specimens from New Zealand cited in this paper originated from plants propagated from the material imported in the 1980s. It is possible that the disease was introduced with these plants as an anthracnose disease of persimmon had not been recorded in New Zealand previously (Pennycook 1989). It is possible that only one or a few of the newly imported cultivars was susceptible but we have no information on which cultivars were infected. Kitagawa & Glucina (1984) noted that anthracnose is one of the most serious diseases of persimmon in Japan, but that large differences in resistance occur between cultivars.

In addition to *C. horii* two other *Colletotrichum* species have been isolated from persimmon fruit in New Zealand. *C. gloeosporioides* Group A (sensu Johnston & Jones 1997) has been found on ripe persimmon fruit. In New Zealand this fungus is commonly associated with bitter rot disease of apples and as a ripe rot on several other fruits (Johnston & Jones 1997). A collection from persimmon from Japan (CBS 272.51 = IMI 086556 = ICMP 17941) belongs in the same clade, but is genetically slightly different (2 bp in GPDH) to the New Zealand isolates. *C. acutatum* has also been isolated from ripe persimmon fruit. Neither of these other two fungi causes a problem with persimmon in New Zealand.

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