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Ceratocystis uchidae, a new species on Araceae in Hawaii and Fiji

Qian Li [a], Thomas C. Harrington [b,*], Douglas McNew [b], Jianqiang Li [a,c]

[a] Department of Plant Pathology, China Agricultural University, Beijing, 100193, PR China
[b] Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA, 50014, USA
[c] Beijing Engineering Research Center of Seed and Plant Health/Beijing Key Laboratory of Seed Disease Testing and Control, Beijing, 100193, PR China

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ABSTRACT

An examination of the isolates of *Ceratocystis fimbriata* from corms of Araceae (*Colocasia esculenta*, taro, and *Xanthosoma sagittifolium*) from Hawaii and Fiji found that they represent a new species, *Ceratocystis uchidae*. Phylogenetic analyses showed that the new species is in the Asian-Australian clade of the *C. fimbriata* complex, and it is most closely related to *C. polychroma* from Indonesia and *C. cercfabiensis* from China. *Ceratocystis uchidae* differs from these species in its shorter perithecial necks, longer ostiolar hyphae, and reduced growth at 32 °C compared to 30 °C. An unnamed species on *Eucalyptus* sp. from Yunnan, China was similar to *C. uchidae*, but *C. uchidae* forms shorter perithecial necks, and the two species were not interfertile in mating tests. Crossing of mating testers demonstrated that *C. uchidae* and *C. polychroma* also were not interfertile. Isolates of *C. cercfabiensis* showed substantial variation in mycelial morphology, with frequent sectoring. Perithecial necks of *C. cercfabiensis* varied greatly in length, and perithecia with the longest necks failed to exude ascospores. Also, tester strains of *C. cercfabiensis* were partially interfertile with tester strains of *C. uchidae*. *Ceratocystis cercfabiensis* appears to be an aberrant species and may be a hybrid between two other species of *Ceratocystis*.

1. Introduction

In spite of only minor morphological variation, the taxonomic concept of *Ceratocystis fimbriata* Ellis & Halsted (Webster and Butler 1967) has been elevated to the genus level, as *Ceratocystis*, and now includes more than two dozen species of plant pathogens (De Beer et al. 2014). Four geographic clades have been recognized within the genus. The Latin American clade (LAC) is represented by *C. fimbriata* as originally described from sweet potato (*Ipomoea batatas* (L.) Lam.) (Harrington 2000; Engelbrecht and Harrington 2005); the North American clade (NAC) is represented by *C. variospora* R. W. Davidson & C. Moreau (Johnson et al. 2005); the African clade (AFC) is represented by *C. albifundus* M.J. Wingf., de Beer & M. J. Morris (Heath et al. 2009; Mbenoun et al. 2014); and the Asian clade (referred to here as the Asian-Australian clade, AAC) was

* *Corresponding author*. Fax: +1 515 294 9420.
E-mail address: tcharrin@iastate.edu (T.C. Harrington).
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initially represented by *C. pirilliformis* I. Barnes & M.J. Wingf. and strains on fig (*Ficus carica* L.) in Japan and taro (*Colocasia esculenta* (L.) Schott) (Johnson et al. 2005; Thorpe et al. 2005). The four geographic clades represent the purported natural origins of the respective species, but humans have moved strains around the world on vegetative plant propagative material, and the native range of many of the species of *Ceratocystis* is unclear (Harrington 2013; Harrington et al. 2014b; Li et al. 2016). For instance, a number of species described from Africa, such as *C. polyconidia* R.N. Heath & Jol. Roux, appear to be phylogenetically related to members of the AAC (Heath et al. 2009; Mbenoun et al. 2014), which also has been called the Indo-Pacific clade (Mbenoun et al. 2014).

Members of the plant family Araceae are commonly propagated by corms or vegetative cuttings, and a study by Thorpe et al. (2005) focused on Araceae strains of the *C. fimbriata* complex that appear to have been moved by humans to new continents and islands in plant propagative material. Among these Araceae strains, two isolates from taro corms in Hawaii and an isolate from a corm of *Xanthosoma sagittifolia* (L.) Schott in Fiji were shown to be closely related to two specimens on taro corms exported from China based on sequences of the internal transcribed spacer regions of the rRNA genes (ITS rDNA) (Thorpe et al. 2005). This undescribed species was found to be similar to *C. pirilliformis* in its isozyme profile (Johnson et al. 2005) and sequences of the ITS rDNA, and it was speculated that the fungus was brought to Fiji and Hawaii from Asia on taro corms by Polynesian settlers (Thorpe et al. 2005). Another Hawaiian isolate from the American aroid *Syngonium podophyllum* Schott was shown to be related to isolates from *X. sagittifolium* in the Caribbean Region and placed in the Latin American clade (Thorpe et al. 2005). Other *C. fimbriata* strains of the LAC have been reported on sweet potato and ohia (*Metrosideros polymorpha* Gaud.) in Hawaii (Keith et al. 2015; Li et al. 2016).

The Hawaiian taro isolates and the isolate from Fiji are closely related to Asian and Australian species of *Ceratocystis* (Thorpe et al. 2005), but species boundaries of the AAC remain unclear. *Ceratocystis pirilliformis* was reported from Australia and later from Africa as a wound colonizer (Barnes et al. 2003; Kamgan Nkuekam et al. 2009; Lee et al. 2016). Species of the AAC have been distinguished from *C. pirilliformis* by phylogenetic analyses of DNA sequences and minor differences in morphology, but some of these species are doubtful and others may be African (De Beer et al. 2014; Mbenoun et al. 2014). A few of the species of the AAC have been associated with crown dieback and death of trees, but the *Ceratocystis* sp. was not necessarily the cause of the mortality. *Ceratocystis ficicola* Kajitani & Masuya causes a lethal canker disease on fig trees in Japan (Kajitani and Masuya 2011). *Ceratocystis polychroma* M. van Wyk & M.J. Wingf. was associated with a wood borer attacking clove (*Syzygium aromaticum* (L.) Merr. & L.M. Perry) in Indonesia (Van Wyk et al. 2004), and *C. atrox* M. van Wyk & M.J. Wingf. was associated with a wood borer on *Eucalyptus grandis* Labill. in Australia (Van Wyk et al. 2007). Other described species from Asia and Australia have been isolated from freshly cut stumps or wounds of plantation forest trees. These include *C. larium* M. van Wyk & M.J. Wingf., *C. corymbicola* Kamgan-Nkuek. & Jol. Roux, *C. colliformis* F.F. Liu, M.J. Wingf. & S.F. Chen, and *C. cercfabiensis* F.F. Liu, Jol. Roux & S.F. Chen (Van Wyk et al. 2009; Kamgan Nkuekam et al. 2012; Chen et al. 2013; Liu et al. 2015). *Ceratocystis fimbriata* was reported to cause a lethal wilt disease on *Eucalyptus* spp. in Yunnan (Li et al. 2014), but Li et al. (2016) suggested that this was an undescribed species closely related to *C. cercfabiensis*.

The Hawaiian and Fijian fungus reported by Thorpe et al. (2005) has not been closely compared to the recently described species of the AAC. Thus, the aim of this study was to compare the undescribed species from Hawaii and Fiji with other members of the AAC using phylogenetic analyses of DNA sequences, morphology, growth at different temperatures, and mating tests. These criteria were used to evaluate whether the taro/*Xanthosoma* fungus should be recognized as a new species.

2. Materials and methods

2.1. Isolates and DNA extraction

Representative isolates of the four geographic clades of the *C. fimbriata* complex (i.e., *Ceratocystis*) were used for phylogenetic comparisons with the new putative species (Table 1). Sequences of representative members of the AAC and LAC, especially of isolates from China and Hawaii, were included in the phylogenetic analyses, as well as sequences of *C. albifundus* (African clade) and sequences from an isolate of *C. variospora* (North American clade, which was used as an outgroup). Chinese isolates included six isolates of *C. cercfabiensis* from two *Eucalyptus* stumps in Guangdong and five isolates of *C. cercfabiensis* from one *Eucalyptus* stump in Hainan (Liu et al. 2015; Li et al. 2016). Six isolates of an unidentified species (*Ceratocystis* sp. Y) obtained from leaves or twigs of three *Eucalyptus* trees on the campus of the Yunnan Agricultural University in Kunming were also studied (Li et al. 2014, 2016). Isolate C2240 (= CBS 115775) from the paratype of *C. polychroma* from Indonesia was also included in the analyses, as well as isolate C1355 (= KFCF 9001) of *C. ficicola*.

There is some ambiguity surrounding two isolates from the Centraalbureau voor Schimmelcultures (CBS; currently Westerdijk Fungal Biodiversity Institute), that may have been incorrectly deposited or later mislabeled. Isolate C2239 was obtained in 2005 from CBS as CBS 118128, *C. pirilliformis*, but the ITS rDNA sequence of this isolate (KY995151) differs from other deposited ITS rDNA sequences of *C. pirilliformis* (e.g., NR119452, deposited as the ITS rDNA sequence of ex-holotype culture, PREM 57323 = CMW 6579 = CBS 118128). At the time of the original deposit of CBS 118128, isolate CBS 118126 was deposited as *C. fimbriata* from clove in Sulawesi, Indonesia, a host and location consistent with the later described *C. polychroma* (Van Wyk et al. 2004). A deposited ITS rDNA sequence (KC493165) of CBS 118126 differs somewhat from the ITS rDNA sequence (KY528970) of other *C. polychroma* isolates (e.g., C2240 = CBS 115775 = CMW 11449 = PREM 57821; C2242 = CBS 115778 = PREM 57818) but is identical to the ITS rDNA sequence of isolate C2239. Isolate C2239 and CBS 118126 are thus presumed to be of the same species, treated here as an unidentified species (*Ceratocystis* sp. X) of the AAC.

Template for polymerase chain reaction (PCR) was extracted from isolates grown on malt yeast extract agar

Table 1 – Collection numbers, location, host, ITS rDNA, mating type genes, and translation elongation factor 1 alpha GenBank accession numbers for representative isolates of *Ceratocystis uchidae*, *C. cercfabiensis* and other *Ceratocystis* species.

Species	Isolate (specimen) no.[a]	Location	Host	ITS rDNA	MAT1-1-2	MAT1-2-1	TEF-1α
C. uchidae	C1714 = CBS 115164, (BPI 843732), ex-holotype	Hawaii, USA	*Colocasia esculenta*	AY526306	KY322693	—	KY982680
	C1715 = CBS 114720, (BPI 843733)	Hawaii, USA	*C. esculenta*	AY526307	—	KY322700	KY982681
	C1931 = DAR 58902, (BPI 843739)	Fiji	*Xanthosoma sagittifolium*	AY526308	—	—	HM569618
C. cercfabiensis	C3301 = C3353 = CERC2548	Guangdong, China	*Eucalyptus* sp.	KY306679	—	—	—
	C3302 = C3354 = CERC2549	Guangdong, China	*Eucalyptus* sp.	KY306680	KY322694	KY322701	KP727623
	C3305 = CERC2552	Guangdong, China	*Eucalyptus* sp.	KY306681	—	—	KP727624
	C3358 = CERC2168	Hainan, China	*Eucalyptus* sp.		—	—	KP727630
Ceratocystis sp. Y	C3372 = E2-1-2	Yunnan, China	*Eucalyptus* sp.	KY306682	KY322695	KY322702	KY982682
	C3374 = E2-2-2	Yunnan, China	*Eucalyptus* sp.	KJ511481	—	—	—
	(BPI 596161)	China	*C. esculenta*	AY526304	—	—	—
	(BPI 596162)	China	*C. esculenta*	AY526305	—	—	—
Ceratocystis sp. X	C2239	Unknown	Unknown	KY995151	KY322696	KY322703	KY316542
C. polychroma	C2240 = CBS 115775	Indonesia	*Syzygium aromaticum*	AY528972	KY322697	KY322704	KY316543
C. ficicola	C1355 = KFCF 9001, MAFF 625119, (BPI 843724)	Japan	*Ficus carica*	KY306683	—	—	KY316544
C. albifundus	C1060 = CMW4079	South Africa	*Acacia mearnsii*	AF043605	KY322698	KY322705	HM569619
C. fimbriata	C1476 = ICMP 8579	Papua New Guinea	*Ipomoea batatas*	AY157957	KF482992	KF483000	HM569615
C. platani	C1317 = CBS 115162	North Carolina, USA	*Platanus occidentalis*	AY157958	KF482995	KF483003	HM569617
	C1717 = CBS 114719	Hawaii, USA	*Syngonium podophyllum*	AY526294	KY322699	KY322706	—
C. colombiana	C1543 = CBS 135861	Colombia	*Coffea arabica*	AY157961	KF482994	KF483002	—
C. cacaofunesta	C1004 = CBS 153.62	Ecuador	*Theobroma cacao*	AY157950	KF482993	KF483001	—
C. variospora	C1963 = CBS 135862	Iowa, USA	*Prunus* sp.	AY907042	KF482996	KF483004	KR347450

[a] Isolate and specimen numbers are those of the corresponding author or the Centraalbureau voor Schimmelcultures (CBS, now Westerdijk Fungal Biodiversity Institute), China Eucalypt Research Centre (CERC), Forestry and Agricultural Biotechnology Institute, University of Pretoria (CMW), Agricultural Institute, New South Wales, Australia (DAR), International Collection of Microorganisms from Plants, Landcare Research, New Zealand (ICMP), Fukuoka Agricultural Research Center, Japan (KFCF), Genetic Resources Center, National Agriculture and Food Research Organization, Japan (MAFF) or the National Fungus Collection, USA (BPI).

(MYEA; 2% malt extract, 0.2% yeast extract, 2% agar) for 8–10 d at room temperature using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI).

2.2. ITS rDNA sequences

Newly generated ITS rDNA sequences were determined using primers ITS1-F and ITS-4 as previously described (Harrington et al. 2014b). New ITS sequences from some isolates of *C. cercfabiensis* were also generated by direct sequencing, but some of the *C. cercfabiensis* isolates were difficult to read, so the PCR products were cloned into the TGEM easy vector for sequencing with vector primers (Li et al. 2016).

The generated sequences were compared to a large dataset of ITS rDNA sequences of more than 800 isolates of the *C. fimbriata* complex collected from throughout the world (Harrington et al. 2014b; Li et al. 2016). Representatives of the respective clades and populations of *Ceratocystis* were included in the analyses. In addition, BLAST searches (National Center for Biotechnology Information, Bethesda, Maryland) with the ITS rDNA sequence of isolate C1714 from Hawaii were used to identify other similar sequences. These ITS rDNA sequences were downloaded from GenBank and aligned by eye with those of the new species, but the ITS1 sequences of *C. collisensis* (KP727578 and KP727580) and *C. larium* (EU881906–7), as well as those of the African species *C. albifundus* (DQ520638, AF388947), could not be aligned, and the sequences of these taxa had to be excluded from further ITS rDNA analysis.

The aligned ITS dataset (TreeBase 20647) was 740 characters long, including gaps, but the last 168 of the aligned characters were eliminated because of ambiguous alignment. Of the remaining 572 characters, 366 were constant, 41 characters were parsimony uninformative, and 165 characters were parsimony informative. Gaps were treated as a new state (5th character), the characters were unordered and treated with equal rate. The aligned dataset was analyzed by maximum parsimony using PAUP 4.0b10 (Sinauer Associates, Sunderland, Massachusetts), with settings as described in Harrington et al. (2014b). Bootstrapping was also performed in PAUP. Posterior probability estimates for branches were performed in MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003), with 1,000,000 generations, diagnosis frequency = 5000, and sample frequency = 500, and burninfrac = 0.25.

2.3. TEF-1α gene sequences

The primers EFCF1a (5′ -AGTGCGGTGGTATCGACAAGCG) and EFCF6 (5′ -CATGTCACGGACGGCGAAAC) were used for amplification and sequencing, and two additional primers (EFCF2 = 5′ -TGCTCAACGGGTCTGGCCAT, EFCF3 = 5′ - ATGGCCAGACCCGTGAGCA) were used to sequence about a 1600 bp region of *TEF-1α* that included portions of three exons and two introns (Oliveira et al. 2015b). Thermocycler settings for amplifying the *TEF-1α* region included: initial denaturation at 85 °C for 2 min followed by 94 °C for 95 s; with 36 cycles of 60 °C for 1 min, 72 °C for 90 s, and 94 °C for 35 s; followed by final extension of 72 °C for 15 min. The fragments were purified and sequenced as described above.

The sequenced *TEF-1α* region was aligned with GenBank accessions of other members of the AAC generated in other studies, but for most of the accessions, the partial sequences included only the first half of the new sequences. New *TEF-1α* sequences of *C. albifundus* and *C. variospora* were included, with the latter as an outgroup taxon. The aligned 763 bp region (TreeBase 20648) included two introns positions, where most of the polymorphisms were found. Gaps were treated as a new state. Of these 763 aligned characters, 646 were constant, 55 were parsimony uninformative, and 62 were parsimony informative. The alignment was analyzed as described for the ITS dataset.

2.4. Mating type gene sequences

Most field isolates of species in the *C. fimbriata* complex are self-fertile, and self-fertile isolates have both MAT1 and MAT2 genes in the mating type locus. Two closely linked segments of the mating type locus (portions of the *MAT1-1-2* and *MAT1-2-1* genes) were amplified and sequenced for phylogenetic analyses. The *MAT1-1-2* region (1022 bp) was amplified and sequenced with CFMAT1-F (5′-CAGCCTCGATTGAKGGTATGA) and CFMAT1-R (5′-GGCATTTTTACGCTGGTTAG). The *MAT1-2-1* region (1102 bp) was amplified with the primer X9978a (5′-GCTAACCTTCACGCCAATTTTGCC) or X9978R1R (5′- GCTA ACCTTCACGCCAATTT) and CFM2-1F (5′- AGTTACAAGT-GTTCCCAAAAG). Sequences of the MAT2 PCR products of some representatives of the AAC were poor with the X9978R1R and CFM2-1F primers, so new primers for PCR and sequencing were developed (AsiaCFM2F: 5′- AGTTACAAGTKTTCCCA-AAAT and AsiaCFM2R: 5′- GCTAACCTKSACGCCAATTTC). The reaction mixture and cycling conditions were as outlined in Harrington et al. (2014b).

The aligned dataset (TreeBase 20646) of 2185 characters (gap = newstate) of the mating type genes showed 1458 characters constant, 417 characters parsimony uninformative, and 310 characters parsimony informative. The aligned dataset was analyzed as described above.

2.5. Morphological characteristics

Eighteen isolates of representatives from the AAC were grown on MYEA for 8–10 d at room temperature (approximately 23 °C) and lighting. Perithecia, ascospores, conidiophores, aleurioconidia (aleuriospores; cf., Kirk et al. 2008) and cylindrical conidia were measured after 10 d. For microscopic examination of cultures and specimens, material was mounted in cotton blue or 20% lactic acid. For each structure, 25 observations were recorded per isolate.

Isolates also were grown at 30 °C and 32 °C in the dark on three replicate MYEA plates. Plugs of mycelium from the advancing edge of a MYEA colony were taken with a No. 3 cork borer and placed in the center of the plates. Colony diameters (two per plate) were measured after 6 d.

2.6. Interfertility tests

Most field isolates of the *C. fimbriata* complex are MAT2 and are self-fertile, so mating tests use self-sterile sectors of MAT2 isolates that have lost femaleness (protoperithecia) as male testers (Harrington and McNew 1997, 1998; Oliveira et al. 2015b). Self-sterile MAT2 testers are frequently recovered as

sectors during the cultivation of field isolates. Suitable male, MAT2 testers were derived from one of the Hawaii taro isolates (C1714), the Fiji isolate (C1931), two isolates of *C. cercfabiensis* (C3358 and C3354), and one tester (C4148) of an isolate of an unidentified species (sp. Y) from *Eucalyptus* in Yunnan (Li et al. 2016). All five male, MAT2 tester strains retained the *MAT1-2-1* gene according to a PCR test (Witthuhn et al. 2000; Oliveira et al. 2015b) and thus could function as a MAT2 strain in a cross. The 12 female, MAT1 tester strains were mostly obtained by recovering single-ascospore progeny that were self-sterile but still produced protoperithecia, and thus could serve as females.

The female, MAT1 tester strain was first grown on MYEA for 7 d at room temperature and lighting. The male testers were grown on MYEA for 7 d, and then a suspension of conidia, conidiophores and mycelial fragments was scraped from the surface in sterile water. An aliquot of 1–2 mL of the suspension was dispersed over the colony of the female tester. The plates were incubated at room temperature and lighting for 7 d and examined through a dissecting scope for the presence of perithecia and ascospores that accumulated at the tip of the perithecial necks. Ascospores were examined and rated by making a microscope slide of ascospore masses from three perithecia mounted in cotton blue and examining at 1125× with Nomarski interference microscopy. Mounts of normal ascospore masses had greater than 90% uniform ascospores, while abnormal ascospore masses had 10% or more of the spores misshapen or without cytoplasm, and there was generally debri within the spore mass, assumed to be aborted asci.

Germination of the ascospores from the crosses was evaluated by placing an ascospore mass from atop one perithecium into 25 μL of light oil (Isopar M) on a MYEA plate. Small amounts of the oil suspension were taken with a wire loop (bacterial loop) and thoroughly streaked across each of two additional plates of MYEA. The remaining oil suspension on the original plate also was thoroughly spread with the wire loop. Germination was evaluated on the three plates after 6–8 d incubation at room temperature. If the colonies on the three plates were too numerous to count (TNTC), then the germination percentage was considered high. If fewer than 40 total colonies developed on the three plates, the germination was considered low. If there were 40 or more colonies total on the three plates, but sill countable, then the germination was considered medium. The phenotypes of the colonies after incubating at room temperature for 1–2 wk were also evaluated for each of the plates to confirm that a cross had occurred, that is, that the progeny colonies were from a genetic recombination between the two parent strains and not a selfing. The streaks of ascospore masses from an interfertile cross resulted in colonies of differing phenotype, and some of the colonies produced perithecia and ascospores (self-fertile, MAT2), and others produced no perithecia.

3. Results

3.1. ITS rDNA sequences

The sequenced ITS1 region of two Asian species, *C. collisensis* and *C. larium*, differed greatly from the other members of the *C. fimbriata* complex, but otherwise the sequences of species of the Asian clade were similar to each other and alignable (Fig. 1). The ITS sequence of *C. albifundus* also had large insertions and was not alignable with the other sequences. For the dataset of the other taxa, six most parsimonious trees of 379 steps were found, with consistency index (CI) = 0.7203, homoplasy index (HI) = 0.2797, HI excluding uninformative characters = 0.3174, retention index (RI) = 0.9466, and rescaled consistency index (RC) = 0.6819. Bootstrap values (for 1000 replications) and probability estimates from Bayesian analyses (after 1,500,000 replications, average standard deviation of split frequencies = 0.008113) supported some, but not all branches (Fig. 1).

The ITS rDNA sequences for the two taro isolates from Hawaii and the Fiji isolate differed by one base substitution, and these three isolates grouped with 95% bootstrap support. This group was sister to a group of isolates from *Eucalyptus* and other hosts from Yunnan, China, referred to here as *Ceratocystis* sp. Y (Fig. 1). Sequences of cloned PCR products identified two different ITS rDNA sequences from some isolates of *C. cercfabiensis*, as reported in earlier studies (Liu et al. 2015; Li et al. 2016). These *C. cercfabiensis* sequences were similar to those of the earlier described *C. polychroma* (from Indonesia), *C. corymbicola* (from Australia), *C. atrox* (from Australia), and two isolates referred to here as *Ceratocystis* sp. X (Fig. 1). This *C. polychroma* cluster had good posterior probability support. The Australian *C. pirilliformis* and three African species (*C. obpyriformis*, *C. polyconidia*, and *C. zombamontana*, but not *C. albifundus*) grouped in another strongly supported clade, while the Japanese species, *C. ficicola*, grouped separately. The *C. polychroma* cluster, the *C. pirilliformis* cluster, and *C. ficicola* comprised the Asian-Australian clade (AAC) with a posterior probability support value of 0.93.

Members of the LAC grouped separately in a well-supported clade that included Chinese isolates of *C. fimbriata* from *Eucalyptus* and other hosts (Li et al. 2016), as well as a Hawaiian isolate from sweet potato (Fig. 1). The LAC sequences included that of an isolate from *Syngonium podophyllum* in Hawaii (Thorpe et al. 2005), which grouped with the sequence of an isolate of *C. platani* from North Carolina, USA (Fig. 1).

3.2. TEF-1α gene sequences

The analysis of the 763 bp alignment resulted in six most parsimonious trees of 163 length with CI = 0.8221 (0.7157 excluding uninformative characters), HI = 0.1779 (0.2843 excluding uninformative characters), RI = 0.8543, and RC = 0.7023. There was little variation among the sequences of representatives of the AAC (Fig. 2). Posterior probability analyses in MrBayes (5 million generations, for a probability of the split frequencies = 0.004172) and the bootstrap values of the maximum parsimony branches showed little support for most of the groupings (Fig. 2). There was bootstrap support for the AAC, and there was strong support for the *C. pirilliformis* cluster (*C. obpyriformis*, *C. zombamontana*, *C. polyconidia* and *C. pirilliformis*), as found in the ITS rDNA analysis. The *TEF-1α* analysis grouped *C. collisensis* with *C. larium*, and *C. ficicola* and *C. atrox* were distinct, but there was no support for the other branches within the AAC (Fig. 2). The *TEF-1α* sequences for the

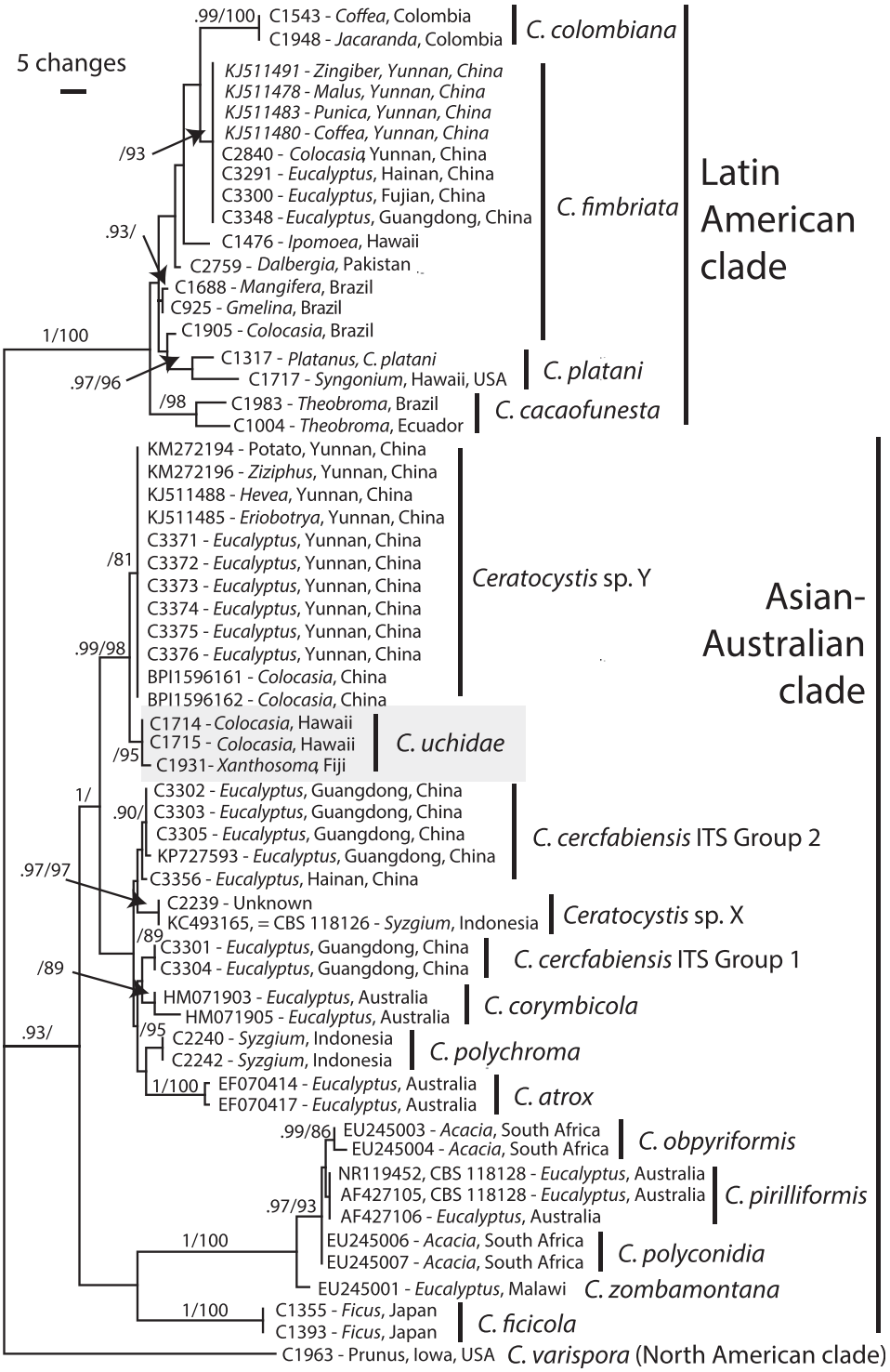

Fig. 1 – One of six most parsimonious trees of 379 steps based on the ITS rDNA sequences of representative isolates of the Latin American and Asian-Australian clades of *Ceratocystis*. The tree is rooted to *C. variospora* of the North American clade. Isolate numbers or GenBank accession numbers are given, along with the host genus and location. Posterior probability values equal to or greater than 0.9 and bootstrap values equal to or greater than 80% and are given above the appropriate branches.

Hawaiian taro isolates and the Fijian isolate were identical and most similar to the sequences for the other members of the *C. polychroma* cluster (*C. corymbicola*, *Ceratocystis* sp. Y, and *C. cercfabiensis*).

3.3. *Mating type genes*

Six most parsimonious trees of 912 steps were found using the combined *MAT1-1-2* and *MAT1-2-1* gene regions, with a CI of

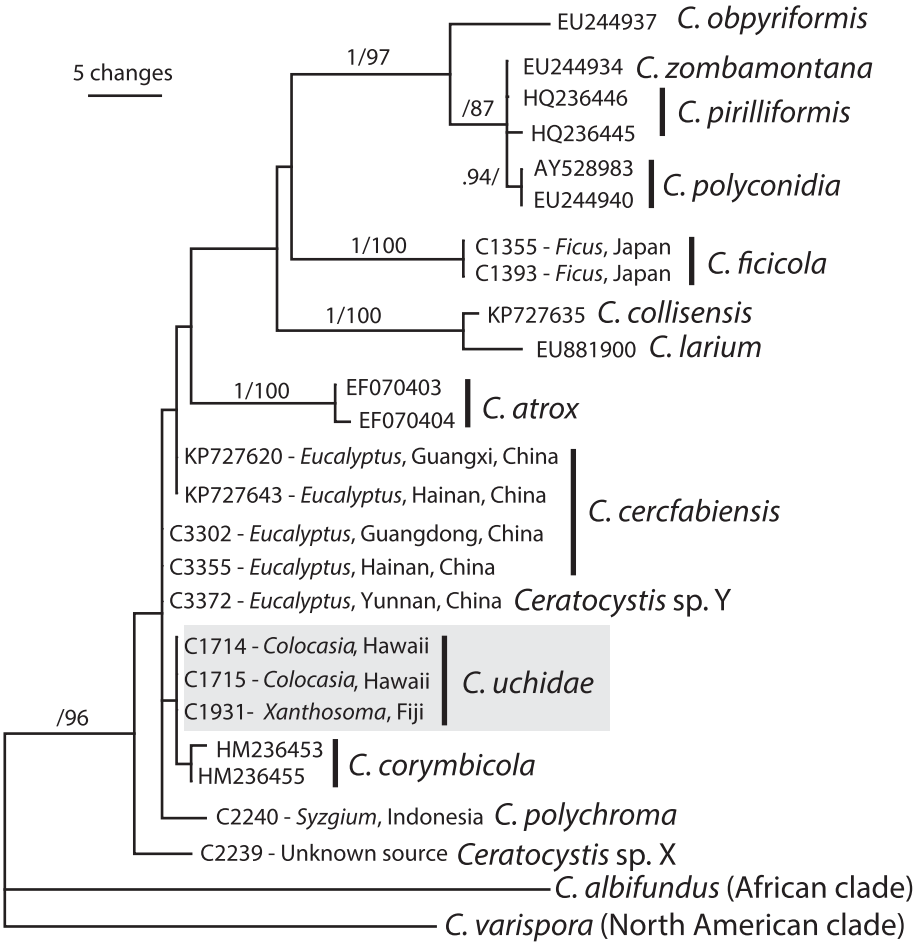

Fig. 2 – One of six most parsimonious trees of 163 steps based on the *TEF-1α* sequences of representative isolates of species in the Asian-Australian clade of *Ceratocystis*. The tree is rooted to *C. variospora* of the North American clade and also includes the African species, *C. albifundus*. Isolate numbers or GenBank accession numbers are given, along with the host genus and location. Posterior probability values equal to or greater than 0.9 and bootstrap values equal to or greater than 80% and are given above the appropriate branches.

0.9145 (0.8271 excluding uninformative characters), HI of 0.0855 (0.1729 excluding uninformative characters), RI of 0.9588 and RC of 0.8768. The posterior probability estimates for the branches were estimated from 1 million generations in MrBayes, and the topology of the likelihood tree from that analysis (not shown) was identical to the maximum parsimony tree shown in Fig. 3.

The members of the *C. polychroma* cluster within the AAC showed very little variation in mating type gene sequences (Fig. 3). The Hawaiian isolates from taro had the identical MAT1/MAT2 sequence as *Ceratocystis* sp. Y from *Eucalyptus* isolates in Yunnan, and this sequence grouped with the Fijian isolate. The representatives of *C. cercfabiensis* also had MAT1/MAT2 sequences similar to the Hawaiian/Fijian species, *Ceratocystis* sp. X, and *C. polychroma*.

The sequences of the Asian isolates grouped separately from the African *C. albifundus*, the North American *C. variospora*, and the strongly-supported LAC (*C. colombiana*, *C. platani*, *C. cacaofunesta* and *C. fimbriata*) (Fig. 3). Some Chinese isolates from *Eucalyptus* and taro had MAT1/MAT2 sequences that grouped within *C. fimbriata* sensu stricto of the LAC, and the *Syngonium* isolate from Hawaii had a sequence very similar to that of *C. platani*.

3.4. *Interfertility tests*

Almost all of the crosses between male, MAT2 and female, MAT1 testers resulted in perithecia and production of some ascospores at the tips of the necks, and when ascospores were produced, there was at least some germination of the ascospores after streaking on MYEA. In streaks of ascospores from the tip of perithecia produced from all intraspecific crosses, and in most cases of interspecific crosses, there was some variation in the mycelial phenotype (colony texture and in presence/absence of perithecia) among the resulting colonies, indicating that the male and female tester strains had crossed. However, microscopic examination indicated that there was substantial variation among interspecific crosses in the abundance and proportion of misshapen ascospores, and in most cases there was only low to medium percentages of ascospore germination (Table 2).

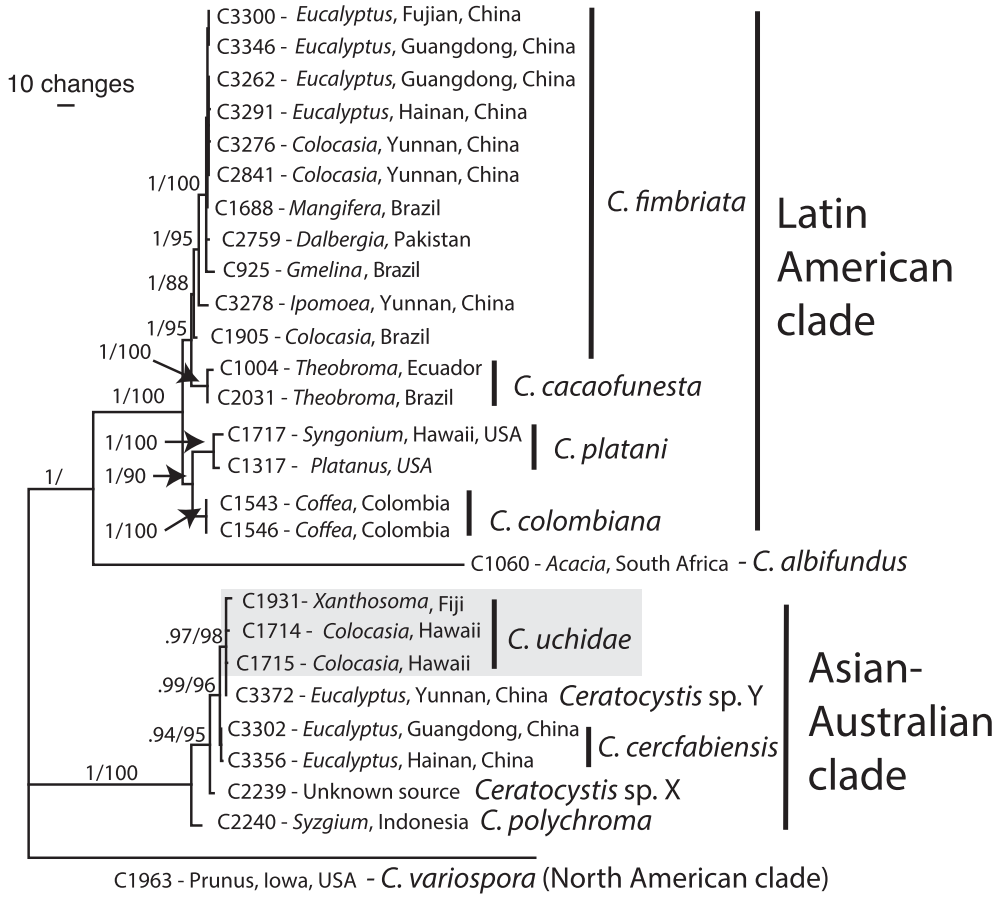

Fig. 3 – One of six most parsimonious trees 912 steps based on sequences of portions of the mating type genes MAT1-1-2 and MAT1-2-1 of representative isolates of the Latin American and Asian clades of *Ceratocystis*. The tree is rooted to *C. variospora* of the North American clade and also includes the African species, *C. albifundus*. Isolate numbers or GenBank accession numbers are given, along with the host genus and location. Posterior probability values equal to or greater than 0.9 and bootstrap values equal to or greater than 80% are given above the appropriate branches.

Of the 12 female, MAT1 testers, only the two Hawaiian testers (C3459 from field isolate C1714 and C3460 from field isolate C1715) were fully interfertile with both male, MAT2 testers of the putative new species (C4181 from field isolate C1714 and C1931 from Fiji) (Table 2). In each of the four combinations, large, creamy ascospore masses with normal ascospores were seen in microscopic examination, and high germination rates were seen when the spores were streaked on MYEA plates.

Each of the four *C. cercfabiensis* female, MAT1 testers formed normal ascospores with the Hawaiian tester C4181, and one of the four *C. cercfabiensis* MAT1 testers formed normal ascospores when paired with the Fiji tester C1931. However, there was a reduced germination for ascospores collected from perithecia in all but one of these five pairings. Either no perithecia or abnormal ascospores with low germination were seen after the male, MAT2 testers of the new species spermatized the female, MAT1 testers of *C. polychroma*, *Ceratocystis* sp. Y or *Ceratocystis* sp. X (Table 2).

Both of the male, MAT2 testers of *C. cercfabiensis* successfully spermatized each of the four female, MAT1 testers of *C. cercfabiensis*. The two MAT2 testers of *C. cercfabiensis* also successfully spermatized the two female, MAT1 testers of the new putative species, but one of these four crosses with the new species produced ascospores with only a medium germination percentage (Table 2). The crosses between the *C. cercfabiensis* male, MAT2 testers and the other *Ceratocystis* spp. produced abnormal ascospores with medium to low germination (Table 2).

The male, MAT2 tester of *Ceratocystis* sp. Y produced normal ascospores with high germination rates with only the four female, MAT1 testers of *Ceratocystis* sp. Y (Table 2). However, it produced normal ascospores of low germination rate with one of the female, MAT1 testers of *C. cercfabiensis*. When the male tester of *Ceratocystis* sp. Y was paired with the MAT1 testers of the new putative species, only abnormal ascospores with low germination percentages were produced (Table 2).

3.5. *Growth on MYEA*

The two taro isolates from Hawaii grew similarly on MYEA and produced abundant conidia and perithecia (Fig. 4A, B). However, the isolate from Fiji grew much slower, with fluffy, aerial mycelium and delayed production of perithecia and ascospores, as well as reduced conidia production (Fig. 4C).

Table 2 – Mating reactions (normal or abnormal ascospores/high, medium or low germination percentages) between female, MAT1 testers and male, MAT2 testers of *Ceratocystis uchidae*, *C. cercfabiensis*, *C. polychroma* and unidentified *Ceratoycstis* species.

	Female, MAT1	Male, MAT2				
		C. uchidae		*C. cercfabiensis*		*Ceratocystis* sp. Y
		C4181 (from C1714)	C1931	C3358	C3354	C4148 (from C3371)
C. uchidae	C3459 (C1714)	Normal/high[a]	Normal/high	Normal/high	Normal/high	Abnormal/low
	C3460 (C1715)	Normal/high	Normal/high	Normal/medium	Normal/high	Abnormal/low
C. cercfabiensis	C3454 (C3301)	Normal/medium	Normal/low	Normal/high	Normal/high	Abnormal/low
	C4143 (C3302)	Normal/high	Abnormal/medium	Normal/high	Normal/high	Abnormal/medium
	C4145 (C3305)	Normal/medium	Abnormal/medium	Normal/high	Normal/high	Normal/low
	C3456 (C3356)	Normal/medium	Abnormal/low	Normal/high	Normal/high	Abnormal/medium
Ceratocystis sp. Y	C4163 (C3371)	Abnormal/low	Abnormal/low	Abnormal/medium	Abnormal/medium	Normal/high
	C3457 (C3372)	Abnormal/low	Abnormal/low	Abnormal/medium	Abnormal/medium	Normal/high
	C3458 (C3376)	Abnormal/low	Abnormal/low	Abnormal/medium	Abnormal/medium	Normal/high
	C4167 (C3374)	Abnormal/low	Abnormal/low	Abnormal/medium	Abnormal/medium	Normal/high
C. polychroma	C4116 (C2240)	Abnormal/low	Abnormal/low	Abnormal/medium	Abnormal/medium	Abnormal/low
Ceratocystis sp. X	C3461 (C2239)	No perithecia	Abnormal/low	Abnormal/medium	Abnormal/low	No perithecia

[a] The most compatible reaction in two replicate pairings. Microscopic examination classified ascospore masses as normal (>90% normal ascospores) or abnormal (more than 10% of the ascospores misshapen or empty, and typically with ascus or ascospore debri). Ascospore masses with high germination percentages produced colonies too numerous to count after an ascospore mass was streaked onto three plates of malt yeast extract agar, medium germination had 40 or more colonies on the three plates, and low germination had fewer than 40 colonies on the three plates.

Isolates of *Ceratocystis* sp. Y from China (Fig. 4D–I) were similar to the Hawaiian taro isolates in growth rate and mycelial morphology. The colony morphology of isolates of *C. cercfabiensis* varied greatly in growth rate and pigmentation, with irregular margins and frequent sectoring (Fig. 4J–V). The culture of *Ceratocystis* sp. X (Fig. 4W) and *C. polychroma* (Fig. 4X) were similar in morphology to the new putative species and *Ceratocystis* sp. Y.

At 30 °C, the Hawaiian taro isolates grew somewhat faster than the other tested isolates, but the Fiji isolate grew substantially slower (Table 3). The growth of the Hawaiian isolates was reduced substantially at 32 °C, as was that of the isolates of *Ceratocystis* spp. X and Y. In contrast, the growth of isolates of *C. cercfabiensis* and the isolate of *C. polychroma* was reduced only slightly at 32 °C (Table 3).

Microscopic observations found little variation in the dimensions of ascospores among the studied isolates (Table 3), and the conidia also varied little in dimensions among the species. More variation was found in the characteristics of perithecia. The perithecial necks of the Hawaiian taro and Fijian isolates were shorter and the ostiolar hyphae longer than those of *C. polychroma* and *Ceratocystis* sp. X. Isolates of *Ceratocystis* sp. Y had longer perithecial necks than those of the Hawaiian taro and Fijian isolates, but the ostiolar hyphae of isolates of these two species were longer than those of the other studied isolates (Table 3). The length of perithecia necks and ostiolar hyphae of the Chinese taro herbarium specimens were similar to those produced by isolates of *Ceratocystis* sp. Y (Table 3).

Ceratocystis cercfabiensis was reported to have very large perithecia, especially perithecial necks, and somewhat larger ascospores than many of the other species in the AAC (Liu et al. 2015). Perithecial necks of *C. cercfabiensis* were reported to be 830–1400 μm long (Liu et al. 2015), vs. 325–520 μm long necks in Hawaiian cultures of *C. uchidae* (Table 3). However, the perithecial necks in the cultures of *C. cercfabiensis* that we examined varied greatly in size, even among different perithecia produced in one Petri plate of MYEA. Perithecial necks were only 250–400 μm in C3305 (CERC2552), similar to the necks of the new species, but other cultures (e.g., C3357 = CERC2167 and C3358 = CERC2168) of *C. cercfabiensis* produced necks 560–1320 μm long, similar to those described from the holotype of *C. cercfabiensis* (Table 3). The perithecia of *C. cercfabiensis* with the longest necks failed to developed ostioles or ostiolar hyphae, and no ascospore drops were seen at the tip of the necks. However, ascospores were seen within the large, aberrant perithecia when they were crushed by a cover slip for microscopic examination. Ascospores of *C. cercfabiensis* and the new species produced in culture were similar, but the ostiolar hyphae of *C. cercfabiensis* isolates were shorter: 16–48 μm vs. 35–78 μm in the new species (Table 3).

Ceratocystis uchidae Q. Li, D. McNew & T. C. Harrin., sp. nov. Fig. 5.

MycoBank no.: MB 820129.

Diagnosis: *Ceratocystis uchidae* has shorter perithecial necks, longer ostiolar hyphae and grows slower at 32 °C than the closely related species *C. cercfabiensis* and *C. polychroma*.

Type: USA, Hawaii, Oahu, corm of *Colocasia esculenta* cv. *Bun long*, Feb 1991, J. Uchida (holotype: BPI 843732; dried culture of CBS 115164, C1714).

Gene sequences ex-holotype culture: AY526306 (ITS rDNA), KY322693 (*MAT1-1-2*), KY982680 (*TEF-1α*).

Etymology: Named for Janice Uchida, a plant pathologist at the University of Hawaii and the collector of the Hawaiian taro isolates.

Colonies on MYEA gray, turning to pale brown-gray after 7–10 d at 25 °C, reaching 60–65 mm diam at 7 d; odor sweet,

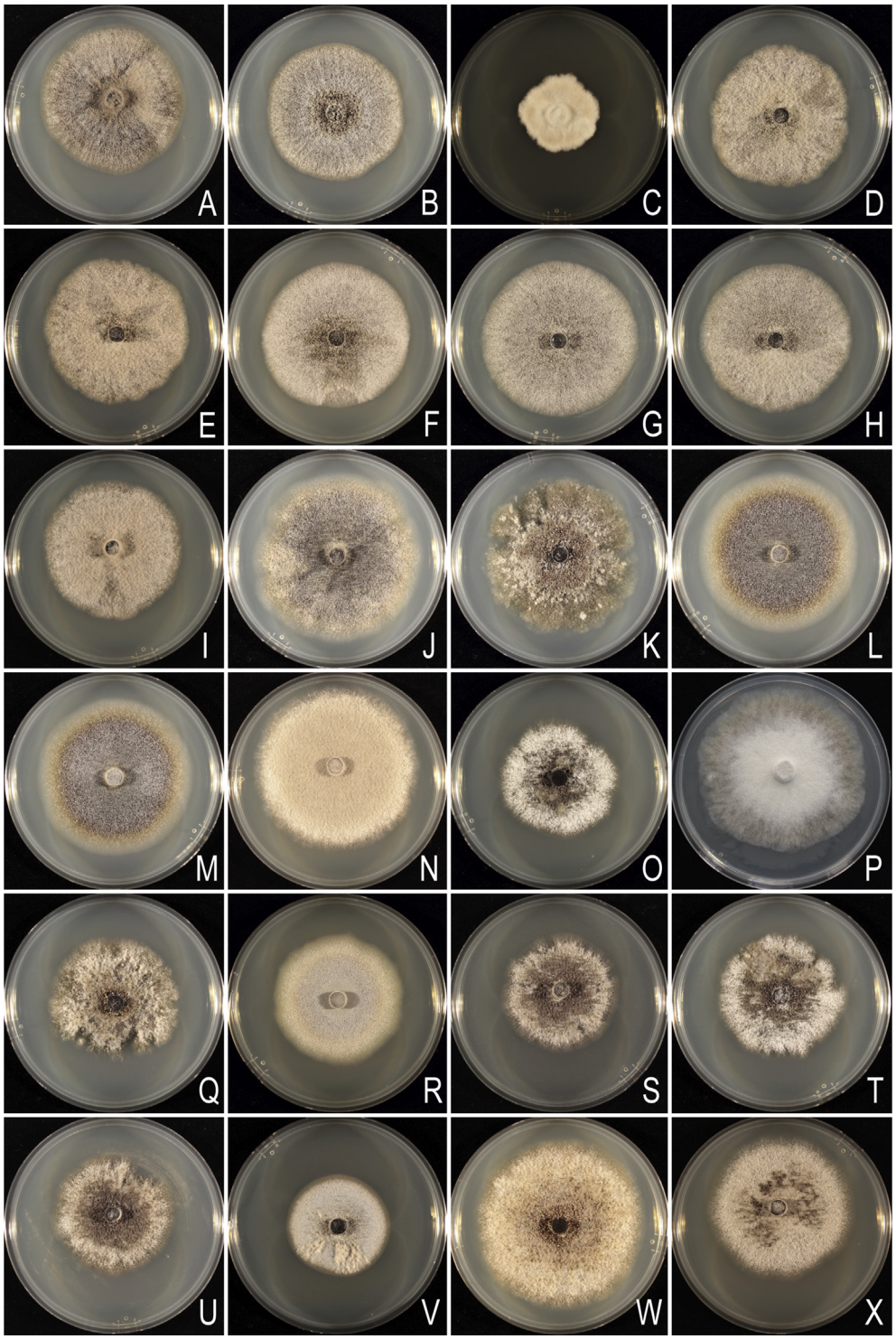

Fig. 4 – Isolates of *Ceratocystis uchidae* and related species grown on malt yeast extract agar for 7 d at 25 °C. A, B: *C. uchidae* C1714 and C1715, from taro in Hawaii. C: *C. uchidae* C1931, from *Xanthosoma sagittifolium* in Fiji. D–I: *Ceratocystis* sp. Y C3371–C3376, from *Eucalyptus* spp. in Yunnan, China. J–V: *C. cercfabiensis* from *Eucalyptus* spp. J–N: C3355–C3359, from Hainan, China. O, Q, S–V: C3301–C3305, and C3352 from Guangdong, China. P: C3353, MAT2 tester from C3301. R: C3354, MAT2 tester of C3302. W: C2239 of *Ceratocystis* sp. X. X: C2240 of *C. polychroma*.

with banana scent; undersurface turning dark green when older, especially under areas where perithecia produced. Perithecial bases superficial or immersed, black with undifferentiated hyphae, mostly obpyriform, 95–190 μm diam, possessing a collar at the base of the neck. Perithecial necks black, 325–520 μm long, 25–35 μm diam at base and 15–25 μm at the tip. Ostiolar hyphae hyaline, 40–75 μm long, tapering to blunt at tips. Asci with ascospores not seen. Ascospores in

Table 3 – Morphological features (range) and growth at 30 °C and 32 °C for isolates of *Ceratocystis uchidae*, *C. cercfabiensis* and other *Ceratocystis* species in the Asian-Australian clade of the *C. fimbriata* complex.

Species	Host/location	Isolate (specimen) no.	Perithecia		Ostiolar hyphae (length, μm)	Ascospores (μm)	Growth (diam, mm) on MYEA after 6 d[b]	
			Base (diam, μm)	Neck (length, μm)			30 °C	32 °C
C. uchidae	*Colocasia*/Hawaii, USA	C1714, CBS 115164, ex-holotype	(83–)130–184	325–500	38–75	4–6 × 2–3	45.5–49.5	7.5–10
		C1715, CBS 114720	96–190	430–520	40–70	4.5–6 × 2.5–3.5	35.5–38	8–10
	Xanthosoma/Fiji	C1931, DAR 58902	90–182	180–350	35–78	4.5–5.5 × 3–3.5	8.5–13	6
C. cercfabiensis	*Eucalyptus*/Guangdong, China	CERC2175, Holotype[a]	(80–)137–231(–286)	(473–)829–1400(–1756)	(32–)48–70(–82)	4–7.5 × 2.5–4.5	ND	ND
		C3301, CERC2548	90–122	300–480	20–36	5–6.5 × 3–3.5	22.5–28	13.5–16.5
		C3302, CERC2549	82–153	300–520	20–35	4.5–6.5 × 2.5–3.5	25.5–28.5	21–22.5
		C3304, CERC2551	90–165	325–540	20–48	5–6 × 3–3.5	20.5–25	17.5–19
		C3305, CERC2552	85–123	252–400	20–38	4.5–6 × 3–3.5	30–32	21.5–28
		C3352, CERC2547	92–172	262–1080	16–45	4.5–6.5 × 2.5–4	29.5–32	18–19
	Eucalyptus/Hainan, China	C3356, CERC2168	88–176	320–520	20–45	4.5–6.5 × 2.5–3	34.5–35	18.5–22
		C3355, CERC2165	90–170	420–650	None	5–6.5 × 2.5–3.5	ND	ND
		C3357, CERC2167	86–160	800–1320	None	5–6.5 × 2.5–3.5	26–34	12–13.5
		C3358, CERC2168	90–152	560–1150	None	5.5–6.5 × 3–3.5	24.5–31	9–19
Ceratocystis sp. Y	*Eucalyptus*/Yunnan, China	C3371	148–220	500–652	40–80	4.5–6 × 2.5–3.5	12–25.5	6.5–8.5
		C3372	120–208	420–700	38–80	4.0–6 × 3–4	8–23	6–9.5
		C3373	120–220	512–860	40–80	5–5.5 × 3–3.5	6–17	6.5–7.5
		C3375	147–190	483–620	40–80	5–6 × 3–3.5	8.5–23.5	8
	Colocasia/China	(BPI 596163)	140–250	650–820	40–80	4–5 × 2.5–3	ND	ND
		(BPI 596161)	180–280	350–840	ND	4–5.5 × 3–3.5	ND	ND
		(BPI 596162)	160–220	700–840	ND	ND	ND	ND
Ceratocystis sp. X	Unknown	C2239	120–181	450–767	22–55	4–5.5 × 2–3	17–33	7.5–11.5
C. polychroma	*Syzygium*/Indonesia	C2240, CBS 115775, ex-paratype	110–182	502–835	26–48	5–6 × 3–3.5	24–37.5	18–24.5

[a] Measurements from the original description of the holotype.
[b] Range of three replicate plates, with two diameter measurements per plate. ND = not determined.

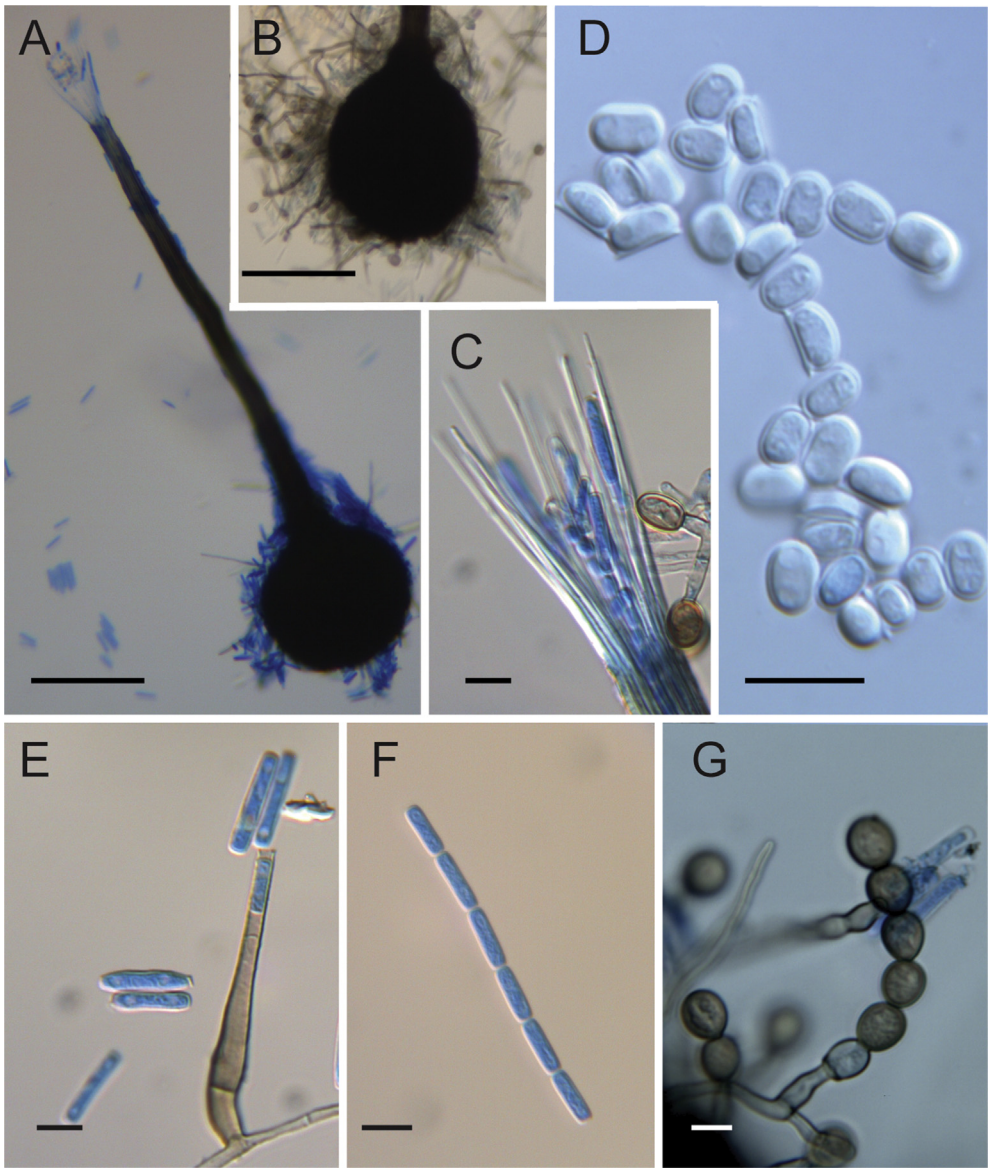

Fig. 5 – Perithecia, ascospores, and conidia of *Ceratocystis uchidae* (ex-holotype, C1714, CBS 115164). A: Perithecium. B: Pyriform base of perithecium with swelling at perithecial neck. C: Divergent ostiolar hyphae with ascospores emerging through the mouth of the neck. D: Hat-shaped ascospores in side and top view. E: Deep-seated phialide on a short conidiophore producing cylindrical conidia. F: Chain of cylindrical conidia. G: Chain of thick-walled, pigmented conidia (aleurioconidia) produced basipetally from a conidiogenous cell. Material in all photos stained in cotton blue. *Bars*: A, B 100 μm; C–G 10 μm.

side view 4.0–6.0 × 2.0–3.5 μm, with outer cell wall forming a hat-shaped brim, in top view 4.5–6.0 × 3.0–4.5 μm. Conidiophores with deep-seated phialides, hyaline to pale brown, septate, 60–80 μm long. Phialides 35–50 μm long, 4.5–6.0 μm wide at base, tapering to 2.5–4.0 μm at the mouth, producing chains of hyaline, aseptate, cylindrical conidia (10–25 × 2.0–4.0 μm). Thick-walled conidia (aleurioconidia) produced singly or basipetally in chains from simple conidiogenous cells, pale brown to dark brown, subglobose, smooth, thick-walled, 7.5–12.5 × 7.0–9.5 μm.

Other specimens examined: USA, Hawaii, Kauai, Nualolokai Valley, corm of *Colocasia esculenta*, Oct 1988, J. Uchida, (BPI 843733, dried culture of CBS 114720, C1715). FIJI (Intercepted by quarantine inspector in Australia), corm of *Xanthosoma sagittifolium*, May 1987, D. Holbein. (BPI 843739, dried culture of DAR 58902, C1931).

The above description is based primarily on the two Hawaiian isolates. The Fiji isolate grows slower than the two Hawaiian isolates (Fig. 5), it produces fewer conidia and perithecia, the perithecia are produced more slowly, and the perithecia have shorter necks than the Hawaiian isolates (Table 3). All three cultures of *C. uchidae* produce aleurioconidia (aleuriospores, referred to as chlamydospores in some papers), but wide-mouthed phialides producing barrel-shaped (doliform) conidia were not seen. *Ceratocystis polychroma* reportedly produces barrel-shaped conidia (Liu et al.

2015) and also can be distinguished from *C. uchidae* by longer perithecial necks, shorter ostiolar hyphae and faster growth at 32 °C (Table 3). *Ceratocystis cercfabiensis* is capable of producing much larger perithecia and necks than *C. uchidae* (Liu et al. 2015), although neck length varies greatly among isolates of *C. cercfabiensis* (Table 3). *Ceratocystis cercfabiensis* produces shorter ostiolar hyphae and grows nearly as fast at 32 °C as at 30 °C, while growth of *C. uchidae* is notably slower at 32 °C (Table 3). In comparison to *C. uchidae*, *C. corymbicola* has larger perithecial bases and necks and barrel-shaped conidia (Kamgan Nkuekam et al. 2012), and *C. atrox* produces no aleurioconidia in culture and has shorter ostiolar hyphae (Van Wyk et al. 2007; Liu et al. 2015).

Among the unidentified isolates in the *C. polychroma* cluster, isolates from *Eucalyptus* trees in Yunnan, China, referred to here as *Ceratocystis* sp. Y, are most similar to isolates of *C. uchidae* in colony morphology (Fig. 4), growth at 32 °C, and in length of ostiolar hyphae (Table 3). However, the perithecial necks of *Ceratocystis* sp. Y are longer than those of *C. uchidae* (Table 3). The perithecial necks on the three taro corm specimens from China (BPI 596161, BPI 596162, and BPI 596163) were similar to those of *Ceratocystis* sp. Y and longer than those of *C. uchidae* (Table 3). The ITS rDNA sequences for two of the corm specimens were identical to those of *Ceratocystis* sp. Y (Fig. 1).

Outside of the *C. polychroma* cluster, other AAC members can be distinguished from *C. uchidae* by minor morphological characters: *C. pirilliformis* has a prominent collar at base of the neck and barrel-shaped conidia (Barnes et al. 2003), *C. obpyriformis* has no aleurioconidia (Heath et al. 2009), *C. polyconidia* and *C. zombamontana* (considered a synonym of *C. pirilliformis* by De Beer et al. 2014) have barrel-shaped conidia and no aleurioconidia (Heath et al. 2009), and *C. larium* and *C. collisensis* have shorter ostiolar hyphae (Van Wyk et al. 2009; Liu et al. 2015). The perithecial bases, necks, ostiolar hyphae and ascospores of *C. ficicola* are larger than those of the other members of the AAC (Kajitani and Masuya 2011).

4. Discussion

Morphological, physiological and intersterility differences support delimitation of *C. uchidae* as a new species in spite of limited phylogenetic support. The taxonomy of the Asian Australian clade and the species concept for the genus *Ceratocystis* as a whole are in need of closer scrutiny, as illustrated by several disputed species in the LAC (Harrington et al. 2011, 2014b; Fourie et al. 2014; Oliveira et al. 2015a; Li et al. 2016). The new species is most closely related to *C. cercfabiensis*, with which it shows partial interfertility, but *C. uchidae* can be distinguished morphologically and physiologically from *C. cercfabiensis*, and the ITS rDNA sequences of the two species differ.

Two groups of very closely related or synonymous species are evident in the AAC: the *C. pirilliformis* cluster and *C. polychroma* clusters. *Ceratocystis pirilliformis* was originally described from *Eucalyptus* wounds in Australia and later reported in Africa (Barnes et al. 2003; Kamgan Nkuekam et al. 2009; Lee et al. 2016), and *C. polychroma* was described from clove trees in Indonesia (Van Wyk et al. 2004). Phylogenetic analyses place *C. uchidae* in the *C. polychroma* cluster, but it was not interfertile with *C. polychroma* in mating tests. Also, *C. uchidae* grows slower at 32 °C and lacks the doliform (barrel-shaped) conidia reported for *C. polychroma*, *C. corymbicola* and *C. atrox* (Van Wyk et al. 2007; Kamgan Nkuekam et al. 2012; Liu et al. 2015).

Another closely related species in the *C. polychroma* cluster is *C. cercfabiensis*, a recently described species from *Eucalyptus* stumps in South China (Liu et al. 2015). This species is highly variable in culture morphology and sectors frequently. The sizes of perithecial necks also vary greatly, and it has intragenomic variation in ITS rDNA sequences, which may indicate that it is a hybrid between two species or distant populations of the same species (Harrington et al. 2014b; Liu et al. 2015). It is known only from fresh stumps of *Eucalyptus* trees felled by chain saw (Liu et al. 2015), so it is possible that strains of one or more *Ceratocystis* spp. were spread and intermixed by chain saws in the harvesting of these *Eucalyptus* trees. Mixtures of different strains in intraspecific or interspecific hybrids might explain the extreme variation in the size of perithecia, culture morphology, intragenomic variation in ITS rDNA sequences and ambiguous interfertility between *C. cercfabiensis* and *C. uchidae*. Thus, *C. cercfabiensis* could be an unstable, interspecific hybrid rather than a true species, and perhaps a species like *C. uchidae* or *C. polychroma* is one of the parents of the hybrid. A few of the cultures of *C. cercfabiensis* that we examined produced the very large perithecial necks reported by Liu et al. (2015), but in our cultures, the perithecia with long necks did not produce ostioles, and no ascospore mass could be seen at the top of the large perithecial necks. Some of the cultures of *C. cercfabiensis* that we studied produced perithecial necks as short as those of *C. uchidae*, and the ostiolar hyphae of our cultures of *C. cercfabiensis* were shorter than those in the original description (Liu et al. 2015). Aside from the great range in morphology seen in cultures of *C. cercfabiensis*, *C. uchidae* cultures can be distinguished from cultures of *C. cercfabiensis* by their longer ostiolar hyphae and slower growth at 32 °C.

Two unnamed species in the *C. polychroma* cluster were also studied: *Ceratocystis* spp. X and Y, respectively. Our isolate C2239 was thought to have been deposited in CBS as *C. pirilliformis*, but DNA sequences place this isolate in the *C. polychroma* cluster, along with the ITS rDNA sequence (KC493165) of CBS 118126, which was deposited as *C. fimbriata* from clove in Indonesia. These isolates appear to represent an undescribed species (*Ceratocystis* sp. X) in the *C. polychroma* cluster, and their ITS rDNA sequence is unique. Cultures of *Ceratocystis* sp. Y from *Eucalyptus* trees in Kunming, Yunnan, China (Li et al. 2016) were morphologically indistinguishable from *C. uchidae* except in its slightly longer perithecial necks. Growth rates of *C. uchidae* and *Ceratocystis* sp. Y were similar at 32 °C. *Ceratocystis uchidae* and *Ceratocystis* sp. Y could be considered conspecific, but these two "biological species" had low interfertility in mating tests and differed slightly in DNA sequences. The taro specimens from China (BPI 1596161 and BPI 11596162) studied by Thorpe et al. (2005) had ITS rDNA sequences identical to *Ceratocystis* sp. Y, and they have perithecia indistinguishable from perithecia of *Ceratocystis* sp. Y. Many other accessions in GenBank of Yunnan strains, deposited as *C. fimbriata*, have the ITS rDNA sequence of

Ceratocystis sp. Y (Li et al. 2014). These include cultures from numerous host plant species, but it is not clear if these strains are aggressive on those hosts. However, other Yunnan isolates of *Ceratocystis* pathogenic on pomegranate, taro and loquat have been shown to be LAC strains of *C. fimbriata* (Huang et al. 2008; Harrington et al. 2014a; Li et al. 2016), and similar *C. fimbriata* strains have been recovered from *Eucalyptus* stumps in South China, along with *C. cercfabiensis* (Li et al. 2016). Thus, both LAC and AAC strains of *Ceratocystis* have been identified in China, but only the Latin American strains of *C. fimbriata* have been shown to be serious plant pathogens (Li et al. 2016).

Ceratocystis ficicola causes an important disease on fig in Japan (Kajitani and Masuya 2011), but in general, the importance of species in the AAC as plant pathogens is unclear. Most of the described AAC species were from stumps or artificially wounded trees, though inoculation studies suggest that they may be somewhat pathogenic wound colonizers (Van Wyk et al. 2009).

Ceratocystis uchidae was isolated from black rotted corms of taro and *Xanthosoma sagittifolium*, and the two Hawaiian isolates were somewhat pathogenic to taro and two other aroid species: *Caladium bicolor* (Aiton) Venten. and *Syngonium podophyllum* (Thorpe et al. 2005). However, many strains and species of *Ceratocystis* are capable of causing black rot on aroids, and the natural biology of the new species is not known. Thorpe et al. (2005) speculated that isolates of this new species were dispersed long ago on taro corms used for vegetative propagation by Polynesians. The natural distribution of *Colocasia esculenta* is thought to be from India to South China to Australia and Melanesia (Matthews and Nguyen Du 2015), and it is possible that *C. uchidae* was brought to Hawaii from Southeast Asia by Polynesians on taro corms prior to European settlement of the Pacific (Matthews 1995). One of the Hawaiian isolates of *C. uchidae* was from an old, abandoned taro pit in a remote location on Kauai. Thus, *C. uchidae* may have been present in Hawaii for centuries, and DNA sequences clearly distinguish it from more recent arrivals of LAC strains of *C. fimbriata* that are pathogenic on *Ipomoea*, *Syngonium* and *Metrosideros polymorpha* (Thorpe et al. 2005; Keith et al. 2015; Li et al. 2016).

Disclosure

The authors declare no conflicts of interest. All experiments undertaken in this study comply with the current laws of the USA.

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