Three new *Ceratocystis* spp. in the *Ceratocystis moniliformis* complex from wounds on *Acacia mangium* and *A. crassicarpa*

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Abstract

The genus *Ceratocystis* includes many important tree pathogens and agents of sap stain. These fungi have a global distribution and commonly infect wounds on trees. During a survey of wound-infecting pathogens in the genus *Ceratocystis* on plantation-grown *Acacia mangium* trees in Indonesia, several isolates resembling *Ceratocystis moniliformis sensu lato* (*s.l.*) were obtained. The aim of this study was to identify these isolates and to test their pathogenicity on commercially grown Acacia spp. in the country. Use was made of morphology and comparisons of DNA sequence data for the ITS, β-tubulin, and transcription elongation factor 1-α gene regions to identify the isolates. Three previously undescribed species in the *C. moniliformis s.l.* species complex were identified and are described here as *C. inquinans* sp. nov., *C. sumatrana* sp. nov., and *C. microbasis* sp. nov. Pathogenicity trials on *Acacia mangium* and *A. crassicarpa* in the greenhouse and in the field indicated that all three species have the potential to infect *A. mangium* and *A. crassicarpa*, although they are not considered important pathogens.

Introduction

Fungal pathogens represent a serious limiting factor to the success and long-term sustainability of commercial plantations in Kalimantan and Sumatra (Indonesia). These plantations, made up especially of nonnative *Acacia mangium* Willd. and *A. crassicarpa* A. Cunn. *ex* Benth., are vulnerable because of their homogeneous nature. The trees have also been separated from their natural enemies, and these are likely to appear and proliferate in time (Old *et al*. 2000, 2003). Similarly, some native pathogens will likely adapt to infect these trees, as has happened elsewhere with *Acacia* spp. (Roux *et al*. 2007). The uniform distribution of these plantation-grown trees will also enhance their vulnerability, as has already been found in the case of diseases that affect *Eucalyptus* plantations (Old et al. 2003).

Acacia mangium trees grown in plantations in Indonesia are commonly wounded during their cultivation. They tend to have multiple leader stems (Srivastava 1993; Lee and Arentz 1997), and it is standard practice in pulpwood plantations to prune or “single” the stems at 4–8 months of age (Beadle *et al*. 2007). In this regard, pruning wounds on young *A. mangium* trees in Indonesian plantations have been recognized as becoming infected with pathogens causing lesions that expand

substantially beyond the physical damage associated with pruning. Various fungi are known to infect pruning wounds on trees, and *Ceratocystis* spp. represent well-recognized examples of wound-infecting pathogens (Zalasky 1965; Teviotdale and Harper 1991; Kile 1993).

The genus *Ceratocystis* is well known to accommodate species that infect wounds on trees (Kile 1993). These species include a number of economically important pathogens, mostly residing in the *Ceratocystis fimbriata s.l.* species complex. Examples of tree-infecting pathogens include *C. albifundus* M.J. Wingf., De Beer & Morris, a pathogen of Australian *Acacia* spp. resulting in wilt and death of *A. mearnsii* De Wild. and *A. decurrens* Willd. trees in Africa (Morris *et al*. 1993; Wingfield *et al*. 1996; Roux and Wingfield 1997; Roux *et al*. 2005), and *C. fimbriata s.l.*, the causal agent of canker and wilt of *A. decurrens* in Brazil (Ribeiro *et al*. 1988). Other *Ceratocystis* spp., such as those in the *C. monilformis s.l.* species complex, are not known to cause disease and death of trees but may result in staining of the timber (Hedgcock 1906).

The taxonomy of *C. moniliformis s.l.* and fungi similar to it has presented considerable challenges in the past and especially before the advent of DNA sequence comparisons. Traditionally, all *Ceratocystis* spp. with hat-shaped ascospores, with conical spines on their ascomatal bases, and in which the bases of the ascomatal necks represented a disk-like structure that easily becomes detached from the ascomatal bases were classified as *C. moniliformis* (Hedgc.) C. Moreau (Hedgcock 1906; Hunt 1956; Van Wyk *et al*. 2004, 2006a). It is, however, now recognized that many of the fungi identified as *C. moniliformis* in the past represent distinct species (Van Wyk *et al*. 2004, 2006a). Thus, the *C. moniliformis s.l.* species complex includes at least seven distinct species, occurring on a broad range of hosts worldwide. Accurate identification of species in this complex relies on a combination of multigene sequence comparisons and morphology (Van Wyk *et al*. 2006a).

The aim of this study was to identify a collection of *Ceratocystis* isolates, which based on morphology resembled species in the *C. moniliformis s.l.* species complex. These isolates were collected from the stems of *A. mangium* trees that had been artificially wounded in a study to obtain information on the *Ceratocystis* spp. present on *Acacia* spp. in Indonesia. Identifications were based on morphology and comparisons of DNA sequence data. Furthermore, their ability to infect *A. mangium* and *A. crassicarpa* was evaluated using pathogenicity trials in the greenhouse and in the field.

Materials and Methods

Fungal isolates

Wounding trials were established on 1-year-old *A. mangium* and *A. crassicarpa* trees in the Teso area, Riau Province, during the dry and wet periods from December 2006 to March 2007, following the method described by Barnes *et al*. (2003). Fifteen trees of each species were randomly selected and wounded. Five weeks after wound establishment, samples of discolored wood and bark were collected from the wounds, wrapped in newspaper to maintain a moist environment, and taken to the laboratory for examination.

Fungi were isolated from the wood and bark specimens by lifting spore masses from the apices of ascomata and transferring these to 2% (w/v) malt extract agar (MEA; 15 g agar, 20 g malt extract) (Biolab, Midrand, South Africa). When no fruiting bodies were observed, wood tissue was incubated in sealed plastic bags containing moistened tissue paper for 4–10 days, until sporulation was observed, after which single spore masses were lifted from the tips of the ascomata. All isolates collected in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Representative

isolates have been deposited in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Delft, Netherlands and dried specimens were deposited at the National Herbarium of South Africa (PREM), Pretoria.

DNA extractions

Nine *Ceratocystis* isolates were selected randomly, based on differences in culture morphology and grown on 2% MEA at 22°C for 1 week. Using sterilized scalpels, the mycelium was scraped from the surface of the agar and transferred to 1.5-ml Eppendorf tubes and lyophilized overnight. The lyophilized fungal mycelium was placed into liquid nitrogen and crushed to a fine powder using a glass rod or mechanical grinder (Ball Mills machine; Retsch, Haan, Germany). The DNA was extracted using the method described by Van Wyk *et al*. (2006a).

PCR amplification, sequencing, and analyses

Polymerase chain reaction (PCR) amplification was carried out for three gene regions: these included the internal transcribed spacer regions (ITS), including the 5.8S gene of the ribosomal RNA (rRNA) operon, and portions of the β-tubulin (βt) and transcription elongation factor 1-α (EF1-α) regions. Each gene region was amplified using the primers ITS1 and ITS4 (White *et al*. 1990), βt1a and βt1b (Glass and Donaldson 1995), and EF1-728F and EF1-986R (Carbone and Kohn 1999), respectively. The PCR reaction mixtures were 25 µl in total, consisting of 0.5 µl 10 mM forward and reverse primers, 2 µl 10 mM dNTP mixture [2.5 mM of each deoxynucleotide triphosphate (dNTP)], 0.5 µl (2 U) DNA Taq enzyme (Roche Molecular Biochemicals, Almeida, CA, USA), 2.5 µl 10 × concentration buffer containing $MgCl_2$ (3.5 mM), 2 µl DNA (2–10 ng), and 17 µl deionized water. The PCR program, electrophoresis conditions, and DNA purification procedures were as described by Van Wyk *et al*. (2006a).

Sequencing of purified PCR amplicons was carried out in both directions using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit, following the manufacturer's protocols (Applied Biosystems, Foster City, CA, USA). Sequencing of each gene region was achieved using the same primers as those used for the PCR reactions. After cleaning, the final sequence products were run on an ABI PRISM 3100 auto sequencer (Applied Biosystems). Sequence electropherograms were analyzed using Sequence Navigator version 1.0.1 (Applied Biosystems).

BLAST analyses were used to compare the sequences obtained with those of *Ceratocystis* spp. that are available in GenBank (The National Centre for Biological Information; http://www.ncbi.nlm.nih.gov/). Thereafter, sequences published for *Ceratocystis* spp. were incorporated into the data sets for each gene region, together with those for the isolates from wounds on the Acacia spp., and aligned using the online interface MAFFT version 6 (http://align.bmr.kyushu-u.ac.jp/mafft/software/source.html). Alignments were confirmed manually and deposited into TreeBASE (Treebase S2353).

After alignment, sequence data for the Indonesian *Ceratocystis* spp. were analyzed using various programs. A partition homogeneity test was run using PAUP (phylogenetic analysis using parsimony) version 4.0b10* to determine whether the data from the three gene regions could be combined (Swofford 2002). Data for each data set were analyzed using PAUP version 4.0b10* (Swofford 2002). Gaps were treated as "newstate", and trees were obtained via stepwise addition of 1000 replicates. The Mulpar option was in effect. Bootstrap confidence intervals using 1000 replicates were calculated. *Ceratocystis virescens* (R.W. Davidson) C. Moreau was used as the outgroup taxon.

The model of nucleotide substitution for each gene region was determined using MrModeltest 2.2

(Nylander 2004), and the models obtained were used in Bayesian analysis with MrBayes version 3.1.1. (Ronquist and Huelsenbeck 2003). Bayesian probabilities of Markov Chain Monte Carlo (MCMC) algorithms combining each model test obtained were used to calculate support for the phylogenetic trees. Following the MCMC procedure, 1 million random trees were generated with four chains and sampled every 100th generation. Samples were taken only from trees after convergence after the trees outside the point of convergence had been discarded.

Culture characteristics and morphology

Two, or where available three, isolates representing each of the groups identified using DNA sequence comparisons were grown on 2% MEA for 1 week at 22°C. These isolates were then used for growth comparisons. Mycelial discs taken from the edge of an actively growing culture were cut using a 5-mm cork borer and placed at the centers of 90-mm Petri dishes containing 2% MEA. Discs representing each isolate were placed on five plates for each treatment, and these were incubated at 4°C, and between 10 and 35°C at 5°C intervals. Two diameter growth measurements for each colony, at right angles to each other, were taken 2 days after incubation, and averages were computed. The experiment was repeated once.

The morphology of the *Ceratocystis* isolates was described from 1-week-old cultures grown on 2% MEA. The color of cultures was described using the color charts of Rayner (1970). Fruiting structures were mounted in lactic acid (85%) for observation and measurement. Fifty measurements of characteristic structures for each isolate representing the main groups, and 10 measurements for one additional isolate in each group, were made using a Carl Zeiss microscope and a Zeiss Axiovision camera system (Zeiss, Oberkochen, Germany). The average (mean), standard deviation (SD), maximum (max), and minimum (min) measurements were calculated and are presented as (min−) mean minus SD − mean plus SD (−max).

Pathogenicity tests

Greenhouse inoculations

Pathogenicity tests were conducted on the stems of 1-year-old *A. mangium* and *A. crassicarpa* seedlings (15-mm-diameter size) grown in 20-cm-diameter plastic bags containing a mixture of topsoil and compost. The seedlings were placed in a greenhouse where the temperature and humidity was adjusted for optimum growth of the seedlings (30°C). One to three isolates representing the various groups of *Ceratocystis* (group 1 = CMW 21106; group 2 = CMW 21109, CMW 21111, and CMW 21113; group 3 = CMW 21115, CMW 21117, and CMW 21118) identified using DNA sequence data were used for the inoculations. Wounds were made on the stems of the seedlings (~30 cm above soil level) using a sterilized cork borer, and inoculations were carried out by inserting an agar disc taken from the edge of an actively growing colony grown on 2% MEA into the wound, with the mycelium facing the exposed cambium. Parafilm (Pechiney, Menasha, WI, USA) was used to seal the wounds after inoculation to avoid contamination and desiccation. Five seedlings of each *Acacia* sp. were inoculated with each of the test fungi, and five seedlings were similarly inoculated with sterile MEA plugs to serve as controls.

Ten days after inoculation, the Parafilm was removed from the inoculation sites and the lengths of the lesions in the xylem were measured. A piece of symptomatic stem tissue was taken from the inoculation site for five seedlings representing each test isolate and placed into moist chambers to induce the production of fruiting structures. Spores from these structures were then transferred to 2% MEA to verify the identity of the inoculated isolate and to fulfill the requirements of Koch's postulates. Analysis of variance (ANOVA) was calculated for all data obtained using SAS statistical analyses (SAS Version 8.2, 2001).

Field inoculations

Pathogenicity trials were conducted on 1-year-old *A. mangium* and *A. crassicarpa* trees in commercial plantations in Indonesia. Trees ranged from 70 to 90 mm in diameter at breast height. The same isolates as those used in the greenhouse inoculations were used to inoculate 20 trees of each of the *Acacia* species for each *Ceratocystis* isolate. An additional 20 trees of each *Acacia* species were inoculated with sterile MEA plugs to serve as controls. For inoculation, a wound was made on the tree stems using a sterilized cork borer (10-mm diameter) and inoculations were carried out by inserting an agar disc of the same size, taken from the edges of actively growing colonies on 2% MEA. These discs were placed into the inoculation points, with the mycelium facing the exposed cambium. The wounds were sealed with wrapping tape after inoculation to avoid contamination and desiccation of the inoculum and wounds.

Six weeks after inoculation, tree diameters at the inoculation points and the lengths of lesions produced on the stems were measured. Reisolations were made by taking a piece of symptomatic tissue from the area associated with the inoculation points for five trees of each isolate and placing these in moist chambers to induce sporulation. Spore masses were then taken from the tips of fruiting structures and plated on 2% MEA to verify the presence of the inoculated fungus. Analysis of variance (ANOVA) was calculated for all data obtained using SAS statistical analyses (SAS Version 8.2, 2001). The entire field experiment was repeated once.

Results

Fungal isolates

Fourteen *Ceratocystis* isolates, resembling *C. moniliformis s.l.*, were obtained from 3 of the 15 *A. mangium* trees wounded in this study. No isolates were obtained from wounded *A. crassicarpa*. Of the 14 isolates obtained, 4 were collected from one tree (CMW 21105–21108), 6 isolates (CMW 21109–21114) were collected from a second tree, and 4 isolates (CMW 21115–21118) were obtained from a third tree. Within 2 weeks, all isolates produced mature perithecia containing hat-shaped ascospores and a *Thielaviopsis* anamorph. At the time of sample collection, staining of the vascular tissue was observed on the trees in the vicinity of the wounds.

PCR amplification, sequencing, and analyses

PCR amplification resulted in fragments of ~500 base pairs (bp) in size for the ITS and βt gene regions; for the EF1-α gene region, amplification resulted in fragments ~300 bp in size. The partition homogeneity test on the three data sets resulted in a P value of 0.02, which is an acceptable level to combine the data (Cunningham 1997; Barker and Lutzoni 2002). Thus, all data sets were combined for further analyses.

The combined data set consisted of 1368 characters including gaps, of which 791 characters were constant, 8 were parsimony uninformative, and 569 were parsimony informative. Twelve most parsimonious trees were obtained after analyses, 1 of which was selected for presentation (Fig. 1). This tree had a length of 840; consistency index (CI) = 0.867, homoplasy index (HI) = 0.133, retention index (RI) = 0.931, and rescaled consistency index (RC) = 0.825.

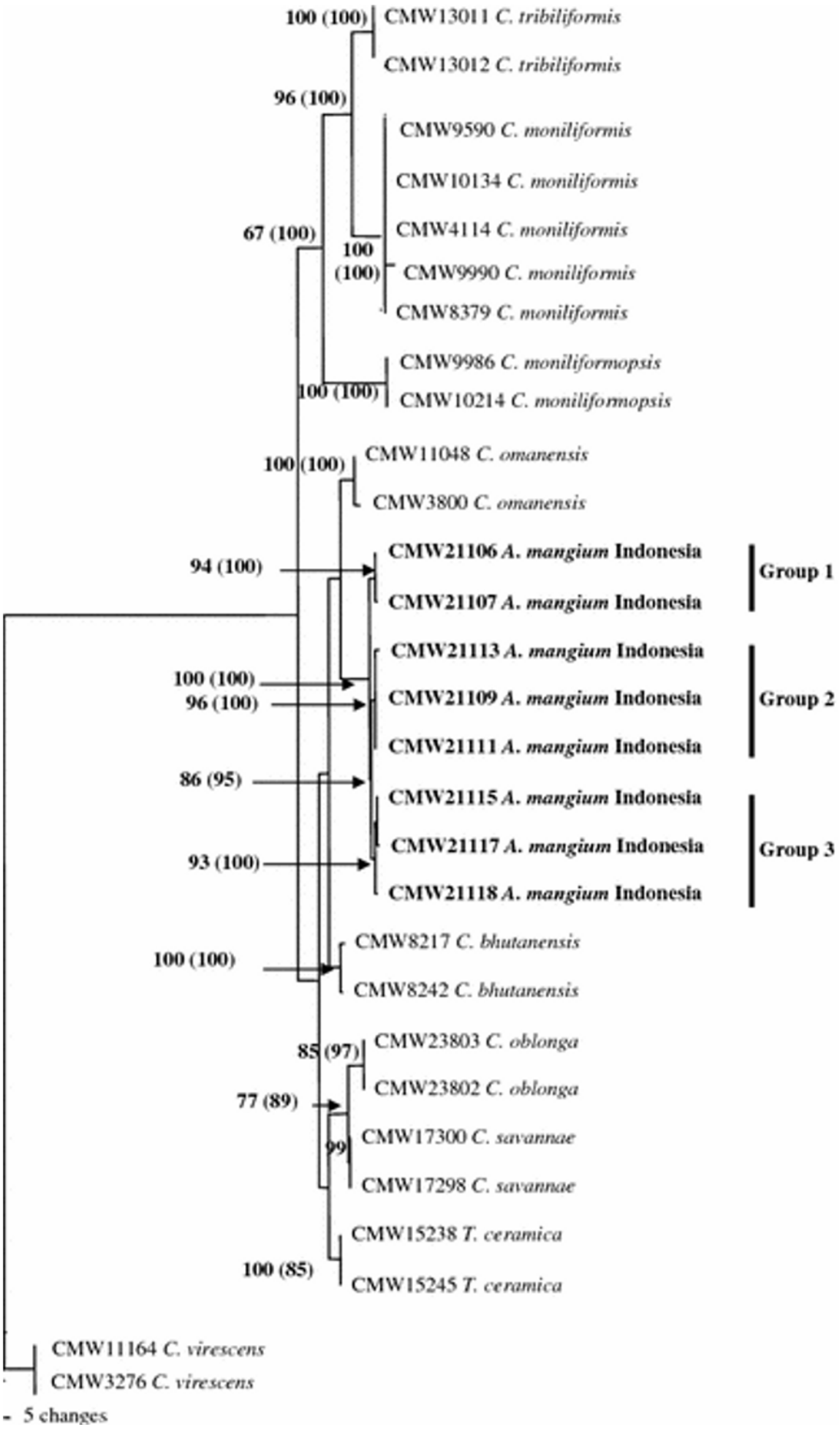
100 (100)
CMW13011 C. tribiliformis
CMW13012 C. tribiliformis
96 (100)
CMW9590 C. moniliformis
CMW10134 C. moniliformis
67 (100)
CMW4114 C. moniliformis
100
(100)
CMW9990 C. moniliformis
CMW8379 C. moniliformis
CMW9986 C. moniliformopsis
100 (100)
CMW10214 C. moniliformopsis
100 (100)
CMW11048 C. omanensis
CMW3800 C. omanensis
94 (100)
CMW21106 A. mangium Indonesia
CMW21107 A. mangium Indonesia
Group 1
CMW21113 A. mangium Indonesia
100 (100)
96 (100)
CMW21109 A. mangium Indonesia
CMW21111 A. mangium Indonesia
Group 2
86 (95)
CMW21115 A. mangium Indonesia
CMW21117 A. mangium Indonesia
93 (100)
CMW21118 A. mangium Indonesia
Group 3
CMW8217 C. bhutanensis
100 (100)
CMW8242 C. bhutanensis
85 (97)
CMW23803 C. oblonga
CMW23802 C. oblonga
77 (89)
CMW17300 C. savannae
99
CMW17298 C. savannae
CMW15238 T. ceramica
100 (85)
CMW15245 T. ceramica
CMW11164 C. virescens
CMW3276 C. virescens
- 5 changes

Fig. 1. Phylogenetic tree based on the combined sequence data of three gene regions (ITS, βt, and EF1-α) showing relationships between *Ceratocystis moniliformis s.l.* isolates used in this study. Isolates shown in bold were isolated from *Acacia mangium* in Indonesia and sequenced as part of this study. The phylogram was obtained using the heuristic search option based on parsimony. Bootstrap values are indicated, and Bayesian values follow in brackets. Two isolates of *C. virescens* were selected as the outgroup.

Model test analysis produced a GTR + G model [Prset statefreqpr = dirichlet (1, 1, 1, 1); Lset nst = 6 rates = gamma] for both the ITS and βt data sets; for EF1-α, it produced a HKY + I model [Prset statefreqpr = dirichlet (1, 1, 1, 1); Lset nst = 2 rates = propinv]. Burn-in was taken at 1000 trees. Bootstrap values followed by Bayesian values in brackets (see Fig. 1) were attached to the branch nodes of the combined datasets. Bayesian values provided strong support to the bootstrap values obtained.

Three distinct groups residing in the *C. moniliformis s.l.* complex were recognized: these were designated group 1 (CMW 21106, 21107), group 2 (CMW 21109, 21111, 21113), and group 3 (CMW 21115, 21117, 21118). All three groups formed well-resolved clades with posterior probability values for the branch nodes of 94% (100%), 96% (100%), and 93% (100%) for group 1, group 2, and group 3, respectively (Fig. 1). All these isolates were phylogenetically most closely related to *C. omanensis* Al-Subhi, M.J. Wingf., M. van Wyk & Deadman. These results indicated that the three groups representing *C. moniliformis s.l.* from *A. mangium* in Riau, Sumatra, represent distinct and undescribed species.

Culture characteristics and morphology

All three *Ceratocystis* groups emerging from the phylogenetic analyses showed similar growth rates and temperature optima. All isolates showed optimum growth at 30°C, with group 1 isolates reaching an average diameter of 67 mm, group 2 isolates reaching an average diameter of 56 mm, and group 3 reaching an average diameter of 63 mm in 2 days at 30°C. For all isolates, growth was reduced at 35, 20, and 15°C. Very slow growth was observed at 10°C, and no growth was found at 4°C.

Cultures representing group 1 had hair brown (17””i) aerial mycelium whereas those in group 2 and group 3 had cream-buff (19’’d) to hair brown (17””i) aerial mycelium. Within 1–2 weeks, both teleomorph and anamorph structures were produced in all cultures. The ascomatal bases were brown, or dark brown to black, in color and globose to subglobose, with ornamentations in the form of conical spines. The ascomatal bases of group 3 isolates were much smaller and lighter in color than those of group 1 and group 2 isolates. All isolates, irrespective of the group to which they belonged, had necks with disc-like bases. They also all had divergent ostiolar hyphae at the tips of the ascomatal necks, from which hat-shaped ascospores were produced. The ostiolar hyphae of group 3 isolates were shorter than those of group 1 and group 2 isolates. Both primary and secondary phialides were present in the isolates, except for group 3, which only has primary phialides. Primary phialides produced cylindrical conidia, either in chains or singly, whereas secondary phialides produced barrel-shaped conidia, also in chains or singly. None of the isolates representing any of the three groups produced chlamydospores (Table 1).

Table 1. Morphological characteristics of Indonesian *Ceratocystis* species collected in this study compared to *C. omanensis* (Al-Subhi *et al*. 2006).

	C. inquinans CMW 21106	*C. sumatrana* CMW 21113	*C. microbasis* CMW 21117	*C. omanensis* CMW 11048
Cultures				
Color	Hair brown (17''''i)	Cream-buff (19''d-) to hair brown (17''''i-)	Cream-buff (19''d-) to hair brown (17''''i)	Wood brown (17'')
Growth rate	Opt. at 25–30°C Good growth at 35°C, no growth at 4°C	Opt. at 25–30°C Good growth at 35°C, no growth at 4°C	Opt. at 25–30°C Good growth at 35°C, no growth at 4°C	Opt. at 30–35°C No growth at 4°C
Hyphae	Smooth	Smooth	Smooth	Smooth
Ascomatal base				
Color	Dark brown to black	Dark brown to black	Light brown or brown	Dark brown to black
Length	(116–)149–205(–236) µm	(148–)168–218(–293) µm	(65–)82–122(–162) µm	(154–)206–254(–279) µm
Width	(130–)161–217(–270) µm	(158–)187–235(–296) µm	(82–)100–146(–185) µm	NA
Shape	Globose to subglobose	Globose to subglobose	Globose to subglobose	Globose
Ornamentation	Spines with hyphal hairs	Spines with hyphal hairs	Spines with hyphal hairs	Spines with hyphal hairs
Spines				
Color	Dark brown to black	Dark brown to black	Dark brown to black	Dark brown to black
Length	(18–)20–30(–37) µm	(5–)6–12(–18) µm	(4–)6–12(–19) µm	(4–)9–19(–26) µm
Ascomatal necks				
Color	Dark brown to black	Dark brown to black	Light brown to brown	Dark brown to black
Disc form at base	Present	Present	Present	Present
Length	(347–)393–575(–687) µm	(323–)390–574(–687) µm	(185–)301–499(–574) µm	(385–)443–819(–1097) µm
Width (base)	(66–)83–119(–141) µm	(67–)81–103(–128) µm	(41–)57–81(–95) µm	(30–)43–57(–64) µm
Width (tip)	(14–)16–20(–23) µm	(12–)15–23(–32) µm	(8–)10–14(–16) µm	(14–)16–22(–26) µm
Ostiolar hyphae				
Orientation	Divergent	Divergent	Divergent	Divergent
Length	(20–)24–34(–38) µm	(21–)24–32(–35) µm	(9–)14–22(–25) µm	(10–)18–36(–50) µm
Asci	Not seen	Not seen	Not seen	Not seen
Ascospores				
Color	Hyaline	Hyaline	Hyaline	Hyaline
Shape (side view)	Cucullate	Cucullate	Cucullate	Hat-shaped
Measurements				
With sheath	5–7 × 2–4 µm	5–7 × 3–4 µm	5–7 × 2–4 µm	5–7 × 2–4 µm
Without sheath	4–6 × 2–4 µm	4–6 × 3–4 µm	4–6 × 2–4 µm	4–6 × 2–4 µm
Primary conidiophores				
Length	(18–)22–32(–44) µm	(17–)21–31(–38) µm	(18–)22–36(–41) µm	(19–)22–36(–56) µm
Width (base)	3–5 µm	2–4(–5) µm	2–4(–5) µm	(1–)2–4(–5) µm
Width (middle)	2–4 µm	(2–)2–4(–5) µm	2–4 µm	NA
Width (tip)	1–3 µm	1–3 µm	1–2 µm	1–3 µm
Shape	Phialides	Phialides	Phialides	Phialides
Secondary conidiophores				
Length	(19–)23–35(–43) µm	(23–)23–37(–47) µm	None	NA
Width (base)	2–4(–5) µm	2–4 µm	None	NA
Width (tip)	2–4(–5) µm	3–5 µm	None	NA
Shape	Phialides	Phialides	None	NA
Primary conidia				
Shape	Cylindrical	Cylindrical	Cylindrical	Cylindrical
Length	(5–)6–8(–11) µm	5–(–8) µm	(3–)4–6(–11) µm	6–8(–9) µm
Width	(2–)3–5(–7) µm	2–4(–5) µm	1–3 µm	2–3 µm
Secondary conidia				
Shape	Barrel-shaped	Barrel-shaped	None	Barrel-shaped
Length	(4–)5–7(–8) µm	(4–)5–7(–8) µm	None	(5–)6–8(–10) µm
Width	1–3 µm	1–3 µm	None	3–5 µm
Chlamydospores	Absent	Absent	Absent	Absent

Opt. optimal

Pathogenicity tests

Greenhouse inoculations

All isolates inoculated in this study produced lesions on *A. crassicarpa* and *A. mangium* seedlings within 10 days of inoculation. The lesion lengths ranged from 21 to 67 mm on *A. crassicarpa* and 27–66 mm on *A. mangium*. Isolate CMW 21106 (group 1) consistently produced significantly longer lesions when compared with the controls on *A. crassicarpa* and *A. mangium*. Isolates residing in group 2 and group 3 also produced longer lesions than the controls; however, only one isolate of each group, namely, CMW 21113 (group 2) and CMW 21115 (group 3), produced lesions that were significantly different from the controls [$P = 0.05$; $r^2 = 0.31$; coefficient of variation (CV) = 94.1; root mean square error (MSE) = 35.5]. Reisolations from the lesions consistently yielded *C. moniliformis s.l.* isolates, and these were never found associated with the control inoculations.

Field inoculations

All *Ceratocystis* isolates produced lesions on *A. mangium* and *A. crassicarpa* trees within 6 weeks of inoculation (Fig. 2). Lesion lengths ranged from 90 to 170 mm on *A. crassicarpa* and 140–250 mm on *A. mangium* trees (Fig. 3). Lesion lengths were significantly smaller on *A. crassicarpa* trees when compared to those on *A. mangium*. All isolates, other than CMW 21113, when inoculated on *A. crassicarpa* resulted in lesions that differed significantly from the controls ($P = 0.05$; $r^2 = 0.37$; CV = 42.5; root MSE = 6.41). Reisolations from the lesions failed to yield isolates of the inoculated fungi. Trees diameters ranged from 70 to 90 mm and had no impact on lesion length ($P = 0.05$; $r^2 = 0.40$; CV = 14.34; root MSE = 1.24).

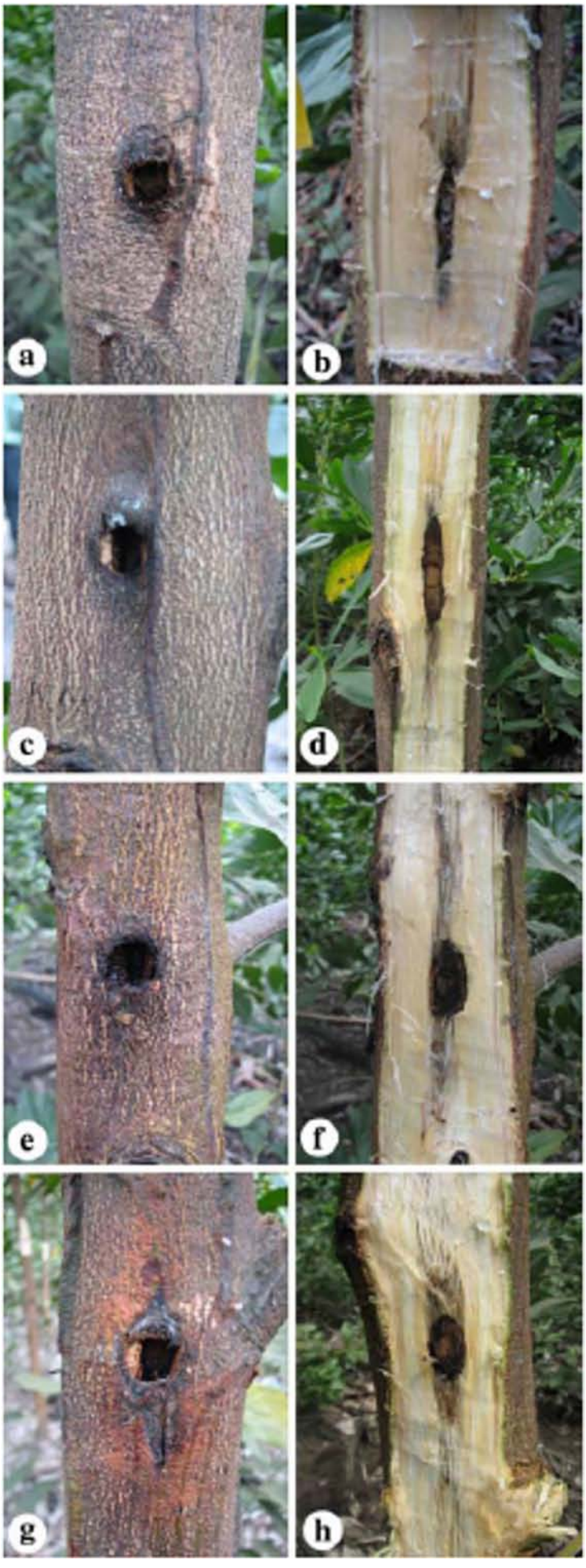
a
b
c
d
e
f
g
h

Fig. 2. Lesions on the bark and the cambium of 1-year-old *Acacia mangium* trees in Indonesia, 6 weeks after inoculation with *Ceratocystis inquinans* (CMW 21106), *C. sumatrana* (CMW 21113), and *C. microbasis* (CMW 21117). a. External/bark lesion for control inoculation. b. Internal/xylem lesion for control inoculation. c. External/bark lesion caused by *C. inquinans*. d. Internal/xylem lesion caused by *C. inquinans*. e. External/bark lesion caused by *C. sumatrana*. f. Internal/xylem lesion caused by *C. sumatrana*. g. External/bark lesion caused by C. microbasis. h. Internal/xylem lesion caused by *C. microbasis*.

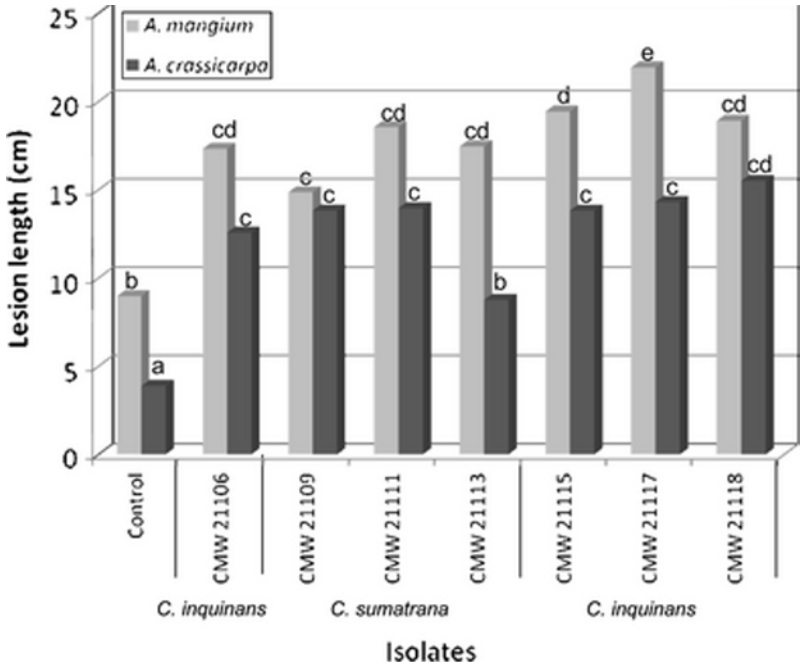

Fig. 3. Results of inoculations using *Ceratocystis inquinans* (CMW 21106), *C. sumatrana* (CMW 21109, CMW 21111, CMW 21113), and *C. microbasis* (CMW 21115, CMW 21117, CMW 21118) on 1-year-old *Acacia mangium* and *A. crassicarpa* trees in an Indonesian plantation, 6 weeks after inoculation. Bars on the graph indicated with the same letter are not significantly different from each other (P = 0.05).

Taxonomy

Results of this study have shown that the three *Ceratocystis* spp. belonging to the *C. moniliformis s.l.* species complex isolated from wounded *A. mangium* in Indonesia represent previously undescribed taxa. They are consequently described as follows.

Ceratocystis inquinans Tarigan, M. van Wyk & M.J. Wingf., sp. nov. Fig. 4.

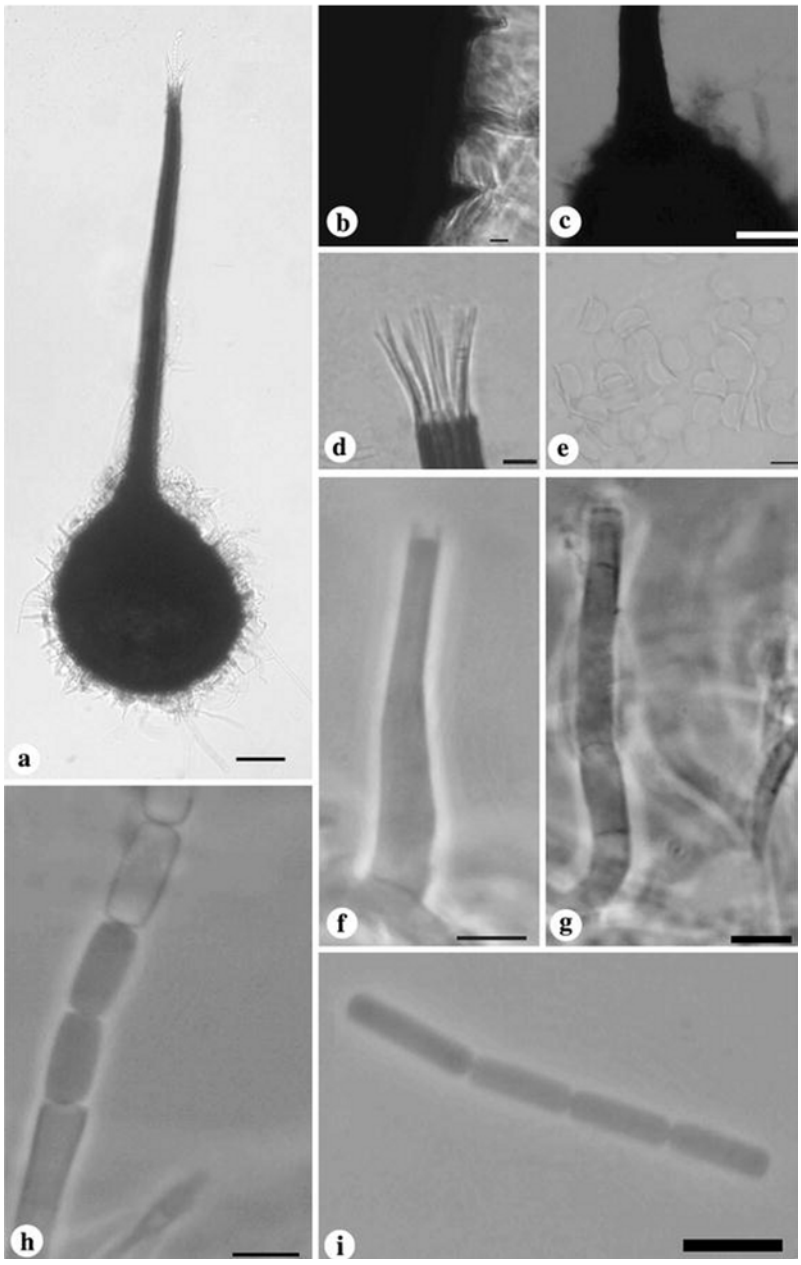

Fig. 4. Morphological characteristics of *Ceratocystis inquinans* (CMW 21106). a. Globose ascoma with long neck. b. Ascomatal base with conical spines and hyphal ornamentation. c. Ascomatal neck with disc-shaped attachment at base. d. Divergent ostiolar hyphae. e. Hat-shaped ascospores in side view. f. Primary phialide. g. Secondary phialide. h. Barrel-shaped conidia. i. Cylindrical conidia. Bars a, c, e 50 µm; d 10 µm; b, f–i 5 µm.

MycoBank no.: MB 508828

Etymology: The name refers to the ability of this fungus to cause stain. Name derived from the Latin inquinans meaning "staining."

Anamorph: *Thielaviopsis* sp.

Coloniae crinobrunneae. Mycelium aerium. Hyphae laeves, septis non constrictis. Bases ascomatum atrobrunneae vel nigrae, globosae vel subglobosae (116–)149–205(–236) µm longae, (130–)161–217(–270) µm latae, spinis hyphisque ornatae; spinae atrobrunneae vel nigrae (18–)20–30(–37) µm longae. Colla ascomatum atrobrunnea vel nigra, apicem versus pallentia (347–)393–575(–687) µm longa, basi (66–)83–119(–141) µm, apice (14–)16–20(–23) µm lata, basi discoidea. Hyphae

ostiolares divergentes, hyalinae, (20–)24–34(–38) µm longae. Asci non visi. Ascosporae in massis fulvo-flavescentibus mucosis in apicibus collorum ascomatum. Ascosporae lateraliter visae cucullatae hyalinae, non septatae, vaginis inclusae, 5–7 × 2–4 µm cum vagina, 4–6 × 2–4 µm sine illa.

Anamorpha *Thielaviopsis*: Conidiophorae primariae in mycelio singulae, hyalinae, basi incrassatae, apicem versus angustatae, (18–)22–32(–44) µm longae, basi 3–5 µm, medio 2–4 µm, apice 1–3 µm latae. Conidiophorae secondariae in mycelio singulae hyalinae (19–)23–35(–43) µm longae, basi apiceque (2–)2–4(–5) µm latae. Conidia biformia: primaria hyalina, cylindrica, non septata, (5–)6–8(–11) × (2–)3–5(–7) µm; secondaria hyalina, doliiformia, (4–)5–7(–8) × 1–3 µm.

Colonies hair brown (17””i) in color. Mycelium aerial. Optimal temperature for growth 25–30°C, no growth at 4°C, but growth observed at 35°C. Hyphae smooth, not constricted at septa. Ascomatal bases dark brown to black, globose to subglobose, (116–)149–205(–236) µm high, (130–)161–217(–270) µm wide, ornamented with spines and hyphae, spines dark brown to black, (18–)20–30(–37) µm long. Ascomatal necks dark brown to black becoming lighter toward the apices, (347–)393–575(–687) µm long, (66–)83–119(–141) µm wide at the base, (14–)16–20(–23) µm wide at the apex, with a disc-like base. Ostiolar hyphae divergent, hyaline, (20–)24–34(–38) µm long. Asci not observed. Ascospores accumulating in buff-yellow (19d) mucilaginous masses at the apices of the ascomatal necks. Ascospores cucullate (hat-shaped) in side view, aseptate, hyaline, invested in sheath, 5–7 × 2–4 µm with sheath, 4–6 × 2–4 µm without sheath.

Thielaviopsis anamorph: Primary conidiophores occurring singly on mycelium, hyaline, swollen at the bases, tapering toward the apices, (18–)22–32(–44) µm long, 3–5 µm wide at the bases, 2–4 µm wide at the middle, 1–3 µm wide at the apices. Secondary conidiophores occurring singly on mycelium, hyaline, (19–)23–35(–43) µm long, 2–4(–5) µm wide at the bases, 2–4(–5) µm wide at the apices. Conidia of two types: primary conidia hyaline, aseptate, cylindrical (5–)6–8(–11) × (2–)3–5(–7) µm; and secondary conidia hyaline, aseptate, barrel-shaped (4–)5–7(–8) × 1–3 µm. Chlamydospores absent.

Habitat: *Acacia mangium*.

Known distribution: Sumatra, Indonesia.

Holotype: Indonesia. Sumatra. Isolated from *Acacia mangium*, 2005, M. Tarigan, (PREM 59866); living culture: CMW 21106 (CBS 124388).

Additional specimen examined: Indonesia, Sumatra. Isolated from *Acacia mangium*, 2005, M. Tarigan, (PREM 59867); living culture: CMW 21107 (CBS 124009).

Ceratocystis sumatrana Tarigan, M. van Wyk & M.J. Wingf., sp. nov. Fig. 5.

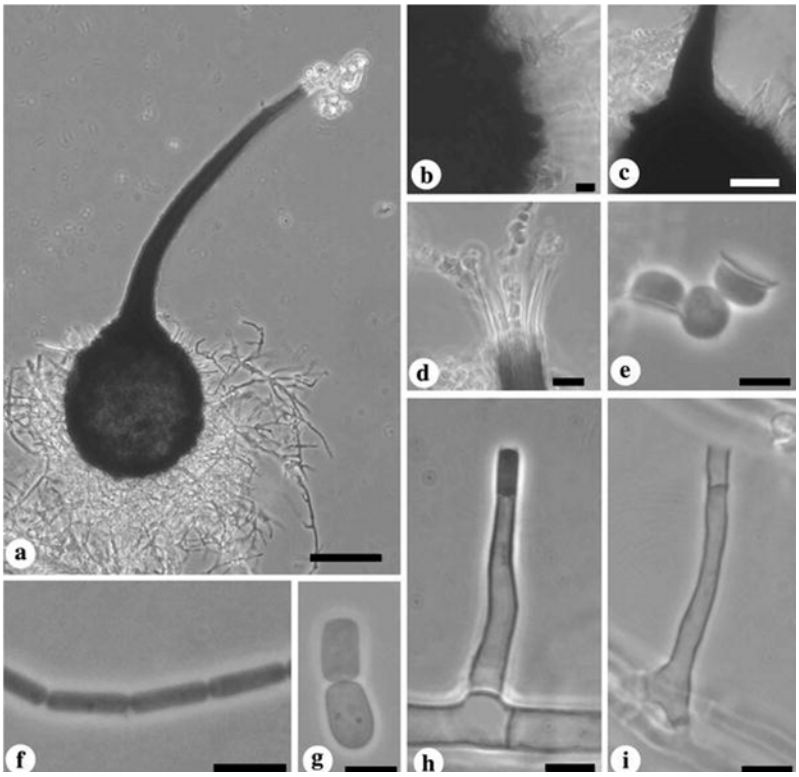

Fig. 5. Morphological characteristics of *Ceratocystis sumatrana* (CMW 21113). a. Globose ascoma with long neck. b. Ascomatal base with conical spines and hyphal ornamentation. c. Ascomatal neck with disc-shaped attachment at base. d. Divergent ostiolar hyphae. e. Hat-shaped ascospores in side view. f. Cylindrical conidia. g. Barrel-shaped conidia. h. Primary phialide. i. Secondary phialide. Bars a 100 µm; c, d 10 µm; b, e–i 5 µm.

MycoBank no.: MB 508829

Etymology: Name refers to the Indonesian island of Sumatra where this species was discovered.

Anamorph: *Thielaviopsis* sp.

Coloniae cremeo-fulvae vel crinobrunneae. Mycelium aerium. Hyphae laeves, septis non constrictis. Bases ascomatum atrobrunneae vel nigrae, globosae vel subglobosae (148–)168–218(–293) µm longae, (158–)187–235(–296) µm latae, spinis hyphisque ornatae; spinae atrobrunneae vel nigrae (5–)6–12(–18) µm longae. Colla ascomatum atrobrunnea vel nigra, apicem versus pallentia (323–)390–574(–687) µm longa, basi (67–)81–103(–128) µm, apice (12–)15–23(–32) µm lata, basi discoidea. Hyphae ostiolares divergentes hyalinae, (21–)24–32(–35) µm longae. Asci non visi. Ascosporae in massis fulvo-flavescentibus mucosis in apicibus collorum ascomatum. Ascosporae lateraliter visae cucullatae hyalinae, non septatae, vaginis inclusae, 5–7 × 3–4 µm cum vagina, 4–6 × 3–4 µm sine illa.

Anamorpha *Thielaviopsis*: Conidiophorae primariae in mycelio singulae, hyalinae, basi incrassatae, apicem versus angustatae, (17–)21–31(–38) µm longae, basi 2–4(–5) µm, medio 2–4(–5) µm, apice 1–3 µm latae. Conidiophorae secondariae in mycelio singulae, hyalinae, 23–37(–47) µm longae, basi 2–4 µm, apice 3–5 µm latae. Conidia biformia: primaria hyalinae, cylindrica, non septata, 5–7(–8) × 2–4(–5) µm; secondaria hyalina doliiformia, non septata, (4–)5–7(–8) × 1–3 µm.

Colonies cream-buff (19’’d) to hair brown (17’’’’i) in color. Mycelium aerial. Optimal temperature for growth 25–30°C, no growth at 4°C, but growth observed at 35°C. Hyphae smooth, not constricted at septa. Ascomatal bases dark brown to black, globose to subglobose, (148–)168–218(–

293) µm high, (158–)187–235(–296) µm wide, ornamented with spines and hyphae, spines dark brown to black, (5–)6–12(–18) µm long. Ascomatal necks dark brown to black and becoming lighter toward the apices, (323–)390–574(–687) µm long, (67–)81–103(–128) µm wide at the bases, (12–)15–23(–32) µm wide at the apices, with a disc-like base. Ostiolar hyphae divergent, hyaline, (21–)24–32(–35) µm long. Asci not observed. Ascospores accumulating in buff-yellow (19d) mucilaginous masses at the apices of the ascomatal necks. Ascospores cucullate (hat-shaped) in side view, aseptate, hyaline, invested in sheath, 5–7 × 3–4 µm with sheath, 4–6 × 3–4 µm without sheath.

Thielaviopsis anamorph: primary conidiophores occurring singly on mycelium, hyaline, swollen at the bases, tapering toward the apices, (17–)21–31(–38) µm long, 2–4(–5) µm wide at the bases, 2–4(–5) µm wide at the middle, 1–3 µm wide at the apices. Secondary conidiophores occurring singly on mycelium, hyaline, 23–37(–47) µm long, 2–4 µm wide at the bases, 3–5 µm wide at the apices. Conidia of two types: primary conidia hyaline, aseptate, cylindrical 5–7(–8) × 2–4(–5) µm, secondary conidia hyaline, aseptate, barrel-shaped (4–)5–7(–8) × 1–3 µm. Chlamydospores absent.

Habitat: *Acacia mangium*.

Known distribution: Sumatra, Indonesia.

Holotype: Indonesia. Sumatra. *Acacia mangium*, 2005, M. Tarigan, (PREM 59870); living culture: CMW 21113 (CBS 124010).

Additional specimens examined: Indonesia, Sumatra. *Acacia mangium*, 2005, M. Tarigan, (PREM 59868); living culture: CMW 21109 (CBS 124011). Indonesia, Sumatra. *Acacia mangium*, 2005, M. Tarigan, (PREM 59869); living culture: CMW 21111 (CBS 124012).

Ceratocystis microbasis Tarigan, M. van Wyk & M.J. Wingf., sp. nov. Fig. 6.

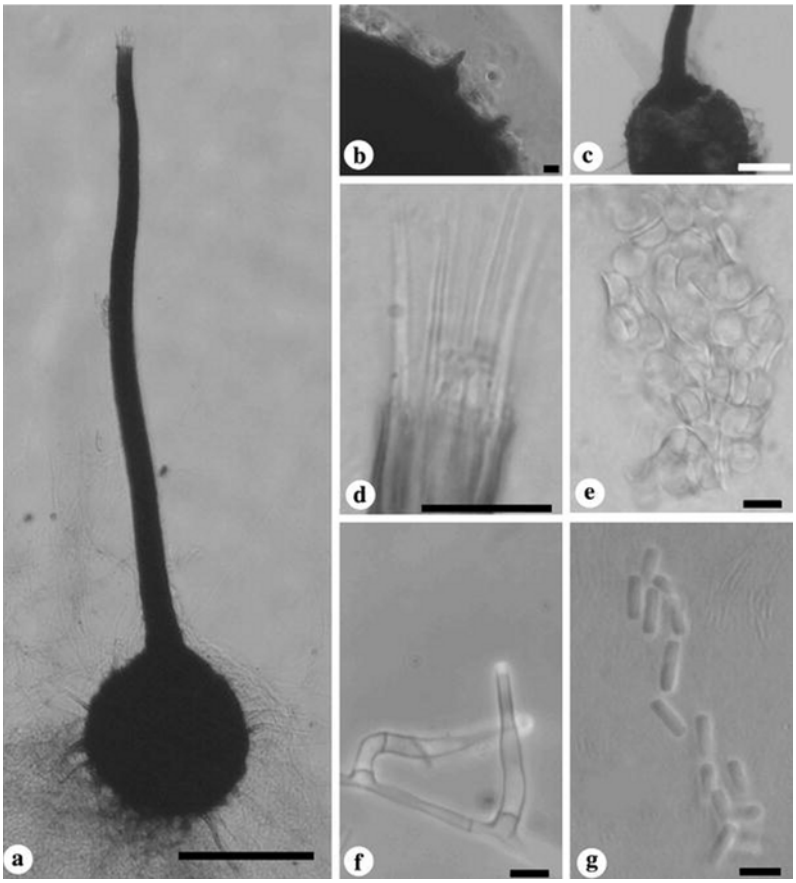

Fig. 6. Morphological characteristics of *Ceratocystis microbasis* (CMW 21117). a. Globose ascoma with long neck. b. Ascomatal base with conical spines and hyphal ornamentation. c. Ascomatal neck with disc-shaped attachment at base. d Divergent ostiolar hyphae e. Hat-shaped ascospores in side view. f. Conidiophore/phialide. g. Cylindrical conidia. Bars a 100 µm; c 50 µm; d 10 µm; b, e–g 5 µm.

MycoBank no.: MB 508945

Etymology: the name refers to the small ascomatal bases of this fungus.

Anamorph: *Thielaviopsis* sp.

Coloniae cremeo-fulvae vel crinobrunneae. Mycelium aerium. Hyphae laeves, septis non constrictis. Bases ascomatum pallide brunneae, atrobrunneae vel nigrae, globosae vel subglobosae (65–)82–122(–162) µm longae, (82–)100–146(–185) µm latae, spinis hyphisque ornatae; spinae atrobrunneae vel nigrae (4–)6–12(–19) µm longae. Colla ascomatum atrobrunnea vel nigra, apicem versus pallentia (185–)301–499(–574) µm longa, basi (41–)57–81(–95) µm, apice (8–)10–14(–16) µm lata, basi discoidea. Hyphae ostiolares divergentes hyalinae, (9–)14–22(–25) µm longae. Asci non visi. Ascosporae in massis fulvo-flavescentibus mucosis in apicibus collorum ascomatum. Ascosporae lateraliter visae cucullatae hyalinae, non septatae, vaginis inclusae, 5–7 × 2–4 µm cum vagina, 4–6 × 2–4 µm sine illa.

Anamorpha *Thielaviopsis*: Conidiophorae in mycelio singulae, hyalinae, basi incrassatae, apicem versus angustatae, (18–)22–36(–41) µm longae, basi 2–4(–5) µm, medio 2–4 µm, apice 1–2 µm latae. Conidia hyalina, cylindrica, non septata, (3–)4–6(–11) × 1–3 µm.

Colonies cream-buff (19’’d) to hair brown (17’’’’i) in color. Mycelium aerial. Optimal temperature for growth 25–30°C, no growth at 4°C, but growth observed at 35°C. Hyphae smooth, not constricted at septa. Ascomatal bases light brown or dark brown to black, globose to sub globose,

(65–)82–122(–162) µm high, (82–)100–146(–185) µm wide, ornamented with spines and hyphae, spines dark brown to black, (4–)6–12(–19) µm long. Ascomatal necks dark brown to black and becoming lighter toward the apices, (185–)301–499(–574) µm long, (41–)57–81(–95) µm wide at the bases, (8–)10–14(–16) µm wide at the apices, with disc-like bases. Ostiolar hyphae divergent, hyaline, (9–)14–22(–25) µm long. Asci not observed. Ascospores accumulating in buff-yellow (19d) mucilaginous masses at the apices of the ascomatal necks. Ascospores cucullate (hat-shaped) in side view, aseptate, hyaline, invested in sheath, 5–7 × 2–4 µm with sheath, 4–6 × 2–4 µm without sheath.

Thielaviopsis anamorph: primary conidiophores occurring singly on mycelium, hyaline, swollen at the bases, tapering toward the apices, (18–)22–36(–41) µm long, 2–4(–5) µm wide at the bases, 2–4 µm wide at the middle, 1–2 µm wide at the apices. Secondary conidiophores, absent. Primary conidia hyaline, aseptate, cylindrical (3–)4–6(–11) × 1–3 µm. Secondary conidia, absent. Chlamydospores absent.

Habitat: *Acacia mangium*.

Known distribution: Sumatra, Indonesia.

Holotype: Indonesia. Sumatra. *Acacia mangium*. 2005, M. Tarigan, (PREM 59872); living culture: CMW 21117 (CBS 124017).

Additional specimens examined: Indonesia. Sumatra. *Acacia mangium*. 2005, M. Tarigan, (PREM 59871); living culture: CMW 21115 (CBS 124015). Indonesia. Sumatra. *Acacia mangium*. 2005, M. Tarigan, (PREM 59873), living culture: CMW 21118.

Discussion

In this study, three previously undescribed *Ceratocystis* spp. belonging to the *C. moniliformis s.l.* species complex were found associated with artificially induced wounds on *A. mangium* in Indonesia. These three fungi were recognized as undescribed taxa based on DNA sequence comparisons. Their unique nature was also confirmed based on morphology. Furthermore, we were able to show that these fungi are able to infect wounded *A. mangium* and *A. crassicarpa* trees in Indonesia. However, the inability to reisolate them from inoculated trees and the relatively limited lesions produced in pathogenicity tests suggests that they are not important pathogens.

The *Ceratocystis* spp. recognized in this study are all morphologically similar to species in the *C. moniliformis* species complex. In this respect, they all have long necks from which hat-shaped ascospores exude. More importantly, they all have globose bases ornamented with spines and their necks attach to the ascomatal bases with a distinct plate-like structure that easily becomes detached from the bases (Davidson 1935). Until recently, all species of *Ceratocystis* with these characteristics were broadly grouped within *C. moniliformis*. However, with the advent of DNA sequence comparisons, *C. moniliformis* has come to be recognized as an aggregate of several cryptic species for which some have been provided with names (Yuan and Mohammed 2002; Van Wyk *et al*. 2006b; Heath *et al*. 2009).

The three new species of *Ceratocystis* from Indonesia are morphologically very similar to each other. They all produce both teleomorph and anamorph structures in culture rapidly, typically within 1 week. However, the ability to produce these structures diminishes over time; this appears to be a common feature of species in the *C. moniliformis* complex (Van Wyk *et al*. 2004).

The three species described in this study can be relatively easily distinguished from each other (Table 1). *Ceratocystis inquinans* has hair brown aerial mycelium, which differs from *C. sumatrana*

and *C. microbasis*, both of which have cream-buff to hair brown aerial mycelium. *Ceratocystis inquinans* and *C. sumatrana* have ascomatal bases, ascomatal necks and spines that are dark brown to black, whereas the ascomata of *C. microbasis* are lighter in color. *Ceratocyctis microbasis* can, furthermore, be distinguished from the other two species by the much smaller size of its teleomorph structures. The anamorph structures of the three new species are practically indistinguishable; however both *C. inquinans* and *C. sumatrana* have secondary conidiophores whereas *C. microbasis* has no secondary conidiophores.

Phylogenetically, the species from Indonesia are most closely related to *C. omanensis*, *C. savannae* Kamgan & Jol. Roux, and *C. bhutanensis* M. van Wyk, M.J. Wingf. & Kirisits (Table 2). This relationship is also supported by the similarity of some morphological characteristics of these new species with *C. omanensis* (Al-Subhi *et al*. 2006), *C. savannae* (Kamgan *et al*. 2008), *C. bhutanensis* (Van Wyk *et al*. 2004), *C. oblonga* Heath & Jol. Roux (Heath *et al*. 2009), and *T. ceramica* Heath & Jol. Roux (Heath *et al*. 2009). However, clear differences exist between these species. *Ceratocystis omanensis* and *C. savannae* have much longer ascomatal necks compared to Indonesian species and *C. bhutanensis* (Van Wyk *et al*. 2004; Al-Subhi *et al*. 2006; Kamgan *et al*. 2008). Species from Indonesia have hair brown or cream-buff to hair brown colonies, whereas *C. omanensis* has wood brown colonies, *C. savannae* has smoke grey colonies, and *C. bhutanensis* has cream-buff to dark olive to black colonies (Van Wyk *et al*. 2004; Al-Subhi *et al*. 2006; Kamgan *et al*. 2008). The secondary conidia of *C. oblonga* have a very characteristic oblong shape with truncated ends that distinguishes it from all other species in the *C. moniliformis* complex, and it has oblong ascomatal bases (Heath et al. 2009). Ascomata of *T. ceramica* have not been seen (Heath *et al*. 2009) and distinguishing it from the newly described species must rely largely on phylogenetic inference, although the species described in this study produce ascomata readily, and this feature might also be considered diagnostic.

Table 2. Isolates of *Ceratocystis* (*C.*) included in the phylogenetic analyses.

Species	Isolate no.	GenBank accession no.	Host	Geographic origin	Collector
C. bhutanensis	CMW 8217	AY528957	*Picea spinulosa*	Bhutan	T Kirisits and DB Chhetri
	CBS 114289	AY528962			
	PREM 57807	AY528952			
C. bhutanensis	CMW 8242	AY528956	*P. spinulosa*	Bhutan	T Kirisits and DB Chhetri
	CBS 112907	AY528961			
	PREM 57809	AY528951			
C. inquinans [a]	**CMW 21106**	**EU588587**	***Acacia mangium***	**Indonesia**	**M Tarigan**
		EU588666			
		EU588674			
C. inquinans	**CMW 21107**	**EU588588**	***A. mangium***	**Indonesia**	**M Tarigan**
	CBS 124009	**EU588667**			
		EU588675			
C. microbasis	**CMW 21115**	**EU588592**	***A. mangium***	**Indonesia**	**M Tarigan**
	CBS 124015	**EU588671**			
		EU588679			
C. microbasis	**CMW 21117**	**EU588593**	***A. mangium***	**Indonesia**	**M Tarigan**
	CBS 124013	**EU588672**			
		EU588680			
C. microbasis	**CMW 21118**	**EU588594**	***A. mangium***	**Indonesia**	**M Tarigan**
		EU588673			
		EU588681			
C. monilifornis	CMW 4114	AY528997	*Schizolobium parahybum*	Ecuador	MJ Wingfield
	CBS 118181	AY528986			
		AY529007			
C. monilifornis	CMW 9590	AY431101	*Eucalyptus grandis*	South Africa	J Roux
	CBS 116452	AY528985			
		AY529006			
C. monilifornopsis	CMW 9986	AY528998	*E. obliqua*	Australia	ZQ Yuan
	CBS 109441	AY528987			
		AY529008			
C. monilifornopsis	CMW 10214	AY528999	*E. sieberi*	Australia	MJ Dudzinski
	CBS 115792	AY528988			
		AY529009			
C. oblonga	CMW 23802	EU245020	*Acacia mensii*	*South Africa*	RN Heath
	CBS 122820	EU244992			
		EU244952			
C. oblonga	CMW 23803	EU245019	*A. mensii*	*South Africa*	RN Heath
	CBS 122291	EU244991			
		EU244951			
C. omanensis	CMW 11048	DQ074742	*Mangifera indica*	Oman	AO Al Adawi
	CBS 115780	DQ074732			
	PREM 57815	DQ074737			
C. omanensis	CMW 11046	DQ074739	*M. indica*	Oman	AO Al Adawi
	CBS 118112	DQ074729			
	PREM 57814	DQ074734			
C. savannae	CMW 17300	EF408551	*Acacia nigrescens*	South Africa	GN Kamgan & J Roux
	PREM 59423	EF408565			
		EF408572			
C. savannae	CMW 17298	EF408551	*Terminalia sericea*	South Africa	GN Kamgan & J Roux
	CBS 121022	EF408551			
		EF408551			
C. sumatrana	**CMW 21109**	**EU588589**	***A. mangium***	**Indonesia**	**M Tarigan**
	CBS 124011	**EU588668**			
		EU588676			
C. sumatrana	**CMW 21111**	**EU588590**	***A. mangium***	**Indonesia**	**M Tarigan**
	CBS 124012	**EU588669**			
		EU588677			
C. sumatrana	**CMW 21113**	**EU588591**	***A. mangium***	**Indonesia**	**M Tarigan**
	CBS 124010	**EU588670**			
		EU588678			
C. tribilformis	CMW 13012	AY529002	*Pinus merkusii*	Indonesia	MJ Wingfield
	CBS 118242	AY528992			
	PREM 57826	AY529013			
C. tribilformis	CMW 13013	AY529003	*P. merkusii*	Indonesia	MJ Wingfield
	PREM 57827	AY528993			
	CBS 115866	AY529014			
C.virescens	CMW 3276	DQ061281	*Quercus* sp.	USA	T Hinds
	CBS 123216	AY528990			
		AY529011			
C. virescens	CMW 11164	DQ520639	*Fagus americanum*	USA	D Houston
	CBS 123166	EF070441			
		EF070413			
T. ceramica	CMW 15245	EU245022	*E. grandis*	Malawi	RN Heath
	CBS 122299	EU244994			
		EU244926			
T. ceramica	CMW 15248	EU245024	*E. grandis*	Malawi	RN Heath
	CBS 122300	EU244996			
		EU244928			

[a] Sequences in boldface are the new species described in this article, and were the only ones sequenced in this study; the rest of the sequences were obtained from previous studies

The *Ceratocystis* spp. described in this study all have temperature optima at 30°C, which is consistent with the area in which they are found, where temperatures range between 25° and 30°C throughout the year. All isolates also grew well at 35°C, but they showed substantially reduced growth at temperatures below 20°C and failed to grow at 4°C. These growth trends are similar to those reported for *C. omanensis* and *C. savannae* (Al-Subhi *et al*. 2006; Kamgan *et al*. 2008), other species originating from areas with warm climates. In contrast, they differ markedly from *C. bhutanensis* (Van Wyk *et al*. 2004), *C. tribiliformis* (Van Wyk et al. 2006b), *C. moniliformis* (Hedgcock 1906), C. moniliformopsis (Yuan and Mohammed 2002), *C. oblonga* (Heath *et al*. 2009), and *T. ceramica* (Heath *et al*. 2009), which have lower temperature optima for growth and most of which originate from areas with cooler climates.

The three new *Ceratocystis* spp. from wounds on *A. mangium* were able to cause relatively short lesions on inoculated trees that were significantly different from the controls. In the field inoculations, the lesions produced on *A. mangium* trees were longer than those on *A. crassicarpa*, which is consistent with the fact that *A. mangium* is sensitive to wounding (Schmitt *et al*. 1995). However, infections appeared to be short lived in the stems of trees, and we were not able to reisolate them from lesions. This result, together with the fact that no signs of disease were observed in either the greenhouse and field inoculations or on the trees from which the fungi were isolated, suggests that they are not significant pathogens. This finding is also consistent with what has been observed for other members of the *C. moniliformis* species complex (Davidson 1935; Bakshi 1951; Hunt 1956; Al-Subhi *et al*. 2006).

This study resulted in the description of three previously unknown *Ceratocystis* spp. from a limited geographic area in Indonesia. These results, and the fact that three new species were isolated from three trees with one species per tree, clearly emphasize the general lack of information on fungal diversity in general, even in relatively well studied environments.

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