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RESEARCH ARTICLE

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Ceratocystis larium sp. nov., a new species from *Styrax benzoin* wounds associated with incense harvesting in Indonesia

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Key words

Ophiostomatoid fungi
phylogenetic inference
vascular staining

Abstract *Styrax benzoin* trees, native to the island Sumatra, Indonesia are wounded to produce resin that is collected and burned as incense. These wounds on trees commonly develop into expanding cankers that lead to tree death. The aim of this study was to consider whether Ophiostomatoid fungi, typically associated with wounds on trees might be associated with resin harvesting on *S. benzoin*. Samples were collected from the edges of artificially induced wounds, and particularly where cankers and staining of the vascular tissue was evident. Tissue samples were incubated in moist chambers and carrot baiting was also used to detect the presence of *Ceratocystis* spp. Fruiting structures with morphology typical of species in the *C. fimbriata* s.l. species complex and species in the anamorph genus *Thielaviopsis* were found, on both the incubated wood and the carrot baits. DNA sequences were generated for the Internal Transcribed Spacer regions 1 and 2 including the 5.8S rRNA gene, part of the β-tubulin and the Transcription Elongation Factor 1-α gene regions. These data were compared with those of other species in the *C. fimbriata* s.l. species complex and *Thielaviopsis* using phylogenetic analysis. Morphology of the isolates in culture as well as phylogenetic inference showed that the *Thielaviopsis* sp. present on the wounds was *T. basicola.* The *Ceratocystis* sp. from *S. benzoin* represents a new taxon in the *C. fimbriata* s.l. complex described here as *C. larium* sp. nov.

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INTRODUCTION

Trees in the genus *Styrax* are native to the Northern Hemisphere including eastern and south-eastern Asia and South America, where they occur in warm temperate areas (Burkill 1935). There are about 150 species of *Styrax* and many are used to produce resin that is aromatic when burned. *Styrax benzoin* trees in Indonesia, specifically Sumatra: commonly referred to as Sumatra Benzoin are tapped for resin, which is collected and dried. The dried resin produces fragrant aromas when burned and is thus a valuable source of incense, which is believed to have magical properties (Wheatley 1959). More than 18 000 families in northern Sumatra alone are dependant on benzoin production (Wollenberg et al. 2004).

Wounds on *S. benzoin* trees often develop into cankers that can eventually girdle and kill them. Such wounds are commonly associated with vascular staining, typical of that resulting from infection by ophiostomatoid fungi (Wingfield et al. 1993, Zhou et al. 2008). These fungi and particularly species of *Ceratocystis* s.l. have the capacity to infect wounds and kill trees (Bretz 1952, Norris 1953, de Vay et al. 1963, Kile 1993).

Ceratocystis s.l. represents a diverse species complex with distinct groups of taxa separated by clear phylogenetic, morphological and ecological boundaries. These groups are in the process of being assigned generic status. Many of these fungi infect wounds on trees but some are also symbionts of conifer infesting bark beetles. Various *Ceratocystis* spp. have been found infecting wounds on trees made during agronomic practices or bark harvesting, often resulting in serious disease problems (de Vay et al. 1963, Kile 1993, Marin et al. 2003).

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The aim of this study was to consider whether wounds made on *S. benzoin* trees in the resin harvesting process might be infected with *Ceratocystis* spp. and to identify these fungi based on morphology and phylogenetic analyses.

MATERIALS AND METHODS

Isolates

Wounds made on *S. benzoin* trees (Fig. 1) were inspected and samples were taken where vascular staining and gummosis was evident (Fig. 1). Samples were wrapped in newspaper and transported to the laboratory. Wood samples were incubated in a moist environment and inspected directly for fungal growth (Fig. 1). Spores produced by fungal structures on the wood surface were transferred onto 2 % malt extract agar (MEA: 20 % w/v; Biolab, Midrand, South Africa) supplemented with 100 mg/L streptomycin sulphate (SIGMA). Pieces of wood were also placed between two slices of 10 mm carrot pieces that were initially treated with streptomycin sulphate to bait for species of *Ceratocystis* (Moller & de Vay 1968a). Pure cultures were obtained (Fig. 1) and these were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), The University of Pretoria, South Africa. Representative isolates were also lodged with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. Representative cultures were dried and deposited with the National Herbarium of South Africa (PREM).

Phylogenetic analyses

DNA was extracted, as described by van Wyk et al. (2006) for six selected isolates representing two morphological groups. PCR reactions for the Internal Transcribed Spacer regions (ITS) 1 and 2 including the 5.8S rDNA, the β-tubulin and the Transcription Elongation Factor 1α (EF-1α) were prepared as

Fig. 1 Isolation of species of fungi from *Styrax benzoin* in Indonesia. a. Triangular wounds created with a knife for gum exudation; b. exposed wound from *S. benzoin* trees illustrating gummosis and wood discolouration; c. fungal growth on collected pieces of wood that were sampled; d. pure culture of a *Ceratocystis fimbriata* s.l. species; e. pure culture of a *Thielaviopsis* species.

described by van Wyk et al. (2006). The conditions for the PCRs were as described by van Wyk et al. (2006) with the annealing temperature at 55 °C for all three gene regions. The primers used to amplify the DNA for these three regions were those of White et al. (1990), Glass & Donaldson (1995) and Jacobs et al. (2004), respectively.

An ABI PRISM™ Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Applied BioSystems, Foster City, California, USA) was used to prepare the PCR amplicons for sequencing. An ABI PRISM™ 3100 Autosequencer (Applied BioSystems, Foster City, California, USA) was used to run the sequencing reactions. Sequences were analysed with Chromas Lite 2.01 (http://www.technelysium.com.au). The sequences obtained were subjected to Blast analysis in the National Centre for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) to confirm the identity of the genera present. This showed the presence of isolates representing the *C. fimbriata* s.l. species complex and others of a *Thielaviopsis* species.

The sequences obtained, together with other closely related species, obtained from GenBank (Table 1) were aligned using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/software/source.html) (Katoh et al. 2002) for each dataset. The alignments were manually inspected and corrected where necessary. Sequences were analysed using Phylogenetic Analysis Using Parsimony (PAUP) v. 4.0b10 (Swofford 2002). A partition homogeneity test (Swofford 2002) was run to determine whether sequence

Table 1 Isolates of *Ceratocystis* spp. used in this study.

Species	Isolate no.	GenBank accession no.			Host	Geographical origin
Ceratocystis albifundus	CMW 4068	DQ520638	EF070429	EF070400	*Acacia mearnsii*	RSA
	CMW 5329	AF388947	DQ371649	EF070401	*Acacia mearnsii*	Uganda
C. atrox	CMW 19383; CBS 120517	EF070414	EF070430	EF070402	*Eucalyptus grandis*	Australia
	CMW 19385; CBS 120518	EF070415	EF070431	EF070403	*Eucalyptus grandis*	Australia
C. cacaofunesta	CMW 15051; CBS 152.62	DQ520636	EF070427	EF070398	*Theobroma cacao*	Costa Rica
	CMW14809; CBS 115169	DQ520637	EF070428	EF070399	*Theobroma cacao*	Ecuador
C. caraye	CMW 14793; CBS 114716	EF070424	EF070439	EF070412	*Carya cordiformis*	USA
	CMW 14808; CBS 115168	EF070423	EF070440	EF070411	*Carya ovata*	USA
C. colombiana	CMW 9565; CBS 121790	AY233864	AY233870	EU241487	Soil	Colombia
	CMW 5751; CBS 121792	AY177233	AY177225	EU241493	*Coffea arabica*	Colombia
	CMW 9572	AY233863	AY233871	EU241488	Mandarin	Colombia
C. fimbriata s.str.	CMW 15049; CBS 141.37	DQ520629	EF070442	EF070394	*Ipomaea batatas*	USA
	CMW 1547	AF264904	EF070443	EF070395	*Ipomaea batatas*	Papua New Guinea
C. fimbriatomima	CMW 24174; CBS 121786	EF190963	EF190951	EF190957	*Eucalyptus* sp.	Venezuela
	CMW 24176; CBS 121787	EF190964	EF190952	EF190958	*Eucalyptus* sp.	Venezuela
C. laurium*	**CMW 25434; CBS 122512**	**EU881906**	**EU881894**	**EU881900**	***Styrax benzoin***	**Indonesia**
	CMW 25435; CBS 122606	**EU881907**	**EU881895**	**EU881901**	***Styrax benzoin***	**Indonesia**
	CMW 25436; CBS 122607	**EU881908**	**EU881896**	**EU881902**	***Styrax benzoin***	**Indonesia**
	CMW 25437	**EU881909**	**EU881897**	**EU881903**	***Styrax benzoin***	**Indonesia**
C. manginecans	CMW 13851; CBS 121659	AY953383	EF433308	EF433317	*Mangifera indica*	Oman
	CMW 13852; CBS 121660	AY953384	EF433309	EF433318	*Hypocryphalus mangifera*	Oman
C. neglecta	CMW 17808; CBS 121789	EF127990	EU881898	EU881904	*Eucalyptus* sp.	Colombia
	CMW 18194; CBS 121017	EF127991	EU881899	EU881905	*Eucalyptus* sp.	Colombia
C. obpyriformis	CMW 23807; CBS 122608	EU245004	EU244976	EU244936	*Acacia mearnsii*	South Africa
	CMW 23808; CBS 122511	EU245003	EU244975	EU244935	*Acacia mearnsii*	South Africa
C. papilata	CMW 8857	AY233868	AY233878	EU241483	*Annona muricata*	Colombia
	CMW 8856; CBS 121793	AY233867	AY233874	EU241484	Citrus lemon	Colombia
	CMW 10844	AY177238	AY177229	EU241481	*Coffea arabica*	Colombia
C. pirilliformis	CMW 6569	AF427104	DQ371652	AY528982	*Eucalyptus nitens*	Australia
	CMW 6579; CBS 118128	AF427105	DQ371653	AY528983	*Eucalyptus nitens*	Australia
C. platani	CMW 14802; CBS 115162	DQ520630	EF070425	EF070396	*Platanus occidentalis*	USA
	CMW 23918	EF070426	EF070397	EU426554	*Platanus* sp.	Greece
C. polychroma	CMW 11424; CBS 115778	AY528970	AY528966	AY528978	*Syzygium aromaticum*	Indonesia
	CMW 11436; CBS 115777	AY528971	AY528967	AY528979	*Syzygium aromaticum*	Indonesia
C. polyconidia	CMW 23809; CBS 122289	EU245006	EU244978	EU244938	*Acacia mearnsii*	South Africa
	CMW 23818; CBS122290	EU245007	EU244979	EU244939	*Acacia mearnsii*	South Africa
C. populicola	CMW 14789; CBS 119.78	EF070418	EF070434	EF070406	*Populus* sp.	Poland
	CMW 14819; CBS 114725	EF070419	EF070435	EF070407	*Populus* sp.	USA
C. smalleyi	CMW 14800; CBS 114724	EF070420	EF070436	EF070408	*Carya cordiformis*	USA
	CMW 26383; CBS 114724	EU426553	EU426555	EU426556	*Carya cordiformis*	USA
C. tanganyicensis	CMW 15991; CBS 122295	EU244997	EU244969	EU244929	*Acacia mearnsii*	Tanzania
	CMW 15999; CBS 122294	EU244998	EU244970	EU244939	*Acacia mearnsii*	Tanzania
C. tsitsikammensis	CMW 14276; CBS 121018	EF408555	EF408569	EF408576	*Rapanea melanophloeos*	South Africa
	CMW 14278; CBS 121019	EF408556	EF408570	EF408577	*Rapanea melanophloeos*	South Africa
C. variospora	CMW 20935; CBS 114715	EF070421	EF070437	EF070409	*Quercus alba*	USA
	CMW 20936; CBS 114714	EF070422	EF070438	EF070410	*Quercus robur*	USA
C. virescens	CMW 11164	DQ520639	EF070441	EF070413	*Fagus americanum*	USA
	CMW 3276	AY528984	AY528990	AY529011	*Quercus robur*	USA
C. zombamontana	CMW 15235	EU245002	EU244974	EU244934	*Eucalyptus* sp.	Malawi
	CMW 15236	EU245000	EU244972	EU244932	*Eucalyptus* sp.	Malawi

* Isolates indicated in **bold** face are described in this study.

data for three gene regions could be combined. In PAUP, gaps were treated as a fifth character and trees were obtained via stepwise addition of 1 000 replicates, the Mulpar option was in effect and the heuristic search option based on parsimony with stepwise addition was selected. Confidence intervals using 1 000 bootstrap replicates were calculated. *Ceratocystis virescens* was the designated outgroup for the dataset containing the *C. fimbriata* s.l. species. *Ceratocystis fimbriata* s.str. was designated as the outgroup for the *Thielaviopsis* dataset. All sequences derived from this study were deposited in GenBank (Table 1 and 2).

Morphology and cultural characteristics

Cultures were grown on 2 % MEA for 2 wk prior to assessment of morphological characters of the unknown *Ceratocystis* sp. Fungal structures were mounted on glass slides in lactic acid and these were examined using a Zeiss Axio Vision microscope. Fifty measurements were made for each taxonomically relevant structure. Ranges, averages and standard deviations (SD) were determined for each of these characters. Colours of structures and cultures were assessed using the mycological colour charts of Rayner (1970).

To determine the optimum temperature for growth of isolates, growth studies were performed on three isolates representing the unknown *Ceratocystis* sp. A 5 mm plug from the margin of an actively growing culture (2-wk-old) was placed at the centres of 90 mm 2 % MEA Petri dishes. There were five replicates for each isolate at each temperature and growth was assessed between 5–35 °C at 5 °C intervals after 7 d. The entire study was repeated once.

Table 2 Isolates of *Thielaviopsis* and associated *Ceratocystis* spp. used in this study.

Species	Isolate no.	GenBank accession no.			Host	Geographical origin
Thielaviopsis australis / *Ceratocystis australis*	CMW 2333	FJ411325	FJ411351	FJ411299	*Nothofagus cunninghamii*	Australia
	CMW 2653	FJ411326	FJ411352	FJ411300	*Nothofagus cunninghamii*	Australia
T. eucalypti / *C. eucalypti*	CMW 3254	FJ411327	FJ411353	FJ411301	*Eucalyptus sieberi*	Australia
	CMW 4453	FJ411328	FJ411354	FJ411302	*Eucalyptus sieberi*	Australia
T. basicola	CMW 6714	FJ411331	FJ411357	FJ411305	Carrots	Australia
	CMW 7625; CBS 117828	FJ411332	FJ411358	FJ411306	Chicory	South Africa
T. basicola*	**CMW 25438**	**FJ411333**	**FJ411359**	**FJ411307**	***Styrax benzoin***	**Indonesia**
	CMW 25439	**FJ411334**	**FJ411360**	**FJ411308**	***Styrax benzoin***	**Indonesia**
	CMW 25440	**FJ411335**	**FJ411361**	**FJ411309**	***Styrax benzoin***	**Indonesia**
T. neocaledoniae	CMW 3270	FJ411329	FJ411355	FJ411303	Unknown	USA
	CMW 26392; CBS 149.83	FJ411330	FJ411356	FJ411304	*Coffea robusta*	USA
T. ovoidea	CMW 22733; CBS 354.76	FJ411343	FJ411369	FJ411317	Fire wood	Netherlands
T. paradoxa / *C. paradoxa*	CMW 8779	FJ411324	FJ411349	FJ411298	Coconut	Indonesia
	CMW 8790	FJ411323	FJ411350	FJ411297	Coconut	Indonesia
T. populi	CMW 26387; CBS 484.71	FJ411336	FJ411362	FJ411310	*Populus robusta*	Belgium
	CMW 26388; CBS 486.71	FJ411337	FJ411363	FJ411311	*Populus gelrica*	Belgium
T. punctulata / *C. radicicola*	CMW 26389; CBS 167.67	FJ411338	FJ411368	FJ411316	*Lawsonia inermis*	Europe
	CMW 1032; CBS 114.47	FJ411339	FJ411364	FJ411312	*Phoenix dactylifera*	USA
	CMW 6728	FJ411340	FJ411365	FJ411313	*Daucus carrota*	Australia
T. quercina / *C. fagacearum*	CMW 2039	FJ411344	FJ411370	FJ411318	*Quercus* sp.	USA
	CMW 2658	FJ411345	FJ411371	FJ411319	*Quercus* sp.	USA
T. thielavioides	CMW 22736; CBS 148.37	FJ411342	FJ411367	FJ411315	*Lupinus albus*	Italy
	CMW 22737; CBS 180.75	FJ411341	FJ411366	FJ411314	*Populus* sp.	Belgium
T. ungeri / *C. coerulescens*	CMW 26364	FJ411321	FJ411347	FJ411295	*Picea* sp.	USA
	CMW 26365; CBS 140.37	FJ411322	FJ411348	FJ411296	*Picea abies*	Germany
	CMW 26366; CBS 489.80	FJ411320	FJ411346	FJ411294	*Picea abies*	Finland
C. fimbriata s.str.	CMW 15049; CBS 141.37	DQ520629	EF070442	EF070394	*Ipomaea batatas*	USA
	CMW 1547	AF264904	EF070443	EF070395	*Ipomaea batatas*	Papua New Guinea

* Isolates indicated in **bold** face are described in this study.

RESULTS

Isolates

Fresh fungal structures were found on the wood surface of the samples collected from wounded *S. benzoin* trees in Indonesia. The fungal structures were characteristic of two different fungi, one with perithecia similar to those of *Ceratocystis* spp. in the *C. fimbriata* s.l. species complex and the other, a *Thielaviopsis* sp. with septate chlamydospores. Sixteen isolates were collected of which six represented a *Thielaviopsis* sp. and the remaining cultures were of a *Ceratocystis* sp.

Phylogenetic analyses

For the *C. fimbriata* s.l. isolates, amplicons of ± 500 bp (ITS and β-tubulin) and ± 800 bp (EF-1α) were obtained. A P-value of 0.01 was obtained for the PHT showing that the three datasets could be combined (Sullivan 1996, Cunningham 1997). This combined dataset consisted of 1 988 characters, of which 1 102 were constant, 46 were parsimony uninformative and 840 were parsimony informative. Seven most parsimonious trees were obtained, one of which was selected for presentation (Fig. 2). The tree is described as follows; Tree length (TL) = 2 030 steps, Consistency Index (CI) = 0.7, Retention Index (RI) = 0.9 and Rescaled Consistency Index (RC) = 0.6.

The isolates representing *C. fimbriata* s.l. grouped phylogenetically separate from all other described species in this species complex with 100 % statistical support. The species phylogenetically closest to the isolates from *S. benzoin* was *C. albifundus* (Fig. 2). All posterior probabilities were high, supporting the separate species within the *C. fimbriata* s.l. species complex.

MrModeltest2.2 selected the HKY+I+G model for the ITS gene region as the most suited. For the β-tubulin gene region, the GTR+G model was selected while the HKY+I+G model were selected for the EF-1α gene region. The selected models were incorporated into the Bayesian analysis. Two thousand trees were discarded to exclude any trees that were drawn outside of the point of convergence. All posterior probabilities that were obtained with parsimony were confirmed with the Bayesian analyses (Fig. 2).

In the case of the *Thielaviopsis* isolates, amplicons of ± 500 bp (ITS and β-tubulin) and ± 800 bp (EF-1α) were obtained. A P-value of 0.01 was obtained for the PHT which suggested combinability of the datasets (Sullivan 1996, Cunningham 1997). The *Thielaviopsis* dataset consisted of 1 956 characters, of which 1 206 were constant, 54 were parsimony uninformative and 696 were parsimony informative. One most parsimonious tree was obtained and presented (Fig. 3). The tree is described as follows: TL = 1 730 steps, CI = 0.7, RI = 0.9 and RC = 0.6. The *Thielaviopsis* sp. grouped phylogenetically close to *Thielaviopsis basicola* with a high bootstrap support (100 %).

The models obtained from MrModeltest2.2 for the ITS, β-tubulin gene region and the EF1-α gene region were the GTR+G, GTR+I+G and GTR+I+G, respectively. Two thousand trees were discarded. All posterior probabilities that were obtained with parsimony were confirmed with the Bayesian analyses (Fig. 3).

Morphology and cultural characteristics

Thielaviopsis basicola is a very well-known fungus with characteristic and distinct segmented chlamydospores. An isolate (CMW 25438) was selected randomly to confirm that morphologically these isolates are representatives of *T. basicola*. Dark clumps of conidiophores were evident in cultures. The very distinct chlamydospores of *T. basicola* were also present.

For the *C. fimbriata* s.l. isolates: one isolate (CMW 25434) was chosen to represent the fungus and three additional isolates (CMW 25435, 25436 and 25437) were chosen as additional specimens for description purposes. The cultures of *C. fimbriata* s.l. isolates had a light greyish olive (21""b) colour (Rayner 1970). These isolates were slow growing. No growth was

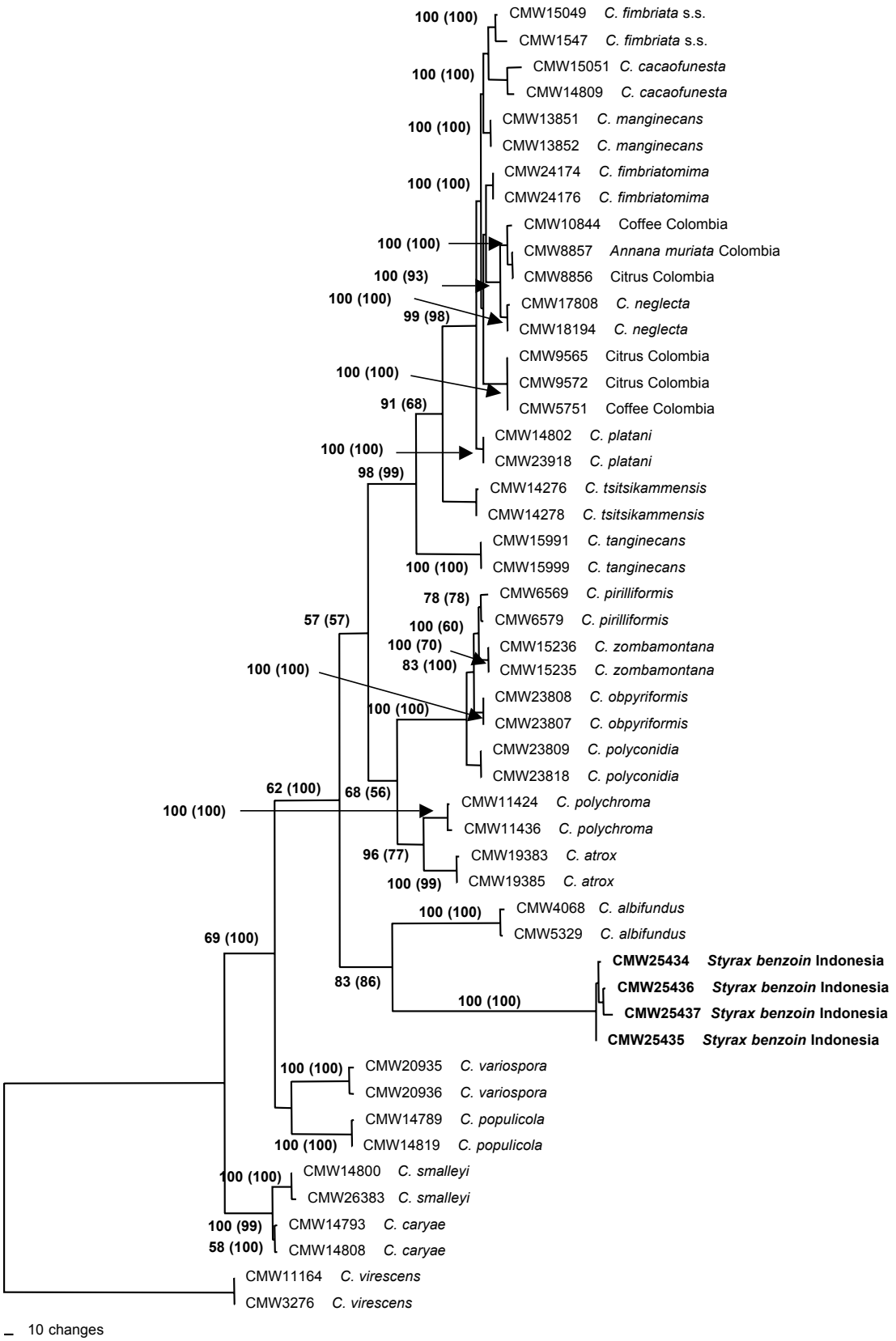

Fig. 2 One of seven most parsimonious phylogenetic trees, based on the combined regions of the ITS, β-tubulin and EF-1α for *Ceratocystis larium* and other species in the *C. fimbriata* s.l. species complex. *Ceratocystis virescens* represents the outgroup taxon. Bootstrap values are indicated at the branch nodes and Bayesian values in parentheses.

observed at 4 °C and 35 °C. Limited growth was observed at 10 °C (5 mm), 15 °C (10 mm) and 30 °C (6.5 mm). Intermediate growth was observed at 20 °C (12.4 mm) with optimal growth at 25 °C (13.5 mm) in 7 d. The cultures had a strong banana odour similar to that of many *Ceratocystis* spp. Micro-morphological characteristics distinct for the isolates from Indonesia included the pirilliform ascomatal bases and both the cylindrical and barrel-shaped conidia were of variable size. Similarly variable sizes were observed for the chlamydospores.

The *Ceratocystis* isolates from wounds on *S. benzoin* trees are phylogenetically and morphologically distinct from all other *Ceratocystis* spp. residing in the *C. fimbriata* s.l. clade. These isolates are therefore described as representing a new species as follows:

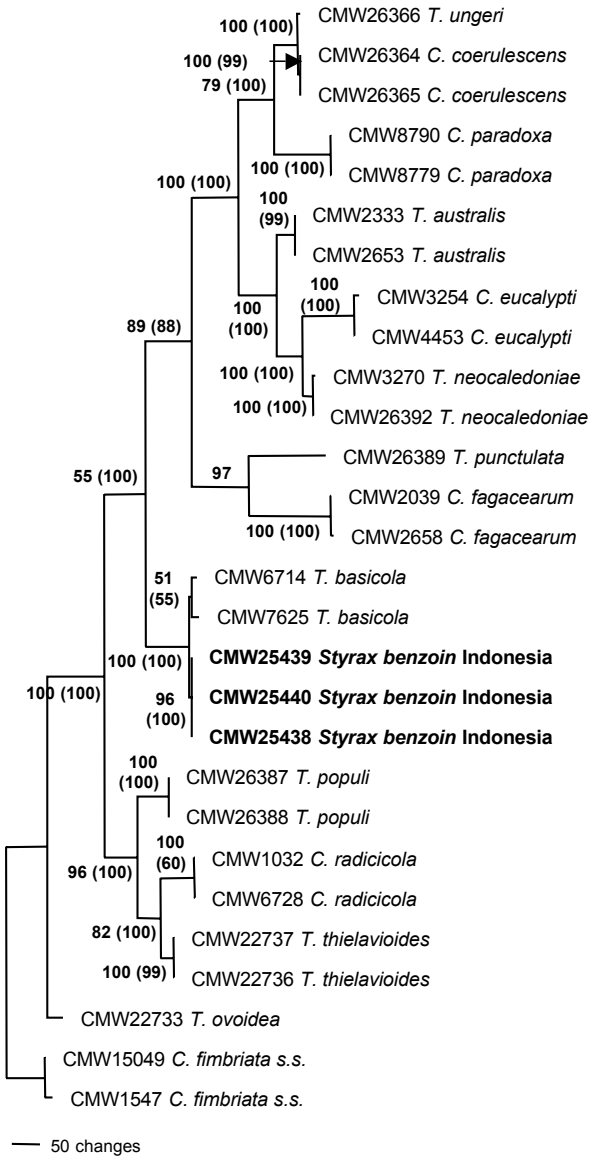

Fig. 3 Most parsimonious tree based on the combined regions of the ITS, β-tubulin and EF-1α for *T. basicola* and other species in the *Thielaviopsis* genus. *Ceratocystis fimbriata* s.str. represents the outgroup taxon. Bootstrap values are indicated at the branch nodes and Bayesian values in parentheses.

Ceratocystis larium M. van Wyk & M.J. Wingf., *sp. nov.* — MycoBank MB512564; Fig. 4

Anamorph. *Thielaviopsis* sp.

Bases ascomatum fuscae pirilliformes inornatae (101–)120–184(–243) µm latae (113–)139–201(–254) µm longae. Conidia primaria cylindrica vel oblonga apicibus truncatis (8–)11–21(–28) µm longa (2–)3–5(–6) µm lata. Conidia secondaria doliiformia vel obtusa, (6–)7–9(–13) µm longa 4–6(–7) µm lata. Chlamydosporae badiae, prolate sphaeroideae vel perprolatae (8–)9–13(–16) µm longae (7–)8–10(–11) µm latae.

Etymology. The name refers to the guardian spirits of a home or town and reflects the spiritual properties the incense obtained from *S. benzoin* trees.

Ascomatal bases dark, pirilliform, unornamented (101–)120–184(–243) µm wide, (113–)139–201(–254) µm long. *Ascomatal necks* dark at bases becoming hyaline at the apices, (222–)347–573(–808) µm long, apices (10–)13–19(–25) µm wide, bases (19–)24–36(–44) µm wide. *Ostiolar hyphae* hyaline, divergent, (18–)22–30(–35) µm long. *Ascospores* hyaline, hat-shaped in side view, invested in sheath, 2–4 µm long, 3–5 µm wide excluding sheath, 4–7 µm wide including sheath, accumulating in buff-yellow masses at tips of ascomatal necks. *Primary conidiophores* phialidic, flask-shaped, (52–)64–98(–141) µm long, (2–)3–5 µm wide at the apices, 4–6(–7) µm wide at broadest points and (3–)4–6(–7) µm wide at bases. *Secondary conidiophores* phialidic, apices wide, (44–)50–86(–99) µm long, 4–6 µm wide at the apices, 3–5(–6) µm wide at bases. *Primary conidia* cylindrical to oblong with truncated apices in shape, (8–)11–21(–28) µm long, (2–)3–5(–6) µm wide. *Secondary conidia*, barrel-shaped to obtuse, (6–)7–9(–13) µm long, 4–6(–7) µm wide. *Chlamydospores* hair-brown (17""""i), prolate spheroidal to perprolate, (8–)9–13(–16) µm long, (7–)8–10(–11) µm wide.

Habitat — Wounds on *Styrax benzoin* trees.

Known distribution — Northern Sumatra, Indonesia.

Specimens examined. Indonesia, Tele, isolated from the wounds created when tapping resin from *S. benzoin* trees, March 2007, *M.J. Wingfield*, holotype Herb. PREM 60193, culture ex-type CMW 25434 = CBS 122512; ditto, paratype Herb. PREM 60194, culture ex-paratype CMW 25435 = CBS 122606; ditto, paratype Herb. PREM 60195, culture ex-paratype CMW 25436 = CBS 122607; ditto, paratype Herb. PREM 60196, culture ex-paratype CMW 25437.

DISCUSSION

Two species of *Ceratocystis* s.l. were isolated from wounds on *S. benzoin* trees in this study. These fungi were identified based on morphology and phylogenetic inference and included *Thielaviopsis basicola* and an undescribed species of *Ceratocystis* residing in the *C. fimbriata* s.l. species complex and which has been given the name *C. larium*. Both fungi were commonly found on the surface of wounds on *S. benzoin* trees and *C. larium* was also easily collected from stained tissue using carrot baiting.

Thielaviopsis basicola is a well-known soil-borne pathogen of many root crops (Nag Raj & Kendrick 1975, Geldenhuis et al. 2006) and its presence on the surface of wounds on trees might seem unusual. However, it has been identified as being associated with insects that vector the conidia and/or chlamydospores (Labuschagne & Kotze 1991, Stanghellini et al. 1999). It is thus possible that insects, for example ants that live in the soil, are attracted by the aromatic gum that accumulates at the wound sites of the trees, thereby carrying the soil-borne fungus to the sites from which it was isolated in this study. Because it is also a carrot pathogen (Geldenhuis et al. 2006), it can be found on carrot baits used to isolate *Ceratocystis* spp., but in the case of this study it was found sporulating on the surface of wounds and had no association with carrots.

The presence of a *Ceratocystis* sp. associated with wounds on *S. benzoin* trees is not surprising as these fungi are commonly found on wounds on trees (Kile 1993). Indeed, various species of *Ceratocystis* have been trapped from the environment by artificially wounding trees (Barnes et al. 2003). In this case, wounds are visited by sap-feeding insects that are also attracted to the fruity aromas produced by many *Ceratocystis* spp. (Moller & de Vay 1968b). We hence assume that *C. larium* was carried to wounds on *S. benzoin* by such insects.

Ceratocystis larium represents a discrete taxon. Based on phylogenetic inference for the ITS, β-tubulin and the EF-1α gene regions, *C. larium* is most closely related to *C. albifundus*. *Ceratocystis albifundus* is most distinct from all the other species within the *C. fimbriata* s.l. species complex with no species phylogenetically closely related to it. *Ceratocystis larium*, residing in a phylogenetically sister group to *C. albifundus,* is thus also clearly distinct from all other species in the *C. fimbriata* s.l. species complex.

Morphologically, *C. larium* is similar to other species in the *C. fimbriata* s.l. species. In this regard, it has a grey to green colony colour and a fruity odour. Similar to *C. pirilliformis* (Barnes et al. 2003) and *C. obpyriformis* (Heath et al. 2009), it has pirilliform ascomatal bases. However, the cylindrical conidia in *C. larium*

Fig. 4 Morphological characteristics of *Ceratocystis larium*. a. Hat-shaped ascospores; b. various shapes of the primary conidia, mainly cylindrical in shape; c. secondary conidia, barrel-shaped to obtuse; d. divergent ostiolar hyphae; e. flask-shaped primary conidiophores; f. secondary conidiophores with emerging barrel-shaped conidia; g. ascomata with pirilliform base; h. chlamydospores of various shapes; i. numerous chlamydospores visible in culture. — Scale bars = 10 µm.

differ substantially in size and shape from each other and this distinct variation is also true for the barrel-shaped conidia. Although variation is expected within a species, there is no other species in the *C. fimbriata* s.l. species complex that displays this remarkable variability in size and shape of the conidia. Chlamydospores in *C. larium* are also variable in shape, ranging from prolate spheroidal to perprolate and these structures are also abundant in this species.

Ceratocystis larium is clearly an opportunistic fungus that infects wounds made to tap the resin of *S. benzoin* trees. Nothing is known regarding the pathogenicity of this fungus or *T. basicola* on these trees. However, many wounds made to the trees develop into significant cankers that appear to eventually lead to tree death. Pathogenicity of these fungi should thus be tested and if they are contributing to the death of trees, efforts should be made to restrict their presence.

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REFERENCES

Barnes I, Roux J, Wingfield BD, Dudzinski MJ, Old KM, Wingfield MJ. 2003. Ceratocystis pirilliformis, a new species from Eucalyptus nitens in Australia. Mycologia 95: 865–871.

Bretz TW. 1952. The ascigerious stage of the oak wilt fungus. Phytopathology 42: 435–437.

Burkill IH. 1935. Styrax. In: A dictionary of the economic products of the Malay Peninsula. The Government of the Straits Settlements and Federated Malay States. Vol. II: 2101–2108. The Crown Agents for the Colonies, London.

Cunningham CW. 1997. Can three incongruence tests predict when data should be combined? Molecular Biology and Evolution 14: 733–740.

Geldenhuis MM, Roux J, Cilliers AJ, Wingfield BD, Wingfield MJ. 2006. Clonality in South African isolates and evidence for a European origin of the root pathogen Thielaviopsis basicola. Mycological Research 110: 306–311.

Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Applied and Environmental Microbiology 61: 1323–1330.

Heath RN, Wingfield BD, Wingfield MJ, Meke G, Mbaga A, Roux J. 2009. Ceratocystis spp. on Acacia mearnsii and Eucalyptus spp. in eastern and southern Africa including six new species. Fungal Diversity 34: 41–68.

Jacobs K, Bergdahl DR, Wingfield MJ, Halik S, Seifert KA, Bright DE, Wingfield BD. 2004. Leptographium wingfieldii introduced into North America and found associated with exotic Tomicus piniperda and native bark beetles. Mycological Research 108: 411–418.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059–3066.

Kile GA 1993. Plant diseases caused by species of Ceratocystis sensu stricto and Chalara. In: Wingfield MJ, Seifert KA, Webber JF (eds), Ceratocystis and Ophiostoma: Taxonomy, ecology, and pathogenicity: 173–183. APS Press, St. Paul, Minnesota.

Labuschagne N, Kotze JM. 1991. Incidence of Chalara elegans in groundnut seed samples and seed transmission of blackhull. Plant Pathology 40: 639–642.

Marin M, Castro B, Gaitan A, Preisig O, Wingfield BD, Wingfield MJ. 2003. Relationships of Ceratocystis fimbriata isolates from Colombian coffee-growing regions based on molecular data and pathogenicity. Journal of Phytopathology 151: 395–405.

Moller WJ, Vay JE de. 1968a. Carrot as a species-selective isolation medium for Ceratocystis fimbriata. Phytopathological Notes 58: 123–126.

Moller WJ, Vay JE de. 1968b. Insect transmission of Ceratocystis fimbriata in deciduous fruit orchards. Phytopathology 58: 1499–1508.

Nag Raj TR, Kendrick B. 1975. A monograph of Chalara and allied genera. Wilfrid Laurier University Press, Waterloo.

Norris DN. 1953. Insect transmission of oak wilt in Iowa. Plant Disease Report 37: 417–418.

Rayner RW. 1970. A mycological colour chart. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey.

Stanghellini ME, Rasmussen SL, Kim DH. 1999. Aerial transmission of Thielaviopsis basicola, a pathogen of corn-salad, by adult shore flies. Phytopathology 89: 476–479.

Sullivan J. 1996. Combining data with different distributions of among-site variation. Systematic Biology 45: 375–380.

Swofford DL. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, USA.

Vay JE de, Lukezic FL, English WH, Trujillo EE. 1963. Ceratocystis canker of stone fruit trees. Phytopathology 53: 873.

Wheatley P. 1959. Geographical notes on some commodities involved in Sung maritime trade. Journal of the Malayan Branch of the Royal Asiatic Society 32: 1–140. Tien Wah Press Ltd., Singapore.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR protocols: A sequencing guide to methods and applications: 315–322. Academic Press, San Diego, USA.

Wingfield MJ, Seifert KA, Webber JF. 1993. Ceratocystis and Ophiostoma: Taxonomy, ecology, and pathogenicity. APS Press, St. Paul, Minnesota.

Wollenberg E, Belcher B, Sheil D, Dewi S, Moeliono M. 2004. Governance brief 2004. Why are forest areas relevant to reducing poverty in Indonesia? 4: 1–6. (http://www.cifor.cgiar.org/Publications/).

Wyk M van, Roux J, Barnes I, Wingfield BD, Wingfield MJ. 2006. Molecular phylogeny of the Ceratocystis moniliformis complex and description of C. tribiliformis sp. nov. Fungal Diversity 21: 181–201.

Zhou XD, Jacobs K, Kirisits T, Chhetri DB, Wingfield MJ. 2008. Leptographium bhutanense sp. nov., associated with the root collar weevil Hylobitelus chenkupdorjii on Pinus wallichiana in Bhutan. Persoonia 21: 1–8.