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

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Key words
Ophiostomatoidei 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 Ophiostomatoidei 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 1α 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.

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 (Burrill 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 dependent 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 ophiostomatoidei 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).

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 (Möller & de Vay 1986a). 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
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.

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 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
Morphological 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 1  Isolates of Ceratocystis spp. used in this study.

| Species | Isolate no. | GenBank accession no. | Host | Geographical origin |
|---------|-------------|-----------------------|------|---------------------|
| C. 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 | Eucalyptus grandis | Australia |
| C. caecofunestia | CMW 15051; CBS 152.62 | DQ520636 EF070427 EF070398 | Theobroma cacao | Costa Rica |
| | CMW 14809; CBS 115169 | DQ520637 EF070428 | Theobroma cacao | Ecuador |
| C. caraye | CMW 14793; CBS 114716 | EF070424 EF070439 | Carya cordiformis | USA |
| | CMW 14608; CBS 115168 | EF070423 EF070440 | Carya ovata | USA |
| C. colombiana | CMW 9665; CBS 121790 | AY233864 AY233870 EU241487 | Soil | Colombia |
| | CMW 5751; CBS 121792 | AY177233 AY177225 EU241493 | Coffee arabica | Colombia |
| | CMW 9572 | AY233863 AY233871 EU241488 | | |
| C. fimbriata s.str. | CMW 15049; CBS 141.37 | DQ520629 EF070442 | Ipomoea batatas | USA |
| | CMW 1547 | AY264904 EF070443 | Ipomoea batatas | Papua New Guinea |
| C. fimbriotomima | CMW 24174; CBS 121786 | EF190963 | Eucalyptus sp. | Venezuela |
| | CMW 24176; CBS 121787 | EF190964 | Eucalyptus sp. | Venezuela |
| C. laurium* | CMW 25434; CBS 122512 | EU819106 EU881894 EU881900 | Styrox benzoin | Indonesia |
| | CMW 25435; CBS 122606 | EU819107 EU881895 EU881901 | Styrox benzoin | Indonesia |
| | CMW 25436; CBS 122607 | EU819108 EU881896 EU881902 | Styrox benzoin | Indonesia |
| | CMW 25437 | EU819109 EU881897 EU881903 | Styrox benzoin | Indonesia |
| C. manginecans | CMW 13851; CBS 121659 | AY953383 EF433308 EF433317 | Mangifera indica | Oman |
| | CMW 13852; CBS 121660 | AY953384 EF433309 EF433318 | Hypocypalus mangifera | Oman |
| C. neglecta | CMW 17808; CBS 121789 | EF127990 EF088198 | Eucalyptus sp. | Colombia |
| | CMW 18194; CBS 121017 | EF127991 EF881899 | 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 8657 | AY233868 AY233878 EU241483 | Annona muricata | Colombia |
| | CMW 8656; CBS 121793 | AY233867 AY233874 EU241484 | Citrus lemon | Colombia |
| | CMW 10844 | AY177238 AY177229 EU241481 | Coffee arabica | Colombia |
| C. pirilloformis | CMW 6596 | AF427104 DQ371652 AY528982 | Eucalyptus nitens | Australia |
| | CMW 6579; CBS 181128 | AF427105 DQ371653 AY528983 | Eucalyptus nitens | Australia |
| C. platani | CMW 14802; CBS 115162 | DQ520630 EF070425 EF070396 | Plataneus occidentalis | USA |
| | CMW 23918 | EF070426 EF070397 EU246554 | Plataneus sp. | Greece |
| C. polychroma | CMW 11424; CBS 115778 | AYS28970 AYS28966 AYS28978 | Syzygium aromaticum | Indonesia |
| | CMW 11436; CBS 115777 | AYS28971 AYS28967 AYS28979 | Syzygium aromaticum | Indonesia |
| C. polycionia | 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 | Populus sp. | Poland |
| | CMW 14619; CBS 114725 | EF070419 | Populus sp. | USA |
| C. smallleyi | CMW 14800; CBS 114724 | EF070420 | Carya cordiformis | USA |
| | CMW 26383; CBS 114724 | EU246555 EU246556 | Carya cordiformis | USA |
| C. tanganyicensis | CMW 15991; CBS 122295 | EU244969 EU244939 | Acacia mearnsii | Tanzania |
| | CMW 15999; CBS 122294 | EU244968 EU244970 | Acacia mearnsii | Tanzania |
| C. tsetsikamennis | CMW 14276; CBS 121018 | EF408555 EF408569 EF408576 | Raphanea melanophloeos | South Africa |
| | CMW 14278; CBS 121019 | EF408556 EF408570 EF408577 | Raphanea melanophloeos | South Africa |
| C. variospora | CMW 20935; CBS 114715 | EF070421 EF070437 EF070409 | Quercus alba | USA |
| | CMW 20936; CBS 114714 | EF070422 | Quercus robur | USA |
| C. virescens | CMW 11164 | DQ520639 EF070441 EF070413 | Fagus americana | USA |
| | CMW 3276 | AYS28984 AYS28990 AYS29011 | 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).
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 was 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. albifun-dus* (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 EF-1α 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 septate chlamydospores. An isolate (CMW 25438) was selected randomly to confirm that morphological and associated species 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

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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 | FJ411125 FJ4111351 FJ4111299 | *Nothofagus cunninghamii* | Australia |
| CMW 2653 | FJ411126 FJ4111352 FJ4111300 | *Nothofagus cunninghamii* | Australia |
| T. eucalypti / *C. eucalypti* | | | |
| CMW 3254 | FJ411127 FJ4111353 FJ4111301 | *Eucalyptus sieberi* | Australia |
| CMW 4453 | FJ411128 FJ4111354 FJ4111302 | *Eucalyptus sieberi* | Australia |
| T. basicola | | | |
| CMW 6714 | FJ411131 FJ4111357 FJ4111305 | Carrots | Australia |
| CMW 7625; CBS 117828 | FJ411132 FJ4111358 FJ4111306 | Chicory | South Africa |
| T. basicola* | | | |
| CMW 25438 | FJ411133 FJ4111359 FJ4111307 | *Styrax benzoin* | Indonesia |
| CMW 25439 | FJ411134 FJ4111360 FJ4111308 | *Styrax benzoin* | Indonesia |
| CMW 25440 | FJ411135 FJ4111361 FJ4111309 | *Styrax benzoin* | Indonesia |
| T. neocaledoniana | | | |
| CMW 3270 | FJ411129 FJ4111355 FJ4111303 | Unknown | USA |
| CMW 26392; CBS 149.83 | FJ411130 FJ4111356 FJ4111304 | Coffea robusta | USA |
| T. ovoidea | | | |
| CMW 22733; CBS 354.76 | FJ411134 FJ4111369 FJ4111317 | Fire wood | Netherlands |
| T. paradoxa / *C. paradoxa* | | | |
| CMW 8779 | FJ411124 FJ4111349 FJ4111298 | Coconut | Indonesia |
| CMW 8790 | FJ411123 FJ4111350 FJ4111297 | Coconut | Indonesia |
| T. populi | | | |
| CMW 26387; CBS 484.71 | FJ411136 FJ4111362 FJ4111310 | *Populus robusta* | Belgium |
| CMW 26388; CBS 486.71 | FJ411137 FJ4111363 FJ4111311 | *Populus gelrica* | Belgium |
| T. punctulata / *C. radicicola* | | | |
| CMW 26389; CBS 167.67 | FJ411138 FJ4111368 FJ4111316 | *Lawsonia inermis* | Europe |
| CMW 1032; CBS 114.47 | FJ411139 FJ4111364 FJ4111312 | *Phoenix dactylifera* | USA |
| CMW 6728 | FJ4111340 FJ4111365 FJ4111313 | *Daucus carota* | Australia |
| T. quercina / *C. fagacearum* | | | |
| CMW 2039 | FJ4111344 FJ4111370 FJ4111318 | Quercus sp. | USA |
| CMW 2658 | FJ4111345 FJ4111371 FJ4111319 | Quercus sp. | USA |
| T. thielavioides | | | |
| CMW 22736; CBS 148.37 | FJ4111342 FJ4111367 FJ4111315 | *Lupinus albus* | Italy |
| CMW 22737; CBS 180.75 | FJ4111341 FJ4111366 FJ4111314 | *Populus sp.* | Belgium |
| T. ungeri / *C. coerulescens* | | | |
| CMW 26364 | FJ4111321 FJ4111347 FJ4111295 | *Picea sp.* | USA |
| CMW 26365; CBS 140.37 | FJ4111322 FJ4111348 FJ4111296 | *Picea abies* | Germany |
| CMW 26366; CBS 489.80 | FJ4111320 FJ4111346 FJ4111294 | *Picea abies* | Finland |
| C. fimbriata s.str. | | | |
| CMW 15049; CBS 141.37 | DQ520629 EF074044 EF070394 | *Ipomaea batatas* | USA |
| CMW 1547 | AF264904 EF074043 EF070395 | *Ipomaea batatas* | Papua New Guinea |

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

CMW15049 C. fimbriata s.s.
CMW1547 C. fimbriata s.s.
CMW15051 C. cacaofunesta
CMW14809 C. cacaofunesta
CMW13851 C. manginecans
CMW13852 C. manginecans
CMW24174 C. fimbriotamimin
CMW24176 C. fimbriotamimin
CMW10844 Coffee Colombia
CMW8857 Annara muriata Colombia
CMW8856 Citrus Colombia
CMW17808 C. neglecta
CMW18194 C. neglecta
CMW9565 Citrus Colombia
CMW9572 Citrus Colombia
CMW5751 Coffee Colombia
CMW14802 C. platani
CMW23918 C. platani
CMW14276 C. taisiakammmensis
CMW14278 C. taisiakammmensis
CMW15991 C. tanginecans
CMW15999 C. tanginecans
CMW6569 C. pirilliformis
CMW6579 C. pirilliformis
CMW15236 C. zambamontana
CMW15235 C. zambamontana
CMW23808 C. obpyriformis
CMW23807 C. obpyriformis
CMW23809 C. polycondia
CMW23818 C. polycondia
CMW11424 C. polychroma
CMW11436 C. polychroma
CMW19383 C. atrox
CMW19385 C. atrox
CMW4068 C. abfilundus
CMW5329 C. abfilundus
CMW25434 Styrax benzoin Indonesia
CMW25436 Styrax benzoin Indonesia
CMW25437 Styrax benzoin Indonesia
CMW25435 Styrax benzoin Indonesia

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.
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 fimбриata 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 fusce pirliformes inornatae (101–)120–184 (–243) μm latae. Conidia primaria doliformia vel obtusa, (6–)7–9 (–13) μm longa 4–6 (–7) μm lata. Chlamydosporae concolorae, (44–)50–86 (–99) μm longa, 4–6 μm lata. Conidia ascosporae oblongae, (8–)11–21 (–28) μm longa, (2–)3–5 (–6) μm lata. Conidiali phialidic, apices wide, (44–)50–86 (–99) μm longa, 4–6 (–7) μm lata. Chromyosporae hair-brown (17°), prolate spheroidal to perprolatae, (8–)9–14 (–16) μm longa, (7–)8–10 (–11) μm lata.

Habitat — Wounds on Styrax benzoin trees. Known distribution — Northern Sumatra, Indonesia.

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. fimбриata 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 caroten 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 taxa. 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. fimбриata s.l. species complex with no species phylogenically 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. fimбриata s.l. species complex.

Morphologically, C. larium is similar to other species in the C. fimбриata 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 ascospatal bases. However, the cylindrical conidia in C. larium 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. Chlamydosporae hair-brown (17°), prolate spheroidal to perprolatae, (8–)9–14 (–16) μm longa, (7–)8–10 (–11) μm lata.

Habitat — Wounds on Styrax benzoin trees. Known distribution — Northern Sumatra, Indonesia.
Ceratocystis larium 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 *Styrax 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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