Rostraureum tropicale gen. sp. nov. (Diaporthales) associated with dying Terminalia ivorensis in Ecuador

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Terminalia ivorensis, a tree of central African origin, is planted in several tropical countries for timber and veneer production. During the course of a recent disease survey, an unknown fungus was found associated with basal cankers on dying T. ivorensis in Ecuador. The fungus has orange fruiting structures and septate, fusoid ascospores, similar to those of Cryphonectria, a well-known genus of canker pathogens. The aim of this study was to identify the fungus and to assess its pathogenicity. Identification was based on morphological characteristics as well as DNA sequence data. DNA sequence data from the ITS regions of the rDNA operon and two regions of the b-tubulin gene, were compared with published sequences of Cryphonectria species and the closely related genera Endothia and Chrysoporthe. Pathogenicity tests were conducted on T. superba saplings. Morphological characterisations revealed that the conidiomata of the fungus from T. ivorensis, differed from those typical of Cryphonectria in being superficial and rostrate. Only Cryphonectria longirostris was similar to the fungus from T. ivorensis, but could be distinguished from it based on conidial size. Phylogenetic analyses showed that the fungus from T. ivorensis grouped closely with species of Cryphonectria, Chrysoporthe and Endothia, yet formed a distinct clade. Pathogenicity tests on T. superba provided evidence that the fungus is able to cause distinct stem cankers. We conclude that the pathogenic fungus from T. ivorensis represents a new genus and new species in the Diaporthales and we provide the name Rostraureum tropicale for it. The genus is typified by R. tropicale. Furthermore, C. longirostris is transferred to Rostraureum.

INTRODUCTION

Terminalia ivorensis (Combretaceae, Myrtales) is native to the rainforests of Central Africa (Lamb & Ntima 1971). A similar species, T. superba, also occurs in tropical central Africa (Groulez & Wood 1985). Both trees are planted in the tropics as a source of high quality solid timber and veneer. These trees grow rapidly, have straight stems, are self-pruning and have tended to display a natural resistance to pests and pathogens (Lamb & Ntima 1971, Groulez & Wood 1985).

Few pathogens have been reported from Terminalia spp. A Sphaeronaema sp. has been associated with dieback of T. ivorensis in nurseries (Lamb & Ntima 1971) and an Endothiella sp. has also been found on cankers on T. ivorensis in Ghana (Ofosu Siedu & Cannon 1976).

In Brazil, Korinomyces terminaliae causes leaf spots on seedlings and young T. ivorensis plants (Hodges & Fereira 1981), and Auerswaldiella parvispora causes black blotches on leaves (Farr 1989). Root rot caused by species of Rosellinia and Phytophthora, leads to dieback of T. ivorensis in Panama and Costa Rica (Kapp, Beer & Lujan 1997). Some foliage diseases caused by unidentified species of Cercospora, Ramularia, Irenina and Spaceloma have been reported from T. superba in Africa (Groulez & Wood 1985).

T. ivorensis and T. superba are cultivated in Ecuador where both perform well, although T. ivorensis trees are prone to unexplained deaths. This study emerged from surveys aimed at gaining an understanding of these deaths. A possible causal agent of basal cankers on dying T. ivorensis trees was sought and identified based on morphological characteristics and DNA sequence analyses.
Table 1. Isolates of Rostraureum tropicale, Chrysoporthe, Cryphonectria and Endothia spp. used for DNA sequence comparisons and growth study.

<table>
<thead>
<tr>
<th>Culture number&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Alternative number&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Identification</th>
<th>Host</th>
<th>Origin</th>
<th>Genbank accession number&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
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<tr>
<td>CMW 8756</td>
<td>–</td>
<td>Chrysoporthe cubensis</td>
<td>Eucalyptus sp.</td>
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<td>AF 046896, AF 273077, AF 285165</td>
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<tr>
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<td>–</td>
<td>C. cubensis</td>
<td>E. marginata</td>
<td>Australia</td>
<td>AF 046893, AF 273078, AF 375607</td>
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<td>CMW 8757</td>
<td>–</td>
<td>C. cubensis</td>
<td>E. grandis</td>
<td>Venezuela</td>
<td>AF 046897, AF 273069, AF 273464</td>
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<tr>
<td>CMW 8758</td>
<td>–</td>
<td>C. cubensis</td>
<td>E. grandis</td>
<td>Venezuela</td>
<td>AF 046898, AF 273068, AF 273463</td>
</tr>
<tr>
<td>CMW 1853</td>
<td>–</td>
<td>C. cubensis</td>
<td>Syzygium aromaticum</td>
<td>Brazil</td>
<td>AF 046891, AF 273070, AF 273465</td>
</tr>
<tr>
<td>CMW 8755</td>
<td>–</td>
<td>C. australiacafricana</td>
<td>E. grandis</td>
<td>South Africa</td>
<td>AF 292040, AF 273064, AF 273458</td>
</tr>
<tr>
<td>CMW 2113</td>
<td>CBS 112916</td>
<td>C. australiacafricana</td>
<td>E. grandis</td>
<td>South Africa</td>
<td>AF 046892, AF 273067, AF 273462</td>
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<tr>
<td>CMW 7047</td>
<td>ATCC 48197, E5</td>
<td>Cryphonectria parasitica</td>
<td>Quercus</td>
<td>MS, USA</td>
<td>AF 368329, AF 273073, AF 273469</td>
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<tr>
<td>CMW 7048</td>
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<td>Q. virginiana</td>
<td>VA, USA</td>
<td>AF 368330, AF 273076, AF 273470</td>
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<tr>
<td>CMW 10477</td>
<td>CBS 240.54, E76</td>
<td>C. radicalis</td>
<td>Castanea sativa</td>
<td>Italy</td>
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<td>CBS 238.54, E42</td>
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<td>C. dentata</td>
<td>Italy</td>
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<td>CBS 112919, E53</td>
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<td>Quercus sp.</td>
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<td>AF 452118, AF 525706, AF 525713</td>
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<td>CBS 112920, E54</td>
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<td>Terminalia</td>
<td>Ecuador</td>
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<td>CBS 115727</td>
<td>R. tropicale</td>
<td>T. iorensis</td>
<td>Ecuador</td>
<td>AY 167429, AY 167434, AY 167439</td>
</tr>
<tr>
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<td>ATCC 48192, E13</td>
<td>Endothia gyrosa</td>
<td>Q. palustris</td>
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<td>AF 368325, AF 368337, AF 368336</td>
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<td>VA, USA</td>
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<td>CBS 112921, E58</td>
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<td>CO, USA</td>
<td>AF 368323, AF 368333, AF 368332</td>
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<tr>
<td>CMW 5288</td>
<td>CBS 112900</td>
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<td>Malus domestic</td>
<td>South Africa</td>
<td>AF 543817, AF 543819, AF 543821</td>
</tr>
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<td>CMW 5587</td>
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<td>M. domesticia</td>
<td>South Africa</td>
<td>AF 543818, AF 543820, AF 543822</td>
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</table>

<sup>a</sup> Culture collection of the Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria.
<sup>b</sup> Isolates sequenced in this study are in bold. The other sequences were obtained from Myburg et al. (1999, 2002b, 2004) and Venter et al. (2002).
<sup>c</sup> Numbers preceded with E are designated numbers in the collection of R. Jay Stipes, incorporated in the culture collection of FABI; ATCC, American Type Culture Collection (Manassas); CBS, Centraalbureau voor Schimmelcultures (Utrecht).
<sup>d</sup> CMW 9972, CMW 10796 were obtained from the holotype specimen PREM 57519; CMW 9971 was obtained from specimen PREM 583301.

MATERIALS & METHODS

Disease symptoms and specimens

Dead and dying Terminalia iorensis trees were inspected in plantations in the lowland tropics of Ecuador. All trees were mature and ranged in age from 13–15 yr. Trees appeared to have declined relatively rapidly and diffuse cankers were present in the root collar region. A fungus with yellow to orange fruiting structures was abundant on the surface of the dead tissue. The fungus was also found on the stumps of recently felled T. superba, but these could not be positively connected with a disease problem.

Specimens of the fungus were collected on bark from the surface of cankers and transported to the laboratory. Single conidial and ascospore suspensions were made by suspending spore masses in sterile water, and spreading these onto the surface of malt extract agar [MEA, 20 g l⁻¹ malt extract agar (Biolab, Merck, Midrand, South Africa)]. Single germ tubes emerging from the spores were transferred to new MEA plates and incubated at 25 °C. Pure cultures have been preserved at 5 °C in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI; University of Pretoria, Pretoria; Table 1), and representative cultures have been deposited in the collection of the Centraalbureau voor Schimmelcultures (CBS; Utrecht). Bark specimens bearing fruiting structures were preserved for morphological comparisons and have been deposited in PREM (National Collection of Fungi, Pretoria; Table 2).

Morphology

Fruiting structures were cut from the bark and boiled in water for 1 min to rehydrate the cells. The structures were then embedded in Leica mountant and sectioned with a Leica CM1100 cryostat (Setpoint Premier, Johannesburg). Sectioning was carried out at −20 °C. Sections 12–16 μm thick, were dropped in water, transferred to microscope slides and mounted in lactophenol. For the holotype specimen, 50 measurements in lactophenol or 3% KOH were taken of ascospores, asci, conidia and conidiophores, and are presented as...
### Table 2. Specimens used in morphological comparisons.

<table>
<thead>
<tr>
<th>Reference collectiona</th>
<th>Identity</th>
<th>Name of specimen</th>
<th>Host</th>
<th>Origin</th>
<th>Date</th>
<th>Collector</th>
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<tr>
<td>PREM 57519 (holotype)b</td>
<td>Rostranceum tropicale</td>
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<td>Terminalia</td>
<td>Ecuador</td>
<td>2001</td>
<td>M. J. Wingfield</td>
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<tr>
<td>PREM 583301b</td>
<td>R. tropicale</td>
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<td>T. ivoirensis</td>
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<td>2001</td>
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<td>PREM 583302</td>
<td>R. tropicale</td>
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<td>T. ivoirensis</td>
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<td>R. tropicale</td>
<td>–</td>
<td>T. ivoirensis</td>
<td>Ecuador</td>
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</tr>
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<td>T. ivoirensis</td>
<td>Ecuador</td>
<td>2001</td>
<td>M. J. Wingfield</td>
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<td>NY 4350</td>
<td>R. longirostris</td>
<td>Cryphonectria longirostris</td>
<td>Fallen tree</td>
<td>Puerto Rico</td>
<td>1900</td>
<td>A. Heller</td>
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<td>NY 617</td>
<td>R. longirostris</td>
<td>C. longirostris</td>
<td>–</td>
<td>Puerto Rico</td>
<td>1923</td>
<td>F. J. Seaver &amp; C. E. Chardon</td>
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<td>R. longirostris</td>
<td>C. longirostris</td>
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<td>1915</td>
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<td>NY 3320</td>
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<td>C. longirostris</td>
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<td>PDD 28477</td>
<td>Unknown</td>
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<td>BPI 631857 (holotype)</td>
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<td>Cuba</td>
<td>1916</td>
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<td>Sri Lanka</td>
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<td>Japan</td>
<td>1954</td>
<td>T. Kobayashi</td>
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<td>–</td>
<td>Q. phellos</td>
<td>Raleigh, USA</td>
<td>1997</td>
<td>L. Grand</td>
</tr>
</tbody>
</table>

a PREM, National Collection of Fungi (Pretoria); NY, New York Botanical Garden (Bronx, New York); PDD, Landcare Research (Mt Albert, Auckland); IMI, CABI Bioscience (Egham, Surrey); BPI, US National Fungus Collections (Beltsville); K, Royal Botanic Gardens (Kew, Surrey); TFM, FPH, Forestry and Forest Products Research Institute (Dunchi-Nai, Japan); CUP, Plant Pathology Herbarium (Cornell University, Ithaca, New York).

b CMW 9972, CMW 10796 obtained from the holotype specimen PREM 57519; CMW 9971 were obtained from specimen PREM 583301.

(min–)(average – s.d.) – (average + s.d.)(–max) μm. A range of measurements was obtained from at least ten structures for the anamorph and teleomorph stromata and perithecia respectively, and at least ten anamorph and teleomorph structures were sectioned to study their internal morphology. Standard colour notations provided by Rayner (1970) were used to describe various elements of the fungus.

The fungus associated with basal cankers on *T. ivoirensis* in Ecuador clearly had characteristics similar to those of species of *Cryphonectria* and *Endothia* (shared anamorph: *Endothiella*), and *Chrysoporthe* (anamorph: *Chrysoporthea*). *Chrysoporthea* is a newly described genus accommodating the fungus previously known as *Cryphonectria cubensis* (Gryzenhout et al. 2004). The fungus from *T. ivoirensis* was thus compared with specimens representing species of *Cryphonectria*, *Endothia* and *Chrysoporthe*. One species, *Cryphonectria longirostris* (Earle) Micales & Stipes, was found to be superficially similar to the fungus from *T. ivoirensis*. Additional specimens of this fungus were thus included in this study for comparative purposes (Table 2). These specimens originated from dead plant material in Puerto Rico, Trinidad and New Zealand and were obtained from various herbaria. Specimens connected to the *Endothiella* species reported from cankers on *T. ivoirensis* in Ghana (Ofosu Siedu & Cannon 1976), as well as another specimen labelled as *C. gyrosea* from *T. ivoirensis* in Kenya, were also examined (Table 2).

Growth in culture of isolates CMW 9973 and CMW 10796 (Table 1), was assessed. CMW 10796 originated from the holotype specimen. These studies were conducted on MEA (20 g l−1 malt extract agar; Biolab, Midrand) as described by Venter et al. (2002). Growth tests were conducted in the dark at temperatures ranging from 15–35 °C, at 5 °C intervals.

### DNA isolations and PCR amplifications

DNA was isolated from isolates using the method described by Rayner (1970). Two β-tubulin gene regions were amplified using the primer pairs Bt1a/Bt1b and Bt2a/Bt2b respectively (Glass & Donaldson 1995). The ITS1 and ITS2, as well
as the conserved 5.8S gene of the ribosomal RNA operon, were amplified using primers ITS-1 and ITS-4 (White et al. 1990). PCR reactions were done according to Myburg et al. (1999) for the ribosomal operon, and Myburg et al. (2002b) for the β-tubulin genes. PCR amplifications were performed on a Perkin Elmer GeneAmp PCR System 9700 thermocycler (Perkin-Elmer Applied Biosystems, Foster City, CA). Sizes of PCR products were verified on 1% agarose-ethidium bromide gels using a UV light source.

**Sequencing and analysis of sequence data**

PCR products were cleaned using a QIAquick PCR Purification kit (Qiagen, Hilden). These were sequenced in both directions using the same primer pairs that were used in the amplification reactions. Sequencing reactions were conducted using an ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington). DNA sequences were determined using an ABI PRISM 3100™ automated DNA sequencer.

Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems) was used to edit the raw sequence data and sequences were manually aligned to already existing datasets from Myburg et al. (2004). Phylogenetic analyses were done using PAUP version 4.0 b (Swofford 1998). To test whether the ITS and β-tubulin datasets were homogenous in phylogenetic analyses and thus combinable, a 500 replicate partition homogeneity test (PHT) (Farris et al. 1994) were done on the two partitions. Results were confirmed with the Templeton Nonparametric Wilcoxon Signed Ranked test (Kellogg, Appels & Mason-Gamer 1996). Alignments were analysed using parsimony and heuristic searches with TBR (tree-bisection-reconnection) and MULTREES (saving all optimal trees) options effective, and random additions set to 100. Uninformative sites were excluded and base pairs were reweighted according to their CI. Gaps inserted during manual sequence alignment, were treated as missing in the heuristic searches. Alignments were also subjected to distance analyses and the appropriate distance model for the datasets were determined with MODELTEST version 3.5 (Posada & Crandall 1998). The confidence levels of the branching points were determined by a 70% bootstrap analysis of 1000 replications (Felsenstein 1985). *Diaporthe ambigua* isolates, which also resides in the *Diaporthales* (Castelbury et al. 2002), were used as outgroup taxon to root the phylogenetic tree. Sequences were deposited in GenBank and accession numbers are listed in Table 1.

**Pathogenicity tests**

Pathogenicity of the fungus from *Terminalia ivorensis* could not be tested on *T. ivorensis* as trees of this species are rare and difficult to obtain in Ecuador. For this reason, 20 saplings of the related *T. superba* were inoculated in February 2000. An isolate of *Chrysosporthe cubensis* (syn. *Cryphonectria cubensis*) an important pathogen of *Eucalyptus* spp. (Hodges et al. 1976, Sharma, Mohanan & Florence 1985) and clove (*Syzygium aromaticum*, Hodges, Alfenas & Ferreira 1986), was also inoculated onto *T. superba* for comparative purposes.

The saplings for inoculations were approx. 2 yr-old at the time of inoculation. Using a metal punch, approx. 5 mm diam, bark was removed from the stems of trees, approx. 10 cm above ground level. Discs of agar bearing mycelium of the fungus were taken from the actively growing edges of a culture and placed, mycelium side downwards, into the wounds. An equal number of plants were inoculated with sterile agar to serve as controls. Wounds were covered with masking tape to prevent desiccation. Inoculated plants were allowed to grow for six weeks before examination. Masking tape was then removed and lesion lengths were measured. The measurements were subjected to a one-way ANOVA analysis and differences between the inoculation sets were determined using a Bonferroni test (SYSTAT 1996).

**RESULTS**

**Morphology**

The fungus from *Terminalia ivorensis* is typically diaporthalean, with periphysate ostiolar canals, no paraphyses present, and ununiticate asci with refractive apical rings (Barr 1978). The orange to yellow fruiting structures are reminiscent of those in *Cryphonectria*, *Chrysosporthe* and *Endothia* (Shear, Stevens & Tiller 1917, Barr 1978, Micales & Stipes 1987, Gryzenhout et al. 2004). The ascospores are one-septate, fusoid to ellipsoid, and similar to those in species of *Cryphonectria* and *Chrysosporthe*, but different from those in *Endothia* (Shear et al. 1917, Barr 1978, Micales & Stipes 1987).

Although the stromata are peripherally similar on the bark, the fungus from *T. ivorensis* is distinctly different to species of *Endothia, Cryphonectria* and *Chrysosporthe*. The perithecial necks of the fungus from *T. ivorensis* are not lodged within well-developed stromatic tissue, as is found in *Cryphonectria* (Barr 1978, Micales & Stipes 1987, Myburg et al. 2004). Instead, the only tissue development, made visible through longitudinal sections, is a sheath of white tissue covered with an orange to luteous-pure yellow layer around the perithecial necks (Figs 1B–C, 2B). In some cases, orange remnant tissue of the anamorph was present on top of the perithecia or orange, rudimentary stromatic tissue occurred between the necks (Figs 1B, 2B). This tissue structure is similar to that found in *Chrysosporthe*, but perithecial necks protruding from the stromatal surfaces in species of *Chrysosporthe* appear fuscous-black (Myburg et al. 2003, 2004, Gryzenhout et al. 2004), while those in the *Terminalia* fungus are orange.
Another distinct difference between *Cryphonectria* and the *Terminalia* fungus is that the anamorph of *Cryphonectria* is usually semi-immersed, pulvinate, convoluted, unilocular to multilocular stromatic (Shear et al. 1917, Kobayashi 1970, Myburg et al. 2004). The anamorph of the fungus from *T. ivorensis* is superficial to slightly immersed, clavate or rostrate, with long, attenuated necks, unilocular and convoluted at the base (Figs 1G–H, 2D–E). The *Chrysoporthella* ana-

morp of *Chrysoporthe* has similar conidiomata, but

the structures of *Chrysoporthella* are fuscous-black and pyriform (Gryzenhout et al. 2004).

One species of *Cryphonectria*, *C. longirostris*, had

the same stromatal characteristics as the fungus from

*T. ivorensis*. Specimens of *C. longirostris* from Puerto Rico (NY 4340, NY 816, NY 617, NY 266417, NY
6576) had the same orange, superficial, rostrate conidiomata with attenuated necks (Figs 3A, 3E, 4D–E). These occur singly or on top of the teleomorph stromata (Figs 3A–B, 3E, 4A, 4D–E). The necks of the perithecia are also surrounded with a white sheath of tissue covered with an orange layer (Figs 3C, 4B). Furthermore, perithecia are umber to fulvous in both *C. longirostris* and the fungus from *T. ivorensis*.

Although similar, the fungus from *T. ivorensis* could be distinguished from *C. longirostris* based on a number of morphological characteristics. The conidia of *C. longirostris* (Figs 3L, 4F) are shorter (3–3.5 μm) than those of the fungus from *T. ivorensis* ((3–)3.5–5(–6) μm; Figs 1L, 2F). Although variation associated with different hosts and environments might contribute to the following differences (Shear et al. 1917, Hodges et al. 1986, Myburg et al. 2003), structures of *C. longirostris* are also more complex than those of the fungus from *T. ivorensis*. The pulvinate structures that usually contain the perithecial necks of

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**Fig. 2.** Schematic drawings of *Rostraureum tropicale* (from holotype PREM 57519). (A) Ascomata on bark. (B) Section through ascoma. (C) Asci and ascospores. (D) Conidiomata on bark. (E) Section through conidioma. (F) Conidiophores, conidiogenous cells and conidia. Bars: A–B and D–E = 100 μm; and C and F = 10 μm.
C. longirostris, frequently have strongly convoluted pycnidial locules in the upper parts with extensive tissue development, and with perithecial bases lodged in the bark, in the lower parts. The anamorph structures of the fungus from T. ivorensis are less convoluted and perithecia and conidial locules are lodged in little to no stromatic tissue. Conidiomata on the C. longirostris specimens were generally larger, bases 600–1300 μm high, 270–880 μm wide, and necks 1011–2050 μm long, than the conidiomata on the specimens from T. ivorensis, base 400–600 μm high, 150–500 μm wide, and neck 900–1450 μm long.

The cells giving rise to the conidiophores in the pycnidial cavities of the fungus from T. ivorensis and C. longirostris frequently contained orange crystals (Figs 3F–G). Thus the linings of the pycnidial locules are bright orange, in comparison to the remainder of the stromatic tissues (Fig. 3F). Other crystals, different
in form and colour, could also be found in the stromatic tissue. This was found to be a variable characteristic and crystals were not present in all specimens.

The fungal structures on the specimen from *T. ivorensis* in Ghana (IMI 187898), which were connected to the report of Ofosu Siedu & Cannon (1976), were different from those of the fungus on *T. ivorensis* in Ecuador. The specimen from Ghana had orange, pulvinate conidiomata with no elongated necks, and ascomata were clearly stromatic without the characteristic sheath of tissue around the perithecial necks. The specimen (IMI 288729) from *T. ivorensis* in Kenya was also different from the Ecuador samples, since it contained ascostromata without sheaths of tissue around the perithecial necks. These African specimens (IMI 187898, IMI 288729) had uniseptate, fusoid to ellipsoid ascospores and minute, cylindrical conidia and could possibly reside in *Cryphonectria*. Ascospores of the two specimens have overlapping ascospore dimensions (9.5–)10–11(–12) × (3–)3.5–4(–4.5) μm for IMI 187898,
and (8–)8.5–10(–11.5) × (3–)3.5–4 μm for IMI 288729. Spore dimensions in these specimens thus resembled those published for C. havanensis (Brunner 1916, Kobayashi 1970, Roane 1986a). However, more extensive comparisons would need to be carried out to verify the identity of these specimens.

A number of specimens labelled C. longirostris were examined in this study and clearly do not represent this fungus. Specimen NY 3360 from Trinidad had pulvinate to conical conidiomata with bright orange exteriors, scarlet to rust interiors and the linings of the pycnidial locules were pale luteous. Specimen NY 3098, also from Trinidad, had conical conidiomata with hazel to rust tissue surrounding an orange interior. Ascomata of this specimen were hazel to rust. A specimen from Puerto Rico (NY 511), had pulvinate, semi-immersed, multilocular conidiomata different from the superficial and clavate conidiomata of C. longirostris, with small, orange ascomata. The sheath of tissue around the perithecial necks, characteristic of C. longirostris, was also absent. Another specimen from Puerto Rico (NY 1053) had orange, oval, superficial conidiomata. Another C. longirostris specimen mentioned by Roane (1986a), PDD 28477 from New Zealand, also lacked the clavate anamorph, but had pulvinate, semi-immersed, multilocular conidiomata. These fungi are not treated further in this study, but we believe that they probably represent undescribed taxa closely related to Cryphonectria and its allies.

Sequencing and analysis of sequence data

The datasets consisted of 22 taxa of which the two Diaporthe ambigua isolates were defined as the outgroup. Results generated with the PHT analyses (P = 0.03) and Templeton Nonparametric Wilcoxon Signed Ranked test indicated that the rDNA and β-tubulin sequence data sets were significantly incongruent and could not be combined as one data set in the phylogenetic analyses. These data sets were consequently analysed separately. The ribosomal DNA sequence alignment consisted of 557 characters of which 339 were constant, 13 were parsimony-uninformative and 205 were parsimony-informative. The dataset showed significant phylogenetic signal (gI = −1.148).

The heuristic search resulted in one most parsimonious tree (tree length = 306, CI = 0.838, RI = 0.912). The Kimura-2 parameter model (Kimura 1980) with a Gamma distribution shape parameter (G) of 0.1979 and the proportion of invariable sites (I) as 0.5437, was suitable for the dataset. The tree obtained with parsimony essentially showed the same groupings as the tree obtained with distance analyses, thus only the tree obtained with distance methods was chosen for presentation (Fig. 6).

The phylogenetic trees obtained from the ribosomal DNA and β-tubulin datasets all showed the same number of well-supported clades, although the relationships between clades differed (Figs 5–6). The first clade in the phylogram typified Cryphonectria (bootstrap support 100% in Fig. 5, 74% in Fig. 6). This group of fungi has been the subject of intensive study in recent years (Myburg et al. 2002a, b, 2003, Gryzenhout et al. 2004). The clade representing the genera Cryphonectria (bootstrap support 94% in Fig. 5, 81% in Fig. 6) was defined by C. parasitica, C. radicalis, C. nitschkei and C. macrospora. Endothia gyrosa and E. singularis represented the genus Endothia, although not always forming a well-defined clade. Myburg et al. (2004) previously considered the taxonomy and DNA-based phylogeny of these species.

The unknown fungus isolated from Terminalia ivorensis grouped separately from Cryphonectria, Cryphonectria and Endothia in all analyses based on different areas of the genome (Figs 5–6). Although grouping separately, evolutionary relationships between the different clades differed in the analyses based on ribosomal DNA and β-tubulin genes. This separate grouping is supported by a bootstrap value of 100% for both areas sequenced, indicating that new genus and species designations should be considered for the fungus from T. ivorensis. Regrettably, no isolates representing Cryphonectria longirostris or the fungi from T. ivorensis in Africa, exist and comparisons with the new fungus are impossible at present.

Pathogenicity

Within 6 wk, Terminalia superba plants inoculated with the fungus from dying T. ivorensis showed well-developed stem cankers (Figs 7A–B). Cankers were 36–84 mm long and were clearly in the process of girdling the stems. ANOVA showed that lesion lengths associated with the inoculated and control plants were significantly different to each other (P < 0.0001). The Bonferroni test showed that lesions caused by Cryphonectria cubensis (Fig. 7C) were significantly smaller than those caused by the fungus from T. ivorensis (P < 0.0001). Wounds used to make control inoculations were covered with callus and stem discoloration was equal in length to the size of the original inoculation wound (Fig. 7D).
Morphological characteristics and phylogenetic data provide good evidence supporting the view that the fungus from *Terminalia ivorensis* represents an undescribed species that should reside in a new genus in the Diaporthales. *Cryphonectria longirostris* is similar to this fungus and should be transferred to the new genus as a second species. *C. longirostris* can be distinguished from the fungus from *T. ivorensis* by its smaller conidia.

**TAXONOMY**

Fig. 5. Distance phylogram showing phylogenetic relationships between *Rostraureum*, *Cryphonectria*, *Chrysoporthe* and *Endothia* spp. based on ITS1/ITS2 DNA sequence of the ribosomal operon. The phylogram was obtained with the Kimura 2 parameter model (G = 0.1979). Bootstrap values greater than 70% (1000 replicates) are indicated at the branch nodes. The *Diaporthe ambigua* isolates were used as outgroup taxa to root the phylogenetic tree.
and larger conidiomata. The fungus from *T. ivorensis* is provided as the type of this new genus, since isolates and DNA sequences are available for this fungus. A more complete and illustrated description of *C. longirostris* is also provided, where features relevant to the new genus in which it now resides, are emphasized.

**Rostraureum** Gryzenh. & M. J. Wingf., **gen. nov.**

*Etym.*: *rostrum* (a beak); and *aureus* (golden), so a golden beak.

*Ascostromata* flava vel aurantiaca, textura stromatali carenti vel praesenti. Colla *perithecialia* vagina texturae porrectae

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**Fig. 6.** Distance phylogram showing phylogenetic relationships between *Rostraureum, Cryphonectria, Chrysoporethe* and *Endothia* spp. based on β-tubulin DNA sequence. The phylogram was obtained with the Tamura Nei parameter model (I = 0.5437, G = 0.7905). Bootstrap values greater than 70% (1000 replicates) are indicated at the branch nodes. The *Diaporthe ambigu* isolates were used as outgroup taxa to root the phylogenetic tree.


Ascostromata erumpent, luteous-pure yellow to orange, consisting of perithecia embedded in bark tissue, with necks erumpent and volsoid, occasionally occurring underneath active pycnidial locules, stromatal tissue absent or present between the necks. Perithecia umber to fulvous, bases globose to subglobose, necks periphysate, surrounded by sheath of white textura porrecta, cells on outside of erumpent perithecial necks of textura globulosa and orange to luteous-pure yellow. Asci fusoides, non-stipitate, unitunicate, with non-amyloid refractive apical ring, octasporous. Ascospores fusoid to ellipsoid with rounded apices, hyaline, one-septate.

Conidiomata eustromatic, clavate or rostrate, superficial to slightly immersed, unilocular, even to strongly convoluted lining, luteous-pure yellow to orange, one to three attenuated necks, base tissue of textura epidermoidea, neck tissue of textura porrecta with thickened cells at surface. Conidiophores hyaline, consisting of a basal cell, branched irregularly at the base or above into cylindrical cells, delimited by septa or not. Conidiogenous cells phialidic, determinate, apical or lateral on branches beneath the septum. Conidia cylindrical, hyaline, aseptate.

Rostraureum tropicale Gryzenh. & M. J. Wingf., sp. nov. (Figs 1–2)

Etym.: tropicus (tropics), refers to the discovery of this fungus in the tropics.


Ascostromata semi-immersed with pulvinate appearance under dissection microscope (Figs 1A–B, 2A), 544–711 μm wide above bark surface where necks converge, stromatal tissue between perithecia (Fig. 1B) usually absent in sections (Fig. 2B), luteous-pure yellow when young, orange when older. Up to 11 perithecia, in volsoid configuration, bases 310–460 μm wide, globose to sub-globose, surrounded by host tissue, umber to fulvous, wall 15–28 μm thick (Figs 1B, 2B). Perithecial necks 45–90 μm wide, periphysate, umber, surrounded by host tissue, umber to fulvous, wall 15–28 μm thick (Figs 1B, 2B).
by tissue sheath with the cells next to the perithecial neck white, of *textura porrecta*, and the cells on the outer edge of the sheath orange to luteous-pure yellow, of *textura globulosa* (Fig. 1C), neck with surrounding tissue 143–225 μm wide and 250–700 μm long when it emerges above bark surface. *Asci* (23–)27–32(–35.5) × (5.5–)6–7.5(–10.5) μm, fusoid, free when mature, non-stipitate, uniloculate, with non-amyloid apical ring (Figs 1D, 2C). *Ascopores* 8 per ascus, (4–)6–8.5(–9) × 2–3(–3.5) μm, hyaline, fusoid to ellipsoid, sometimes slightly curved, apices rounded, single septum median or off-median (Figs 1E, 2C).

**Conidiomata** eustromatic, clavate or rostrate with neck attenuated or not (Figs 1G–H, 2D), base 400–600 μm high, 150–500 μm wide, neck 900–1450 μm long, 100–200 μm wide, superficial to slightly immersed, unilocular, even to convoluted lining, perithecia occasionally forming underneath conidiomata, luteous-pure yellow when young, orange when mature. **Locules** 40–280 μm at widest point, usually single conidial locule in center opening through neck, longitudinal sections at edge of base reveal more than one locule due to convoluted lining (Figs 1H, 2E). Basal tissue of *textura epidermoidea* (Fig. 1F), tissue at the junction between neck and base of *textura intricata* and neck tissue of *textura porrecta* with thicker cells at edges of neck (Fig. 1I). *Conidiophores* hyaline, with a globular to rectangular basal cell, (3–)3.5–5.5–7(–) × 2–12.5–4.5(–)6 μm, branched irregularly at the base or above into cylindrical cells, cells delimited by septa or not, total length of conidiophore (12–)15–21(–24.5) μm (Figs 1J–K, 2F). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath the septum, cylindrical to flask-shaped with attenuated apices, 1.5–2(–2.5) μm wide, collarette and periclinal thickening inconspicuous (Figs 1J–K, 2F). *Conidia* (3–)3.5–5(–6) × 1.5–2 μm, cylindrical, asperate, hyaline, exuded as brick red spore droplets (Figs 1L, 2F).

**Cultures** on MEA suppressed with sparse aerial hyphae when young, remaining suppressed when older, young cultures creamy white with a luteous interior, older cultures are orange to luteous with or without white margins, margins even, conidiomata occasionally produced in mature cultures, optimum growth from 25–30 °C, isolates covering 90 mm plates after 6 days at optimum growth temperatures.

**Ecology:** Bark of *Terminalia ivorensis* and *T. superba*

**Distribution:** Ecuador.

**Additional material examined.** **Ecuador:** Pichincha: Rio Pitzara (0° 15′ 27″ N, 79° 7′ 43″ W, 350 meters above sea level), bark of *Terminalia ivorensis*, Nov. 2001, M. J. Wingfield (PREM 583301, PREM 583302, PREM 583303, PREM 583304, living culture CMW 9971 from PREM 583301).

**Rostraureum longirostris** (Earle) Gryzenh. & M. J. Wingf., comb. nov. **(Figs 3–4)**


**Synonym:** *Diatrype radicalis* (Schwein.: Fr.) Fr., *Ann. Sci. Nat. (Bot.)* 4: 123 (1855).


**Ascomata** semi-immersed, pulvinate, 700–950 μm wide above bark surface, orange, prosenchymatous stromatal tissue usually present in erumpent part of stromata and containing conidial locules and perithecial necks, perithecial bases at base of structures surrounded by host tissue (Figs 3A–B, 4A–B). Up to 15 perithecia per structure, valsid, bases (250–)285–408(–420) μm wide, globose to sub-globose, umbra to fulvous, wall 13–20(–25) μm thick (Figs 3B, 4B). Perithecial necks (50–)52–78(–90) μm wide, periphysate, umbra, surrounded by tissue sheath with the cells alongside the perithecial necks white, of *textura porrecta*, and cells at the outer edge of sheath luteous-pure yellow to orange, of *textura globulosa* (Fig. 3C); neck with surrounding tissue (140–)156–205(–213) μm wide and (400–)450–600(–650) μm long where they emerge above bark surface (Fig. 3A). No intact *asci* were observed, but according to Earle (1901) *asci* are spindle-shaped, thin-walled, 25–30 × 6 μm with no paraphyses (Fig. 4C). *Ascopores* 8 per ascus, (5–)6–7.5(–9) × 2–3(–3.5) μm, fusoid to ellipsoid, apices rounded, hyaline, single septum median or off-median (Figs 3D, 4C).

**Conidiomata** eustromatic, clavate or rostrate with necks attenuated or not, bases 600–1300 μm high, 270–880 μm wide, necks 1011–2050 μm long, 175–288 μm wide, superficial to slightly immersed, unilocular and convoluted, occurring alone or with teleomorph structures forming below, orange (Figs 3A–B, 3E, 4D–E). Locules 230–1500 μm wide at widest point, usually single pycnidial locule at center, opening through neck, length sections at edges of base reveal more than one locule due to convoluted lining (Figs 3E–F, 4E). Base tissue of *textura epidermoidea* (Fig. 3H), tissue where neck and base join of *textura intricata* (Fig. 3H), neck tissue of *textura porrecta* with thicker cells at edges of neck (Fig. 3I). *Conidiophores* hyaline, with a globular to rectangular basal cell, (2–)3–5(–7.5) × (1.5–)2.5–3.5(–4.5) μm, branched irregularly at the base or above into cylindrical cells, cells delimited by septa or not, total length of conidiophore (13–)15–19.5(–22.5) μm (Figs 3J–K, 4F). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath the septum, cylindrical to flask-shaped with attenuated apices, 1.5–2(–2.5) μm wide, collarette and periclinal thickening inconspicuous (Figs 3J–K, 4F). *Conidia* (3–)3.5–5(–6) × 1.5–2 μm, cylindrical, asperate, hyaline, exuded as brick red spore droplets (Figs 3L, 4F).

**Ecology:** dead logs and branches

**Distribution:** Puerto Rico, French Guiana, and Trinidad and Tobago.

**Material examined:** **Puerto Rico:** east of Santurce, bark of fallen tree, 19 Jan. 1900, A. A. Heller (NY4340 – holotype);
DISCUSSION

In this study, we have shown that the fungus associated with basal cankers on *Terminalia ivorensis* in Ecuador represents a new genus and species of Diaporthales, for which we have provided the name *Rostraureum tropicale*. The decision to place this fungus in a distinct genus is strongly linked to phylogeny based on DNA sequence data. Here, we have shown that isolates of *R. tropicale* formed a clade distinct from species of *Endothia*, *Cryphonectria* and *Chrysoporthe*, the genera that it most closely resembles.

Robust morphological features support the distinct phylogenetic grouping of isolates of *R. tropicale*. The primary distinguishing feature of the genus is the orange, superficial, rostrate, eustromatic conidiomata. This is in contrast to species of *Cryphonectria* that have semi-immersed, pulvinate eustromatic conidiomata (Shear et al. 1917, Micales & Stipes 1987, Myburg et al. 2004), and *Chrysoporthe* spp., which have superficial, black, pulvinate conidiomata (Hodges 1980, Gryzenhout et al. 2004, Myburg et al. 2004). Species of *Endothia* has large, pulvinate and superficial conidiomata (Shear et al. 1917, Micales & Stipes 1987, Myburg et al. 2004).

One species of *Cryphonectria*, *C. longirostris*, exhibits similar characteristics to *R. tropicale*. For this reason, we have transferred *C. longirostris* to the new genus as *R. longirostris*. It is unfortunate that cultures are not available for *C. longirostris* and at the present time, it is impossible to determine whether our decision to transfer it to *Rostraureum* as a second species, will be supported by phylogenetic data. However, to avoid confusion, we have elected to rely on morphology to support our decision.

The morphology of anamorph, as opposed to teleomorph structures, has recently been shown to provide important taxonomic features in the classification of *Cryphonectria* and *Endothia* (Myburg et al. 2004). Thus, species that would have been treated in *Cryphonectria* based on teleomorph morphology, but that have anamorphs different to the pulvinate, semi-immersed, unilocular to multilocular eustromata of *Cryphonectria*, grouped outside the clade representing *Cryphonectria* based on phylogenetic comparisons (Myburg et al. 2004). For example, isolates of *Chrysoporthe* with blackened, pyriform eustromatic anamorphs with attenuated necks, and a group of isolates from New Zealand with an orange conical anamorph, grouped outside *Cryphonectria* (Myburg et al. 2004). Results of the present study further support the view that anamorph morphology provides a strong indicator of generic status for diaporthalean fungi with orange stromatic tissue.

Observation of various forms of crystals in the stromata and linings of the conidial locules in *R. tropicale* and *R. longirostris*, was an unusual finding in this study. Various pigments have been reported for *Cryphonectria* spp. and these have been clearly summarised by Roane (1986b). These pigments are bisanthraquinones, and include skyrin, skyrinol, oxyskyrin and regulosin (Roane & Stipes 1978, Roane 1986b). A phenolic compound known as endothine red or pigment B, also forms red crystals in the mycelium of some *Endothia* and *Cryphonectria* spp., and imparts a purple colour to growth media (Roane & Stipes 1978, Roane 1986b). Furthermore, species of *Endothia* and *Cryphonectria* turn 3% KOH purple and lactic acid yellow (Castlebury et al. 2002). It is clear that species of *Cryphonectria* and *Endothia*, and other fungi closely related to them, produce different, brightly coloured pigments in culture and in their fruiting structures. It is possible that these compounds could be linked to the crystals observed in *R. tropicale* and *R. longirostris*.

Various specimens examined in this study could not be identified as belonging to an existing taxon. Herbarium specimens labelled as *C. longirostris* were found to represent at least four different fungi. These fungi all have orange-coloured fruiting structures and conidia or ascospores similar to those of *C. longirostris*. These general characteristics and the Caribbean origin of these specimens undoubtedly led to their identification as *C. longirostris*. The fungi, however, all exhibited unique morphological features different to those that characterise species in existing genera such as *Cryphonectria*, *Rostraureum*, *Chrysoporthe* and *Endothia*. Although the fungi are most probably related to these genera, we expect that they represent undescribed taxa. The acquisition of additional collections and preferably cultures that can be used in DNA sequence comparisons will be useful in providing names for them.

Specimens associated with *T. ivorensis* in Africa represent fungi different from *R. tropicale*. Although the orange stromata found on the African specimens are similar to those of *Rostraureum*, these fungi do not have the typical rostrate conidiomata of *Rostraureum* spp. Characteristics of these specimens resembled those in descriptions of *Cryphonectria havanensis*, a fungus reported from Cuba and Florida (USA) on various hosts, including *Eucalyptus* spp. (Bruner 1916, Barnard et al. 1987). It is, however, also possible that these specimens represent undescribed taxa in *Cryphonectria*. The correct identity of these specimens will be difficult to determine in the absence of additional specimens, especially those linked to isolates. Their identification is, however, of considerable interest as they appear to be associated with disease of *Terminalia* spp. in Africa.

The discovery of *R. tropicale* emerged from an interest in dying *T. ivorensis* trees in Ecuador. The fungus...
is common on basal cankers of dying trees, but we are not convinced that it is the sole cause of tree death. Although we were not able to obtain T. ivorensis trees for inoculation in this study, results of inoculations on T. superba showed that the fungus is at least, a significant pathogen of this tree after inoculation. We have, however, not found any evidence of natural infections on T. superba that led to disease, although intensive surveys have not been undertaken. In the future, we hope to undertake further studies of the death of T. ivorensis in Ecuador. It will then hopefully also be possible to obtain trees of this species for inoculation studies with R. tropicale.

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*Rostraureum tropicale* gen. sp. nov.

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