CHAPTER 7

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

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

Marieka Gryzenhout[1], Henrietta Myburg[2], Brenda D. Wingfield[2], Fernando Montenegro[3] & Michael J. Wingfield[1]

[1]*Department of Microbiology and Plant Pathology,* [2]*Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa.*

[3]*Fundacion Forestal, Grupo Juan Manuel Durini, Quito, Ecuador.*

Abstract: *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 β-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*.

Taxonomic novelties: *Rostraureum* Gryzenh. & M. J. Wingf. gen. nov., *Rostraureum tropicale* Gryzenh. & M. J. Wingf. sp. nov., *Rostraureum longirostris* (Earle) Gryzenh. & M. J. Wingf. comb. nov.

Key words: *Cryphonectria*, *Cryphonectria longirostris*, *Diaporthales*, Ecuador, *Rostraureum*, *Terminalia*

INTRODUCTION

Terminalia ivorensis (*Combretaceae*, *Myrtales*) is native to the rainforests of Central Africa (Lamb & Ntima 1971). A similar species, *Terminalia 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 die-back 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, *Korunomyces terminaliae* Hodges & F.A. Ferreira causes leaf spots on seedlings and young *T. ivorensis* plants (Hodges & Fereira 1981), and *Auerswaldiella parvispora* M. L. Farr causes black blotches on leaves (Farr 1989). Root rot caused by species of *Rosellinia* and *Phytophthora*, leads to die-back of *T. ivorensis* in Panama and Costa Rica (Kapp *et al*. 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).

Terminalia 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.

MATERIALS & METHODS

Disease symptoms and specimens

Dead and dying *Terminalia ivorensis* trees were inspected in plantations in the lowland tropics of Ecuador. All trees were mature and ranged in age from 13–15 yrs. 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 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 the herbarium (PREM) of the National Collection of Fungi, Pretoria, South Africa (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 (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. ivorensis* in Ecuador clearly had characteristics similar to those of species of *Cryphonectria* and *Endothia* (shared anamorph: *Endothiella*), and *Chrysoporthe* (anamorph: *Chrysoporthella*). *Chrysoporthe* is a newly described genus accommodating the fungus previously known as *Cryphonectria cubensis* (Bruner) Hodges (Gryzenhout *et al*. 2004/Chapter 1 in this thesis). The fungus from *T. ivorensis* 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. ivorensis*. 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. ivorensis* in Ghana (Ofosu Siedu & Cannon 1976), as well as another specimen labelled as *C. gyrosa* (Berk. & Broome) Sacc. from *T. ivorensis* 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 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 ºC, at 5 ºC intervals.

DNA isolations and PCR amplifications

DNA was isolated from isolates using the method described in Myburg *et al.* (1999). 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 an UV light source.

Sequencing and analysis of sequence data

PCR products were cleaned using a QIAquick PCR Purification kit (Qiagen, Hilden, Germany). 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, UK). 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 4.0b (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 *et al.* 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* Nitschke isolates, which also resides in the *Diaporthales* (Castlebury *et al*. 2002), were used as outgroup taxa 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 *T. ivorensis* could not be tested on *T. ivorensis* due to the fact that 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 *Chrysoporthe cubensis* (Bruner) Gryzenh. & M. J. Wingf. (syn. *Cryphonectria*

cubensis (Bruner) Hodges) an important pathogen of *Eucalyptus* spp. (Hodges *et al.* 1976, Sharma *et al.* 1985) and clove (*Syzygium aromaticum,* Hodges *et al.* 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 *T. ivorensis* is typically diaporthalean, with periphysate ostiolar canals, no paraphyses present, and unitunicate asci with refractive apical rings (Barr 1978). The orange to yellow fruiting structures are reminiscent of those in *Cryphonectria*, *Chrysoporthe* and *Endothia* (Shear *et al.* 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 *Chrysoporthe*, 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 *Chrysoporthe*. 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 *Chrysoporthe*, but perithecial necks protruding from the stromatal surfaces in species of *Chrysoporthe* 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* anamorph 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 *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 (IMI 187898) from *T. ivorensis* in Ghana, 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* (Bruner 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 *D. 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 (g1 = –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 was used in the distance analyses. The trees obtained with the distance and parsimony analyses showed the same clades of isolates, although the relatedness of these

groups with each other varied. The tree obtained with the distance analyses is shown in Fig. 5.

The β-tubulin DNA sequence alignment contained significant phylogenetic signal (g1 = –0.887) according to Hillis & Huelsenbeck (1992) and included a total of 951 characters of which 562 were constant, 15 were parsimony-uninformative and 374 were parsimony-informative. The heuristic search resulted in one most parsimonious tree (tree length = 659, CI = 0.819, RI = 0.924). MODELTEST indicated that the Tamura-Nei parameter model (Tamura & Nei 1993), with the Gamma distribution shape parameter set to 0.7905 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 *Chrysoporthe* (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, 2002b, 2003, 2004, Gryzenhout *et al*. 2004). The clade representing the genera *Cryphonectria* (bootstrap support 94 % in Fig. 5, 81 % in Fig. 6) was defined by *Cryphonectria parasitica* (Murrill) M. E. Barr, *Cryphonectria radicalis* (Schwein.: Fr.) Fr., *Cryphonectria nitschkei* (G. H. Otth) M. E. Barr and *Cryphonectria macrospora* (Tak. Kobay. & Kaz. Itô) M. E. Barr. *Endothia gyrosa* (Schwein.: Fr.) Fr. and *Endothia singularis* (Syd. & P. Syd.) Shear & N. E. Stevens 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 *T. ivorensis* grouped separately from *Chrysoporthe*, *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 comparisions with the new fungus are impossible at present.

Pathogenicity

Within six weeks, *T. 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 *Chr. 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).

Taxonomy

Morphological characteristics and phylogenetic data provide good evidence supporting the view that the fungus from *T. 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. *Cryphonectria longirostris* can be distinguished from the fungus from *T. ivorensis* by its smaller conidia 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.**

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

Ascostromata flava vel aurantiaca, textura stromatali carenti vel praesenti. Colla *perithecialia* vagina texturae porrectae albae circumcincta, cellulae in superficie exteriori collorum perithecialium aurantiacae vel flavae. *Asci* fusoides. *Ascosporae* fusoides vel ellipsoides, hyalinae, semel septatae. *Conidiomata* clavata, superficialia, unilocularia, luteo-flava vel aurantiaca, collis singulis, binis vel ternis attenuatis. *Conidiophora* hyalina, cellulae basales irregulariter ramosae, phialides cylindricas proferentes, septis divisas an non. *Conidia* cylindrica, hyalina, non septata.

Typus: *Rostraureum tropicale* Gryzenh. & M. J. Wingf. 2005.

Ascostromata erumpent, luteous-pure yellow to orange, consisting of perithecia embedded in bark tissue, with necks erumpent and valsoid, 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* fusoid, 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

Etymology: Latin, *tropicus* (tropics), refers to the discovery of this fungus in the tropics.

Ascostromata flava vel aurantiaca, textura stromatali inter perithecia in sectionibus plerumque carenti. *Perithecia* valsoidea, umbrina. *Colla perithecialia* umbrina, cellulis vaginae juxta collum perithecii albis, cellulis exterioris luteo-flavis vel aurantiacis. *Asci* fusoidei. *Ascosporae* 8 in quoque asco, hyalinae, fusoideae vel ellipsoideae, semel septatae. *Conidiomata* flava vel aurantiaca, clavata vel rostrata collis

attenuatis vel non, superficialia, unilocularia, perithecia interdum sub conidiomatibus formantia. *Conidiophora* hyalina, cellulae basales irregulariter ramosae, phialides cylindricas proferentes, septis divisas an non, collare et inspissatio periclinalis inconspicuae. *Conidia* cylindrica, non septata, hyalina, guttulae sporarum exsudatae testaceae. In MEA *culturae* opprimuntur; culturae juvenes albae, intus luteae, seniores aurantiacae, crescunt optime ad 25–30 °C, in temperaturis optimis coloniae tegunt 90 mm in 6 diebus.

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 valsoid 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 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, unitunicate, 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 (Fig. 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 (Figs 1I). *Conidiophores* hyaline, with a globular to rectangular basal cell, (3–)3.5–6.5(–7) × (2–)2.5–4.5(–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, aseptate, hyaline, exuded as brick red spore droplets (Figs 1L, 2F).

Cultural characteristics: 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.

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

Distribution: Ecuador.

Specimens examined. **Ecuador,** Pichincha, Río Pitzara (0° 15′ 27″ N 79° 7′ 43″ W, 350 meters above sea level), *Terminalia ivorensis*, Nov. 2001, M. J. Wingfield, **holotype** PREM 57519, ex-type cultures CMW 9972, CMW 10796 = CBS 115757; 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

Basionym: *Endothia longirostris* Earle, *Muehlenbergia* **1**: 14 (1901).

≡ *Cryphonectria longirostris* (Earle) Micales & Stipes, Phytopathology 77: 651. 1987.

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, valsoid, bases (250–)285–408(–420) µm wide, globose to sub-globose, umber to fulvous, wall 13–20(–25) µm thick (Figs 3B, 4B). Perithecial necks (50–)52–78(–90) µm wide, periphysate, umber, 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* (Figs 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 × 1.5 µm, cylindrical, aseptate, hyaline (Figs 3L, 4F), exuded as brick red spore droplets.

Substrate: dead logs and branches

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

Specimens examined: **Puerto Rico**, east of Santurce, bark of fallen tree, 19 Jan. 1900, A. A. Heller, **holotype** NY4340; bark, 24 Jan.–5 Apr. 1923, F. J. Seaver & C. E. Chardon,

NY 617; Naguabo, fallen bark, 25 March 1915, N. Wille, NY 816; Rio Piedras, 18 June 1917, J. A. Stevenson & R. C. Rose, NY 6576. **Trinidad & Tobago,** Ortoire river, Guayaguayare road, bark, 25 March 1921, F. J. Seaver, NY 3320.

DISCUSSION

In this study, we have shown that the fungus associated with basal cankers on *T. ivorensis* in Ecuador represents a new genus and species of *Diaporthales*, for which we have provided the name *R. 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* (Bruner) M. E. Barr, 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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Table 1. Isolates of *Rostraureum tropicale*, *Chrysoporthe*, *Cryphonectria* and *Endothia* spp. used for DNA sequence comparisons and growth study.

Culture number [a]	Alternative number[b]	Identification	Host	Origin	Genbank accession number [d]
CMW 8756	–	*Chrysoporthe cubensis*	*Eucalyptus* sp.	Indonesia	AF 046896, AF 273077, AF 285165
CMW 2632	–	*C. cubensis*	*Eucalyptus marginata*	Australia	AF 046893, AF 273078, AF 375607
CMW 8757	–	*C. cubensis*	*Eucalyptus grandis*	Venezuela	AF 046897, AF 273069, AF 273464
CMW 8758	–	*C. cubensis*	*E. grandis*	Venezuela	AF 046898, AF 273068, AF 273463
CMW 1853	–	*C. cubensis*	*Syzygium aromaticum*	Brazil	AF 046891, AF 273070, AF 273465
CMW 8755	–	*Chrysoporthe austroafricana*	*E. grandis*	South Africa	AF 292040, AF 273064, AF 273458
CMW 2113	CBS 112916	*C. austroafricana*	*E. grandis*	South Africa	AF 046892, AF 273067, AF 273462
CMW 7047	ATCC 48197, E5	*Cryphonectria parasitica*	*Quercus virginiana*	MS, USA	AF 368329, AF 273073, AF 273469
CMW 7048	ATCC 48198, E9	*C. parasitica*	*Q. virginiana*	VA, USA	AF 368330, AF 273076, AF 273470
CMW 10477	CBS 240.54, E76	*Cryphonectria radicalis*	*Castanea sativa*	Italy	AF 368328, AF 368347, AF 368346
CMW 10455	CBS 238.54, E42	*C. radicalis*	*C. dentata*	Italy	AF 452113, AF 525705, AF 525712
CMW 10518	CBS 112919, E53	*Cryphonectria nitschkei*	*Quercus* sp.	Japan	AF 452118, AF 525706, AF 525713
CMW 10463	CBS 112920, E54	*Cryphonectria macrospora*	*Castanopsis cupsidata*	Japan	AF 368331, AF 368351, AF 368350

CMW 9971 [c]	CBS 115725	*Rostraureum tropicale*	*Terminalia ivorensis*	Ecuador	AY 167425, AY 167430, AY 167435
CMW 9972 [c]	–	*R. tropicale*	*T. ivorensis*	Ecuador	AY 167426, AY 167431, AY 167436
CMW 9973	CBS 115726	*R. tropicale*	*T. ivorensis*	Ecuador	AY 167427, AY 167432, AY 167437
CMW 9975	CBS 115727	*R. tropicale*	*T. ivorensis*	Ecuador	AY 167429, AY 167434, AY 167439
CMW 10796 [c]	CBS 115757	*R. tropicale*	*T. ivorensis*	Ecuador	AY 167428, AY 167433, AY 167438
CMW 2091	ATCC 48192, E13	*Endothia gyrosa*	*Q. palustris*	VA, USA	AF 368325, AF 368337, AF 368336
CMW 10442	E27	*E. gyrosa*	*Q. palustris*	VA, USA	AF 368326, AF 368339, AF 368338
CMW 10465	CBS 112921, E58	*Endothia singularis*	–	CO, USA	AF 368323, AF 368333, AF 368332
CMW 5288	CBS 112900	*Diaporthe ambigua*	*Malus domestica*	South Africa	AF 543817, AF 543819, AF 543821
CMW 5587	CBS 112901	*D. ambigua*	*M. domestica*	South Africa	AF 543818, AF 543820, AF 543822

[a] Culture collection of the Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria.

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).

[b] 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).

[c] CMW 9972, CMW 10796 were obtained from the holotype specimen PREM 57519; CMW 9971 was obtained from specimen PREM 583301.

[d] Given as sequences from the ITS region, and two regions from the β-tubulin genes amplified with primers 1a/1b and 2a/2b respectively.

Table 2. Specimens used in morphological comparisons.

Reference collection[a]	Identity	Name on specimen	Host	Origin	Date	Collector
PREM 57519 (holotype)[b]	*Rostraureum tropicale*	–	*Terminalia ivorensis*	Ecuador	2001	M.J. Wingfield
PREM 583301[b]	*R. tropicale*	–	*T. ivorensis*	Ecuador	2001	M.J. Wingfield
PREM 583302	*R. tropicale*	–	*T. ivorensis*	Ecuador	2001	M.J. Wingfield
PREM 583303	*R. tropicale*	–	*T. ivorensis*	Ecuador	2001	M.J. Wingfield
PREM 583304	*R. tropicale*	–	*T. ivorensis*	Ecuador	2001	M.J. Wingfield
NY 4340 (holotype) and NY 266417	*Rostraureum longirostris*	*Cryphonectria longirostris*	Fallen tree	Puerto Rico	1900	A. Heller
NY 617	*R. longirostris*	*C. longirostris*	–	Puerto Rico	1923	F.J. Seaver & C.E. Chardon
NY 816	*R. longirostris*	*C. longirostris*	Fallen bark	Puerto Rico	1915	N. Wille
NY 6576	*R. longirostris*	*C. longirostris*	–	Puerto Rico	1917	J.A. Stevenson & R.C. Rose
NY 3320	*R. longirostris*	*C. longirostris*	Bark	Trinidad & Tobago	1921	F.J. Seaver
NY 1053	Unknown	*C. longirostris*	Sticks	Puerto Rico	1923	F.J. Seaver & C.E. Chardon
NY 511	Unknown	*C. longirostris*	unknown	Puerto Rico	1923	F.J. Seaver & C.E. Chardon
NY 3360	Unknown	*C. longirostris*	Forest	Trinidad & Tobago	1921	F.J. Seaver
NY 3098	Unknown	*C. longirostris*	Forest	Trinidad & Tobago	1921	F.J. Seaver
PDD 28477	Unknown	*C. longirostris*	*Coriaria* sp.	New Zealand	1958	J.M. Dingley

IMI 187898	Unknown	*Cryphonectria* sp.	*T. ivorensis*	Ghana	–	P.F. Cannon
IMI 288729	Unknown	*Cryphonectria gyrosa*	*T. ivorensis*	Kenya	1984	D. Pawsey
BPI 631857 (holotype)	*Chrysoporthe cubensis*	*Diaporthe cubensis*	*Eucalyptus botryoides*	Cuba	1916	S.C. Bruner
PREM 57297	*C. cubensis*	*Cryphonectria cubensis*	*Eucalyptus* sp.	Indonesia	2001	M.J. Wingfield
K 109809	*Cryphonectria gyrosa*	Unknown (#290)	Bark	Sri Lanka	–	G.H.K. Thwaites
TFM: FPH 1045 (holotype)	*Cryphonectria nitschkei*	*Endothia nitschkei*	*Quercus grosseserrata*	Japan	1954	T. Kobayashi
CUP 2926	*Cryphonectria parasitica*	*Diaporthe parasitica*	*Castanea dentata*	New York, USA	1907	W.A. Murrill
PREM 56218	*Endothia gyrosa*	*E. gyrosa*	*Q. phellos*	Raleigh, USA	1997	L. Grand

[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 (Danchi-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.

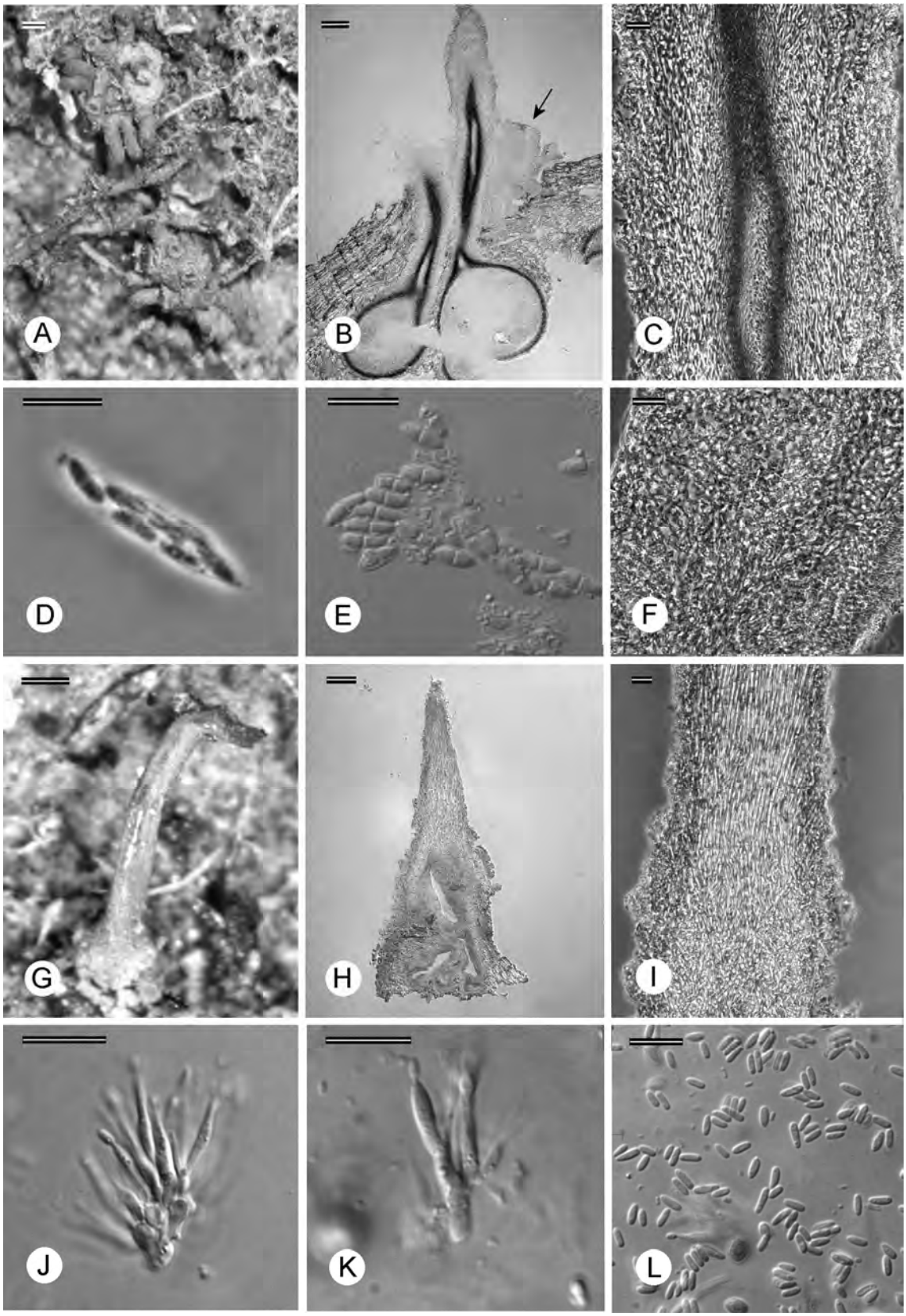

Fig. 1. Fruiting structures of *Rostraureum tropicale* (from holotype PREM 57519). A. Ascomata on bark. B. Longitudinal section through ascoma also showing stromatic tissue (indicated by arrow). C. Tissue of perithecial neck. D. Ascus. E. Ascospores. F. Tissue at base of conidioma. G. Conidioma on bark and sectioned (H). I. Tissue of conidioma where neck begins. J, K. Conidiophores. L. Conidia. Scale bars A, G = 200 µm; B, H = 100 µm; C

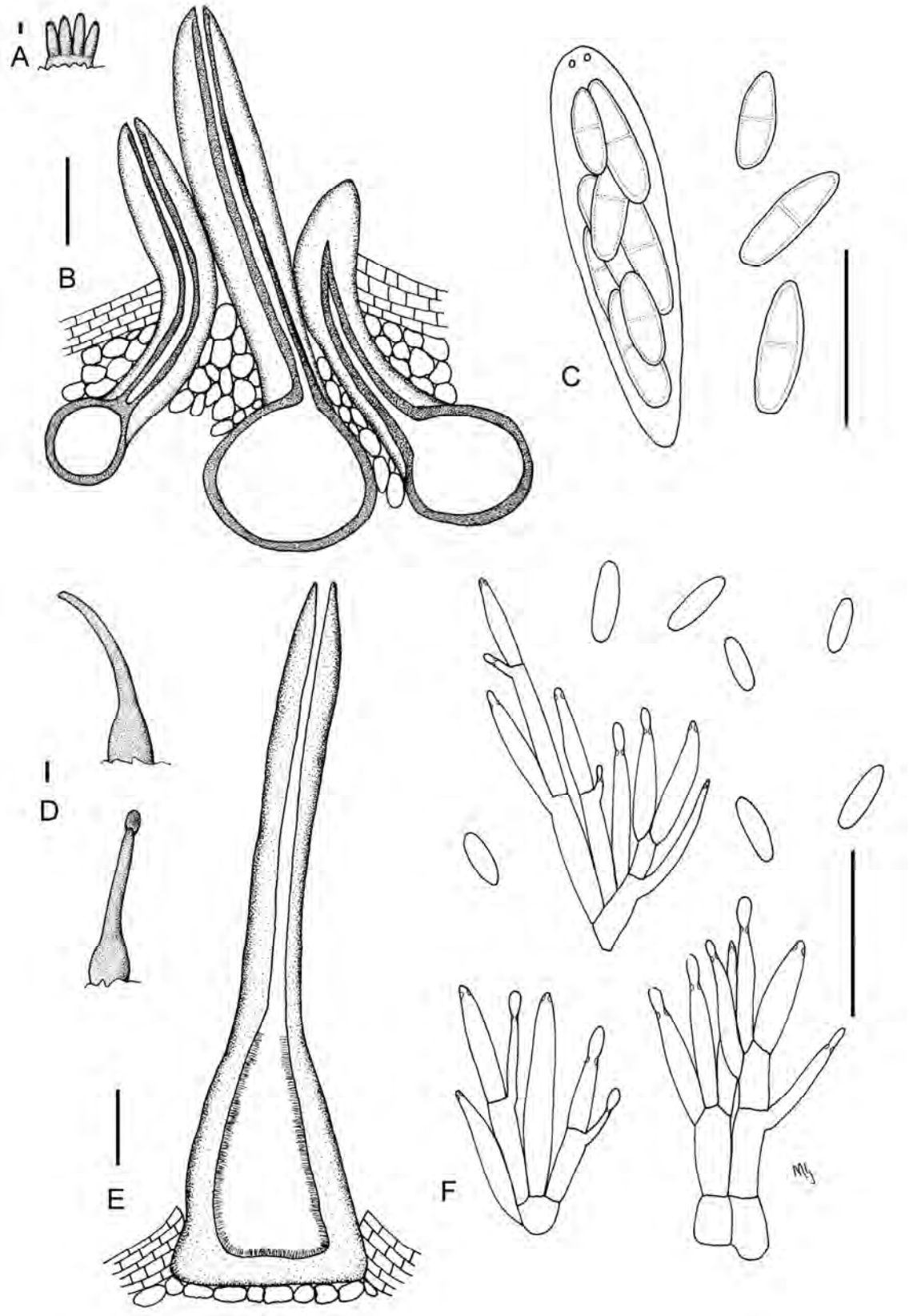

Fig. 2. Schematic drawings of *Rostraureum tropicale* (from holotype PREM 57519). A. Ascomata on bark. B. Section through ascoma. C. Asci and ascospores. D. Conidiomata on bark. E. Section through conidioma. F. Conidiophores, conidiogenous cells and conidia. Scale bars A–B, D–E = 100 µm; C, F

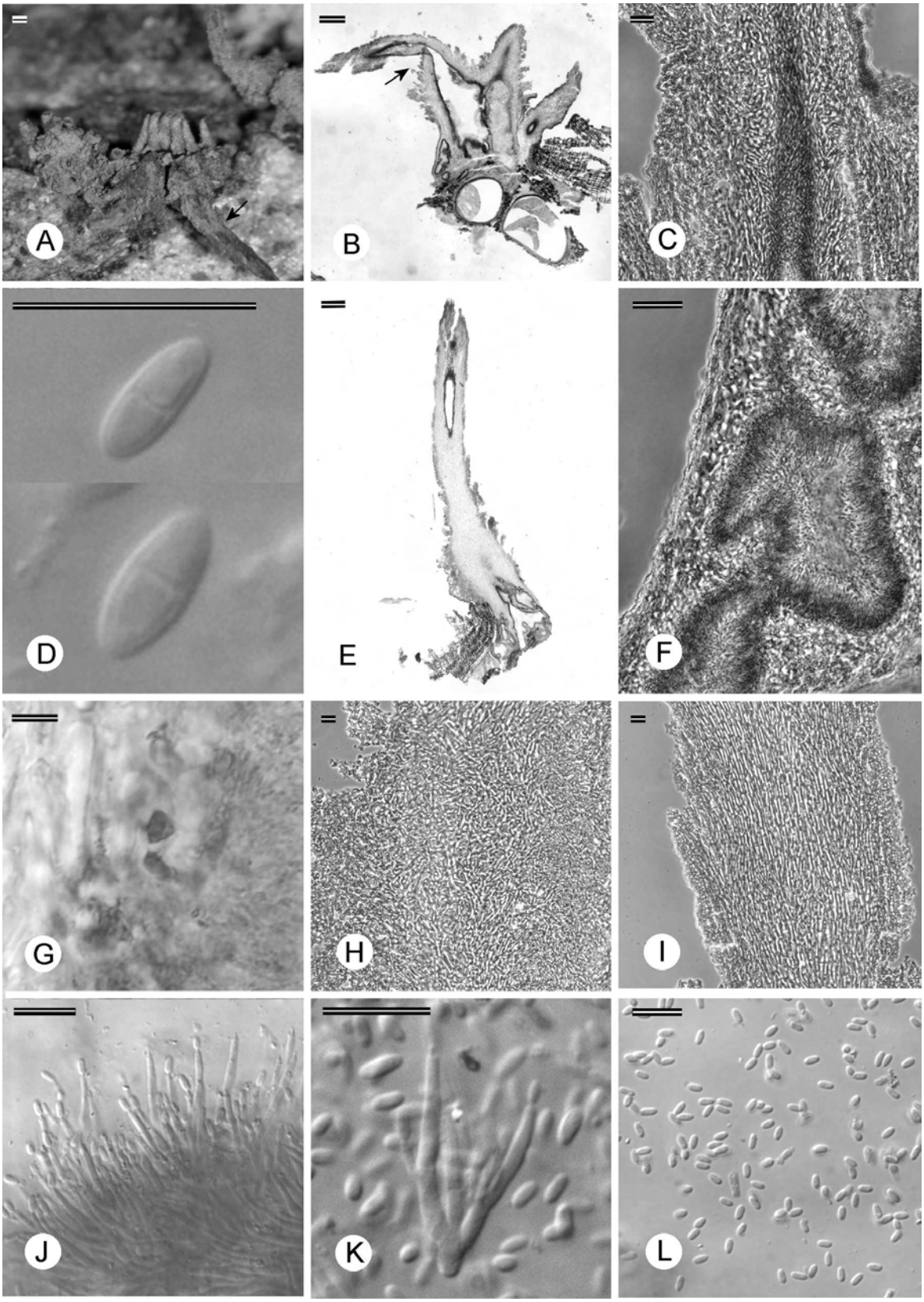

Fig. 3. Fruiting structures of *Rostraureum longirostris* (from holotype NY 4340). A. Ascoma with conidioma attached (indicated with arrow). B. Longitudinal section through ascoma and conidioma (indicated with arrow). C. Tissue of perithecial neck. D. Ascospores. E. Longitudinal section of conidioma. F–G. Crystals in lining of conidial cavity. H. Tissue of conidiomal base where neck begins. I. Tissue of conidiomal neck. J–K. Co rs of A–B, E = 200 µm; C, F, H–I = 20 µm; D, G, J–L

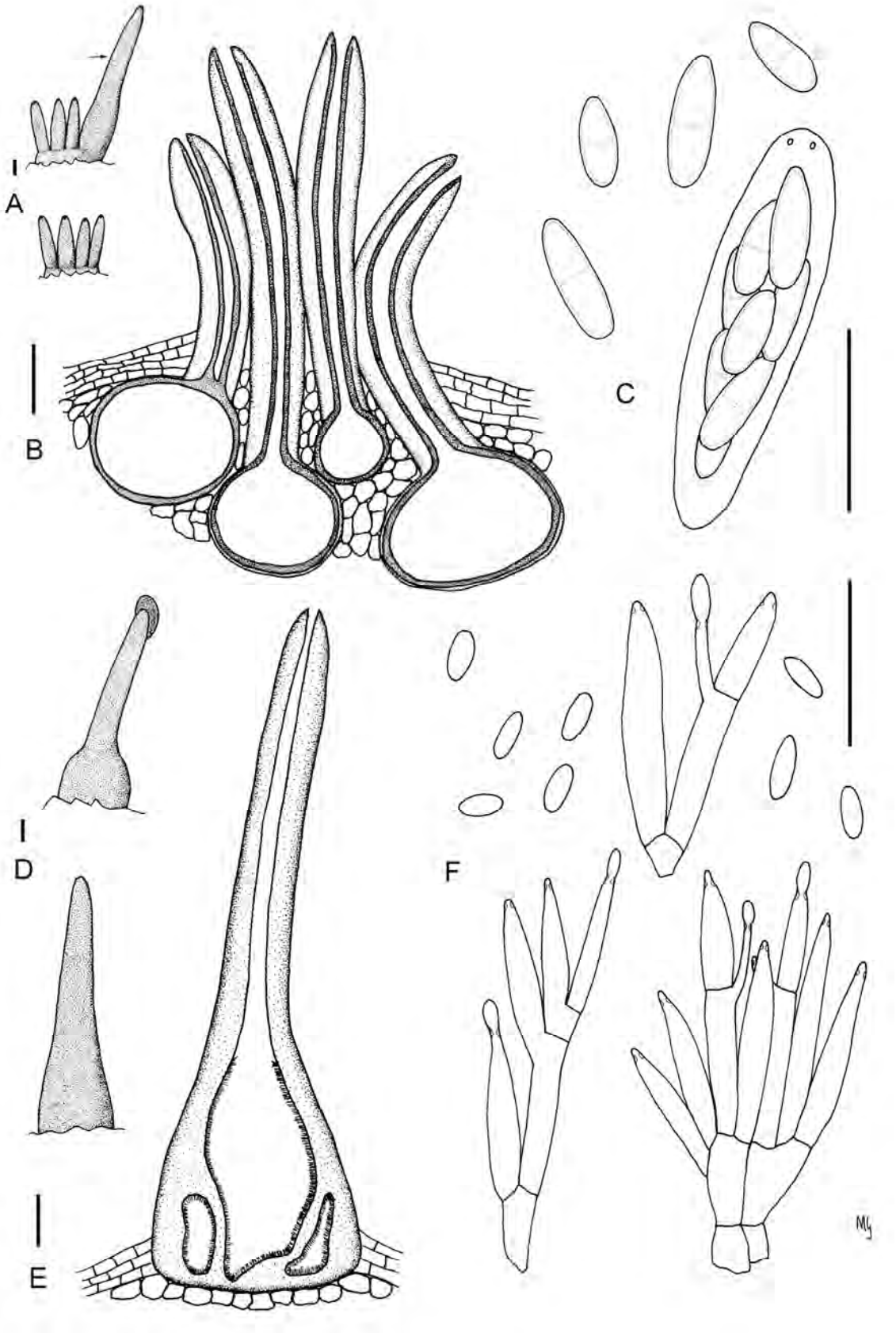

Fig. 4. Schematic drawings of *Rostraureum longirostris* (from holotype NY 4340). A. Ascomata on bark with conidioma (indicated with arrow). B. Section through ascoma. C. Asci and ascospores. D. Conidiomata on bark. E. Section through conidioma. F. Conidiophores, conidiogenous cells and conidia. Scale bars A–B, D–E =

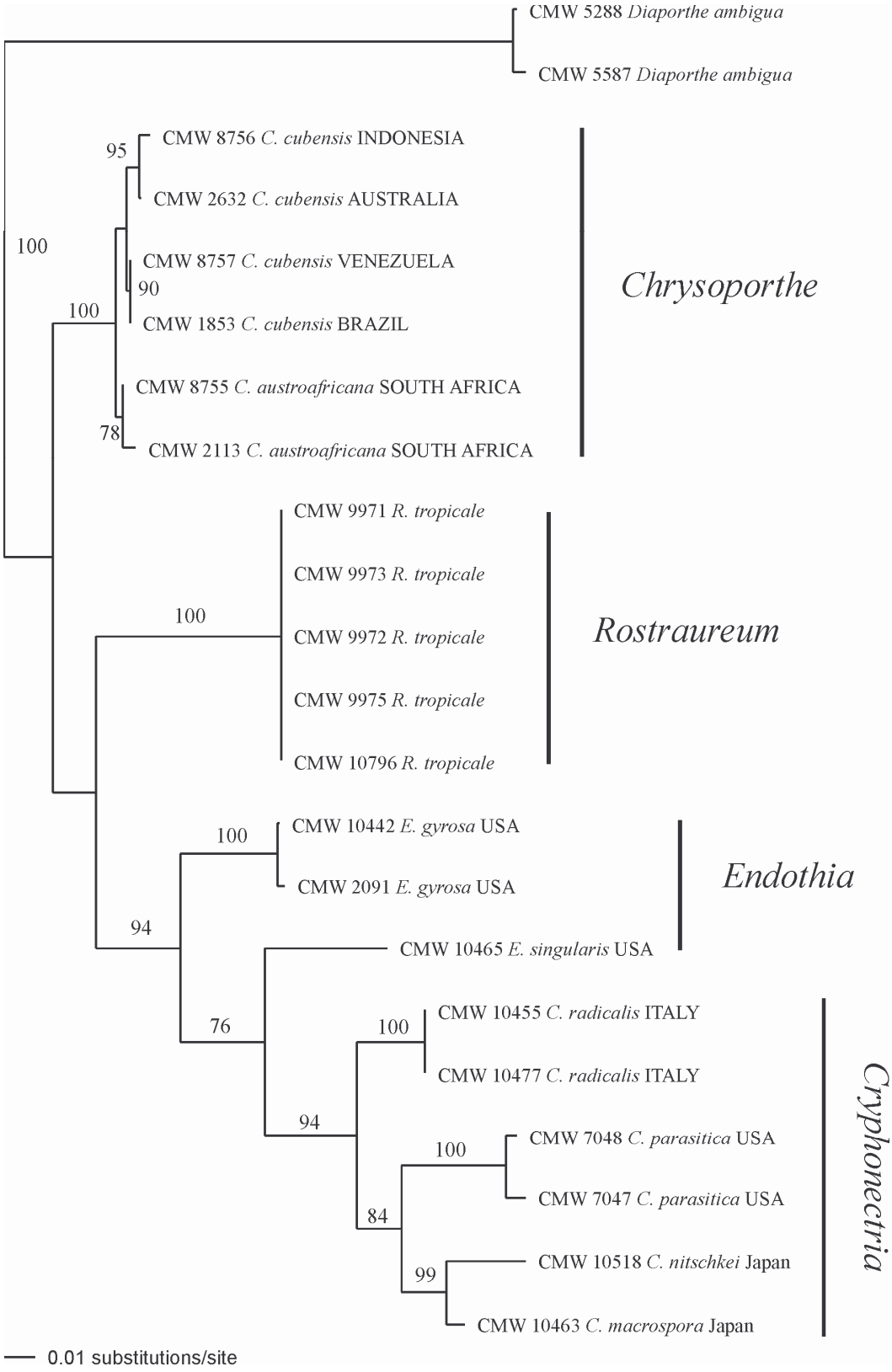

Fig. 5. Distance phylogram showing phylogenetic relationships between *Rostraureum*, *Cryphonectria*, *Chrysoporthe* and *Endothia* spp. based on ITS1/ITS2 DNA sequence of the ribosomal operon. The phylogram was obtained with the Kimura 2 parameter model (G = 0.1979). Bootstrap values greater than 70 % (1000 replicates) are indicated at the branch nodes. The *Diaporthe ambigua* isolates were used as outgroup taxa to root the phylogenetic tree.

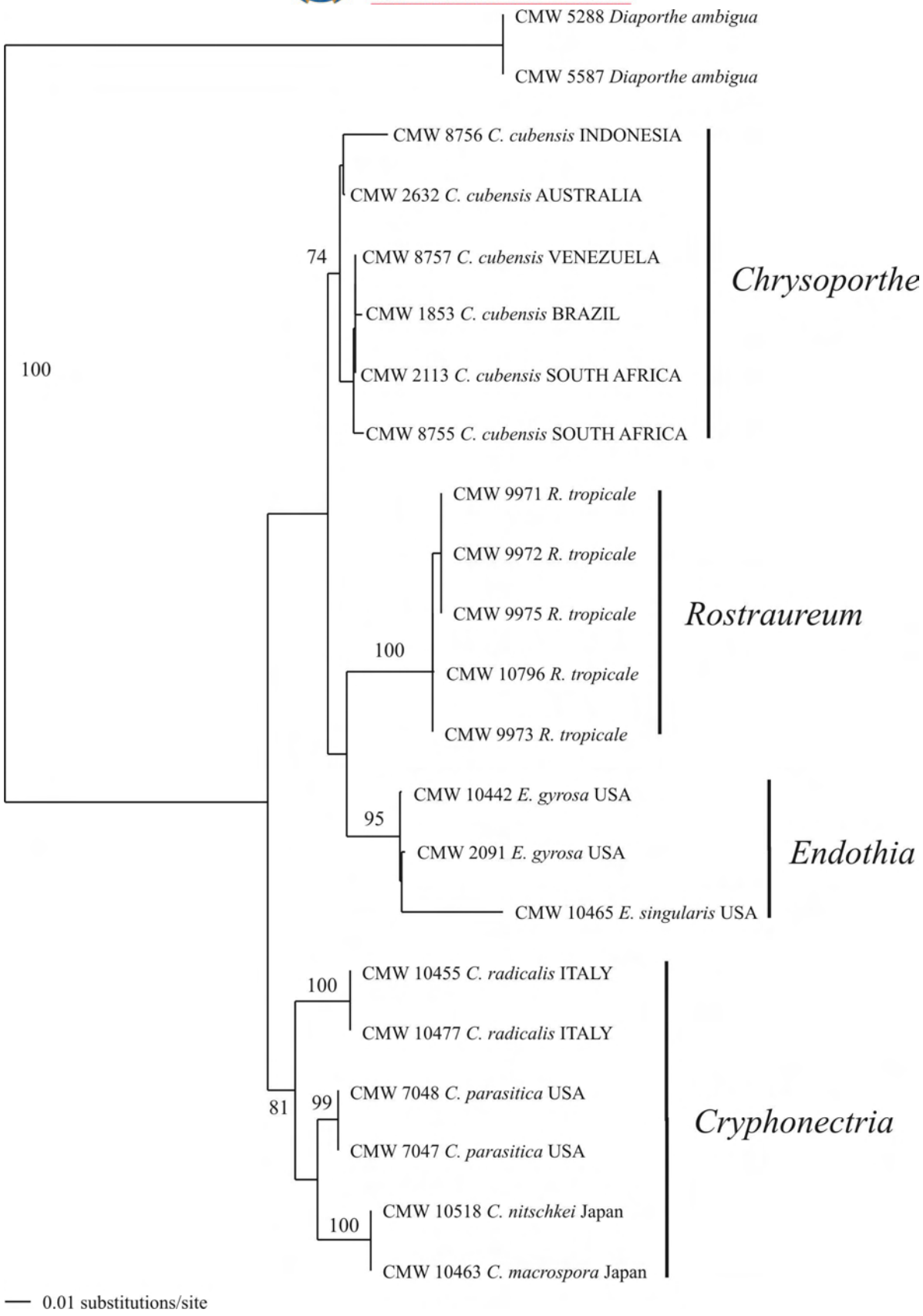

Fig. 6. Distance phylogram showing phylogenetic relationships between *Rostraureum*, *Cryphonectria*, *Chrysoporthe* and *Endothia* spp. based on β-tubulin DNA sequence. The phylogram was obtained with the Tamura Nei parameter model (I = 0.5437, G = 0.7905). Bootstrap values greater than 70 % (1000 replicates)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

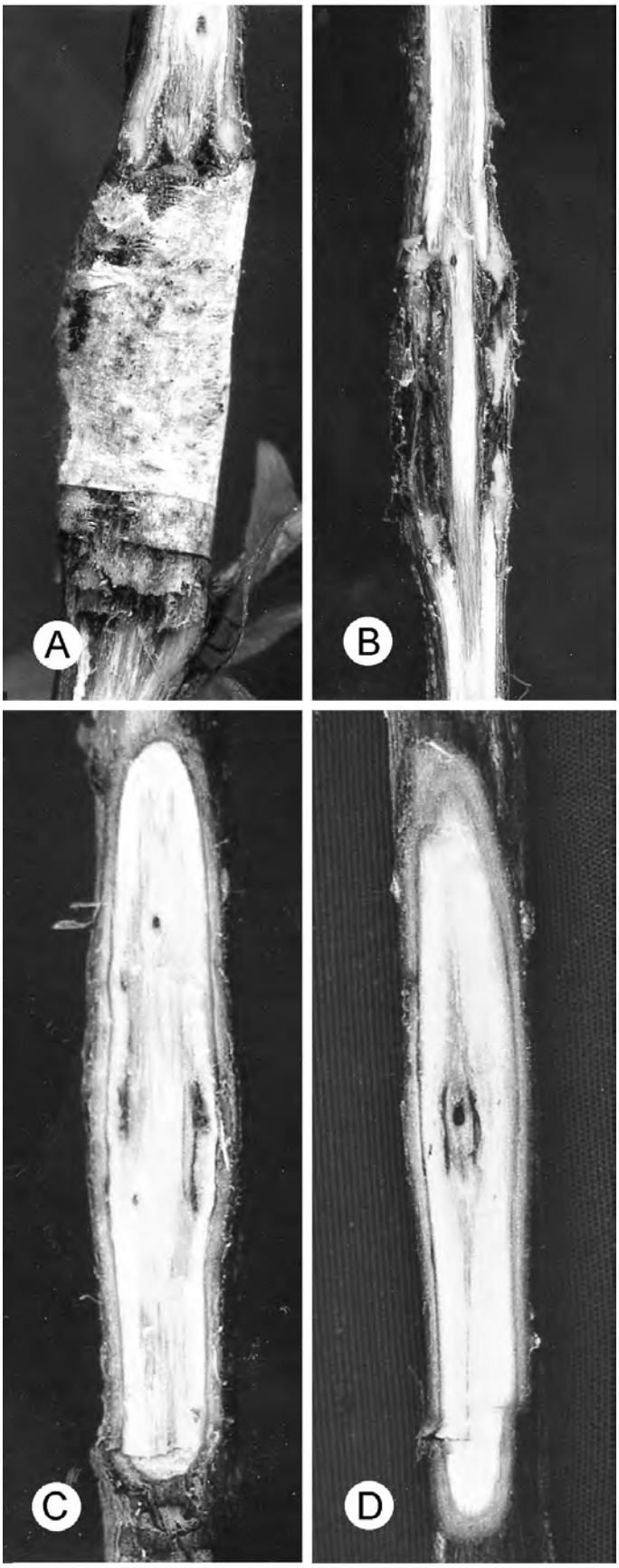

Fig. 7. Lesions associated with inoculation of the newly described *Rostraureum tropicale* and *Chrysoporthe cubensis* on *Terminalia ivorensis* in Ecuador. A. Fruiting structures formed on the canker resulting from inoculation with *R. tropicale*. B. Lesions associated with *R. tropicale*. C. Lesion development associated with *Chr. cubensis*. D. Control inoculation.