Mycologia, 98(1), 2006, pp. 105–115.

Aurapex penicillata gen. sp. nov. from native *Miconia theaezans* and *Tibouchina* spp. in Colombia

Marieka Gryzenhout[1]

Department of Microbiology and Plant Pathology, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa

Henrietta Myburg

Department of Genetics, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa

Carlos A. Rodas[2]

Department of Microbiology and Plant Pathology, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa

Brenda D. Wingfield

Department of Genetics, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa

Michael J. Wingfield

Department of Microbiology and Plant Pathology, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa

Abstract: Conidiomata of a fungus resembling *Chrysoporthe cubensis*, a serious canker pathogen of *Eucalyptus* spp. (Myrtaceae, Myrtales) in tropical and subtropical parts of the world, was found on *Eucalyptus grandis* in Colombia. Fruiting structures of the fungus could be distinguished from those of *C. cubensis* by their distinctly orange conidiomatal necks. This fungus also was found on several plant species native to Colombia including *Tibouchina urvilleana, T. lepidota* and *Miconia theaezans* (Melastomataceae, Myrtales). Morphological comparisons, as well as those based on sequences of the ITS1/ITS2 region of the ribosomal DNA repeat and the β-tubulin gene, were used to characterize this fungus. Its pathogenicity was assessed on various plants from which it has been collected, either in field or greenhouse trials. Phylogenetic analyses showed that isolates reside in a clade distinct from the four clades accommodating *Chrysoporthe, Cryphonectria, Endothia* and *Rostraureum*. Members of this clade are distinguished by the presence of orange conidiomatal necks with black bases and a unique internal stromatal structure. No teleomorph has been found for this fungus, for which we have provided the name *Aurapex penicillata* gen. sp. nov. *A. penicillata* produced only small lesions after inoculation on young *T. urvilleana, M. theaezans* and *E. grandis* trees and appears not to be a serious pathogen.

Key words: *Chrysoporthe*, Diaporthales, *Eucalyptus*, *Melastomataceae*

INTRODUCTION

The Melastomataceae represents a family of flowering plants common to neotropical America and Hawaii (Everett 1981). This family resides in the Myrtales, which also accommodates the Myrtaceae (Conti et al 1996). The Myrtaceae includes the genus *Eucalyptus*, many species of which are grown as a source of pulp and timber in plantations around the world (Turnbul 2000).

Chrysoporthe cubensis (Bruner) Gryzenh. & M. J. Wingf. (formerly *Cryphonectria*) is a serious canker pathogen of *Eucalyptus* spp. (Boerboom and Maas 1970, Hodges 1980, Sharma et al 1985, Wingfield 2003) and *Syzygium aromaticum* (L.) Murr. & Perry (clove, also Myrtaceae) (Hodges et al 1986), in the tropics and subtropics. Intriguingly, this pathogen recently has been shown to cause disease on members of the Melastomataceae such as *Miconia theaezans* (Bonpl.) Cogn. (niguito) and *Miconia rubiginosa* (Bonpl.) DC. (mortiño) in Colombia (Rodas et al 2005). A second fungus, *Chrysoporthella hodgesiana* Gryzenh. & M. J. Wingf., a species of *Chrysoporthe* based on phylogenetic data but known only by its anamorph, also occurs on Colombian Melastomataceae such as *M. theaezans* (Rodas et al 2005), *Tibouchina urvilleana* Cogn., *T. lepidota* Baill. and *T. semidecandra* Cogn. (Gryzenhout et al 2004). Recognition of *C. cubensis* on hosts residing in the Melastomataceae has substantially altered views regarding the origin and distribution of this important tree pathogen (Wingfield et al 2001, Wingfield 2003, Rodas et al 2005).

During the course of surveys in Colombia to assess the occurrence of *Chrysoporthe* spp. on trees other than *Eucalyptus* spp. a fungus similar to *C. cubensis* and *Chrysop. hodgesiana* was found on *Tibouchina* spp. These trees were planted as ornamentals in parks or on farms. The unknown fungus produced only conidiomata that were black and pyriform, in a shape

Accepted for publication 12 Dec 2005.

[1] Corresponding author. E-mail: Marieka.Gryzenhout@fabi.up.ac.za

[2] Current address: Smurfit Cartón de Colombia, Investigación Forestal, Carrera 4 Nu. 10–44, oficina 1106 Edificio Plaza de Caicedo, Cali, Valle, Colombia.

reminiscent of *Chrysoporthe* spp. The fruiting bodies, however, differed from those of *Chrysoporthe* spp. in that the apices of the conidiomatal necks were orange. Subsequent surveys led to the discovery of the fungus on native *M. theaezans* as well as on *E. grandis*. The aims of this study were to define the phylogenetic position of this fungus using DNA sequence comparisons as well as to produce a taxonomic description and generic key. In addition the pathogenicity of the new fungus was assessed in greenhouse and field inoculation experiments.

MATERIALS AND METHODS

Symptoms and collection of samples.—Structures of the unknown fungus were found first in 1996 on *T. urvilleana* and *T. lepidota* in Colombia. These trees were growing in La Culebra Park in El Peñol, on a private farm near Granada (Antioquia Province), and on the Argentina farm of Smurfit Cartón de Colombia near Riosucio (Caldas Province). In a subsequent disease survey in 1998, similar fruiting structures were found on *Miconia theaezans* occurring in native vegetation on La Selva farm near Pereira (Risaralda Province). In 2002 this fungus was discovered for the first time on basal cankers on *E. grandis* on the Libano farm near Pereira as well as on *M. theaezans* at the same location.

The fruiting structures of the unknown fungus occurred around the periphery of cankers on the stems and branches of trees, which occasionally led to branch die-back. In some cases fruiting structures of *C. cubensis* and *Chrysop. hodgesiana* occurred on the same plant. On *E. grandis* fruiting structures of the fungus also were found on branches that were in the process of senescence, and the fungus also appeared to colonize branch stubs.

Bark specimens containing conidiomata were collected from cankers and taken to the laboratory for isolation. Single conidial isolates were obtained from spore suspensions on 2% malt-extract agar (MEA) (20 g Biolab malt extract, 15 g Biolab agar, 1 L water, Merck, Midrand, South Africa) and incubated at 25 C. The cultures have been maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, and representative isolates have been deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands (TABLE I). Original bark specimens from which isolations had been made were used for morphological characterization and have been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).

Morphology.—Conidiomata were cut from bark specimens, rehydrated for 1 min in boiling water and sectioned at −20 C with a Leica CM1100 Cryostat after embedding in Leica mountant (Setpoint Premier, Johannesburg, South Africa). Sections approximately 12 µm thick were mounted on microscope slides in lactophenol. Fifty measurements of ascospores, asci, conidia and conidiophores were taken for the holotype specimen and these are presented as (min−)(average–SD)−(average+SD)(−max) µm. Ten structures were sectioned to observe the internal morphology of the fruiting bodies and a range was obtained for the eustromatic bases, necks and conidial locules. Micrographs were taken with a HRc Axiocam digital camera and accompanying Axiovision 3.1 software (Carl Zeiss Ltd., Germany). The color charts of Rayner (1970) were used to define colors of cultures and morphological structures.

Culture morphology of the ex holotype strain CMW 10030 and CMW 11296 (TABLE I), was characterized on MEA (20 g/L malt-extract agar, Biolab, Merck). These studies were conducted using the technique described by Venter et al (2002). Growth tests were conducted at 15–35 C at 5 C intervals, and cultures were grown in the dark.

DNA sequence comparisons.—Representative isolates of the fungus from Colombia were used in the DNA sequence comparisons (TABLE I). Sequences of *C. cubensis* isolates from *Miconia* and *Eucalyptus* spp. in Colombia (Gryzenhout et al 2004, Rodas et al 2005), and *Chrysop. hodgesiana* isolates from *Miconia* and *Tibouchina* spp. in Colombia (Wingfield et al 2001, Gryzenhout et al 2004, Rodas et al 2005) also were used. Sequences for isolates of *C. cubensis* from other parts of the world, those of *C. austroafricana* (Myburg et al 2002a, 2003) and those for recognized members of *Cryphonectria* and *Endothia*, which are closely related to *Chrysoporthe* (Venter et al 2002, Myburg et al 2004a, b), also were included (TABLE I). Sequences from the recently described *Rostraureum tropicale* Gryzenh. & M. J. Wingf., a pathogen of *Terminalia* in Ecuador (Gryzenhout et al 2005), were included in the dataset. *Rostraureum* contains the fungus previously known as *Cryphonectria longirostris* (Earle) Micales & Stipes (Gryzenhout et al 2005). Two *Diaporthe ambigua* Nitschke isolates, which also reside in the Diaporthales but have been shown to reside in a different family to that of *Cryphonectria* and related taxa (Castlebury et al 2002), were used as a single outgroup to root the phylogenetic tree generated in this study. The sequence matrix (study accession number = S1128, matrix accession number = M1935) from Myburg et al (2004a) was used as template for the alignment.

DNA was isolated from the fungi as described in Myburg et al (1999). PCR amplification of the ITS1, conserved 5.8S and ITS2 regions of the rRNA operon as well as two regions in the β-tubulin gene was performed as described respectively in Myburg et al (1999) and Myburg et al (2002a). Primer pairs ITS1/ITS4 (White et al 1990) were used for the ITS1/ITS2 region, and primer pairs Bt1a/Bt1b and Bt2a/Bt2b respectively (Glass & Donaldson 1995) were used to amplify two β-tubulin gene regions. The PCR products were purified with a QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany). The purified PCR products were sequenced in both directions with the same primers that were used in the amplification reactions. Sequencing reactions were performed with a PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer,

TABLE I. Isolates of *Chrysoporthe, Cryphonectria, Endothia, Rostraureum* and *Aurapex penicillata* used in this study. Isolates sequenced in this study are in bold

Identification	Culture No.[a]	Alternative isolate number[a]	Host	Origin	Collector	Genbank Accession No.[b]
Chrysoporthe cubensis	CMW 2632	–	*Eucalyptus marginata*	Australia	E Davison	AF 046893, AF 273078, AF 375607
Chrysoporthe cubensis	CMW 1856	–	*Eucalyptus* sp.	Kauai, Hawaii	–	AY 083999, AY 084010, AY 084022
Chrysoporthe cubensis	CMW 11290	CBS 115738	*Eucalyptus* sp.	Indonesia	MJ Wingfield	AY 214304, AY 214232, AY 214268
Chrysoporthe cubensis	CMW 10774	–	*Syzygium aromaticum*	Zanzibar, Tanzania	–	AF 492130, AF 492131, AF 492132
Chrysoporthe cubensis	CMW 9432	CBS 115724	*Eucalyptus grandis*	Mexico	MJ Wingfield	AY 692321, AY 692324, AY 692323
Chrysoporthe cubensis	CMW 8757	–	*Eucalyptus* sp.	Venezuela	MJ Wingfield	AF 046897, AF 273069, AF 273464
Chrysoporthe cubensis	CMW 1853	–	*Syzygium aromaticum*	Brazil	–	AF 036891, AF 273070, AF 273465
Chrysoporthe cubensis	CMW 10639	CBS 115747	*Eucalyptus grandis*	Colombia	CA Rodas	AY 263419, AY 263420, AY 263421
Chrysoporthe cubensis	CMW 9993	CBS 115728	*Miconia theaezans*	Colombia	CA Rodas	AY 214298, AY 214226, AY 214262
Chrysoporthe cubensis	CMW 10024	CBS 115739	*Miconia rubiginosa*	Colombia	CA Rodas	AY 262390, AY 262394, AY 262398
Chrysoporthella hodgesiana	CMW 9927	–	*Tibouchina urvilleana*	Colombia	CA Rodas, MJ Wingfield	AF 265653, AF 292034, AF 292037
Chrysoporthella hodgesiana	CMW 9995	CBS 115730	*Tibouchina urvilleana*	Colombia	R Arbelaez	AY 956969, AY 956977, AY 956978
Chrysoporthella hodgesiana	CMW 10625	CBS 115744	*Miconia theaezans*	Colombia	CA Rodas	AY 956970, AY 956979, AY 956980
Chrysoporthella hodgesiana	CMW 10641	CBS 115854	*Tibouchina semidecandra*	Colombia	R Arbaleaz	AY 692322, AY 692326, AY 692325
Chrysoporthe austroafricana	CMW 2113	CBS 112916	*Eucalyptus grandis*	South Africa	MJ Wingfield	AF 046892, AF 273067, AF 273462
Chrysoporthe austroafricana	CMW 8755	–	*Eucalyptus grandis*	South Africa	MJ Wingfield	AF 292040, AF 273064, AF 273459
Rostraureum tropicale	CMW 9973	CBS 115726	*Terminalia ivorensis*	Ecuador	MJ Wingfield	AY 167427, AY 167432, AY 167437
Rostraureum tropicale	CMW 9975	CBS 115727	*Terminalia ivorensis*	Ecuador	MJ Wingfield	AY 167429, AY 167434, AY 167439
Rostraureum tropicale	CMW 10796	CBS 115757	*Terminalia ivorensis*	Ecuador	MJ Wingfield	AY 167428, AY 167433, AY 167438
Aurapex penicillata	**CMW 10030** (ex-type designated here)	CBS 115740	*Miconia theaezans*	Colombia	CA Rodas	AY 214311, AY 214239, AY 214275,
Aurapex penicillata	**CMW 10031**	–	*Miconia theaezans*	Colombia	CA Rodas	AY 994511, AY 994513, AY 994514
Aurapex penicillata	**CMW 10034**	–	*Miconia theaezans*	Colombia	CA Rodas	AY 994512, AY 994515, AY 994516
Aurapex penicillata	**CMW 10035**	CBS 115742	*Miconia theaezans*	Colombia	CA Rodas	AY 214313, AY 214241, AY 214277,
Aurapex penicillata	**CMW 11296**	CBS 115801	*Miconia theaezans*	Colombia	CA Rodas	AY 214315, AY 214243, AY 214279
Cryphonectria radicalis	CMW 10455	CBS 238.54	*Quercus suber*	Italy	A Biraghi	AF 452113, AF 525705, AF 525712
Cryphonectria parasitica	CMW 7047	ATCC 48197	*Quercus virginiana*	USA	RD Wolfe	AF 368329, AF 273073, AF 273469
Cryphonectria nitschkei	CMW 13742	MAFF 410570	*Quercus grosseserrata*	Japan	T Kobayashi	AY 697936, AY 697961, AY 697962
Cryphonectria macrospora	CMW 10463	CBS 112920	*Castanopsis cuspidata*	Japan	T Kobayashi	AF 368331, AF 368351, AF 368350
Endothia gyrosa	CMW 2091	ATCC 48192	*Quercus palustris*	USA	RJ Stipes	AF 046905, AF 368337, AF 368336
Endothia gyrosa	CMW 10442	–	*Quercus palustris*	USA	RJ Stipes	AF 368326, AF 368339, AF 368338
Diaporthe ambigua	CMW 5288	CBS 112900	*Malus domestica*	South Africa	WA Smit	AF 543817, AF 543819, AF 543821
Diaporthe ambigua	CMW 5587	CBS 112901	*Malus domestica*	South Africa	WA Smit	AF 543818, AF 543820, AF 543822

[a] CMW = Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; ATCC = American Type Culture Collection, Manassas, USA; MAFF = Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan.

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

Warrington, UK). Nucleotide sequence data were generated with an ABI PRISM 3100™ automated DNA sequencer. The raw sequence data were manipulated with the Sequence Navigator version 1.0.1 software package (Perkin-Elmer Applied BioSystems, Foster City, California).

Nucleotide sequences were aligned manually by inserting gaps. Gaps were treated as newstate in the parsimony analyses and as missing in the distance analyses. Phylogenetic analyses were performed with PAUP* (phylogenetic analysis using parsimony) version 4.0b10 (Swofford 2002). A partition homogeneity test (Farris et al 1994) was used to determine whether the ribosomal rRNA (ITS1, 5.8S, ITS2) partition and β-tubulin partition could be combined in the phylogenetic analyses. The aligned sequences were analyzed with parsimony by heuristic searches, with the tree-bisection-reconnection (TBR) and MULTREES options (saving all optimal trees) in effect, and sequences added randomly (100 additions). Uninformative sites were excluded and sites were reweighted according to their individual consistency indices (CI) to reduce the number of trees obtained. A distance analysis was performed with the Tamura-Nei parameter model (Tamura and Nei 1993) with adjusted settings (proportion of invariable site [I] = 0.4334; gamma distribution [G] = 0.4592; base frequency 0.2044, 0.3191, 0.2551; rate matrix 1.00, 2.2953, 1.00, 1.00, 4.5168). This model was chosen as suggested by Modeltest version 3.5 (Posada and Crandall 1998). Tree branch supports were assessed with a 1000-replicate bootstrap analysis. GenBank accession numbers of sequences generated in this study as well as those from previous phylogenetic studies are listed (TABLE I). The DNA sequence alignment has been deposited in TreeBASE (study accession number = 1489, matrix accession number = M2674).

Pathogenicity tests.—Two isolates of the newly described fungus (CMW 10031, CMW 10034) from *M. theaezans* were compared with isolates of *Chrysoporthe* spp. in a contained pathogenicity trial. One *Chrysoporthe* isolate (CMW 2113) represented *C. austroafricana* and has been used in previous pathogenicity tests (Myburg et al 2002b, van Heerden and Wingfield 2002). The other *Chrysoporthe* isolate (CMW 10639) represented *C. cubensis* from *E. grandis* in Colombia (Gryzenhout et al 2004).

Pathogenicity of the isolates was compared on 10 seedlings of *T. urvilleana* and a susceptible *E. grandis* clone (ZG14) in a custom-built phytotron. These trees were approximately 1.5 m tall and 7 mo old, and they were exposed to natural light conditions and an average daily temperature of ~25 C. Ten trees were inoculated at a constant height (~30 cm above the ground) with sterile water agar (WA, Biolab, Merck) plugs to serve as negative controls. Wounds were made on stems with a cork borer (6 mm diam) to expose the cambium. Disks of the same size were taken from the actively growing edges of colonies and inserted into the wounds with the mycelium facing inward. Wounds then were covered with plastic film to prevent desiccation and contamination. Trees were inoculated in Jun 2002 and lesion development was evaluated after 6 wk by measuring lesion lengths below the outer bark.

A field trial to consider the pathogenicity of the fungus under natural conditions was carried out on *M. theaezans* trees and a susceptible clone (018) of *E. grandis* on the Libano farm, Pereira, Risaralda, Colombia. Twenty *E. grandis* and the same number of *M. theaezans* trees were inoculated with isolate CMW 10031 from *M. theaezans* in Colombia. Trees of the *E. grandis* clone were approximately 18 mo old (~9 m high), while the *M. theaezans* trees formed part of the natural vegetation growing near the *E. grandis* trees and were of unknown age (~4–6 m high). Ten trees of each species were inoculated with WA as negative controls. Inoculations were carried out in the same way as those described for the phytotron inoculation, except that the inoculation wounds were 4 mm diam and covered with masking tape. The trees were inoculated in Jun 2002 and results were evaluated 12 wk later in Sep 2002. The lengths of the lesions produced in the cambium were measured and compared after removal of the bark. Data were compared using a one-way analysis of variance (ANOVA) computed with the SAS software package, v. 6 (2002). Mean lesion sizes together with 95% confidence limits were presented graphically.

RESULTS

Morphology.—Conidiomata of the fungus on bark specimens of *M. theaezans, T. urvilleana* and *E. grandis* were pyriform and superficial with fuscous black bases and most similar to fruiting structures of *C. cubensis* (Hodges 1980, Myburg et al 2002a, 2003, Gryzenhout et al 2004). These structures thus were different to the anamorph structures of all genera in the Diaporthales with orange fruiting structures (i.e. *Cryphonectria, Endothia* and *Rostraureum*), all of which have completely orange conidiomata (Myburg et al 2004a, Gryzenhout et al 2005). Conidiomata had fuscous-black, globose bases with long, slender necks (FIGS. 1A, 2A) that might be confused with those of *C. cubensis* in the absence of the orange neck apices. The conidiomatal base cells were *textura globosa*, umber to sienna when sectioned, with thick walls, while the inner cells were prosenchymatous (FIG. 1C). This is similar to the basal tissue of conidiomata of *C. cubensis* (Myburg et al 2002a, 2003; Gryzenhout et al 2004). The minute ([2.5−]3−4[−4.5] × 1−1.5[−2] μm), aseptate conidia (FIGS. 1I, 2C) also were similar to those of *C. cubensis* (Hodges 1980; Myburg et al 2002a, 2003; Gryzenhout et al 2004).

The fungus from *M. theaezans, T. urvilleana* and *E. grandis* in Colombia could be distinguished from *C. cubensis* by various unique characteristics of the conidiomata as well as on the basis of cultural morphology (TABLE II). Despite having basal tissue similar to that of *C. cubensis* tissue surrounding the conidiomatal locules is dark, consisting of larger cells than those of the adjacent prosenchyma (FIG. 1C).

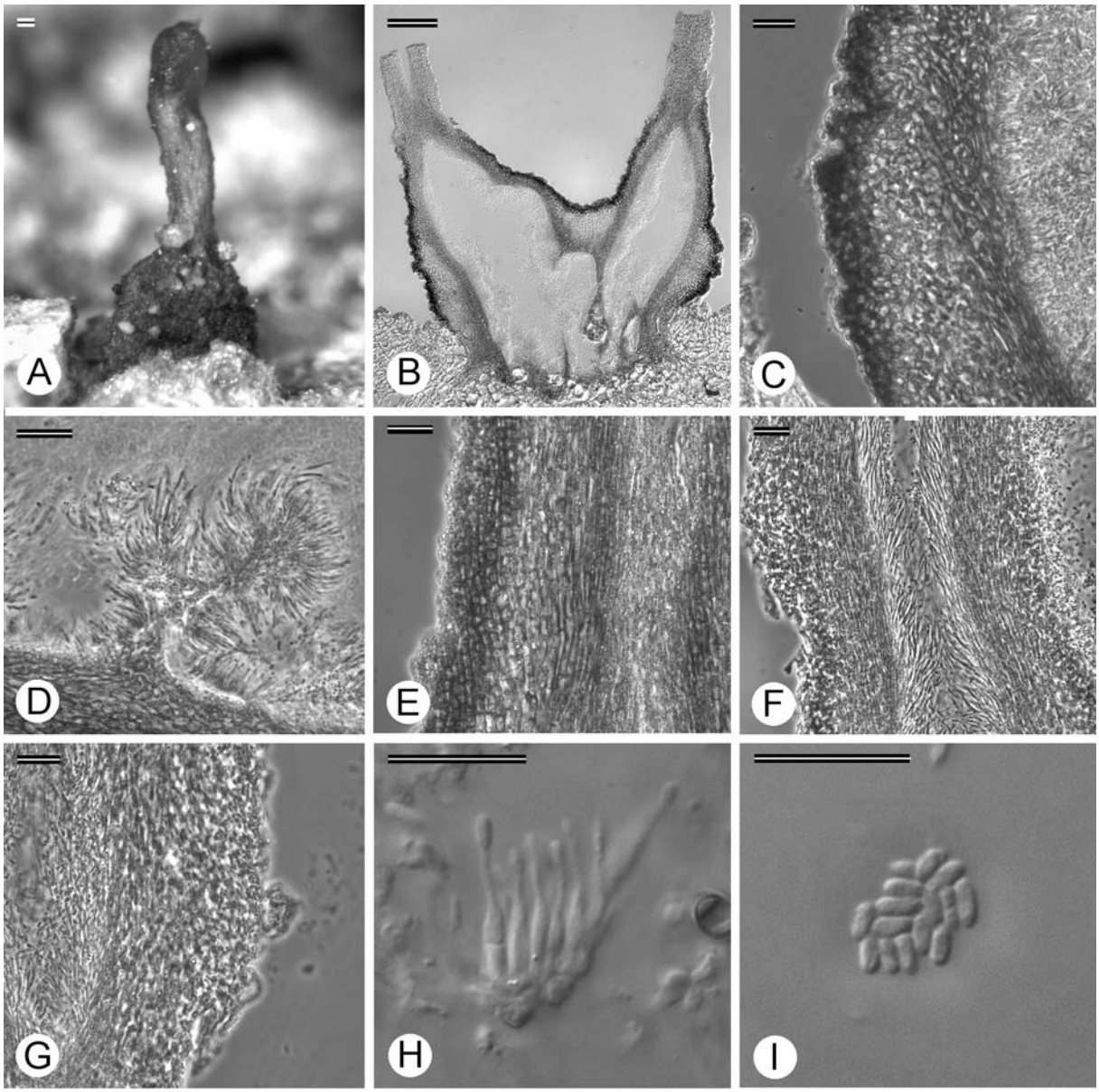

FIG. 1. Fruiting structures of *Aurapex penicillata*. A. Conidiomata on bark and in section (B). C. Tissue at base of conidioma. D. Protrusions in locule lining. E. Tissue of neck and periphyses in ostiolar canal (F). G. Tissue of neck apex. H. Conidiophores. I. Conidia. Bars: A–B = 100 µm, C–G = 20 µm, H–I = 10 µm.

Neck tissue differed from that found in *C. cubensis* and consisted of square cells at the outer edge, with *textura porrecta* cells at the center and thinner *textura porrecta* cells lining the ostiolar canals (FIGS. 1E, F). The tissue at the tips of the conidiomatal necks was orange and contained orange crystals (FIGS. 1A, G; 2A). Long, sterile hyphae similar to perithecial periphyses occurred in the ostiolar canals (FIG. 1F), but these are absent in *C. cubensis*. Unique protrusions consisting of three to several cell layers also were formed within the conidiomatal locule lining (FIGS. 1B, D; 2B). Cultures of the fungus from *Tibouchina* and *Miconia* spp. had an olivaceous to isabelline interior that differed from the creamy white cultures patched with cinnamon produced by *C. cubensis*.

No ascomata were observed that were known to have been produced by the newly discovered fungus. A few ascomatal structures were observed on specimen PREM 58575 from *M. theaezans*. However these structures stained brown in 3% KOH, not purple as was the case for the conidiomata of the fungus being studied and other members of this group in the Diaporthales (Castlebury et al 2002). These ascomata probably represent a species of *Valsa* co-infecting this particular host because the ascospores were allantoid and aseptate.

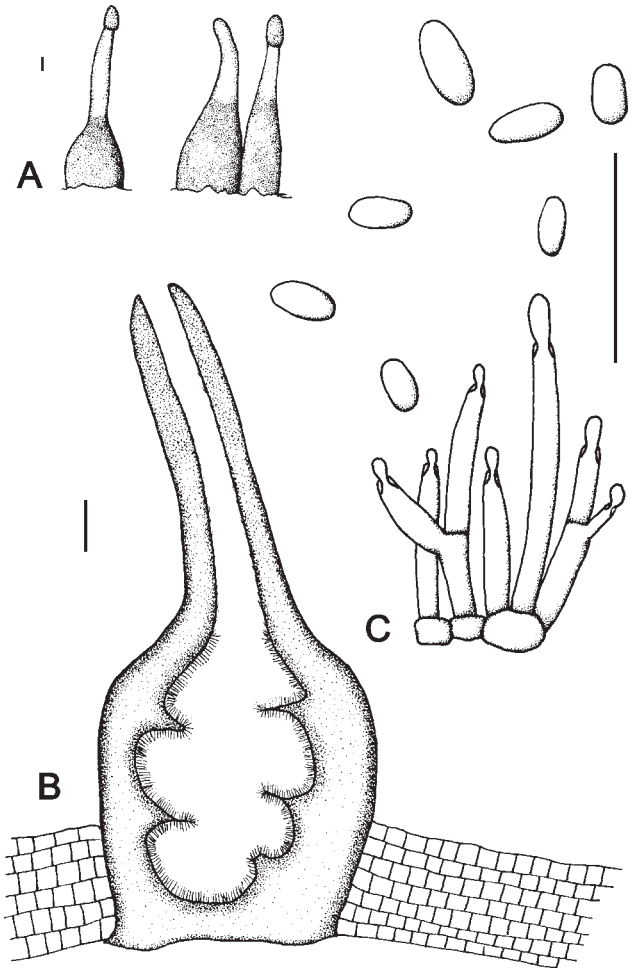

FIG. 2. Schematic drawings of *Aurapex penicillata*. A. Conidiomata on bark. B. Section through conidioma. C. Conidiophores and conidia. Bars: A–B = 100 µm, C = 10 µm.

DNA sequence comparisons.—The PCR products generated for the ribosomal and two β-tubulin gene regions were respectively 550–600 bp in size. The PHT test ($P = 0.182$) indicated no significant conflict between the two datasets for these gene regions, which thus were combined in the phylogenetic analyses. There were also no strongly supported conflicts between the trees obtained for the two gene regions separately. The sequence dataset included 32 taxa of which the two *D. ambigua* isolates represented a single outgroup taxon. The β-tubulin dataset (total 952 bp including both regions) consisted of 543 constant, 32 variable parsimony uninformative and 377 variable and parsimony informative characters (g1 = −0.780817). The ITS dataset (total 573 bp) consisted of 338 constant, 29 variable parsimony uninformative and 206 variable and parsimony-informative characters (g1 = −0.863275). The combined set amounted to a total of 1525 characters. The heuristic search produced 10 trees (tree length = 1095.3 steps, consistency index of = 0.805, retention index of = 0.918) that differed only in branch length for isolates. The tree obtained with distance analyses showed the same clades as the trees obtained with parsimony, one of which was chosen for presentation (FIG. 3). The same groupings of isolates with equally high bootstrap support also were obtained when ambiguously aligned portions, which mostly represented the introns of the β-tubulin dataset and the ITS1 region, were excluded from the analyses.

The isolates of the anamorphic fungus on *M. theaezans* formed a distinct clade (bootstrap support 100%) among the other clades in the phylogenetic tree, although it was apparent that some degree of variation existed between isolates, some originating from the same location but different fruiting structures. The clade representing the anamorphic fungus was most closely related to *Cryphonectria* (FIG. 3). The other clades represented different closely related genera, namely *Cryphonectria, Endothia* and *Rostraureum.* Therefore the anamorphic fungus grouped separately from the isolates of *C. cubensis* and *Chrysop. hodgesiana* from *Miconia* and *Tibouchina* spp. in Colombia, which grouped within the *Chrysoporthe* clade (FIG. 3).

TABLE II. Comparison of morphological features distinguishing *Aurapex penicillata* from *Chrysoporthe* spp

Morphological features	*Chrysoporthe* spp.[a]	*Aurapex penicillata*
Stromatic tissue	Cells surrounding locule similar to those in center.	Cells surrounding locule darker, larger.
Neck	Uniformly fuscous-black.	Orange apex.
Neck tissue	Cells at edge *textura globulosa.*	Cells at edge square.
Ostiolar canal	Contains no periphyses.	Contains periphyses.
Locule lining	Even to convoluted.	Forms protrusions consisting of three to several cell layers.
Culture morphology	Creamy white with cinnamon patches.	Creamy white with olivaceous to isabelline interior.

[a] According to Myburg et al (2002a, 2003), Gryzenhout et al (2004).

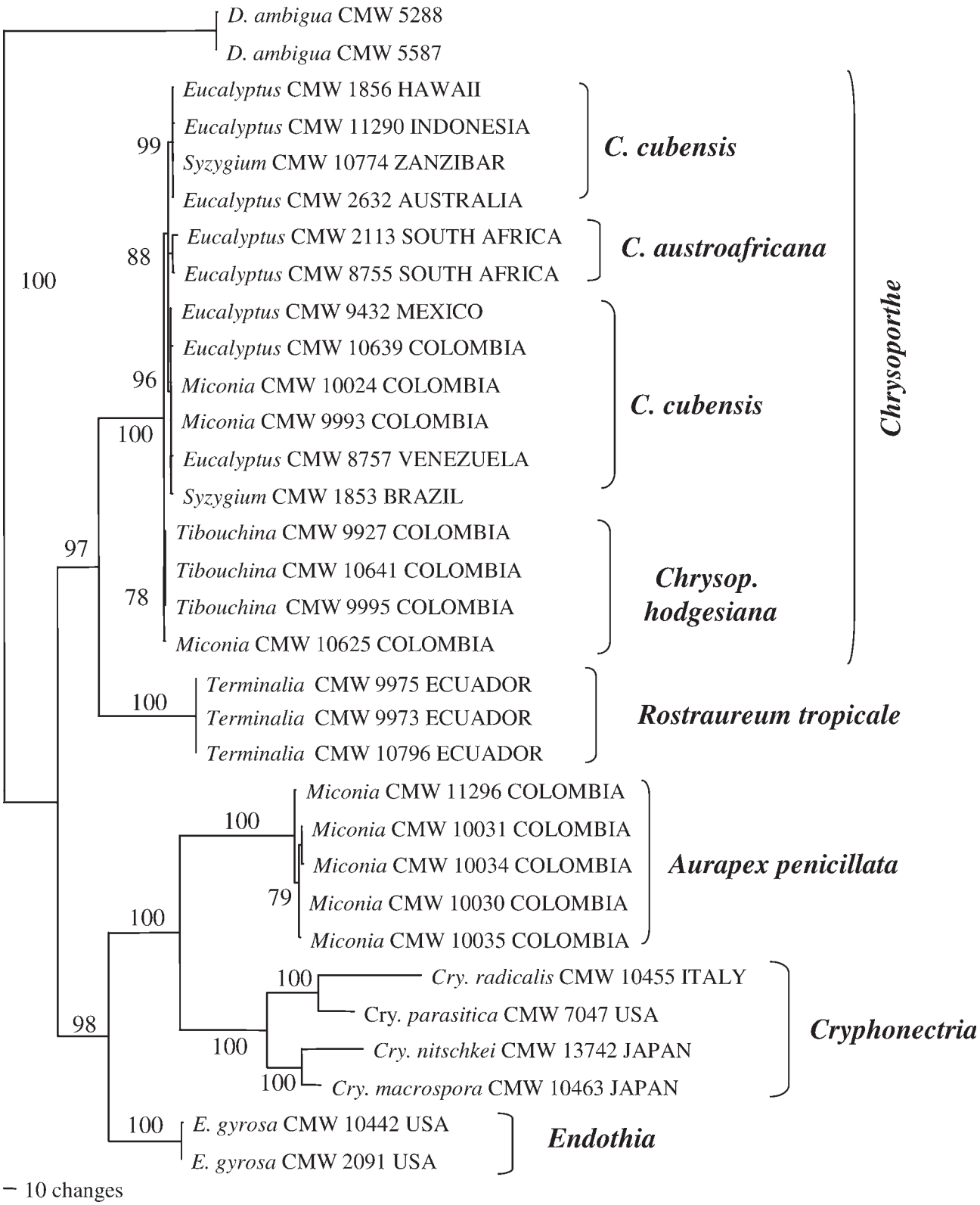

FIG. 3. One of 10 most parsimonious trees obtained from a combined dataset comprising ribosomal and β-tubulin gene sequences (tree length = 1095.3 steps, consistency index = 0.805, retention index = 0.918). Isolates representing the genera *Chrysoporthe, Rostraureum, Aurapex, Cryphonectria* and *Endothia* are represented. Confidence levels >70% determined by a 1000 replicate bootstrap analysis are indicated on the tree branch nodes. Two sequences for *Diaporthe ambigua* were used as outgroup.

TAXONOMY

The fungus found on *M. theaezans, T. urvilleana* and *E. grandis* from Colombia has clearly defined morphological features that distinguish it from *Chrysoporthella*, the anamorph genus of *Chrysoporthe*, to which it is morphologically most similar (TABLE II). These features are unlike those of any other coelomycete genus. The fuscous black conidiomata are also different from the uniformly orange fruiting structures of *Cryphonectria, Endothia* and *Rostraureum*, although the necks of the undescribed fungus are orange. These differences are supported by DNA sequence data showing that isolates of the morphologically distinct fungus from Colombia group separately from those representing *Chrysoporthe, Cryphonectria, Endothia* and *Rostraureum*.

No teleomorph was found for the fungus considered in this study. The distinct grouping of the fungus from Colombia, however, indicates that the fungus represents a distinct genus and not the anamorph of an already existing genus. Phylogenetic data present clear evidence of its affinity to members of *Cryphonectria* and allied genera residing in the Diaporthales. In the absence of a teleomorph the fungus from Colombia cannot be described in an ascomycete genus (ICBN, Art. 59.2, Greuter et al 2000). It thus is described as a new species in a new mitosporic genus, and the following description is provided.

Aurapex Gryzenh. & M. J. Wingf., gen. nov.

Etymology. aureus, golden, and *apex*, top, refers to the golden colored tips of the conidiomata.

Conidiomata globosa vel pyriformia, basibus fusconigris collis aurantiacis, superficialia. Collum e textura porrecta factum, cellulis parietalibus ostioli gracilioribus, in ora colli quadratis, intra canales ostiolares cum filamentis non septatis. Conidiophorae cylindricae vel ampulliformes, hyalinae. Cellulae conidiogenae phialidicae. Conidia obtusa, hyalina, non septata.

Conidiomata eustromatic, globose to pyriform base with one to several, long, cylindrical to attenuated necks with orange tips, superficial to slightly immersed, fuscous-black. *Tissue* at the edges of conidiomal bases of *textura globulosa*, with elongated cells adjacent to conidial lining and prosenchymatous tissue occurring in the center of the basal tissue. Tissue of necks made up of *textura porrecta* with cells lining the ostiole thinner, cells at edge of necks consisting of square cells. *Conidiophores* cylindrical to flask-shaped, hyaline, occasionally septate with or without lateral branches. *Conidiogenous cells* phialidic. *Conidia* obtuse, hyaline, aseptate.

Species typica: Aurapex penicillata

Aurapex penicillata Gryzenh. & M. J. Wingf., sp. nov. FIGS. 1–2

Etymology. penicillus, a painter's brush, refers to the brush-like protrusions formed by the lining of the conidial locules.

Conidiomata pyriformia cum collis, superficialia, basibus fusconigris collis aurantiacis, textura parietali loculorum prominentia e 3-circiter 15 cellulis formans. Textura colli in ora e cellulis quadratis, intus e textura porecta facta, intra canales ostiolares cum filamentis non septatis. Conidiophorae cylindricae vel ampulliformes apicibus attenuatis, hyalinae. Cellulae conidiogenae phialidicae. Conidia (2.5−)3−4(−4.5) × 1−1.5(−2) μm, obtusa, non septata, hyalina, in forma guttarum sporarum coccinearum exsudata. Coloniae cum hyphis aeriis sparsis, albocremet, intus atro-olivaceae vel isabellinae, celeriter crescentes. temperatura optima 25 C.

Conidiomata single or aggregated, eustromatic, with globose to pyriform bases and attenuated or cylindrical necks, base 120–400 μm high, 300–700 μm wide above bark surface, necks up to ~800 μm long depending on environmental conditions, 80–225 μm wide, conidiomata superficial to slightly immersed, bases fuscous-black with tips of necks orange (FIGS. 1A, B; 2A, B). Unilocular or multilocular (FIGS. 1B, 2B), locules up to 360 μm diam at widest point, locule lining producing conidiophores forming protrusions consisting of 3 to ~15 cells (FIGS. 1B, D; 2B), locules opening through 1–3 necks, each either connected to a single locule or to more than one locule. *Tissue* of base complex with thick-walled cells, *textura globulosa*, umber to sienna at edge, cells around the locules sienna to hazel, larger and more elongated, and almost white prosenchymatous tissue occurring between the edge and the locule (FIG. 1C). Neck tissue consisting of hazel, double-walled, square cells at the edge, with the cells lining the ostiole thinner and those at the center of *textura porrecta* tissue (FIG. 1E, F), long, aseptate filaments, similar to periphyses, occurring inside the ostiolar canals (FIG. 1F), tip of necks of *textura epidermoidea*, containing orange crystals (FIG. 1G). *Conidiophores* (6−) 7.5−13.5(−18.5) × (0.5−)1−1.5(−2) μm, cylindrical or flask-shaped with attenuated apices, occasionally with separating septa and branching, hyaline (FIGS. 1H, 2C). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches, collaret and periclinal thickening inconspicuous (FIGS. 1H, 2C). *Conidia* (2.5−)3−4(−4.5) × 1−1.5(−2) μm, obtuse, aseptate, hyaline (FIGS. 1I, 2C), exuded as scarlet spore droplets.

Culture morphology fluffy with few aerial hyphae, creamy white with a dark olivaceous to isabelline interior, margins even, conidiomata occasionally pro-

duced in mature cultures, optimum growth at 25 C, isolates covering the surface of 90 mm plates on Day 6 at the optimum temperature.

Holotype. COLOMBIA. RISARALDA: Pereira, Libano farm, 75°35′49″W and 4°43′13″N, 2102 msal. Bark of *Miconia theaezans*, Sep 2002, *C.A. Rodas* (PREM 57520, ex-type culture CMW 10030 = CBS 115740, additional cultures CMW 10031, CMW 10034, CMW 10035 = CBS 115742).

Additional material examined. COLOMBIA. QUINDIO: Salento, Andes farm, 75°33′16″W and 4°41′08″N, 2102 masl. Bark of *Miconia theaezans*, May 2000, *M.J. Wingfield* (PREM 58576, living cultures CMW 11296 = CBS 115801). RISARALDA: Pereira, La Selva farm, 75°35′34″W and 4°47′26″N, 2048 msal. Bark of *Miconia theaezans*, Nov 1998, *C.A. Rodas* (PREM 58572). Libano farm, 75°35′49″W and 4°43′13″N, 2102 msal. Bark of *Eucalyptus grandis*, Sep 2002, *C.A. Rodas* (PREM 58578). ANTIOQUIA: Granada, Granada farm, 75°8′10″W and 6°6′52″N, 2050 msal. Bark of *Tibouchina urvilleana*, Nov 1998, *C.A. Rodas* (PREM 58573). CALDAS: Riosucio, La Argentina farm, 75°44′55″W and 5°22′25″N, 2247 msal. Bark of *Tibouchina urvilleana*, Nov 1998, *C.A. Rodas* (PREM 58574, PREM 58575). VALLE, Darien, Cedral farm, 76°26′06″W and 3°57′06″N, 1825 masl. Bark of *Eucalyptus grandis*, Dec 2001, *C.A. Rodas* (PREM 58577).

Known distribution. Colombia

Habitat. Miconia theaezans, Tibouchina urvilleana, Tibouchina lepidota, Eucalyptus grandis

This key is provided to differentiate *Aurapex* from closely related genera.

KEY TO *CRYPHONECTRIA, ENDOTHIA, CHRYSOPORTHELLA, ROSTRAUREUM* AND *AURAPEX*

1. Base of anamorph fruiting structures fuscous-black 2
1. Anamorph fruiting structures completely orange 3
   2. Conidiomata uniformly fuscous-black, locule lining even. . . . . *Chrysoporthella*
   2. Conidiomata fuscous-black but with tip of neck orange, locule lining have brush-like protrusions . . . . . *Aurapex*
      3. Conidiomata rostrate, superficial . . . . . *Rostraureum*
      3. Conidiomata pulvinate 4
         4. Conidiomata semi-immersed, ascospores uniseptate . . . . . *Cryphonectria*
         4. Conidiomata superficial, ascospores aseptate . . . . . *Endothia*

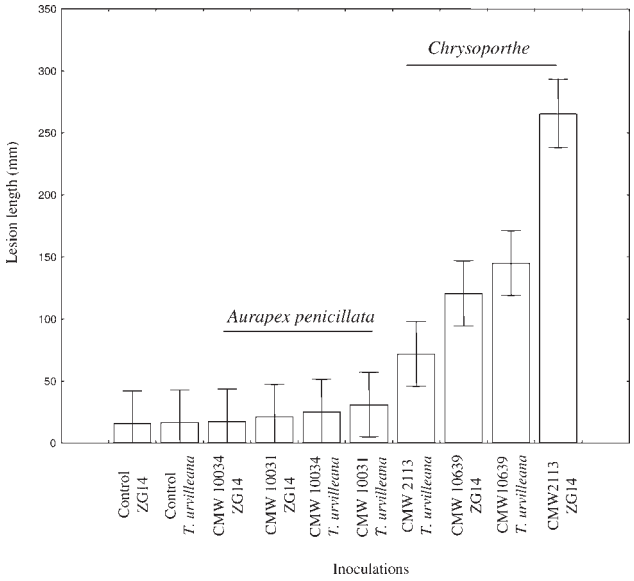

FIG. 4. Mean lesion length in *Tibouchina urvilleana* and a ZG14 clone of *Eucalyptus grandis* resulting from greenhouse inoculations with *Aurapex penicillata* (CMW 10031, CMW 10034), *Chrysoporthe cubensis* (CMW 10639), *Chrysoporthe austroafricana* (CMW 2113) and a negative control. Means are shown with 95% confidence limits.

Pathogenicity.—Isolates of *A. penicillata* (CMW 10031, CMW 10034) produced small lesions on both *T. urvilleana* and the *E. grandis* clone in the phytotron trial. These lesions did not differ significantly from the control inoculations (FIG. 4). Conidiomata were produced abundantly on the surfaces of the lesions. Lesions associated with *A. penicillata* were significantly smaller ($P < 0.0001$) than those associated with *C. cubensis* and *C. austroafricana* on *E. grandis* and *T. urvilleana* with those of *C. austroafricana* (CMW 2113) on *E. grandis* longest (FIG. 4). The latter isolate was also the only one observed to girdle inoculated stems resulting in the production of epicormic shoots below the sites of inoculation.

In the Colombian field trial the isolate of *A. penicillata* (CMW 10031) gave rise to small lesions on *M. theaezans* that did not differ significantly from control inoculations (FIG. 5). Lesions produced on the *E. grandis* clone did not differ significantly from those on *M. theaezans* ($P = 0.0217$) and were extremely variable (FIG. 5). Control inoculations on the *E. grandis* clone also gave rise to small lesions, possibly due to endophytes naturally present in the stems of trees (FIG. 5).

DISCUSSION

This study treats the discovery of a new mitosporic genus in the Diaporthales, which thus far contains

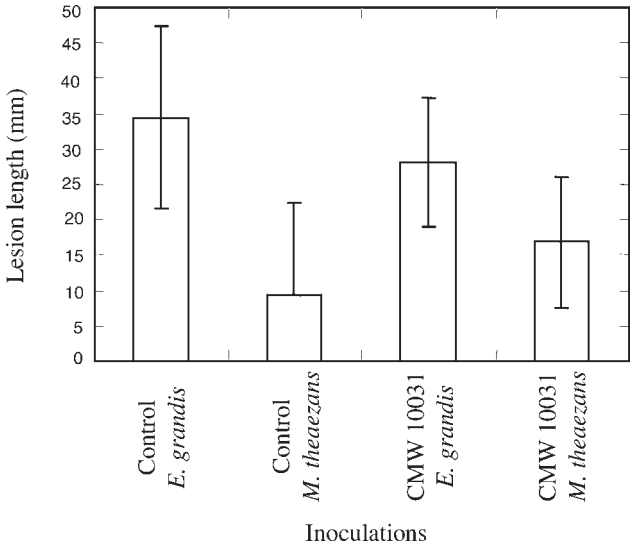

FIG. 5. Comparison of mean lesion length resulting from field inoculations with *Aurapex penicillata* (CMW 10031) and a negative control in *Miconia theaezans* and a susceptible clone (018) of *Eucalyptus grandis*. Means are shown with 95% confidence limits.

a single species, *Aurapex penicillata*. All indications are that this fungus is native to South America where it occurs naturally on *Miconia* and *Tibouchina* spp. *Aurapex penicillata* is closely related to *Cryphonectria* and *Endothia* and the recently described genera *Chrysoporthe* (anamorph *Chrysoporthella*) and *Rostraureum*, which contain species previously in *Cryphonectria*. This was established through DNA sequence comparisons of the ITS region of the ribosomal repeat and β-tubulin genes.

The distinction of *A. penicillata* based on DNA sequence comparisons as a genus separate from *Cryphonectria, Chrysoporthe, Endothia* and *Rostraureum*, and not an anamorph genus of one of the existing genera, is well supported by morphological characteristics. Although the teleomorph is unknown the morphological characteristics of the asexual state differ substantially from those of its closest relatives. Conidiomata of *A. penicillata* are fuscous-black, superficial and pyriform with attenuated necks. These resemble the conidiomata of *Chrysoporthella*, the anamorph of *Chrysoporthe* that also has fuscous black conidiomata. *Aurapex*, however, can be distinguished easily from *Chrysoporthella* based on the distinctly orange tips of the necks and by its unique stromatal tissue. This new fungus also can be distinguished from the conidiomata of *Cryphonectria, Endothia* and *Rostraureum* spp., which are completely orange. These anamorph differences are consistent with those found in previous studies (Venter et al 2002, Myburg et al 2004a, Gryzenhout et al 2005). These studies showed that stromatic and anamorph morphology are the most informative morphological characters that support the different phylogenetic assemblages, even in the absence of sexual structures. Recognition of these phylogenetic assemblages as different genera is strongly supported by the fact that they are morphologically distinct and they would not comfortably reside in a single genus.

Aurapex penicillata could be confused with the serious pathogens *C. cubensis* and *Chrysop. hodgesiana*, which occur on the same hosts and in the same region. This is especially so when the characteristic orange necks of *A. penicillata* become dislodged from their conidiomatal bases. Moreover fruiting structures of these different fungi can occur on the same piece of bark. Of these three fungi only *C. cubensis* is known to cause serious disease on *Eucalyptus* (Wingfield 2003, Gryzenhout et al 2004) although *Chrysop. hodgesiana* also can infect *Eucalyptus* trees in artificial inoculations (Wingfield et al 2001, Gryzenhout et al 2004).

Aurapex penicillata gave rise to lesions on *E. grandis* in pathogenicity tests, but there was no evidence of significant pathogenicity, at least in comparison to *C. cubensis*. Although *A. penicillata* occurs on *E. grandis* under natural conditions, the pathogen appears to be mainly associated with dead branch stubs. Because of differences in pathogenicity and importance it is necessary to identify *C. cubensis, Chrysop. hodgesiana* and *A. penicillata* correctly in *Eucalyptus* plantation disease surveys.

Pathogenicity tests conducted in this study should be seen as preliminary because they were limited by the lack of a complete series of known hosts of *A. penicillata*. Our primary objective was to assess pathogenicity, especially given the fact that the highly pathogenic *C. cubensis* now has been found to cause severe disease on Melastomataceae native to South America (Rodas et al 2005). Although preliminary our results show that *A. penicillata* probably poses no threat to *Eucalyptus* or *Melastomatacea*. Furthermore the common occurrence of the fungus on native Melastomataceae in Colombia adds substance to our view that it is native to hosts in that family. Its ability to infect *Eucalyptus* spp. is probably opportunistic and related to the high levels of inoculum on surrounding native vegetation in Colombia.

ACKNOWLEDGMENTS

We thank Dr B.E. Eisenberg for the statistical analyses presented in this study as well as Liliana Perafan for additional statistical support. Dr H.F. Glen of the National Botanical Institute of Pretoria, Pretoria, provided the Latin description and assisted us in choosing a name for the new fungus. Dr Luis F. Osorio and Mr Ramon Arbelaez provided

technical assistance during sampling and field trials in Colombia. This study was made possible through financial support of Smurfit Carton de Colombia, as well as the National Research Foundation (NRF), members of the Tree Pathology Co-operative Programme (TPCP), the THRIP support programme of the Department of Trade and Industry and the Department of Science and Technology/ NRF Centre of Excellence in Tree Health Biotechnology (CTHB), South Africa.

LITERATURE CITED

Boerboom JHA, Maas PWT. 1970. Canker of *Eucalyptus grandis* and *E. saligna* in Surinam caused by *Endothia havanensis*. Turrialba 20:94–99.

Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN. 2002. A preliminary overview of the Diaporthales based on large subunit nuclear ribosomal DNA sequences. Mycologia 94:1017–1031.

Conti E, Litt A, Sytsma K. 1996. Circumscription of Myrtales and their relationships with other rosids: evidence from RBCL sequence data. American J Bot 83:221–233.

Everett TH. 1981. The New York Botanical Garden Illustrated Encyclopaedia of Horticulture. Vol. 7. New York: Garland Publishing. p 2196–2197.

Farris JS, Källersjö M, Kluge AG, Bult C. 1994. Testing significance of incongruence. Cladistics 10:315–319.

Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environm Microbiol 61:1323–1330.

Greuter W, McNeill J, Barrie FR, Burdet HM, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth DL. 2000. International Code of Botanical Nomenclature (Saint Louis Code). XVI International Botanical Congress, St Louis, Missouri, Jul–Aug 2000. Königstein, Germany: Koeltz Scientific Books.

Gryzenhout M, Myburg H, van der Merwe NA, Wingfield BD, Wingfield MJ. 2004. *Chrysoporthe*, a new genus to accommodate *Cryphonectria cubensis*. Stud Mycol 50:119–142.

———, ———, Wingfield BD, Montenegro F, Wingfield MJ. 2005. *Rostraureum tropicale* gen. sp. nov. (*Diaporthales*) associated with dying *Terminalia ivorensis* in Ecuador. Mycol Res 109:1029–1044.

Hodges CS. 1980. The taxonomy of *Diaporthe cubensis*. Mycologia 72:542–548.

———, Alfenas AC, Cordell CE. 1986. The conspecificity of *Cryphonectria cubensis* and *Endothia eugeniae*. Mycologia 78:334–350.

Myburg H, Gryzenhout M, Heath RN, Roux J, Wingfield BD, Wingfield MJ. 2002b. Cryphonectria canker on *Tibouchina* in South Africa. Mycol Res 106:1299–1306.

———, ———, Wingfield BD, Milgroom MG, Shigeru K, Wingfield MJ. 2004b. DNA sequence data and morphology define *Cryphonectria* species in Europe, China, and Japan. Can J Bot 82:1730–1743.

———, ———, ———, Stipes RJ, Wingfield MJ. 2004a. Phylogenetic relationships of *Cryphonectria* and *Endothia* species, based on DNA sequence data and morphology. Mycologia 96:990–1001.

———, ———, ———, Wingfield MJ. 2002a. β-tubulin and Histone *H3* gene sequences distinguish *Cryphonectria cubensis* from South Africa, Asia and South America. Can J Bot 80:590–596.

———, ———, ———, ———. 2003. Conspecificity of *Endothia eugeniae* and *Cryphonectria cubensis*: A re-evaluation based on morphology and DNA sequence data. Mycoscience 104:187–196.

———, Wingfield BD, Wingfield MJ. 1999. Phylogeny of *Cryphonectria cubensis* and allied species inferred from DNA analysis. Mycologia 91:243–250.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

Rayner RW. 1970. A Mycological Colour Chart. Kew, Surrey, U.K.: Commonwealth Mycological Institute.

Rodas CA, Gryzenhout M, Myburg H, Wingfield BD, Wingfield MJ. 2005. Discovery of the *Eucalyptus* canker pathogen *Chrysoporthe cubensis* on native *Miconia* (Melastomataceae) in Colombia. Plant Pathol 54:460–470.

SAS Statistical Software. 2000. SAS/STAT Users Guide. Version 6. 4th ed. vols. 1 and 2. SAS Institute: Cary, North Carolina.

Sharma JK, Mohanan C, Florence EJM. 1985. Disease survey in nurseries and plantations of forest tree species grown in Kerala. KFRI Research Report 36. Kerala, India: Kerala Forest Research Institute.

Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sunderland, Massachusetts: Sinauer Associates.

Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526.

Turnbul JW. 2000. Economic and social importance of eucalypts. In: Keane PJ, Kile GA, Podger FD, Brown BN, eds. Diseases and pathogens of eucalypts. Australia: CSIRO Publishing. p 1–9.

van Heerden SW, Wingfield MJ. 2002. Effect of environment on the response of *Eucalyptus* clones to inoculation with *Cryphonectria cubensis*. For Pathol 32:395–402.

Venter M, Myburg H, Wingfield BD, Coutinho TA, Wingfield MJ. 2002. A new species of *Cryphonectria* from South Africa and Australia, pathogenic on *Eucalyptus*. Sydowia 54:98–119.

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

Wingfield MJ. 2003. Daniel McAlpine Memorial Lecture. Increasing threat of diseases to exotic plantation forests in the Southern Hemisphere: lessons from Cryphonectria canker. Australas Plant Pathol 23:133–139.

———, Rodas C, Myburg H, Venter M, Wright J, Wingfield BD. 2001. Cryphonectria canker on *Tibouchina* in Colombia. Fors Pathol 31:297–306.