CHAPTER 9

Microthia, *Holocryphia* and *Ursicollum*, three new genera on *Eucalyptus* and *Coccoloba* for fungi previously known as *Cryphonectria*

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Microthia, *Holocryphia* and *Ursicollum*, three new genera on *Eucalyptus* and *Coccoloba* for fungi previously known as *Cryphonectria*

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Abstract: *Cryphonectria havanensis* is a fungus associated with *Eucalyptus* species in Cuba and Florida (U.S.A.). Until recently, there have been no living cultures of *C. havanensis* and it has thus not been possible to assess its taxonomic status. Isolates thought to represent this fungus have, however, emerged from surveys of *Eucalyptus* in Mexico and Hawaii (U.S.A.). Results of this study showed that these isolates represent *C. havanensis* but reside in a genus distinct from *Cryphonectria sensu stricto*, which is described here as *Microthia*. Isolates of an unidentified fungus occurring on *Myrica faya* in the Azores and Madeira also grouped in *Microthia* and were identical to other *M. havanensis* isolates. *Cryphonectria coccolobae*, a fungus occurring on sea grape (*Coccoloba uvifera*) in Bermuda and Florida, was found to be morphologically identical to *Microthia* and is transferred to this genus, but as a

distinct species. Surveys for *M. coccolobae* on sea grape in Florida, yielded a second diaporthalean fungus from this host. This fungus is morphologically and phylogenetically distinct from *M. coccolobae* and other closely related taxa and is described as *Ursicollum fallax* gen. et sp. nov. Phylogenetic analyses in this study have also shown that isolates of *C. eucalypti*, a pathogen of *Eucalyptus* in South Africa and Australia, group in a clade separate from all other groups including that representing *Cryphonectria sensu stricto*. This difference is supported by the fact that *Cryphonectria eucalypti* has ascospore septation different to that of all other *Cryphonectria* species. A new genus, *Holocryphia*, is thus erected for *C. eucalypti*.

Taxonomic novelties: *Microthia* Gryzenh. & M. J. Wingf. gen. nov., *Microthia havanensis* (Bruner) Gryzenh. & M. J. Wingf. comb. nov., *Microthia coccolobae* (Vizioli) Gryzenh. & M. J. Wingf. comb. nov., *Holocryphia* Gryzenh. & M. J. Wingf. gen. nov., *Holocryphia eucalypti* (M. Venter & M. J. Wingf.) Gryzenh. & M. J. Wingf. comb. nov., *Ursicollum* Gryzenh. & M. J. Wingf. gen. nov., *Ursicollum fallax* Gryzenh. & M. J. Wingf. sp. nov.

Key words: *Cryphonectria coccolobae, Cryphonectria eucalypti, Cryphonectria havanensis, Diaporthales*, Phylogeny

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INTRODUCTION

Cryphonectria havanensis (Bruner) M.E. Barr was first described from *Eucalyptus* spp. (*Myrtaceae, Myrtales*) in Cuba (Bruner 1916). Bruner (1916) found this fungus on bark of dead, injured or healthy *Eucalyptus* trees, but it did not appear to cause disease. *Cryphonectria havanensis* was also found on dead branches of mango

(*Mangifera indica*, *Anacardiaceae*, *Sapindales*) and avocado (*Persea gratissima*, *Lauraceae*, *Laurales*) lying on the ground in the vicinity of the *Eucalyptus* trees (Bruner 1916). Besides these exotic hosts, fruiting structures of *C. havanensis* were also found on the bark of jobo (*Spondias mombin*, *Anacardiaceae*, *Sapindales*), a plant native to Cuba (Bruner 1916).

Barnard *et al.* (1987) found *C. havanensis* on *Eucalyptus* plantations in Florida. The fungus was, however, reported as *Cryphonectria gyrosa* (Berk. & Broome) Sacc., a name previously used for the species (Kobayashi 1970, Hodges 1980). The identification of the fungus as *C. havanensis* was based on the presence of orange stromata, as well as conidial and ascospore dimensions that resembled those of the type specimen from Cuba. *Chrysoporthe cubensis* (Bruner) Gryzenh. & M.J. Wingf., a fungus previously known as *Cryphonectria cubensis* (Bruner) Hodges (Gryzenhout *et al.* 2004) and a serious pathogen of *Eucalyptus* spp. (Wingfield 2003), was also found in the same plantations (Barnard *et al.* 1987). *Cryphonectria havanensis* was mainly associated with dead coppice shoots in stands of *Eucalyptus grandis* while *Chr. cubensis* was the causal agent of basal cankers and death of coppice shoots (Barnard *et al.* 1987).

Other than reports from tropical or sub-tropical areas of the world such as Cuba and Florida, the name *C. havanensis* has also been used for collections of a fungus from *Eucalyptus globulus* in Japan (Kobayashi & Itô 1956, Kobayashi 1970). The fungus referred to as *C. havanensis* in Japan is also known from other host genera besides *Eucalyptus* (Kobayashi 1970), namely species of *Quercus* (*Fagaceae*, *Fagales*), *Betula* (*Betulaceae*, *Fagales*) and *Pyrus* (*Rosaceae*, *Rosales*). A recent study employing DNA sequence comparisons (Myburg *et al.* 2004a) showed that the fungus referred to as *C. havanensis* in Japan is the same as *Cryphonectria nitschkei*

(G. H. Otth) M.E. Barr. The study by Myburg *et al.* (2004a) did not, however, consider whether *C. nitschkei* is the same as the fungus referred to as *C. havanensis* from Cuba, where *C. havanensis* was originally described (Bruner 1916).

Cryphonectria havanensis and four other fungi in the *Diaporthales* with orange stromatic tissue are known from islands in the Caribbean Sea and Atlantic Ocean (Fig. 1). *Chrysoporthe cubensis* is well-known from several countries in Central and South America (Gryzenhout *et al.* 2004), including Cuba (Bruner 1917) where *C. havanensis* was first discovered. *Cryphonectria coccolobae* (Vizioli) Micales & Stipes occurs as a saprobe on twigs, branches and seeds of *Coccoloba uvifera* (sea grape, *Polygonaceae*, *Polygonales*) from Bermuda (Vizioli 1923) and Florida (Micales & Stipes 1987, Barnard *et al.* 1993). In the Azores and Madeira, an unidentified species of *Cryphonectria* has been associated with cankers on *Myrica faya* (*Myricaceae*, *Myricales*) (Gardner & Hodges 1990, Hodges & Gardner 1992). Another closely related species, *Cryphonectria longirostris* (Earle) Micales & Stipes, occurs in Puerto Rico and Trinidad (Earle 1901, Roane 1986). This fungus is saprobic and has recently been transferred to the new genus *Rostraureum* (Gryzenhout *et al.* 2005a). *Rostraureum* also includes a second new species, *Rostraureum tropicale* Gryzenh. & M.J. Wingf., which is a pathogen of *Terminalia ivorensis* trees in Ecuador (Gryzenhout *et al.* 2005a).

The correct identity of *C. havanensis* and its phylogenetic relationship with species of *Cryphonectria* and closely related genera remained unresolved (Myburg *et al.* 2004b). This is largely due to the absence of isolates that could, with reasonable certainty, be attributed to this species. The same problem was true for *C. coccolobae* (Myburg *et al.* 2004b), which has been suspected to be a synonym of *C. havanensis* (Hodges & Gardner 1990). The relationship between *C. havanensis* and the fungus

attributed to this species from Japan (Myburg *et al.* 2004a) also remains to be resolved.

Recently, fungi closely resembling *C. havanensis* were found on *Eucalyptus* spp. in Mexico and Hawaii, where this fungus had not been known previously. These collections included cultures and specimens on bark and enabled us to reconsider questions relating to the identity and the phylogenetic position of *C. havanensis*.

MATERIALS & METHODS

Symptoms and collection of samples

Fruiting structures thought to represent *C. havanensis* were collected from cankers and dead trees on the stems of *E. grandis* and an unidentified *Eucalyptus* sp. on the island of Kauai (Hawaii, U.S.A.). Fruiting structures of *Chr. cubensis* were also found on the stems of the same *Eucalyptus* spp. in the plantation, but were associated with cankers on living trees. *Chrysoporthe cubensis* was also common on cankered *E. grandis* trees on the island of Hawaii. Specimens of this fungus previously examined from the Hawaiian Islands were all from Kauai (Hodges *et al.* 1979, Myburg *et al.* 2003), and collections made in this study represent the first record of *Chr. cubensis* from the island of Hawaii.

Bark tissue bearing orange fruiting structures resembling *C. havanensis* was also collected from cankers on *E. grandis* in Las Chiapas, Mexico. An additional isolate from Mexico was received from Dr. E.L. Barnard (Florida Division of Forestry, FDACS, Gainesville, Florida). An isolate (ATCC 60862 = CMW 14332) representing *C. havanensis* (collected as *C. gyrosa*) from *Eucalyptus* plantations in Florida, linked to the study of Barnard *et al.* (1987), was acquired from the American

Type Culture Collection (ATCC). Isolates and specimens (Tables 1–2) linked to the report of a *Cryphonectria* species from *M. faya* in the Azores (Gardner & Hodges 1990) were also included in this study. This collection also included authentic isolates (Table 1) of *C. parasitica* from *Castanea sativa* in the Azores (Gardner & Hodges 1990). Unfortunately, no isolates of *C. havanensis* could be obtained from Cuba despite surveys aimed at re-collecting the fungus in that country.

During surveys for *C. coccolobae* on *Co. uvifera* in Florida, a fungus with distinctive orange fruiting structures was found in the vicinity of Fort Lauderdale, Key Biscayne, Dania and Oakland Park (Tables 1–2). This fungus was fruiting profusely on branches and twigs, but was not associated with disease symptoms. It was included in this study to determine whether it represents *C. coccolobae.*

Isolations from fungal structures on bark specimens were made from single conidia and ascospores collected from the apices of pycnidia and perithecia, respectively. The isolates used in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa and representative isolates not originally obtained from internationally recognised collections have been deposited with the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands (Table 1). The original bark specimens from which cultures were made have been deposited in the National Collection of Fungi (PREM), Pretoria, South Africa (Table 2).

DNA sequence comparisons

DNA was extracted from isolates grown in malt extract broth (20 g/L malt extract, Biolab, Midrand, South Africa) as described by Myburg *et al.* (1999). DNA sequences were derived for the internal transcribed spacer (ITS) regions ITS1 and

ITS2, including the conserved 5.8S gene of the ribosomal RNA (rRNA) operon, using primer pair ITS1/ITS4 (White *et al.* 1990), and β-tubulin genes using the primer pairs Bt1a/Bt1b and Bt2a/Bt2b respectively (Glass & Donaldson 1995). For these, the protocols of Myburg *et al.* (1999) and Myburg *et al.* (2002), respectively, were followed. Purification of PCR products for subsequent sequence reactions was done using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). Sequence reactions were performed with the same primers used in the PCR reactions, using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase (Perkin-Elmer, Warrington, UK). The sequencing reactions were run on an ABI PRISM 3100™ automated DNA sequencer. Nucleotide sequences were analysed using Sequence Navigator v. 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, California, U.S.A.) software.

New sequences were submitted to GenBank (Table 1). These also included sequences obtained in this study of additional *Cryphonectria eucalypti* M. Venter & M.J. Wingf. isolates to strengthen the *C. eucalypti* clade presented by Myburg *et al.* (2004b). This fungus is a pathogen of *Eucalyptus* trees in South Africa (Van der Westhuizen *et al.* 1993, Gryzenhout *et al.* 2003) and Australia (Walker *et al.* 1985, Yuan & Mohammed 2000). The sequences were compiled into a matrix using a modified data set (TreeBASE accession numbers S1128, M1935) of Myburg *et al.* (2004b) as a template. Additional sequences from other studies were also added to the data matrix. These included sequences of *Chrysoporthella hodgesiana* Gryzenh. & M.J. Wingf. (Gryzenhout *et al.* 2004, Rodas *et al.* 2005), and those of *Cryphonectria parasitica* (Murrill) M.E. Barr, *Cryphonectria macrospora* (Tak. Kobay. & Kaz. Itô) M.E. Barr and *C. nitschkei* from Japan, including those of isolates referred to as *C. havanensis* (Myburg *et al.* 2004b). Sequences representing *R.*

tropicale (Gryzenhout *et al.* 2005a) and *Amphilogia gyrosa* (Berk. & Broome) Gryzenh. & M.J. Wingf., the new genus that now contains *Cryphonectria gyrosa* (Gryzenhout *et al.* 2005b), were also added. The resultant dataset was deposited with TreeBASE (S***, M***).

The alignment was obtained using the web interface (http://timpani.genome.ad.jp/%7Emafft/server/) of the alignment program MAFFT v. 5.667 (Katoh *et al.* 2002). Phylogenetic analyses were made using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). A 500 replicate partition homogeneity test (PHT) was done on the rRNA and β-tubulin gene sequence data sets (after the exclusion of uninformative sites) to determine whether they could be analysed collectively (Farris *et al.* 1994). Phylogenetic analyses included parsimony and distance methods. Maximum parsimony (MP) was inferred using the heuristic search option with the tree-bisection-reconnection (TBR) branch swapping and MULTREES options (saving all optimal trees) effective and a 100 random additions. Gaps inserted during manual sequence alignment were treated as fifth character (NEWSTATE) in the heuristic searches, and missing in distance analyses. Uninformative characters were excluded and remaining characters were reweighted according to the individual Consistency Indices (CI) to reduce the number of trees. For the distance analyses, the correct model for the datasets was found with MODELTEST v. 3.5 (Posada & Crandall 1998). This model was the Tamura-Nei model (TrN+G+I) (Tamura & Nei 1993) with the Gamma distribution shape parameter (G) set to 0.9717 and frequency of invariable sites (I) 0.4643; base frequencies of 0.1903, 0.3411, 0.2301 and 0.2385; and rate matrix of 1, 3.1147, 1, 1, 4.1643, 1. Support for the branch nodes in the various phylogenetic trees was tested with a 1000 replicate bootstrap analysis and is presented as a 70 % majority rule tree.

Morphology

A large number of specimens from different species, hosts and geographical areas were included in the morphological comparisons (Table 2). These included the type specimen of *C. havanensis* (BPI 614275). Conidiomata and ascostromata were cut from bark specimens, rehydrated (1 min) in boiling water and sectioned with a Leica CM1100 cryostat at –20 ºC, 12–14 µm thick. For embedding, Leica mountant (Setpoint Premier, Johannesburg, South Africa) was used, which was dissolved in water after sectioning. Lactic acid (85 %) was used to prepare semi-permanent slides. Hand sections were made with a razor blade to more closely study conidiophore morphology. Fruiting structures were also mounted in 3 % KOH when conidiophores and asci could not easily be observed. Twenty measurements of ascospores, asci, conidia and conidiophores suspended in lactic acid or KOH, were taken for the specimens and these are presented as (min–)(average – std. dev.) – (average + std. dev.)(–max) µm. For the eustromata and perithecia, a size range from the largest and smallest structures was obtained. Colours were assigned to structures using the charts of Rayner (1970).

For growth studies, colony growth was assessed on 90 mm diam plates of MEA (20 g/L malt extract agar, Biolab, Merck, Midrand, South Africa). Four plates were inoculated per isolate. The cultures were grown in the dark at temperatures ranging from 15–35 ºC. Two measurements were taken daily for each plate until the plates were fully covered.

RESULTS

DNA sequence comparisons

The sequence data set consisted of 51 taxa with sequences from two isolates of *Diaporthe ambigua* Nitschke (*Diaporthales*), which reside in a different family in the *Diaporthales* (Castlebury *et al.* 2002), as outgroup. The ribosomal DNA dataset (571 bp) consisted of 335 constant, 10 parsimony-uninformative and 226 parsimony-informative characters (g1 = –0.4143), and the β-tubulin DNA sequence set (966 bp) consisted of 516 constant, 32 parsimony-uninformative and 418 parsimony-informative characters (g1 = –0.3582). Results generated with the PHT analyses (P = 0.004) indicated that trees obtained with the different gene regions were incongruent. This was because the relationship of the *Cryphonectria sensu stricto* clade with the other clades was different in each gene tree. Each tree, however, showed the same clades, which were always highly supported with bootstrap values between 90 and 100%. For this reason we combined the data. The resultant dataset contained 1537 characters.

The heuristic search resulted in six most parsimonious trees (tree length = 1101.9, CI = 0.736, Retention index/RI = 0.943), which differed only in the lengths of the branches. The trees obtained with the distance and parsimony analyses showed identical clades grouping isolates. The same groups of isolates, with high bootstrap values, were obtained when the more variable regions, and thus potentially ambiguously aligned sequences of the introns and ITS1 region, were excluded. The tree obtained with distance analysis based on the complete dataset is presented in Fig. 2.

The isolates thought to represent *C. havanensis* from *E. grandis* in Mexico (CMW 14550, CMW 11297, CMW 11298), Florida (CMW 14332) and Kauai (CMW 10879, CMW 10885), grouped together (Fig. 2) and formed a discrete clade (bootstrap support 100 %) separate from the clades representing species of *Cryphonectria* (Sacc.) Sacc., *Endothia* Fr., *Chrysoporthe* Gryzenh. & M.J. Wingf., *Rostraureum* Gryzenh. & M.J. Wingf. and *Amphilogia* Gryzenh., Glen & M.J. Wingf.. The *C. havanensis* clade (Fig. 2) also included the isolates from *M. faya* in the Azores (CMW 11301) and Madeira (CMW 14551, CMW 11300). *Cryphonectria havanensis* isolates from Kauai grouped separately from *Chr. cubensis* isolates from Kauai (CMW 1856, CMW 11006, CMW 11008) and Hawaii (CMW 10889). The latter isolates all grouped (bootstrap support 100 %) in the South East Asian sub-clade (Myburg *et al.* 2002) of *Chr. cubensis* (Fig. 2).

Isolates from Japan and previously assigned to *C. havanensis* (CMW 10910, CMW 11294), grouped with *C. nitschkei* isolates (CMW 10785, CMW 13747) in the *Cryphonectria* clade (bootstrap support 100%; Fig. 2), as previously reported (Myburg *et al.* 2004a). They thus resided in a clade separate from isolates believed to represent *C. havanensis*. Isolates derived from cankers on *Castanea sativa* (Gardner & Hodges 1990) from the Azores (CMW 14547, CMW 14548) grouped with other *C. parasitica* isolates (CMW 7048, CMW 13749) in the *Cryphonectria* clade (bootstrap support 100 %; Fig. 2).

The *C. eucalypti* isolates formed a discrete clade (bootstrap support 100 %) separate from the clade defining *Cryphonectria s. str.* (Fig. 2). This clade was also separated from the clades representing other genera. The isolates obtained from *Co. uvifera* in Florida also formed a clade distinct from those representing the other

genera (bootstrap support 100 %), and did not group with the isolates representing *C. havanensis*.

Morphology

Fruiting structures on the specimens connected to the isolates from Mexico, Florida and Kauai (Fig. 3) were indistinguishable from those on the type specimen of *C. havanensis* from Cuba. Ascospores [(5.5–)7–9(–10) × (2–)2.5–3(–4) µm], asci [(26.5–)29.5–34.5(–37) × (5–)5.5–7(–8) µm] and conidia [(2.5–)3–4(–5) × 1–1.5 µm] also fell within the range of those reported for the type specimen (Bruner 1916). We are thus confident that the collections from Mexico and Hawaii represent *C. havanensis*, although the phylogenetic relationship between the fungus in Cuba and the isolates from Mexico and Kauai could not be determined due to the lack of isolates from Cuba. Fruiting structures on herbarium specimens of *M. faya* from the Azores and Madeira, linked to isolates (CMW 11300, CMW 11301, CMW 14551) that also grouped with those from Mexico and Kauai, were similar to those from Cuba, Hawaii and Mexico (Fig. 3). A specimen from Puerto Rico (NY 511), annotated as *C. longirostris* but shown by Gryzenhout *et al.* (2005a) not to represent this species, was also morphologically similar to *C. havanensis*.

Clear differences could be seen between the specimens that represent *C. havanensis* (originating from Cuba, Florida, Mexico, Puerto Rico, Kauai, Madeira and the Azores), and those previously labeled as *C. havanensis* from Japan. Non-confluent stromata in the *C. havanensis* specimens were much smaller (200–650 µm diam above level of bark) than those on specimens from Japan (250–1630 µm diam above the level of the bark). Longitudinally sectioned stromata of the *C. havanensis* specimens also tended to be more superficial with reduced tissue development (Fig.

3C, H), while structures on specimens from Japan were distinctly semi-immersed with strongly developed, erumpent tissue. Ascostromata on the *C. havanensis* specimens (Fig. 3A–B) occasionally had long extending perithecial necks (up to 370 µm long) while those from Japan were consistently short (up to 130 µm long). The conidiogenous cells on the *C. havanensis* specimens also had characteristically long, cylindrical conidiophores up to 57 µm long, with the longest of these being sterile, resembling paraphyses (Fig. 3F–K). These structures differed from conidiophores of the Japanese specimens that were up to 29 µm long. Although the structural differences could also be attributed to different hosts, there are also differences, e.g. the presence of paraphyses, that cannot be attributed to hosts. Thus these differences more likely represent robust characteristics to support the distinct phylogenetic grouping (Fig. 2) of specimens representing *C. havanensis s. str.* from those of *Cryphonectria s. str.* and other closely related genera.

Structures of *C. coccolobae* on *Co. uvifera* (Fig. 4) on various specimens were similar to those thought to represent *C. havanensis*. Conidia [(2.5–)3–4.5(–5.5) × 1–1.5 µm] and ascospores [(6.5–)7.5–9(–10.5) × (2.5–)3–3.5(–4) µm] were similar to those of *C. havanensis*, and similar long (up to 62 µm) and cylindrical conidiophores, with the longest sterile, were observed (Fig. 4H–J). A specimen with conidia of (3–)3.5–4.5(–5) × 1–1.5 µm and labeled *C. coccolobae* from bark of *Conocarpus erecta* (CUP 35081) in Bermuda, also contained structures similar to those of the other *C. coccolobae* specimens. Fruiting structures on seed, however, differed from those on bark (Table 2) in being superficial and not semi-immersed.

Asci measured for the different *C. coccolobae* specimens [(32.5–)34.5–39(–41) × (5–)7–9.5(–10.5) µm] were longer and wider than those measured for the majority of *C. havanensis* specimens [(26.5–)29.5–34.5(–37) × (5–)5.5–7(–8) µm].

Ascus size was, however, a variable character since specimen PREM 57518, linked to isolate CMW 11298 grouping with the other *C. havanensis* isolates, had asci of similar size [(31.5–)32–39(–44.5) × (5.5–)6–7.5(–8.5) µm] to those of the *C. coccolobae* specimens and thus longer than the other *C. havanensis* specimens.

The newly collected specimens from *Co. uvifera* in Florida were morphologically different from those representing *C. coccolobae*. Conidiomata were pyriform to rostrate, often having a globose base with a long to tapered cylindrical neck or more than one neck (Figs 5A–D, 6A–B). This was different from conidiomata of *C. coccolobae* which are pulvinate without long necks (Fig. 4E–F). Furthermore, necks of the conidiomata were often covered with short hairs (Fig. 5F). Conidial locules of the Florida specimens (Figs 5D, 6B) also did not contain the long, sterile paraphyses commonly found in locules of *C. coccolobae* (Fig. 4H–J). No teleomorph was observed for the Florida specimens on the bark.

The conidiomata of the Florida specimens did not resemble the anamorphs of *Cryphonectria*, *Endothia*, *Rostraureum*, *Amphilogia* or *Chrysoporthe* although that fungus was closely related to these genera in the DNA sequence comparisons. The conidiomata of the fungus from Florida resembled the rostrate conidiomata of *Rostraureum* (Gryzenhout *et al*. 2005a) most closely, but could be distinguished from *Rostraureum* based on conidiomata that are more pyriform in shape, and with necks more cylindrical. Conidiomata of the newly collected fungus from *Co. uvifera* in Florida also lacked the distinct *textura intricata* tissue at the junction between neck and base in the conidiomata of *Rostraureum* (Gryzenhout *et al.* 2005a). Furthermore, the conidiomatal neck tissue was prosenchymatous (Fig. 5F), and not of *textura porrecta* as is found in *Rostraureum* (Gryzenhout *et al*. 2005a).

One of the specimens labeled as *C. coccolobae* (CUP 34657) contained a fungus morphologically different from *C. coccolobae*, but with orange stromatic tissue. This fungus was erroneously illustrated by Waterston (1947) to represent *C. coccolobae*, an illustration previously used by Seaver & Waterston (1940) in their description of a fungus named *Gnomonia pulcherrima* Seaver & Waterston. These structures occurred on petioles and twigs of *Co. uvifera* from Bermuda (Table 2). The fungus differs from *C. coccolobae* because the perithecial necks extending from the orange stromata are black and not orange as is the case for *C. coccolobae*. Ascospores are also cylindrical, 1–2-septate, guttulate and 11.5–14.5(–16) × (2.5–)3–4(–5) µm. Fruiting structures of this fungus did not colour purple in KOH and yellow in lactic acid, similar to structures of *C. coccolobae*. Previously *G. pulcherrima* was cited as a synonym (Roane 1986) of *C. coccolobae*, but these are clearly distinct fungi.

Taxonomy

Results of the phylogenetic analyses and morphological comparisons have shown clearly that cultures and specimens believed to represent *C. havanensis* do not reside in *Cryphonectria s. str.* but represent a distinct taxonomic group. Based on morphological characteristics, *C. havanensis* most closely resembles *Cryphonectria s. str.*, but it can be distinguished from species in *Cryphonectria s. str.* by its smaller and more superficial stromata, and long paraphyses between the conidiophores. Based on our observations of the material representing *C. havanensis* in this study, we transfer the fungus to a new genus that is closely related to *Cryphonectria*. The following description is provided.

Microthia Gryzenh. & M.J. Wingf., **gen. nov.** MycoBank MB xx.

Etymology: Greek, *micros*, small, and *this*, a heap, thus referring to the small and pulvinate stromata.

Ascostromata subimmersa vel superficialia, pulvinata, aurantiaca. Ascosporae fusoideae vel ellipsoideae, hyalinae, semel septatae. Stromata anamorpha subimmersa vel superficialia, pulvinata, aurantiaca. Conidiophora cylindrica, subcontracta, saepe longa, cellulis longissimis paraphyses fingentibus. Conidia hyalina, cylindrica, non septata.

Ascostromata semi-immersed to superficial, pulvinate, orange, tissue predominantly prosenchymatous but pseudoparenchymatous at edges. *Perithecia* dark-walled, with globose to sub-globose bases and slender periphysate necks that emerge at the stromatal surface as black ostioles in papillae covered with orange stromatal tissue. *Asci* fusiform, floating freely in the perithecial cavity, unitunicate with non-amyloid, refractive apical rings. *Ascospores* fusoid to ellipsoid, hyaline, 1-septate, often with a slight constriction at the septum.

Anamorphic stromata semi-immersed to superficial, pulvinate, orange, uni- to multilocular and convoluted, locules often occurring in the same stroma that contains perithecia. *Conidiophores* cylindrical, slightly tapering, often septate with or without lateral branches beneath the septum, hyaline, often long with longest cells sterile and representing paraphyses, conidiogenous cells phialidic. *Conidia* hyaline, cylindrical, aseptate, expelled through opening at stromatal surface as orange droplets or tendrils.

Typus: *Microthia havanensis* (Bruner) Gryzenh. & M.J. Wingf., comb. nov.

Microthia havanensis (Bruner) Gryzenh. & M.J. Wingf., **comb. nov.** MycoBank MB xx. Fig. 3.

Basionym: *Endothia havanensis* Bruner, Mycologia 8: 241–242. 1916.

≡ *Cryphonectria havanensis* (Bruner) M.E. Barr, Mycologia Mem. 7: 143. 1978.

Specimens examined: **Cuba**, Santiago de las Vegas, *Eucalyptus* sp., 15 Feb. 1916, S.C. Bruner, **holotype** BPI 614275, BPI 614273; *Eucalyptus botryoides*, 25 Mar. 1916, C.L. Shear, BPI 614278; *Spondias* sp., 28 Mar. 1916, C.L. Shear, BPI 614282; Earle' s Herradura, *Spondias myrobalanus*, 5 Apr. 1916, C.L. Shear, BPI 614283, BPI 614284; Santiago de las Vegas, *Mangifera indica*, 6 Apr. 1916, C.L. Shear, BPI 614279, BPI 614280, 26 Mar. 1916, C.L. Shear, BPI 614281. **Mexico**, Las Chiapas, *Eucalyptus saligna*, 26 Feb. 1998, C.S. Hodges, PREM 57518, living culture CMW 11298. **Puerto Rico**, 1923, F.J. Seaver & C.E. Chardon, NY 511. **U.S.A.**, Hawaii, Kauai, *Eucalyptus* sp., Sept. 2002, M.J. Wingfield, PREM 57521, living culture CMW 10879 = CBS 115758, PREM 57522, living culture CMW 10885 = CBS 115760. Florida, Near Palmdale, Glades Co., *Eucalyptus robusta*, 1984, E.L. Barnard & K.M. Old, FLAS 54261, ATCC 60862; *Eucalyptus grandis*, 1984, E.L. Barnard & K.M. Old, FLAS 54263. **Madeira**, Machico, *Myrica faya*, 8 May 2000, C.S. Hodges, PREM 57523, living culture CMW 14551 = CBS 115841. **Azores**, Island of São Miguel, Mosteiro, *M. faya*, C.S. Hodges & D.E. Gardner, PREM 57524, living culture from same locality CMW 11301; Island of Pico, *M. faya*, 30 Jul. 1992, C.S. Hodges & D.E. Gardner, PREM 57525, living culture from same locality CMW 11301; Island of Pico, *M. faya*, 31 May 1985, C.S. Hodges & D.E. Gardner, PREM 58810, living culture from same locality CMW 11301; Island of São Miguel, *M. faya*, 2 Aug. 1992,

C.S. Hodges & D.E. Gardner, PREM 58811, living culture from same locality CMW 11301; Island of Terceiro, *M. faya*, 31 May 1987, C.S. Hodges & D.E. Gardner, PREM 58812, living culture from same locality CMW 11301; Island of Faial, *M. faya*, 27 May 1985, C.S. Hodges, PREM 58813, living culture from same locality CMW 11301.

Notes: Microthia havanensis and *A. gyrosa* have been considered as synonyms when the latter fungus was still known as *C. gyrosa* (Kobayashi 1970, Hodges 1980). *Cryphonectria gyrosa* has also been known as *Endothia tropicalis* Shear & N.E. Stevens during the time that *Cryphonectria* was considered synonymous to *Endothia* (Shear *et al*. 1917, Kobayashi & Itô 1956, Kobayashi 1970, Roane 1986). *Amphilogia gyrosa* is, however, a distinct fungus from *M. havanensis*, as shown clearly in this study.

Specimens of *C. coccolobae* resemble those of *Mi. havanensis* closely and clearly reside in the same genus. Based on the similar spore dimensions, it is also probable that *C. coccolobae* is conspecific with *Mi. havanensis*. However, in the absence of isolates that can be used to confirm the phylogenetic relationship of *C. coccolobae*, we propose that *C. coccolobae* retain its independent taxonomic status for the present. Specimens representing *C. coccolobae* are, however, transferred to *Microthia* since this species clearly does not reside in *Cryphonectria s. str*.

Microthia coccolobae (Vizioli) Gryzenh. & M.J. Wingf., **comb. nov.** MycoBank MB xx. Fig. 4.

Basionym: *Endothia coccolobae* Vizioli, Mycologia 15: 115. 1923 (as *E. coccolobii*).

≡ *Cryphonectria coccolobae* (Vizioli) Micales & Stipes, Phytopathology 77: 651. 1987 (as *C. coccolobii*).

Specimens examined: **Bermuda**, Grape Bay, fruit of *Coccoloba uvifera*, 11 Dec. 1921, H.H. Whetzel, **holotype** CUP 128; Grape Bay, fruit of *Co. uvifera*, 11 Dec. 1921, H.H. Whetzel, **isotypes** BPI 613756, NY 147, other specimen CUP 30512; Elbow Beach, fruit of *Co. uvifera*, 28 Jan. 1926, Whetzel, Seaver & Ogilvie, CUP 34658; South Shore, bark of *Co. uvifera*, 25 Nov. 1940, F.J. Seaver & J.M. Waterston, CUP 57366; Devonshire, *Calophyllum calaba*, 2 Feb. 1926, Seaver, Whetzel & Ogilvie, CUP 35078; Devonshire Bay, *Conocarpus erecta*, 5 Feb. 1926, Seaver, Whetzel & Ogilvie, CUP 35081.

The fungus collected from *Co. uvifera* in Florida as part of this study clearly does not represent *Mi. coccolobae*. DNA sequence and morphological comparisons showed that a new genus should be provided for it and the appropriate description is presented below. No teleomorph could be found on the material, but based on DNA sequence comparisons the fungus clearly belongs to the *Diaporthales* and is closely related to *Cryphonectria* and allied genera. It is, however, described as an anamorphic fungus following Art. 59.2 of the International Code of Botanical Nomenclature (Greuter *et al.* 2000).

Ursicollum Gryzenh. & M.J. Wingf., **gen. nov.** MycoBank MB xx.

Etymology: Latin, *ursus*, a bear, and latin, *collus*, neck. Referring to the hairy neck of the conidioma that reminds of that of a bear.

Conidiomata eustromatica, pyriformia vel rostrata, superficialia, aurantiaca, cum collis uno vel tribus, textura pseudoparenchymatosa sed in collo prosenchymatosa. Conidiophora cylindrica. Conidia cylindrica, hyalina, non septata.

Conidiomata eustromatic, pyriform or rostrate, superficial to slightly immersed in bark, unilocular, internally strongly convoluted, orange, with one to three attenuated or cylindrical necks, tissue pseudoparenchymatous but prosenchymatous in the neck. *Conidiophores* hyaline, delimited by septa or not, cylindrical, conidiogenous cells phialidic, apical or lateral on branches beneath the septum. *Conidia* cylindrical, hyaline, aseptate.

Typus: *Ursicollum fallax* Gryzenh. & M.J. Wingf., sp. nov.

Ursicollum fallax Gryzenh. & M.J. Wingf., **sp. nov.** MycoBank MB xx. Figs 5–6.

Etymology: Latin, *fallax*, false. Refers to the conidiomata that appear to be false ascostromata.

Conidiomata eustromatica, pyriformia vel rostrata, aurantiaca, cum collis attenuatis uno vel tribus, superficialia vel subimmersa. Textura basalis pseudoparenchymatosa, textura collorum prosenchymatosa. Conidiophora cylindrica, apice attenuata an non. Conidia (2.5–)3–4(–5.5) × (1–)1.5(–2) µm, cylindrica, non septata, hyalina.

Conidiomata orange, eustromatic, pyriform to rostrate, with one to three attenuated or cylindrical necks (Figs 5A–B, 6A–B), base 120–400 µm high, 190–550 µm diam, neck up to 400 µm long, 90–180 µm wide, superficial to slightly immersed, unilocular, internally convoluted (Figs 5B–C, 6B). Basal tissue predominantly

pseudoparenchymatous (Fig. 5E), neck tissue prosenchymatous (Fig. 5F). *Conidiophores* hyaline, cylindrical with or without attenuated apex, cells delimited by septa or not, total length of conidiophore (4.5–)5.5–19(–39) µm (Figs 5G–H, 6C). *Conidiogenous cells* phialidic, 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 5G–H, 6C). *Conidia* (2.5–)3–4(–5.5) × (1–)1.5(–2) µm, cylindrical, aseptate, hyaline, exuded as orange droplets (Figs 5I, 6C).

Cultural characteristics: on MEA white, fluffy, margins even, optimum for growth 25–30 °C, isolates covering 90 mm diam plates after 5–6 d at optimum growth temperatures.

Substratum: Bark of *Coccoloba uvifera.*

Distribution: Florida (U.S.A.).

Specimens examined. **U.S.A.**, Florida, Fort Lauderdale, *Coccoloba uvifera*, 8 Mar. 2005, C.S. Hodges, **holotype** PREM 58840, culture ex-type CMW 18119 = CBS 118663; Key Biscayne, *Coccoloba uvifera*, 10 Mar. 2005, C.S. Hodges, PREM 58841, PREM 58842, living cultures CMW 18115 = CBS 118660, CMW 18124 = CBS 118662; Oakland Park, *Coccoloba uvifera*, 11 Mar. 2005, C.S. Hodges, PREM 58843, living culture CMW 18114 = CBS 118661; Dania, *Coccoloba uvifera*, 11 Mar. 2005, C.S. Hodges, PREM 58844, living culture CMW 18110 = CBS **.

Phylogenetic analyses based on the collection of isolates treated in this study and that of Gryzenhout *et al*. (2006), showed that isolates representing *C. eucalypti* from Australia and South Africa form a clade distinct from other species in *Cryphonectria s. str*. This phylogenetic grouping is supported by discrete morphological

characteristics such as aseptate ascospores and small stromata, which are different to those found in *Cryphonectria*. Results of this study provide us with strong justification to erect a new genus for *C. eucalypti*, and a description is provided as follows:

Holocryphia Gryzenh. & M.J. Wingf., **gen. nov.** MycoBank XX.

Etymology: Greek, *holo*, undivided, *crypho*-, secret, referring to undivided ascospores and the semi-immersed nature of the stromata.

Ascostromata subimmersa, pulvinata, aurantiaca. Ascosporae cylindricae, interdum allantoideae, hyalinae, non septatae. Stromata anamorpha subimmersa, pulvinata, aurantiaca. Conidiophora cylindrica, basibus inflatis an non, attenuatae; paraphyses inter conidiophora adsunt. Conidia hyalina, cylindrica, non septata.

Ascostromata semi-immersed, pulvinate, orange, pseudoparenchymatous tissue at the edge of stromata, prosenchymatous tissue in the centre. *Perithecia* dark-walled, with globose to sub-globose bases and slender periphysate necks that emerge at the stromatal surface as black ostioles in papillae covered with orange stromatal tissue. *Asci* fusiform, floating freely in the perithecial cavity, unitunicate with non-amyloid, refractive apical rings. *Ascospores* cylindrical, occasionally allantoid, hyaline, aseptate.

Anamorphic stromata erumpent, semi-immersed, pulvinate, orange, uni- to multilocular and convoluted, locules often occurring in same stroma that contains perithecia. *Conidiophores* cylindrical with or without inflated bases, tapering, often septate with or without lateral branches beneath a septum, hyaline, paraphyses occurring between conidiophores, conidiogenous cells phialidic. *Conidia* hyaline,

cylindrical, aseptate, expelled through an opening at the stromatal surface as orange droplets or tendrils.

Typus: *Holocryphia eucalypti* (M. Venter & M.J. Wingf.) Gryzenh. & M.J. Wingf., comb. nov..

Holocryphia eucalypti (M. Venter & M.J. Wingf.) Gryzenh. & M.J. Wingf., **comb. nov.** MycoBank XX.

Basionym: *Cryphonectria eucalypti* M. Venter & M. J. Wingf., Sydowia 54: 113–115. 2002.

Specimens examined: **South Africa**, Northern Kwazulu-Natal, Mtubatuba, Nyalazi estate, bark of GC747 clone of *Eucalyptus*, 25 Feb. 1998, M. Venter, **holotype**, PREM 56211, ex-type culture CMW 7034; Dukuduku estate, bark of *Eucalyptus grandis*, Oct. 1998, M. Venter, PREM 56214, PREM 56216; KwaMbonambi, Amangwe estate, bark of *E. grandis*, Oct. 1998, M. Venter, **epitype designated here** PREM 56215, living culture CMW 7033 = CBS 115842; Mpumalanga, Sabie, bark of *E. grandis*, Aug. 1998, J. Roux, PREM 56212; Limpopo, Tzaneen, bark of *Eucalyptus saligna*, 6 Feb. 1999, M. Venter, PREM 56305, living culture CMW 7035. **Australia**, Western Australia, Perth, *Eucalyptus globulus*, 1997, M.J. Wingfield, PREM 56217, living culture CMW 7038 = CBS ***.

DISCUSSION

In this study, we describe three new genera that are closely related to *Cryphonectria*. *Microthia* includes the fungi previously known as *C. havanensis* and *C. coccolobae*, while *Holocryphia* represents the *Eucalyptus* pathogen previously known as *C. eucalypti*. *Ursicollum* is a new genus that was discovered on *Co. uvifera* in Florida while attempting to locate fresh specimens of *Mi. coccolobae*. The description of these new genera is justified based primarily on the phylogenetic grouping of the isolates, which are distinct from *Cryphonectria* and other closely related genera such as *Endothia*, *Chrysoporthe* and *Rostraureum.*

Microthia, *Holocryphia* and *Ursicollum* are defined by the following morphological characteristics. The pulvinate and semi-immersed stromata of *Microthia* and *Holocryphia* are similar to those of *Cryphonectria* but are much smaller. Stromata of *Microthia* also tend to be more superficial on the substrate than those found in *Cryphonectria*. Another interesting and unique feature, shared by *Microthia* and *Holocryphia*, is that the conidiomata of both fungi contain exceptionally long cells between the conidiophores. These cells, previously referred to as paraphyses (Venter *et al.* 2002), do not produce conidia. *Microthia* and *Holocryphia* are thus morphologically quite similar but can be distinguished from each other based on ascospore morphology. *Microthia* has single-septate ascospores, while those of *Holocryphia* are aseptate. *Ursicollum* is morphologically distinct from the anamorphs of *Microthia*, *Holocryphia* and other related genera because of its unique orange, pyriform to globose conidiomata with cylindrical to attenuated necks.

Holocryphia eucalypti was previously known as *Endothia gyrosa* (Schwein. : Fr.) Fr. (Venter *et al*. 2002). The fungus was described as a species of *Cryphonectria*

because phylogenetic analyses indicated that isolates of this fungus grouped more closely with *Cryphonectria* than with *Endothia*, the only two genera that it resembled at that time (Venter *et al.* 2001, 2002). This phylogenetic grouping was supported morphologically by the semi-immersed stromata similar to those of *Cryphonectria.* Consequently, the new species was placed in *Cryphonectria,* despite the fact that its single-celled ascospores were different from the two-celled ascospores characteristic of all other *Cryphonectria* species. Phylogenetic studies (Myburg *et al.* 2004b) including more genera and species than those considered by Venter *et al.* (2002) did not provide convincing evidence to separate *H. eucalypti* from other *Cryphonectria* species. It was necessary to include the isolates of additional taxa presented in this study and that of Gryzenhout *et al*. (2006), which are morphologically similar to those of *H. eucalypti*, to reveal the distinction between *H. eucalypti* and species in the *Cryphonectria sensu stricto* clade. The unusual and contradictory fact that *H. eucalypti* (as *C. eucalypti*) had single-celled ascospores different from all species in *Cryphonectria s. str.* with two-celled ascospores, could thus be resolved.

The newly recognised taxonomic position of *Microthia* is well defined because numerous isolates of *Mi. havanensis* could be subjected to DNA sequence comparisons in this study. Although careful examination of the herbarium specimens of *Mi. coccolobae* have led us to suspect that this fungus is a synonym of *Mi. havanensis*, the taxonomic position of the former fungus has yet to be defined precisely. In the past, morphological characteristics such as spore size (Hodges & Gardner 1992), constriction at the ascospore septa and stromatal size (Roane 1986), the length of the perithecial necks (Vizioli 1923, Hodges & Gardner 1992), and the small number of perithecia in the stromata (Vizioli 1923) have been used to distinguish *C. coccolobae* from other species in *Cryphonectria*. These features are,

however, quite variable in specimens. For example, constricted ascospores were seen in specimens of both *Mi. havanensis* and *Mi. coccolobae*, and stromatal morphology varied greatly. Size variation of these characteristics between samples was also observed. For example, asci in specimen PREM 57518 were larger than those in other specimens of *Mi. havanensis.* This was despite the fact that isolate CMW 11298, linked to PREM 57518, grouped with isolates linked to the other specimens of the same species based on DNA sequence data. Another feature that may have convinced previous authors that *Mi. coccolobae* represents a distinct taxon is the superficial fruiting structures on *Co. uvifera* seeds. We believe that this is related to the substrate, since stromatal morphology on the seeds (Vizioli 1923) was superficial, while on bark it is semi-immersed (Micales & Stipes 1987, Gardner & Hodges 1990).

While the morphology of *Mi. coccolobae* and *Mi. havanensis* is very similar, the pathogenicity and ecology of these two species have been reported to be different. In studies to determine the identity of the *Cryphonectria* sp. on *M. faya* (Hodges & Gardner 1992), an isolate of *Mi. coccolobae* from Bermuda failed to colonise freshly-cut branch sections of *M. faya* as successfully as isolates obtained from *M. faya*, which have been shown in this study to represent *Mi. havanensis.* Likewise, the fungus from *M. faya* did not grow in freshly-cut branch sections of *Co. uvifera*, although the *Mi. coccolobae* isolate was able to colonise this substrate. No inoculations were made on living trees of either host (Hodges & Gardner 1992). Reciprocal inoculations on various hosts such as *Co. uvifera*, *Quercus* spp. and *Eucalyptus* spp. with several isolates including *Mi. havanensis* from *Eucalyptus* and *Mi. coccolobae*, showed that the *Mi. coccolobae* isolates alone were able to infect *Co. uvifera* resulting in cankers (Barnard *et al.* 1993). These differences in pathogenicity to *Co. uvifera* may indicate that the two species are distinct, despite their similar

morphology. Another unusual characteristic that distinguishes *Mi. coccolobae* from other closely related fungi is its prolific colonization of fruits of *Co. uvifera*, often while they are still green. In contrast, other species of *Microthia*, *Cryphonectria* and allied genera have been found only on bark. It is for these reasons that we have chosen not to synonymise these species before isolates of *Mi. coccolobae* can be obtained for DNA sequence comparisons.

While searching for fresh material of *C. coccolobae* (now *Mi. coccolobae*) on sea grape in Florida, another morphologically similar fungus, *U. fallax,* was found on this host. This fungus represents a new genus and species, which is closely related to *Cryphonectria* and allied genera, although no teleomorph structures were found for the fungus. Morphological comparisons with *Mi. coccolobae* showed that *U. fallax* is distinctly different from *Mi. coccolobae*. Two closely related and morphologically similar fungi thus occur on *Co. uvifera*, although it could also be possible that previous reports of *Mi. coccolobae* in Florida actually represent *U. fallax*. This will complicate continuing surveys searching for *Mi. coccolobae* on this host in order to obtain isolates for later phylogenetic comparisons.

It has previously been suggested that the fungus referred to as *C. havanensis* in Japan, represents *C. nitschkei* (Myburg *et al.* 2004a). At the time of that study, it was not possible to determine whether *C. nitschkei* was the same as *C. havanensis* in Cuba (Myburg *et al.* 2004a). For the present study, we had at our disposal a substantial collection of isolates linked to additional specimens that we feel confident to have the fungus previously known as *C. havanensis*. We were thus able to conduct morphological and phylogenetic comparisons to show clearly that the type of *Mi. havanensis* represents a fungus different from that of *C. nitschkei* from Japan. The fungus now known as *Mi. havanensis* thus does not occur in Japan.

Microthia havanensis appears to occur saprotrophically on *Eucalyptus* and other hosts. Bruner (1916) described the fungus on dead branches and twigs. Barnard *et al.* (1987) also reported it as a saprotroph on *E. grandis* in Florida, while *Chr. cubensis* was the cause of canker disease in the same plantations. In Mexico and Kauai the fungus was found only on dead, suppressed trees of *Eucalyptus*, and was not associated with cankers. Similarly, although *Mi. havanensis* was associated with cankers on *M. faya* trees in the Azores (Gardner & Hodges 1990), it also occurs on dead trees, and may only play a saprotrophic role on cankers (Hodges & Gardner 1992).

Microthia havanensis frequently occurs on *Eucalyptus* in the same locality as trees infected with *Chr. cubensis*. This is consistent with the fact that both *Chr. cubensis* and *Mi. havanensis* were first described from Cuba in the same locality (Bruner 1916, 1917) and both occurred in the same plantations in Florida (Barnard *et al.* 1987) and Kauai. Clearly the pathogenicity of *Mi. havanensis*, factors that influence its pathogenicity and the ecological relationship between *Mi. havanensis* and *Chr. cubensis*, deserves further consideration.

This study emphasizes the fact that several closely related and morphologically similar fungi, all with orange stromatic tissue, occur on *Eucalyptus* trees worldwide. These fungi previously resided in the single genus *Cryphonectria*, but most have now been transferred to new genera. *Microthia havanensis* and *H. eucalypti* have been newly described in this study. *Cryphonectria nitschkei* occurs on *Eucalyptus* spp. in Japan, and *C. parasitica* and an unknown *Cryphonectria* sp. have also been reported from *Eucalyptus* spp. in Japan (Old & Kobayashi 1988). Lastly, *Chrysoporthe* species, previously treated as the single species *Cryphonectria*

cubensis, also occur on *Eucalyptus* spp. and have been observed in the same geographic regions as *H. eucalypti* and *Mi. havanensis* (Gryzenhout *et al.* 2004).

The various *Cryphonectria* spp. and related fungi occur on *Eucalyptus* spp. in different parts of the world (Fig. 1). Thus *C. nitschkei*, *C. parasitica* and the undescribed *Cryphonectria* sp. on *Eucalyptus* are known from the Far East, *H. eucalypti* occurs in Australia and South Africa, and *Mi. havanensis* is now known from Mexico, Cuba, Puerto Rico, Florida, Hawaii, Azores and Madeira. Furthermore, the different species of *Chrysoporthe* occur in different tropical and sub-tropical countries of the world (Gryzenhout *et al.* 2004). For example, *Chr. austroafricana* occurs specifically in South Africa and *Chr. cubensis* occurs in Hawaii, Central and South America, Central Africa, South East Asia and Australia (Gryzenhout *et al.* 2004).

Cryphonectria, *Chrysoporthe*, *Microthia* and *Holocryphia* differ significantly in their pathogenicity to *Eucalyptus* spp., which is an ecologically important tree that also forms the basis of large forestry industries. *Chrysoporthe* spp. and *H. eucalypti* are considered the most important pathogens in this group. *Mi. havanensis* and the different *Cryphonectria* spp. are mild pathogens or saprophytes. Although the geographical range of *C. nitschkei*, *Mi. havanensis* and *H. eucalypti* is not currently known to overlap (Fig. 1), it is possible that these fungi could be introduced into new areas. It has been hypothesized that *H. eucalypti* has already moved from Australia, where it is presumed to be native due to the widespread occurrence of *H. eucalypti* in native *Eucalyptus* forests in Australia (Walker *et al.* 1985, Old *et al.* 1986), into *Eucalyptus* plantation areas of South Africa (Nakabonge *et al.* 2005). Because of the importance of some of these fungi as pathogens, every effort must be made to identify

collections accurately. This underpins efforts to monitor the spread of diseases and to manage their impact.

The following key is provided to facilitate the distinction between different diaporthalean genera with orange stromatic tissue, some of which occur on *Eucalyptus*:

1a. Conidiomata pyriform to clavate; ascostromata with reduced stromatic tissue ……..…….....……..... 2

1b. Conidiomata pulvinate; ascostromata well-developed…… 4

2a. Conidiomata black; orange ascostroma with black perithecial necks …….. ***Chrysoporthe***

2b. Conidiomata orange…… 3

3a. Conidiomata rostrate with tapered necks; orange stroma with orange perithecial necks ……. ***Rostraureum***

3b. Conidiomata pyriform or rostrate or globose with more cylindrical necks; teleomorph unknown……..…..……...….... ***Ursicollum***

4a. Ascospores septate……... 5

4b. Ascospores aseptate……. 6

5a. Ascostromata large, well-developed, semi-immersed; paraphyses absent in conidial locules ……...…… ***Cryphonectria***

5b. Ascostromata small to medium size, usually superficial; conidial locules containing paraphyses ……***Microthia***

6a. Ascostromata large, well-developed, superficial……...…… ***Endothia***

6b. Ascostromata small to medium size, semi-immersed …….. ***Holocryphia***

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Table 1. Isolates sequenced in this study (in bold) and previously published sequences.

Species identity	Isolate number[a]	Alternative isolate number[a]	Host	Origin	Collector	GenBank accession numbers[b]
Microthia havanensis	**CMW 14332**	ATCC 60862	*Eucalyptus grandis*	Florida (U.S.A.)	E.L. Barnard & K. Old	DQ368734, DQ368739, DQ368740
	CMW 14550	CBS 115855	*Eucalyptus saligna*	Mexico	C.S. Hodges	DQ368735, DQ368741, DQ368742
	CMW 11297	CBS 115765	*Eucalyptus* sp.	Mexico	E.L. Barnard	AY 214319, AY 214247, AY 214283
	CMW 11298	-	*Eucalyptus* sp.	Mexico	C.S. Hodges	AY 214320, AY 214248, AY 214284
	CMW 11301	-	*Myrica faya*	Azores	C.S. Hodges & D.E. Gardner	AY 214323, AY 214251, AY 214287
	CMW 11300	-	*M. faya*	Madeira	C.S. Hodges	AY 214322, AY 214250, AY 214286
	CMW 14551	CBS 115841	*M. faya*	Madeira	C.S. Hodges	DQ368736, DQ368743, DQ368744
	CMW 10879	CBS 115758	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	DQ368737, DQ368745, DQ368746
	CMW 10885	CBS 115760	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	DQ368738, DQ368747, DQ368748
Amphilogia gyrosa	CMW 10469	E67, CBS 112922	*Elaeocarpus dentatus*	New Zealand	G. Samuels	AF 452111, AF 525707, AF 525714
	CMW 10470	E68, CBS 112923	*El. dentatus*	New Zealand	G. Samuels	AF 452112, AF 525708, AF 525715
Chrysoporthe cubensis	CMW 10639	CBS 115747	*E. grandis*	Colombia	C.A. Rodas	AY 263419, AY 263420, AY 263421
	CMW 10669	CBS 115751	*Eucalyptus* sp.	Republic of Congo	J. Roux	AF 535122, AF 535124, AF 535126
	CMW 11006	CBS 115732	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	DQ368719, DQ368723, DQ368724
	CMW 11008	CBS 115733	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	DQ368718, DQ368721, DQ368722

	CMW 10889	CBS 118666		Hawaii, Hawaii (U.S.A.)	M.J. Wingfield	DQ368720, DQ368725, DQ368726
	CMW 1856	-	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	–	AY 083999, AY 084010, AY 084022
	CMW 8651	CBS 115718	*Syzygium aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084002, AY 084014, AY 084026
Chrysoporthella hodgesiana	CMW 9994	CBS 115729	*Tibouchina semidecandra*	Colombia	R. Arbelaez	AY 956968, AY 956975, AY 956976
	CMW 10641	CBS 115854	*T. semidecandra*	Colombia	R. Arbaleaz	AY 692322, AY 692326, AY 692325
Chrysoporthe austroafricana	CMW 2113	CBS 112916	*E. grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462
	CMW 9327	CBS 115843	*Tibouchina granulosa*	South Africa	M.J. Wingfield	AF 273473, AF 273060, AF 273455
Rostraureum tropicale	CMW 9971	CBS 115725	*Terminalia ivorensis*	Ecuador	M.J. Wingfield	AY 167426, AY 167431, AY 167436
	CMW 10796	CBS 115757	*Te. ivorensis*	Ecuador	M.J. Wingfield	AY 167428, AY 167433, AY 167438
Holocryphia eucalypti	CMW 7036	CRY62, CBS***	*Eucalyptus* sp.	South Africa	I. van der Westhuizen	AF 232878, AF 368341, AF 368340
	CMW 7037	CRY45, CBS **	*Eucalyptus delegatensis*	Australia	K.M. Old	AF 232880, AF 368343, AF 368342
	CMW 7038	CRY909, CBS **	*Eucalyptus globulus*	Australia	M.J. Wingfield	AF 232881, AF 368345, AF 368344
	CMW 14545	CRY 103, CBS 115852	*Eucalyptus* sp.	South Africa	I. van der Westhuizen	AF 232877[c], DQ368730, DQ368731
	CMW 14546	CRY 287, CBS 115838	*Eucalyptus* sp.	South Africa	H. Smith	AF 232879[c], DQ368732, DQ368733
	CMW 7033	CBS 115842	*E. grandis*	South Africa	M. Venter	DQ368727, DQ368728, DQ368729
Ursicollum fallax	CMW 18110	CBS **	*Coccoloba uvifera*	Florida (U.S.A.)	C. S. Hodges	-
	CMW 18114	CBS 118661	*Co. uvifera*	Florida (U.S.A.)	C. S. Hodges	-

	CMW 18115	CBS 118660	*Co. uvifera*	Florida (U.S.A.)	C. S. Hodges	DQ368756, DQ368760, DQ368761
	CMW 18119	CBS 118663	*Co. uvifera*	Florida (U.S.A.)	C. S. Hodges	DQ368755, DQ368758, DQ368759
	CMW 18124	CBS 118662	*Co. uvifera*	Florida (U.S.A.)	C. S. Hodges	DQ368757, DQ368762, DQ368763
Cryphonectria parasitica	CMW 13749	MAFF 410158 TFM:FPH Ep1	*Castanea mollisima*	Japan	Unknown	AY 697927, AY 697943, AY 697944
	CMW 7048	ATCC 48198, E9	*Quercus virginiana*	USA	F.F. Lombard	AF 368330, AF 273076, AF 273470
	CMW 14547	CBS 115845	*Castanea sativa*	Azores	D.E. Gardner	DQ368749, DQ368751, DQ368752
	CMW 14548	CBS 115846	*Ca. sativa*	Azores	D.E. Gardner	DQ368750, DQ368753, DQ368754
Cryphonectria radicalis	CMW 10455	CBS 238.54, E42	*Castanea dentata*	Italy	A. Biraghi	AF 452113, AF 525705, AF 525712
	CMW 10477	CBS 240.54, E76	*Quercus suber*	Italy	M. Orsenigo	AF 368328, AF 368347, AF 368346
	CMW 10436	CBS 165.30, E14	*Quercus suber*	Portugal	B. d'Oliviera	AF 452117, AF 525703, AF 525710
	CMW 10484	E83, CBS 112918	*Castanea sativa*	Italy	A. Biraghi	AF 368327, AF 368349, AF 368349
Cryphonectria macrospora	CMW 10463	E54, CBS 112920	*Castanopsis cuspidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350
	CMW 10914	TFM: FPH E55	*Castanopsis cuspidata*	Japan	T. Kobayashi	AY 697942, AY 697973, AY 697974
Cryphonectria nitschkei	CMW 10785	09494	*Quercus* sp.	China	M. Milgroom & K. Wang	AF 140246, AF 140252, AF 140258
	CMW 13747	MAFF 410569 TFM:FPH E25	*Quercus serrata*	Japan	T. Kobayashi	AY 697937, AY 697963, AY 697964
	CMW 10910[d]	TFM:FPH E11	*Eucalyptus globulus*	Japan	T. Kobayashi	AY 697941, AY 697971, AY 697972
	CMW 11294[d]	TFM:FPH E57	*Quercus grosseserrata*	Japan	T. Kobayashi	AY 214211, AY 214213, AY 214215
Endothia gyrosa	CMW 2091	ATCC 48192, E13	*Quercus palustris*	USA	R.J. Stipes	AF 046905, AF 368337, AF 368336
	CMW 10442	E27	*Q. palustris*	USA	R.J. Stipes	AF 368326, AF 368339, AF

						368338
Diaporthe ambigua	CMW 5288	CBS 112900	*Malus domestica*	South Africa	W.A. Smit	AF 543817, AF 543819, AF 543821
	CMW 5587	CBS 112901	*M. domestica*	South Africa	W.A. Smit	AF 543818, AF 543820, AF 543822

[a] **CMW, CRY** = Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; **ATCC** = American Type Culture Collection, Manassas, USA; **CBS** = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **TFM:FPH** = Forestry and Forest Products Research Institute, Danchi-Nai, Ibaraki, Japan, E or Ep refers to an isolate; (**09494**) = isolates used in Liu *et al.* (2003); **MAFF** = Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan; **E** = from the culture collection of Prof. R.J. Stipes (Department of Plant Pathology, Virginia Polytechnic Institute & State University, Blacksburg, Virginia, U.S.A.) now housed in the culture collection (CMW) of FABI.

[b] Accession numbers refer to sequence data of the ITS, β-tubulin 1 (primers Bt1a/1b) and β-tubulin 2 (primers Bt2a/2b) regions, respectively.

[c] Only the β-tubulin sequences were obtained in this study, while the ITS sequences were obtained from Venter *et al*. (2001).

[d] Previously labelled *Cryphonectria havanensis*.

Table 2. Herbarium specimens examined in this study.

Species identity	Herbarium number[a]	Linked isolate[b]	Host	Origin	Collector	Date
Microthia havanensis	BPI 614275 (holotype)	-	*Eucalyptus* sp.	Santiago de las Vegas, Cuba	S.C. Bruner	15 Feb. 1916
	BPI 614273		*Eucalyptus* sp.	Santiago de las Vegas, Cuba	S.C. Bruner	15 Feb. 1916
	BPI 614278		*Eucalyptus botryoides*	Santiago de las Vegas, Cuba	C.L. Shear	25 Mar. 1916
	BPI 614282	-	*Spondias* sp.	Santiago de las Vegas, Cuba	C.L. Shear	28 Mar. 1916
	BPI 614283	-	*Spondias myrobalanus*	Earle's Herradura, Cuba	C.L. Shear	5 Apr. 1916
	BPI 614284	-	*S. myrobalanus*	Earle's Herradura, Cuba	C.L. Shear	5 Apr. 1916
	BPI 614279	-	*Mangifera indica*	Santiago de las Vegas, Cuba	C.L. Shear	6 Apr. 1916
	BPI 614280	-	*Ma. indica*	Santiago de las Vegas, Cuba	C.L. Shear	Apr. 1916
	BPI 614281	-	*Ma. indica*	Santiago de las Vegas, Cuba	C.L. Shear	26 March 1916
	PREM 57518	CMW 11298	*Eucalyptus saligna*	Las Chiapas, Mexico	C.S. Hodges	26 Feb. 1998
	NY 511	-	Unknown	Puerto Rico	F.J. Seaver & C.E. Chardon	1923
	PREM 57521	CMW 10897	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	Sep. 2002
	PREM 57522	CMW 10885	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	Sep. 2002
	FLAS 54261	ATCC 60862	*Eucalyptus robusta*	Near Palmdale, Glades Co., Florida (U.S.A.)	E.L. Barnard & K. Old	1984
	FLAS 54263	-	*Eucalyptus grandis*	Glades Co., Florida (U.S.A.)	E.L. Barnad & K. Old	1984
	PREM 57523	CMW 14551	*Myrica faya*	Machico, Madeira	C.S. Hodges	8 May 2000
	PREM 57524	CMW 11301[c]	*M. faya*	Mosteiro, Island of São Miguel, Azores	C.S. Hodges & D.E. Gardner	
	PREM 57525	CMW 11301[c]	*M. faya*	Island of Pico, Azores	C.S. Hodges & D.E. Gardner	30 Jul. 1992
	PREM 58810	CMW 11301[c]	*M. faya*	Island of Pico, Azores	C.S. Hodges & D.E.	31 May 1985

					Gardner	
	PREM 58811	CMW 11301 [c]	*M. faya*	Island of São Miguel, Azores	C.S. Hodges & D.E. Gardner	2 Aug. 1992
	PREM 58812	CMW 11301 [c]	*M. faya*	Island of Terceiro, Azores	C.S. Hodges & D.E. Gardner	31 May 1987
	PREM 58813	CMW 11301 [c]	*M. faya*	Island of Faial, Azores	C.S. Hodges	27 May 1985
Microthia coccolobae	CUP 128 (holotype)	-	Fruit of *Coccoloba uvifera*	Grape Bay, Bermuda	H.H. Whetzel	11 Dec. 1921
	BPI 613756 (isotype)	-	Fruit of *Co. uvifera*	Grape Bay, Bermuda	H.H. Whetzel	11 Dec. 1921
	NY 147 (isotype)	-	Fruit of *Co. uvifera*	Grape Bay, Bermuda	H.H. Whetzel	11 Dec. 1921
	CUP 30512	-	Fruit of *Co. uvifera*	Grape Bay, Bermuda	H.H. Whetzel	11 Dec. 1921
	CUP 35078	-	*Calophyllum calaba*	Devonshire, Bermuda	Seaver, Whetzel & Ogilvie	2 Feb. 1926
	CUP 57366 (nr. 326)	-	Bark of *Co. uvifera*	South Shore, Bermuda	F.J. Seaver & J.M. Waterston	25 Nov. 1940
	CUP 35081	-	*Conocarpus erecta*	Devonshire Bay, Bermuda	Seaver, Whetzel & Ogilvie	5 Feb. 1926
	CUP 34658	-	Fruit of *Co. uvifera*	Elbow Beach, Bermuda	Whetzel, Seaver & Ogilvie	28 Jan. 1926
Unknown	CUP 34657	-	Petioles of *Co. uvifera*	Hungry Bay, Bermuda	Seaver & Whetzel	14 Jan. 1926
Ursicollum fallax	PREM 58840	CMW 18119	*Co. uvifera*	Fort Lauderdale, Florida (U.S.A.)	C. S. Hodges	Mar. 2005
	PREM 58841	CMW 18124, CMW 18115	*Co. uvifera*	Crandon Park, Key Biscayne, Florida (U.S.A.)	C. S. Hodges	Mar. 2005
	PREM 58842	CMW 18124, CMW 18115	*Co. uvifera*	Key Biscayne, Florida (U.S.A.)	C. S. Hodges	Mar. 2005
	PREM 58843	CMW 18114	*Co. uvifera*	Oakland Park, Florida (U.S.A.)	C. S. Hodges	Mar. 2005
	PREM 58844	CMW 18110	*Co. uvifera*	Oakland Park, Florida (U.S.A.)	C. S. Hodges	Mar. 2005
Holocryphia eucalypti	PREM 56211 (holotype)	CMW 7034	GC747 clone of *Eucalyptus*	Mtubatuba, South Africa	M. Venter	25 Feb. 1998
	PREM 56214	-	*Eucalyptus grandis*	Mtubatuba, South Africa	M. Venter	Oct. 1998
	PREM 56216	-	*Eucalyptus grandis*	Mtubatuba, South Africa	M. Venter	Oct. 1998

	PREM 56215 (epitype designated here)	CMW 7033	*E. grandis*	KwaMbonambi, South Africa	M. Venter	Oct. 1998
	PREM 56212	-	*E. grandis*	Sabie, South Africa	J. Roux	Aug. 1998
	PREM 56305	CMW 7035	*E. saligna*	Tzaneen, South Africa	M. Venter	6 Feb. 1999
	PREM 56217	CMW 7038	*Eucalyptus globulus*	Perth, Australia	M.J. Wingfield	1997
Chrysoporthe cubensis	PREM 58814	CMW 11006, CMW 11008	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	Sep. 2002
	PREM 58815	CMW 10889	*Eucalyptus* sp.	Hawaii, Hawaii (U.S.A).	M.J. Wingfield	Sep. 2002
Cryphonectria parasitica	CUP 2926	CMW 10790	*Castanea dentata*	New York, U.S.A.	W.A. Murrill	1907
Cryphonectria nitschkei	TFM: FPH 1045 (holotype)	CMW 10518	*Quercus grosseserrata*	Japan	T. Kobayashi	1954
Cryphonectria havanensis [d]	TFM:FPH 633	CMW 10910	*Eucalyptus globulus*	Meguro, Japan	T. Kobayashi	1954
	TFM:FPH 2300	-	*Betula* sp.	Yoshiwara, Japan	Zinno	1963
	TFM:FPH 1270	CMW 13736	*Pyrus sinensis*	Inagi, Japan	T. Kobayashi	1960
	TFM:FPH 1203	-	*Quercus variabilis*	Seto, Japan	T. Kobayashi	1953
	TFM:FPH 1047	-	*Quercus glandulifera*	Japan	T. Kobayashi	1954

[a] **BPI**, U.S. National Fungus Collections, Systematic Botany and Mycology, Beltsville, U.S.A.; **PREM**, National Collection of Fungi, Pretoria, South Africa; **CUP**, Plant Pathology Herbarium, Plant Pathology Department, Cornell University, Ithaca, New York, U.S.A.; **FLAS**, Mycological Herbarium, Department of Plant Pathology, University of Florida, Gainesville, U.S.A.; **NY**, William and Lynda Steere Herbarium, New York Botanical Garden, Bronx, New York, USA; **TFM: FPH**, Forestry and Forest Products Research Institute, Norin Kenkyu, Danchi-Nai, Ibaraki, Japan.

[b] **CMW** = Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

[c] Isolates originating from same locality and host, but are not necessarily linked to specific specimen.

[d] Specimens labeled as *C. havanensis* but actually representing *C. nitschkei*.

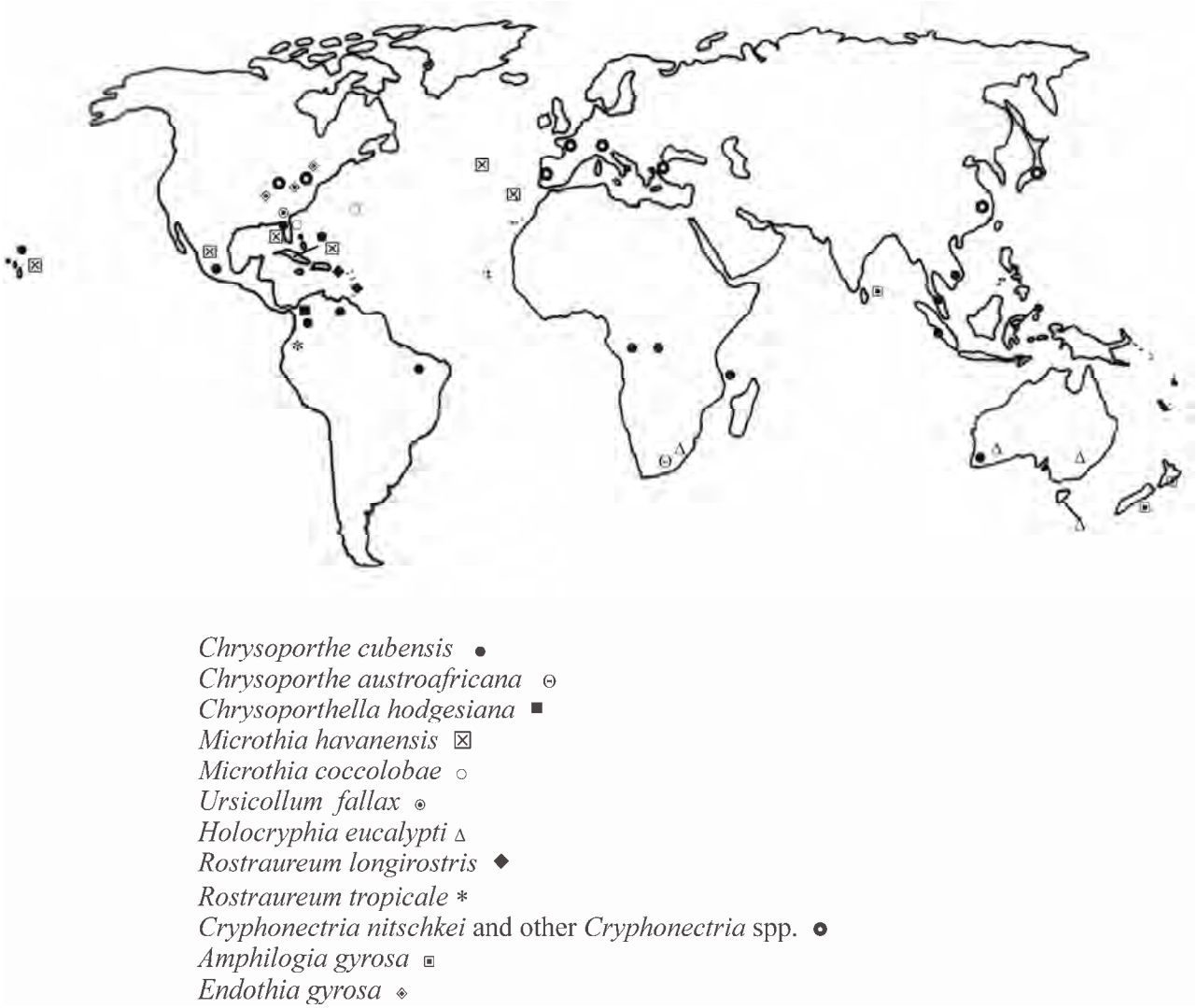

Fig. 1. Map showing the distribution of the various taxa in the *Diaporthales* with orange stromata. Only locations verified with sequence data are shown.

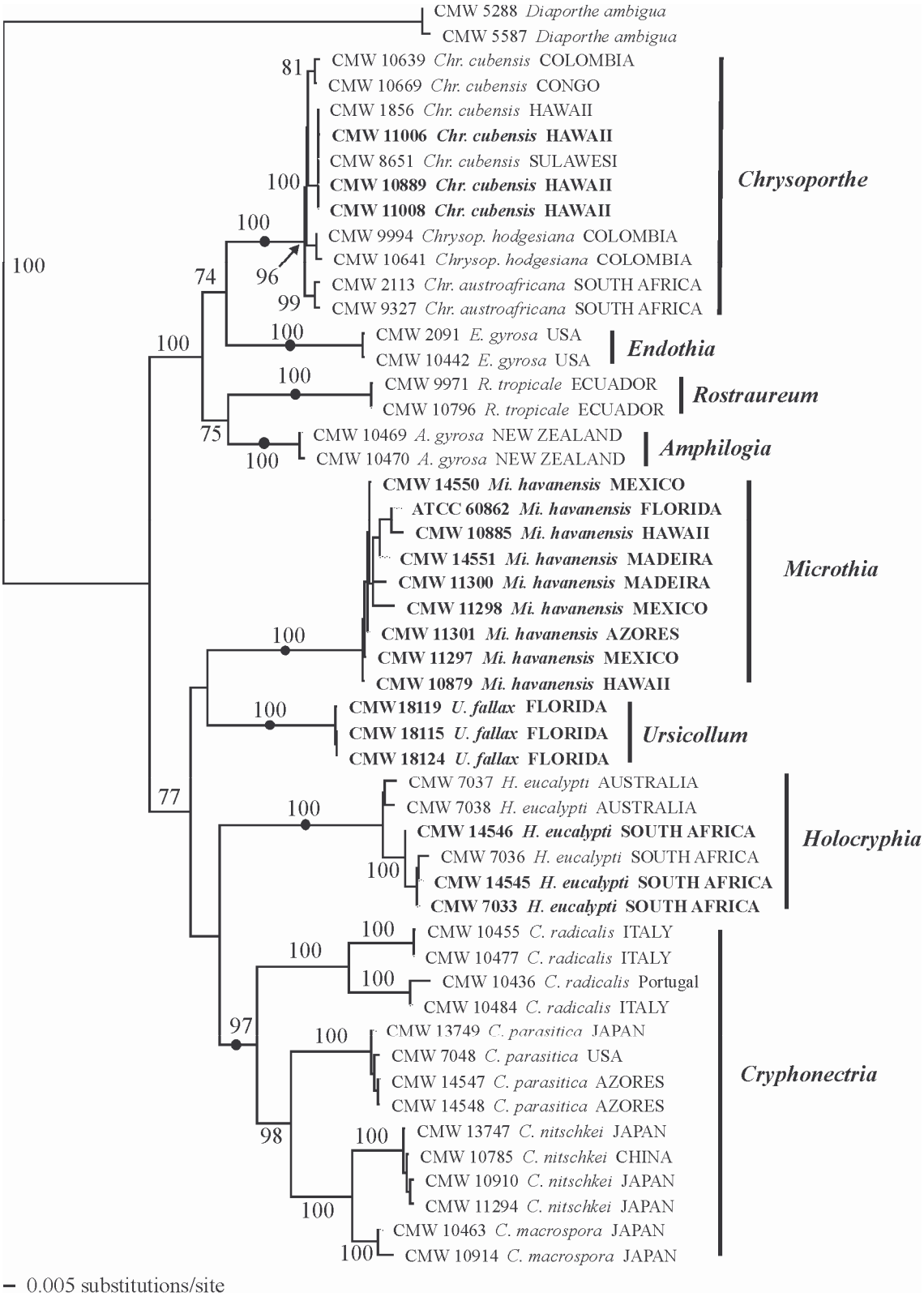

Fig. 2. A phylogenetic tree obtained with distance analyses (TrN+G+I model, G = 0.9717, I = 0.4643, base frequencies 0.1903, 0.3411, 0.2301, 0.2385; rate matrix 1, 3.1147, 1, 1, 4.1643, 1) from a combined DNA sequences dataset of the ITS1, 5.8S rRNA gene and ITS2 regions of the ribosomal operon, and β-tubulin genes. Bootstrap confidence levels (>70%) are indicated on th senting genera are marked with a dot. The outgroup ta

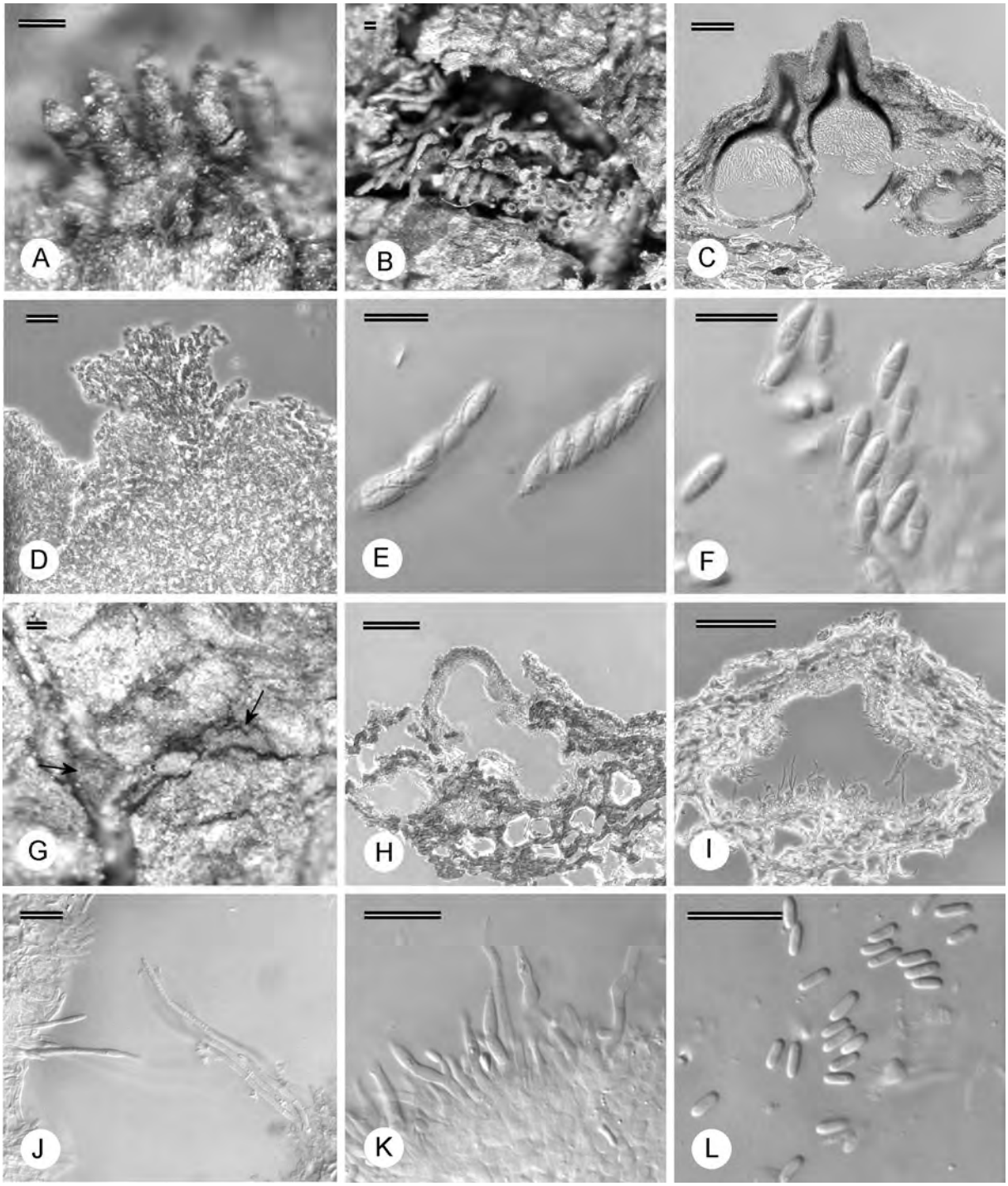

Fig. 3. Fruiting structures of *Microthia havanensis*. A–B. Stereomicrographs of ascomata C. Longitudinal section through ascoma. D. Stromatic tissue. E. Asci. F. Ascospores. G. Conidiomata on bark (arrows). H. Longitudinal section of conidioma. I–J. Long conidiophores and sterile paraphyses. K. Conidiophores. L. Conidia. Scale bars A–C, G–I = 100 µm; D = 20 µm; E–F, J–L = 10 µm.

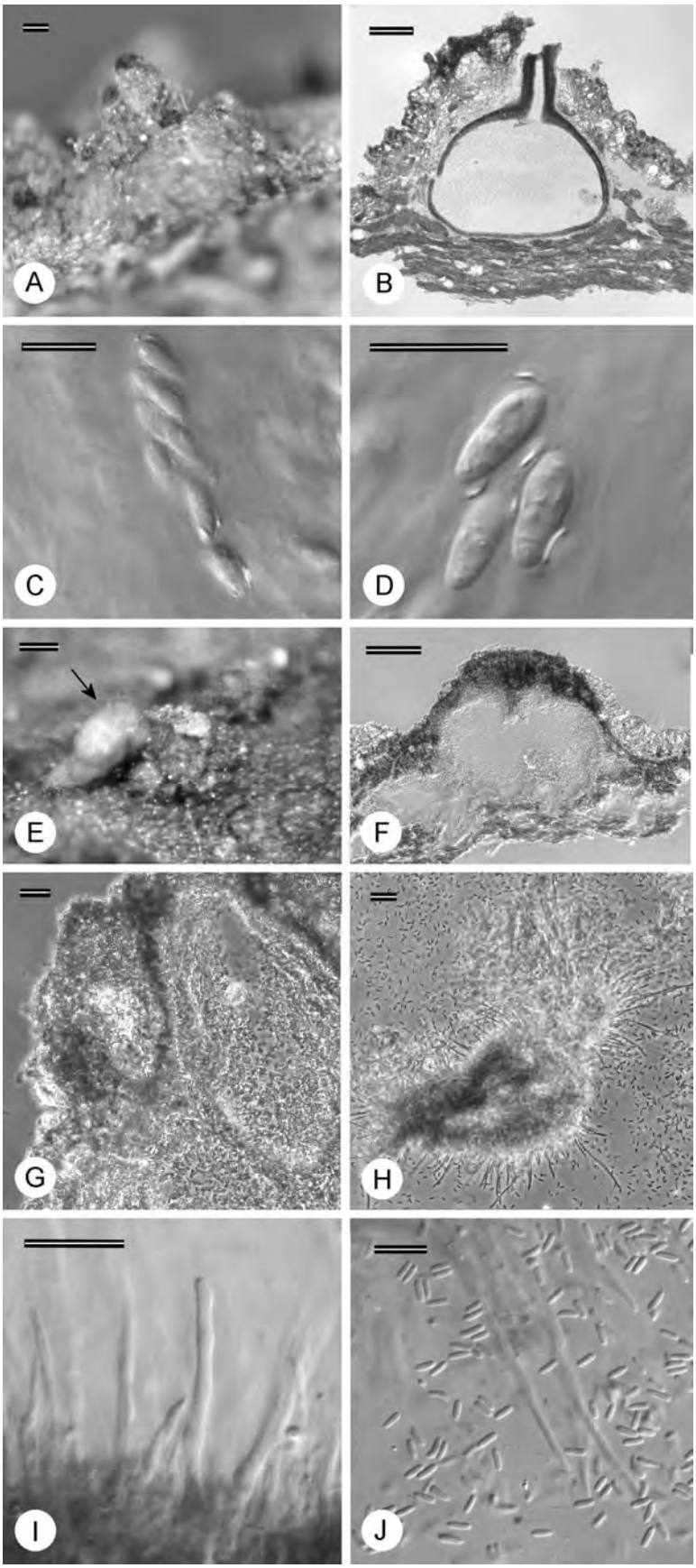

Fig. 4. Fruiting structures of *Microthia coccolobae*. A. Ascoma on bark. B. Longitudinal section through ascoma. C. Ascus. D. Ascospores. E. Conidioma on bark with spore mass (arrow). F. Longitudinal se sue. H. Conidiophores and long paraphyses. I. Conidio Scale bars A–B, E–F = 100 µm; G–H = 20 µm; C–D, I–

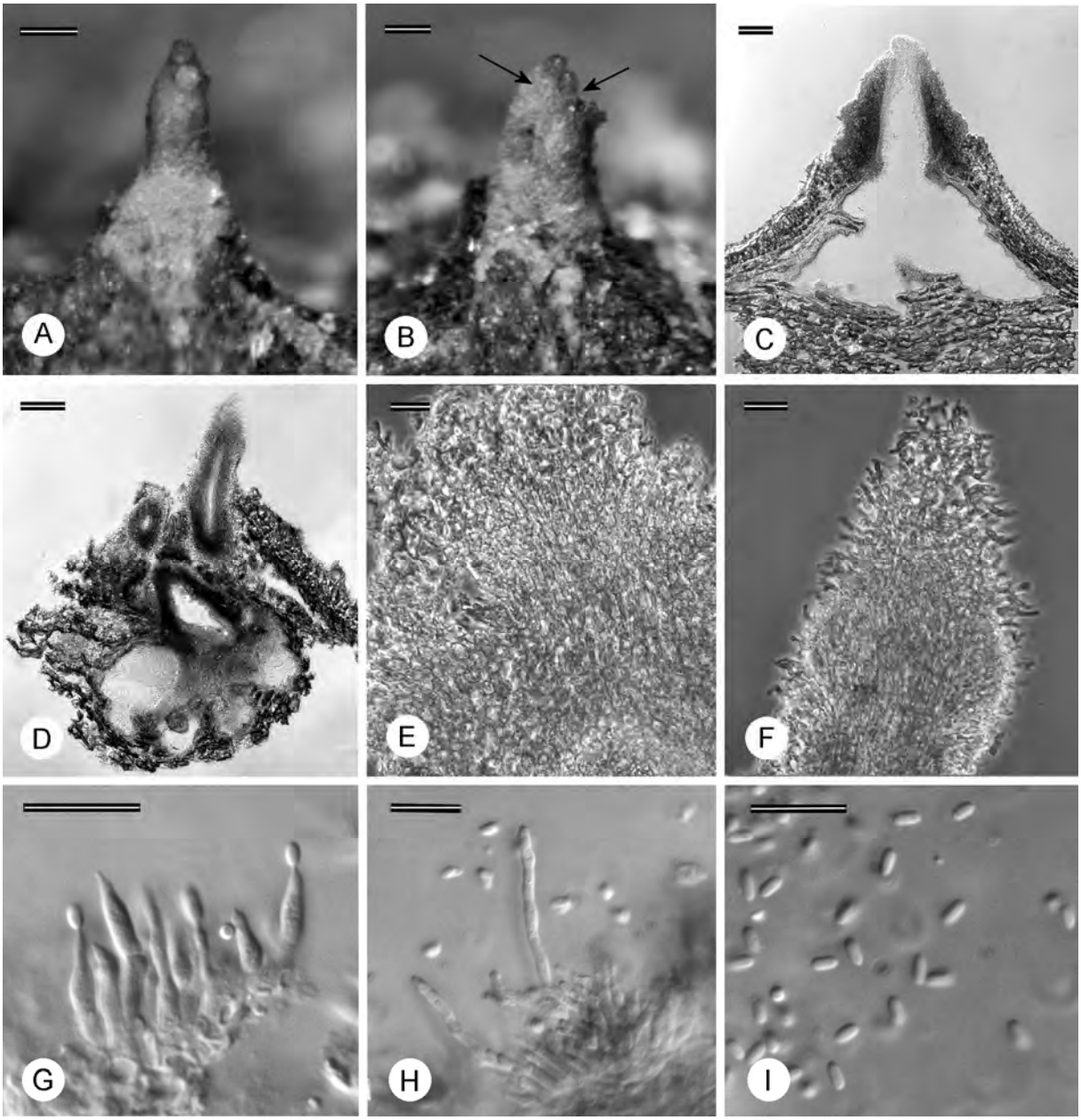

Fig. 5. Fruiting structures of *Ursicollum fallax*. A–B. Conidiomata on bark (necks indicated with arrows). C–D. Longitudinal section through conidioma. E. Tissue at base of conidioma. F. Tissue of neck. G–H. Conidiophores. I. Conidia. Scale bars A–D = 100 µm; E–F = 20 µm; G–I = 10 µm.

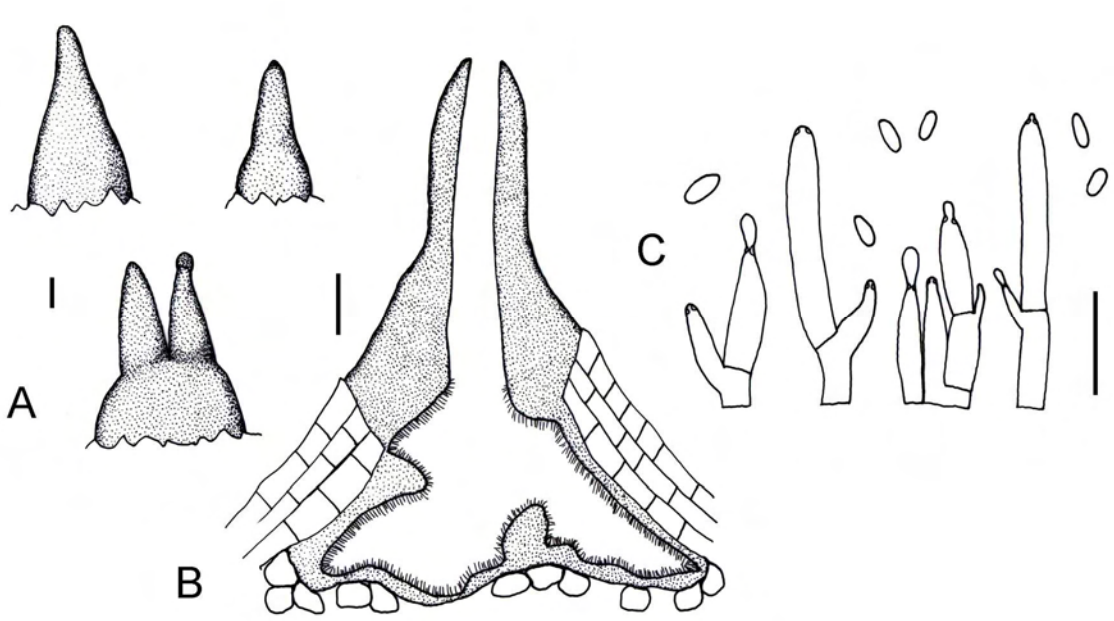

Fig. 6. Line drawings of *Ursicollum fallax*. A. Conidiomata on bark. B. Longitudinal section through conidioma. C. Conidiophores and conidia. Scale bars A–B = 100 µm; C = 10 µm.