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# *Eucalyptus* microfungi known from culture. 1. *Cladoriella* and *Fulvoflamma* genera nova, with notes on some other poorly known taxa

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**Abstract:** A study of microfungi associated with living *Eucalyptus* leaves and leaf litter revealed several novel and interesting taxa. *Cladoriella eucalypti* gen. et sp. nov. is described as a *Cladosporium*-like genus associated with litter collected in South Africa, while *Fulvoflamma eucalypti* gen. et. sp. nov. is newly described from leaf litter collected in Spain. Beta-conidia are newly reported for species of *Pestalotiopsis*, namely *Pestalotiopsis disseminata* in New Zealand, and a *Pestalotiopsis* sp. from Colombia. *Satchmopsis brasiliensis* is reported from litter in Colombia and Indonesia, while *Torrendiella eucalypti* is reported from leaf litter in Indonesia, and shown to have a *Sporothrix*-like anamorph. *Leptospora rubella* is reported from living *Eucalyptus* leaves in Colombia, where it is associated with leaf spots of *Mycosphaerella longibasalis*, while *Macrohilum eucalypti* is reported from leaf spots of *Eucalyptus* in New Zealand.

**Taxonomic novelties:** *Cladoriella eucalypti* Crous gen. et sp. nov., *Fulvoflamma eucalypti* Crous gen. et sp. nov.
**Key words:** *Cladosporium, Eucalyptus*, *Leptospora*, *Macrohilum*, microfungi, *Pestalotiopsis*, *Satchmopsis*, systematics.

## INTRODUCTION

The genus *Eucalyptus* (*Myrtaceae*) contains approximately 700 species (Potts & Pederick 2000), most of which are known to host a range of incredibly diverse and interesting microfungi (Crous *et al*. 1989, Sankaran *et al.* 1995). In recent years there have been numerous papers listing and describing the plant-pathogenic fungi occurring on eucalypts in the various countries where these trees are grown as ornamentals, or planted in plantations for timber and paper fibre (Old & Davison 2000, Park *et al*. 2000). As the majority of the plant-pathogenic fungi are known from culture, this has enabled plant pathologists to revise numerous important pathogen complexes such as Mycosphaerella leaf blotch (Crous 1998, Crous *et al*. 2000, 2001, 2004a, Hunter *et al*. 2004), Cylindrocladium leaf blight (Crous 2002, 2004b), Cryphonectria canker (Gryzenhout *et al*. 2004), Botryosphaeria canker (Slippers *et al*. 2004a–c), *Coniella* (Van Niekerk *et al*. 2004), *Cytospora* (Adams *et al*. 2005), and *Harknessia* leaf spots (Lee *et al.* 2004), to name but a few. In contrast, however, the saprobic microfungi have largely been neglected, and in spite of checklists and descriptions, very few are in fact known from culture, or are represented in freely accessible culture collections. As such, many of these diverse genera will never be represented in international initiatives like Assembling the Tree of Life (AToL), or the Consortium for the Barcoding of Life (CBoL), and biologists will remain ignorant as to their distribution, host range, importance and various ecological roles.

Because the eucalypt microbial community is so rich and diverse, and appears to harbour numerous undescribed and relatively unstudied fungal species, it was decided to focus on this host substrate to obtain cultures for inclusion in larger projects and international initiatives such as those cited above. The current paper represents the first in a series aimed at describing eucalypt microfungi from culture, and recollecting and culturing those already known (Sankaran *et al*. 1995), to help elucidate their taxonomy, and resolve their phylogenetic relationships.

## MATERIALS AND METHODS

### Isolates

Leaf litter as well as living, symptomatic leaves were chosen for study. Leaves were incubated in moist chambers (Petri dishes with moist filter paper on the laboratory bench), and inspected daily for microfungi. Hyphomycetes and coelomycetes were cultured on 2 % malt extract agar (MEA) plates (Gams *et al.* 1989) by obtaining single conidial colonies as explained in Crous (2002). Single germinating ascospores were obtained and cultured using the technique as explained in Crous (1998). Colonies were sub-cultured onto fresh MEA, oatmeal agar (OA), cornmeal agar (CMA) and carnation leaf agar (CLA) plates (Gams *et al.* 1989) and incubated at 25 °C under continuous near-ultraviolet light, to promote sporulation.

### DNA amplification and sequence analysis

Genomic DNA was isolated from fungal mycelium grown on malt extract agar plates following the protocol of Lee & Taylor (1990). The primers ITS1 and ITS4 (White *et al*. 1990) were used to amplify part (ITS) of the nuclear rRNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene (LSU). PCR conditions and protocols were treated and generated

as explained in Crous *et al*. (2004a). Part of the 18S rRNA gene was amplified and sequenced as explained in Braun *et al*. (2003) and part of the 28S rRNA gene as explained in Lee *et al*. (2004). ITS sequences were subjected to a nucleotide-nucleotide BLAST (Altschul *et al*. 1997) of the NCBI sequence database (BLAST-N 2.2.11; http://www.ncbi.nlm.nih.gov/). The LSU and / or SSU sequences were also used in cases where ITS sequences did not provide adequate BLAST results.

### Taxonomy

Fungal structures were mounted in lactic acid or in water when stated. The extremes of spore measurements (30 observations) are given in parentheses. Colony colours (surface and reverse) were rated after 7–14 d on MEA and OA at 25 °C in the dark, using the colour charts of Rayner (1970). All cultures obtained in this study are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands (Table 1), and type specimens in the mycology herbarium (PREM) at the Biosystematics Division of the Plant Protection Research Institute, Agricultural Research Council of South Africa.

## RESULTS AND DISCUSSION

### Sequence analysis

Sequence data obtained from the amplification products were deposited in GenBank (Table 1). BLAST searches resulted in associations with known fungal species or orders. These results are discussed in the descriptive notes below each of the treated species.

### Taxonomy

***Cladoriella*** Crous, **gen. nov.** MycoBank MB500799.

*Etymology*: Resembling species accommodated in *Cladosporium*.

Genus anamorphosis, hyphomyceticum. Devriesiae simile, sed chlamydosporis carens. Hila conidiorum inspissata, fuscata, refringentia, poro centrali minuto praedita.

*Typus*: *Cladoriella eucalypti* Crous, sp. nov.

*External hyphae* coiling on the leaf surface, medium to dark brown, thick-walled, smooth to finely verruculose, branched, septate, with swollen cells giving rise to conidiophores; hyphododium-like structures present, simple, intercalary. *Conidiophores* separate, erect, medium to dark brown, smooth to finely verruculose, thick-walled, subcylindrical, straight, septate. *Conidiogenous cells* terminal or intercalary, monotretic or polytretic, sympodial, with 1–2 conspicuous loci, thickened, darkened, refractive, with a minute central pore, not protruding as in the case of *Cladosporium s. str*. *Conidia* frequently remaining attached in long acropetal chains, simple or branched, narrowly ellipsoidal to cylindrical or fusoid, 0–1-septate, medium brown, thick-walled, finely verruculose, apical conidium with rounded apex, additional conidia with 1–2 truncate, conspicuous hila; thickened, darkened, refractive, with a minute central pore. *Colonies* on MEA producing abundant amounts of diffusing red pigment. *Chlamydospores* absent.

***Cladoriella eucalypti*** Crous, **sp. nov.** MycoBank MB500800. Figs 1–2.

Devriesiae thermoduranti similis, sed conidiis 0–1-septatis, (11–)13–15(–22) × (2.5–)3–3.5(–4) µm, hilo conspicuo, inspissato, fuscato, refringente, 1.5–2 µm diam, praeditis distinguenda; porus hili centralis 0.5 µm latus; coloniae in agaro malti pigmentum rubrum formantes; chlamydosporae absentes.

*Hyphae* internal and external; external hyphae coiling on the leaf surface, medium to dark brown, thick-walled, smooth to finely verruculose, branched, septate, 2.5–3.5 µm wide, frequently forming a swollen cell which gives rise to a conidiophore; hyphododium-like structures present, simple, intercalary, 2.5–3.5 µm diam. *Conidiophores* separate, erect, medium to dark brown, smooth to finely verruculose, thick-walled, subcylindrical, straight, 1–4-septate, 15–60 × 5–7 µm. *Conidiogenous cells* terminal or intercalary, monotretic or polytretic, sympodial, usually with 1–2 conspicuous loci, 1.5–2 µm wide, thickened, darkened, refractive, with a minute central pore, 0.5–1 µm wide, scar usually within the cell outline, and not protruding as in the case of *Cladosporium s. str*., finely verruculose, medium brown, 10–17 × 4–5 µm. *Conidia* frequently remaining attached in long acropetal chains, simple or branched, narrowly ellipsoidal to cylindrical or fusoid, 0–1-septate, (11–)13–15(–22) × (2.5–)3–3.5(–4) µm, medium brown, thick-walled, finely verruculose, apical conidium with rounded apex, additional conidia with 1–2 truncate, conspicuous hila, 1.5–2 µm wide, thickened, darkened, refractive, with a minute central pore, 0.5 µm wide.

*Cultural characteristics*: Colonies on MEA producing abundant amounts of diffusing red pigment that changes the colour of the medium to red; colonies irregular,

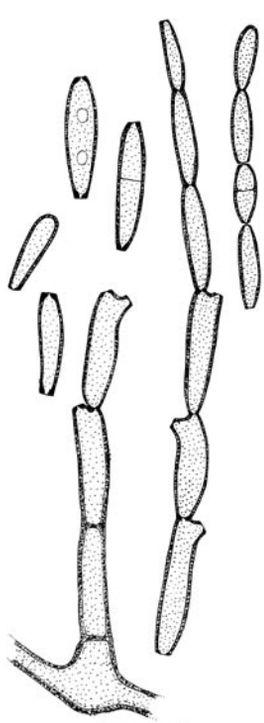

**Fig. 1.** *Cladoriella eucalypti*. Conidiophore and conidia. Scale bar = 10 µm.

erumpent, with smooth, irregular margins; surface iron-grey; reverse greenish black.

*Substrate and distribution*: *Eucalyptus* sp., South Africa (Western Cape Province).

*Specimen examined*: **South Africa**, Western Cape Province, Stellenbosch Mountain, on *Eucalyptus* leaf litter, 13 Dec. 2003, P.W. Crous, CBS H-18043, **holotype**, cultures ex-type CPC 10953–10955 = CBS 115898–115890.

*Notes*: The genus *Cladosporium* Link contains 772 names (Dugan *et al*. 2004), many of which represent elements not congeneric with the type species, *C. herbarum* (Pers.: Fr.) Link, which is an anamorph of *Davidiella* Crous & U. Braun (Braun *et al*. 2003). The recent description of *Devriesia* Seifert & N.L. Nickerson (Seifert *et al*. 2004) for a group of heat-resistant, chlamydospore forming species with slightly thickened conidial scars proves this point. *Cladoriella* resembles *Devriesia* in general morphology, but lacks chlamydospores, forms a distinct red pigment in culture, and clusters apart from the *Cladosporium* complex (*Mycosphaerellaceae*), the *Cladophialophora* Borelli complex (*Herpotrichiellaceae*), or the *Pseudocladosporium* U. Braun complex (*Venturiaceae*). BLAST results of the ITS sequence of this species had an E-value of 1e-90 with ITS sequences of

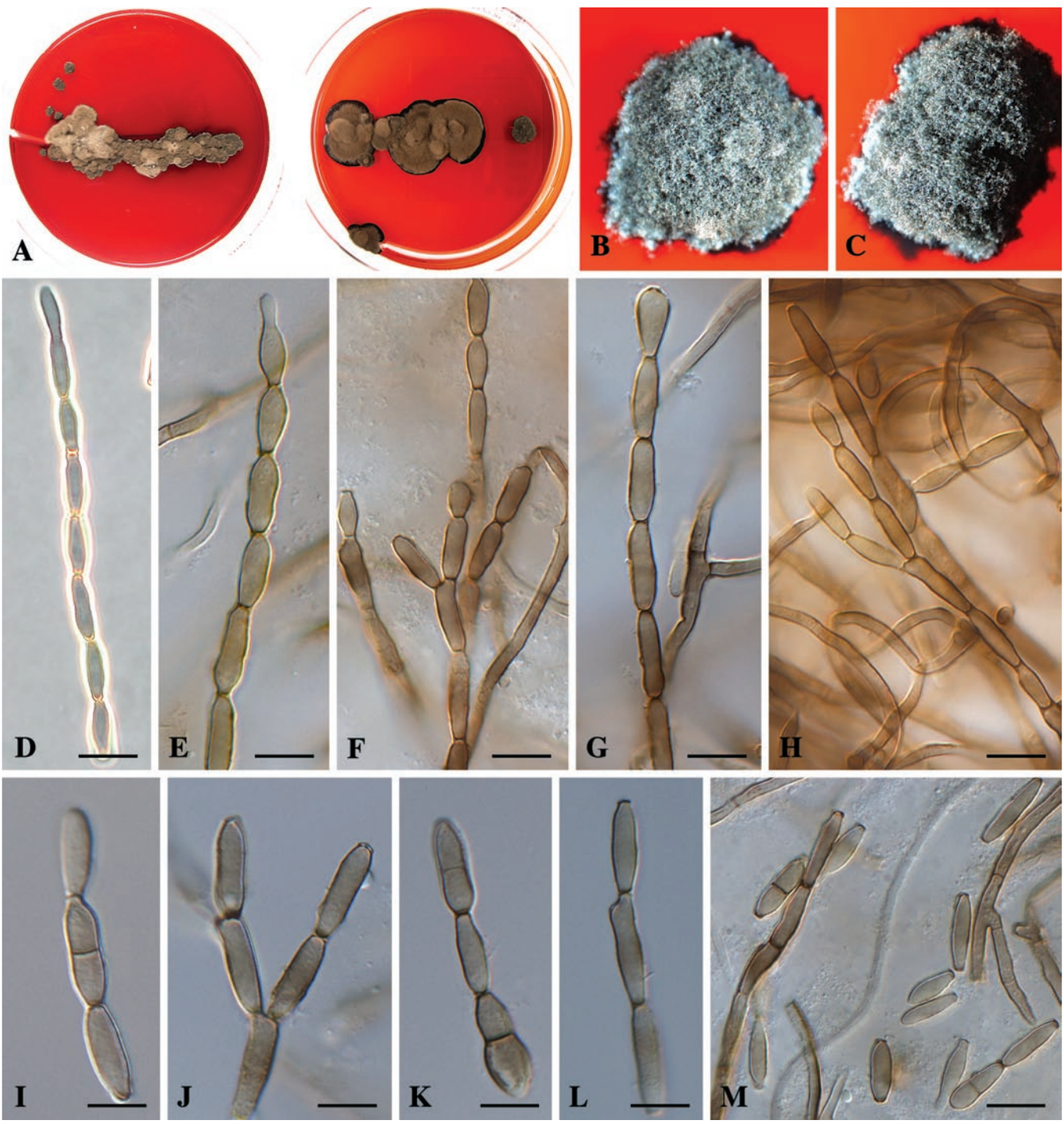


**Fig. 2.** *Cladoriella eucalypti*. A–B. Colonies on MEA, with diffuse red pigment visible in agar. D–M. Conidiophores and conidia. Scale bars: D, F, H–K, M = 10 µm, E, G, L = 6 µm.

**Table 1.** Isolates used for DNA sequence analysis.

| Species | Accession number[1] | Host | Country | Collector | GenBank numbers[2] (ITS, LSU, SSU) |
|---|---|---|---|---|---|
| *Cladoriella eucalypti* | CBS 115898; CPC 10953 | *Eucalyptus* sp. | South Africa | P.W. Crous | DQ195778, DQ195790, DQ195801 |
| *Fulvoflamma eucalypti* | CBS 118549; CPC 11243 | *Eucalyptus* sp. | Spain | M.J. Wingfield | DQ195779, DQ195791, DQ195802 |
| *Leptospora rubella* | CBS 118550; CPC 11006 | *Eucalyptus* sp. | Colombia | M.J. Wingfield | DQ195780, DQ195792, DQ195803 |
| *Macrohilum eucalypti* | CBS 118551; CPC 10945 | *Eucalyptus* sp. | New Zealand | J.A. Stalpers | DQ195781, DQ195793, DQ195804 |
| *Pestalotiopsis disseminata* | CBS 118552; CPC 10950 | *Eucalyptus botryoides* | New Zealand | M.A. Dick | DQ195782, DQ195794, DQ195805 |
| *Pestalotiopsis* sp. | CBS 118553; CPC 10969 | *Eucalyptus eurograndis* | Colombia | M.J. Wingfield | DQ195783, DQ195795, DQ195806 |
| *Satchmopsis brasiliensis* | CBS 420.93 | *Pimenta dioica* | Cuba | R.F. Castañeda | DQ195784, DQ195796, DQ195807 |
| | CPC 10972 | *Eucalyptus* sp. | Colombia | M.J. Wingfield | DQ195785, DQ195797, DQ195808 |
| | CBS 118554; CPC 11017 | *Eucalyptus* sp. | Indonesia | M.J. Wingfield | DQ195786, DQ195798, DQ195809 |
| *Torrendiella eucalypti* | CBS 115326; CPC 11049 | *Eucalyptus* sp. | Indonesia | M.J. Wingfield | DQ195787, DQ195799, DQ195810 |
| | CBS 115326; CPC 11050 | *Eucalyptus* sp. | Indonesia | M.J. Wingfield | DQ195788, DQ195800, DQ195811 |
| | CBS 115326; CPC 11051 | *Eucalyptus* sp. | Indonesia | M.J. Wingfield | DQ195789, —, — |

[1]CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS.

[2]ITS: internal transcribed spacer region, LSU: partial 28S rDNA gene, SSU: partial 18S rDNA gene.

*Claviceps* Tul., *Calonectria* De Not. (*Hypocreales*) and *Gaeumannomyces* Arx & D.L. Olivier (*Sordariomycetes incertae sedis*). A number of similarities with an E-value of 0.0 were obtained from the LSU data: *Hysteropatella clavispora* (Peck) Seaver (*Hysteriales*), *Candelariella vitellina* (Hoffm.) Müll. Arg. (*Lecanorales*), *Polysporina simplex* (Davies) Vězda (*Lecanorales*), *Botryosphaeria ribis* Grossenb. & Duggar (*Dothideales, incertae sedis*), and others. A number of similarities with an E-value of 0.0 were also obtained from the SSU data: *Sarcinomyces petricola* Wollenz. & de Hoog (*Chaetothyriales*), *Scytalidium dimidiatum* (Penz.) B. Sutton & Dyko, *Botryosphaeria ribis* (*Dothideales incertae sedis*), *Fusicladium convolvulorum* Ondřej (*Pleosporales*) and others.

***Fulvoflamma*** Crous, **gen. nov.** MycoBank MB500801.

*Etymology*: Named after its characteristic conidiomata and spore masses that appear as orange candle flames once plant material is incubated in moist chambers.

Genus anamorphosis coelomyceticum. Satchmopsi similis, sed proliferatione sympodiali cellularum conidiogenarum et setis marginalibus hyalinis tenuitunicatis et conidiis cylindricis distinguenda.

*Typus*: *Fulvoflamma eucalypti* Crous, sp. nov.

*Mycelium* immersed, consisting of smooth, hyaline, branched, septate hyphae, forming brown stromata that give rise to conidiomata. *Conidiomata* sporodochial, appearing as erect, orange, fusoid structures; basal region consisting of pale brown *textura angularis* to *textura epidermoidea*, giving rise to thick-walled, pale brown cells of *textura porrecta,* becoming thin-walled, hyaline, and radiating outwards from the narrower, semi-cylindrical sporodochial base, branching sympodially to give rise to hyaline, smooth, thin-walled setae with bluntly rounded ends; inner conidiomatal layer consisting of a mixture of setae and conidiogenous cells. *Conidiogenous cells* hyaline, smooth, subcylindrical, proliferating blastically and sympodially. *Conidia* subcylindrical, straight or slightly curved with obtuse ends, septate, hyaline, smooth, guttulate.

***Fulvoflamma eucalypti*** Crous, **sp. nov.** MycoBank MB500802. Fig. 3.

Conidiomata sporodochialia, erecta, aurantiaca, flammam candelae fingentia. Cellulae conidiogenae hyalinae, leves, subcylindricae, 7–15 x 1.5–2.5 µm, sympodialiter proliferentes. Conidia subcylindrica, recta vel modice curvata, 3-septata, hyalina, levia, (35–)43–55(–60) × 1.5–2 µm.

*Mycelium* immersed, consisting of smooth, hyaline, branched, septate hyphae, 1–1.5 µm wide; aggregating in the epidermis to form a pale to dark brown stroma, up to 50 µm wide, which gives rise to a conidioma. *Conidiomata* sporodochial, appearing as erect, orange, fusoid structures on the leaf surface (like the flame of a candle), up to 100 µm diam and 200 µm high; basal region consisting of pale brown cells of *textura angularis* to *textura epidermoidea*, 3–7 × 2–3 µm, giving rise to thick-walled, pale brown cells of *textura porrecta*, 6–15 × 2–3 µm, becoming thin-walled, hyaline, and radiating outwards from the narrower, semi-

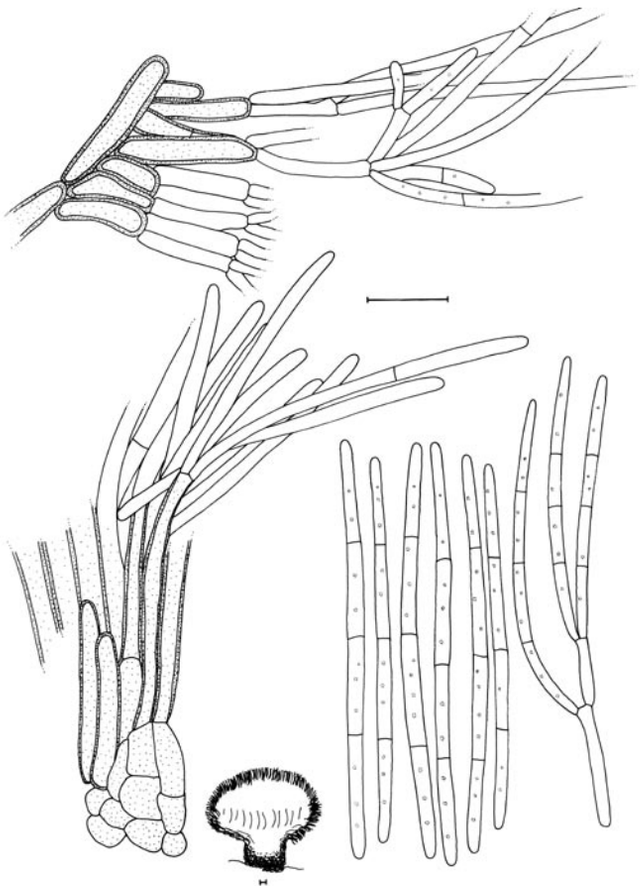

**Fig. 3.** *Fulvoflamma eucalypti*. Conidioma, conidia, conidiogenous cells and setae. Scale bars = 10 µm.

cylindrical sporodochial base, branching sympodially to give rise to hyaline, smooth, thin-walled setae that terminate in bluntly rounded, obtuse ends, and give the conidiomatal margin a feathery appearance; the inner layer of the conidioma gives rise to a mixture of setae and conidiogenous cells. *Conidiogenous cells* hyaline, smooth, subcylindrical, 7–15 × 1.5–2.5 µm, proliferating sympodially, with inconspicuous scars, giving rise to additional conidiogenous cells, or to conidia. *Conidia* subcylindrical, straight or slightly curved, 3-septate, hyaline, smooth, guttulate, widest in the middle, with obtusely rounded ends, (35–)43–55(–60) × 1.5–2 µm.

*Cultural characteristics*: Colonies on MEA spreading, erumpent, folded, with sparse aerial mycelium; surface pale luteous to buff, with diffuse strips of red; reverse luteous. On OA colonies slimy with no aerial mycelium, spreading, appearing to grow more in the agar than on the surface, pale luteous; colonies sporulated when freshly isolated, but became sterile upon first transfer.

*Substrate and distribution*: *Eucalyptus* sp., Spain.

*Specimen examined*: **Spain**, on *Eucalyptus* leaf litter, Apr. 2004, M.J. Wingfield, CBS H-18045, **holotype**, cultures ex-type CPC 11243 = CBS 118549, CPC 11244–11245.

*Notes*: *Fulvoflamma* is similar to other genera with sporodochial conidiomatal such as *Satchmopsis* B. Sutton & Hodges, *Stevensonula* Petr., *Shawiella* Hansf. and *Zelosatchmopsis* Nag Raj (Sutton 1975, Saikawa *et al*. 1991). It is easily distinguished, however, by its unique conidiophores, mode of conidiogenesis, presence of marginal, thin-walled setae and its cylindrical conidia. BLAST results of the ITS sequence of this species had an E-value of 5e-130 with the ITS sequence of a foliar endophyte of *Picea glauca*. Similarities with known species include *Potebniamyces pyri* (Berk. & Broome) Dennis (7e-123; *Rhytismatales*), *Phacidiopycnis* sp. (2e-120; *Rhytismatales*) and *Pseudeurotium desertorum* Mouch. (2e-117; *Pseudeurotiaceae*). A number of similarities with an E-value of 0.0 were obtained from the LSU data: *Crinula caliciiformis* Fr. (*Helotiales*), *Leuconeurospora pulcherrima* (G. Winter) Malloch & Cain (*Hypocreales*), *Pseudeurotium zonatum* F.H. Beyma (*Pseudeurotiaceae*), *Aleurodiscus farlowii* Burt (*Stereales*), *Cudoniella clavus* (Alb. & Schwein.) Dennis (*Helotiales*) and others. A number of similarities with an E-value of 0.0 were also obtained from the SSU data: *Phacidium coniferarum* (G.G. Hahn) DiCosmo, Nag Raj & W.B. Kendr. (*Helotiales*), *Bulgaria* spp. (*Helotiales*), *Neofabraea malicorticis* H.S. Jacks. (*Helotiales*) and others.

***Leptospora rubella*** (Pers.: Fr.) Fr., Herb. mycol., ed. 2: no. 532. 1857.

≡ *Sphaeria rubella* Pers., Syn. meth. fung. (Göttingen): 63. 1801, sanctioned by Fries, Syst. Mycol. 2: 506. 1823.

Ascomata indistinct on host, intermingled with those of *Mycosphaerella longibasalis* Crous & M.J. Wingf. Description based on sporulation obtained on CLA. *Ascomata* dark brown to black, up to 400 µm high and 300 µm wide, flask-shaped with an elongated red-brown neck up to 70 µm long. *Asci* numerous, cylindrical, bitunicate, with a prominent foot cell, 120–160 × 4–6 µm. *Pseudoparaphyses* hyaline, septate, constricted at the septa, 2–3.5 µm wide, not extending beyond the asci. *Ascospores* somewhat spiralled or twisted in the asci, pale brown, subcylindrical, with tapering to subobtuse ends, multiseptate (septa at approx. 10 µm intervals), 130–165 × 1–1.5 µm.

*Cultural characteristics*: Colonies spreading on MEA, slightly erumpent with moderate aerial mycelium and feathery margins; surface on PDA and OA pale mouse grey to mouse grey; reverse chestnut on MEA, iron-grey on OA. Cultures were sterile on MEA, but perithecial initials formed on OA, and fertile perithecia was obtained on CLA.

*Newly observed substrate and distribution*: *Eucalyptus* sp., Colombia.

*Specimen examined*: **Colombia**, on *Eucalyptus* leaf spots, associated with lesions of *Mycophaerella longibasalis* Crous & M.J. Wingf., 16 Feb. 2004, M.J. Wingfield, CBS H-18046, culture CPC 11006 = CBS 118550.

*Notes*: Shoemaker (1976) listed numerous hosts for *L. rubella* (as *Ophiobolus rubellus* (Pers.: Fr.) Sacc.), and stated that it is often recognized by the red-purple stain it induces on the host substrate, and the red-brown colour of the apical part of the ascomatal neck. Furthermore, he reported that the fungus is common in Canada, and is suspected to be the teleomorph of *Phoma exigua* Desm. var. *foveata* Foister. BLAST results

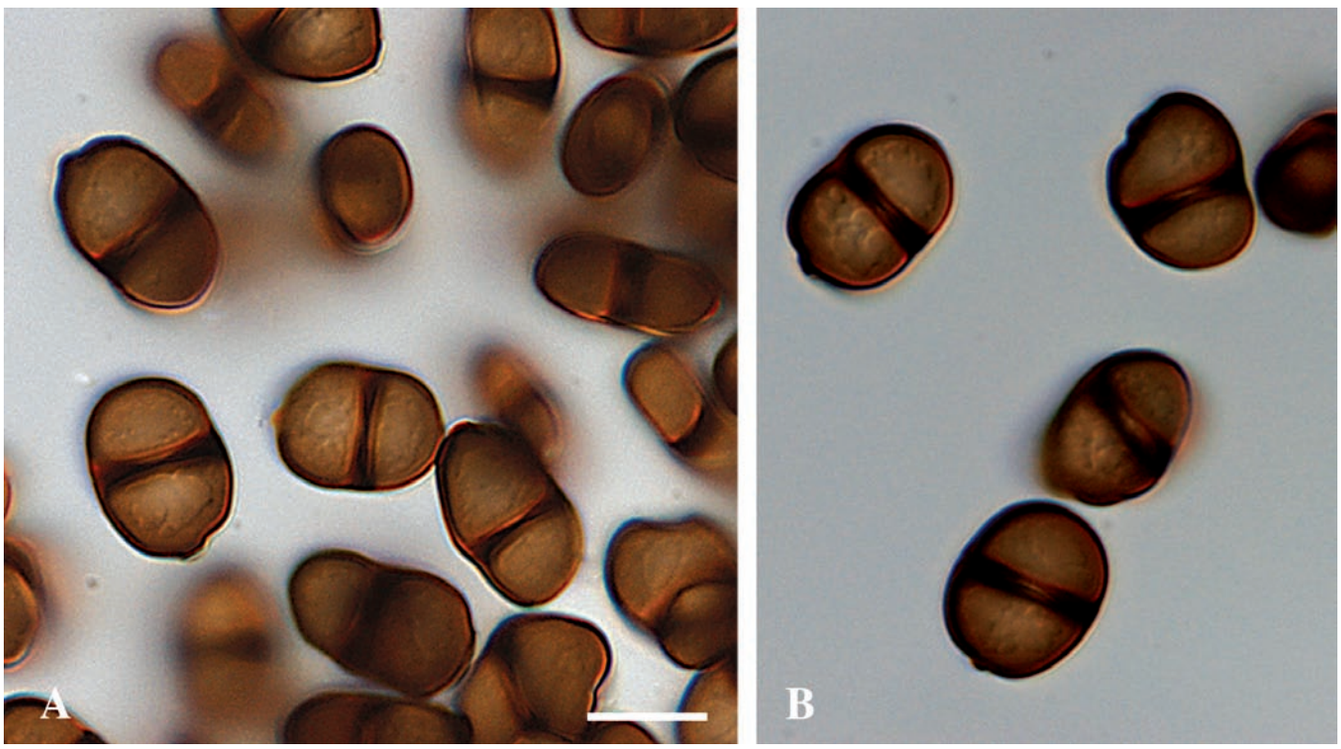


**Fig. 4.** A–B. Conidia of *Macrohilum eucalypti*. Scale bar = 10 µm.

of the ITS sequence of this species had an E-value of 0.0 with an ITS sequence of *Leptospora rubella* on GenBank (AF383951; 99 % similarity). Similarities with *Phaeosphaeria* spp. (*Pleosporales*) ranged from 9e-175 to 4e-109. A number of similarities with an E-value of 0.0 were obtained from the LSU data: *Phaeosphaeria avenaria* (G.F. Weber) O.E. Erikss., *Setomelanomma holmii* M. Morelet, *Setosphaeria monoceras* Alcorn (*Pleosporales*) and others. A number of similarities with an E-value of 0.0 were also obtained from the SSU data: *Phaeosphaeria avenaria*, *Paraphaeosphaeria* spp., *Septoria nodorum* (Berk.) Berk., *Ophiobolus fulgidus* (Cooke & Peck) Sacc. (*Pleosporales*) and others.

***Macrohilum eucalypti*** H.J. Swart, Trans. Br. Mycol. Soc. 90: 288. 1988. Fig. 4.

A single conidioma was observed on the host, from which a culture was obtained, and thus the description is based on features *in vitro*. *Conidiomata* were sparingly formed on MEA, medium brown, globose, up to 400 µm diam. *Conidiogenous cells* pale brown, cylindrical, proliferating percurrently near the apex, 10–15 × 3–5 µm. *Conidia* medium to dark brown, ovoid, smooth, guttulate, developing a single supramedian septum, thick-walled, frequently constricted at the septum, apex obtuse, base truncate with a visible scar, 2–3 µm wide, (15–)17–19(–20) × (8–)10–12(–13) µm.

*Cultural characteristics*: Colonies flat on MEA, spreading, with moderate aerial mycelium and submerged, smooth margins. Surface pale luteous on MEA, cream to pale white on OA; reverse with patches of luteous to umber on MEA, pale luteous on OA; fertile on MEA.

*Substrate and distribution*: *Eucalyptus* sp., New Zealand; also known from *Eucalyptus* spp. in Australia (Sankaran *et al*. 1995).

*Specimen examined*: **New Zealand**, on *Eucalyptus* sp., 2004, J.A. Stalpers, CPC 10945 = CBS 118551.

*Notes*: As far as we could establish, *M. eucalypti* has not previously been known from culture (Swart 1988). BLASTn results of the ITS sequence of this species had an E-value of 9e-98 with an ITS sequence of *Valsa sordida* Nitschke (*Diaporthales*). Similarities with known species include *Phomopsis* spp. (1e-96), *Diaporthe helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov (1e-96; *Diaporthales*) and *Monilinia* sp. (6e-96; *Helotiales*). A number of similarities with an E-value of 0.0 were obtained from the LSU data: *Diaporthe* spp. (*Diaporthales*), *Cryphonectria* spp. (*Diaporthales*), *Harknessia* spp. (*Diaporthales*) and others. A number of similarities with an E-value of 0.0 were also obtained from the SSU data: *Leucostoma persoonii* (Nitschke) Höhn. (*Diaporthales*), *Cryphonectria* spp., *Prosopidicola mexicana* Crous & C.L. Lennox, *Endothia gyrosa* (Schwein.) Fr. (*Diaporthales*, *incertae sedis*) and others.

***Pestalotiopsis disseminata*** (Thüm.) Steyaert, Bulletin Jard. Bot. I Etat Bruxelles 19: 319. 1949. Fig. 5.

≡ *Pestalotia disseminata* Thüm., Inst. Rev. Sci. Coimbra 18: 501. 1880.

*Conidiomata* developing from 10–14 d (none after 7 d) mainly on the surface of the colony. *Conidia* broadly fusoid to fusoid-clavate, straight or somewhat curved, 5-celled, upper cell conical to cylindrical, hyaline, fairly thin-walled, apical setulae central, (2–)3(–4), rather stout, up to 1.2 µm wide, 11–20 µm long, with a blunt tip, three intermediate cells concolorous or the upper

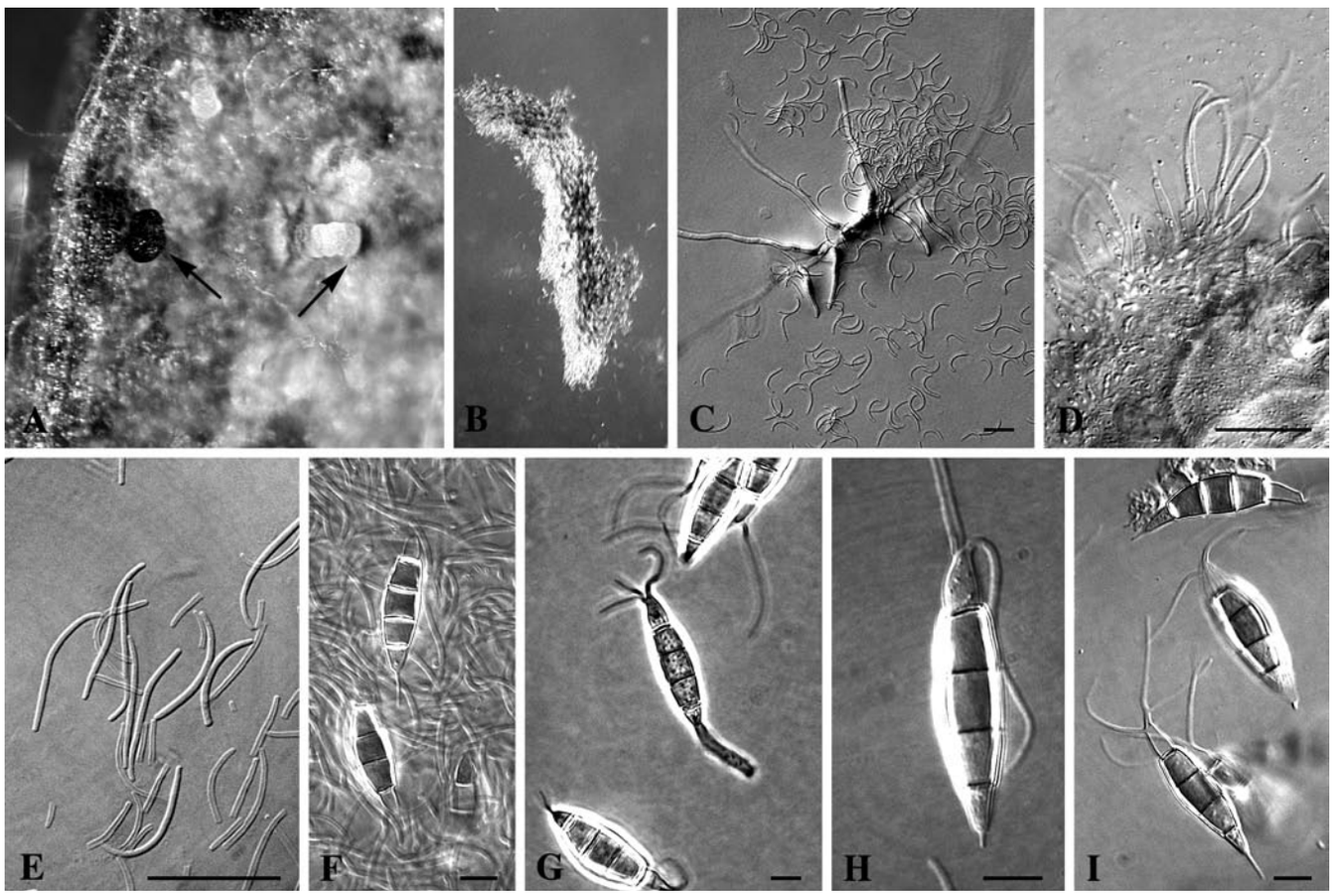


**Fig. 5.** *Pestalotiopsis disseminata*. A. Conidomata with exuding alpha- (black) and beta- (cream) conidial masses (arrowed). B. Conidial cirrus containing back (alpha-) and hyaline (beta-) conidia. C. Germinating alpha-conidium, among infertile beta-conidia on MEA plate. D. Conidiogenous cells giving rise to beta-conidia. E. Beta-conidia. F–I. Alpha-conidia. Scale bars: C–E = 10 µm, F–I = 7 µm.

two intermediate cells slightly darker, dull olivaceous-brown to vinaceous-brown, contents guttulate, walls smooth, slightly constricted at the septa when mounted in water, and thickened up to 1 µm especially in the upper two intermediate cells and in the septa, basal cell hyaline, thin-walled, tapering into a filiform pedicel (2–)2.5–4.5(–5) µm long; conidium body (18–)20–24(–25) × 6.5–7(–8) µm (OA).

*Cultural characteristics*: Colonies on OA reaching 52–54 mm diam in 7 d with an even, glabrous, colourless margin; immersed mycelium colourless, aerial mycelium pure white, fluffy, covering most of the colony surface, and very dense and high in the centre and in concentric zones after 7 d; reverse in the centre buff. Colonies on CMA reaching 52–55 mm diam after 7 d, as on OA, but aerial mycelium less well-developed, and reverse colourless. Colonies on MEA reaching 56 mm diam in 7 d, with an even or slightly undulating colourless margin; immersed mycelium colourless, but surface of the colony completely covered by a high, dense mat of pure white, in the centre yellowish, fluffy aerial mycelium, the margin also covered by a diffuse layer of aerial hyphae; reverse with a faint cinnamon tinge.

*Substrate and distribution*: *Eucalyptus botryoides*, New Zealand (North Island).

*Specimen examined*: **New Zealand**, North Island, Kerikeri, living leaves of *Eucalyptus botryoides*, 17 Oct. 2003, M.A. Dick, CPC 10950 = CBS 118552, CPC 10951.

*Notes*: BLASTn results of the ITS sequence of this species had an E-value of 0.0 with ITS sequences of *Pestalotiopsis* spp.

***Pestalotiopsis* sp.**

*Conidiomata* developing on agar surface and in the aerial mycelium after 3–5 d (OA, MEA & CMA). *Conidia* narrowly fusoid to fusoid-clavate, straight or somewhat curved, 5-celled, upper cell conical to cylindrical, hyaline, fairly thin-walled, without visible cellular contents, bearing (2–)3(–4) rather stout central apical appendages, 10–19 µm long, up to 1.2 µm wide, with a blunt tip, three intermediate cells concolorous or the upper two intermediate cells slightly darker, dull olivaceous-brown to vinaceous-brown, contents guttulate, walls smooth, thickened up to 1 µm especially in the upper two intermediate cells and in the septa, basal cell hyaline, thin-walled, tapering into a filiform pedicel (3–)4–5(–6) µm long; conidium body (19–)20–24(–27) × (5.2–)5.5–6 µm (OA).

*Cultural characteristics*: Colonies on OA reaching 50–53 mm diam in 7 d with an even to undulating, glabrous, colourless margin; immersed mycelium colourless, aerial mycelium pure white, woolly-cottony, covering most of the colony surface without distinct concentrical zonations, almost absent in the marginal zone after 7 d; reverse concolorous, in the centre buff (where sporulation occurs). Colonies on CMA reaching 50 mm diam after 7 d, as on OA, but colony margin undulating

to ruffled, and aerial mycelium less well-developed. Colonies on MEA reaching 49–55 mm diam in 7 d, with an irregularly undulating, colourless, glabrous margin; immersed mycelium colourless, but surface of the colony completely covered by a moderately high, densely woolly mat of pure white, locally faintly sulphur-yellow, aerial mycelium; reverse ochreous to fulvous, brown where conidiomata develop.

*Substrate and distribution*: *Eucalyptus eurograndis*?, Colombia.

*Specimen examined*: **Colombia**, living leaves of *Eucalyptus eurograndis*, 2004, M.J. Wingfield, CBS H-18044, cultures CPC 10969 = CBS 118553, CPC 10970–10971.

*Notes*: BLASTn results of the ITS sequence of this species had an E-value of 0.0 with ITS sequences of *Pestalotiopsis* spp., including *Pestalotiopsis disseminata* and *Pestalotiopsis uvicola* (Speg.) Bissett (both 99 % similar).

The primary reason for the inclusion of these *Pestalotiopsis* spp. in the present paper is the presence of a synanamorph, which has never before been reported for species of *Pestalotiopsis* in the literature (Nag Raj 1993). According to unpublished notes in the CBS database, this has once before been observed for a culture of a *Pestalotiopsis* sp. in the collection. Conidiomata were observed in host tissue to exude a mixture of black and hyaline spores in a typical cirrhus associated with *Pestalotiopsis* conidiomata. The cirrhus consisted of two conidial types, namely typical *Pestalotiopsis* conidia (alpha), and long, narrow, bent, needle-like cylindrical conidia (beta) resembling the beta conidia observed in species of *Phomopsis*, or the conidia typically associated with *Libertella* anamorphs. Conidia were 25–30 ×1–1.5 µm, widest in the middle, tapering to a subobtuse apex, and a truncate base. Conidia were formed on slightly tapering, hyaline, subcylindrical conidiogenous cells that terminated in an apex with 1–2 loci which gave rise to conidia in a sympodial arrangement. In some cases the conidiogenous cells were situated on 1–3-septate conidiophores that were 10–20 × 2–3 µm.

Beta-conidia were initially observed in the collection obtained from Colombia. Although they occurred in the same conidioma, none could be induced to germinate on MEA (observed over 2 wk), while all alpha conidia germinated within 1–2 d. The second collection which had a mixture of both conidial types was obtained from New Zealand. Again, the beta-conidia could not be induced to germinate, and thus their ecological role as potential conidia, or spermatia, still needs to be resolved. None of the colonies derived from alpha conidia could be induced to form beta conidia on MEA, OA or CLA. In this regard it is interesting to note that, contrary to common opinion, it has only recently been proven that beta-conidia of *Phomopsis* spp. do, in fact, germinate in culture (Sergeeva *et al.* 2003).

***Satchmopsis brasiliensis*** B. Sutton & Hodges, Nova Hedwigia 26: 3. 1975. Fig. 6.

*Conidiomata* cupulate, superficial, up to 180 µm wide and 100 µm deep, dark brown, attached centrally to a stroma of dark brown cells that occupy the stomatal chamber; wall consisting of two regions, the lower region having thick-walled dark-brown cells, up to 5 layers thick, the upper region consisting of thin-walled, paler cells, up to 5 layers thick. *Conidiogenous cells* restricted to the lower part of the basal wall, 3–7 × 2–3 µm, doliiform to lageniform, phalidic with periclinal thickening, hyaline, with an indistinct collarette. *Conidia* hyaline, aseptate, guttulate, subcylindrical, predominantly straight, with obtuse ends, 11–17 × 1–1.5 µm.

*Cultural characteristics*: Colonies spreading on MEA, flat with sparse aerial mycelium and smooth margins; surface sienna to umber with patches of white, and dark brown conidiomata; reverse umber (centre) to sienna (margins); on OA umber with no aerial mycelium, and dark brown conidiomata.

*Substrate and distribution*: *Eucalyptus* spp., Colombia, Indonesia.

*Specimens examined*: **Colombia**, on *Eucalyptus* leaf litter, Feb. 2004, M.J. Wingfield, CBS H-18048, cultures CPC 10972–10974. **Indonesia**, on *Eucalyptus* leaf litter, Mar. 2004, M.J. Wingfield, CBS H-18049, cultures CPC 11017 = CBS 118554, CPC 11018–11019.

*Notes*: The collections from Indonesia and Colombia are morphologically similar. Colonies appear similar on MEA, and conidia of the Indonesian collection (11–17 × 1–1.5 µm) are similar to those of the Colombian collection (12–14 × 1–1.5 µm), and fit within the range given for the species, namely 11.5–15.5 × 1–1.5 µm (Sutton 1975). However, from the sequence data (data not shown) it is clear that there are some base pair differences between these isolates, suggesting that these strains may in fact represent different species. The only obvious morphological difference observed was that conidiomata of the Colombian collection were pale brown, with cells at the margin of the wall being up to 5 µm wide. In contrast, conidiomata from the Indonesian collection were darker brown, with cells at the margins being narrower, namely 3–4 µm wide. Whether these morphological differences can be related to the differences observed in the DNA sequences, can only be resolved once further collections have been obtained. BLASTn results of the ITS sequence of this species has E-values of 5e-167 to 1e-115 with ITS sequences of unidentified leaf litter and mycorrhizal ascomycetes. The closest known species include *Pezicula frangulae* (Pers.) Fuckel (2e-110), *Pezicula ocellata* (Pers.) Seaver (9e-107; *Helotiales*), and *Cryptosporiopsis* spp. (4e-106; *Helotiales*). A number of similarities with an E-value of 0.0 were obtained from the LSU data: *Crinula caliciiformis* Fr. (*Helotiales*), *Leuconeurospora pulcherrima* (G. Winter) Malloch & Cain (*Hypocreales*), *Vibrissea albofusca* G.W. Beaton (*Helotiales*) and others. A number of similarities with an E-value of 0.0 were also obtained from the SSU data: *Phacidium coniferarum* (G.G. Hahn) DiCosmo, Nag Raj & W.B. Kendr., *Bulgaria* spp., *Neofabraea malicorticis* H.S. Jacks. (all *Helotiales*) and others.

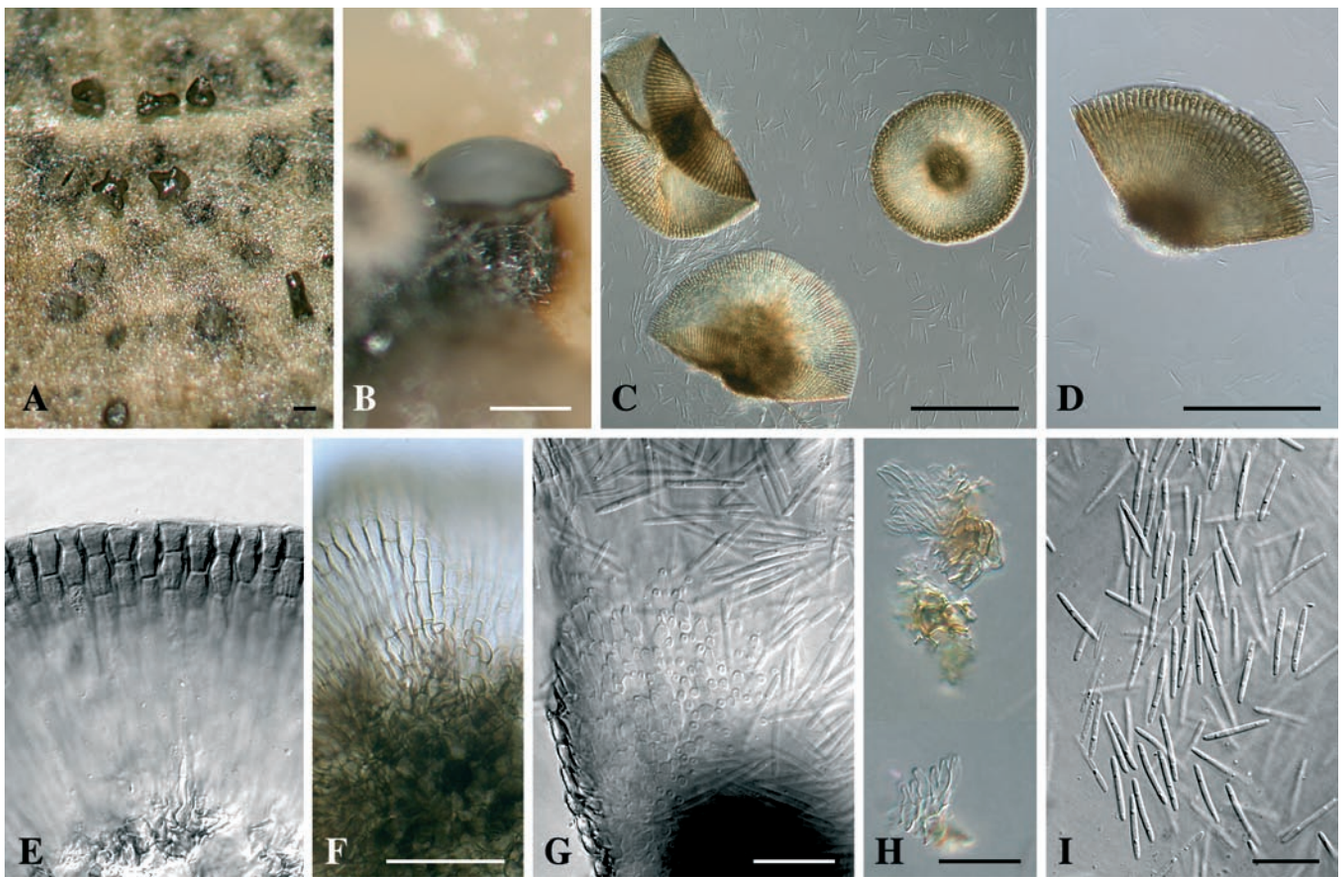


**Fig 6.** *Satchmopsis brasiliensis.* A–D. Conidiomata. E–F. Conidiomatal wall. G. Upper view of conidioma, showing aggregated conidiogenous cells. H. Conidiogenous cells. I. Conidia. Scale bars: A–D = 90 µm, E–I = 15 µm.

***Torrendiella eucalypti*** (Berk.) Spooner, Bibl. Mycol. 116: 322. 1987. Figs 7–8.

≡ *Peziza eucalypti* Berk., Flora Tasman. 2: 274. 1860.

*Apothecia* on host scattered or gregarious in large groups, erumpent, stipitate, arising from a subepidermal stroma visible around the stipe as a dark discoloration. *Disc* plane to convex, greyish brown to olivaceous, smooth, 0.4–1.5 mm diam. *Receptacle* cupulate, concolorous but usually darker than the hymenium, bearing dark brown to reddish brown setae. *Stipe* central, smooth and dark brown, 0.4–1.8 mm high. *Setae* mostly 20–50 per apothecium, (150–)200–250 µm long, smooth, with dark brown walls thickened up to 1.5 µm, septate, paler at the blunt top, attenuated and bent at the base. *Asci* cylindrical-clavate, apex conical-rounded, the apical apparatus blueing in Melzer's reagent, croziers present, 8-spored, 75–100 × 7–9 µm; *ascospores* fusoid, 0-septate, narrowly rounded at both ends, contents guttulate, hyaline, each end provided with a central, everted (umbrella-shaped) mucelaginous appendage, 17–25 × 3–4 µm; sometimes producing ellipsoid microspores 3–5.5 × 1.5–2 µm directly from apertures at one or both ends. *Paraphyses* simple or branched near the base, obtuse, hyaline, somewhat inflated and up to 3.5 µm wide at the top.

*Cultural characteristics*: *Colonies* on OA reaching a diam of 15–20(–30) mm in 14 d, with an even to slightly ruffled, glabrous and colourless margin; immersed mycelium at first colourless, then very faintly yellowish (primrose) or reddish (apricot), after 10–20 d gradually developing a mixture of several tinges, pale hazel, ochreous and amber, in the centre sometimes also greyish to olivaceous buff, most of the surface almost glabrous and without aerial mycelium, locally with patches of woolly, pure-white aerial mycelium. Colonies on MEA reaching 33–37 mm diam in 14 d, with a ruffled, glabrous, colourless margin; most of the colony surface covered by a fairly dense, woolly but low mat of pure white aerial mycelium; reverse in centre ochreous to umber, fading to the colourless margin.

*Apothecia* formed on OA after about 10 wk, mostly on the agar surface, most very similar in shape and size to those formed *in planta*, but with less setae; however, large abnormally shaped apothecia are also formed: hymenium convex, protruding from the agar surface as a greyish-black, globular mass with a smooth surface, 1–2.5 mm diam, receptacle reduced, hairs present or absent, lacking a stipe.

*Anamorph in vitro*: *Conidiophores* developing on the surface of globular ascomatal initials after 2–3 wk, smooth-walled, variable, simple, but mostly branched near the base, 15–30 × 2–4(–5) µm thick, hyaline or somewhat yellowish brown, conidiogenesis blastic, sympodial, sometimes seemingly retrogressive, apertures mostly terminal but also immediately below septa (acropleurogenous), scars visible but not thickened or protruding; *conidia* hyaline, ellipsoid, broadly rounded at the top, slightly attenuated into a blunt base, with one or two small guttules, 4–5.2(–6) × (1.5–)1.8–2 µm.

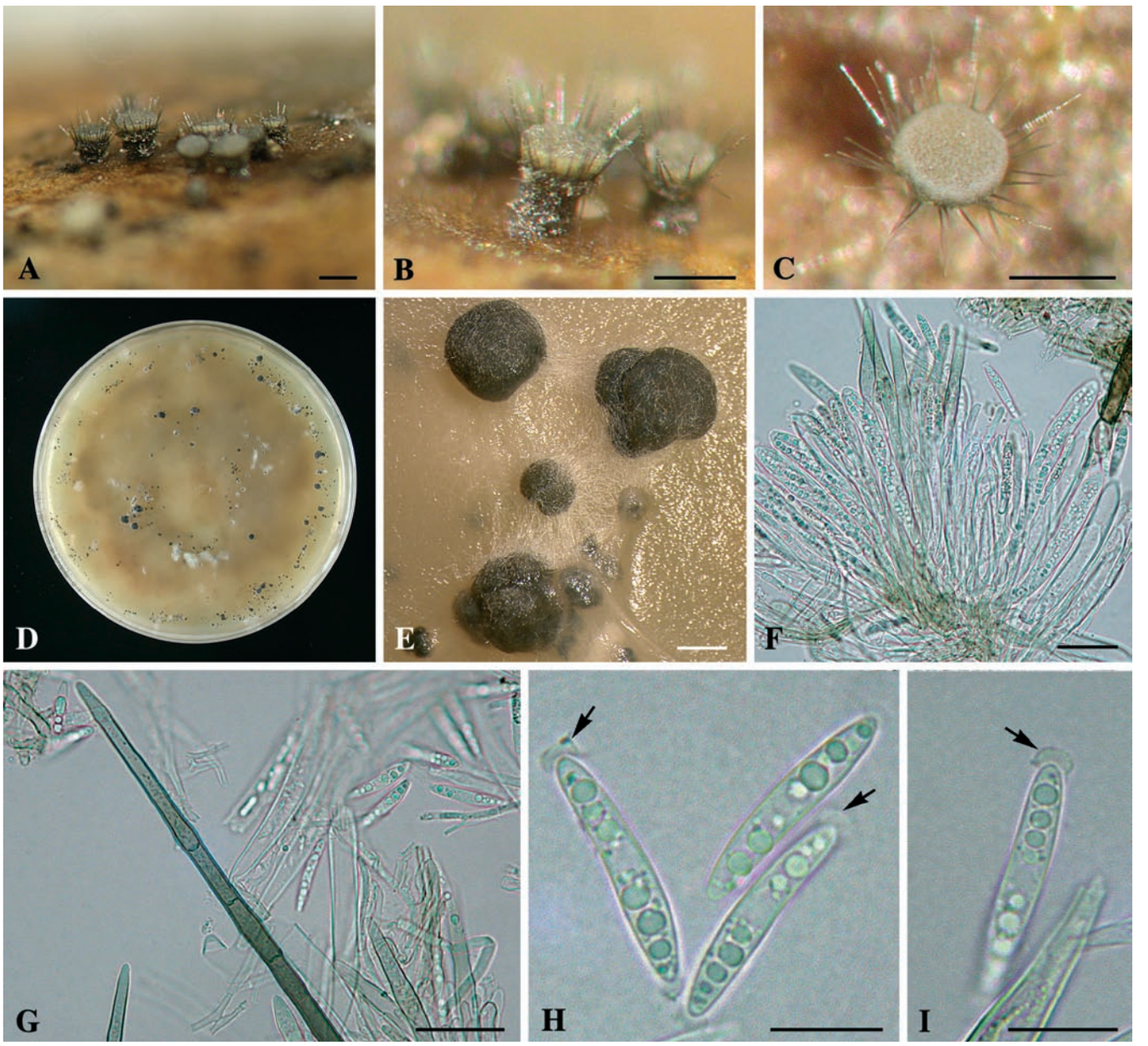


**Fig. 7.** *Torrendiella eucalypti*. A–C. Apothecia on the plant. D–E. in culture on OA. D. 15-wk-old culture with apothecia. E. Abnormally shaped apothecia. F. Asci and ascospores. G. Apothecial hair. H–I. Ascospores with apical appendages (arrows). Scale bars: A–C = 1.5 mm, E = 100 µm, F–G = 25 µm, H–I = 10 µm.

*Substrate and distribution*: *Eucalyptus* sp., Indonesia.

*Specimen examined*: **Indonesia**, on *Eucalyptus* leaf litter, in association with *Coccomyces antillarum* Sherwood, M.J. Wingfield, Mar. 2004, CBS H-18041, single-ascospore isolates, CPC 11049 = CBS 115326, CPC 11050–11051.

*Notes*: The material used in this study generally agrees well with the description given by Spooner (1987). There are, however, some additional observations that were not reported by this author, particularly, the presence of apical appendages on the ascospores, and the production of microspores from liberated ascospores. We observed 3–8 large guttules in ascospores of *T. eucalypti*, while Spooner reported only two or three guttules per spore. After drying, the guttules in our material often merged into larger bodies, and this could explain the difference between our obervations and those of Spooner, which were based on herbarium specimens. After drying of our specimen, the

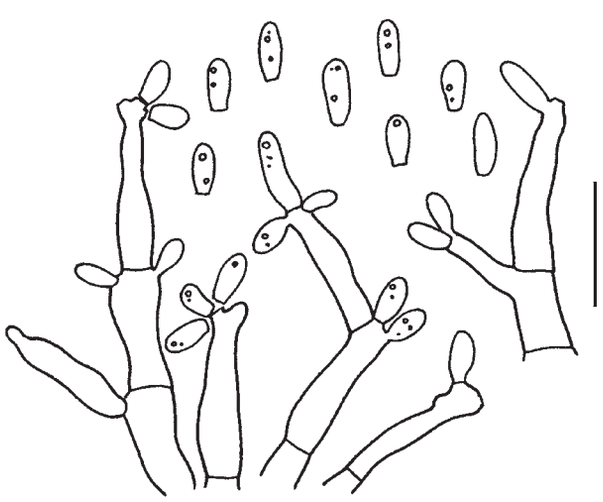

**Fig. 8.** Anamorph of *Torrendiella eucalypti*. Conidiophores and conidia on OA. Scale bar = 10 µm.

appendages of the ascospores were barely visible. The present study is also the first to report on observtions in pure culture. The anamorph was only observed in culture, and showed plasticity in conidiogenesis making it very difficult to assign it to a particular anamorph genus. It could be circumscribed as *Sporothrix*-like, although it lacks the denticles characteristic of that anamorph, and it also differs by branched and septate conidiophores. *Sporothrix schenkii* Hektoen & C.F. Perkins, the type species of *Sporothrix* Hektoen & C.F. Perkins, is commonly isolated from *Eucalyptus* wood, but is linked to *Ophiostoma* Syd. & P. Syd. BLASTn results of the ITS sequence of this species had an E-value of 0.0 with ITS sequences of *Torrendiella eucalypti* and *Torrendiella madsenii* (G.W. Beaton & Weste) Spooner (both 94 % similar). Similarities with known species include *Cyathicula coronata* (Bull.) De Not. (2e-135), *Hymenoscyphus fructigenus* (Bull.) Fr. (8e-135) and *Pezizella amenti* (Batsch) Dennis (6e-96; allHelotiales). A number of similarities with an E-value of 0.0 were obtained from the LSU data: *Hymenoscyphus scutula* (Pers.) W. Phillips, *Cudoniella clavus* (Alb. & Schwein.) Dennis (both *Helotiales*) and others.

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