Eucalyptus microfungi known from culture. 2. *Alysidiella*, *Fusculina* and *Phlogicylindrium* genera nova, with notes on some other poorly known taxa

Brett A. Summerell[1], Johannes Z. Groenewald[2], Angus J. Carnegie[3], Richard C. Summerbell[2] and Pedro W. Crous[2*]

[1]Royal Botanic Gardens and Domain Trust, Mrs. Macquaries Road, Sydney, NSW 2000, Australia
[2]Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
[3]Forest Resources Research, NSW Department of Primary Industries, PO Box 100, Beecroft, NSW 2119, Australia

Summerell, B.A., Groenewald, J.Z., Carnegie, A., Summerbell, R.C. and Crous, P.W. (2006). *Eucalyptus* microfungi known from culture. 2. *Alysidiella*, *Fusculina* and *Phlogicylindrium* genera nova, with notes on some other poorly known taxa. Fungal Diversity 23: 323-350.

Although numerous microfungi have been described from *Eucalyptus* in recent years, this plant genus remains a rich substrate colonized by numerous undescribed species. In the present study several species and genera of ascomycetes were collected from symptomatic leaves or from leaf litter of this host in Australia, South Africa and Europe. New genera include those encompassing *Alysidiella parasitica* and *Phlogicylindrium eucalypti* genera et spp. nov. (hyphomycetes), and *Fusculina eucalypti* gen. et sp. nov. (a coelomycete). New species include *Colletogloeopsis blakelyi*, *C. considenianae*, *C. dimorpha*, *Elsinoë eucalyptorum*, *Harknessia rhabdosphaera*, *Neofusicoccum corticosae* and *Staninwardia suttonii*. A new combination is proposed for *Microsphaeropsis eucalypti* in *Readeriella*, while new cultures, hosts and distribution records are reported for *Cytospora diatrypelloidea*, *Mycosphaerella swartii*, *Plectosphaera eucalypti* and *Valsa fabianae*.

Key words: ITS rDNA sequence data, *microfungi*, *morphology*, *pure culture*, *systematics*.

Introduction

Eucalyptus (*Myrtaceae*) appears to host an incredibly diverse range of microfungi (Sankaran *et al*., 1995; Crous *et al*., 2006b,d), most of which have never been grown in pure culture. For some fungal groups this deficiency is being addressed, and thus many complexes of plant pathogenic fungi are now beginning to become known from DNA sequence data. Some of the more

*Corresponding author: Pedro Crous; e-mail: crous@cbs.knaw.nl

important groups include those responsible for Mycosphaerella leaf blotch (Cortinas *et al*., 2006, Crous 1998, Crous *et al*., 2000, 2001, 2004a,b, 2006e; Hunter *et al*., 2006), Cylindrocladium leaf blight (Crous 2002, Crous *et al*., 2004c, 2006a), Cryphonectria canker (Gryzenhout *et al*., 2004, 2006, Nakabonge *et al*., 2006), Botryosphaeria canker (Slippers *et al*., 2004a-c, Crous *et al*., 2006c), Cytospora canker (Adams *et al*., 2005), *Coniella* (Van Niekerk *et al*., 2004), *Phomopsis* (Van Niekerk *et al*., 2005; Van Rensburg, 2006), *Quambularia* (de Beer *et al*., 2006) 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 known from culture or represented in freely accessible culture collections. Biologists are largely ignorant about their distribution, host range, importance and various ecological roles.

The present study is part of a series aimed at describing eucalypt microfungi from culture, and recollecting and culturing known species (Sankaran *et al*. 1995) so that their taxonomy and phylogeny can be resolved.

Materials and methods

Isolates

Symptomatic *Eucalyptus* leaves were chosen for study, as was leaf litter showing signs of fungal colonization. Excised lesions with ascomata were soaked in water for approximately 2 h, then placed in the bottom of Petri dish lids, with the top half of the dish containing 2% malt extract agar (MEA) (Biolab, Midrand, South Africa). Germination patterns of ballistically deposited ascospores on the overhanging medium were examined after 24 h, and single-ascospore and -conidial cultures were established as described by Crous (1998). Leaves were also incubated in moist chambers (Petri dishes with moist filter paper inside them, incubated on the laboratory bench), and inspected daily for microfungi. Hyphomycetes and coelomycetes were cultured on MEA (Gams *et al*., 1989) by obtaining single conidial colonies as explained in Crous (2002). Colonies were subcultured onto fresh MEA, oatmeal agar (OA), cornmeal agar (CMA) and potato-dextrose agar (PDA) plates (Gams *et al*., 1989) and incubated at 25°C under continuous near-ultraviolet light, to promote sporulation.

DNA isolation, amplification and phylogeny

Fungal colonies were established on MEA plates, and genomic DNA was isolated following the protocol of Lee and Taylor (1990). The primers V9G

(Hoog and Gerrits van den Ende, 1998) and ITS4 (White *et al*., 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S rDNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rDNA gene, the second ITS region and the 5' end of the 28S rDNA gene (LSU). PCR conditions and protocols were treated and generated as explained in Crous *et al*. (2004a).

Taxonomy

Slide preparations, based on material *in vivo* and *in vitro*, were mounted in lactic acid for microscopic examination. Thirty observations (×1000) were made of each structure, and 95% intervals were determined in order to generate standardized conidial and ascospore measurements, with the excluded extremes given in parentheses. Colony colours (surface and reverse) were classified using the colour charts of Rayner (1970). Descriptions and nomenclatural details were deposited in MycoBank (www.MycoBank.org), and cultures and herbarium specimens were accessioned in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands.

Results

DNA phylogeny

Sequence data were deposited in GenBank. Accession numbers for each species are given with the description. The phylogenetic placement suggested by the sequences is discussed in the descriptive notes below each of the treated species.

Taxonomy

Alysidiella Crous, **gen. nov.** MycoBank MB510004

Etymology: *Alysidi-* from *Alysidium*, indicating its morphological similarity to this genus.

Hyphomycetes dematiacei sporodochiales, inter *Alysidium* (conidiis aseptatis) et *Heteroconium* (conidiis multiseptatis).

Hyphomycetous, foliicolous. *Conidiomata* sporodochial, consisting of brown, verrucose, thick-walled, branched, septate, hyphae. *Conidiogenous cells* holoblastic, scars indistinct to thickened along the rim, not darkened nor refractive. *Setae* and *hyphopodia* absent. *Conidia* dry, in branched or simple acropetal chains, ellipsoidal to subcylindrical, medium brown, thick-walled, verruculose, aseptate to multiseptate.

Alysidiella parasitica Crous, **sp. nov.** Fig. 1
MycoBank MB510005

Etymology: Named after the severe leaf spotting associated with infections of this fungus.

Conidiomata sporodochialia, brunnea, ad 90 µm diam. Cellulae conidiogenae integratae, indistinctae, terminales, 4–13 × 4–6 µm. Conidia catenulata, sicca, ellipsoidea vel subcylindrica, medio-brunnea, crassitunicata, verruculosa 0–13-septata, 8–30 × 5–7 µm.

Leaf spots predominently hypophyllous, but some also epiphyllous, mostly not extending through the leaf lamina, circular, slightly raised, 1–6 mm diam; margins chlorotic or red-purple; spots also occurring on leaf petioles, somewhat reminiscent of *Aulographina eucalypti*. *Colonies* sporulating on dark brown, mature lesions. *Conidiomata* sporodochial, brown, up to 90 µm diam, consisting of brown, verrucose, thick-walled, branched, septate, 4–6 µm wide hyphae. *Conidiogenous cells* integrated, indistinct, terminal on hyphal cells in sporodochium, 4–13 × 4–6 µm, giving rise to conidia in acropetal chains; scars 2–3 µm wide, indistinct to thickened along the rim, not darkened nor refractive. *Setae* and *hyphopodia* absent. *Conidia* dry, in branched or simple acropetal chains, ellipsoidal to subcylindrical with rounded ends, medium brown, thick-walled, verruculose, 0–13-septate, 8–30 × 5–7 µm; conidia constricted at septa, which eventually separate to form additional arthroconidia; aseptate conidia 5–7 µm long; 1-septate conidia 7–10 µm long; 2-septate conidia 10–15 µm long.

Cultural characteristics: Colonies on PDA erumpent, with sparse to moderate aerial mycelium, margins smooth but feathery; surface and reverse greenish black; colonies reaching 10 mm diam after 2 mo on PDA at 25°C; colonies fertile.

Specimen examined: **South Africa**, Western Cape Province, Stellenbosch Mountain, on leaves of *Eucalyptus* sp., Jan. 2006, P.W. Crous, CBS H-19742, **holotype**, culture ex-type CPC 12835 = CBS 120088, CPC 12836-12837.

Notes: This collection has been placed in a new genus, *Alysidiella*, because it could not be accommodated in *Alysidium*, which has aseptate conidia, *Heteroconium*, which has multiseptate conidia, or *Taeniolella*, which has multiseptate conidia and which generally lacks aerial mycelium. Furthermore, in all three genera listed above, nucleotide sequence data (Crous, unpublished) have clearly shown that each genus is polyphyletic, and in fact represents numerous distinct genera that belong to different families and orders. None of these genera has a type species that is closely phylogenetically related to the fungus described here, nor does any known name currently in synonymy apply to this species. This fungus appears to be quite an important pathogen of *Eucalyptus* and is thus a high priority for a description compatible with modern phylogenetic standards. BLASTn results of the ITS sequence of this species

Fig. 1. *Alysidiella parasitica* (CBS H-19742). **A, B.** Leaf and stem lesions. **C–J.** Conidiophores and conidia *in vivo*. **K.** Colony on PDA. **L, M.** Catenulate conidia *in vitro*. Scale bar = 10 µm.

(GenBank DQ923525) had an E-value of 3e-79 with the ITS sequence of a leaf litter ascomycete AF502855, and with *Caloplaca maritima* AF353948 (Lecanoromycetes, *Teloschistaceae*). Similarities with other known species include *Guignardia mangiferae* (1e-78; *Botryosphaeriaceae*), *Coniosporium*

apollinis (1e-78) and *Guignardia citricarpa* AF374371 (5e-78; *Botryosphaeriaceae*).

Colletogloeopsis blakelyi Crous & Summerell, **sp. nov.** Fig. 2
MycoBank MB510006

Etymology: Named after its host species, *Eucalyptus blakelyi*.

Coniothyrio ovato similis, sed conidiis anguste ellipsoideis, (8–)9–10(–12) × 3(–4) µm.

Leaf spots pale brown, irregular, amphigenous, up to 7 mm diam; associated with wasp damage. *Conidiomata* amphigenous, pycnidial, subepidermal, substomatal, globose, brown, up to 90 µm diam, exuding conidia in black masses; wall consisting of 2–3 cell layers of brown cells of *textura angularis*. *Conidiogenous cells* pale brown, verruculose, ampulliform, proliferating percurrently near the apex, 5–7 × 3–4 µm. *Conidia* pale brown, verruculose, frequently bi-guttulate, characteristically narrowly ellipsoidal, apex subobtuse, base subtruncate, predominantly straight, with inconspicuous, minute marginal frill, (8–)9–10(–12) × 3(–4) µm.

Cultural characteristics: Colonies on MEA reaching 20 mm diam after 2 months at 25°C; colonies erumpent with sparse aerial mycelium; surface cream to smoke-grey, with prominent superficial ridges; margin feathery, reverse sepia; agar discoloring to vinaceous-brick due to a diffuse pigment exuding from the colonies.

Specimen examined: **Australia**, New South Wales, on leaves of *Eucalyptus blakelyi* Maiden, 13.5 km along Glen Davis road from Capertee. Central Tablelands NSW, Australia, 33 08 13 S 150 04 46 E, Alt: 554 metres. Generally E to SE aspect in gully. Open forest of *Eucalyptus moluccana*, *E. albens*, *E. blakelyi*, *E. cannonii*, *E. fibrosa*, *Brachychiton populneus*, *Callitris endlicheri*, *Acacia buxifolia*, *A. verniciflua*, *A. ixiophylla*, *Exocarpos cupressiformis*, *Bursaria spinosa*, etc. Rocky sandy loam, orange-red in colour; over sandstone and some limestone, Mar. 2006, B. Summerell, CBS H-19743, **holotype**, culture ex-type CPC 12837 = CBS 120089, CPC 12838-12839, GenBank DQ923526.

Notes: Although there is some overlap in conidial dimensions between the *Colletogloeopsis* species described to date, *C. blakelyi* can readily be distinguished from other species (Crous 1998, Crous *et al*., 2004a, 2006e) based on its characteristic narrowly ellipsoidal conidia. This species is also distinct from others in the genus based on its nucleotide sequence data. BLASTn results of the ITS sequence of *C. blakelyi* (GenBank DQ923526) had an E-value of 0.0 with the ITS sequence of *Mycosphaerella vespa* AY534227 (96% identical; Mycosphaerellaceae), and was 95% identical to other members of Mycosphaerellaceae such as *C. zuluensis* AY244421, *Mycosphaerella ambiphylla* AY150675 and *Mycosphaerella molleriana* AF449102.

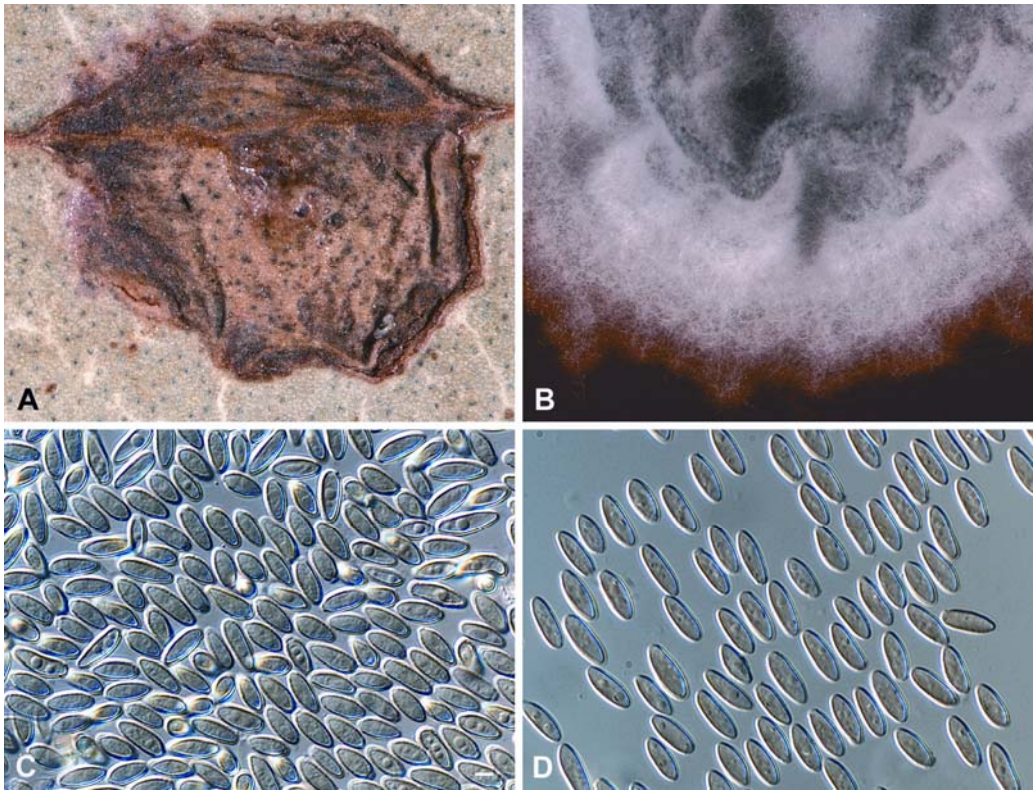

Fig. 2. *Colletogloeopsis blakelyi* (CBS H-19743). **A.** Leaf lesion. **B.** Colony on MEA. **C, D.** Conidia *in vitro*. Scale bar = 10 µm.

Colletogloeopsis considenianae Crous & Summerell, **sp. nov.** Fig. 3
MycoBank MB510007

Etymology: Named after its host species, *Eucalyptus consideniana*.

Coniothyrio ovato similis, sed conidiis ellipsoideis, (6–)7–9(–10) × 3(–4) µm.

Leaf spots amphigenous, circular, medium brown, 1–4 mm diam, surrounded by a prominent red-purple margin. *Conidiomata* amphigenous, pycnidial, globose, brown, up to 90 µm diam, exuding conidia in black masses; wall consisting of 2–3 cell layers of brown cells of *textura angularis*. *Conidiogenous cells* medium brown, finely verruculose, doliiform to ampulliform, proliferating percurrently near the apex, 3–6 × 4–5 µm. *Conidia* medium brown, verruculose, ellipsoidal, apex obtuse, base subtruncate to truncate, straight to slightly curved, with inconspicuous, minute marginal frill, (6–)7–9(–10) × 3(–4) µm.

Cultural characteristics: Colonies on MEA reaching 15 mm diam after 2 months at 25°C; colonies erumpent with sparse aerial mycelium; surface with

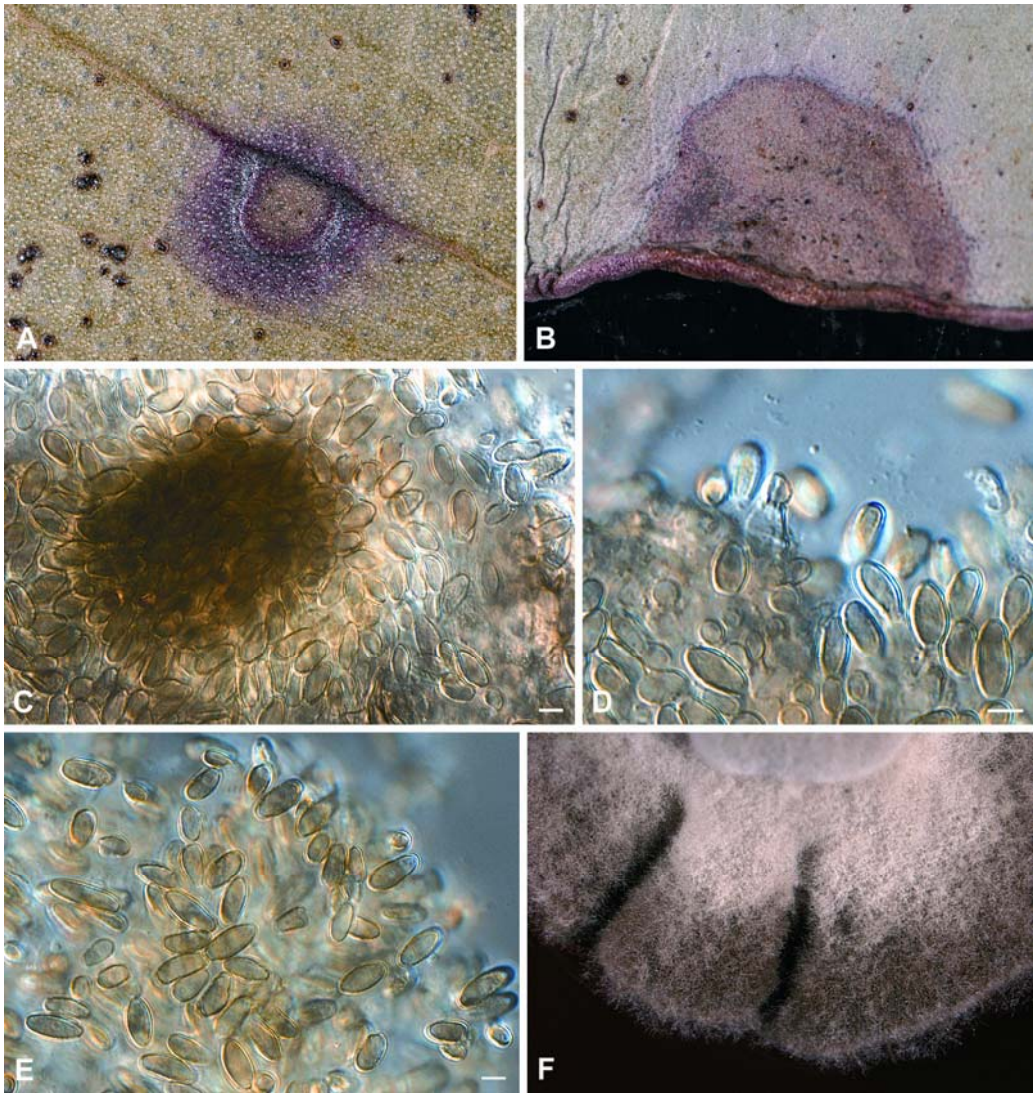

Fig. 3. *Colletogloeopsis considenianae* (CBS H-19744). **A, B.** Leaf spots. **C.** Transverse section through a pycnidium. **D, E.** Conidia and conidiogenous cells. **F.** Colony on MEA. Scale bars = 10 µm

prominent ridges, centre pale olivaceous-grey, outer region olivaceous-grey; margins regular, but uneven due to ridges; reverse iron-grey.

Specimen examined: **Australia**, New South Wales, Blaxland, on leaves of *Eucalyptus consideniana* Maiden, in Blaxland War Memorial Park, opposite Blaxland Public School, intersection of Wilson Way and Great Western Highway, Central Coast NSW, 33 44 14 S 150 36 19 E, Alt: 255 metres; Ridgetop, gentle E-SE facing aspect, shale cap - sandstone transition

zone, over sandstone; light brown rocky loam; site cleared of understorey for parkland. Disturbed remnant open forest of *Eucalyptus sparsifolia*, *Syncarpia glomulifera*, *Corymbia eximia* and some *E. consideniana*. Some regrowth of shrubs such as *Kunzea ambigua*, *Grevillea sericea*; old cultivated plantings of various species evident. Small tree, ca. 10 m tall; locally occasional; Mar. 2006, B. Summerell, CBS H-19744, **holotype**, culture ex-type CPC 12840 = CBS 120087, CPC 12841-12842, GenBank DQ923527.

Notes: *Colletogloeopsis considenianae* closely resembles *C. ovatum* in symptomatology, but is distinct in having conidiogenous cells and conidia smaller than those found in *C. ovatum*. Conidiogenous cells of *C. ovatum* are 3–5(–14) × 5(–7) µm, and conidia (6–)7–9(–14) × 3–3.5(–6) µm. BLASTn results of the ITS sequence of *C. considenianae* (GenBank DQ923527) had an E-value of 0.0 with the ITS sequence of *Mycosphaerella ambiphylla* AY150675 (96% identical; *Mycosphaerellaceae*), *C. zuluensis* AY244419 (97%), *Mycosphaerella vespa* AY045500 (96%), and *Mycosphaerella molleriana* AF449102 (96%).

Colletogloeopsis dimorpha Crous & Carnegie, **sp. nov.** Fig. 4
MycoBank MB510008

Etymology: Named after its two types of conidia commonly observed in culture.

Coniothyrio ovato similis, sed conidiis dimorphicis et microcyclo propagantibus, (7–)9–11(–13) × (3–)4(–5) µm.

Leaf spots amphigenous, medium to dark brown, irregular to angular, with a raised border, 2–5 mm diam. *Conidiomata* pycnidioid, amphigenous, brown on leaves, up to 150 µm; wall consisting of 2–4 layers of brown *textura angularis*. *Conidiogenous cells* lining the inner cavity, doliiform to subcylindrical, medium brown, finely verruculose, proliferating percurrently near apex, 7–15 × 3–5 µm. *Conidia* (7–)9–11(–13) × (3–)4(–5) µm, medium brown, finely verruculose, guttulate, ellipsoidal to fusiform, straight, apex subobtuse, widest in middle if fusiform, or in lower third of conidium if ellipsoidal, tapering towards a subtruncate base, 1–1.5 µm wide; with age some conidia become median septate, usually at the onset of microcyclic conidiation, though this is not a requirement, and it can occur without a septum; microcyclic conidiation occurs from one or both ends, either via percurrent or sympodial proliferation; conidial hilum mostly without a marginal frill.

Cultural characteristics: Colonies on MEA spreading, with moderate aerial mycelium, margins even but feathery; surface olivaceous-grey, margins and reverse iron-grey; on PDA iron-grey with moderate aerial mycelium; colonies fertile.

Specimen examined: **Australia**, New South Wales, Rosewood, on leaves of *Eucalyptus* sp., native regeneration within *Pinus radiata* D. Don plantation, Carabost State Forest, Downfall Road, about 3 km north-west of Rosewood, Southern Highlands, Jan. 2006, A. Carnegie, CBS H-19739, **holotype**, DAR 77443 isotype, culture ex-type CPC 12919 = CBS 120086; New South Wales, Laurel Hill, on *Eucalyptus nitens* (Deane & Maid.) Maid., in

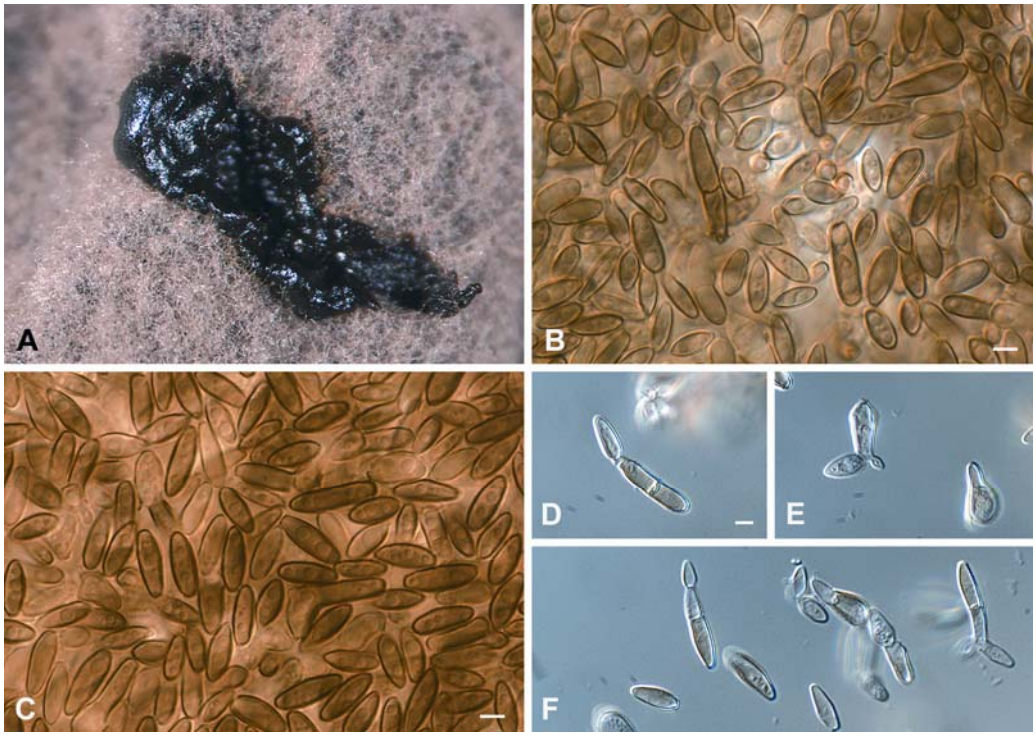

Fig. 4. *Colletogloeopsis dimorpha* (CBS H-19739). Sporulating colony on MEA. **B–F.** Dimorphic conidia, and conidia undergoing microcyclic conidiation in culture. Scale bars = 10 µm.

eucalypt species trial established within *Pinus radiata* D. Don plantation, Bago State Forest, 20 km north of Tumbarumba, Southern Highlands, Jan. 2006, A. Carnegie, DAR 77444, culture CPC 12798 = CBS 120085.

Notes: *Colletogloeopsis dimorpha* is easily distinguishable from other species of *Colletogloeopsis* by its dimorphic conidia, as well as in its ITS sequence. BLASTn results of the ITS sequence of this species (GenBank DQ923528 and DQ923529) had an E-value of 0.0 with the ITS sequence of *Mycosphaerella ambiphylla* AY150675 (96% identical; *Mycosphaerellaceae*), *Mycosphaerella vespa* AY045500 (96%), *C. zuluensis* AY244421 (96%) and *Mycosphaerella molleriana* AF449102 (96%). The ITS sequence is 95% identical to that of *C. blakelyi* and 96% to that of *C. considenianae*.

Elsinoë eucalyptorum Crous & Summerell, **sp. nov.** Fig. 5
MycoBank MB510009

Etymology: Named after its host plant genus, *Eucalyptus*.

Ascomata sparsa, separate, pulvinata, subcuticularia, ad 200 µm diam. Asci ovoidei vel globosi, crassitunicati, 8-spori, 19–30 × 16–20 µm. Ascosporae hyalinae, leves, tenuitunicatae,

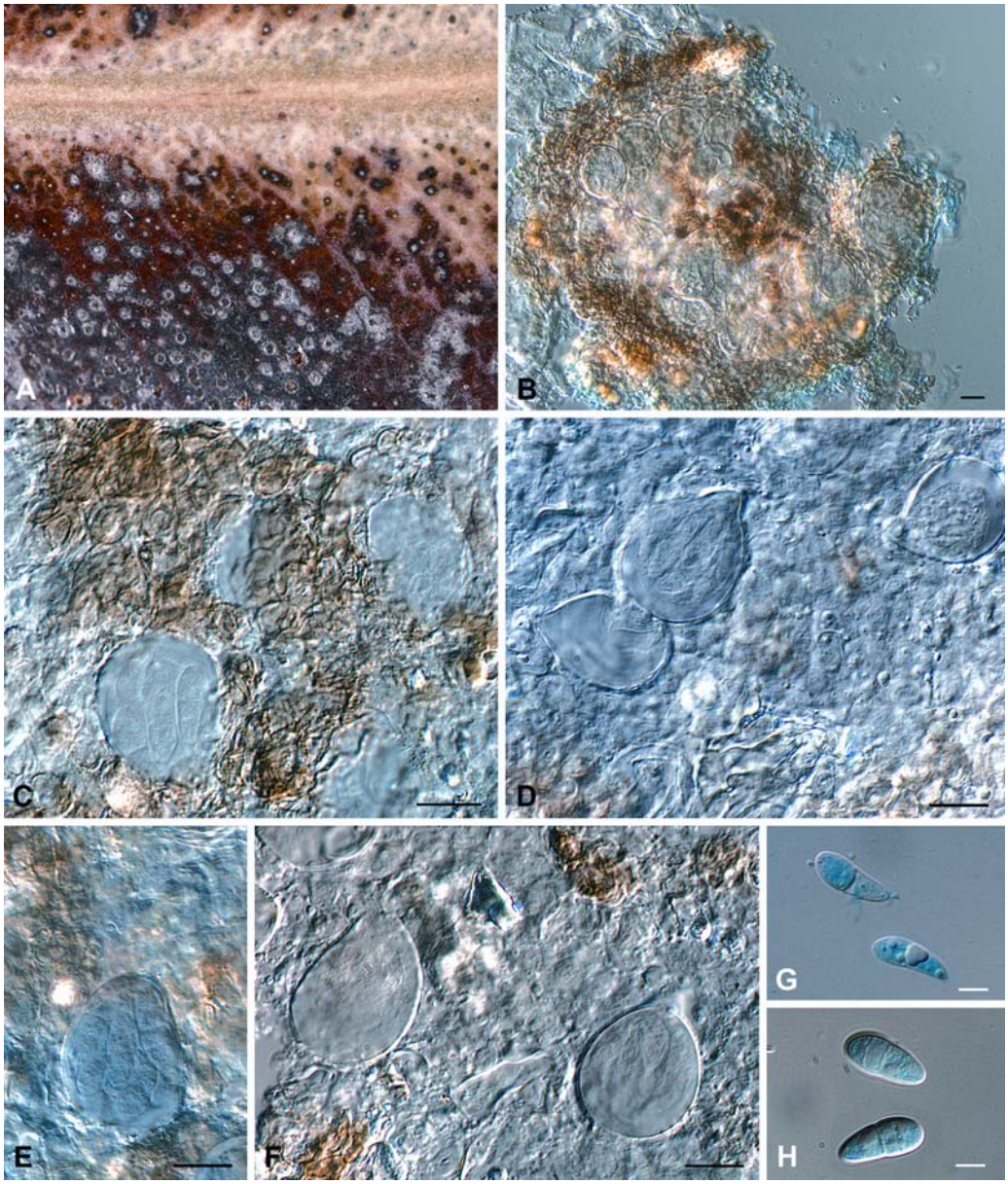

Fig. 5. *Elsinoë eucalyptorum* (CBS H-19746). **A.** Leaf spot. **B.** Section through an ascoma. **C–F.** Asci. **G, H.** Ascospores. Scale bars = 10 µm.

late ellipsoideae, utrinque rotundatae, uno (–tribus) septis transversalibus et 1–2 septis longitudinalibus divisae, (11–)13–15 × (4–)5(–6) µm.

Leaf spots not extending through the leaf lamina, epiphyllous, brown, with patches of grey due to lifting of the cuticle, margins discrete to somewhat

confluent, not raised; spots elongated, ellipsoidal to irregular, up to 7 mm wide, and 25 mm long. *Ascomata* scattered, separate, pulvinate, subcuticular, becoming erumpent, appearing dark brown and circular on the surface; margin grey due to the lifting of the cuticle; composed of medium brown pseudoparenchymatic *textura angularis*, up to 200 µm wide. *Asci* distributed irregularly throughout ascomata, ovoid to globose, with rounded apex and slightly flattened base, thick-walled, 8-spored, sessile, hyaline, 19–30 × 16–20 µm. *Ascospores* hyaline, smooth, thin-walled, broadly ellipsoidal with rounded ends, with 1(–3) transverse septa, and 1–2 vertical or oblique septa; constricted at median septum, (11–)13–15 × (4–)5(–6) µm.

Cultural characteristics: Colonies on PDA slow-growing, erumpent, round to somewhat irregular, aerial mycelium absent, margin smooth, growing down into the agar, causing a sunken zone around the colony; colonies dirty white, reaching 4 mm diam after 2 wks on PDA at 25°C.

Specimen examined: **Australia**, New South Wales, Kew, on leaves of *Eucalyptus propinqua* H. Deane & Maiden, 0.9 km west of Pacific Highway on Middle Brother Road, ca. 11 km south of Kew. North Coast NSW, 31 42 38 S 152 42 20 E, Alt: 40 metres; Low ridge in undulating country, tall open forest with *Eucalyptus propinqua*, *E. microcorys*, *Corymbia intermedia*, *Allocasuarina torulosa*, *Lantana camara*, *Breynia oblongifolia*, *Polyscias sambucifolia*, *Acacia melanoxylon*, *Lophostemon confertus*, *Kennedia rubicunda*, *Imperata cylindrica*, etc.; fine mid brown clay loam over fine grained volcanic rock; locally abundant tall straight-trunked tree to 35 m × 20 m, Feb. 2006, B. Summerell, CBS H-19746, culture CPC 13052 = CBS 120084, CPC 13053-13-54.

Notes: *Elsinoë eucalypti* Hansf., which was described from a *Eucalyptus* sp. on Kangaroo Island, differs from *E. eucalyptorum* by having amphigenous leaf spots, larger asci (up to 45 × 28 µm), and larger ascospores (20–28 × 7–8 µm) (Park *et al*., 2000). BLASTn results of the ITS sequence of this species (GenBank DQ923530) had an E-value of 2e-139 with the ITS sequence of *Elsinoë australis* U28057 (91% identical; Dothideomycetes, *Elsinoaceae*) and *Elsinoë fawcettii* AF097577.

Fusculina Crous & Summerell, **gen. nov.** MycoBank MB510010.

Etymology: *Fuscu-* from *Fusicoccum*, *-lina* from *Disculina*, indicating its morphological position between these two genera.

Fusicocco et Disculinae simile genus, sed conidiomatibus pycnidialibus, conidia subcylindrica proliferatione percurrente et conidia ellipsoidea holoblastica proliferatione sympodiali formans.

Fusculina eucalypti Crous & Summerell, **sp. nov.** Fig. 6
MycoBank MB510011

Etymology: Named after its host genus, *Eucalyptus*.

Conidiomata pycnidialia, ad 250 µm diam. Cellulae conidiogenae primariae hyalinae, leves, ampulliformes vel subcylindricae, percurrenter proliferentes, 5–15 × 4–8 µm; conidia

Fig. 6. *Fusculina eucalypti* (CBS H-19747). **A.** Leaf spot. **B.** Conidiomata in agar. **C, D.** Chlamydospores. **E, F.** Conidiogenous cells. **G, H.** Primary and secondary conidia. Scale bars: B = 250 µm, C = 10 µm.

primaria hyalina, levia, subcylindrica, sursum obtusa, deorsum truncata, (16–)18–20(–22) × 3–4 µm. Cellulae conidiogenae secundariae holoblasticae, sympodialiter proliferentes, 5–10 × 3–4 µ; conida secundaria hyalina, levia, ellipsoidea vel subcylindrica, 5–8 × 3–4 µm.

Leaf spots amphigenous, circular, medium brown with a raised border, 2–6 mm diam. *Mycelium* predominantly internal, but external hyphae brown, thick-walled, 3–5 µm wide, rough, giving rise to chlamydospore-like cells; conidia exuding in a hyaline mass; two conidial types other than chlamydospores present on host material as well as in culture. *Conidiomata* amphigenous, black on leaves, pycnidial, immersed, becoming slightly

erumpent, up to 250 μm diam, brown under light microscope, but upper region appearing crust-like, dark-brown; opening by irregular rupture; wall consisting of 2–4 layers of brown cells of *textura angularis* that give rise to 2–4 layers of hyaline cells. *Conidiomata* forming on OA in culture, exuding creamy masses of conidia, with visible strands of dark-brown, thick-walled chlamydospore-like cells formed intercalary in aerial mycelium; upper, outer region of conidiomata crust-like, dark-brown, distinct from brown, smooth conidiomatal body; conidiomata opening by irregular rupture; apical region containing long, subcylindrical, hyaline, septate periphysoids, up to 50 μm long, 2–3 μm wide, extending half way down the conidioma, where they are replaced by conidiogenous cells of two types. *Primary conidiogenous cells* hyaline, smooth, ampulliform to subcylindrical, proliferating several times percurrently near apex, 5–15 × 4–8 μm, giving rise to subcylindrical primary conidia. *Primary conidia* hyaline, granular, smooth, straight to somewhat irregular, subcylindrical, apex obtuse, base truncate, (16–)18–20(–22) × 3–4 μm. *Secondary conidiogenous cells* integrated among primary conidiogenous cells, hyaline, smooth, proliferating sympodially, 5–10 μm long and 3–4 μm wide. *Secondary conidia* hyaline, smooth, granular, ellipsoid with rounded ends, or subcylindrical with obtuse apex and truncate base, 5–8 × 3–4 μm.

Cultural characteristics: Colonies on MEA slow-growing, reaching 8 mm diam after 2 mo at 25°C; colonies erumpent, with no to sparse aerial mycelium; margins irregular, feathery; colonies cream (surface), dark brick (reverse); on PDA also slow-growing, with white aerial mycelium, reaching 5 mm diam after 2 wks; on OA similar to PDA, but fertile, sporulating after 2 wks.

Specimen examined: **Australia**, New South Wales, Wollemi National Park, on leaves of *Eucalyptus* sp., Jan. 2006, B. Summerell, CBS H-19747, **holotype**, culture ex-type CPC 12946 = CBS 120083, CPC 12987-12988, GenBank DQ923531.

Notes: *Fusculina* is somewhat reminiscent of species in the *Fusicoccum* complex, having hyaline, subcylindrical to somewhat fusoid conidia produced on percurrently proliferating conidiogenous cells. The genus is also similar to *Disculina*, which has hyaline, aseptate conidia arising from percurrent proliferating conidiogenous cells. It is distinct, however, in forming two conidial types, and in having pycnidia, while the other genera discussed have stromatic conidiomata. Based on its ITS sequence, it is not allied to either genus, and therefore a new name is introduced. BLASTn results of the ITS sequence of this species (GenBank DQ923531) had an E-value of 3e-80 with the ITS sequence of a *Berkleasmium* sp. DQ280266 (*Pleosporales*) and *Lophiostoma caulium* AF383953 (1e-73; *Pleosporales*, *Lophiostomataceae*).

Harknessia rhabdosphaera Crous & Summerell, **sp. nov.** Fig. 7
MycoBank 510017

Etymology: rhabdos = stripe in Greek.

Harknessiae globosae similis, sed conidiis striatis (13–)15–17 × (13–)14–15 µm; appendice basilari ad 5 × 2–3 µm.

Leaf spots angular, confined by leaf veins, amphigenous, medium brown with a raised, dark brown border, 2–5 mm diam; primarily associated with *Phaeophleospora* sp. *Conidiomata* sparse on leaf, globose, immersed, becoming erumpent, opening by irregular rupture, up to 300 µm diam. *Conidiogenous cells* lining the inner layer of the cavity, ampulliform to subcylindrical, 0–1-septate, proliferating several times percurrently near the apex, 7–15 × 4–6 µm. *Conidia* globose to somewhat irregular, thick-walled, granular, not guttulate, with longitudinal striations covering the length of the conidium; base subtruncate, 2–3 µm wide, with a visible scar if basal appendage is detached, (13–)15–17 × (13–)14–15 µm; basal appendage hyaline, tubular, mostly absent on mature conidia, present on young, developing conidia, up to 5 µm long, 2–3 µm wide.

Cultural characteristics: *Colonies* on MEA spreading, fluffy, with a dirty cream aerial mycelium; surface buff to honey; reverse cinnamon with dark brick outer margins; cultures fertile, sporulating in black masses; no fruity odour detected, which is typical of many *Harknessia* species.

Specimen examined: **Australia**, New South Wales, on leaves of *Corymbia henryi* (S.T. Blake) K.D. Hill & L.A.S. Johnson (= *Eucalyptus henryi*), Grafton-Nymboida Road, 1.6 km north of junction with Orara Way, North Coast NSW, 29 44 56 S 152 55 59 E, Alt: 65 metres; gently undulating country, mostly cleared for agriculture/residential (acreages); remnant trees of *Corymbia henryi*, *Eucalyptus siderophloia*, *E. fibrosa*; red-brown clay loam over shale; locally dominant tree 30 m × 20 m, Mar. 2006, B. Summerell, CBS H-19748, **holotype**, culture ex-type CPC 12922 = CBS 120082, CPC 12923-12924, GenBank DQ923532.

Notes: Presently there are no species of *Harknessia* that have globose, striate conidia and short, basal appendages of these dimensions (Nag Raj 1993, Lee *et al*. 2004). BLASTn results of the ITS sequence of *H. rhabdosphaera* (GenBank DQ923532) had an E-value of 0.0 with the ITS sequence of *H. syzygii* AY720738 (99% identical; Diaporthales), *H. uromycoides* AY720739 (98%), *H. eucalyptorum* AY720747 (98%) and *H. karwarrae* AY720748 (98%).

Neofusicoccum corticosae Crous & Summerell, **sp. nov.** Fig. 8
MycoBank MB510012

Synanamorph: *Dichomera* sp.

Etymology: Named after its host plant, *Eucalyptus corticosa*.

Dichomerae eucalypti simile, sed conidiis maioribus, (10–)13–14(–15) × (9–)10(–11) µm.

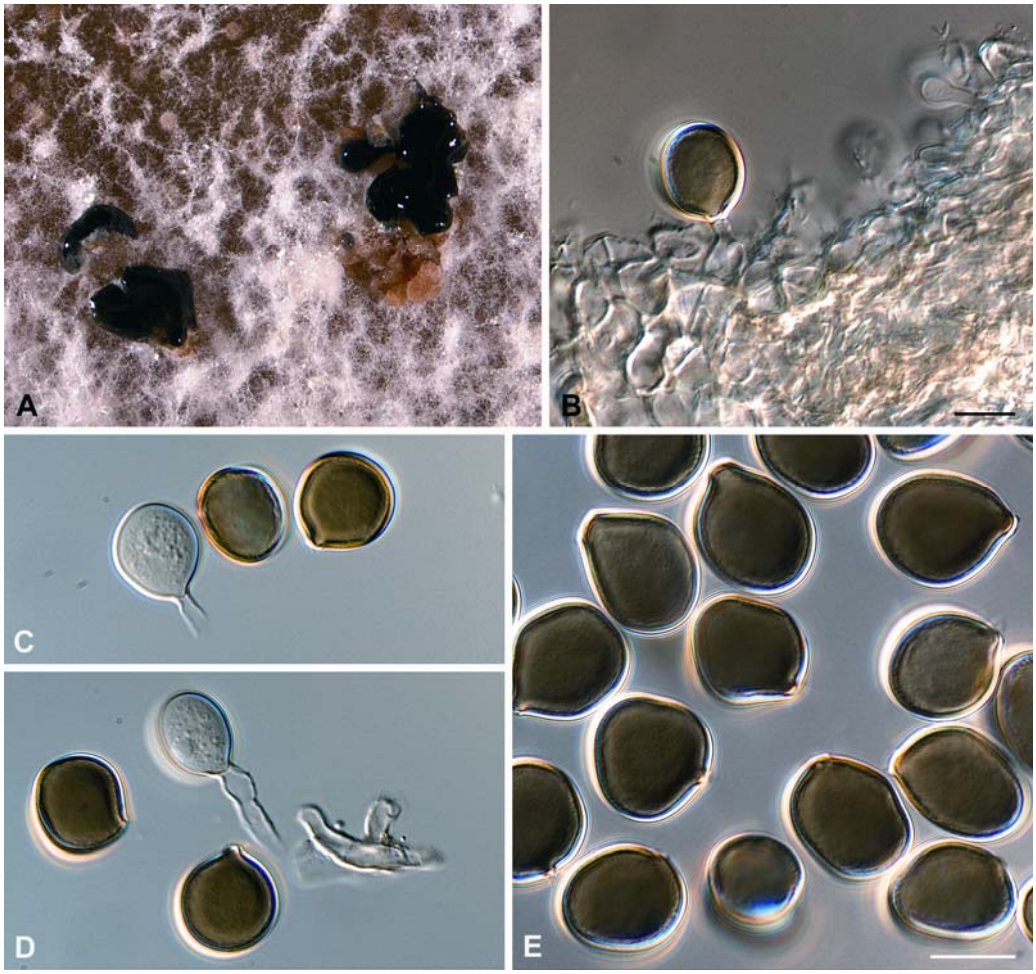

Fig. 7. *Harknessia rhabdosphaera* (CBS H-19748). **A.** Conidiomata sporulating on MEA. **B.** Conidium with conidiogenous cell. **C–E.** Developing conidia attached to conidiogenous cells, and mature conidia without appendages. Scale bars = 10 µm.

Leaf spots brown, amphigenous, irregular, raised, 2–4 mm diam, with a thin, red-purple to chlorotic margin. *Conidiomata* amphigenous, pycnidioid, stromatic, brown, up to 250 µm diam, ostiolate, or opening via irregular rupture in apex; wall of 3–4 layers of brown *textura angularis*. *Conidiogenous cells* lining the inner layer of the conidioma, phialidic, hyaline, smooth, ampulliform to subcylindrical, 10–15 × 4–8 µm; proliferating once or twice percurrently, or with minute periclinal thickening. *Conidia* hyaline, smooth, thin-walled, granular, ellipsoidal, apex obtuse, base subtruncate, somewhat flattened, at times with minute marginal frill, (8–)15–17(–18) × (7–)8 µm. *In vitro*

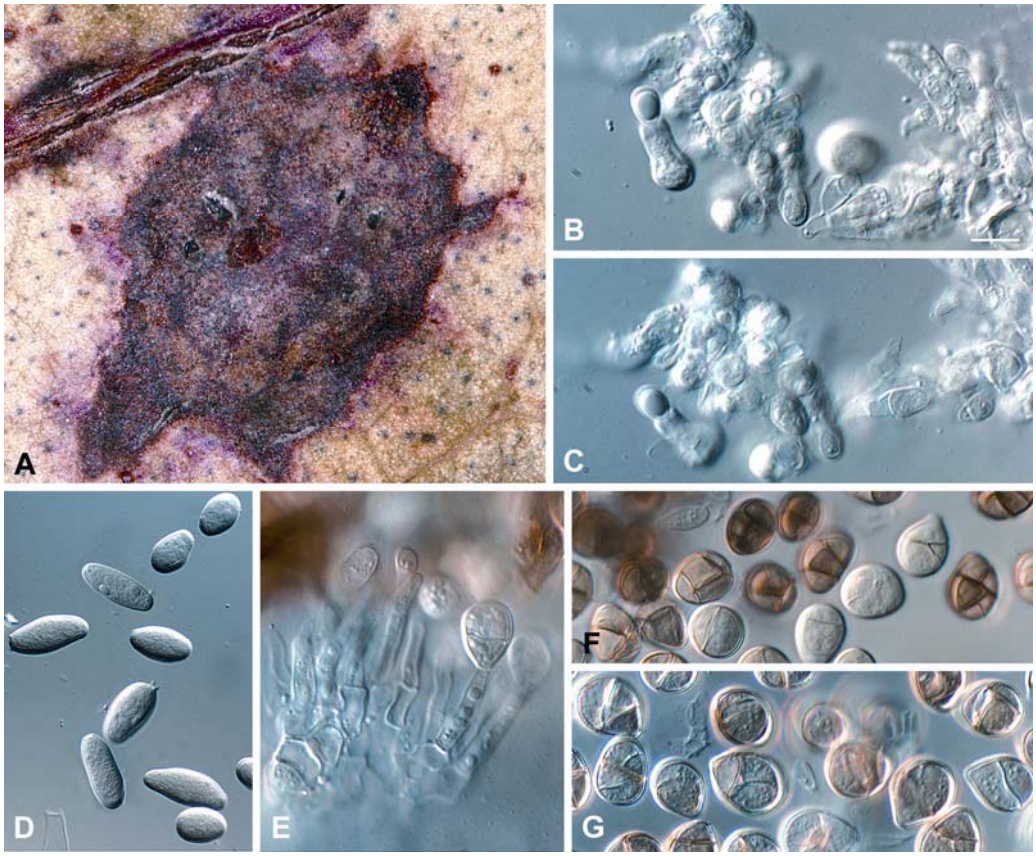

Fig. 8. *Neofusicoccum corticosae* (CBS H-19745). **A.** Leaf spot. **B, C.** Conidiogenous cells giving rise to *Neofusococcum* conidia *in vivo*. **D.** *Neofusicoccum* conidia *in vivo*. **E.** Conidiogenous cells giving rise to *Dichomera* conidia *in vitro*. **F, G.** *Dichomera* conidia *in vitro*. Scale bars = 10 µm.

producing brown *Dichomera* conidiomata up to 200 µm diam. *Conidiogenous cells* hyaline, smooth, subcylindrical, 10–30 × 3–4 µm, proliferating once or twice percurrently, or with minute apical periclinal thickening. *Conidia* globose, obpyriform or obovoid, brown at maturity, smooth, thin-walled, with 1–2 transverse and 1–3 vertical or oblique septa, (10–)13–14(–15) × (9–)10(–11) µm.

Cultural characteristics: Colonies on MEA fluffy, spreading, iron-grey, with abundant aerial mycelium; fertile on MEA and on WA with pine needles, forming the *Dichomera* state in culture.

Specimen examined: **Australia**, New South Wales, Wollemi National Park, on leaves of *Eucalyptus corticosa* L.A.S. Johnson, 3.5 km E of final turnoff to Dunn's Swamp (ca. 5 km E of Currant Mountain Gap), Wollemi National Park, Central Tablelands NSW, 32 51 28 S 150

14 11 E. Alt: 740 metres, gentle NE sloping gully; open forest with *Eucalyptus rossii*, *E. parramattensis*, *E. corticosa*; *understorey with Leucopogon muticus*, *Persoonia myrtilloides*, *Patersonia sp*., *Grevillea evansiana*, *Acacia buxifolia*, *A. caesiella*, *Pteridium* sp., *Lomandra longifolia*, etc.; deep white sand over sandstone; locally frequent tree, Feb. 2006, B. Summerell, CBS H-19745, culture CPC 12925 = CBS 120081, CPC 12926–12927.

Notes: Using the key of Barber *et al*. (2005), *Neofusicoccum corticosae* is closest to *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips and *N. ribis* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips. However, morphologically its *Neofusicoccum* state has smaller conidia. In ITS sequence data it is closest to *D. eucalypti* (G. Winter) B. Sutton, though its *Dichomera* conidia are larger, and its *Neofusicoccum* conidia narrower than those of *D. eucalypti*. BLASTn results of the ITS sequence of this species (GenBank DQ923533) had an E-value of 0.0 with the ITS sequence of *N. arbuti* AY819725 (99% identical, *Botryosphaeriaceae*; but *Neofusicoccum* conidia do not overlap in size, being 16.8–30.5 × 4.6–7.8 µm; Farr *et al*. 2005), *Botryosphaeria dothidea* AY662403 (98%), *N. parva* AY228103 (98%) and *N. vitifusiforme* AY343383 (99%). The ITS sequence of this species is 98% identical to *Dichomera eucalypti* AY744373.

Phlogicylindrium Crous, Summerb. & Summerell, **gen. nov.** MycoBank MB510013.

Etymology: Named after its cylindrical conidia, and characteristic flame-like conidiomata.

Conidiomata erecta, massa mucida conidiorum hyalinorum. Cellulae conidiogenae hyalinae vel brunneae, leves, ampulliformes, e collo elongato percurrenter proliferentes. Conidia hyalina, cylindrica, sursum obtusa, basi subtruncata, 0–1-septata.

Phlogicylindrium eucalypti Crous, Summerb. & Summerell, **sp. nov.** MycoBank MB510014 Fig. 9

Etymology: Named after its host plant, *Eucalyptus*.

Conidiomata erecta, massa mucida conidiorum hyalinorum, ad 200 µm diam et 300 µm alta. Cellulae conidiogenae hyalinae vel brunneae, leves, ampulliformes, collo elongato percurrenter proliferente, 15–30 × 3–4 µm. Conidia hyalina, cylindrica, sursum obtusa, basi subtruncata, 0–1-septata, 26–35 × 2–3 µm (in vivo).

Occurring on lesions of living leaves in association with *Mycosphaerella* spp. *Conidiomata* visible as slimy, erect tufts of hyaline conidial masses, resembling candle flames, up to 200 µm wide and 300 µm tall, synnematous, indeterminate; conidiomata gradually turn brown with age due to the mucus binding the conidial mass turning brown. *Conidiophores* consisting of an intricate network of brown, smooth, branched cells, 3–6 µm wide, constricted at septa, with irregular patches of dark brown pigment within certain zones of older cells, appearing thick-walled in these areas; becoming uniformly medium brown and smooth towards the conidiogenous region. *Conidiogenous cells*

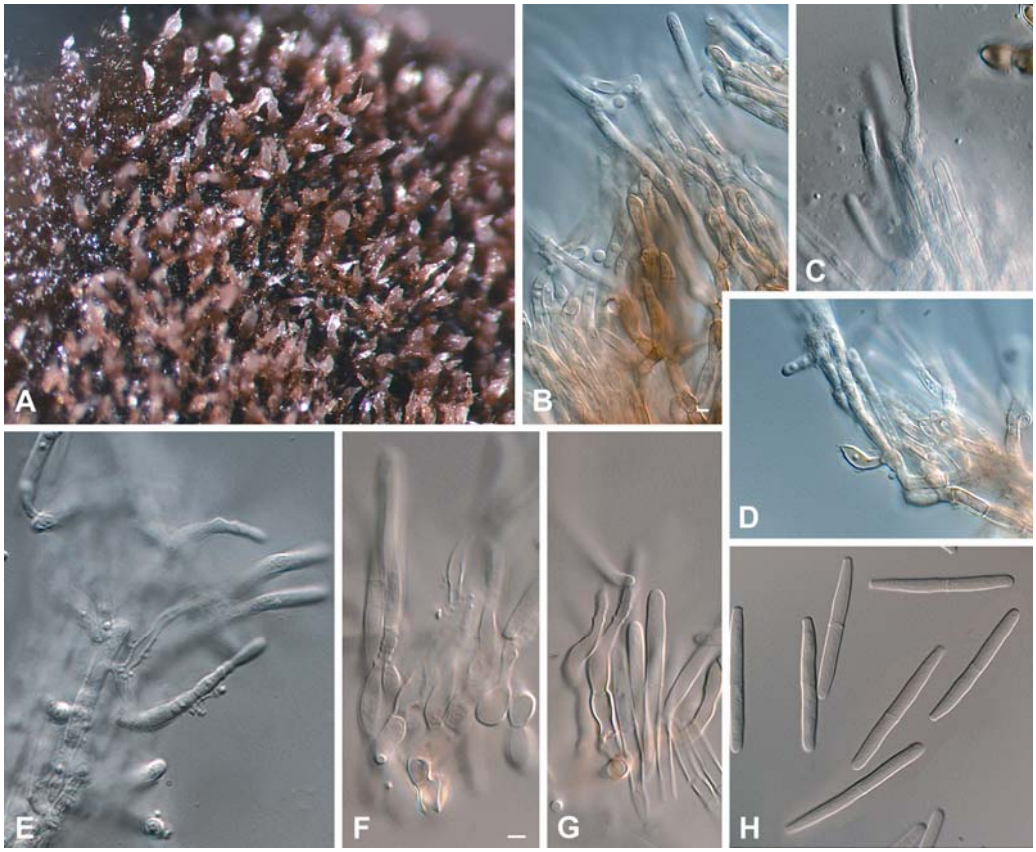

Fig. 9. *Phlogicylindrium eucalypti* (CBS H-19749). **A.** Colony sporulating on MEA. **B–E.** Conidiophores and conidiogenous cells *in vitro*. **F–H.** Conidiogenous cells (note percurrent proliferation) and conidia *in vivo*. Scale bars = 10 µm.

hyaline, smooth, becoming pale brown with age, ampulliform with elongated necks on which percurrent proliferations are clearly visible; in culture the basal part of the conidiogenous cell can become elongated with irregular swellings, indicative of further growth, 15–30 × 3–4 µm. *Conidia* formed apically on conidiogenous cells, though in culture some proliferations are somewhat irregular, and can create the impression of sympodial development; conidia forming along the sides and apex of the conidioma; conidia hyaline, cylindrical with obtusely rounded apex, aseptate to 1-septate, lower cell tapering slightly to a somewhat long, obconically subtruncate base; predominantly straight, regular in width, guttulate, (23–)26–30(–35) × (2–)3 µm in culture; (26–)28–33(–35) × (2–)3 µm in vivo; conidia anastomosing while still in aggregated in mucus on the conidiophore.

Cultural characteristics: Colonies on MEA slow-growing, reaching 8 mm diam after 2 months at 25°C; aerial mycelium sparse, colonies erumpent; margins irregular, feathery; colonies on MEA cinnamon (surface), sepia (reverse); on oatmeal agar brown-vinaceous. Colonies sterile on MEA, but fertile on OA.

Specimen examined: **Australia**, Victoria, on leaves of *Eucalyptus globulus*, Sept. 2005, I. Smith, CBS H-19749, **holotype**, culture ex-type CPC 12409 = CBS 120080, CPC 12410–12411, GenBank DQ923534.

Notes: As far as we could establish, there is presently no genus available to accommodate this fungus, characterised by erect flame-like conidiomatal tufts, and cylindrical, hyaline conidia forming on brown, percurrently proliferating conidiogenous cells. BLASTn results of the ITS sequence of this species (GenBank DQ923534) had an E-value of 2e-154 with the ITS sequence of an uncultured ascomycete AY969434. Similarities with known species include *Seiridium unicorne* AF377299 (3e-116; Xylariales; Amphisphaeriaceae) and species of *Pestalotiopsis*, for example *Pestalotiopsis gracilis* AY687312 (2e-114; Xylariales; Amphisphaeriaceae).

Readeriella eucalypti (Gonz. Frag.) Crous, **comb. nov.** Fig. 10

MycoBank MB510018.

≡ *Coniothyrium olivaceum* Bonord. var. *eucalypti* Gonz. Frag., Bolm Soc. Broteriana, Sér. 2 2: 59. 1924. 1923.

≡ *Microsphaeropsis eucalypti* (Gonz. Frag.) B. Sutton, Mycol. Pap. 123: 37. 1971.

In vivo: Associated with brown, subcircular leaf spots on living leaves. *Pycnidia* up to 100 µm diam, hypophyllous, scattered, dark-brown to black, *erumpent*; wall consisting of 3–6 layers of dark-brown *textura angularis*. *Conidiogenous cells* mono- to polyphialidic, or indeterminate, proliferating percurrently near the apex, dolliform to ampulliform to cylindrical, hyaline, smooth, 4–9 × 3–5 µm. *Conidia* globose, subglobose to pyriform, base truncate, thick-walled, dark-brown, smooth-walled, 6–7 × 5–6 µm; in older cultures conidia are frequently more pyriform in shape (onset of germination?), and also vary from being smooth to rough.

Cultural characteristics: Colonies on MEA spreading, with sparse to moderate aerial mycelium, and smooth, feathery margins; reaching 6 cm diam after 1 month at 25°C; surface hazel to fucous-black, reverse fucous-black; colonies fertile.

Specimens examined: **Portugal**, Taboaco, *E. globulus*, Nov. 1922, IMI 151356, **holotype**. **Spain**, *Eucalyptus globulus*, Apr. 2004, M.J. Wingfield, CBS H-19741, **epitype designated here**, cultures ex-epitype CPC 11184 = CBS 120079, CPC 11185–11186; *E. globulus*, 2004, J.P.M. Vazquez, 13/11 sample 4, CPC 11735–11737.

Notes: In his treatment of the genus *Readeriella* Syd. & P. Syd., Sutton (1971) stated that conidiogenous cells appeared to be monophialidic, but were

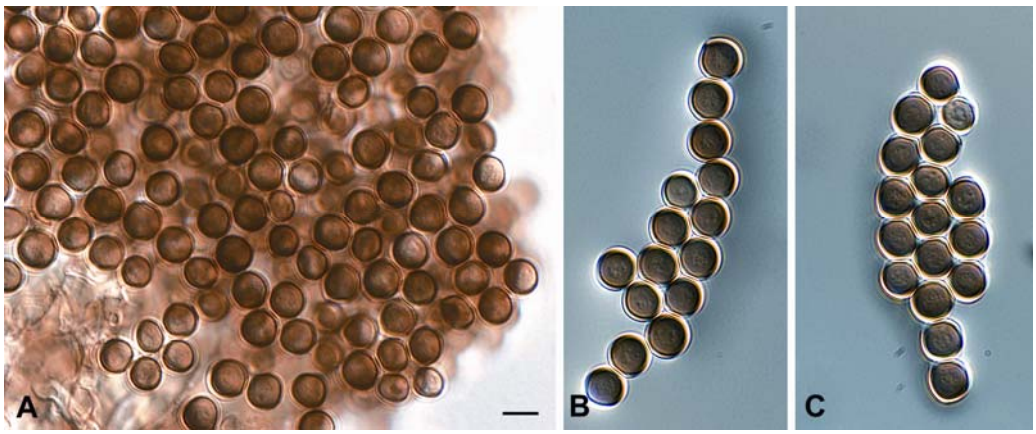

Fig. 10. *Readeriella eucalypti* (CBS H-19741). **A–C.** Conidia *in vitro*. Scale bar = 10 µm.

frequently also polyphialidic, and even annellidic. This observation was recently confirmed by Crous *et al*. (2004a) for the type species, *R. mirabilis* Syd. & P. Syd. In his treatment of *Microsphaeropsis eucalypti* (Gonz. Frag.) B. Sutton, however, Sutton (1971) did not observe any annellations, though he mentions and illustrates polyphialides. In recent collections and cultures obtained of this species, we observed it also to proliferate via annellides. BLASTn results of the ITS sequence of this species (GenBank DQ303092 and DQ303093) had an E-value of 0.0 with the ITS sequence of *Mycosphaerella readeriellophora* AY725577 (99% identical, *Mycosphaerellaceae*; though clearly distinct in conidial shape and size; Crous *et al*. 2004a), *R. novaezelandiae* AY725578 (97%) and *R. mirabilis* AY725529 (98%). This species clusters in the *Readeriella* clade within *Mycosphaerella* based on its ITS sequence. A new name is therefore proposed for this fungus in *Readeriella*.

Staninwardia suttonii Crous & Summerell, **sp. nov.** Fig. 11
MycoBank MB510016.

Etymology: Named after B. Sutton, a mycologist from the International Mycological Institute, who described the genus.

Staninwardiae breviusculae similis, sed conidiis maioribus, (8–)10–14(–17) × (3–)4(–5) µm.

Leaf spots amphigenous, dark brown to black, circular, 2–5 mm diam, rarely extending through the leaf lamina, margins diffuse, slightly chlorotic. *Conidiomata* amphigenous, black, erumpent acervuli up to 400 µm diam; basal stroma consisting of dense, aggregated hyaline cells giving rise to conidiophores. *Conidiophores* hyaline, cylindrical, densely aggregated, covered in mucous, 0–2-septate, unbranched or branched below, 6–16 × 3–4 µm.

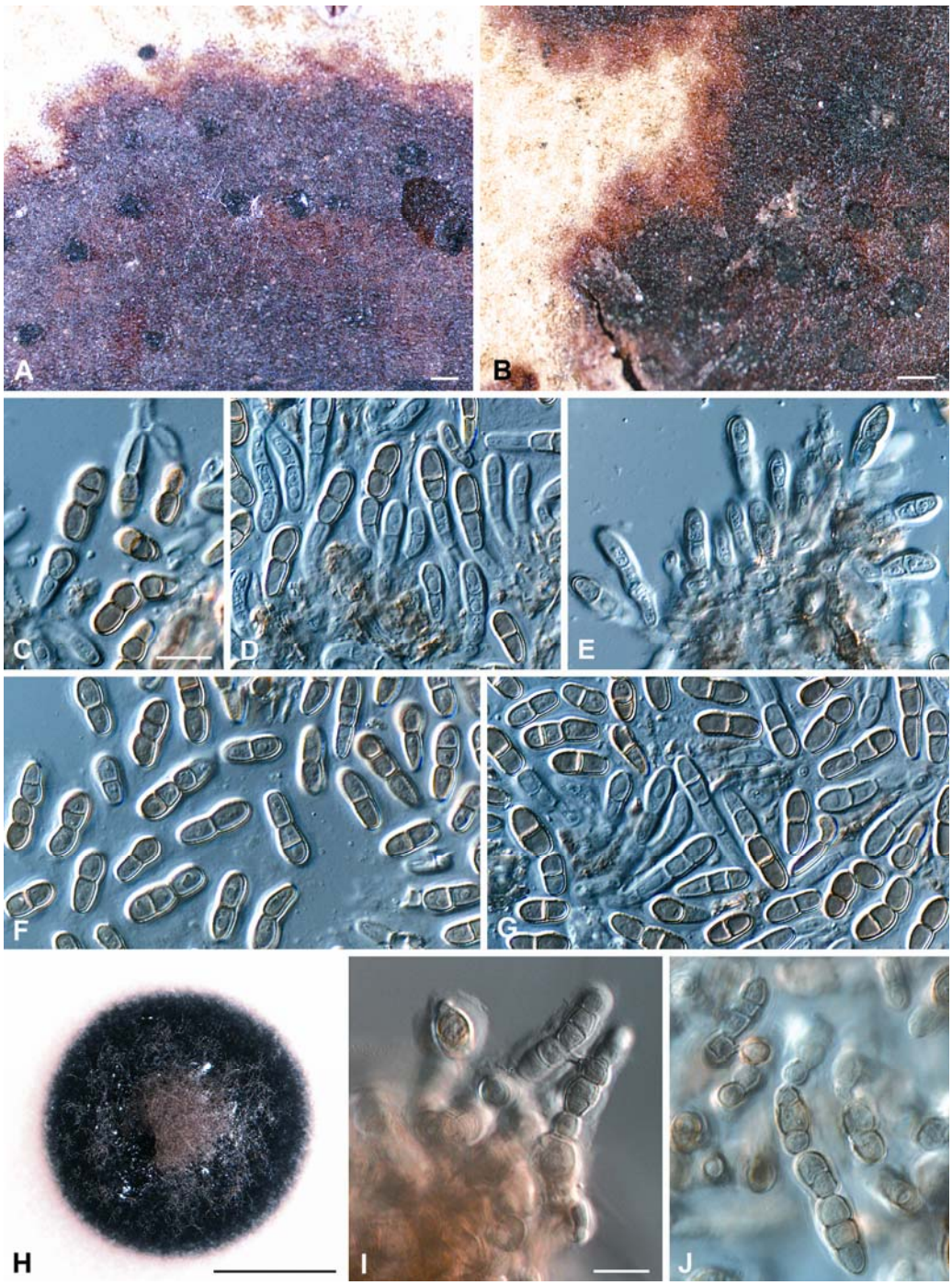
A
B
C
D
E
F
G
H
I
J

Fig. 11. *Staninwardia suttonii* (CBS H-19750). **A, B.** Leaf spots with conidiomata. **C–G.** Conidiophores giving rise to pigmented, septate conidia. **H.** Colony on MEA**. I, J.** Conidiogenous cells giving rise to conidia *in vitro* (note persistent mucilaginous sheath). Scale bars: A, B = 400 µm, C = 10 µm, H = 4 mm, I = 10 µm.

Conidiogenous cells integrated, terminal, hyaline, smooth, covered in mucus, proliferating several times percurrently near apex, 3–8 × 3–4 µm. *Conidia* aggregated in mucus, fusoid-ellipsoidal with rounded apex and truncate base, brown, verruculose, predominantly 1-septate, but each cell can develop an additional septum with age (though the first is observed in the apical cell), constricted at septa, (8–)10–14(–17) × (3–)4(–5) µm, basal cell shorter and narrower (4–)5–7 × 3–4 µm than the apical cell (4–)5–8 × (3–)4–5 µm; conidia remain covered in a mucilaginous sheath (up to 4 µm wide), and also form short conidial chains. In culture conidial chains are more prominent, and conidia can become up to 20 µm long and 7 µm wide.

Cultural characteristics: Colonies extremely slow-growing, black, reaching 2 mm diam on MEA after 2 months at 25°C.

Specimen examined: **Australia**, New South Wales, on leaves of *Eucalyptus robusta* Sm., 1.4 km along Lakes Way from intersection with Green Point Drive, then ca. 500 m NW from Lakes Way along dirt access road, ca. 10 km S of Forster, Booti Booti National Park, North Coast NSW, 32 15 45 S 152 31 33 E, Alt: 5 metres; low-medium height coastal heath (wallum) on deep pale grey sand; with stunted emergents of *Melaleuca quinquenervia*, *Angophora costata* and *Eucalyptus robusta*. Shrubs consists of *Banksia aemula*, *Dillwynia retorta*, *Leptospermum laevigatum*, *L. polygalifolium*, *Eriostemon australasius*, *Boronia pinnata*, *B. falcifolia*, *Persoonia katerae*, etc.; small stunted trees, in scattered clumps, to ca. 4 m tall and 4 m wide, Mar. 2006, collected by B. Summerell, CBS H-19750, **holotype**, culture ex-type CPC 13055 = CBS 120061, CPC 13056–13057, GenBank DQ923535.

***Notes*:** The genus *Staninwardia* has hitherto proven to be monotypic, with *S. breviuscula* B. Sutton known from India, Mauritius and Sri Lanka (Sutton 1980). The genus is very characteristic, forming acervuli on brown leaf spots, with brown, catenulate conidia covered in a mucilaginous sheath. *Staninwardia suttoniae* differs from *S. breviuscula* in both size and development of conidia. Its conidia are larger than those of *S. breviuscula*, and develop more septa than the single septum seen in that species.

In the present study it proved extremely difficult to cultivate *S. suttonii*, but conidia were eventually induced to germinate on PDA, yielding very slow-growing colonies that sporulated on OA after 2 months. BLASTn results of the ITS sequence of *Staninwardia suttonii* (GenBank DQ923535) had an E-value of 2e-108 with the ITS sequence of a melanized limestone ascomycete AY559345 (94% identical over the 5.8s rDNA). Similarities with known species include *Hortaea werneckii* AY820141 (2e-102; Dothideales), *Mycosphaerella bellula*

AY260092 (2e-102; Mycosphaerellaceae), a *Capnobotryella* sp. AJ971406 (2e-102) and *Taeniolella stilbospora* AY843127 (5e-100; *Botryosphaeriaceae*).

Newly cultured species and new records

Mycosphaerella swartii R.F. Park & Keane, Trans. Brit. Mycol. Soc. 83: 99. 1984.

Anamorph: *Sonderhenia eucalyptorum* (Hansf.) H.J. Swart & J. Walker, Trans. Brit. Mycol. Soc. 90: 640. 1988.

≡ *Hendersonia eucalyptorum* Hansf., Proc. Linn. Soc. N. S. W. 79: 135. 1954.

Specimen examined: **Australia**, Tasmania, on leaves of *Eucalyptus coccifera*, Jan. 2006, C. Mohammed, CPC 12553 = CBS 120220, CPC 12554–12555, GenBank DQ923536.

Notes: Although this species is known to occur on *E. coccifera* in Australia (Crous, 1998), and has been cultured in previous studies (Park and Keane, 1984), this to our knowledge is the first isolate subjected to sequence analysis. As predicted by Swart and Walker (1988), it is closely related to *Sonderhenia eucalypticola* (A.R. Davis) H.J. Swart & J. Walker. BLASTn results of the ITS sequence of this species (GenBank DQ923536) had an E-value of 0.0 with the ITS sequence of *Mycosphaerella walkeri* AF309616 (99% identical; *Mycosphaerellaceae*), *M. colombiensis* AF309612 (95%) and *M. irregulariramosa* AF468878 (94%).

Cytospora diatrypelloidea G.C. Adams & M.J. Wingf., Stud. Mycol 52: 121. 2005.

Specimen examined: **Australia**, Western Australia, Perth, King's Park, on leaves of *Eucalyptus* sp., 25 Sept. 2005, A. van Iperen, CPC 12453 = CBS 120062, CPC 12454, GenBank DQ923537.

Notes: Adams *et al*. (2005) described this fungus from dead branches of *E. globulus* in Victoria. This is the first record of C. *diatrypelloidea* from Western Australia, where it was found to occur on leaf litter of a *Eucalyptus* sp. BLASTn results of the ITS sequence of this species (GenBank DQ923537) had an E-value of 0.0 with the ITS sequence of *C. diatrypelloidea* AY347368 (99% identical; *Diaporthales*, *Valsaceae*), *Valsa cinereostroma* AF260267 (98%), *C. austromontana* AY347362 (98%), *V. fabianae* AY347373 (98%) and *C. berkeleyi* AY347351 (98%).

Plectosphaera eucalypti (Cooke & Massee) H.J. Swart, Trans. Br. Mycol. Soc. 76: 91. 1981.

≡ *Trabutia eucalypti* Cooke & Massee, Grevillea 17: 42. 1888.

≡ *Phyllachora eucalypti* (Cooke & Massee) Theiss. & Syd., Annls Mycol. 13: 539. 1915.

≡ *Placostroma eucalypti* (Cooke & Massee) Hansf., Proc. Linn. Soc. N. S. W. 81: 29. 1956.

? *Phyllachora maculata* Cooke, Grevillea 20(93): 4. 1891.

Cultural characteristics: Colonies on MEA spreading, flat, with sparse aerial mycelium; margins smooth, catenate, submerged; surface cinnamon, outer zone with patches of smoke grey due to black ascomatal development in the outer region of the colony; reverse cinnamon with patches of olivaceous-grey; colonies reaching 3 cm diam after 2 mo on MEA at 25°C.

Specimen examined: **Australia**, Olive Pink Botanic Gardens, Alice Springs, Northern Territory, on leaves of *Eucalyptus oblifolia*, Oct. 2005, B. Summerell, CBS H-19751, culture CPC 12955 = CBS 120063, CPC 12956, GenBank DQ923538.

Notes: As far as we could establish, this is the first time that this fungus has been cultured. Pascoe (1990) reported on the pseudoclypeus and apparent bitunicate nature of its asci, and speculated about its correct classification. BLASTn results of the ITS sequence of this species (GenBank DQ923538) had an E-value of 1e-122 with the ITS sequence of *Pestalotiopsis microspora* AF377291 (Xylariales; Amphisphaeriaceae) and an E-value of 7e-121 with other species of *Pestalotiopsis*, for example *Pestalotiopsis gracilis* AY687312. Based on ITS sequence data, *P. eucalypti* thus appears to be allied to the *Amphisphaeriaceae*.

Valsa fabianae G.C. Adams, M.J. Wingf. & Jol. Roux, Stud. Mycol. 52: 85. 2005.

Anamorph: *Cytospora eucalypticola* van der Westh., S. African For. J. 54: 8. 1965, *emend*. G.C. Adams & M.J. Wingf., Stud. Mycol. 52: 85. 2005.

Specimen examined: **Australia**, Tasmania, on leaves of *Eucalyptus nitens* H. Deane & Maiden, 9 Nov. 2005, C. Mohammed, CPC 12524 = CBS 120064, CPC 12525–12526, GenBank DQ923539.

Notes: *Valsa fabiana* was recently described from cankered branches of *E. nitens* collected in Tasmania (Adams *et al*., 2005). This is, however, the first report associating this fungus with leaf spots. BLASTn results of the ITS sequence of this species (GenBank DQ923539) confirms the identity of this species with 100% similarity to the ITS sequence of *Valsa fabianae* AY347358.

Acknowledgements

We are grateful to several technicians who have assisted with this project, namely A. van Iperen (cultures), M. Vermaas (photo plates), and M. Starink (DNA sequencing). This study would not have been possible without the numerous specimens placed at our disposal, for which we are eternally grateful to Drs I. Smith, M.J. Wingfield, J.P.M. Vazquez, C. Mohammed and I. van Iperen.

References

Adams, G.C., Wingfield, M.J., Common, R. and Roux, J. (2005). Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (*Ascomycota*, *Diaporthales*, *Valsaceae*) from *Eucalyptus*. Studies in Mycology 52: 1-147.

Barber PA, Burgess T, Hardy G St J, Slippers B, Keane PJ and Wingfield MJ (2005). *Botryosphaeria* species from *Eucalyptus* in Australia are pleoanamorphic, producing *Dichomera* synanamorphs in culture. Mycological Research 109: 1347-1363.

Beer, Z.W. de, Begerow, D., Bauer, R., Pegg, G.S., Crous, P.W. and Wingfield, M.J. (2006). Phylogeny of the *Quambalariaceae* fam. nov., including important *Eucalyptus* pathogens in South Africa and Australia. Studies in Mycology 55: 289-298.

Cortinas, M.-N., Crous, P.W., Wingfield, B.D. and Wingfield, M.J. (2006). Multi-gene phylogenies and phenotypic characters distinguish two species within the *Colletogloeopsis zuluensis* complex associated with *Eucalyptus* stem cankers. Studies in Mycology 55: 133-146.

Crous, P.W. (1998). *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. Mycologia Memoir 21: 1-170.

Crous, P.W. (2002). *Taxonomy and pathology of Cylindrocladium (Calonectria) and allied genera*. APS Press.

Crous, P.W., Aptroot, A., Kang, J.-C., Braun, U. and Wingfield, M.J. (2000). The genus *Mycosphaerella* and its anamorphs. Studies in Mycology 45: 107-121.

Crous, P.W., Groenewald, J.Z., Mansilla, J.P., Hunter, G.C. and Wingfield, M.J. (2004a). Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. Studies in Mycology 50: 195-214.

Crous, P.W., Groenewald, J.Z., Pongpanich, K., Himaman, W., Arzanlou, M. and Wingfield, M.J. (2004b). Cryptic speciation and host specificity among *Mycosphaerella* spp. occurring on Australian *Acacia* species grown as exotics in the tropics. Studies in Mycology 50: 457-469.

Crous, P.W., Groenewald, J.Z., Risède, J.-M., Simoneau, P. and Hywel-Jones, N.L. (2004c). *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. Studies in Mycology 50: 415-430.

Crous, P.W., Groenewald, J.Z., Risède, J.-M., Simoneau, P. and Hyde, K.D. (2006a). *Calonectria* species and their *Cylindrocladium* anamorphs: species with clavate vesicles. Studies in Mycology 55: 213-226.

Crous, P.W., Kang, J.-C. and Braun, U. (2001). A phylogenetic redefinition of anamorph genera in *Mycosphaerella* based on ITS rDNA sequence and morphology. Mycologia 93: 1081-1101.

Crous, P.W., Rong, I.H., Wood, A., Lee, S., Glen, H., Botha, W., Slippers, B., de Beer, W.Z., Wingfield, M.J. and Hawksworth, D.L. (2006b). How many species of fungi are there at the tip of Africa? Studies in Mycology 55: 13-33.

Crous, P.W., Slippers, B., Wingfield, M.J., Rheeder, J., Marasas, W.F.O., Phillips, A.J.L., Alves, A., Burgess, T., Barber, P. and Groenewald, J.Z. (2006c). Phylogenetic lineages in the *Botryosphaeriaceae*. Studies in Mycology 55: 235-253.

Crous, P.W., Verkley, G.J.M. and Groenewald, J.Z. (2006d). *Eucalyptus* microfungi known from culture. 1. *Cladoriella* and *Fulvoflamma* genera nova, with notes on some other poorly known taxa. Studies in Mycology 55: 53-63.

Crous, P.W., Wingfield, M.J., Mansilla, J.P., Alfenas, A.C. and Groenewald, J.Z. (2006e). Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. II. Studies in Mycology 55: 99-131.

Farr, D.F., Elliott, M., Rossman, A.Y. and Edmonds, R.L. (2005). *Fusicoccum arbuti* sp. nov. causing cankers on Pacific madrone in western North America with notes on *Fusicoccum dimidiatum*, the correct name for *Scytalidium dimidiatum* and *Nattrassia mangiferae*. Mycologia 97: 730-741.

Gams, W., Hoekstra, E.S. and Aptroot, A. (eds) (1998). *CBS course of mycology*. 4th ed. Centraalbureau voor Schimmelcultures, Baarn, the Netherlands.

Gryzenhout, M., Myburg, H., Hodges, C.S., Wingfield, B.D. and Wingfield, M.J. (2006). *Microthia*, *Holocryphia* and *Ursicollum*, three new genera on *Eucalyptus* and *Coccoloba* for fungi previously known as *Cryphonectria*. Studies in Mycology 55: 35-52.

Gryzenhout, M., Myburg, H., Merwe, N.A. van der, Wingfield, B.D. and Wingfield, M.J. (2004). *Chrysoporthe*, a new genus to accommodate *Cryphonectria cubensis*. Studies in Mycology 50: 119-142.

Hoog, G.S. de and Gerrits van den Ende, A.H.G. (1998). Molecular diagnostics of clinical strains of filamentous Basidiomycetes. Mycoses 41: 183-189.

Hunter, G.C., Wingfield, B.D., Crous, P.W. and Wingfield, M.J. (2006). A multi-gene phylogeny for species of *Mycosphaerella* occurring on *Eucalyptus* leaves. Studies in Mycology 55: 147-161.

Lee, S., Groenewald, J.Z. and Crous, P.W. (2004). Phylogenetic reassessment of the coelomycete genus *Harknessia* and its teleomorph *Wuestneia* (*Diaporthales*), and the introduction of *Apoharknessia* gen. nov. Studies in Mycology 50: 235-252.

Lee, S.B. and Taylor, J.W. (1990). Isolation of DNA from fungal mycelia and single spores. In: *PCR Protocols*: *a guide to methods and applications* (eds. M.A. Innis, D.H. Gelfand, J.J. Sninisky and T.J. White). Academic Press, San Diego, USA: 282-287.

Nag Raj TR (1993). *Coelomycetous anamorphs with appendage-bearing conidia*. Mycologue Publications, Waterloo, Ontario.

Nakabonge, G., Gryzenhout, M., Roux, J., Wingfield, B.D. and Wingfield, M.J. (2006). *Celoporthe dispersa* gen. et sp. nov. from native *Myrtales* in South Africa. Studies in Mycology 55: 255-267.

Niekerk J.M. van, Groenewald, J.Z., Farr D.F., Fourie P.H., Halleen, F. and Crous, P.W. (2005). Reassessment of *Phomopsis* species on grapevines. Australasian Plant Pathology 34: 27–39.

Niekerk, J.M. van, Groenewald, J.Z., Verkley, G.J.M., Fourie, P.H., Wingfield, M.J. and Crous, P.W. (2004). Systematic reappraisal of *Coniella* and *Pilidiella*, with specific reference to species occurring on *Eucalyptus* and *Vitis* in South Africa. Mycological Research 108: 283-303.

Park, R.F. and Keane, P.J. (1984). Further *Mycosphaerella* species causing leaf diseases of *Eucalyptus*. Transactions of the British Mycological Society 83: 93-105.

Park, R.F., Keane, P.J., Wingfield, M.J. and Crous, P.W. (2000). Fungal diseases of eucalypt foliage. In: *Diseases and pathogens of eucalypts* (eds P.J. Keane, G.A. Kile, F.D. Podger and B.N. Brown). CSIRO Publishing, Australia: 153-239.

Pascoe, I.G. (1990). Observations on ascus structure of *Plectosphaera eucalypti* (*Phyllachoraceae*). Mycological Research 94: 675-684.

Rayner, A.W. (1970). *A Mycological Colour Chart*. Commonwealth Mycological Institute, Kew.

Rensburg, J.C.J. van, Lamprecht, S.C., Groenewald, J.Z., Castlebury, L.A. and Crous, P.W. (2006). Characterisation of *Phomopsis* spp. associated with die-back of rooibos (*Aspalathus linearis*) in South Africa. Studies in Mycology 55: 65-74.

Sankaran, K.V., Sutton, B.C. and Minter, D.W. (1995). A checklist of fungi recorded on *Eucalyptus*. Mycological Papers 170: 1-376.

Slippers, B., Crous, P.W., Denman, S., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. (2004a). Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia 96: 83-101.

Slippers, B., Fourie, G., Crous, P.W., Coutinho, T.A., Wingfield, B.D., Carnegie, A.J. and Wingfield, M.J. (2004b). Speciation and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees in Australia and South Africa. Studies in Mycology 50: 343-358.

Slippers, B., Fourie, G., Crous, P.W., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. (2004c). Multiple gene sequences delimit *Botryosphaeria australis* sp. nov. from *B. lutea*. Mycologia 96: 1028-1039.

Sutton, B.C. (1971). *Staninwardia* gen. nov. (Melanconiales) on *Eucalyptus*. Transactions of the British Mycological Society 57: 539-542.

Sutton, B.C. (1980). *The Coelomycetes*. *Fungi Imperfecti with Pycnidia*, *Acervuli and Stromata*. Commonwealth Mycological Institute: England.

Swart, H.J. and Walker, J. (1988). Australian leaf-inhabiting fungi XXVIII. *Hendersonia* on *Eucalyptus*. Transactions of the British Mycological Society 90: 633-641.

White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols*: *a guide to methods and applications* (eds. M.A. Innis, D.H. Gelfand, J.J. Sninisky and T.J. White). Academic Press, San Diego, USA: 315-322.

(Received 6 June 2006; accepted 7 September 2006)