
Eucalyptus* microfungi known from culture. 3. *Eucasphaeria* and *Sympoventuria* genera nova, and new species of *Furcaspora*, *Harknessia*, *Heteroconium* and *Phacidiella

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Members of the genus *Eucalyptus* represent a substrate richly colonized by numerous undescribed fungal species. Several species and genera of ascomycetes were collected from leaves or from leaf litter of this host genus in Australia and South Africa in the present study. New genera include *Eucasphaeria capensis* and *Sympoventuria capensis* (ascomycetes), genera et spp. nov. New species include *Furcaspora eucalypti*, *Harknessia ipereniae*, *H. gibbosa*, *Heteroconium kleinziensis* and *Phacidiella eucalypti*.

Key words: ITS, 28S rDNA sequence data, microfungi, morphology, pure culture, systematics.

Introduction

Although numerous microfungi are known to colonise species of *Eucalyptus* (*Myrtaceae*) (Sankaran *et al.*, 1995; Crous *et al.*, 2006c, e; Summerell *et al.*, 2006), only few are known from culture and DNA sequence data. Economically important groups such as those species associated with *Mycosphaerella* stem cankers and leaf blotch disease (Cortinas *et al.*, 2006; Crous, 1998; Crous *et al.*, 2000, 2001, 2004a, b, 2006f; 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 (Crous *et al.*, 2006d; Slippers *et al.*, 2004a–c, 2007), *Cytospora* canker (Adams *et al.*, 2005), *Coniella* (Van Niekerk *et al.*, 2004), *Phomopsis* (Van Niekerk *et al.*, 2005; Van Rensburg *et al.*, 2006), *Quambalaria*

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(de Beer *et al.*, 2006) and *Harknessia* leaf spots (Lee *et al.*, 2004), have been studied to some extent. The saprobic microfungi, however, have been poorly studied, and very few are available from culture collections. The distribution, host range, and relative importance of these fungi remain largely unknown. The present study is the third in a series aimed at describing eucalypt microfungi from culture, with the aim of resolving their taxonomy and DNA phylogeny.

Materials and Methods

Isolates

Eucalyptus leaves and leaf litter showing signs of fungal colonization were chosen for study. Leaf tissue with ascomata were soaked in water for approximately 2 hours, then placed in the bottom of Petri dish lids, with the top half of the dish containing 2% malt-extract agar (MEA) (Sigma). 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. Anamorphs were cultured on MEA (Gams *et al.*, 1998) 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.*, 1998) and incubated at 25°C under continuous near-ultraviolet light, to promote sporulation.

DNA isolation, amplification and phylogeny

Genomic DNA was isolated from colonies established on MEA plates following the protocol of Lee and Taylor (1990). The primers V9G (Hoog and Gerrits van den Ende, 1998) and LR5 (Vilgalys and Hester, 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S rDNA (SSU), the first internal transcribed spacer (ITS1), the 5.8S rDNA, the second ITS region and the 5' end of the 28S rDNA (LSU). PCR conditions and protocols were treated and generated as explained in Crous *et al.* (2006f). The primers ITS4 (White *et al.*, 1990) and LR0R (Rehner and Samuels, 1994) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon.

Taxonomy

Fungal specimens were mounted on slides in lactic acid for microscopic examination. Thirty observations (with oil lens at ×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. Colours of colony 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

Eucasphaeria Crous, **gen nov.**

MycoBank: 501093.

Etymology: *Euca* = *Eucalyptus* + *Sphaeria* = globose ascomata.

Plectosphaeriae simile, sed clypeo carens, ascis unitunicatis, sporas vi per anellum apicalem liberantibus; anamorphem *Ascochytopsidi* similem formans in vitro.

Morphologically similar to *Plectosphaera*, but lacking a clypeus, and having unitunicate asci with an apical discharge mechanism, producing an *Ascochytopsis*-like anamorph in culture.

Eucasphaeria capensis Crous, **sp. nov.**

(Fig. 1)

MycoBank 501094.

Anamorph: *Ascochytopsis*-like

Etymology: Named after the Cape Province of South Africa, where it was collected.

Ascomata subepidermalia, globosa, ad 250 μm diam. Asci cylindrici, crassitunicati, unitunicati, annulo apicali J+, 40–70 \times 6–8 μm . Ascosporae hyalinae, guttulate, fusoido-ellipsoideae, curvatae, 1-septatae, (17–)19–25(–28) \times 3–)3.5(–4) μm .

Ascomata subepidermal, medium brown, globose, up to 250 μm diam, with a single central ostiole, up to 10 μm wide; upper region of ascoma with 5–6 layers of hyaline cells that give rise to short, hyaline, cylindrical periphysoids, 5–10 μm long; ascomatal wall consisting of 2–3 layers of brown cells of *textura angularis*. *Asci* cylindrical, thick-walled, unitunicate, apex bluntly rounded, apical ring visible (J+); stipitate, fasciculate, aparaphysate, 40–70 \times 6–8 μm .

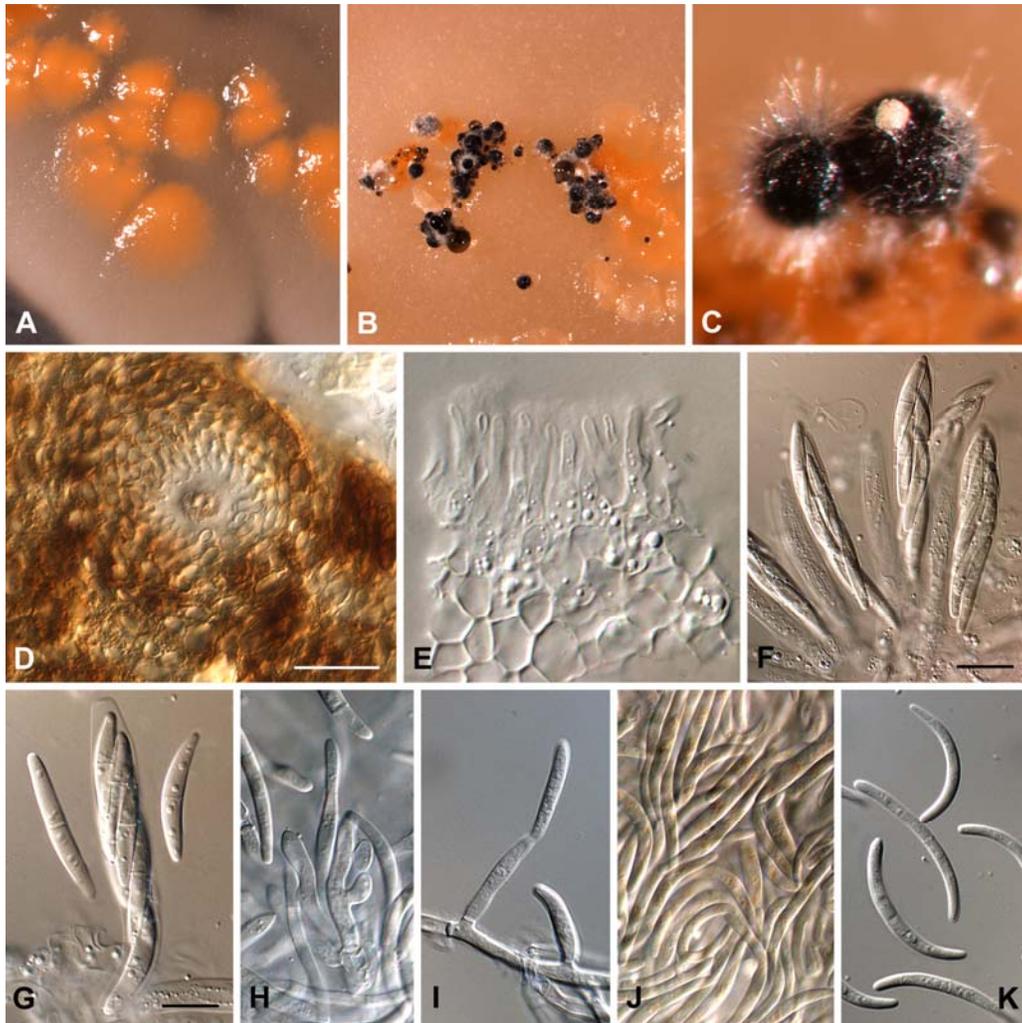


Fig. 1. *Eucasphaeria capensis* (CBS-H19764, CBS 120027). **A–C.** Sporulation on PDA. **D.** Ostiolar region. **E.** Periphyses. **F, G.** Asci. **H–K.** Conidia and conidiogenous cells. Scale bars = 10 μm .

Ascospores hyaline, guttulate, fusoid-ellipsoidal, mostly curved, 1-septate, not constricted at median septum, widest at septum, tapering towards both subobtuse ends, multiseriate, (17–)19–25(–28) \times (3–)3.5(–4) μm . *Conidiomata* subepidermal, opening by irregular ruptures, acervuloid, up to 150 μm diam; wall consisting of 5–6 layers of brown cells of *textura angularis*, becoming hyaline towards inner conidiogenous region. *Conidiophores* hyaline, subcylindrical, branched apically, 1–2-septate, 10–20 \times 3–4 μm , giving rise to

1–2 conidiogenous cells. *Conidiogenous cells* phialidic, hyaline, fusoid-ellipsoidal, straight to curved, tapering towards a subtruncate apex with visible periclinal thickening, $5\text{--}15 \times 2\text{--}3 \mu\text{m}$. *Conidia* hyaline, minutely guttulate, predominantly falcate, widest in middle, aseptate, base subtruncate, apex subobtusely rounded, $(16\text{--})20\text{--}22(\text{--}27) \times (2\text{--})2.5(\text{--}3) \mu\text{m}$ on host; in culture sporulating by means of inconspicuous sporodochia, conidia 0–2-septate, $(22\text{--})25\text{--}30(\text{--}40) \times 3(\text{--}4) \mu\text{m}$; conidia covered in mucus.

Cultural characteristics: Colonies spreading on PDA, with sparse to no aerial mycelium; margins smooth but regular; surface ochreous, outer zone cream; reverse ochreous.

Specimens examined: **South Africa**, Western Cape Province, On N7 from Malmesbury towards Cape Town, on living leaves and leaf litter of *Eucalyptus* sp., Jan. 2006, P.W. Crous, CBS-H19764, **holotype**, cultures ex-type CPC 12796 = CBS 120028 = GenBank EF110618, CPC 12797; Malmesbury, on leaf litter of *Eucalyptus* sp., Jan. 2006, P.W. Crous, CPC 12823 = CBS 120027 = GenBank EF110619, CPC 12824–12825.

Notes: The teleomorph is somewhat reminiscent of *Plectosphaera eucalypti*, but again distinct in that ascomata lack a clypeus-like structure, and have an apical discharge mechanism in their unitunicate asci. The anamorph is *Ascochytopsis*-like, but differs in that the conidiomata are immersed, and not superficial or stipitate (Sutton, 1980). After 2 months ascomata also formed on OA cultures established from single ascospores, proving the species to be homothallic. Ascomata were separate or aggregated in clusters, exuding spores in white masses. The upper region around the periphysate ostiole had patches of darker pigment, appearing like an irregular, dark-brown crust. Furthermore, in culture ascomata also developed setae in the apical region; setae were brown, 1–2-septate, smooth, up to $100 \mu\text{m}$ long, with pointed to bluntly rounded apices. BLASTn results of the ITS sequence of *Eucasphaeria capensis* did not reveal close relatives, except for distant similarity to species of *Paecilomyces* (*Hypocreales*). The partial 28S rRNA sequence revealed it to be allied to *Niesslia exilis*, species of *Chaunopycnis*, and *Fusarium lichenicola* (*Hypocreales*).

***Furcaspora eucalypti* Crous & Verkley, sp. nov.**

(Figs 2, 3)

MycoBank 501109.

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

Furcasporae pinicolae similis, sed conidiorum ramis ad $1.5 \mu\text{m}$ latis et appendicibus non-spathulatis differens.

Conidiomata associated with brown leaf spots of *Mycosphaerella cryptica*, amphigenous, subepidermal, pycnidiod, globose, unilocular, pale brown, up to $800 \mu\text{m}$ diam, only visible on surface by exuding pale brown to cream conidial cirrhi; conidiomatal wall of thin-walled, pale brown *textura angularis*. *Conidiophores* lining the inner conidiomatal cavity, branched,

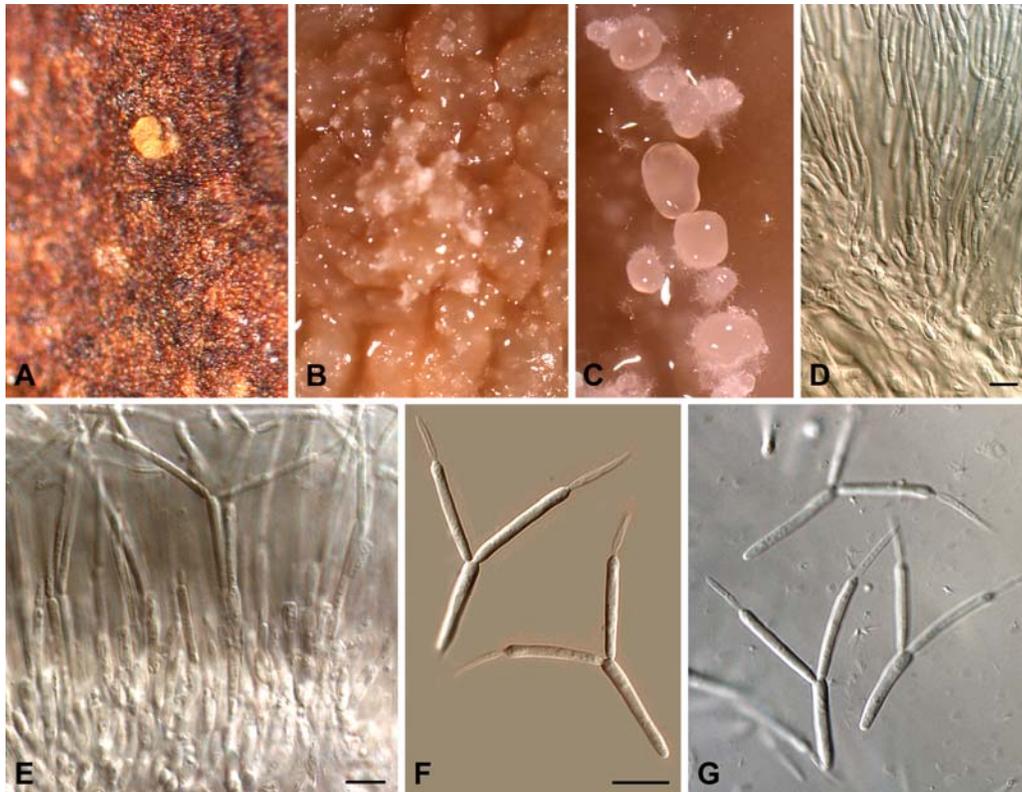


Fig. 2. *Furcaspora eucalypti* (CBS-H 19761, CBS 119111). **A.** Oozing conidial mass on host tissue. **B, C.** Sporulating colonies on CMA. **D, E.** Conidia attached to conidiophores. **F, G.** Conidia. Scale bars = 10 μ m.

septate, hyaline, thin-walled, smooth, embedded in mucus, 5–15 \times 2–3.5 μ m. *Conidiogenous cells* subcylindrical, hyaline, smooth, terminal, polyblastic, proliferating sympodially, 5–7 \times 2–3.5 μ m. *Conidia* tri-radiate, arms cylindrical, bearing an appendage; upper two arms (15–)18–20(–22) \times 1.5 μ m, separated from the main cylindrical, vertical axis (13–)15–17 \times 2(–3) μ m by a septum; appendages cellular, separated from arms by septa, fusiform, (6–)8–10(–12) μ m long and 1 μ m wide near base (CMA).

Cultural characteristics: Colonies on CMA spreading, flat, aerial mycelium absent, sporulating in concentric circles, colonies and conidiomata cream to pale brown in colour.

Specimen examined: **Australia**, Victoria, *Eucalyptus globulus*, 3 Oct. 2005, I. Smith, CBS-H19761, **holotype**, cultures ex-type CPC 12556 = CBS 119111, CPC 12557–12558, GenBank EF110613.

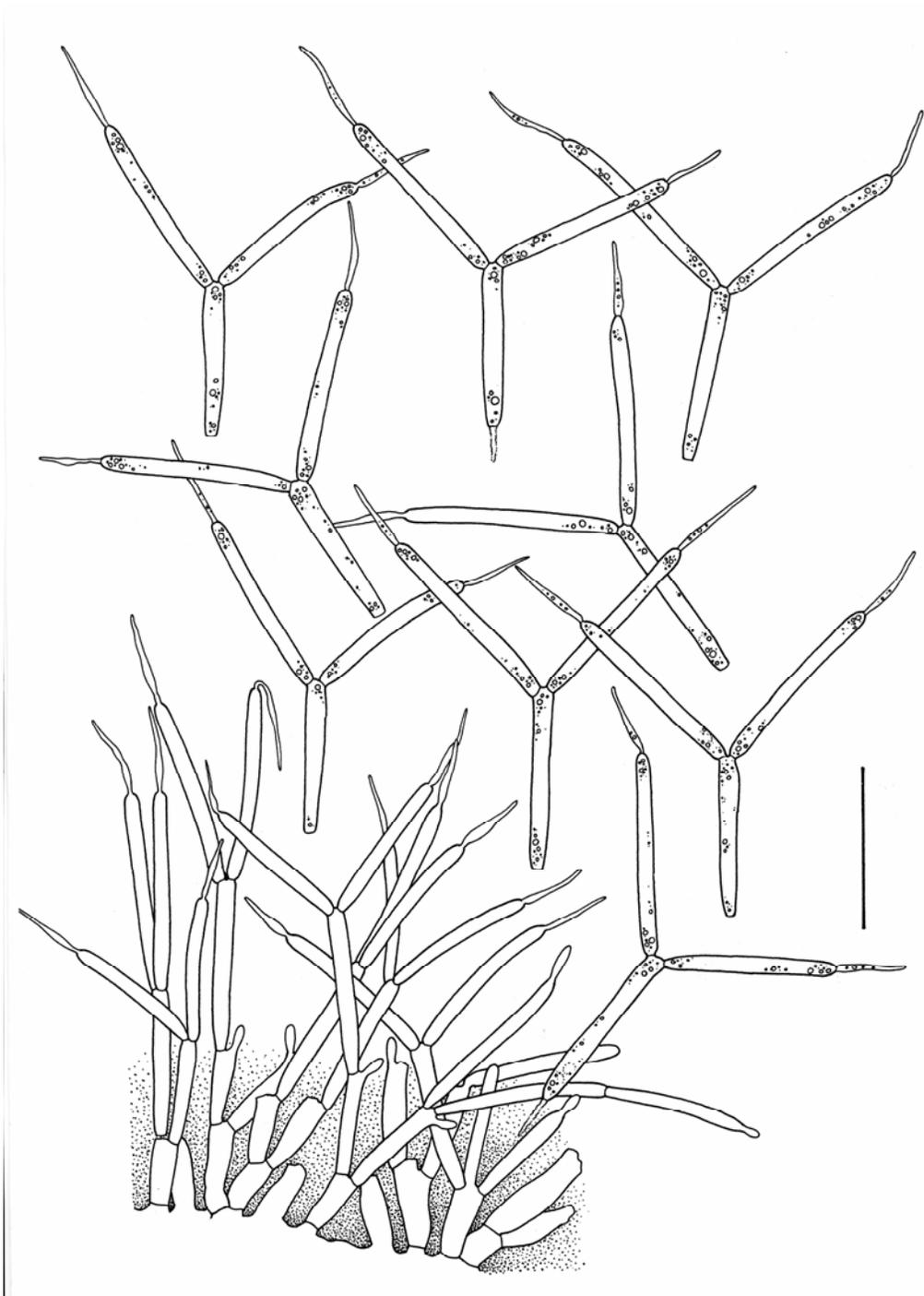


Fig. 3. *Fuscaspora eucalypti* (CBS 119111). Conidia and conidiophores produced in culture on CMA. Scale bar = 20 μ m.

Notes: *Furcaspora* is known from three species, of which one, *F. pinicola* is currently recognized (Nag Raj, 1993). *Furcaspora eucalypti* is easily distinguished by its narrower conidial arms, and characteristic fusiform shape of its conidial appendages. In *F. pinicola* the conidial arms are up to 2.5 µm wide, and the apical appendages are spatulate in shape (Nag Raj, 1993). BLASTn results of the ITS sequence of *F. eucalypti* did not reveal close relatives, except for distant similarity to species of *Lanzia*, species of *Sclerotinia* and *Monilinia* (*Helotiales*). The partial 28S rRNA sequence revealed it to be allied to species of *Xanthoria* (*Teloschistales*), *Porpidia* (*Lecanorales*) and *Umbilicaria* (*Lecanoromycetidae*).

***Harknessia ipereniae* Crous, sp. nov.**

(Fig. 4)

MycoBank 501104.

Etymology: Named after its collector, Arien van Iperen.

Harknessiae spermatoideae similis, sed conidiis majoribus, (26–)30–35(–37) × (9–)10–11(–12) µm, differens.

Conidiomata caulicolous, pycnidoid, scattered to gregarious, immersed in host tissue, but becoming erumpent, ovoid, up to 500 µm diam; unilocular, area of dehiscence irregular, with a wide border of furfuraceous cells; wall of 2–3 cell layers of brown *textura angularis*. *Conidiophores* aseptate, hyaline, arising from the inner layer of the conidioma, ampulliform to lageniform, proliferating once or twice percurrently, 5–15 × 4–7 µm. *Conidia* subcylindrical to ellipsoid with a truncate base, medium brown, apex pale brown, thick-walled, smooth, without striations, but with a longitudinal band of lighter pigment, (26–)30–35(–37) × (9–)10–11(–12) µm; basal appendage tubular, thin-walled, smooth, 85–150 × 2–3 µm.

Cultural characteristics: Colonies on PDA fast-growing, completely covering plates within 2 wk; surface fluffy, white, with abundant fluffy aerial mycelium that collapses with age, giving a smooth, slimy appearance; odour sweet and fruity; reverse creamy-white; colonies sterile on PDA, MEA and OA.

Specimen examined: **Australia**, Western Australia, Perth, King's Park, *Eucalyptus* leaf litter, 20 Sept. 2005, A. van Iperen, CBS-H 19759, **holotype**, cultures ex-type CPC 12480 = CBS 120030, CPC 12481–12482, GenBank EF110614.

Notes: Conidia of *H. ipereniae* resemble those of *H. spermatoidea* (Nag Raj, 1993; Lee *et al.*, 2004) in shape, and the presence of a longitudinal band of lighter pigment. They differ, however, in being larger, and having longer appendages, as well as lacking striations, which were observed to be present on the type specimen of *H. spermatoidea*. BLASTn results of the ITS sequence of *H. ipereniae* had high similarity to other species of *Harknessia* (*Diaporthales*), the closest species being *H. uromycoides* and *H. weresubiae*.

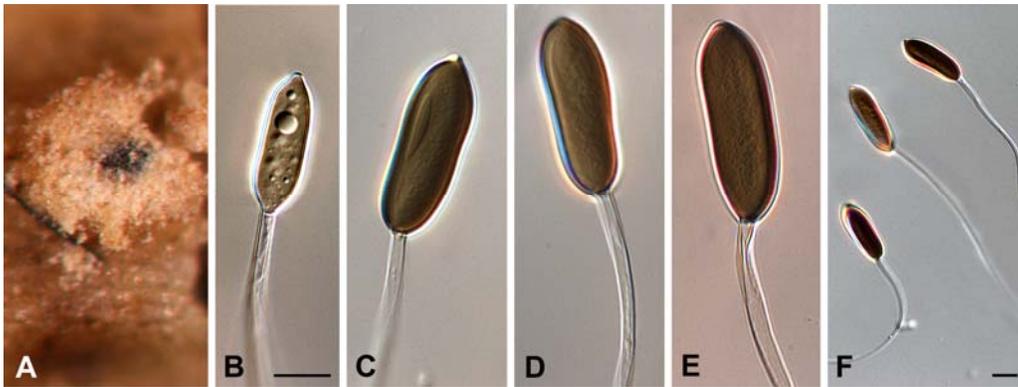


Fig. 4. *Harknessia ipereniae* (CBS-H 19759). **A.** Erumpent ostiolar region of conidioma on host tissue. **B.** Juvenile conidium. **C–F.** Mature conidia. Scale bars = 10 μm .

Harknessia gibbosa Crous & C. Mohammed, **sp. nov.** (Fig. 5)
Mycobank 501095.

Etymology: Named after its characteristic gibbose conidia.

Harknessiae gunnerae similis, sed conidiis majoribus, (13–)17–19(–20) \times (10–)11–12 μm , differens.

Conidiomata foliicolous, pycnidiod, scattered, immersed in host tissue, subepidermal, globose, up to 350 μm diam; unilocular, area of dehiscence irregular; wall of 2–3 cell layers of brown *textura angularis*. *Conidiophores* arising from the inner layer of the conidioma, aseptate, hyaline, ampulliform to lageniform, proliferating once or twice percurrently near the apex, 5–10 \times 4–6 μm . *Conidia* ellipsoid in front view, gibbose in side view, apex bluntly rounded to apiculate and pale brown, thick-walled, smooth, but with longitudinal striations in restricted areas, frequently with a large central guttule, (13–)17–19(–20) \times (10–)11–12 μm ; basal appendage tubular, thin-walled, cylindrical, hyaline, smooth, 3–6(–10) \times 2–4 μm .

Cultural characteristics: Colonies on PDA covering plates within 2 wk; surface fluffy, white, with abundant fluffy aerial mycelium; odour sweet and fruity; reverse creamy-white.

Specimen examined: **Australia**, Tasmania, Lake St. Clair, *Eucalyptus delegatensis*, 15 Nov. 2005, C. Mohammed, CBS-H 19760, **holotype**, cultures ex-type CPC 12473 = CBS 120033, CPC 12474–12475, GenBank EF110615.

Notes: Conidia of *H. gibbosa* (13–20 \times 10–12 μm) resemble those of *H. gunnerae* (11–15.5 \times 6.5–8 μm) (Nag Raj, 1993) in shape, but are easy to distinguish by being larger. BLASTn results of the ITS sequence of *H. gibbosa* had high similarity to other species of *Harknessia* (Diaporthales), the closest species being *Wuestneia molokaiensis* and *H. leucospermi*.

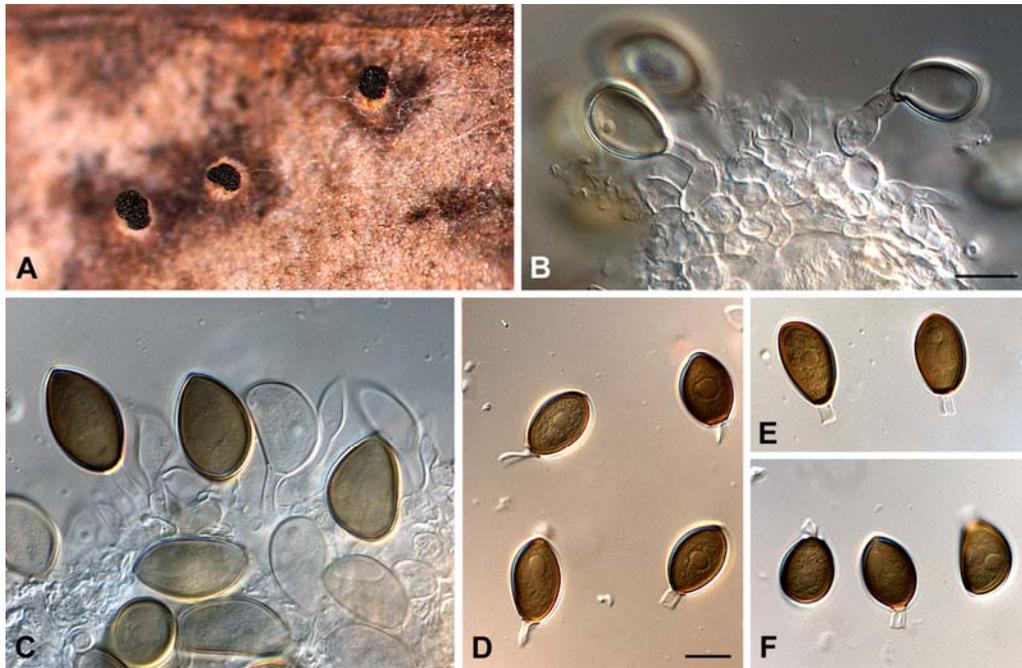


Fig. 5. *Harknessia gibbosa* (CBS-H 19760, CBS 120033). **A.** Oozing conidial masses on host tissue. **B, C.** Conidia attached to conidiogenous cells. **D–F.** Conidia. Scale bars = 10 μ m.

***Heteroconium kleinziense* Crous & Z.A. Pretorius, sp. nov.** (Fig. 6)
Mycobank 501237.

Etymology: Named after the locality where it was collected in South Africa, Kleinzee.

Conidiophora brunnea, verruculosa, crassitunicata, 1–3-septata, 20–40 \times 7–8 μ m. Cellulae conidiogenae integratae, terminales, percurrenter proliferantes, 7–10 \times 7–8 μ m. Conidia subcylindrica vel ellipsoidea, brunnea, verruculosa, aseptata vel nonnullis septis transversalibus divisa, 10–60 \times 7–8 μ m.

Leaf spots amphigenous, irregular to subcircular, 2–4 mm diam, medium brown, with a slightly raised, concolorous border; margin thin, chlorotic. *Mycelium* predominantly internal, hypophyllous, consisting of branched, septate, brown, verruculose hyphae, 3–4 μ m wide; hyphae forming a brown, superficial, radiating stroma up to 250 μ m diam, giving rise to conidiophores. *Conidiophores* brown, verruculose, thick-walled, 1–3-septate, 20–40 \times 7–8 μ m. *Conidiogenous cells* integrated, terminal, brown, verruculose, proliferating percurrently, frequently with a long, brown, thick collarete that encloses the conidial initial, 7–10 \times 7–8 μ m. *Conidia* subcylindrical to ellipsoid, brown, verruculose, thick-walled, non to transversely multiseptate, disto- and euseptate,

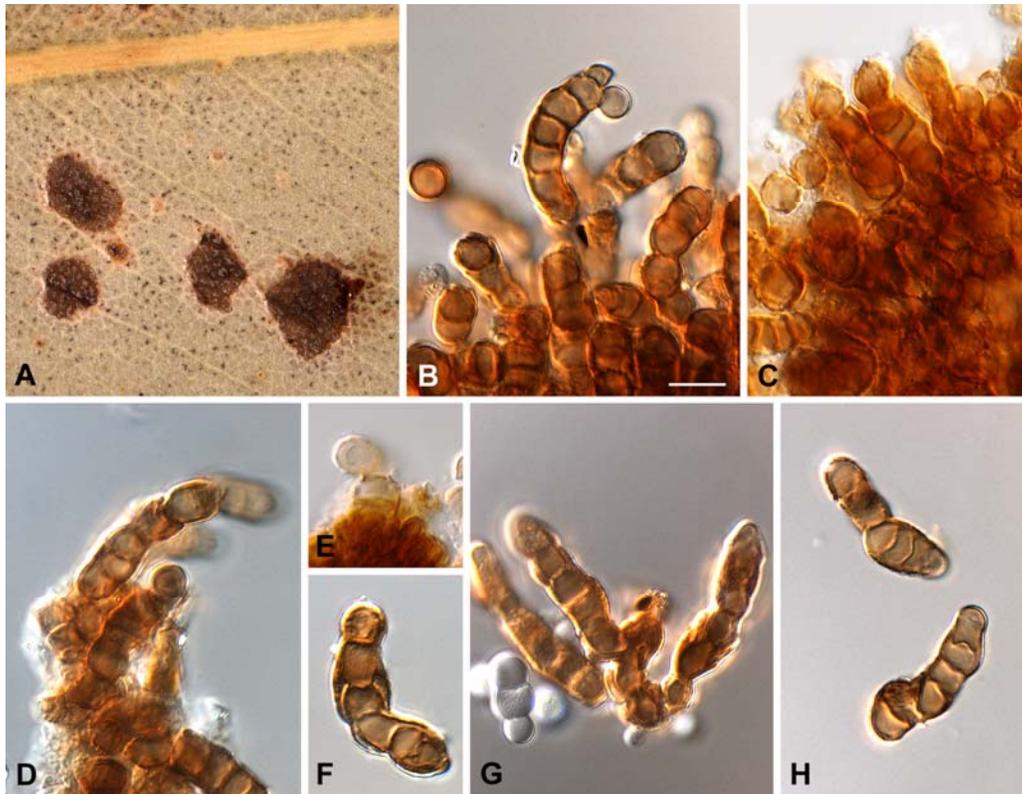


Fig. 6. *Heteroconium kleinzii* (CBS-H 19767). **A.** Leaf spots. **B–E, G.** Conidia attached to conidiogenous cells. **F, H.** Conidia. Scale bar = 10 μm .

occurring solitarily or in chains that are predominantly unbranched, frequently remaining attached to the conidiogenous cells, $10\text{--}60 \times 7\text{--}8 \mu\text{m}$.

Cultural characteristics: Colonies on PDA slow-growing, reaching 3 mm diam after 2 wk at 25°C; aerial mycelium absent; colonies erumpent, margins irregular, feathery; surface and reverse olivaceous-black.

Specimen examined: **South Africa**, Northern Cape Province, Kleinzee, on leaves of *Eucalyptus* sp., Apr. 2005, Z.A. Pretorius, CBS-H 19767, **holotype**, cultures ex-type CPC 12174 = CBS 120138, CPC 12175–12176, GenBank EF110616.

Notes: Based on morphology, the present fungus resembles members of the genus *Stigmina*, having prominent percurrent proliferations at the apices of its conidiogenous cells. However, its DNA sequence data suggests that it is not allied to the *Mycosphaerellaceae*, but is a member of the genus *Heteroconium*, sharing a 94% DNA sequence similarity to *H. eucalypti* DQ885893 (*Chaetothyriales*; *Herpotrichiellaceae*; Crous *et al.*, 2006b).

***Phacidiella eucalypti* Crous, sp. nov.**

(Fig. 7)

MycoBank 501238.

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

Conidiophora subhyalina, levia, cylindrica, ramosa praecipue in parte basilari, 1–3-septata, $5\text{--}15 \times 2.0\text{--}2.5 \mu\text{m}$. Cellulae conidiogenae polyblasticae, sympodialiter proliferantes, $3\text{--}6 \times 2.0\text{--}2.5 \mu\text{m}$. Conidia subhyalina vel dilute brunnea, levia, subcylindrica vel doliiformia, ad quinque catenulata, $(4\text{--})5\text{--}6(\text{--}7) \times (2\text{--})2.5 \mu\text{m}$.

Conidiomata dark brown to black, up to 250 μm diam, amphigenous, subepidermal, becoming erumpent, associated with necrotic leaf tissue on living leaves. *Conidiophores* subhyaline, smooth, cylindrical, predominantly branched below, densely aggregated, 1–3-septate, $5\text{--}15 \times 2\text{--}2.5 \mu\text{m}$. *Conidiogenous cells* terminal and lateral, integrated, subhyaline, smooth, polyblastic, proliferating sympodially, $3\text{--}6 \times 2\text{--}2.5 \mu\text{m}$. *Conidia* subhyaline to pale brown, smooth, subcylindrical to barrel-shaped, ends bluntly rounded, occurring in disarticulating, unbranched, short chains, $(4\text{--})5\text{--}6(\text{--}7) \times (2\text{--})2.5 \mu\text{m}$.

Cultural characteristics: Colonies erumpent on PDA, with sparse to moderate white aerial mycelium, sectoring with smooth, but uneven margins; surface and reverse cream, reaching 20 mm diam after 1 mo at 25°C; colonies exuding drops of mucus, sterile on PDA and MEA after 2 mo.

Specimens examined: **South Africa**, Western Cape Province, Stellenbosch Mountain, on leaves of *Eucalyptus* sp., 10 Jan. 2006, P.W. Crous, CBS-H 19768, **holotype**, cultures ex-type CPC 12745 = CBS 120255, CPC 12746–12747, GenBank EF110620; Malmesbury, on *Eucalyptus* sp., Jan. 2006, P.W. Crous, cultures CPC 12742 = CBS 120038, CPC 12743–12744.

Notes: The genus *Phacidiella* is characterized by having acervular conidiomata that produce disarticulating chains of hyaline, smooth, aseptate subcylindrical conidia (Sutton, 1980). *Phacidiella eucalypti* fits this morphological concept, though in mass conidia show some pigmentation, which is absent in *Phacidiella*. Presently no other species of *Phacidiella* is known from culture, and therefore it is impossible to determine if the *Eucalyptus* fungus is also phylogenetically related to *P. salicina*, the type species, which was described from *Salix* twigs collected in Europe. Apparently several species of the genus are known to occur on *Eucalyptus* in Australia, awaiting formal description (I. Pascoe, pers. comm.). BLASTn results of the ITS sequence of *Phacidiella eucalypti* did not reveal any close relatives, except for distant similarity to *Stictis* and *Conotrema* spp. (Ostropales; Stictidaceae), as well as *Mycosphaerella walkeri* and *Septoria betulae* (Mycosphaerellaceae). The partial 28S rRNA gene sequence revealed it to be allied to members of the Stictidaceae (Ostropales), such as *Stictis radiata*, *Carestiella socia*, *Schizoxylon albescens* and *Conotrema populorum*.

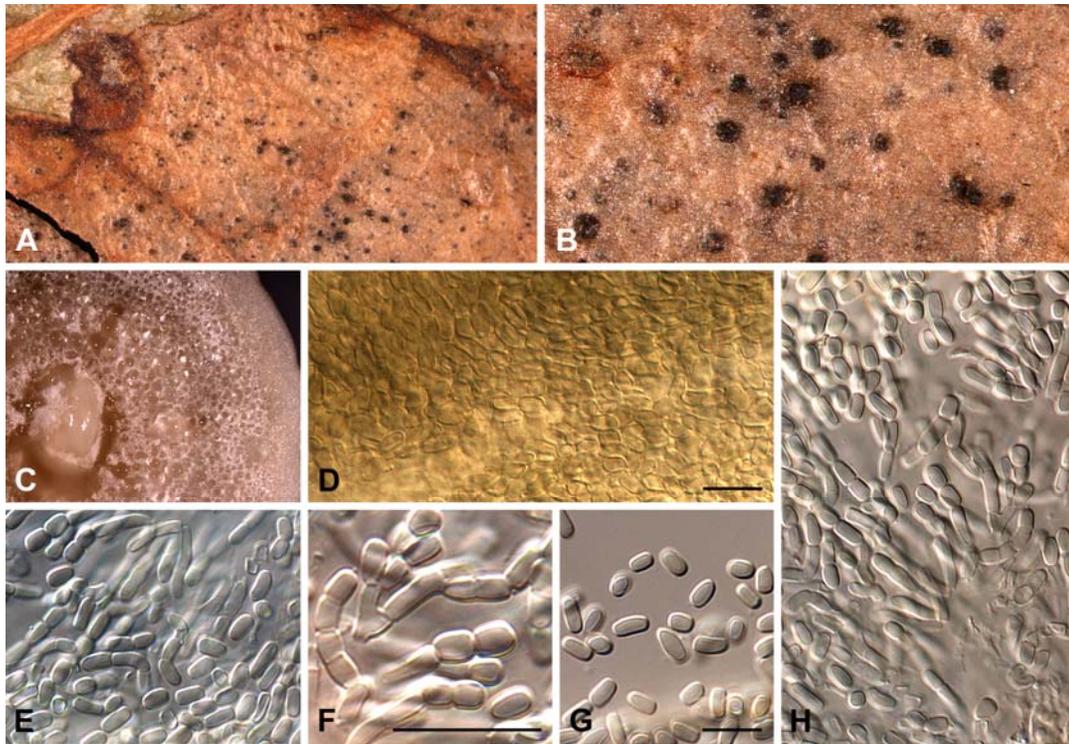


Fig. 7. *Phacidiella eucalypti* (CBS-H 19768, CBS 120038). **A, B.** Leaf spots with conidiomata. **C.** Colony on PDA. **D–H.** Conidia and conidiogenous cells. Scale bars = 10 μm .

***Sympoventuria* Crous & Seifert, gen. nov.**

(Fig. 8)

Mycobank MB501002.

Anamorph: *Sympodiella*-like

Etymology: Named after its sympodial conidiogenesis, and morphological similarity to *Venturia*.

Venturiae et *Caproventuriae* simile genus, sed ascosporis hyalinis, setis absentibus et anamorphe *Sympodiella* distinctum.

Saprobic on leaf litter; *Ascomata* pseudothecial, subglobose, immersed, black, inconspicuous, papillate, ostiolate. *Asci* hyaline, subcylindrical, stipitate, 8-spored. *Pseudoparaphyses* hyaline, septate, constricted at septa, anastomosing, extending above the asci. *Ascospores* hyaline, fusoid-ellipsoidal, constricted at median septum. Forming a *Sympodiella*-like anamorph in culture.

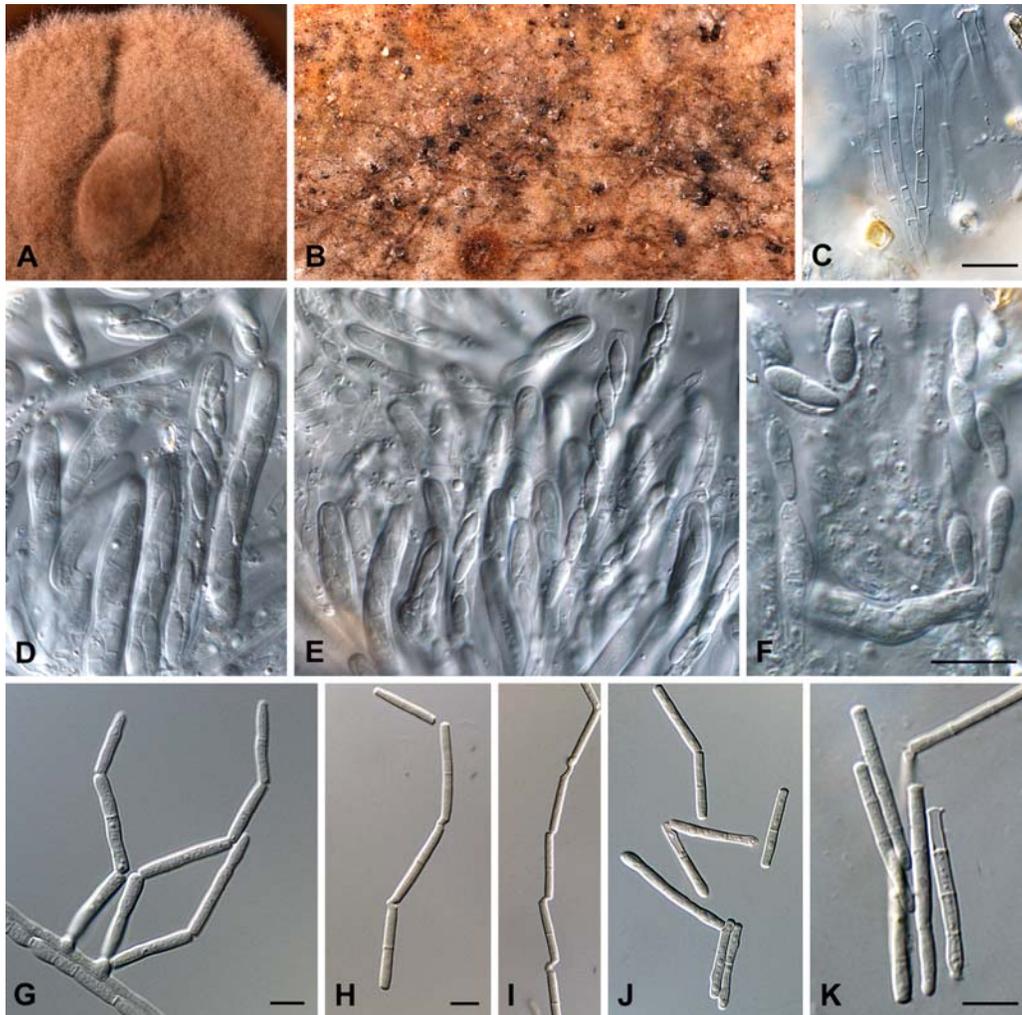


Fig. 8. *Sympoventuria capensis* (CBS-H 19757, CBS 120136). **A.** Colony on PDA. **B.** Ascomata in host tissue. **C.** Pseudoparaphyses. **D–F.** Asci and ascospores. **G–K.** Catenulate conidia and conidiogenous cells. Scale bars = 10 μ m.

***Sympoventuria capensis* Crous & Seifert, sp. nov.**

Mycobank MB501003.

Anamorph: *Sympodiella*-like

Etymology: Named after the Cape Province of South Africa, from where this fungus was collected.

Ascomata pseudothecialia, subglobosa, substomatalia, ad 150 μ m diam. Asci hyalini, subcylindrici, 8-spori, 35–55 \times 5–6 μ m. Ascosporae hyalinae, fusoido-ellipsoideae, ad septum medianum constrictae, (8–)9–10(–13) \times (2.5–)3–4(–5) μ m.

Saprobic on leaf litter; *Ascomata* pseudothecial, on adaxial leaf surface, subglobose, substomatal, subepidermal, black, inconspicuous, raising the cuticle at maturity, up to 150 µm diam, papillate, ostiolate; wall consisting of 2–3 layers of brown *textura angularis*. *Asci* hyaline, subcylindrical, stipitate, 8-spored, 35–55 × 5–6 µm. *Pseudoparaphyses* hyaline, septate, constricted at septa, anastomosing, 2–3 µm wide, extending above the asci. *Ascospores* 1-septate, hyaline, fusoid-ellipsoidal, constricted at median septum, widest in middle of the apical cell, guttulate, (8–)9–10(–13) × (2.5–)3–4(–5) µm. Anamorph produced in culture. *Mycelium* composed of brown, septate, thin- to thick-walled, smooth, 3–5 µm wide hyphae. *Conidiogenous cells* integrated, terminal, mono- or polyblastic and sympodial, (10–)15–30 × (3–)4–5 µm, giving rise to disarticulating chains of arthroconidia; scars inconspicuous, or sometimes slightly refractive with phase contrast optics. *Conidia* in unbranched acropetal chains, cylindrical with truncate ends, but the apical conidium having an obtuse apex and truncate base, pale brown, smooth, thin-walled, scars inconspicuous, not thickened nor darkened, chains remaining attached for some time, individual conidia frequently anastomosing, finely guttulate or not, (1–)3(–5)-septate; 1-septate conidia (10–)20–25 × (2.5–)3 µm, 3-septate conidia (27–)30–35(–40) × (3–)3.5–4 µm, 5-septate conidia (40–)55–65 × 4–5 µm.

Cultural characteristics: Colonies on PDA reaching 22 mm diam after 3 wk at 25°C; centre hazel because of fluffy aerial mycelium; outer margin smooth, entire, isabelline to sepia; reverse sepia to fuscous-black; colonies fertile on most media, producing the anamorph.

Specimen examined: **South Africa**, Western Cape Province, Malmesbury, *Eucalyptus* leaf litter, Jan. 2006, P.W. Crous, CBS-H 19757, **holotype**; cultures ex-type derived from single ascospores, CPC 12838 = CBS 120136, CPC 12839–12840 (GenBank DQ885904–DQ885906).

Notes: Single-ascospore cultures of *S. capensis* produced an anamorph in culture resembling members of the *Sympodiella/Parasympodiella* complex. The connections between the conidia are often asymmetrical, giving a false appearance of clamp connections. However, a comparison of the internal transcriber spacer (ITS) region of the rDNA with that of *P. laxa*, which typifies *Parasympodiella*, revealed that these fungi are not congeneric, and that although the anamorph resembles *Parasympodiella*, true species of the genus, allied with *P. laxa*, would not have *Symptoventuria* teleomorphs. Presently there are no known teleomorph connections for *Parasympodiella* or *Sympodiella sensu stricto*. BLASTn results of the ITS sequence of *Symptoventuria capensis* did not reveal close relatives. The partial 28S rRNA gene sequence revealed *S. capensis* (GenBank DQ885904–DQ885906) to be allied to the *Venturiaceae*, and the *Tubeufiaceae*. Morphologically *Symptoventuria* is typical of the *Venturiaceae*, though the anamorph formed in culture is not.

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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.
- 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 Cyndrocladium (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 *Cyndrocladium* 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 *Cyndrocladium* anamorphs: species with clavate vesicles. *Studies in Mycology* 55: 213-226.
- Crous, P.W., Groenewald, J.Z. and Wingfield, M.J. (2006b). *Heteroconium eucalypti*. *Fungal Planet* No. 10.
- 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. (2006c). 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. (2006d). Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* 55: 235-253.
- Crous, P.W., Verkley, G.J.M. and Groenewald, J.Z. (2006e). *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. (2006f). Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. II. *Studies in Mycology* 55: 99-131.
- 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 *Apharknessia* 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. Sninsky 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.
- Rayner, A.W. (1970). *A Mycological Colour Chart*. Commonwealth Mycological Institute, Kew.
- Rehner, S.A. and Samuels, G.J. (1994). Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* 98: 625-634.
- 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.
- Slippers, B., Smit, W.A., Crous, P.W., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. (2007). Taxonomy, phylogeny and identification of Botryosphaeriaceae associated with pome and stone fruit trees in South Africa and other regions of the world. Plant Pathology 56: 128-139.
- Summerell, B.A., Groenewald, J.Z., Carnegie, A.J., 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.
- Sutton, B.C. (1980). *The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stromata*. Commonwealth Mycological Institute: England.
- Vilgalys, R. and Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238-4246.
- 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. Sninsky and T.J. White). Academic Press, San Diego, USA: 315-322.

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