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

Pedro W. Crous¹*, Caroline Mohammed², Morag Glen², Gerard J.M. Verkley¹ and Johannes Z. Groenewald¹

¹Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
²CSIRO Forestry and Forest Products, GPO Box 252-12, Hobart 7001, Tasmania


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

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

Plectosphaeriales similis, sed clypeo carens, ascis unitunicatis, sporas vi per annellum apicalem liberantibus; anamorphem *Ascochytopsidi* similis 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 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 cylindric, thick-walled, unitunicate, apex bluntly rounded, apical ring visible (J+); stipitate, fasciculate, aporaphysate, 40–70 × 6–8 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) × (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 × 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, 5–15 × 2–3 µm. *Conidia* hyaline, minutely guttulate, predominantly falcate, widest in middle, aseptate, base subtruncate, apex subobtusely rounded, (16–)20–22 (–27) × (2–)2.5 (–3) µm on host; in culture sporulating by means of inconspicuous sporodochia, conidia 0–2-septate, (22–) 25–30 (–40) × 3 (–4) µm; 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.


**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 µm 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 *Nieslilia 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 conidiorn ramis ad 1.5 µm latis et appendicibus non-spathulatis differens.

*Conidiomata* associated with brown leaf spots of *Mycosphaerella cryptica,* amphigenous, subepidermal, pycnidial, globose, unilocular, pale brown, up to 800 µm diam, only visible on surface by exuding pale brown to cream conidial cirrhí; 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 µm.

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

Fig. 3. *Furcaspora eucalypti* (CBS 119111). Conidia and conidiophores produced in culture on CMA. Scale bar = 20 µm.
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 spermatoidea* similis, sed conidiis majoribus, (26–)30–35(–37) × (9–)10–11(–12) µm, differens.

*Conidiomata* caulicolous, pycnidial, 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.


*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) × (10–)11–12 µm, differens.*

*Conidiomata* foliicolous, pycnidioi, 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 × 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) × (10–)11–12 µm; basal appendage tubular, thin-walled, cylindrical, hyaline, smooth, 3–6(–10) × 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.


*Notes:* Conidia of *H. gibbosa* (13–20 × 10–12 µm) resemble those of *H. gunnerae* (11–15.5 × 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*.

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 × 7–8 µm. Cellulae conidiogenae integratae, terminales, percurrenter proliferentes, 7–10 × 7–8 µm. Conidia subcylindrica vel ellipsoidea, brunnea, verruculosa, aseptata vel nonnullis septis transversalibus divisa, 10–60 × 7–8 µm.

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 µm wide; hyphae forming a brown, superficial, radiating stroma up to 250 µm diam, giving rise to conidiophores. Conidiophores brown, verruculose, thick-walled, 1–3-septate, 20–40 × 7–8 µm. Conidiogenous cells integrated, terminal, brown, verruculose, proliferating percurrently, frequently with a long, brown, thick collarette that encloses the conidial initial, 7–10 × 7–8 µm. Conidia subcylindrical to ellipsoid, brown, verruculose, thick-walled, non to transversely multiseptate, disto- and euseptate,
occurring solitarily or in chains that are predominantly unbranched, frequently remaining attached to the conidiogenous cells, 10–60 × 7–8 µm.

*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, 5–15 × 2.0–2.5 μm. Cellulae conidiogenae polyblasticae, sympodialiter proliferantes, 3–6 × 2.0–2.5 μm. Conidia subhyalina vel dilute brunnea, levia, subcylindrica vel doliiformia, ad quinque catenulata, (4–)5–6(–7) × (2–)2.5 μm.

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, 5–15 × 2–2.5 μm. Conidiogenous cells terminal and lateral, integrated, subhyaline, smooth, polyblastic, proliferating sympodially, 3–6 × 2–2.5 μm. Conidia subhyaline to pale brown, smooth, subcylindrical to barrel-shaped, ends bluntly rounded, occurring in disarticulating, unbranched, short chains, (4–)5–6(–7) × (2–)2.5 μm.

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.


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, Caretiella socia, Schizoxylon albescens and Conotrema populorum.
**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. 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 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.

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.


*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 *Sympoventuria* telemorphs. Presently there are no known telemorph connections for *Parasympodiella* or *Sympodiella sensu stricto*. BLASTn results of the ITS sequence of *Sympoventuria 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 *Sympoventuria* is typical of the *Venturiaceae*, though the anamorph formed in culture is not.
Acknowledgements

The authors gratefully acknowledge A. van Iperen for assisting with the cultures, M. Vermaas for making the photo plates, and M. Starink for DNA sequencing. Dr I. Pascoe is thanked for his opinions and the generic affinity of Phacidiella eucalypti. This study would not have been possible without the numerous specimens placed at our disposal, for which we are eternally grateful to Dr I.W. Smith, Prof. Z.A. Pretorius and Mrs I. van Iperen.

References


Fungal Diversity


(Received 14 November 2006; accepted 11 January 2007)