



Genera of diaporthean coelomycetes associated with leaf spots of tree hosts

P.W. Crous¹, B.A. Summerell², A.C. Alfenas³, J. Edwards⁴, I.G. Pascoe⁴, I.J. Porter⁴, J.Z. Groenewald¹

Key words

leaf spot disease
molecular phylogeny
systematics

Abstract Four different genera of diaporthean coelomycetous fungi associated with leaf spots of tree hosts are morphologically treated and phylogenetically compared based on the DNA sequence data of the large subunit nuclear ribosomal DNA gene (LSU) and the internal transcribed spacers and 5.8S rRNA gene of the nrDNA operon. These include two new Australian genera, namely *Auratiopycnidiella*, proposed for a leaf spotting fungus occurring on *Tristaniopsis laurina* in New South Wales, and *Disculoides*, proposed for two species occurring on leaf spots of *Eucalyptus* leaves in Victoria. Two new species are described in *Aurantiosacculus*, a hitherto monotypic genus associated with leaf spots of *Eucalyptus* in Australia, namely *A. acutatus* on *E. viminalis*, and *A. eucalyptorum* on *E. globulus*, both occurring in Tasmania. Lastly, an epitype specimen is designated for *Erythrogloeum hymenaeae*, the type species of the genus *Erythrogloeum*, and causal agent of a prominent leaf spot disease on *Hymenaea courbaril* in South America. All four genera are shown to be allied to *Diaportheales*, although only *Aurantiosacculus* (*Cryphonectriaceae*) could be resolved to family level, the rest being *incertae sedis*.

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INTRODUCTION

The present study reports on four different diaporthean genera of coelomycetes associated with leaf spots of different tree hosts recovered by the authors as part of extensive surveys collecting fungi associated with foliar diseases of woody hosts (Summerell et al. 2006, Crous et al. 2007a, b, 2009b, Cheewangkoon et al. 2009, 2010). Genera of plant pathogenic coelomycetes are commonly associated with leaf spots of tree hosts (Sutton 1980), several of which are regarded as economically important (Park et al. 2000). Because of their relatively simple morphology, genera of coelomycetous fungi are notoriously difficult to identify. The problem is further exemplified by the fact that very few of these taxa are represented in culture collections, or known from DNA sequence data (Crous & Groenewald 2005). This is particularly true for genera of diaporthean fungi, where numerous genera remain poorly known in spite of their importance as plant pathogens (Castlebury et al. 2002, Adams et al. 2005, Gryzenhout et al. 2006, Mostert et al. 2005).

One such genus is *Auratiopycnidiella*, which is thus far only known from Australia, where it is associated with leaf spots of *Eucalyptus baxteri*, *E. incrassata* and *E. obliqua* (Dyko et al. 1979, Marshall 1997). The genus is characteristic in that it has bright orange conidiomata, and hyaline, aseptate, scolecosporous conidia with thickened hila. Based on its characteristic conidiomata, Dyko et al. (1979) speculated that it could be allied to hypocrealean fungi. The genus is monotypic, based on *A. eucalypti*, is not known from culture, and thus far its phylogenetic affinity remains unresolved.

The genus *Erythrogloeum* is monotypic, based on *Erythrogloeum hymenaeae*, a fungus first invalidly described as "*Phyllosticta hymenaeae*" and later validated by Petrak (1953) as *E. hymenaeae*. The fungus is associated with a severe anthracnose of apical plant parts of *Hymenaea* spp., causing seedling mortality in nurseries during rainy periods (Ferreira et al. 1992). The disease is well established in the Dominican Republic and Costa Rica, and has in recent years also spread throughout the states of Pará, Maranhão, Espírito Santo, Minas Gerais and the Federal District of Brazil. Although an important pathogen of *Hymenaea* spp., the genus *Erythrogloeum* is insufficiently known, and in the absence of phylogenetic data, has been regarded as *incertae sedis*.

Two genera are also newly described from Australia, one proposed for a leaf spotting fungus occurring on *Tristaniopsis laurina*, a member of *Myrtaceae* closely related to *Eucalyptus*, and another for two species occurring on leaf spots of *Eucalyptus* leaves. The aim of the present study was thus to clarify the phylogenetic affinity of *Auratiopycnidiella* and *Erythrogloeum*, and also to resolve the taxonomy of novel leaf spotting fungi isolated from *Tristaniopsis* and *Eucalyptus*.

MATERIALS AND METHODS

Isolates

Symptomatic leaves were placed in damp chambers, and incubated at room temperature for 1–2 d. Single conidial colonies were established from sporulating conidiomata on Petri dishes containing 2 % malt extract agar (MEA; Crous et al. 2009c) as described earlier (Crous et al. 1991). Colonies were subcultured onto potato-dextrose agar (PDA), oatmeal agar (OA), MEA (Crous et al. 2009c), autoclaved pine needles on synthetic nutrient poor agar (PNA) (Crous et al. 2006), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Reference strains are maintained in the CBS-KNAW Fungal Biodiversity Centre (CBS) Utrecht, The Netherlands.

¹ CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; corresponding author e-mail: p.crous@cbs.knaw.nl.

² Royal Botanic Gardens and Domain Trust, Mrs Macquaries Road, Sydney, NSW 2000, Australia.

³ Departamento de Fitopatologia, Universidade Federal de Viçosa, 36.570 Viçosa, MG, Brazil.

⁴ Biosciences Research Division, Department of Primary Industries, P. Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia.

Table 1 Fungal isolates included in the morphological and/or phylogenetic analyses.

Species	Culture accession numbers ¹	Substrate	Country	Collector	GenBank accession numbers ²			
					ITS	LSU	TUB	CAL
<i>Aurantiosacculus acutatus</i>	CPC 13704; CBS 132181	Leaves of <i>Eucalyptus viminalis</i>	Australia, Tasmania	B.A. Summerell & P. Summerell	JQ685514	JQ685520		
<i>Aurantiosacculus eucalyptorum</i>	CPC 13229; CBS 130826	Leaves of <i>Eucalyptus globulus</i>	Australia, Tasmania	C. Mohammed & M. Glen	JQ685515	JQ685521		
<i>Auratioporicidiella tristaniopsis</i>	CPC 16371; CBS 132180	Leaves of <i>Tristaniopsis laurina</i>	Australia, New South Wales, North Washpool State forest	B.A. Summerell	JQ685516	JQ685522	JQ685526	JQ685529
<i>Disculoides eucalypti</i>	CPC 17650; CBS 132183	Leaves of <i>Eucalyptus</i> sp.	Australia, Victoria, Melbourne	P.W. Crous, J. Edwards, I.J. Porter & I.G. Pascoe	JQ685517	JQ685523	JQ685527	JQ685530
<i>Disculoides eucalyptorum</i>	CPC 17648; CBS 132184	Leaves of <i>Eucalyptus viminalis</i>	Australia, Victoria, Woorndoo	P.W. Crous, J. Edwards, I.J. Porter & I.G. Pascoe	JQ685518	JQ685524	JQ685528	JQ685531
<i>Erythrogloeum hymenaeae</i>	CPC 18819; CBS 132185	Leaves of <i>Hymenaea coubaril</i>	Brazil, Minas Gerais, Viçosa	A. C. Afenias	JQ685519	JQ685525		

¹ CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS.

² LSU: partial 28S nrRNA gene; ITS: internal transcribed spacer regions 1 & 2 including 5.8S nrRNA gene; TUB: partial beta-tubulin gene; CAL: partial calmodulin gene.

DNA isolation, amplification and analyses

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocol. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S nrRNA gene, the first internal transcribed spacer (ITS1), the 5.8S nrRNA gene, the second ITS region (ITS2) and approximately 900 bp of the 5' end of the 28S nrRNA gene. The primers ITS4 (White et al. 1990) and LSU1Fd (Crous et al. 2009a) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. For species delimitation, the ITS region was used and in some cases supplemented with the partial gene sequences for calmodulin (CAL), determined using the primers CAL-228F (Carbone & Kohn 1999) and CAL-737R (Carbone & Kohn 1999) or CAL2Rd (Quaedvlieg et al. 2011) and beta-tubulin (TUB), amplified and sequenced using the primers T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995). Amplification conditions followed Lee et al. (2004).

The sequence alignment and subsequent phylogenetic analyses for the LSU and ITS data were carried out using methods described by Crous et al. (2006). Gaps were treated as 'fifth state' data in the parsimony analysis. Sequence data were deposited in GenBank (Table 1) and the alignments and trees in TreeBASE (<http://www.treebase.org>). Remaining sequence data is discussed under the species notes below.

Morphology

Slide preparations were mounted in clear lactic acid from colonies sporulating on MEA, PDA or OA. Observations were made with a Zeiss V20 Discovery stereo-microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an AxioCam MRc5 camera and software. Colony characters and pigment production were noted after 1 mo of growth on MEA and OA (Crous et al. 2009c) incubated at 25 °C. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970). Nomenclatural novelties and descriptions were deposited in MycoBank (Crous et al. 2004).

RESULTS

Phylogeny

Amplicons of approximately 1 600 bases were obtained for ITS (including the first approx. 900 bp of LSU) of the isolates listed in Table 1. The manually adjusted LSU alignment contained 34 sequences (including the outgroup sequence) and 762 characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analyses; 68 of these were parsimony-informative, 42 were variable and parsimony-uninformative, and 652 were constant. Neighbour-joining analyses using three substitution models on the sequence alignment yielded identical tree topologies to those of the parsimony analysis (Fig. 1a). Thirteen equally most parsimonious trees were saved, the first of which is shown in Fig. 1a (TL = 187 steps; CI = 0.647; RI = 0.796; RC = 0.515).

Bayesian analysis was conducted on the same aligned LSU dataset using a general time-reversible (GTR) substitution model with inverse gamma rates and dirichlet base frequencies. The Markov Chain Monte Carlo (MCMC) analysis of two sets of 4 chains started from a random tree topology and lasted 1 121 000 generations, after which the split frequency reached less than 0.01. Trees were saved each 1 000 generations, resulting in 2 242 saved trees. Burn-in was set at 25 %, leaving

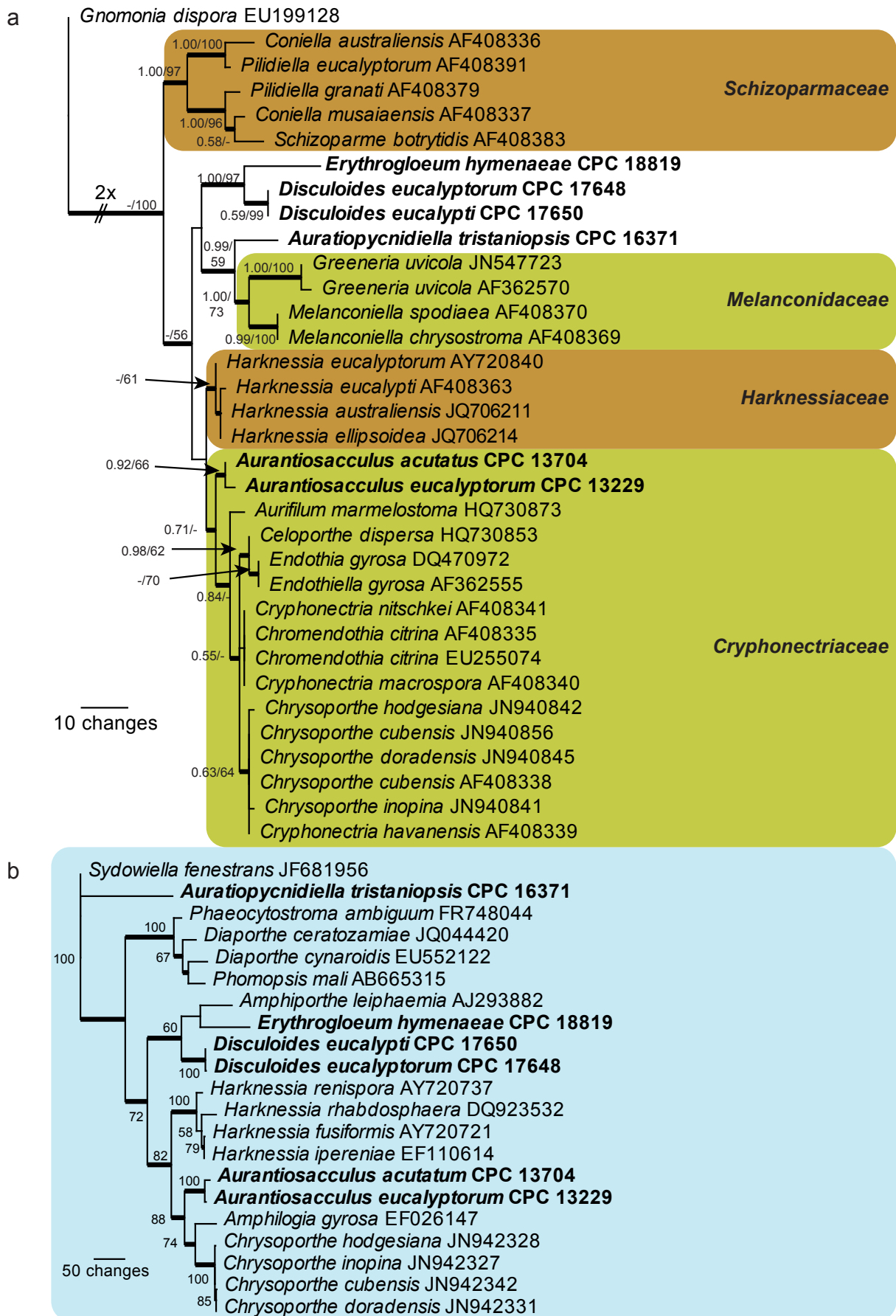


Fig. 1 Molecular phylogenetic trees generated in this study. a. The first of 13 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 10 changes, and posterior probability (PP) and maximum parsimony (MPBS) bootstrap support values from 1 000 replicates are shown at the nodes (PP/MPBS). Families are indicated to the right of the tree. Branches present in the parsimony strict consensus tree are thickened and novel sequences indicated in **bold**. The tree was rooted to a sequence of *Gnomonia dispora* (GenBank accession EU199128); b. the first of six equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the ITS sequence alignment. The scale bar shows 50 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Branches present in the parsimony strict consensus tree are thickened and novel sequences indicated in **bold**. The tree was rooted to a sequence of *Sydowiella fenestrans* (GenBank accession JF681956).

1 682 trees from which the consensus tree and posterior probabilities (PPs) were calculated.

A comparison between the tree topologies obtained through the Bayesian, parsimony and distance analyses yielded mostly the same terminal clades, corresponding to the families as they are delimited in Fig. 1a. The only difference was that the *Harknessia* spp. were collapsed to a basal polytomy in the Bayesian analysis. *Erythrogloeum* and *Disculoides* formed a separate clade in all the analyses and *Auratiopycniidiella* was always sister to the clade containing *Greeneria* and *Melanconiella* (both *Melanconidaceae*). *Aurantiosacculus* always clustered as a basal sister clade to *Cryphonectriaceae*.

The manually adjusted ITS alignment contained 21 sequences (including the outgroup sequence) and 585 characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analyses; 209 of these were parsimony-informative, 69 were variable and parsimony-uninformative and 307 were constant. Neighbour-joining analyses using three substitution models on the sequence alignment yielded identical tree topologies to one another but differed from the parsimony analysis (Fig. 1b) with regard to the placement of some of the deeper nodes, for example the position of *Amphilogia gyrosa* as either a sister to the *Chrysosporthe* clade or the *Aurantiosacculus* clade. Six equally most parsimonious trees were saved, the first of which is shown in Fig. 1b (TL = 767 steps; CI = 0.671; RI = 0.723; RC = 0.485).

Taxonomy

Auratiopycniidiella Crous & Summerell, *gen. nov.* — MycoBank MB564733

Type species. *Auratiopycniidiella tristaniopsis* Crous & Summerell, sp. nov.

Etymology. Named after its characteristic orange pycnidia.

Follicolous, conidiomata associated with leaf spots, amphigenous, globose, orange on leaves with dark brown border, pycnidial with irregular central opening; wall of 4–7 layers of pale brown *textura angularis*. *Paraphyses* hyaline, cellular,

subcylindrical, branched or not, with obtuse apex, septate, constricted at septa. *Conidiophores* reduced to conidiogenous cells, hyaline, smooth, lageniform to ampulliform, with terminal truncate locus, frequently somewhat thickened, and in a few cases appearing to proliferate percurrently. *Conidia* ellipsoid, smooth, solitary, medianly 1-septate, constricted at septum (disarticulating in some cases) apex obtuse, base truncate, thickened, at times with marginal frill, becoming golden brown during germination, germinating with solitary, brown, wavy germ tubes, 90° to the long axis on the spore.

Auratiopycniidiella tristaniopsis Crous & Summerell, *sp. nov.* — MycoBank MB564734; Fig. 2

Etymology. Named after the host genus from which it was collected, *Tristaniopsis*.

Conidiomata sporulating on brown leaf spots, intermingled with a *Pseudocercospora* sp.; conidiomata amphigenous, globose, up to 200 µm diam, orange on leaves with dark brown border, delimiting it from host tissue, pycnidial with irregular central opening; wall of 4–7 layers of pale brown *textura angularis*, up to 25 µm thick, exuding orange conidial masses on host, but luteous on OA and PNA. *Paraphyses* hyaline, cellular, subcylindrical, branched or not, with obtuse apex, 2–6-septate, constricted at septa, up to 50 µm long, 3–5 µm diam. *Conidiophores* reduced to conidiogenous cells, hyaline, smooth, lageniform to ampulliform, 10–25 × 3–6 µm, with terminal truncate locus, 1–1.5 µm diam, frequently somewhat thickened, and in a few cases appearing to proliferate percurrently. *Conidia* (11–)13–15(–16) × (4–)5–5.5(–6) µm, ellipsoid, smooth, solitary, medianly 1-septate, constricted at septum (disarticulating in some cases) apex obtuse, base truncate, 2 µm diam, thickened, at times with marginal frill, becoming golden brown during germination, germinating with solitary, brown, wavy germ tubes, 90° to the long axis on the spore.

Culture characteristics — After 1 mo at 25 °C in the dark, covering the plate, flat, spreading, with lobate, feathery margins, and moderate, ropey aerial mycelium. On PDA pale luteous on surface and reverse; on OA dirty white to pale luteous; on MEA ochreous on surface, umber in reverse; fertile on OA and PNA.



Fig. 2 *Auratiopycniidiella tristaniopsis* (GPC 16371). a. Leaf spot symptoms; b. close-up of orange pycnidia; c. colony forming conidioma on OA; d–f. conidiogenous cells giving rise to conidia; g, h. conidia; i. disarticulating conidial cells; j. germinating conidium. — Scale bars = 10 µm.

Specimen examined. AUSTRALIA, New South Wales, North Washpool State Forest, S29°08'43.9" E152°24'90", on leaves of *Tristaniopsis laurina*, 27 Apr. 2009, B.A. Summerell (CBS H-20932 holotype, cultures ex-type CPC 16371 = CBS 132180).

Notes — We are presently not aware of any genus that has this combination of unique characters among those treated by Sutton (1980), namely subepidermal, orange, pycnidial conidiomata, giving rise to hyaline, tightly aggregated holoblastic conidiogenous cells that at times proliferate percurrently, with or without a thickened scar, giving rise to hyaline, ellipsoid, 1-septate conidia that can disarticulate with age, have a thickened hilum or minute marginal frill, and turn brown upon germination, germinating with a thin, wavy germ tube, 90° to the long axis of the spore. Based on its LSU sequence, the species appears to be distantly allied to *Melanconidaceae*, but this family is paraphyletic in *Diaporthales* (Lamprecht et al. 2011), and therefore it would be best to treat this genus as *incertae sedis* pending the availability of more molecular data of the families in the order. A megablast search of NCBI's GenBank nucleotide database using the ITS, TUB and CAL sequences only retrieved sequence identities of less than 90 % with *Harknessia* (ITS and TUB) and 80 % with *Amphilogia* and *Cryptosporella* (CAL), confirming its association with *Diaporthales*.

Aurantiosacculus Dyko & B. Sutton, *Mycologia* 71: 922. 1979.

Type species. *Aurantiosacculus eucalypti* (Cooke & Masee) Dyko & B. Sutton, *Mycologia* 71: 924. 1979.

Typus. AUSTRALIA, Victoria, Melbourne, on leaves of *Eucalyptus incrassata*, Reader no. 24 (holotypus K).

Associated with amphigenous, brown leaf spots. *Conidiomata* amphigenous, eustromatic, subepidermal, becoming erumpent, rupturing epidermis, appearing bright orange; subglobose to flattened, with ostiole central, opening via irregular flaps in upper layer of conidioma. *Conidiophores* subcylindrical, septate, hyaline, smooth, lining the inner layer of cavity, unbranched or branched below. *Conidiogenous cells* lageniform to subcylindrical, hyaline, smooth, integrated, determinate; apex with minute periclinal thickening and collarette. *Conidia* hyaline, smooth, aseptate, sigmoid, apex obtuse to subobtuse, base swollen, obtuse with central, thickened, somewhat refractive scar, at times with marginal frill.

Notes — Presently species of *Aurantiosacculus* have only been reported from Australia (Dyko et al. 1979), where they are associated with leaf spots on *Eucalyptus baxteri*, *E. incrassata* and *E. obliqua* (Dyko et al. 1979, Marshall 1997). The genus

has characteristic leaf spots, with bright orange conidiomata, and aseptate conidia with swollen bases and thickened scars. Based on its bright orange conidiomata, Dyko et al. (1979) originally speculated that it could be allied to hypocrealean fungi. As shown in the present study, however, *Aurantiosacculus* belongs to *Diaporthales*, and erumpent, orange conidiomata with brown furfuraceous tissue is not uncommon in the order, with groups like the *Harknessia*-complex (Lee et al. 2004), or *Crysoporthe* (*Cryphonectriaceae*) (Gryzenhout et al. 2004).

Aurantiosacculus acutatus Crous & Summerell, *sp. nov.* — MycoBank MB564735; Fig. 3

Typus. AUSTRALIA, Tasmania, Crescent Bay, S43°11'13.9", E147°50'50.7", on leaves of *Eucalyptus viminalis*, 14 Oct. 2006, B.A. Summerell & P. Summerell (CBS H-20933 holotypus, cultures ex-holotype CPC 13704 = CBS 132181).

Etymology. Named after its acutely tapered conidia.

Leaf spots amphigenous, pale brown, irregular to subcircular, frequently situated along leaf margins, up to 5 cm long, with thin, dark brown border. *Conidiomata* amphigenous, eustromatic, subepidermal, becoming erumpent, rupturing epidermis, appearing bright orange; subglobose to flattened, up to 600 µm diam; ostiole central, but opening via irregular flaps in upper layer of conidioma. *Conidiophores* subcylindrical, 0(–2)-septate, hyaline, smooth, lining the inner layer of cavity, at times branched below, 10–20 × 2.5–3.5 µm. *Conidiogenous cells* lageniform to subcylindrical, hyaline, smooth, integrated, determinate, 7–15 × 2.5–3 µm; apex with minute periclinal thickening and collarette. *Conidia* hyaline, smooth, aseptate, sigmoid, apex subobtuse, base swollen, obtuse with central, thickened, somewhat refractive scar, 1–1.5 µm diam, at times with marginal frill, (40–)50–57(–67) × (2–)2.5(–3) µm.

Culture characteristics — Colonies after 2 wk in the dark up to 50 mm diam, with even, lobate margins and moderate aerial mycelium. On PDA, OA and MEA white on surface, salmon in reverse. Fertile on PNA and OA.

Notes — Conidia of *A. acutatus* are shorter and narrower than those of *A. eucalypti* (51–81 × 2–3 µm; Dyko et al. 1979) and *A. eucalyptorum* (48–67 × 2.5–4 µm), and are quite distinct in having a more prominent apical taper. Based on the LSU sequences of the species included in this study, the genus appears to be allied with *Cryphonectriaceae*. A megablast search of NCBI's GenBank nucleotide database using the ITS sequence confirms the placement of these species with approximately 95 % identity to sequences of *Cryphonectria* and *Chrysosporthe*.

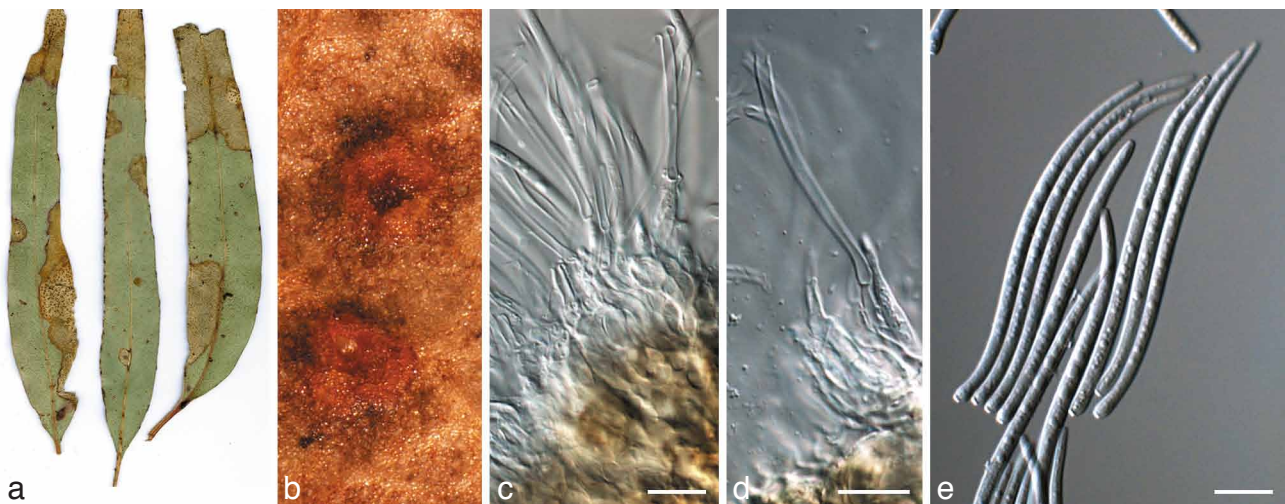


Fig. 3 *Aurantiosacculus acutatus* (CPC 13704). a. Leaf spot symptoms; b. close-up of orange pycnidia; c, d. conidiogenous cells giving rise to conidia; e. conidia. — Scale bars = 10 µm.

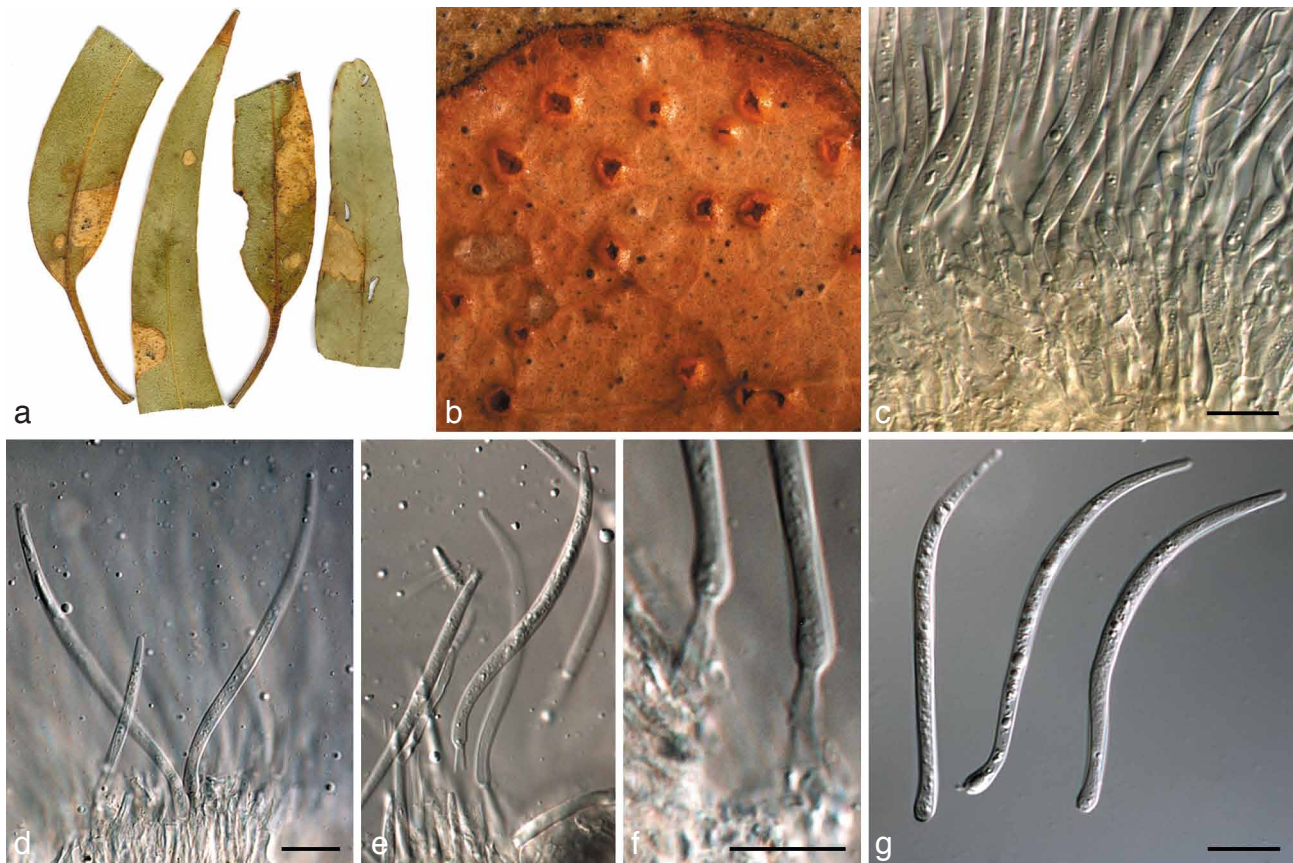


Fig. 4 *Aurantiosacculus eucalyptorum* (CPC 13229). a. Leaf spot symptoms; b. close-up of orange pycnidia; c–f. conidiogenous cells giving rise to conidia; g. conidia. — Scale bars = 10 µm.

Aurantiosacculus eucalyptorum Crous & C. Mohammed,
sp. nov. — MycoBank MB564736; Fig. 4

Etymology. Named after the host genus from which it was collected, *Eucalyptus*.

Leaf spots amphigenous, pale brown, irregular to subcircular, 2–35 mm diam, with thin, dark brown border. *Conidiomata* amphigenous, eustromatic, subepidermal becoming erumpent, rupturing epidermis, appearing characteristically bright orange, somewhat subglobose to flattened, up to 800 µm diam; ostiole central, but frequently opening via irregular slits giving rise to flaps. *Conidiophores* subcylindrical, 0(–2)-septate, hyaline, smooth, lining the inner layer of cavity, at times branched below, 5–20 × 2.5–4 µm. *Conidiogenous cells* lageniform to subcylindrical, hyaline, smooth, integrated, determinate, 12–30 × 2.5–3 µm; apex with minute periclinal thickening and collarette. *Conidia* hyaline, smooth, aseptate, sigmoid, apex subobtuse, base swollen, obtuse with central, thickened, somewhat refractive scar (2 µm diam), at times with marginal frill, (48–)55–60(–67) × (2.5–)3–3.5(–4) µm.

Culture characteristics — Colonies after 2 wk in the dark up to 70 mm diam, with even, lobate margins and sparse to moderate aerial mycelium. On PDA dirty white, on OA saffron, on MEA salmon on surface and reverse. Fertile on PNA and OA.

Specimen examined. AUSTRALIA, Tasmania, on leaves of *Eucalyptus globulus*, 31 Aug. 2006, C. Mohammed & M. Glen (CBS H-20934 holotypus, cultures ex-holotype CPC 13229 = CBS 130826).

Notes — Conidia of *A. eucalyptorum* (48–67 × 2.5–4 µm) are shorter and wider than those of *A. eucalypti* (51–81 × 2–3 µm; Dyko et al. 1979). Based on ITS sequences, *A. acutatus* and *A. eucalyptorum* differ by 10 nucleotide differences (Identities = 716/726 (99 %), Gaps = 4/726 (1 %)). Unfortunately, there are presently no cultures available of *A. eucalypti* and thus it could not be included in these analyses.

Disculoides Crous, Pascoe, I.J. Porter & Jacq. Edwards, gen.
nov. — MycoBank MB564737

Type species. *Disculoides eucalypti* Crous, Pascoe, I.J. Porter & Jacq. Edwards, sp. nov.

Etymology. Named after its morphological similarity to the genus *Discula*.

Follicolous, associated with leaf spots. *Conidiomata* brown to black, amphigenous, subepidermal, acervular, opening by irregular rupture; wall of 2–3 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity of conidioma, hyaline to olivaceous, smooth, subcylindrical to ampulliform, tapering to a long, thin neck, proliferating several times percurrently near apex, with flaring collarettes. *Conidia* olivaceous, smooth, guttulate, ellipsoid to fusoid to somewhat obclavate, straight to curved, apex subobtuse, base truncate, with prominent marginal frill, up to 1 µm long.

Disculoides eucalypti Crous, Pascoe, I.J. Porter & Jacq.
Edwards, sp. nov. — MycoBank MB564738; Fig. 5

Etymology. Named after the host genus from which it was collected, *Eucalyptus*.

Leaf spots amphigenous, subcircular to irregular, 1–18 mm diam, pale to medium brown, with prominent, wide red-purple margin. *Conidiomata* brown to black, amphigenous, subepidermal, acervular, opening by irregular rupture, up to 350 µm diam; wall of 2–3 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity of conidioma, hyaline to olivaceous, smooth, subcylindrical to ampulliform, tapering to a long, thin neck, 8–25 × 3–5 µm, proliferating several times percurrently near apex, with flaring collarettes. *Conidia* olivaceous, smooth, guttulate, ellipsoid to fusoid to somewhat obclavate,



Fig. 5 *Disculoides eucalypti* (CPC 17650). a. Leaf spot symptoms; b. close-up of lesion; c–e. conidiogenous cells giving rise to conidia; f. conidia with basal marginal frill; g. conidia. — Scale bars = 10 μ m.

straight to curved, (13–)14–16(–17) \times (4–)5–6(–6.5) μ m in vivo, (16–)17–20(–23) \times (5–)6–6.5(–7) μ m in vitro on PNA, apex subobtuse, base truncate, 1–1.5 μ m diam with prominent marginal frill, up to 1 μ m long.

Culture characteristics — Colonies spreading, flat with sparse to moderate aerial mycelium, and smooth to feathery, lobate margins; covering dish after 1 mo. On MEA surface

dirty white to pale luteous, reverse umber in middle, luteous in outer region. On OA luteous with patches of umber due to conidiomatal development, lacking aerial mycelium. On PDA surface and reverse luteous, lacking aerial mycelium.

Specimen examined. AUSTRALIA, Victoria, Melbourne, S38°24'3.1" E144°59'36.9", on leaves of *Eucalyptus* sp., 12 Oct. 2009, P.W. Crous, J. Edwards, I.J. Porter & I.G. Pascoe (CBS H-20935 holotype, cultures ex-type CPC 17650 = CBS 132183).

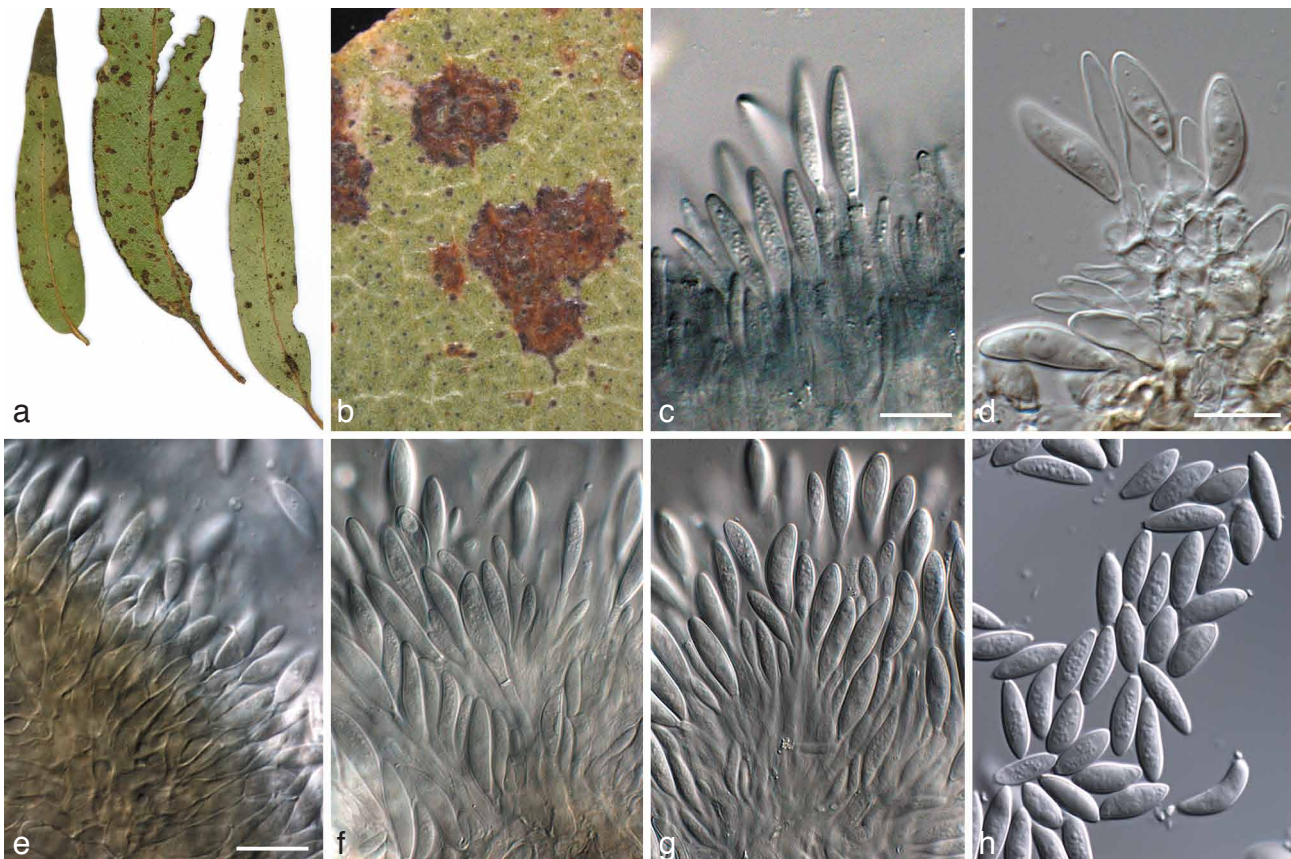


Fig. 6 *Disculoides eucalyptorum* (CPC 17648). a. Leaf spot symptoms; b. close-up of lesions; c–g. conidiogenous cells giving rise to conidia; h. conidia. — Scale bars = 10 μ m.

Notes — Based on its LSU sequence, the species clusters in *Diaporthales* distinct from the other families currently known from sequence, and therefore it would be best to treat this genus as *incertae sedis* within the order (Fig. 1).

Disculoides eucalyptorum Crous, Pascoe, I.J. Porter & Jacq. Edwards, *sp. nov.* — MycoBank MB564739; Fig. 6

Etymology. Named after the host genus from which it was collected, *Eucalyptus*.

Leaf spots amphigenous, subcircular to irregular, 1–8 mm diam, brown to red-brown with raised margin and chlorotic border. *Conidiomata* brown to black, amphigenous, subepidermal, acervular, opening by irregular rupture, up to 250 µm diam; wall of 2–3 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity of conidioma, hyaline to olivaceous, smooth, subcylindrical to ampulliform, tapering to a long, thin neck, 10–15 × 3–6 µm, proliferating several times percurrently near apex, with flaring collarettes. *Conidia* olivaceous, smooth, guttulate, ellipsoid to obclavate to fusoid, straight to curved, (12–)14–16(–18) × (4–)5–6(–6.5) µm *in vivo*, (12–)14–18(–20) × (4.5–)5(–5.5) µm *in vitro* on PNA, apex subobtuse, base truncate, 1–1.5 µm diam with prominent marginal frill, up to 1 µm long.

Culture characteristics — Colonies spreading, with moderate aerial mycelium and lobate, smooth to feathery margins. On MEA surface dirty white in centre, cream in outer region, underneath umber with patches of luteous. On OA luteous to ochreous with dirty white aerial mycelium. On PDA aerial mycelium sparse, surface orange to luteous, reverse saffron; colonies covering dish after 1 mo on OA and MEA, but reaching only 60 mm diam on PDA.

Specimen examined. AUSTRALIA, Victoria, Woorndoo, S37°53'29.3" E142°47'51.1", on leaves of *Eucalyptus viminalis*, 17 Oct. 2009, P.W. Crous, J. Edwards, I.J. Porter & I.G. Pascoe (CBS H-20936 holotype, cultures ex-type CPC 17648 = CBS 132184).

Notes — The genus *Disculoides* resembles *Discula*, but clusters related to the *Harknessia*-complex/*Cryphonectriaceae*/*Schizoparmaceae* (Fig. 1), whereas *Discula* is allied to *Gnomo-*

niaceae, *Diaporthales* (Castlebury et al. 2002). Furthermore, although conidia of *Disculoides* appeared to be hyaline on host material, they tended to become olivaceous when sporulating on PNA. *Disculoides eucalypti* can be distinguished from *D. eucalyptorum* in that the latter has smaller conidia. Based on nucleotide sequence comparisons, *D. eucalypti* and *D. eucalyptorum* are genetically highly similar based on their ITS sequences (ITS: Identities = 692/693 (99 %), Gaps = 0/693 (0 %), but they can be distinguished based on their CAL and TUB sequences (CAL: Identities = 702/706 (99 %), Gaps = 0/706 (0 %); TUB: Identities = 835/841 (99 %), Gaps = 0/841 (0 %)).

Erythrogloeum Petr., Sydowia 7: 378. 1953.

Type species: *Erythrogloeum hymenaeae* Gonz. Frag. & Cif. ex Petr., Sydowia 7: 379. 1953.

Follicolous, associated with leaf spots. *Conidiomata* acervular, epiphyllous, eustromatic, subepidermal, separate, rupturing surface by means of irregular splits; wall of thin-walled *textura angularis*, but sides appearing dark brown to black, exuding luteous to orange conidial masses. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, smooth, phialidic with periclinal thickening, discrete, lageniform to cylindrical, lining the inner walls of cavity. *Conidia* hyaline, smooth, guttulate or not, thin-walled, ellipsoid to ovoid, apex obtusely rounded, tapering to a truncate base.

Erythrogloeum hymenaeae Gonz. Frag. & Cif. ex Petr., Sydowia 7: 379. 1953. — Fig. 7

Leaf spots solitary, irregular to subcircular, medium brown, 2–25 mm diam. *Conidiomata* acervular, epiphyllous, eustromatic, subepidermal, separate, up to 250 µm diam, rupturing surface by means of irregular splits; wall of thin-walled *textura angularis*, but sides appearing dark brown to black, exuding luteous to orange conidial masses. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, smooth, phialidic with periclinal thickening, discrete, lageniform to cylindrical, lining the inner walls of cavity, 5–10 × 2.5–4 µm. *Conidia*

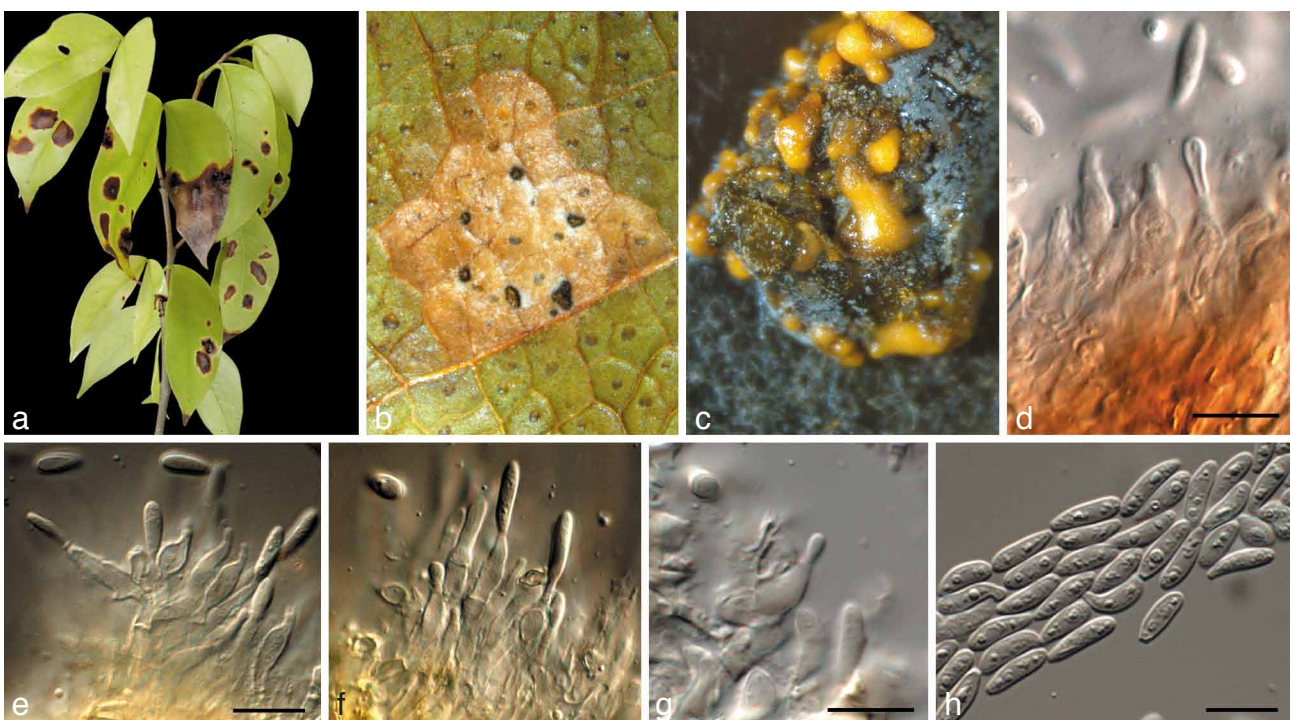


Fig. 7 *Erythrogloeum hymenaeae* (CPC 18819). a. Leaf spot symptoms; b. close-up of leaf spot; c. colony with yellow sporulation in culture on PDA; d–g. conidiogenous cells giving rise to conidia; h. conidia. — Scale bars = 10 µm.

hyaline, smooth, guttulate or not, thin-walled, ellipsoid to ovoid, apex obtusely rounded, tapering to a truncate base (1–1.5 µm diam), (4.5–)7–9(–10) × 2.5–3(–3.5) µm in vitro.

Culture characteristics — After 1 mo at 25 °C in the dark, reaching 70 mm diam on OA, 50 mm on MEA and 25 mm diam on PDA; fertile on all media. On PDA flat, spreading, umber on surface and underneath, with sparse, pale luteous aerial mycelium and smooth, lobate margins, sporulating with bright orange exuding conidial masses. On MEA somewhat erumpent, bay in centre due to profuse sporulation, dirty white in outer region, sparse aerial mycelium, smooth, lobate margins, bay underneath, becoming pale luteous towards outer region. On OA bay in centre due to profuse sporulation, moderate aerial mycelium, buff to honey to amber; margin smooth, lobate.

Specimens examined. BRAZIL, Minas Gerais, Viçosa, Nursery (José Geraldo – Paraíso), on leaves of *Hymenaea courbaril*, 11 Oct. 2010, A.C. Alfenas (CBS H-20937 epitype designated here, cultures ex-epitype CPC 18819 = CBS 132185). – DOMINICAN REPUBLIC, Haina, on leaves of *Hymenaea courbaril*, Oct. 1925, R. Ciferri (no. 4486), syntype BPI 352761; Moca, National Agronomy Station, on leaves of *H. courbaril*, 7 July 1929, R. Ciferri, BPI 352760; La Vega, Road Jarabacoa, on leaves of *H. courbaril*, 7 Oct. 1926, R. Ciferri, BPI 352762.

Notes — The name *Erythrogloeum hymenaeae* was published by Petrak (1953), based on a basionym (*Phyllosticta hymenaeae*) that was never validly published, and hence the publication of Petrak was accepted as the first valid publication of the epithet. The disease on *Hymenaea* is common in the Dominican Republic, and has also been observed in Costa Rica, and in recent years has spread in the states of Pará, Maranhão, Espírito Santo, Minas Gerais and the federal District. The fungus, which causes leaf spots and anthracnose of *Hymenaea* spp., is especially serious in the rainy season (Ferreira et al. 1992). The syntype specimen was located in BPI and examined (BPI 352761), which is morphologically identical to the epitype specimen designated here. Based on its LSU sequence, *Erythrogloeum* should be accommodated in *Diaporthales*, but without a clear family affinity (Fig. 1a). A megablast search of NCBI's GenBank nucleotide database using the ITS sequence confirms the placement of the species in *Diaporthales* but, as with the LSU sequence, does not reveal a significant association with any genera currently available in the nucleotide database.

DISCUSSION

The present study treats four different genera of coelomycetous fungi that were found to be associated with leaf spot symptoms on diverse tree hosts. Surprisingly, all four genera were shown to be members of the order *Diaporthales*. Although the phylogeny of *Diaporthales* is still poorly resolved in most phylogenetic studies published on the subject (Castlebury et al. 2002), the order includes some well known canker pathogens such as members of the *Cryphonectria* canker complex (Gryzenhout et al. 2004, 2006), *Discula* anthracnose (Green & Castlebury 2006), *Cytospora* canker (Adams et al. 2005), *Diaporthe* cankers (Mostert et al. 2001, van Niekerk et al. 2005, van Rensburg et al. 2006, Santos et al. 2011, Thompson et al. 2011), decline disease pathogens such as *Phaeoacremonium* (Groenewald et al. 2001, Mostert et al. 2003, 2006, Essakhi et al. 2008), some of which are also associated with phaeohyphomycosis in humans (Mostert et al. 2005), and leaf pathogens such as *Pilidiella* and *Coniella* (van Niekerk et al. 2004, Rossman et al. 2007) and *Harknessia* (Crous et al. 1993, Lee et al. 2004, Crous et al. 2007a), to name but a few.

Aurantiosacculus has thus far been regarded as an obscure genus, originally reported on leaves of *Eucalyptus obliqua* in Australia (Dyko et al. 1979), and later also recorded from leaf

spots on *E. baxteri* (Marshall 1997). Thus far all known records are from the state of Victoria (Australia), and the two species added to the genus in the present study represent the first reports from Tasmania, and may indicate that this genus is restricted to temperate parts of southern Australia. Of interest is the fact that although this genus was originally suspected to be hypocrealean due to its bright conidiomata, it in fact turned out to be a member of the *Cryphonectriaceae* (Gryzenhout et al. 2006), which is well known for its erumpent conidiomata with bright brown furfuraceous margins (Rossman et al. 2007). The genus *Erythrogloeum* represents yet another obscure genus of coelomycetes, initially described to accommodate *E. hymenaeae*, a well-known pathogen of *Hymenaea* spp. in South America. Since this pathogen was first described from leaves of *Hymenaea courbaril* in the Dominican Republic (Petrak 1953), the pathogen has spread to neighbouring countries such as Costa Rica and Brazil, where it causes a serious leaf spot and anthracnose disease of *Hymenaea* spp. (Ferreira et al. 1992). The phylogenetic position of this rather obscure genus has been unknown to date, and hence it is surprising to find it to represent a member of *Diaporthales*, albeit with an unclear family affiliation. A second species was later added to the genus, namely *E. pini-acicola*, described from needles of *Pinus oocarpa* in Nicaragua (Evans 1984), though in the absence of cultures, it is presently unknown if this species is phylogenetically allied to *E. hymenaeae*.

The description of *Aurantiopycniella* and *Disculoides* add yet a further two genera to the *Diaporthales*, although they could not be placed in any currently circumscribed family. Both genera are associated with leaf spots of woody hosts in Australia. Although the erumpent, bright orange conidiomata of *Aurantiopycniella* suggest an affinity to other families in *Diaporthales* like the *Harknessia*-complex or *Cryphonectriaceae*, this is not the case for *Disculoides*, which is more obscure, and seems to be morphologically more allied to taxa presently accommodated in the *Discula* complex (*Gnomoniaceae*). Even though it is possible that there are older 'forgotten' genera that could accommodate *Aurantiopycniella* and *Disculoides*, we were not able to resolve their generic placement during the present study. These genera are thus introduced here as new, hoping that this would add further information to aid in our understanding of the numerous families and genera that occur in *Diaporthales*.

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