# **CHAPTER 6**

#### NEW AND INTERESTING EUCALYPT MICROFUNGI

#### **6.1 Introduction**

The family *Myrtaceae* represents close to 150 genera of evergreen, dicotyledon, woody plants known to produce a range of essential oils (Wilson *et al.*, 2001). Within the *Myrtaceae*, species belonging to the genera *Corymbia*, *Eucalyptus* and *Syzygium* are widespread in tropical and temperate regions of the Southern Hemisphere (Wilson *et al.*, 2001). *Eucalyptus* spp. are particularly abundant and have a wider range of distribution than other myrtaceous genera, as they are frequently grown as exotics in commercial plantations (Ball, 1995). Many members of the *Myrtaceae* contain a range of substrates and oils that support a highly diverse fungal community, making them favourable hosts to numerous plant pathogenic and saprobic fungi (Sankaran *et al.*, 1995a; Crous, 1999; Crous *et al.*, 1995c, 2001a, 2006f, 2007f–h; Sivanesan and Shivas, 2002; van Niekerk *et al.*, 2004; van Wyk *et al.*, 2004; Pavlic *et al.*, 2004, 2007; de Beer *et al.*, 2006; McKenzie *et al.*, 2006; Summerell *et al.*, 2006; Carnegie *et al.*, 2007).

Extending the distribution of *Myrtaceae* species (particularly by means of exotic plantations) will consequently increase the opportunity for these fungi to enter new habitats and explore new hosts, also accelerating their evolution. Furthermore, because the *Myrtaceae* represents such a large family, the majority of the fungi that occur on these hosts remain unstudied and undescribed, or have not yet been properly documented (Crous *et al.*, 2006d; Hyde *et al.*, 2007). Many cryptic fungal species were named (and even grouped) based on only wide and/or unspecific morphological characteristics. Recent developments in molecular techniques such as DNA sequence analysis allow mycologists to accurately distinguish these fungi and the various morphs in their lifecycles, thereby allowing a more precise classification (Hawksworth, 2004; Crous and Groenewald, 2005; Damm *et al.*, 2007; Phillips *et al.*, 2007; Shenoy *et al.*, 2007; Seifert,

2009), even though they may be similar in morphology (Crous *et al.*, 2001b, 2004c; Alves *et al.*, 2008). While the implementation of molecular techniques has led to a re-classification and integration of anamorph and teleomorph states, it also led to the recognition of numerous cryptic species (Crous *et al.*, 2006i).

Many fungi exhibit host specificity, indicating their dependency on a particular host species or group of related species from which they derive nutrients (Wong and Hyde, 2001; Zhou and Hyde, 2001). Within the host-specific fungi, many are able to switch their nutritional modes from being endophytic or pathogenic on living plants, to being saprobic on detached/dead plant tissues during host senescence (Zhou and Hyde, 2001, Hyde *et al.*, 2007; Promputtha *et al.*, 2007; Hyde and Soytong, 2008). Fungal pathogens may even grow as saprobes on non-host tissues that have been infected by other primary pathogenic species (Roy, 2001; Crous *et al.*, 2008). This contrasts with the suggestion by Ehrlich and Raven (1964) that pathogens generally colonise closely related hosts only. In order to distinguish fungi with different life styles, Roy (2001) proposed the use of two terms: 'host shift' for fungi shifting to closely related hosts. The host-changing ability can influence their genetic behavior and makeup, such as recombination (*Ophiostoma novo-ulmi*, Brasier, 2001) or hybridisation (*Phytophthora* sp., Brasier *et al.*, 1999; Brasier, 2000).

Thus far, fungi occurring on *Myrtaceae* have proven to be largely host family specific, and only a few examples are known to occur on different species or genera of *Myrtaceae*, or unrelated hosts. Presently these examples include species of *Harknessia* (Sutton and Pascoe, 1989; Crous *et al.*, 1993c, 2007f; Crous and Rogers, 2001; Lee *et al.*, 2004), *Cryphonectria cubensis* (Conradie *et al.*, 1990; van Zyl *et al.*, 1999; Gryzenhout *et al.*, 2006a; Nakabonge *et al.*, 2006), *Puccinia psidii* (Coutinho *et al.*, 1998), *Calonectria* (Victor *et al.*, 1997; Schoch *et al.*, 1999; Kang *et al.*, 2001a, b; Crous, 2002; Crous *et al.*, 2004d, 2006a), *Mycosphaerellaceae* and *Teratosphaeriaceae* (Crous and Wingfield, 1996; Crous, 1999; Crous *et al.*, 2004c, 2006i, 2007h, 2008, 2009b, d; Hunter *et al.*, 2004, 2006a, b; Carnegie *et al.*, 2007; Cheewangkoon *et al.*, 2008) and

*Botryosphaeriaceae* (Crous *et al.*, 2006d; Slippers *et al.*, 2004a – c; Pavlic *et al.*, 2007; Slippers and Wingfield, 2007; Phillips *et al.*, 2008; Marincowitz *et al.*, 2008), among others.

The present study examines and describes the morphology of several novel species of microfungi occurring on *Myrtaceae*, and also comments on their host range and distribution where several collections of these fungi are known from literature.

#### 6.2 Materials and methods

#### **6.2.1 Isolates**

Symptomatic Myrtaceae leaves were chosen for study. Leaf pieces bearing ascomata were soaked in water for approximately 2 h, after which they were placed in the bottom of Petri dish lids, with the top half of the dish containing 2 % malt extract agar (MEA; Oxoid, Hampshire, England) (Crous et al., 2009b). Ascospore germination patterns were examined after 24 h, and single ascospore and conidial cultures established as described earlier (Crous et al., 1991; Crous, 1998). Leaves were incubated in moist chambers for up to 2 wk, and inspected daily for microfungi, and single conidial colonies of hyphomycetes and coelomycetes established on MEA (Crous, 2002). Colonies were sub-cultured onto 2 % potato-dextrose agar (PDA), synthetic nutrient-poor agar (SNA), MEA, oatmeal agar (OA), carnation-leaf agar (CLA) (Crous et al., 2006a, 2009c), and pine needle agar (PNA) (2 % tap water agar, with sterile pine needles) (Crous et al., 2006e), and incubated under continuous near-ultraviolet light at 25 °C to promote sporulation. Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org; Crous et al., 2004c). All cultures obtained in this study are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands, and the working collection (CPC) of P.W. Crous (Table 6.1).

## 6.2.2 DNA isolation, amplification and analyses

Genomic DNA was isolated from fungal mycelium grown on MEA, using the UltraClean® Microbial DNA Isolation Kit (Mo-Bio Laboratories, Inc., Solana Beach,

Species	Strain number <sup>1</sup>	Substrate	Country	GenBank Accession
r	<i>1</i>			
Antennariella placita	CPC 13706; CBS 124785	Eucalyptus placita	Cessnock, Australia	GQ303268, GQ303299
Bagadiella lunata	CPC 13655; CBS 124762	Eucalyptus delegatensis	Tasmania, Australia	GQ303269, GQ303300
Bagadiella sp.	CPC 16622; CBS 124763	Eucalyptus dives	New South Wales, Australia	GQ303270, GQ303301
Blastacervulus eucalypti	CPC 13956; CBS 124759	Eucalyptus robertsonii	Mullion Creek, Australia	GQ303271, GQ303302
		subsp. hemisphaerica		
Cladoriella paleospora	CPC 14646; CBS 124761	Eucalyptus oblonga	Menai, Australia	GQ303272, GQ303303
Cladoriella rubrigenum	CPC 13751; CBS 124760	Eucalyptus globulus	Tasmania, Australia	GQ303273, GQ303304
Cyphellophora eucalypti	CPC 13412; CBS 124764	Eucalyptus sp.	Queensland, Australia	GQ303274, GQ303305
Elsinoë eucalypticola	CPC 13318; CBS 124765	Eucalyptus sp.	Cairns, Australia	GQ303275, GQ303306
Foliocryphia eucalypti	CPC 12494; CBS 124779	Eucalyptus coccifera	Tasmania, Australia	GQ303276, GQ303307
Leptoxyphium madagascariensis	CPC 14623; CBS 124766	Eucalyptus camaldulensis	Morondavo, Madagascar	GQ303277, GQ303308
Minimedusa obcoronata	CPC 13495, CBS 120605	Eucalyptus camaldulensis	Chachoengsao, Thailand	GQ303278, GQ303309
Neofabraea eucalypti 🕜 🕒	CPC 13755; CBS 124810	Eucalyptus globulus	Otway, Australia	GQ303279, GQ303310
Parasympodiella elongata	CPC 13285; CBS 124768	Eucalyptus sp.	Queensland, Australia	GQ303280, GQ303311
Parasympodiella elongata	CPC 13288	Eucalyptus sp.	Queensland, Australia	GQ303281, GQ303312
Parasympodiella elongata	CPC 13498	Eucalyptus sp.	Queensland, Australia	GQ303282, GQ303313
Parasympodiella elongata	CPC 533; CBS 522.93	Syzygium cordatum	Sabie, South Africa	GQ303283, GQ303314
Parasympodiella eucalypti	CPC 13397; CBS 124767	Eucalyptus camaldulensis	Venezuela	GQ303284, GQ303315
Parasympodiella laxa	CBS 102698	Camellia japonica	Auckland, New Zealand	GQ303285, GQ303316
Penidiella corymbia	CPC 14640; CBS 124769	Corymbia foelscheana	Emerald Springs, Australia	GQ303286, GQ303317
Polyscytalum algarve	CPC 14936; CBS 124770	Eucalyptus sp.	Algarve, Portugal	GQ303287, GQ303318
Pseudocercospora	CPC 13387; CBS 124771	Syzygium sp.	Moubray Park, Australia	GQ303288, GQ303319
palleobrunneae				
Pseudoramichloridium henryii	CPC 13121; CBS 124775	Corymbia henryii	New South Wales, Australia	GQ303289, GQ303320
Quambalaria simpsonii	CPC 14499; CBS 124772	Eucalyptus tintinnans	Edith Falls, Australia	GQ303290, GQ303321
Quambalaria simpsonii	CBS 124773	Eucalyptus sp.	Lamphoon, Thailand	GQ303291, GQ303322

 Table 6.1 Isolates of microfungi used for DNA analysis and morphological studies.

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Table 6.1 Isolates of microfungi us	ed for DNA analysis and morr	phological studies. (continued)		
Species	Strain number <sup>1</sup>	Substrate	Country	GenBank Accession number (ITS, LSU) <sup>2</sup>
Rachicladosporium americana	CPC 14045; CBS 124774	Leaf litter of unknown host	Fort Royal, USA	GQ303292, GQ303323
Selenophoma australiense	CPC 14582; CBS 124776	Eucalyptus mineata	Edith Falls, Australia	GQ303293, GQ303324
Sphaceloma tectifica	CPC 14594; CBS 124777	Eucalyptus tectifica	Northern Territory,	GQ303294, GQ303325
			Australia	
Strelitziana australiense	CPC 13421; CBS 124778	Eucalyptus sp.	Queensland, Australia	GQ303295, GQ303326
Sydowia eucalypti	CPC 14028	Eucalyptus sp.	New South Wales, Australia	GQ303296, GQ303327
Sydowia eucalypti	CPC 14927	Eucalyptus sp.	Algave, Faro, Portugal	GQ303297, GQ303328
Zeloasperisporium eucalyptorum	CPC 14603; CBS 124809	Eucalyptus tectifica	Northern Territory,	GQ303298, GQ303329
			Australia	
<sup>1</sup> CBS: CBS Fungal Biodiversity Ce	entre, Utrecht, The Netherland	s; CPC: Culture collection of Per	dro Crous, housed at CBS.	
<sup>2</sup> ITS: Internal transcribed spacers 1	and 2 together with 5.8S nrD	NA; LSU: 28S nrDNA.		

CA, USA) according to the manufacturer's protocols. The primers V9G (de Hoog and Gerrits van den Ende, 1998) and LR5 (Vilgalys and Hester, 1990) were used to amplify part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the first 900 bases at the 5' end of the 28S rRNA gene (LSU). 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. The PCR conditions, sequence alignment and subsequent phylogenetic analysis followed the methods of Crous *et al.* (2006a). Sequences were compared with the sequences available in NCBI's GenBank nucleotide (nr) database using a megablast search and results are discussed in the relevant species notes where applicable. Alignment gaps were treated as new character states. Sequence data were deposited in GenBank (Table 6.1) and alignments in TreeBASE (www.treebase.org).

#### 6.2.3 Morphology

Preparations from fungal cultures were mounted on glass slides with clear lactic acid for microscopic examination. Sections of ascomata were made by hand for examination purposes. Measurements of all taxonomically relevant parameters were made at  $\times 1~000$  magnification by Nikon NIS-Elements D3.0 Imaging software, with 30 measurements per structure where possible. Colony colours on MEA (surface and reverse) were determined using the colour charts of Rayner (1970) after 2 wk at 25 °C in the dark.

## 6.3 Results

# 6.3.1 Phylogenetic analysis

Approximately 1 700 bases, spanning the ITS and LSU regions, were obtained for isolates listed in Table 1. The LSU region was used in the phylogenetic analysis for the generic placement and ITS to determine species-level relationships. Due to the inclusion of shorter GenBank sequences such as *Pseudoramichloridium brasilianum* EU041854,



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**Fig. 6.1** One of 27 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment using PAUP v4.0b10. The scale bar shows 10 changes, and bootstrap support values higher than 70 % from 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and novel sequences are printed in **bold**. The tree was rooted to *Saccharomyces cerevisiae* (GenBank accession Z73326). AGA = *Agaricomycetes*, EXO = *Exobasidiomycetes*, EUR = *Eurotiomycetes*, LEO = *Leotiomycetes*, SOR = *Sordariomycetes*, DOT = *Dothideomycetes*, all others are incertae sedis.

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*Pringsheimia smilacis* FJ150970, *Endothia eugeniae* AF277142, *Endothia gyrosa* AY194115 and *Cryphonectria parasitica* AF277132, it was not possible to use the complete length of the determined LSU sequences in the analysis.

The manually adjusted LSU alignment contained 98 taxa (including the outgroup sequence) and, of the 479 characters used in the phylogenetic analysis, 294 were parsimony-informative, 33 were variable and parsimony-uninformative and 152 were constant. Twenty-seven equally most parsimonious trees were obtained from the heuristic search, the first of which is shown in Fig. 1 (TL = 1831, CI = 0.356, RI = 835, RC = 297). The phylogenetic tree of the LSU region (Fig. 6.1) showed the isolates obtained in this study to cluster in several classes, including *Agaricomycetes, Exobasidiomycetes, Eurotiomycetes, Sordariomycetes* and *Dothideomycetes*. Further results are discussed under the species notes below where applicable.

## 6.3.2 Taxonomy

Several taxonomic novelties were found that do not match any species presently described, or linked to the sequences available in GenBank. These genera and species are described as new below.

Antennariella placitae Cheewangkoon & Crous, sp. nov. – MycoBank MB513839; Fig. 6.2

Teleomorph. Unknown.

Pycnidia globosa vel subovoidea, ex ramulis erectis hypharum oriunda, intercalaria, lateralia vel terminalia,  $(30-)40-60(-120) \times (22-)30-40(-65)$  µm. Cellulae conidiogenae phialidicae, subcylindraceae vel lageniformes, hyalinae,  $(5-)8-10(-13) \times 4-5.5$  µm. Conidia hyalina, aseptata, globosa vel subglobosa,  $(2.3-)2.5-3(-3.8) \times (2-)$  2.5–2.8(-3.2) µm.

Etymology: Named after the host species on which it occurs, Eucalyptus placita.

*Mycelium* superficial or immersed, pale to medium brown, septate, branched; hyphae mostly smooth, thin-walled, septate, 3.5-5 µm wide, darker and wider when around conidiomata, 3.5-8.5 µm wide, hyphal cells regular in width, constricted at septa, wall 0.9–1.3 µm thick, with a mucilaginous outer wall layer, up to 3.5 µm thick.



**Fig. 6.2** Antennariella placitae. a, b. Colony on MEA; c–f. pycnidia; g–i. conidiogenous cells; j. conidia; k–n. hyphae in culture. — Scale bars: a, b = 1 cm; c–n = 30  $\mu$ m, g applies to g–j, k applies to k–n.

*Conidiomata* pycnidial, superficial or immersed, globose to subovoid, medium to dark grey-brown, intercalary, lateral or terminal on erect hyphal branches, meristogenous

in development, pseudoparenchymatous, thin-walled, 1–2 cell layers of *textura angularis*,  $(30-)40-60(-120) \times (22-)30-40(-65) \ \mu\text{m}$ . Ostiole absent, or not well-developed, mostly releasing conidia by means of irregular rupture. Conidiophores absent. Conidiogenous cells phialidic, subcylindrical to lageniform, hyaline, invested among mucilage, formed from the inner cells of the pycnidial wall,  $(5-)8-10(-13) \times 4-5.5 \ \mu\text{m}$ . Conidia hyaline, aseptate, globose to subglobose, base subtruncate, with 1–3 minute guttules, smooth, thin-walled,  $(2.3-)2.5-3(-3.8) \times (2-)2.5-2.8(-3.2) \ \mu\text{m}$ .

Culture characteristics — Colonies reaching 2 cm diam after 2 wk at 25 °C on MEA, flat, folded in the middle, with ovary-white conidial masses on the surface, and entire edge with medium to dark brownish grey woolly aerial mycelium; greyish fucousblack (reverse).

Specimen examined. Australia, New South Wales, Cessnock S 32°50'45", E 151°17'07" on *Eucalyptus placita*, 14 Oct. 2006, coll. *B.A. Summerell*, isol. *P.W. Crous*, CBS H-20277 holotype, culture ex-type CPC 13706 = CBS 124785.

Notes — The genus *Antennulariella* is a teleomorph genus of sooty molds which has *Capnodendron* and *Antennariella* synanamorphs (Hughes, 1976). *Antennariella placitae* resembles other *Antennariella* spp. which produce meristogeneous pycnidia, that are intercalary or terminal on the hyphae, and give rise to aseptate conidia. *Antennariella placitae* also has a characteristic hyaline mucilaginous outer hyphal wall layer, which is a typical characteristic of sooty molds (Hughes, 1976). Conidia of *A. placitae* are globose, while those of other *Antennariella* spp. are more or less ellipsoidal (Hughes, 1976). Phylogenetically *A. placitae* is closely related to the sooty molds *Capnodium coffeae* (DQ247800; 97 % identical), *Microxyphium citri* (AY004337; 96 % identical) and *Fumagospora capnodioides* (EU019269; 95 % identical) based on its LSU sequence data. All four taxa grow superficially on the cuticle of their plant hosts. *Cladorrhino* simile, sed conidiis lunaribus, monophialibidus et conidiophores in rosulis suprastomatalibus.

Type species. Bagadiella lunata Cheewangkoon & Crous

*Etymology*. Named after the standard diet enjoyed at CBS over weekends from the automated dispenser, an apple cake (B1 = Ba) and a packet of wine gums (A7 = Ag).

Mycelium immersed, becoming superficial when incubated in moist chambers, pale to medium brown, consisting of septate, branched, smooth hyphae. Chlamydospores absent. *Caespituli* suprastomatal, pale brown, compact, arising from pseudoparenchymatal tissue in the substomatal cavity, forming a rosette with a central, basal point of attachment, giving rise to conidiophores with a slimy conidial mass, up to 110 µm high and 130 µm diam. Conidiophores micronematous, arranged in a rosette, cylindrical, mostly dichotomously branched, slightly thick-walled, medium to pale greybrown, straight or slightly flexuous. Conidiogenous cells terminal, monophialidic, branched, subcylindrical to lageniform, at times constricted at base of conidiogenous cell, tapering toward the apex, pale brown, paler toward the apex, with a terminal, narrow, pale olivaceous, vase-shaped, flaring collarette, constricted beneath the collarette, thickened and slightly darkened at the conidiogenous regions. Conidia borne in Conidiophores and conidiogenous cells, showing collarates, slimy heads, lunate, curved, apex rounded, with slight taper towards the subtruncate base, hyaline.

Bagadiella lunata Cheewangkoon & Crous, sp. nov. — MycoBank MB513841; Fig. 6.3

# Teleomorph. Unknown.

*Conidiophora* in rosulis compactis suprastomatalibus, ad 110  $\mu$ m alta, 80–130  $\mu$ m diam. *Cellulae conidiogenae* plerumque terminales, monophialidicae, ramosae, subcylindraceae vel lageniformes, (8.5–)11–13(–15) × 2.5–3.3  $\mu$ m. *Conidia* in capitulis mucosis, curvata, apice rotundato, basi obconice truncata, hyalina, (15–)16–18(–22) × (1.3–)1.5(–1.7)  $\mu$ m.

Etymology. Named after the characteristic lunate shape of its conidia.



**Fig. 6.3** *Bagadiella lunata.* a. Caespituli on leaf; b. pseudoparenchymatal tissue in substomatal cavity; c, d. rosette of conidiophores; e. conidia; f–i. conidia. — Scale bars: a =  $300 \mu$ m; b–d =  $30 \mu$ m; e–h =  $20 \mu$ m, b applies to b–d, e applies to e–i.

Mycelium immersed, becoming superficial upon incubation in moist chambers, pale to medium brown, consisting of septate, branched, smooth, 2–4  $\mu$ m wide hyphae. Chlamydospores absent. Caespituli pale brown (appearing whitish under the stereo microscope when young), suprastomatal, pseudoparenchymatal cells in substomatal cavity giving rise to a compact rosette of conidiophores, attached via a central, basal point, with a slimy conidial mass on top, up to 110  $\mu$ m high, 80–130  $\mu$ m diam. Conidiophores micronematous, cylindrical, mostly dichotomously branched in apical region, slightly thick-walled, pale to medium grey-brown, straight or slightly flexuous, up

to 115 µm long, 2.5–4 µm wide. Conidiogenous cells predominantly terminal, monophialidic, branched, subcylindrical to lageniform,  $(8.5-)11-13(-15) \times 2.5-3.3$  µm, at times constricted at the base, pale brown, paler toward the apex, with a hyaline, vase-shaped, flaring collarette that is constricted at the base,  $1.5-2.5(-5) \times 1.5-2$  µm, thickened and slightly darkened at the conidiogenous region. Conidia borne in slimy heads, lunate, curved, with a rounded apex, tapering toward a subtruncate base, hyaline,  $(15-)16-18(-22) \times (1.3-)1.5(-1.7)$  µm. Conidia mostly fail to germinate, but when they do, it happens via an appressorium-like structure forming in the centre of the conidium.

Culture characteristics — Colonies reaching 5 cm diam on MEA after 1 wk at 25 °C, flat, irregular, greenish grey, with sparse aerial mycelium, slightly folded at the centre, olivaceous-grey to buff (surface), with white margin, yellow-brown (reverse).

Specimens examined. Australia, Tasmania, Mount Wellington Park S 42°55'0", E 147°15'0" on Eucalyptus delegatensis, 10 Oct. 2006, coll. B.A. Summerell, isol. P.W. Crous, CBS H-20281 holotype, culture ex-type CPC 13655, CPC 13656 = CBS 124762; New South Wales, Paddy's River, S 34°37'47.2", E 150°10'06.2", on E. dives, 23 Mar. 2009, coll. B.A. Sum-*merell*, isol. *P.W. Crous*, CBS H-20308, cultures CPC 16622– 16624, CBS 124763; New South Wales, Paddy's River, S 34°37'45", E 150°10'00", on *E. dives*, 24 Mar. 2009, *B.A. Summerell*, CBS H-20306; New South Wales, Southern Highlands, S 34°29'54.9", E 150°20'29.3", on *E. dives*, 23 Mar. 2009, *B.A. Summerell*, CBS H-20307; New South Wales, North Washpool State Forest, S 29°06'50.6", E 150°25'08.6", on *E. campanulata, B.A. Summerell*, 23 Mar. 2009, CBS H-20309.

Notes — The genus *Bagadiella* is similar to the genus *Cladorrhinum* in having pigmented hyphae and a pustular-like aggregation of conidiophores (Mouchacca & Gams 1993). *Bagadiella* can be distinguished from *Cladorrhinum* species by its lunate conidia, those of *Cladorrhinum* species being dactyroid to ellipsoid (Mouchacca and Gams, 1993), its monophialides, and conidiophores which form in suprastomatal rosettes. The genus *Cladorrhinum* has teleomorphs in *Apiosordaria*, which is related to, but not congeneric with, *Bagadiella*. Based on several bp differences observed the ITS DNA

sequence data of CPC 16622 and 13655, these collections appear to represent a different taxon to that typified by the ex-type strain.

Blastacervulus eucalypti H.J. Swart, Trans. Brit. Mycol. Soc.90: 289. 1988 — Fig. 6.4

Teleomorph. Unknown.

Leaf lesions prominent on leaf tips, amphigenous, subcircular to irregular, discrete to influent, up to 2 mm diam, medium brown at the middle, darker at the border, with a leaves incubated in moist chambers. — Scale bars:  $a = 150 \mu m$ ;  $b = 100 \mu m$ ;  $c = 30 \mu m$ ;  $d-i = 15 \mu m.red$ -purple margin, with amphigenous, dark conidiomata at the margin, surrounded by indistinct border, not vein-limited. Mycelium immersed, rarely superficial, visible below the cuticular layer, septate, branched, medium brown, thick-walled,  $\leq 0.8$ μm wide, somewhat constricted at septa, 2.5–5 μm wide. Conidiomata acervular, single, 5-15 per lesion, developing subcuticular or between the epidermal cells, becoming erumpent with age, often surrounded by remnants of the epidermis, circular to slightly oblong, containing 1-2 cell layers of textura angularis, up to 80 µm high and 280 µm diam, producing masses of medium to dark brown conidia. Conidiophores absent. Conidiogenous cells formed from the upper stromatic cells, determinate, shortsubcylindrical to ampulliform or subglobose, pale brown to hyaline, slightly vertuculose, thin-walled, mostly monoblastic,  $3.5-5.5 \times 4.5-8.5 \mu m$ . Conidia pale to medium brown, aseptate,  $5-7 \times 5-8$  µm, mostly subglobose to broadly ovoid, slightly obtuse to truncate at the base, thick-walled, 1-1.5 µm, forming branched chains of acropetal conidia; ramoconidia with up to three hila,  $\leq 1 \mu m$  wide.

Culture characteristics — Colonies reaching 1 cm diam after 3 wk at 25 °C; erumpent with moderate reddish brown aerial mycelium and paler in the outer region; margins smooth, regular; reverse olivaceous-black; colonies fertile. *Specimen examined*. Australia, New South Wales, Mullion Creek, S 33°06'48", E 149°08'45", on *Eucalyptus robertsonii* subsp. *hemisphaerica*, 1 Jan. 2007, coll. *B.A. Summerell*, isol. *P.W. Crous*, CBS H-20278, culture CPC 13956 = CBS 124759.



**Fig. 6.4** *Blastacervulus eucalypti.* a. Leaf spot; b. cross section of sporodochium; c–f. conidiogenous cells and conidia; g–i. conidia in chains, developing onleaves incubated in moist chambers. — Scale bars:  $a = 150 \mu m$ ;  $b = 100 \mu m$ ;  $c = 30 \mu m$ ;  $d-i = 15 \mu m$ , d applies to d–i.

Specimen examined. Australia, New South Wales, Mullion Creek, S 33°06'48", E 149°08'45", on *Eucalyptus robertsonii* subsp. *hemisphaerica*, 1 Jan. 2007, coll. *B.A. Summerell*, isol. *P.W. Crous*, CBS H-20278, culture

CPC 13956 = CBS 124759.Notes — The present collection closely matches *Blastacervulus eucalypti*, which is the only member of the genus knownto date (Swart, 1988). Based on its DNA phylogeny, it appears closely related to *Alysidiella parasitica* and some '*Heteroconium*' species with catenulate, multiseptate conidia (Crous *et al.*, 2006b, 2007f; Summerell *et al.*, 2006).

Cladoriella paleospora Cheewangkoon and Crous, sp. nov.— MycoBank MB513842; Fig. 6.5

Teleomorph. Unknown.

Cladoriellae eucalypti similis, sed conidiis minoribus,  $6-10 \times 3.5-4 \mu m$ , in cultura sine pigmento.

Etymology. Named after its pale brown conidia.

*Mycelium* pale to medium brown, smooth to finely vertuculose, branched, septate, (1.5-)2.2-3(-3.5) µm wide, thin-walled to somewhat thickened, sterile hyphae usually paler and narrower. Conidiophores micro- to macronematous, arising from creeping mycelium, solitary, erect, cylindrical, sometimes reduced to conidiogenous cells, straight to slightly curved, medium to dark brown, somewhat thick-walled, smooth, finely vertuculose, at times produced on swollen hyphal cells,  $(10-)18-25(-87) \times 3-3.5(-4)$ µm. Conidiogenous cells terminal, cylindrical, tapering to a truncate apex, not denticulate, dark to medium brown, paler towards the apex,  $(15-)20-25(-35) \times 3.3-4$  $\mu$ m, with 1–3 conspicuous loci, with thickened, slightly darkened scars, 1.5–2  $\mu$ m wide. *Conidia* catenulate, in simple to loosely branched chains that frequently remain attached; ramoconidia cylindrical to subfusoid,  $12-15(-18) \times 3.5-4.2 \mu m$ , tapering to both truncate ends, 0-1-septate, unconstricted at septa, smooth to finely vertuculose, pale brown; intercalary conidia cylindrical, ellipsoid to fusoid,  $11-15 \times 3.3-4 \mu m$ , pale brown, 0–1-septate, tapering towards both truncate ends; *terminal conidia* obovoid, pale brown, paler towards the apex, aseptate, with truncate ends,  $6-10 \times 3.5-4 \mu m$ ; scars thickened along the rim, reflective, somewhat darkened, not protruding,  $1.5-2 \mu m$  wide.

Culture characteristics — Colonies on MEA reaching 15 mm diam after 3 wk, irregular, erumpent in the centre, folded, with moderate iron-grey aerial mycelium, and irregular margins; olivaceous-grey (surface); dark greenish olivaceous (reverse).



**Fig. 6.5** *Cladoriella paleospora.* a. Caespituli on leaf; b. colony on SNA; c–g. conidial chain, conidiogenous cells and conidiophores; h. conidia. — Scale bars:  $a = 120 \mu m$ ; c–h = 20  $\mu m$ , c applies to c–e, h applies to f–h.

Specimen examined. Australia, New South Wales, Menai, S 34°00'38", E 151°00'57", on *Eucalyptus oblonga*, 22 Sept. 2007, coll. *B.A. Summerell*, isol. *P.W. Crous*, CBS H-20280 holotype, culture ex-type CPC 14646 = CBS 124761, CPC 14647, 14648.

Notes — *Cladoriella paleospora* is morphologically similar to *C. eucalypti* and *C. rubrigena*, having brown conidiophores with relatively few conidial loci that are thickened, darkened and reflective, giving rise to long conidial chains that frequently remain attached (Crous *et al.*, 2006f). *Cladoriella paleospora* is distinct from *C. eucalypti* and *C. rubrigena* in having smaller conidia, and by not producing any pigment in culture. Phylogenetically the three species form a well supported clade (Fig. 1).

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Cladoriella rubrigena Cheewangkoon and Crous, sp. nov.— MycoBank MB513843; Fig. 6.6

Teleomorph. Unknown.

*Cladoriellae eucalypti* similis, sed conidiophoris brevioribus, saepe in cellulis conidiogenis reductis.

*Etymology*. Named after the diffuse red pigment that this species forms in culture.

*Mycelium* pale to medium brown, thick-walled, smooth to finely verruculose, branched, septate, (1.5-)2-3(-3.5) µm wide, sterile hyphae usually paler and narrower. *Conidiophores* mononematous, separate, erect, subcylindrical, straight, medium to dark brown, smooth to finely verruculose, thick-walled, 0–1-septate, frequently reduced to conidiogenous cells,  $5-10 \times 3.5-4.8$  µm. *Conidiogenous cells* terminal, monotretic, subcylindrical, dark to medium brown, with a truncate apex,  $(12-)14-16(-18) \times (3.4-)$  4(-4.7) µm, with a single, terminal conspicuous scar, 1.5-2 µm wide, darkened, refractive, and thickened along the rim. *Conidia* subcylindrical to fusoid, 0–1-septate, slightly constricted at the middle, guttulate, medium brown, thickwalled, finely verruculose, apical conidium with rounded apex and truncate, not protruding base; conidia frequently remaining attached in long acropetal chains (-15), which are simple or branched,  $(11-)14-17(-20) \times 3.5-4.2$  µm; hila darkened, slightly thickened along the rim.

Culture characteristics — Colonies on MEA reaching 13 mm diam after 2 wk, producing a diffuse pigment that changes the colour of the media to orange-red; colonies irregular, erumpent in the middle, folded, with sparse aerial mycelium, and irregular margins; brown to greenish grey (surface); brownish green (reverse).

*Specimen examined*. Australia, Tasmania, Bruny Island, Adventure Bay Beach, S 43°20'55.3", E 147°19'21.8" on *Eucalyptus globulus*, 10 Nov. 2006, coll. *B.A. Summerell*, isol. *P.W. Crous*, CBS H-20279 holotype, culture extype CPC 13751 = CBS 124760.



**Fig. 6.6** *Cladoriella rubrigena*. a, b. Colony on MEA; c, d. conidial chains; e. conidiophores and conidiogenous cells. — Scale bars: a = 20 mm; c,  $e = 15 \mu \text{m}$ ;  $d = 10 \mu \text{m}$ .

Notes — *Cladoriella rubrigena* is similar to *Cladoriella eucalypti* in conidial dimensions, and both produce a red pigment in agar (Crous *et al.*, 2006f). They can be distinguished, however, based on their conidiophores and conidiogenous cells. *Cladoriella rubrigena* has short conidiophores which are usually reduced to conidiogenous cells, whereas conidiophores of *C. eucalypti* can be up to 60  $\mu$ m tall, and are slightly wider (5–7  $\mu$ m). Phylogenetically *C. rubrigena* clusters with *C. eucalypti*, but differs by 12 nucleotides in the ITS region.

Cyphellophora eucalypti Cheewangkoon and Crous, sp. nov.— MycoBank MB513844; Fig. 6.7

Teleomorph. Unknown.

Cyphellophorae indicae similis, sed conidiis 1–3-septatis, plus minusve 15–20 µm longis.

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

Mycelium dense, superficial, partly immersed, smooth, loosely septate, predominantly thin-walled, branched, hyaline to pale brown, 1.5-2.5 µm wide. Conidiophores absent. Conidiogenous cells intercalary or terminal on erect hyphal branches, solitary, subcylindrical to pyriform or lageniform, straight to slightly curved, widest in the lower third or in the middle,  $(5-)7-10(-12) \times 3-5 \mu m$ , pale to medium brown, thick-walled, smooth, proliferating percurrently, with 1-2 annellations, and funnel-shaped collarettes, 2.5-4.5 µm long and 2.5-3.5 µm wide, constricted and somewhat darkened and thickened below the collarette. Conidia clavate and 1-septate when young, becoming slightly sigmoid-fusiform, 1–3-septate, hyaline to pale brown, apex obtusely rounded, base minutely truncate or slightly protruding, 0.5-0.8 µm wide, thin-walled, slightly thickened along the rim, refractive, aggregating in a slimy mass, (8–)  $15-20(-25) \times 2-2.5(-3)$  µm. Culture characteristics — Colonies reaching 4 cm diam after 2 wk at 25 °C in the dark, circular, flat, medium to dark brown; margin entire, consisting of dense, immersed mycelium; aerial mycelium loose, cottony, pale grey-brown (surface), appearing minutely orange-brown due to slimy conidial masses on mycelium, medium yellowish brown (reverse).

*Specimen examined.* Australia, Kuranda Kennedy Highway, Queensland, on *Eucalyptus* sp., 26 Aug. 2006, *P.W. Crous*, CBS H-20282 holotype, culture ex-type CPC 13412 = CBS 124764, CPC 13413, 13414.



**Fig. 6.7** *Cyphellophora eucalypti.* a. Colony on MEA; b–e. conidial bundles on conidiogenous cells; f–j. conidiogenous cells conidiogenous cells giving rise toconidia, with visible collarattes; k–l. conidia. — Scale bars =  $10 \mu m$ , b applies to b–d, I applies to i–j.

Notes — *Cyphellophora eucalypti* has dark colonies and forms large, flared collarettes on well-developed phialides, which are characteristic of the genus *Cyphellophora* (Decock *et al.*, 2003, Crous *et al.*, 2007g, 2009a). Using the key of Crous et al. (2009a), *C. eucalypti* is most similar to *C. indica* and *C. pluriseptata*, but is distinct in having 1–3-septate conidia, with an average length of 15–20 µm. Phylogenetically it is also closely related to *C. laciniata* (EU035416 (ITS), 91 % identical and (LSU), 97 % identical) (Fig. 6.1).

*Elsinoë eucalypticola* Cheewangkoon and Crous, *sp. nov.* — MycoBank MB513845; Fig. 6.8

Anamorph. Sphaceloma sp.

*Elsinoes eucalyptorum* et *E. eucalypti* similes, sed amplitudine conidiorum intermedia,  $20-28 \times 7-8 \mu m$ .

Etymology. Named after the host genus on which it occurs, Eucalyptus.

*Leaf spot* amphigenous, separate, subcircular to ellipsoidal, white-grey, with raised dark definite border, occasionally surrounded by an irregular red-purple margin,  $\leq$  1.5 mm diam, becoming long-irregular when confluent with 2–4 spots; with 1–3 minute, black ascomata erupting through host tissue in the middle of the lesion. *Ascomata* scattered, separate, pulvinate,

subcuticular; wall composed of dark brown to black pseudoparenchymatic *textura* angularis,  $150-200 \times 55-65 \mu m$ . Asci distributed irregularly throughout the ascomata, subglobose to broadly obovoid, thick-walled, 8-spored, sessile, hyaline,  $30-47 \times 24-30 \mu m$ . Ascospores hyaline to pale brown, broadly ellipsoid with rounded ends, with more prominent taper towards the base, with 4-transverse septa, and 0-3 vertical septa, and sometimes with oblique septa; mostly slightly constricted at the median septum,  $(16-)17-18(-20) \times (6.5-)7-8 \mu m$ . Sphaceloma

state not observed.

Culture characteristics — Colonies reaching up to 1.5 cm diam on MEA after 1 mo at 25 °C in the dark, almost circular, high convex, becoming 3–4 mm high in the middle, with raised, concave edge, and slightly lobate edge, frequently folded, with ruptures on the colony surface, yellow-brown, with sparse pale grey aerial mycelium.

Specimen examined. Australia, Queensland, Cairns, *Eucalyptus* sp., 26 Sept. 2006, *P.W. Crous*, CBS H-20283 holotype, culture ex-type CPC 13318 = CBS 124765, CPC 13319, 13320.



**Fig. 6.8** *Elsinoë eucalypticola*. a, b. Lesions on leaf; c. colony on MEA; d–g. asci; h–l. ascospores. — Scale bars: b = 10 mm; d-l = 10 µm, d applies to d–e, f applies to f–g, h applies to h–l.

Notes — Presently there are two species of *Elsinoë* that have been recorded on *Eucalyptus*, namely *E. eucalypti* and *E. eucalyptorum*. Ascospores of *E. eucalypticola*  $(16-20 \times 6.5-8 \ \mu\text{m})$  are intermediate in size between those of *E. eucalyptorum*  $(11-15 \times 4-6 \ \mu\text{m})$  (Summerell *et al.*, 2006) and *E. eucalypti*  $(20-28 \times 7-8 \ \mu\text{m})$  (Park *et al.*, 2000). Both *E. eucalypti* and *E. eucalyptorum* form larger leaf spots than those associated with *E. eucalypticola*. Phylogenetically *E. eucalypticola* is closely related to *E. centrolobi* (Fig. 6.1), which has smaller ascospores  $(12-15 \times 4-6 \ \mu\text{m})$  (Bitancourt and Jenkins, 1949).

Foliocryphia Cheewangkoon & Crous, gen. nov. — MycoBank MB513846

Differt a generibus diversis familiae (*Cryphonectriaceae*) stromatibus purpurascentibus in 3 % KOH vel acido lactario nullis et phylogenetice manifeste divergenti.

Type species. Foliocryphia eucalypti Cheewangkoon and Crous, sp. nov.

*Etymology. Folium* (L.) = leaf, crypho (Greek) = hidden; referring to its foliicolous habit and inconspicuous or hidden nature.

*Conidiomata* eustromatic, amphigenous, separate, subsuperficial, pulvinate, subglobose, with or without ostiole; stromatic tissue of *textura angularis*, pale to medium brown, with convoluted inner surface, uni- to multilocular. *Conidiophores* consisting of basal subglobular to angular cells, that branch irregularly, becoming cylindrical, transversely septate. *Conidiogenous cells* enteroblastic, determinate, integrated or decrete, phialidic, cylindrical, tapering to a thinner apical part, with visible collarette and periclinal thickening. *Conidia* hyaline, aseptate, smooth, ellipsoid, straight to irregularly curved.



**Fig. 6.9** *Foliocryphia eucalypti.* a. Pycnidia on OA; b. cross section of conidioma; c–e. conidiophores and conidiogenous cells; f. conidia. — Scale bars:  $a = 450 \mu m$ ;  $b = 100 \mu m$ ;  $c-f = 10 \mu m$ .

*Foliocryphia eucalypti* Cheewangkoon and Crous, *sp. nov.* — MycoBank MB513847; Fig. 6.9

Teleomorph. Unknown.

Conidiomata foliicola, eustromatica, amphigena, subglobosa vel horizontaliter late ellipsoidea,  $300-370 \times 320-590 \ \mu m$ , interdum multilocularia. Cellulae conidiogenae

enteroblasticae, determinatae, integratae vel discretae, phialidicae, cylindricae,  $(7.5-)12-15.5(-20) \times 2.8-3.8 \mu m$ . Conidia hyalina, aseptata, ellipsoidea, recta vel irregulariter curvata, apice obtuso, basi abrupte attenuata in hilis protrusis, cicatricibus, laevia, tenuitunicata,  $(8.5-)9-10(-11.5) \times 3.3-4.2 \mu m$ .

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

Colonies on OA effuse, yellowish brown, with dark greybrown margin, producing numerous umber to dark brown or fucous-black conidiomata. Mycelium mostly immersed, aerial mycelium sparse, whitish, 1.5-2.3 µm wide. Conidiomata eustromatic, amphigenous on leaf, separate, subsuperficial, pulvinate, subglobose to horizontally broadly ellipsoid,  $300-370 \times 320-590 \mu m$ , with or without ostiole; stromatic tissue of textura angularis, pale to medium brown, somewhat darker and thicker-walled at the outer region; covered with pale brown mycelium as outer layer; conidiomata with convoluted inner surface, occasionally multilocular. Conidiophores consisting of basal subglobular to angular cells, formed from the inner cells of the locular walls, hyaline to medium brown, slightly thick-walled, irregularly branched, transversely septate, forming cylindrical cells,  $7-16(-22) \times 3-4.5 \mu m$ . Conidiogenous cells enteroblastic, determinate, integrated or decrete, phialidic, cylindrical, tapering to a narrowly cylindrical part in the apical region,  $(7.5-)12-15.5(-20) \times 2.8-3.8 \,\mu\text{m}$ ; collarette tubular, with visible periclinal thickening. Conidia hyaline, aseptate, ellipsoid, straight to irregularly curved, apex obtuse, baseabruptly tapered to a flat protruding scar, which can be basal or somewhat off-centre, smooth, thin-walled,  $(8.5-)9-10(-11.5) \times 3.3-4.2 \,\mu\text{m}$ .

Culture characteristics — Colonies on OA reaching 5 cm after 2 wk at 25 °C in the dark, subcircular, effuse, yellowish brown, with dark grey-brown, even margin; aerial mycelium sparse, producing numerous umber to dark brown or fucousblack semiimmersed conidiomata.

*Specimen examined*. Australia, Tasmania, on *Eucalyptus coccifera*, 1 Feb. 2007, coll. *C. Mohammed*, isol. *P.W. Crous*, CBS H-20299 holotype, culture ex-type CPC 12494 = CBS 124779, CPC 12495, 12496.

Notes — Phylogenetically *Foliocryphia* resides within the *Cryphonectriaceae* clade, but appears to not fit into any presently circumscribed genus of this family. *Foliocryphia* produces aseptate conidia in eustromatic conidiomata as do other *Cryphonectriaceae* members. However, *Foliocryphia* lacks the main characteristics of the *Cryphonectriaceae*, namely that its stromata do not turn purple in 3 % KOH, or yellow in lactic acid (Gryzenhout *et al.*, 2006a). Based on its distinct morphological characteristics and DNA phylogeny, *Foliocryphia* is described here as a new foliicolous genus within the *Cryphonectriaceae*.

*Leptoxyphium madagascariense* Cheewangkoon and Crous, *sp. nov.* — MycoBank MB513848; Fig. 6.10

Teleomorph. Unknown.

Differt a speciebus diversis *Leptoxyphii* conidiis  $4.5-5 \times 3-3.5 \mu m$ .

Etymology. Named after Madagascar, the island from which it was collected.

*Mycelium in vitro* superficial and immersed, dark grey-brown, septate, constricted at septa, loosely branched, smooth to slightly verruculose, thick-walled,  $\leq 1 \mu m$  wide, frequent septate and wider in hyphae around conidiomata, irregular in width, 3–6  $\mu m$ wide, with prominent mucilaginous outer hyphal layer, 2–4.5  $\mu m$  wide. *Conidiomata* determinate synnematal, superficial, arising from hyphal ropes; stipe composed of unbranched, parallel synnematous hyphae, sometimes with a helical twist, or not enclosed in mucilage, or occasionally producing 2–3 synnemata on a single hyphal rope; cylindrical part (200–)250(–300)  $\mu m$  high, (8–)10–12(–15)  $\mu m$  wide, expanding to a funnel-shaped hyphal apex, 35–50  $\mu m$  high, 35–60  $\mu m$  wide. *Conidiophores* cylindrical, subulate, septate, slightly thick-walled, consisting of several aggregated, synnematous hyphae that diverge close to the apex; hyphae 3–4.5  $\mu m$  wide, flaring in apical part, appearing like a terminal hyphal fringe, terminating in rounded apices. *Conidiogenous cells* integrated, formed from the inner cell surface, intercalary, never terminal, monophialidic, denticlelike, with a truncate apex,  $\leq 1 \mu m$  high and up to 2.8  $\mu m$  wide. *Chlamydospores* subglobose to subsphaerical, multiseptate, dark grey-brown, thickwalled, formed on the lateral side of hyphae, not enclosed in a mucilaginous layer, or in a very thin layer if present,  $25-30 \times 25-35 \mu m$ . *Conidia* rod-shape, with rounded ends, 1-celled, 1–3 guttules,  $4.5-5 \times 3-3.5 \mu m$ , gathered in a slimy mass at the apex of synnemata; conidia not becoming pigmented, anastomosed or septate at maturation.



**Fig. 6.10** Leptoxyphium madagascariense. a. Colony on MEA; b–d. synemata; e, f. conidiogenous cells; g. conidia; h–j. hyphae and chlamydospores in culture. — Scale bars:  $a = 500 \mu m$ ;  $b-d = 50 \mu m$ ;  $e-j = 10 \mu m$ , e applies to e–f, h applies to h–j.

Culture characteristics — Colonies becoming up to 2.5 cm diam at 25 °C on MEA after 5 d in the dark; colonies flat, with entire edge, and sparse, medium to dark brownish grey aerial mycelium; producing numerous, superficial, dark synnemata with ovary-white apical conidial masses.

*Specimen examined.* Madagascar, Morondavo, on leaves of *Eucalyptus camaldulensis*, Aug. 2007, coll. *M.J. Wingfield*, isol. *P.W. Crous*, CBS H-20284 holotype, culture ex-type CPC 14623, CPC 14624 = CBS 124766.

Notes — *Leptoxyphium madagascariense* has elongated synnemata with a stout base, a long, narrow neck and a terminal conidiogenous zone. It produces conidia from phialidic openings on the inner surface of its conidiogenous hyphae. These characteristics are typical of the genus *Leptoxyphium* (Hughes, 1976). *Leptoxyphium madagascariense* can be distinguished with other known *Leptoxyphium* species by its conidial dimensions (Batista & Ciferri 1963). It does not produce any conidial pigment or septation during conidial maturation, unlike many other *Leptoxyphium* species (Batista and Ciferri, 1963; Hughes, 1976). Phylogenetically it is also clusters in the *Capnodiales* (Schoch *et al.*, 2006) with other sooty mould species such as *Microxyphium citri* (AY004337; 98 % identical), *Leptoxyphium fumago* (AB441707; 98 % identical), *Capnodium coffeae* (DQ247800; 96 % identical) and *Fumagospora capnodioides* (EU019269; 93 % identical) (Fig. 6.1).

*Minimedusa obcoronata* (B. Sutton, Kuthub. and Muid) Diederich, Lawrey and Heylen, Mycol. Progr. 6: 76. 2007 — Fig. 6.11

*Basionym. Pneumatospora obcoronata* B. Sutton, Kuthub. and Muid, Trans. Brit. Mycol. Soc. 83: 423. 1984.

*= Tricellulortus peponiformis* (*'pepoformis'*) Matsush., in Matsushima, Matsushima Mycological Memoirs 8: 39. 1995.

Teleomorph. Unknown.

*Mycelium* superficial, consisting of septate, branched, pale brown to hyaline, 2.5–6 µm wide hyphae. *Conidiophores* macronematous, consisting of three cylindrical hyphae with swollen base (L-shaped), pale brown to hyaline, smooth, erect, closely gathered and parallel to each other, elongating outwards; an additional central core hypha was formed laterally during sporulation; septa appearing due to maturation, 0–2 (mostly 1 median) septum. *Conidiogenous cells* integrated, composite, determinate, cylindrical, 5–7

× (11–)15–25(–28)  $\mu$ m, up to 8.5  $\mu$ m wide at the base. *Conidia* solitary, a single propagule formed from the four hyphae composing the conidiophore, smooth or finely verruculose, orange-brown, pumpkin-shaped, 15 – 30  $\mu$ m wide × 8–13  $\mu$ m high (excluding basal projection), consisting of two layers; a central hexagonal cell, surrounded by six peripheral cells in each layer, with additional four connecting cells which formed between the lower layer and the conidiogenous cells; three exterior connecting cells finally become spike-like cells after enlarging and detaching from the conidiogenous cells; base 7–10  $\mu$ m wide × 10–15  $\mu$ m long.





**Fig. 6.11** *Minimedusa obcoronata.* a. Sporodochia on leaf; b–d. conidia, conidiogenous cells and conidiophores; e, f, h, i. conidia (underneath); g, j. conidia (surface). — Scale bars:  $a = 100 \mu m$ ;  $b-j = 10 \mu m$ , b applies to b–d, e applies to e–j.

Specimen examined. Thailand, Thatakiab, Chachoengsao, on *Eucalyptus* camaldulensis, 1 Jan. 2006, coll. *W. Himaman*, isol. *P.W. Crous*, culture CBS 120605 = CPC 13495, CPC 13496, 13497.

Notes — *Pneumatospora* and *Tricellulortus* were transferred to the genus *Minimedusa* based on the conical bulbil-like structures observed on their conidia (Diederich & Lawrey 2007). DNA sequence data of the LSU region support this decision, confirming the close relationship to *Minimedusa obcoronata* (Lawrey *et al.*, 2007), and placing the genus in the *Cantharellales*.

*Neofabraea eucalypti* Cheewangkoon and Crous, sp. nov. — MycoBank MB513849; Fig. 6.12

Anamorph. Unknown.

Differt a speciebus diversis *Neofabraeae* ascis brevioribus,  $(35-)40-45(-52) \times 10-12 \mu m$ , et ascosporis brevioribus,  $10-14 \times 4-6 \mu m$ .

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

Ascomata apothecial, sessile to subsessile, short-stalked, gregarious, sometimes confluent, clustering on a basal stroma, partly immerged, with 3–12 apothecia per group, merged into irregular complexes, up to 0.3 mm high and 0.5 mm diam, medium to dark brown, with soft flesh, lacking a pseudoparenchymatous ectal excipulum; disc becoming turbinate, bearing filamentous, sparse white aerial mycelium at the base of apothecia, 2–3  $\mu$ m wide, up to 200  $\mu$ m long; producing pale brown; rigid pale brown setae-like structures surrounding the apothecia, cylindrical, up to 6  $\mu$ m wide, 45–60  $\mu$ m long, 2–3-septate, straight or very slightly curved, slightly enlarged at the truncate apex. *Basal stroma* subsuperficial, up to 50  $\mu$ m thick, partly immersed in host tissue, composed of irregular, pale to medium brown cells. *Asci* clavate to cylindrical-clavate, apex rounded, short pedicellate, base truncate, hyaline to very pale brown, 8-spored, ascospores discharging through apical pore, (35–)40–45(–52) × 10–12  $\mu$ m. *Paraphyses* mostly 2.5  $\mu$ m wide, up to 65  $\mu$ m high, cylindrical, slender, wider at the base, 2–3(–5)-septate, apex round, hyaline to pale brown, flexuous, numerous. *Ascospores* fusoid to ellipsoid,

aseptate, hyaline, ends rounded, unequal, straight or slightly curved, thin-walled, guttulate,  $10-14 \times 4-6 \mu m$ .

Culture characteristics — Colonies on OA reaching 3 cm after 2 wk at 25 °C in the dark, subcircular, raised, with even margin and slightly folded surface, with dense, white aerial mycelium, partly submerged, buff to white. Apothecia formed after about 4 wk, mostly on the agar surface, black, asci and ascospores mostly similar in shape and size to those formed on PNA (Crous *et al.*, 2006d).



**Fig. 6.12** *Neofabraea eucalypti.* a, b. Ascomata on pine needle agar; c. pycnidia on OA; d–f. paraphyses, asci and setae-like structures (arrows); f. basal stroma; g. asci; h. ascospores. — Scale bars:  $a-c = 100 \mu m$ ;  $d-h = 10 \mu m$ , d applies to d–e, g applies to g–h.

Specimen examined. Australia, Otway, on *Eucalyptus globulus*, 15 Feb. 2007, coll. *I. Smith*, isol. *P.W. Crous*, CBS H-20285 holotype, culture ex-type CPC 13755 = CBS 124810, CPC 13756, 13757.

Notes — *Neofabraea eucalypti* is morphologically similar to species of *Neofabraea* and *Pezicula*. Both genera have apothecia that develop from an immersed

stroma, and a similar ascal shape, and 1-celled ascospores (Verkley, 1999). However, *Neofabraea eucalypti* is better accommodated in *Neofabraea* as revealed by its characteristic fused apothecial discs (Verkley, 1999). This species is different from other known species based on its shorter asci and distinct ascospore dimensions. Phylogenetically it is also well supported as a species of *Neofabraea*, but does not match any presently described species.

Parasympodiella elongata Crous, M.J. Wingf. and W.B. Kendr., Can. J. Bot. 73: 228. 1995 — Fig. 6.13

Synanamorph. Stylaspergillus sp.

# Teleomorph. Unknown.

Colonies on OA effuse, brownish grey. Mycelium superficial or submerged, consisting of branched, septate, smooth, pale to dark brown, (2.5-)4-6 µm wide hyphae. Conidiophores solitary, micro- to macronematous, cylindrical, unbranched; sterile part with semi-thickened walls, medium to dark grey-brown, 7-10 µm wide, up to 700 µm long, with up to 17 septa; fertile part with thinner walls, pale brown, becoming paler toward the apex, up to 500 µm long, comprising up to 9 conidiogenous cells. Conidiogenous cells holoblastic, terminal and intercalary, integrated, indeterminate, proliferating sympodially, smooth, pale grey-brown, becoming hyaline toward the apex,  $35-50 \times 6-10 \mu m$ . Conidia thallic-arthric, hyaline to pale brown, dry, smooth, guttulate, thin-walled, cylindrical,  $(35-)40-50(-65) \times 6-8 \mu m$ , (0-)1(-2)-septate, apex and base of intercalary conidia truncate, with a punctiform septal plug at each end, apical conidia with obtuse or rounded apex, occurring in unbranched conidial chains. *Chlamydospores* formed in vegetative hyphae, terminal or intercalary, solitary or in chains, dark brown, sphaerical, limoniform or fusiform, thin-walled, smooth, guttulate,  $(25-)30-40(-45) \times$ (15-)20-35(-45) µm. Stylaspergillus state. Conidiophores micro- or macronematous, formed directly from submersed mycelium, or as lateral branch from the same conidiophores giving rise to the Parasympodiella state, medium to darkbrown, thinwalled, 70-100(-180) µm tall, 9-10 µm diam, with a clavate to subglobose versicle-like

apical cell,  $14-17 \times 15-20 \ \mu\text{m}$ , occasionally giving rise to secondary conidiophores from these apical cells. *Conidiogenous cells*  $6-9 \times 5-7 \ \mu\text{m}$ , formed terminally on the vesicle-like apical cell, supported by one short metula-like structure, rarely branched, ampulliform, lageniform or subcylindrical, uniseriate, with tubular collarettes. *Conidia* subulate, aseptate, hyaline, curved, tapening towards the apex, with a slightly truncate base, thickened,  $(9-)12-17(-22) \times 1-1.7 \ \mu\text{m}$ ; produced in mucoid masses.



Fig. 6.13 Parasympodiella elongata. a. Colony on OA; b. conidiogenous cells and conidia; c. conidia; d. Stylaspergillus sp. synanamorph on Parasympodiella

conidiophores; e–h. conidiophores, conidiogenous cells and conidia of *Stylaspergillus* sp.; i, j. chlamydospores. — Scale bars:  $a = 400 \mu m$ ;  $b-j = 10 \mu m$ .

Specimens examined. Australia, Queensland, Cairns, on *Eucalyptus* sp., 26 Aug. 2006, *P.W. Crous*, CBS H-20287, culture CPC 13285–13287, CBS 124768; Queensland, Cairns, on *Eucalyptus* sp., 26 Aug. 2006, *P.W. Crous*, CPC 13288, 13289; Queensland, Cairns, on *Eucalyptus* sp., 26 Aug. 2006, *P.W. Crous*, CPC 13498 — South Africa, Mpumalanga, Sabie, on leaves of *Syzygium cordatum*, Nov. 1992, coll. *M.J. Wingfield*, isol. *P.W. Crous*, holotype PREM 5190, ex-type cultures CPC 553 = CBS 522.93.

Notes — The Australian collections had conidia similar to *P. elongata*, though slightly longer than those originally reported for this species  $(20-40 \times 6-12 \mu m)$  (Crous *et al.*, 1995c), and with punctiform septal plugs at each end. Furthermore, isolates produced a previously unreported *Stylaspergillus* state in culture. The *Stylaspergillus* state of *P. elongata* differs from *S. laxus* by having branched conidiophores, metula-like structures, shorter conidia, and less dense conidiogenous cells on its apical vesicle. However, only isolate CPC 13285 and CPC 13288 produced the *Stylaspergillus* synanamorph in culture. Phylogenetically these collections are identical to *P. elongata*, and closely related to *P. laxa* and *P. eucalypti* (Fig. 6.1).

Parasympodiella eucalypti Cheewangkoon and Crous, sp. nov. — MycoBank MB513850; Fig. 6.14

Synanamorph. Stylaspergillus sp.

Teleomorph. Unknown.

*Parasympodiellae elongatae* similis, sed conidiis longioribus, (25-)40-50 (-65) × 8–11 µm, et conidiophoris brevioribus, ad 700 µm longis.

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

*Colonies* on OA effuse, medium to dark grey, chlamydospores absent. *Mycelium* immersed and superficial, consisting of branched, septate, smooth, hyaline to pale brown,  $(3-)5-7 \mu m$  wide hyphae. *Conidiophores* solitary, micro- to macronematous, cylindrical, unbranched; sterile part thicker walled, medium to dark grey-brown,  $5-8 \mu m$  wide, up to

700 µm long, with up to 20 septa; fertile part thinner walled, pale grey-brown at basal region, paler toward the apex, up to 500 µm long, comprising up to 6 conidiogenous cells. Conidiogenous cells holoblastic, terminal and intercalary, integrated, indeterminate, proliferating sympodially, with one conidiogenous locus per cell, smooth, pale greybrown, becoming hyaline toward the apex,  $(35-)45-65 \times 8-12$  µm. Conidia thallicarthric, hyaline to very pale brown, dry, smooth, guttulate, thin-walled, cylindrical, (25-)  $40-50(-65) \times 8-11 \ \mu\text{m}, (0-)1(-2)$  septate, somewhat swollen in the apical cells, up to 14 µm wide, apex and base of intercalary conidia truncate, with a punctiform septal plug at each end, apical conidia with obtuse or rounded apex; conidia occurring in unbranched conidial chains. Stylaspergillus state. Conidiophores macro- or mononematous, mostly formed as a lateral branch from the same conidiophore giving rise to the Parasympodiella state, medium to dark brown, thin-walled, branched, 50-70(-100) µm high, 6-8 µm wide, with a clavate to subglobose vesicle-like apical cell, variable in length, narrower than the main conidiophores,  $10-14 \times 12-17$  µm. Conidiogenous cells terminal or intercalary, thin-walled, smooth, medium to dark brown, slightly paler toward the apex, formed terminally on half of the vesicle-like apical cell or intercalary, ampulliform, lageniform or subcylindrical, forming loosely, with uniseriate phialides, and a tubular collarette, 5–8  $\times$ 4-6 µm. Conidia subulate, aseptate, hyaline, curved, with an attenuated end, and slightly truncate base, thickened,  $(8-)10-12(-15) \times 0.8-1.2 \mu m$ , produced in mucoid masses.

Specimen examined. Venezuela, on *Eucalyptus camaldulensis*, 1 Jan. 2006, coll. *M.J. Wingfield*, isol. *P.W. Crous*, CBS H-20286 holotype, culture ex-type CPC 13397 = CBS 124810.

Notes — Parasympodiella eucalypti is most similar to P. elongata, but it has longer conidia and shorter conidiophores (Crous et al., 1995b). In culture P. eucalypti forms a typical Stylaspergillus synanamorph. The Stylaspergillus state of P. elongate differs from S. laxus by its branched conidiophores, metulalike structures, shorter conidia, and less dense conidiogenous cells on its apical vesicle. Phylogenetically it clusters close to Parasympodiella laxa and P. eucalypti (Fig. 6.1).



**Fig. 6.14** *Parasympodiella eucalypti.* a, b. Conidiogenous cells; c. conidial chain; d. conidia; e. *Stylaspergillus* sp. synanamorph on *Parasympodiella* conidiophore; f–i. conidiophores, conidiogenous cells and conidia of *Stylaspergillus* sp. — Scale bars = 10  $\mu$ m, a applies to a–b, c applies to c–d, e applies to e–i.

*Parasympodiella laxa* (Subram. and Vittal) Ponnappa, Trans. Brit. Mycol. Soc. 64: 344. 1975 — Fig. 6.15

Synanamorph. *Stylaspergillus laxus* B. Sutton, Alcorn and P.J. Fisher, Trans.Brit. Mycol. Soc.

Teleomorph. Unknown.

*Mycelium* on PNA superficial, consisting of branched, septate, smooth, hyaline to pale brown hyphae, 6–10  $\mu$ m wide. *Conidiophores* micro- to macronematous, scattered, erect, solitary, unbranched, cylindrical; sterile part dark brown, with a somewhat thickened wall, 8–10  $\mu$ m wide, up to 500  $\mu$ m long, with up to 8 septa; swollen base 12–17  $\mu$ m wide; fertile part grey-brown, later becoming paler toward the apex, up to 500  $\mu$ m long, comprising up to 8 conidiogenous cells. *Conidiogenous cells* holoblastic, terminal and intercalary, integrated, indeterminate, with sympodial proliferation, and one conidiogenous locus per cell, smooth, pale grey-brown, becoming hyaline toward the apex, 35–50(–75) × 8–12  $\mu$ m between conidiogenous

loci. *Conidia* thallic-arthric, forming in loose chains, hyaline to pale brown, dry, smooth, guttulate, thin-walled, cylindrical,  $(25-)35-50(-60) \times 8-9 \mu m$ , (0-)3(-7)-septate, apex andbase of intercalary conidia truncate, with a punctiform septal plug at each end, apical conidia with obtuse or rounded apex, occurring in unbranched conidial chains. *Stylaspergillus* state. *Conidiophores* macro- or mononematous, formed as a lateral branch or intercalary from the same conidiophores giving rise to the *Parasympodiella* state, or arising separately from the same mycelium, medium to dark brown, generally branched, sometimes giving rise to 2 apical branches,  $60-80(-120) \mu m$  high,  $6-9 \mu m$  wide, with a clavate apical cell,  $10-12 \times 10-18 \mu m$ . *Conidiogenous cells* terminal or intercalary, crowded in the upper half of the apical cell, ampulliform or lageniform, phialidic, inwardly curved, thin-walled, smooth, pale brown, slightly paler toward the apex,  $5-9 \times 4-6 \mu m$ . *Conidia* subulate, aseptate, hyaline, curved, with an attenuated end and slightly truncate base, thickened,  $(12-)15-20(-25) \times 0.8-1.8 \mu m$ , produced in mucoid masses.

Specimen examined. New Zealand, Auckland, on Camellia japonica, C.F.Hill, CBS 102698.

Notes — The present isolate closely matches the original description of *Parasympodiella laxa* based on its conidial morphology and that of its reported synanamorph, *S. laxus* (Sutton *et al.*, 1982). Phylogenetically it clusters close to *P. longispora* and *P. elongata* (Fig. 6.1).



**Fig. 6.15** *Parasympodiella laxa*. a, b. Conidiophores, conidiogenous cells and conidia; c. conidia; d. conidiophores and conidiogenous cells of *Stylaspergillus laxus* synanamorph; e. *Stylaspergillus laxus* synanamorph on *Parasympodiella* conidiophores. — Scale bars = 10  $\mu$ m, a applies to a–b, d applies to d–e.

*Penidiella corymbia* Cheewangkoon and Crous, sp. nov. —MycoBank MB513851; Fig. 6.16

Teleomorph. Unknown.

Differt a speciebus diversis *Penidiellae* hyphis manifeste constrictis et conidiis septatis.

Etymology. Named after its host genus, Corymbia.

*Mycelium* consisting of branched, septate, smooth to slightly vertuculose, pale to dark brown,  $2-3 \mu m$  wide hyphae, swollen cells up to 6.5  $\mu m$  wide, with semi-thickened walls; hyphae becoming constricted at septa, darker and thicker-walled in wider hyphae. *Conidiophores* micronematous to semi-macronematous, arising from creeping mycelium, mostly from narrow hyphae, solitary, erect, cylindrical, somewhat constricted at septa, straight to slightly curved, medium to dark brown, slightly thick-walled, smooth to finely

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verruculose,  $(15 - )25 - 35(-40) \times 3 - 3.5(-4)$  µm. Conidiogenous cells terminal, rarely intercalary, cylindrical, tapering to a flattened apical region, finely vertuculose, medium brown, paler toward the apex,  $(8.5-)13-20(-25) \times 3-3.5 \mu m$ , with up to two conidiogenous loci, often apical, sometimes situated on small lateral shoulders, loci truncate, not denticulate; scars slightly thickened, darkened, 2.5–3 µm wide, visible as small dark circles when viewed directly from above. Ramoconidia subcylindrical or obovoid, 0-2-septate, basesubtruncate to slightly rounded, but not coronate, mostly with 2 apical hila, pale to medium brown, finely vertuculose, slightly thick-walled,  $\leq 1 \mu m$ ,  $(7-)10 - 12(-14) \times 2.5-3 \mu m$ ; scars thickened and darkened, minute marginal frill present on basal end of some conidia; basal hila  $2.5-3 \mu m$  wide, apical hila  $1.5-2 \mu m$  wide. Conidia in branched acropetal chains, broadly fusiform to obovoid, 0-1-septate, pale to medium brown,  $7-9(-12.5) \times 2.5-3(-3.5)$  µm; terminal conidia obovoid, aseptate, pale brown, paler towards the apex, mostly smooth, base truncate,  $4.5-6.5 \times 2-3$  µm. Microcyclic conidiation observed, original conidia become swollen, darker and thickwalled, constricted at septa, up to 5 µm wide; wall 1-1.5 µm thick. Chlamydospores globose to subovoid, dark brown, thin-walled,  $7-9 \times 7-9(-14)$  µm, terminal or intercalary, mostly 1-celled, rarely septate (up to 3 horizontal septa), produced from narrow hyphae.

Culture characteristics — Colonies on MEA reaching 1.5 cm diam after 10 d at 25 °C in the dark; margin feathery, colonies erumpent, spreading, with moderate aerial mycelium. Surface pale brown to olivaceous, reverse olivaceous-black.

Specimen examined. Australia, Northern Territory, Emerald Springs, S 13°37'13.3", E 131°36'40", on *Corymbia foelscheana*, 22 Sept. 2007, coll. *B.A.* Summerell, isol. P.W. Crous, CBS H-20288 holotype, culture ex-type CPC 14640 = CBS 124769, CPC 14641, 14642.

Notes — *Penidiella corymbia* is a typical member of the genus *Penidiella* in having solitary conidiophores with a branching system consisting of ramoconidia, intercalary and terminal conidia, and lacking a rachis (Crous *et al.*, 2007a; Cheewangkoon *et al.*, 2008). *Penidiella corymbia* is different from most other *Penidiella* 

species by having prominently constricted hypha and septate conidia. It is similar to *P. rigidophora* based on its macronematous conidiophores and conidial dimensions. Phylogenetically it clusters with other members of *Penidiella* (Fig. 6.1), but is distinct from all other species known to date based on ITS sequence data (Table 6.1).



**Fig. 6.16** *Penidiella corymbia.* a–h. Conidiophores, conidiogenous cells and conidial chains; i–l. chlamydospores; m–o. conidiogenous cells with prominent scars; p–r. hyphae; s, t. conidia. — Scale bars =  $10 \mu m$ , a applies to a–h, i applies to i–n, p applies to p–r, s applies to s–t.

Polyscytalum algarvense Cheewangkoon and Crous, sp. nov. — MycoBank MB513852; Fig. 6.17

Teleomorph. Unknown.

*Polyscytalo fuegiano* simile, sed conidiis brevioribus et latioribus, 11–13.5 (–15)  $\times$  2–2.5 µm.

*Etymology*. Named after the Algarve Province in Portugal, where this fungus was collected.

Colonies on OA. Conidiomata consisting of a dark brown, submerged, sclerotium-like structure, which give rise to white, blush-like caespituli consisting of conidiophores and conidial chains, up to 200 µm tall and 380 µm diam. Mycelium immersed, dense, subcylindrical, medium to dark brown, thick-walled, frequently somewhat constricted at septa, up to 6 µm wide; aerial mycelium hyaline, smooth,  $\leq 2$  µm wide. Conidiophores erect, solitary, cylindrical, hyaline, straight to slightly flexuous, simple or with two lateral branches, smooth,  $45-60(-90) \times 3-3.5$  µm, up to 5-septate, thickened at septa, usually swollen and slightly brown at the base. Conidiogenous cells terminal, integrated, cylindrical, slightly tapering to a flat apex, or short and broad denticles, hyaline,  $13-16(-23) \times 2-2.5$  µm wide. Conidia elongating acropetally, or branching di- or trichotomously, forming long conidial chains that remain attached, cylindrical, with slight taper towards both ends, smooth, hyaline, with minute guttulates, aseptate,  $11-13.5(-15) \times 2-2.5$  µm, with two conidiogenous loci in ramoconidia; scars flat to slightly rounded, thickened, slightly refractive, 1.5-2 µm wide.

Culture characteristics — Colonies obtaining 3 cm diam on MEA after 1 wk at 25 °C in the dark; flat, with sparse aerial mycelium, and entire margins; yellow-buff (surface), and similar in reverse. Colonies on OA flat, appearing dark brown, with moderate, white aerial mycelium, and irregular margins. *Specimen examined*. Portugal, Faro, Algarve, on *Eucalyptus* sp., 24 Jan. 2007, *P.W. Crous*, CBS H-20289 holotype, culture ex-type CPC 14936 = CBS 124770, CPC 14937, 14938.



**Fig. 6.17** *Polyscytalum algarvensea*–c. Sporodochia on OA; d–f. conidiophores, conidiogenous cells and conidial chains; g–i. conidia; j. conidiogenous cell; k. creeping hyphae. — Scale bars:  $a = 300 \ \mu\text{m}$ ;  $b = 200 \ \mu\text{m}$ ;  $c = 150 \ \mu\text{m}$ ;  $d-k = 10 \ \mu\text{m}$ , d applies to e–f, g apllies to g–i, j applies to j–k.

Notes — *Polyscytalum algarvense* closely resembles other members of the genus *Polyscytalum* in forming conidiophores on swollen hyphal cells, cylindrical, polyblastic conidiogenous cells with denticles, and catenate, acropetal chains of cylindrical, hyaline conidia (Ellis, 1971). Three species of *Polyscytalum* have thus far been reported from *Eucalyptus*, namely *P. gracilisporum* (Sutton and Hodges, 1977; Crous and van der Linde, 1993), *P. hareae* (Sutton, 1978; Kirk, 1981) and *P. truncatum* (Sutton and Hodges, 1977), which differ in conidium and conidiophore morphology. *Polyscytalum algarvensense* is morphologically most similar to *P. fuegianum* (Gamundí *et al.*, 1977), in mostly producing simple conidiophores, conidiogenous cells with only a few terminal loci, and aseptate conidia. However, *P. algarvensense* has shorter and wider conidia in vivo than that of *P. fuegianum* (15.4–19.2 × 1.4–2 µm). Phylogenetically *P. algarvensense* is allied to *P. fecundissimum* (GenBank EU035441) (Fig. 6.1).

*Pseudocercospora palleobrunnea* Cheewangkoon and Crous, *sp. nov.* — MycoBank MB513853; Fig. 6.18

Teleomorph. Unknown.

Differt a speciebus *Pseudocercosporae myrtacearum* conidiis subcylindraceis, 1– 6-septatis,  $(35-)40-55(-85) \times (2.5-)3-4(-4.5) \mu m$ .

Etymology. Named after its pale brown conidia.

*Mycelium* internal and external, pale brown, consisting of septate, branched, smooth hyphae, 4–6 µm wide. *Caespituli* pale to medium brown, 80–130 µm high and up to 230 µm wide. *Conidiophores* fasciculate, densely aggregated, arising from the upper cells of a well-developed sub-superficial stroma; stroma dark brown, up to 70 µm high and 180 µm wide; conidiophores medium brown, paler toward the apex, slightly verruculose, 2–4-septate, subcylindrical, straight or slightly sinuous, rarely branched,  $(40-)50-65(-80) \times 3-4.5$  µm. *Conidiogenous cells* terminal, unbranched, mono- to polyblastic, sympodial, subcylindrical, pale brown, slightly verruculose to smooth, terminating in truncate or bluntly rounded loci,  $(6-)10-15(-22) \times (2.7-)3-4(-4.8)$  µm. *Conidia* solitary, subcylindrical, tapering to a bluntly rounded apex and truncate base, thick-walled, subhyaline to pale brown, guttulate, curved, mostly widest above the middle, 1–6-septate,  $(35-)40-55(-85) \times (2.5-)3-4(-4.5)$  µm; hila 2–3 µm wide, not darkened, but slightly thickened along the rim.

Culture characteristics — Colonies reaching 17 mm diam on MEA after 1 mo at 25 °C in the dark; colonies circular, convex, with entire margin and medium aerial mycelium; pale greenish grey (surface), fuscous-black (reverse).

*Specimen examined.* Australia, Queensland, Moubray Park, on *Syzygium* sp., 27 Aug. 2006, *P.W. Crous*, CBS H-20290 holotype, culture ex-type CPC 13387 = CBS 124771, CPC 13388, 13389.

Notes — Several cercosporoid species have been recorded on *Myrtaceae* (Crous and Wingfield, 1997; Sutton and Crous, 1997; Crous, 1998, 1999; Braun 2001a; Braun and Dick, 2002; Crous *et al.*, 2004b, 2006f, 2007e; Hunter *at al.*, 2006a; Carnegie *et al.*, 2007). Of these, *P. palleobrunnea* resembles *P. syzygii-cumini* (conidia 1–6-septate,

subcylindrical to obclavate,  $25-60 \times 2-3.5 \ \mu\text{m}$ ), *P. syzygiicola* (conidia cylindrical, 1–11septate,  $40-80 \times 2-3 \ \mu\text{m}$ ), and *P. syzygiigena* (conidia 1–5-septate, subcylindricalfiliform,  $15 - 60 \times 1.5-3 \ \mu\text{m}$ ), but can be distinguished from them based on its conidial shape, septation and dimensions. Based on ITS sequence data (Table 1) *P. palleobrunnea* is phylogenetically closely related to *Mycosphaerella fori*, the *Pseudocercospora* state of which is quite distinct, having conidia that are 1–3-septate,  $50-100 \times 2-3.5 \ \mu\text{m}$  (Hunter *et al.*, 2006b).



**Fig. 6.18** *Pseudocercospora palleobrunnea.* a. Sporodochium; b–e. conidiophores, conidiogenous cells and conidia; f. conidia. — Scale bars:  $a = 35 \mu m$ ; b–f = 10  $\mu m$ , b applies to b–f.

*Pseudoramichloridium* Cheewangkoon and Crous, *gen. nov.* — MycoBank MB513854

*Ramichlorodio* simile, sed coloniis in cultura (MEA) atro-olivaceis et tarde crescentibus, cicatricibus et hilis leviter incrassatis, fuscatis et refractivis.

Type species. Pseudoramichloridium henryi Cheewangkoon and Crous, sp. nov.

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

*Mycelium* consisting of submerged and aerial hyphae; submerged hyphae pale to medium olivaceous-brown, thin- to slightly thick-walled; aerial hyphae smooth or verrucose, narrower and darker than the submerged hyphae. *Conidiophores* unbranched, slightly thick-walled, darker than the subtending hyphae, arising vertically from submerged or creeping aerial hyphae, with additional thin septa. *Conidiogenous cells* integrated, terminal, proliferating sympodially, giving rise to a long rachis with crowded, polyblastic scars that are protruding, somewhat prominent, thickened along the rim, slightly reflective, somewhat darkened. *Conidia* obovoid to fusiform, thin-walled, smooth to verruculose, aseptate, pale brown, solitary, aseptate, subhyaline to pale brown, smooth to slightly verruculose, with truncate base; hilum thickened, slightly reflective, somewhat darkened; conidial secession schizolytic. Colonies are dark olivaceous and slow-growing on MEA, and exophiala-like states are absent.

*Pseudoramichloridium henryi* Cheewangkoon and Crous, *sp. nov.* — MycoBank MB513855; Fig. 6.19

Teleomorph. Unknown, Teratosphaeriaceae.

*Pseudoramochloridio brasiliano* simile, sed conidiis longioribus,  $6-8(-9) \times (2-)$ 2.5–3 um.

Etymology. Named after the host species on which it occurs, Corymbia henryi.

*Mycelium* consisting of submerged and aerial hyphae; narrow hyphae submerged, hyaline to subhyaline, thin-walled, smooth to slightly vertuculose, 2-3 µm wide; fertile hyphae submerged, partly erumpent, become wider, thicker and darker-walled, up to 5

μm wide, constricted at septa, forming an erumpent, darkened stroma; aerial hyphae mostly produced on setae-like structures among conidiophores, smooth to slightly verruculose, thick-walled, pale to medium brown, becoming thinner-walled and paler toward the apex,  $80-120(-150) \times 2.5-3$  μm. *Conidiophores* mono- and macronematous, produced on stroma-like structures, not on creeping hyphae, or arising from thickened, darkened hyphae, not swollen at the base, cylindrical, straight, unbranched, thick-walled, medium brown, up to 90 μm long, 2.5–3 μm wide, 3–8-septate. *Conidiogenous cells* integrated, terminal, polyblastic, smooth, thick-walled, medium to pale brown, thinner and paler toward the apex, apical part subhyaline, proliferating sympodially, straight, with conspicuous conidiogenous loci, (5–)10–15(–20) × 2.5–3 μm; scars scattered, somewhat thickened and slightly pigmented, flat, 0.5–0.8 μm wide, sometimes prominent denticles, up to 1.5 μm high. *Conidia* solitary, aseptate, hyaline to very pale brown, smooth, thin-walled, obovoid to ellipsoidal, with truncate base, 6–8(–9) × (2–)2.5–3 μm;



**Fig. 6.19** *Pseudoramichloridium henryi.* a. Colony on MEA; b. colony on SNA; c, d. conidiophores, conidiogenous cells and conidia; e, f. conidia. — Scale bars =  $10 \mu m$ , c applies to c–f.

hilum prominently thickened along the rim, slightly reflective, somewhat darkened,  $1-1.2 \mu m$  wide.

Culture characteristics — Colonies on MEA reaching 15 mm diam after 14 d at 25 °C; circular, convex, with a slightlyundulate, smooth margin, and moderate aerial mycelium; pale greenish grey to pale olivaceous-grey (surface); olivaceousblack (reverse).

*Specimen examined*. Australia, New South Wales, on *Corymbia henryi*, 16 Feb. 2006, coll. *A.J. Carnegie*, isol. *P.W. Crous*, CBS H-20293 holotype, culture ex-type CPC 13121 = CBS 124775, CPC 13122, 13123.

Notes — Morphologically *Pseudoramichloridium (Teratophaeriaceae)* resembles the genus *Ramichloridium (Mycosphaerellaceae)* by having well-differentiated, pigmented, unbranched, sympodially proliferating rachi producing aseptate, pigmented conidia, and lacking exophiala-like states (de Hoog, 1977; Arzanlou *et al.*, 2007). *Pseudoramichloridium* can be distinguished from *Ramichloridium* by having colonies that are dark olivaceous and slow-growing on MEA, and conidial scars and hila that are faintly thickened, darkened and somewhat refractive. Conidia of *Pseudoramichloridium henryi* are longer,  $6-8(-9) \times (2-)2.5-3$  µm, than those of *Pseudoramichloridium brasilianum*,  $(4-)5-6(-8.5) \times 2-2.5(-3)$  µm.

*Pseudoramichloridium brasilianum* (Arzanlou and Crous) Cheewangkoon and Crous, *comb. nov.* — MycoBank MB513586

Basionym. Ramichloridium brasilianum Arzanlou and Crous, Stud. Mycol. 58: 72. 2007.

Description and illustrations — Arzanlou et al. (2007).

*Quambalaria simpsonii* Cheewangkoon and Crous, sp. nov. — MycoBank MB513857; Fig. 6.20

Teleomorph. Unknown.

*Quambalariae coyrecup* similis, sed coloniis in cultura (MEA et PDA) constanter albidis.

*Etymology*. Named after the Australian mycologist, Dr J.A. Simpson, who introduced the genus *Quambalaria*.

*Mycelium* superficial, partly immersed; aerial hyphae hyaline, smooth, thinwalled, loosely septate, branched, 1.5–2.2 µm wide. *Conidiogenous cells* scattered, cylindrical, similar to hyphae, terminal or integrated in short side branches, (9–)18–40 (–53) × (1.5–)1.8–2(–2.2) µm, widest at swollen apex, which forms conidia via sympodial growth, 2–3.5(–4.2) µm wide, often elongating, giving rise to another conidiogenous cell at a higher level; conidiogenous loci denticulate, inconspicuous, pointed or flattened. *Conidia* aseptate, hyaline, smooth, thin-walled, continuous; *ramoconidia* fusiform or ellipsoid, with tapered base, (4.6–)5.5–8.5(–10.4) × (2.5–)3 (–3.5) µm, usually giving rise to one or several obovoid to fusiform secondary conidia, (2.5–)3–4.5(–6.2) × (1.7–)2–2.5(–2.9) µm, sometimes giving rise to 1–4 obovoid, tertiary conidia, (2–)2.5(–3.4) × (1.3–)1.5–1.8(–2.3) µm.

Culture characteristics — Colonies on MEA reaching up to 25 mm diam after 7 d, finely floccose, becoming powdery, white (surface), yellow-brown (reverse), with an odour reminiscent of ripe bananas.

Specimens examined. Australia, Northern Territory, Edith Falls S 14°05'20", E 132°05'12"E, on *Eucalyptus tintinnans*, 1 Jan. 2007, coll. *B.A. Summerell*, isol. *P.W. Crous*, CBS H-20291 holotype, culture ex-type CPC 14499 = CBS 124772, CPC 14500, 14501. — Thailand, Ban Hong, Lamphoon, *Eucalyptus* sp., 26 June 2007, *R. Cheewangkoon*, CBS 124773.

Notes — Of the *Quambalaria* species known to date (Walker and Bertus, 1971; de Hoog and de Vries, 1973; Wingfield *et al.*, 1993; Braun 1998; Simpson, 2000; de Beer *et al.*, 2006), *Q. simpsonii* closely resembles *Q. coyrecup* in conidial dimensions (Paap *et al.*, 2008). However, colonies of *Q. simpsonii* remain white, whereas those of *Q. coyrecup* are reported as turning yellowish white or pale orange on MEA and PDA (Paap *et al.*, 2008). Phylogenetically *Q. simpsonii* is more closely related to

*Q. cyanescens* and *Q. eucalypti* than *Q. coyrecup* based on LSU and ITS sequences (Fig. 6.1, Table 6.1).



**Fig. 6.20** *Quambalaria simpsonii*. a. Colony on MEA; b–i. hyphae, conidiogenous cells and conidia; j. conidia. — Scale bars =  $10 \mu m$ , b applies to b–g, h applies to h–i.

**Rachicladosporium americanum** Cheewangkoon and Crous, *sp. nov.* — MycoBank MB513858; Fig. 6.21

Teleomorph. Unknown.

Rachicladosporio luculiae simile, sed conidiis longioribus.

Etymology. Named after the USA, where it was collected.

*Mycelium* septate, not constricted at septa, which are not thickened nor darkened; hyphae smooth, slightly vertuculose, pale to medium brown, loosely branched, walls semi-thickened, 4.5-6(-7.5) µm wide. *Conidiophores* arising laterally from creeping hyphae, micronematous to semi-macronematous, erect, straight to slightly flexuous, cylindrical, neither geniculate nor nodulose, occasionally short-branched, up to 95 µm long, 5–6.5 µm wide, 4–11-septate, pale to medium brown, smooth to slightly verruculose, walls slightly thickened. *Conidiogenous cells* integrated, mostly terminal, sometimes intercalary, cylindrical,  $4-6.5 \times (7.5-)9.5-12(-15)$  µm, conidiogenesis holoblastic, proliferation sympodial, with a single or up to three conidiogenous loci, often at the apex, sometimes situated on small lateral shoulders, loci truncate, not denticulate, 1.5–2.5 µm wide, thickened and darkened, visible as small dark circles when viewed directly from above. *Ramoconidia* cylindrical,  $(13-)16-18(-23) \times (3-)3.5-4$  µm, 1–2septate, slightly constricted at septa, concolorous with conidiophores, walls semithickened, smooth to slightly verruculose, apically with up to 3 truncate hila, 2–2.5 µm wide, thickened and darkened, not refractive. *Conidia* catenate, in loosely branched chains, ellipsoid, fusiform to subcylindrical, tapering towards both ends, (10-)12-16 $(-18) \times (3-)3.5-4$  µm, walls semi-thickened, 0–1-septate, mostly with 1-median septum, slightly constricted at septum; hila truncate, 1–2 µm wide, thickened, somewhat darkened, not refractive; terminal conidia ellipsoid, paler towards apex, with rounded apex, thin-walled, 0–1-septate, 8–11 × 3–3.5 µm; base with truncate hilum that is thickened, darkened, but not refractive.

Culture characteristics — Colonies on MEA reaching 2 cm diam after 10 d at 25 °C in the dark, flat, elevated at centre; colonies felty, with dense sporulation and sparse aerial mycelium; brownish olivaceous in the centre, grey-olivaceous at the margin (surface), dark brown (reverse).

Specimen examined. USA, Virginia, Fort Royal, on leaf litter of unknown host, 1 May 2007, *P.W. Crous*, CBS H-20292 holotype, culture ex-type CPC 14045 = CBS 124774, CPC 14046, CPC 14047.

Notes — Using the key to cladosporioid genera provided by Crous *et al.* (2007b), *R. americanum* is a typical member of the genus *Rachicladosporium*, except that it lacks an apical rachis (though this feature is not considered diagnostic, and hence not used in the key). Morphologically, *R. americanum* can also be distinguished from *R. luculiae* by its longer conidia. Phylogenetically the two species cluster together, suggesting that the rachis observed in the type species, *R. luculiae*, is probably not a feature of generic importance.



**Fig. 6.21** *Rachicladosporium americanum.* a–e. Conidiophores, conidiogenous cells and conidia; f. conidiogenous cells with prominent scars. — Scale bars =  $10 \mu m$ , a applies to a–e.

Selenophoma australiensis Cheewangkoon and Crous, sp. nov. — MycoBank MB513859; Fig 6.22

Teleomorph. Unknown.

Selenophomae eucalypti similis, sed conidiis minoribus,  $(5.5-)6-6.5(-7) \times 3-3.5$ 

μm.

*Etymology*. Named after its country of origin, Australia. *Selenophoma* state. *Conidiomata* on CLA pycnidial, dark brown, subepidermal to erumpent, globose, 70–110  $\times$  80–110 µm; wall consisting of 2–3 layers of medium to dark brown *textura angularis*, thick-walled. *Conidiophores* not uniform, short, barrel- shaped or subobovoid, simple, medium brown, thick-walled, composed of 1–3 cells, tapering toward the conidiogenous

cell, occasionally reduced to conidiogenous cells,  $7-10 \times 5-7$  µm. Conidiogenous cells subglobose, obpyriform or obovoid, phialidic, with apical periclinal thickening, (5-)6-7.5 $\times$  (4–)6–7(–8) µm. Conidia aseptate, hyaline, ellipsoidal to obovoid, thinwalled, guttulate,  $(5.5-)6-6.5(-7) \times 3-3.5$  µm. Hormonema state. Mycelium immersed and superficial; hyphae hyaline, thinwalled, smooth to slightly vertuculose, loosely septate; brown hyphae (type 1) thick-walled, slightly verruculose, densely septate, mostly constricted at septa, phialides integrated in hyphal cells, loci  $\leq 1.5 \mu m$  wide, producing hyaline, aseptate conidia,  $4 - 5.5 \mu m$  wide; brown hyphae (type 2), thin-walled, smooth, loosely septate, not constricted at septa, producing endoconidia, 5-8 µm wide, aggregating in masses in the centre of colonies. Conidiogenous cells undifferentiated from creeping hyphae, intercalary or terminal on brown hyphae,  $4-5 \times 5-6(-8) \mu m$ , producing 1-2 conidia basipetally, with prominent loci, and visible collarette after conidial seccession, apex 1-1.5 µm wide. Conidia producing synchronously, along hyphae and on short lateral branches, aseptate, hyaline, ellipsoid to obovoid, smooth, tapering to  $\leq 1.5 \mu m$  wide truncate base, slightly thick-walled, turning brown and thicker walled when mature, occasionally becoming 1-medianly septate, slightly constricted at septum,  $(2.7-)3-3.5(-4) \times (5.5-)7-8(-8.5)$  µm. Arthroconidia ellipsoid, medium brown, thick-walled, medianly septate, or with slightly longer basal cell, conspicuously constricted at septum, with broadly rounded ends; hilum with 1-2 µm wide; conidia 4.8- $5.5 \times (8.5-)9-10(-11.5) \mu m$ , sometimes producing secondary conidia via microcyclic conidiation,  $7-7.5 \times 3.5-4.5 \mu m$ . Endoconidia produced in thick- and thin-walled hyphae,

0-2-septate, ellipsoidal, constricted at septa, thick-walled. *Chlamydospores* multiseptate, brown, composed of subglobose cells, thick-walled, constrict at septa, irregular in shape,  $10-17 \times 10-13 \mu m$ .

Specimen examined. Australia, Northern Territory, Edith Falls S 14°05'20", E 132°05'12", on *Eucalyptus mineata*, 22 Sept. 2007, coll. *B.A. Summerell*, isol. *P.W. Crous*, CBS H-20294 holotype, culture ex-type CPC 14582 = CBS 124776, CPC 14583, 14584.

Notes — Considerable confusion surrounds the delimitation of *Aureobasidium* and *Hormonema*, complicating species identification in these genera (see discussion under *Sydowia eucalypti* below). For this reason, we prefer to name the current species in *Selenophoma*, which possesses a similar yeast synanamorph as observed in *S. eucalypti* (Crous *et al.*, 1995a).



**Fig. 6.22** Selenophoma australiensis. a. Colony on SNA; b, c. hyphae; d–i. conidia produced from hyphal cells; j, k. endoconidia; l. chlamydospores; m. cross section through pycnidium on canation leaf agar; n, o. conidiogenous cells; p. conidia. — Scale bars:  $a = 200 \mu m$ ;  $b-p = 10 \mu m$ , f applies to f–g, j applies to j–k, l applies to l–m.

Sphaceloma tectificae Cheewangkoon and Crous, sp. nov. — MycoBank MB513860; Fig. 6.23

Synanamorph. Sporotrichum sp.

Teleomorph. Unknown.

Cellulae conidiogenae phialidicae, hyalinae, laeviae, non ramosae, ex hyphis lateraliter oriundae, obclavatae vel cylindraceae,  $(4-)8-10 \times 2.5-3(-5) \mu m$ . Conidia hyalina, acrogena, ellipsoidea vel brevicylindracea vel obovoidea, aseptata,  $4-4.5 \times 2-2.5 \mu m$ .

Etymology. Named after the host species on which it occurs, Eucalyptus tectifica.

Sphaceloma state. Acervular conidiomata not observed in culture. Conidiogenous cells phialidic, hyaline, smooth, unbranched, occurring as lateral tips on hyphae, sometime with one basal supporting cell, obclavate to cylindrical, tapering sharply to a truncate apex,  $(4-)8-10 \times 2.5-3(-5)$  µm. Conidia acrogenous, ellipsoid to short cylindrical or obovoid, aseptate,  $4-4.5 \times 2-2.5 \mu m$ , hyaline, minutely guttulate, hila slightly rounded to subtruncate; germinating conidia become slightly elongated and swollen, thicker walled, guttulate, but remain hyaline. Sporotrichum synanamorph. Mycelium consisting of branched, septate, smooth, hyaline to pale brown hyphae, minutely guttulate, occasionally constricted at septa, 2-3(-5) µm wide, somewhat aggregated in bundles, densely septate. Conidiophores macronematous, arising from creeping mycelium, sometimes reduced to conidiogenous cells, pale brown, darker towards conidiogenous cells, cylindrical, simple or branched, 0-4-septate, (12-)20-30  $(-40) \times 2-2.5$  µm. Conidiogenous cells terminal, integrated, smooth to slightly verruculose, thin-walled, straight or geniculate, somewhat swollen to irregular, (7–)15–  $20(-30) \times (3-)4-5(-6)$  µm, with crowded conidiogenous loci in an apical rachis, denticles  $\leq$  1 µm high, flat tipped, with minutely thickened and reflective scars, visible as a circle when viewed from directly above, 1-1.3 µm diam. Conidia in short, branched chains; ramoconidia cylindrical to ellipsoid, tapering toward both ends, sometimes swollen at the crowded conidiogenous loci, aseptate, thin-walled, smooth to slightly vertuculose, pale to medium brown,  $7-9(-11) \times 2.5-3(-4)$  µm; hila thickened along the rim, refractive, not

darkened; *intercalary conidia* ellipsoid to fusiform, aseptate, pale to medium brown, 6–8(–9.5) × 2.2–3.3 µm; *terminal conidia* obovoid, pale brown, paler toward the apex, (2.5–)3.5–5 × 2–2.5 µm. *Chlamydospores* globose to subglobose, thin-walled, 0– multicellular, hyaline, muriformly septate,  $5-8 \times 8-10$  µm. *Microcyclic conidiation* present.



**Fig. 6.23** *Sphaceloma tectificae.* a. Colony on MEA; b–h. *Sporotrichum* synanamorph; b–f. conidiophores, conidiogenous cells and conidia; g. *Sporotrichum* producing from *Sphaceloma* conidial anastomosis; h, i. *Sphaceloma* sp. — Scale bars: a = 2 cm;  $b-i = 20 \mu \text{m}$ , b applies to b–i.

Culture characteristics — Colonies on MEA reaching 15 mm diam after 15 d at 25 °C in the dark; colonies irregular, centre strongly folded, convoluted, with sparse, pale, orangegrey aerial mycelium, turning greenish grey and woolly when sporulating; margin feathery, producing a diffuse pigment that changes the colour of the media to reddish orange.

Specimen examined. Australia, Northern Territory, road to Robin Falls, S 14°10'20", E 131°07'15" on Eucalyptus tectifica, 23 Sept. 2007, coll. B.A. Summerell,

isol. *P.W. Crous*, CBS H-20296 holotype, culture ex-type CPC 14594 = CBS 124777, CPC 14595, 14596.

Notes — Sphaceloma tectificae produces both a Sphaceloma and Sporotrichum state in culture, as illustrated by Doidge and Butler (1924) for the anamorphs of Elsinoë fawcettii, confirming that these genera are actually synanamorphs. Although S. tectificae is phylogenetically closely related to E. fawcettii, they still differ in 45 nucleotides based on their ITS sequence data (Table 1). Morphologically S. tectificae produces smaller conidia than E. fawcettii, and its Sporotrichum synanamorph also has larger and more densely branched conidiophores. Phylogenetically S. tectificae clusters apart from E. eucalyptorum (Summerell et al., 2006) and E. eucalypticola.

Strelitziana australiensis Cheewangkoon and Crous, sp. nov. — MycoBank MB513861; Fig. 6.24

Teleomorph. Unknown.

Strelitzianae africanae similis, sed conidiis ad apicem cum appendice mucosa.

Etymology. Named after its country of origin, Australia.

*Mycelium* superficial, partly immersed, hyaline to pale brown, septate, branched, smooth, 2.8–4 µm wide, constricted at septa, which tend to be more frequent closer to the conidiogenous cells. *Chlamydospores* mostly subglobose or ellipsoid, medium to dark brown, thick-walled, 1–4-septate, somewhat constrict at septa,  $(8-)10-14(-18) \times 7-11$  µm. *Conidiophores* mostly reduced to conidiogenous cells, rarely 1–2-septate, subcylindrical, with an apical conidiogenous cell. *Conidiogenous cells* irregular in shape, subglobose to obovoid, somewhat curved,  $(5-)7-10 \times (3.5-)7-11$  µm, intercalary, rarely terminal, polyphialidic, conidial succession rhexolytic, with remnants of the separating cell visible on conidiogenous cells, collarettes  $1.5-2 \times 1.5-4$  µm, appearing as open denticles, up to 12 per conidiogenous cell. *Conidia* cylindrical, rounded apex, smooth, hyaline to very pale brown, minutely guttulate, 4–8-septate,  $(30-)50-60(-73) \times 2.8-3.2$  µm, with a small, globose, hyaline, apical mucilaginous appendage, 4.5-5.5 µm; base obconically subtruncate; sometimes remnants of the separating cell remain attached to the

conidial hilum as a minute marginal frill, up to 1  $\mu$ m long, 1.5–2  $\mu$ m wide; microcyclic conidiation observed in culture.

Culture characteristics — Colonies reaching up to 2.5 cm diam on MEA after 2 wk at 25 °C in the dark; colonies irregular, flat, raised at the middle, slightly folded, with irregular margin, and sparse, smoky-grey aerial mycelium; medium to dark brown (surface); dark brown (reverse).



**Fig. 6.24** *Strelitziana australiensis.* a. Colony on MEA; b–e. conidiogenous cells and conidia; f. conidiogenous cells; g. young conidia arising from conidiogenous cells; h. chlamydospores; i. connection cells on conidiogenous cells; j. apical conidial mucilaginous appendage; k. conidia and microcyclic conidiation. — Scale bars: b–e, h, i,  $k = 20 \mu m$ ; f, g, j = 40  $\mu m$ , b applies to b–d, e, i, k, f applies to f–g, j.

*Specimen examined.* Australia, Queensland, Kennedy Highway, on *Eucalyptus* sp., 26 Aug. 2006, *P.W. Crous*, CBS H-20297 holotype, culture ex-type CPC 13421 = CBS 124778, CPC 13422, CPC 13423.

Notes — *Strelitziana australiensis* can be classified in *Strelitziana* based on its rhexolytic conidiation, polyphialides, pigmented structures, and unthickened conidial scars (Arzanlou and Crous, 2006). Although *S. africana* is presently the only member of this genus, it has similar conidial dimensions to *S. australiensis*. However, *S. africana* lacks an apical mucilaginous appendage, chlamydospores and has obclavate conidia, making it easy to distinguish from *S. africana*. Phylogenetically, the two species also cluster together in *Strelitziana* (Fig. 6.1).

*Sydowia eucalypti* (Verwoerd & du Plessis) Crous, Sydowia 55: 143. 2003 — Fig. 6.25

Basionym. Sphaerulina eucalypti Verwoerd and du Plessis, S. Afr. J. Sci. 28: 296. 1931.

Anamorph. Selenophoma eucalypti Crous, C.L. Lennox and B. Sutton, Mycol. Res. 99: 648. 1995.

Synanamorphs. Coniothyrium-like and Hormonema-like.Descriptions — Verwoerd and du Plessis (1931), Crous *et al.* (1995a, 2003).

*Coniothyrium*-like synanamorph on PNA: *Conidiomata* pycnidial to avervular, dark brown, semi-thick-walled, up to 120  $\mu$ m diam and 200  $\mu$ m high. *Conidiophores* reduced to conidiogenous cells, annellidic, integrated, indeterminate, formed from the inner cells of the conidiomatal wall, hyaline to pale brown, smooth, slightly thick-walled, short-cylindrical to narrowly ampulliform, slightly tapered toward the apex, (2.5–)3.5–5.5 × 1.8–3.3. *Conidia* aseptate, medium brown to olivaceous-brown, ellipsoid to ovoid, not guttulate, thin-walled, 6–8(–10) × (2.3–)3–5.5.

Specimens examined. Australia, New South Wales, on *Eucalyptus* sp., 1 Mar. 2007, coll. *B. Wiecek*, isol. *P.W. Crous*, H-20295, culture CPC 14028 = CPC 14029–

14030. – Portugal, Algarve, Faro, on *Eucalyptus* sp., 22 Jan. 2008, *P.W. Crous*, CPC 14927 = CPC 14928, 14929.



**Fig. 6.25** *Sydowia eucalypti.* a. Conidiomata giving rise to spore masses on host leaf; b. conidia of *Selenophoma*; c. colony on MEA; d. colony on pine needle agar; e, f. conidia of *Coniothyrium*-like synanamorph; g. conidiogenous cell; h–j. asci and ascospores. — Scale bars: a, f, g = 10 mm; b, e = 20  $\mu$ m; d = 100  $\mu$ m; h–j = 40  $\mu$ m, g applies to g–j.

Notes — Morphologically, *Sydowia eucalypti* (anamorph *Selenophoma eucalypti*) has characteristics of *Aureobasidium* and *Hormonema* in culture (de Hoog and Yurlova, 1994, Crous *et al.*, 1995a, 2003; Bills *et al.*, 2004; Zalar *et al.*, 2008). However, the connection between *Aureobasidium* and *Selenophoma* was commented on by Ramaley (1992). Thus far only *Sydowia polyspora* and *Hormonema dematioides* have been

suggested as representing a potential anamorph-teleomorph relationship (Robak, 1952; Butin, 1964). Several other genera in the *Dothideomycetes* (*Sydowia, Pringsheimia, Dothidea, Dothiora*) produce hormonema-like anamorphs in culture (Froidevaux, 1972; Sivanesan, 1984). The taxonomic status of *Aureobasidium* and *Hormonema* remains controversial, however, as these two genera are not well-differentiated using molecular techniques and physiological characteristics (de Hoog and Yurlova, 1994; Yurlova *et al.*, 1996). Phylogenetically *Selenophoma eucalypti* clusters with species of *Aureobasidium* and *Hormonema*. The coniothyrium-like synanamorph reported here for *Sydowia eucalypti* is frequently isolated from *Eucalyptus* leaves in nature, leading to confusion when a yeast-like growth appears in culture. We therefore hope that this relationship between the *Sydowia* teleomorph, *Selenophoma* anamorph, coniothyriumlike synanamorph and the *Hormonema* yeast has now been clarified.

*Zeloasperisporium eucalyptorum* Cheewangkoon and Crous, *sp. nov.* — MycoBank MB513862; Fig. 6.26

Teleomorph. Unknown.

*Zeloasperisporio hyphopodioidi* simile, sed conidiis latioribus et brevioribus, (15–)17–22(–25) × 4.5–6(–7)  $\mu$ m.

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

*Mycelium* internal to superficial, consisting of sparingly branched, loosely septate, pale brown, smooth or minutely vertuculose, thin-walled,  $(1.5-)2.5-3.5 \mu m$  wide hyphae. *Conidiophores* reduced to conidiogenous cells, micronematous, arising as lateral hyphal branches, erect, straight, subcylindricalor conical, not geniculate, unbranched,  $(17-)20-25(-31) \times 3-3.5(-4.5) \mu m$ , tapering towards the apex, pale to medium brown, smooth or minutely vertuculose, slightly thick-walled, somewhat constricted at the apex below the conidiogenous loci. *Conidial proliferation* sympodial, with one to several subdenticulate

to flat conidiogenous loci, mostly crowded at the apex, protuberant; conidial scars thickened-refractive, appearing as thickened circles when viewed from directly above,  $\leq 1 \mu$  m wide. *Conidia* solitary, straight to curved, fusiform, tapered towards the apex,

1-septate, distinctly constricted at the median septum, pale to medium brown, verruculose, somewhat thickwalled,  $(15-)17-22(-25) \times 4.5-6(-7) \mu m$ ; apex subhyaline, thinner and smoother than the rest of the conidial body, at times forming a globose, apical mucoid appendage; base truncate or slightly rounded, tapering toward a protruding scar, which is

somewhat thickened and darkened-refractive,  $0.8-1.2 \ \mu m$  wide; microcyclic conidiation observed in culture. *Micronematous* synanamorph. *Conidiogenous cells* short-cylindrical, brown, smooth, as lateral pegs on hyphae,  $2-4 \times 1.5 \ \mu m$ , with minute apical scars. *Conidia* not observed.



**Fig. 6.26** Zeloasperisporium eucalyptorum. a. Colony on pine needle agar; b–f. conidia on conidiogenous cells; g. microcyclic conidiation; h, i. conidia; j, k. micronematous conidiogenous cells. — Scale bars:  $a = 200 \mu m$ ;  $b-f = 15 \mu m$ ;  $g-k = 10 \mu m$ , b applies to b–k.

Culture characteristics — Colonies reaching up to 15 mm diam on MEA after 2 wk at 25 °C in the dark; subcircular to irregular, convex, with a slightly folded,

undulate surface, and pale brownish grey aerial mycelium; surface pale brownish olivaceous-grey; reverse dark olivaceous-brown.

Specimen examined. Australia, Northern Territory, road to Robin Falls, S 14°10'20", E 131°07'15", on *Eucalyptus tectifica*, 23 Sept. 2007, coll. *B.A. Summerell*, isol. *P.W. Crous*, CBS H-20298 holotype, culture ex-type CPC 14603 = CBS 124809, CPC 14604, 14605.

Notes — Zeloasperisporium eucalyptorum is very similar to Z. hyphopodioides in conidiogenesis and conidial shape (Castañeda *et al.*, 1996; Crous *et al.*, 2007d), but conidia of Z. *eucalyptorum* are wider and shorter. Phylogenetically Z. *eucalyptorum* clusters close to Z. hyphopodioides (ITS region 93 % identical), but the two species still differ by 40 nucleotides (Fig. 6.1).

#### 6.4 Discussion

The present study treats 26 fungal species representing 22 different genera, including genera that harbour well-known saprobes, plant pathogens, or both. Based on the high number of novel species encountered, we questioned the aspect of host specificity within the various genera treated. In other words, although described as novel from *Eucalyptus*, could expect to isolate the same fungus from another genus in the *Myrtaceae*, or even from a totally unrelated plant family?

Based on the various literature sources cited in this paper, it was clear that host ranges associated with these fungal genera are highly variable, with some genera being reported to occur on one to more than 100 different plant families. However, few records are available of individual species having the ability to undergo host-shifting and/or host-jumping. *Myrtaceae*, and particularly *Eucalyptus*, support an extremely high number of diverse fungal genera (Sankaran *et al.*, 2005a). It is therefore possible that when members of *Myrtaceae* are introduced into a new habitat outside their natural range, the fungi with potentially high host-shifting/jumping ability can colonise *Myrtaceae* plantations from surrounding native plant hosts. On the other hand, if all those fungi are naturally occurring on *Myrtaceae*, these plantations could act as sources of fungi that could

shift/jump to other, nearby native plants. After host-shifting/jumping, a particular fungal species might progressively become adapted and specialised to its new host, leading to further speciation (Brasier, 2000; Roy, 2001; Munday *et al.*, 2004; Giraud, 2006; Faucci *et al.*, 2007). To determine the possible extent in which this is happening, however, a detailed survey of the fungal diversity present on *Myrtaceae* in native forests and in plantations, as well as on and in the surrounding flora, would be required, which was beyond the scope of the present study.

Of the fungal novelties described in the present study, species of Quambalaria appear to be restricted to Eucalyptus and Corymbia (Myrtaceae) (Simpson, 2000; de Beer et al., 2006; Langrell et al., 2008; Paap et al., 2008; Pegg et al., 2008; Zhou et al., 2008). Quambalaria cyanensis is the only exception, being reported from both eucalypts (Paap et al., 2008) and human skin, though the latter is believed to be an opportunistic infection (de Hoog and de Vries, 1973). Although species from the genus Cladoriella have thus far only been reported from Eucalyptus (Crous et al., 2006f; present study), this is in contrast to the morphologically similar genus, Pseudocercospora. The latter contains more than a 1 000 species, spread over a wide range of host families. In spite of this, however, molecular data to prove that these species occur on hosts in diverse genera is still outstanding. Similar to Pseudocercospora, species of Elsinoë are also able to colonise > 60 plant families, though most species appear to be specialised to specific hosts (Sinclair & Lyon 2005). Some species appear to have the ability to occur on other hosts within the same family, e.g. E. araliae on Aralia, Fatsia, Hedera and Schefflera (Araliaceae), and E. fawcettii on Citrus, Clausena, Fortunella, Lablab and Poncirus (Rutaceae). In a few cases where there have been reports of species with wider host ranges (Spaulding, 1961; Crous et al., 1989a; Taylor et al., 2001b), these lack conclusive molecular evidence to back up these observations.

Most *Sydowia* and *Selenophoma* species are potentially able to grow on a wide range of unrelated plant families (Park and Sprague, 1953; Sutton, 1980; Crous *et al.*, 2000b), but once again molecular data is lacking to substantiate these observations. Sooty molds like species of *Antennulariella* and *Leptoxyphium* spp. can colonise various unrelated plant families (Adhikari, 1990; Singh and Rawat, 1990). This is not surprising, however, as they usually grow on insect secretions, and colonise surfaces of living plants, rather than interact directly with their hosts. Based on these data, it would appear that the most plant pathogenic fungi treated here are host-specific. To complicate matters further, even some saprobes appear to exhibit a high level of host specificity. The only feasible way to address these issues would be to either intensively sample all hosts in a specific region, or to use new DNA sequencing technologies to determine all taxa occurring on selected hosts. Given the fact that the considering the 1.5 M species of fungi to be a vast underestimate, and that this study only know around 10 % of this number to date, and approximately 16 % of this fraction is known from culture, and even less represented in GenBank, the present inadequacy of fungal DNA databases make it impossible to accurately assess host specificity (Hawksworth, 2004; Crous *et al.*, 2006d). Based on these findings it is clear that further in-depth studies are urgently called for, as these data could hold serious consequences not only for import and export of agricultural and forestry produce, but also for devising effective strategies for biodiversity conservation.

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