CHAPTER 4

TAXONOMIC STATUS OF *CRYPTOSPORIOPSIS EUCALYPTI* AND ITS ASSOCIATED TELEOMORPH

4.1 Introduction

Cryptosporiopsis eucalypti is a host-specific pathogen of *Eucalyptus* species that occurs over a wide geographical range varying from dry to very humid zones including those in Australia, India, Hawaii (Sankaran *et al*., 1995), New Zealand (Gadgil and Dick, 1999), Brazil (Ferreira *et al*., 1998), Japan, Laos, Indonesia, Sri Lanka, Thailand and Vietnam (Old and Yuan, 1994; Old *et al*., 2003). The fungus can be associated with various disease symptoms including leaf spots, shoot blight, cankers on woody tissue, defoliation and even tree death.

The leaf spots develop on both sides of leaves and vary in size, shape, and colour among *Eucalyptus* species (Sharma, 1994; Sankaran *et al*., 1995; Old *et al*., 2002, 2003). The fungus proliferates by producing a vast number of spores from conidiomata that develop on infected leaves and shoots. After causing death of shoot tips or small branches, repeated infection can occur over extended periods of time. Leaf blight and other foliar diseases induced by *C. eucalypti* can easily be confused with those caused by other plant-pathogenic fungi, such as *Mycosphaerella* spp. and their anamorphs (Cheewangkoon *et al*., 2008, 2009; Crous, 2009g), and Calonectria (Crous *et al*., 2004e, 2006a; Lombard *et al*., 2009, 2010c).

Although infection by *C. eucalypti* can eventually lead to yield reduction of *Eucalyptus* plantations, the biology of this pathogen is not well understood. Infection often appears to be associated with minor mechanical, insect or wind damage (Ciesla *et al*., 1996), or with lesions caused primarily by *Calonectria* spp. (Park *et al*., 2000; Crous, 2002). Old and Yuan (1999) also reported the cooccurrence of *C. eucalypti* with *Pilidiella* species (as *Coniella*; Van Niekerk *et al*., 2004) on *E. camaldulensis*, showing serious defoliation in the North Queensland region of Australia. Cryptosporiopsis foliar disease develops under conditions of high humidity, and the

optimum temperature for its growth and sporulation on agar is 25–26°C, while temperatures of 32°C or above appear to limit disease development. In contrast, low ambient temperatures may be a predisposing factor for initiation of disease (Sankaran *et al*., 1995). Spread of *C. eucalypti* is probably through wind and rain splash dissemination, and it is unknown whether the fungus can be spread via contaminated seed or chaff commonly found in seed lots (Ciesla *et al*., 1996).

Cryptosporiopsis eucalypti was first described by Sankaran *et al*. (1995). Verkley (1999) suggested that it differs from typical *Cryptosporiopsis* anamorphs by only having acervuloid conidiomata with discrete conidiogenous cells, lacking any stromatic tissue in culture. In contrast many species of *Cryptosporiopsis* s. str. as typified by *C. scutellata* (syn. *C. nigra*), anamorph of *Pezicula ocellata*, form integrated conidiogenous cells on conidiophores, and in culture, are always associated with stromatic tissue.

Cryptosporiopsis eucalypti was nonetheless accepted in *Cryptosporiopsis* by Verkley (1999) based on its morphological characteristics. Species of *Cryptosporiopsis* have known teleomorphs in *Pezicula* and *Neofabraea* (Dermateaceae, Helotiales; Sutton, 1980; Verkley, 1999), though presently no teleomorph has yet been linked to *C. eucalypti*.

During routine surveys of *Eucalyptus* leaf diseases, an unknown ascomatal fungus was found associated with leaf spots resembling those caused by *C. eucalypti*. Because single ascospore isolates produced typical *C. eucalypti* colonies in culture, these strains were included in a phylogenetic study pursuing the hypothesis that it might represent the teleomorph of *C. eucalypti*. Furthermore, based on preliminary phylogenetic data for *C. eucalypti* and similar fungi, we concluded that these taxa could not be accommodated in the Dermateaceae (Helotiales), but rather that they represented a novel clade in the Diaporthales (unpubl. data).

The aim of this study was to consider the phylogenetic relationships among *C. eucalypti*-like fungi collected from *Eucalyptus* leaves and twigs in many parts of the world. This was achieved by employing sequences of the internal transcribed spacer (ITS) sequences of the nuclear ribosomal DNA operon (ITS1, 5.8 S nrDNA and ITS2) and the ß-tubulin (TUB) gene. Furthermore, to resolve their higher order phylogeny, sequences were generated from the 28 nrRNA (LSU) gene. For

morphological comparisons, isolates were studied on a range of culture media and growth conditions.

4.2 Materials and methods

4.2.1 Isolates

Lesions bearing ascomata were cut from *Eucalyptus* leaves, soaked in distilled water for 2 h, and then attached to the inside lower surfaces of Petri dish lids, with the top halves of dishes containing 2% malt extract agar (MEA; Oxoid, Hampshire, England). Germinating ascospores on the agar surface were examined after 24 h, and single ascospore cultures were established as described earlier (Crous *et al*., 1991; Crous, 1998).

Eucalyptus leaves were incubated in moist chambers for up to 2 wk, and single conidial colonies established from sporulating conidiomata (Crous, 2002). Colonies were sub-cultured onto 2% potato-dextrose agar (PDA), synthetic nutrient-poor agar (SNA), MEA, oatmeal agar (OA; Crous *et al*., 2009g), and pine needle agar (2% tap water agar, with sterile pine needles) (PNA; Crous *et al*., 2006c), and incubated under continuous near-ultraviolet light at 25°C to promote sporulation. Nomenclatural novelties with their descriptions were recorded in Myco- Bank (www.MycoBank.org; Crous *et al*., 2004e). All cultures obtained in this study are maintained in the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS) in Utrecht, the Netherlands, and/or the working collection (CPC) of P.W. Crous (Table 4.1).

4.2.2 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, CA, USA) following 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

Table 4.1 Isolates of *Cryptosporiopsis* and *Pseudoplagiostoma* used for DNA analysis and morphological studies.

Species	Accession number[1]	Host	Location	GenBank No. (ITS, EF,TUB, LSU)			
Cryptosporiopsis californiae	CPC 13819, CBS 124819*	*Eucalyptus* sp.	California, USA	GU973504,	—,	GU973567,	GU973597
C. caliginosa	CPC 14048, CBS 124806*	*E.caliginosa*	Australia	GU973505,	—,	GU973568,	GU973598
Cryptosporiopsis sp.	CBS 433.75	*Eucalyptus* sp.	Australia	GU973506,	—,	GU973569,	GU973599
Cryptosporiopsis versiformis eucalypti	CBS 286.39	*Rhamnus* sp.	Berkenbrück; Germany	—,	—,	—,	GU973600
	CPC 12280	*Eucalyptus* sp.	Hawaii, USA	GU973507,	GU973537,	GU973570,	GU973601
	CPC 12292	*E.camaldulensis*	Bhutan	GU973515,	GU973545,	GU973578,	—,
	CPC 12998	*E.tereticornus*	Australia	GU973516,	GU973546,	GU973579,	—,
	CPC 13023	*E.longifolia*	Australia	GU973521,	GU973551,	GU973584,	—,
	CPC 13341, CBS 124807*	*E.urophylla*	Venezuela	GU973512,	GU973542,	GU973575,	GU973606
	CPC 13344	*E.urophylla*	Venezuela	GU973511,	GU973541,	GU973574,	GU973605
	CPC 13396	*Eucalyptus* sp.	Venezuela	GU973523,	GU973553,	GU973586,	—,
	CPC 13471	*E.camaldulensis*	Thailand	GU973529,	GU973559,	GU973592,	—,
	CPC 13473	*E.camaldulensis*	Thailand	GU973530,	GU973560,	GU973593,	—,
	CPC 14075	*E.urophylla*	China	GU973519,	GU973549,	GU973582,	—,
	CPC 14154	*E.urophylla*	Australia	GU973527,	GU973557,	GU973590,	—,
	CPC 14156	*E.saligna*	Australia	GU973524,	GU973554,	GU973587,	—,
	CPC 14157	*E.saligna*	Australia	GU973525,	GU973555,	GU973588,	—,
	CPC 14158	*E.pellita*	Australia	GU973528,	GU973558,	GU973591,	—,
	CPC 14159	*E.pellita*	Australia	GU973526,	GU973556,	GU973589,	—,
	CPC 14160	*E.camaldulensis*	Vietnam	GU973522,	GU973552,	GU973585,	—,
	CPC 14161	*E.camaldulensis*	Vietnam	GU973510,	GU973540,	GU973573,	GU973604
	CPC 14162	*E.camaldulensis*	Vietnam	GU973531,	GU973561,	GU973594,	—,
	CPC 14163	*E.globulus*	Uruguay	GU973517,	GU973548,	GU973581,	—,

Table 4.1 Isolates of *Cryptosporiopsis* and *Pseudoplagiostoma* used for DNA analysis and morphological studies. (continued)

Species	Accession number[1]	Host	Location	GenBank No. (ITS, EF,TUB, LSU)			
	CPC 1253, CBS 111063	Unknown	Malaysia	GU973508,	GU973538,	GU973571,	GU973602
	CPC 2974, CBS 112116	*Angophora* sp.	Australia	GU973513,	GU973543,	GU973576	GU973607
	CPC 5320, CMW 10604, CBS 115743	*E.globulus*	Uruguay	GU973509,	GU973539,	GU973572,	GU973603
	CMW 13311, CBS 115788	*E.camaldulensis*	Thailand	GU973532,	GU973562,	GU973595,	—,
	CBS 116335	*E.camaldulensis*	Vietnam	GU973520,	GU973550,	GU973583,	—,
	CMW 13310, CBS 116382	*E.camaldulensis*	Thailand	GU973514,	GU973544,	GU973577,	GU973608
	CBS 117840	*E.camaldulensis*	Vietnam	GU973533,	GU973563,	GU973596,	—,
	CMW 13309, CBS 118840	*E.camaldulensis*	Thailand	GU973517,	GU973547,	GU973580,	—,
Ps. oldii	CPC 14155, CMW 6675, CBS 124808*	*E.camaldulensis*	Australia	GU973534,	GU973564,	GU993862	GU973609
	CMW 6674, CBS 115722	*E.camaldulensis*	Australia	GU973535,	GU973565,	GU993863	GU973610
Ps. variabile	CPC 5321, CBS 113067*	*E.globulus*	Uruguay	GU973536,	GU973566,	GU993864	GU973611

[1] CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of P.W. Crous, housed at CBS, and representative strains at Chiang Mai University, Thailand; CMW: Culture collection of M.J. Wingfield, housed at FABI, University of Pretoria, South Africa.
* Ex-type cultures.

part of the nuclear rDNA operon spanning the 3′ end of the 18 S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8 S rRNA gene, the second ITS region (ITS2) and the first 900 bases at the 5′ end of the 28 S 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. To resolve species identities, the ITS region was supplemented with sequences of the ß-tubulin gene (TUB) using the primers T1 (O'Donnell and Cigelnik, 1997) and Bt-2b (Glass and Donaldson, 1995). The PCR conditions, sequence alignment and subsequent phylogenetic analyses followed the methods of Crous *et al*., (2006a). Sequences were compared with those available in NCBI's GenBank nucleotide (nr) database using a megablast search and results are provided in the relevant species notes where applicable. Alignment gaps were treated as fifth character states. Sequence data were deposited in GenBank (Table 4.1) and alignments in TreeBASE (www.treebase.org).

4.2.3 Morphology

Isolates were plated onto fresh MEA, OA, PDA and PNA plates, and subsequently incubated at 25°C under nearultraviolet light to promote sporulation. Fungal structures were mounted on glass slides in clear lactic acid for microscopic examination. Sections of ascomata were made by hand for examination purposes. Measurements of all taxonomically relevant characters were made at 1,000× magnification by Nikon NIS-Elements D3.0 Imaging Software, with 50 measurements per structure where possible. Colony colours on MEA (surface and reverse) were determined using the colour charts of Rayner (1970) after 1–2 wk at 25°C in the dark.

4.3 Results

4.3.1 Phylogenetic analysis

Approximately 1,700 bases, spanning the ITS and LSU regions, were obtained for isolates listed in Table 4.1. The LSU region was used in the phylogenetic analysis to determine generic or family placements and ITS sequences were used to determine

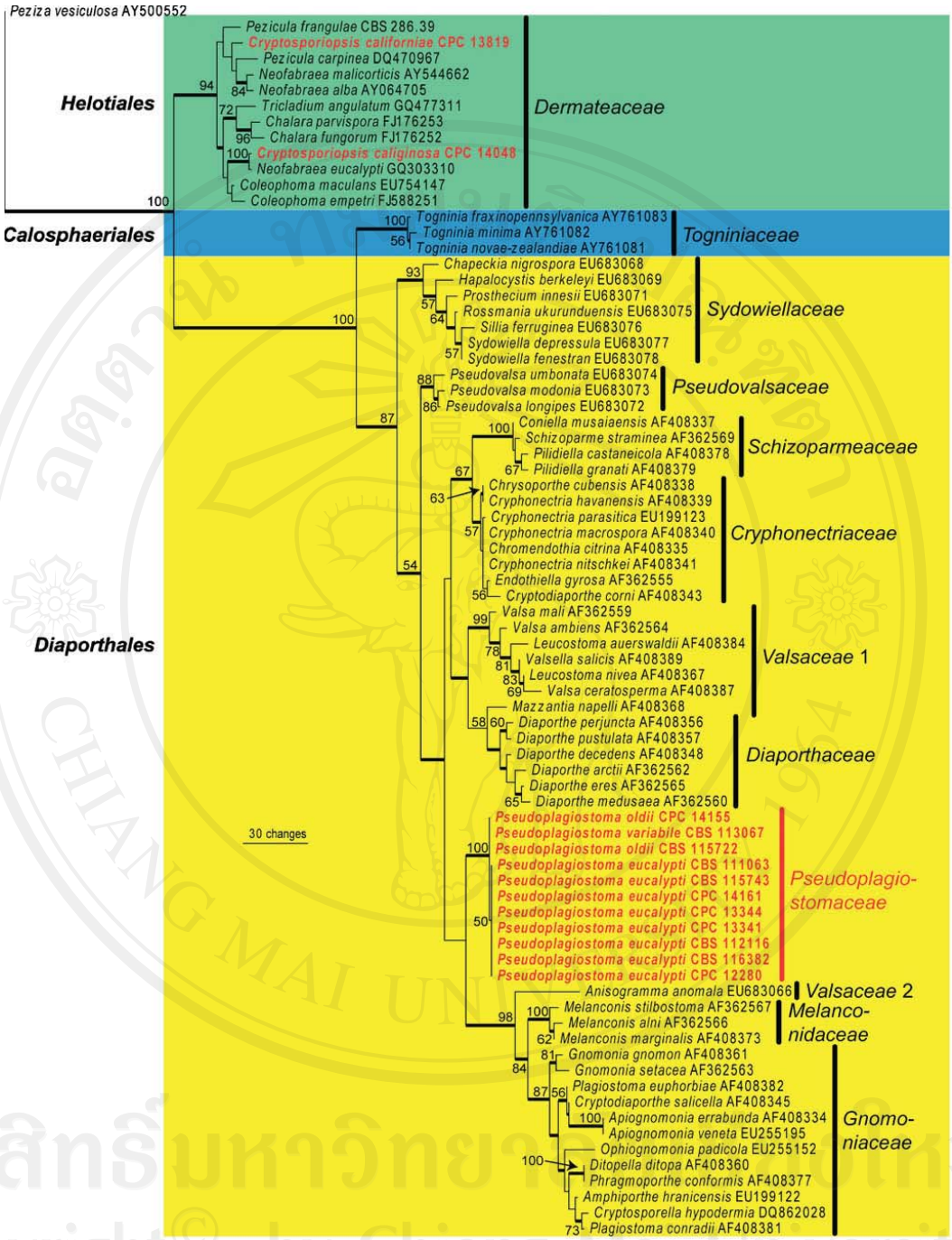

Fig. 4.1 The first of 1,000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 30 changes, and bootstrap support values from 1,000 replicates are shown at the nodes. Novel species and families described in this study are shown in red. Branches present in the strict consensus tree are thickened. Orders are indicated to the left and families to the right of the tree. The tree was rooted to a sequence of *Peziza vesiculosa* (GenBank accession AY500552)

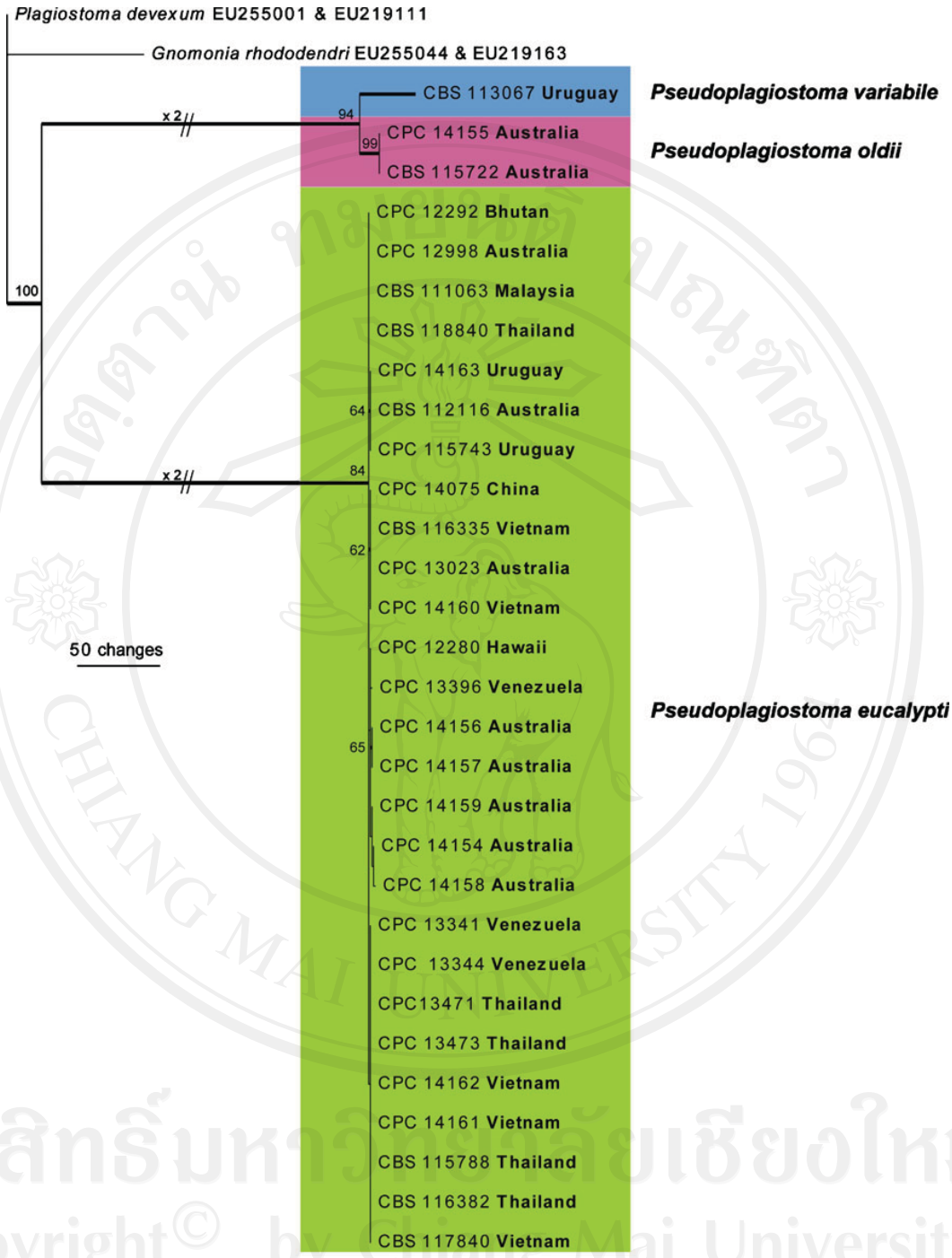

Fig. 4.2 The first of 212 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined ITS and TUB sequence alignment. The scale bar shows 50 changes, and bootstrap support values from 1,000 replicates are shown at the nodes. Branches present in the strict consensus tree are thickened. The tree was rooted to sequences of *Plagiostoma devexum* (GenBank accession ITS: EU255001; TUB: EU219111) and *Gnomonia rhododendri* (GenBank accession ITS: EU255044; TUB: EU219163)

species-level relationships. The LSU alignment contained 78 taxa (including the outgroup sequence) and, of the 753 characters used in the phylogenetic analysis, 214 were parsimony-informative, 53 were variable and parsimony-uninformative and 486 were constant. The first 1,000 equally most parsimonious trees were kept from the heuristic search, the first of which is shown in Fig. 4.1 (TL=784, CI=0.490, RI=0.883, RC=0.433). The phylogenetic tree for the LSU region (Fig. 1) revealed the family relationships for the isolates within Diaporthales and Helotiales. Isolates that had been tentatively identified as *C. eucalypti* did not reside in any existing family, and a new genus and family is introduced below to accommodate them.

A second alignment of sequences for the *C. eucalypti* isolates based on ITS and TUB sequences included a combined set of 1,256 characters (incl. alignment gaps) (number of included characters: ITS: 525 and TUB: 731). Of the 32 sequences used (including the outgroup), 386 characters were parsimony-informative, 91 were variable and parsimony-uninformative, and 779 were constant. A total of 212 equally most parsimonious trees were obtained from the heuristic search, the first of which is shown in Fig. 2 (TL=524, CI=0.987, RI=0.984, RC=0.971). Isolates originally identified as "*C. eucalypti*" were found to represent two novel species of *Cryptosporiopsis*, and three novel species that represented a new genus and family (Figs. 4.1, 4.2). Further results are discussed in the species notes sections below where applicable.

4.3.2 Taxonomy

Results of this study led to the discovery of two novel species of *Cryptosporiopsis* s.str. associated with leaf spots on *Eucalyptus* spp. Furthermore, single ascospore isolates of a diaporthalean fungus produced colonies typical of *C. eucalypti* in culture. Phylogenetic analyses of sequence data showed that this collection represents a previously undescribed genus and family, which are treated below.

Cryptosporiopsis californiae Cheewangkoon, Denman & Crous, sp. nov. — MycoBank MB516493; Fig. 4.3

Etymology. Named for the state of California, USA where the fungus was collected.

Maculae amphigenae, subcirculares ad irregulares, brunneae. *Conidiomata* pycnidialia ad acervularia, superficialia vel pro parte immersa, brunnea ad atrobrunnea, discreta vel confluentia, 80–130 µm diam, 45–70 µm alta. *Conidiophora* nulla vel ad 1–2 cellulis brevibus reducta sunt. *Cellulae conidiogenae* discretae, phialidicae, incrassatae, cylindricae, plerumque infra apice leniter inflatae, hyalinae, (4–)8–11(–16)×2.5–3.5 µm. *Conidia* elongate ellipsoidea, recta vel leniter curvata, nonnulla inaequilateralia, apex obtusus vel late acutus, basi abrupte angustata hilo leniter protrudente, 1.5–2 µm lato, aseptata, hyalina, crassitunicata, guttulis 5–30 minutis, (12.5–)15–18(–27.5)×(4.2–)4.5–5.2(–5.8) µm.

Leaf spots amphigenous, subcircular to irregular, medium brown. On PNA: mycelium immersed, consisting of branched, hyaline to very pale brown, 2.5–3.5 µm wide hyphae. *Conidiomata* pycnidial to acervular, superficial or partly immersed, medium to dark brown, with cream conidial masses; separate or confluent, 80–130 µm diam, 45–70 µm high; wall dark brown, pseudoparenchymatous, thick, composed of irregular, medium brown cells that become pale brown towards the inner region, 8–15 µm thick; stroma weakly developed, 5–10 µm thick, paler in non-pycnidial conidiomata, consisting of numerous sterile hyphae. *Conidiophores* absent, or reduced to 1–2 short supporting cells. *Conidiogenous cells* arise from the inner cells of the cavity, discrete, phialidic, thickened, cylindrical, mostly slightly enlarged below the apex, hyaline, (4–) 8–11(–16)×2.5–3.5 µm. *Conidia* elongate ellipsoidal, straight or slightly curved, some inaequilateral, apex obtuse or broadly acute, tapering abruptly to a slightly protruding scar at the base, 1.5–2 µm wide; aseptate, hyaline, thick-walled, with 5–30 min guttules per conidium, (12.5–)15–18(–27.5)×(4.2–)4.5–5.2 (–5.8) µm.

Culture characteristics — Colonies reaching 4 cm diam on MEA after 1 wk at 25°C, slightly raised, olivaceous-grey to buff (surface), with white margin, and dense white aerial mycelium; yellow-brown (reverse). Numerous black pycnidia are produced on the colony surface that is partly submerged, irregular.

Fig. 4.3 *Cryptosporiopsis californiae*. a. Colony on MEA. b. Conidiomata on MEA. c–k. Conidia and phialidic conidiogenous cells. l. Conidia. Scale bars: b=150 µm, c–k=15 µm, l=10 µm; d applies to d–k

Specimen examined. USA, California, on *Eucalyptus* sp., Mar. 2009, S. Denman, holotype CBS H-20302, culture ex-type CPC 13819=CBS 124819, CPC 13820, 13821.

Notes — Numerous pycnidia are formed on OA after about 3 wk, which become fertile after 5 wk. Conidia are mostly similar in shape and size to those formed on PNA, but slightly shorter. Based on conidial size, *C. californiae* (12.5–27.5×4.2–5.8 µm) is easily distinguished from *C. edgertonii* (30–48×12–15 µm), which also occurs on *E.*(Edgerton 1908). Although *C. californiae* may occur on other hosts, we were unable to locate a name for it, and BLAST results for its ITS sequences did not reveal its presence in GenBank. The ITS sequence of this species had an E-value of 0.0 with the ITS sequences of *Pezicula* spp. and *Cryptosporiopsis* spp. such as *P. carpinea* (AF141197; 95 % identical), *P. heterochroma* (AF141167;

95 % identical), *P. sporulosa* (AF141172; 94 % identical), *C. radicicola* (AF141193; 95 % identical), *C. melanigena* (AF141196; 94 % identical) and others.

Cryptosporiopsis caliginosa Cheewangkoon, Summerell & Crous, sp. nov. — MycoBank MB516494; Fig. 4

Etymology. Name refers to *E.caliginosa*, on which the fungus was collected.

Maculae amphigenae, subcirculares ad irregulares, brunneae. *Conidiomata* in foliis acervularia, subcuticularia ad epidermalia, pallide brunnea, discreta, 2–3 strata texturae angularis composita, ad 200 μm diam, 150–200 μm alta. *Conidiophora* nulla. *Cellulae conidiogenae* discretae, phialidicae, cylindricae, hyalinae, rectae vel leniter curvatae, glabrae, (14.5–)16–18(–20)×4.5–6 μm. *Conidia* elongate ellipsoidea, plerumque recta, apice late obtuso, basi abrupt angustata in hilum leniter protrudens, aseptata, hyalina, crassitunicata, minute guttulata, (8.5–)15–17(–19) × (3.5–)4.5–5.5 μm.

Leaf spots amphigenous, subcircular to irregular, medium brown. *Conidiomata* on leaves acervular, subcuticular to epidermal, pale brown, separate, consisting of 2–3 layers of textura angularis, up to 200 μm diam, 150–200 μm high; dehiscence irregular, by rupture of the overlying host tissues. *Conidiophores* absent. *Conidiogenous cells* arise from the inner cells of the cavity, discrete, phialidic, cylindrical, hyaline, straight to slightly curved, smooth, (14.5–)16–18(–20)×4.5–6 μm. Conidia elongate ellipsoidal, mostly straight, broadly obtuse at the apex, tapering abruptly to a slightly protruding basal scar, aseptate, hyaline, thick-walled, minutely guttulate, (8.5–)15–17(–19)×(3.5–) 4.5–5.5 μm.

Cultural characteristics — Colonies on OA reaching 3 cm after 1 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, conidia not formed in culture.

Specimen examined. Australia, New South Wales, Northern Tablelands, Mt Mackenzie Nature Reserve (290504S; 1515805E) on *E.caliginosa*, 1 Feb. 2007, B.A. Summerell, holotype CBS H-20301, culture extype CPC 14048=CBS 124806, CPC 14049, 14050.

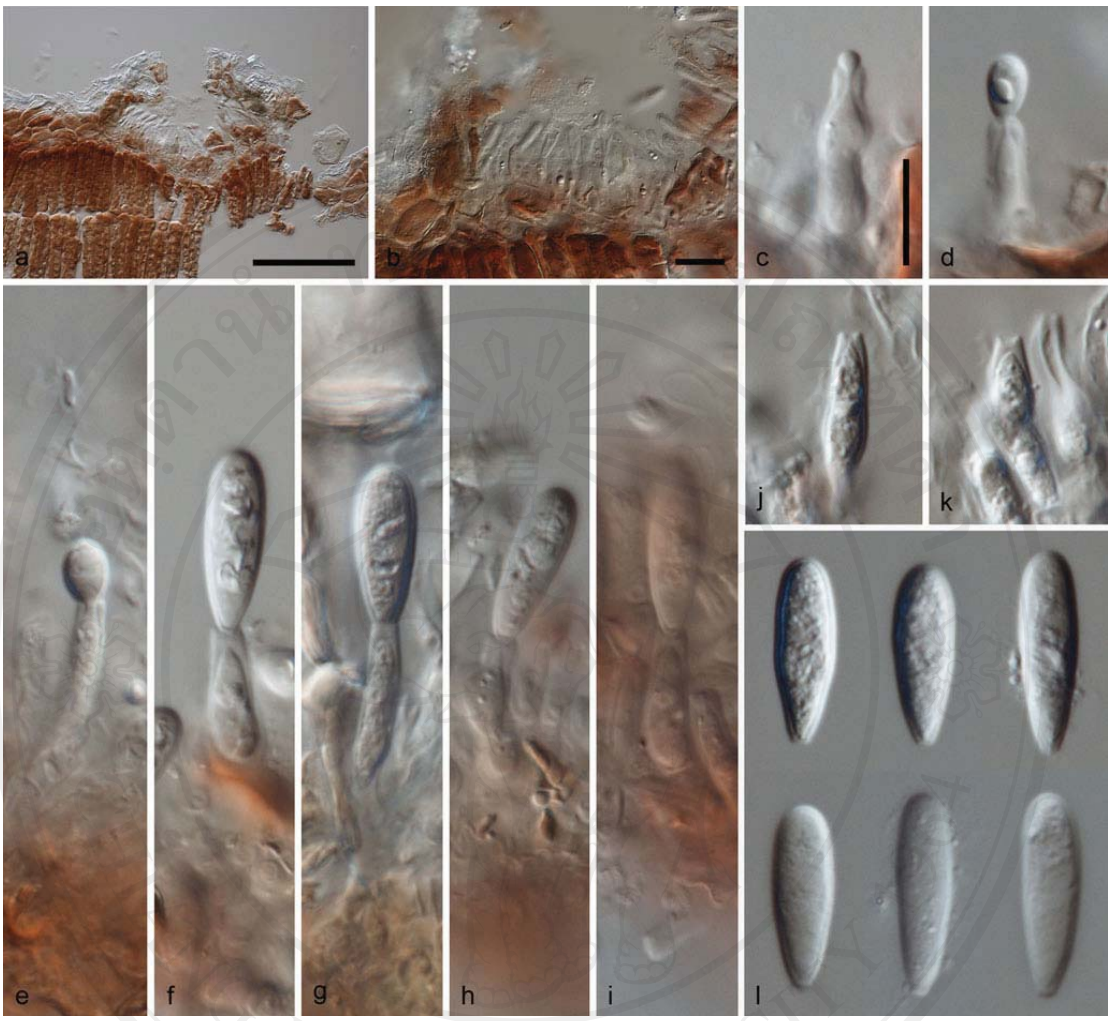

Fig. 4.4 *Cryptosporiopsis caliginosa*. a, b. Conidiomata on host substrate. c–i. Conidia attached to phialidic conidiogenous cells. j, k. Conidiogenous cells. l. Conidia. Scale bars: a=100 µm, b=20 µm, c–l=10 µm; c applies to c–l

Notes — *Cryptosporiopsis caliginosa* (conidia 8.5–19 µm long) is easily distinguishable from *C. californiae*, which has longer conidia (12.5–27.5 µm). BLAST results for the ITS sequence of this species had an E-value of 0.0 with the ITS sequences of *Neofabraea eucalypti* (GQ303279; 97 % identical), *Gloeosporium* sp. (EF672242; 92 % identical), *Coleophoma empetri* (FJ480134; 92 % identical) and others.

Pseudoplagiostomaceae Cheewangkoon, M.J. Wingf. & Crous, fam. nov. — MycoBank MB516495.

Perithecia immersa, obliqua vel horizontalia; subglobosa vel elliptica; rostrum excentricum vel laterale, stroma non formatum. *Asci* unitunicati, annulo subapicali nonamyloideo, aparaphysati. *Ascosporae* uniseptatae, hyalinae, appendicibus terminalibus elongatis hyalinis.

Members of the Diaporthales having morphological characters of the genus Pseudoplagiostoma. Immersed, oblique to horizontal perithecia in host tissue; depressed globose or elliptical; beak eccentric to lateral; stromatic tissue not formed. Asci unitunicate, with non-amyloid subapical ring, lacking paraphyses. Ascospores hyaline,1-septate, with terminal, elongate, hyaline appendages.

Type genus: *Pseudoplagiostoma* Cheewangkoon, M.J. Wingf. & Crous

Notes — Of the families presently known from the Diaporthales (Wehmeyer, 1975; Castlebury *et al*., 2002; Gryzenhout *et al*., 2006b; Rossman *et al*., 2007; Voglmayr and Jaklitsch, 2008), the Pseudoplagiostomaceae most closely resembles the Gnomoniaceae in the morphological characters of its teleomorph, such as solitary, thin-walled, immersed ascomata with lateral beaks lacking stromata, asci with a distinct ring, and medianly 1-septate ascospores less than 25 mm long (Monod, 1983; Castlebury *et al*., 2002; Sogonov *et al*., 2008). Phylogenetically, Pseudoplagiostromaceae is closer to families with well-developed stromatic tissue such as Diaporthaceae and Pseudovalsaceae, or families with stromatic and non-stromatic tissues such as Valsaceae and Sydowiellaceae.

Pseudoplagiostoma Cheewangkoon, M.J. Wingf. & Crous, gen. nov. — MycoBank MB516496.

Etymology. Named reflects morphological similarity to *Plagiostoma*.

Ascomata perithecia, immersa, obliqua ad horizontalia, subglobosa vel elliptica, atrobrunnea ad nigra; rostrum vulgo in epiphyllo erumpens, excentricum ad laterale; ostiolum periphysatum; peridium coriaceum, stroma non formatum. *Asci* subcylindrici ad elongate obovoidei, aparaphysati, unitunicati, annulo subapicali nonamyloideo. *Ascosporae* hyalinae, ellipsoideae, utrinque rotundatae, plerumque rectae, in medio uniseptatae, glabrae, appendicibus terminalibus elongatis hyalinis.

Conidiomata acervularia ad pycnidialia, subcuticularia ad epidermalia, paries texturae angularis compositus. *Conidiophora* nulla. *Cellulae conidiogenae* cylindricae ad ampulliformes, enteroblasticaliter proliferentes, tunica periclinaliter incrassata colluloque, vel parte apicali percurrenter proliferentes. *Conidia* holoblastica, ellipsoidea, apice obtuso et basi hilo plano protrudente, continua.

Ascomata perithecial, immersed in host tissue, oblique to horizontal, depressed globose or elliptical, dark brown to black; beak usually erumpent epiphyllously, eccentric to lateral; ostiole lined with periphyses; peridium coriaceous, with sparse hyphae visible growing into the host tissue; stromatic tissue not formed. *Asci* subcylindrical to long obovoid, lacking paraphyses, unitunicate, with non-amyloid subapical ring, wedge-shaped, refractive, with canal leading to the apex. *Ascospores* hyaline, ellipsoidal, tapering towards rounded ends, usually straight, medianly 1-septate, wall smooth, with terminal, elongate, hyaline appendages. *Conidiomata* acervular to pycnidial, subcuti cular to epidermal, wall composed of textura angularis. *Conidiophores* absent. *Conidiogenous cells* cylindrical to ampulliform, proliferating enteroblastically with periclinal thickening and collarette, or percurrently proliferating in the apical part. *Conidia* holoblastic, ellipsoid, with obtuse apex and a flat protruding scar at the base, 0-septate.

Type species: *Pseudoplagiostoma eucalypti* Cheewangkoon, M.J. Wingf. & Crous

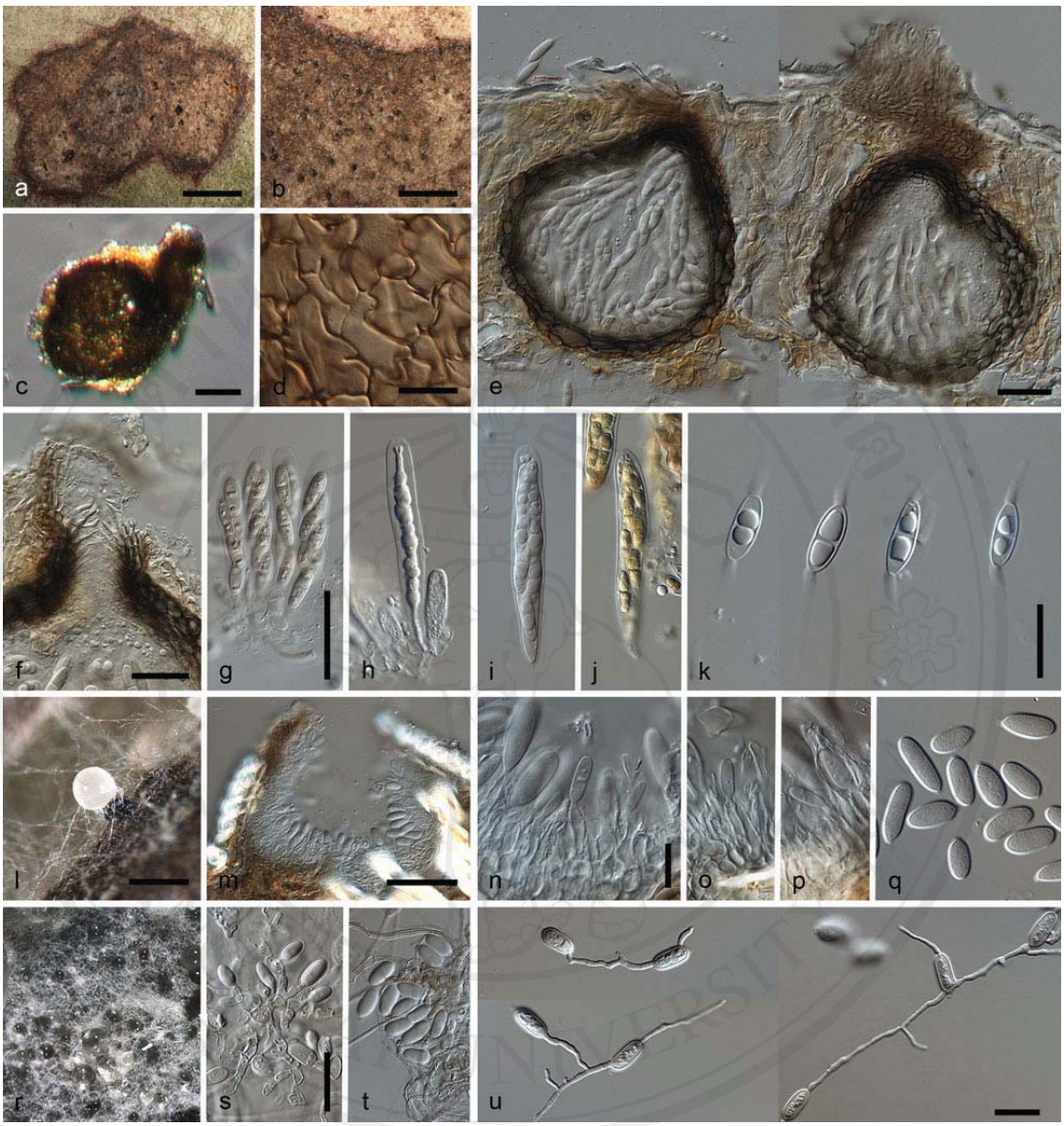

Fig. 4.5 *Pseudoplagiostoma eucalypti*. a. leaf spot. b, c. Ascomata. d. Ascomatal wall. e. Cross section though ascomata. f. Ostiole. g. Asci. h. Young ascus. i. Mature ascus. j. Ascus strained in Melzer's reagent, showing non-amyloid subapical ring. k. Ascospores. l. Conidiomata. m. Cross section though conidiomata. n–p. Conidia attached to conidiogenous cells with percurrent proliferation. q. Conidia. r. Colony on MEA. s, t. Conidia and conidiogenous cells. u. Microcyclic conidiation. a–k: From E.leaves. l–q: From PNA. r–u: From MEA. Scale bars: a=5 mm, b=1 mm, c, e=50 µm, d=5 µm, f–j= 30 µm, k, s–u=20 µm, l=200 µm, m=70 µm, n–q=15 µm; g applies to g–j; n applies to n–q; s applies to s–t

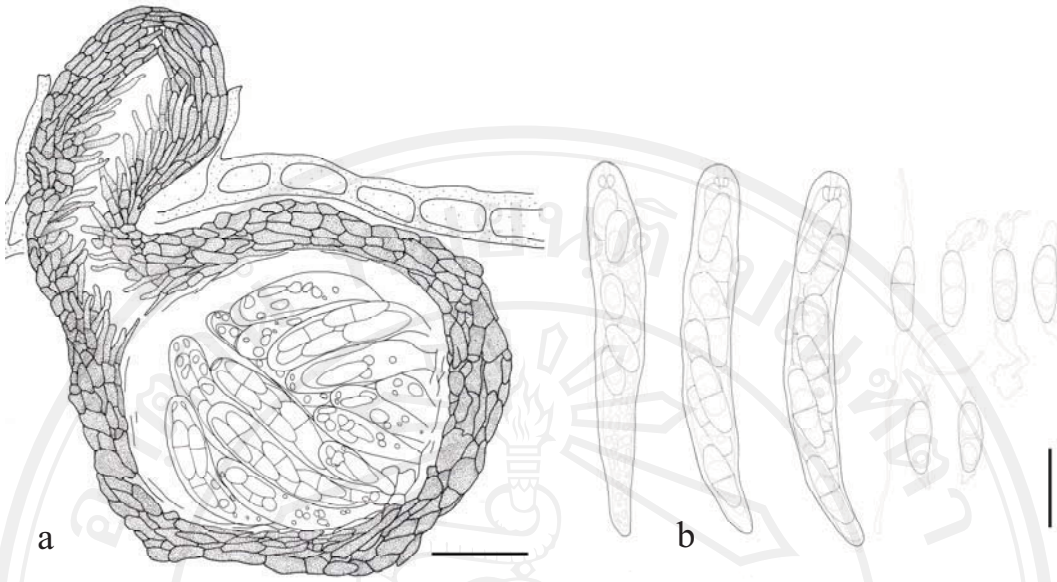

Fig. 4.6 Line drawing. *Pseudoplagiostoma eucalypti*. a. Cross section though ascoma. b. Asci; c. Ascospores. Scale bars: a=30 µm, b–c= 15; c applies to b–c

Pseudoplagiostoma eucalypti Cheewangkoon, M.J. Wingf. & Crous, sp. nov. — MycoBank MB516497; Figs. 4.5, 4.6

Anamorph: "*Cryptosporiopsis*" *eucalypti* Sankaran & B. Sutton, Mycol. Res. 99: 828. 1995.

Maculae amphigenae, subcirculares ad irregulares, brunneae et atrobrunneae. *Ascomata* epigena immersa ad semiimmersa, intraepidermalia vel subepidermalia, subglobosa vel elliptica, coriacea, (90–)100–130(–170) µm lata, (120–)130–150 (–190) µm alta, atrobrunnea ad nigra; ostiolum laterale, rostratum (50–)60–65(–70) µm latum, papillatum, usqua ad 105 µm longum, periphysatum. *Peridium* 2–4 strata texturae angularis atrobrunneae compositum. *Asci* aparaphysati, unitunicati, octospori, apice rotundati, subcylindrici ad elongate obovoidei, annulo subapicali nonamyloideo, (60–)65–70(–80)×(10–) 11–13(–14) µm. *Ascosporae* ellipsoideae, utrinque rotundatae, septo latissimae, hyalinae, in medio uniseptatae; (15–)17–19(–21)× (5–)6(–7) µm; maturitate appendicibus cylindricis terminalibus elongatis, 5.5–7 µm latis, (8–)15–20(–30) µm longis. *Conidiomata* brunnea ad atrobrunnea, acervularia ad pycnidialia, subglobosa ad late ovoidea, subcuticularia ad epidermalia, discreta, 2–4 strata texturae angularis medio brunneae composita, (170–)180–200(–230) µm lata, (150–)170–190(–220) µm alta. *Conidiophora* nulla. *Cellulae conidiogenae* enteroblasticaliter proliferentes, phialidis similes tunica periclinaliter incrassate

colluloque, vel parte apicali percurrenter proliferentes, hyalinae, glabrae, cylindricae ad ampulliformes, rectae vel leniter curvatae, (6–) 8–12(–15) × 2–4(–6) µm. *Conidia* holoblastica, hyalina, guttulata, glabra, cassitunicata, ellipsoidea, continua, apice obtuso, leniter curvata, basi hilo plano protrudente angustata, (15–)17–19(–23) × (6.5–) 7–8(–8.5) µm.

Etymology. Name refers to the fact that the fungus occurs on *Eucalyptus*.

Leaf spots amphigenous, subcircular to irregular, medium brown with blackish brown, reverse medium brown, 3–20 mm diam, surrounded by a purple-brown margin, which is dark brown in reverse. Mycelium immersed, consisting of smooth, septate, branched, medium brown, 2–3.5 µm wide hyphae. *Ascomata* epigenous immersed to semi-immersed, intra- or subepidermal, visible as minute ostiolar dots, depressed globose or elliptical, coriaceous, (90–)100–130(–170) µm wide, (120–) 130–150(–190) µm high, dark brown to black; ostiole lateral, beaked, (50–)60–65 (–70) µm wide, papillate, up to 105 µm long, periphysate; wall consisting of 2–4 layers of dark brown textura angularis. *Asci* aparaphysate, unitunicate, 8-spored, apically rounded, subcylindrical to long obovoid, sessile or subsessile in young asci, slightly curved, with non-amyloid subapical ring, (60–)65–70(–80)×(10–)11–13(–14) µm. *Ascospores* ellipsoid, tapering to rounded ends, widest at septum, hyaline, bi- to tri-seriate overlapping, fasciculate, medianly 1-euseptate; not constricted at the septum, with 1–2 large guttules in each cell, thin-walled, straight, (15–)17–19 (–21)×(5–)6(–7) µm; with hyaline, cylindrical appendages at both polar ends at maturity, expanded at the base, tapering towards the apex, 5.5–7 µm wide, (8–)15–20(–30) µm long. *Conidiomata* medium to dark brown, acervular to pycnidial, with pale yellow drops of exuding conidia (at times forming a short cirrus); subglobose to broadly ovoid, subcuticular to epidermal, separate, consisting of 2–4 layers of medium brown textura angularis, (170–)180–200(–230) µm wide, (150–)170–190 (–220) µm high; wall 15–20 µm thick, with central rupture, breaking through plant tissue, (50–)60–80(–100) µm wide. *Conidiophores* absent. *Conidiogenous cells* proliferating enteroblastically, appearing as phialides with periclinal thickening and collarette, or with percurrent proliferation in the apical part; discrete, arising from the inner cell layer, hyaline, smooth, cylindrical to ampulliform, wider at the base, straight or slightly curved, (6–)8–12(–15)×2–4(–6) µm. *Conidia* holoblastic, hyaline,

guttulate, smooth, thick-walled, ellipsoid, aseptate, slightly curved, frequently slightly narrow at the middle, with obtuse apex; base tapering to flat protruding scar, (15–) 17–19(–23)×(6.5–)7–8(–8.5) µm; on MEA, (14–)16–19(–22)×(6–)7–9(–11) µm.

Ascospore germination — Ascospores germinate from the apical cell, with primary germ tubes forming near the apex; secondary germ tubes form later from the second cell, remaining hyaline; cell wall becoming slightly thicker, but not constricted at the septum, showing no distortion.

Cultural characteristics — Characteristics on MEA, PDA and OA of all three species of *Pseudoplagiostoma* are compared in Table 4.2 and Figs. 4.9, 4.10.

Specimens examined. Venezuela, on living leaves of *E.urophylla*, Oct. 2006, M.J. Wingfield, holotype of Ps. eucalypti, CBS H-20303, cultures ex-type CPC 13341=CBS 124807, CPC 13342, 13343. HAWAII, Kauai, on *E.grandis*, 23 May 1978, C.S. Hodges, holotype of *Cryptosporiopsis eucalypti*, IMI 237416 f.

Pseudoplagiostoma oldii Cheewangkoon, M.J. Wingf. & Crous, sp. nov. — MycoBank MB 516498; Fig. 4.7

Etymology. Named for Australian forest pathologist, Dr Ken Old, who contributed substantially to an understanding of E.diseases including the *Cryptosporiopsis* disease complex.

Ascomata non vidimus. Species haec a *Ps. eucalypti* et *Ps. variabili* differt conidiomatibus (265–)285–300(–330) µm latis et (200–)220–250(–270) µm altis et conidiis maturitate brunneis in agaro extracto malti, (15–)17–20(–23)×(6–)7–8(–9) µm.

Leaf spots amphigenous, subcircular to irregular, medium brown. *Ascomata* not observed. On PNA dark brown conidiomata appeared after 15 d in the dark; conidiomata acervular to pycnidial, with pale grey masses of conidia, subglobose to broadly ovoid, subcuticular to epidermal, separate, consisting of 3–5 layers of dark brown textura angularis, (265–)285–300(–330) µm wide, (200–)220–250 (–270) µm high; central opening, (90–)110–120(–140) µm wide, wall 20–30 µm thick. *Conidiophores* absent. *Conidiogenous cells* discrete, phialidic with periclinal thickening, or 1–3 apical percurrent proliferations; cylindrical to ampulliform, arising from the inner cell layer, hyaline but at maturity brown on MEA, straight or slightly

curved, wider at the base, smooth, (8.5–)15–20(–26)×2–3(–4.5) µm. *Conidia* holoblastic, hyaline, guttulate, smooth, thickwalled, ellipsoidal, aseptate, slightly curved, apex obtuse, base tapering to a flat, protruding scar, (15–)17–20(–23)×(6–)7–8(–9) µm; on MEA, (11–)14–17(–20)×(6–) 7–9(–11) µm.

Specimens examined. Australia, Queensland, Lannercost, on *E.camaldulensis*, 6 Jan. 2007, K. Old, holotype CBS H-20300, cultures ex-type CBS 124808=CMW 6675, CPC 14155; on E. camaldulensis, Jan. 2007, K. Old, CBS 115722.

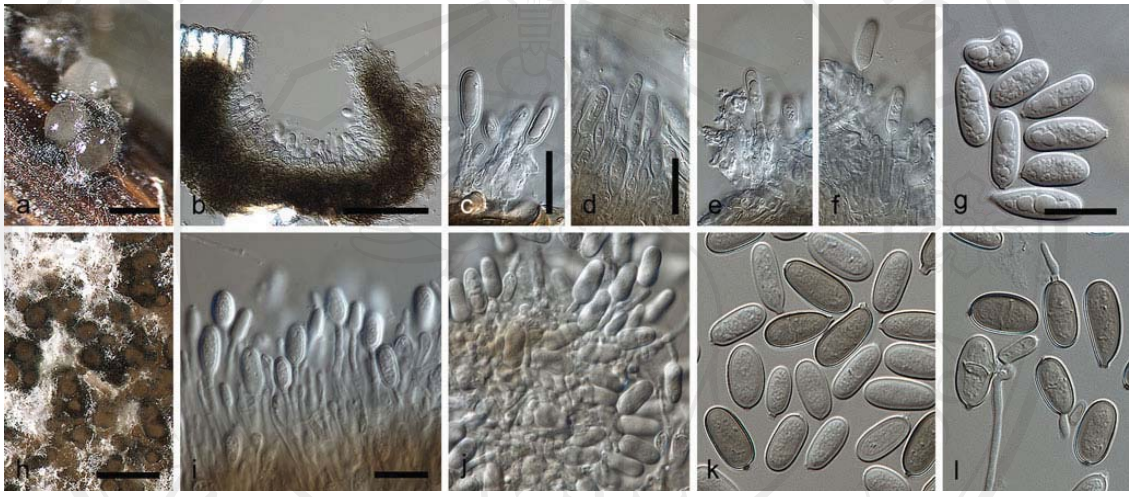

Fig. 4.7 *Pseudoplagiostoma oldii*. a. Conidiomata. b. Cross section though conidiomata; c–f. Conidia attached to conidiogenous cells with percurrent proliferation; g. Conidia; h. Conidiomata; i–j. Conidia and conidiogenous cells; k. Conidia; l. Germinating conidia. a–g: on PNA. h–l: on MEA. Scale bars: a, h=800 µm, b=100 µm, c–g, k–l=20 µm, i–j=15 µm; d applies to d–f; g applies to g, k–l; i applies to i–j

Pseudoplagiostoma variabile Cheewangkoon, M.J. Wingf. & Crous, sp. nov. —MycoBank MB516499; Fig. 4.8

Etymology. Name reflects the variable conidial shape in this fungus.

Ascomata non vidimus. Species haec a *Ps. eucalypti* et *Ps. oldii* differt conidiomatibus (145–)170–190(–245) µm latis et (130–)160–180(–230) µm altis, et conidiis unitunicatis, (12.5–)15.5–17.5(–23.5)×(5.5–)6.5–8(–9) µm.

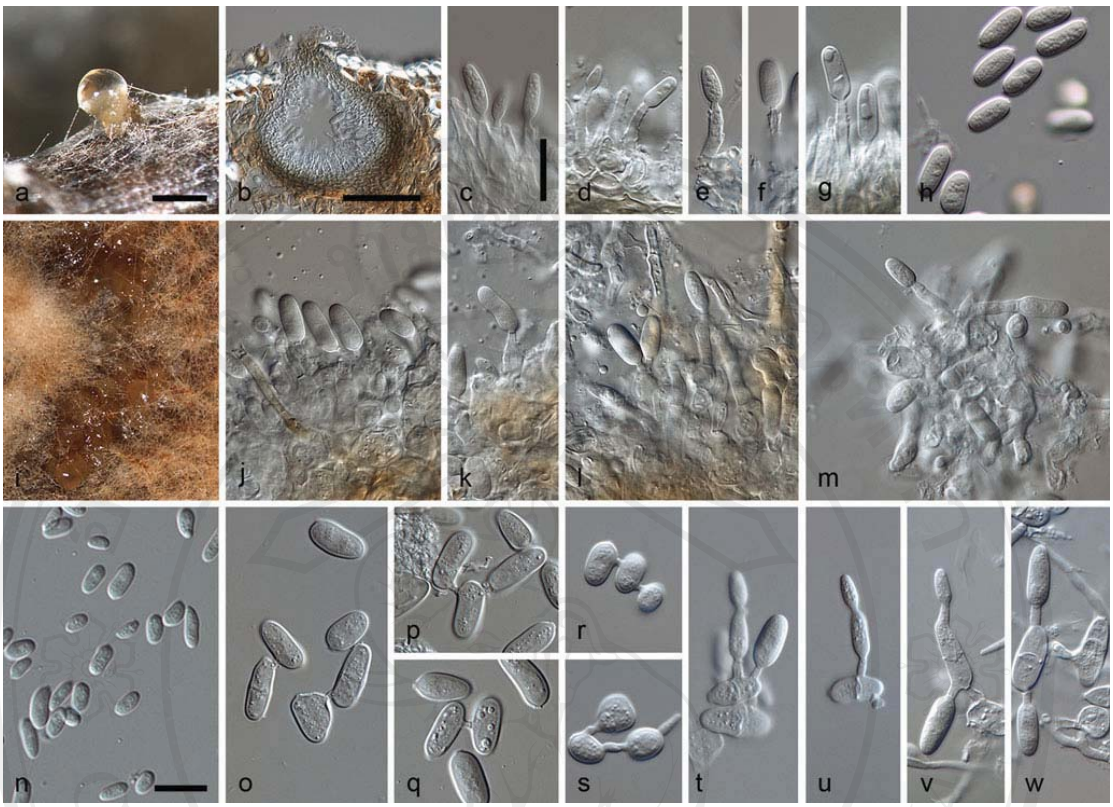

Fig. 4.8 *Pseudoplagiostoma variabile*. a. Conidiomata; b. Cross section through conidiomata; c–g. Conidia attached to conidiogenous cells with percurrent proliferation; h. Conidia; i. Conidiomata; j–m. Conidia and conidiogenous cells; n. Conidia; o–s. Conidial anastomosis; t–w. Microcyclic conidiation. a–h: on PNA. i–w: on MEA. Scale bars: a=800 µm, b=100 µm, c–w=20 µm, c applies to c–m, o–w.

Leaf spots amphigenous, subcircular to irregular, medium brown. *Ascomata* not observed. On PNA medium to dark brown pycnidial conidiomata appeared after 15 d of incubation in the dark, exuding pale yellow conidial masses; conidiomata subglobose to broadly ovoid, subcuticular to epidermal, separate, consisting of 2–4 layers of medium brown textura angularis, (145–)170–190(–245) µm wide, (130–) 160–180(–230) µm high, apical ostiole central, (60–) 70–90(–110) µm wide; wall 15–25 µm thick. *Conidiophores* absent. *Conidiogenous cells* discrete, phialidic with periclinal thickening, or 1–5 apical percurrent proliferations; cylindrical to ampulliform, arising from the inner cell wall, hyaline, straight or slightly curved, wider at the base, smooth, (12–)15–20(–23)×2–3 (–4.5) µm. *Conidia* holoblastic, hyaline, guttulate, smooth, thin to slightly thick-walled, ellipsoid, aseptate, slightly curved, frequently constricted in the middle, apex obtuse, base tapering to flat

protruding scar, (12.5–)15.5–17.5(–23.5)×(5.5–)6.5–8(–9) µm; on MEA, (6.5–) 15.5–17(–19)×(6.5–)7.5–9(–10.5) µm.

Specimen examined. Uruguay, on *E.globulus*, 5 Aug. 2002, M.J. Wingfield, holotype CBS H-20304, cultures ex-type CBS 113067=CPC 5320, CPC 5321.

Key to species of *Pseudoplagiostoma**

1. Conidia turn brown at maturity, (11–)14–17(–20)×(6–) 7–9(–11) µm, ratio (1.9–) 2.3–2.5:1 (l:w)……. *Ps. oldii*
1. Conidia remain hyaline at maturity, ratio 2-2.1:1 (l:w).....…….....2
2. Conidia ellipsoid, (14–)16–19(–22)×(6–)7–9(–11) µm, ratio 2.1:1 (l:w)……..……...……....*Ps. eucalypti*
2. Conidia variable in shape, subglobose to bean-shaped, (6.5–)15.5–17(–19)×(6.5) 7.5–9(–10.5) µm, ratio 2:1 (l:w) …….……...…… *Ps. variabile*

*Sporulating on MEA in culture.

Table 4. 2 Culture characteristics of *Pseudoplagiostoma* spp. after 15d of incubation at 25°C in the dark.

Species	Culture characteristics on MEA			
	Colonies	Surface colour	Growth rate	Conidial shape and dimensions
Ps. eucalypti	Flat; moderate aerial mycelium; smooth margin	Greenish white to greenish olivaceous	fast 8–9 cm diam.	Ellipsoid (14–)16–19(–22) × (6–)7–9(–11) µm; 1.9:1 (l:w)
Ps. oldii	Flat; sparse aerial mycelium; smooth margin	Greenish olivaceous	Moderate-fast 6–7 cm diam	Ellipsoid (11–)14–17(–20) × (6–)7–9(–11) µm; 2.1:1 (l:w)
Ps. variabile	Umbonate, folded; sparse aerial mycelium; irregular margin	Yellow-brown, becoming paler at the margin	Slow-moderate 3–4 cm diam	Subglobose, ellipsoid to reniform (6–)15.5–17(–19) × (6.5–)7.5–9(–10.5) µm 2:1 (l:w)

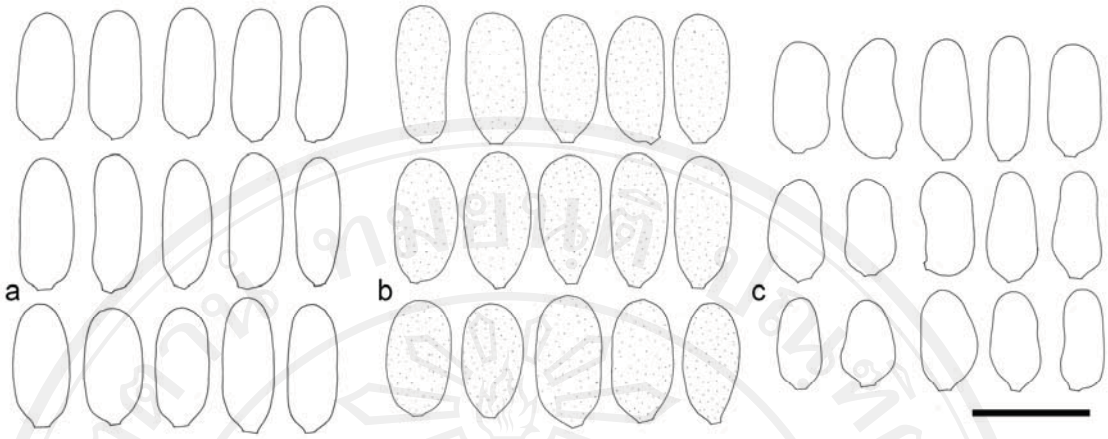

Fig. 4.9 Line drawing. Conidia of *Pseudoplagiostoma* spp. on MEA. a. *Ps. eucalypti*; b. *Ps. oldii*. c. *Ps. variabile*. Scale bar:=10 μm

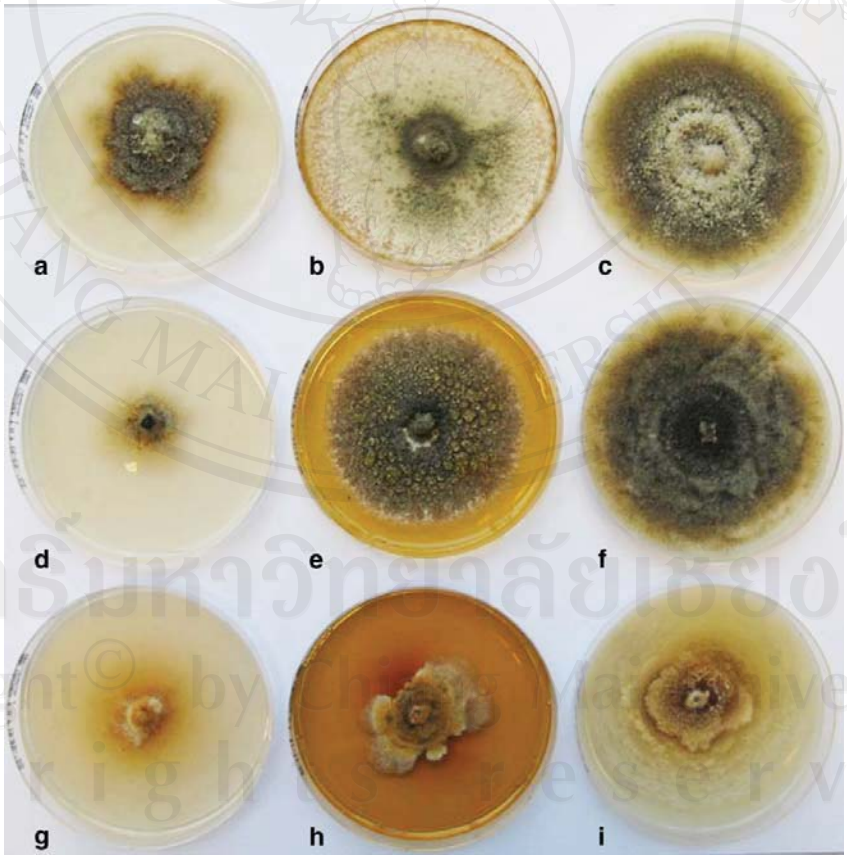

Fig. 4.10 *Pseudoplagiostoma* spp. in culture after 15 d. a–c. *Ps. eucalypti* (CBS 115788). a. On OA. b. On MEA. c. On PDA. d–f. *Ps. oldii* (CBS 124808). d. On OA. e. On MEA; f. On PDA. g–i. *Ps. variabile* (CBS 113067). g. On OA; h. On MEA; i. On PDA; g–i

4.4 Discussion

Results of this study have elucidated considerable confusion that has surrounded the taxonomy of one of the fungal pathogens most commonly encountered on leaves of *Eucalyptus* in plantations globally. Phylogenetic inference of DNA sequence data thus showed that the fungus known as *Cryptosporiopsis eucalypti* and encountered in many treatments of *Eucalyptus* diseases (Sharma 1994; Sankaran *et al*., 1995; Old *et al*., 2002, 2003) is the anamorph of a member of the Diaporthales (99% bootstrap support), and not the Dermateaceae (Helotiales) along with *Cryptosporiopsis* s. str. The *Eucalyptus* pathogen that has been treated as *C. eucalypti* since 1995 has thus been placed in a novel genus as *Pseudoplagiostroma eucalypti*.

This study includes 32 isolates collected from *Eucalyptus* in plantations on four continents and from 10 countries. The combined sequence data sets for this collection of isolates delineate three distinct species within a monophyletic lineage. The major clade (*P. eucalypti*) includes 27 isolates, while the second clade (*P. oldii*) includes two isolates (CBS 124808 and CBS 115722) and the third clade (*P. variabile*) consists of a single isolate, CBS 113067. The monophyly of *Pseudoplagiostoma* is strongly supported by morphological characteristics. While all three species are very similar on OA, PDA, and PNA, they can easily be distinguished in culture on MEA. The conidial wall of *Ps. oldii* turns brown at maturity, suggesting that this feature can be used to distinguish them (also on PNA and OA, but not on PDA). Colonies of *Ps. variabile* grow more slowly than those of *Ps. eucalypti* and *Ps. oldii*. It produces fewer conidia on MEA, undergoes microcyclic conidiation, and its conidia are not uniform, ranging from subglobose to ellipsoid. These features should make this widely distributed group of fungi easy to identify in *E.*disease surveys.

Within the Diaporthales, Pseudoplagiostoma is more similar to members of the Gnomoniaceae based on the morphological characters of its teleomorph, such as solitary, thin-walled, immersed ascomata with lateral beaks lacking stromata, asci with a distinct ring, and medianly 1-septate ascospores less than 25 mm long (Monod, 1983; Barr, 1978; Samuels and Blackwell, 2001; Castlebury *et al*., 2002; Sogonov *et al*., 2008). In contrast, in the Valsaceae and Sydowiellaceae, stromatic and nonstromatic tissues are present (Wehmeyer, 1975; Rossman *et al*., 2007). Also, in

other families of Diaporthales such as Cryphonectriaceae, Diaporthaceae, Melanconidaceae and Pseudovalsaceae, the stromatic tissues are often welldeveloped (Castlebury *et al*., 2002; Gryzenhout *et al*., 2006b; Voglmayr and Jaklitsch, 2008). The coelomycetous anamorph of *Pseudoplagiostoma* (previously reported as *C. eucalypti*) also has acervular to pycnidial conidiomata without a well-developed stroma, phialidic and annellidic conidiogenous cells, and aseptate conidia, which are features typical of the Diaporthales (Rossman *et al*., 2007).

Pseudoplagiostoma is morphologically most similar to *Plagiostoma* in the Gnomoniaceae. It is, however, distinct from *Plagiostoma* and other members of the Gnomiaceae in having a truly lateral instead of a marginal neck, and distinct appendages at both ends of its ascospores. However, it shares some features with *Plagiostoma*, such as oblate perithecia with a single neck, but lacking a clypeus, and thin-walled asci with a conspicuous apical ring containing medianly 1-septate ascospores (Sogonov *et al*., 2008). *Pseudoplagiostoma* developed Gnomoniaceae-like morphological characters, which can be the result of convergent evolution. Phylogenetically, *Pseudoplagiostroma* is more closely related to families with well developed stromatic tissue such as Diaporthaceae and Pseudovalsaceae; or families with stromatic and nonstromatic tissues such as Valsaceae and Sydowiellaceae. This indicates that the presence (or absence) of stromata and its development should not be over emphasised when distinguishing families within Diaporthales. Castlebury *et al*. (2002) also emphasised that stromatal development and thickness of the ascospore wall are of less importance than formerly suggested by Barr (1987, 1990).

Phylogenetic analysis based on LSU sequences indicated that *Pseudoplagiostoma* does not reside with *Plagiostoma* or any genus in the Gnomoniaceae, but represents a distinct clade in the Diaporthales. The genus *Pseudoplagiostoma* contains teleomorphic fungi with horizontal, dark, soft textured perithecial ascomata lacking stromatic tissues, but with a lateral ostiolar neck; distinct non-amyloid asci with a refractive apical ring; eight medianly 1-septate ascospores, which have elongated appendages at both ends, but lacking true paraphyses. A new family, Pseudoplagiostomaceae, is thus described to accommodate *Pseudoplagiostoma* in the Diaporthales.

Anamorphs of Diaporthales are generally coelomycetous, producing phialidic, often annellidic conidiogenous cells, and usually have aseptate conidia in acervular or pycnidial conidiomata, with or without a well-developed stroma (Rossman *et al*., 2007). Cryptonectriaceae, Diaporthaceae, Gnomoniaceae, Schizoparmeaceae and Valsaceae anamorphs produce phialides, while only Melanconidaceae and Pseudovalsaceae produce annellidic conidiogenous cells. Sydowiellaceae includes taxa with both phialidic and annellidic conidiogenous cells. According to the descriptions by Verkley (1999), *Cryptosporiopsis* species generally have acervular or eustromatic conidiomata. Their conidiogenous cells are determinate and phialidic, with no proliferation or formation of consecutive conidia at progressive levels. These leave a series of scars on the cell apex, with periclinal thickening often seen in vitro. *Pseudoplagiostoma* anamorphs are difficult to distinguish morphologically from *Cryptosporiopsis* s. str. based on this widely-used generic concept. In this study, the three species of *Pseudoplagiostoma* produced conidiogenous cells that proliferated percurrently, with conidia seceding at the same level or higher, and lacking the swollen structure observed below the conidiogenous loci seen in *Cryptosporiopsis* anamorphs linked to *Pezicula* (Verkley, 1999). This difference in conidiogenesis could, therefore, be used to distinguish anamorphs of *Pseudoplagiostoma* from other similar coelomycetous genera in the Diaporthales, and from those in the Helotiales.

Moreover, based on LSU and ITS sequence data, three species of *Cryptosporiopsis* (*C. californiae*, *C. caliginosa* and *Cryptosporiopsis* sp.) clustered with other members of *Pezicula* and *Cryptosporiopsis* within the Dermateaceae (Helotiales). Thus far, only one true other *Cryptosporiopsis* species (*C. edgertonii*) has been reported from *Eucalyptus* samples in New Zealand (Gadgil, 2005), which has much larger conidia (30–48×12–15 μm; Edgerton, 1908) than these taxa.

Phenotypic plasticity remains a major factor leading to taxonomic uncertainty in the classification and identification of diaporthalean fungi. Castlebury *et al*. (2002) noted that the delimitation of diaporthalean families varied considerably among specialists, and that their morphological characters could easily lead to confusion for nonspecialists. Nine diaporthalean families were previously established based on phylogenetic analysis, because it highlighted the specific differences observed among species at molecular level (Rossman *et al*., 2007).

For Pseudoplagiostomaceae, we found that certain morphological characters are more valuable for species distinction, such as conidia, conidiogenous cells and conidiomata of anamorphs. However, only the ascomatal neck and asci-forming positions could be used to distinguish these teleomorphs from those in other families. It should be noted though, that the phylogeny of the Diaporthales is still not fully resolved (Castlebury *et al*. 2002). The addition of new taxa and description of potential new genera may result in changes in relative relatedness between families. This may also indicate differences in the importance of certain morphological characteristics to delineate families.

This study has resolved the taxonomy of one of the most commonly encountered fungi emerging from *Eucalyptus* disease surveys. The results will contribute substantially to a better understanding of these fungi and their role in *Eucalyptus* leaf diseases in many different parts of the world. A priority at this stage will be to compare the pathogenicity of the three new species of *Pseudoplagiostoma* that have previously been treated as the single species, *C. eucalypti*. The temptation to assume that they are all pathogens should be avoided until Koch's postulates have been proven. Furthermore, it would be useful to have information regarding the pathogencity of *Cryptosporiopsis* spp. (*C. californiae*, *C. caliginosa* and *Cryptosporiopsis* sp.) which have never been experimentally shown to be pathogens of *Eucalyptus*.