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RESEARCH ARTICLE

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Reassessing *Vermisporium* (*Amphisphaeriaceae*), a genus of foliar pathogens of eucalypts

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Key words

Australia
Eucalyptus
foliar pathogen
Seimatosporium
taxonomy

Abstract The genus *Vermisporium* presently accommodates 13 species, 11 of which are associated with leaf spots of eucalypts in the Southern Hemisphere. *Vermisporium* is chiefly distinguished from *Seimatosporium* (*Amphisphaeriaceae*) on the basis of a short exogenous basal appendage, and the absence of a recognisable apical appendage. Due to the increasing importance of these species in native forests, and confusion pertaining to their taxonomy, a revision of the genus was undertaken based on fresh collections and dried herbarium specimens. Results from DNA sequence data analyses of the nrDNA-ITS and 28S nrRNA genes for species of *Vermisporium* indicated the genus to be a synonym of *Seimatosporium*. New combinations are introduced in *Seimatosporium* for several species: *S. acutum*, *S. biseptatum*, *S. brevicentrum*, *S. obtusum*, *S. orbiculare*, *S. verrucisporum* and *S. walkeri*. An updated key to all species occurring on eucalypts is also provided.

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INTRODUCTION

Some of the most commonly encountered leaf-infecting fungi on eucalypts in native forests belong to the genus *Vermisporium.* Swart & Williamson (1983) proposed the generic name *Vermisporium* (based on *V. walkeri*) for a group of leaf-spotting fungi found on *Eucalyptus*, some of which had previously been described in the genus *Seimatosporium* (Shoemaker 1964, Swart 1982). These fungi were considered to differ from those in the genus *Seimatosporium* by producing conidia which appeared free of pigment under the microscope, were uniformly thin-walled and 10–20 times as long as they were wide; the basal cell invariably carried a short exogenous appendage, and there was no recognisable appendage present on the apical cell (Swart & Williamson 1983). When they made attempts to group the various *Vermisporium* specimens into species it was found that traditional measurements of conidium length and width were inadequate for this purpose. However, a number of distinct forms could be separated on the basis of relative lengths of conidial cells. This approach enabled them to clearly define six species within the genus *Vermisporium.*

Nag Raj (1993) reviewed the assemblage of species disposed in *Seimatosporium*, and rearranged them into five groups under extant generic names based on their conidial features. In doing so, *S. cylindrosporum*, *S. eucalypti* and *S. falcatum* – species that were retained in *Seimatosporium* by Swart & Williamson (1983) – were transferred to *Vermisporium.* According to Nag Raj (1993), *Seimatosporium* s.str. should be limited to coelomycetes that have a mixture of appendaged and non-appendaged conidia, appendages which are tubular, filiform and possibly enucleate and an excentric basal appendage, while *Vermisporium* should have conidia that are falcate to elongate-fusiform, thin-septate, have pigmented cells, a beak-like apical cell and a podiform, tubular, unbranched, excentric basal appendage which is possibly nucleate.

Thirteen species of *Vermisporium* have been described worldwide, 11 of these from eucalypt leaves collected in Australia. Collections of this group of fungi on *Eucalyptus* date back to the 1800s when Cooke (1891) described *Stagonospora orbicularis* (syn. *V. orbiculare*) from dead eucalypt leaves collected in Victoria. A 1903 collection from young, diseased leaves of *E. melliodora* at Dandenong Creek (Victoria) was originally described as *Cylindrosporium eucalypti* by McAlpine (1903), later renamed *Seimatosporium eucalypti* by Swart (1982), and more recently *Vermisporium eucalypti* by Nag Raj (1993). The majority of collections and published accounts of this genus have been made from *Eucalyptus* material collected in Australia (Cooke 1891, McAlpine 1903, Sutton 1963, 1980, Marks et al. 1982, Swart 1982, Swart & Williamson 1983, Nag Raj 1993, Simpson & Grgurinovic 1996), although there have been some collections from New Zealand (Dick 1990, Gadgil & Dick 1999) and South Africa (Crous et al. 1990).

Two *Vermisporium* species have been recorded on hosts other than eucalypts. *Vermisporium quercinum* was found on the bark of *Quercus suber* (*Fagaceae*) in Sardinia, Italy (Franceschini et al. 1995), while *V. tenzingii* was reported from leaves of *Osbeckia crinita* (*Melostomataceae*, *Myrtales*) from Darjeeling, India and *Osbeckia stellata* in Kaltani, Nepal (Wu & Sutton 1996).

Several species of *Vermisporium* were found during disease surveys in Australian native forests conducted for the present study. No species were found during surveys of plantations and nearby native forests in the Green Triangle, a region in the south-west of Victoria and south-east of South Australia which has been a major focus of the expansion of *E. globulus* plantations in Australia, with a number of foliar pathogens belonging to the genus *Mycosphaerella* recorded during disease surveys

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Table 1 Collection details and GenBank accession numbers of isolates for which novel sequences were generated in this study.

Species	Strain no.[1]	Country	Substrate	Collector(s)	GenBank Acc. No.	
					ITS[2]	LSU[2]
Seimatosporium biseptatum	CPC 13584	Australia: New South Wales	*Eucalyptus oresbia*	A.E. Orme & R. Johnstone	JN871199	JN871208
Seimatosporium eucalypti	CPC 156; CBS 115131	South Africa: Mpumalanga	*Eucalyptus smithii*	P.W. Crous	JN871200	JN871209
	CPC 157; CBS 110733	South Africa: Mpumalanga	*Eucalyptus smithii*	P.W. Crous	JN871201	JN871210
	CPC 158; CBS 110734	South Africa: Mpumalanga	*Eucalyptus smithii*	P.W. Crous	–	JN871211
	CPC 159; CBS 114876	South Africa: Mpumalanga	*Eucalyptus smithii*	P.W. Crous	JN871202	JN871212
Seimatosporium falcatum	CPC 12992	Australia: New South Wales	*Eucalyptus* sp.	A. Carnegie	JN871203	–
	CPC 13578	Australia: New South Wales	*Eucalyptus alligatrix*	R. Johnstone & A.E. Orme	JN871204	JN871213
	CPC 13580	Australia: New South Wales	*Eucalyptus alligatrix*	R. Johnstone & A.E. Orme	JN871205	JN871214
Seimatosporium obtusum	CPC 12935	Australia: New South Wales	*Corymbia henryi*	B. Summerell	JN871206	JN871215
Seimatosporium walkeri	CPC 17644	Australia: Victoria	*Eucalyptus* sp.	P.A. Barber	JN871207	JN871216

[1] CBS: CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of P.W. Crous, housed at CBS.
[2] ITS: Internal transcribed spacers 1 and 2 together with 5.8S nrDNA; LSU: partial 28S nrDNA.

(Barber et al. 2005, 2008). However, specimens were collected from native forests near to blue gum plantations in other regions of Victoria and New South Wales. Due to the association of fungi such as *V. falcatum* with disease epidemics in native forests (Felton 1981), their increasingly common occurrence in hardwood plantations (Yuan 1999) and confusion over their taxonomy (Nag Raj 1993), a review of the genus was undertaken from fresh material collected from native forests and plantations in various states in Australia, and dried herbarium material from Australia, New Zealand and the United Kingdom.

MATERIAL AND METHODS

Sample collection and isolation of strains

This study is based on the type specimens and original herbarium material lodged in DAR, K, MELU, NZFRI, VPRI and fresh leaf samples of diseased *Eucalyptus*. Diseased foliage of eucalypts growing in native forests was specifically collected over a 3 yr period between spring 1999 and autumn 2002. In addition, sporadic sampling was carried out in subsequent years in native forests from eastern Victoria across to south-eastern South Australia and in New South Wales. Diseased leaves were placed into plastic bags, brought back to the laboratory and stored in a cold room (4 °C) until further examination. Symptomatic leaves were incubated in moist chambers for 3–7 d and inspected daily for *Vermisporium* and single conidial colonies established on 2 % malt extract agar (MEA; Crous et al. 1991). Colonies were subcultured onto 2 % potato-dextrose agar (PDA) and oatmeal agar (OA) (Crous et al. 2009d) and incubated under continuous near-ultraviolet light at 25 °C to promote sporulation.

DNA isolation, amplification and analyses

Genomic DNA was isolated from fungal mycelium grown on MEA, using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocols. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the internal transcribed spacer 1, the 5.8S rRNA gene, the internal transcribed spacer 2 (ITS) and the first 900 bases at the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White et al. 1990) and LSU1Fd (Crous et al. 2009b) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The sequence alignment and subsequent phylogenetic analysis followed the methods of Crous et al. (2006, 2009a). Sequences were compared with the sequences available in NCBIs GenBank nucleotide (nr) database using a megablast search and results are discussed in the relevant species notes where applicable. The LSU sequences were added to an alignment modified from that of Tanaka et al. (2011). Alignment gaps were treated as new character states. Sequence data were deposited in GenBank (Table 1) and alignments in TreeBASE (www.treebase.org).

Morphology

Isolates were transferred onto fresh PDA, MEA and OA plates, and subsequently incubated at 25 °C under near-ultraviolet light to promote sporulation. Morphological observations, however, were based on conidiomata sporulating on host material. Structures were removed with a needle and mounted in lactic acid glycerol on a microscope slide. Investigation of these squash mounts was carried out using a Wild Leitz M20 compound microscope. Size ranges of spores were derived from at least 50 spores and the extremes given in parentheses. Sections of sporocarps were prepared using a sharp razor blade and mounted in lactic acid glycerol. Structures were measured and drawn using a drawing tube attached to a Wild Leitz M20 compound microscope. Original drawings were then digitized and final copies prepared using the methods described by Barber & Keane (2007). Squash mounts, sections and spores were photographed on a Nikon Optiphot microscope with either brightfield or differential interference contrast (DIC) objectives and an Olympus DP10 digital camera. Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004). Colony colours on PDA, MEA and OA (surface and reverse) were determined using the colour charts of Rayner (1970) after 2 wk at 25 °C in the dark. Reference strains are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS-KNAW), Utrecht, the Netherlands, or the working collection (CPC) of P.W. Crous (Table 1). Specimens cited in the working collection of P.B. have been deposited in PERTH.

RESULTS

Phylogenetic analysis

Amplicons of approximately 1 700 bases were obtained for ITS (including the first approx. 900 bp of LSU) for most of the isolates listed in Table 1. The LSU sequences were added to an alignment modified from Tanaka et al. (2011) for generic placement (Fig. 1), while the ITS was used for species identification (not shown; discussed in species notes where applicable). The manually adjusted LSU alignment contained 56 sequences (including the outgroup sequence) and 793 characters including alignment gaps (available in TreeBASE), which were used in the phylogenetic analysis; 73 of these were parsimony-informative, 39 were variable and parsimony-uninformative and 681 were constant. Neighbour-joining analyses using three substitution models on the sequence alignment yielded trees with identical topologies to one another and support the same clades as obtained from the parsimony analysis. Only the first 1 000 equally most parsimonious trees were saved (TL = 241

Fig. 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 20 changes and bootstrap support values from 1 000 replicates are shown at the nodes. Novel sequences generated in this study are indicated in **bold** and the *Seimatosporium* clade in an orange rectangle. Branches present in the strict consensus tree are thickened and the tree was rooted to a sequence of *Pestalotiopsis versicolor* (GenBank accession AF382357).

steps; CI = 0.544; RI = 0.829; RC = 0.451). The phylogenetic results obtained (Fig. 1) are discussed where applicable in the descriptive notes below.

Taxonomy

Vermisporium spp. were collected from eucalypts growing predominantly in native forests from various regions throughout Australia. Eight of the 11 species of *Vermisporium* described from eucalypts were collected in the present study. Three species, *V. orbiculare*, *V. samuelii* and *V. verrucisporum*, were not collected, and therefore herbarium specimens were borrowed from herbaria (DAR, K, VPRI) to allow comparison with the other species in the genus. In addition, herbarium specimens of *V. acutum*, *V. cylindrosporum*, *V. eucalypti*, *V. falcatum* and *V. walkeri* were borrowed from various herbaria (K, MELU, NZFRI(M), VPRI) to help identify characters that could be used to accurately distinguish species. As shown in the phylogenetic study (Fig. 1), species of *Vermisporium* appeared to cluster in several subclades within *Seimatosporium* and hence the genus is herewith reduced to synonymy:

Seimatosporium Corda, in Sturm, Deutschl. Fl., Abt. 3, Pilze Deutschl.: 79. 1833.

= *Vermisporium* H.J. Swart & M.A. Will., Trans. Brit. Mycol. Soc. 81, 3: 491. 1983.

Additional synonyms listed in Nag Raj (1993).

Type species. *Seimatosporium rosae* Corda, 1833, in Sturm's Deutschl. Fl., Abt. 3, Pilze Deutschl.: 79. 1833.

Fig. 2 Disease symptoms on *Eucalyptus camaldulensis* associated with *Seimatosporium acutum.* a. Older necrotic lesions along leaf lamina; b. younger necrotic lesions along leaf lamina; c. upper surface of younger partly necrotic lesion showing orange acervuli (arrowed) in the centre; d. abaxial surface of older necrotic lesion showing few black acervuli; e. adaxial surface of older necrotic lesion showing many black acervuli.

Seimatosporium acutum (H.J. Swart & M.A. Will.) Barber & Crous, *comb. nov*. — MycoBank MB560630; Fig. 2, 3

Basionym. Vermisporium acutum H.J. Swart & M.A. Will., Trans. Brit. Mycol. Soc. 81: 495. 1983.

Leaf spots circular to subcircular, up to 15 mm diam, often with an oblong protrusion on one side, positioned somewhat regularly along the leaf lamina, usually centred between the mid-rib and edge of the leaf lamina; pale brown, becoming grey with age, not vein-limited, developing a distinct dark brown to purple margin when older (Fig. 2). *Conidiomata* stromatic, acervular, amphigenous, mainly epiphyllous, scattered, subepidermal to intra-epidermal, oval in outline, 190–410 µm wide, 110–150 µm deep, glabrous when young, orange to brown. *Stroma* 20–30 µm thick, of *textura angularis*, pale brown to almost hyaline. *Conidiophores* mostly reduced to conidiogenous cells, branched, hyaline, up to 15 µm long. *Conidiogenous cells* lageniform, hyaline, annellidic, 5–14 µm long. *Conidia* narrowly fusiform, straight or curved, (2–)3(–4)-septate, hyaline, orange in mass, slightly or not constricted at septa, (39–)45–61(–66) × 3–4.5(–5) (av. = 51.5 × 3.8) µm; apical cell narrowly conical, attenuated to an acute apex, (11–)13–22 (av. = 16.1) µm long; second cell from apex cylindrical to subcylindrical, (10–)11–17 (av. = 13.4) µm long; third cell from apex cylindrical to subcylindrical, 9–15 (av. = 11.7) µm long; basal cell with a truncate base, (7–)9–12(–13) (av. = 10.2) µm long; basal appendage excentric, single, narrowly cuneiform, 2–7 (av. = 4.1) µm long; mean conidium length to width ratio = 13.6 : 1. Relative cell lengths from base to apex = 1.0 : 1.15 : 1.31 : 1.58. *Microconidia* not seen.

Previously known host — *Eucalyptus* sp. (Swart & Williamson 1983, Gadgil & Dick 1999).

Recorded host in this study — *E. camaldulensis* (GR 0.05).

Specimens examined. Australia, Victoria, Victoria Valley, Grampians National Park, on *E. camaldulensis*, 6 Oct. 1999, *P.A. Barber* (GR 0.05); Victoria, Rutherglen, on *Eucalyptus* sp., 1903, Collector's initials illegible (VPRI 2156). – New Zealand, Wellington, Botanic Gardens, on *Eucalyptus* sp., 31 Oct. 1996, *B.J. Rogan* (NZFRI-M 3644).

Notes — *Seimatosporium acutum* was observed on foliar lesions of an *E. camaldulensis* sapling growing within an open, mature (> 200 yr) *E. camaldulensis* native woodland in the Grampians National Park (Vic.). This is the first record of *S. acutum* on *E. camaldulensis*. Conidial dimensions of the type specimen examined in the present study were found to fall within the range of those described by Swart & Williamson (1983) and Nag Raj (1993). The specimen collected in the

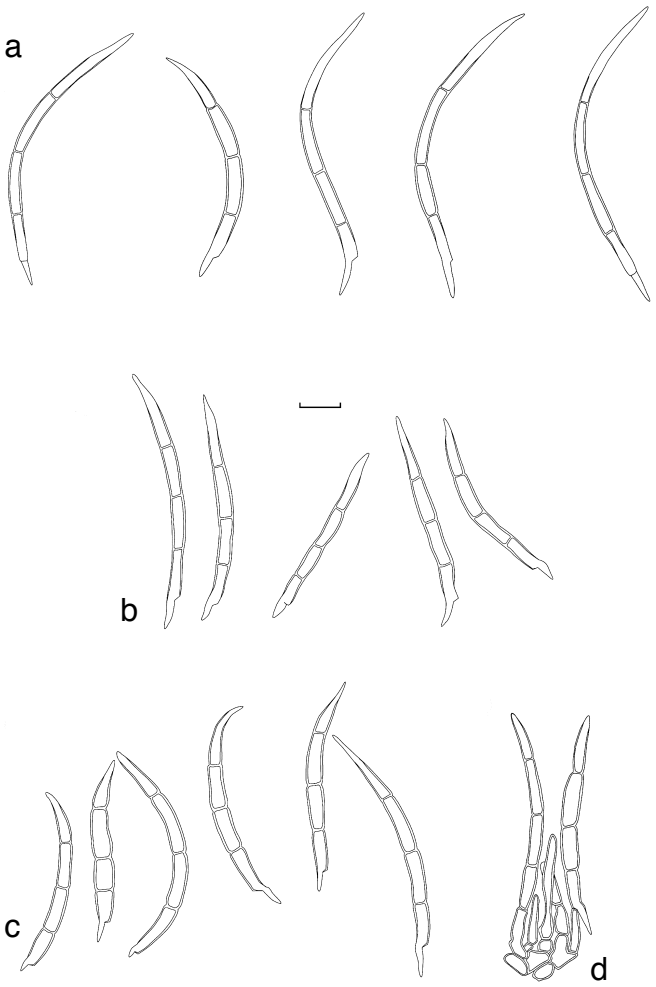

Fig. 3 *Seimatosporium acutum*. a. Type specimen VPRI 2156 conidia; b. NZFRI 3644 conidia; c. GR 0.05 conidia; d. GR 0.05 developing conidia attached to conidiogenous cells. — Scale bar = 10 µm.

present study (GR 0.05) had some shorter and wider conidia than previously described specimens of *V. acutum* (Table 2, 3, Fig. 3). The relative cell lengths of all specimens examined in the present study agreed with those given by Nag Raj (1993), with the cells increasing in length from the base to the apex (Table 2). *Seimatosporium acutum* can best be distinguished from *S. cylindrosporum* and *S. eucalypti* by its complete lack of pigmentation of its conidial median cells and the lack of an apical appendage, which *S. eucalypti* and *S. cylindrosporum* usually have.

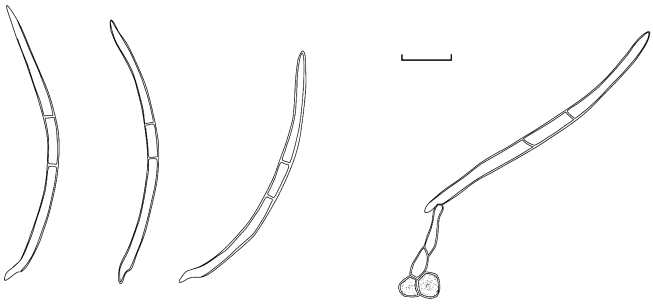

Fig. 4 *Seimatosporium biseptatum* conidia and conidiogenous cells from PAB 99.12. — Scale bar = 10 µm.

Seimatosporium biseptatum (H.J. Swart & M.A. Will.) Barber & Crous, *comb. nov*. — MycoBank MB560631; Fig. 4, 5

Basionym. Vermisporium biseptatum H.J. Swart & M.A. Will., Trans. Brit. Mycol. Soc. 81: 492. 1983.

Leaf spots circular, oval to angular, 2–12 mm diam, somewhat vein-limited, grey, necrotic, surrounded by a brown margin, and bearing numerous acervuli mostly on the abaxial surface (Fig. 5). *Conidiomata* stromatic, acervular, hypophyllous, scattered to gregarious, occasionally confluent, subepidermal, oval to irregular in outline, 250–1000 µm wide and 90–200 µm deep, orange to brown becoming dark brown with age. *Stroma* 20–30 µm thick, of *textura angularis*, cells pale brown to almost hyaline. *Conidiophores* arising from the upper layer of the stroma, reduced to conidiogenous cells. *Conidiogenous cells* lageniform, hyaline, annellidic, 5–15 µm long. *Conidia* narrowly cylindrical to acerose, straight, curved or sigmoid, 2-septate, hyaline, orange in mass, not constricted at the septa, septa faint, 45–62(–69) × (1.5–)2–2.5 (av. = 54.6 × 2.0) µm; apical cell subcylindrical, tapering to a blunt apex, (17–)20–27 (av. = 23.2) µm long; middle cell short cylindrical, (6–)7–11(–13) (av. = 9.3) µm long; basal cell cylindrical with a truncate base, (17–)20–29 (av. = 23.5) µm long; basal appendage excentric, single, podiform, 1.5–3 (av. = 2.2) µm long; mean conidium length to width ratio = 27.3 : 1. Relative cell lengths from base to apex = 1 : 0.40 : 1. *Microconidia* not seen.

Culture characteristics — Colonies erumpent, spreading, with sparse to moderate aerial mycelium and feathery margins, reaching 45 mm diam after 2 wk. Colonies salmon on MEA, OA and PDA.

Previously known hosts — *E. baxteri*, *E. foecunda*, *E. globulus*, *E. macrorhyncha*, *E. melliodora*, *E. regnans*, *E. rostrata* (syn: *E. camaldulensis*), *E. viminalis* (Swart & Williamson 1983).

Recorded host in this study — *E. camaldulensis* (PAB 99.01, PAB 99.12), *E. oresbia* (CBS H-20743).

Table 2 Conidial measurements of *Seimatosporium acutum* as recorded by various authors and in the present study (in **bold** type).

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
Swart & Williamson 1983 (description from type VPRI 2156)	53–75	2–3	4–8	–	–
Nag Raj 1993 (description from type VPRI 2156)	52–75	3–4	5–7	18 : 1	1.0 : 1.22 : 1.43 : 1.83
Gadgil & Dick 1999 (description from NZFRI 3644)	53–68	2–4	7–8	–	–
VPRI 2156 type	59–72	3–4	5–11	20.2 : 1	1.0 : 1.39 : 1.50 : 2.0
NZFRI 3644	42–62	3–4	3–6	14.5 : 1	1.0 : 1.14 : 1.22 : 1.73
GR 0.05	39–66	3–5	2–7	13.6 : 1	1.0 : 1.15 : 1.31 : 1.58

Table 3 Comparison of conidial measurements between *Seimatosporium acutum* (top compartment), *S. obtusum* (middle compartment), and *S. orbiculare* (lower compartment), (* indicates that these cell ratios described by Nag Raj (1993) are incorrect). Only those authors stating relative cell lengths have been included.

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
S. acutum					
Nag Raj 1993 (type)	52–75	3–4	5–7	18 : 1	1.0 : 1.22 : 1.43 : 1.83
NZFRI 3644	42–62	3–4	3–6	14.5 : 1	1.0 : 1.14 : 1.22 : 1.73
VPRI 2156 type	59–72	3–4	5–11	20.2 : 1	1.0 : 1.39 : 1.50 : 2.0
GR 0.05	39–66	3–5	2–7	13.6 :1	1.0 : 1.15 : 1.31 : 1.58
S. obtusum					
Nag Raj 1993	52–81	2–4.5	2–10	19 : 1	1.0 : 1.07 : 1.18 : 1.71
Yuan 1999	55–75	2.5–3.5	3–8	19.6 : 1	1.0 : 1.08 : 1.25 : 1.59
PAB 99.13	67–103	2.5–3.5	4–11	27.2 : 1	1.0 : 1.08 : 1.11 : 1.37
PAB 02.31	49–78	3–4.5	2–6	18.5 : 1	1.0 : 1.10 : 1.21 : 1.52
S. orbiculare					
Nag Raj 1993 * (incl. type)	42–71	3–4.5	3–7	15 : 1	1.0 : 0.89 : 1.11 : 1.11
Present study (type)	49–66	4–4.5	3–8	13.7 : 1	1.0 : 1.21 : 1.24 : 1.56

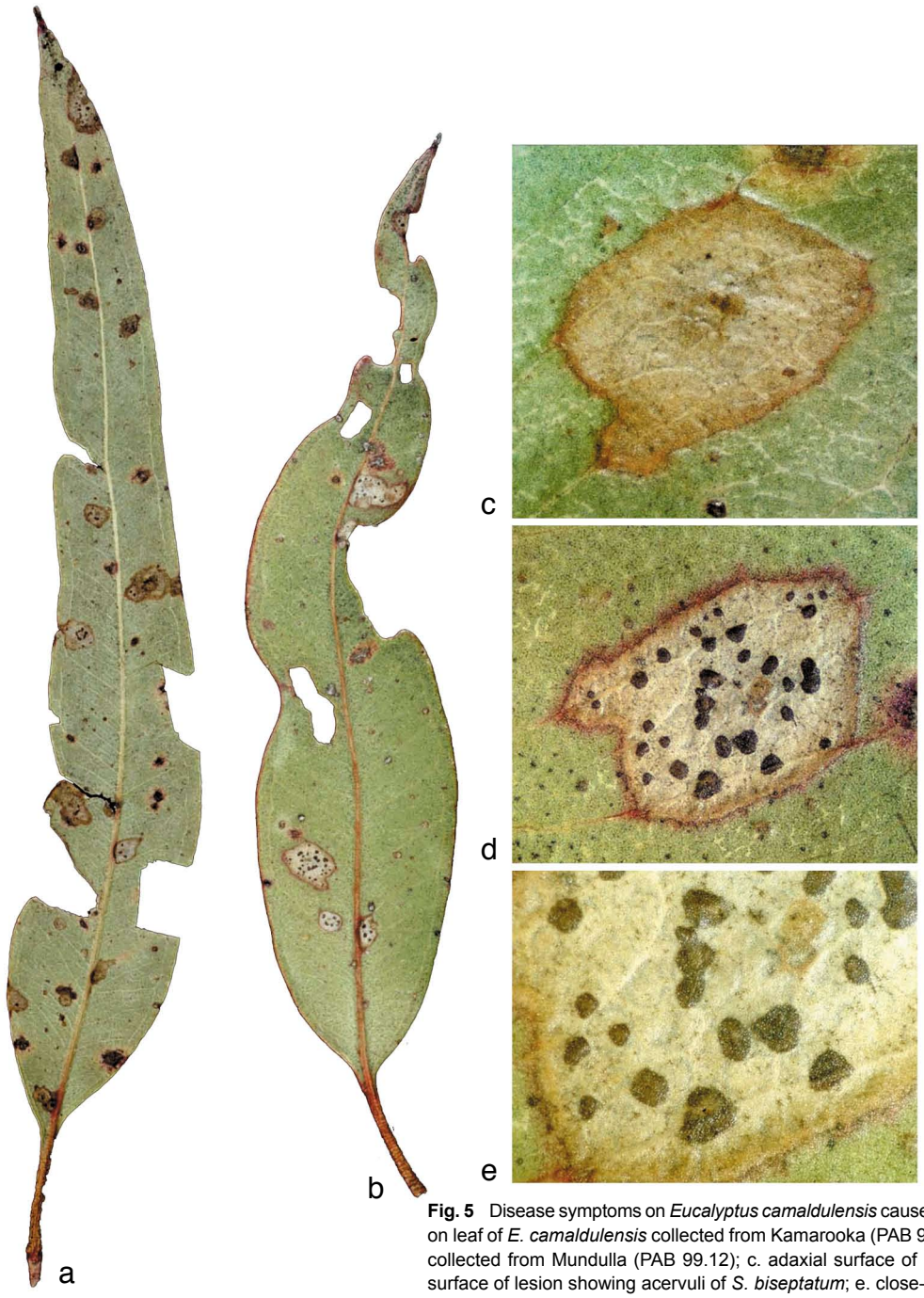

Fig. 5 Disease symptoms on *Eucalyptus camaldulensis* caused by *Seimatosporium biseptatum*. a. Lesions on leaf of *E. camaldulensis* collected from Kamarooka (PAB 99.01); b. lesions on leaf of *E. camaldulensis* collected from Mundulla (PAB 99.12); c. adaxial surface of lesion caused by *S. biseptatum*; d. abaxial surface of lesion showing acervuli of *S. biseptatum*; e. close-up of acervuli of *S. biseptatum.*

Table 4 Conidial measurements of *Seimatosporium biseptatum* as recorded by various authors and in the present study (in **bold** type).

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
Swart & Williamson 1983 (incl. type)	40–70	1.5–2.5	1–3	–	–
Nag Raj 1993 (incl. holotype)	45–70	1.5–2.5	1–3	24.0 : 1	1.0 : 0.48 : 1.15
PAB 99.01	45–65	1.5–2.5	1–3	25.7 : 1	1.0 : 0.33 : 0.92
PAB 99.12	48–69	2–2.5	1.5–3	27.5 : 1	1.0 : 0.44 : 1.03

Specimens examined. Australia, Victoria, Tennyson, on *E. camaldulensis*, 26 Oct. 1999, *P.A. Barber* (PAB 99.01); South Australia, Mundulla, on *E. camaldulensis*, 27 Aug. 1999, *P.A. Barber* (PAB 99.12); New South Wales, Northern Tablelands, 7.5 km E of Nundle on road to Hanging Rock (c. 100 m E of Hanging Rock track turnoff), S31°28'31" E151°10'59", alt. 1090 m, 20 July 2006, *A.E. Orme* & *R. Johnstone* NSW 732739, on *E. oresbia*, CBS H-20743, culture CPC 13584–13586 = CBS 131116.

Notes — *Seimatosporium biseptatum* was found on foliar lesions of *E. camaldulensis*. These leaves were collected from old road-side trees near Tennyson (Vic.) and Mundulla (S.A.). Conidial dimensions of PAB 99.01 and PAB 99.12 observed in the present study fall within the range of those described by both Nag Raj (1993) and Swart & Williamson (1983) (Table 4). As noted by Swart & Williamson (1983), "by being the only species with three-celled conidia, *V. biseptatum* is easily identified within the genus". These conidia also lack pigment and an apical appendage. The relative lengths of the conidial cells is also distinctive, with the basal and apical cells being similar and the median cell being only a third to a half of their length (Fig. 4).

Seimatosporium brevicentrum (H.J. Swart & M.A. Will.) Barber & Crous, *comb. nov*. — MycoBank MB560632; Fig. 6–8

Basionym. Vermisporium brevicentrum H.J. Swart & M.A. Will., Trans. Brit. Mycol. Soc. 81: 493. 1983.

Leaf spots large, irregular, vein-limited, pale-brown to grey with an indistinct margin when young, becoming dark red-brown

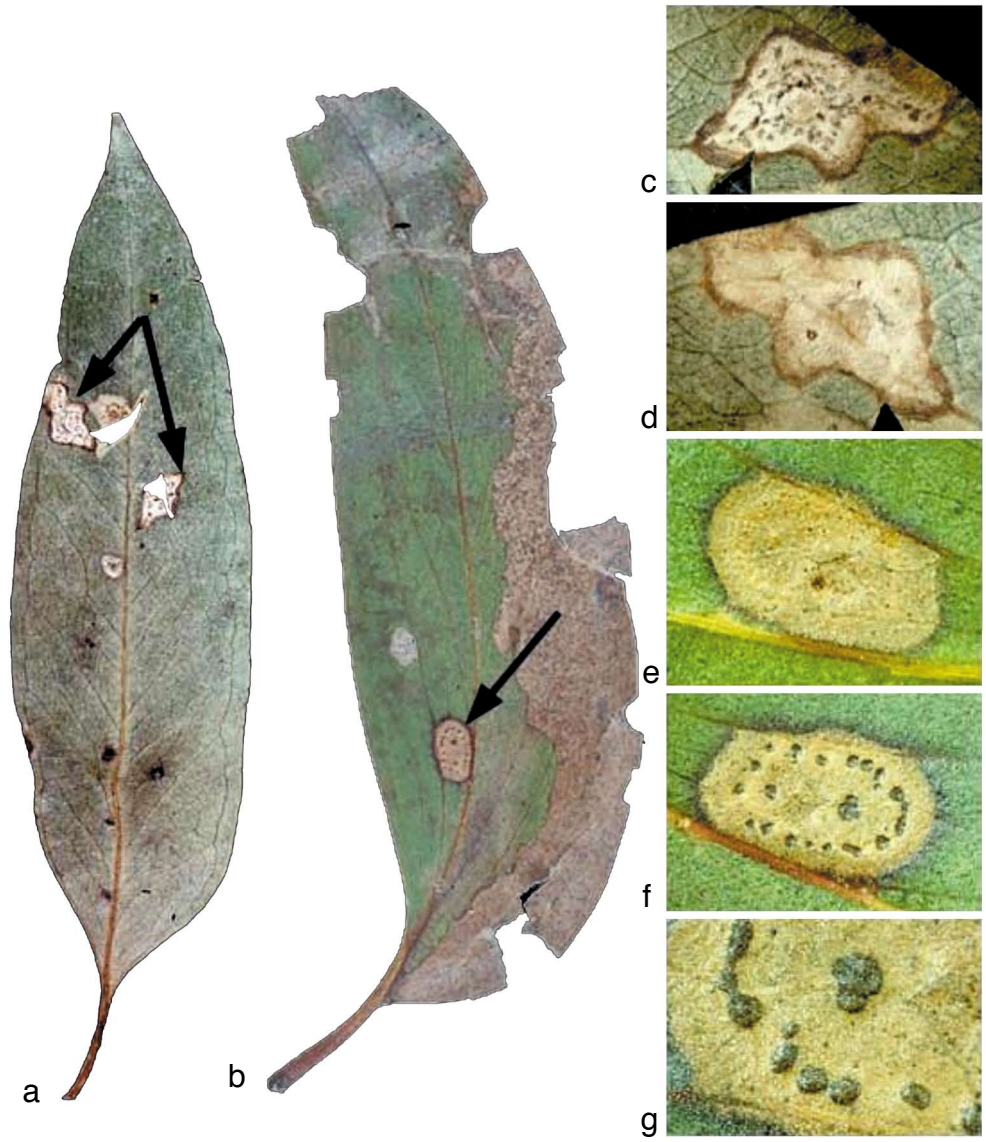

Fig. 6 Disease symptoms on *Eucalyptus ovata* and *E. serraensis* associated with *Seimatosporium brevicentrum.* a. Lesions (arrowed) on leaf of *E. ovata*; b. lesion on leaf of *E. serraensis*; c. adaxial surface of lesion on *E. ovata* showing acervuli; d. abaxial surface of lesion on *E. ovata*; e. abaxial surface of lesion on *E. serraensis* showing insect exit hole (arrowed); f. adaxial surface of lesion on *E. serraensis* showing acervuli; g. higher magnification of acervuli of *S. brevicentrum* on *E. serraensis.*

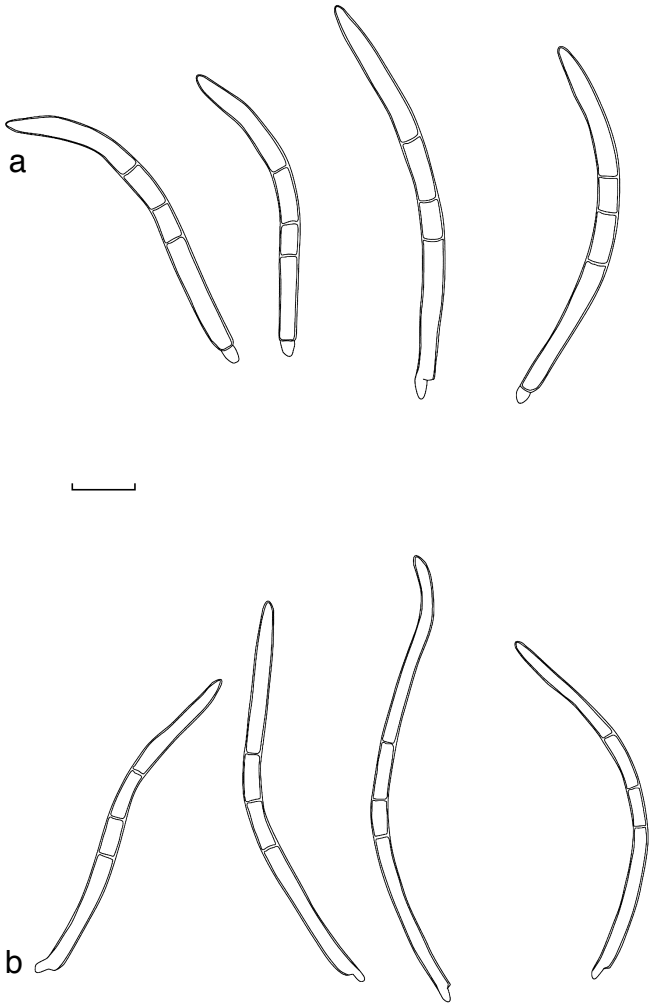

Fig. 7 Conidia of *Seimatosporium brevicentrum*. a. PAB 99.11; b. GR 0.02. — Scale bar = 10 µm.

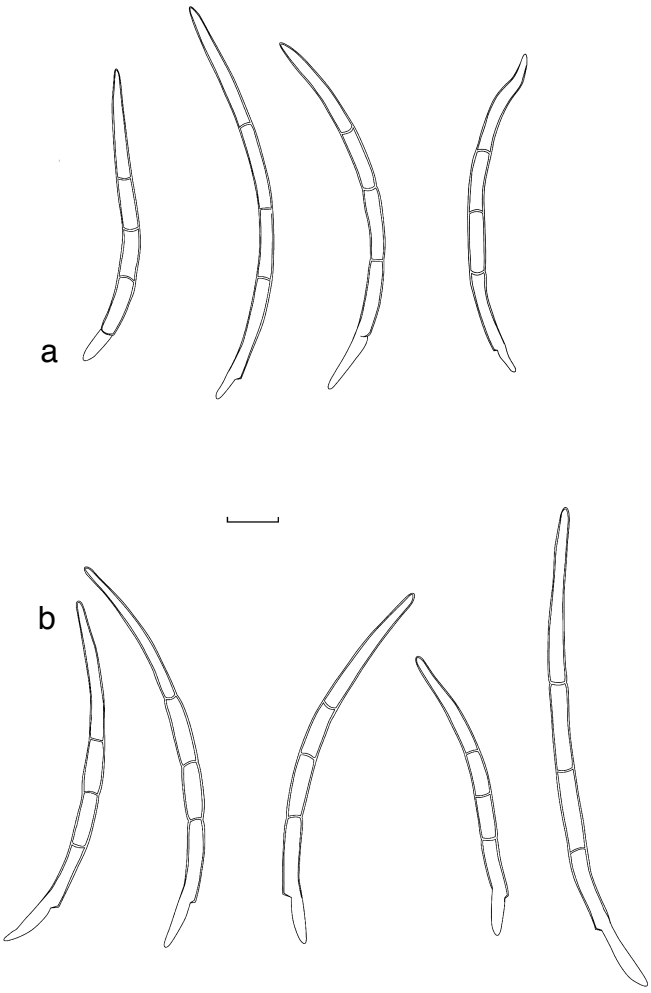

Fig. 8 Conidia of *Seimatosporium walkeri* incorrectly described as *S. brevicentrum* in Gadgil & Dick (1999). a. NZFRI (M) 3645; b. NZFRI (M) 3756. — Scale bar = 10 µm.

when older and more necrotic, up to 11 mm diam, bearing numerous acervuli more commonly on the adaxial surface, with thin dark brown margins on *E. ovata* (Fig. 6)*;* lesions on *E. serraensis*, in contrast to those on *E. ovata*, were not vein-limited, lacked a distinct brown margin and bore acervuli in a concentric arrangement within the lesion (Fig. 6); acervuli were epiphyllous on both hosts which bears similarities to *V. acutum* but differs from *V. biseptatum*. Evidence of an insect association was present in some lesions. *Conidiomata* stromatic, acervular, epiphyllous, scattered to gregarious, often confluent, subepidermal, oval to irregular in outline, 225–575 µm wide and 90–135 µm deep, gelatinous when moistened, glabrous, orange becoming dark brown with age. *Stroma* up to 15 µm thick, of *textura angularis*, cells hyaline. *Conidiophores* arising from the upper layer of the stroma, reduced to conidiogenous cells. *Conidiogenous cells* lageniform, hyaline, annellidic, 5–10 µm long. *Conidia* narrowly cylindrical, straight, curved or sigmoid, 3-septate, hyaline, smooth walled, hyaline or almost hyaline in mass, not constricted at the septa, 39–70 × 2–3.5 (av. = 54.3 × 2.8) µm; apical cell cylindrical with an obtuse apex, (13–)18–25(–31) (av. = 21.3) µm long; second cell from apex cylindrical, 5–10(–12) (av. = 7.3) µm long; third cell from apex cylindrical, (4–)5–9(–10) (av. = 6.7) µm long; basal cell with a truncate base, (9–)13–25(–28) (av. = 19.1) µm long; basal appendage excentric, single, cuneiform to podiform with an obtuse tip, 1–3(–3.5) (av. = 2.1) µm long; mean conidium length to width ratio = 19.4 : 1. Relative cell lengths from base to apex = 1.0 : 0.35 : 0.38 : 1.12. *Microconidia* not seen.

Previously known hosts — *E. dumosa*, *E. ovata*, *E. viminalis* (Swart & Williamson 1983), *E. fastigata*, *E.* sp. (Gadgil & Dick 1999) (refer to Notes).

Recorded hosts in this study — *E. ovata* (PAB 99.11), *E. serraensis* (GR 0.02).

Specimens examined. Australia, Victoria, Whittlesea, on *E. ovata*, 11 Oct. 1999, *P.A. Barber* (PAB 99.11); Mt Burchell, Grampians National Park, on *E. serraensis*, 12 Aug. 2000, *P.A. Barber* (GR 0.02). – New Zealand, Wellington, Karori Cemetary, on *Eucalyptus* sp., 14 Nov. 1996, *B. Rogan* (NZFRI-M 3645); Wellington, Catchpool Forest, on *E. fastigata*, 16 Oct. 1997, *B.J. Rogan* (NZFRI-M 3756).

Notes — *Seimatosporium brevicentrum* was examined in the present study on leaves collected from *E. ovata* from road-side vegetation in Whittlesea (Vic.) and from a single leaf of *E. serraensis* from the summit of Mt Burchell in the Serra Ranges of the Grampians National Park (Vic.). No fungi have previously been recorded from *E. serraensis* and hence, this is a new host record for *V. brevicentrum*.

Conidial dimensions of specimens collected in the present study generally fell within the ranges of those described for *S. brevicentrum* by Swart & Williamson (1983) and Nag Raj (1993) (Table 5). Although the general conidial morphology of this species bears some similarities to that of *S. biseptatum*, it differs in having an additional septum, giving two shorter, median cells instead of the one in *S. biseptatum*. *Seimatosporium walkeri* is the only other *Seimatosporium* species with two median cells shorter than both the apical and basal cells. However, the two median cells are somewhat longer in *S. walkeri.* The median cells in *S. brevicentrum* are only about

Table 5 Conidial measurements of *Seimatosporium brevicentrum* as recorded by various authors and in the present study (in **bold** type); species described by Gadgil & Dick 1999b is actually *S. walkerii*.

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
Swart & Williamson 1983 (incl. holotype)	46–70	2–3	2–3	–	–
Nag Raj 1993 (incl. holotype)	45–68	2.5–3	1.5–3	20.7 : 1	1.0 : 0.30 : 0.40 : 1.18
Gadgil & Dick 1999 (description from NZFRI 3645 and NZFIR 3756)	60–72	3–4	10–11	–	–
NZFRI 3645	48–74	2.5–3.5	2–11	18.9 : 1	1.0 : 0.76 : 0.87 : 1.75
NZFRI 3756	48–82	3–4	5–13	17.2 : 1	1.0 : 0.75 : 0.76 : 1.79
PAB 99.11	43–69	2.5–3.5	1–3	19.2 : 1	1.0 : 0.36 : 0.38 : 1.10
GR 0.05	49–70	2–3	1–3.5	26.7 : 1	1.0 : 0.35 : 0.40 : 1.10

half as long as the basal cell, whereas in *S. walkeri* the median cells are nearly as long as the basal cell (Table 5, Fig. 7, 8). In both these species, conidia lack pigmentation and an apical appendage. However, *S. brevicentrum* has a significantly shorter basal appendage and the conidia of *S. walkeri* taper more at the apex and basal appendage.

Gadgil & Dick (1999) described *S. brevicentrum* from *E. fastigata* and an *Eucalyptus* sp., but examination of the herbarium specimens (NZFRI (M) 3645 and NZFRI (M) 3756) during the present study revealed that the causal pathogen had been misidentified, and is in fact *S. walkeri*. This would explain the variation in conidial measurements (Table 5). From these findings we can conclude that *S. brevicentrum* has not been described from New Zealand or *E. fastigata* and that *S. walkeri* is newly recorded from New Zealand and *E. fastigata*.

Seimatosporium cylindrosporum H.J. Swart, Trans. Brit. Mycol. Soc. 78: 267. 1982 — Fig. 9–11

≡ *Vermisporium cylindrosporum* (H.J. Swart) Nag Raj, in Nag Raj, Coelomycetous anamorphs with appendage-bearing conidia: 965. 1993.

Leaf spots circular, 1–8 mm diam when not confined to the leaf margins, or irregular and up to 23 mm in length when confined to the leaf margins, vein-limited, grey to pale brown in the centre, becoming brown towards the margins; margins carmine red on smaller lesions, becoming red-brown on larger lesions; acervuli amphigenous, at times with evidence of insect association (Fig. 9). *Conidiomata* stromatic, acervular, amphigenous, scattered to gregarious, subepidermal, roughly circular in outline, 130–500 µm wide and 70–110 µm deep, brown to dark brown. *Stroma* 15–25 µm thick, of *textura angularis*, cells pale brown to almost hyaline. *Conidiophores* arising from the upper layer of the stroma, reduced to conidiogenous cells. *Conidiogenous cells* cylindrical, lageniform to ampulliform, hyaline, annellidic, up to 5–11 µm long. *Conidia* cylindrical to acerose, straight or curved, 3-septate, distinct, constricted at the septa, median cells pale brown, apical and basal cells pale brown, periclinal wall smooth, pale brown in mass, (40–)43–56(–58) × 3.5–4.5(–5) (av. = 49.7 × 4.0) µm; apical cell subcylindrical, pale brown, terminating in a discernible, hyaline, conical appendage up to 7 µm long, total length including the appendage (12–)13–20 (av. = 16.1) µm long; second cell from apex cylindrical, pale brown, 10–15(–17) (av. = 12.2) µm long; third cell from apex cylindrical, pale brown, (8–)10–13(–14) (av. = 11.3) µm long; basal cell subcylindrical to narrowly obconic with a truncate base and basal appendage, pale brown, 8–12(–13) (av. = 10.3) µm long; basal appendage single, excentric, cuneiform to podi-

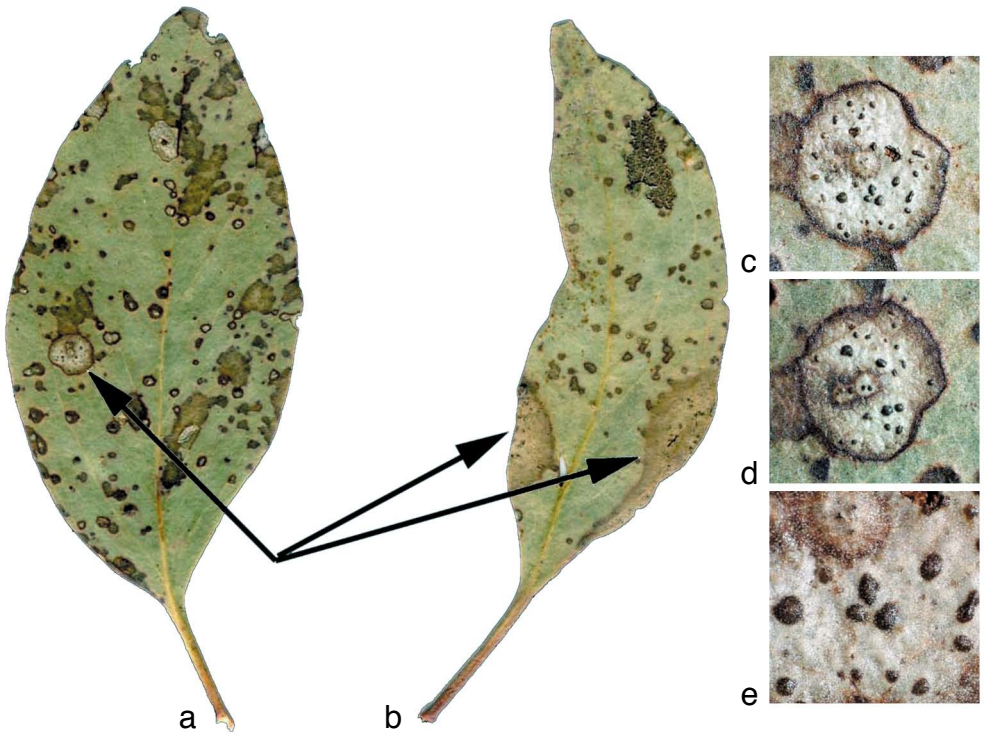

Fig. 9 Disease symptoms on *Eucalyptus behriana* associated with *Seimatosporium cylindrosporum*. a. Smaller lesion on leaf lamina (arrowed); b. larger blight along the edge of the leaf lamina (arrowed); c. adaxial surface of the lesion carmine red margin and distinct dark brown acervuli; d. abaxial surface of the lesion showing carmine red margin and distinct dark brown acervuli; e. higher magnification of adaxial surface of lesion showing roughly circular, dark brown acervuli.

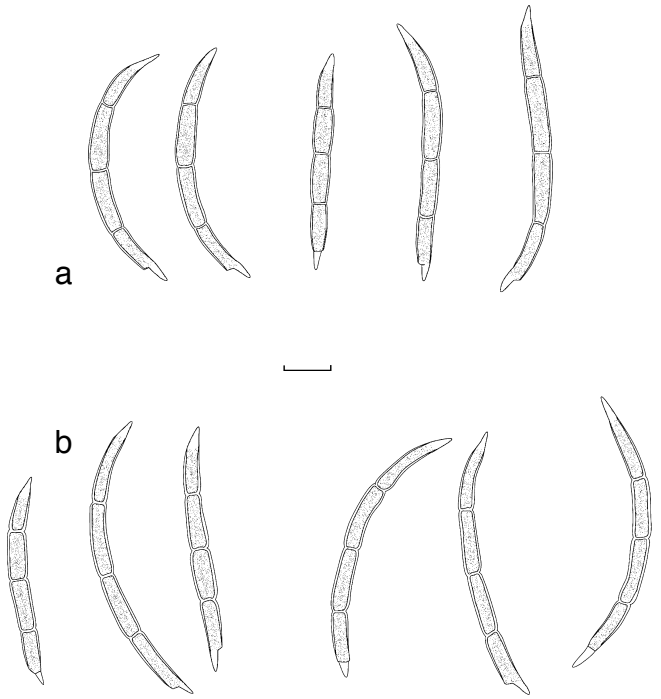

Fig. 10 Conidia of *Seimatosporium cylindrosporum*. a. PAB 99.06; b. isotype specimen c77.04. — Scale bar = 10 µm.

form, attenuated to a point, 2–7(–9) (av. = 4.7) µm long; mean conidium length to width ratio = 12.5 : 1. Relative cell lengths from base to apex = 1.0 : 1.10 : 1.19 : 1.58. *Microconidia* not seen.

Previously known hosts — *E. behriana* (Swart 1982), *E. diversifolia* (MELU specimen, Harry Swart collection HJS 76.07), *E. radiata*, *E. regnans*, *E. saligna*, *Eucalyptus* sp. (Dick 1990), *E. globulus*, *E. nitens* (Yuan 1999) (see Notes).

Recorded host in this study — *E. behriana* (PAB 99.06).

Specimens examined. Australia, Victoria, Kamarooka State Forest, on *E. behriana*, 26 Oct. 1999, *P.A. Barber* (PAB 99.06); Victoria, Melton, on *E. behriana*, 19 Mar. 1977, *I. Pascoe* (MELU 2002-5-3 isotype). – New Zealand, Tokoroa, Kinleith Forest, on *E. radiata* ssp. *radiata*, Oct. 1986, (NZFRI-M 3167); Tokoroa, Kinleith Forest, on *E. regnans*, July 1985, (NZFRI-M 3156); Tokoroa, Kinleith Forest, on *E. saligna*, May 1988, (NZFRI-M 3259); Tokoroa, Kinleith Forest, on *Eucalyptus* sp., Nov. 1982, (NZFRI-M 3155).

Notes — *Seimatosporium cylindrosporum* was found associated with lesions on *E. behriana* collected on the road-side in the Kamarooka State Forest, approximately 1.5 h north-east of Melbourne, Victoria. Examination of the isotype showed conidial dimensions that fell within the range of those described by Swart (1982) and Nag Raj (1993) (Table 6). The isolate collected in the present study was similar to the isotype in conidial length,

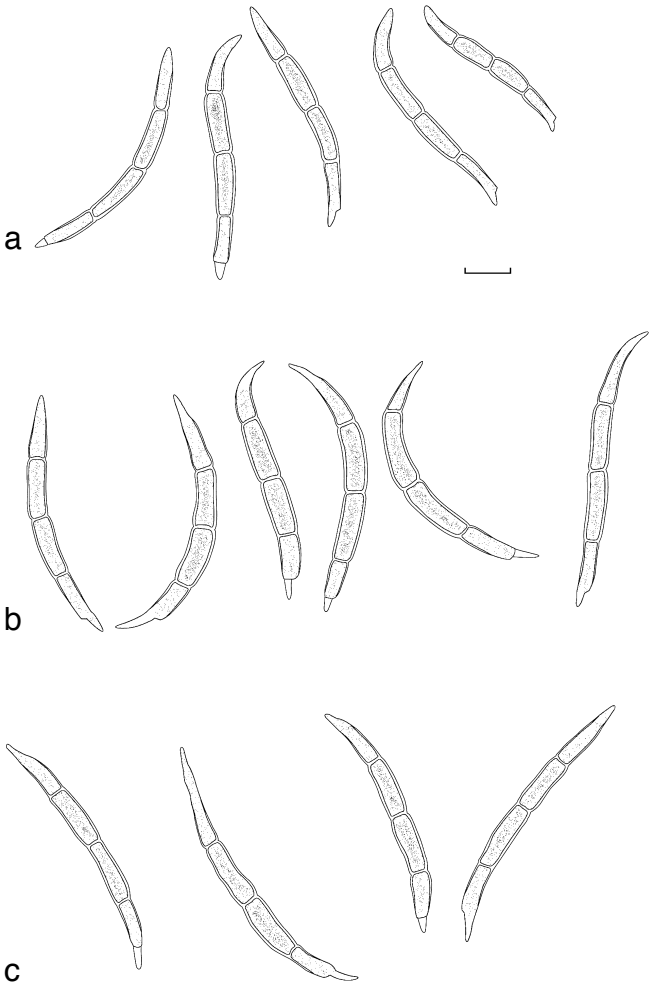

Fig. 11 Conidia of *Seimatosporium* aff. *cylindrosporum*. a. NZFRI 3155; b. NZFRI 3156; c. NZFRI 3167. — Scale bar = 10 µm.

width, cell ratios, appendage lengths and pigmentation (Fig. 10, Table 6). Our findings agree with those of Swart (1982) – conidia were evenly pigmented throughout their length up until the hyaline apical and basal appendages (Fig. 10). These appendages are distinct and attenuate to a point.

This species is most similar to *S. falcatum* and *S. eucalypti*; all three species have pigmented cells to some degree; however, *S. cylindrosporum* is the only species with median and terminal cells pigmented to the same degree, with only the terminal appendages being distinctly hyaline. Both *S. falcatum* and *S. eucalypti* have pigmented median cells but often the terminal cells are more lightly pigmented than the median cells or become less pigmented towards the ends of the cells (Fig. 15,

Table 6 Conidial measurements of *Seimatosporium cylindrosporum* as recorded by various authors and in the present study (in **bold** type).

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of apical appendage (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
Swart 1982 (type)	43–70	3–4	up to 5	up to 9	–	–
Dick 1990 (description from NZFRI 3155, 3156 and 3167)	34–50	3–5	up to 6	2–7	–	–
Nag Raj 1993 (ex paratype)	30–57	3–4	up to 5	2.5–5	12.4 : 1	1.0 : 1.08 : 1.20 : 1.39
Yuan 1999	35.7–55	3.5–4.5	–	2.5–3.8	11.1 : 1	1.0 : 1.08 : 1.16 : 1.46
c77.04 (isotype)	40–56	3.5–4.5	up to 7	2–7	12.7 : 1	1.0 : 1.11 : 1.18 : 1.67
PAB 99.06	40–58	3.5–5	up to 7	3–9	12.2 : 1	1.0 : 1.09 : 1.19 : 1.48
NZFRI (M) 3155	40–57	3.5–4	–	2–4	12.2 : 1	1.0 : 1.21 : 1.19 : 1.27
NZFRI (M) 3156	41–61	3.5–5	up to 6	2–6	12.4 : 1	1.0 : 1.35 : 1.37 : 1.42
NZFRI (M) 3167	47–60	4–5	–	3–7	13.1 : 1	1.0 : 1.32 : 1.28 : 1.69

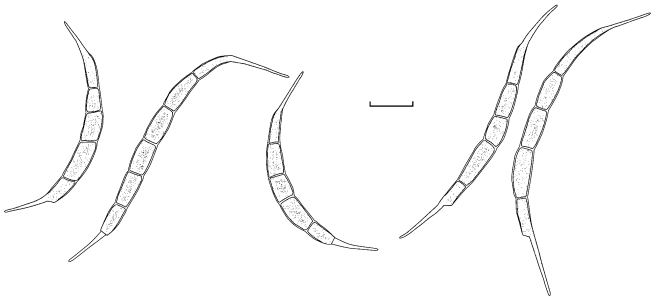

Fig. 12 Conidia of *Diploceras leptospermi* incorrectly identified as *Seimatosporium cylindrosporum* from specimen NZFRI 3259. — Scale bar = 10 µm.

16, 19, 22). Conidial cells of *S. cylindrosporum* always increase in length from the base to the apex and the second cell from the base is approximately only 10 % longer than the basal cell, compared with *S. falcatum* and *S. eucalypti* where it is always more than 20 % longer than the basal cell.

Examination of the diseased material on *E. saligna* (NZFRI 3259) revealed that the causal pathogen was actually *Diploceras leptospermi*, not *S. cylindrosporum.* These two species differ markedly in their conidial morphology, with *D. leptospermi* having 1 or 2 additional septa and long, tubular apical and basal appendages (Fig. 12). We can therefore conclude that *S. cylindrosporum* has not been recorded on *E. saligna* and this is the first record of *D. leptospermi* on *Eucalyptus.*

Seimatosporium eucalypti (McAlpine) H.J. Swart, Trans. Brit. Mycol. Soc. 78: 268. 1982 — Fig. 13–17, 19

Basionym. Cylindrosporium eucalypti McAlpine, Proc. Linn. Soc. New South Wales 28: 97. 1903.

≡ *Vermisporium eucalypti* (McAlpine) Nag Raj, in Nag Raj, Coelomycetous anamorphs with appendage-bearing conidia: 966. 1993.

Leaf spots definite, cream to grey, generally with a distinct raised, ruddy brown margin, not vein-limited, usually circular but sometimes angular or irregular, isolated or confluent, on both surfaces of the leaf, ultimately thin, brittle and cracking, variable in size, 2–25 mm diam; frequently associated with insect damage or other fungi (Fig. 13, 14, 17). *Conidiomata*

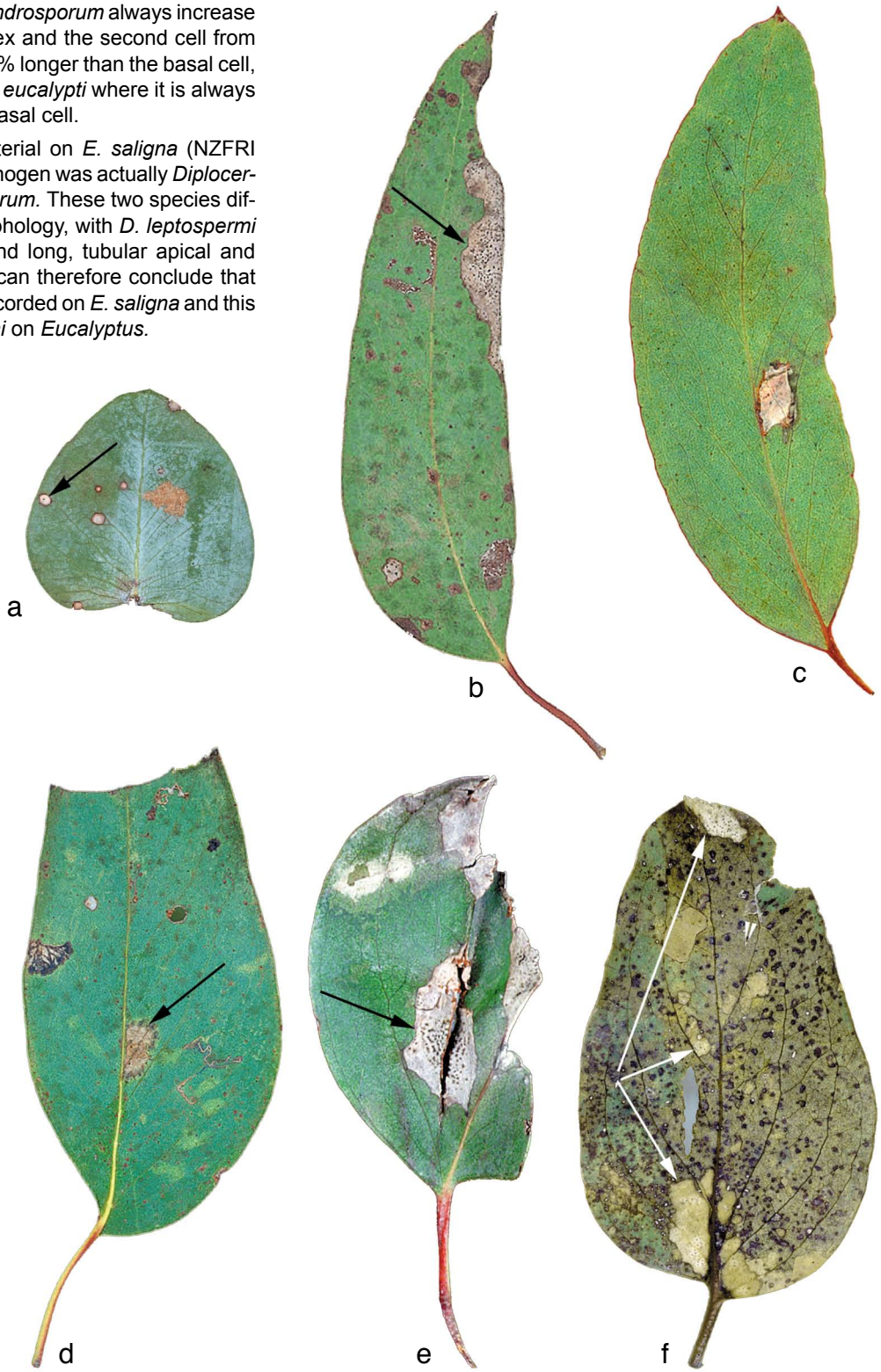

Fig. 13 Disease symptoms on various eucalypt hosts associated with *Seimatosporium eucalypti.* a. Lesion (arrowed) on *Eucalyptus cinerea*; b. lesion (arrowed) associated with *Seimatosporium eucalypti* and *Phylacteophaga* sp. on *E. delegatensis*; c. lesion on *E. delegatensis*; d. lesion (arrowed) associated with *S. eucalypti* and *Pachysacca samuelii* on *E. delegatensis*; e. lesion (arrowed) associated with *S. eucalypti* and *Mycosphaerella cryptica* on *E. oblique*; f. lesions (arrowed) on *E. pauciflora.*

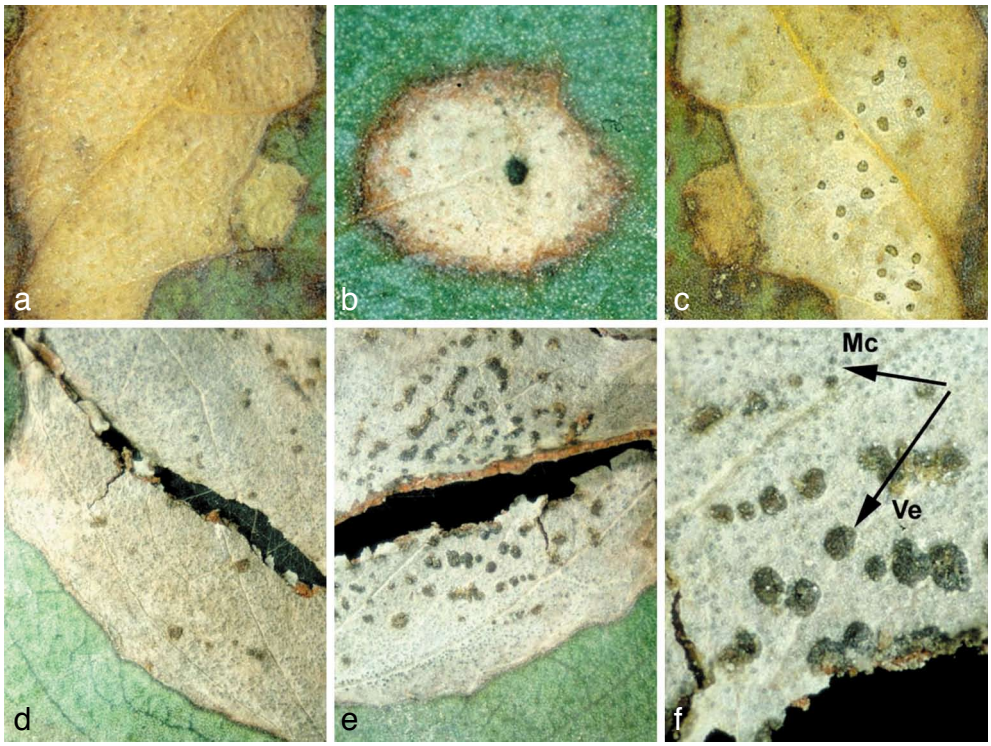

Fig. 14 Lesions on *Eucalyptus cinerea*, *E. obliqua* and *E. pauciflora* associated with *Seimatosporium eucalypti.* a. Abaxial surface of lesion on *E. cinerea* showing single acervulus of *S. eucalypti*; b. abaxial surface of lesion on *E. pauciflora*; c. adaxial surface of lesion on *E. pauciflora* showing acervuli of *S. eucalypti* surrounded by pale zones; d. abaxial surface of lesion on *E. obliqua* showing few acervuli of *S. eucalypti*; e. adaxial surface of lesion on *E. obliqua* showing many acervuli of *S. eucalypti*; f. higher magnification of adaxial surface of lesion on *E. obliqua* showing large erumpent acervuli (arrowed Ve) of *S. eucalypti* and small, substomatal pseudothecia (arrowed Mc) of *Mycosphaerella cryptica.*

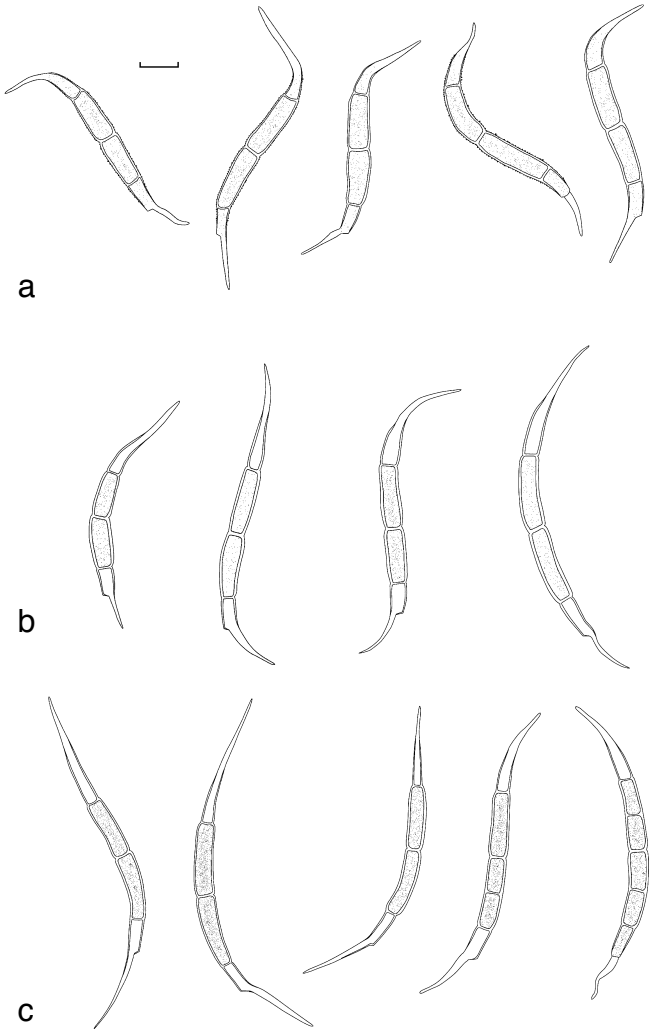

Fig. 15 Conidia of *Seimatosporium eucalypti.* a. MB 0.14b; b. MB 0.02 in vivo; c. MB 0.02 in vitro. — Scale bar = 10 µm.

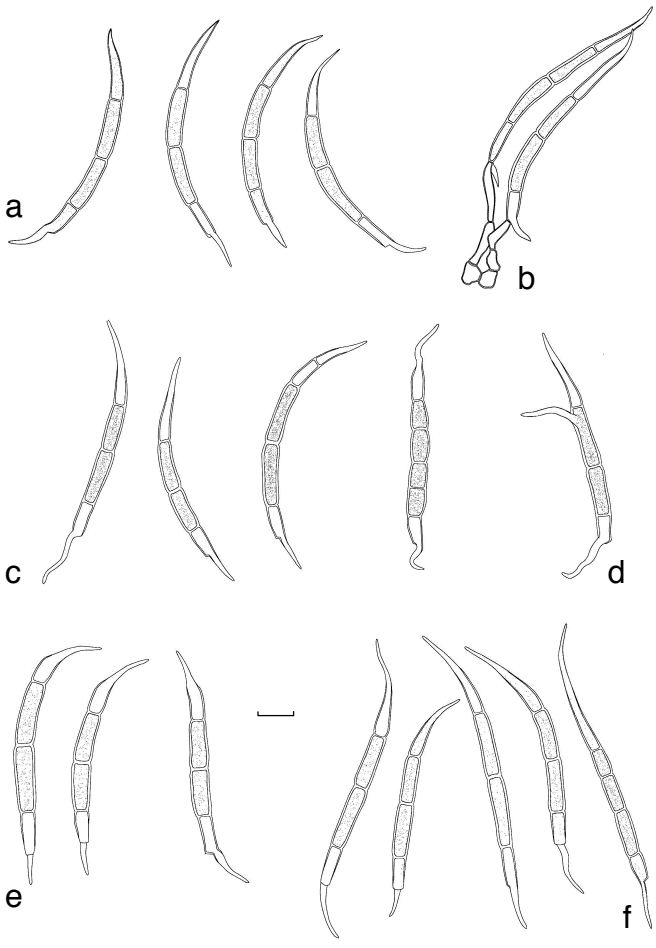

Fig. 16 Conidia of *Seimatosporium eucalypti.* a. MB 0.29; b. conidiogenous cells and conidia of MB 0.29; c. MB 0.33; d. germinating conidium of MB 0.33; e. MB 0.35; f. MB 0.38. — Scale bar = 10 µm.

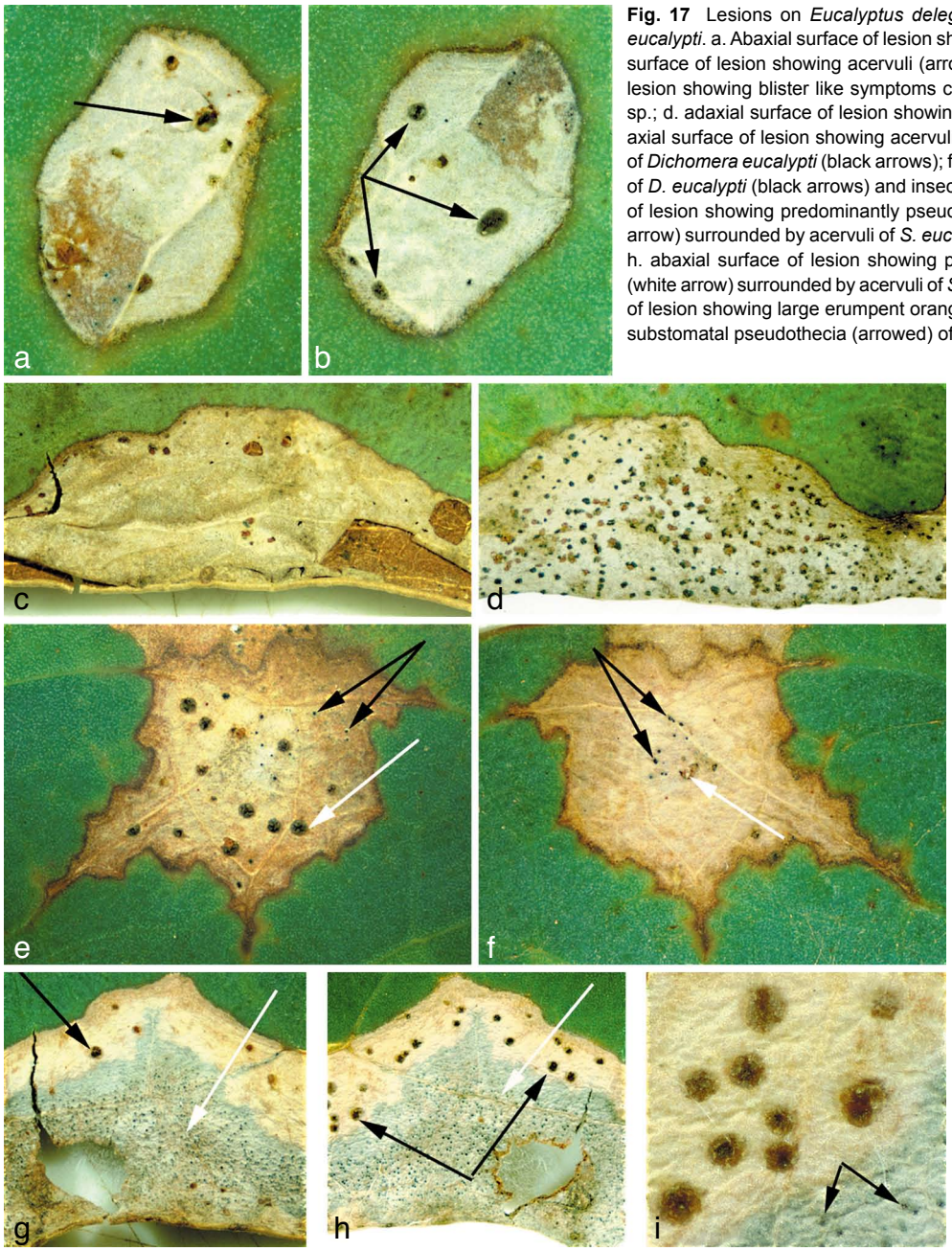

Fig. 17 Lesions on *Eucalyptus delegatensis* associated with *Seimatosporium eucalypti*. a. Abaxial surface of lesion showing insect exit hole (arrowed); b. adaxial surface of lesion showing acervuli (arrowed) of *S. eucalypti*; c. abaxial surface of lesion showing blister like symptoms commonly associated with *Phylacteophaga* sp.; d. adaxial surface of lesion showing numerous acervuli of *S. eucalypti*; e. adaxial surface of lesion showing acervuli of *S. eucalypti* (white arrow) and pycnidia of *Dichomera eucalypti* (black arrows); f. abaxial surface of lesion showing pycnidia of *D. eucalypti* (black arrows) and insect exit hole (white arrow); g. adaxial surface of lesion showing predominantly pseudothecia of *Mycosphaerella cryptica* (white arrow) surrounded by acervuli of *S. eucalypti* (black arrow) on outer edge of lesion; h. abaxial surface of lesion showing predominantly pseudothecia of *M. cryptica* (white arrow) surrounded by acervuli of *S. eucalypti* (black arrows); i. abaxial surface of lesion showing large erumpent orange-brown acervuli of *S. eucalypti* and small substomatal pseudothecia (arrowed) of *M. cryptica*.

stromatic, acervular, amphigenous, scattered to gregarious, intra-epidermal, oval to irregular in outline, 240–560 µm wide and 100–230 µm deep, glabrous, orange to brown becoming dark brown with age. *Stroma* 10–25 µm thick, of *textura angularis*, cells brown to almost hyaline. *Conidiophores* arising from the upper layer of the stroma, reduced to conidiogenous cells. *Conidiogenous* cells subcylindrical to lageniform, hyaline, occasionally branched, commonly septate, annellidic, up to 30 µm long. *Conidia* falcate, fusiform or sigmoid, 3(–4)-septate, rarely 5-septate, distinct, slightly constricted at the septa, guttulate or not guttulate, median cells pale brown to almost hyaline, apical and basal cells almost hyaline to hyaline, periclinal wall smooth or minutely verruculose, slightly thicker in the median cells, pale brown in mass, (35–)46–76(–82) × (3.5–)4–5(–6) (av. = 61.3 × 4.5) µm; apical cell subcylindrical, almost hyaline to hyaline, upper half hyaline, attenuated to an acute to sharply acute apex or sometimes into a discernible conical or sometimes tubular appendage up to 22 µm, total length including the appendage (11–)12–30(–32) (av. = 20.4) µm long; second cell from apex cylindrical, pale brown to almost hyaline, (9–)10–20(–22) (av. = 15.4) µm long; third cell from apex cylindrical, pale brown to almost hyaline, (8–)11–19(–21) (av. = 15.2) µm long; basal cell elongate-obconic with a truncate base and basal appendage, almost hyaline to hyaline, lower half hyaline, (6–)7–12 (av. = 9.2) µm long; basal appendage single, excentric, cuneiform to plectronoid or tubular and flexuous, attenuated to a point, (3–)4–15(–17) (av. = 9.3) µm long; mean conidium length to width ratio = 13.6 : 1. Relative cell lengths from base to apex = 1.0 : 1.65 : 1.67 : 2.22. *Microconidia* not seen.

Culture characteristics — Colonies erumpent, spreading, with sparse to moderate aerial mycelium and lobate, even margins, reaching 55 mm diam after 2 wk. Colonies salmon on MEA, OA and PDA.

Previously known hosts — *E. camaldulensis*, *E. nitens* (Nag Raj 1993), *E. delegatensis*, *E. saligna* (Gadgil & Dick 1999), *E. maculata*, *E. maidenii*, *E. smithii* (Crous et al. 1990), *E. melliodora* (McAlpine 1903).

Recorded hosts in this study — *E. cinerea* (IAS 01/149-1), *E. delegatensis* (MB 0.02; MB 0.06; MB 0.29; MB 0.33; MB 0.35; MB 0.36; MB 0.38; MB 0.39), *E. obliqua* (PAB 02.35), *E. pauciflora* (MB 0.14b).

Specimens examined. Australia, Victoria, Dandenong Creek, on *E. melliodora*, 16 Nov. 1902, *C. French Jr.* (VPRI 5927a Type specimen); Victoria, Benalla, on *E. cinerea*, 29 Oct. 2001, *I.W. Smith* (VPRI 30218); Victoria, Mt

Buffalo, on *E. delegatensis*, 8 May 2000, *P.J. Keane* (MB 0.02); Victoria, Mt Buffalo, on *E. delegatensis*, 8 May 2000, *P.J. Keane* (MB 0.06); Victoria, Mt Buffalo, on *E. delegatensis*, 8 May 2000, *P.J. Keane* (MB 0.29); Victoria, Mt Buffalo, on *E. delegatensis*, 8 May 2000, *P.J. Keane* (MB 0.33); Victoria, Mt Buffalo, on *E. delegatensis*, 8 May 2000, *P.J. Keane* (MB 0.35); Victoria, Mt Buffalo, on *E. delegatensis*, 8 May 2000, *P.J. Keane* (MB 0.36); Victoria, Mt Buffalo, on *E. delegatensis*, 8 May 2000, *P.J. Keane* (MB 0.38); Victoria, Mt Buffalo (The Hump), on *E. delegatensis*, 8 May 2000, *P.J. Keane* (MB 0.39); Victoria, Mt Buffalo, on *E. pauciflora*, 8 May 2000, *P.J. Keane* (MB 0.14b). – New Zealand, Rotoehu Forest, on *E. saligna*, 12 Aug. 1997, *K. Dobbie* (NZFRI-M 3740); Waimea Forest, on *E. delegatensis*, 11 Aug. 1998, *P. Bradbury* (NZFRI-M 3867). – South Africa, Mpumalanga, Sabie, Sabie Forest Station, on *E. smithii*, Sept. 1989, *P.W. Crous*, PREM 50457, cultures CPC 156 = CBS 115131, CPC 157 = CBS 110733, CPC 158 = CBS 110734, CPC 159 = CBS 114876.

Notes — A number of collections of *S. eucalypti* were also made on *E. delegatensis* at Mt Buffalo, Victoria. All diseased material was collected from juvenile and intermediate foliage of young re-growth along the road-side. These trees were directly adjacent to mature stands of *E. delegatensis.* A large proportion of the acervuli of the species found on *E. delegatensis* were associated with insect damage, other fungal species (*Dichomera eucalypti*, *Pachysacca samuelii*, *Teratosphaeria cryptica*), or both. Study of the type confirmed that there was no distinct apical appendage and the apical cell gradually tapered to an acute apex (Fig. 19a). A number of specimens collected in the present study had significantly longer or wider conidia than those previously described (Table 7, Fig. 15, 18). Relative conidial cell lengths vary between specimens, increasing from base to apex in some, while others have median cells of the same or very similar length; in some specimens the second cell from the apex was shorter than the third cell from the apex (Table 7).

Pigmentation of spores varied considerably between specimens (Fig. 15, 16, 19). One specimen collected in the present study, MB 0.14b, had conidia with roughened walls (Fig. 15a). This is the only recorded specimen of *S. eucalypti* with verruculose spores and the only collection known from *E. pauciflora.* Another variable feature was the number of septa, with some specimens having 1 or 2 additional septa either in the median cells or in the apical cell or in both, resulting in up to 5 septa. This feature was relatively rare, and was also seen in a number of the other collections of this species made in the present study (Fig. 15c, 16c, f).

As mentioned previously, this species is most similar to *S. cylindrosporum*, *S. falcatum* and *S. verrucisporum* in all having pigmented spores. *Seimatosporium eucalypti* differs from *S. cylindrosporum* in the features outlined above. The major differences between *S. eucalypti* and *S. falcatum* include the overall length of the spores and the degree of verrucosity. Conidia of *S. eucalypti* are generally longer, although there does appear to be a degree of overlap between the two species (Table 8). The shorter and often wider conidia of *S. falcatum* have a length

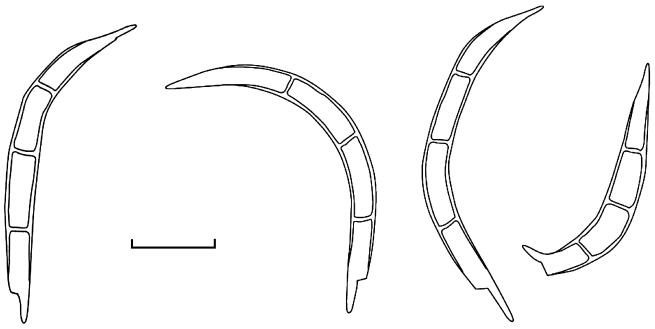

Fig. 18 Conidia of NZFRI (M) 3740 described as *S. eucalypti.* — Scale bar = 10 µm.

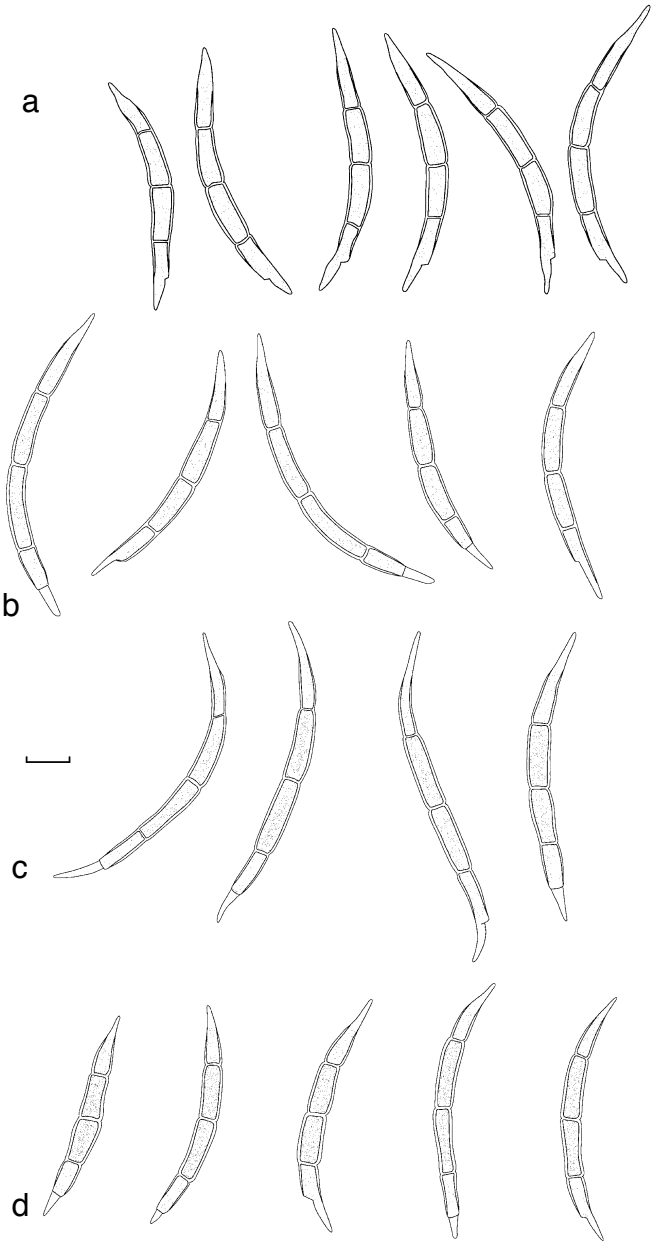

Fig. 19 Conidia of *Seimatosporium eucalypti.* a. VPRI 5927a Type specimen; b. VPRI 30218; c. NZFRI (M) 3867; d. PAB 02.35. — Scale bar = 10 µm.

to width ratio less than 10 : 1 compared to a ratio greater than 11 : 1 for *S. eucalypti* (Table 8).

Spores of *S. eucalypti* rarely show any degree of roughening of the walls (Table 8), whereas those of *S. falcatum* are commonly rough-walled (Table 8), although they are known sometimes to be smooth as described by Swart (1982) and Nag Raj (1993). There appears to be considerable overlap between the two species when considering the relative lengths of the conidial cells of the two species (Table 8).

We can tentatively suggest from findings in the present study that conidia of *S. eucalypti* are generally smooth-walled with a length to width ratio greater than 11 : 1, while conidia of *S. falcatum* are either smooth or rough-walled with a length to width ratio less than 10 : 1. All other characteristics, including relative conidial cell lengths, overlap between the two species. The ITS sequences of these two species, however, are clearly distinct (Identities = 673/715 (94 %), Gaps = 30/715 (4 %)).

Examination of herbarium specimen NZFRI 3740 from *E. saligna* collected in the North Island of New Zealand revealed that the fungus found did not accurately fit the description of *S. eucalypti* or any other *Seimatosporium* species described to

Table 7 Conidial measurements of *Seimatosporium eucalypti* as recorded by various authors and in the present study (in **bold** type); # indicates that the specimen does not accurately match the description of *S. eucalypti*.

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of apical appendage (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
McAlpine 1903	50–56	3.5–4	–	–	–	–
Swart 1982 (type)	33–53	3.5–5	–	3–8	–	–
Crous et al. 1990	32–55	2.5–5.5	–	2–7	–	–
Nag Raj 1993 (incl. type)	24–62	3.5–4.5	–	3–9	13 : 1	1.0 : 1.39 : 1.39 : 1.83
Gadgil & Dick 1999b (description from NZFRI 3867 and 3740)	55–62	2–4	–	5.5–7.5	–	–
Yuan 1999	25–60	4–5.2	–	2.5–9.5	11 : 1	1.0 : 1.29 : 1.32 : 1.66
VPRI 30218	48–64	4–4.5	up to 13	4–12	13.4 : 1	1.0 : 1.44 : 1.55 : 1.87
VPRI 5927a type	46–57	4–5	–	4–8	11.1 : 1	1.0 : 1.23 : 1.32 : 1.86
MB 0.02 (in vivo)	57–82	4–5.4	up to 20	8–17	14.9 : 1	1.0 : 1.78 : 1.84 : 2.68
MB 0.02 (in vitro)	61–82	4–5	up to 23	11–23	17.0 : 1	1.0 : 1.92 : 1.95 : 3.22
MB 0.29	56–74	3.5–5	up to 12	6–11	15.4 : 1	1.0 : 1.73 : 1.74 : 2.14
MB 0.33	41–76	4–5	up to 22	7–21	14.8 : 1	1.0 : 1.61 : 1.73 : 2.38
MB 0.35	51–66	4.5–6	up to 12	6–12	11.3 : 1	1.0 : 1.51 : 1.51 : 2.09
MB 0.38	53–74	4–5	up to 15	8–16	13.9 : 1	1.0 : 1.78 : 1.85 : 2.78
PAB 02.35	35–57	3.5–5	up to 8	3–8	13.9 : 1	1.0 : 1.42 : 1.42 : 1.58
MB 0.14b	54–68	4–6	up to 13	8–15	12.8 : 1	1.0 : 1.87 : 1.81 : 2.38
NZFRI 3867	51–64	4.5–5	up to 9	3–9	12.6 : 1	1.0 : 1.66 : 1.59 : 2.01
NZFRI 3740 #	29–43	3–4	–	2–6	11.3 : 1	1.0 : 0.94 : 1.0 : 1.49

Table 8 Comparison of conidial characteristics between *Seimatosporium eucalypti* (top portion of table) and *S. falcatum* (bottom portion of table) as recorded by various authors and in the present study, (# not stated whether apical appendage included in description, + smooth walls, ++ minutely verruculose walls, +++ verruculose walls).

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of apical appendage (µm)	Length of basal appendage (µm)	Roughness	Length : width ratio	Relative cell lengths (base → apex)
S. eucalypti							
McAlpine 1903	50–56	3.5–4	–	–		–	–
Swart 1982 (type)	33–53	3.5–5	–	3–8		–	–
Crous et al. 1990	32–55	2.5–5.5	–	2–7		–	–
Nag Raj 1993 (incl. type)	24–62	3.5–4.5	–	3–9		13 : 1	1.0 : 1.39 : 1.39 : 1.83
Gadgil & Dick 1999b (description from NZFRI 3867 and 3740)	55–62	2–4	–	5.5–7.5		–	–
Yuan 1999	25–60	4–5.2	–	2.5–9.5		11 : 1	1.0 : 1.29 : 1.32 : 1.66
VPRI 30218	48–64	4–4.5	up to 13	4–12	+	13.4 : 1	1.0 : 1.44 : 1.55 : 1.87
VPRI 5927a type	46–57	4–5	–	4–8	+	11.1 : 1	1.0 : 1.23 : 1.32 : 1.86
MB 0.02 (in vivo)	57–82	4–5.4	up to 20	8–17	+	14.9 : 1	1.0 : 1.78 : 1.84 : 2.68
MB 0.02 (in vitro)	61–82	4–5	up to 23	11–23	+	17.0 : 1	1.0 : 1.92 : 1.95 : 3.22
MB 0.29	56–74	3.5–5	up to 12	6–11	+	15.4 : 1	1.0 : 1.73 : 1.74 : 2.14
MB 0.33	41–76	4–5	up to 22	7–21	+	14.8 : 1	1.0 : 1.61 : 1.73 : 2.38
MB 0.35	51–66	4.5–6	up to 12	6–12	+	11.3 : 1	1.0 : 1.51 : 1.51 : 2.09
MB 0.38	53–74	4–5	up to 15	8–16	+	13.9 : 1	1.0 : 1.78 : 1.85 : 2.78
PAB 02.35	35–57	3.5–5	up to 8	3–8	+	13.9 : 1	1.0 : 1.42 : 1.42 : 1.58
MB 0.14b	54–68	4–6	up to 13	8–15	++	12.8 : 1	1.0 : 1.87 : 1.81 : 2.38
NZFRI 3867	51–64	4.5–5	up to 9	3–9	+	12.6 : 1	1.0 : 1.66 : 1.59 : 2.01
S. falcatum							
Sutton 1963 type	28–37.5 #	4–5	6–7	6.5–10		–	–
Swart 1982 (HJS 75.07)	–	4.5–5.5	8–22	7–20		–	–
HJS 75.07	38–52	4.5–5.5	8–17	7–16	++	8.7 : 1	1.0 : 1.48 : 1.45 : 3.21
Dick 1990 (description from NZFRI 3153, 3158 and 3209)	23–34 #	6–8	7–12	7–12		–	–
Nag Raj 1993	24–60	4–6	–	2–17		7.3 : 1	1.0 : 1.25 : 1.25 : 1.75
PAB 01.03	35–54	4–5.5	6–13	6–13	++	9.1 : 1	1.0 : 1.33 : 1.28 : 2.04
NZFRI 3153	34–50	5–6	7–14	4–13	+++	7.8 : 1	1.0 : 1.39 : 1.31 : 2.45
NZFRI 3209	31–46	4.5–6	6–11	3–9	++	7.4 : 1	1.0 : 1.34 : 1.25 : 2.10
NZFRI 3158	34–59	4–5	9–25	4–17	+++	9.8 : 1	1.0 : 1.37 : 1.24 : 2.96

date. This specimen was referred to by Gadgil & Dick (1999) as *S. eucalypti*, although examination of the specimen in the present study found the characteristics of spores such as length, width and cell ratios did not fall within the boundaries of their description of the species (Table 8). A number of acervuli from various lesions were sampled. Even though spores of this specimen roughly resemble the shape of those seen in some other isolates of *S. eucalypti*, they lack pigmentation, are short (29–43 µm), and have median cells roughly equal in length to the basal cell (Table 8, Fig. 18). Therefore, we conclude that this fungus is not *S. eucalypti* and may actually be a new species. It is possible that we failed to find the fungus used in the description by Gadgil & Dick (1999) on the specimen examined.

Seimatosporium falcatum (B. Sutton) Shoemaker, Canad. J. Bot. 42: 416. 1964 — Fig. 20–22

Basionym. Cryptostictis falcata B. Sutton, Mycol. Pap. 88: 25. 1963.

≡ *Vermisporium falcatum* (B. Sutton) Nag Raj, in Nag Raj, Coelomycetous anamorphs with appendage-bearing conidia: 969. 1993.

Leaf spots small (2 mm diam), amphigenous, more or less circular but occasionally irregular, separate, neither marginal nor terminal, ash-grey with a thin dark brown, raised margin (Fig. 20). *Conidiomata* stromatic, acervular, amphigenous, scattered to gregarious or roughly concentric in orientation, subepidermal, oval to irregular to rounded or irregular in outline, 180–360 µm wide and 90–180 µm deep, glabrous, pale brown to dark brown or black. *Stroma* 10–15 µm thick, of *textura angularis*, cells hyaline to almost hyaline to pale brown. *Conidiophores* arising from the upper layer of the stroma, reduced to conidiogenous cells. *Conidiogenous* cells cylindrical to subcylindrical to lageniform, hyaline, aseptate or septate and occasionally branched, annellidic, up to 25 µm long. *Conidia* falcate, fusiform or sigmoid, 3(–4)-septate, rarely 5-septate, distinct, slightly to strongly constricted at the septa, guttulate or not guttulate, median cells brown to pale brown, apical and basal cells pale brown to almost hyaline to hyaline, periclinal wall verruculose or minutely verruculose, slightly thicker in the median cells, pale brown to dark brown in mass, (31–)34–51(–59) × 4–6 (av. = 42.4 × 5.0) µm; apical cell subcylindrical to narrowly conic, pale brown to almost hyaline, upper half hyaline, attenuated into a discernible conical or tubular appendage up to 25 µm, total length including the appendage, (9–)11–24(–31) (av. = 16.7) µm long; second cell from apex cylindrical to subcylindrical, brown to pale brown, (6–)7–12(–13) (av. = 9.0) µm long; third cell from apex cylindrical to subcylindrical, brown to pale brown, (5–)7–12(–13) (av. = 9.6) µm long; basal cell obconic with a truncate base and basal appendage, pale brown to almost hyaline, lower half hyaline, 5–9(–10) (av. = 7.1) µm long; basal appendage single, excentric, plectronoid to tubular and flexuous, attenuated to a point, (3–)4–14(–17) (av. = 8.5) µm long; mean conidium length to width ratio = 8.5 : 1. Relative cell lengths from base to apex = 1.0 : 1.35 : 1.27 : 2.35. *Microconidia* not seen.

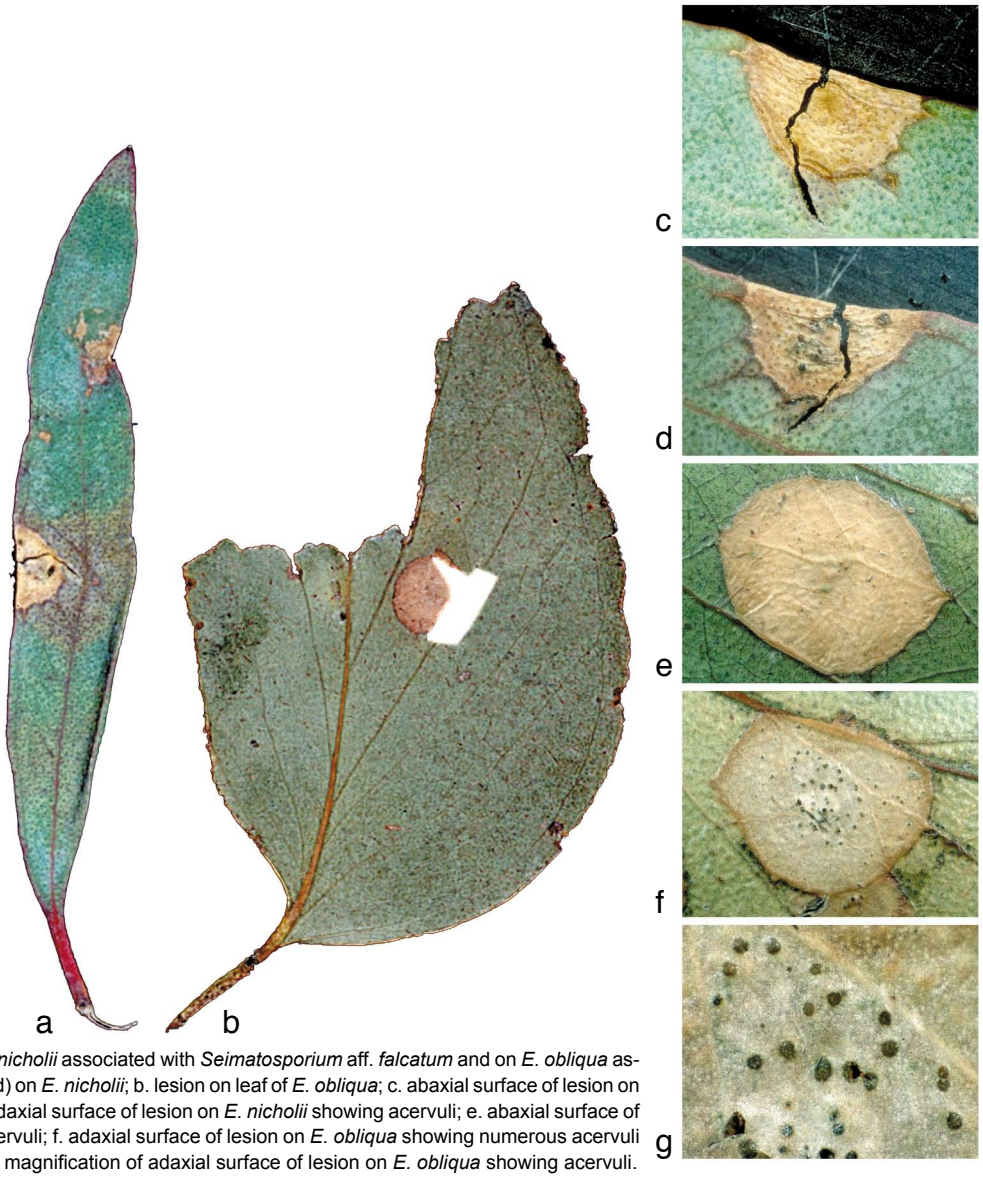

Fig. 20 Disease symptoms on *Eucalyptus nicholii* associated with *Seimatosporium* aff. *falcatum* and on *E. obliqua* associated with *S. falcatum*. a. Lesion (arrowed) on *E. nicholii*; b. lesion on leaf of *E. obliqua*; c. abaxial surface of lesion on *E. nicholii* showing absence of acervuli; d. adaxial surface of lesion on *E. nicholii* showing acervuli; e. abaxial surface of lesion on *E. obliqua* showing absence of acervuli; f. adaxial surface of lesion on *E. obliqua* showing numerous acervuli in a somewhat concentric pattern; g. higher magnification of adaxial surface of lesion on *E. obliqua* showing acervuli.

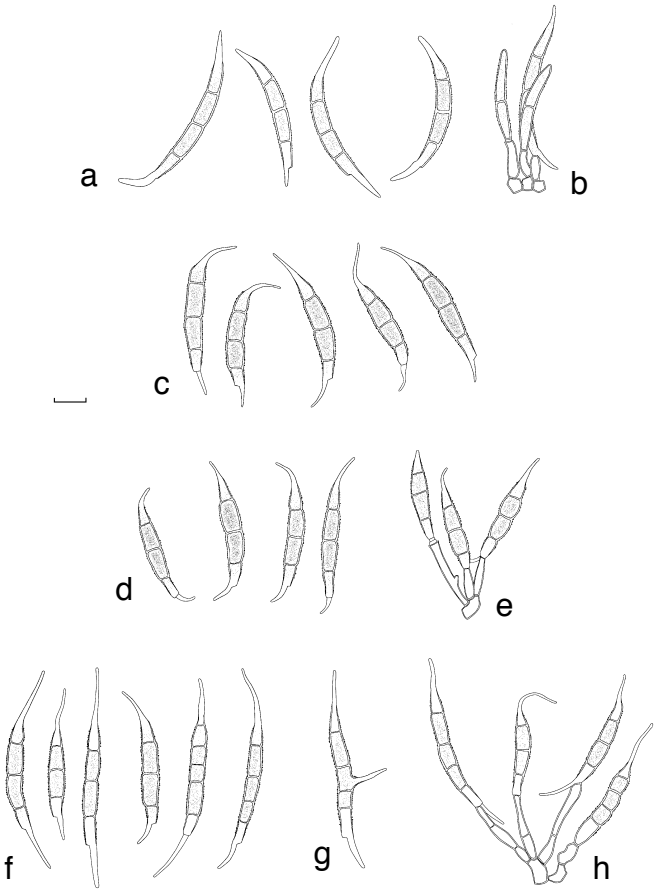

Fig. 21 *Seimatosporium falcatum*. a. Conidia in PAB 01.03; b. conidiogenous cells and conidia in PAB.03; c. conidia in NZFRI 3153; d. conidia in NZFRI 3209; e. conidiogenous cells and conidia in NZFRI 3209; f. conidia in NZFRI 3158; g. germinating conidia in NZFRI 3158; h. conidiogenous cells and conidia in NZFRI 3158. — Scale bar = 10 µm.

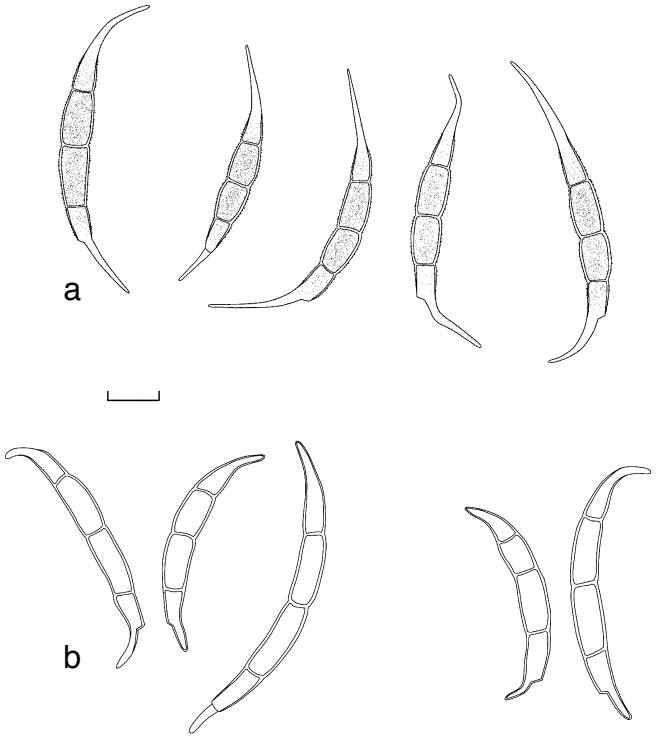

Fig. 22 Conidia. a. *Seimatosporium falcatum* (c75.07); b. *Seimatosporium* aff. *falcatum* (PAB 02.51 – VPRI 30233a). — Scale bar = 10 µm.

Culture characteristics — Colonies erumpent, spreading, with sparse aerial mycelium and smooth, lobate margins, reaching 55 mm diam after 2 wk. Colonies salmon on MEA, OA and PDA.

Previously known hosts — *Eucalyptus* sp. (Sutton 1963), *E. crebra*, *E. delegatensis*, *E. dives*, *E. radiata* (Swart 1982), *E. delegatensis*, *E. regnans* (Dick 1990), *E. parviflora* (type as *Eucalyptus* sp. by Sutton 1963) (Nag Raj 1993), *E. nitens*, *E. obliqua* (Yuan 1999).

Recorded host in this study — *E. obliqua* (PAB 01.03), *E. alligatrix* (CBS H-20744).

Specimens examined. Australia, Victoria, Kinglake West, on *E. obliqua*, 7 Nov. 2001, *P.A. Barber* (PAB 01.03); Victoria, Hoddles Creek, 'Andrews Farm', on *E. nicholii*, 7 July 2002, *P.A. Barber* (VPRI 30233a); Victoria, on *E. radiata*, 1975, (MELU 2002-4-1 (HJS 75.07)); NSW, Central Tablelands, c. 200 m WSW of 'Coomber' homestead, on Coomber property, c. 8 km SW of Rylstone, S32°50'04" E149°56'13", alt. 600 ± 10 m, 17 Aug. 2006, *R. Johnstone* & *A.E. Orme*, NSW 734259, on *E. alligatrix*, (CBS H-20744), culture CPC 13578 = CBS 131117, CPC 13579, CPC 13580. – New Zealand, Westland, Hochstetter Forest, on *E. delegatensis*, 18 Dec. 1981, *A. Holloway* (NZFRI-M 3158); Westland, Mawhero Forest, on *E. delegatensis*, 1 Jan. 1985, *A. Holloway* (NZFRI-M 3209); Tokoroa, Kinleith Forest, on *E. regnans*, Sept. 1982 (NZFRI-M 3153).

Notes — In the present study, a fungus we called *Seimatosporium* aff. *falcatum* was found associated with a lesion on *E. nicholii*, collected from an ornamental foliage farm at Hoddles Creek, Victoria. Also, a fungus bearing a much tighter affinity to *S. falcatum* was collected from *E. obliqua.* Conidia of all specimens examined within the present study, fell within the range of lengths given by previous authors (Table 9). The width of the conidia measured here varied between specimens but was generally between 4 and 6 µm, as described by Nag Raj (1993). We can conclude from examination of specimens in the present study and conidial dimensions published by previous authors that the length of apical appendages is very variable both within and between specimens and ranges from 6–25 µm in length. Conidia from most collections examined here had either minutely verruculose or verruculose walls (Table 9, Fig. 21, 22). The only exception was specimen PAB 02.51 (= VPRI 30233a) with smooth walls. However, this isolate does not accurately match the description of *S. falcatum* as outlined below. The single collection that accurately matched the description of *S. falcatum* had conidia with minutely verruculose walls (PAB 01.03) (Fig. 21a). Therefore, from our findings and those of other researchers, we conclude that this characteristic is variable both within and between specimens and conidia can range from smooth walled through to verruculose.

The type specimen described by Sutton (1963) had pale brown to olivaceous median cells and hyaline end cells. It is evident from the present study that all specimens with the exception of PAB 02.51 (= VPRI 30233a) were pigmented to some degree (Fig. 21, 22) with the median cells being more darkly pigmented than the end cells; the conidia vary from pale brown, to almost hyaline becoming hyaline at the extremities including the appendages. Collections NZFRI 3153, NZFRI 3209 and HJS 75.07 were more pigmented than the other isolates examined here (Fig. 21c, d, 22a, respectively). It was also evident that pigmentation varied not only between but within collections (Fig. 21, 22).

The length to width ratio of conidia varied between collections but all had a ratio of less than 10 : 1 (Table 9). Examination of specimens in this study found the median cells to be roughly equal in length with a tendency for the second cell from the apex to be shorter than the third cell from the apex (Table 9). The apical cell was significantly longer than all other cells in all specimens examined in this study with the exception of PAB 02.51 (= VPRI 30233a). The apical cell and two median cells were equal in length in PAB 02.51 (= VPRI 30233a). This

Table 9 Conidial measurements of *Seimatosporium falcatum* as recorded by various authors and in the present study (in **bold** type); * not distinguishable, † does not accurately fit the description of *S. falcatum*, †† does not accurately fit the description of *S. falcatum* according to Yuan (1999), [a] as measured by present author, [n] not stated whether apical appendage included in dimensions,+ smooth, ++ minutely verruculose, +++ verruculose).

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of apical appendage (µm)	Length of basal appendage (µm)	Roughness	Length : width ratio	Relative cell lengths (base → apex)
Sutton 1963 type	28–37.5[n]	4–5	6–7	6.5–10		–	–
Swart 1982 (HJS 75.07)	–	4.5–5.5	8–22	7–20		–	–
HJS 75.07[a]	38–52	4.5–5.5	8–17	7–16	++	8.7 : 1	1.0 : 1.48 : 1.45 : 3.21
Dick 1990 (description from NZFRI 3153, 3158 and 3209)	23–34[n]	6–8	7–12	7–12		–	–
Nag Raj 1993	24–60	4–6	–	2–17		7.3 : 1	1.0 : 1.25 : 1.25 : 1.75
Yuan 1999 ††	27–50	4.5–5.5	–	5–14		8.0 : 1	1.0 : 1.37 : 1.42 : 2.64
PAB 01.03	35–54	4–5.5	6–13	6–13	++	9.1 : 1	1.0 : 1.33 : 1.28 : 2.04
PAB 02.51 †	30–56	4.5–6	*	3–9	+	9.1 : 1	1.0 : 1.55 : 1.51 : 1.51
NZFRI 3153	34–50	5–6	7–14	4–13	+++	7.8 : 1	1.0 : 1.39 : 1.31 : 2.45
NZFRI 3209	31–46	4.5–6	6–11	3–9	++	7.4 : 1	1.0 : 1.34 : 1.25 : 2.10
NZFRI 3158	34–59	4–5	9–25	4–17	+++	9.8 : 1	1.0 : 1.37 : 1.24 : 2.96

contrasts with all other collections of *S. falcatum* and other species of *Seimatosporium.* In addition, this specimen differs from the collections of *S. falcatum* examined in the present study by lacking rough walls and, in lacking pigmented conidial cells walls, it is not consistent with previous descriptions of *S. falcatum* that refer to the fungus as having pigmented conidia. This is the only *Seimatosporium* species to be isolated from *E. nicholii* and there is evidence that it may be a new species.

Another variable feature was the number of septa, with collection NZFRI 3158 having 1 or 2 additional septa either in the median cells or in the apical cell or in both, resulting in up to 5 septa (Fig. 21f–h). This feature was relatively rare, and was also seen in a number of collections of other species made in the present study as outlined above (Fig. 15c, 16c, f).

Seimatosporium obtusum (H.J. Swart & M.A. Will.) Barber & Crous, *comb. nov*. — MycoBank MB560633; Fig. 23–25

Basionym. Vermisporium obtusum H.J. Swart & M.A. Will., Trans. Brit. Mycol. Soc. 81: 499. 1983.

Leaf spots roughly circular, 7–16 mm diam on *E. obliqua*, 3–21 mm on *E. regnans*, distinct, grey in the centre and brown towards the outer edge with a distinct brown margin up to 1 mm diam, bearing brown, amphigenous but predominantly epiphyllous acervuli either scattered, or in a circular arrangement within the lesion (Fig. 23, 24). Acervuli were also evident on tissue that appeared to be infected by the fungus but was yet to become necrotic. These infected areas were somewhat circular

Fig. 23 Disease symptoms on *Eucalyptus regnans* associated with *Seimatosporium obtusum.* a. Small, necrotic lesions (arrowed i) and larger, necrotic blights (arrowed ii) on leaf lamina; b. adaxial surface of a small lesion showing acervuli in the centre; c. abaxial surface of a small lesion showing scattered acervuli; d. adaxial surface of a large blight showing acervuli in a somewhat circular arrangement; e. abaxial surface of a large blight showing few, scattered acervuli; f. higher magnification of adaxial surface of lesion showing acervuli.

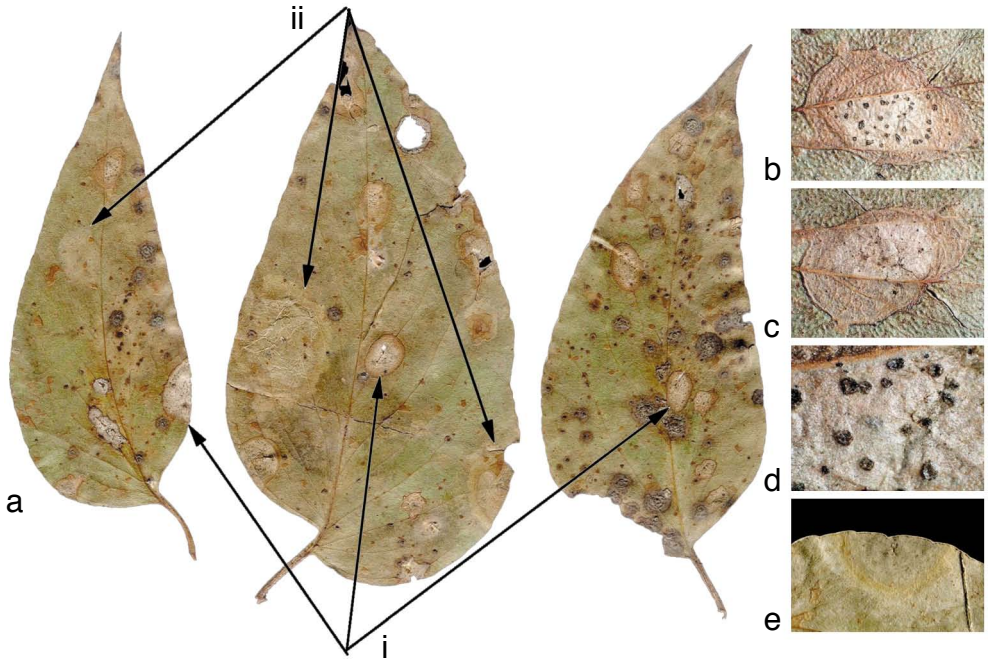

Fig. 24 Disease symptoms on *Eucalyptus obliqua* associated with *Seimatosporium obtusum*. a. Small, necrotic lesions (arrowed i) and larger, developing lesions (arrowed ii) on green tissue of leaf lamina; b. adaxial surface of a small lesion showing acervuli in the centre; c. abaxial surface of a small lesion showing lack of acervuli; d. adaxial surface of a lesion showing dark brown acervuli; e. adaxial surface of a developing lesion showing acervuli in the centre of the lesion.

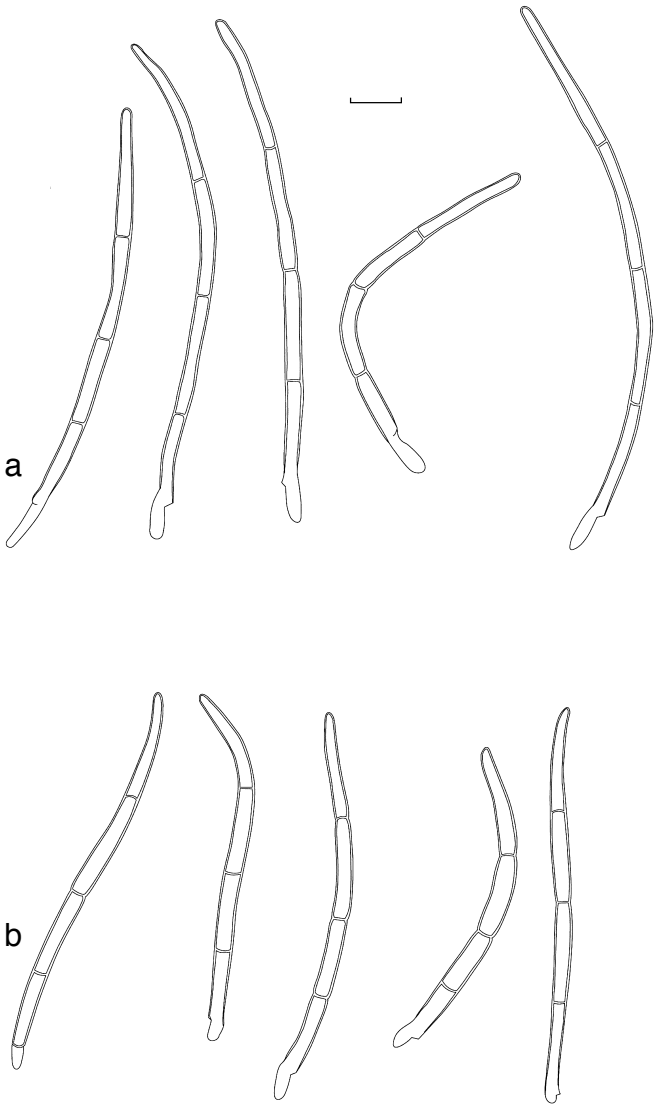

Fig. 25 Conidia of *Seimatosporium obtusum*. a. PAB 99.13; b. PAB 02.31. — Scale bar = 10 µm.

and extending up to 32 mm diam. Insect emergent holes were present in some of the smaller, necrotic lesions. *Conidiomata* stromatic, acervular, amphigenous, scattered to gregarious or roughly concentric in orientation, subepidermal, immersed to erumpent, oval, rounded or irregular in outline, 150–400 µm wide and 100–250 µm deep, glabrous, orange becoming brown when old. *Stroma* 15–25 µm thick, of *textura angularis*, cells hyaline to pale brown. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lageniform, hyaline, 10–15 µm long. *Conidia* ellipsoid-fusiform to subcylindrical, straight, slightly curved or slightly sigmoid, 3(–4)-septate, hyaline, smooth-walled, hyaline in mass, not or slightly constricted at the septa, (49–)54–92(–103) × (2.5–)3–4(–4.5) (av. = 70.6 × 3.3) µm; apical cell subcylindrical, slightly attenuated to an obtuse tip, (14–)20–31 (av. = 21.8) µm; second cell from apex cylindrical, 13–25(–27) (av. = 17.6) µm; third cell from apex cylindrical, (10–)12–21(–25) (av. = 16.3) µm; basal cell subcylindrical with a narrow, truncate base, (8–)11–20(–24) (av. = 15.0) µm; basal appendage tubular, single, excentric, cuneiform to podiform, often somewhat swollen in the middle, 2–9(–11) (av. = 5.1) µm long; mean conidium length to width ratio = 21.4 : 1. Relative cell lengths from base to apex = 1.0 : 1.09 : 1.18 : 1.47.

Culture characteristics — Colonies erumpent, spreading, with moderate aerial mycelium and even, lobate margins, reaching 60 mm diam after 2 wk. Colonies dirty white on surface, salmon in reverse on MEA, OA and PDA.

Previously known hosts — *E. baxteri*, *E. delegatensis*, *E. macrorhyncha*, *E. obliqua*, *E. pauciflora*, *E. radiata*, *E. regnans* (Swart & Williamson 1983), *E. delegatensis*, *E. fraxinoides*, *E. regnans* (Dick 1990).

Recorded hosts in this study — *Corymbia henryi* (CBS H-20745), *E. obliqua* (PAB 02.31), *E. regnans* (PAB 99.13).

Specimens examined. Australia, Victoria, Toolangi, on *E. regnans*, 16 July 1999, *P.A. Barber* (PAB 99.13); Victoria, Gellibrand, Otways State Forest, on *E. obliqua*, 1 May 2002, *P.A. Barber* (PAB 02.31); New South Wales, Australian Botanic Garden, Mt Annan, on *Corymbia henryi*, 3 Mar. 2006, *B. Summerell* (CBS H-20745), culture CPC 12935 = CBS 131118, CPC 12936.

Notes — In the present study, *S. obtusum* was associated with lesions on a sapling of *E. regnans* in native forest at Tool-

Table 10 Conidial measurements of *Seimatosporium obtusum* as recorded by various authors and in the present study (in **bold** type).

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
Swart & Williamson 1983 (incl. type)	50–80	2–3.5	2–10	–	–
Dick 1990	45–80	3–6	3–5	–	–
Nag Raj 1993 (incl. type)	52–81	2–4.5	2–10	19 : 1	1.0 : 1.07 : 1.18 : 1.71
Yuan 1999	55–75	2.5–3.5	3–8	19.6 : 1	1.0 : 1.08 : 1.25 : 1.59
Elton 2002	45–75	2.5–4	2–7	–	–
PAB 99.13	67–103	2.5–3.5	4–11	27.2 : 1	1.0 : 1.08 : 1.11 : 1.37
PAB 02.31	49–78	3–4.5	2–6	18.5 : 1	1.0 : 1.10 : 1.21 : 1.52

angi, Victoria and a sapling of *E. obliqua* in native forest adjacent to Wait-a-While Rd in the Otway State Forest. The findings in the present study and by previous authors indicate conidial dimensions are quite variable within the species. Specimen PAB 99.13, collected in the present study, contained conidia up to 22 µm longer than any other specimen previously described (Table 10). Host effect can not explain this difference as *S. obtusum* has been described from *E. regnans* previously by other authors (Swart & Williamson 1983, Dick 1990, Elton 2002). Although this is a large difference in length, other characters such as the obtuse tip of the apical cell (Fig. 25a), the blunt basal appendage (Fig. 25a) and the relative cell ratio (Table 10) indicate that this isolate is *S. obtusum* and not another species. It is notable though that this increase in overall length is associated with slightly smaller relative cell lengths (Table 10). Swart & Williamson (1983) have described such variation in the relative cell lengths of *S. obtusum*, especially when grown in culture.

Previous authors (Swart & Williamson 1983, Nag Raj 1993, Yuan 1999) have described this species as being most similar to *S. walkeri.* The findings from this study support those of Swart & Williamson (1983), who stated that *S. obtusum* differs from *S. walkeri* in not only having a shorter basal cell, but also an apical cell that at most is one and a half times the length of the second cell, whereas in *S. walkeri* it is at least twice as long. Nag Raj (1993), in his key to *Seimatosporium* species, has an incorrect basis for differentiating between *S. walkeri* and *S. obtusum*, stating that *S. walkeri* has "two central cells of the conidium equal in length but longer than the basal cell and shorter than the apical cell". This contrasts with his description of *S. obtusum* as having "conidial cells from the base up progressively elongated". These two species are clearly distinct based on their respective ITS sequences (Identities = 413/431 (96 %), Gaps = 4/431 (1 %)). Nag Raj's drawing of *S. obtusum* on p. 971 (f. 132.7) clearly shows conidia with basal cells longer than the two median cells and shorter than the apical cell. This drawing is a better representation of *S. walkeri*, a species that will be discussed later in more detail.

Seimatosporium orbiculare (Cooke) Barber & Crous, *comb. nov*. — MycoBank MB560634; Fig. 26

Basionym. *Stagonospora orbicularis* Cooke, Grevillea 20: 6. 1891.

≡ *Vermisporium orbiculare* (Cooke) H.J. Swart & M.A. Will., Trans. Brit. Mycol. Soc. 81: 497. 1983.

Leaf spots small, orbicular, pallid, to pallid grey or greyish brown in the centre (5–10 mm diam), amphigenous, delineated by a brown margin. *Conidiomata* stromatic, acervular, predominantly epiphyllous, scattered to gregarious, subepidermal, immersed to erumpent, oval or rounded in outline, 180–250 µm wide and 80–150 µm deep, brown. *Stroma* 15–25 µm thick, of *textura angularis*, hyaline to pale brown. *Conidiophores* arising from the upper layer of the stroma, branched, hyaline, septate, 5–10 µm long. *Conidiogenous cells* lageniform, hyaline, 8–17 µm long. *Conidia* cylindrical to acerose, curved or occasionally sigmoid, 3-septate, hyaline, constricted at the septa, (49–)53–66 × 4–4.5 (av. = 57.6 × 4.2) µm; apical cell tapering well above the middle to form a conical, acute apex, (15–)16–20(–23) (av. = 17.9) µm long; second cell from the apex cylindrical to subcylindrical, (11–)12–16(–18) (av. = 14.3) µm long; third cell from the apex cylindrical to subcylindrical, (10–)12–15(–18) (av. = 13.9) µm long; basal cell subcylindrical with a truncate base, 10–13(–14) (av. = 11.5) µm long; basal appendage excentric, single, cuneiform to podiform often with a slightly swollen median part, (3–)4–7(–8) (av. = 5.6) µm long; mean conidium length to width ratio = 13.7 : 1. Relative cell lengths from base to apex = 1 : 1.21 : 1.24 : 1.56. *Microconidia* not seen.

Previously known hosts — *Eucalyptus* sp. (Cooke 1891), *E. macrorhyncha*, *E. obliqua* (Swart & Williamson 1983).

Recorded hosts in this study — No records.

Specimen examined. Australia, Victoria, on *Eucalyptus* sp., 24 May 1886, *F.M.C.* (K (M) 104759 Type specimen).

Notes — Examination of the type specimen in the present study found conidial dimensions within the range described by Nag Raj (1993), but somewhat longer and thicker than those described by Swart & Williamson (1983) (Table 11). Swart & Williamson (1983) noted that different strains of *S. orbiculare* differed quite noticeably in their conidium measurements but all showed the relative lengths of conidial cells, with the apical cell being the longest and the second and third cells somewhat similar in length (with the second cell from the apex more often longer than the third cell from the apex) with the basal cell the shortest. In contrast, Nag Raj (1993) described *S. orbiculare* as having the second cell (from the base) shorter than basal cell, the third cell (from base) and fourth cell (apical cell) equal in length but longer than the other two (Table 11). Our examination of the type specimen confirms the relative cell length pattern

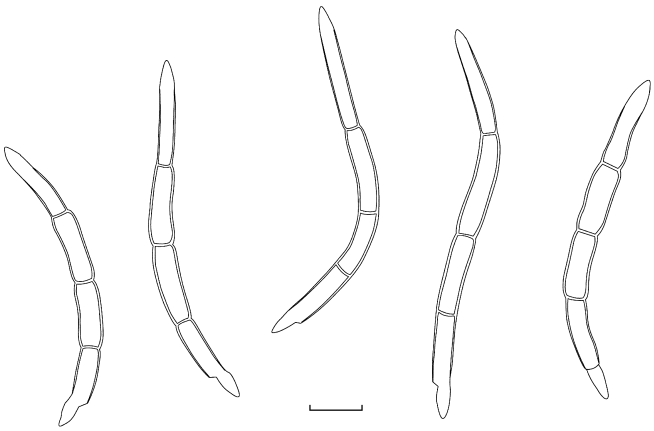

Fig. 26 Conidia of *Seimatosporium orbiculare* type specimen. — Scale bar = 10 µm.

Table 11 Conidial measurements of *Seimatosporium orbiculare* as recorded by various authors and in the present study.

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
Cooke 1891b	60–70	8	–	–	–
Swart & Williamson 1983 (incl. type)	53–59	3.5–4	4–6	–	–
Nag Raj 1993 (incl. type)	42–61	3–4.5	3–7	15 : 1	1.0 : 0.89 : 1.11 : 1.11
Present study (type)	49–66	4–4.5	3–8	13.7 : 1	1.0 : 1.21 : 1.24 : 1.56

described by Swart & Williamson (1983). Nag Raj's drawing of *S. obiculare* on p. 973 (f. 132.8) shows the relative cell lengths as he described them. It is interesting to note that this drawing is of a specimen (ADW 3743 ex holotype) not examined by Swart and Williamson. In addition to examining the type and this specimen, Nag Raj has examined an additional specimen (ADW 4583) from the same host and location as ADW 3743 when describing the species. It is possible that these two specimens have relative cell lengths markedly different from that of the type and when combined, have given the contrasting cell lengths. If this is the case then it is likely that those specimens are new taxa; examination of them is required to determine whether this is the case.

Our findings suggest that *S. orbiculare* is somewhat morphologically similar to *S. eucalypti.* Both species either have cells that increase in length from the base to the apex or have the two median cells roughly equal in length (Table 11). The most notable difference between these species is the lack of pigmentation in cells of *S. orbiculare* (Fig. 26).

Our findings suggest that *S. orbiculare* is most similar to *S. acutum* and *S. obtusum* in having i) median cells longer than the basal cell; and ii) cells that lack pigmentation. However, it appears that *S. orbiculare* differs from both these species in having median cells that are somewhat equal in length (Table 11) compared with both these species where the second cell from the base is usually substantially shorter than the third cell from the base. *Seimatosporium orbiculare* also differs from *S. obtusum* by having an apical cell and basal appendage with a slightly acute tip (Fig. 24, 26). Observations in the present study support the conclusion by Swart & Williamson (1983) that *S. orbiculare* and *S. acutum* differ in the way the apical cell tapers to a point (Fig. 3, 26). However, without the additional finding that the relative cell lengths differ, we would be inclined to believe that these two taxa are conspecific. Therefore, the relative cell lengths are an important characteristic differentiating these two species (Table 11).

Seimatosporium samuelii (Hansf.) J. Walker & H.J. Swart, Trans. Brit. Mycol. Soc. 90: 287. 1988 — Fig. 27

Basionym. *Cylindrosporium samuelii* Hansf., Proc. Linn. Soc. New South Wales 81: 46. 1956.

≡ *Vermisporium samuelii* (Hansf.) J.A. Simpson & Grgur., Muelleria 9: 239. 1996.

Leaf spots mainly epiphyllous, sometimes showing below as indefinite brown areas, brown, not secedent, up to 4 mm diam, rounded or irregular, surrounded by a dark brown, thin border. *Conidiomata* stromatic, acervular, epiphyllous, scattered to gregarious, subepidermal, immersed, oval or rounded in outline, up to 200 µm wide and 120 µm deep, brown. *Stroma* 10–20 µm thick, of *textura angularis*, pale brown. *Conidiophores* arising from the upper layer of the stroma, reduced to conidiogenous cells. *Conidiogenous cells* cylindrical to subcylindrical to lageniform, aseptate or septate, hyaline, up to 20 µm long. *Conidia* subcylindrical, curved or occasionally sigmoid, 3-septate, septa relatively inconspicuous, hyaline, constricted or somewhat constricted at the septa, guttulate, hyaline to almost hyaline, white to yellowish in mass, (66–)69–85(–90) × (4.5–)5–6.5 (av. = 76.4 × 5.5) µm; apical cell subcylindrical tapering to form a distinct knob-like appendage up to 5 µm, total length including the appendage, (19–)26–31(–33) (av. = 27.4) µm long; second cell from apex cylindrical to subcylindrical, (11–)12–20 (av. = 15.0) µm long; third cell from apex cylindrical to subcylindrical, 10–14(–16) (av. = 12.3) µm long; basal cell obconic with a truncate base and basal appendage, (18–)20–25(–28) (av. = 22.4) µm long; basal appendage single, excentric, cuneiform to podiform with an obtuse or somewhat truncate tip, 1–4 (av. = 2.9) µm long; mean conidium length to width ratio = 13.9 : 1. Relative cell lengths from base to apex = 1.0 : 0.55 : 0.67 : 1.22. *Microconidia* not seen.

Previously known host — *Eucalyptus* sp. (Hansford 1956).

Recorded hosts in this study — No records.

Specimen examined. Australia, South Australia, on *Eucalyptus* sp., Sept. 1924, *G. Samuel* (ADW 3840 Type specimen).

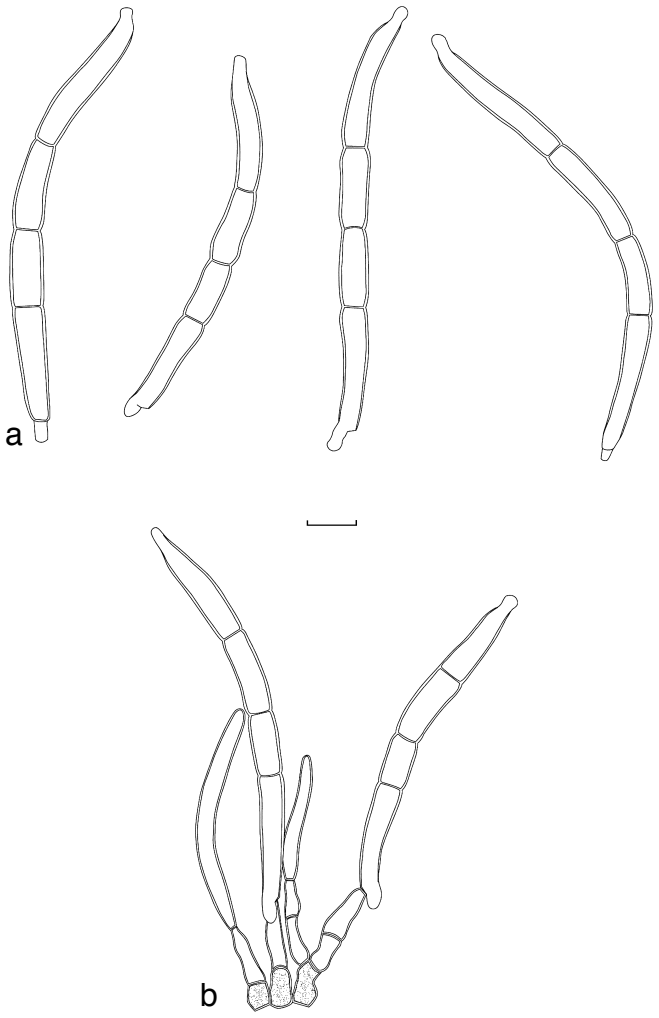

Fig. 27 *Seimatosporium samuelii* type specimen in ADW 3840. a. Conidia; b. conidia developing attached to conidiogenous cells. — Scale bar = 10 µm.

Table 12 Conidial measurements of *Seimatosporium samuelii* type specimen as recorded by Hansford (1956) and in the present study.

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of apical appendage (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
Hansford 1956	60–95	4–5	–	–	–	–
Present study	66–90	4.5–6.5	1–5	1–4	13.9 : 1	1.0 : 0.55 : 0.67 : 1.22

Table 13 Comparison of conidial characteristics between *Seimatosporium samuelii*, *S. brevicentrum* and *S. walkerii* as recorded by various authors and in the present study. Only those authors stating relative cell lengths of conidia have been included for the purpose of this table.

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of apical appendage (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
S. samuelii						
Present study	66–90	4.5–6.5	1–5	1–4	13.9 : 1	1.0 : 0.55 : 0.67 : 1.22
S. brevicentrum						
Nag Raj 1993 (incl. holotype)	45–68	2.5–3	–	1.5–3	20.7 : 1	1.0 : 0.30 : 0.40 : 1.18
PAB 99.11	43–69	2.5–3.5	–	1–3	19.2 : 1	1.0 : 0.36 : 0.38 : 1.10
GR 0.02	49–70	2–3	–	1–3.5	26.7 : 1	1.0 : 0.35 : 0.40 : 1.10
S. walkerii						
Nag Raj 1993 (part of type)	33–76	3–4	–	5–10	17.4 : 1	1.0 : 1.17 : 1.17 : 1.75
PAB 00.03	43–67	3–4	–	4–11	16.6 : 1	1.0 : 0.75 : 0.80 : 1.76
PAB 00.04	47–67	3–4	–	6–13	16.9 : 1	1.0 : 0.80 : 0.90 : 2.10
PAB 01.05	61–87	3–4	–	5–11	23.2 : 1	1.0 : 0.68 : 0.72 : 1.70
PAB 01.07	43–80	3–5	–	6–15	15.6 : 1	1.0 : 0.74 : 0.84 : 2.10
NZFRI 3756	48–82	3–4	–	5–13	17.2 : 1	1.0 : 0.75 : 0.76 : 1.79

Notes — Hansford (1956) originally described this fungus from foliage of a *Eucalyptus* sp. at Pinnaroo, South Australia. This is the only known collection of this fungus. No collections of this fungus were made in this study and hence, no new descriptions of disease symptoms are made here. Examination of the type in the present study found the conidial length of *S. samuelii* to be within the range described by Hansford (1956), but with a greater conidial width (Table 12). Hansford did not note the presence of an apical appendage, but noted that the base was "rounded or slightly pedicellate", indicating that a basal appendage may have been observed. We, like Swart (1988), observed a distinct knob-like appendage on the apical cell and a basal appendage (Fig. 27). These appendages were similar in length (Fig. 27, Table 12) and quite similar in appearance (Fig. 27) sometimes making it hard to distinguish between them. The median cells, like those of *S. brevicentrum* and *S. walkeri*, are much shorter than both the apical and basal cells, the apical cell being the longest (Table 12).

Swart (1988) described *S. samuelii* as being most similar to *S. cylindrosporum* and *S. walkeri.* It is easily distinguishable from *S. cylindrosporum* in lacking any real pigmentation (Fig. 27), having a very different looking apical appendage (Fig. 27) and having completely different relative cell lengths. Our findings suggest that it is most similar to *S. walkeri* and *S. brevicentrum*, all three species lacking pigmentation of the spores and having a similar cell ratio in the conidia (Table 13). However, *S. samuelii* is distinguished from *S. walkeri* in having a distinct knob-like apical appendage (Fig. 27) (*S. walkeri* lacks an apical appendage) and a short cuneiform to podiform basal appendage (Fig. 27). The basal appendage of *S. brevicentrum* is somewhat similar to that of *S. samuelii*, but it also lacks an apical appendage and the conidia are markedly narrower (Table 13).

Seimatosporium verrucisporum (Nag Raj) Barber & Crous, *comb. nov*. — MycoBank MB560635; Fig. 28

Basionym. Vermisporium verrucisporum Nag Raj, in Nag Raj, Coelomycetous anamorphs with appendage-bearing conidia: 972. 1993.

Leaf spots irregular, vein-limited, grey, becoming brown toward the outer edge, 3–20 mm and coalescing to form blights up to 50 mm in length, with a distinct brown, raised margin up to 1 mm thick, bearing dark brown to black, amphigenous acervuli; no insect association was evident. *Conidiomata* stromatic, acervular, amphigenous, scattered, intra-epidermal to subepidermal, immersed or erumpent, oval to irregular or rounded in outline, 200–430 µm wide and 70–140 µm deep, glabrous, black. *Stroma* 15–25 µm thick, of *textura angularis*, cells pale brown to brown. *Conidiophores* arising from the upper layer of the stroma, reduced to conidiogenous cells. *Conidiogenous cells* cylindrical to subcylindrical to lageniform, hyaline, annellidic, aseptate or septate and occasionally branched, annellidic, up to 20 µm long. *Conidia* falcate, fusiform or sigmoid, 3(–4)-septate, rarely 5-septate, distinct, slightly to strongly constricted at the septa, guttulate or not guttulate, median cells brown to pale brown, apical and basal cells pale brown to almost hyaline to hyaline, periclinal wall verruculose or minutely verruculose, slightly thicker in the median cells, pale brown to dark brown in mass, (20–)33–58(–63) × (3.5–)4–6(–6.5) (av. = 48.0 × 5.0) µm; apical cell subcylindrical to narrowly conic, pale brown to almost hyaline, upper half hyaline, attenuated into a discernible conical or tubular appendage up to 24 µm, total length including the appendage, (7–)11–24(–36) (av. = 18.7) µm long; second cell from apex cylindrical to subcylindrical, brown to pale brown, (4–)8–14(–15) (av. = 10.2) µm long; third cell from apex cylindrical to subcylindrical, brown to pale brown, (5–)7–14(–15) (av. = 10.5) µm long; basal cell obconic with a truncate base and basal appendage, pale brown to almost hyaline, lower half hyaline, (4–)5–9(–10) (av. = 7.1) µm long; basal appendage single, excentric, plectronoid to tubular and flexuous, attenuated to a point, (2–)4–15(–25) (av. = 11.3) µm long; mean conidium length to width ratio = 9.8 : 1. Relative cell lengths from base to apex = 1.0 : 1.48 : 1.43 : 2.93.

Previously known host — *E. regnans* (Nag Raj 1993).

Recorded host in this study — *E. delegatensis* (NZFRI 4047a, 4047b, 4315).

Table 14 Conidial measurements of *Seimatosporium verrucisporum* as described by Nag Raj (1993) and as determined in the present study.

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of apical appendage (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
Nag Raj 1993 (ADW 1784)	29–55	5–6.5	13–24	5–12	6.5 : 1	1.0 : 1.43 : 1.37 : 2.38
VPRI 1932b (isotype)	46–63	5–6	7–17	4–17	10.2 : 1	1.0 : 1.61 : 1.62 : 2.78
NZFRI 4047a	36–49	3.5–5	3–7	2–8	10.0 : 1	1.0 : 1.40 : 1.38 : 1.65
NZFRI 4047b	30–65	4–6	6–24	5–20	10.2 : 1	1.0 : 1.49 : 1.35 : 3.49
NZFRI 4315	36–63	4.5–6.5	9–22	8–25	8.6 : 1	1.0 : 1.40 : 1.38 : 3.80

Specimens examined. Australia, Victoria, on *Eucalyptus* sp., 1915, *C. French Jr.* (VPRI 1932b Isotype). – New Zealand, Taupo, Kaingaroa Forest: on *E. delegatensis*, 6 Oct. 2000, *R.F. Thum* (NZFRI-M 4047); Taupo, Kaingaroa Forest, on *E. delegatensis*, 6 Oct. 2000, *J.A. Bartram* (NZFRI-M 4315).

Notes — Nag Raj (1993) described this species associated with leaf spots on *E. regnans* collected from Miles Creek in Victoria, Australia. This is the only published record of this fungus and the only known account of it in Australia. Some specimens lodged at NZFRI as *V. verrucisporum* were also studied. Both specimens (NZFRI 4047 and 4315) were found on *E. delegatensis* and had similar symptoms.

Examination of the isotype (VPRI 1932b) showed dimensions to be noticeably different from those described previously for the type, with longer conidia, a significantly greater length to width ratio, and longer median cells of equal length (Table 14).

Nag Raj (1993) described spores of *S. verrucisporum* (from the holotype ADW 1784) as distinctly more verruculose and having noticeably darker median cells than conidia of *S. falcatum*. Our findings after examining the isotype of *S. verrucisporum* and comparing these conidia with those of a number of specimens

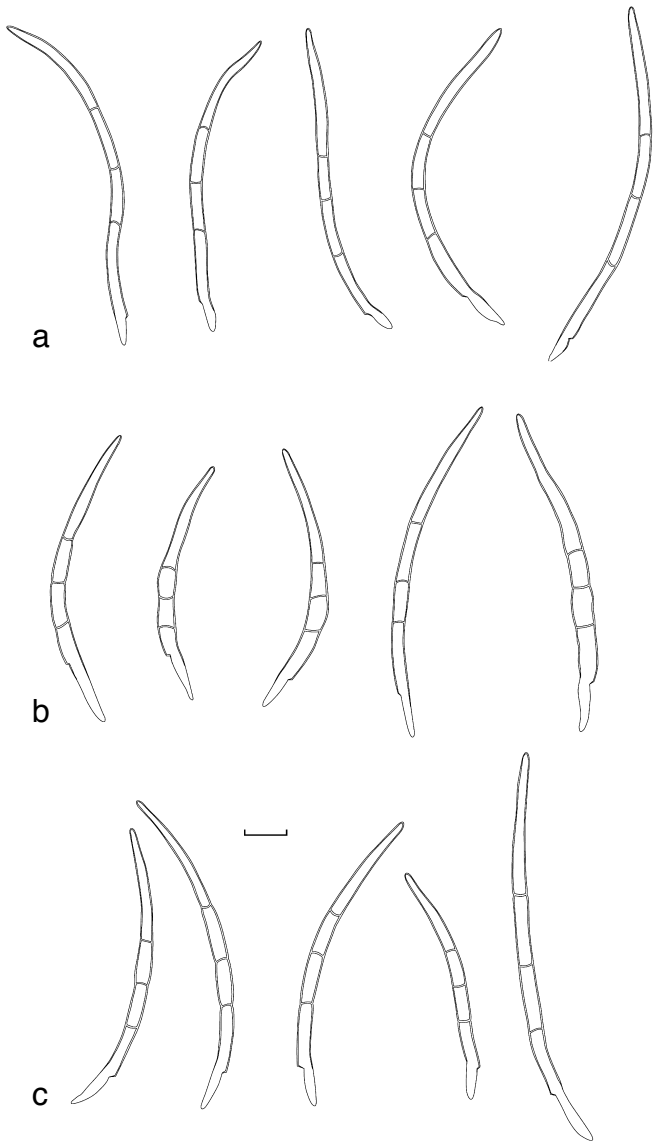

Fig. 28 Conidia of *Vermisporium walkeri*. a. PAB 01.05; b. PAB 01.07; c. NZFRI 3756 described as *V. brevicentrum* in Gadgil & Dick (1999). — Scale bar = 10 µm.

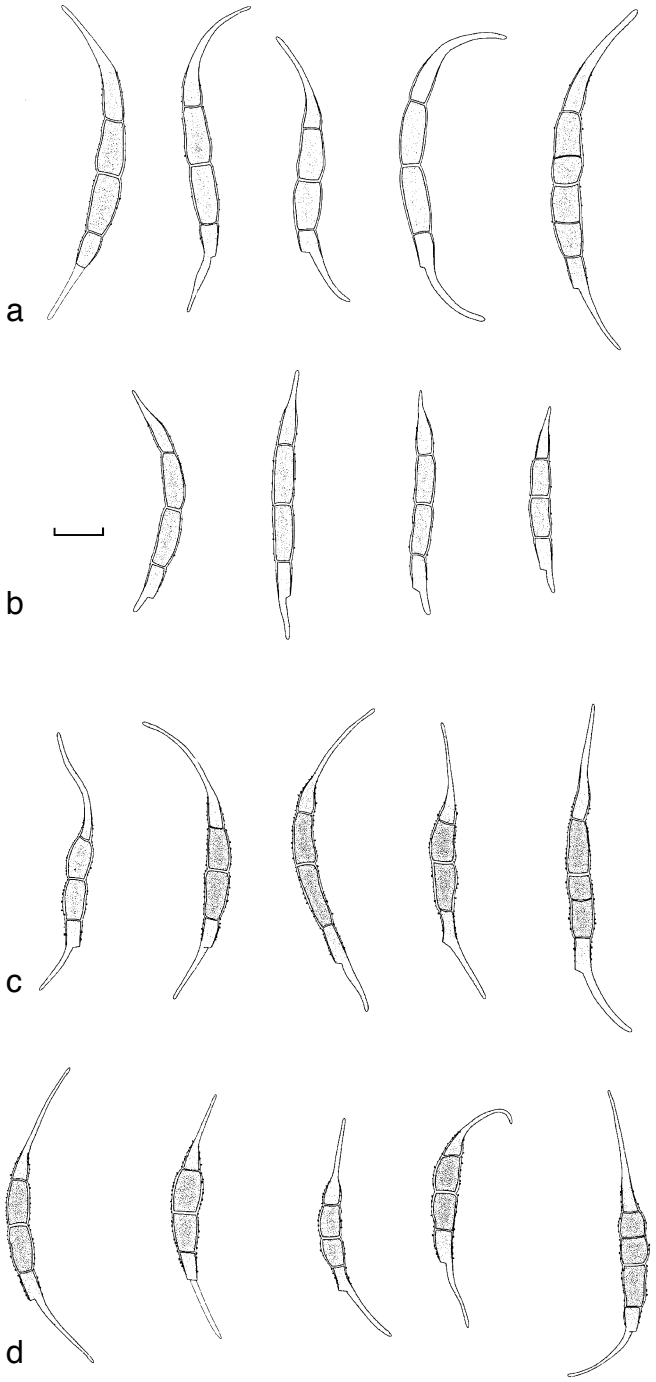

Fig. 29 Conidia of *Seimatosporium verrucisporum*. a. VPRI 1932b (isotype); b. NZFRI 4047a; c. NZFRI 4047b; d. NZFRI 4315. — Scale bar = 10 µm.

Table 15 A comparison between conidial measurments of *Seimatosporium verrucisporum* described by by Nag Raj (1993) and in the present study *S. falcatum* as described by various authors and in the present study ([a] as measured in the present study, [n] not stated whether apical appendage included in dimensions).

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of apical appendage (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
S. verrucisporum						
Nag Raj 1993 (ADW 1784)	29–55	5–6.5	13–24	5–12	6.5 : 1	1.0 : 1.43 : 1.37 : 2.38
VPRI 1932b (isotype)	46–63	5–6	7–17	4–17	10.2 : 1	1.0 : 1.61 : 1.62 : 2.78
NZFRI 4047a	36–49	3.5–5	3–7	2–8	10.0 : 1	1.0 : 1.40 : 1.38 : 1.65
NZFRI 4047b	30–65	4–6	6–24	5–20	10.2 : 1	1.0 : 1.49 : 1.35 : 3.49
NZFRI 4315	36–63	4.5–6.5	9–22	8–25	8.6 : 1	1.0 : 1.40 : 1.38 : 3.80
S. falcatum						
Sutton 1963 type	28–37.5[n]	4–5	6–7	6.5–10	–	–
Swart 1982 (HJS 75.07)	–	4.5–5.5	8–22	7–20	–	–
HJS 75.07[a]	38–52	4.5–5.5	8–17	7–16	8.7 : 1	1.0 : 1.48 : 1.45 : 3.21
Dick 1990 (description from NZFRI 3153, 3158 and 3209)	23–34[n]	6–8	7–12	7–12	–	`–
Nag Raj 1993	24–60	4–6	–	2–17	7.3 : 1	1.0 : 1.25 : 1.25 : 1.75
PAB 01.03	35–54	4–5.5	6–13	6–13	9.1 : 1	1.0 : 1.33 : 1.28 : 2.04
NZFRI 3153	34–50	5–6	7–14	4–13	7.8 : 1	1.0 : 1.39 : 1.31 : 2.45
NZFRI 3209	31–46	4.5–6	6–11	3–9	7.4 : 1	1.0 : 1.34 : 1.25 : 2.10
NZFRI 3158	34–59	4–5	9–25	4–17	9.8 : 1	1.0 : 1.37 : 1.24 : 2.96

of *S. falcatum* suggest that conidia of *S. verrucisporum* are neither distinctly more verruculose nor more darkly pigmented than many of the specimens of *S. falcatum* examined (Fig. 21, 22). As also noted for *S. falcatum*, the characteristics of pigmentation and verrucosity appear to differ both within specimens and between them, indicating that this is a variable feature of the species.

Another variable feature was the number of septa in conidia for particular specimens. The isotype (VPRI 1932b), NZFRI 4047b and NZFRI 4315 all had conidia with additional septa in the median cells (Fig. 29a, c, d), with some conidia being up to 5-septate (Fig. 29a). This feature was uncommon but by no means rare.

In Nag Raj's description and drawings of *S. verrucisporum*, many similarities can be seen with *S. falcatum*. The conidia of both species overlap in their dimensions and shape. Nag Raj separated these two species based on the conidia of *S. verrucisporum* having overall thin septa, darker coloured median cells, more verrucosities and differing conidial cell ratios than those of *S. falcatum*. The large variation within both species, and the close similarities between them (Table 15), make them difficult to clearly distinguish. Fresh collections, cultures and DNA data would greatly help resolve their possible synonymy.

Seimatosporium walkeri (H.J. Swart & M.A. Will.) Barber & Crous, *comb. nov*. — MycoBank MB560636; Fig. 28, 30–33

Basionym. Vermisporium walkeri H.J. Swart & M.A. Will., Trans. Brit. Mycol. Soc. 81: 495. 1983.

Leaf spots roughly circular, 5–10 mm diam, not vein-limited, grey on the upper surface, pale brown on the lower surface, with a brown margin; no insect association evident (Fig. 30–32). *Conidiomata* stromatic, acervular, amphigenous but predominantly epiphyllous, scattered or gregarious in a somewhat concentric arrangement, subepidermal, immersed to erumpent, rounded to oval to irregular in outline, up to 460 µm wide and 210 µm deep, orange to pale brown becoming dark brown with age. *Stroma* 10–25 µm thick, of *textura angularis*, pale brown. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lageniform, annellidic, up to 15 µm long. *Conidia* subcylindrical to fusiform, falcate or sigmoid, 3(–4)-septate, hyaline, slightly or not constricted at the septa, pale orange or pale brown in mass, (43–)48–82(–87) × 3–4.5(–5) (av. = 61.9 × 3.5) µm; apical cell narrowly conic tapering to an obtuse apex, 20–35(–36) (av. = 25.9) µm long; second cell from apex cylindrical to subcylindrical, (7–)8–15(–20) (av. = 11.3) µm long; third cell cylindrical to subcylindrical, (7–)8–14(–18) (av. = 10.4) µm long; basal cell cylindrical to subcylindrical with a narrow truncate base and basal appendage, (7–)10–22(–25) (av. = 14.2) µm long; basal appendage single, excentric, cuneiform to podiform often with a slightly swollen median part and bluntly acute to somewhat obtuse tip, (4–)6–12(–15) (av. = 8.3) µm long; mean conidium length to width ratio = 17.2 : 1. Relative cell lengths from base to apex = 1.0 : 0.75 : 0.76 : 1.79. *Microconidia* acicular with a truncate base and acute apex, straight or falcate, unicellular, hyaline, 16–29 × 1–1.5 (av. = 22.8 × 1.2) µm.

Culture characteristics — Colonies erumpent, spreading, fluffy, with moderate aerial mycelium and smooth, lobate margins, reaching 50 mm diam after 2 wk. Colonies salmon on MEA and OA, but pale luteous on PDA.

Previously known hosts — *E. baxteri*, *E. macrorhyncha*, *E. obliqua*, *E. pauciflora* (Swart & Williamson 1983), *E. fastigata* (Gadgil & Dick 1999) (see Notes).

Recorded hosts in this study — *E. fastigata* (NZFRI 3756), *E. obliqua* (PAB 00.03, PAB 01.05, PAB 01.07), *E. sieberi* (PAB 00.04), *Eucalyptus* sp. (CBS H-20746).

Specimens examined. Australia, Victoria, Yanakie, on *E. obliqua*, 7 Mar. 2000, *P.A. Barber* (PAB 00.03); Victoria, Wilson's Promontory, Squeky Beach Lookout, on *E. sieberi*, 7 Mar. 2000, *P.A. Barber* (PAB 00.04); Victoria, Kinglake West, on *E. obliqua*, 7 Nov. 2001, *P.A. Barber* (PAB 01.05); Victoria, Kinglake West, on *E. obliqua*, 7 Nov. 2001, *P.A. Barber* (PAB 01.07); Victoria, Melbourne, 'Lamatina's Farm', S38°24'26.2", E144°55'9", on *Eucalyptus* sp., 12 Oct. 2009, *P.W. Crous*, CBS H-20746, culture CPC 17644 = CBS 131119, CPC 17645. – New Zealand, Wellington, Catchpool Forest, on *E. fastigata*, 16 Oct. 1997, *B.J. Rogan* (NZFRI-M 3756).

Notes — *Seimatosporium walkeri* was found in this study associated with varying symptoms on a number of hosts in the native forests of Victoria. It was found on a coastal form of *E. obliqua* on the road-side opposite the Yanakie Caravan Park in Yanakie, Victoria. Insect emergence holes were present in some lesions. Other fungi such as *Teratosphaeria* sp. and *Phyllachora* sp. were occasionally present on the same lesion.

Findings by previous authors and in the present study indicate conidial dimensions to be variable within the species. Measure-

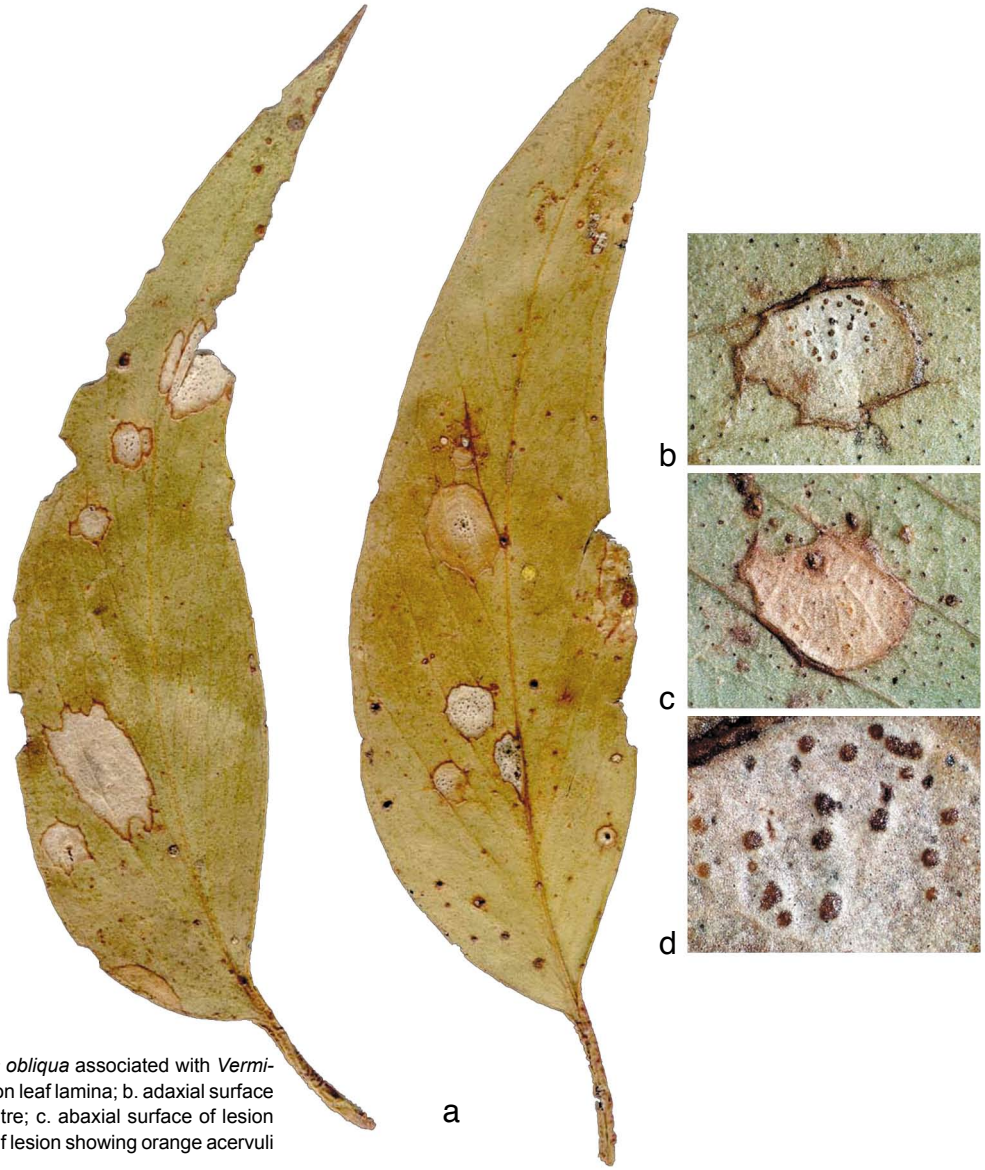

Fig. 30 Disease symptoms on *Eucalyptus obliqua* associated with *Vermisporium walkeri*. a. Brown and grey lesions on leaf lamina; b. adaxial surface of lesion showing acervuli towards the centre; c. abaxial surface of lesion showing lack of acervuli; d. adaxial surface of lesion showing orange acervuli becoming brown with age.

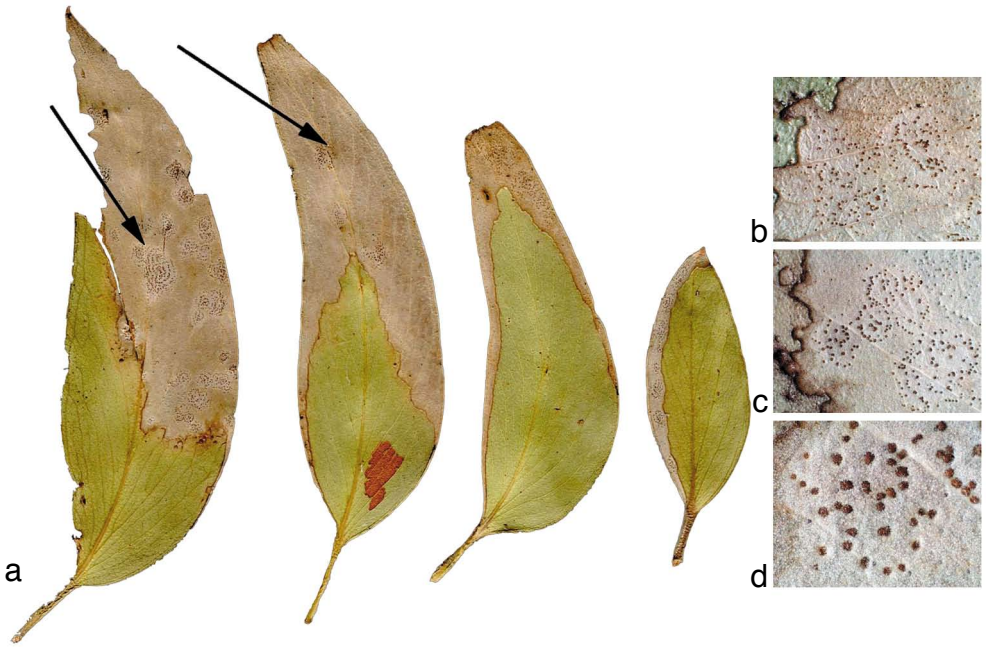

Fig. 31 Disease symptoms on *Eucalyptus sieberi* associated with *Vermisporium walkeri*. a. Large blights extending inward from the leaf margin; b. abaxial surface of the blight showing acervuli in concentric rings; c. adaxial surface of the blight showing acervuli in concentric rings; d. adaxial surface of the blight showing orange acervuli becoming brown with age.

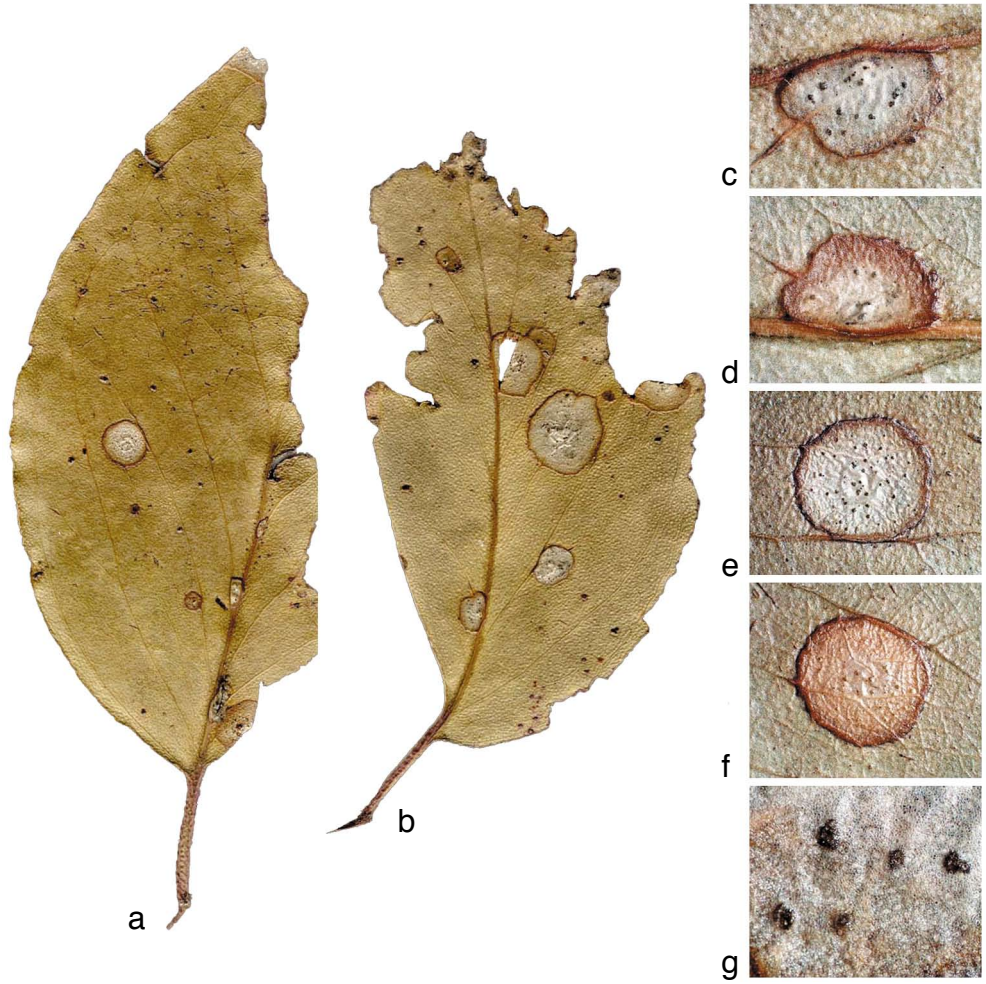

Fig. 32 Disease symptoms on *Eucalyptus obliqua* associated with *Vermisporium walkeri.* a, b. Necrotic lesions on leaf lamina; c. adaxial surface of an angular, vein-limited lesion showing acervuli; d. abaxial surface of an angular, vein-limited lesion showing few acervuli; e. adaxial surface of a circular lesion showing many acervuli; f. abaxial surface of a circular lesion showing lack of acervuli; g. abaxial surface of a lesion showing dark brown acervuli.

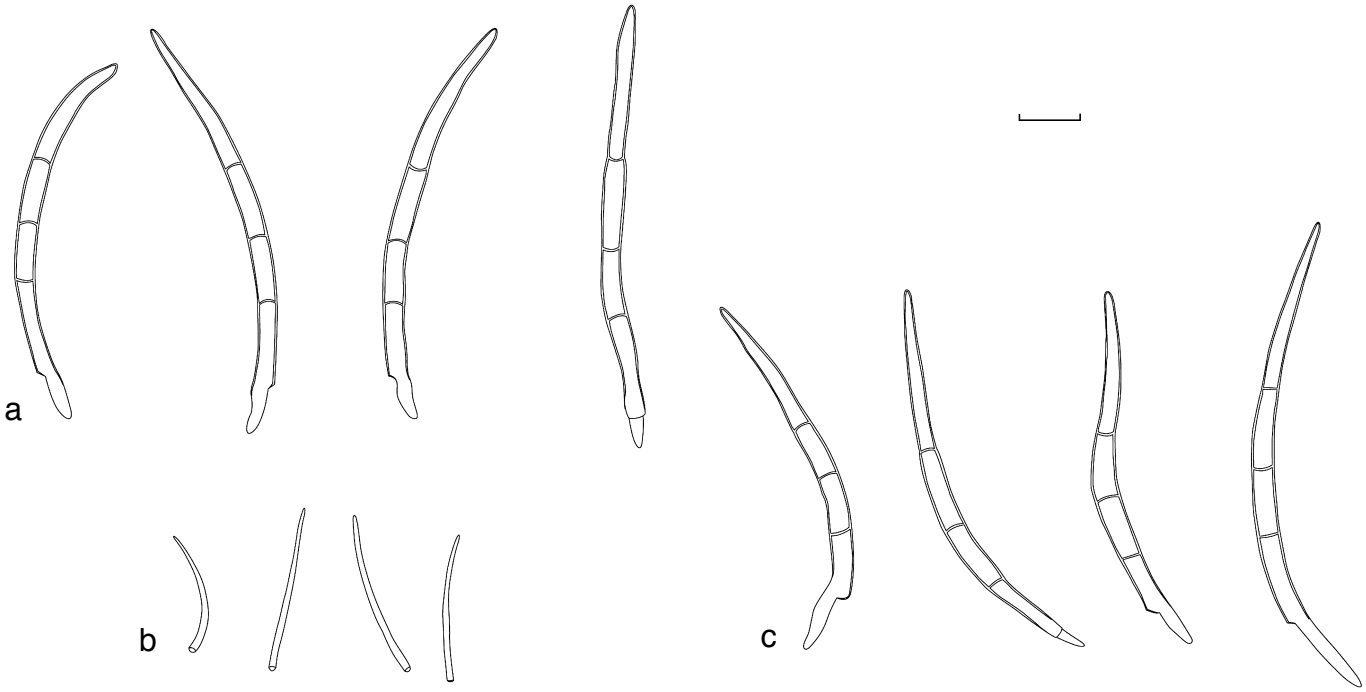

Fig. 33 *Vermisporium walkeri.* a. PAB 00.03 conidia; b. PAB 00.03 microconidia; c. PAB 00.05 conidia. — Scale bar = 10 µm.

Table 16 Conidial measurements of *Seimatosporium walkerii* described by Swart & Williamson (1983), Nag Raj (1993) and in the present study (in **bold** type) (* specimen incorrectly indentified as *S. brevicentrum* in Gadgil & Dick 1999b).

Author / Specimen	Length of conidia (µm)	Width of conidia (µm)	Length of basal appendage (µm)	Length : width ratio	Relative cell lengths (base → apex)
Swart & Williamson 1983 (incl. type)	42–74	2–3.5	3–10	–	–
Nag Raj 1993 (part of type)	33–76	3–4	5–10	17.4 : 1	1.0 : 1.17 : 1.17 : 1.75
PAB 00.03	43–67	3–4	4–11	16.6 : 1	1.0 : 0.75 : 0.80 : 1.76
PAB 00.04	47–67	3–4	6–13	16.9 : 1	1.0 : 0.80 : 0.90 : 2.10
PAB 01.05	61–87	3–4	5–11	23.2 : 1	1.0 : 0.68 : 0.72 : 1.70
PAB 01.07	43–80	3–5	6–15	15.6 : 1	1.0 : 0.74 : 0.84 : 2.10
NZFRI 3756*	48–82	3–4	5–13	17.2 : 1	1.0 : 0.75 : 0.76 : 1.79

ments made in the present study from freshly collected material agree with the description of Swart & Williamson (1983), and disagree with Nag Raj (1993). Specimens collected in the present study showed conidia to be variable, some with a somewhat obtuse tip (Fig. 33a) and some with a rather acute tip (Fig. 28b). The present study found conidia up to 11 µm longer, 1 µm thicker and with a basal appendage up to 5 µm longer than those previously described (Table 16).

Seimatosporium walkeri and *S. brevicentrum* are distinctly different from all other *Seimatosporium* species in having the median cells shorter than both the apical and basal cells. Swart & Williamson (1983), in their key to species, state that the length of each central cell in *S. walkeri* is only slightly exceeded by that of the basal cell, c.f. *S. brevicentrum*, where the length of each central cell is less than half that of both the apical and basal cells.

Based on our examination, specimen NZFRI-M 3756 previously described as *S. brevicentrum* (Gadgil & Dick 1999) is morphologically identical to *S. walkeri*, and is therefore the first record of *S. walkeri* from New Zealand and on *E. fastigata.*

KEY TO *SEIMATOSPORIUM* SPP. OCCURRING ON EUCALYPTS

1. Conidia 3-celled, central cell very short . . . . *S. biseptatum*
1. Conidia 4-celled 2
2. Each central cell shorter than both the apical and the basal cell 3
2. Each central cell longer than the basal cell; all cells lacking pigmentation 4
2. Each central cell longer than the basal cell; median cells showing some degree of pigmentation 5
3. Length of each central cell roughly half that of the basal cell; apical cell with knob-like appendage . . . . . *S. samuelii*
3. Length of each central cell less than half that of both the apical and the basal cell . . . . . *S. brevicentrum*
3. Length of each central cell only slightly exceeded by that of the basal cell . . . . . *S. walkeri*
4. Apical cell gradually tapering to a pointed tip; second cell from the base shorter than the third cell from the base . . . . . *S. acutum*
4. Apical cell tapering well above the middle to a relatively short, pointed, conical tip; median cells roughly equal in length . . . . . *S. orbiculare*
4. Conidium apex and basal appendage both rounded; second cell from the base shorter than the third cell from the base . . . . . *S. obtusum*
5. All cells equally pigmented with hyaline apical and basal appendages; cells increasing in length from base to apex; second cell from the base roughly 10 % longer than the basal cell . . . . . *S. cylindrosporum*
5. Median cells more pigmented than both the apical and basal cells; walls usually smooth; second cell from the base more than 20 % longer than the basal cell; length to width ratio greater than 11 : 1 . . . . . *S. eucalypti*
5. Median cells more pigmented than both the apical and basal cells; walls usually verruculose; second cell from the base more than 20 % longer than the basal cell; length to width ratio less than 11 : 1 . . . . . *S. falcatum*

The key presented here for *Seimatosporium* spp. occurring on eucalypts relies on conidial morphology, which can be easily observed by making squash mounts from either fresh or dried specimens. It has been kept as simple and concise as possible to avoid confusion. When using the key it should be noted that each conidium has an apical cell, one or two central cells and a basal cell. All isolates carry a basal appendage but not all carry an apical appendage. The measured length of the apical cell includes the apical appendage if present; the measured length of the basal cell excludes the basal appendage, which is always clearly demarked by an abrupt narrowing of the basal cell. This method has been adopted because the basal appendage is easily distinguishable in comparison to the apical appendage, which may sometimes not be detectable in some conidia of a single isolate. The main characters used to distinguish between species were the relative cell lengths of conidia and the presence or absence of conidial pigmentation. Where required, additional characters such as appendage shape, cell shape and length to width ratio have been used.

DISCUSSION

Vermisporium, *Discosia* and *Seimatosporium* are coelomycetes that have several morphological features in common. *Discosia* has subhyaline conidia with bipolar appendages attached to the concave side of the conidium. *Seimatosporium* sensu Nag Raj (1993) has conidia with pigmented median cells, with or without one apical and basal appendage. The present finding that species of *Vermisporium*, characterised by their falcate to elongate-fusiform conidia with pigmented cells, a beak-like apical cell, and a podiform, tubular, unbranched, excentric basal appendage cluster in *Seimatosporium*, was rather unexpected. Furthermore, this finding also questions the value of appendage types sensu Nag Raj (1993) as informative features at generic level. Species of *Seimatosporium* have teleomorphs in *Discostroma* and are saprobic or plant pathogenic members of the *Amphisphaeriaceae* (*Xylariales*) (Tanaka et al. 2011). Although none have been reported to date, if teleomorphs of '*Vermisporium*' species were ever to be collected, we expect them to be members of *Discostroma.* We thus accept the generic circumscription of *Seimatosporium* as defined by Sutton (1980) and reject the division as suggested by Swart & Williamson (1983) and Nag Raj (1993).

Jeewon et al. (2002) showed in their cladogram and neighbour-joining tree that *Seimatosporium leptospermi* and *S. grevillea* were closely related. It is interesting to note that Nag Raj (1993) reclassified both these species into separate genera: *S. leptospermi* as *Diploceras leptospermi* and *S. grevilleae* as *Sarcostroma grevilleae*. Nag Raj's treatment of *S. leptospermi* is confusing, as on p. 842 he again excludes the species from *Seimatosporium* as '*Vermisporium leptospermi*', an opinion clearly supported by his illustration of the fungus on p. 292. However, as it clusters within *Seimatosporium*, Lee et al. (2006) correctly referred to the taxon as *S. leptospermi*, showing in f. 9 that it resides in the *Seimatosporium* clade. Given the confusion that relates to genera within this complex, there appears to be a clear need for a detailed molecular analysis of genera within *Amphisphaeriaceae* (Jeewon et al. 2002, Lee et al. 2006, Tanaka et al. 2011).

Nag Raj (1993) transferred three species of *Seimatosporium* to *Vermisporium*, described a new species (*V. verrucisporum*) from herbarium material and produced a key to the species based on conidial characters. Significant problems were encountered when trying to use this key to identify the specimens found in the present study, prompting further analysis that lead to a review of the genus on *Eucalyptus.* One of the main characters used by Nag Raj (1993) for distinguishing species was the length to width ratio, even though this had previously been found to be inadequate by Swart & Williamson (1983). Taxonomists have traditionally placed a great deal of emphasis on the length and width of conidia (length/width ratio) when distinguishing species in *Pestalotiopsis* (Jeewon et al. 2003), a genus sharing many similarities with *Seimatosporium.* Spore length has been used as a key character, with many new species of *Pestalotiopsis* being described based on subtle differences in spore size (Mordue 1985, 1986, Nag Raj 1985, 1986, Venkatasubbaiah et al. 1991). Molecular studies have found, however, that spore size is homoplasious and that this character has been given too much weight in the past when describing new species of *Pestalotiopsis* (Jeewon et al. 2003). These findings would suggest that caution is necessary when grouping species of *Seimatosporium* based on length to width ratios.

Relative cell lengths of conidia were described by Nag Raj (1993) only for *V. acutum* and used as a distinguishing character only between *V. falcatum* and *V. verrucisporum.* The shape of the apical and basal cells and appendages were also compared. Pigmentation was largely ignored by Nag Raj (1993) and was only discussed when distinguishing between *V. falcatum* and *V. verrucisporum.* Despite arguments over the reliability of pigmentation as a taxonomic character, findings from molecular studies (Jeewon et al. 2003) have proven pigmentation to be a sound diagnostic character for species differentiation in *Pestalotiopsis.* Our findings suggest that pigmentation is a reliable character for species differentiation in *Seimatosporium.*

Findings in the present study indicate that there are six easily distinguishable species of *Seimatosporium* on eucalypts based on conidial characters, in particular relative cell ratios and pigmentation. These are *S. biseptatum*, *S. brevicentrum*, *S. cylindrosporum*, *S. obtusum*, *S. samuelii* and *S. walkeri.* The remaining species treated are distinguishable but require closer analysis of particular characteristics as discussed previously.

This genus is a classic example of one that requires additional cultures and further molecular studies to determine how robust the conidial characters are in distinguishing between taxa. It is imperative, however, that the DNA sequence data generated is matched by a high level of expertise in classical taxonomy to accurately describe associated morphological characters (Bensch et al. 2010). Failure to follow this ideal will no doubt result in an increasing number of incorrect identifications and widespread confusion and chaos. The value of the information in a DNA database is only as good as the original classical identification of the fungus. The identity of some fungal DNA sequences deposited in public databases has recently been contested (Crous 2002, Deckert et al. 2002, Bridge et al. 2003, Bidartondo et al. 2008), highlighting the importance of carrying out detailed morphological studies to supplement DNA data to aid in the accurate identification and description of fungi (Seifert & Rossman 2010). In addition, depositing the cultures from which these sequences are derived in public culture collections is essential to form a basis for future research on these species. This also ensures that all additional sequence data generated by other researchers are derived from the exact same culture, irrespective of any prospective name changes this culture will undergo as taxonomic knowledge of the genus increases.

Although *Seimatosporium* spp. are very common as foliar pathogens of eucalypts, they are not considered to be as important as some other species of foliar fungi such as *Mycosphaerella* and *Teratosphaeria* (Crous et al. 2009c, Barber et al. 2003). Two of the species found in this study, *S. brevicentrum* and *S. eucalypti*, were associated with insects presumably as secondary invaders of leaf tissue damaged by the insects or as endophytes sporulating in necrotic tissue. Two species, *S. orbiculare* and *S. samuelii*, were not collected in this study and the remaining species, *S. acutum*, *S. biseptatum*, *S. cylindrosporum*, *S. falcatum*, *S. obtusum* and *S. walkeri*, were not observed associated with any biotic damage of leaves (e.g. by insects). It therefore appears that these *Seimatosporium* species may be primary pathogens on various eucalypt species. None of the species examined in this study were observed or collected from *E. globulus* plantations during surveys even though some specimens were collected from other host species in surrounding native forests. These results would suggest that species of *Seimatosporium* are not likely to be a serious problem in commercial *E. globulus* plantations in the future.

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