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RESEARCH ARTICLE

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Fungal pathogens of *Proteaceae*

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Key words

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Abstract Species of *Leucadendron*, *Leucospermum* and *Protea* (*Proteaceae*) are in high demand for the international floriculture market due to their brightly coloured and textured flowers or bracts. Fungal pathogens, however, create a serious problem in cultivating flawless blooms. The aim of the present study was to characterise several of these pathogens using morphology, culture characteristics, and DNA sequence data of the rRNA-ITS and LSU genes. In some cases additional genes such as TEF 1-α and CHS were also sequenced. Based on the results of this study, several novel species and genera are described. Brunneosphaerella leaf blight is shown to be caused by three species, namely *B. jonkershoekensis* on *Protea repens*, *B. nitidae* sp. nov. on *Protea nitida* and *B. protearum* on a wide host range of *Protea* spp. (South Africa). *Coniothyrium*-like species associated with Coniothyrium leaf spot are allocated to other genera, namely *Curreya grandicipis* on *Protea grandiceps*, and *Microsphaeropsis proteae* on *P. nitida* (South Africa). *Diaporthe leucospermi* is described on *Leucospermum* sp. (Australia), and *Diplodina microsperma* newly reported on *Protea* sp. (New Zealand). Pyrenophora blight is caused by a novel species, *Pyrenophora leucospermi*, and not *Drechslera biseptata* or *D. dematoidea* as previously reported. *Fusicladium proteae* is described on *Protea* sp. (South Africa), *Pestalotiopsis protearum* on *Leucospermum cuneiforme* (Zimbabwe), *Ramularia vizellae* and *R. stellenboschensis* on *Protea* spp. (South Africa), and *Teratosphaeria capensis* on *Protea* spp. (Portugal, South Africa). Aureobasidium leaf spot is shown to be caused by two species, namely *A. proteae* comb. nov. on *Protea* spp. (South Africa), and *A. leucospermi* sp. nov. on *Leucospermum* spp. (Indonesia, Portugal, South Africa). Novel genera and species elucidated in this study include *Gordonomyces mucovaginatus* and *Pseudopassalora gouriqua* (hyphomycetes), and *Xenoconiothyrium catenata* (coelomycete), all on *Protea* spp. (South Africa).

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INTRODUCTION

Proteaceae is one of the Southern Hemisphere's most prominent flowering plant families and is amongst the oldest groups of flowering plants. Records show that they existed in Gondwanaland at least 300 million years ago (Vogts 1982). Today, long after the movement of continental plates caused Gondwanaland to break up these plants still survive. The highest degree of species richness occurs in eastern and western Australia, and the Western Cape of South Africa with the greatest diversity of *Proteaceae*, occurring in Australia, which has representatives of all seven subfamilies. More than 800 species representing 45 genera are found in Australia, of which 550 species are found mainly in the south-western part of that country (Rebelo 2001). Africa, the second most species-rich continent, has members of only two of the subfamilies (Paterson-Jones 2000). Approximately 330 species (representing 14 genera) are confined to the Cape Floristic Region (CFR), making this area exceptional in its natural diversity of *Proteaceae*, and also in their associated fungi (Crous et al. 2006a). The Fynbos biome is a major part of the Cape Floral Kingdom and the *Proteaceae* forms one of the main components of the Cape Floristic Region along with ericoids, restioids and geophytes (Cowling & Richardson 1995).

The South African *Proteaceae* comprises 14 genera, of which 7 genera are commercially utilised (Vogts 1982, Littlejohn 1999, Paterson-Jones 2000, Rebelo 2001). Three of these, *Leucadendron*, *Leucospermum* and *Protea* are of the greatest commercial value and are grown for their exotic, brightly coloured and textured flowers or bracts, which are in high demand on the world floriculture market (Coetzee & Littlejohn 2001). Fungal pathogens, however, create a serious problem in cultivating flawless blooms. Several groups of fungal pathogens of *Proteaceae* have in recent years been characterised phylogenetically, e.g. Botryosphaeria stem cankers (Denman et al. 1999, 2000, 2003, Crous et al. 2006b, Marincowitz et al. 2008b), Armillaria and Cylindrocladium root rot (Schoch et al. 1999, Coetzee et al. 2003, Lombard et al. 2010a–c), Elsinoë scab disease (Swart et al. 2001) Phomopsis cankers (Mostert et al. 2001a, b), and leaf spots caused by species of *Mycosphaerella* and *Teratosphaeria* (Crous et al. 2008, 2009a, b, 2011b). Several other pathogenic fungi on *Proteaceae* however, have never been studied from a phylogenetic perspective based on DNA analyses, and in light of new knowledge are now suspected to represent species complexes.

The aim of the present study, therefore, was to recollect and culture as many of the fungi associated with diseases of *Proteaceae* as possible, to facilitate DNA phylogenetic studies and

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characterisation based on DNA analyses, culture characteristics and morphology.

MATERIALS AND METHODS

Isolates

Leaves and stems of *Proteaceae* with cankers or leaf spots were chosen for study. Single conidial colonies were established from sporulating conidiomata on Petri dishes containing 2 % malt extract agar (MEA; Crous et al. 2009c) as described earlier (Crous et al. 1991). Excised lesions containing ascomata were soaked in water for approximately 2 h, after which they were placed on the underside of Petri dish lids, with the top half of the dish containing MEA, allowing spores to be deposited on the MEA in the dish above. Ascospore germination patterns were examined after 24 h, and single ascospore cultures established as described by Crous (1998). Colonies were subcultured onto 2 % potato-dextrose agar (PDA), synthetic nutrient-poor agar (SNA), MEA, and oatmeal agar (OA) (Crous et al. 2009c), and incubated under continuous near-ultraviolet light at 25 °C to promote sporulation. Reference strains are maintained in the CBS-KNAW Fungal Biodiversity Centre (CBS) Utrecht, The Netherlands (Table 1). Nomenclatural novelties and descriptions were deposited in MycoBank (Crous et al. 2004b).

DNA phylogeny

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocol. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene. The primers ITS4 (White et al. 1990) and LSU1Fd (Crous et al. 2009a) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon.

For the genera *Drechslera* and *Brunneosphaerella*, the partial gene sequences for translation elongation factor 1-α (TEF) were determined using the primers EF1-728F (Carbone & Kohn 1999) and EF1-986R (Carbone & Kohn 1999) or EF-2 (O'Donnell et al. 1998) as described by Crous et al. (2006b) and Bensch et al. (2010). In addition sequences of the chitin synthase (CHS) gene were obtained using the primers CHS-79F and CHS-354R (Carbone & Kohn 1999) following the above amplification protocol. For *Diplodina microsperma*, TEF was amplified and sequenced as described above; in addition beta-tubulin was amplified and sequenced using the primers T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995).

The sequence alignment and subsequent phylogenetic analyses for all the above were carried out using methods described by Crous et al. (2006b). Gaps longer than 10 bases were coded as single events for the phylogenetic analyses; the remaining gaps were treated as 'fifth state' data. Sequence data were deposited in GenBank (Table 1) and the alignment and trees in TreeBASE (http://www.treebase.org).

Taxonomy

A minimum of 30 measurements ($\times$ 1 000 magnification) were made of conidia and ascospores mounted in lactic acid, with the extremes of spore measurements given in parentheses. Ranges of the dimensions of other characters are given. Colony colours (surface and reverse) were assessed on MEA, OA and PDA at 25 °C in the dark for different periods as stated below, using the colour charts of Rayner (1970).

RESULTS

DNA phylogeny

28S nrDNA generic overview

Amplicons of approximately 1 700 bases were obtained ITS (including the first approximately 900 bp of LSU) for the isolates listed in Table 1. The LSU sequences were used to obtain additional sequences from GenBank, which were added to the alignment (Fig. 1) and the ITS to determine species identification (not shown; discussed in species notes where applicable). The manually adjusted LSU alignment contained 136 sequences (including the outgroup sequence) and 759 characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analysis; 326 of these were parsimony-informative, 35 were variable and parsimony-uninformative, and 398 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 = 1567 steps; CI = 0.419; RI = 0.911; RC = 0.381). The phylogenetic results obtained (Fig. 1) are discussed where applicable in the descriptive notes below.

Brunneosphaerella combined ITS, TEF and CHS

Amplicons of approximately 1 700 bases were obtained ITS (including the first approximately 900 bp of LSU), 560–630 bp for TEF and 300 bp for CHS for the isolates listed in Table 1. The manually adjusted combined alignment contained 16 sequences (including the outgroup sequence) and 1 305 (510, 534 and 261 for ITS, TEF and CHS, respectively) characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analysis; 61 of these were parsimony-informative, 545 were variable and parsimony-uninformative, and 699 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. The parsimony analysis yielded a single most parsimonious tree (TL = 640 steps; CI = 0.988; RI = 0.962; RC = 0.950). All three species treated can be identified by unique sequence differences in all three sequenced loci (data not shown). The phylogenetic results obtained (Fig. 2) are discussed where applicable in the descriptive notes below. A partition homogeneity test indicated that all three loci were combinable (P value = 0.222).

Pyrenophora combined ITS, TEF and CHS

Amplicons of approximately 1 700 bases were obtained ITS (including the first approximately 900 bp of LSU), 660–750 bp for TEF and 300 bp for CHS for the isolates listed in Table 1. The manually adjusted combined alignment contained 23 sequences (including the outgroup sequence) and 1 374 (566, 534 and 274 for ITS, TEF and CHS, respectively) characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analysis; 133 of these were parsimony-informative, 550 were variable and parsimony-uninformative, and 691 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. The parsimony analysis yielded a single most parsimonious tree (TL = 792 steps; CI = 0.992; RI = 0.972; RC = 0.964). The TEF alignment was found to provide the highest resolution, followed by CHS.

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 Accession number[2]				
					ITS	LSU	TEF	CHS	TUB
Brunneosphaerella jonkershoekensis	CPC 13902	South Africa	Leaves of *Protea repens*	P.W. Crous	JN712439	JN712503	JN712571	JN712609	–
	CPC 13905	South Africa	Leaves of *Protea repens*	P.W. Crous	GU214623	GU214394	JN712572	JN712610	–
	CPC 13908	South Africa	Leaves of *Protea repens*	P.W. Crous	JN712440	JN712504	JN712573	JN712611	–
	CPC 13911; CBS 130594	South Africa	Leaves of *Protea repens*	P.W. Crous	JN712441	JN712505	JN712574	JN712612	–
	CPC 15237	South Africa	*Protea nitida*	L. Mostert	JN712442	JN712506	JN712575	JN712613	–
	CPC 16850	South Africa	*Protea repens*	J.E. Taylor	JN712443	JN712507	JN712576	JN712614	–
	CPC 16851	South Africa	*Protea repens*	J.E. Taylor	JN712444	JN712508	JN712577	JN712615	–
	CPC 18297	South Africa	Living leaves of *Protea repens*	P.W. Crous	JN712445	JN712509	JN712578	JN712616	–
	CPC 18301	South Africa	Living leaves of *Protea repens*	P.W. Crous	JN712446	JN712510	JN712579	JN712617	–
Brunneosphaerella nitidae	CPC 13914; CBS 130596	South Africa	*Protea nitida*	P.W. Crous	GU214624	GU214395	JN712580	JN712618	–
	CPC 15231; CBS 130595	South Africa	Leaf litter of *Protea nitida*	L. Mostert	GU214625	GU214396	JN712581	JN712619	–
Brunneosphaerella protearum	CPC 16338; CBS 130597	South Africa	Leaves of *Protea* sp.	P.W. Crous	GU214626	GU214397	JN712582	JN712620	–
	CPC 16849	South Africa	Living leaves of *Protea magnifica*	J.E. Taylor	JN712447	JN712511	JN712583	JN712621	–
	CPC 18308; CBS 130598	South Africa	Leaves of *Protea coronata*	P.W. Crous	JN712448	JN712512	JN712584	JN712622	–
	CPC 18328	South Africa	Leaves of *Protea mundii*	P.W. Crous	JN712449	JN712513	JN712585	JN712623	–
Coccomyces proteae	CPC 1727; CBS 111704	South Africa	Leaves of *Protea* sp.	S. Denman	JN712450	JN712514	–	–	–
	CPC 1730; CBS 111703	South Africa	Leaves of *Protea* sp.	S. Denman	JN712451	JN712515	–	–	–
Coniothyrium nitidae	CPC 1476; CBS 111322	South Africa	Leaves of *Protea nitida*	S. Denman	JN712452	JN712516	–	–	–
	CPC 1477; CBS 111321	South Africa	Leaves of *Protea nitida*	S. Denman	JN712453	JN712517	–	–	JN712647
	CPC 1478; CBS 111302	South Africa	Leaves of *Protea nitida*	S. Denman	JN712454	JN712518	–	–	–
	CPC 1532; CBS 111380	South Africa	Leaves of *Protea nitida*	S. Denman	JN712455	JN712519	–	–	–
Curreya grandicipis	CPC 1852; CBS 114272	South Africa	Leaves of *Protea grandiceps*	J.E. Taylor & S. Denman	JN712456	JN712520	–	–	–
	CPC 1853; CBS 111702	South Africa	Leaves of *Protea grandiceps*	J.E. Taylor & S. Denman	JN712457	JN712521	–	–	–
Curvularia trifolii	CPC 2941; CBS 114135	Australia	*Leucospermum* sp.	P.W. Crous	JN712458	JN712522	–	–	–
	CPC 2995; CBS 111997	Australia	*Leucospermum* sp.	P.W. Crous	JN712459	JN712523	–	–	–
Diaporthe leucospermi	CPC 2956; CBS 111980	Australia	Leaves of *Leucospermum* sp.	P.W. Crous & B. Summerell	JN712460	JN712524	–	–	–
Diplodina microsperma	CPC 2336; CBS 114545	New Zealand	Leaves of *Protea* sp. (intercepted specimen of flowers exported to California, USA)	M.A. Abdelshife & M.E. Palm	JN712461	JN712525	JN712586	–	JN712648
Drechslera biseptata	CBS 307.69	Germany	*Lolium multiflorum*	U.G. Schlösser	–	JN712526	–	–	–
	CBS 599.71	Netherlands	Leaf of *Zea mays*	H.A. van Kesteren	–	JN712527	–	–	–
	CBS 205.60	–	–	W.B. Kendrick	JN712462	JN712528	–	JN712624	–
	CBS 306.69	Germany	*Lolium multiflorum*	U.G. Schlösser	JN712463	JN712529	JN712587	JN712625	–
	CBS 308.69	Germany	*Lolium* sp.	U.G. Schlösser	JN712464	JN712530	JN712588	JN712626	–
Drechslera dematioidea	CBS 108962	British Columbia	Overwintered grass	G. Zhang	JN712465	JN712531	JN712589	JN712627	–
	CBS 108963	British Columbia	Overwintered grass	G. Zhang	JN712466	JN712532	JN712590	JN712628	–
Pyrenophora leucospermi	CPC 1293; CBS 111083	South Africa	Leaves of *Leucospermum cordifolium*	L. Swart	JN712467	JN712533	JN712591	JN712629	–
	CPC 1294; CBS 111084	South Africa	Leaves of *Leucospermum cordifolium*	L. Swart	JN712468	JN712534	JN712592	JN712630	–
	CPC 1295; CBS 111085	South Africa	Leaves of *Leucospermum cordifolium*	L. Swart	JN712469	JN712535	JN712593	JN712631	–
	CPC 1296; CBS 111180	South Africa	Leaves of *Leucospermum cordifolium*	L. Swart	JN712470	JN712536	JN712594	JN712632	–
	CPC 1297; CBS 111086	South Africa	Leaves of *Leucospermum cordifolium*	L. Swart	JN712471	JN712537	JN712595	JN712633	–
	CPC 1298; CBS 111087	South Africa	Leaves of *Leucospermum cordifolium*	L. Swart	JN712472	JN712538	JN712596	JN712634	–
	CPC 13777	Spain: Tenerife	*Leucospermum* sp.	P.W. Crous	JN712473	JN712539	JN712597	JN712635	–
	CPC 13786	Spain: Tenerife	*Leucospermum* sp.	P.W. Crous	JN712474	JN712540	JN712598	JN712636	–
	CPC 16268	Portugal	*Leucadendron succeisus*	J.J. Morais	JN712475	JN712541	JN712599	JN712637	–
	CPC 1785; CBS 111505	South Africa	*Leucospermum cordifolium*	S. Denman	JN712476	JN712542	JN712600	JN712638	–
	CPC 2195; CBS 111862	USA	*Leucospermum* sp., cloud bunk	P.W. Crous	JN712477	JN712543	JN712601	JN712639	–
	CPC 2196; CBS 111863	USA	*Leucospermum* sp., cloud bunk	P.W. Crous	JN712478	JN712544	JN712602	JN712640	–
	CPC 2200; CBS 114493	USA	*Leucospermum* sp.	P.W. Crous	JN712479	JN712545	JN712603	JN712641	–
	CPC 2836; CBS 114131	South Africa	*Leucospermum* sp.	L. Swart	JN712480	JN712546	JN712604	JN712642	–
	CPC 2837; CBS 114033	South Africa	*Leucospermum* sp.	L. Swart	JN712481	JN712547	JN712605	JN712643	–
	CPC 2839; CBS 114032	South Africa	*Leucospermum* sp.	L. Swart	JN712482	JN712548	JN712606	JN712644	–
	CPC 5215; CBS 115178	Spain	*Leucadendron* sp.	S. Denman	JN712483	JN712549	JN712607	JN712645	–
	CPC 5238; CBS 115397	–	*Leucadendron* sp.	S. Denman	JN712484	JN712550	JN712608	JN712646	–

Fusicladium proteae	CPC 18282; CBS 130599	South Africa	Leaves of *Protea* sp., in association with *Vizella interrupta*	P.W. Crous	JN712485	JN712551	–	–	–
Gordonomyces mucovaginatus	CMW 22212; CBS 127273; CPC 18172	South Africa	Leaf litter of *Leucadendron laureolum*	S. Marincowitz	JN712486	JN712552	–	–	–
Aureobasidium leucospermi	CPC 15081	Portugal	Leaves of *Leucospermum* cv. 'Tango'	P.W. Crous	JN712487	JN712553	–	–	–
	CPC 15099	Indonesia	*Leucospermum* sp.	P.W. Crous	JN712488	JN712554	–	–	–
	CPC 15180; CBS 130593	South Africa	Leaves of *Leucospermum conocarpodendron*	F. Roets	JN712489	JN712555	–	–	–
Aureobasidium proteae	CPC 13701	–	*Protea* sp. intercepted in the Netherlands	P.W. Crous	JN712490	JN712556	–	–	–
	CPC 2824; CBS 114273	South Africa	*Protea* cv. 'Sylvia' (*Protea eximia* × *Protea susannae*)	S. Denman	JN712491	JN712557	–	–	–
	CPC 2825; CBS 111973	South Africa	*Protea* cv. 'Sylvia' (*Protea eximia* × *Protea susannae*)	S. Denman	JN712492	JN712558	–	–	–
	CPC 2826; CBS 111970	South Africa	*Protea* cv. 'Sylvia' (*Protea eximia* × *Protea susannae*)	S. Denman	JN712493	JN712559	–	–	–
Leptosphaerulina australis	CPC 3712; CBS 116307	Kenya	Leaves of *Protea* sp.	–	JN712494	JN712560	–	–	–
Microsphaeropsis proteae	CPC 1423; CBS 111303	South Africa	Leaves of *Protea nitida*	S. Denman	JN712495	JN712561	–	–	–
	CPC 1424; CBS 111320	South Africa	Leaves of *Protea nitida*	S. Denman	JN712496	JN712562	–	–	JN712649
	CPC 1425; CBS 111319	South Africa	Leaves of *Protea nitida*	S. Denman	JN712497	JN712563	–	–	JN712650
Pestalotiopsis protearum	CPC 1765; CBS 114178	Zimbabwe	Living leaves of *Leucospermum cuneiforme* cv. 'Sunbird'	L. Swart	JN712498	JN712564	–	–	–
Pseudopassalora gouriqua	CPC 1811; CBS 101954	South Africa	Leaves of *Protea susanne*	L. Dryer	–	JN712565	–	–	–
Ramularia stellenboschensis	CPC 18294; CBS 130600	South Africa	Leaves of *Protea* sp.	P.W. Crous	JN712499	JN712566	–	–	–
Ramularia vizellae	CPC 18283; CBS 130601	South Africa	Leaves of *Protea* sp., in association with *Vizella interrupta* (secondary?)	P.W. Crous	JN712500	JN712567	–	–	–
Teratosphaeria capensis	CPC 13981	Portugal	Leaves of *Protea repens*	M.F. Moura	EU707887	JN712568	–	–	–
	CPC 18299; CBS 130602	South Africa	Living leaves of *Protea* sp.	P.W. Crous	JN712501	JN712569	–	–	–
Xenoconiothyrium catenata	CMW 22113; CBS 128994	South Africa	Twig litter of *Protea laurifolia*	S. Marincowitz	JN712502	JN712570	–	–	–

[1] CBS: CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; CMW: Culture collection of FABI, University of Pretoria, South Africa; 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; TEF: partial translation elongation factor 1-alpha gene; CHS: partial chitin synthase gene; TUB: partial beta-tubulin gene.

On ITS, it was not possible to distinguish between *Drechslera biseptata* and *Pyrenophora leucospermi* (data not shown). The phylogenetic results obtained (Fig. 3) are discussed where applicable in the descriptive notes below. A partition homogeneity test indicated that all three loci were combinable (P value = 0.093).

Taxonomy

During the course of the present study several previously described species were either newly collected, or found to represent new species. These taxa are subsequently treated below.

Aureobasidium leaf spot

Although species of the genus *Aureobasidium* are generally regarded as saprobes, several taxa have the ability to form *Kabatiella* synanamorphs that generally cause leaf spots, and are considered plant pathogens (Taylor & Crous 2000, Zalar et al. 2008). *Aureobasidium* is an important pathogen of *Proteaceae*, and has (as *Kabatiella* synanamorph) been reported from South African material intercepted by the USDA Animal and Plant Health Inspection Service (APHIS) in the USA (Taylor 2001). *Kabatiella proteae* has recently been recorded from *Protea* in Australia (Crous et al. 2000), and a *Kabatiella* state of a species of *Aureobasidium* is quite abundant as a leaf spot pathogen on *Leucospermum* spp. in the Canary Islands and Portugal (P.W. Crous, unpubl. data). In accordance with the Amsterdam Declaration to integrating different morphs of pleomorphic fungi into a single generic name (Hawksworth et al. 2011), preference is given to the older generic name *Aureobasidium* (1891), rather than the younger, lesser-known *Kabatiella* (1907).

Aureobasidium leucospermi Crous, *sp. nov*. — MycoBank MB560556; Fig. 4

Aureobasidium proteae morphologice similis, sed conidiis majoribus, 6–15 × 4–8 µm.

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

Leaf spots subcircular irregular, amphigenous, necrotic, sunken, pale to medium brown with a raised, dark brown margin. *Mycelium* immersed. *Conidiomata* acervular to sporodochial, amphigenous, substomatal, subepidermal, pulvinate, dry or crystaline in appearance, pale brown, discrete, 60–100 µm diam. *Stroma* visible in substomatal cavity, dark brown, mainly consisting of elongated pseudo-parenchymatous cells with large lumina, becoming hyaline, thinner-walled at the apex, 80–130 × 50–100 µm. *Conidiogenous cells* cylindrical, clavate or globose, integrated, terminal, conidial ontogeny holoblastic, with numerous synchronously produced conidia, 15–30 × 4–11 µm. *Conidia in vitro* solitary, aseptate, ellipsoidal to spherical, occasionally with a slightly truncate base, hyaline, thin-walled, smooth, (6–)8–11(–15) × 4–5(–8) µm.

Culture characteristics — Colonies flat, spreading, lacking aerial mycelium, with feathery margins, covering the dish in 3 wk. On MEA rosy-buff, reverse buff; on OA surface buff; on PDA sectors of leaden-black, with patches of buff, similar in reverse.

Specimens examined. Indonesia, on leaves of *Leucospermum* sp., 16 Apr. 2008, *P.W. Crous*, CPC 15099–15101. – Portugal, on leaves of *Leucospermum* cv. 'Tango', 1 Mar. 2008, *P.W. Crous*, CPC 15081–15083. – South Africa, Western Cape Province, Stellenbosch, J.S. Marais Garden, on leaves of *Leucospermum conocarpodendron*, 20 Apr. 2008, *F. Roets*, holotype CBS H-20669, culture ex-type CPC 15180–15182 = CBS 130593.

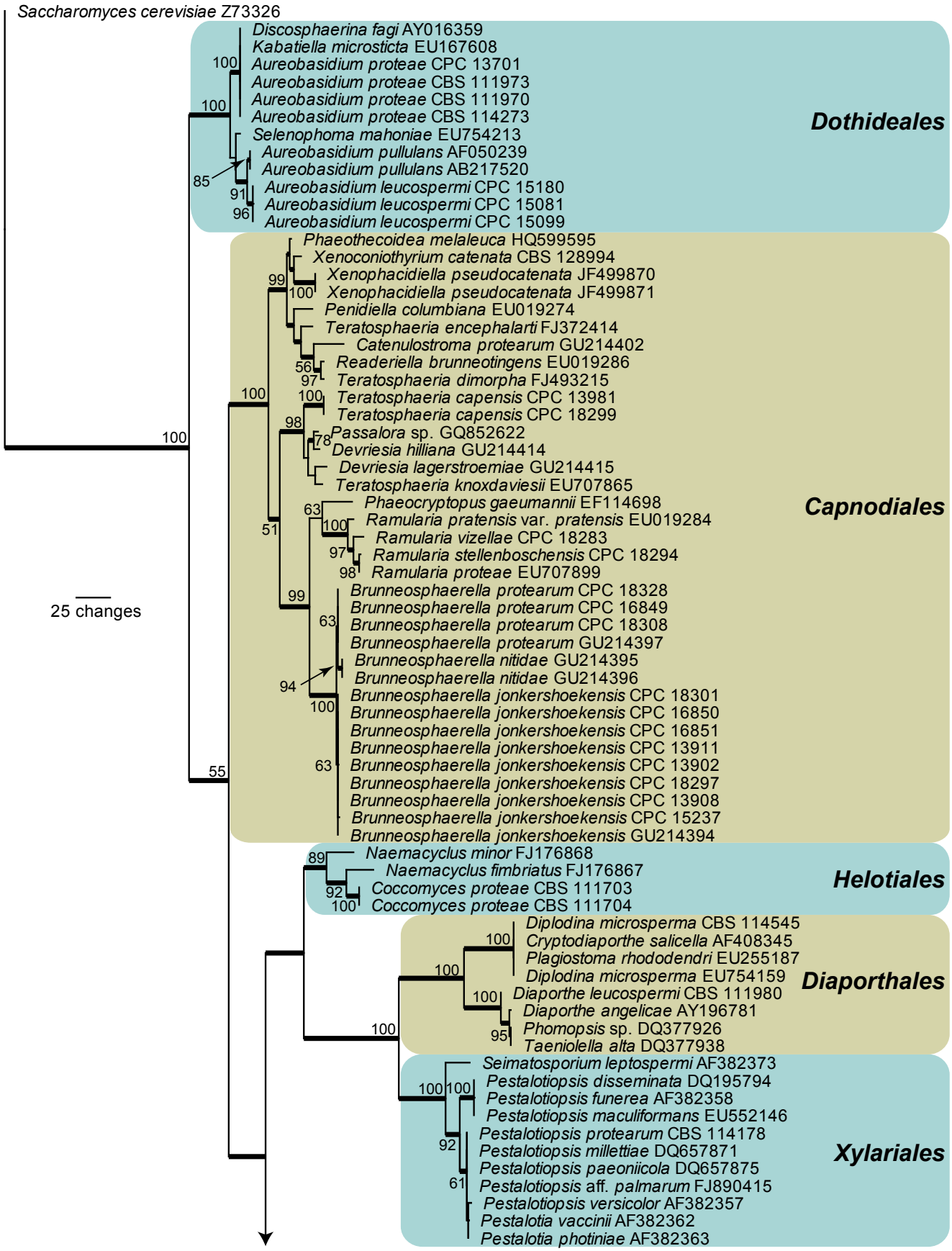

Fig. 1 The first of 1 000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 25 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Orders are indicated to the right of the tree. Branches present in the strict consensus tree are thickened and the tree was rooted to a sequence of *Saccharomyces cerevisiae* (GenBank accession Z73326).

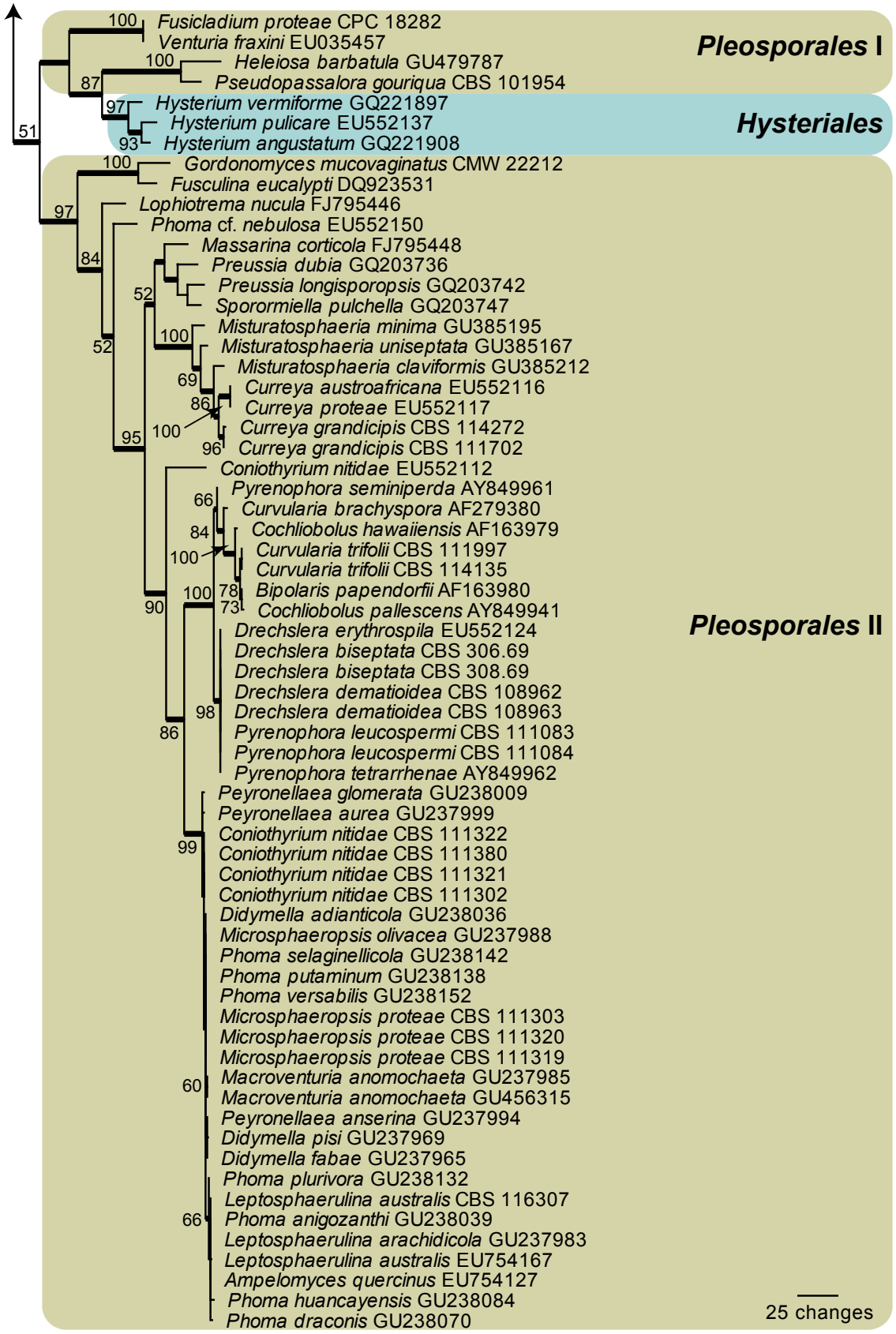
Pleosporales I
Hysteriales
Pleosporales II
Fusicladium proteae CPC 18282
Venturia fraxini EU035457
Heleiosa barbatula GU479787
Pseudopassalora gouriqua CBS 101954
Hysterium vermiforme GQ221897
Hysterium pulicare EU552137
Hysterium angustatum GQ221908
Gordonomyces mucovaginatus CMW 22212
Fusculina eucalypti DQ923531
Lophiotrema nucula FJ795446
Phoma cf. nebulosa EU552150
Massarina corticola FJ795448
Preussia dubia GQ203736
Preussia longisporopsis GQ203742
Sporormiella pulchella GQ203747
Misturatosphaeria minima GU385195
Misturatosphaeria uniseptata GU385167
Misturatosphaeria claviformis GU385212
Curreya austroafricana EU552116
Curreya proteae EU552117
Curreya grandicipis CBS 114272
Curreya grandicipis CBS 111702
Coniothyrium nitidae EU552112
Pyrenophora seminiperda AY849961
Curvularia brachyspora AF279380
Cochliobolus hawaiiensis AF163979
Curvularia trifolii CBS 111997
Curvularia trifolii CBS 114135
Bipolaris papendorfii AF163980
Cochliobolus pallescens AY849941
Drechslera erythrospila EU552124
Drechslera biseptata CBS 306.69
Drechslera biseptata CBS 308.69
Drechslera dematioidea CBS 108962
Drechslera dematioidea CBS 108963
Pyrenophora leucospermi CBS 111083
Pyrenophora leucospermi CBS 111084
Pyrenophora tetrarrhenae AY849962
Peyronellaea glomerata GU238009
Peyronellaea aurea GU237999
Coniothyrium nitidae CBS 111322
Coniothyrium nitidae CBS 111380
Coniothyrium nitidae CBS 111321
Coniothyrium nitidae CBS 111302
Didymella adianticola GU238036
Microsphaeropsis olivacea GU237988
Phoma selaginellicola GU238142
Phoma putaminum GU238138
Phoma versabilis GU238152
Microsphaeropsis proteae CBS 111303
Microsphaeropsis proteae CBS 111320
Microsphaeropsis proteae CBS 111319
Macroventuria anomochaeta GU237985
Macroventuria anomochaeta GU456315
Peyronellaea anserina GU237994
Didymella pisi GU237969
Didymella fabae GU237965
Phoma plurivora GU238132
Leptosphaerulina australis CBS 116307
Phoma anigozanthi GU238039
Leptosphaerulina arachidicola GU237983
Leptosphaerulina australis EU754167
Ampelomyces quercinus EU754127
Phoma huancayensis GU238084
Phoma draconis GU238070
25 changes

Fig. 1 (cont.)

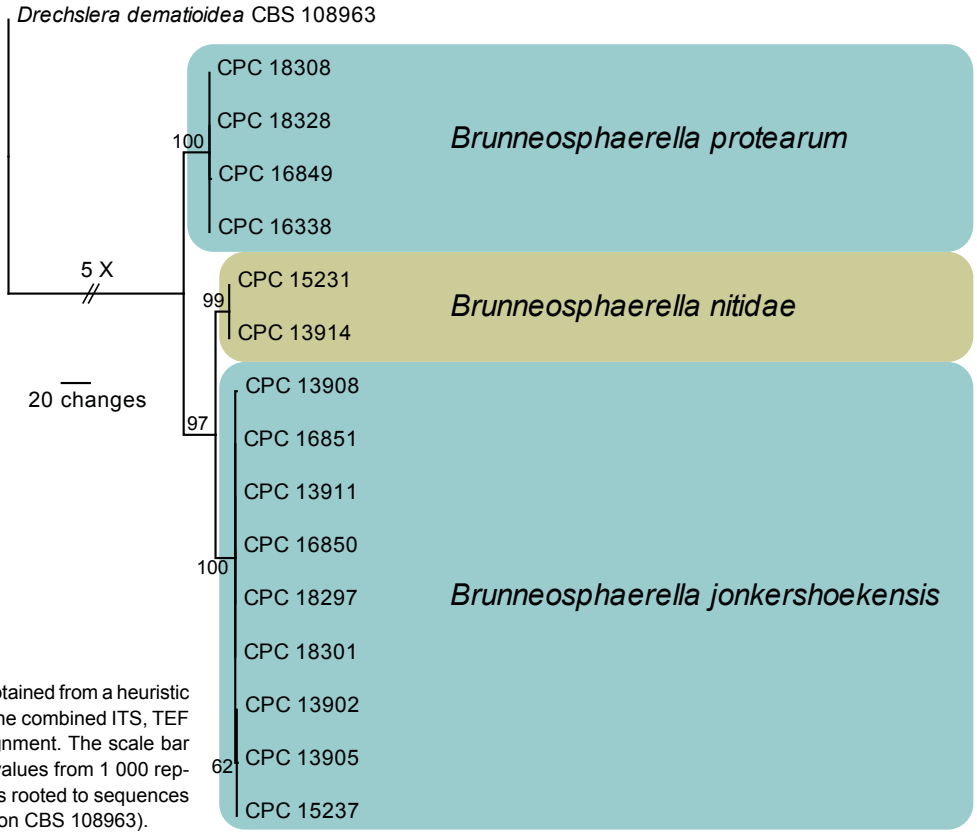

Fig. 2 The single most parsimonious tree obtained from a heuristic search with 100 random taxon additions of the combined ITS, TEF and CHS *Brunneosphaerella* sequence alignment. The scale bar shows 20 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. The tree was rooted to sequences of *Drechslera dematioidea* (Culture accession CBS 108963).

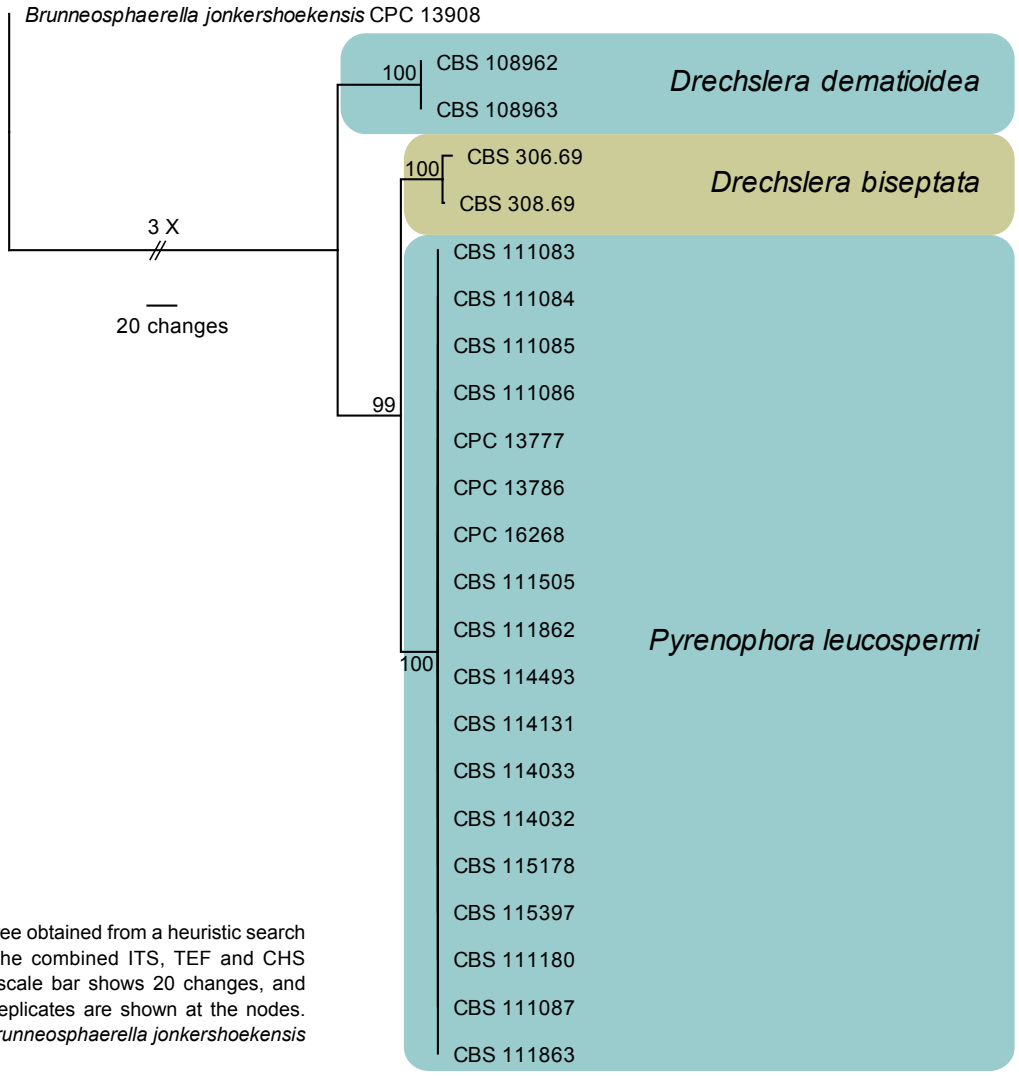

Fig. 3 The single most parsimonious tree obtained from a heuristic search with 100 random taxon additions of the combined ITS, TEF and CHS *Drechslera* sequence alignment. The scale bar shows 20 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. The tree was rooted to sequences of *Brunneosphaerella jonkershoekensis* (Culture accession CPC 13908).

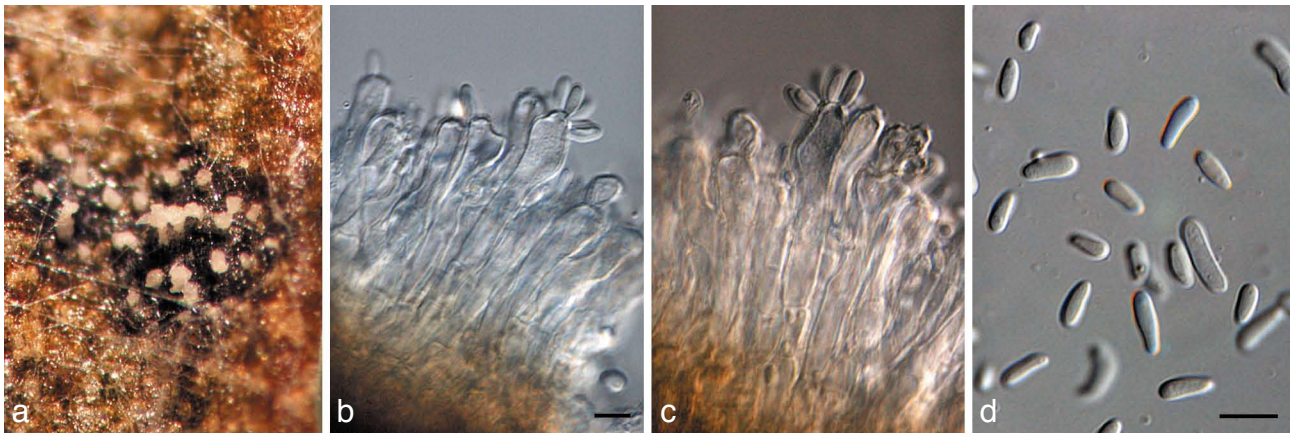

Fig. 4 *Aureobasidium leucospermi* (CBS H-20669). a. Leaf spot with sporulating conidiomata; b, c. conidiogenous cells giving rise to conidia; d. hyaline conidia. — Scale bars = 10 µm.

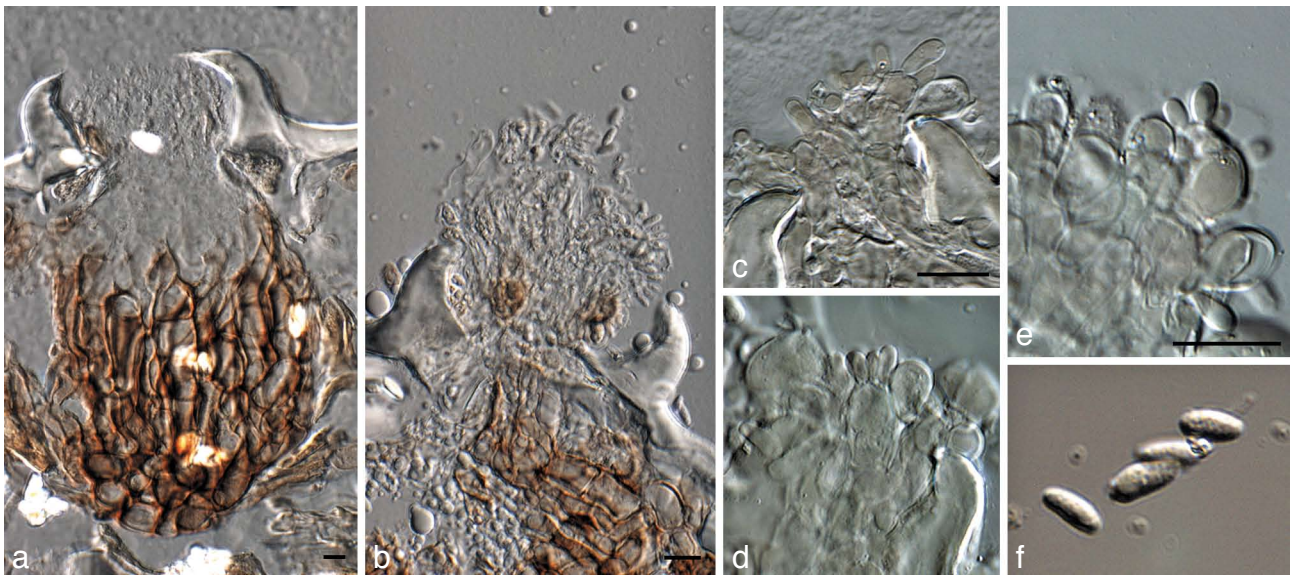

Fig. 5 *Aureobasidium proteae* (CBS 114273). a. Sporulating conidiomata in leaf tissue; c–e. conidiogenous cells giving rise to conidia; f. hyaline conidia. — Scale bars = 10 µm.

Aureobasidium proteae (Joanne E. Taylor & Crous) Joanne E. Taylor & Crous, *comb. nov.* — MycoBank MB560557; Fig. 5

Basionym. Kabatiella proteae Joanne E. Taylor & Crous, Mycol. Res. 104: 619. 2000.

Leaf spots irregular, occurring on the petiole and base of lamina, extending up the leaf, necrotic, sunken, pale to medium brown with a raised, dark brown margin; areas where sporodochia occur often darkened. *Mycelium* immersed. *Conidiomata* acervular to sporodochial, amphigenous, substomatal, subepidermal, pulvinate, dry or crystaline in appearance, pale brown, discrete, 60–100 µm diam. *Stroma* visible in substomatal cavity, dark brown, mainly consisting of elongated pseudo-parenchymatous cells with large lumina, becoming hyaline, thinner-walled at the apex, (80–)101–121(–125) × (50–)72–92(–100) µm. *Conidiogenous cells* cylindrical, clavate or globose, integrated, terminal, conidial ontogeny holoblastic, with numerous synchronously produced conidia, (13–)16–20(–27) × (4–)6–8(–11) µm. *Conidia in vivo* solitary, aseptate, ellipsoidal to spherical, occasionally with a slightly truncate base, hyaline, thin-walled, smooth, often with small guttules, (5–)6.5–7.5(–10) × (2–)2.5–3(–3.5) µm; *conidia in vitro* similar, (4–)6–7(–9) × (2–)2.5–3(–3.5) µm.

Culture characteristics — Colonies with moderate to sparse aerial mycelium on MEA, surface olivaceous-grey, reverse olivaceous-grey to iron-grey, radial striations appearing in the agar and visible from underneath.

Specimens examined. South Africa, Somerset West, Hilly Lands Farm, on a leaf of a *Protea cynaroides* seedling, 21 July 1998, *S. Denman & J.E. Taylor*, JT338 (holotype PREM 56192); on leaves of *Protea* cv. 'Sylvia' (*P. eximia* × *P. susannae*), 19 July 1999, *S. Denman*, epitype designated here as CBS H-20668, cultures ex-epitype CPC 2824 = CBS 114273, CPC 2825 = CBS 111973, CPC 2826 = CBS 111970, CPC 2827. – Unknown origin, on leaves of *Protea* sp., intercepted in the Netherlands, 24 Feb. 2006, *P.W. Crous*, CPC 13701–13703.

Notes — Leaf spots caused by *A. leucospermi* appear very similar to those of *A. proteae*, except that the former is so far only known from species of *Leucospermum*. The two species are also morphologically similar and phylogenetically closely related (Fig. 1). They can be distinguished morphologically, however, in that conidia of *A. leucospermi* (6–15 × 4–8 µm) are larger than those of *A. proteae* (4–9 × 2–3.5 µm).

Brunneosphaerella leaf blight

Crous et al. (2009a) recently introduced the genus *Brunneosphaerella* to accommodate *Leptosphaeria protearum*, which is a major leaf spot and blight pathogen of *Protea* spp. (Knox-Davies et al. 1987). This pathogen occurs naturally on native protea in South Africa and is damaging to commercially cul-

tivated crops there, but it also causes severe losses in other countries where South African proteas are cultivated (Taylor & Crous 2000, Taylor et al. 2001a, b, d, Crous et al. 2004a). Although *Brunneosphaerella* was recognised as distinct from *Leptosphaeria*, additional collections and molecular data were required to resolve the species complex represented by isolates identified as *B. protearum*. Based on their distinct phylogeny (Fig. 2), morphology and host ranges, three species of *Brunneosphaerella* are now recognised and described below. Although highly similar in ITS, the three *Brunneosphaerella* species treated in this study can be resolved easily based on their diagnostic TEF and CHS sequences (Fig. 2).

Brunneosphaerella jonkershoekensis (Marinc., M.J. Wingf. & Crous) Crous, Stud. Mycol. 64: 31. 2009 — Fig. 6

Basionym. Leptosphaeria jonkershoekensis Marinc., M.J. Wingf. & Crous, in Marincowitz et al., Microfungi occurring on Proteaceae in the fynbos: 62. 2008.

Leaf spots amphigenous, up to 15 mm diam, pale brown, with a raised, red-brown border. *Ascomata* perithecioid, subepidermal, amphigenous, remaining immersed, obpyriform in section, 180–205 × 160–235 µm, with a papillate ostiole. *Peridium* 20–30 µm thick, composed of relatively large cells, 11–15 × 2.5–5.5 µm, cells arranged in three strata; outer stratum consisting of 3–5 layers of dark brown, very thick-walled cells; middle stratum transient, consisting a few layers of pale brown, thick-walled, compressed cells; inner stratum consisting of 1–2 layers of thin-walled, very compressed cells. *Hamathecium* not observed in mature ascomata. *Asci* bitunicate, inflated cylindrical to clavate, 70–95 × 12–15 µm, ocular chamber dome-shaped, indistinct. *Ascospores* pale brown, finely verruculose, fusoid to ellipsoid, tapering towards the base, (25–)27–34(–37) × (5–)6–7(–9) µm (av. 31 × 6.7 µm), apical cell the shortest, upper hemispore slightly bigger than lower, at times slightly curved, 3-septate, smooth, guttulate, with each cell containing a large central guttule, prominently constricted at median septum, with globose mucoid caps up to 3 µm diam at each end; ascospores widest in second cell from apex.

Culture characteristics — After 2 mo on OA flat, spreading with moderate to sparse aerial mycelium; surface olivaceous-grey with patches of iron-grey and pale olivaceous-grey and smoke-grey; margins lobate, smooth, reaching 35 mm diam. On PDA flat, spreading, with moderate aerial mycelium and uneven surface and feathery, lobate margins; surface olivaceous-grey with patches of pale olivaceous-grey to smoke-grey; reverse iron-grey, reaching 35 mm diam. On MEA flat, spreading, with sparse aerial mycelium and even, lobate margins; surface dirty white with patches of smoke-grey; reverse iron-grey, reaching 35 mm diam.

Specimens examined. South Africa, Western Cape Province, Jonkershoek Nature Reserve, on leaf litter of *Protea repens*, 6 June 2000, *S. Marincowitz*, holotype PREM 59447; Jonkershoek Nature Reserve, S33°59'11.2" E18°57'14.7", on leaves of *P. repens*, 1 Apr. 2007, *P.W. Crous*, epitype designated here as CBS H-20333, cultures ex-epitype CPC 13902–13907; CBS H-20332, cultures CPC 13908–13910; CBS H-20331, cultures CPC 13911–13913 = CBS 130594; Stellenbosch, J.S. Marais Garden, S33°55'59.3" E18°52'22.5", on living leaves of *P. repens*, 6 May 2010, *P.W. Crous*, CBS H-20670, culture CPC 18297; CBS H-20671, CPC 18301; Jonkershoek Nature Reserve, on leaves of *P. nitida*, 12 Apr. 2008, *L. Mostert*, CBS H-20672, culture CPC 15237; Jonkershoek Nature Reserve, on leaves of *P. repens*, 28 Jan. 1999, *J.E. Taylor*, CPC 16850, 16851.

Notes — *Brunneosphaerella jonkershoekensis* was originally described from leaf litter of *Protea repens* collected in Jonkershoek (Marincowitz et al. 2008a), but no cultures were available for study until now. *Brunneosphaerella jonkershoekensis* appears to be a serious pathogen, particularly of *P. repens*, but is presently only known from the Stellenbosch-Jonkershoek area of the Western Cape Province of South Africa. This spe-

Fig. 6 *Brunneosphaerella jonkershoekensis* (CBS H-20333). a, b. Leaf spots; c. globose ascomata visible on lesion surface; d. substomatal ascoma with central ostiole; e. vertical section through ascoma wall of *textura angularis*; f. germinating ascospore; g–i. asci; j. ascospores. — Scale bars = 10 µm.

cies has been largely overlooked and incorrectly identified as *B. protearum*. *Brunneosphaerella jonkershoekensis* is morphologically similar to *B. protearum*, but distinct in having much larger ascospores (Marincowitz et al. 2008a).

Brunneosphaerella nitidae Crous, *sp. nov.* — MycoBank MB560558; Fig. 7

Brunneosphaerellae protearum similis, sed ascosporis longioribus et angustioribus, (20–)24–28(–30) × (3–)5–6(–7) µm.

Etymology. Named after the host on which it was collected, *Protea nitida*.

Leaf spots circular to irregular, discrete to confluent, variable in size, up to 2 cm diam, medium brown, with a raised, red-brown border. *Ascomata* amphigenous, immersed to semi-immersed, becoming erumpent when mature, black, single, gregarious, 180–300 µm diam; in section, substomatal, subepidermal, pyriform or globose with a papillate, periphysate ostiole, frequently opening by means of irregular rupture when mature. *Peridium* consisting of three strata of slightly compressed *textura angularis*, an outer stratum of dark brown, thick-walled cells, becoming paler in the central stratum, and hyaline, thin-walled in the inner stratum, altogether (20–)24.5–30(–40) µm thick. *Asci* clavate to cylindro-clavate, often curved, tapering to a pedicel, narrowing slightly to a rounded apex with an indistinct ocular chamber, 8-spored, bitunicate with fissitunicate dehiscense, 65–80 × 13–15 µm. *Ascospores* biseriate, fusiform, broader at the apical end, initially hyaline and 1-septate, becoming yellow-brown and 3-septate at maturity, slightly constricted at median septum, with large central guttule per cell, widest in second cell from the apex, and having terminal globose mucoid caps, 3 µm diam, (20–)24–28(–30) × (3–)5–6(–7) µm (av. 26 × 5.5 µm).

Culture characteristics — On PDA and OA spreading, flat with moderate aerial mycelium; margins lobate, smooth; surface smoke-grey with submerged, iron-grey margin; reverse iron-grey. On MEA surface smoke-grey; reverse iron-grey. Colonies reach 20 mm diam after 2 mo on all three media.

Specimens examined. South Africa, Western Cape Province, Jonkershoek Nature Reserve, on leaf litter of *Protea nitida*, 12 Apr. 2008, *L. Mostert*, holotype CBS H-20334, culture ex-type CPC 15231 = CBS 130595; Jonkershoek Nature Reserve, on leaves of *P. nitida*, 1 Apr. 2007, *P.W. Crous*, CBS H-20330, culture CPC 13914 = CBS 130596.

Notes — *Brunneosphaerella nitidae* is only known from the Jonkershoek Valley in South Africa, where it occurs on *P. nitida*. It is morphologically similar to *B. protearum* (ascospores av. 24.5 × 6.5 µm), but can be distinguished in that on average it has longer and narrower ascospores (av. 26 × 5.5 µm). In culture (on MEA and PDA) ascomatal initials developed after 2–3 mo that formed a few asci and ascospores, suggesting that this species is homothallic. Additionally, this species can be differentiated from the other species of *Brunneosphaerella* by comparison of their TEF and CHS sequences.

Brunneosphaerella protearum (Syd. & P. Syd.) Crous, Stud. Mycol. 64: 31. 2009 — Fig. 8

Anamorph. '*Coniothyrium*' *protearum* Joanne E. Taylor & Crous, IMI Descriptions of Fungi and Bacteria No. 1343. 1998.

Basionym. *Leptosphaeria protearum* Syd. & P. Syd., Ann. Mycol. 10: 441. 1912.

Leaf spots circular to irregular, discrete to confluent, variable in size, under conditions favourable to disease symptoms more similar to a blight than a leaf spot, necrotic, sunken with a raised dark brown margin and with conspicuous black ascomata in the dead tissue, 4–30 mm diam. *Ascomata* amphigenous, immersed to semi-immersed, not erumpent, black, single, gregarious, 180–320 µm diam; in section, substomatal, subepidermal, pyriform or globose with a papillate, periphysate ostiole, immersed in a stroma consisting of deteriorated host mesophyll

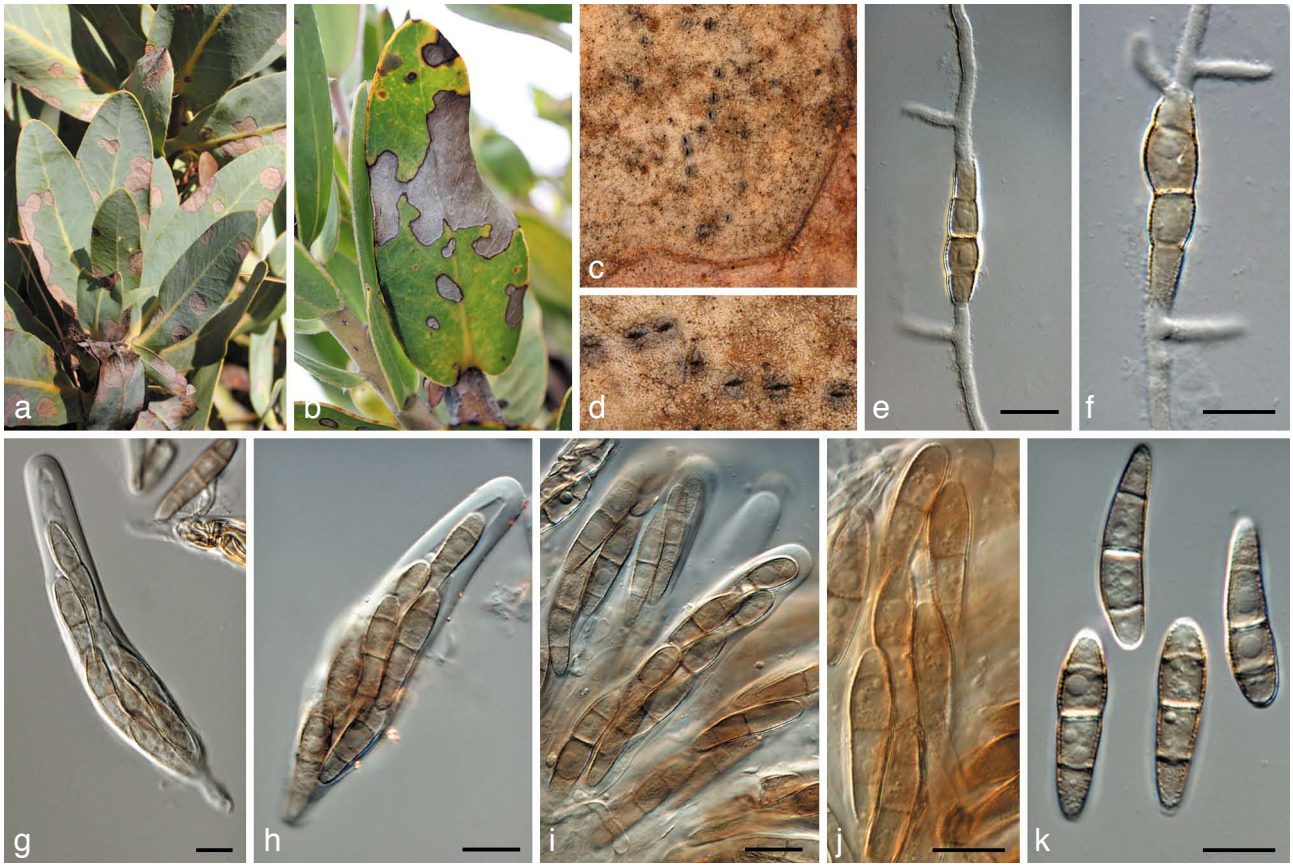

Fig. 7 *Brunneosphaerella nitidae* (CBS H-20334). a, b. Leaf spots; c, d. ascomata visible on lesion surface (note rupture in central ostiole); e, f. germinating ascospores; g–j. asci; k. ascospores. — Scale bars = 10 µm.

Fig. 8 *Brunneosphaerella protearum* (CBS H-20335). a, b. Leaf spots; c, d. globose ascomata visible on lesion surface (note rupture in central ostiole); e, f. germinating ascospores; g–j. asci; k. ascospores. — Scale bars = 10 µm.

cells filled with fungal hyphae, (210–)230–264(–288) µm high, (180–)200–255(–300) µm diam. *Peridium* consisting of three strata of slightly compressed *textura angularis*, an outer stratum of dark brown, thick-walled cells, becoming paler in the central stratum, and hyaline, thin-walled in the inner stratum, altogether (20–)24.5–37.5(–50) µm thick. *Asci* clavate to cylindro-clavate, often curved, tapering to a pedicel, narrowing slightly to a rounded apex with an indistinct ocular chamber, 8-spored, bitunicate with fissitunicate dehiscense, (70–)80–87.5(–105) × (13.5–)14.5–16(–21.5) µm. *Ascospores* biseriate, fusiform, broader at the apical end, initially hyaline and 1-septate, becoming yellow-brown and 3-septate at maturity, slightly constricted at median to supra-median septum, with large central guttule per cell, widest in second cell from the apex, and having globose mucoid caps, 2 µm diam, (20–)23–26(–30) × (5–)6–7(–8) µm (av. 24.5 × 6.5 µm). *Conidiomata* barely visible and interspersed between ascomata, pycnidial, subepidermal, substomatal, separate, globose to pyriform, occasionally with well-developed papilla, dark brown, < 200 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* discrete, smooth, hyaline, doliiform to ampulliform, holoblastic, proliferating 1–2 times percurrently, 4–6 × 3–4 µm. *Conidia* pale brown to medium brown, thick-walled on maturity, smooth to finely verruculose, eguttulate, ellipsoidal to globose, often truncate at one end, 5–10 × 3–7 µm.

Culture characteristics — On OA spreading, flat with moderate aerial mycelium; margins lobate, smooth; surface smoke-grey with submerged, iron-grey margin. On PDA similar, surface smoke-grey with broad, iron-grey margin; reverse iron-grey. On MEA surface folded, smoke-grey with patches of dirty white and olivaceous-grey and submerged iron-grey margin; reverse iron-grey. Colonies reach 35 mm diam after 2 mo on all three media.

Specimens examined. South Africa, Western Cape Province, Wellington, on leaves of *Protea lepidocarpodendron* (as *P. melaleuca*), 22 Feb. 1912, *E.M. Doidge*, holotype PREM 2061; Kirstenbosch Botanical Garden, on leaves of *Protea* sp., 13 Jan. 2009, *P.W. Crous*, epitype designated here as CBS H-20335, culture ex-epitype CPC 16338 = CBS 130597; Kirstenbosch Botanical Garden, on leaves of *P. coronata*, 8 May 2010, *P.W. Crous*, CBS H-20673, culture CPC 18308 = CBS 130598; Harold Porter Botanical Garden, Betties Bay, on leaves of *P. mundii*, 4 May 2010, *P.W. Crous*, CBS H-20683, culture CPC 18328; Stellenbosch, J.S. Marais Garden, S33°55'59.3" E18°52'22.5", on living leaves of *P. magnifica*, 1 Apr. 1998, *J.E. Taylor*, culture CPC 16849.

Notes — *Brunneosphaerella protearum* appears to have a broad host range, and is widely distributed on *Protea* hosts in South Africa (Crous et al. 2004a). Although highly similar in ITS, the three *Brunneosphaerella* species treated in this study can be resolved easily based on their diagnostic TEF and CHS gene sequences (Fig. 2).

Coniothyrium leaf spot

Coniothyrium-like species are commonly associated with necrotic spots at the tips or margins of leaves of *Proteaceae* (Swart et al. 1998). As such, they are regularly intercepted during phytosanitary inspections. Many of these species have no known teleomorph, or are anamorphs of *Teratosphaeriaceae*, or other species belonging to *Dothideomycetes* (Schoch et al. 2006, 2009, Crous et al. 2007a, 2009a, b, Zhang et al. 2009). Recent studies have shown that many species of *Phoma* produce conidia that become dark and thick-walled with age, appearing *Coniothyrium*-like in morphology (Aveskamp et al. 2009, 2010, de Gruyter et al. 2009, 2010). For that reason the taxonomy of the *Coniothyrium*-like species occurring on *Proteaceae* was reevaluated.

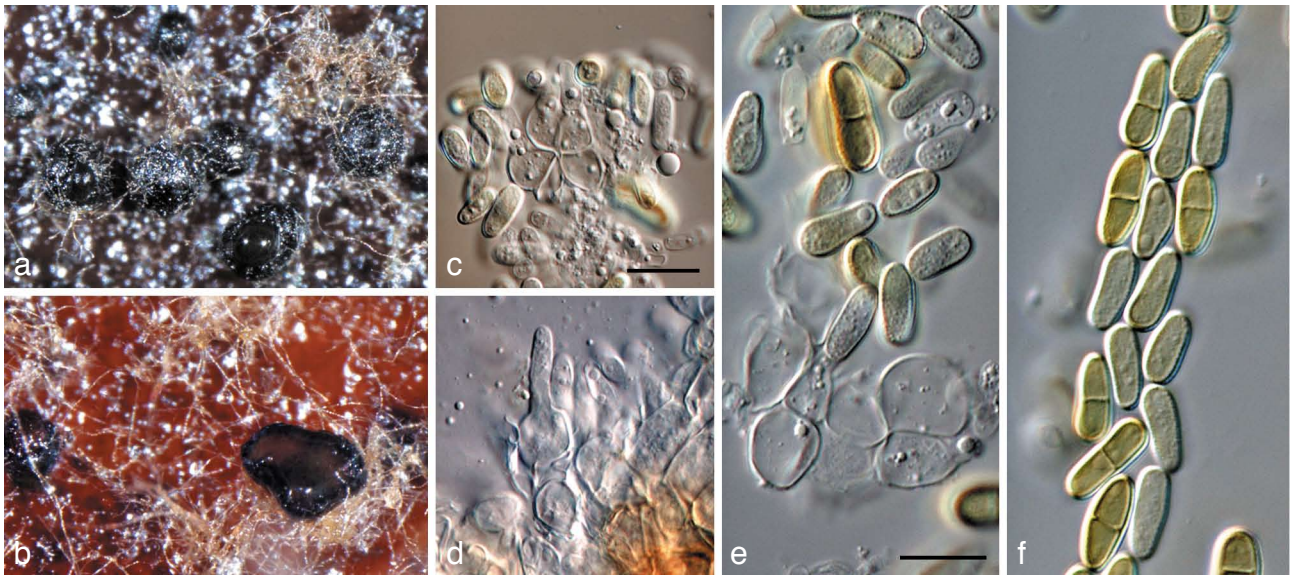

Fig. 9 *Coniothyrium nitidae* (CBS 111322). a, b. Colonies sporulating on MEA; c–e. conidiogenous cells giving rise to conidia; f. pigmented, verruculose, 1-septate conidia. — Scale bars = 10 µm.

Coniothyrium nitidae Crous & Denman, S. African J. Bot. 64: 138. 1998 — Fig. 9

Specimen examined. South Africa, Western Cape Province, Hermanus, on leaves of *Protea nitida*, 29 Aug. 1996, *S. Denman*, holotype PREM 55346, cultures ex-type CPC 1476 = CBS 111322, CPC 1477 = CBS 111321, CPC 1478 = CBS 111302, CPC 1532 = CBS 111380.

Notes — *Coniothyrium nitidae* is not a member of *Coniothyrium* s.str in the *Leptosphaeriaceae* (Zhang et al. 2009), but clusters in *Didymellaceae*. However, given the nature of its conidia, becoming 1-septate, dark brown and verruculose with age, it does not fit into any genus within the family as discussed by Aveskamp et al. (2010). We thus retain this species under its current name until this generic complex has been better resolved.

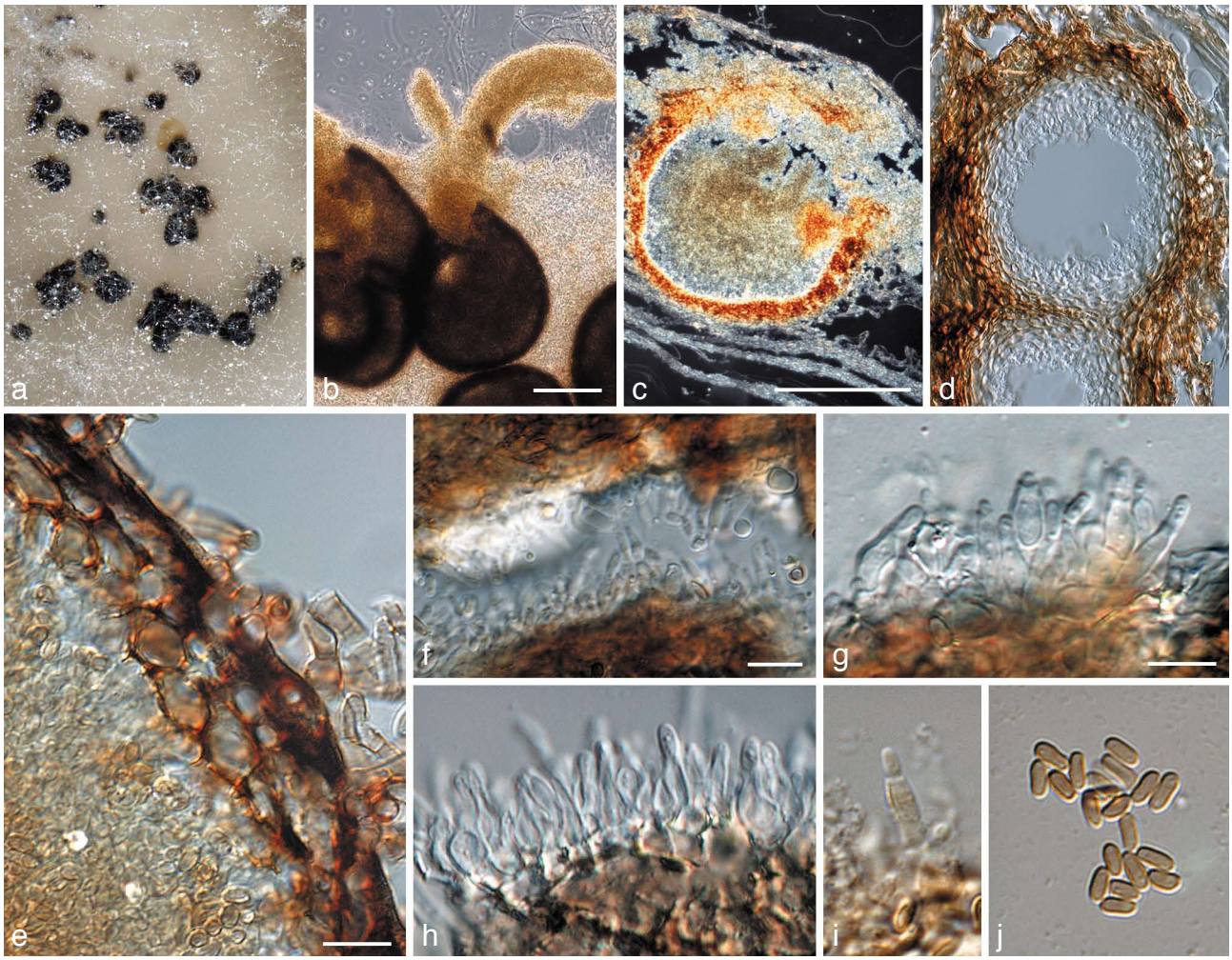

Fig. 10 *Curreya grandicipis* (CBS 114272). a, b. Colonies sporulating on OA; c, d. vertical sections through conidiomata; e. conidiomatal wall of *textura angularis*; f–i. conidiogenous cells giving rise to conidia; j. pigmented conidia. — Scale bars: b, c = 150 µm, all others = 10 µm.

Curreya grandicipis (Joanne E. Taylor & Crous) Joanne E. Taylor & Crous, *comb. nov*. — MycoBank MB560559; Fig. 10

Basionym. *Coniothyrium grandicipis* Joanne E. Taylor & Crous, In Crous et al., Cultivation and diseases of Proteaceae: Leucadendron, Leucospermum and Protea: 60. 2004.

Specimen examined. South Africa, Western Cape Province, Elgin, on leaves of *Protea grandiceps*, 20 July 1998, *J.E. Taylor & S. Denman*, holotype PREM 56616, cultures ex-type CPC 1852 = CBS 114272, CPC 1853 = CBS 111702.

Notes — The type species of *Coniothyrium*, *C. palmarum*, is allied to *Leptosphaeriaceae* (Zhang et al. 2009). The generic type of *Curreya* is *C. conorum* (*Cucurbitariaceae*), which is reported to have a *Coniothyrium*-like anamorph (von Arx & van der Aa 1983). Marincowitz et al. (2008a) also induced *Coniothyrium*-like anamorphs for species of *Curreya* in culture, and according to Fig. 1 it appears that '*Coniothyrium' grandicipis* is best placed in *Curreya*, though the distinction between *Curreya* and *Misturatosphaeria* (Mugambi & Huhndorf 2009) is less clear at this stage (Fig. 1). Furthermore, von Arx & van der Aa (1983) list several *Curreya*-like teleomorph genera that have *Coniothyrium*-like anamorphs, revealing this generic complex to be in need of urgent taxonomic revision.

Microsphaeropsis proteae (Crous & Denman) Crous & Denman, *comb. nov*. — MycoBank MB560560; Fig. 11

Basionym. *Coniothyrium proteae* Crous & Denman, S. African J. Bot. 64: 139. 1998.

Specimen examined. South Africa, Western Cape Province, Hermanus, on leaves of *Protea nitida*, *S. Denman*, 29 Aug. 1996, holotype PREM 55347, cultures ex-type CPC 1423 = CBS 111303, CPC 1424 = CBS 111320, CPC 1425 = CBS 111319.

Notes — *Coniothyrium proteae* produces thin-walled conidia, 5–8 × 3.5–4 µm in vivo, 3–4 × 2–2.5 µm in vitro (Swart et al. 1998), that become brown with age, and phialidic conidiogenous cells that proliferate with periclinal thickening or with percurrent proliferation on conidiogenous cells (in old conidiomata), appearing *Coniothyrium*-like. Phylogenetically it is closely allied to species in the *M. olivacea* complex.

Diaporthe and Diplodina leaf spots and cankers

To date *Phomopsis saccharata* has been the only species of *Phomopsis* (teleomorph: *Diaporthe*) described from *Proteaceae*. The fungus causes a severe canker and dieback disease on *Protea repens* both in natural and cultivated stands in the Western and Eastern Cape Province of South Africa (Orffer & Knox-Davies 1989). However a number of other records of *Diaporthe* associated with *Proteaceae* exist, for example the *Phomopsis* state of a *Diaporthe* sp. was recorded in South Africa by Benic (1986) on dead *P. repens* mistbed cuttings showing basal and tip dieback and necrosis of leaves, as well as on seeds of different species of *Proteaceae*. A Diaporthe shoot and stem canker of *Protea* spp. was recorded in Queensland, Australia, and that fungus was reported to enter through wounds and cause sunken lesions which result in death of branches and entire plants (Greenhalgh 1981). Moura & Rodrigues (2001) reported a *Diaporthe* sp. on stems of *Protea cynaroides* in Madeira, Portugal. *Diaporthe*/*Phomopsis* spp. have also been recorded as endophytes on *Proteaceae* (Swart et al. 2000, Taylor et al. 2001c), but until now the significance of this occurrence has not been given context on *Proteaceae*. In accordance with the Amsterdam Declaration for pleomorphic fungi (Hawksworth et al. 2011), preference is given to the older generic name *Diaporthe* (1870), rather than the younger *Phomopsis* (1905) (see Crous et al. 2011a).

Diaporthe leucospermi Crous & Summerell, *sp. nov.* — MycoBank MB560561; Fig. 12

Alpha conidia aseptata, guttulata, ellipsoidea, (6–)7(–8) × (2.5–)3 µm. Beta conidia aseptata, fusiformia, (20–)25–30(–35) × (1–)1.5 µm.

Etymology. Named after the host on which it occurs, *Leucospermum*.

On OA. *Conidiomata* pycnidial, dark brown, imbedded, solitary to aggregated, opening via a central ostiole, exuding a creamy white conidial cirrhus; pycnidia up to 300 µm diam; wall consisting of several layers of dark brown *textura angularis*. *Conidiophores* lining the inner cavity, subcylindrical, hyaline (though pale brown at base), smooth, reduced to conidiogenous cells, or 1–3-septate, 15–30 × 2–3 µm. *Conidiogenous cells* phialidic, apical or lateral, hyaline, smooth, subcylindrical with apical taper, 10–15 × 2–3 µm; apex with visible periclinal thickening and flaring collarette, 1 µm long. *Alpha conidia* hyaline, smooth, aseptate, with two prominent guttules, ellipsoid, tapering to acutely rounded apex and obtuse to truncate base, (6–)7(–8) × (2.5–)3 µm; hilum with flattened scar, 1 µm diam. *Beta conidia* hyaline, smooth, aseptate, spindle-shaped, prominently hooked in apical part, apex acute, base truncate, (20–)25–30(–35) × (1–)1.5 µm.

Culture characteristics — Colonies spreading, flat, with sparse to moderate aerial mycelium, covering dish in 2 wk; on OA growing with concentric zones, middle olivaceous-grey, with alternating zones of smoke-grey and olivaceous-buff; on PDA pale olivaceous-grey to smoke-grey, reverse olivaceous-grey; on MEA surface dirty white with patches of olivaceous-buff to smoke-grey, reverse olivaceous.

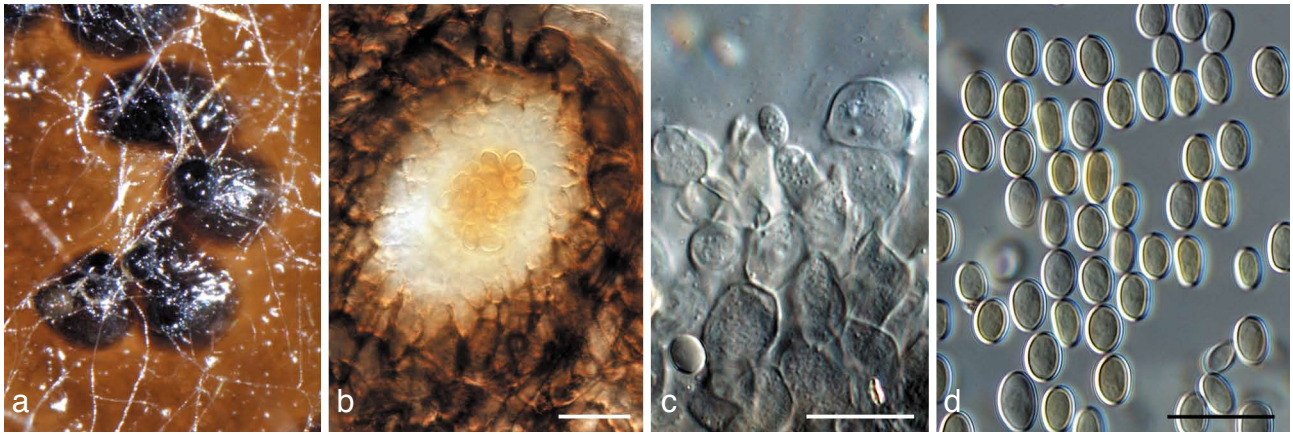

Fig. 11 *Microsphaeropsis proteae* (CBS 111303). a. Colonies sporulating on OA; b. central ostiole with oozing conidia; c. conidiogenous cells giving rise to conidia; d. pigmented conidia. — Scale bars = 10 µm.

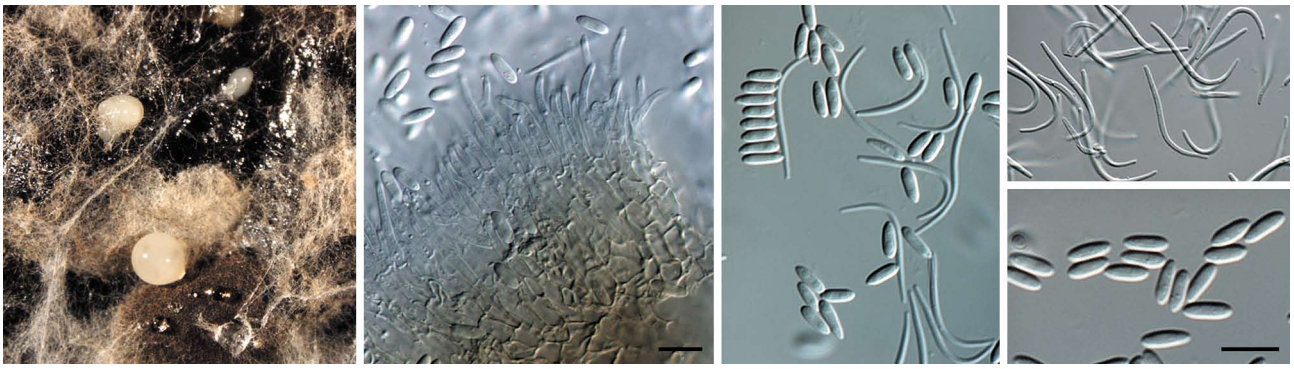

Fig. 12 *Diaporthe leucospermi* (CBS 111980). a. Colonies sporulating on PDA; b. conidiogenous cells giving rise to conidia; c. alpha and beta conidia; d. beta conidia; e. alpha conidia. — Scale bars = 10 µm.

Specimen examined. Australia, New South Wales, the Blue Mountains Botanic Gardens, Mount Tomah, on leaves of *Leucospermum* sp., Aug. 1999, *P.W. Crous & B. Summerell*, CBS H-20674 holotype, culture ex-type CPC 2956 = CBS 111980.

Notes — The ITS sequence of this species is 100 % identical to *Diaporthe* sp. (GenBank GQ250223) from *Hydrangea macrophylla* in Portugal, *Diaporthe* sp. (GenBank EU002916) isolated as a fruit endophyte from *Coffea arabica* in Hawaii and *Diaporthe* sp. (GenBank GQ250207) from *Acer negundo* in Portugal. Whether this species represents an endophyte with a broader host range remains to be tested.

Diplodina microsperma (Johnst.) B. Sutton, Mycol. Pap. 141: 69. 1977 — Fig. 13

Basionym. *Stilbospora microsperma* Johnst., in Johnston, A Flora of Berwick-upon-Tweed 2: 192. 1831.

= *Sphaeria apiculata* Wallr., Fl. Crypt. Germ. 2: 778. 1833.

≡ *Metasphaeria apiculata* (Wallr.) Sacc., Syll. Fung. 2: 166. 1883.

≡ *Gnomonia apiculata* (Wallr.) G. Winter, Rabenh., Kryptog.- Fl., ed. 2, vol. 1, 2: 589. 1887.

≡ *Diaporthe spina* Fuckel var. *apiculata* (Wallr.) Rehm, Ann. Mycol. 7: 404. 1909.

≡ *Cryptodiaporthe apiculata* (Wallr.) Petr., Ann. Mycol. 19: 177. 1921.

≡ *Plagiostoma apiculatum* (Wallr.) L.C. Mejía, Stud. Mycol. 68: 219. 2011.

Associated with leaf spots, and initially suspected to represent a species of *Diaporthe*. *Conidiomata* brown, multilocular, up to 400 µm diam, globose to depressed, immersed on MEA, superficial on PNA, opening by means of irregular rupture. *Conidiophores* lining the cavity, base pale brown, becoming hyaline towards apex, smooth, densely aggregated, irregularly branched, 1–3-septate, subcylindrical, 10–25 × 2–3 µm. *Conidiogenous cells* phialidic, pale brown to hyaline, smooth, terminal or lateral, doliiform to ampulliform, tapering towards a truncate apex with visible periclinal thickening, 5–12 × 2–2.5 µm. *Conidia* hyaline, smooth, guttulate, thick-walled when mature, fusiform, straight to curved, medianly 1-septate, apex acutely rounded, base truncate, (10–)12–14(–17) × (2–)3–3.5(–4) µm.

Culture characteristics — Colonies on MEA and OA covering the plate within 1 mo; colonies on MEA with abundant aerial mycelium, cream to dirty white with patches of sienna and umber; similar on OA, but also with patches of olivaceous-grey.

Specimen examined. New Zealand, on leaves of *Protea* sp., intercepted specimen of flowers exported to California, USA, LA143956, carrier UA 842, 11 Mar. 1999, *M.A. Abdelshife & M.E. Palm*, CBS H-20675, culture CPC 2336 = CBS 114545.

Notes — The genus *Diplodina* has *Plagiostoma* (= *Cryptodiaporthe*) teleomorphs (Mejía et al. 2011). The ITS, partial beta-tubulin, and TEF sequences of the isolate from *Protea* is identical to *Diplodina microsperma* (= *Plagiostoma apiculatum*) from *Salix dasyclados* in France (Sogonov et al. 2008, Mejía et al. 2011) (GenBank ITS: GU367068, *Cryptodiaporthe apiculata*) and *Salix sitchensis* from Washington, USA (GenBank ITS: GU367066, as *Cryptodiaporthe apiculata*; GenBank TUB: GU367009, as *Cryptodiaporthe apiculata*; GenBank TEF:

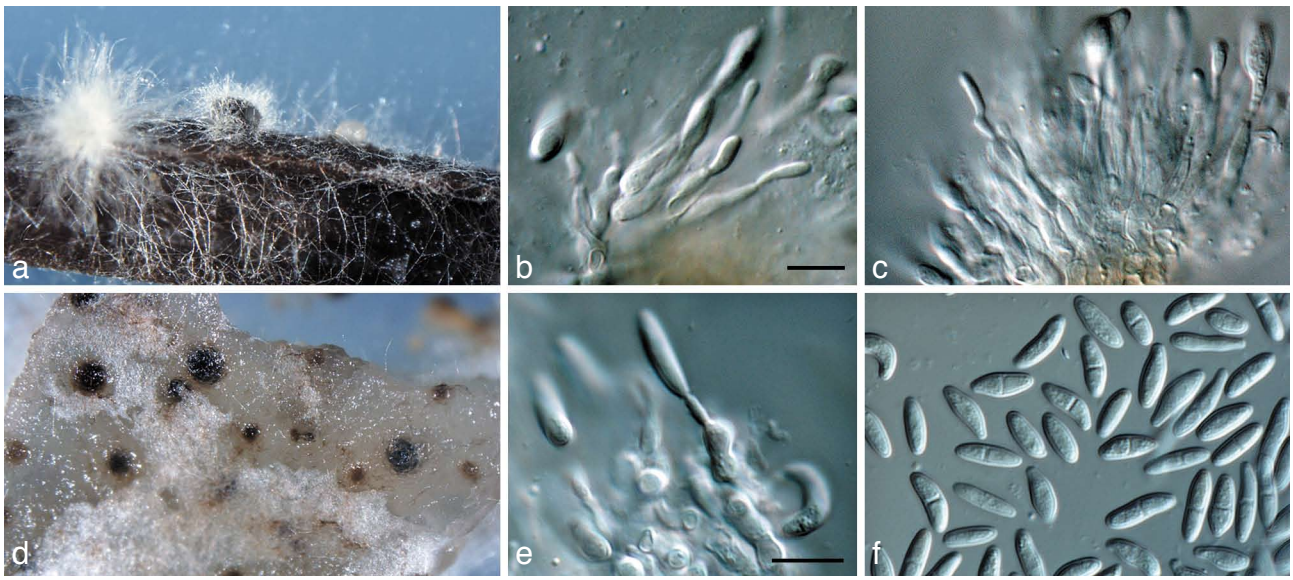

Fig. 13 *Diplodina microsperma* (CBS 114545). a. Colonies sporulating on sterile pine needles; b, c. conidiogenous cells giving rise to conidia; d. conidiomata forming on OA; e. conidiogenous cells giving rise to conidia; f. 0–1-septate conidia. — Scale bars = 10 µm.

GU353991, as *Cryptodiaporthe apiculata*). The ITS is also identical to *Cryptodiaporthe salicella* from *Salix* sp. in Austria (GenBank ITS: DQ323529), but differs on TUB (GenBank GU367008; identities = 718/725 (99 %), Gaps = 6/725 (1 %)) and TEF (GenBank EU221916; very little identity). *Diplodina microsperma* is a pathogen of *Salix*, thus it is unusual to find this species occurring on *Proteaceae*. Although conidia of the present isolate appear somewhat smaller than that known for *D. microsperma* (Sogonov et al. 2008, Mejía et al. 2011), more collections are required, and cross pathogenicity trials will need to be conducted to determine if this is simply a chance occurrence, or a real pathogen of *Proteaceae*.

Fusicladium leaf spot

Fusicladium proteae Crous, *sp. nov.* — MycoBank MB560562; Fig. 14

Conidiophora solitaria, erecta, subcylindrica, recta vel curvata, 1–6-septata, 20–70 × 3–4 µm. Cellulae conidiogenae integratae, terminales, 15–40 × (3–)4–5 µm, cicatricibus conidialibus in parte apicali, marginaliter fuscatis et incrassatis, 1.5–2 µm diam. Conidia solitaria, obpyriformia, inequaliter 1-septatis, (13–)17–22(–30) × 4(–5) µm.

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

Mycelium consisting of smooth to finely verruculose, medium brown, septate, branched, 2.5–4 µm diam hyphae. *Conidiophores* medium brown, smooth, solitary, erect, subcylindrical, straight to curved, or once geniculate, 1–6-septate, 20–70 × 3–4 µm. *Conidiogenous cells* integrated, terminal on medium brown, smooth, subcylindrical, straight to geniculate-sinuous, 15–40 × (3–)4–5 µm; scars aggregated in apical part, darkened and thickened along the rim, 1.5–2 µm diam. *Conidia* solitary, pale to medium brown, smooth, guttulate, obpyriform, widest at obconically truncate base, that tapers abruptly to a darkened, thickened hilum, 1.5–2.5 µm; unequally 1-septate, with septum in lower third of the conidium (6–9 µm from base), tapering to an acutely rounded apex, (13–)17–22(–30) × 4(–5) µm.

Culture characteristics — Colonies flat, spreading, with moderate aerial mycelium (on PDA and MEA, sparse on OA), with even, lobate margins, reaching 40 mm diam after 2 mo. On OA olivaceous-grey with patches of smoke-grey; on PDA smoke-grey with patches of olivaceous-grey; on MEA surface with patches of smoke-grey and iron-grey, reverse iron-grey with smoke-grey in outer region.

Specimen examined. South Africa, Western Cape Province, Hermanus, Fernkloof Nature Reserve, on leaves of *Protea* sp., in association with *Vizella interrupta*, 5 May 2010, *P.W. Crous*, holotype CBS H-20677, culture ex-type CBS 130599 = CPC 18282.

Notes — No other species of *Fusicladium* has thus far been reported from *Proteaceae* (Schubert et al. 2003). DNA sequence data (ITS) of *Fusicladium proteae* are not identical to any other species of *Fusicladium* presently in GenBank. Although the genus *Fusicladium* (1851) is older than its teleomorph, *Venturia* (1882), the latter is far more commonly used, and a proposal will have to be prepared to conserve *Venturia* over *Fusicladium*.

Pestalotiopsis leaf spot

Two species of *Pestalotiopsis* have been described from *Proteaceae*. *Pestalotiopsis montellicoides* was isolated from *Protea cynaroides* leaves from South Africa (Mordue 1986), and a *Pestalotiopsis* sp., the anamorph of *Pestalosphaeria leucospermi*, was described from living leaves of a *Leucospermum* sp. in New Zealand (Samuels et al. 1987). In Zimbabwe, a species of *Pestalotiopsis* was recorded causing leaf spots on several *Protea* and *Leucospermum* hosts (Swart et al. 1999), and has subsequently been intercepted at quarantine inspection points (Taylor 2001). This species is named as new below. The anamorph genus *Pestalotiopsis* (1949) is more commonly used, and older than the teleomorph genus *Pestalosphaeria* (1975), and hence has precedence.

Pestalotiopsis protearum Crous & L. Swart, *sp. nov.* — MycoBank MB560563; Fig. 15

Pestalotiopsis montellicoidis similis, sed conidiis minoribus, in medio tricellularibus, cellulis (14–)16–17(–18) × (6.5–)8–9(–10) µm.

Etymology. Named after its occurrence on *Proteaceae*.

Leaf spots irregular, necrotic, associated with leaf margins or causing tip dieback; slightly sunken, pale brown with red-brown margins that are mostly raised and distinct, rarely diffuse, 2–35 mm diam. *Conidiomata* amphigenous, pycnidioid to acervular, immersed, becoming erumpent, unilocular, dark brown to black, dehiscing by irregular splits in the apical wall and the overlying host tissue, scattered, (100–)195–240(–400) µm; in section pyriform or conical, with applanate base, intra-epidermal in origin (125–)138–165(–180) µm wide, and (125–)138–165(–180) µm diam. *Peridium* comprising two strata of *textura angularis*, an outer stratum of pale brown, thick-walled cells becoming hyaline in the inner layer, apical and lateral walls composed of slightly compressed, thinner-walled cells; basal wall (13–)17–21(–23) µm, apical wall (7–)11–17(–19) µm thick. *Conidiophores* peripheral, reduced to conidiogenous cells, invested in mucus. *Conidiogenous cells* discrete, ampulliform, hyaline, smooth, (4–)5.5–6.5(–8) × (2–)4–5(–6) µm; conidiogenesis initially holoblastic,

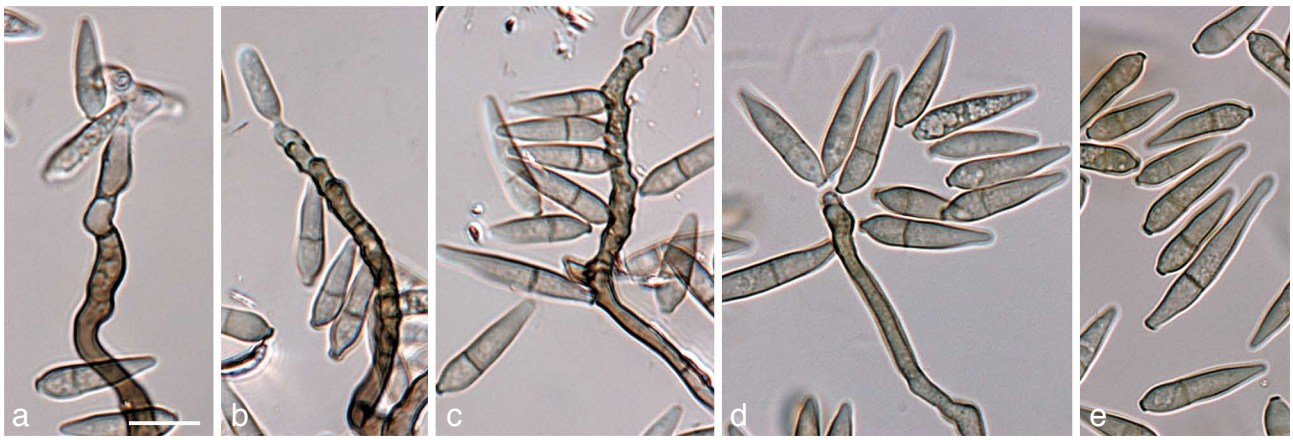

Fig. 14 *Fusicladium proteae* (CBS H-20677). a–d. Conidiophores with conidiogenous cells giving rise to conidia; e. 1-septate conidia. — Scale bar = 10 µm.

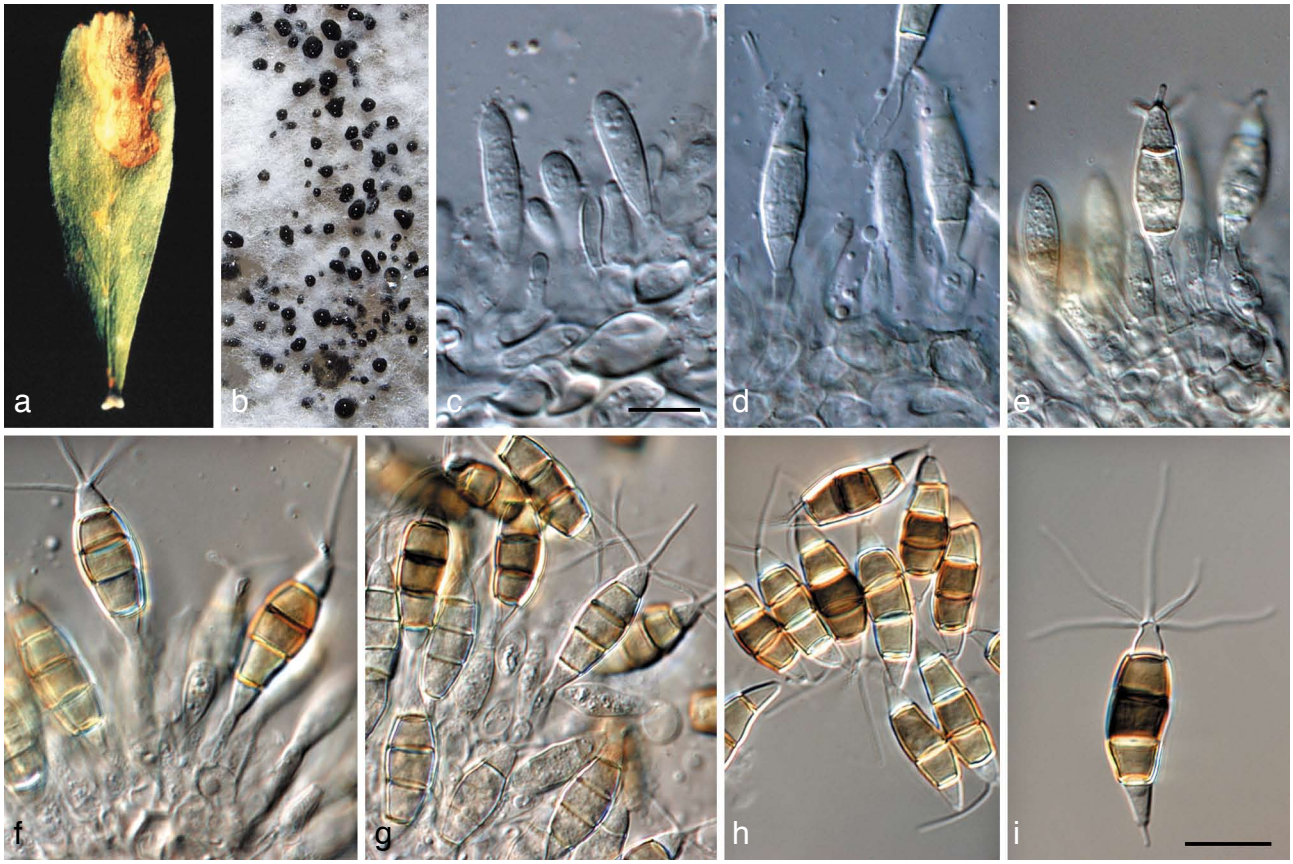

Fig. 15 *Pestalotiopsis protearum* (CBS 114178). a. Leaf spot on *Leucospermum cuneiforme*; b. colony sporulating on PDA; c–g. conidiogenous cells giving rise to conidia; h, i. appendaged conidia. — Scale bars = 10 µm.

with up to two enteroblastic, percurrent proliferations to produce additional conidia at slightly higher levels. *Conidia* ellipsoidal to obovoid, 4-euseptate, the second and third septa often darkened and indistinct, cells unequal, without constrictions at the septa, versicoloured, bearing appendages; basal cell obconic with a truncate base, bearing minute marginal frills, hyaline below, thin-walled, (3.5–)5–6(–7.5) × 4–4.5(–5) µm; second cell from base subcylindrical, pale brown, verruculose, (4–)5–5.5(–6) µm, third and fourth cells doliiform to subcylindrical, dark red-brown, verruculose, (4–)5–6(–7) µm and (4–)5.5–6(–7) µm long, respectively, combined dimensions of 3 median cells (14–)16–17(–18) × (6.5–)8–9(–10) µm; apical cell subconical, hyaline, collapsed at maturity, thin-walled, smooth, (3–)3.5–4.5(–6) × (3–)3.5–4(–5) µm; 2–4 appendages arising apically, tubular, branched or not, straight to flexuous, tip rounded, (15–)26–32(–43) µm long; basal appendage occasionally absent, filiform, flexuous, slender, centric, (2–)4.5–6(–9) µm. *Conidia in vitro* ellipsoidal to obovoid, 4(–6)-euseptate, the second and third septa often darkened and indistinct, cells unequal, without constrictions at the septa, versicoloured, bearing appendages; basal cell obconic with a truncate base, bearing minute marginal frills, hyaline below, thin-walled, (4–)5–6(–8) × 4–4.5(–5) µm; second cell subcylindrical, pale brown, faintly verruculose, third and fourth cells doliiform to subcylindrical, medium brown, verruculose, combined lengths of median cells (14.5–)16–17(–19) × (6–)7–7.5(–8) µm [length of second cell from base (5–)5.5–6(–7) µm; central cell, (4–)5–5.5(–7) µm; fourth cell, (4.5–)5–5.5(–6) µm] apical cell subconical, hyaline, collapsed at maturity, thin-walled, smooth, (3.5–)4–4.5(–5) × 3.5–4(–5) µm; appendages tubular, branched, straight to flexuous; (1–)2–4(–5) appendages arising apically, tip rounded but often absent due to frequent breakage of appendage, (10–)15–17(–22) µm long; basal appendage occasionally absent, filiform, flexuous, slender, centric, (2–)3–3.5(–5) µm.

Culture characteristics — Colonies circular with undulate margins; mycelium of medium density, woolly, with white aerial mycelium, white in reverse; 69 mm diam after 7 d at 25 °C. Conidiomata fertile after 12 d at 25 °C under white light, with conidiomata developing over the entire surface of the colony and producing black, wet spore masses.

Specimen examined. Zimbabwe, Harare, Aveley Farm, on living leaves of *Leucospermum cuneiforme* cv. 'Sunbird', 6 Mar. 1998, *L. Swart*, holotype PREM 56186, culture ex-type CPC 1765 = CBS 114178.

Notes — *Pestalotiopsis montellicoides* differs from *P. protearum* from Zimbabwe in the larger dimensions of the conidia of the former, specifically the size of three median cells (26–35 × 7.5–10.5 µm). The *Pestalotiopsis* anamorph of *Pestalosphaeria leucospermi* from *Leucospermum* (Samuels et al. 1987) however, has conidia of similar dimensions to *P. protearum*, but the conidia of the former have concolorous median cells and more cylindrical conidiogenous cells (11–18 × 2–2.5 µm). In culture, *Pestalosphaeria leucospermi* becomes green-yellow (also noted for some Zimbabwean isolates, e.g. CPC 1783), and produces conidiomata in distinct concentric rings all over the surface of the colony. The combination of these features indicates that the collections in the present study represent a distinct species. When compared to the *Pestalotiopsis* species in the key provided by Nag Raj (1993), the species from Zimbabwe does not correspond to any previously described species, although it is most similar in dimensions and morphology to *P. macrospora*. In a recent study of *Pestalotiopsis* and allied genera (Jeewon 2002), several isolates from *Proteaceae* were included and identified as follows: *Pestalotiopsis longisetula*, *Leucospermum* sp., (CPC 1771) (Sicily); *Pestalotiopsis aquatica*, *Leucospermum* sp., (JT 615) (USA, Hawaii); *Pestalotiopsis leucothoës*, *Telopea* sp., (JT 551) (USA, Hawaii); *Pestalotiopsis sydowiana*, endophyte from *P. neriifolia*, (JT 258) (South Africa); *Pestalotiopsis theae*, endophyte from *Protea neriifolia*, (JT 258)

(South Africa); *Pestalotiopsis vismiae*, *Leucospermum* sp., (JT 694) (USA, Hawaii). Attempts to use the ITS sequences of these isolates in a blast search of NCBIs GenBank nucleotide database were inconclusive with regard to species identification as several species with near identical sequences were obtained. Also, studying the GenBank ITS sequences of strains associated with the obtained species names did not yield a conclusive barcode representative of each of those species. The genus *Pestalotiopsis* requires major revision, incorporating type studies with multi-locus DNA sequence data and cultures.

Pyrenophora blight

Two *Drechslera* morphs of *Pyrenophora* species have been reported from *Proteaceae*, namely *D. biseptata* and *D. dematioidea*. Shoemaker (1998) placed *D. biseptata* and *D. dematioidea* in a new genus, *Marielliottia*, based on their unusual conidial morphology, while a phylogenetic study by Zhang & Berbee (2001) concluded that *Marielliottia* was best retained in *Drechslera*. In accordance with the Amsterdam Declaration for pleomorphic fungi (Hawksworth et al. 2011), preference is given to the older generic name *Pyrenophora* (1849), rather than the younger *Drechslera* (1930).

In spite of published reports, the present study revealed the disease to be mainly associated with a single species, described here as *Pyrenophora leucospermi.* This pathogen causes a severe blight of current-season leaves, stems, and flower heads, particularly during growth flushes. The disease is characterised by discrete leaf lesions and slowly expanding stem cankers that result in death of young shoot tips, or as moderately expanding lesions that are restricted to a few leaves or to leaf tips only. The leaf lesions are yellow, grey or brown and irregularly shaped with distinct red margins, which rapidly enlarge, often covering the entire leaf (von Broembsen, 1986, 1989, Forsberg 1993). The presence of *Neofusicoccum* spp., which act as important secondary pathogens of the stem cankers caused by *P. leucospermi*, results in significantly larger lesions than with either alone.

Pyrenophora leucospermi Crous & L. Swart, *sp. nov.* — MycoBank MB560564; Fig. 16

Drechslerae dematoideae similis, sed conidiis 3–4(–7)-septatis.

Etymology. Named after its occurrence on *Leucospermum*.

Conidiophores on host material erect, medium yellow-brown, base slightly enlarged, 7–9(–15) µm, stalk nearly straight, septa approximately 15 µm apart, scars few (1–5), close and dark brown, apex truncate, 22–135 × 4–8 µm. *Conidiogenous cells* tretic, integrated, terminal, proliferating sympodially, cylindrical, cicatrised. *Conidia* mostly obovoid to nearly cylindrical, finely verruculose, if 4-septate, widest at the third cell from the base, with hemi-spherical apex, hemi-ellipsoidal base, pale to medium yellow-brown, but the basal cell paler brown, 3–4(–7)-septate, slightly constricted at the basal septum, with non-protruding scar, (1.5–)3 µm wide, (27–)30–38(–50) × (10–)11–13(–15) µm on SNA.

Culture characteristics — Colonies on MEA after 1 mo at 25 °C in the dark spreading, flat, with patches of scarlet and olivaceous-grey (surface), and iron-grey in reverse. On OA iron-grey with olivaceous-grey aerial mycelium.

Specimens examined. South Africa, Western Cape Province, Stellenbosch, Elsenburg Farm, on leaves of *Leucospermum cordifolium*, 7 Mar. 1996, *L. Swart*, CBS H-20676 holotype, cultures ex-type CPC 1293 = CBS 111083, CPC 1294 = CBS 111084, CPC 1295 = CBS 111085, CPC 1296 = CBS 111080, CPC 1297 = CBS 111086, CPC 1298 = CBS 111087.

Notes — Pyrenophora blight is the most important disease of commercially cultivated *Leucospermum*, particularly the cultivars developed from *Lsp. cordifolium*. Some *Leucadendron* and *Mimetes* spp. are also susceptible to blight (von Broembsen 1989). Although '*D.*' *biseptata* is reported as the main cause of disease of *Leucospermum* in the southern states of Australia and Queensland, both '*D.*' *biseptata* and '*D.*' *dematoidea* are reported to cause blight on *Leucospermum* in Hawaii (Boesewinkel 1986, Forsberg 1993). A different *Pyrenophora* sp. that causes leaf spot and blight was reported from the North Island of New Zealand (Soteros 1986), while Shoemaker (1998) also found material of the *Bipolaris* anamorph of *Cochliobolus australiensis* to be present in *Pyrenophora*-like lesions, and concluded that more species were involved in this disease complex. In spite of several collections investigated in the present study (Table 1), no authentic cultures of '*D.*' *biseptata* or '*D.*' *dematoidea* were obtained from symptomatic *Proteaceae* in South Africa (Fig. 2). Furthermore, the species commonly associated with Pyrenophora blight in South Africa, which was also collected in Europe, appeared to represent a novel species, described here as *P. leucospermi* (Fig. 2). *Pyrenophora leucospermi* can be distinguished from '*D.*' *biseptata* and '*D.*' *dematoidea* by having conidia that are 3–4(–7)-septate, and

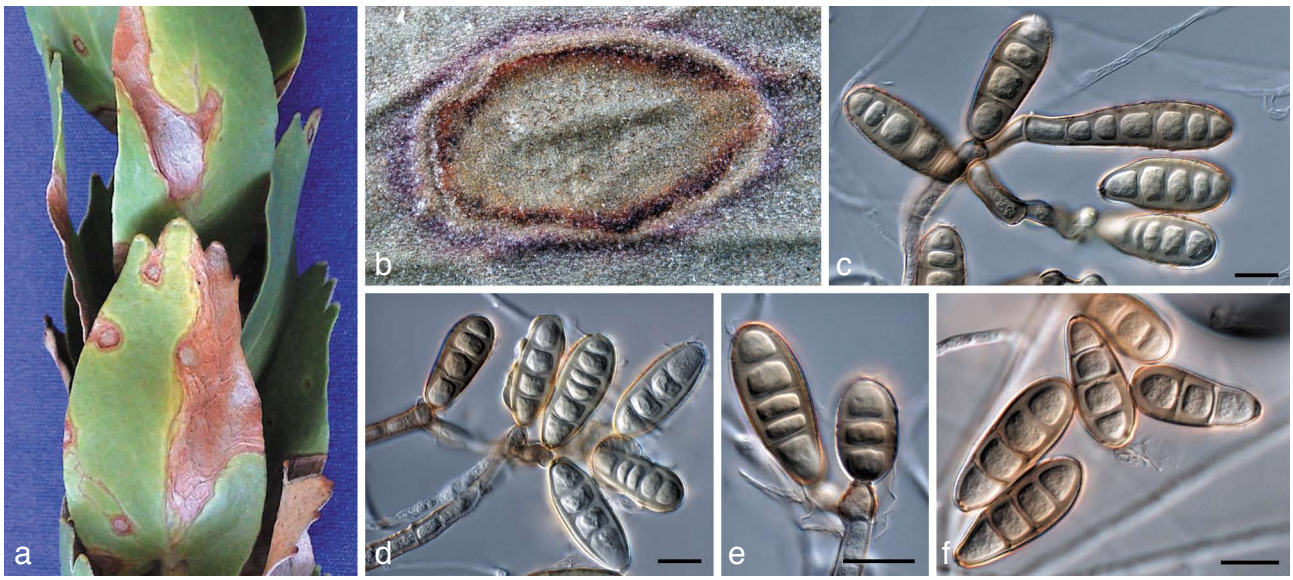

Fig. 16 *Pyrenophora leucospermi* (CBS 111083). a. Leaf spot on *Leucospermum cordifolium*; b. close-up of leaf spot with red-brown margin; c–e. conidiogenous cells giving rise to conidia; f. conidia. — Scale bars = 10 µm.

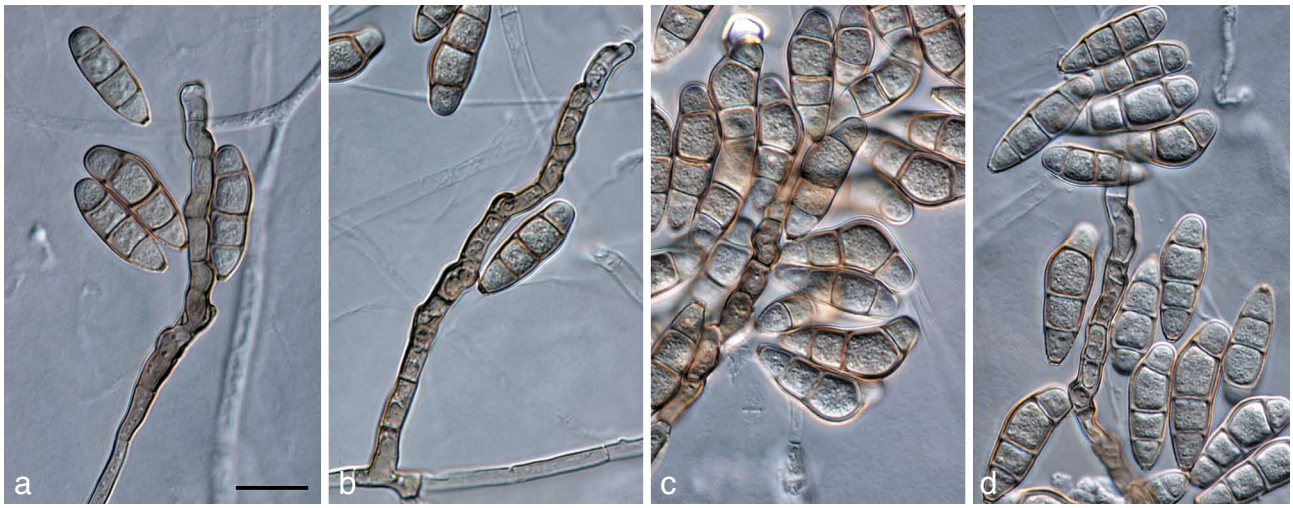

Fig. 17 *Curvularia trifolii* (CBS 111997). a–d. Conidiogenous cells giving rise to conidia on SNA. — Scale bar = 10 µm.

by differences in the TEF region (Fig. 2). Furthermore, collections from leaf spots of *Leucospermum* spp. in Australia (CBS 111997, 114135), resulted in *Curvularia trifolii* (CPC 2941 = CBS 114135 and CPC 2995 = CBS 111997; Fig. 17) being newly reported from *Proteaceae* in the present study.

Ramularia leaf spot

Species of *Ramularia* represent anamorphs of *Mycosphaerella* (*Mycosphaerellaceae*) (Verkley et al. 2004, Crous et al. 2009b, Koike et al. 2011), and most are assumed to be host specific (Braun 1998). Presently only a single species, *R. proteae*, has been reported associated with a leaf spot disease on *P. longifolia* in Tasmania (Crous et al. 2000). The newly described species below represent the first species of *Ramularia* described from *Proteaceae* in South Africa. The generic name *Ramularia* (1833) predates that of *Mycosphaerella* (1884), and should be used for this genus, as the latter is a confused concept that has been incorrectly applied to numerous genera (Crous et al. 2009b).

Ramularia stellenboschensis Crous, *sp. nov.* — MycoBank MB560565; Fig. 18

Ramulariae proteae morphologice similis, sed conidiis subcylindraceis et majoribus, (12–)15–17(–20) × (2–)2.5–3(–3.5) µm.

Etymology. Named after the town of Stellenbosch, where this fungus was collected.

On SNA: *Mycelium* consisting of septate, branched, smooth, hyaline, 1.5–2 µm diam hyphae. *Conidiophores* solitary on hyphae, erect, smooth, hyaline, subcylindrical, straight to flexuous, terminal or intercalary on hyphae, reduced to conidiogenous cells, or up to 3-septate, 3–60 × 2–2.5 µm. *Conidiogenous cells* terminal or lateral, subcylindrical, smooth, hyaline, 3–20 × 1.5–2 µm, smooth, hyaline; scars thickened, darkened, refractive, 0.5–1 µm diam. *Ramoconidia* 0(–1)-septate, hyaline, smooth to finely verruculose, subcylindrical, (12–)15–17(–20) × (2–)2.5–3(–3.5) µm. *Intercalary conidia* in branched chains of up to 6, hyaline, smooth to finely verruculose, subcylindrical to narrowly ellipsoid-fusoid, (6–)7–9(–10) × (2–)2.5(–3) µm. *Terminal conidia* narrowly ellipsoid-fusoid, hyaline, smooth to finely verruculose, aseptate, 5–6(–7) × (1.5–)2 µm; hila thickened, darkened, refractive, 0.5–1 µm diam.

Culture characteristics — Colonies erumpent, spreading, surface folded, with sparse aerial mycelium, and smooth, lobate margins. On PDA surface pale vinaceous-grey (centre), vinaceous-grey in outer region, purplish grey underneath. On MEA surface pale vinaceous-grey, reverse iron-grey. On OA white in centre due to profuse sporulation, vinaceous-grey in outer region. Colonies reach 10–15 mm diam after 2 wk at 25 °C on all media tested.

Specimen examined. South Africa, Western Cape Province, Stellenbosch, J.S. Marais Botanical Garden, on leaves of *Protea* sp., associated with leaf spots of *Vizella interrupta*, 6 May 2010, *P.W. Crous*, holotype CBS H-20678, cultures ex-type CPC 18294 = CBS 130600.

Ramularia vizellae Crous, *sp. nov.* — MycoBank MB560566; Fig. 19

Ramulariae pratensis var. *pratensis* morphologice similis, sed conidiis minoribus et subcylindraceis-obclavatis vel ellipsoideis.

Etymology. Named after the fungal genus *Vizella*, from whose lesions it was isolated as potential secondary coloniser.

On SNA: *Mycelium* consisting of septate, branched, smooth, hyaline, 1.5–2.5 µm diam hyphae. *Conidiophores* solitary, erect on hyphae, terminal and lateral, smooth, hyaline, 1–4-septate, or reduced to conidiogenous cells, 2–100 × 1.5–2 µm, cylindrical, straight to curved. *Conidiogenous cells* smooth, hyaline, terminal and lateral on conidiophores, 2–25 × 1.5–2 µm; scars thickened, darkened, refractive, 0.5–1 µm diam. *Ramoconidia* subcylindrical to obclavate or ellipsoid, 0(–1)-septate, hyaline, smooth to finely verruculose, (8–)10–12(–23) × (2.5–)3–3.5(–5) µm, with 1–3 apical loci. *Intercalary conidia* hyaline, smooth, aseptate, ellipsoid, smooth to finely verruculose, (5–)6–7 × (2.5–)3(–3.5) µm. *Terminal conidia* in branched chains of up to 6, hyaline, smooth, aseptate, ellipsoid, smooth to finely verruculose, 4–5(–5.5) × (2–)3(–3.5) µm; hila thickened, darkened, refractive, 0.5–1 µm diam.

Culture characteristics — Colonies erumpent, spreading, surface folded, with moderate aerial mycelium; margins smooth, lobate. On PDA surface dirty cream, reverse apricot. On MEA surface pale olivaceous-grey, with patches of scarlet; reverse olivaceous-grey with patches of apricot. On OA surface pale olivaceous-grey to dirty cream, with patches of apricot. Colonies reach 12–15 mm diam after 2 wk on all media tested.

Specimen examined. South Africa, Western Cape Province, Hermanus, Fernkloof Nature Reserve, on leaves of *Protea* sp., in association with *Vizella interrupta* (secondary?), 2 May 2010, *P.W. Crous*, holotype CBS H-20679, cultures ex-type CPC 18283 = CBS 130601.

Notes — *Ramularia vizellae*, obtained from leaves of *Protea* sp. infected with *Vizella interrupta*, appears to be genetically related to *Ramularia pratensis* var. *pratensis* (on *Polygonaceae*; Braun 1998). This could have been a chance encounter on the

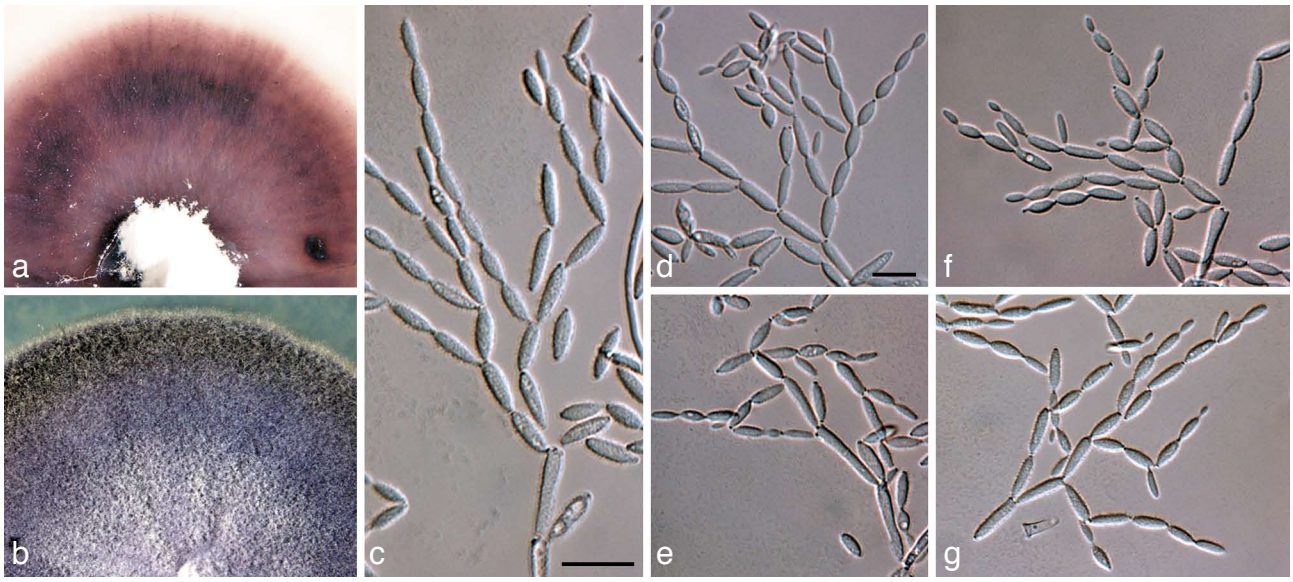

Fig. 18 *Ramularia stellenboschensis* (CBS H-20678). a. Colony sporulating on OA; b. colony sporulating on PDA; c–g. branched conidial chains formed on SNA. — Scale bars = 10 µm.

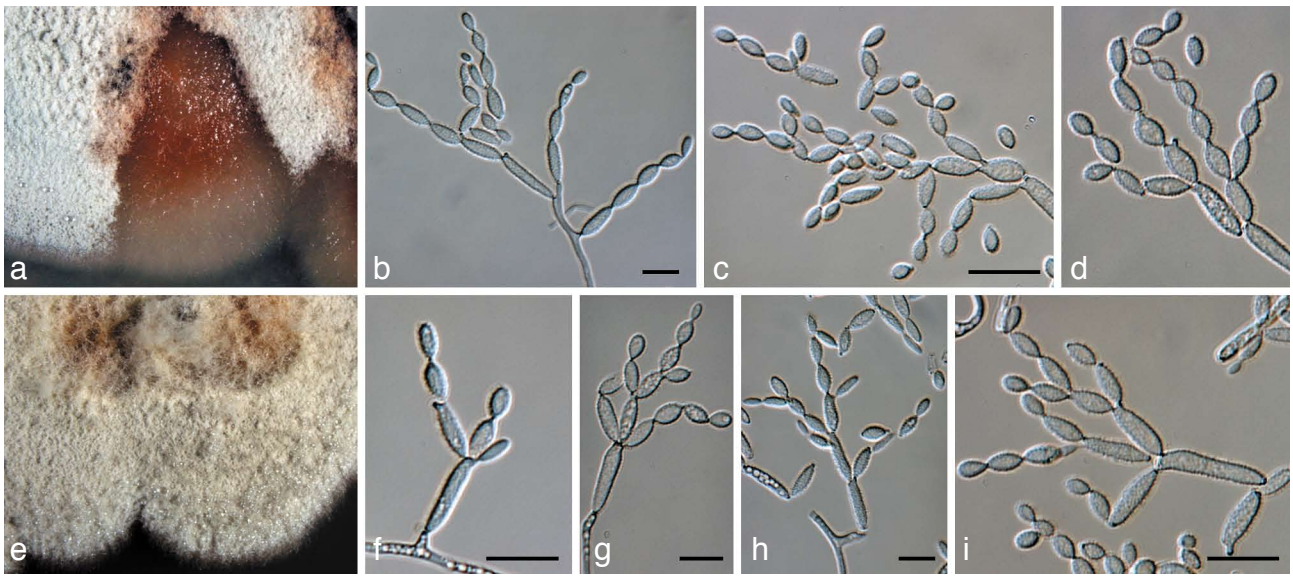

Fig. 19 *Ramularia vizellae* (CBS H-20679). a. Colony sporulating on OA; e. colony sporulating on MEA; b–d, f–i. branched conidial chains formed on SNA. — Scale bars = 10 µm.

Protea leaf, as no sporulation was observed on the leaf itself. Morphologically, however, conidia of *R. pratensis* var. *pratensis* are somewhat larger, ellipsoid-ovoid, subcylindrical-fusoid, (6–)8–25(–35) × (1.5–)2–4(–5) µm. The ITS sequence of *R. vizellae* is not identical to that of *R. pratensis* var. *pratensis* (GenBank EU019284.2; Identities = 564/583 (97 %), Gaps = 6/583 (1 %)).

The three species of *Ramularia* occurring on *Proteaceae* can easily be distinguished based on conidial size, with conidia of *R. proteae* being the smallest, subcylindrical-fusoid, 5–8(–10) × 1–1.5(–2) µm, while the subcylindrical conidia of *R. stellenboschensis* are larger, (12–)15–17(–20) × (2–)2.5–3(–3.5) µm. Finally, conidia of *R. vizellae* are more subcylindrical to obclavate or ellipsoid in shape, (8–)10–12(–23) × (2.5–)3–3.5(–5) µm.

Teratosphaeria leaf spot

Crous et al. (2004a) listed 13 species of *Mycosphaerella* (incl. *Teratosphaeria*) and 18 associated anamorph species occurring on *Proteaceae*. Since this date, however, numerous additional species have been described from this host family (Crous et al. 2007a, 2008, 2009b). Species of *Teratosphaeria* are commonly associated with leaf spots and blotches on *Proteaceae*, though not much is known about their host specificity (Crous & Groenewald 2005).

Teratosphaeria capensis Crous, *sp. nov.* — MycoBank MB560567; Fig. 20

Teratosphaeriae bellulae similis, sed ascosporis sine vagina gelatinosa, ascosporis quidem in asco brunnescentibus.

Etymology. Named after the Cape Province, South Africa, where this species was collected.

Leaf spots amphigenous, subcircular to circular, up to 2.5 cm diam, medium brown with a raised, dark brown border. *Ascomata* amphigenous, black, immersed, substomatal, up to 100 µm diam, along the margins of leaf spots, with *Brunneosphaerella protearum* ascomata in the centre (which is probably the primary pathogen). This suggests that *T. capensis* was either endophytic or opportunistic. The ascomatal wall consists of 2–3 layers

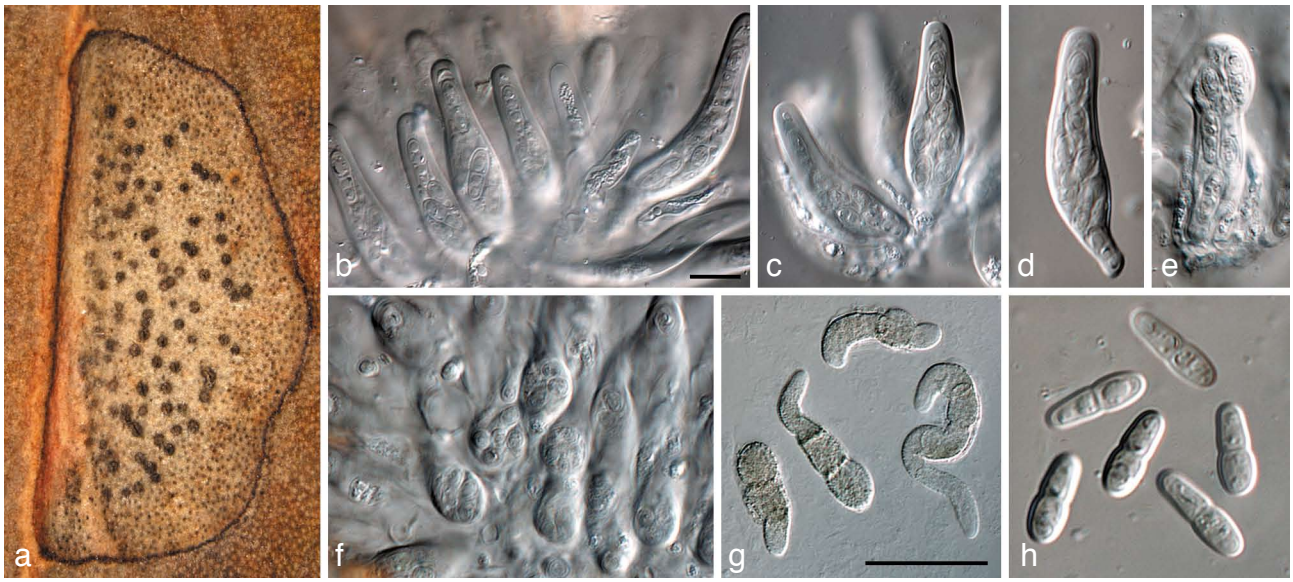

Fig. 20 *Teratosphaeria capensis* (CBS H-20680). a. Leaf spot of *Brunneosphaerella protearum* (prominent, large, semi-erumpent ascomata), surrounded by inconspicuous, substomatal ascomata of *T. capensis*; b–f. asci; g. germinating ascospores on MEA; h. ascospores. — Scale bars = 10 µm.

of medium brown *textura angularis*. *Ostiole* central, 5–10 µm diam, lined with hyaline, 0–1-septate periphyses, up to 10 µm long, 2 µm diam. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, obovoid, straight to curved, 8-spored, 35–50 × 8–12 µm. *Ascospores* tri- to multiseriate, overlapping, hyaline, prominently guttulate, thick-walled, straight, fusoid-ellipsoidal with obtuse ends, widest in middle of apical cell, prominently constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (8–)10–11(–13) × 3.5–4 µm. Germinating ascospores on MEA become brown, verruculose and distorted, with one to several germ tubes that grow at irregular angles to the long axis of the spore, which becomes up to 8 µm wide.

Cultural characteristics — Colonies erumpent, spreading, with moderate aerial mycelium and smooth, lobate margins, reaching 15 mm diam after 1 mo at 25 °C in the dark on all media tested. On MEA olivaceous-grey, reverse iron-grey; on OA olivaceous-grey; on PDA olivaceous-grey, reverse iron-grey; colonies sporulating on MEA after 2 mo, homothallic, ascospores 10–16 × 3–5 µm.

Specimens examined. Portugal, on leaves of *P. repens*, 1 Jan. 2007, *M.F. Moura*, CPC 13981. – South Africa, Western Cape Province, Stellenbosch, J.S. Marais Garden, S33°55'59.3" E18°52'22.5", on living leaves of *Protea* sp., 6 May 2010, *P.W. Crous*, holotype CBS H-20680, culture ex-type CPC 18299 = CBS 130602.

Notes — *Teratosphaeria capensis* generally is found on necrotic leaf spots of *Brunneosphaerella protearum* in South Africa. However, it was the sole pathogen associated with necrotic leaf spots of a *P. repens* in Portugal (CPC 13981) (Crous et al. 2008). The varying niche ecology demonstrated by this species suggests that it is adaptive to its host in different environments. Since *P. repens* was brought to Portugal this fungus may well have been introduced as an endophyte. Morphologically *T. capensis* is similar to several species occurring on *Proteaceae* based on its ascospore dimensions and germination patterns, and would be difficult to distinguish without DNA sequence data. Presently this is the only species of *Teratosphaeria* known to be homothallic and form fertile ascomata in culture.

ADDENDUM

Several species were encountered during this study that appeared to be saprobic rather than plant pathogenic. These taxa are thus treated in the addendum below.

Coccomyces proteae Marinc., M.J. Wingf. & Crous, in Marincowitz et al., Microfungi occurring on Proteaceae in the fynbos: 32. 2008.

Specimens examined. South Africa, Western Cape Province, Jonkershoek Nature Reserve, on leaf litter of *Protea nitida*, *S. Marincowitz*, 6 June 2000, holotype PREM 59439; KwaZulu-Natal, Drakensberg Mountains, on leaves of *Protea* sp., 21 Mar. 1998, *S. Denman*, epitype designated here as CBS H-20681, cultures ex-epitype CBS 111703 = CPC 1730, CBS 111704 = CPC 1727.

Notes — Both collections of this fungus have been from leaf litter, and thus far nothing is known about its potential role as pathogen of *Proteaceae*, though this seems unlikely.

Gordonomyces Crous & Marinc., *gen. nov.* — MycoBank MB560568

Phaeophleosporae morphologice similis, sed conidiomatibus erumpentibus, pariete externo laevi et nigro, conidiis juvenilibus cum vagina mucosa.

Type species. *Gordonomyces mucovaginatus* Crous & Marinc.

Etymology. Named after Gordon's Bay, a harbour town in the Western Cape Province of South Africa, which was in turn named after Robert Jacob Gordon (1743–1795), the Dutch explorer of Scottish descent.

Conidiomata up to 600 µm diam, globose, separate to aggregated in clusters, black, outer wall smooth; wall of *textura angularis*, up to 60 µm thick, outer region of 3–8 layers of dark brown cells, inner region of 3–7 layers of thick-walled, hyaline cells. *Paraphyses* intermingled among conidiogenous cells, cylindrical, branched below, septate; at times becoming fertile. *Conidiogenous cells* subcylindrical to ampulliform, mostly reduced to single cells, with apical percurrent proliferation. *Conidia* medium to golden-brown, verruculose, obovoid to semi-clavate, medianly 1-euseptate, apex obtuse, base subtruncate, conidia covered in mucoid sheath, 2–3 µm wide.

Gordonomyces mucovaginatus Crous & Marinc., *sp. nov.* — MycoBank MB560559; Fig. 21

Conidia (12–)13–15(–17) × (4–)4.5–5 µm, medio- vel aureobrunnea, verruculosa, ovoidea, in medio 1-euseptata, cum vagina mucosa, 2–3 µm lata.

Etymology. Named after the prominent mucoid sheath that surrounds the apices of its conidia.

Sporulating on PDA. *Conidiomata* up to 600 µm diam, globose, erumpent on agar, separate to aggregated in clusters, black,

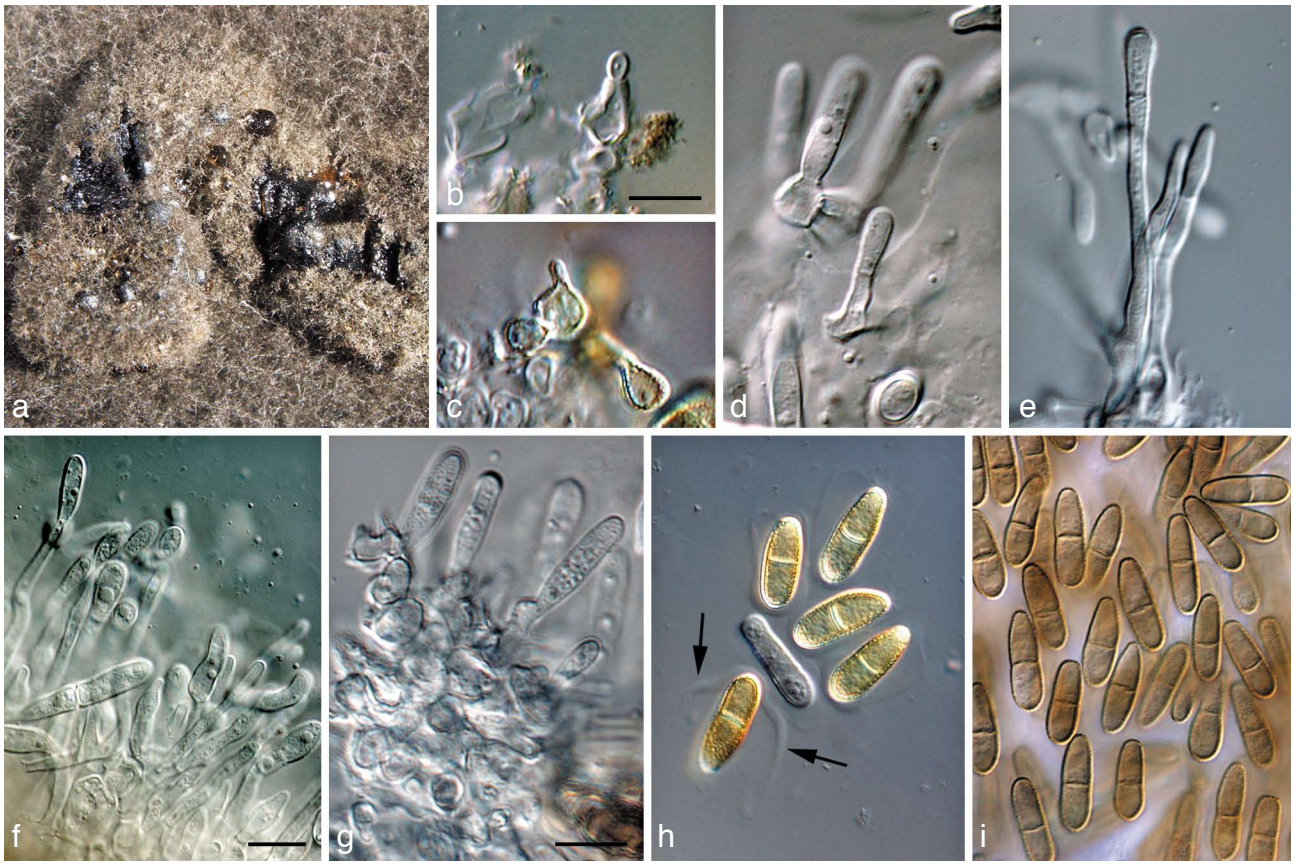

Fig. 21 *Gordonomyces mucovaginatus* (PREM 59592). a. Colony sporulating on PDA; b–d, g. conidiogenous cells giving rise to conidia; e, f. paraphyses; h. conidia with mucoid appendages (arrows); i. mature conidia. — Scale bars = 10 µm.

outer wall smooth; conidiomata filled with a brown-black mucoid mass containing numerous conidia; wall of *textura angularis*, up to 60 µm thick, outer region of 3–8 layers of dark brown cells, inner region of 3–7 layers of thick-walled, hyaline cells. *Paraphyses* intermingled among conidiogenous cells, cylindrical, branched below, 1–6-septate, 30–70 × 3–4 µm, with obtuse ends, but at times becoming fertile, giving rise to conidia, terminal and lateral, by means of inconspicuous percurrent proliferation. *Conidiogenous cells* subcylindrical to ampulliform, mostly reduced to single cells, with apical percurrent proliferation, 5–15 × 3.5–4 µm. *Conidia* medium to golden-brown, verruculose, obovoid to semi-clavate, medianly 1-euseptate, apex obtuse, base subtruncate, conidia covered in mucoid sheath, 2–3 µm wide, (12–)13–15(–17) × (4–)4.5–5 µm.

Culture characteristics — Colonies on PDA spreading, with smooth, lobed margins, and moderate aerial mycelium; surface sepia (middle), fuscous-black in outer region and underneath, up to 30 mm diam after 2 wk at 25 °C. On OA spreading, flat, with smooth margin, lacking aerial mycelium, rosy-buff, up to 40 µm diam after 2 wk. On MEA spreading, with smooth, lobed margin and moderate aerial mycelium; surface iron-grey with patches of white; reverse fuscous-black; colonies reaching 40 mm diam after 2 wk.

Specimen examined. South Africa, Western Cape Province, Gordon's Bay, on leaf litter of *Leucadendron laureolum*, 26 June 2000, *S. Marincowitz*, holotype PREM 59592, culture ex-type CMW 22212 = CBS 127273 = CPC 18172.

Notes — *Gordonomyces* is *Phaeophleospora-Kirramyces*-like in morphology (Crous et al. 1997, Andjic et al. 2007), but distinct in having erumpent conidiomata with a smooth, black outer wall, and juvenile conidia that are encased in a mucoid sheath.

Leptosphaerulina australis McAlpine, in McAlpine, Fungus diseases of stone-fruit trees in Australia and their treatment: 103. 1902 — Fig. 22

For synonyms see MycoBank.

On SNA: *Ascomata* pseudothecial, solitary to aggregated in clusters, brown, superficial on agar medium, obpyriform to subglobose, 100–150 × 150–200 µm; ostiole central, up to 30 µm diam; outer wall covered with short, brown hyphal setae, 5–15 × 3–5 µm, with obtuse ends. *Asci* 100–120 × 35–45 µm, 8-spored, hyaline, obovoid, bitunicate with strongly developed apical chamber, 5–7 × 2–3 µm. *Ascospores* multiseriate in asci, hyaline, smooth, with mucoid sheath, 4 transverse septa, and 2–3 vertical, and 1–2 oblique septa, constricted at second vertical septum from apex, ellipsoid to obovoid, tapering from middle of upper part of ascospore (widest point) to an acutely rounded apex, base obtusely rounded; hamathecial tissue dissolving among asci, and pseudoparaphyses not observed, (32–)33–27(–40) × (12–)13–14(–15) µm.

Culture characteristics — Colonies spreading, flat, with sparse aerial mycelium and smooth, lobate margins, reaching 22 mm diam after 1 wk at 25 °C. On MEA centre dirty white, outer region sienna, reverse sienna; on PDA centre dirty white, outer region olivaceous-grey, reverse iron-grey; on OA centre dirty white, outer region olivaceous-grey to iron-grey.

Specimen examined. Kenya, on leaves of *Protea* sp., 1999, culture CPC 3712 = CBS 116307.

Notes — The ITS sequence data and morphology of this isolate matches that of other isolates of the species. *Leptosphaerulina australis* was isolated from leaf spots of a *Protea* sp. collected in Kenya, and as such is the first record from *Proteaceae*. Although species of *Leptosphaerulina* are well-known plant pathogens (Graham & Luttrell 1961), the pathogenicity

Fig. 22 *Leptosphaerulina australis* (CBS 116307). a. Colony sporulating on PDA; b. ascomata forming on SNA; c, d. ascomata showing ostiolar region; e–h; asci; i. muriformly septate, hyaline ascospores with mucoid sheath. — Scale bars: a, c = 200 µm, d = 30 µm, all others = 10 µm.

status of *L. australis* is uncertain (Mitkowski & Browning 2004). Notwithstanding this, the fungus has been intercepted on exported agricultural produce, namely on leaf spots of *Brassica oleracea* var. *capitata* from China (Hyun et al. 2005), and could be relevant to trade in agricultural produce.

Pseudopassalora Crous, *gen. nov.* — MycoBank MB560570

Passalorae similis, sed hyphis et cellulis conidiogenis hyalinis, conidiis vetustis verrucosis.

Type species. *Pseudopassalora gouriqua* Crous.

Etymology. Named after its morphological similarity to *Passalora*.

Mycelium consisting of smooth, hyaline, septate, branched hyphae. *Conidiophores* solitary on hyphae, micronematous, reduced to conidiogenous loci, or erect, cylindrical, straight to flexuous, hyaline, smooth, 1–2-septate. *Conidiogenous cells* integrated, reduced to loci on hyphae, or terminal on short conidiophores, cylindrical, hyaline, smooth, containing a solitary terminal, truncate locus, or at times polyblastic with 1–3 phialidic loci that are thickened along the rim. *Conidia* solitary, fusoid-ellipsoidal, brown, verruculose to warty, widest in the middle of the upper third, tapering to a subobtose apex, aseptate or medianly 1-septate; base truncate, at times slightly thickened along the rim.

Pseudopassalora gouriqua Crous, *sp. nov.* — MycoBank MB560571; Fig. 23

Conidia solitaria, fusoida-ellipsoidea, brunnea, verruculosa vel verrucosa, aseptata vel in medio 1-septata, (14–)20–25(–35) × (3–)3.5–4(–4.5) µm; basi truncata, interdum marginaliter leviter incrassata.

Etymology. Named after the location where it was collected. The word 'Gouriqua' stems from the Khoi-Khoi, who lived in this area about five hundred years ago.

Mycelium consisting of smooth, hyaline, septate, branched, 2–3 µm diam hyphae. *Conidiophores* solitary on hyphae, micronematous, reduced to conidiogenous loci, 1 × 1.5 µm, or erect, cylindrical, straight to flexuous, hyaline, smooth, 1–2-septate, 10–35 × 2–3 µm. *Conidiogenous cells* integrated, reduced to loci on hyphae, or terminal on short conidiophores, cylindrical, hyaline, smooth, 1–20 × (1.5–)2–3 µm; conidiogenous cells containing a solitary terminal, truncate locus, 1.5–3 µm diam, or at times conidiogenous cell polyblastic with 1–3 loci that are thickened along the rim, 0.5–1 µm diam. *Conidia* solitary, fusoid-ellipsoidal, brown, verruculose to warty, widest in the middle of the upper third, tapering to a subobtose apex, aseptate or medianly 1-septate, (14–)20–25(–35) × (3–)3.5–4(–4.5) µm; base truncate, at times slightly thickened along the rim.

Culture characteristics — Colonies flat, spreading, with sparse aerial mycelium, and even, smooth margins, reaching 20 mm diam after 2 mo on all media tested. On OA sepia, with margins concolorous with agar medium; on PDA surface cinnamon, reverse honey; on MEA surface dirty white, with patches of greyish sepia, cinnamon and honey, reverse cinnamon.

Specimen examined. South Africa, Western Cape Province, Mossel Bay, Gouriqua, Rein's Coastal Nature Reserve, on leaves of *Protea susannae*, 1 Apr. 1998, *L. Dryer*, holotype CBS H-20682, cultures ex-type CBS 101954 = CPC 1811.

Notes — *Pseudopassalora* resembles *Retroconis*, but is distinct in producing solitary conidia (Seifert et al. 2011). It is somewhat reminiscent of *Fusicladium* (*Venturia*) (Beck et al. 2005, Crous et al. 2007b), but clusters apart from this genus, and

Fig. 23 *Pseudopassalora gouriqua* (CBS 101954). a–g. Conidiogenous cells on superficial hyphae giving rise to brown, warty, 1-septate conidia. — Scale bars = 10 µm.

has somewhat thickened loci, and hyaline hyphae. It also resembles *Passalora* in having somewhat thickened loci, but is again distinct in having hyaline hyphae and conidiogenous cells, and conidia that become warty with age.

Presently very little is known of the ecology of *P. gouriqua*. Although occurring on leaves of *P. susanne*, it was at the time of isolation suspected to be a possible saprobe.

Xenoconiothyrium Crous & Marinc., *gen. nov.* — MycoBank MB560572

Coniothyrio morphologice simile, sed conidiis primo brevicatenatis, cum poris conspicuis ad extremum singulum vel ad extrema.

Type species. *Xenoconiothyrium catenata* Crous & Marinc.

Etymology. Mature conidia resembling that of the genus *Coniothyrium*, but distinct when young, thus (*Xeno-*) *Coniothyrium*.

Mycelium consisting of septate, branched, olivaceous-brown to brown, thick-walled, warty hyphae, covered in a mucoid layer, giving rise to pycnidial conidiomata. *Conidiophores* reduced to phialidic conidiogenous cells that line the cavity, ampulliform, hyaline, tapering to an abrupt apex. *Conidia* initially in short chains 3(–4), attached via a small locus, eventually breaking free, with minute loci visible on conidial ends, ellipsoid to subcylindrical, thick-walled, initially hyaline, smooth, becoming verruculose, dark brown, 0(–1)-septate, not or constricted at septum, apex obtuse, widest at septum, tapering abruptly to truncate hilum (when viewed directly from above, it appears to have a central pore, which can be basal, or present at both conidial ends); not thickened nor darkened. *Spermatia* at times formed in same conidioma, hyaline, bacilliform.

Xenoconiothyrium catenata Crous & Marinc., *sp. nov.* — MycoBank MB560573; Fig. 24

Conidia ellipsoidea-subcylindracea, crassitunicata, primo hyaline, laevia, deinde verrucolosa, atrobrunnea, 0(–1)-septata, (7–)8–10(–12) × (4–)5 µm, primo brevicatenata, 3(–4), adherentia per locum parvum, ad 0.5 µm diam.

Etymology. Named after its conidia, which occur in chains.

Mycelium consisting of septate, branched, olivaceous-brown to brown, thick-walled, warty, 3–5 µm diam hyphae, covered in a mucoid layer, giving rise to pycnidial conidiomata up to 250 µm diam. *Conidiophores* reduced to phialidic conidiogenous cells that line the cavity, ampulliform, hyaline, 8–15 × 5–6 µm, tapering to an abrupt apex, locus 0.5 µm diam. *Conidia* (7–)8–10(–12) × (4–)5 µm, initially in short chains, 3(–4), attached via a small locus up to 0.5 µm diam, eventually breaking free, with minute loci visible on conidial ends, ellipsoid to subcylindrical, thick-walled (1 µm diam), initially hyaline, smooth, becoming verruculose, dark brown, 0(–1)-septate, not or constricted at septum, apex obtuse, widest at septum, tapering abruptly to truncate hilum (when viewed directly from above, it appears to have a central pore, which can be basal, or present at both conidial ends), 0.5 µm diam; not thickened nor darkened. *Spermatia* at times formed in same conidioma, hyaline, bacilliform, 3–7 × 3–4 µm.

Culture characteristics — Colonies spreading, erumpent with sparse aerial mycelium and even margin; surface folded, reaching 25 mm diam after 2 mo on all media tested; on MEA surface olivaceous-grey, reverse iron-grey; on OA surface iron-grey.

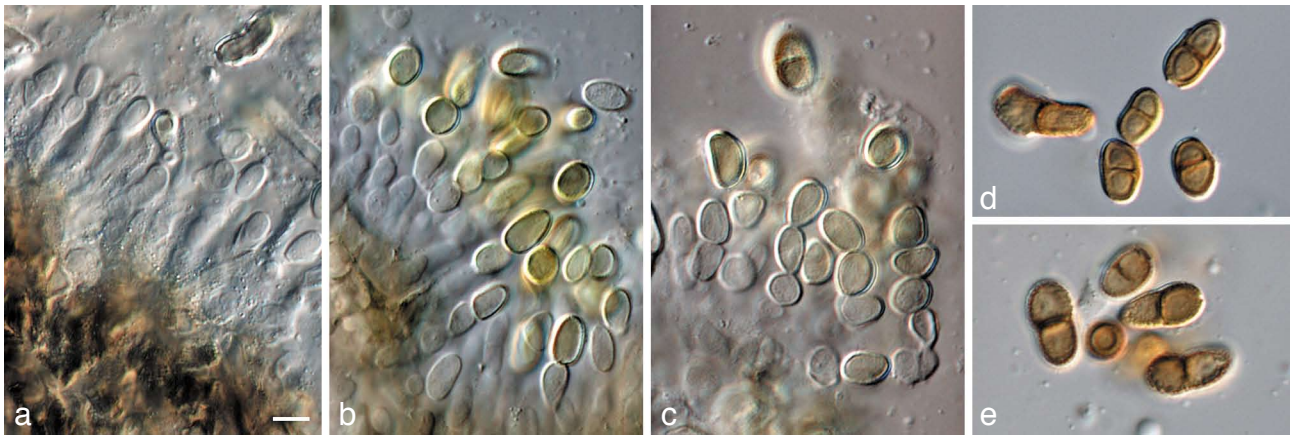

Fig. 24 *Xenoconiothyrium catenata* (CBS 128994). a, b. Conidiogenous cells giving rise to conidial chains; c. conidia forming in short chains; d, e. mature, brown, verruculose, 1-septate conidia with basal and/or apical attachment loci (apical, basal, or both). — Scale bar = 10 µm.

Specimen examined. South Africa, Western Cape Province, Helderberg Nature Reserve, on twig litter of *Protea laurifolia*, 14 Aug. 2000, *S. Marincowitz*, holotype PREM 59486, culture ex-type CMW 22113 = CBS 128994.

Notes — Based on the dimensions of its septate, brown conidia, this fungus was originally identified as *Coniothyrium septatum* by Marincowitz et al. (2008a). It is distinct from the genus *Coniothyrium*, however, in that conidia occur in short chains when young, and have pores visible at one or either end. Based on its LSU sequence it is closest to *Xenophacidiella pseudocatenata* (GenBank JF499870; Crous & Groenewald 2011) and *Phaeothecoidea melaleuca* (GenBank HQ599595; Crous et al. 2010) in *Teratosphaeriaceae*. It was originally collected from twig litter of *Protea laurifolia*, and nothing is thus known about its potential role as pathogen of *Proteaceae*.

DISCUSSION

The Cape Floristic Region (CFR) is located in the south-western part of South Africa, and includes 42 % of the vascular plants occurring in the Southern African subcontinent (Goldblatt & Manning 2000, Rouget et al. 2003). The CFR comprises components of fynbos and five other biomes (Cowling & Holms 1992). As a shrub land, the fynbos has four different plant growth forms. These are tall *Protea* shrubs with large leaves (proteoids), heath-like shrubs (ericoids), wiry reed-like plants (restioids) and bulbous herbs (geophytes) (Cowling & Richardson 1995, Rebelo 2001).

Very little is known, however, about the fungi occurring in the CFR, and recent studies have highlighted the unusual diversity that occur in saprobic (Schubert et al. 2007, Visagie et al. 2009, Bensch et al. 2010), and plant pathogenic fungi (Crous et al. 2009a, 2011b). Mycological exploration in South Africa commenced in the late 1700s, and thus later than plant collections, which began in the 1600s (Eicker & Baxter 1999, Rong & Baxter 2006). For more than a century, both collections and studies on fungi were largely conducted by European visiting scientists. It was only in the late 1800s that the first resident mycologists were appointed in South Africa. A third generation mycologist, Dr Ethel M. Doidge, compiled the list of fungi and lichens collected in South Africa to the end of 1945. In this compilation, about 30 fungi are listed associated with members of *Proteaceae* (Doidge 1950), while based on the known plant biodiversity of South Africa, Crous et al. (2006a) estimated the potential fungal biodiversity as approximately 200 000 species, many of which would occur on *Proteaceae*.

The first fungus described from *Proteaceae* in the Western Cape was *Pseudocercospora protearum* (as *Cercospora protearum*) (Cooke 1883). After many decades of work, the plant pathogenic fungi associated with the family, especially on *Protea*, *Leucospermum* and *Leucadendron* was published (Crous et al. 2004a), as well as the common saprobic fungi occurring on twig and leaf litter (Marincowitz et al. 2008a).

We now have a fragmentary amount of information about fungi in the fynbos, which clearly shows that a unique, endemic mycoflora exists. Nevertheless, the fact that the present study can add an additional 12 species and three genera to the number of known taxa occurring on *Proteaceae*, many of them obvious and clearly plant pathogenic, underlines the fact that more attention needs to be directed towards studying the fungal biodiversity of the CFR. This region not only represents an important part of natural resource-based ecotourism, that also generates thousands of jobs for the local community, many involved with the sale and export of cut-flowers, but is also a key biogeographic region that is the focus of intensive study to better understand evolutionary processes. Adding a mycological component to these studies will greatly benefit our understanding of evolutionary processes in fungi.

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