



Host specificity and speciation of *Mycosphaerella* and *Teratosphaeria* species associated with leaf spots of Proteaceae

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Key words

ITS

Leucadendron
Leucospermum
Mycosphaerella
Protea
Teratosphaeria

Abstract Species of *Mycosphaerella* and *Teratosphaeria* represent important foliicolous pathogens of Proteaceae. Presently approximately 40 members of these genera (incl. anamorphs) have been recorded from Proteaceae, though the majority are not known from culture, and have never been subjected to DNA sequence analysis. During the course of this study, epitypes were designated for several important species, namely *Batcheloromyces leucadendri*, *B. proteae*, *Catenulostroma macowanii*, *Mycosphaerella marksii*, *Teratosphaeria bellula*, *T. jonkershoekensis*, *T. parva*, and *T. proteae-arboreae*. Several species were also newly described, namely *Batcheloromyces sedgfieldii*, *Catenulostroma wingfieldii*, *Dissoconium proteae*, *Teratosphaeria persoonii*, *T. knoxdavesii*, and *T. marasasii*. Although accepted as being highly host specific, some species were shown to have wider host ranges, such as *M. communis* (*Eucalyptus*, *Protea*), *M. konae* (*Leucospermum*, *Eucalyptus*), *M. marksii* (*Eucalyptus*, *Leucadendron*), *T. associata* (*Eucalyptus*, *Protea*), and *T. parva* (*Eucalyptus*, *Protea*), which in most cases were found to co-occur with other species of *Mycosphaerella* or *Teratosphaeria* on Proteaceae. Furthermore, earlier records of *T. jonkershoekensis* on Proteaceae in Australia were shown to be representative of two recently described species, *T. associata* and *T. maxii*. A phenomenon of underdeveloped, or micro-ascospores was also newly observed in asci of *T. maculiformis* and *T. proteae-arboreae*. The exact purpose of asci with two distinct types of ascospores remains to be clarified, as both types were observed to germinate on agar.

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INTRODUCTION

Several genera of South African Proteaceae, especially *Protea*, *Leucospermum* and *Leucadendron* are routinely cultivated for the local and export cut-flower industry. Due to popular demand, these flowers are also now being cultivated in various countries around the world (Crous et al. 2004a). In spite of the popularity of these crops, fungal pathogens still represent a serious impediment to their cultivation. Several groups of fungal pathogens of Proteaceae have in recent years been characterised morphologically as well as phylogenetically, such as the Botryosphaeriaceae stem cankers (Denman et al. 1999, 2000, 2003, Crous et al. 2006b), Armillaria and Cyliodrocladium root rot (Schoch et al. 1999, Crous 2002, Coetzee et al. 2003), Elsinoë scab disease (Swart et al. 2001) and Phomopsis cankers (Mostert et al. 2001a, b). However, this is generally not true for the pathogens associated with leaf diseases, as most have not been studied in culture.

Species of *Mycosphaerella* and *Teratosphaeria* are widespread on Proteaceae, and cause leaf spots and blights on numerous plant hosts in this family (Swart et al. 1998, Crous & Palm 1999, Crous et al. 2000a, Taylor & Crous 2000). In the compendium of Proteaceae diseases, Crous et al. (2004a) listed 13 species of *Mycosphaerella* (incl. *Teratosphaeria*), and 18 associated anamorph species, while Crous & Groenewald (2006a, b)

recently described a further two *Teratosphaeria* spp. from *Protea*. Although several studies have focused on the distribution of *Mycosphaerella* spp. of Proteaceae in native and exotic habitats (Crous et al. 2000b, 2004a, Taylor & Crous 2000, Taylor et al. 2001a, b), their phylogenetic relationships have remained largely unresolved (Taylor et al. 2003).

The genus *Mycosphaerella* includes more than 3 000 names (Aptroot 2006), which together with names in associated anamorph genera probably represent close to 10 000 names (Crous et al. 2000a, 2001, 2004a, b, 2006a–c, 2007a–c, Crous & Braun 2003, Arzanlou et al. 2007). Although previous phylogenetic studies based on the ITS rDNA region have suggested *Mycosphaerella* to be monophyletic (Crous et al. 2000a, 2001, Goodwin et al. 2001), recent studies employing LSU sequence data have refuted this (Hunter et al. 2006), and split off several genera such as *Davidiella* (Davidiellaceae, Braun et al. 2003, Schoch et al. 2006, Crous et al. 2007b, Schubert et al. 2007), *Schizothyrium* (Schizothyriaceae, Batzer et al. 2008), and *Teratosphaeria* (Teratosphaeriaceae, Crous et al. 2007a). Although Crous et al. (2004a) listed eight species of *Mycosphaerella* from Proteaceae in South Africa, Crous et al. (2007a) have recently placed several of these in *Teratosphaeria*.

The genus *Teratosphaeria* is separated from *Mycosphaerella* s.str. based on several characters such as the presence of superficial stromatic tissue, ascospores that darken in their asci, remnants of the hamathecial tissue, ascospores that are frequently covered by a mucoid sheath, asci with a multi-layered endotunica, and the presence of ostiolar periphyses (Crous et al. 2007a). Presently 12 anamorph genera have been linked to *Teratosphaeria* (see Crous et al. 2007a for key), with the majority

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Table 1 Details of isolates included for morphological and / or molecular examination in this study. The GenBank accession numbers of isolates for which ITS sequences were generated for the first time are printed in **bold** face.

Teleomorph	Anamorph	Accession number ¹	Host	Country	Collector	GenBank Accession number
<i>Mycosphaerella buckinghamiae</i>		CBS 111996; CPC 3006*	<i>Buckinghamia</i> sp.	Australia	P.W. Crous & B. Summerell	EU707855
<i>M. communis</i>	<i>Dissoconium commune</i>	CBS 112175; CPC 3360	<i>Buckinghamia</i> sp.	–	–	EU707856
		CBS 112889; CPC 3359	<i>Protea magnifica</i>	Australia	P.W. Crous	AY725539
<i>M. holualoana</i>		CBS 114238; CPC 10440*	<i>Eucalyptus globulus</i>	Spain	J.P. Mansilla	AY725541
<i>M. konae</i>	<i>Pseudocercospora</i> sp.	CBS 110698; CPC 2126*	<i>Leucospermum</i> sp.	USA: Hawaii	P.W. Crous & M.E. Palm	AY260087
		CBS 111261; CPC 2123*	<i>Leucadendron</i> sp.	USA: Hawaii	P.W. Crous & M.E. Palm	AY260086
		CBS 111028; CPC 2125	<i>Leucadendron</i> sp.	USA: Hawaii	P.W. Crous & M.E. Palm	AY260085
<i>M. marksii</i>		CBS 120748; CPC 13469	<i>E. camaldulensis</i>	Thailand	W. Himaman	EF394842
		CBS 110942; CPC 982*	<i>E. botryoides</i>	Australia	A.J. Carnegie	AF309589
		CBS 110974; CPC 984	<i>E. botryoides</i>	Australia	A.J. Carnegie	–
		CBS 115501; CPC 5358	<i>Leucadendron tinctum</i>	Australia	A.J. Carnegie	–
<i>M. stromatosa</i>	<i>Pseudocercospora stromatosa</i>	CBS 101953; CPC 1731*	<i>Protea</i> sp.	Madeira Islands	S. Denman	DQ302979
<i>M. waimeana</i>	<i>Stenella</i> sp.	CBS 110697; CPC 2179*	<i>Leucospermum</i> sp.	South Africa	S. Denman	AY167598
<i>Teratosphaeria alstairii</i>	<i>Batcheloromyces</i> sp.	CBS 120035; CPC 12730*	<i>P. repens</i>	USA: Hawaii	P.W. Crous & M.E. Palm	AY260083
<i>T. associata</i>		CBS 112224; CPC 3116	<i>P. lepidocarpodendron</i>	South Africa	P.W. Crous & A. Smith	DQ885901
		CBS 112627; CPC 3115	<i>P. lepidocarpodendron</i>	Australia	P.W. Crous & B. Summerell	DQ302968
		CBS 114165; CPC 3117	<i>P. lepidocarpodendron</i>	Australia	P.W. Crous & B. Summerell	EU707857
		CBS 120730; CPC 13119*	<i>Corymbia henryii</i>	Australia	P.W. Crous & B. Summerell	EU707858
		CBS 120731; CPC 13128	<i>C. variegata</i>	Australia	A.J. Carnegie	EF394826
		CBS 120732; CPC 13108	<i>E. dunii</i>	Australia	A.J. Carnegie	EF394827
<i>T. bellula</i>		CBS 111699; CPC 1816	<i>Leucospermum</i> sp.	South Africa	J.E. Taylor	EF394824
		CBS 111700; CPC 1821	<i>P. eximia</i>	South Africa	J.E. Taylor	EU707859
		CBS 114145; CPC 2795	<i>Leucadendron</i> sp.	South Africa	J.E. Taylor	EU019301
<i>T. fibrillosa</i>		CPC 14908	<i>Protea</i> sp.	South Africa	L. Swart	EU707860
		CBS 121707; CPC 13960*	<i>Protea</i> sp.	South Africa	P.W. Crous	EU707861
		CPC 1876	<i>P. nitida</i>	South Africa	P.W. Crous & L. Mostert	EU707862
		CPC 13969	<i>Protea</i> sp.	South Africa	J.E. Taylor	AY260094
<i>T. jonkershoekensis</i>		CBS 122897; CPC 13984*	<i>Protea</i> sp.	South Africa	P.W. Crous	EU707863
<i>T. knoxdavesii</i>		CBS 122898; CPC 14960*	<i>Protea</i> sp.	South Africa	P.W. Crous & L. Mostert	EU707864
		CPC 14905	<i>Protea</i> sp.	South Africa	P.W. Crous & M. Crous	EU707865
		No culture available	<i>Protea</i> sp.	South Africa	P.W. Crous & M. Crous	EU707866
<i>T. maculiformis</i>		CBS 122899; CPC 14889*	<i>Protea</i> sp.	South Africa	P.W. Crous & K.L. Crous	EU707867
<i>T. marasasii</i>		CBS 112231; CPC 3321	<i>Protea</i> sp.	South Africa	P.W. Crous & M. Crous	EU707868
<i>T. maxii</i>		CBS 112232; CPC 3323	<i>Protea</i> sp.	Australia	P.W. Crous & B. Summerell	EU707869
		CBS 112496; CPC 3322	<i>Protea</i> sp.	Australia	P.W. Crous & B. Summerell	EU707870
		CBS 120137; CPC 12805*	<i>P. repens</i>	Australia	P.W. Crous & B. Summerell	EU707871
		CPC 12943	<i>P. repens</i>	South Africa	M. Crous & P.W. Crous	DQ885899
<i>T. microspora</i>	<i>Catenulostroma microsporum</i>	CBS 101951; CPC 1960*	<i>P. cynaroides</i>	South Africa	P.W. Crous	DQ885898
		CBS 110890; CPC 1832	<i>P. cynaroides</i>	South Africa	S. Denman & J.E. Taylor	EU707872
		CBS 111031; CPC 1848	<i>P. cynaroides</i>	South Africa	L. Swart	AY260097
		CBS 111697; CPC 1597	<i>P. cynaroides</i>	South Africa	J.E. Taylor	AY260098
<i>T. parva</i>		CBS 114761; CPC 1217	<i>P. repens</i>	South Africa	P.W. Crous	EU707873
		CBS 122892; CPC 12421*	<i>E. globulus</i>	South Africa	P.W. Crous	EU707874
		CBS 122893; CPC 14898	<i>P. repens</i>	Australia	I. Smith	EU707875
				South Africa	L. Mostert	EU707876

<i>T. persoonii</i>	CBS 122894; CPC 13896	<i>P. nitida</i>	South Africa	P.W. Crous & L. Mostert	EU707877
	CPC 2120	<i>P. repens</i>	South Africa	G. Matthews	AY260091
	CPC 12418	<i>E. globulus</i>	Australia	I. Smith	EU707878
	CPC 12419	<i>E. globulus</i>	Australia	I. Smith	EU707879
	CBS 122895; CPC 13972*	<i>Protea</i> sp.	South Africa	P.W. Crous & L. Mostert	EU707880
	CBS 122896; CPC 14846; STE-U 6389	<i>Euchaetis meridionalis</i>	South Africa	A.R. Wood	EU707881
	CPC 12952*	<i>P. nitida</i>	South Africa	M.K. Crous & P.W. Crous	EU707882
	CPC 12954*	<i>P. nitida</i>	South Africa	M.K. Crous & P.W. Crous	EU707883
	CPC 14963	<i>Protea</i> sp.	South Africa	P.W. Crous & M. Crous	EU707884
	CPC 13917	<i>P. nitida</i>	South Africa	P.W. Crous & L. Mostert	EU707885
<i>T. proteae-arboreae</i>	CPC 13963	<i>P. nitida</i>	South Africa	P.W. Crous & L. Mostert	EU707886
	CPC 13981	<i>P. repens</i>	Portugal	M.F. Moura	EU707887
	CPC 14957	<i>Protea</i> sp.	South Africa	P.W. Crous & M. Crous	EU707888
	CBS 110892; CPC 1837	<i>Leucadendron</i> sp.	South Africa	L. Swart	AY260100
	CBS 111577; CPC 1838*	<i>Leucadendron lauratum</i>	South Africa	L. Swart	AY260101
	CBS 111937; CPC 2822	<i>Leucadendron</i> sp.	South Africa	L. Swart	EU707889
	CBS 114024; CPC 2794	<i>Leucadendron</i> sp.	South Africa	L. Swart	EU707890
	CBS 114144; CPC 2823	<i>Leucadendron</i> sp.	South Africa	L. Swart	EU707891
	CBS 114146; CPC 2820	<i>Leucadendron</i> sp.	South Africa	L. Swart	EU707892
	CBS 119344; CMW 20456; PREM 58041	<i>Leucadendron salignum</i>	South Africa	S. Lee	EU552103
<i>Batcheloromyces leucadendri</i>	CPC 1839	<i>Leucadendron</i> sp.	South Africa	L. Swart	–
	CPC 1840	<i>Leucadendron gandogerii</i>	South Africa	L. Swart	–
	CBS 110696; CPC 1518*	<i>P. cynaroides</i>	South Africa	L. Swart	AY260099
	CPC 1833	<i>P. cynaroides</i>	South Africa	L. Swart	–
	CPC 1834	<i>P. repens</i>	South Africa	L. Swart	–
	CPC 1835	<i>Protea</i> sp.	South Africa	L. Swart	–
	CPC 1836	<i>P. nerifolia</i>	South Africa	L. Swart	–
	CBS 112119; CPC 3026*	<i>P. repens</i>	South Africa	J.E. Taylor	EU707893
	CBS 111030; CPC 1958	<i>P. grandiceps</i>	South Africa	J.E. Taylor & S. Denman	AY260093
	CBS 110756; CPC 1872	<i>P. nitida</i>	South Africa	J.E. Taylor	AY260095
<i>B. sedgefieldii</i>	CBS 111029; CPC 1488	<i>P. nitida</i>	South Africa	P.W. Crous	AY260096
	CBS 122901; CPC 13899*	<i>P. nitida</i>	South Africa	P.W. Crous & L. Mostert	EU707894
	CPC 13966	<i>P. nitida</i>	South Africa	P.W. Crous & L. Mostert	EU707895
	CBS 112163; CPC 2944*	<i>P. nitida</i>	South Africa	J.E. Taylor	EU707896
	CBS 122900; CPC 13853*	<i>Protea</i> sp.	Canary Islands:		
			Tenerife		
	CBS 114129; CPC 2831*	<i>P. repens</i>	South Africa	P.W. Crous	EU707897
			South Africa	S. Denman	EU707898
	CPC 1869	<i>Leucadendron</i> sp.	South Africa	P.W. Crous & S. Denman	AY260089
	CBS 112161; CPC 3075*	<i>P. longifolia</i>	Australia	A. Macfadyen	EU707899
<i>Catenulostroma elginense</i>	CBS 117550; MUC 46082	Leaf litter	French Guiana	–	EU707900
	CBS 778.97; ATCC 201159;				
	CPC 1470; IMI 375230	<i>P. cynaroides</i>	South Africa	L. Viljoen	AY260081
	CPC 5212	<i>Protea</i> sp.	Canary Islands:		
			Tenerife		
			South Africa	S. Denman	AY260082
<i>C. macowanii</i>	CBS 112163; CPC 2944*	<i>P. nitida</i>	South Africa	J.E. Taylor	EU707895
	CBS 122900; CPC 13853*	<i>Protea</i> sp.	Canary Islands:		
			Tenerife		
	CBS 114129; CPC 2831*	<i>P. repens</i>	South Africa	P.W. Crous	EU707897
			South Africa	S. Denman	EU707898
	CPC 1869	<i>Leucadendron</i> sp.	South Africa	P.W. Crous & S. Denman	AY260089
	CBS 112161; CPC 3075*	<i>P. longifolia</i>	Australia	A. Macfadyen	EU707899
	CBS 117550; MUC 46082	Leaf litter	French Guiana	–	EU707900
	CBS 778.97; ATCC 201159;				
	CPC 1470; IMI 375230	<i>P. cynaroides</i>	South Africa	L. Viljoen	AY260081
<i>C. wingfieldii</i>	CPC 5212	<i>Protea</i> sp.	Canary Islands:		
			Tenerife		
			South Africa	S. Denman	AY260082
<i>Phaeothecoidea proteae</i>	CBS 112163; CPC 2944*	<i>P. nitida</i>	South Africa	J.E. Taylor	EU707895
	CBS 122900; CPC 13853*	<i>Protea</i> sp.	Canary Islands:		
			Tenerife		
	CBS 114129; CPC 2831*	<i>P. repens</i>	South Africa	P.W. Crous	EU707897
			South Africa	S. Denman	EU707898
	CPC 1869	<i>Leucadendron</i> sp.	South Africa	P.W. Crous & S. Denman	AY260089
	CBS 112161; CPC 3075*	<i>P. longifolia</i>	Australia	A. Macfadyen	EU707899
	CBS 117550; MUC 46082	Leaf litter	French Guiana	–	EU707900
	CBS 778.97; ATCC 201159;				
	CPC 1470; IMI 375230	<i>P. cynaroides</i>	South Africa	L. Viljoen	AY260081
<i>Pseudocercospora protearum</i>	CPC 5212	<i>Protea</i> sp.	Canary Islands:		
			Tenerife		
			South Africa	S. Denman	AY260082
<i>Ramularia proteae</i>	CBS 112163; CPC 2944*	<i>P. nitida</i>	South Africa	J.E. Taylor	EU707895
	CBS 122900; CPC 13853*	<i>Protea</i> sp.	Canary Islands:		
			Tenerife		
	CBS 114129; CPC 2831*	<i>P. repens</i>	South Africa	P.W. Crous	EU707897
			South Africa	S. Denman	EU707898
	CPC 1869	<i>Leucadendron</i> sp.	South Africa	P.W. Crous & S. Denman	AY260089
	CBS 112161; CPC 3075*	<i>P. longifolia</i>	Australia	A. Macfadyen	EU707899
	CBS 117550; MUC 46082	Leaf litter	French Guiana	–	EU707900
	CBS 778.97; ATCC 201159;				
	CPC 1470; IMI 375230	<i>P. cynaroides</i>	South Africa	L. Viljoen	AY260081
<i>Readerella guyanensis</i>	CPC 5212	<i>Protea</i> sp.	Canary Islands:		
			Tenerife		
			South Africa	S. Denman	AY260082
<i>Septoria protearum</i>	CBS 112163; CPC 2944*	<i>P. nitida</i>	South Africa	J.E. Taylor	EU707895
	CBS 122900; CPC 13853*	<i>Protea</i> sp.	Canary Islands:		
			Tenerife		
	CBS 114129; CPC 2831*	<i>P. repens</i>	South Africa	P.W. Crous	EU707897
			South Africa	S. Denman	EU707898
	CPC 1869	<i>Leucadendron</i> sp.	South Africa	P.W. Crous & S. Denman	AY260089
	CBS 112161; CPC 3075*	<i>P. longifolia</i>	Australia	A. Macfadyen	EU707899
	CBS 117550; MUC 46082	Leaf litter	French Guiana	–	EU707900
	CBS 778.97; ATCC 201159;				
	CPC 1470; IMI 375230	<i>P. cynaroides</i>	South Africa	L. Viljoen	AY260081

¹ ATCC: American Type Culture Collection, Virginia, USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW: Culture collection of Mike Wingfield, housed at FABI, Pretoria, South Africa; CPC: Culture collection of Pedro Crous, housed at CBS; IMI: International Mycological Institute, Egham, Basingstoke, UK; MUC: Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; PREM: National Collection of Fungi, Pretoria, South Africa; STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa.

* Ex-type cultures.

being quite distinct from those found in *Mycosphaerella* s.str. Although the genus *Teratosphaeria* was initially established for species occurring on Proteaceae, the genus remains poorly understood, as many of the taxa are not known from culture, and their phylogenetic position remains uncertain. The aim of the present study was thus to recollect these taxa from Proteaceae, and designate epitype specimens for many of the older names, thereby enabling us to clarify their phylogeny.

MATERIALS AND METHODS

Isolates

Proteaceae leaves bearing ascomata, or with leaf spots were chosen for study. Excised lesions were soaked in water for approximately 2 h, after which they were placed in the bottom of

Petri dish lids, with the top half of the dish containing 2 % malt extract agar (MEA; Oxoid, Hampshire, England). Ascospore germination patterns were examined after 24 h, and single ascospore and conidial cultures established as described by Crous (1998). Colonies were sub-cultured onto 2 % potato-dextrose agar (PDA), synthetic nutrient-poor agar (SNA), MEA, and oatmeal agar (OA) (Gams et al. 2007), and incubated under continuous near-ultraviolet light at 25 °C to promote sporulation. All cultures obtained in this study are maintained in the culture collection of the CBS (Table 1). Nomenclatural novelties, descriptions and trace files of the ITS DNA barcodes were deposited in MycoBank (www.Mycobank.org).

DNA phylogeny

Fungal colonies were established on agar plates, and genomic DNA was isolated following the CTAB-based protocol described

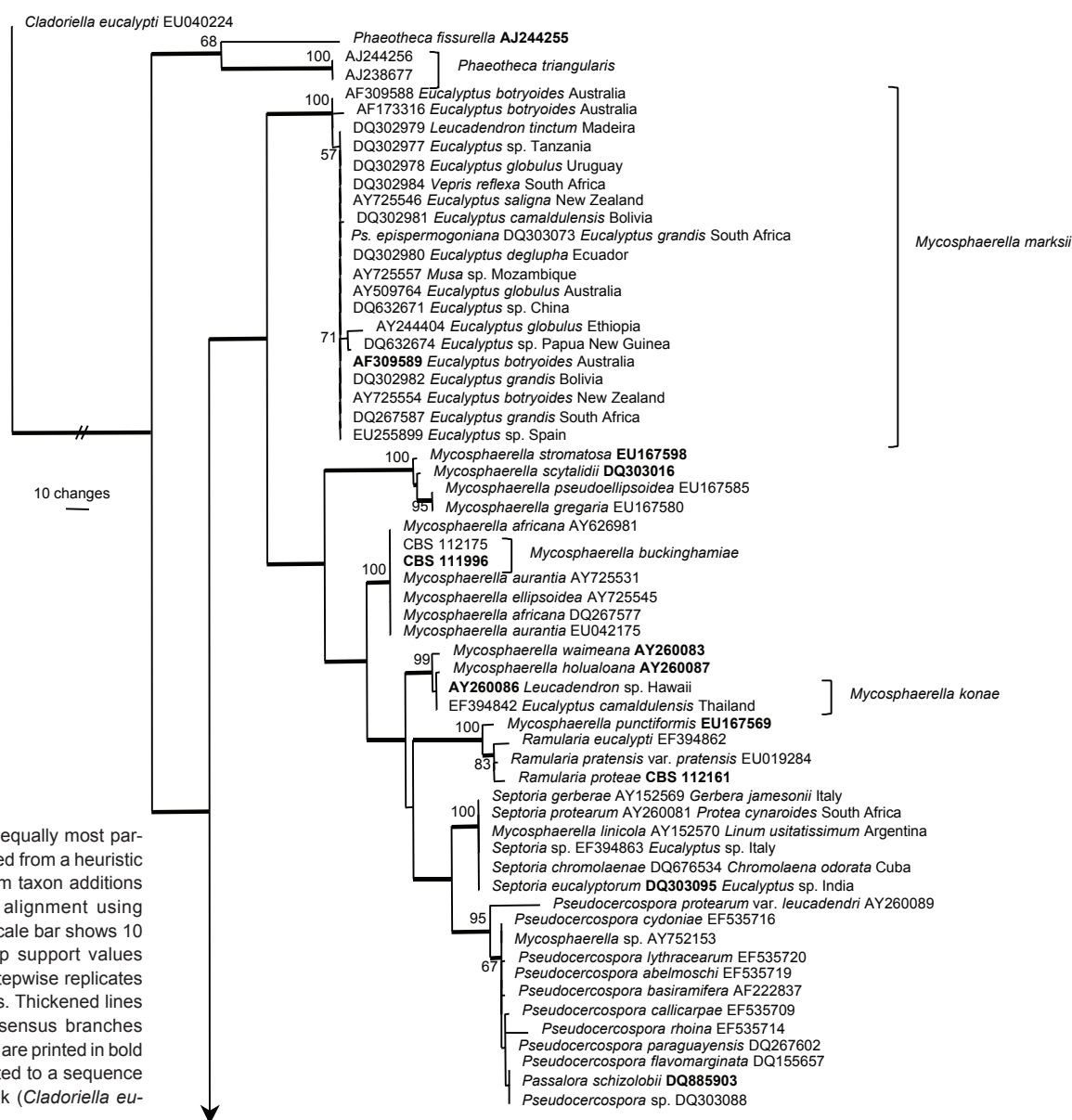


Fig. 1 One of 10 000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the ITS sequence alignment using PAUP v. 4.0b10. The scale bar shows 10 changes and bootstrap support values from 10 000 000 fast stepwise replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and ex-type sequences are printed in bold face. The tree was rooted to a sequence obtained from GenBank (*Cladoriella eucalypti* EU040224).

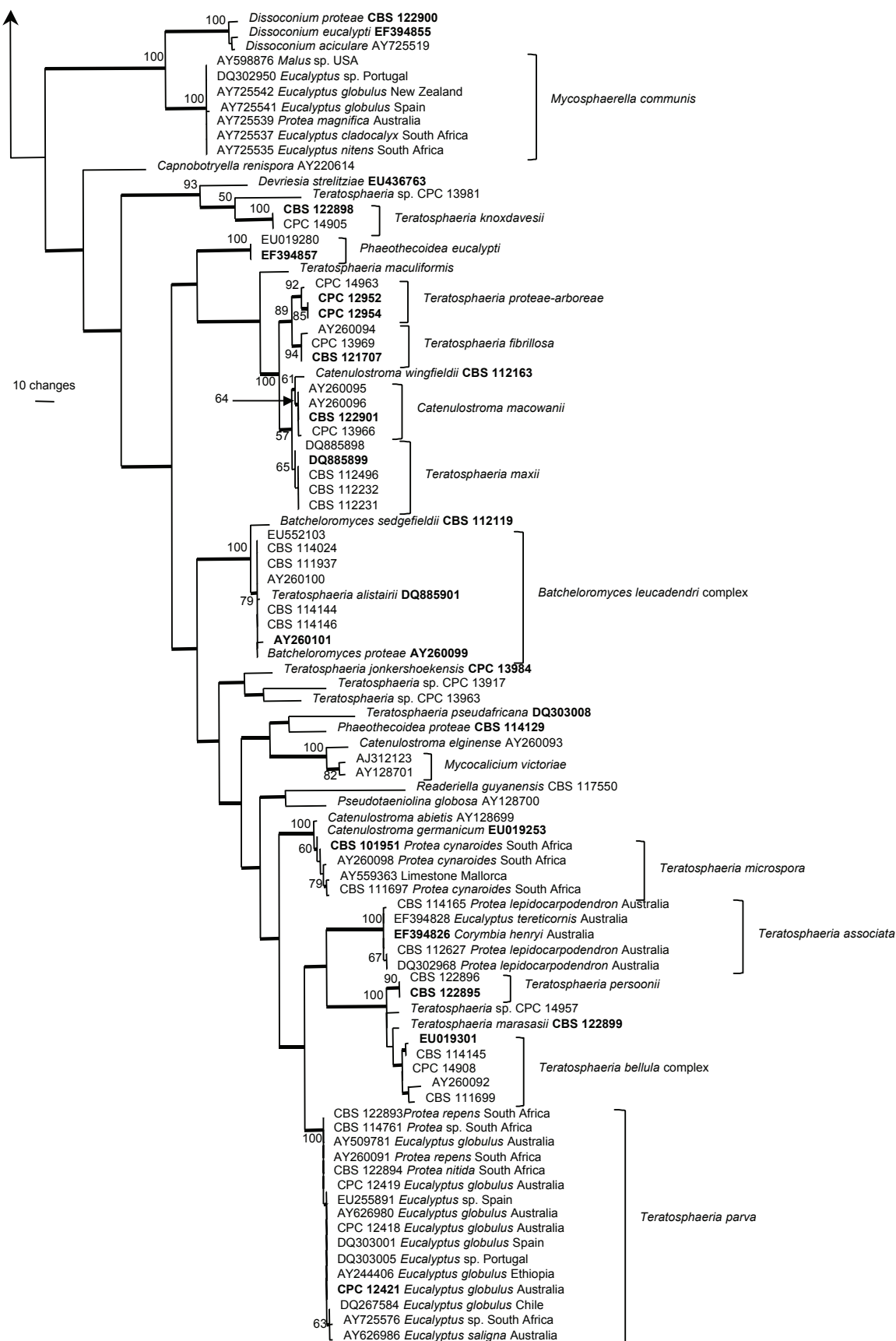


Fig. 1 (cont.)

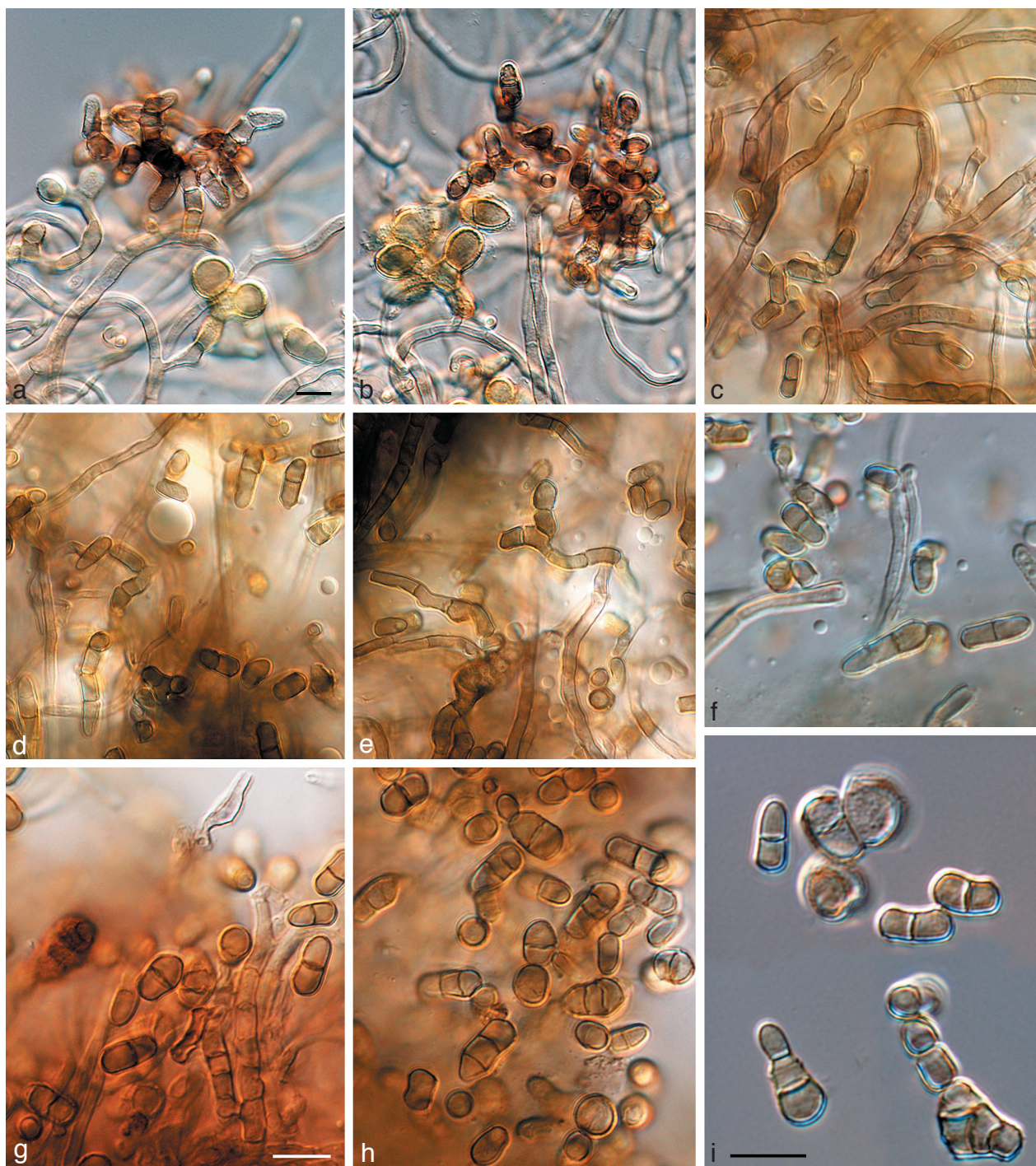


Fig. 2 *Batcheloromyces leucadendri* and *B. proteae*. a–f. *Batcheloromyces leucadendri* (CBS 110892). a, b. Sporodochia formed in culture; c–e. chains of conidia formed in aerial mycelium; f. conidia. — g–i. *Batcheloromyces proteae* (CBS 110696). g, h. conidia formed in aerial mycelium; i. catenulate conidia (note percurrent proliferation on solitary conidiogenous cell). — Scale bars = 10 µm.

in Gams et al. (2007). 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 primer ITS4 (White et al. 1990)

was used in combination with primer V9G for sequencing to ensure good quality overlapping sequences were obtained. The PCR conditions, sequence alignment and subsequent phylogenetic analysis followed the methods of Crous et al. (2006b). The ITS1, ITS2 and 5.8S rRNA gene were sequenced only for those isolates for which these data were not available.

Gaps longer than 10 bases were coded as single events for the phylogenetic analyses; the remaining gaps were treated as missing data. Sequence data were deposited in GenBank (Table 1) and the alignment and trees in TreeBASE (www.treebase.org).

Taxonomy

Wherever possible, 30 measurements ($\times 1\,000$ magnification) were made of structures mounted in lactic acid, with the extremes of spore measurements given in parentheses. Colony colours (surface and reverse) were assessed after 2–8 wk on MEA, OA and PDA at 25 °C in the dark, using the colour charts of Rayner (1970).

RESULTS

DNA phylogeny

Amplicons of approximately 1 700 bases were obtained for the isolates listed in Table 1. The ITS sequences were used to obtain additional sequences from GenBank, which were added to the alignment. The manually adjusted ITS alignment contained 151 sequences (including the outgroup sequence) and 973 characters including alignment gaps (available in TreeBASE). Of the 540 characters used in the phylogenetic analysis, 279 were parsimony-informative, 55 were variable and parsimony-uninformative, and 206 were constant. Neighbour-joining analyses using three substitution models on the sequence alignment yielded trees with identical topologies to one another, except for the position of *Capnobotryella renispora*, which differed when the uncorrected 'p' substitution model was compared with the Kimura 2-parameter and HKY85 models (it is placed basal to *Teratosphaeria* and *Mycosphaerella* in the latter two models). The neighbour-joining trees support the same clades as obtained from the parsimony analysis, but with a different arrangement at the deep nodes, for example the position of the *Dissoconium* and *Phaeotheca* clades. Because of the large number of different strain associations, for example in the *Mycosphaerella marksii*, *Pseudocercospora* and *Teratosphaeria parva* clades (as evident from the strict consensus branches shown in Fig. 1), only the first 10 000 equally most parsimonious trees (TL = 1725 steps; CI = 0.386; RI = 0.870; RC = 0.336) were saved, one of which is shown in Fig. 1. The phylogenetic results obtained are discussed where applicable in the descriptive notes below.

Taxonomy

Although numerous species of *Mycosphaerella*, *Teratosphaeria* and associated anamorphs have been described from Proteaceae (Crous & Wingfield 1993, Crous & Braun 1994, 1996, Swart et al. 1998, Crous & Palm 1999, Crous et al. 2000b, Taylor & Crous 2000, Taylor et al. 2001a–c, 2003, Sivanesan & Shivas 2002), only those known from culture are discussed below.

Batcheloromyces leucadendri P.S. van Wyk, Marasas & Knox-Dav., S. African J. Bot. 51: 344. 1985 — Fig. 2

Descriptions — Taylor et al. (1999), Crous et al. (2004a).

Cultural characteristics — Colonies on MEA iron-grey, reverse olivaceous-black, smooth with irregular margins; aerial mycelium sparse and grey or lacking; moderately slow growing, up to 40 mm in diam after 2 mo.

Specimens examined. SOUTH AFRICA, Western Cape Province, Betty's Bay, living leaves of *Leucadendron gandogerii*, 27 Mar. 1984, P.S. van Wyk, holotype PREM 47423; Cape Town, Kirstenbosch Botanical Gardens, on a living leaf of *Leucadendron coniferum*, 1996, L. Swart, F54, PREM 55954, CPC 1840; Porterville, Osdam Farm, on a living leaf of *Leucadendron* sp., 15 Jan. 1998, J.E. Taylor, JT84, PREM 55940; Stellenbosch, Helderberg Nature Reserve, on a living leaf of *Leucadendron* sp., 19 Jan. 1998, J.E. Taylor, JT107, PREM 55941; Stellenbosch, J.S. Marais Nature Reserve, on a living leaf of *Leucadendron* sp., 1996, L. Swart, F46, CPC 1837 = CBS 110892; Stellenbosch, Protea Heights farm, on a living leaf of *Leucadendron lauratum*, 1996, L. Swart, epitype designated here PREM 55949, cultures ex-epitype, CPC 1838 = CBS 111577; *ibid.*, *Leucadendron* cultivar 'Pisa', F49, PREM 55950; Stellenbosch, J.S. Marais Park, on a living leaf of *Leucadendron salicifolium*, 1996, L. Swart, F50, PREM 55951; *ibid.*, *Leucadendron* sp., F51, PREM 55952, CPC 1839; *ibid.*, *Leucadendron elimense*, F52, PREM 55953; Western Cape Province, *Leucadendron* sp., 27 May 1999, L. Swart, CPC 2820 = CBS 114146; *ibid.*, CPC 2823 = CBS 114144; *ibid.*, CPC 2794 = CBS 114024; *ibid.*, CPC 2822 = CBS 111937; Jonkershoek Nature Reserve, 6 June 2000, leaf litter of *Leucadendron salignum*, S. Marincowitz, S.L.130, PREM 58041, cultures CBS 119344 = CMW 20456.

Batcheloromyces proteae Marasas, P.S. van Wyk, & Knox-Dav., J. S. African Bot. 41: 43. 1975 — Fig. 2

= *Stigmia proteae* (Marasas, P.S. van Wyk, & Knox-Dav.) B. Sutton & Pascoe, Mycol. Res. 92: 214. 1989.

Descriptions — Taylor et al. (1999), Crous et al. (2004a).

Cultural characteristics — Colonies olivaceous, the same in reverse, smooth with irregular margins, sectorial; aerial mycelium lacking; slow-growing, approximately 10–18 mm diam after 2 mo.

Specimens examined. SOUTH AFRICA, Western Cape Province, Stellenbosch, on living leaves of *Protea cynaroides*, 15 Aug. 1973, P.S. van Wyk, holotype PREM 44850; Betty's Bay, Harold Porter Botanical Garden, on a living leaf of *P. magnifica*, 1997, L. Swart, F44, PREM 55947; *ibid.*, *P. nerifolia*, F45, PREM 55948, CPC 1836; Stellenbosch, J.S. Marais Park, on a living leaf of *P. cynaroides*, 28 Feb. 1998, J.E. Taylor, JT119, PREM 55942; *ibid.*, 1996, L. Swart, F42, PREM 55946; *ibid.*, 26 Aug. 1996, L. Swart, F25, PREM 55943, CPC 1833; *ibid.*, *P. repens*, F40, PREM 55944, CPC 1834; *ibid.*, *Protea* sp., F41, PREM 55945, CPC 1835; Stellenbosch, Devon Valley, on a living leaf of *P. cynaroides*, 30 Aug. 1996, L. Swart, epitype designated here CBS H-20087, culture ex-epitype CPC 1518 = CBS 110696.

Notes — Although morphologically distinct, *B. proteae* could not be distinguished phylogenetically from *B. leucadendri* in the present study (Fig. 1).

Batcheloromyces sedgefieldii Crous, *sp. nov.* — MycoBank MB506591; Fig. 3

Batcheloromyces proteae similis, sed in agaro (MEA) coloniis tarde crescentibus, usque ad 5 mm diam post 30 dies 25 °C.

Etymology. Named after Sedgefield, a town in the Southern Cape of South Africa, from where this fungus was collected.

Leaf spots containing black sporodochial plates, not extending through leaf lamina, on both sides of leaf surface, irregular, non-necrotic, becoming somewhat erumpent, up to 7 mm diam.

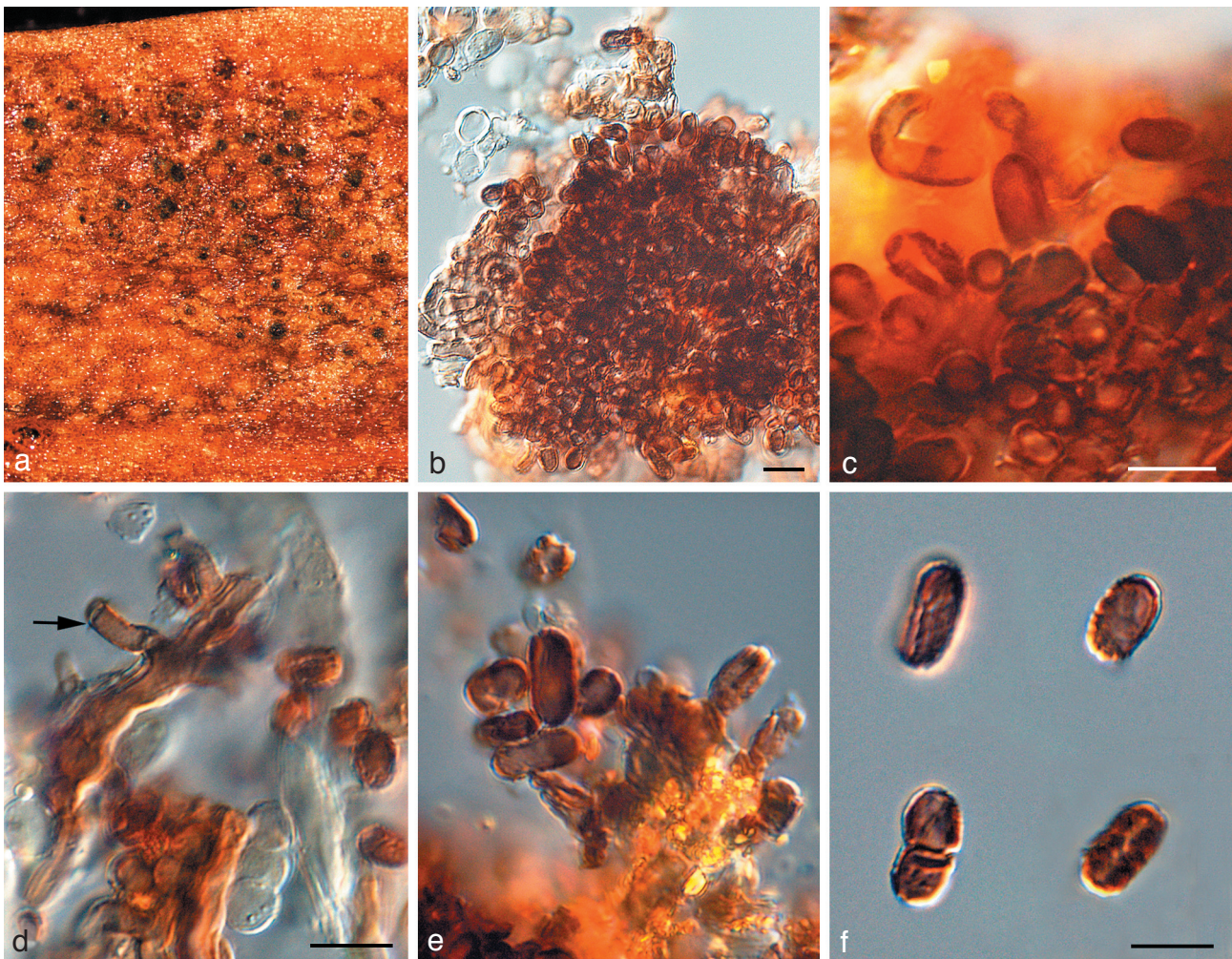


Fig. 3 *Batcheloromyces sedgefieldii* in vivo (CBS H-20088). a. Sporodochia on leaf; b. sporodochium; c–e. conidiogenous cells giving rise to conidia; f. verruculose conidia. — Scale bars = 10 µm.

Conidiomata sporodochial, composed of a single layer of radiating, septate, branched, dark brown, thick-walled hyphae which are formed from a stroma within the substomatal cavity, forming pulvinate plates, 100–120 µm diam; hyphae radiating from sporodochial plates and adhering closely to the host surface or growing into the stomata. *Conidiophores* erect or ascending, short lateral branches on the superficial hyphae, simple, brown, effuse, but occurring mainly towards the centre of the sporodochial plates, above the stomata, terminating to produce conidiogenous cells. *Conidiogenous cells* mainly subcylindrical, but occasionally doliiform, proliferating percurrently resulting in up to three irregular, ragged annellides, 5–10 × 3–5 µm. *Conidia* arising singly from blown out ends of the conidiogenous cells, solitary, unicellular, but often remaining in fragile chains of 2 conidia, ellipsoidal, oblong, with thick, verrucose walls, and a basal marginal frill, (5–)6.5–7(–10) × (3.5–)4–5 µm.

Cultural characteristics — Colonies on MEA olivaceous, with smooth margins, lacking aerial mycelium; extremely slow-growing, up to 5 mm diam after 2 mo.

Specimen examined. SOUTH AFRICA, Western Cape Province, Southern Cape, Sedgefield, on leaves of *Protea repens*, 10 Aug. 1999, J.E. Taylor, holotype CBS H-20088, culture ex-type CPC 3026 = CBS 112119.

Notes — Although phylogenetically distinct, *B. sedgefieldii* is morphologically similar to *B. proteae*. The two species can be distinguished in culture, however, as colonies of *B. proteae* are relatively fast growing compared to those of *B. sedgefieldii*, which only reach 5 mm diam after 2 mo.

Catenulostroma elginense (Joanne E. Taylor & Crous) Crous & U. Braun, Stud. Mycol. 58: 16. 2007

Basionym. *Trimmatostroma elginense* Joanne E. Taylor & Crous, Mycol. Res. 104: 633. 2000.

Descriptions — Taylor & Crous (2000), Crous et al. (2004a).

Specimen examined. SOUTH AFRICA, Western Cape Province, Elgin, Molteno Brothers Farm, on a living leaf of *Protea grandiceps* Tratt., 21 July 1998, J.E. Taylor & S. Denman, holotype PREM 56208, culture ex-type CPC 1958 = CBS 111030.

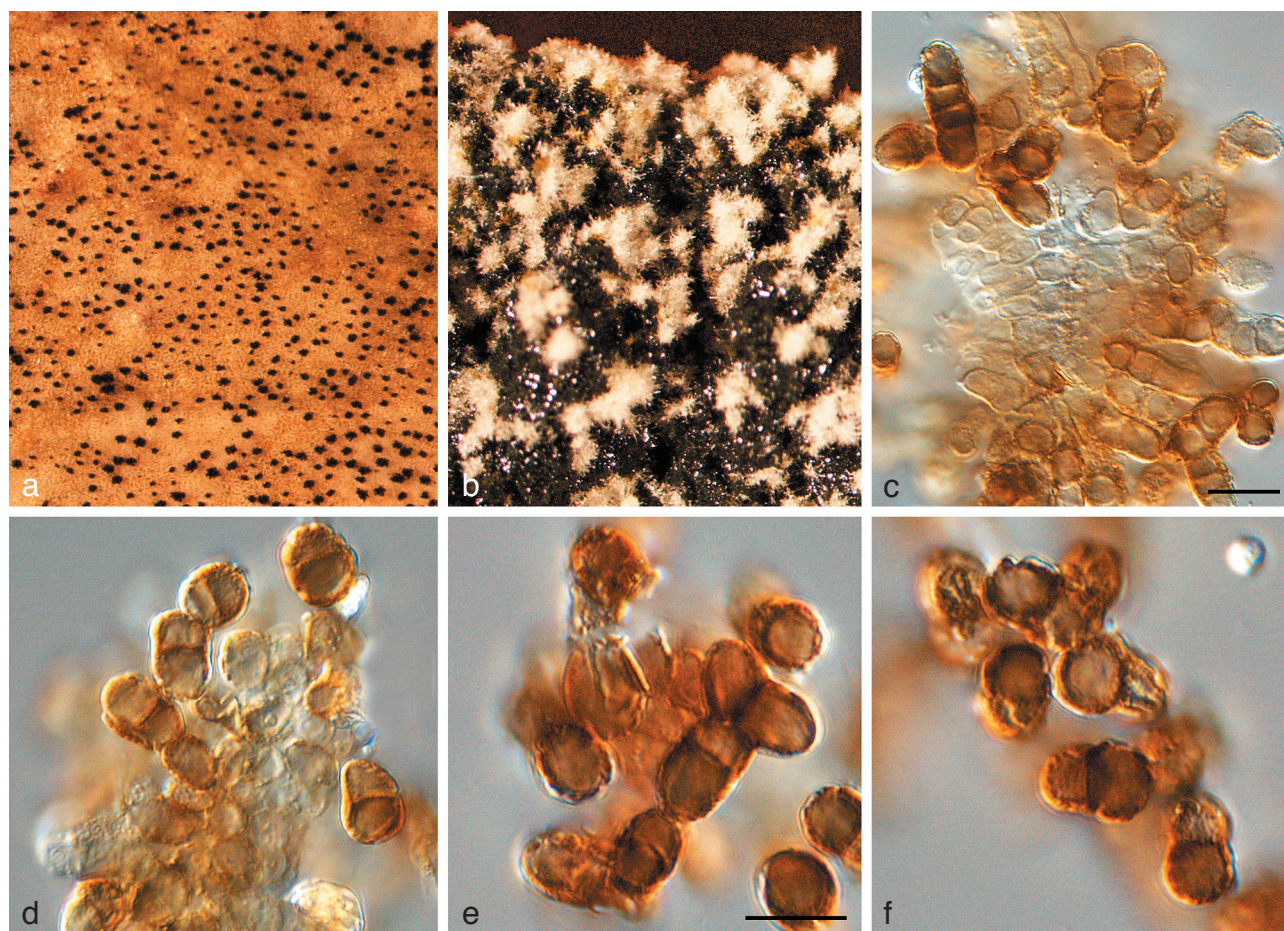


Fig. 4 *Catenulostroma macowanii* (CBS 122901). a. Sporodochia on leaf; b. sporulation on MEA; c, d. conidiogenous cells giving rise to conidia; e, f. conidia. — Scale bars = 10 µm.

Catenulostroma macowanii (Sacc.) Crous & U. Braun, Stud. Mycol. 58: 17. 2007 — Fig. 4

Basionym. *Coniothecium macowanii* Sacc., Syll. Fung. 4: 512. 1886. nom. nov., based on *Coniothecium punctiforme* G. Winter, Hedwigia 24: 33. 1885, non *C. punctiforme* Corda, Icon. Fungorum (Corda) 1: 2. 1837.

≡ *Trimmatostroma macowanii* (Sacc.) M.B. Ellis, *More Dematiaceous Hyphomycetes*: 29. 1976.

Descriptions — Taylor & Crous (1998f), Crous et al. (2004a).

Cultural characteristics — Colonies on MEA erumpent, spreading, margins irregular, smooth, surface with sparse pale olivaceous-grey aerial mycelium, interrupted by black conidial masses bursting through the layer of aerial mycelium; reaching 4 mm diam after 2 wk.

Specimens examined. SOUTH AFRICA, Western Cape Province, Table Mountain, on living leaves of *Protea grandiflora*, P. MacOwan, holotype JE (of *Coniothecium punctiforme* G. Winter); Cederberge, on living leaves of *P. nitida*, 10 May 1998, J.E. Taylor, CPC 1872 = CBS 110756; Hermanus, on a living leaf of *P. nitida*, 13 Aug. 1996, P.W. Crous, CPC 1488 = CBS 111029; Jonkershoek, S33°59'11.2" E18°57'14.7", on living leaves of *P. nitida*, 1 Apr. 2007, P.W. Crous & L. Mostert, epitype designated here CBS H-20089, cultures ex-epitype CPC 13899, 13901, 13900 = CBS 122901; ibid, CPC 13966–13968.

Catenulostroma wingfieldii Crous, *sp. nov.* — MycoBank MB506592; Fig. 5

Catenulostromatis macowanii simile, sed conidiis longioribus, (13–)20–26 (–35) × (5–)6–8 (–12) µm.

Etymology. Named in honour of Prof. M.J. Wingfield, who has made a significant contribution to our knowledge of the fungi occurring on Proteaceae.

Conidiomata sporodochial, pulvinate, punctiform, brown-black, 40–80 µm diam. **Conidiophores** emerging as fascicles through the stomata, hyaline to pale brown, smooth or verruculose, cylindrical. **Conidiogenous cells** holoblastic, basipetal, forming an unconnected chain of conidia delimited by a septum followed by diffuse wall-building below the previous conidium to form the next conidium which is delimited retrogressively, conidia separating by schizolytic secession. **Conidia** formed in simple and branched chains, dry, highly variable in shape, ellipsoidal to subcylindrical, Y-shaped, curved or straight, with rounded apices, and truncate bases, frequently with a prominent marginal frill, transversely 1- to multi-septate, at times with oblique septa, pale to medium-brown, verrucose, (13–)20–26 (–35) × (5–)6–8 (–12) µm.

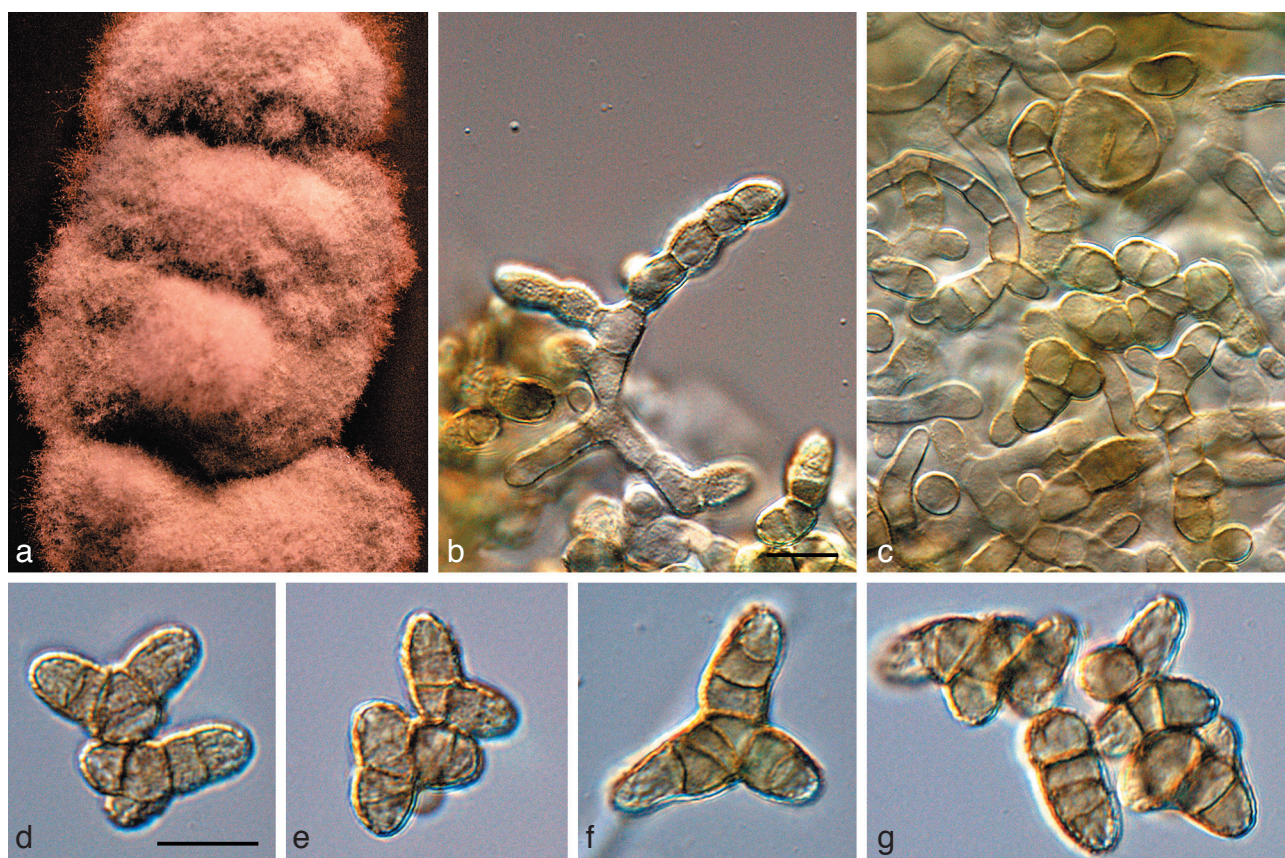


Fig. 5 *Catenulostroma wingfieldii* (CBS 112163). a. Colony growing on MEA; b, c. conidia forming on aerial mycelium; d–g. conidia. — Scale bars = 10 µm.

Cultural characteristics — Colonies on MEA erumpent, with smooth, regular margins, and sparse aerial mycelium, pale olivaceous-grey; colonies reaching 7 mm diam after 2 wk on MEA; on OA erumpent with smooth to feathery margins, sparse to moderate aerial mycelium, pale olivaceous-grey to olivaceous-grey; colonies reaching 7 mm diam after 2 wk at 25 °C.

Specimen examined. SOUTH AFRICA, Western Cape Province, Kirstenbosch Botanical Gardens, on living leaves of *Protea nitida*, 15 Aug. 1999, J.E. Taylor, holotype CBS H-20090, cultures ex-type CPC 2944 = CBS 112163, CPC 2945–2946.

Notes — *Catenulostroma wingfieldii* is similar to *C. macowanii*, but differs in that colonies are pale olivaceous-grey on MEA, while those of *C. macowanii* are iron-grey to black, and lack aerial mycelium. Furthermore, conidia of *C. wingfieldii* are somewhat larger, being (13–)20–26(–35) × (5–)6–8(–12) µm, while those of *C. macowanii* are (10–)15–17(–23) × (6–)6.5–7(–9) µm.

***Dissoconium proteae* Crous, sp. nov.** — MycoBank MB506593; Fig. 6

Dissoconio aciculari simile, sed conidiis primariis minoribus, (9–)10–11(–12) × (3–)3.5(–4) µm, conidiis secundariis quoque minoribus, 7–8(–10) × (3–)3.5(–4) µm.

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

Mycelium internal and external, consisting of branched, septate, smooth, hyaline to pale brown hyphae, 1.5–2 µm wide. **Conidiophores** separate, arising from hyphae, subcylindrical, subulate or lageniform, tapering to a bluntly rounded or truncate apex, straight to gently curved, smooth, hyaline, becoming medium brown with age, aseptate, 10–30 × 3–5 µm; loci terminal and lateral, visible as slightly thickened, darkened scars, 0.5 µm wide. **Conidia** (9–)10–11(–12) × (3–)3.5(–4) µm, solitary, straight to somewhat curved, hyaline to pale olivaceous, smooth, ellipsoid, not to slightly constricted at median septum, apex obtuse, base obconic-truncate, tapering pronounced at somewhat protruding hilum, unthickened, not darkened, 1 µm wide. **Secondary conidia** developing adjacent to primary conidia, hyaline to subhyaline, aseptate, ellipsoid, tapering prominently towards a protruding, truncate base, 7–8(–10) × (3–)3.5(–4) µm; anastomosing with primary conidia after active discharge (in some cases the secondary conidia were observed to germinate, which has never been observed in species with smaller, pyriform secondary conidia).

Cultural characteristics — Colonies on OA spreading, with sparse aerial mycelium, and irregular margins; surface sienna, with patches of white and cinnamon; forming clusters of black sclerotia (remaining infertile) on OA, MEA and PDA; reaching 10 mm diam after 1 mo on OA.

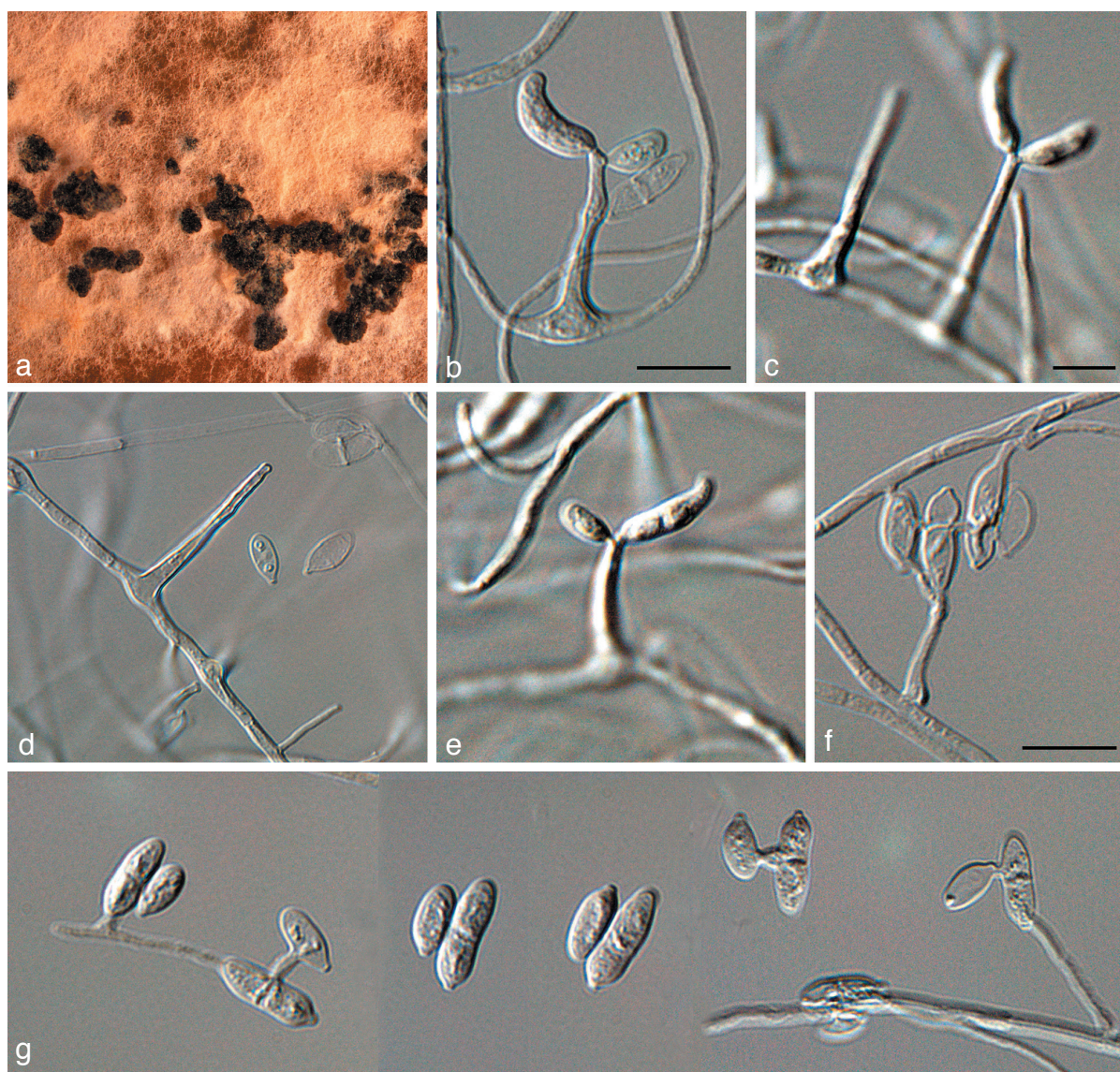


Fig. 6 *Dissoconium proteae* (CBS 122900). a. Sclerotia forming on MEA; b–f. solitary conidiophores giving rise to primary and secondary conidia; g. anastomosing primary and secondary conidia. — Scale bars = 10 μ m.

Specimen examined. CANARY ISLANDS, Tenerife, on leaves of *Protea* sp., 1 Mar. 2007, P.W. Crous, holotype CBS H-20091, culture ex-type CPC 13853 = CBS 122900.

Notes — Of the *Dissoconium* species known to date (Crous et al. 2004b, Zhang et al. 2007, Arzanlou et al. 2008), *D. proteae* is most similar to *D. eucalypti* (primary conidia, $8\text{--}14 \times 4.5\text{--}6$ μ m, and secondary conidia, $4\text{--}7 \times 2.5\text{--}3$ μ m), and *D. aciculare* (primary conidia, $12\text{--}25 \times 3.5\text{--}6$ μ m, and secondary conidia, $7.5\text{--}12 \times 3.5\text{--}6$ μ m), but has smaller and narrower primary and secondary conidia ($9\text{--}12 \times 3\text{--}4$ μ m, and $7\text{--}10 \times 3\text{--}4$ μ m, respectively), than both.

Mycosphaerella buckinghamiae Crous & Summerell, Australas. Pl. Pathol. 29: 272. 2000

Description — Crous et al. (2000b).

Specimen examined. AUSTRALIA, New South Wales, Mangrove Mountain, on leaves of *Buckinghamia* sp., Aug. 1999, P.W. Crous & B. Summerell, holotype DAR 74865, cultures ex-type CPC 3006 = CBS 111996.

Notes — Although the ITS DNA sequence of *M. buckinghamiae* is identical to that of *M. africana* (ascospores $7\text{--}11 \times 2\text{--}3$ μ m, darkening and distorting upon germination), *M. buckinghamiae* has larger ascospores ($9\text{--}13 \times 2.5\text{--}3.5$ μ m), which do not darken at germination, and colonies that contain rose and off-white sectors, which are lacking in *M. africana*, which again has black colonies, forming a brown pigment in MEA (Crous 1998, Crous et al. 2000b).

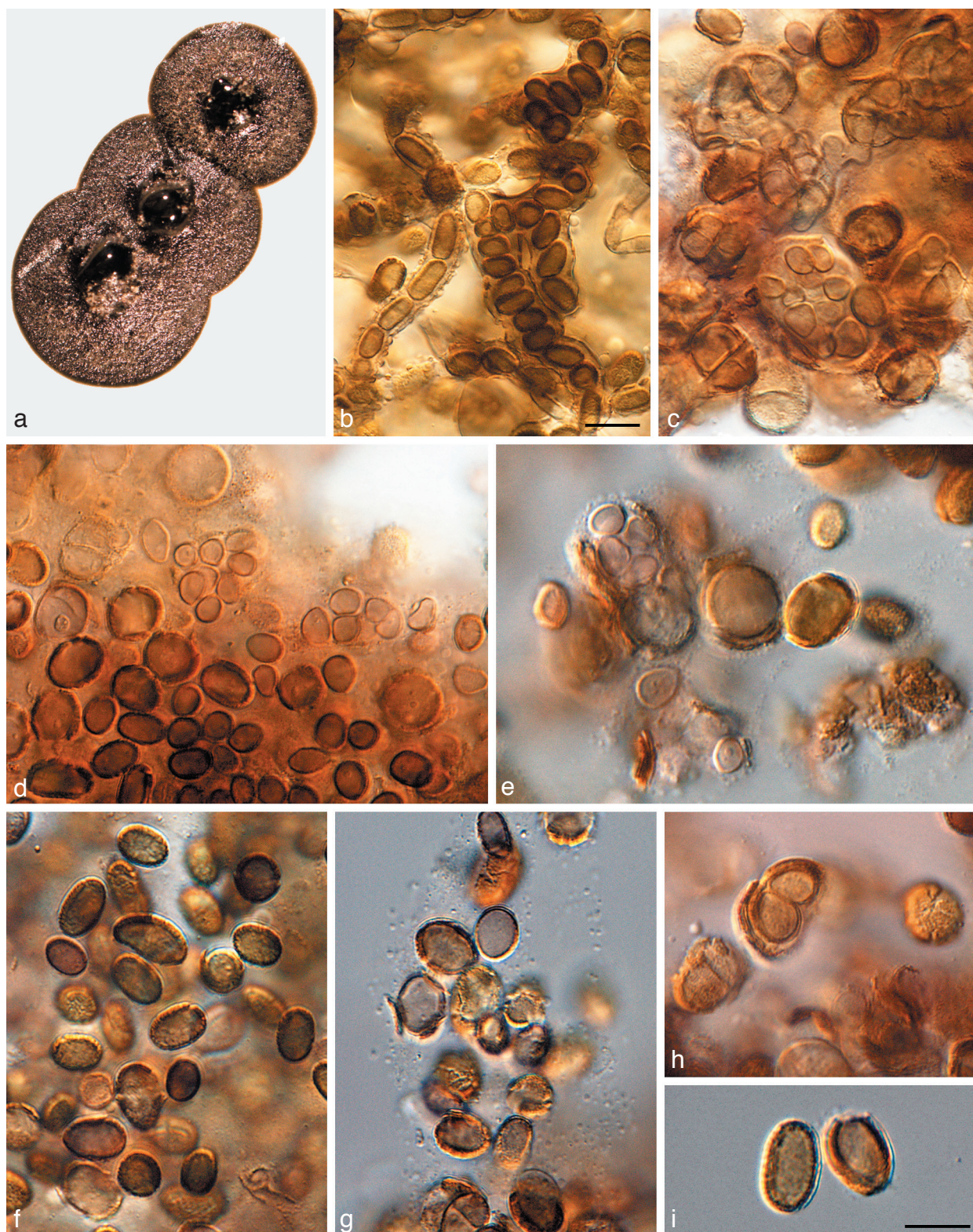


Fig. 7 *Phaeothecoidea proteae* (CBS 114129). a. Colony on OA; b–e. hyphae with endoconidia visible; f–i. released endoconidia become brown and verruculose. — Scale bars = 10 µm.

***Mycosphaerella communis* Crous & Mansilla, Stud. Mycol. 50: 203. 2004**

Anamorph. *Dissoconium commune* Crous & Mansilla, Stud. Mycol. 50: 203. 2004.

Description — Crous et al. (2004b).

Specimens examined. SPAIN, Pontevedra, Lourizán, Areeiro, on leaves of *Eucalyptus globulus*, Dec. 2002, J.P. Mansilla, holotype of *M. communis* and *D. commune* CBS H-9900, culture ex-type CBS 114238 = CPC 10440. — AUSTRALIA, New South Wales, Mount Tomah Botanic Garden, on leaves of *Protea magnifica*, 2002, P.W. Crous, CPC 3359 = CBS 112889.

***Mycosphaerella holualoana* Crous, Joanne E. Taylor & M.E. Palm, Mycotaxon 78: 458. 2001**

Descriptions — Taylor et al. (2001b), Crous et al. (2004a).

Specimen examined. USA, Hawaii, Kona district, Holualoa, on a living leaf of *Leucospermum* sp., 17 Nov. 1998, P.W. Crous & M.E. Palm, holotype PREM 56926, culture ex-type CPC 2126 = CBS 110698.

***Mycosphaerella konae* Crous, Joanne E. Taylor & M.E. Palm, Mycotaxon 78: 459. 2001**

Anamorph. *Pseudocercospora* sp.

Descriptions — Taylor et al. (2001b), Crous et al. (2004a).

Specimens examined. USA, Hawaii, Kona district, Holualoa, on a living leaf on *Leucadendron* 'Safari Sunset', 17 Nov. 1998, P.W. Crous & M.E. Palm, holotype PREM 56921, cultures ex-type CPC 2123 = CBS 111261, CPC 2125 = CBS 111028. — THAILAND, Thatakiab District, Chachoengsao Province, on leaves of *Eucalyptus camaldulensis*, 12 Oct. 2006, W. Hima-man, CPC 13469 = CBS 120748, CPC 13470.

***Mycosphaerella marksii* Carnegie & Keane, Mycol. Res. 98: 414. 1994**

Description — Carnegie & Keane (1994), Crous (1998).

Specimens examined. AUSTRALIA, Victoria, Nowa Nowa, on leaves of *Eucalyptus botryoides*, 11 Nov. 1990, A.J. Carnegie, holotype IMI 353731; Tostaree, on leaves of *E. botryoides*, Oct. 1994, A.J. Carnegie, epitype designated here PREM 51932, cultures ex-epitype CPC 982 = CBS 110942, CPC 984 = CBS 110974. — MADEIRA ISLANDS, Florialis Estate, on leaves of *Leucadendron tinctorum*, 1 Apr. 2000, S. Denman, CPC 5358 = CBS 115501.

***Mycosphaerella stromatosa* Joanne E. Taylor & Crous, Mycol. Res. 104: 625. 2000**

Anamorph. *Pseudocercospora stromatosa* Joanne E. Taylor & Crous, Mycol. Res. 104: 625. 2000.

Description — Taylor & Crous (2000), Crous et al. (2004a).

Specimen examined. SOUTH AFRICA, Kwazulu-Natal, Drakensberg, Dragon's Peak, on a living leaf of *Protea* sp., Jan. 1998, S. Denman, holotype PREM 56204, culture ex-type CPC 1731 = CBS 101953.

***Mycosphaerella waimeana* Crous, Joanne E. Taylor & M.E. Palm, Mycotaxon 78: 463. 2001**

Anamorph. *Stenella* sp.

Descriptions — Taylor et al. (2001b), Crous et al. (2004a).

Specimen examined. USA, Hawaii, Kona district, Waimea, on a living leaf of *Leucospermum* hybrid 24, 17 Nov. 1998, P.W. Crous & M.E. Palm, holotype PREM 56950, culture ex-type CPC 2179 = CBS 110697.

***Phaeothecoidea proteae* Crous, sp. nov. — MycoBank MB506594; Fig. 7**

Phaeothecoidea eucalypti similis, sed conidiis majoribus, (6–)8–10(–13) × (4–)5–6(–11) µm.

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

Hyphae in vitro creeping, brown, verruculose, branched, septate, 3–5 µm wide, becoming swollen, up to 15 µm wide, verruculose, dark brown, or forming a mucoid capsule filled with endoconidia which are former hyphal cells that turn brown and thick-walled; end cells dividing into several endoconidia, which are released upon rupture of the cell wall. *Endoconidia* medium to dark brown, verruculose to verrucose to warty, thick-walled, ellipsoid to obovoid or obclavate, (6–)8–10(–13) × (4–)5–6(–11) µm; after liberation swelling, becoming transversely 1-septate, or with several oblique septa, again forming endoconidia, becoming warty with age, with the outer layer peeling off once endoconidia are released.

Cultural characteristics — Colonies on MEA slimy, erumpent, lacking aerial mycelium, irregular, folded, with smooth, regular margin; surface iron-grey, reverse fuscous-black; colonies reaching 7 mm diam after 2 wk; on OA lacking aerial mycelium, erumpent with smooth margins, black, reaching 6 mm diam after 2 wk; fertile.

Specimen examined. SOUTH AFRICA, Western Cape Province, Stellenbosch, Elsenburg Farm, on leaves of *Protea repens*, 23 July 1999, S. Denman, holotype CBS H-20092, cultures ex-type CPC 2828–2830, 2831 = CBS 114129.

Notes — The present species clusters close to the type of the genus *Phaeothecoidea*, *P. eucalypti* (Crous et al. 2007d), and as it shares the feature of brown, verruculose endoconidia, we chose to describe it as a new species of this genus. It is interesting to note that it was originally isolated as a coelomycete, and based on its yeast-like growth in culture, identified as *Coniothyrium leucospermi* (Swart et al. 1998, Taylor & Crous 2001), which has subsequently been allocated to a new genus, *Coniozyma* (Marincowitz et al. 2008). Unfortunately, the original herbarium specimen could not be located, and thus only the cultural synanamorph can be described here.

***Pseudocercospora protearum* var. *leucadendri* (Cooke) U. Braun & Crous, Mycol. Progr. 1: 22. 2002**

Basionym. *Cercospora protearum* Cooke var. *leucadendri* Cooke, Grevillea 12: 39. 1883.

= *Stigmata protearum* var. *leucadendri* (Cooke) M.B. Ellis, Mycol. Pap. 131: 7. 1972.

= *Cercostigmata protearum* var. *leucadendri* (Cooke) U. Braun & Crous, Sydowia 46: 206. 1994.

= *Passalora protearum* Kalchbr. & Cooke, Grevillea 19: 6. 1890.

Description — Crous et al. (2004a).

Specimens examined. SOUTH AFRICA, Cape Province, Cape of Good Hope, Table Mountain, *Leucadendron argenteum*, MacOwan, No 1457, holotype K; Stellenbosch, Devon Valley, Protea Heights, on leaves of *Leucadendron* sp., 3 Apr. 1998, P.W. Crous & S. Denman, culture CPC 1869.

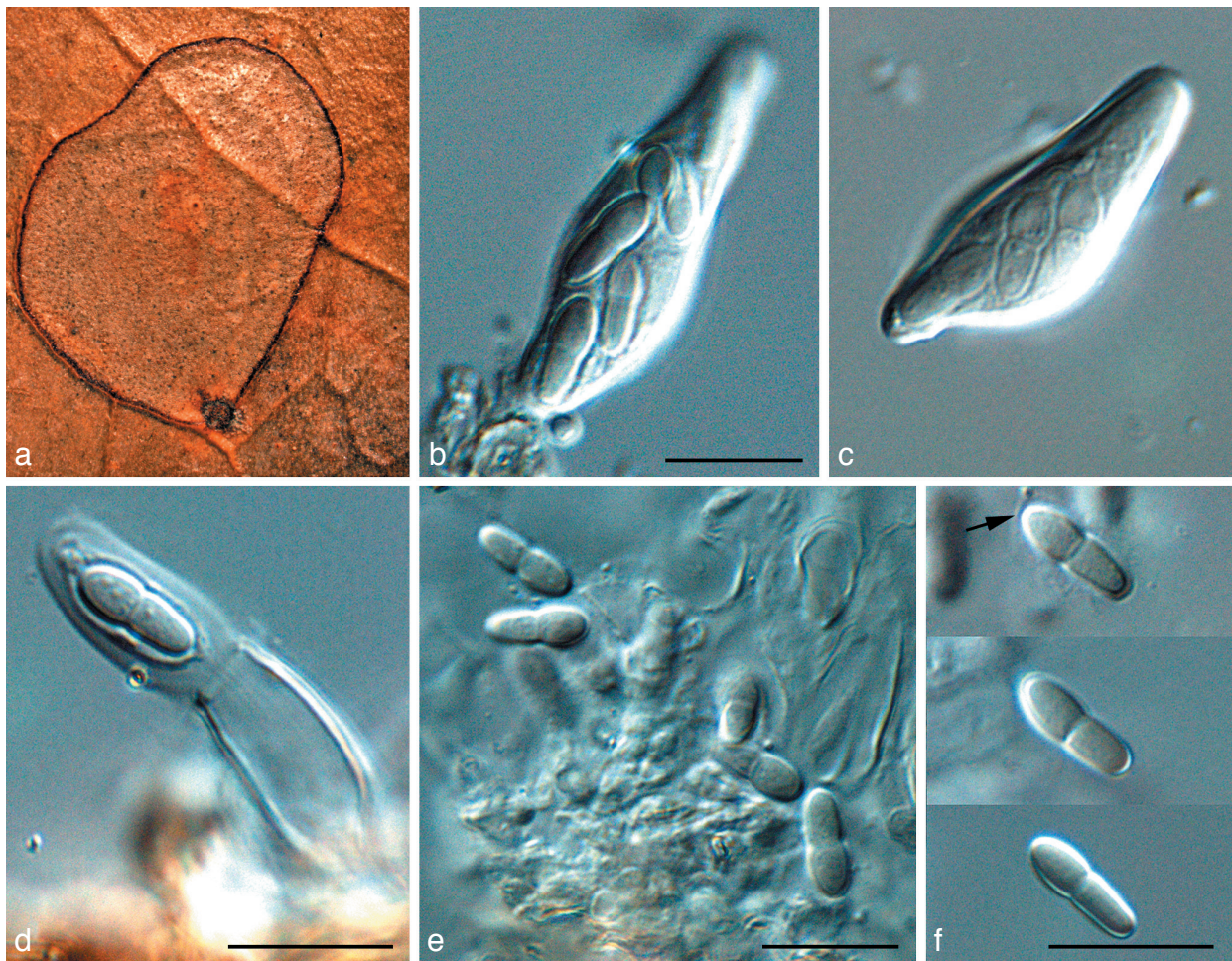


Fig. 8 *Teratosphaeria bellula* (CBS 111699). a. Leaf spot on *Protea eximia*; b, c. asci; d. ascus with jack-in-the-box release of inner sack, showing mucilaginous sheath around ascospore; e, f. ascospores (sheath indicated by arrow). — Scale bars = 10 μ m.

Ramularia proteae Crous & Summerell, Australas. Pl. Pathol. 29: 277. 2000

Description — Crous et al. (2000b).

Specimen examined. AUSTRALIA, Tasmania, Royal Tasmanian Botanical Gardens, Hobart, on leaves of *Protea longifolia*, Aug. 1999, A. Macfadyen, holotype DAR 74883, culture ex-type CPC 3075 = CBS 112161.

Septoria protearum Viljoen & Crous, S. Afr. J. Bot. 64: 144. 1998

Description — Crous et al. (2004a).

Specimens examined. SOUTH AFRICA, Gauteng Province, Pretoria, leaves of *Protea cynaroides*, Sept. 1996, L. Viljoen, holotype PREM 55353, culture ex-type CPC 1470 = IMI 375230 = ATCC 201159 = CBS 778.97. — CANARY ISLANDS, Tenerife, leaves of *Protea* sp., 1 Apr. 2000, S. Denman, CPC 5212.

Teratosphaeria alistairii (Crous) Crous & U. Braun, Stud. Mycol. 58: 9. 2007

Basionym. *Mycosphaerella alistairii* Crous, in Crous & Groenewald, Fungal Planet, No. 4. 2006.

Anamorph. *Batcheloromyces* sp.

Description — Crous & Groenewald (2006a).

Specimen examined. SOUTH AFRICA, Western Cape Province, Hermanus, Rotary Road, close to the Vodacom tower, on leaves of *Protea repens*, 31 Dec. 2005, P. W. Crous & A. Smith, holotype CBS H-19765, cultures ex-type CPC 12730 = CBS 120035, CPC 12731–12732.

Notes. — *Teratosphaeria alistairii* resembles *T. jonkershoekensis* in symptomatology on the host, but is distinct in having smaller ascospores, $(9-10-12(-13) \times (2.5-3-4 \mu\text{m})$, and a *Batcheloromyces* anamorph (Crous & Groenewald 2006a).

Teratosphaeria associata (Crous & Carnegie) Crous & U. Braun, Stud. Mycol. 58: 9. 2007

Basionym. *Mycosphaerella associata* Crous & Carnegie, Fung. Diversity 26: 159. 2007.

Description — Crous et al. (2007d).

Specimens examined. AUSTRALIA, New South Wales, South Grafton, Grafton City Council Landfill Plantation, E152°54'38", S29°46'21", on leaves of *Corymbia henryii*, 16 Feb. 2006, A. J. Carnegie, holotype CBS-H 19833, isotype DAR 78031, cultures ex-type CPC 13119 = CBS 120730, CPC 13120 (occurring with *Lembosina* sp.); NSW, Bungawalbin, Robertson Plantation,



Fig. 9 *Teratosphaeria fibrillosa* in vivo (CBS H-19913). a. Ascomata on leaf surface, linked by stromatic tissue; b, c. germinating ascospores; d–f. asci with darkening ascospores; g–i. ascospores. — Scale bars = 10 µm.

E153°15'39", S29°5'34", on leaves of *Corymbia variegata*, 23 Jan. 2005, A.J. Carnegie, DAR 78032, cultures CPC 13128 = CBS 120731, CPC 13129–13130 (occurring with *Lembosina* sp.); NSW, Bungawalbin, Robertson Plantation, E153°15'39", S29°5'34", on leaves of *Eucalyptus dunnii*, 14 Feb. 2006, A.J. Carnegie, cultures CPC 13108 = CBS 120732, CPC 13109–13110, 13113–13114 (occurring with *M. suberosa*); NSW, Mount Tomah Botanic Gardens, on leaves of *Protea lepidocarpodendron*, Aug. 1999, P.W. Crous & B. Summerell, JT 993, DAR 74867, cultures CPC 3115 = CBS 112627, CPC 3116 = CBS 112224, CPC 3117 = CBS 114165.

Notes — *Teratosphaeria associata* was recently described from *Eucalyptus* in Australia (Crous et al. 2007d), where it occurred in association with several other species (hence the name, *associata*). Crous et al. (2000b) reported the presence of *T. jonkershoekensis* from *Protea* spp. in Australia, where this species was observed to be an important primary pathogen (suggesting *Eucalyptus* is not a primary host of *T. associata*). Based on the results obtained here, it appears that the *Protea* isolates were incorrectly identified, and belong to the recently named *T. associata*. Furthermore, the recent description of *T. alistairii* and *T. maxii* from this host, suggest that there could be several more species that resemble *T. jonkershoekensis* in morphology and symptomatology, but which could represent distinct species.

Teratosphaeria bellula (Crous & M.J. Wingf.) Crous & U. Braun, Stud. Mycol. 58: 10. 2007 — Fig. 8

Basionym. *Mycosphaerella bellula* Crous & M.J. Wingf., Mycotaxon 46: 20. 1993.

Descriptions — Crous & Wingfield (1993), Taylor & Crous (1998b), Crous et al. (2004a).

Specimens examined. SOUTH AFRICA, Western Cape Province, Stellenbosch, Stellenbosch Mountain, living leaves of *Protea repens*, 30 Aug. 1991, P.W. Crous, holotype PREM 51028; Western Cape Province, Stellenbosch, Protea Heights, Devon Valley, on *Leucospermum* sp., 6 Mar. 1998, J.E. Taylor, CBS H-20093, CPC 1815, 1816 = CBS 111699; Western Cape Province, Stellenbosch, J.S. Marais Botanical Garden, on leaves of *Protea eximia*, Apr. 1998, J.E. Taylor, epitype designated here CBS H-20094, culture ex-epitype CPC 1821 = CBS 111700; Western Cape Province, *Leucadendron* sp., 27 May 1999, L. Swart, CPC 2795 = CBS 114145; Western Cape Province, Kirstenbosch Botanical Gardens, on leaves of *Protea* sp., 1 Jan. 2008, P.W. Crous, CPC 14908.

Notes — *Teratosphaeria bellula* is characterised by having ascospores that are strongly constricted at the median septum, with small guttules, and surrounded by a prominent sheath when mounted in water. Attempts to recollect *T. bellula* for

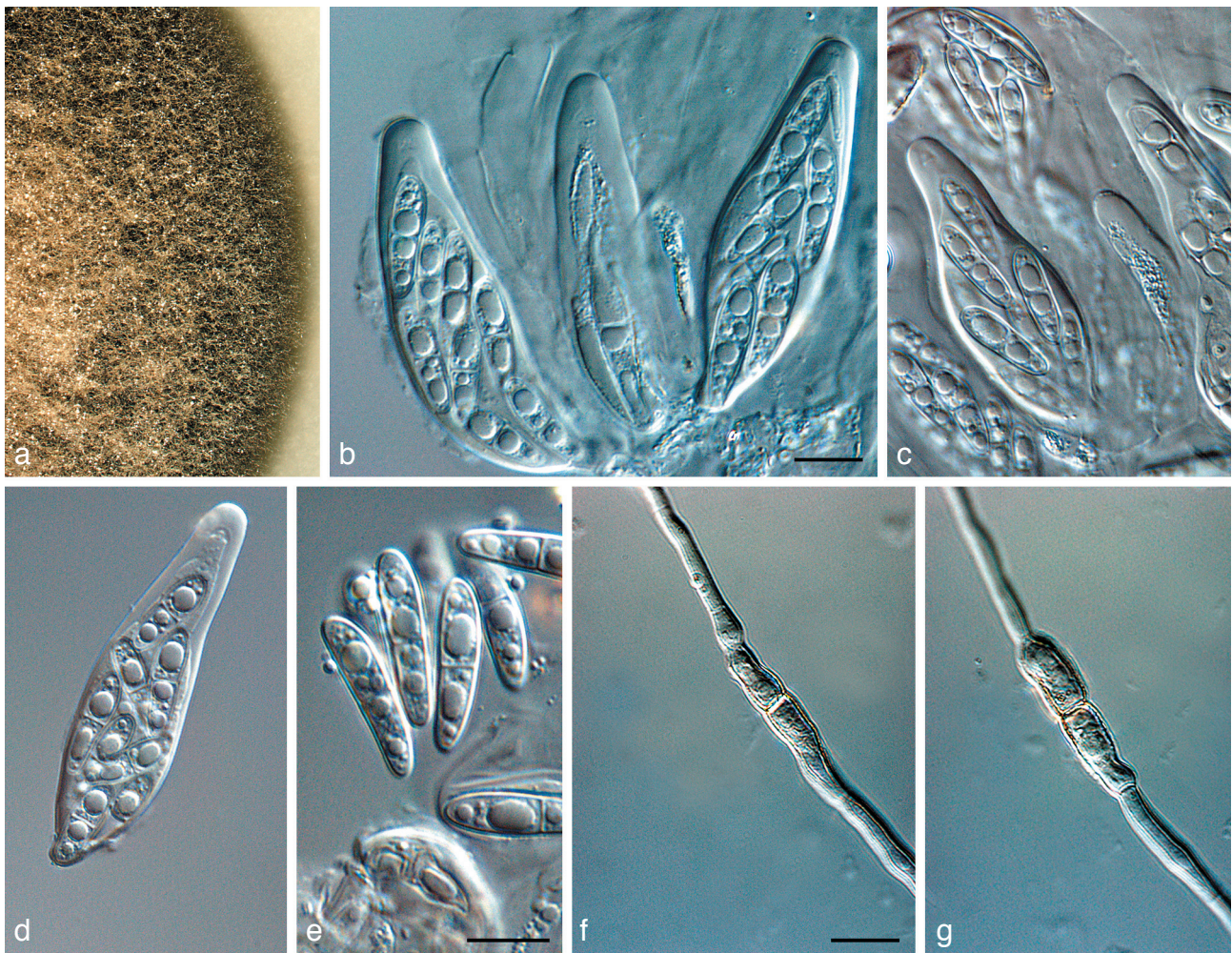


Fig. 10 *Teratosphaeria jonkershoekensis* (CBS 122897). a. Colony on OA; b–d. asci; e. ascospores; f, g. germinating ascospores. — Scale bar = 10 µm.

the purpose of epitypification have revealed it to be a species complex. As the morphology of the strains is quite similar, more isolates from other hosts in the Proteaceae need to be collected to clarify if strains from *Leucadendron* and *Leucospermum* represent *T. bellula* s.str.

Teratosphaeria fibrillosa Syd. & P. Syd., Ann. Mycol. 10: 40. 1912 — Fig. 9

≡ *Mycosphaerella fibrillosa* (Syd. & P. Syd.) Joanne E. Taylor & Crous, Mycol. Res. 107: 657. 2003.

Descriptions — Taylor & Crous (1998d), Crous et al. (2004a, 2007a).

Specimens examined. SOUTH AFRICA, Western Cape Province, Bains Kloof near Wellington, on living leaves of *Protea grandiflora*, 26 Feb. 1911, E.M. Doidge, holotype PREM; Stellenbosch, Jonkershoek valley, S33°59'44.7", E18°58'50.6", on leaves of *Protea* sp., 1 Apr. 2007, P.W. Crous & L. Mostert, epitype CBS H-19913, culture ex-epitype CBS 121707 = CPC 13960; Cederberg, on leaves of *P. nitida*, J.E. Taylor, CPC 1876.

Notes — *Teratosphaeria fibrillosa* is the type species of *Teratosphaeria* (Teratosphaeriaceae), and is characterised by having subepidermal ascomata linked by means of stromatic tissue, apical periphyses, asci with a multi-layered endotunica, ascospores that have a sheath and turn brown and verruculose while still in their asci (Crous et al. 2007a).

Teratosphaeria jonkershoekensis (P.S. van Wyk, Marasas & Knox-Dav.) Crous & U. Braun, Stud. Mycol. 58: 10. 2007 — Fig. 10

Basionym. *Mycosphaerella jonkershoekensis* P.S. van Wyk, Marasas & Knox-Dav., J. S. African Bot. 41: 234. 1975.

Descriptions — Van Wyk et al. (1975), Taylor & Crous (1998a), Crous et al. (2004a).

Cultural characteristics — Colonies on MEA erumpent with feathery margins and radiating superficial ridges; aerial mycelium sparse, fuscous-black (surface and reverse); on OA with smooth, regular margins and moderate pale olivaceous-grey aerial mycelium; outer margin olivaceous-black; reaching 30 mm diam after 1 mo on OA at 25 °C; sterile.

Specimens examined. SOUTH AFRICA, Western Cape Province, Jonkershoek, on living leaves of *Protea repens*, 9 Sept. 1971, P.S. van Wyk, holotype PREM 44830; Jonkershoek, S33°59'4.2" E18°57'16.1", on living leaves of *Protea* sp., 1 Apr. 2007, P.W. Crous & L. Mostert, epitype designated here CBS H-20095, culture ex-epitype CBS 122897 = CPC 13984 (occurring on leaf spots in association with *T. persoonii*).

Notes — No anamorph has thus far been observed for this species. Germ tubes grow parallel to the long axis of the spore, but after 48 h several germ tubes have been produced and germination is irregular. Germinating ascospores become brown, verruculose and constricted at the septum. Optimal ascospore germination occurs at 15 °C, and it is hypothesised that this low temperature requirement is necessary for successful germination and infection of leaf tissue (Swart et al. 1998, Taylor & Crous 1998a). Isolates reported from Australia as representative of *T. jonkershoekensis* (Crous et al. 2000b), are in fact representative of two morphologically similar species

that were recently described in the complex, namely *T. associata* and *T. maxii* (Fig. 1).

Teratosphaeria knoxdavesii Crous, sp. nov. — MycoBank MB506595; Fig. 11

Teratosphaeriae bellulae similis, sed ascosporis cum tubis germinalibus parallelis ad axem longum sporae.

Etymology. Named in honour of Prof. P.S. Knox-Davies, who dedicated a large part of his career to studying fungal pathogens of Proteaceae.

Leaf spots amphigenous, irregular to subcircular, 5–12 mm diam, medium brown with a raised border, and thin, red-purple margin. *Ascomata* amphigenous, black, immersed, substomatal, up to 100 µm diam; wall consisting of 2–3 layers of medium brown *textura angularis*. *Asci* paraphysate, fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid, straight to slightly curved, 8-spored, 35–45 × 8–12 µm. *Ascospores* tri- to multi-seriate, overlapping, hyaline, non-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, (8.5–)10–11(–12) × (3–)3.5(–4) µm; germinating ascospores on MEA become brown and verruculose, germinating from both polar ends, with germ tubes parallel to the long axis of the spore, constricted at septum, but not distorting, 3.5–4 µm wide.

Cultural characteristics — Colonies on MEA erumpent, spreading, folded, with moderate, pale olivaceous-grey aerial mycelium, and smooth, catenulate, olivaceous-grey margins; reverse olivaceous-grey; colonies reaching 10 mm diam on MEA after 1 mo; on OA erumpent, spreading, grey-olivaceous, with moderate aerial mycelium and even margins; sterile.

Specimens examined. SOUTH AFRICA, Western Cape Province, Kirstenbosch Botanical Garden, on living leaves of *Protea* sp., 6 Jan. 2008, P.W. Crous & M. Crous, holotype CBS H-200104, cultures ex-type CBS 122898 = CPC 14960, 14961, 14962 (occurring on leaf spots in association with *Coleroa senniana*); Kirstenbosch Botanical Garden, on living leaves of *Protea* sp., 6 Jan. 2008, P.W. Crous & M. Crous, CPC 14905–14907.

Notes — Although several small-spored species of *Teratosphaeria* are known from Proteaceae, *T. knoxdavesii* is distinct in having a very characteristic ascospore germination pattern, germinating from both polar ends, with spores becoming constricted, brown, and verruculose, germ tubes growing parallel to the long axis of the spore.

Teratosphaeria maculiformis (G. Winter) Joanne E. Taylor & Crous, IMI Descriptions of Fungi and Bacteria No. 1346. 1998 — Fig. 12

Basionym. *Didymella maculiformis* G. Winter, Hedwigia 23: 169. 1884.

≡ *Oligostroma maculiformis* (G. Winter) Doidge, Bothalia 1: 31. 1921.

= *Oligostroma proteae* Syd., Ann. Mycol. 12: 265. 1914.

≡ *Mycosphaerella proteae* (Syd.) Arx, Beitr. Kryptogamenfl. Schweiz 11(2): 357. 1962.

= *Euryachora maculiformis* Nel, Annals of the University of Stellenbosch 20 Ser. A (2): 11. 1942.

Descriptions — Taylor & Crous (1998c), Crous et al. (2004a).

Cultural characteristics — Colonies black and erumpent, devoid of aerial mycelium, slow-growing; 3 mm diam after 6 mo

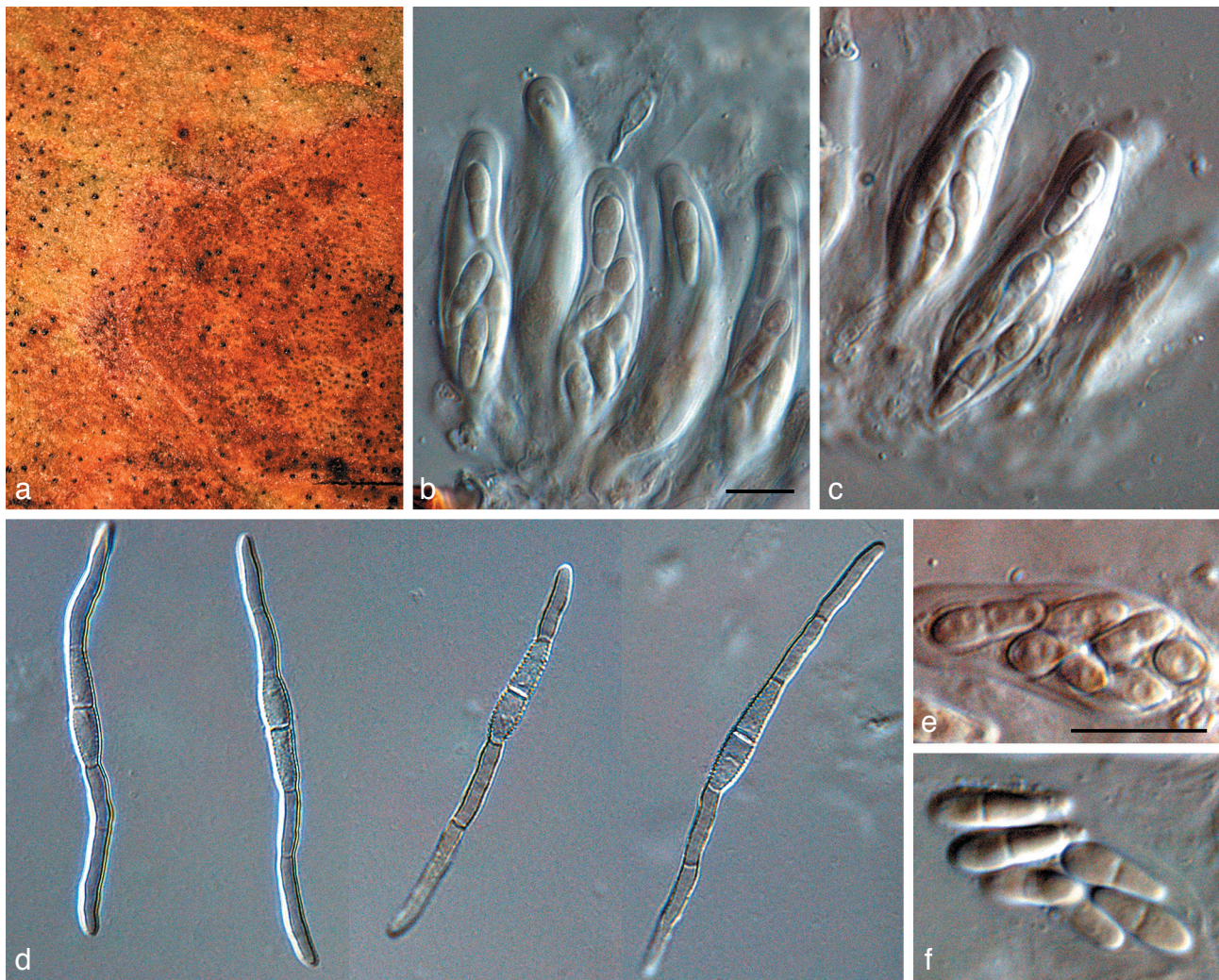


Fig. 11 *Teratosphaeria knoxdavesii* (CBS 122898). a. Leaf spot on *Protea* sp.; b, c. asci; d. germinating ascospores; e, f. close-up of ascospores in asci. — Scale bars = 10 µm.

at 25 °C on PDA; colonies did not survive preservation, and numerous subsequent attempts have been unsuccessful in cultivating it again. Germinating ascospores died on OA, MEA and PCA. Ascospores become brown at germination on PDA, and produce germ tubes parallel to the long axis of the ascospore, but the spore wall does not become verruculose.

Specimens examined. SOUTH AFRICA, Kentani, on *Protea flanaganii*, 17 July 1912, Pegler, 5163 deposited in PREM (type of *Oligostroma proteae*); Cape Town, *Protea grandiflora*, June 1884, MacOwan (Winter, Fungi Eur. Extraeur. Exs. 3056, type of *Didymella maculiformis*); Western Cape Province, Knysna, S34°1'49.9", E23°1'6.0", on leaves of *Protea* sp., 4 Jan. 2008, P.W. Crous & K.L. Crous, CBS H-20096 (used to harvest ascospores for DNA isolation).

Notes — Ascospores were observed to disarticulate at the septum, with each cell frequently becoming 1-septate and germinating (on host tissue) (Crous et al. 2004a). Although the spores are thick-walled, they also seem to get punctured quite easily, in which case cytoplasm leaks out of the damaged

ascospore cell, and the spore sheds this cell by disarticulating relatively easily at the septum. In some cases, this separation was observed even in the absence of ascospore cell damage. This interesting mechanism has not been observed in this group of fungi before, and may be linked to the relatively large ascospore size of *T. maculiformis*. Ascospores often adhere to leaf hairs, with the resulting hyphae growing superficially until they infect the host. Upon germination ascospores become brown and produce germ tubes parallel to the long axis of the ascospore, but the spore wall does not become verruculose. A further interesting phenomenon observed in *T. maculiformis* is the fact that asci frequently have one or two microascospores that appear completely underdeveloped, and much smaller than the 'normal' ascospores. As far as we could establish, these microascospores are viable, and germinate along with the normal, larger ascospores.

Ascospores on the leaf surface are frequently hyperparasitised by a species of *Cladosporium*. A similar *Cladosporium* species



Fig. 12 *Teratosphaeria maculiformis* in vivo (CBS H-20096). a. Leaf spot on *Protea* sp.; b. close-up of substomatal ascomata; c. periphysoids; d–h. asci with ascospores (microascospores arrowed); i. mature ascospores; j. disarticulating ascospores. — Scale bars = 10 µm.



Fig. 13 *Teratosphaeria marasasii* (CBS 122899). a. Leaf spot on *Protea* sp.; b, c. germinating ascospores; d. fasciculate asci viewed from above; e. asci with darkening ascospores; f, g. asci; h. ascospores. — Scale bars = 10 µm.

(a member of the *C. cladosporioides* species complex) was also observed to grow on exuding ascospore masses of *T. proteae-arboreae*. Due to the extremely slow growth of *T. maculiformis*, this species could not be deposited in the CBS culture collection. Ascospores germinate on PDA (though they fail to do so upon MEA, OA or SNA), but although germ tubes elongate and branch, they never form mycelium, and stay recognisable as single germinating ascospores, even 6–8 mo after they started germinating. At a certain point (3–5 mo after the onset of germination), all growth ceases, though the spore and the hyphae do not dissolve, but still appear viable, though they enter a dormant phase. The DNA sequence provided in this study was obtained from harvesting a mass of discharged, germinated ascospores (4 mo after ascospore discharge), and extracting their DNA using a commercial DNA isolation kit (E.Z.N.A. Forensic DNA Isolation Kit, Omega Bio-Tek).

***Teratosphaeria marasasii* Crous, sp. nov.** — MycoBank MB506596; Fig. 13

Teratosphaeria jonkershoekensis similis, sed maculis minoribus et ascosporis brevioribus, (15–)16–18(–22) × (3.5–)4(–5) µm.

Etymology. Named in honour of Prof. W.F.O. Marasas, who was instrumental in naming *T. jonkershoekensis*, which this species closely resembles in morphology and symptomatology.

Leaf spots amphigenous, circular, 2–3 mm diam, pale brown to grey-brown, with a thin, raised, dark brown border. **Ascomata** amphigenous, black, immersed, substomatal, up to 120 µm diam; wall consisting of 2–3 layers of medium brown *textura angularis*. **Asci** paraphysate, fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid, straight to slightly curved, 8-spored, 35–50 × 11–15 µm; with a well-developed ocular chamber, and multi-layered endotunica. **Ascospores** tri- to multi-seriate, overlapping, hyaline, guttulate, thick-walled, straight, fusoid-ellipsoidal with obtuse ends, widest in middle of apical cell, not to slightly constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (15–)16–18(–22) × (3.5–)4(–5) µm, becoming brown and verruculose in the asci; germinating ascospores on MEA become brown and verruculose, distorting, with 2–4 germ tubes, growing irregular to the long axis, 6–10 µm wide.

Cultural characteristics — Colonies after 1 mo on MEA erumpent, spreading, with sparse aerial mycelium, and smooth, entire margins; surface olivaceous-grey in middle, iron-grey in outer region; reverse greenish black, 10–15 mm diam; on OA with moderate aerial mycelium, and smooth, regular margins, olivaceous-grey; sterile.

Specimen examined. SOUTH AFRICA, Western Cape Province, Kirstenbosch Botanical Garden, on living leaves of *Protea* sp., 6 Jan. 2008, P.W. Crous & M. Crous, holotype CBS H-20105, cultures ex-type CBS 122899 = CPC 14889, 14890, 14891 (occurring on leaf spots in association with *Coleroa senniana*).

Notes — *Teratosphaeria marasasii* closely resembles *T. jonkershoekensis* in morphology (ascospores 15–23 × 4–6 µm), and symptomatology. It is distinct, however, by producing smaller leaf spots and having shorter ascospores.

***Teratosphaeria maxii* (Crous) Crous & U. Braun, Stud. Mycol. 58: 10. 2007**

Basionym. *Mycosphaerella maxii* Crous, in Crous & Groenewald, Fungal Planet No. 6. 2006.

Description — Crous & Groenewald (2006b).

Specimens examined. AUSTRALIA, New South Wales, Mount Tomah Botanic Gardens, on leaves of *Protea* sp., Aug. 1999, P.W. Crous & B. Summerell, DAR 74870, CBS H-20097, cultures CPC 3321 = CBS 112231, CPC 3322 = CBS 112496, CPC 3323 = CBS 112232. — SOUTH AFRICA, Western Cape Province, Bettie's Bay, Harold Porter Botanical Garden, on leaves of *Protea repens*, 4 Jan. 2006, M. Crous & P.W. Crous, holotype CBS H-19774, cultures ex-type CPC 12805 = CBS 120137, CPC 12806–12807; Hermanus, Rotary road on top of mountain, on leaves of *P. repens*, 31 Dec. 2005, P.W. Crous, CPC 12943–12945.

Notes — *Teratosphaeria maxii* is associated with leaf spot symptoms reminiscent of those of *T. alistairii*, *T. bellula* and *T. jonkershoekensis*. It is distinct from *T. alistairii* and *T. bellula* in its larger ascospores, (15–)17–19(–22) × 4–5(–6) µm, which closely resemble those of *T. jonkershoekensis* in size. However, ascospores of *T. maxii* do not darken during germination, and colonies have a peculiar, thick-walled, budding aerial mycelium, which eventually form clumps of orange crystals, which has never been observed in *T. jonkershoekensis* (Crous & Groenewald 2006b). It is interesting to note, however, that several strains reported as '*Mycosphaerella jonkershoekensis*' from Australia (Crous et al. 2000b), are in fact *T. maxii*.

***Teratosphaeria microspora* Joanne E. Taylor & Crous, Mycol. Res. 104: 631. 2000**

= *Mycosphaerella microspora* (Joanne E. Taylor & Crous) Joanne E. Taylor & Crous, Mycol. Res. 107: 657. 2003.

Anamorph. *Catenulostroma microsporum* (Joanne E. Taylor & Crous) Crous & U. Braun, Stud. Mycol. 58: 10. 2007.

Basionym. *Trimmatostroma microsporum* Joanne E. Taylor & Crous, Mycol. Res. 104: 631. 2000.

Descriptions — Taylor & Crous (2000), Crous et al. (2004a).

Specimens examined. SOUTH AFRICA, Western Cape Province, Somerset West, Hilly Lands Farm, on a living leaf of a *Protea cynaroides*, 21 July 1998, S. Denman & J.E. Taylor, PREM 56207a, holotype of teleomorph, culture ex-type CPC 1960 = CBS 101951; PREM 56207b, holotype of anamorph; Stellenbosch, J.S. Marais Nature Reserve, on a living leaf of *P. cynaroides*, July 1998, L. Swart, CPC 1832 = CBS 110890; Somerset West, on a living leaf of *P. cynaroides*, July 1998, J.E. Taylor, CPC 1848 = CBS 111031; Stellenbosch, J.S. Marais Nature Reserve, on leaves of *P. cynaroides*, 30 Aug. 1996, P.W. Crous, CPC 1597 = CBS 111697.

Notes — *Teratosphaeria microspora* is presently the only known teleomorph connection for species of *Catenulostroma* (Crous et al. 2007a). *Catenulostroma microsporum* is part of a species complex that resembles *C. abietis*, which is known from needles of various species of Gymnospermae. Ascospores of *T. microspora* germinate on MEA from both cells, and germ tubes grow parallel to the long axis of the spore. There is no constriction at the septum and the spores do not darken or become verruculose upon germination. Isolate CBS 111697 is listed here under *T. microspora*, but has somewhat larger conidia, and probably represents a cryptic species. More collections are required to fully elucidate the variation present in this species complex.

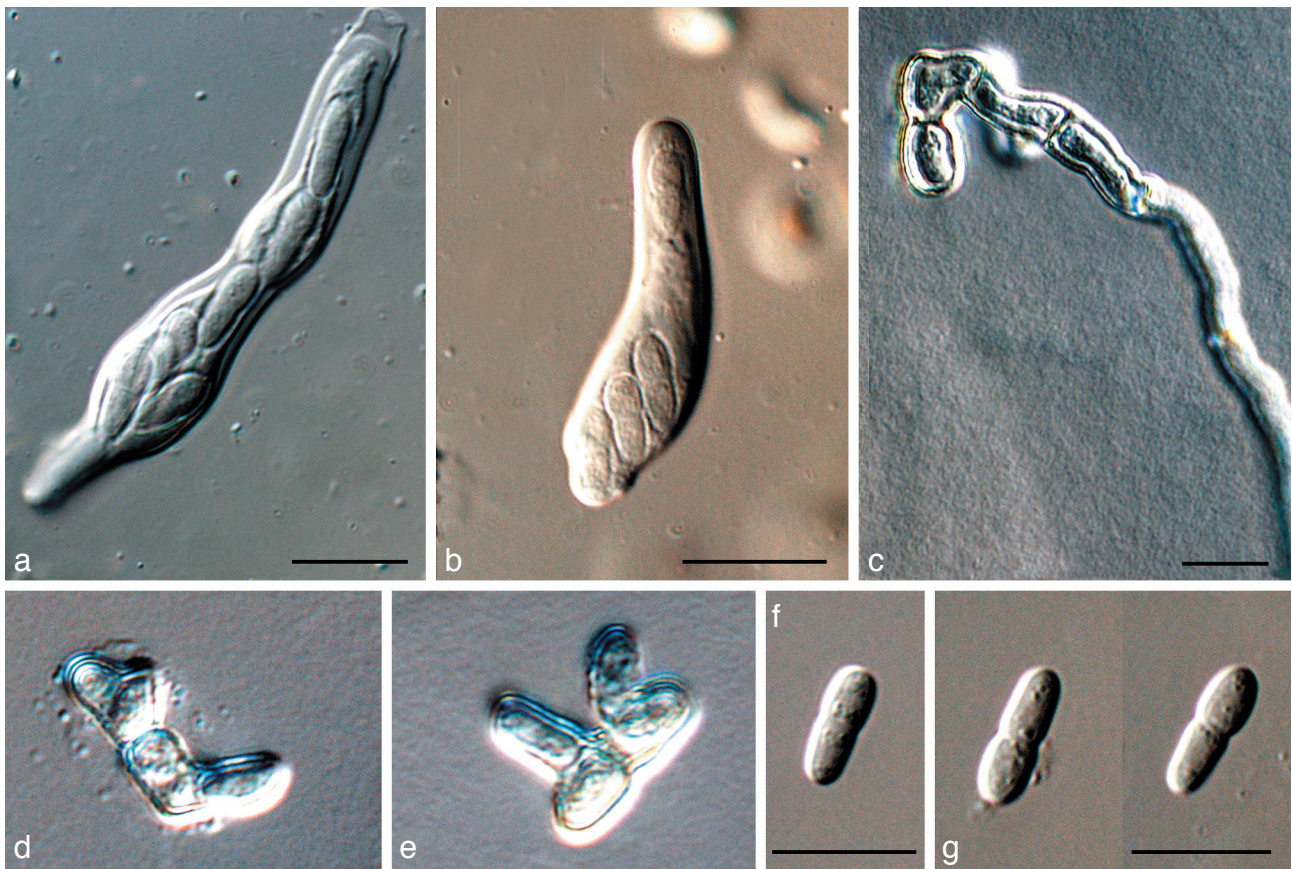


Fig. 14 *Teratosphaeria parva* (CBS 122892). a. Jack-in-the-box separation of layers in bitunicate ascus; b. ascus; c–e. germinating ascospores; f, g. ascospores. — Scale bars = 10 µm.

Teratosphaeria parva (R.F. Park & Keane) Crous & U. Braun, Stud. Mycol 58: 10. 2007 — Fig. 14

Basionym. *Mycosphaerella parva* R.F. Park & Keane, Trans. Brit. Mycol. Soc. 79: 99. 1982.

= *Mycosphaerella grandis* Carnegie & Keane, Mycol. Res. 98: 414. 1994.

Descriptions — Carnegie & Keane (1994), Crous (1998).

Specimens examined. AUSTRALIA, Victoria, Nowa Nowa, on leaves of *Eucalyptus globulus*, July 1981, R.F. Park, holotype IMI 263258 (published as 263358); Victoria, Otway Ranges, (near Gellibrand), latitude: -38.568412, longitude: 143.539586, elevation: 175 m, on leaves of *E. globulus*, Sept. 2005, I. Smith, epitype designated here CBS H-20098, cultures ex-epitype CPC 12421 = CBS 122892, CPC 12422, 12423. — SOUTH AFRICA, Western Cape Province, Stellenbosch, Stellenbosch Mountain, on leaves of *Protea repens*, 20 Sept. 1995, P.W. Crous, CPC 1217 = CBS 114761; Western Cape Province, on leaves of *P. repens*, 12 Nov. 1998, G. Matthews, CBS H-20099, CPC 2118–2120; Western Cape Province, Botmaskop, on leaves of *P. repens*, Nov. 2007, L. Mostert, CBS H-20101, CPC 14898 = CBS 122893, CPC 14899, 14900; Western Cape Province, Jonkershoek, S33°59'11.2" E18°57'14.7", on living leaves of *P. nitida*, 1 Apr. 2007, P.W. Crous & L. Mostert, CPC 13896 = CBS 122894, CPC 13897, 13898.

Teratosphaeria persoonii Crous & L. Mostert, *sp. nov.* — MycoBank MB506597; Fig. 15

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

Etymology. Named after Christiaan Hendrik Persoon (31 Dec. 1761 – 16 Nov. 1836) who was born in South Africa, but left for Europe at the age of 12, never to return. His most important work was *Synopsis Fungorum*, published in 1801, which formed the basis of modern mycology.

Leaf spots amphigenous, subcircular to circular, 2–7 mm diam, pale brown with a raised, pale to medium brown border. **Ascomata** amphigenous, black, immersed, substomatal, up to 120 µm diam; wall consisting of 2–3 layers of medium brown *textura angularis*. **Asci** paraphysate, fasciculate, bitunicate, subsessile, obovoid to ellipsoid, straight to slightly curved, 8-spored, 23–40 × 8–11 µm. **Ascospores** tri- to multi-seriate, overlapping, hyaline, with 1–2 large guttules in every cell, thick-walled, straight to slightly curved, 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, (7–)8–10(–11) × 3–3.5(–4) µm; ascospores commonly observed to turn brown and verruculose in asci. Germinating ascospores on MEA become brown and verruculose, and one to several germ tubes grow at irregular angles to the long axis of the spore.

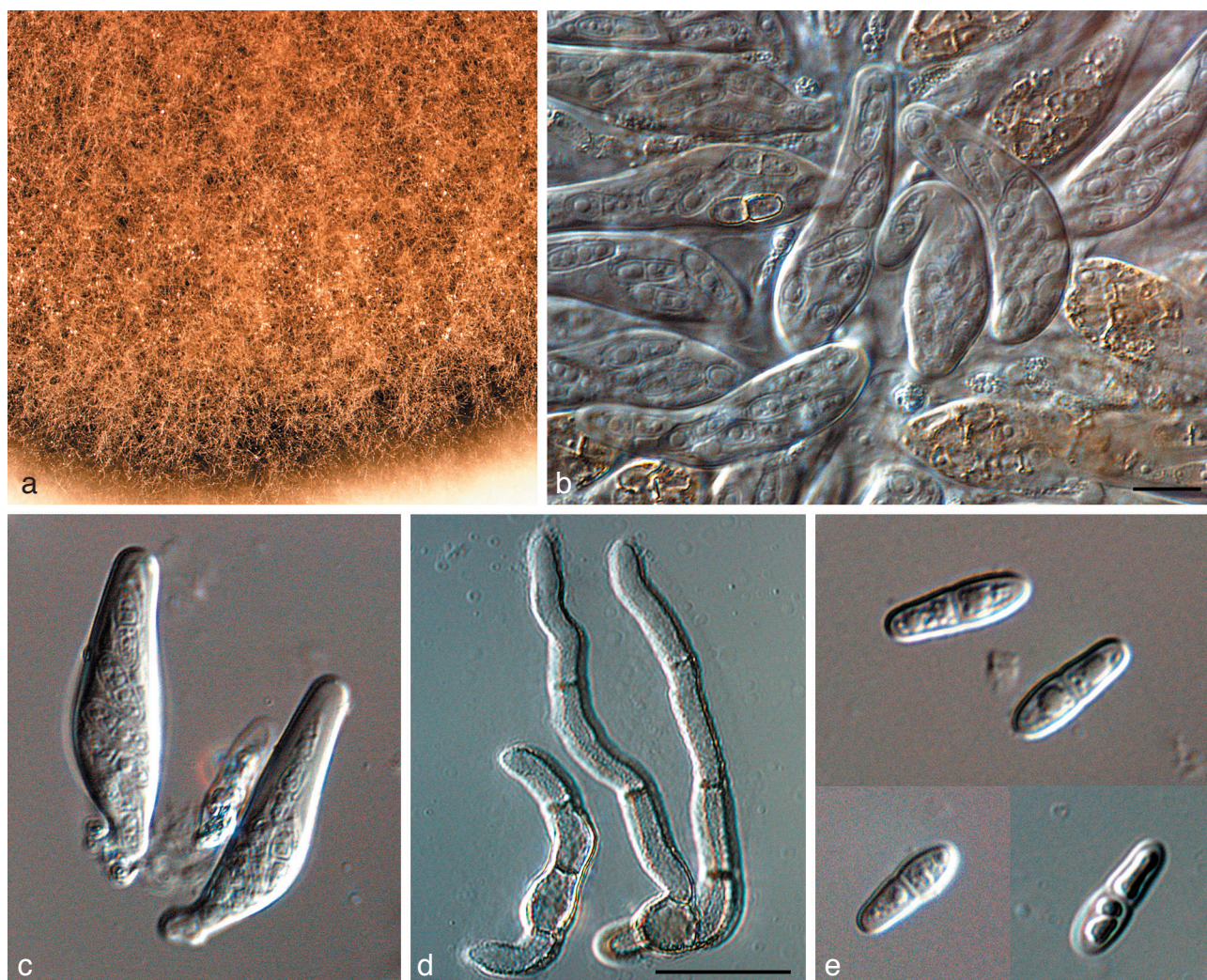


Fig. 15 *Teratosphaeria personii* (CBS 122895). a. Colony on OA; b, c. bitunicate asci; d. germinating ascospores; e. ascospores. — Scale bars = 10 µm.

Cultural characteristics — Colonies on MEA erumpent with moderate aerial mycelium and smooth, regular margins; surface pale grey-olivaceous to grey-olivaceous; reverse grey-olivaceous, reaching 10 mm diam after 2 wk on MEA; sterile.

Specimens examined. SOUTH AFRICA, Western Cape Province, Jonkershoek, S33°59'4.2" E18°57'16.1", on living leaves of *Protea* sp., 1 Apr. 2007, P.W. Crous & L. Mostert, holotype CBS H-20102, cultures ex-type CPC 13972 = CBS 122895, CPC 13973, 13974 (occurring on leaf spots in association with *T. jonkershoekensis*); Western Cape Province, De Hoop Nature Reserve, Bredasdorp, S34°27'33" E20°27'04", on leaves of *Euchaetis meridionalis* (whole leaf turns brown, covered with brown-black mycelium; leaf and stem necrosis often starts where petiole attaches to branch; several fungi appear to be present, incl. species of *Phoma* and *Leptosphaeria*), 29 June 2006, A.R. Wood, CBS H-20103, culture STE-U 6389 = CPC 14846 = CBS 122896.

Notes — Although *T. personii* is morphologically very similar to *T. bellula* with regards to ascospore dimensions and symptomatology, it can be distinguished by having ascospores that have large, prominent guttules, are relatively thick-walled, commonly turn brown and verruculose while still in asci, and lack a mucoid sheath when mounted in water. No anamorph

has been observed. *Teratosphaeria personii* also has a wider host range, occurring on *Protea* as well as *Euchaetis*.

Teratosphaeria proteae-arboreae P.S. van Wyk, Marasas & Knox-Dav., J. S. Afr. Bot. 41: 232. 1975 — Fig. 16

= *Mycosphaerella proteae-arboreae* (P.S. van Wyk, Marasas & Knox-Dav.) Joanne E. Taylor & Crous, Mycol. Res. 107: 657. 2003.

Descriptions — Taylor & Crous (1998e), Crous et al. (2004a).

Leaf spots initially indistinct, chlorotic, raised, circular, with catenulate margins, with substomatal ascomata appearing as black spots in lesions; spots not extending through lamina, becoming black in centre, later extending so that the whole spot appears black, 1–3 mm diam. *Ascomata* epiphyllous or hypophyllous, globose, ostiolate, non-papillate, black, singular, gregarious; in section substomatal, subepidermal, globose to slightly pyriform, periphysoids lining the ostiole and the upper ascoma wall, up to 250 µm diam. *Peridium* consisting of three layers of

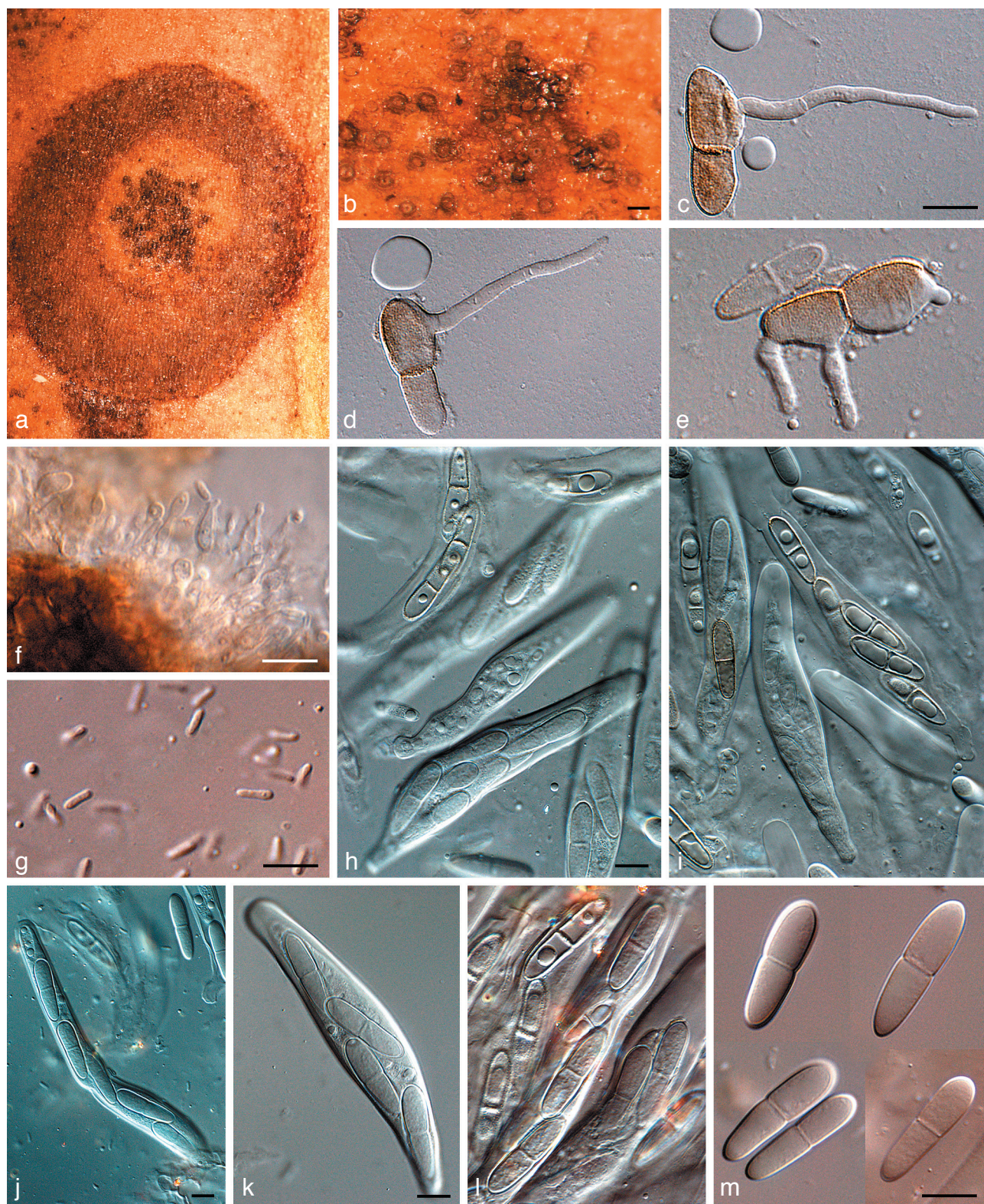


Fig. 16 *Teratosphaeria proteae-arboreae* (CPC 12952). a. Leaf spot on *Protea nitida*; b. close-up of substomatal ascomata; c–e. germinating ascospores; f. spermatophores; g. spermatia; h–l. asci with darkening ascospores; m. ascospores. — Scale bars: b = 200 μ m, all others = 10 μ m.

compressed, brown *textura angularis*. Asci narrowly ellipsoid to obovoid, tapering abruptly to a small pedicel, narrowing to a rounded apex with a distinct ocular chamber, 2–4 µm diam, mainly straight, fasciculate, bitunicate with fissitunicate dehiscence, with a multi-layered endotunica, 80–120 × 14–20 µm; asci predominantly 8-spored, with two ascospores being underdeveloped. Ascospores bi- to tri-seriate, fusoid-ellipsoidal, straight to slightly curved, 1-septate, prominently guttulate, with septum median or slightly supra-median, constricted, broadest in the middle of the apical cell and tapering to the lower end, hyaline, but becoming brown and verruculose in asci, (17–)25–30(–35) × (5–)6–7(–9) µm. *Spermatogonia* intermixed with ascomata, similar in morphology. *Spermatophores* producing spermatia that are bacilliform, smooth, hyaline with obtuse ends, 4–6 × 1.5 µm. Germinating ascospores on MEA after 24 h with one or several germ tubes, germinating from polar ends, parallel to the long axes, or lateral, from sides of spore; ascospores distorting, brown, verruculose, with outer, brown, verruculose layer frequently becoming separate from inner, hyaline layer.

Cultural characteristics — Colonies on MEA erumpent with sparse aerial mycelium and even, catenulate margins; centre hazel, margins isabelline to sepia; reverse fuscous-black; reaching 12 mm diam after 1 mo. On OA erumpent with sparse aerial mycelium and even, catenulate margins, pale olivaceous-grey to olivaceous-grey, reaching 10 mm diam after 1 mo at 25 °C.

Specimens examined. SOUTH AFRICA, Western Cape Province, Jonkershoek, Houttuyn, on living leaves of *Protea arborea*, 21 June 1971, P.S. van Wyk, holotype PREM 44801; Western Cape Province, Bettie's Bay, Harold Porter Botanical Garden, on leaves of *P. nitida*, 4 Jan. 2006, M.K. Crous & P.W. Crous, epitype designated here CBS H-20106, cultures ex-epitype CPC 12952–12954; Western Cape Province, Kirstenbosch Botanical Garden, on living leaves of *Protea* sp., 6 Jan. 2008, P.W. Crous & M. Crous, CBS H-20107, cultures CPC 14963–14965.

Notes — *Teratosphaeria proteae-arboreae* is commonly associated with prominent leaf spots on *Protea nitida*. The present collection was obtained from the type location, and could subsequently be designated as epitype to clarify its phylogenetic relationship to other species of *Teratosphaeria*. It is interesting to note that as observed in *T. maculiformis*, asci frequently have one or two underdeveloped microascospores. The reason for the smaller ascospores occurring in asci along with normally developed ascospores is unknown. It is thus tempting to speculate that microascospores (which frequently aggregate at the ascal apex in *T. proteae-arboreae* and *T. maculiformis*) could have a different ecological role, being the first to be discharged. Alternatively, these could simply be weakly developed ascospores that are not viable. Further collections would be required to clarify this aspect.

Teratosphaeria sp.

Cultural characteristics — Colonies on MEA erumpent, with sparse aerial mycelium and smooth, catenulate margins; grey-olivaceous in centre, olivaceous-grey at margins and underneath; colonies reaching 7 mm diam after 2 wk at 25 °C.

Specimen examined. SOUTH AFRICA, Western Cape Province, Jonkershoek, S33°59'4.2" E18°57'16.1", on living leaves of *Protea nitida*, 1 Apr. 2007, P.W. Crous & L. Mostert, CPC 13917–13919.

Notes — This species, which is closely related to *T. proteae-arboreae*, could not be described here due to insufficient material. It was isolated from small, fusoid-ellipsoidal ascospores, 10 × 3.5 µm, that became constricted and distorted upon germination, and germinated from one end only. Based on its DNA phylogeny and ascospore germination pattern, it clearly represents yet another undescribed species of *Teratosphaeria*.

Teratosphaeria sp.

Cultural characteristics — Colonies on PDA erumpent, irregular, with smooth, catenulate margins and sparse aerial mycelium, iron-grey (surface), olivaceous-black (reverse); on OA spreading with smooth, regular margins; aerial mycelium sparse, iron-grey (surface); reaching 20 mm diam after 1 mo on OA at 25 °C; sterile.

Specimen examined. SOUTH AFRICA, Western Cape Province, Jonkershoek, S33°59'4.2" E18°57'16.1", on living leaves of *Protea nitida*, 1 Apr. 2007, P.W. Crous & L. Mostert, CPC 13963–13965.

Notes — This species, which has ascospores that distort and turn brown upon germination, occurred on spots in association with *T. fibrillosa* and *T. proteae-arboreae*. It could not be described, however, due to paucity of material.

Teratosphaeria sp.

Cultural characteristics — Colonies on MEA erumpent, folded, with sparse to moderate aerial mycelium and smooth, catenulate to feathery margins; grey-olivaceous in centre, olivaceous-grey at margins and underneath; colonies reaching 12 mm diam after 1 mo at 25 °C.

Specimen examined. SOUTH AFRICA, Western Cape Province, Kirstenbosch Botanical Garden, on living leaves of *Protea* sp., 6 Jan. 2008, P.W. Crous & M. Crous, CPC 14957–14959.

Notes — This species was isolated from ascospores that turned brown and distorted upon germination. It could not be described, however, due to paucity of material.

Teratosphaeria sp.

Cultural characteristics — Colonies on PDA erumpent with smooth margins and moderate aerial mycelium; isabelline in middle, becoming olivaceous towards margin; dark mouse-grey underneath. On OA spreading with smooth, catenulate margins and moderate olivaceous-grey aerial mycelium; iron-grey in outer region; colonies reaching 40 mm diam after 1 mo on OA at 25 °C; sterile.

Specimen examined. PORTUGAL, on leaves of *Protea repens*, 1 Jan. 2007, M.F. Moura, CPC 13981.

Notes — This species was isolated from leaf spots on living leaves of *Protea repens*, but could not be described due to paucity of the material. Based on its cultural characteristics and DNA phylogeny, it appears to be distinct from the *Teratosphaeria* spp. presently known from Proteaceae

DISCUSSION

In their treatment of the *Mycosphaerella* diseases associated with Proteaceae, Crous et al. (2004a) listed 13 species of *Mycosphaerella* (incl. *Teratosphaeria*) and 18 associated anamorph species. Since the publication of the compendium, several species have been investigated by means of DNA molecular analyses, showing the morphological species concepts used in the past to have been too wide, obscuring the presence of several novel taxa. A good example of this was the report of *M. jonkershoekensis* from Australia based on symptomatology, morphology, and ascospore germination patterns (Crous et al. 2000b), which based on DNA techniques employed here, was revealed to in fact represent two species that were newly described in the *T. jonkershoekensis* complex, namely *T. associata* and *T. maxii* (Crous & Groenewald 2006a, b). A further significant step has been the acknowledgement of Teratosphaeriaceae as being distinct from Mycosphaerellaceae (Crous et al. 2007a), which made it essential to re-evaluate all species occurring on Proteaceae.

The present study also addressed the problem that many of the older names known from Proteaceae have never been studied in culture, and thus were omitted from previous DNA studies. These species had to be recollected, compared to the holotype specimens, and epitype specimens designated, so that ex-epitype cultures could become available for DNA analyses. This was achieved for most, but not all species, namely *Batcheloromyces leucadendri*, *B. proteae*, *Catenulostroma macowanii*, *Mycosphaerella marksii*, *Teratosphaeria bellula*, *T. jonkershoekensis*, *T. parva*, and *T. proteae-arboreae*. Several species are also newly described, namely *Batcheloromyces sedgefieldii*, *Catenulostroma wingfieldii*, *Dissoconium proteae*, *Teratosphaeria knoxdavesii*, *T. marasasii* and *T. persoonii*.

While *Mycosphaerella* and *Teratosphaeria* species are generally accepted to be host specific (Crous & Braun 2003), several species have now been shown to have wider host ranges than was commonly accepted (Burgess et al. 2007, Crous et al. 2004c, 2007d, Crous & Groenewald 2005). These include *M. communis*, which is known to have a wider host range, including *Eucalyptus* (South Africa, Spain, New Zealand), *Musa* (Trinidad) as well as *Protea magnifica* in Australia; *Mycosphaerella konae* (*Leucospermum*, Hawaii), which also occurs on *Eucalyptus* in Thailand (Crous et al. 2007d), *M. marksii* (*Eucalyptus*, Australia, Bolivia, China, Ecuador, Ethiopia, Papua New Guinea, New Zealand, South Africa, Spain, Tanzania, Uruguay), which also occurs on *Leucadendron* on the Madeira Islands, and *Musa* in Mozambique (Arzanlou et al. 2008). *Teratosphaeria associata*, an apparent opportunist on *Eucalyptus* in Australia, appears to be a primary pathogen on *Protea* in the same country (Crous et al. 2000b, 2007d). *Teratosphaeria parva* (on *Eucalyptus* in Australia, Chile, Ethiopia, Portugal, South Africa and Spain), is also found on *Protea* in South Africa. Wider host ranges may also be applicable to *T. microspora* and *Septoria protearum*, though species concepts in these genera are still unresolved.

Based on DNA analysis of single ascospore cultures of *Mycosphaerella* spp. derived from various hosts and substrates, Crous & Groenewald (2005) introduced the pogo stick hypo-

thesis to explain the fact that well-known plant pathogenic species of *Mycosphaerella* (incl. *Teratosphaeria*) are frequently encountered on 'non-hosts', where they appear to colonise leaf spots of other *Mycosphaerella* species that are primary pathogens on these hosts, to enable them to produce a limited amount of progeny to enable onward dispersal. This 'host jumping' phenomenon appears to be much more common in *Mycosphaerella* and *Teratosphaeria* than generally accepted in literature, revealing these necrotrophic species to be able to grow also as saprobes on dead tissue, enabling them to disperse further in an attempt to locate their ideal hosts.

Many species of *Mycosphaerella* and *Teratosphaeria*, which were commonly accepted as host-specific necrotrophic pathogens, thus appear to also exhibit a facultative saprobic behaviour. This indicates that the definitions of 'necrotroph' or 'saprobe' do not clearly define all species of *Mycosphaerella* and *Teratosphaeria*, as some have obviously retained the ability to also grow on dead tissue when they lose the connection to their real host.

The exact mechanism that allows species of *Mycosphaerella* and *Teratosphaeria* to co-colonise the same host tissue, leading to several species co-occurring in the same leaf spot (Crous & Wingfield 1996, Crous 1998), also deserves further study.

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