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Species of Botryosphaeriaceae occurring on Proteaceae

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Key words

Botryosphaeria
Fusicoccum
Neofusicoccum
Saccharata

Abstract The Botryosphaeriaceae includes several species that are serious canker and leaf pathogens of Proteaceae. In the present study, sequence data for the ITS nrDNA region were used in conjunction with morphological observations to resolve the taxonomy of species of Botryosphaeriaceae associated with Proteaceae. *Neofusicoccum luteum* was confirmed from *Buckinghamia* and *Banksia* in Australia, and on *Protea cynaroides* in South Africa. A major pathogen of *Banksia coccinea* in Australia was shown to be *N. australe* and not *N. luteum* as previously reported. *Neofusicoccum protearum* was previously reported on Proteaceae from Australia, Madeira, Portugal and South Africa, and is shown here to also occur in Hawaii and Tenerife (Canary Islands). Furthermore, several previous records of *N. ribis* on Proteaceae were shown to be *N. parvum*. *Saccharata capensis* is described as a new species that is morphologically similar to *S. proteae*. There is no information currently available regarding its potential importance as plant pathogen and pathogenicity tests should be conducted with it in the future.

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INTRODUCTION

The Proteaceae (proteas) is a prominent Southern Hemisphere plant family consisting of approximately seven subfamilies, 60 genera and 1 400 species (Rebelo 2001). Most proteas are trees or shrubs that can survive under very dry conditions. Several genera are successfully cultivated in tropical, subtropical and temperate regions, in many cases as introduced non-natives. Amongst the commonly cultivated species are the South African *Protea*, *Leucadendron* and *Leucospermum*, which are farmed for fresh cut-flowers, dried flowers and dried foliage. These species are traded globally and are in high demand. Any disease on these products has a direct influence on international and domestic trade and markets. Although many pathogens are associated with proteas (Crous et al. 2004a), some of the most important pathogens from a phytosanitary standpoint are species of the Botryosphaeriaceae. This is chiefly because they exist as latent pathogens in healthy plant tissues, causing serious disease when plants are stressed (Denman et al. 2000, 2003). The species of Botryosphaeriaceae occurring on Proteaceae have recently been circumscribed (Denman et al. 2003), and guidelines to the management and control of the diseases with which they are associated have been published (Crous et al. 2004a, Denman et al. 2004).

Species of Botryosphaeriaceae have a worldwide distribution and they occur on a wide diversity of plant hosts (Denman et al. 2000). They also occupy a wide range of niches and can be primary or opportunistic pathogens, endophytes or saprobes (Denman et al. 2000, Swart et al. 2000, Taylor et al. 2001a–c, Denman 2002, Crous et al. 2004a, Slippers & Wingfield 2007). Ten lineages in the Botryosphaeriaceae were recognised based on sequence data of 28S rDNA (Crous et al. 2006b), with one recently added lineage representing the anamorph genus *Aplosporella* (Damm et al. 2007b). *Botryosphaeria* spp. and similar species are prevalent on proteas under environmental stress, causing stem cankers, dieback or leaf blight (Crous et al. 2004a). A total of 19 species have thus far been reported to be associated with proteas (Table 1), although there are undoubtedly more awaiting discovery (Crous et al. 2006a). Since the DNA-based phylogenetic study conducted on the Botryosphaeriaceae infecting Proteaceae by Denman et al. (2003), several additional isolates have been obtained from Proteaceae cultivated in South Africa and elsewhere in the world. The aim of this study was, therefore, to clarify the taxonomic status of these newly collected isolates by comparing them with reference strains using comparisons of DNA sequence data for the ITS nrDNA region. Furthermore, we aimed to resolve the status of isolates that appeared morphologically distinct from species presently known from this family.

MATERIALS AND METHODS

Isolates

Cultures were obtained by making single spore isolations from mature fruiting bodies present in diseased material as well as by isolating fungi directly from stem cankers and leaf spots. Isolates obtained from asymptomatic protea leaves presumably as endophytes, were also included. Plant tissue was surface disinfested by placing samples in 70 % ethanol for 30 s, 1 % NaOCl for 1 min, 30 s in 70 % ethanol and rinsing in sterile water for 1 min. Spores were allowed to germinate on 2 % malt extract agar (MEA; Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands) plates following the protocols described by Crous (1998).

DNA phylogeny

Genomic DNA was isolated from fungal mycelium grown on MEA, using the FastDNA kit (BIO101, Carlsbad, CA, USA)

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Table 1 Species of Botryosphaeriaceae reported to be associated with the Proteaceae.

Species	Clade[1]	Host[2]	Locality
Botryosphaeria dothidea (anamorph: *Fusicoccum aesculi*)	Clade 2	*Grevillea*[P], *Leucadendron*[P], *Leucospermum*[P], *Protea*[P], *Telopea*[P]	Guatemala, Hawaii, South Africa, USA: California, Zimbabwe
Botryosphaeria gaubae	Petrak 1967	*Grevillea*[P]	Australia
Botryosphaeria sp.	Taylor et al. 2001b	*Grevillea*[P]	USA: Florida
'*Botryosphaeria*' *quercuum*	Clade 8	*Grevillea*[P]	USA: Florida
Diplodia seriata (teleomorph: '*Botryosphaeria*' *obtusa*)	Clade 1	*Protea*[P, E]	South Africa
Diplodia sp.	Clade 1	*Protea*[S]	South Africa
Diplodia sp.	Clade 1	*Grevillea*[P]	USA: Florida
Dothiorella banksiae	Clade 5	*Banksia*[P]	Australia
Dothiorella sp.	Clade 5	*Leucadendron*[S]	South Africa
Dothiorella sp.	Clade 5	*Leucadendron*[P], *Protea*[P]	Hawaii
Fusicoccum spp.	Taylor et al. 2001a, b	*Leucospermum*[P], *Protea*[P], *Telopea*[P]	Hawaii, USA: California
Lasiodiplodia theobromae (teleomorph: '*Botryosphaeria*' *rhodina*)	Clade 1	*Banksia*[P], *Grevillea*[P], *Leucospermum*[P], *Protea*[P], *Telopea*[P]	Australia, Cuba[3], Hawaii, India[3], Madeira, Malawi[3], Uganda
Neofusicoccum australe (teleomorph: '*Botryosphaeria*' *australis*)	Clade 6	*Banksia*[P], *Protea*[P]	Australia, South Africa
Neofusicoccum luteum (teleomorph: '*Botryosphaeria*' *lutea*)	Clade 6	*Banksia*[P], *Buckinghamia*[P], *Protea*[P]	Australia, South Africa
Neofusicoccum protearum (teleomorph: '*Botryosphaeria*' *protearum*)	Clade 6	*Leucadendron*[P,E], *Protea*[P,E]	Australia, Hawaii, Madeira, South Africa
Neofusicoccum cf. *protearum*	Clade 6	*Leucadendron*[S], *Leucospermum*[S], *Protea*[S]	South Africa
Neofusicoccum ribis (teleomorph: '*Botryosphaeria*' *ribis*)	Clade 6	*Banksia*[P], *Buckinghamia*[P], *Grevillea*[P], *Leucadendron*[P], *Leucospermum*[P], *Macadamia*[P], *Protea*[P], *Telopea*[P]	Australia, Hawaii, Malawi, South Africa, Zimbabwe
Saccharata capensis	Clade 9	*Leucospermum*[S], *Mimetes*[S]	South Africa
Saccharata proteae (anamorph: '*Fusicoccum*' *proteae*)	Clade 9	*Leucadendron*[S], *Leucospermum*[P,S], *Protea*[P,S,E]	Australia[5], Hawaii, Madeira[5], Portugal[5], South Africa[4], Tasmania, USA: California

[1] Clade number corresponds to that of Crous et al. (2006b).
[2] S = saprobe, P = pathogen, E = endophyte.
[3] Published as *Botryodiplodia theobromae*.
[4] Published as *Phyllachora proteae*.
[5] Published as *Botryosphaeria proteae*.

according to the manufacturer's protocols. The primers V9G (de Hoog & Gerrits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA operon, including the 3' end of the 18S rRNA gene, the first ITS region, the 5.8S rRNA gene; the second ITS region and the 5' end of the 28S rRNA gene. To resolve taxa in the *N. ribis* complex (Slippers et al. 2004a) the primers EF1-728F and EF1-986R (Carbone & Kohn 1999) were used to amplify part of the translation elongation factor 1-α gene (TEF1) as described in Crous et al. (2004b) where applicable. Sequences for the internal transcribed spacers and 5.8S rDNA of the Botryosphaeriaceae isolates from Proteaceae were subjected to a megablastn search of NCBI's GenBank nucleotide database. Identical and closely related sequences were downloaded manually aligned and added to the outgroup sequences using Sequence Alignment Editor v. 2.0a11 (Rambaut 2002) to create the alignment. Phylogenetic analyses of sequence data were made using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) and consisted of parsimony analyses with alignment gaps treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed using the heuristic search option with 100 random taxa additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the tree(s) obtained was evaluated by 1 000 bootstrap replications (Hillis & Bull 1993). Other measures calculated included tree length, consistency index, retention index and rescaled consistency index (TL, CI, RI and RC). The resulting trees were printed with TreeView v. 1.6.6 (Page 1996). Sequences were deposited in GenBank (Table 2) and the alignment and tree in TreeBASE (www.treebase.org). The TEF1 sequences were compared with the sequences available in NCBI's GenBank nucleotide database using a megablastn search.

Table 2 Isolates investigated in this study.

Fungus	Culture accession No.	GenBank No.		Host[1]	Locality	Collector	Reference
		ITS	TEF1				
Diplodia seriata	CPC 4373	AF452556		*Protea magnifica*	South Africa	S. Denman	Denman et al. 2003
Lasiodiplodia theobromae	CBS 111530 = CPC 2095	FJ150695		*Leucospermum* sp.	Hawaii	J.E. Taylor	Present study
Neofusicoccum australe	CBS 115185 = CPC 5182	FJ150696		*Protea cynaroides*	Spain	S. Denman	Present study
	CPC 4393	AF452548		*Protea cynaroides*	South Africa	L. Swart	Denman et al. 2003 (as *N. luteum*)
	CPC 13783	FJ150697		*Protea* sp.	Tenerife	P.W. Crous	Present study
Neofusicoccum parvum	CBS 111523 = CPC 2051	AF452526		*Leucospermum* sp.	Hawaii	P.W. Crous	Denman et al. 2003
	CBS 111524 = CPC 2057	AF452524	FJ150709	*Protea cynaroides*	Hawaii	P.W. Crous	Denman et al. 2003
	CBS 114472 = CPC 2055	AF452523	FJ150710	*Leucadendron* cv. Safari Sunset	Hawaii	P.W. Crous	Denman et al. 2003
	CPC 4381	AF452522		*Protea cynaroides*	Zimbabwe	C. Saywood	Denman et al. 2003
Neofusicoccum protearum	CBS 111496 = CPC 1772	FJ150698		*Protea* sp.	South Africa	J.E. Taylor	Present study
	CBS 111502 = CPC 1771	FJ150699		*Protea* sp.	South Africa	J.E. Taylor	Present study
	CBS 113071 = CPC 5172	FJ150700		*Protea cynaroides*	Portugal	S. Denman	Present study
	CBS 113076 = CPC 5186	FJ150701		*Leucadendron* cv. Safari Sunset	Portugal	S. Denman	Present study
	CBS 113079 = CPC 5180	FJ150702		*Protea* cv. Pink Ice	Tenerife	S. Denman	Present study
	CBS 114176 = CPC 1775	AF452539		*Leucadendron* cv. Silvan Red	South Africa	S. Denman	Denman et al. 2003
	CBS 115177 = CPC 4357	FJ150703		*Protea magnifica*	South Africa	S. Denman	Present study
	CBS 115480 = CPC 4398	AF452531		*Leucadendron* sp.	Portugal	S. Denman	Denman et al. 2003
	CBS 115481 = CPC 4397	AF452530		*Leucadendron tinctum*	Madeira	S. Denman	Denman et al. 2003
	CBS 115499 = CPC 5171	FJ150704		*Leucadendron* sp.	Portugal	S. Denman	Present study
	CBS 119220 = CMW 20464	EU552144		Leaf litter of *Leucospermum conocarpodendron*	South Africa	S. Marincowitz	Marincowitz et al. 2008
	CPC 2147	AF452534		*Protea cynaroides*	Hawaii	P.W. Crous	Denman et al. 2003
	CPC 2930	AF452528		*Leucadendron* sp.	Australia	P.W. Crous	Denman et al. 2003
	CPC 2988	AF452537		*Protea magnifica*	Australia	P.W. Crous	Denman et al. 2003
	CPC 4360	AF195774		*Protea eximia*	South Africa	S. Denman	Denman et al. 2000
	CPC 4361	AF196295		*Protea magnifica*	South Africa	S. Denman	Denman et al. 2000
	CPC 4367	AF452544		*Protea neriifolia*	South Africa	S. Denman	Denman et al. 2003
	CPC 4369	AF452536		*Protea repens*	South Africa	S. Denman	Denman et al. 2003
	CPC 4384	AF452535		*Protea cynaroides*	South Africa	S. Denman	Denman et al. 2003
	CPC 13780	FJ150705		*Protea* sp.	Tenerife	P.W. Crous	Present study
	CBS 119220 = CMW 20464	EU552144		Twig litter and senescent cone of *Leucadendron xanthoconus*	South Africa	S. Marincowitz	Marincowitz et al. 2008
Neofusicoccum sp.	CBS 115184 = CPC 4379	AF452525	FJ150711	*Protea cynaroides*	Zimbabwe	C. Saywood	Denman et al. 2003
Saccharata capensis	CBS 122693 = CPC 13699 = CMW 22200	EU552130		Leaf litter of *Mimetes cucullata*	South Africa	S. Marincowitz	Present study; Marincowitz et al. 2008 (as *Saccharata* sp.)
	CBS 122694 = CPC 13698 = CMW 22197	EU552129		Leaf litter of *Leucospermum conocarpodendron* subsp. *viridum*	South Africa	S. Marincowitz	Present study; Marincowitz et al. 2008 (as *Saccharata* sp.)
Saccharata proteae	CBS 114569 = CPC 2169	FJ150706	FJ150712	*Protea* sp.	Hawaii	P.W. Crous	Present study
	CBS 114570 = CPC 2273	FJ150707		*Protea* cv. Lady Di	Hawaii	P.W. Crous	Present study
	CBS 115206 = CPC 4378	AF452560		*Protea* sp.	Australia (USDA interception)	M.E. Palm	Denman et al. 2003
	CBS 119218 = CMW 20003	EU552145	FJ150713	Leaf litter of *Protea lepidocarpodendron*	Betty's Bay, South Africa	S. Marincowitz	Marincowitz et al. 2008
	CPC 2269	AF452563		*Protea laurifolia*	Hawaii	P.W. Crous	Denman et al. 2003
	CPC 2271	AF452562		*Protea* cv. Lady Di	Hawaii	P.W. Crous	Denman et al. 2003
	CPC 4355	AF196301		*Protea repens*	South Africa	S. Denman	Denman et al. 2000
	CPC 4358	AF196299		*Protea cynaroides*	South Africa	L. Swart	Denman et al. 2000
	CPC 4399	AF452557		*Protea cynaroides*	Madeira	S. Denman	Denman et al. 2003
	CPC 4400	AF452559		*Protea repens*	Portugal	S. Denman	Denman et al. 2003
	CPC 14856	FJ150708		*Protea* sp.	South Africa	P.W. Crous	Present study

[1] cv. Safari Sunset = *Leucadendron salignum* × *Leucadendron laureolum*, cv. Silvan Red = *Leucadendron laureolum* × *Leucadendron salignum*, cv. Lady Di = *Protea magnifica* × *Protea compacta*.

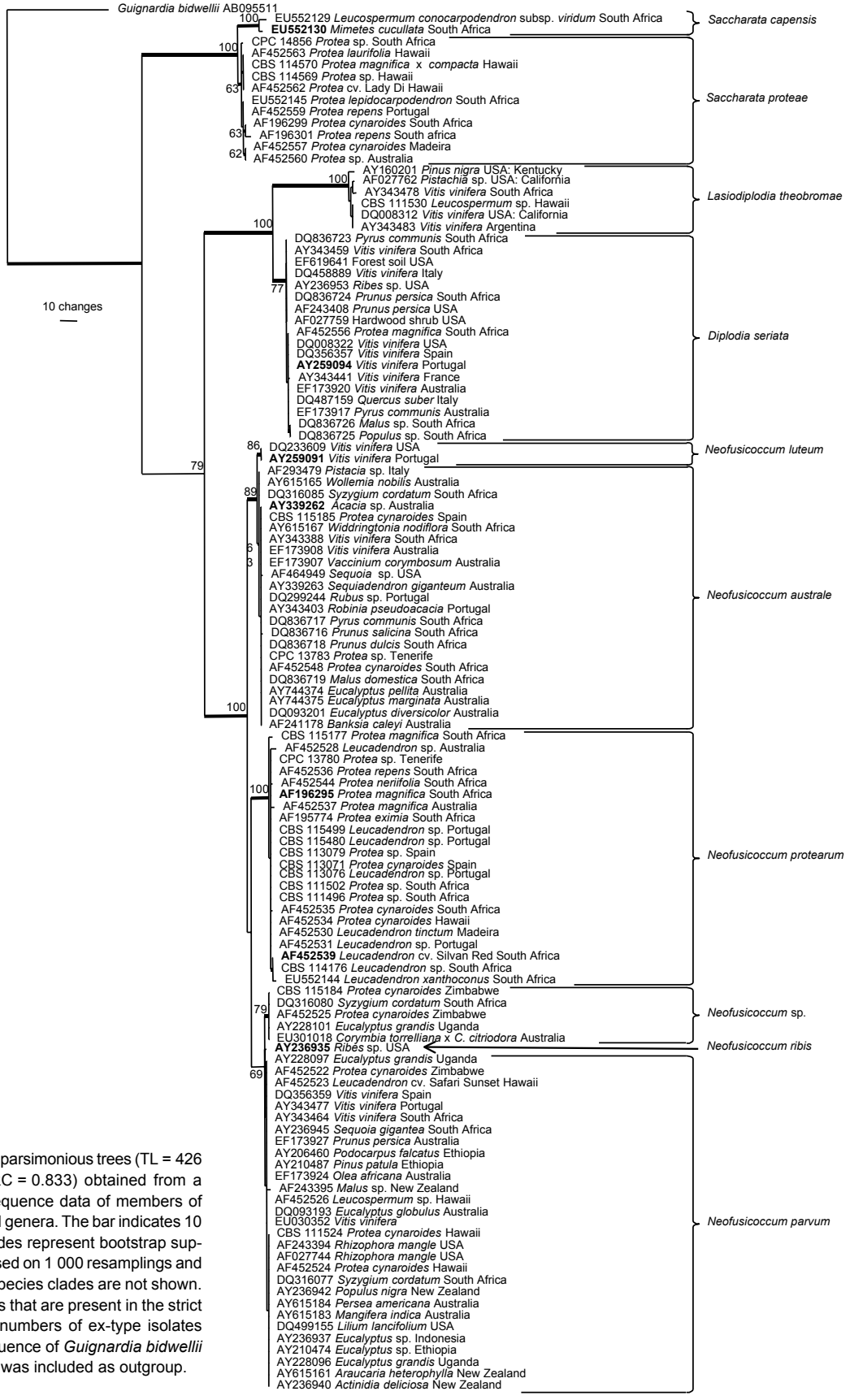

Fig. 1 One of 1 000 equally most parsimonious trees (TL = 426 steps, CI = 0.847, RI = 0.983, RC = 0.833) obtained from a parsimony analysis using ITS sequence data of members of the Botryosphaeriaceae and allied genera. The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values (higher than 60 %) based on 1 000 resamplings and bootstrap support values within species clades are not shown. Thickened lines indicate branches that are present in the strict consensus tree. The accession numbers of ex-type isolates are printed in **bold** face. The sequence of *Guignardia bidwellii* (GenBank accession AB095511) was included as outgroup.

Morphology

Colony colours (surface and reverse) were assessed after growth on MEA and oatmeal agar (OA, Gams et al. 2007) using the colour charts of Rayner (1970). Microscopic observations were made from colonies cultivated on MEA and OA. Preparations were mounted in lactic acid and studied under a light microscope (× 1 000 magnification). The 95 % confidence intervals were derived from 30 observations of spores formed on MEA or OA, with extremes given in parentheses. All cultures obtained in this study are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands, and duplicates have been stored in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute, Pretoria, South Africa or the working collection (CPC) of P.W. Crous (Table 2). Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org) and ITS barcodes and DNA sequence trace files in BOLD (www.barcodinglife.org).

RESULTS

DNA phylogeny

The manually adjusted ITS alignment contained 120 sequences (including the outgroup sequence) and 525 characters were used in the phylogenetic analysis; of these 162 were parsimony-informative, 72 were variable and parsimony-uninformative and 291 were constant. Only the first 1 000 equally most parsimonious trees, one of which is shown in Fig. 1, were saved from the parsimony analysis. Taxonomic novelties are described below and specific taxa are highlighted in the Discussion.

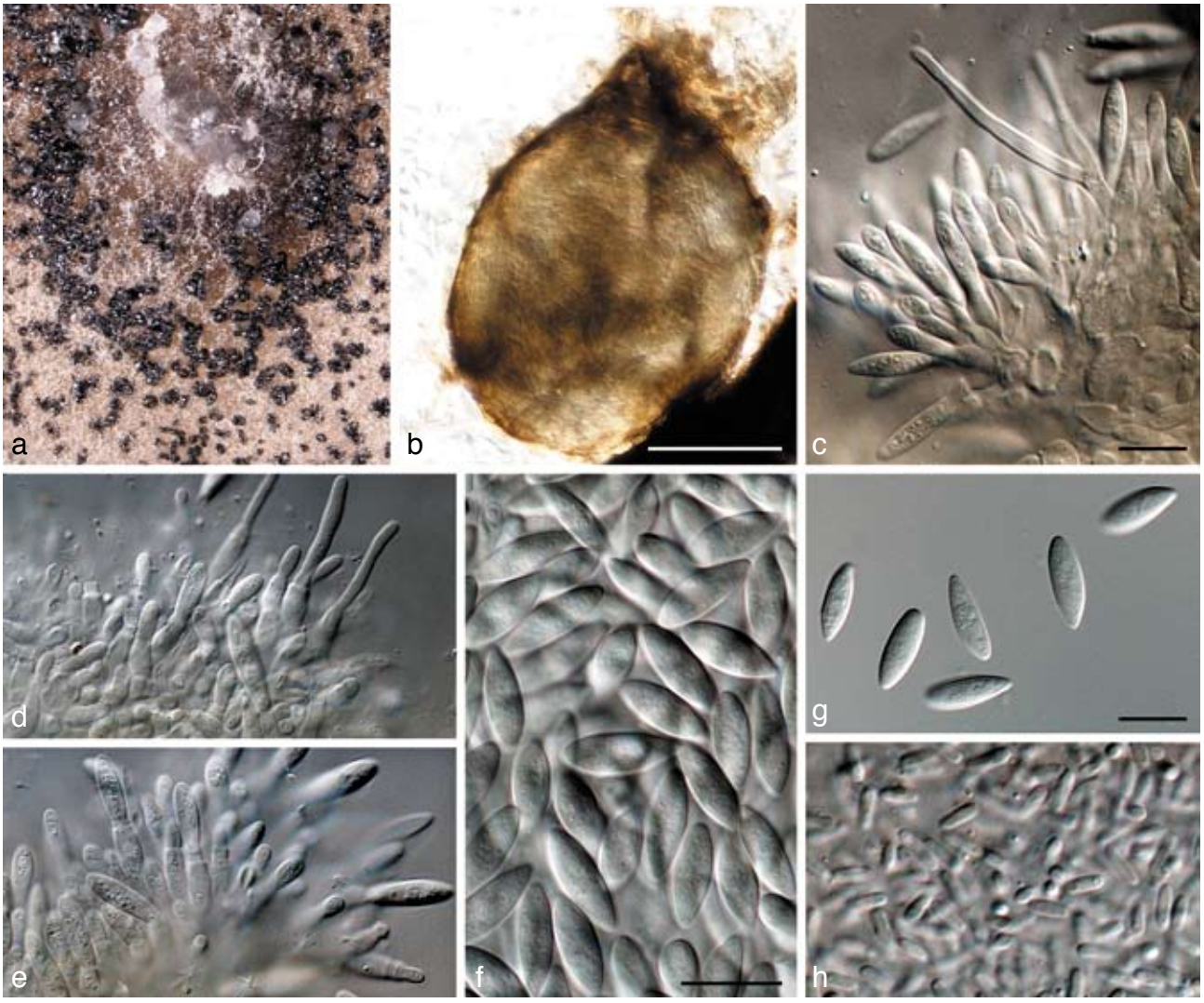

Fig. 2 *Saccharata capensis* on oatmeal agar (OA). a. Colony sporulating on OA; b. pycnidial conidioma; c, d. conidiogenous cells and branched paraphyses; d, e. conidiogenous cells showing percurrent proliferation; f, g. fusoid to ellipsoid conidia; h. spermatia. — Scale bars: b = 250 µm, all others = 10 µm.

Taxonomy

Saccharata capensis Crous, Marinc. & M.J. Wingf., *sp. nov.* — MycoBank MB512395; Fig. 2

Saccharata proteae simile, sed conidiis minoribus, (13–)15–16(–18) × (3.5–)4–5(–5.5) µm, differens.

Etymology. Name refers to the Cape Province of South Africa, where the fungus occurs.

Conidiomata pycnidial, black, up to 250 µm diam, opening by a single, central ostiole, up to 20 µm diam; wall consisting of 2–3 layers of pale dark brown *textura angularis*. *Conidiophores* hyaline, smooth, subcylindrical, branched, lining the inner layer of the cavity, 1–3-septate, 10–20 × 3.5–5 µm, intermingled with hyaline, smooth, subcylindrical paraphyses, 2–3 µm wide, with obtuse ends, extending slightly above the conidia. *Conidiogenous cells* phialidic with minute periclinal thickening, or 1–3 apical, percurrent proliferations, subcylindrical with slight apical taper, 7–12 × 3.5–4.5 µm. *Conidia* hyaline, smooth, thin-walled, aseptate, granular, fusoid-ellipsoid, apex subobtuse, base subtruncate, widest in the middle of the conidium, (13–)15–16(–18) × (3.5–)4–5(–5.5) µm (av. 15.5 × 4.5 µm). *Spermatia* formed in same conidioma as conidia, bacilliform, hyaline with rounded ends, 3–5 × 1–1.5 µm.

Cultural characteristics — *Colonies* sporulating profusely on OA, aerial mycelium sparse to absent, olivaceous-black with zones of grey-olivaceous in outer region; colonies flat, spreading, with irregularly crenate margins.

Specimens examined. South Africa, Western Cape Province, Kleinmond Nature Reserve, leaf litter of *Mimetes cucullata*, 11 July 2000, *S. Marincowitz*, holotype CBS H-20077, culture ex-type CBS 122693 = CMW 22200 = CPC 13699; Kogelberg Nature Reserve, leaf litter of *Leucospermum conocarpodendron* subsp. *viridum*, 11 July 2000, *S. Marincowitz*, CBS H-20078, culture CBS 122694 = CMW 22197 = CPC 13698.

Notes — *Saccharata capensis* is only the second species to be described in this genus (Crous et al. 2004a) and it is most easily distinguished from *S. proteae* by its smaller conidia. When it was originally isolated, a diplodia-like synanamorph, which is also typical of *S. proteae*, was observed in culture. With time, however, the cultures lost the ability to form this synanamorph and hence only the dominant anamorph state could be described here.

DISCUSSION

The present study is the first to revisit the taxonomy of Botryosphaeriaceae since Denman et al. (2003) treated the taxa that occur on Proteaceae. The most significant change to the taxonomy of this group subsequent to the study of Denman et al. (2003) was presented by Crous et al. (2006b). These authors employed LSU sequence data to reveal that the family consists of at least 10 distinct lineages, correlating to a diversity of different anamorphs and teleomorphs, and restricting *Botryosphaeria* to a rather small clade containing *B. dothidea* and *B. corticis*. This study was recently supplemented by Phillips et al. (2008), who used a similar approach to resolve the dark-spored genera of the Botryosphaeriaceae.

When they characterised the members of Botryosphaeriaceae occurring on proteas, Denman et al. (2003) reported the presence of some taxa that have since been shown to represent species complexes. The most significant of these, *Neofusicoccum luteum* (as *Fusicoccum luteum*), was reported from *Buckinghamia* and *Banksia* in Australia (Slippers et al. 2004b), and on *Protea cynaroides* in South Africa. Shearer et al. (1995) described a serious disease of *Banksia coccinea* caused by *N. ribis* (as *B. ribis*) along the south-western coast of Australia, which Denman et al. (2003) believed was *N. luteum* rather than *N. ribis*. In light of the present findings (Fig. 1) it appears that these isolates are more correctly treated as *N. australe* rather than the closely related *N. luteum*.

Neofusicoccum protearum was reported on Proteaceae from Australia, Madeira, Portugal and South Africa, and it is shown here to also occur on this host in Hawaii and Tenerife (Canary Islands). The exclusive association with South African Proteaceae led Denman et al. (2003) to hypothesise that *N. protearum* was indigenous to South Africa. In this case it would have been introduced into these other countries along with protea planting material, which is very plausible because the pathogen exists as endophyte in asymptomatic Proteaceae (Denman 2002, Denman et al. 2004). *Neofusicoccum protearum* causes leaf blight disease of Proteaceae, with lesions extending down the stems (Denman et al. 2003).

Denman et al. (2003) and Crous et al. (2004b) recorded *N. ribis*, from South African and Australian Proteaceae cultivated in Hawaii, and from *P. cynaroides* in Zimbabwe. This report, was largely based on the ITS sequence data available at the time, and the broad morphological circumscription applied to *N. ribis*. In a subsequent study, Slippers et al. (2004a) recollected and epitypified *N. ribis*, and showed that this species could be distinguished from the morphologically similar *N. parvum*, only by means of DNA sequence comparisons of TEF1. Results of the present study using these techniques showed clearly that these isolates from Proteaceae represent *N. parvum* and not *N. ribis* as initially reported. In fact, none of the previous reports of *N. ribis* from Proteaceae such as those on *Grevillea robusta* in Guatemala (Schieber & Zentmyer 1978) and *Leucadendron* in South Africa (Olivier 1951), have been confirmed and they need to be viewed with some circumspection. Results of this study have also shown that the cryptic *Neofusicoccum* species closely related to *N. parvum* and *N. ribis*, occurring on *Eucalyptus* in Uganda, *Protea cynaroides* in Zimbabwe, *Corymbia* in Australia and *Syzygium cordatum* in South Africa, probably represent yet another, undescribed component of the *N. ribis* species complex, which will be resolved elsewhere (B. Slippers, pers. comm.).

Other unconfirmed and doubtful records on Proteaceae include *Botryosphaeria dothidea*, which is reported to cause cankers, leaf infections and seedling dieback or blight of proteas (Crous et al. 2004a). Because of the lack of cultures and sequence data, we cannot at present confirm that *B. dothidea* occurs on Proteaceae. Although *Lasiodiplodia theobromae*, which is associated with dieback and stem cankers of proteas (Crous et al. 2004a) is confirmed from Hawaii (Fig. 1), records from elsewhere remain doubtful. Recent papers focusing on this

pathogen have revealed it to represent a species complex (Pavlic et al. 2004, Burgess et al. 2006, Damm et al. 2007a, Alves et al. 2008), again casting doubt on the identity of the species of *Lasiodiplodia* associated with Proteaceae. While substantial progress has been made towards understanding and managing Botryosphaeriaceae diseases of Proteaceae in recent years (Denman et al. 2004), additional collections from various locations and hosts in this family are required to fully resolve the status of these pathogens on this economically and ecologically important family of plants.

Saccharata proteae appears to be highly host-specific and has been found associated only with South African Proteaceae (Crous et al. 2000b, Taylor et al. 2001a, b). *Saccharata proteae*, which is a well-established endophyte (Swart et al. 2000, Taylor et al. 2001c), causes leaf spots and leaf tip dieback, which is usually associated with insect wounds (Denman et al. 1999, Crous et al. 2004a). The ecology of *S. capensis*, which is newly described here from Proteaceae leaf litter, is unknown. However, it is quite possible that isolates of *S. capensis* have in the past been confused with those of *S. proteae*, and that it could have a similar ecological habitat. Furthermore, there is some variation in the DNA sequence data between the two collections of *S. capensis* (12 nt different), which could suggest that further collections may reveal yet more cryptic taxa in this complex.

Diplodia seriata (= '*Botryosphaeria*' *obtusa*), *Saccharata proteae* and *Neofusicoccum protearum* all have an endophytic habitat (Crous et al. 2004a). Although it is not clear whether *Diplodia seriata* is a protea pathogen, there are reports of this species associated with serious stem cankers on fruit trees and grapevines (Denman et al. 2003, van Niekerk et al. 2004, Damm et al. 2007a). Several species of Botryosphaeriaceae that are pathogens have also been isolated from *Protea* litter, including *S. proteae*, *N. protearum* and species of *Diplodia* and *Dothiorella* (Marincowitz et al. 2008). It is, therefore, not possible to disregard *S. capensis* as a potential pathogen of Proteaceae until pathogenicity studies have been conducted.

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