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Foliicolous microfungi occurring on *Encephalartos*

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Key words

Catenulostroma
Cladophialophora
Dactylaria
ITS nrDNA
LSU nrDNA
Ochroconis
Phaeomoniella
Saccharata
systematics
Teratosphaeria

Abstract Species of *Encephalartos*, commonly known as bread trees, bread palms or cycads are native to Africa; the genus encompasses more than 60 species and represents an important component of the indigenous African flora. Recently, a leaf blight disease was noted on several *E. altensteinii* plants growing at the foot of Table Mountain in the Kirstenbosch Botanical Gardens of South Africa. Preliminary isolations from dead and dying leaves of *E. altensteinii*, *E. lebomboensis* and *E. princeps*, collected from South Africa, revealed the presence of several novel microfungi on this host. Novelties include *Phaeomoniella capensis*, *Saccharata kirstenboschensis*, *Teratosphaeria altensteinii* and *T. encephalarti*. New host records of species previously only known to occur on Proteaceae include *Cladophialophora proteae* and *Catenulostroma microsporum*, as well as a hyperparasite, *Dactylaria leptosphaeriicola*, occurring on ascomata of *T. encephalarti.*

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INTRODUCTION

Encephalartos (Zamiaceae) is a genus of cycads indigenous to Africa. Due to its edible pith, species of *Encephalartos* are commonly referred to as bread trees or bread palms (www.kew.org/plants/). Another interesting aspect that makes *Encephalartos* noteworthy is the fact that it could represent one of the oldest pot-plants in the world. A specimen of *E. altensteinii* was collected in the Eastern Cape Province of South Africa in the early 1770s, and taken to Kew Botanic Gardens in the UK by Francis Masson in 1775, where it is still to be seen in the Palm House today. Although this plant genus is endangered and known to suffer from trunk and root parasites, as well as fungal infections, very few fungi have been described from this host (Doidge 1950, Nag Raj 1993, nt.ars-grin.gov/fungaldatabases/).

Fungal biodiversity has been poorly studied from most African countries, which could explain why so few fungal taxa have thus far been reported from *Encephalartos*. In a recent attempt to estimate how many species of fungi could occur at the tip of Africa, Crous et al. (2006a) concluded that the 1.5 M estimate suggested by Hawksworth (1991) was clearly too conservative. Based on available data, South Africa alone should have at least 200 000 fungal species associated with plant species, without taking into account the number associated with insects, or other ecological habitats such as water and soil.

Because of its extremely hard, leathery leaves, microfungi are not readily observed to colonise foliage of *Encephalartos* species. In January 2008, however, a tip blight disease was observed on several *Encephalartos* palms growing in the Kirstenbosch Botanical Gardens of South Africa, as well as in the KwaZulu-Natal Province. The aim of the present study was therefore to determine if any microfungi could be isolated from these diseased leaves and also investigate symptomatic *Encephalartos* leaf samples collected from elsewhere.

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MATERIALS AND METHODS

Isolates

Dead *Encephalartos* leaves, or leaves with tip blight symptoms, were chosen for study. As none of the collections had leaves that were visibly colonised, leaves were incubated in moist chambers for up to 2 wk, and inspected daily for fungi. Leaf pieces bearing ascomata were subsequently 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 subcultured 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 and descriptions were deposited in MycoBank (Crous et al. 2004b).

DNA phylogeny

Genomic DNA was isolated from fungal mycelium grown on MEA, using the UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocols. The Primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rDNA operon spanning

Table 1 Collection details and GenBank accession numbers for fungal species isolated from *Encephalartos* spp.

Species	Strain no.[1]	Substrate	Collector(s)	GenBank Accession number	
				ITS[2]	LSU[2]
Catenulostroma abietis	CPC 14996	Dead leaf tissue of *E. altensteinii*	P.W. Crous	FJ372387	FJ372404
Cladophialophora proteae	CPC 14902	Dead leaf tissue of *E. altensteinii*	P.W. Crous	FJ372388	FJ372405
Lophiostoma sp.	CPC 15000; CBS 123543	Living leaves of *E. altensteinii*	P.W. Crous et al.	FJ372389	FJ372406
Ochroconis sp.	CPC 15461; CBS 123536	Living leaves of *E. lebomboensis*	A.R. Wood	FJ372390	FJ372407
Phaeomoniella capensis	CPC 15416; CBS 123535	Living leaves of *E. altensteinii*	A.R. Wood	FJ372391	FJ372408
Saccharata kirstenboschensis	CPC 15275; CBS 123537	Living leaves of *E. princeps*	A.R. Wood	FJ372392	FJ372409
Teratosphaeria altensteinii	CPC 15133; CBS 123539	Living leaves of *E. altensteinii*	P.W. Crous et al.	FJ372394	FJ372411
Teratosphaeria encephalarti	CPC 14886; CBS 123540	Living leaves of *E. altensteinii*	P.W. Crous et al.	FJ372395	FJ372412
	CPC 15281; CBS 123544	Living leaves of *E. altensteinii*	A.R. Wood	FJ372396	FJ372413
	CPC 15362; CBS 123541	Living leaves of *E. altensteinii*	A.R. Wood	FJ372397	FJ372414
	CPC 15413; CBS 123545	Living leaves of *E. altensteinii*	A.R. Wood	FJ372398	FJ372415
	CPC 15464; CBS 123546	Living leaves of *E. lebomboensis*	A.R. Wood	FJ372399	FJ372416
	CPC 15465	Living leaves of *E. lebomboensis*	A.R. Wood	FJ372400	FJ372417
	CPC 15466	Living leaves of *E. lebomboensis*	A.R. Wood	FJ372401	FJ372418
Teratosphaeria sp.	CPC 14997	Living leaves of *E. altensteinii*	P.W. Crous et al.	FJ372402	FJ372419

[1] CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS.
[2] ITS: Internal transcribed spacers 1 and 2 together with 5.8S nrDNA; LSU: 28S nrDNA.

the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the first 900 bases at the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White et al. 1990) and LR0R (Rehner & Samuels 1994) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The PCR conditions, sequence alignment and subsequent phylogenetic analysis followed the methods of Crous et al. (2006b). Alignment gaps were treated as new character states. Sequence data were deposited in GenBank (Table 1) and alignments in TreeBASE (www.treebase.org). The ITS sequences were compared with the sequences available in NCBIs GenBank nucleotide database using a megablast search.

Morphology

Colony growth characteristics (surface and reverse) were assessed on MEA, PDA, OA and SNA (Gams et al. 2007), and colours determined using the colour charts of Rayner (1970). Microscopic observations were made from fungal colonies cultivated on different media, as stated with each fungus. Preparations were mounted in lactic acid and studied by means of a light microscope (× 1000 magnification). Microscopic observations were made from hyphomycetes by using the transparent tape or slide culture technique, as respectively explained by Schubert et al. (2007) and Arzanlou et al. (2007). The 95 % confidence intervals were derived from 30 observations of spores formed in culture, 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, or the working collection (CPC) of P.W. Crous (Table 1).

RESULTS

DNA phylogeny

Amplification products of approximately 1 700 bases were obtained for the isolates listed in Table 1. The LSU region of the sequences was used to obtain additional sequences from GenBank, which were added to the alignment. Due to the inclusion of the shorter *Phaeomoniella chlamydospora* (GenBank AB278179) and *Ochroconis* '*humicola*' (GenBank AB161068) sequences in the alignment, it was not possible to subject the full length of the determined LSU sequences (Table 1) to the analyses. The manually adjusted alignment contained 53 sequences (including the outgroup sequence) and, of the 563 characters used in the phylogenetic analyses, 253 were parsimony-informative, 24 were variable and parsimony-uninformative, and 286 were constant. Neighbour-joining analyses using three substitution models on the sequence data yielded trees supporting the same tree topology to one another but differed from the most parsimonious tree shown in Fig. 1 with regard to the placement of the clade containing *Ochroconis* and *Fusicladium* (in the distance analyses, this clade moves to a more basal position). Forty equally most parsimonious trees (TL = 1039 steps, CI = 0.477, RI = 0.833, RC = 0.397), one of which is shown in Fig. 1, were obtained from the parsimony analysis of the LSU alignment. The isolates from *Encephalartos* are distributed across several families and orders and taxonomic novelties are described below and specific taxa are highlighted in the Discussion. Results obtained from the BLAST searches of the ITS sequences are discussed where applicable.

Taxonomy

Several species of fungi which are believed to be new were collected, and are described in genera such as *Phaeomoniella*, *Saccharata* and *Teratosphaeria*. New records for *Encephalartos* include *Catenulostroma microsporum*, *Cladophialophora proteae*, *Dactylaria leptosphaeriicola*, and undescribed species of *Teratosphaeria*, *Lophiostoma* and *Ochroconis*.

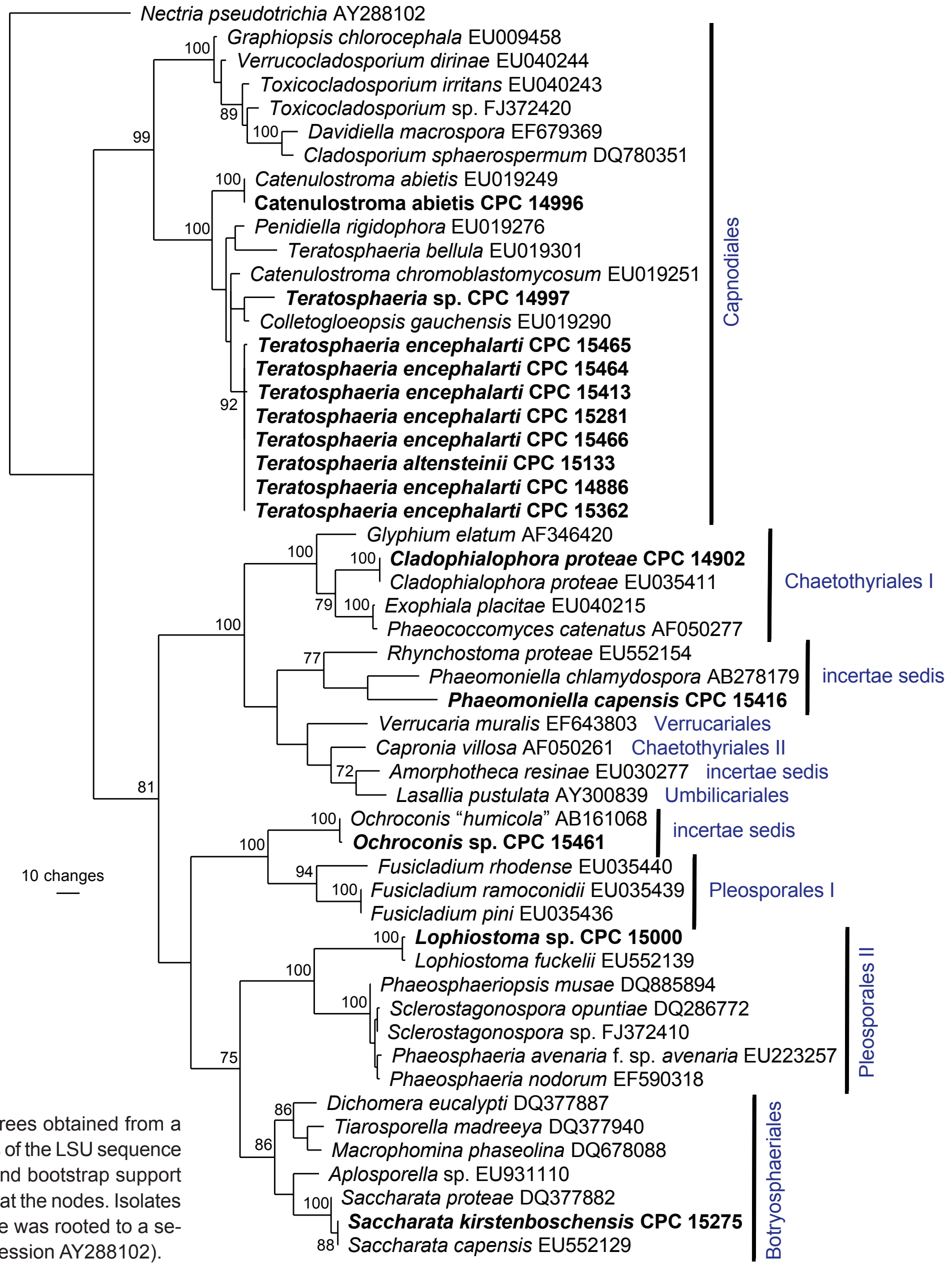

Fig. 1 One of 40 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 10 changes, and bootstrap support values (>70 %) from 1 000 replicates are shown at the nodes. Isolates from *Encephalartos* are shown in **bold**. The tree was rooted to a sequence of *Nectria pseudotrichia* (GenBank accession AY288102).

Phaeomoniella capensis Crous & A.R. Wood, *sp. nov.* — MycoBank MB508007; Fig. 2

Phaeomoniellae chlamydosporae similis, sed conidiis majoribus, (2–)3(–4) × 1–1.5 µm.

Etymology. Name refers to the Cape Province of South Africa, where this fungus was collected.

On SNA. *Mycelium* consisting of septate, branched, hyaline to pale brown, thick-walled hyphae, 1.5–2 µm; developing hyaline, thin-walled, swollen, globose structures. *Conidiomata* pycnidial to acervular, opening by irregular rupture, erumpent, brown, up to 250 µm diam; wall of 3–6 layers of brown *textura angularis*. *Conidiophores* hyaline, smooth, highly variable in morphology, occurring in branched structures, 2–4-septate, or solitary, ampulliform, reduced to phialides. *Conidiogenous cells* 3–10 × 2–3 µm; apical opening with minute periclinal thickening. *Conidia* hyaline, smooth, narrowly ellipsoid, straight, (2–)3(–4) × 1–1.5 µm.

Cultural characteristics — *Colonies* erumpent, spreading, lacking aerial mycelium, slimy, with folded surface and smooth, catenulate margin; on PDA salmon with patches of apricot, and apricot in reverse, reaching 10 mm diam after 1 mo; on OA salmon to flesh with brown patches due to conidiomatal formation, reaching 12 mm diam after 1 mo; on MEA salmon

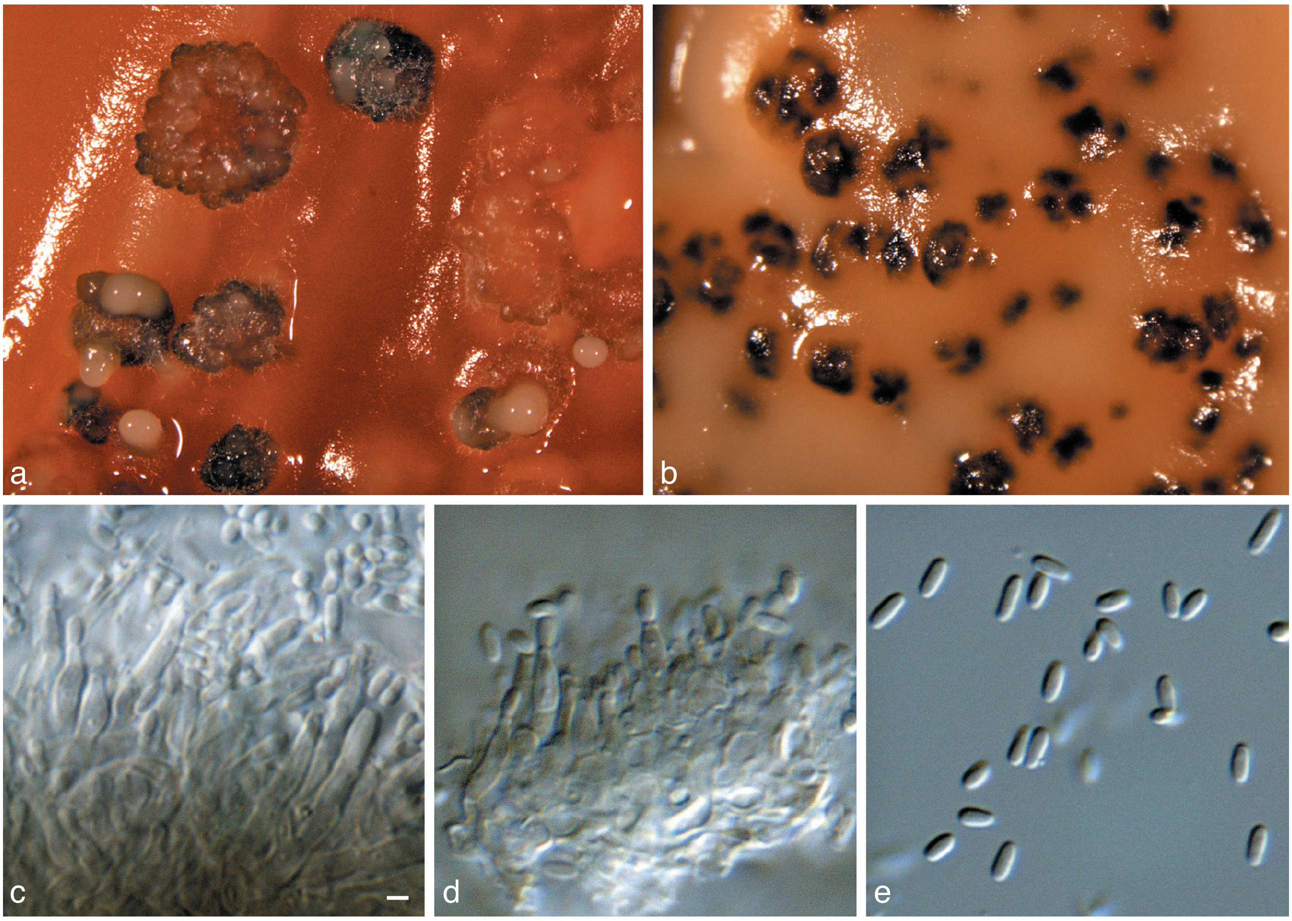

Fig. 2 *Phaeomoniella capensis* in vitro (CBS 123535). a. Colony on OA; b. colony on PDA; c, d. conidiogenous cells and conidia; e. conidia. — Scale bar = 10 µm.

with patches of apricot and flesh, apricot in reverse, reaching 15 mm diam after 1 mo.

Specimen examined. South Africa, Western Cape Province, Kirstenbosch Botanical Garden, on living leaves of *Encephalartos altensteinii*, 22 May 2008, *A.R. Wood*, CBS H-20159, culture ex-type CPC 15416 = CBS 123535, CPC 15417–15418.

Notes — Two fungal species that have previously been described from *Encephalartos* need to be compared with *P. capensis*. *Leptothyrium evansii* forms hypophylous pycnidia with oblong, hyaline conidia, 3.5–5 × 1.5–2 µm, thus larger than observed in *P. capensis* (Sydow & Sydow 1912). The second species, *Phoma encephalarti*, is distinct in having larger, biguttulate conidia, 6.3–7.2 × 2.7–3.6 µm (Negodi 1932).

The fact that the present collection clusters in *Phaeomoniella* (hyphomycetous genus) is somewhat surprising. However, this genus also has a phoma-like synanamorph and a yeast-like growth in culture (Crous & Gams 2000), similar to *P. capensis*. Although further collections may eventually show this complex to represent more than one genus, we presently consider it best to place the *Encephalartos* fungus in *Phaeomoniella* based on current data. BLAST results of the ITS sequence revealed an identity of 89 % with *Phaeomoniella chlamydospora* (GenBank accession AY772237).

Saccharata kirstenboschensis Crous & A.R. Wood, *sp. nov.* — MycoBank MB508008; Fig. 3

Saccharatae proteae similis, sed conidiis minoribus, (16–)18–22(–24) × 3.5–4(–5) µm.

Etymology. Name refers to Kirstenbosch Botanical Gardens, South Africa, where this fungus was collected.

On WA with sterile pine needles. *Conidiomata* pycnidial, black, up to 350 µm diam, with a single, central ostiole; wall consisting of 2–3 layers of brown *textura angularis*. *Conidiophores* subcylindrical, hyaline, smooth, frequently reduced to conidiogenous cells or branched in apical part, 1–2-septate, 10–45 × 2–3.5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, 15–20 × 2–3 µm; apex with periclinal thickening, or with 1–3 percurrent proliferations. *Paraphyses* intermingled among conidiophores, at times arising as lateral branches from conidiophores, or separate, unbranched or branched above, hyaline, smooth, 0–3-septate, 2–3 µm wide, extending above conidiophores. *Conidia* hyaline, smooth, fusiform to narrowly ellipsoid, apex subobtuse, base truncate with minute marginal frill, guttulate, thin-walled, (16–)18–22(–24) × 3.5–4(–5) µm, base 2–3 µm wide.

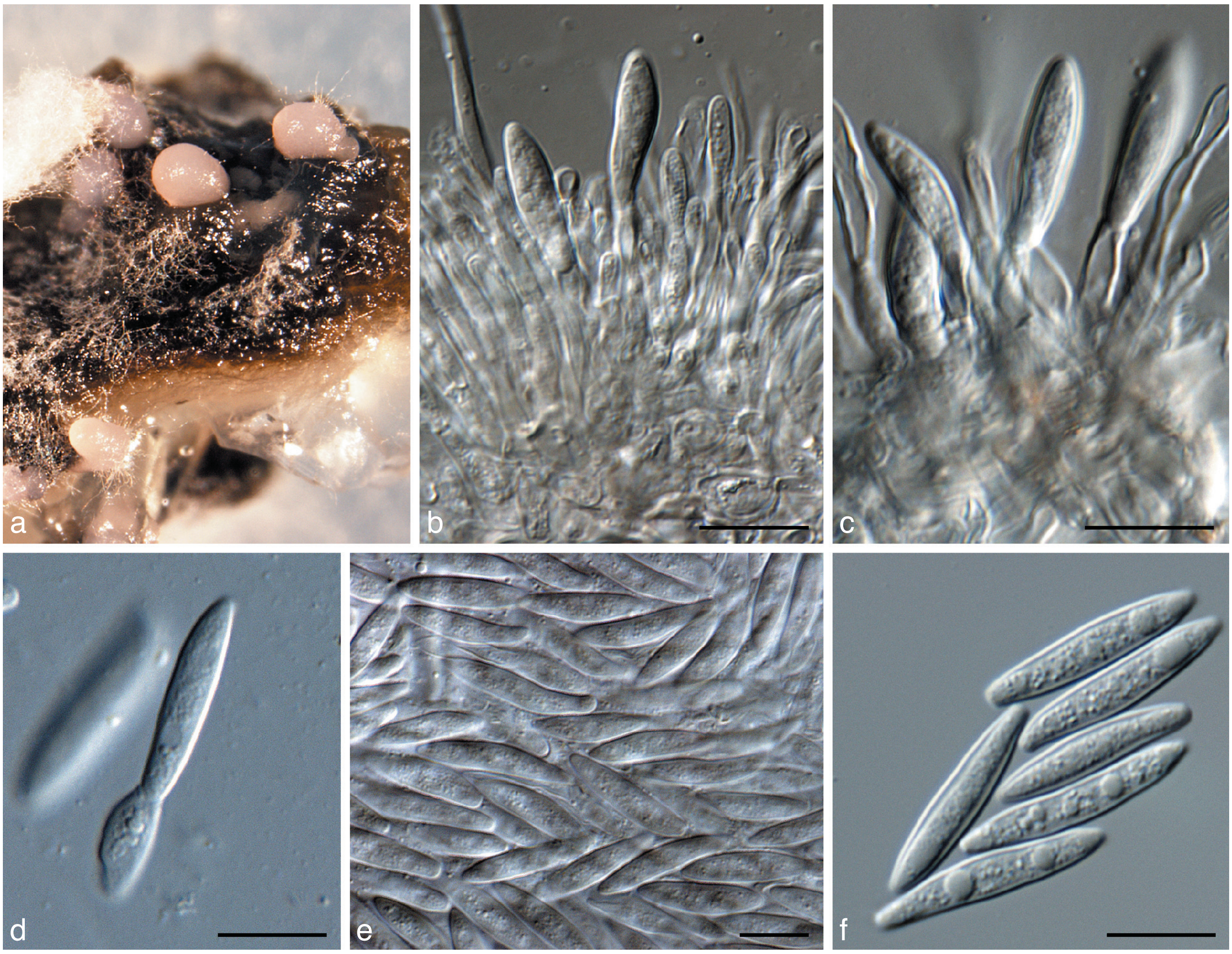

Fig. 3 *Saccharata kirstenboschensis* in vitro (CBS 123537). a. Conidiomata on WA with sterile pine needles; b, c. conidiogenous cells giving rise to conidia; d. conidium attached to conidiogenous cell; e, f. conidia. — Scale bars = 10 µm.

Cultural characteristics — *Colonies* on MEA, PDA and OA spreading, erumpent, with moderate aerial mycelium and uneven, catenulate margins; pale olivaceous-grey with patches of grey and olivaceous-grey; reverse olivaceous-grey; reaching 6 cm diam after 1 mo.

Specimen examined. South Africa, Western Cape Province, Kirstenbosch Botanical Garden, on living leaves of *Encephalartos princeps*, 22 May 2008, *A.R. Wood*, holotype CBS H-20160, culture ex-type CPC 15275 = CBS 123537, CPC 15276–15277.

Notes — The genus *Saccharata* presently consists of two species, namely *S. proteae* (conidia 20–30 × 4.5–6 µm; Denman et al. 1999, Crous et al. 2006b) and *S. capensis* (conidia 13–18 × 3.5–5.5 µm; Marincowitz et al. 2008). *Saccharata kirstenboschensis* represents an intermediate species, having conidia 16–24 × 3.5–5 µm. Furthermore, it is the first species of *Saccharata* known to occur on a host other than Proteaceae, although all taxa described thus far appear to be endemic to South Africa. BLAST results of the ITS sequence revealed an identity of 98 % with *S. proteae* (GenBank accession EU552145; 819 of 830 bases) and *S. capensis* (GenBank accession EU552130; 803 of 816 bases).

Teratosphaeria altensteinii Crous, *sp. nov.* — MycoBank MB508010; Fig. 4

Teratosphaeriae bellulae similis, sed ascosporis minoribus, 7–8(–9) × 2.5–3(–3.5) µm.

Etymology. Name refers to its host species, *Encephalartos altensteinii*.

Leaves with tip-blight symptoms; necrotic tissue grey-brown, separated from healthy tissue by a narrow, dark-brown border. *Ascomata* hypophyllous, black, immersed, substomatal, up to 90 µm diam; ostiole lined with periphyses; wall consisting of 2–3 layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, obovoid, straight to slightly curved, 8-spored, 35–37 × 8–9 µm. *Ascospores* bi- to triseriate, overlapping, hyaline, guttulate, thin-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, 7–8(–9) × 2.5–3(–3.5) µm; germinating ascospores on MEA become brown and verruculose, germinating with multiple germ tubes irregular to the long axis of the spore, constricted at septum and distorting, up to 8 µm wide.

Fig. 4 *Teratosphaeria altensteinii* in vitro (CBS 123539). a, b. Asci; c, d. ascospores; e–g. germinating ascospores on MEA. — Scale bars = 10 µm.

Cultural characteristics — *Colonies* on MEA spreading, somewhat erumpent, with moderate aerial mycelium, and even, catenulate margins; surface iron-grey; reverse greenish black; reaching 20 mm diam after 1 mo; on PDA and OA similar, but olivaceous-grey on surface, and iron-grey in reverse; on MEA and PDA hyphae form terminal clusters of chlamydospore-like cells, which are catenulostroma-like in appearance, and frequently detach under squash mounts.

Specimen examined. South Africa, Western Cape Province, Kirstenbosch Botanical Garden, on living leaves of *Encephalartos altensteinii*, 6 Jan. 2008, *P.W. Crous*, *M.K. Crous*, *M. Crous & K. Raath*, holotype CBS H-20162, culture ex-type CPC 15133 = CBS 123539, CPC 15134–15135.

Notes — *Teratosphaeria altensteinii* is phylogenetically closely related to *T. bellula* (593 of 601 bases when the ITS sequence is compared to GenBank accession EU707861), which is a pathogen of Proteaceae (Crous & Wingfield 1993, Crous et al. 2004a, 2008). Morphologically it has ascospores that are similar in shape, but are distinct in that they lack a prominent sheath and are somewhat smaller (7–9 × 2.5–3.5 µm) than those of *T. bellula* (8–11 × 2–3.5 µm; Crous & Wingfield 1993).

Teratosphaeria encephalarti Crous & A.R. Wood, *sp. nov.* — MycoBank MB508011; Fig. 5

Anamorph. *Penidiella* sp.

Teratosphaeriae bellulae similis, sed ascosporis majoribus, (9–)10–11(–14) × (3–)3.5–4 µm.

Etymology. Name refers to its host genus, *Encephalartos*.

Leaves with tip-blight symptoms; nectrotic tissue grey-brown. *Ascomata* hypophyllous, black, immersed, substomatal, up to 90 µm diam; ostiole lined with periphyses; wall consisting of 2–3 layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid, straight to curved, 8-spored, 30–40 × 10–13 µm. *Pseudoparaphyses* intermingled among asci, branched, septate, hyaline, 2–3 µm wide. *Ascospores* bi- to triseriate, overlapping, hyaline, guttulate, thin-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, (9–)10–11(–14) × (3–)3.5–4 µm; turning brown and verruculose in older asci; germinating ascospores on

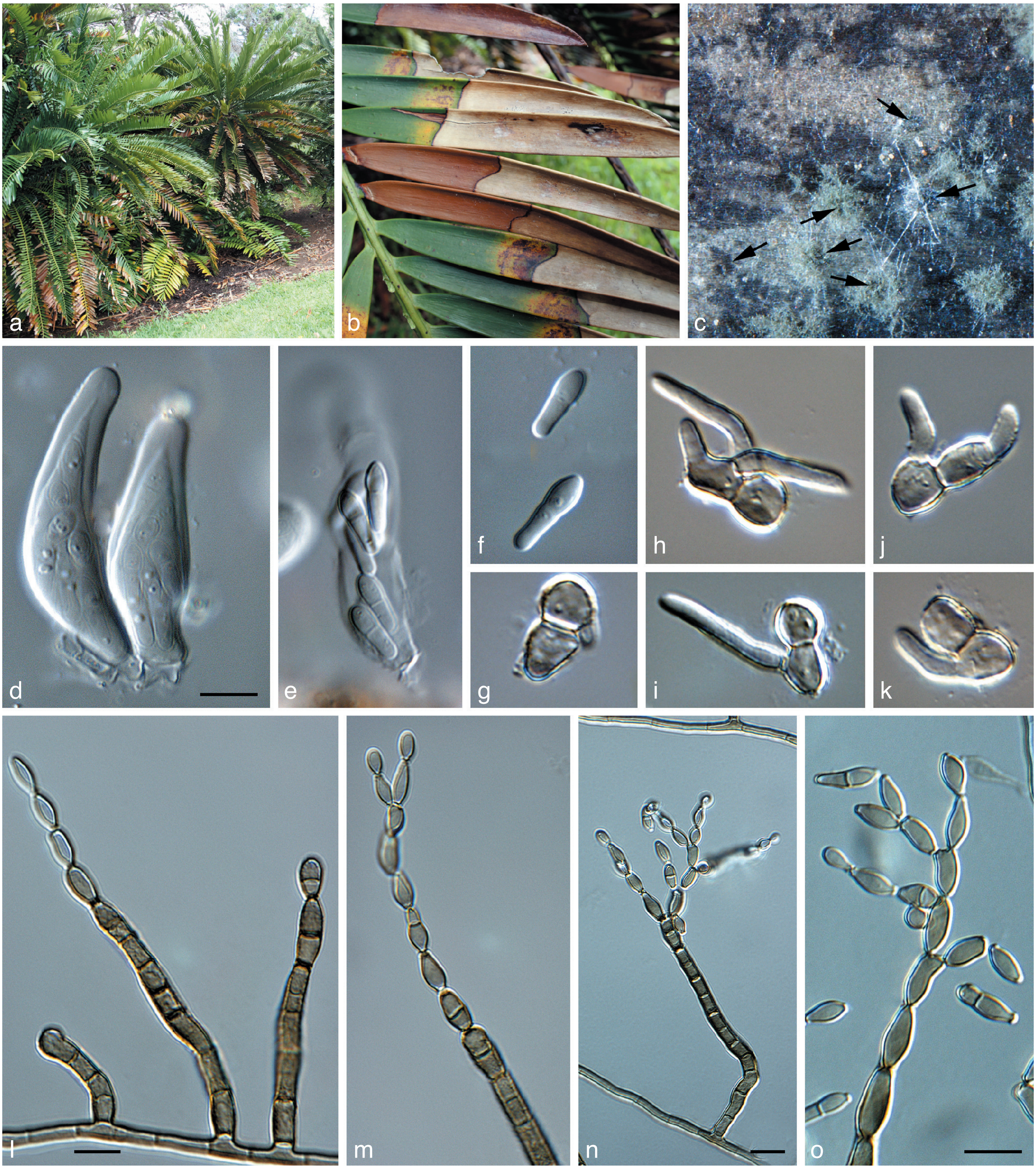

Fig. 5 *Teratosphaeria encephalarti* (CBS 123540). a. Diseased *Encephalartos altensteinii* palms in Kirstenbosch Botanical Gardens, South Africa; b. leaf blight symptoms; c. ascomata on leaves (arrows); d, e. asci; f. ascospores; g–k. germinating ascospores on MEA; l–o. *Penidiella* anamorph with branched conidial chains. — Scale bars = 10 µm.

MEA become brown and verruculose, germinating with several germ tubes irregular to the long axis of the spore, constricted at septum and distorting, up to 7 µm wide. On OA. *Mycelium* consisting of creeping, branched, septate, brown, smooth, 2–3.5 µm wide hyphae. *Conidiophores* solitary, erect, subcylindrical, arising from creeping hyphae, medium brown, thick-walled, smooth to finely verruculose, 1–6-septate, 15–50 × 3–4.5 µm. *Conidiogenous cells* terminal, subcylindrical, medium brown, smooth, up to 4 µm wide; scars somewhat thickened and darkened, up to 2.5 µm wide. *Ramoconidia* 0–1-septate, subcylindrical to elongate-ellipsoid, medium brown, smooth, thick-walled, with 1–3 apical loci, 10–15 × 3–4 µm. *Secondary ramoconidia* 0–1-septate, narrowly ellipsoid, 7–10 × 3–3.5 µm. *Intercalary conidia* in chains of up to 15, aseptate, fusoid-ellipsoid, medium brown, smooth, (5–)6–7(–8) × 2–3(–2.5) µm. *Terminal conidia* aseptate, ellipsoid, pale to medium brown, with truncate base, 3–4 × 2–3 µm; hila slightly thickened and darkened, 0.5–1 µm wide.

Cultural characteristics — *Colonies* on OA, MEA and PDA spreading with moderate aerial mycelium and smooth, catenulate margins; centre olivaceous-grey, outer region and reverse iron-grey; reaching 30 mm diam after 1 mo.

Specimens examined. South Africa, Western Cape Province, Kirstenbosch Botanical Garden, on living leaves of *Encephalartos altensteinii*, 6 Jan. 2008, *P.W. Crous*, *M.K. Crous*, *M. Crous & K. Raath*, holotype CBS H-20163, culture ex-type CPC 14886 = CBS 123540, CPC 14887–14888; 22 May 2008, *A.R. Wood*, culture CPC 15413 = CBS 123545, CPC 15414–15415; CPC 15362 = CBS 123541, CPC 15363–15364; CPC 15281 = CBS 123544, CPC 15282–15283; KwaZulu-Natal, South Coast, Uvongo, Skyline Nature Reserve, arboretum, living leaves of *Encephalartos lebomboensis*, 29 May 2008, *A.R. Wood*, culture CPC 15464 = CBS 123546, CPC 15465–15466.

Notes — *Teratosphaeria encephalarti* appeared to be quite dominant on the dying leaves of *E. altensteinii* in the Western Cape Province and it is possible that this species plays a role in the recently observed leaf blight disease. Inoculation studies are required, however, to confirm its potential role in this disease. Phylogenetically *T. encephalarti* and *T. altensteinii* are distantly related (88 % based on ITS) to *T. associata*, which occurs on *Eucalyptus* and *Protea* spp. (Crous et al. 2007a, 2008). The ITS sequences of the ex-type strains of *T. altensteinii* and *T. encephalarti* have an identity of 91 % with each other (430 of 468 bases).

Undetermined species

Lophiostoma sp.

Cultural characteristics — *Colonies* on MEA, PDA and OA spreading with moderate aerial mycelium, and smooth, catenulate margins; surface olivaceous-grey; reverse iron-grey; reaching 25 mm diam after 1 mo.

Specimen examined. South Africa, Western Cape Province, Kirstenbosch Botanical Garden, on living leaves of *Encephalartos altensteinii*, 6 Jan. 2008, *P.W. Crous*, *M.K. Crous*, *M. Crous & K. Raath*, culture CPC 15000 = CBS 123543, CPC 15001–15002.

Notes — Isolate CBS 123543 is representative of a species of *Lophiostoma* (based on ITS DNA sequence similarity to *L. macrostomum* GenBank accession EU552140). It could not be described, however, due to paucity of material. Ascospores remained hyaline upon germination on MEA, but distort prominently (up to 10 µm wide), becoming constricted, with germ tubes growing down into the agar.

Ochroconis sp. — Fig. 6

On OA. *Colonies* moderately fast-growing, flat with predominantly submerged mycelium. *Mycelium* consisting of branched, septate, hyaline to pale brown, smooth, 2–2.5 µm wide hyphae. *Conidiophores* erect, arising from creeping hyphae, unbranched, 1–6-septate, straight to flexuous, brown, thick-walled, 10–50 × 2.5–3.5 µm. *Conidiogenous cells* terminal, integrated, 10–35 µm long, polyblastic, cylindrical, straight to flexuous, pale to medium brown, with scattered pimple-shaped, subhyaline denticles, 0.5 µm wide and long. *Conidia* (5–)7–9(–10) × (2.5–)3(–3.5) µm, solitary, subhyaline, smooth to verruculose, 1-septate, thin-walled, obovoid to fusiform, apex subobtuse, base narrowly truncate with minute marginal frill, 0.5 µm wide; conidial secession rhexolytic.

Cultural characteristics — *Colonies* on MEA, PDA and OA spreading, flat, with even, smooth margins, and sparse aerial mycelium; surface olivaceous-grey, reverse iron-grey; colonies reaching 25 mm diam after 1 mo.

Specimen examined. South Africa, KwaZulu-Natal, South Coast, Uvongo, Skyline Nature Reserve, arboretum, living leaves of *Encephalartos lebomboensis*, 29 May 2008, *A.R. Wood*, culture CPC 15461 = CBS 123536, CPC 15462–15463.

Notes — Species of *Ochroconis* are known to infect cold blooded vertebrates, or to occur as saprobes on different plant substrates and in soil (de Hoog et al. 2000), suggesting that the species from *Encephalartos* is probably saprobic. Phylogenetically the present collection clusters with a strain identified as *Ochroconis humicola* (CBS 780.83), though conidia of the ex-type strain of *O. humicola* (CBS 116655) are larger and it clusters distant from these strains. Preliminary DNA sequence data suggest that many species of *Ochroconis* in fact represent species complexes, and hence it would be best to treat the *Encephalartos* collection as part of a generic revision (de Hoog et al. in prep).

Teratosphaeria sp.

Cultural characteristics — *Colonies* on MEA, PDA and OA erumpent, fluffy, with abundant aerial mycelium and even, catenulate margins; surface olivaceous-grey with patches of iron-grey and pale olivaceous-grey; reverse iron-grey; reaching 30 mm diam after 1 mo.

Specimen examined. South Africa, Western Cape Province, Kirstenbosch Botanical Garden, on living leaves of *Encephalartos altensteinii*, 6 Jan. 2008, *P.W. Crous*, *M.K. Crous*, *M. Crous & K. Raath*, culture CPC 14997–14999.

Notes — Isolate CPC 14997 could not be described due to paucity of material. Based on the DNA similarity to an ITS sequence of *Batcheloromyces leucadendri* (accession EU552103; 739 of 801 bases identity) deposited in GenBank, however, it appears to represent a species of *Teratosphaeria*. Ascospores germinated from both polar ends with germ tubes growing parallel to the long axis of the spore. Germinating spores became

Fig. 6 *Ochroconis* sp. in vitro (CBS 123536). a, b. Conidial fascicles on MEA with light from above and below, respectively; c–j. conidiophores giving rise to conidia, with visible denticles (arrows); k. conidia. — Scale bars = 10 µm.

prominently constricted and distorted, up to 7 µm wide, pale brown, and somewhat verruculose.

DISCUSSION

Prior to the present study only four fungal species had been described from *Encephalartos*, namely *Leptothyrium evansii*, *Pestalotia encephalartos*, *Phoma encephalarti* and *Phyllosticta encephalarti* (http://nt.ars-grin.gov/fungaldatabases/). A very preliminary examination of four collections during the present study has added a further four species in genera such as *Phaeomoniella*, *Saccharata* and *Teratosphaeria*. Furthermore, due to paucity of fungal material, several other species remain to be described in future studies. At present none of these fungi are confirmed as being pathogenic, and further work is required to determine which species are pathogens of *Encephalartos* and what impact they have on the population dynamics of these plant species. Considering that many of these cycad species are endangered this could have important consequences for their conservation.

What is interesting to note, however, is that some species known from indigenous Proteaceae were also observed for the first time on *Encephalartos*. *Dactylaria leptosphaeriicola* (Fig. 7) was initially described as a hyperparasite of ascomata of *Leptosphaeria protearum* on leaves of *Protea repens*. It is interesting that this fungus was found occurring on ascomata of *Teratosphaeria encephalarti* on *Encephalartos altensteinii* in the present study. As found by Braun & Crous (1992), conidia of this species failed to germinate on MEA or PDA, stressing its close hyperparasitic relationship with its ascomycetous host. It is possible, however, that *D. leptosphaeriicola* is not a true member of *Dactylaria*, but represents yet another undescribed genus resembling *Dactylaria* in morphology. To confirm this, however, DNA will have to be isolated from fresh collections,

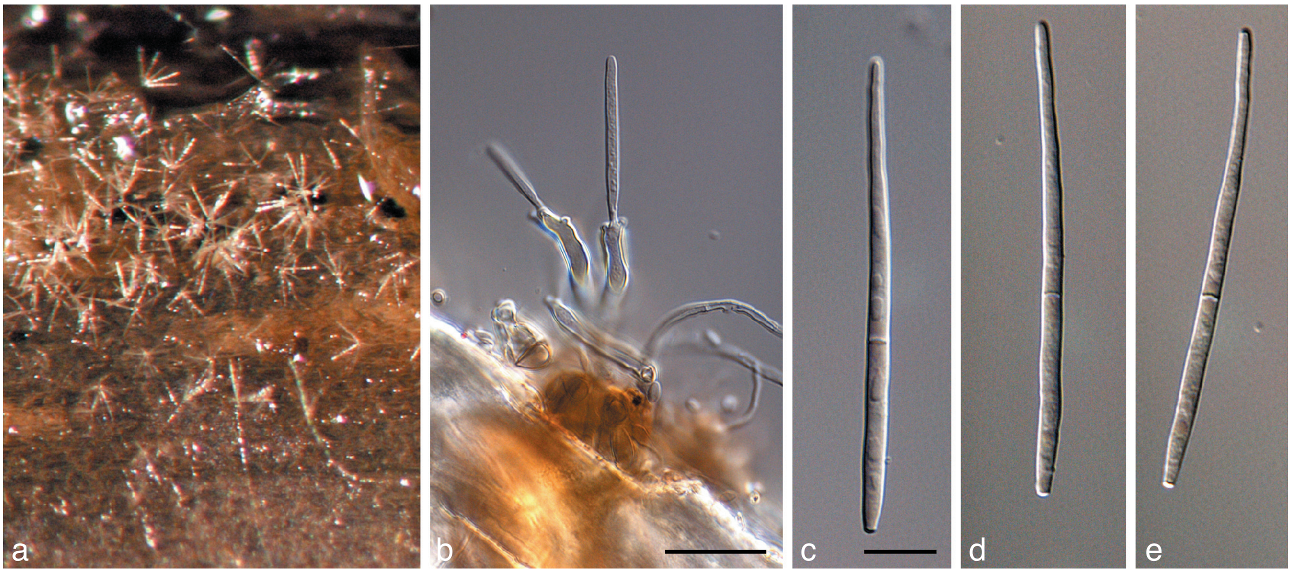

Fig. 7 *Dactylaria leptosphaeriicola* in vivo. a. Conidial fascicles on leaf; b. conidiogenous cells giving rise to conidia; c–e. conidia. — Scale bars = 10 µm.

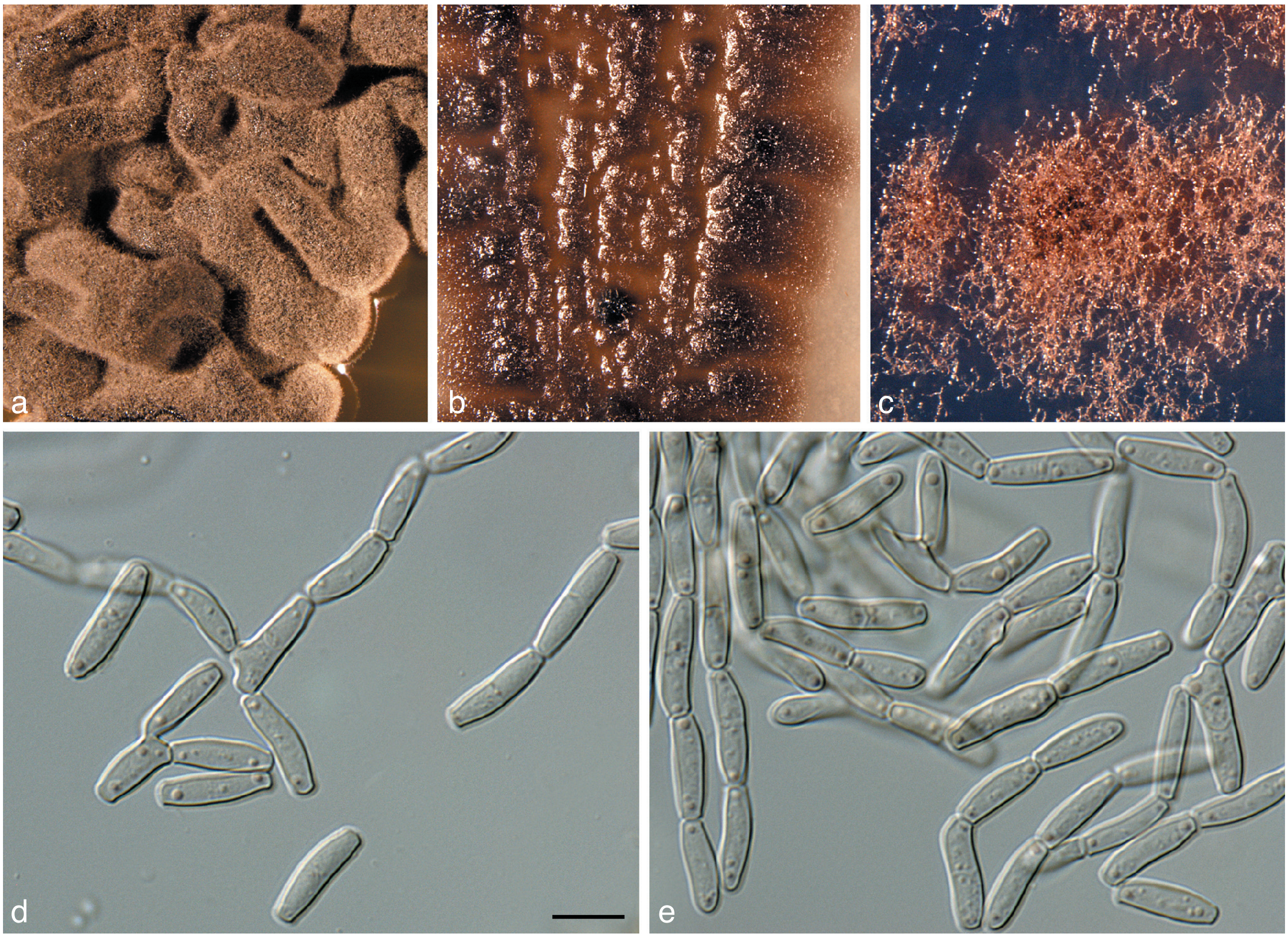

Fig. 8 *Cladophialophora proteae* in vitro (CPC 14902). a–c. Colony on MEA, OA and PDA, respectively; d, e. conidial chains. — Scale bar = 10 µm.

which would be difficult, as fascicles occur in conjunction with ascomata of other fungi, and attempts to cultivate the fungus have thus far proven to be unsuccessful.

Cladophialophora proteae was initially isolated from lesions of *Batcheloromyces proteae* on *Protea cynaroides*, to which it was assumed to be pathogenic, though no inoculation tests have ever been conducted to confirm this hypothesis (Swart et al. 1998). The status of *Cladophialophora* and *Pseudocladosporium* has been an issue of debate, and as *Cladophialophora* was used for taxa pathogenic to humans, Crous et al. (2004a) allocated the species isolated from *Protea* to *Pseudocladosporium*. However, as shown in a subsequent molecular study (Crous et al. 2007b), *Pseudocladosporium* is a synonym of *Fusicladium* (Venturiaceae) while species of *Cladophialophora* (Herpotrichiellaceae) were shown to occur on humans and plant hosts, and thus the name *Cladophialophora proteae* can be used for this fungus (Fig. 8). The fact that this species could also occur on dead leaf tissue of *Encephalartos altensteinii* (CPC 14902–14904) in the Western Cape Province is surprising, however, and again questions its possible ecological role and its potential wider host range.

The link of '*Trimmatostroma'* to '*Mycosphaerella'* was first reported on leaf spots of *Teratosphaeria maculiformis* from *Protea cynaroides* leaves collected in South Africa by Taylor & Crous (2000). After initial data suggesting that *Teratosphaeria* and *Mycosphaerella* represented a single genus (Taylor et al. 2003), a subsequent study demonstrated that these were in fact from two different families and that species of *Teratosphaeria* belonged to the Teratosphaeriaceae, in which the anamorph genus *Catenulostroma* was established for these trimmatostroma-like anamorphs (Crous et al. 2007a). Within *Catenulostroma* there is a species complex surrounding *C. abietis*, which based on DNA sequence data solely of the ITS gene region, is very difficult to distinguish. It is quite possible, therefore, that the *Encephalartos* isolates (CPC 14996), although phylogenetically similar to *Catenulostroma microsporum* (*Teratosphaeria microspora*), may very well still be shown to represent yet another cryptic species in this complex.

Africa is well known to have a high level of botanical diversity. As shown here after an initial cursory look at a few *Encephalartos* leaves, these plants were found to host numerous undescribed species of fungi. Given the high level of endemism found in African flora, it can be expected that an equally high number of these fungal species will be unique species. Unfortunately, indigenous African fungal biodiversity has never been regarded as a research priority and as such this research topic has never been well supported financially. Given the current importance placed on ecotourism and the preservation of unique African flora and fauna, it is clearly timely that more research focus and financial resources be channelled towards documenting, studying ecological roles and impacts, and conserving African mycoflora.

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