RESEARCH ARTICLE

Why everlastings don't last

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Abstract The Cape Floral Region represents one of the world's biodiversity hot spots, with a high level of plant, animal and insect endemism. The fungi occurring in this region, however, remain poorly studied. It is widely postulated that each plant species should harbour at least five to six unique fungal species, a number that we regard to be a huge underestimate. To test this hypothesis, we decided to study a single senescent flower of *Phaenocoma prolifera* ('everlasting'; *Asteraceae*) collected in South Africa, and posed the question as to how many different species of fungi could be isolated and cultivated from 10 leaf bracts. Using a damp chamber technique, numerous microfungi could be induced to sporulate, enabling most of them to be successfully isolated on artificial agar media. Isolates were subsequently subjected to DNA sequencing of the ITS and LSU nrDNA regions. During the course of this study 17 species could be cultivated and identified, of which 11 appeared to be new to science. These include *Catenulostroma hermanusense*, *Cladosporium phaenocomae*, *Devriesia tardicrescens*, *Exophiala capensis*, *Penidiella aggregata*, *P. ellipsoidea*, *Teratosphaeria karinae*, *Toxicocladosporium pseudoveloxum* spp. nov., and *Xenophacidiella pseudocatenata* gen. & sp. nov. Further studies are now required to determine if these fungi also occur as endophytes in healthy flowers. If this trend holds true for other plant hosts from southern Africa, it would suggest that there are many more fungi present in the Cape Floral Region than estimated in previous studies.

INTRODUCTION

Fynbos, which is essentially shrubland vegetation, is the most characteristic vegetation type of the Cape Floral Region, which is the world's richest and most diverse floristic region (Goldblatt 1997). The Cape Floral Region has an extremely high level of species richness and endemism, which has been attributed to either a combination of diverse habitats and steep ecological gradients, or an intermediate level of system stress in the Cape Floral Region (Davis et al. 1994, Goldblatt 1997). Approximately 68 % of the species, 20 % of the genera and six families are endemic to the region (Bond & Goldblatt 1984, Goldblatt 1997). The largest concentration (70 %) of southern Africa's Red Data Book plants, occurs in the Cape Town metropolitan area with 15.1 species per km^2 (Hilton-Taylor 1996).

Taylor (1977) drew attention to the decline of the Cape Floral Region, by reporting a close to 60 % reduction in size of the area due to agricultural development, industry, urbanisation, intrusion of alien invasive plants, deforestation and fragmentation. Retaining the biodiversity in the fynbos is economically important, as a number of species are used in the wildflower industry (Crous et al. 2004a), and for the production of thatching materials (Wessels et al. 1997), and ecotourism (Davis et al. 1994, Cowling et al. 1997).

One unique example of a fynbos species is the monotypic genus *Phaenocoma* (*Asteraceae*). *Phaenocoma* is based on *P. prolifera* (commonly referred to as Cape strawflower, Cape everlasting, or 'Rooi sewejaartjie' in Afrikaans), which is restricted to the Western Cape Province of South Africa. The name *Phaenocoma* refers to the shiny leaf bracts ('*phaino*': to shine, and '*coma*': hair) (Jackson 1990; www.sanbi.org).

These plants are common in the Cape Floristic Region, occurring on sandy soils on mountain slopes and in valleys, at altitudes ranging from sea level to 1 500 m, where they grow as shrubs that can become up to 1.2 m tall. Plants flower from September to January, forming terminal flower heads which contain up to 1 000 individual flowers with bright pink to red bracts. The latter eventually fade, becoming white with age (Jackson 1990, Koekemoer 2002).

During a recent collection trip to the Western Cape Province, several senescent, but still attached flowers of *P. prolifera* were collected on the mountain slopes of the Fernkloof Nature Reserve, Hermanus, which were dirty-white in colour, with blackened stems. The aim of this study was thus to determine which fungi were colonising these senescent flowers. A further aim was to determine if any of these fungi had previously been reported to colonise other hosts in the Cape Floral Region, as has been found for some species occurring on *Protea* (Crous et al. 2008a, Marincowitz et al. 2008a, b) and *Encephalartos* (Crous et al. 2008b). Finally, by choosing a single flower head from this location, and only looking at leaf bracts of this flower, we wanted to know if we would obtain the five to six unique fungal species postulated by Hawksworth (1991) to occur on each species of flowering plants.

MATERIALS AND METHODS

Isolates

Ten flower bracts from a single flower were selected for study. Flower bracts bearing ascomata were soaked in water for approximately 2 h, after which they were placed in the inner side of Petri dish lids, of plates containing 2 % malt extract agar (MEA; Crous et al. 2009c). Ascospore germination patterns were examined after 24 h, and single ascospore and conidial cultures established as described earlier (Crous et al. 1991, Crous 1998). Flower bracts were also incubated in moist chambers for up to 2 wk, and inspected daily for microfungi, and single conidial colonies of hyphomycetes and coelomycetes established on MEA (Crous 2002). Colonies were subcultured

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

Species	Strain no.[1]	Substrate	Country	Collector(s)	GenBank Accession number			
					ITS	LSU	TEF	ACT[2]
Batcheloromyces leucadendri	CPC 18277	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499832	JF499852	–	–
Catenulostroma hermanusense	CBS 128768 = CPC 18276	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499833	JF499853	–	–
Cladosporium cladosporioides	CPC 18230	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499834	JF499854	JF499872	JF499878
Cladosporium perangustum	CPC 18228	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499835	JF499855	JF499873	JF499879
	CPC 18229	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499836	JF499856	JF499874	JF499880
Cladosporium phaenocomae	CBS 128769 = CPC 18223	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499837	JF499857	JF499875	JF499881
	CPC 18221	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499838	JF499858	JF499876	JF499882
Cladosporium ramotenellum	CPC 18224	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499839	JF499859	JF499877	JF499883
Devriesia tardicrescens	CBS 128770 = CPC 18259	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499840	JF499860	–	–
Exophiala capensis	CBS 128771 = CPC 18473	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499841	JF499861	–	–
Penidiella aggregata	CBS 128772 = CPC 18278	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499842	JF499862	–	–
Penidiella ellipsoidea	CBS 128773 = CPC 18317	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499843	JF499863	–	–
Teratosphaeria cf. *bellula*	CPC 18280	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499844	JF499864	–	–
	CPC 18281	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499845	JF499865	–	–
Teratosphaeria karinae	CBS 128774 = CPC 18255	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499846	JF499866	–	–
Toxicocladosporium pseudoveloxum	CBS 128775 = CPC 18257	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499847	JF499867	–	–
	CBS 128777 = CPC 18471	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499848	JF499868	–	–
Xenophacidiella pseudocatenata	CPC 18274	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499849	JF499869	–	–
	CBS 128776 = CPC 18472	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499850	JF499870	–	–
	CPC 18279	leaf bracts of *Phaenocoma prolifera*	South Africa	*K.L. Crous & P.W. Crous*	JF499851	JF499871	–	–

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

onto synthetic nutrient-poor agar (SNA), potato-dextrose agar (PDA), oatmeal agar (OA), MEA (Crous et al. 2009c), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation.

DNA isolation, amplification and analyses

Genomic DNA was isolated from fungal mycelium grown on MEA, using the UltraCleanTM Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocols. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the internal transcribed spacer 1, the 5.8S rRNA gene, the internal transcribed spacer 2 (ITS) and the first 900 bases at the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White et al. 1990) and LSU1Fd (Crous et al. 2009b) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The PCR conditions, sequence alignment (using the online interface of MAFFT (mafft.cbrc.jp/alignment/server/index.html; Katoh et al. 2002), followed by manual correction by eye) and subsequent phylogenetic analysis (using PAUP v4.0b10; Swofford 2003) followed the methods of Crous et al. (2006a, 2009a). Partial actin (ACT) and translation elongation factor 1-alpha sequences were determined for *Cladosporium* spp. as described in Schubert et al. (2007) and Bensch et al. (2010). Sequences were compared with the sequences available in NCBIs GenBank nucleotide (nr) database using a megablast search and results are discussed in the relevant species notes where applicable. Alignment gaps were treated as new character states. Sequences derived in this study were lodged at GenBank, the alignment in TreeBASE (www.treebase.org), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous et al. 2004b).

Morphology

Microscopic preparations were made in clear lactic acid, with 30 measurements determined per structure, and observations made with a Nikon SMZ1500 dissecting microscope, and with a Zeiss Axioscope 2 microscope using differential interference contrast (DIC) illumination. Colony characters and pigment production were noted after 2 wk of growth on MEA, PDA, SNA and OA; Crous et al. 2009c) incubated at 25 °C. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970). Growth characteristics were studied on MEA plates incubated for 1–2 wk in the dark at 25 °C.

RESULTS

Isolations

During the present study a total of 50 taxa were isolated, which eventually were identified as representing 17 different species. A minimum of two single conidial or ascospore isolates was preserved of each isolated taxon in the CPC working collection of P.W. Crous, of which one isolate was subjected to DNA analysis. Reference strains are maintained in the CBS-KNAW Fungal Biodiversity Centre (CBS) Utrecht, The Netherlands. Isolates used for morphological and sequence analyses are presented in Table 1.

Phylogeny

Approximately 1 700 bases, spanning the ITS and LSU regions, were obtained from the sequenced cultures and approximately 450 and 230 bases for TEF and ACT, respectively. Three phylogenetic analyses were performed: 1) an analysis of the LSU region to determine the generic relationship of the obtained
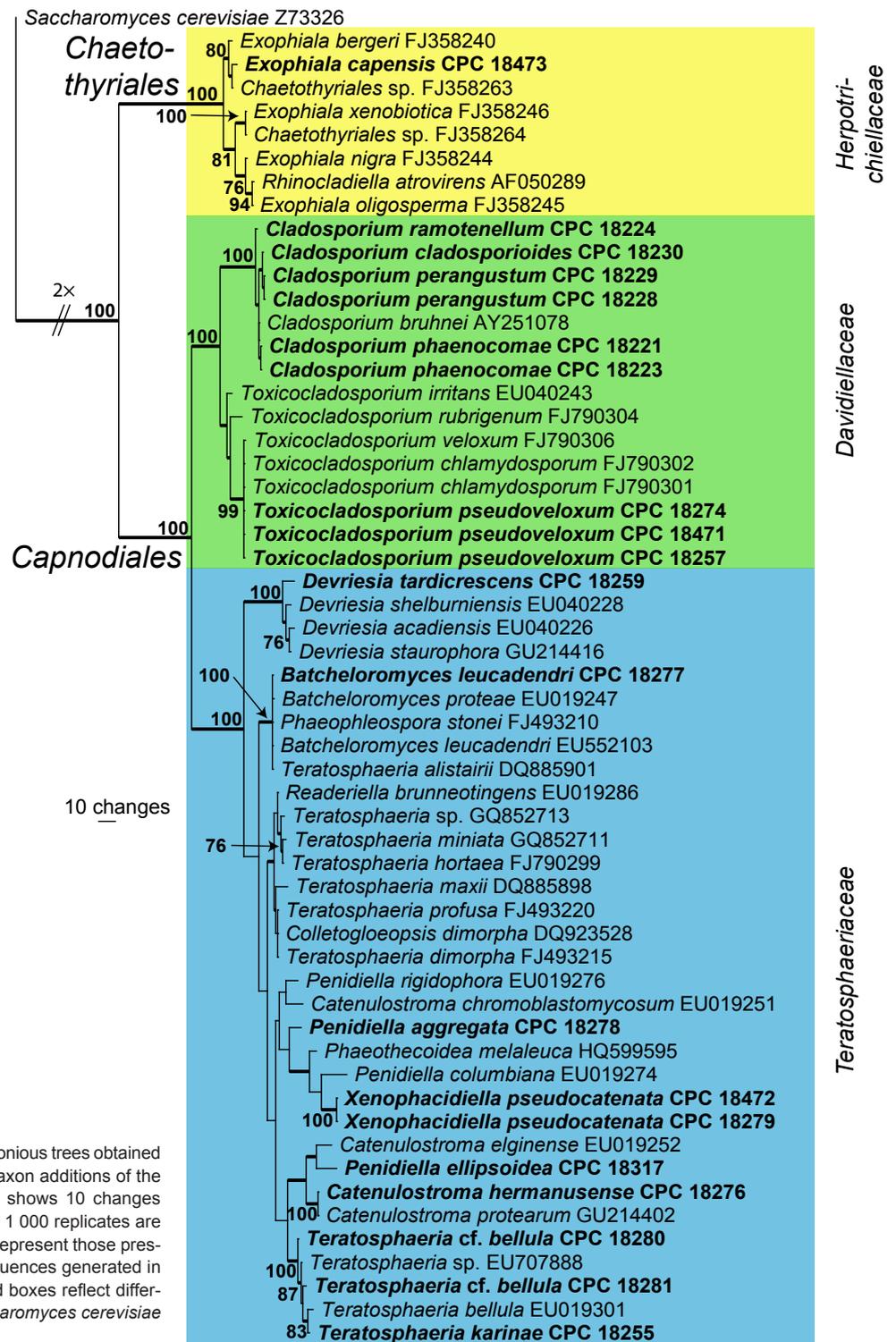
Fig. 1 The first of 153 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 10 changes and bootstrap support values > 75 % from 1 000 replicates are shown at the nodes. Thickened branches represent those present in the strict consensus tree. Novel sequences generated in this study are shown in **bold** and coloured boxes reflect different families. The tree was rooted to *Saccharomyces cerevisiae* (GenBank Z73326).

isolates; 2) an analysis of the ITS sequences clustering in the *Teratosphaeriaceae* to confirm species-level relationships; and 3) a combined ITS, ACT and TEF analysis focussed on the *Cladosporium* isolates.

The manually adjusted LSU alignment contained 57 taxa (including the *Saccharomyces cerevisiae* outgroup sequence, GenBank Z73326) and, of the 840 characters (including alignment gaps) used in the phylogenetic analysis, 209 were parsimony-informative, 63 were variable and parsimony-uninformative and 568 were constant. The first of 153 equally most parsimonious trees retained from the heuristic search is shown in Fig. 1 (TL = 607, CI = 0.636, RI = 0.912, RC = 0.580). The phylogenetic tree of the LSU region (Fig. 1) shows that the obtained sequences cluster in *Chaetothyriales* and *Capnodi-*

ales, with the latter mainly having associations with members of *Davidiellaceae* and *Teratosphaeriaceae*.

The manually adjusted ITS alignment contained 29 taxa (including the *Cladosporium cladosporioides* outgroup sequence, GenBank GU566222) and, of the 499 characters (including alignment gaps) used in the phylogenetic analysis, 167 were parsimony-informative, 56 were variable and parsimony-uninformative and 276 were constant. The first of four equally most parsimonious trees retained from the heuristic search is shown in Fig. 2 (TL = 596, CI = 0.596, RI = 0.757, RC = 0.451). Specific associations based on this tree are discussed, where applicable, under the species notes below.

The manually adjusted combined ITS/ACT/TEF alignment contained 25 taxa (including the *Cercospora beticola* outgroup
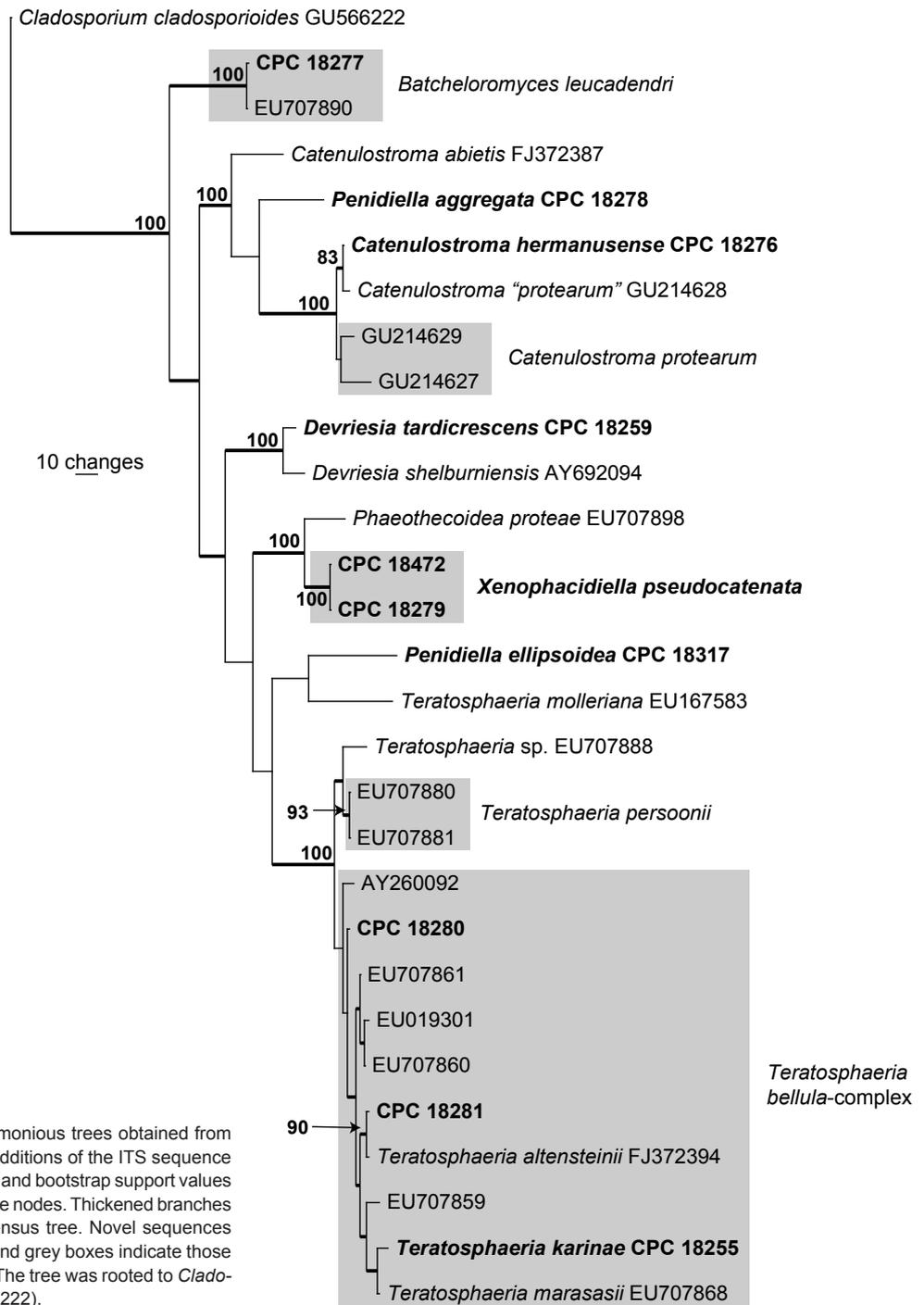
Fig. 2 The first of four equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the ITS sequence alignment. The scale bar shows 10 changes and bootstrap support values > 75 % from 1 000 replicates are shown at the nodes. Thickened branches represent those present in the strict consensus tree. Novel sequences generated in this study are shown in **bold** and grey boxes indicate those species with more than one strain present. The tree was rooted to *Cladosporium cladosporioides* (GenBank GU566222).

sequence, GenBank AY840527, AY840458, AY840494, respectively) and, of the 1 102 characters (including alignment gaps) used in the phylogenetic analysis, 230 were parsimony-informative, 212 were variable and parsimony-uninformative and 660 were constant. The first of six equally most parsimonious trees retained from the heuristic search is shown in Fig. 3 (TL = 596, CI = 0.596, RI = 0.757, RC = 0.451). Specific associations based on this tree are discussed, where applicable, under the species notes below.

Taxonomy

Seventeen fungal species were isolated from the 10 *Phaenocoma* leaf bracts studied. Known species included two species of *Penicillium* (not treated in the present manuscript), namely *P. crocicola* (= *P. thomii*) (DTO 132D6 = DTO 133G5) and a species recently described from fynbos soils, *P. ramulosum* (DTO

133G6 = DTO 133G7) (Visagie et al. 2009). Known species of *Cladosporium* that occurred on the leaf bracts include *C. cladosporioides* (CPC 18230), *C. perangustum* (CPC 18228, 18229), and *C. ramotenellum* (CPC 18224) (Fig. 3; discussed in Bensch et al. 2010). *Teratosphaeria bellula* (CPC 18280, 18281) and *Batcheloromyces leucadendri* (CPC 18277), were also isolated, as well as unknown, potentially undescribed species. These are treated and discussed below.

Catenulostroma hermanusense Crous, *sp. nov.* — MycoBank MB560017; Fig. 4

Catenulostromatis proteatum simile, sed conidiis minoribus, 10–25 × 5–10 µm.

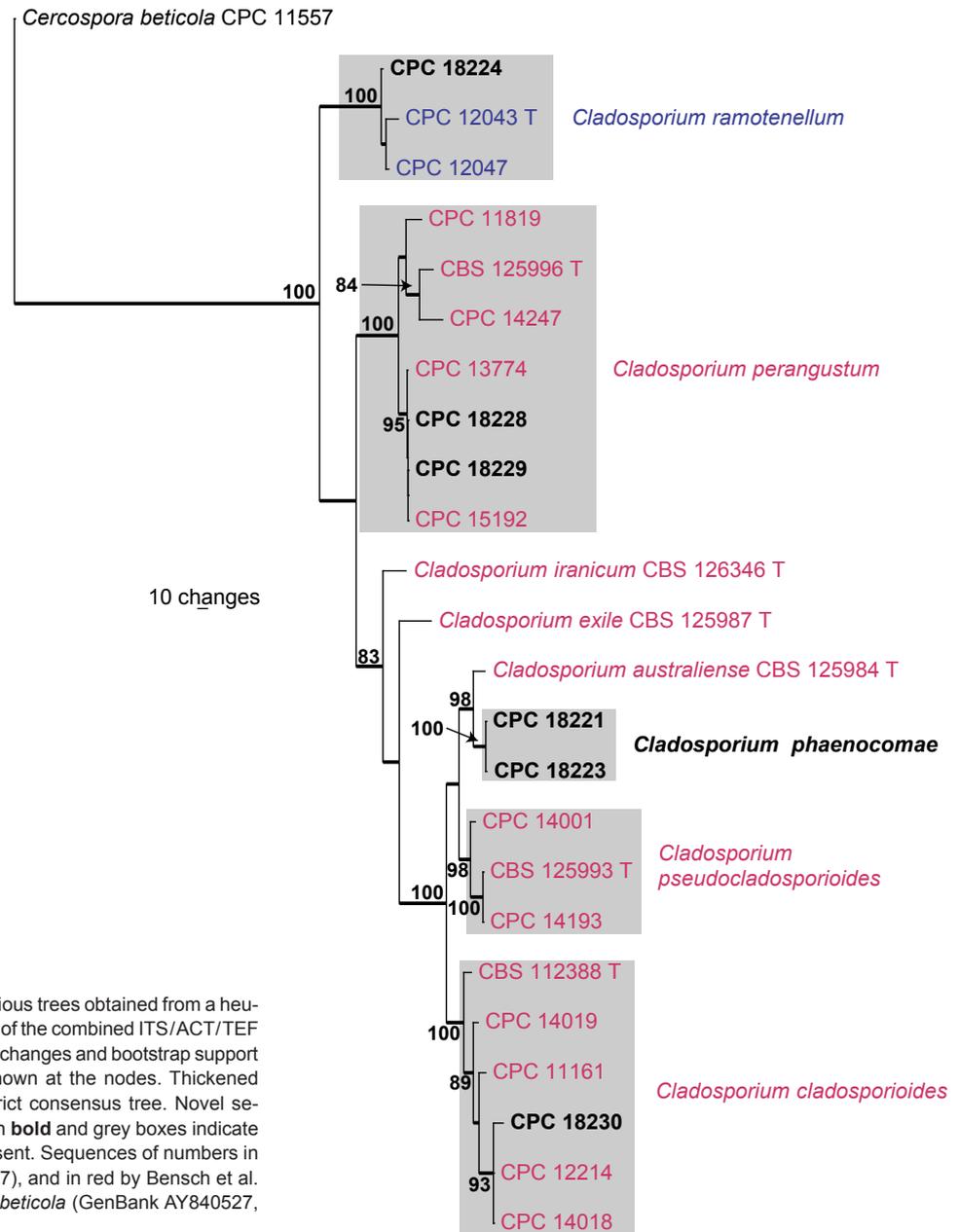*Etymology*. Named after the locality where it was collected, Hermanus, South Africa.

Fig. 3 The first of six equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined ITS/ACT/TEF sequence alignment. The scale bar shows 10 changes and bootstrap support values > 75 % from 1 000 replicates are shown at the nodes. Thickened branches represent those present in the strict consensus tree. Novel sequences generated in this study are shown in **bold** and grey boxes indicate those species with more than one strain present. Sequences of numbers in blue were generated by Schubert et al. (2007), and in red by Bensch et al. (2010). The tree was rooted to *Cercospora beticola* (GenBank AY840527, AY840458, AY840494, respectively).

Colonies sporulating on MEA. *Mycelium* consisting of branched, septate, verruculose to warty, medium to dark brown, 2–4 μm wide hyphae. *Conidiophores* reduced to conidiogenous cells integrated on hyphal ends. *Conidiogenous cells* subcylindrical, unbranched, medium brown, 10–18 × 3–4 μm, thick-walled, with 1(–3) terminal loci; scars inconspicuous, 2–3 μm wide. *Conidia* in simple or branched chains, subcylindrical to ellipsoid, straight to flexuous, (10–)15–20(–25) × 5–8(–10) μm, 0–3 transversely septate, or with 1–2 oblique septa, medium to dark brown, thick-walled, verruculose to warty; hila unthickened, 2–3 μm wide.

Culture characteristics — Colonies spreading, erumpent, with folded surface and sparse aerial mycelium and even, smooth, crenate margins. On PDA surface olivaceous-grey, margin submerged, iron-grey; reaching 15 mm diam after 2 wk. On MEA similar in colour, also reaching 15 mm diam after 2 wk.

Specimen examined. S OUTH A FRICA, Western Cape Province, Hermanus, Fernkloof Nature Reserve, S 34°23'38" E 19°16'9.7", on leaf bracts of *Phaenocoma prolifera*, 2 May 2010, *K.L. Crous & P.W. Crous*, CBS H-20528 holotype, cultures ex-type CPC 18276 = CBS 128768.

Notes — Phylogenetically (Fig. 2), *C. hermanusense* is closely related to *C. protearum* (Crous et al. 2009b), but is morpho-

logically distinct in that it has smaller conidia (10–25 × 5–10 μm) than *C. protearum* (12–45 × 7–25 μm; Crous et al. 2007a). Our ITS sequence of *C. hermanusense* differs with three nucleotides from an isolate from *Hakea* (GenBank GU214628), originally assumed to belong to *C. protearum*. However, based on the current data it is possible that that isolate either belongs to *C. hermanusense* or represents a cryptic species closely related to it rather than to *C. protearum*. More isolates should be collected from both hosts and be subjected to multilocus sequence typing to test this hypothesis.

Cladosporium phaenocomae Crous, *sp. nov.* — MycoBank MB560018; Fig. 5

Cladosporio australiensis phylogenetice simile, sed hyphis angustioribus, microconidiophoris formantibus et conidiis leniter verruculosis.

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

Mycelium immersed and superficial, abundant, 1–2.5 μm wide, septate, subhyaline to pale or medium olivaceous-brown, smooth to verruculose, at times forming hyphal ropes. *Macroconidiophores* macronematous, solitary, arising terminally and
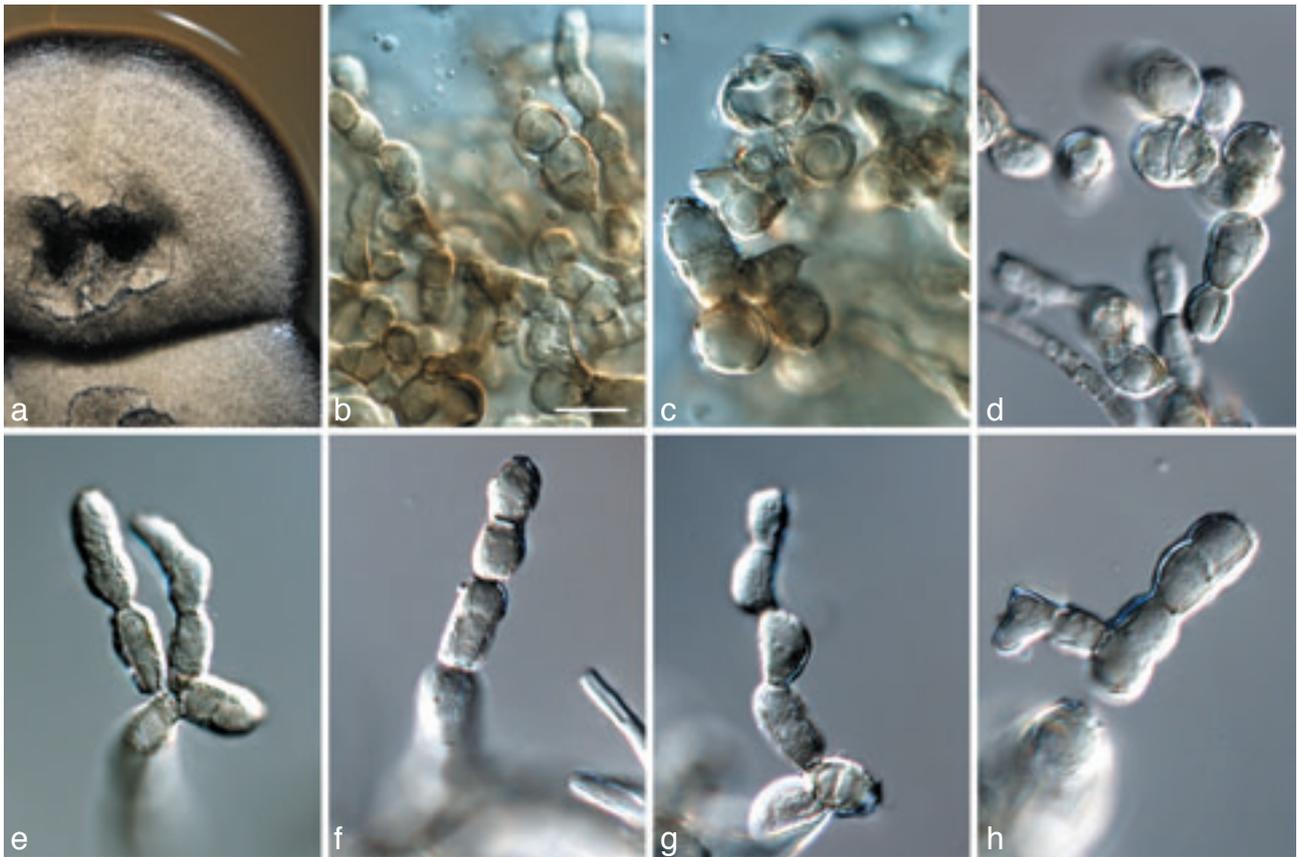
Fig. 4 *Catenulostroma hermanusense* (CPC 18276). a. Colony on MEA; b–h. a series of conidiophores with chains of disarticulating conidia. — Scale bar = 10 μm.
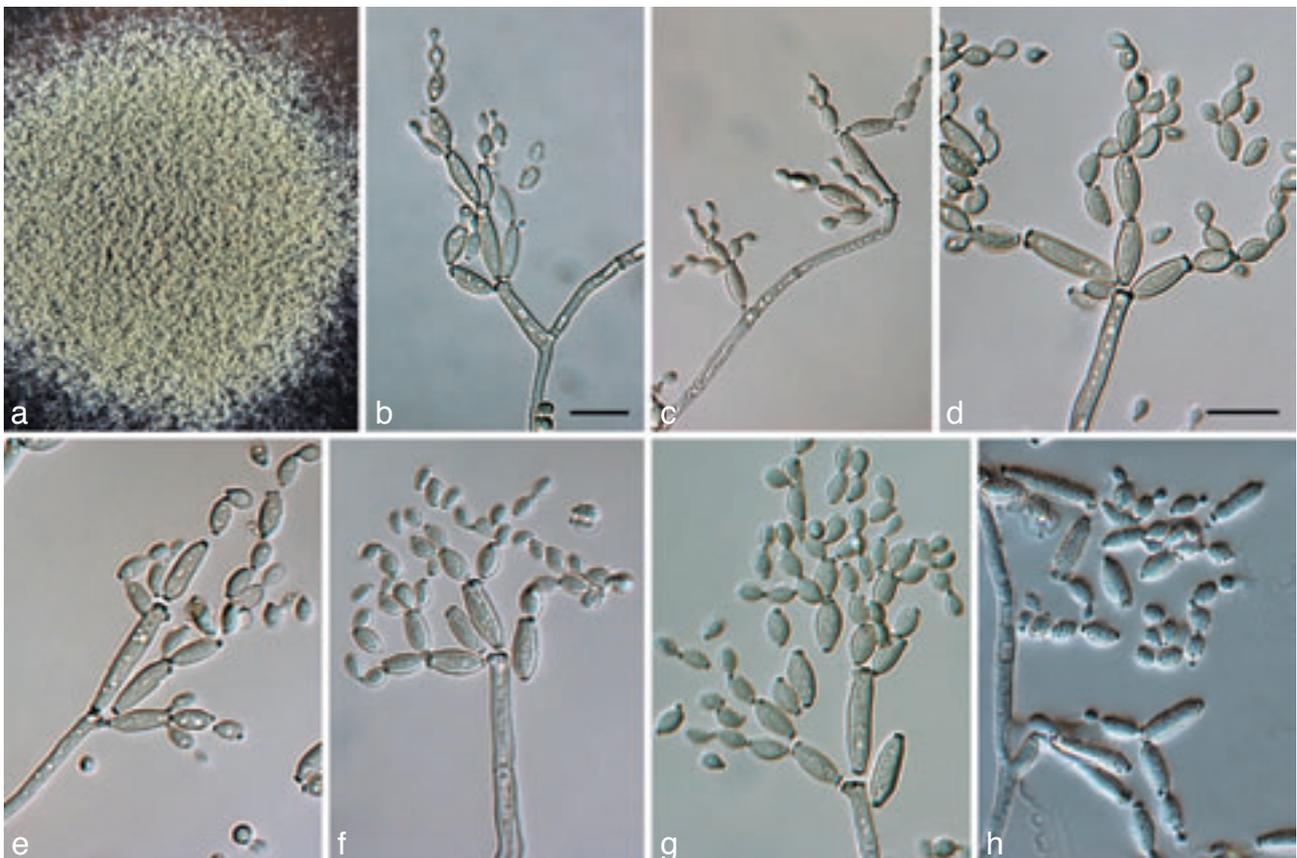

Fig. 5 *Cladosporium phaenocomae* (CPC 18223). a. Colony on MEA; b–h. a series of micro- and macroconidiophores showing conidia in chains. — Scale bars = 10 μm.

laterally from hyphae, erect, slightly flexuous, cylindrical-oblong, 60–100(–200) × 2.5–3 μm, neither geniculate nor nodulose, unbranched or branched below, 2–5-septate, not constricted at septa, pale to medium olivaceous-brown, smooth. *Microconidiophores* erect, intercalary, subcylindrical, smooth to finely verruculose, pale to medium brown, 0–1-septate, 5–20 × 2–3 μm. *Conidiogenous cells* integrated, terminal and intercalary, cylindrical-oblong, neither geniculate nor nodulose, 5–20(–25) × (2–)3(–3.5) μm, with 1–4(–6) loci at the apex or 1–3 loci in intercalary cells with loci situated mostly all at more or less the same level, conspicuous, subdenticulate, 1–1.5 μm diam, somewhat thickened and darkened-refractive. *Ramoconidia* occasionally formed, subcylindrical, 0(–1)-septate, 17–20(–28) × (2–)3(–4) μm. Secondary ramoconidia fusoid-ellipsoid, aseptate, (5–)10–15(–20) × (3–)3.5(–4) μm. *Conidia* pale to olivaceous-brown, finely verruculose, catenate, in branched chains, branching in all directions, up to 2–4 conidia in the terminal unbranched part of the chain; *intercalary conidia* ovoid to ellipsoid, aseptate, 4–5(–10) × (2.5–)3(–3.5) μm, with 1–3 distal hila, somewhat thickened, darkened-refractive, 1–1.5 μm diam; *small terminal conidia* globose, subglobose to obovoid, (3–)4(–5) × 2–3 μm, aseptate, rounded at the apex; microcyclic conidiogenesis not observed.

Culture characteristics — Colonies after 1 wk at 25 °C in the dark, with sparse aerial mycelium and smooth, even margins, reaching 7 cm diam; on OA greenish olivaceous; on MEA dull green (surface and reverse); on PDA grey-olivaceous (surface), and olivaceous-grey in reverse; sporulating profusely on all media.

Specimen examined. SOUTH AFRICA, Western Cape Province, Hermanus, Fernkloof Nature Reserve, S 34°23'38" E 19°16'9.7", on leaf bracts of *Phaenocoma prolifera*, 2 May 2010, *K.L. Crous & P.W. Crous*, CBS H-20529 holotype, cultures ex-type CPC 18221, 18223 = CBS 128769.

Notes — Phylogenetically (Fig. 3), *C. phaenocomae* is closely allied to *C. australiense* (described from *Eucalyptus* in Australia; Bench et al. 2010), but can be distinguished by its narrower hyphae, conidia that are slightly roughened, and the presence of microconidiophores.

Devriesia tardicrescens Crous, *sp. nov.* — MycoBank MB560019; Fig. 6

Devriesiae staurosporae similis, sed in cultura tarde crescent et conidiis longioribus.

Etymology. Named after its slow growth rate in culture.

Colonies sporulating on OA. *Mycelium* consisting of branched, septate, pale brown, smooth, 1.5–2 μm wide hyphae. *Conidiophores* solitary, erect on creeping hyphae, unbranched or branched, medium brown, smooth, flexuous, 30–200 × 1.5–2.5 μm, 2–11-septate. *Conidiogenous cells* terminal or lateral, medium brown, subcylindrical, smooth, 15–25 × 1.5–2 μm; proliferating sympodially, scars flattened, thickened, somewhat darkened, 1–1.5 μm wide. *Conidia* medium brown, smooth, aseptate, subcylindrical to narrowly fusoid-ellipsoidal, apical conidium with obtuse apex, additional conidia with truncate ends, somewhat darkened hila, 0.5–1 μm wide; conidia straight, mostly in branched chains. Ramoconidia with 1–3 apical loci, truncate, subdenticulate, 15–25 × 1.5–2.5 μm. Secondary ramo-
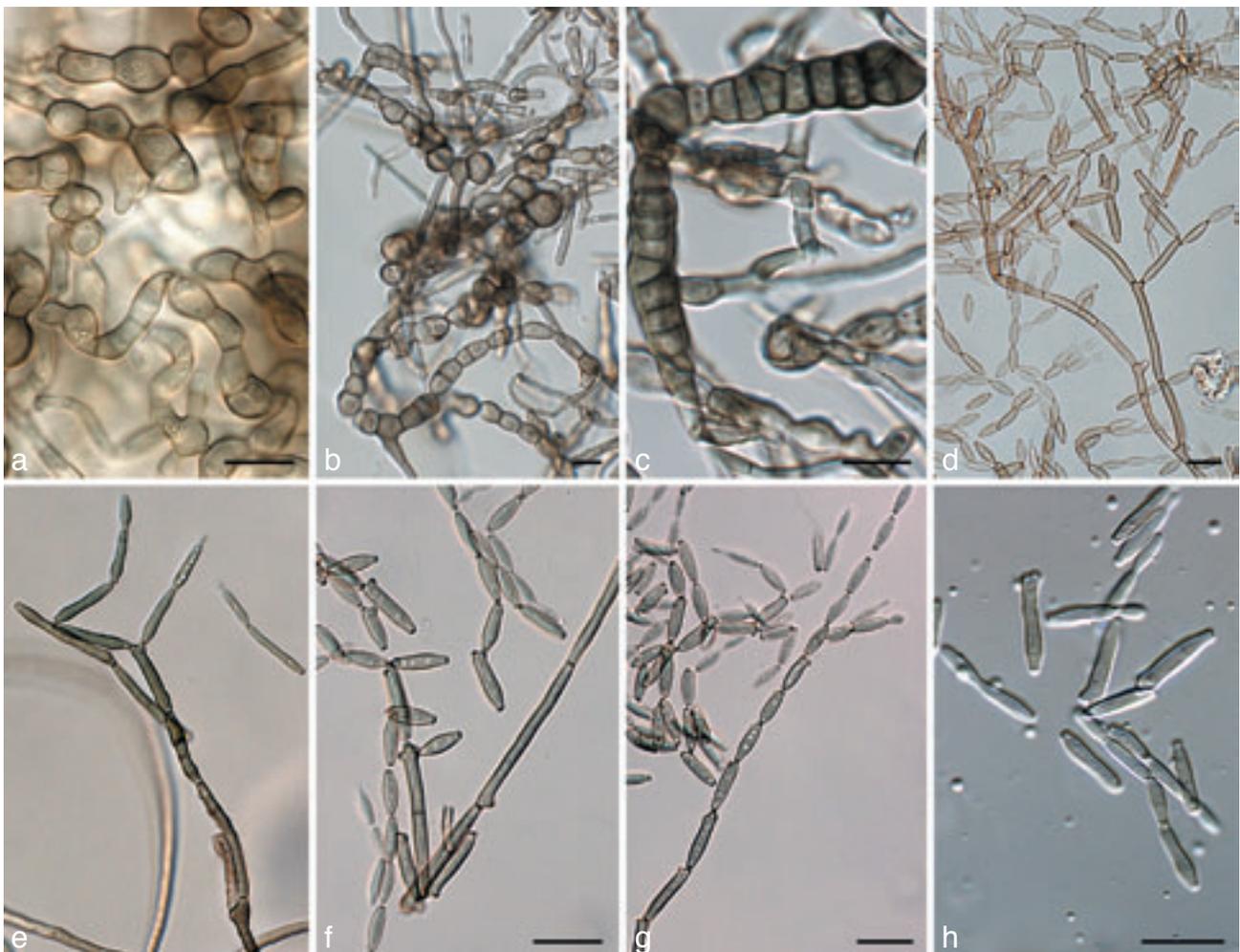

Fig. 6 *Devriesia tardicrescens* (CPC 18259). a–c. Chlamydospore-like structures formed in culture; d–g. conidiophores with conidia in chains; h. conidia. — Scale bars = 10 μm.

conidia with 1–2 apical loci, 7–14 × 1.5–2.5 μm. Intercalary and terminal conidia aseptate, (5–)6–7(–8) × (1.5–)2(–2.5) μm. *Chlamydospores* dark-brown, smooth to verruculose, ellipsoid, 0–1-septate, 10–20 × 7–10 μm; forming additional septa with age, becoming irregular, microsclerotial, up to 25 μm diam.

Culture characteristics — Colonies erumpent, spreading, uneven, with sparse to moderate aerial mycelium, with folded surface and smooth, even, crenate margin. On PDA surface olivaceous-grey, reverse iron-grey; reaching 7 mm diam after 2 wk. On OA surface olivaceous-grey with iron-grey outer margin; reaching 7 mm diam after 2 wk. On MEA surface olivaceous-grey with submerged iron-grey margins, and iron-grey underneath; reaching 10 mm diam after 2 wk.

Specimen examined. SOUTH AFRICA, Western Cape Province, Hermanus, Fernkloof Nature Reserve, S 34°23'38" E 19°16'9.7", on leaf bracts of *Phaenocoma prolifera*, 2 May 2010, *K.L. Crous & P.W. Crous*, CBS H-20530 holotype, cultures ex-type CPC 18259 = CBS 128770.

Notes — Although *D. tardicrescens* is phylogenetically related to *D. staurophora* (Fig. 1) and *D. shelburniensis* (Fig. 1, 2), it has a slower growth rate (10 mm vs > 20–24 mm after 2 wk), and different conidial dimensions than the latter two species (Seifert et al. 2004).

Exophiala capensis Crous, *sp. nov.* — MycoBank MB560020; Fig. 7

Synanamorph: *Cladophialophora* sp.

Exophialae bergeri morphologice similis, sed conidiis majoribus, (2–)3–5(–6) × (2–)3–3.5(–4) μm.

Etymology. Named after the Cape Province, where this fungus was collected.

Mycelium consisting of branched, septate, pale brown, smooth, 2–3 μm wide hyphae. *Conidiophores* dimorphic. *Cladophialophora* state with erect, solitary conidiophores, up to 80 μm tall. *Conidiogenous cells* intercalary and terminal, subcylindrical, 8–12 × 2–3 μm, proliferating sympodially. *Conidia* pale brown, smooth, aseptate to 1-septate, occurring in branched or unbranched chains, ellipsoid to subcylindrical, (7–)8–10(–13) × 2–3(–4) μm, tapering towards truncate ends, 1 μm wide, not thickened nor darkened. *Exophiala* state. *Conidiogenous cells* inconspicuous on hyphae, phialidic, lateral or terminal, subcylindrical to ellipsoid, pale brown, smooth, 5–7 × 2–3 μm; phialidic opening inconspicuous, up to 1 μm wide, with small collarette, up to 0.5 μm tall. *Conidia* pale brown, smooth, ellipsoid to globose, (2–)3–5(–6) × (2–)3–3.5(–4) μm; conidia frequently giving rise to secondary conidia via microcyclic conidiation.

Culture characteristics — Colonies spreading, slightly erumpent, with sparse aerial mycelium and smooth, lobate to regular margins. On MEA surface folded, brown-vinaceous, also in reverse; on potato-dextrose agar irregular with sparse aerial mycelium and even, smooth, crenate margins. On PDA iron-grey in the middle, olivaceous-grey in outer region, iron-grey in reverse. On OA iron-grey, slimy; colonies on all media reaching 7 mm diam after 2 wk.

Specimen examined. SOUTH AFRICA, Western Cape Province, Hermanus, Fernkloof Nature Reserve, S 34°23'38" E 19°16'9.7", on leaf bracts of *Phaenocoma prolifera*, 2 May 2010, *K.L. Crous & P.W. Crous*, CBS H-20531 holotype, cultures ex-type CPC 18473 = CBS 128771.

Notes — *Exophiala capensis* is phylogenetically (Fig. 1) distant to *Exophiala bergeri*, and together they appear to represent a different lineage in the *Chaetothyriales*. For the present, however, we prefer to describe it as a novel species of *Exophiala* with a *Cladophialophora*-like synanamorph.
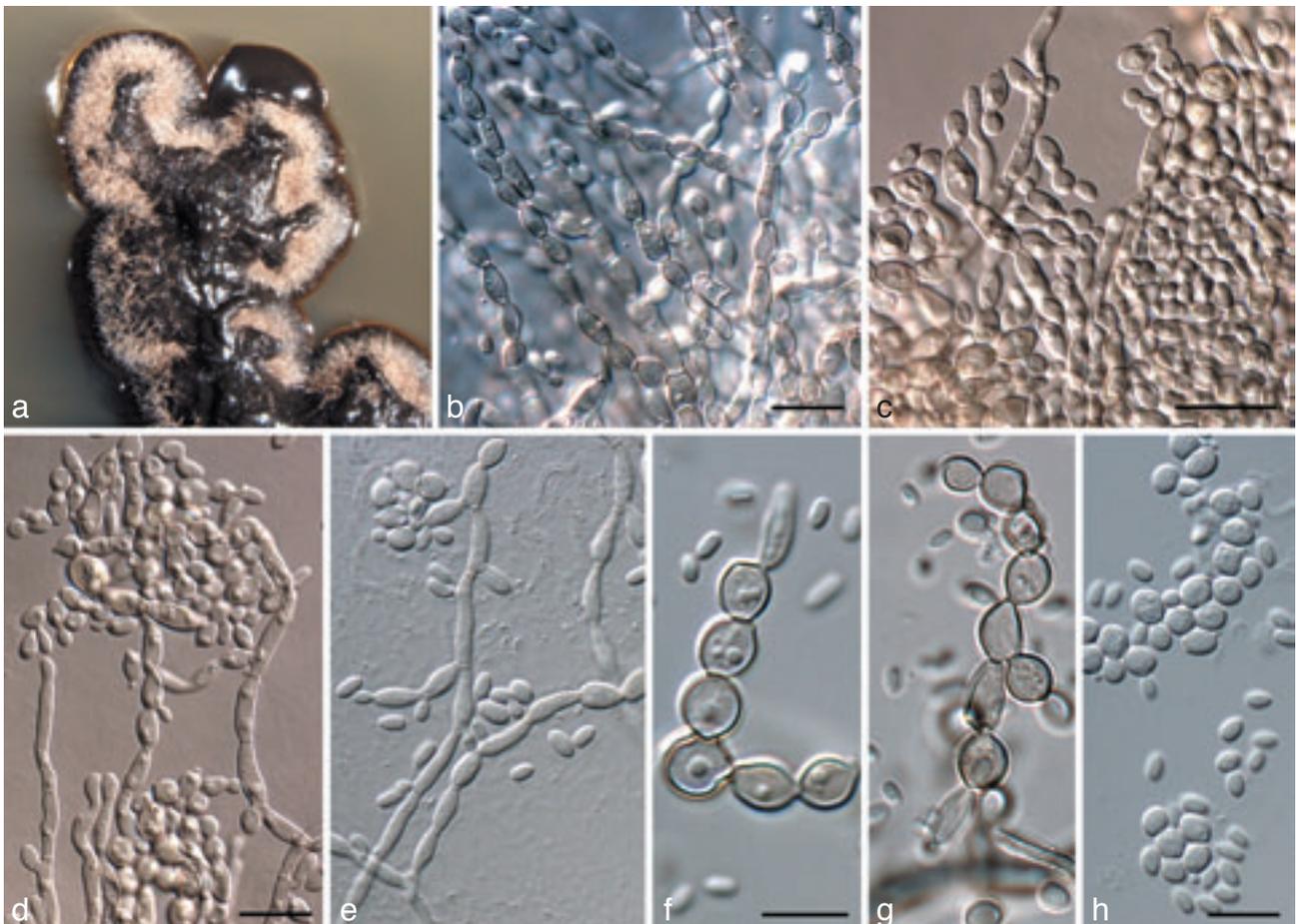

Fig. 7 *Exophiala capensis* (CPC 18473). a. Colony on MEA; b, c. *Cladophialophora*-like state; d–h. *Exophiala* conidiogenous cells, hyphae and conidia. — Scale bars = 10 μm.

Penidiella aggregata Crous, *sp. nov.* — MycoBank MB560022; Fig. 8

Penidiellae rigidophorae morphologice similis, sed conidiis minoribus, (5–)6–8 × (2–)2.5(–3) μm.

Etymology. Named after the densely aggregated scars on the conidiogenous cells.

Colonies sporulating on OA. *Mycelium* consisting of branched, septate, smooth, pale brown, 2–3 μm wide hyphae. *Conidiophores* solitary, arising from superficial mycelium, erect, brown, smooth, up to 60 μm tall, 3–4 μm wide at base, 3–5-septate, straight to irregularly geniculate-sinuous. *Conidiogenous cells* terminal, subcylindrical, unbranched, medium brown, 7–20 × 3–3.5 μm, smooth, tapering to a flattened or rounded apical region, scars unthickened, aggregated, somewhat darkened, not refractive, 0.5–1 μm wide. *Ramoconidia* 0–1-septate, medium brown, smooth, ellipsoidal to obclavate or obovoid, with 1–3 apical hila, 8–15 × 3–4 μm. Intermediate and terminal *conidia* subcylindrical to ellipsoid, 0(–1)-septate, brown, in chains of up to 6, (5–)6–8 × (2–)2.5(–3) μm; hila truncate, unthickened, somewhat darkened, 0.5–1 μm wide.

Culture characteristics — Colonies spreading, erumpent, with sparse aerial mycelium and even, smooth margins. On PDA surface and reverse iron-grey; reaching 12 mm diam after 2 wk. On OA surface iron-grey; reaching 8 mm diam after 2 wk. On MEA surface folded, iron-grey on surface and reverse; reaching 12 mm diam after 2 wk.

Specimen examined. SOUTH AFRICA, Western Cape Province, Hermanus, Fernkloof Nature Reserve, S 34°23'38" E 19°16'9.7", on leaf bracts of *Phaenocoma prolifera*, 2 May 2010, *K.L. Crous & P.W. Crous*, CBS H-20532 holotype, cultures ex-type CPC 18278 = CBS 128772.

Notes — *Penidiella aggregata* is morphologically characterised by having apically aggregated, flattened conidial scars on its conidiogenous cells. It is similar to *P. rigidophora* in its conidial branching patterns (Crous et al. 2007a), but distinct in that conidia are smaller than in *P. rigidophora* (intercalary and terminal conidia 7–12 × 3–5 μm). Phylogenetically it is related to species of *Penidiella* and *Catenulostroma* (Fig. 1, 2).

Penidiella ellipsoidea Crous, *sp. nov.* — MycoBank MB560021; Fig. 9

Penidiellae rigidophorae morphologice similis, sed conidiis majoribus, (14–)20–30(–70) × 4–5(–5.5) μm, 0–7-septatis, saepe in catenis haud ramosis.

Etymology. Named after its typically ellipsoid conidia.

Colonies sporulating on OA. *Mycelium* consisting of branched, septate, verruculose, medium brown, 3–4 μm wide hyphae. *Conidiophores* solitary, arising from superficial mycelium, erect, brown, verruculose, up to 90 μm tall, (3–)5–6 μm wide at base, up to 12-septate. *Conidiogenous cells* terminal, intercalary or lateral, unbranched, brown, 6–15 × 3.5–6 μm, finely verruculose, subcylindrical to somewhat doliiform, tapering to a flattened apical region, scars unthickened, not darkened, nor refractive, 1 μm wide. *Conidia* medium brown, smooth, subcylindrical to ellipsoid, 0–4(–7)-septate, in mostly unbranched chains of up to 10, (14–)20–30(–70) × 4–5(–5.5) μm; hila truncate, unthickened, not darkened, 1–1.5 μm wide.
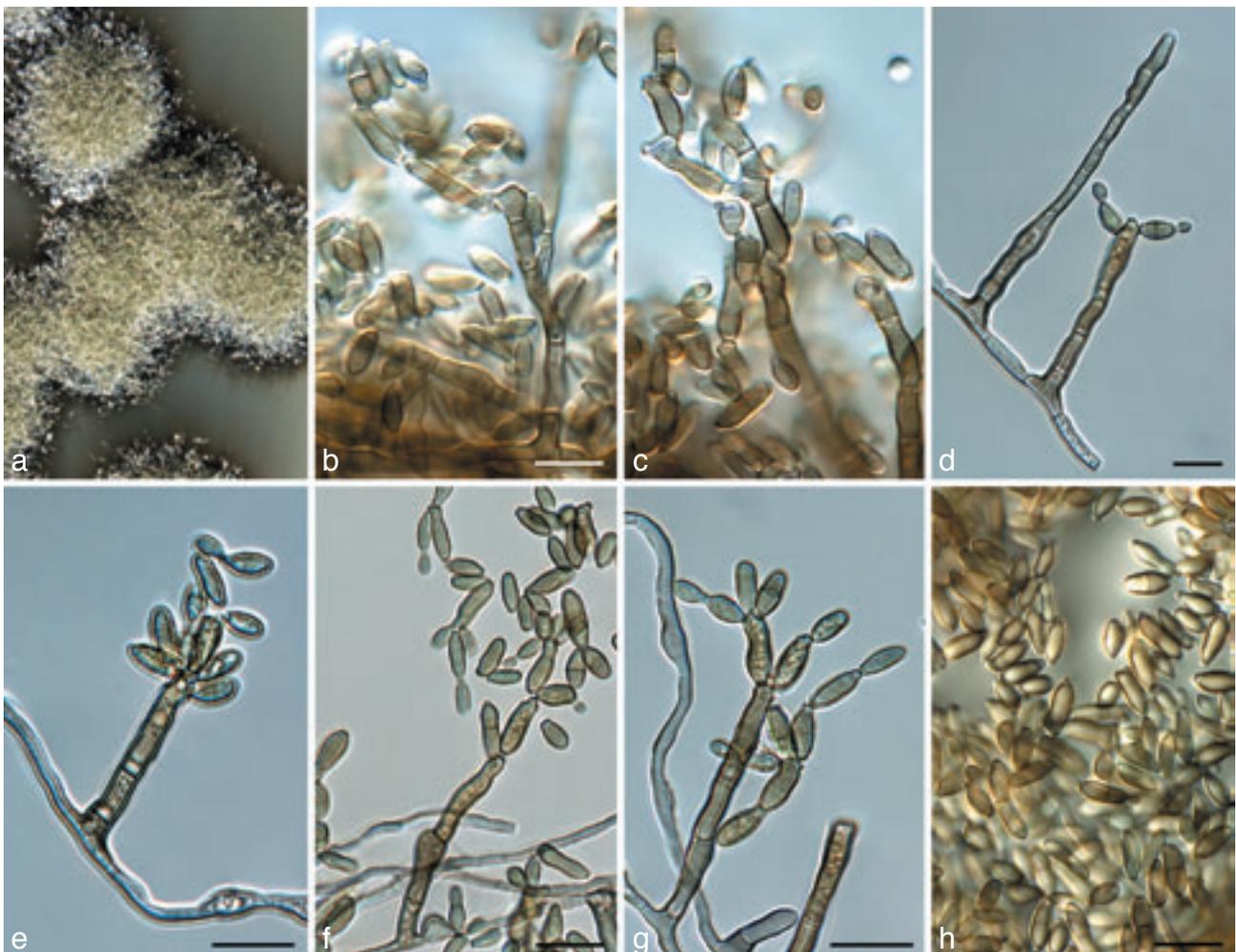

Fig. 8 *Penidiella aggregata* (CPC 18278). a. Colony on MEA; b–g. conidiophores with aggregated conidiogenous loci, and short conidial chains; h. conidia. — Scale bars = 10 μm.

Culture characteristics — Colonies spreading, erumpent with sparse aerial mycelium, and smooth, even, crenate margins. On PDA surface folded, iron-grey on surface and reverse; reaching 10 mm diam after 2 wk. On OA surface iron-grey, reaching 12 mm diam. On MEA surface folded, iron-grey on surface and reverse; reaching 10 mm diam after 2 wk.

Specimen examined. South Africa, Western Cape Province, Hermanus, Fernkloof Nature Reserve, S 34°23'38" E 19°16'9.7", on leaf bracts of *Phaenocoma prolifera*, 2 May 2010, *K.L. Crous & P.W. Crous*, CBS H-20533 holotype, cultures ex-type CPC 18318, 18317 = CBS 128773.

Notes — *Penidiella ellipsoidea* is morphologically similar to *P. rigidophora* (Crous et al. 2007a), but distinct in that conidia mostly occur in unbranched chains, are larger in size and have more septa than conidia of *P. rigidophora* (ramoconidia 10–25 × 3–5 µm, 1–3-septate, intercalary and terminal conidia 7–12 × 3–5 µm). Phylogenetically, it is more related to *Catenulostroma* spp. and *Teratosphaeria bellula* (Fig. 1, 2).
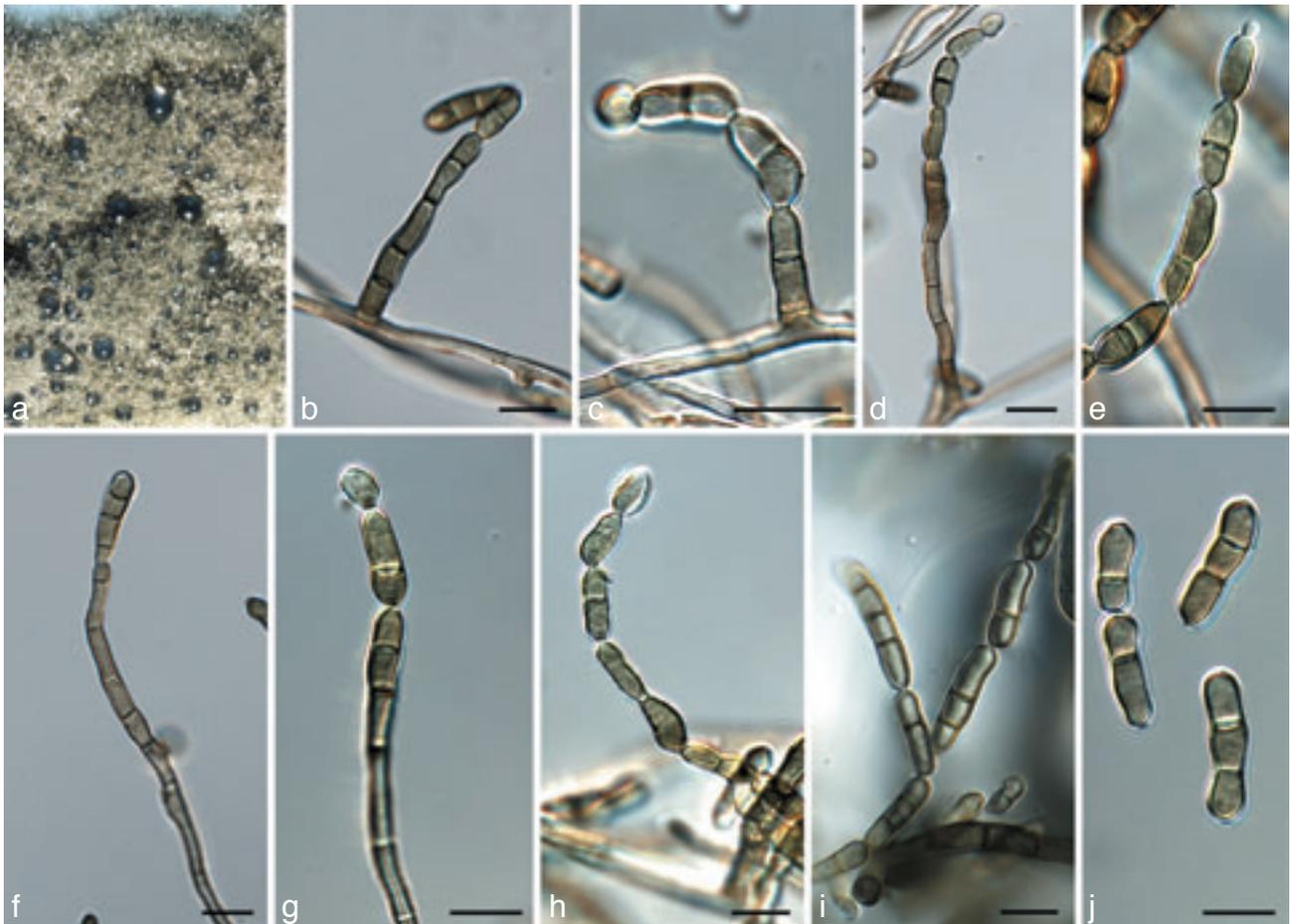

Fig. 9 *Penidiella ellipsoidea* (CPC 18317). a. Colony on PDA; b–i. conidiophores with conidiogenous cells and conidial chains; j. conidia. — Scale bars = 10 µm.

Fig. 10 *Teratosphaeria* cf. *bellula* (CPC 18281). a. Colony on MEA; b, c. asci with ascospores; d. ascospores; e. ascospores germinating on MEA after 24 h of incubation. — Scale bar = 10 µm.

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

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

Descriptions — Crous & Wingfield (1993), Taylor & Crous (1998), Crous et al. (2004a, 2008a).

Culture characteristics — Colonies spreading, erumpent, with sparse to moderate aerial mycelium. On PDA surface folded, olivaceous-grey with thin, submerged, iron-grey margin, reverse iron-grey; reaching 8 mm diam after 2 wk. On OA surface folded, olivaceous-grey; reaching 8 mm diam after 2 wk. On MEA surface folded, olivaceous-grey, with thin, submerged, iron-grey margin, reverse iron-grey; reaching 10 mm diam after 2 wk.

Specimen examined. SOUTH AFRICA, Western Cape Province, Hermanus, Fernkloof Nature Reserve, S 34°23'38" E 19°16'9.7", on leaf bracts of *Phaenocoma prolifera*, 2 May 2010, *K.L. Crous & P.W. Crous*, CPC 18280, 18281.

Notes — Crous et al. (2008a) designated an epitype for *T. bellula*, but also revealed this taxon to represent a species complex occurring on several different hosts, characterised by small ascospores with bluntly rounded ends, surrounded by a mucoid sheath. Furthermore, ascospores germinate at right angles to the long axis, darken, and become roughened upon germination. The present isolates from *Phaenocoma* represent at least two species within this complex (Fig. 1, 2), distinguished morphologically only by lacking a characteristic mucoid sheath. This complex is poorly understood, and hence we have chosen to not name these isolates in the present study, as more gene loci need to be sequenced to resolve their species boundaries.

Teratosphaeria karinae Crous, *sp. nov.* — MycoBank MB560023; Fig. 11

Teratosphaeriae bellulae similis, sed ascosporis subtiliter guttulatis, sine vagina mucoide, haud fuscatis in statu germinanti et tubis germinationis parallelis ad axem sporae.

Etymology. Named after Karina Louise Crous, who collected the specimen of *Phaenocoma prolifera* that formed the basis of this study.

Fig. 11 *Teratosphaeria karinae* (CPC 18255). a, b. Flowers of *Phaenocoma prolifera*; c. colony on MEA; d, e. hyphal network in leaf bracts with ascomata; f–h. asci with ascospores; i, j. ascospores. — Scale bars = 10 μm.

Ascomata black, immersed to erumpent, up to 70 µm diam; wall consisting of 2–3 layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, obovoid, straight to slightly curved, 8-spored, 35–45 × 8–10 µm. *Ascospores* tri- to multiseriate, overlapping, hyaline, finely guttulate, thin-walled, straight, fusoid-ellipsoidal with obtuse ends, widest in the middle of apical cell, not to somewhat constricted at septum, tapering towards both ends, but more prominently towards lower end, (8–)9–10(–11) × (2.5–)3 µm; germinating ascospores on MEA remain hyaline, and germinate from polar ends, with germ tubes parallel to the long axis of the spore.

Culture characteristics — Colonies spreading, erumpent, with moderate aerial mycelium and even, smooth, crenate margins. On PDA surface grey-olivaceous, reverse iron-grey; reaching 13 mm diam after 2 wk. On OA olivaceous-grey, reaching 12 mm after 2 wk. On MEA olivaceous-grey, with a thin, submerged, iron-grey margin, iron-grey underneath; reaching 11 mm diam after 2 wk.

Specimen examined. SOUTH AFRICA, Western Cape Province, Hermanus, Fernkloof Nature Reserve, S 34°23'38" E 19°16'9.7", on leaf bracts of *Phaenocoma prolifera*, 2 May 2010, *K.L. Crous & P.W. Crous*, CBS H-20534 holotype, cultures ex-type CPC 18256, 18255 = CBS 128774.

Notes — The ascospore dimensions of *T. karinae* are similar to that of *T. bellula*, which also occurs on this material. However, ascospores of *T. karinae* are finely guttulate, lack a mucoid sheath (Crous et al. 2008a), and do not darken at germination, with germ tubes being parallel, not 90° to the long axis of the spore, as observed in *T. bellula*. The *T. bellula* complex contains many unresolved cryptic species, but more genes would have to be sequenced to completely resolve their species boundaries (Fig. 1, 2).

Toxicocladosporium pseudoveloxum Crous, *sp. nov.* — MycoBank MB560024; Fig. 12

Toxicocladosporio veloxo simile, sed ramoconidiis brevioribus, 8–15 × 2.5–4 µm.

Etymology. Named after its morphological similarity to *T. veloxum*.

Mycelium on SNA consisting of branched, septate, pale brown, smooth, 1.5–2 µm wide hyphae. *Conidiophores* solitary, macronematous, subcylindrical, straight to geniculous-sinuous, or irregularly curved, unbranched or branched above, 2–5-septate, dark brown, finely verruculose, walls thick, septa dark-brown, 20–50 × 3–4 µm. *Conidiogenous cells* integrated, terminal or lateral, subcylindrical with slight taper towards apex, 10–15 × 3–4 µm; proliferating sympodially with 1–3 apical loci, 1–1.5 µm wide, thickened, darkened and refractive. *Conidia* catenate in branched chains, medium to dark brown, thick-walled, with dark, thick septa, smooth; ramoconidia 0–1-septate, broadly ellipsoid to subcylindrical, 8–15 × 2.5–4 µm; intermediate and terminal conidia ellipsoid, pale to medium brown, aseptate, (6–)7–10(–11) × (2–)2.5(–3) µm; hila protruding, 0.5–1.5 µm wide, thickened, darkened and refractive.

Culture characteristics — Colonies spreading with moderate aerial mycelium and even, smooth margins. On PDA reaching 20 mm diam after 2 wk, olivaceous-grey on surface and reverse. On OA surface olivaceous-grey, reaching 20 mm diam. On MEA surface olivaceous-grey, somewhat folded; reverse iron-grey, reaching 25 mm diam.
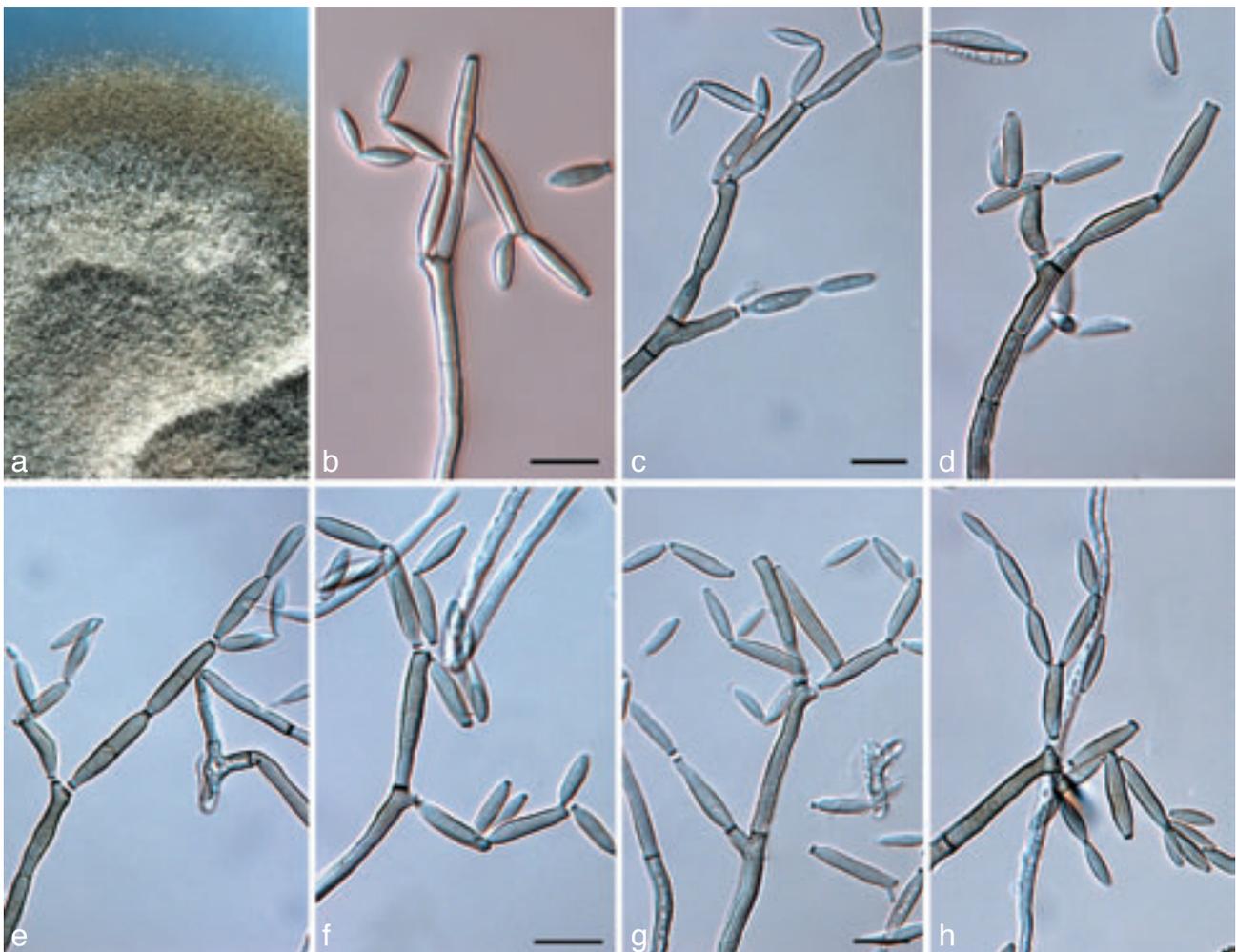

Fig. 12 *Toxicocladosporium pseudoveloxum* (CPC 18257). a. Colony on PDA; b–h. conidiophores with conidiogenous cells and conidial chains. — Scale bars = 10 µm.

Specimen examined. South Africa, Western Cape Province, Hermanus, Fernkloof Nature Reserve, S 34°23'38" E 19°16'9.7", on leaf bracts of *Phaenocoma prolifera*, 2 May 2010, *K.L. Crous & P.W. Crous*, CBS H-20535 holotype, cultures ex-type CPC 18257 = CBS 128775, 18274, 18275, 18471 = CBS 128777.

Notes — Phylogenetically (Fig. 1) and morphologically *T. pseudoveloxum* is similar to *T. veloxum* and other *Toxicocladosporium* spp. (Crous et al. 2009d), but differs in that it has shorter ramoconidia (8–15 × 2.5–4 μm), than *T. veloxum* (15–18 × 2.5–4 μm). Blast searches using the ITS sequence revealed high identity to *T. protearum* (GenBank HQ599586; Identities = 647/653 (99 %), Gaps = 3/653 (0 %)), *T. veloxum* (GenBank FJ790288; Identities = 607/613 (99 %), Gaps = 4/613 (0 %)), *T. chlamydosporum* (GenBank FJ790284; Identities = 604/615 (99 %), Gaps = 6/615 (0 %)) and *T. banksiae* (GenBank HQ599598; Identities = 648/663 (98 %), Gaps = 7/663 (1 %)).

Xenophacidiella Crous, *gen. nov.* — MycoBank MB560056

Phacidiellae morphologice similis, sed conidiomatibus pycnidialibus, conidiis pigmentis, haud hyalinis, binis in catenis falsis, disarticulantibus.

Type species. Xenophacidiella pseudocatenata Crous.

Etymology. Not *Phacidiella*, which it resembles morphologically.

Mycelium consisting of branched, septate, brown, verruculose, 1.5–2 μm wide hyphae. *Conidiomata* eustromatic, pycnidial, multilocular, with several ostioles, erumpent, grey-brown; wall of 2–3 layers of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity of conidioma, embedded in mucoid layer, subcylindrical, hyaline, smooth, proliferating inconspicuously percurrently at truncate apex. *Conidia* medium brown, verruculose, subcylindrical, apex obtusely rounded, base truncate, thin-walled, aseptate, straight to slightly curved, at times occurring in chains of two.

Xenophacidiella pseudocatenata Crous, *sp. nov.* — MycoBank MB560025; Fig. 13

Conidiomata eustromatica, pycnidiales, multiloculares, usque ad 120 μm diam, pariete ex 2–3 stratis texturae angularis. Cellulae conidiogenae subcylindraceae, hyalinae, leves, 4–7 × 2.5–3.5 μm, ad apicem inconspicue percurrente proliferantes. Conidia modice brunnea, verruculosa, subcylindrica, apice obtuse rotundato, basi truncate, tenuitunicata, aseptata, interdum bina in catenis falsis, disarticulantibus, (4–)5–7(–8) × 2(–2.5) μm.

Etymology. Named after the conidia that become septate, separating into two cells, creating the impression of false chains.

Mycelium consisting of branched, septate, brown, verruculose, 1.5–2 μm wide hyphae. *Conidiomata* eustromatic, pycnidial, multilocular, with several ostioles, erumpent, grey-brown on OA, up to 120 μm diam; wall of 2–3 layers of *textura angularis*.

Conidiophores reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity of conidioma, embedded in mucoid layer, subcylindrical, hyaline, smooth, 4–7 × 2.5–3.5 μm, proliferating inconspicuously percurrently at truncate apex. *Conidia* medium brown, verruculose, subcylindrical, apex obtusely rounded, base truncate, thin-walled, aseptate, straight to slightly curved, at times occurring in chains of two, (4–)5–7(–8) × 2(–2.5) μm.

Culture characteristics — Colonies spreading, erumpent, irregular with sparse aerial mycelium and even, smooth, crenate margins. On PDA with folded surface, olivaceous-grey; reverse iron-grey, reaching 8 mm diam after 2 wk. On OA olivaceous-grey, not folded, smooth, reaching 10 mm. On MEA surface folded, olivaceous-grey; reverse iron-grey, reaching 8 mm diam.

Specimen examined. South Africa, Western Cape Province, Hermanus, Fernkloof Nature Reserve, S 34°23'38" E 19°16'9.7", on leaf bracts of *Phaenocoma prolifera*, 2 May 2010, *K.L. Crous & P.W. Crous*, CBS H-20536 holotype, cultures ex-type CPC 18472 = CBS 128776, CPC 18279.

Notes — The genus *Xenophacidiella* resembles *Phacidiella* in having disarticulating chains of conidia. *Phacidiella* is distinct, however, in having acervular conidiomata, and hyaline, smooth, aseptate, subcylindrical conidia (Sutton 1980). The recently described *P. eucalypti* (Crous et al. 2007b), which probably represents yet another genus in this complex, is also phylogenetically distinct from *X. pseudocatenata*, being associated with *Ostropales* whereas *Xenophacidiella* is associated with *Capnodiales.* Based on the LSU and ITS phylogenies (Fig. 1, 2), the closest sister taxa are *Phaeothecoidea protea* and *Penidiella* spp.

DISCUSSION

Knowing the number of species that exist on earth is fundamental to understanding and protecting the world's biodiversity, and thus estimating the number of fungal species has been discussed for a great number of years (Fries 1825, Bisby & Ainsworth 1943, Pirozynski 1972, Pascoe 1990, Hawksworth 1991, 1998, 2001, 2004, Dreyfuss & Chapela 1994, Rossman 1994, Hyde et al. 1997, Fröhlich & Hyde 1999, Crous et al. 2006b). In spite of this, the number that is commonly used to argue for fungal biodiversity is the 1.5 M estimate by Hawksworth (1991), though the fungal biodiversity in the Southern Hemisphere seems to greatly exceed this estimate (Crous et al. 2006b, Marincowitz et al. 2008a, b). Furthermore, recent 454 pyrosequencing DNA-based techniques like those employed by Buée et al. (2009) showed an unexpected high level of novel fungal biodiversity in forest soils, suggesting that former specimen-based estimates were far too conservative.
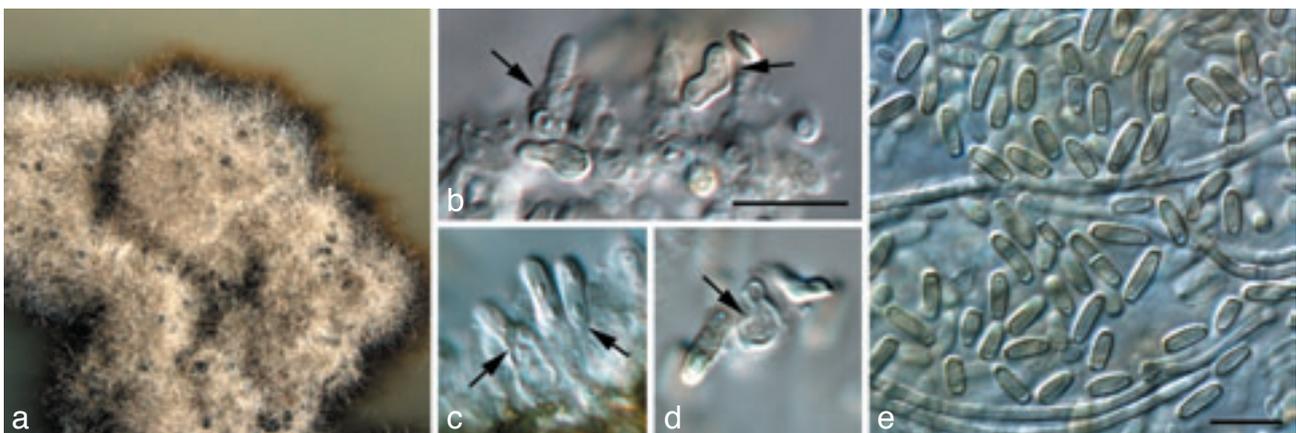

Fig. 13 *Xenophacidiella pseudocatenata* (CPC 18472). a. Colony on MEA with conidiomata exuding black spore masses; b–d. conidiogenous cells (arrows) giving rise to conidia; e. brown, verruculose, cylindrical conidia that disarticulate at the median septum. — Scale bars = 10 μm.
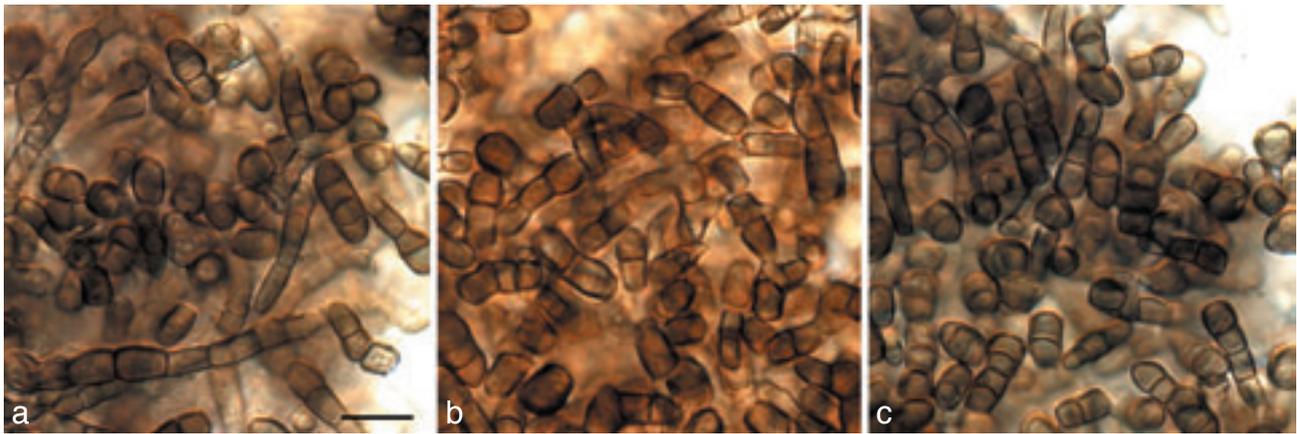
Fig. 14 *Batcheloromyces leucadendri* (CPC 18277). a–c. Colony sporulating in vitro on OA, showing disarticulating conidial chains. — Scale bar = 10 μm.

Without taking species occurring in soil and on insects (Suh et al. 2005) into account, Crous et al. (2006b) estimated that at least 200 000 unique fungal species should occur in southern Africa. In Australia, however, Pascoe (1990) estimated that there could be at least ten times as many fungi as vascular plants. Researchers working in specific niches, tended to have much higher estimates, namely 1 M on tropical plants (Smith & Waller 1992), 1.3 M endophytic fungi (Dreyfuss & Chapela 1994), or a ratio of 33 : 1 fungi per plant species for palm fungi (Fröhlich & Hyde 1999). Hyde et al. (1997) also reported that 75 % of all fungi collected on palms were new to science, followed by Marincowitz et al. (2008b) who reported 43 % of the taxa collected from *Proteaceae* leaf and twig litter to be undescribed, while Crous et al. (2009d) described eight unique species from a single leaf spot of a eucalypt tree growing in Madagascar.

The fungal biodiversity in South Africa has been poorly studied to date, and no species have thus far been described from *Phaenocoma prolifera*. Using the same damp chamber technique as employed here, Crous et al. (1996) described four unique hyphomycetes from *Podocarpus elongatus*, and five from *Syzygium cordatum* (Crous et al. 1995), while later studies added at least eight more species from this host (Sutton & Crous 1997, Pavlic et al. 2004, 2009), with several more host-specific fungi awaiting description.

In spite of the new species described in this study, several other taxa were also isolated that have known, wider host ranges. These include *Teratosphaeria bellula* (ex-type strain of species: CBS 111700; cryptic species isolated here CPC 18280, 18281) and *Batcheloromyces leucadendri* (ex-type strain of species: CBS 111577; isolated here CPC 18277; Fig. 14), both representing well-known pathogens of *Proteaceae* (Crous et al. 2004a, 2008a), which may be moving among different substrates in search of their ideal hosts (e.g. pogostick hypothesis; Crous & Groenewald 2005). Presumed saprobic species included two species of *Penicillium*, namely *P. crocicola* (= *P. thomii*) (DTO 132D6 = DTO 133G5) and *P. ramulosum* (DTO 133G6 = DTO 133G7) (Visagie et al. 2009), and three species of *Cladosporium*, namely *C. cladosporioides* (CPC 18230), *C. perangustum* (CPC 18228, 18229), and *C. ramotenellum* (CPC 18224) (Bensch et al. 2010).

The present study revealed 17 cultivatable species of microfungi to occur on 10 leaf bracts of *Phaenocoma prolifera*, of which nine of the 11 observed novelties could be named. For several taxa only a few isolates were recovered, meaning that the full variation present in populations of these species is not known at present. Furthermore, based on the isolation technique employed here, mostly hyphal ascomycetes (forming ascomata, or sporulating hyphomycetes or coelomycetes) were recovered, leaving out many other fungi that are certainly also present. Undoubtedly, if novel sequencing techniques such as 454 pyrosequencing technology were to be employed, a large portion of uncultivatable and largely unseen fungi would also be detected, which would greatly increase this number. In contrast to the statement of Hawksworth (1991) that each plant species can be expected to have 5–6 novel species of fungi, using these novel techniques one should be able to refine the question as to *how many novel species could be expected per different plant part*. Recent work by Batzer and colleagues dealing with flyspeck and sooty blotch of apples, for instance, have shown the epiphytes on apple fruit surfaces to be different from fungi occurring on leaves and branches of this host (Batzer et al. 2008, Yang et al. 2010).

Based on this initial look at microfungi present in a single flower of one plant species in southern Africa, as well as the observations from southern Africa discussed in Crous et al. (2006b), it seems that the world estimate of 1.5 M (based on 5–6 novel fungal species) is too conservative. However, as this was a senescent flower, and the host specificity of most of the taxa treated remains unknown, it is premature to draw definitive conclusions about species numbers based on these data, as the everlasting flowers could simply act as catch crops for fungi with wider host ranges. This is certainly true for the majority of other taxa discussed here, of which we only suspect members of *Penidiella*, *Teratosphaeria* and *Xenophacidiella* to be host specific based on currently published data. Further in-depth studies involving more hosts, different plant parts, and different growth stages are now required, to see if this trend also holds true for other plant species from the Cape Floral Region.

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