Why everlasting don’t last

P.W. Crous¹, J.Z. Groenewald¹

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 rDNA 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 Catenuolostruma hermanusense, Cladosporium phaenocomae, Devriesia tardicrescens, Exophiala capensis, Penidiella aggregata, P. ellipsoidea, Teratosphaeria curvata, Toxocladosporium pseudooveloxum 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 fungal 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 km² (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 (Vessels 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, Marinowitz 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...
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 NCBI’s 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

<table>
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<tr>
<th>Collection details and GenBank accession numbers of isolates for which novel sequences were generated in this study.</th>
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<tr>
<td><strong>Species</strong></td>
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<tr>
<td>Batcheloromyces leucadendri</td>
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<tr>
<td>Catenulostroma hermanusense</td>
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<td>Cladosporium cladosporioides</td>
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<td>Cladosporium ramotenellum</td>
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<td>Cladosporium ramosissimum</td>
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<td>Exophiala capensis</td>
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<td>Exophiala pseudocatenata</td>
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<td>Teratosphaeria bellula</td>
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<td>Teratosphaeria karinae</td>
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<td>Xenophacidiella pseudocatenata</td>
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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 *Chaeotothyriales* and *Capnodiales*, with the latter mainly having associations with members of *Davidelliaceae* 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. crociola* (= *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.

**Cladosporium cladosporioides** GU566222

*Batcheloromyces leucadendri*

*Catenulostromatis protearum* GU214629

*Catenulostroma cladosporioides* CPC 18277

*Penidiella aggregata* CPC 18278

*Devresia tardicrescens* CPC 18259

*Teratosphaeria bellula* complex

*Phaeothecoidea protearum* EU707898

*Xenophaidiella pseudocatenata* CPC 18472

*Penidiella ellipsoidea* CPC 18317

*Teratosphaeria molleriana* EU167583

*Teratosphaeria sp.* EU707888

*Teratosphaeria persoonii* EU707880

*Teratosphaeria altensteinii* FJ372394

*Teratosphaeria marasasi* EU707868

*Catenulostroma hermanusense* CPC 18276

*Catenulostroma protearum* GU214627

*Devresia shelburniensis* AY692094

*Cladosporium cladosporioides* CPC 18187

*Cladosporium cladosporoides* GU566222

*Cladosporium cladosporioides* CPC 181472

Etymology. Named after the locality where it was collected, Hermanus, South Africa.
Colonies sporing 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 subcyllindrical, 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.


Notes — Phylogenetically (Fig. 2), C. hermanusense is closely related to C. protearum (Crous et al. 2009b), but is morphologically 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 phaenocoma Crous, sp. nov. — MycoBank MB560018; Fig. 5

Cladosporium australiensis phylogenetice simile, sed hyphis angustioribus, microconidiophoris fomantibus et conidiis lenter 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. Microconiophores 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-ellipsoidal, 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 ellipsoidal, 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.


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 microconiophores.

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

Devriesia stauropora similis, sed in cultura tarde crescent et conidia 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-
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, 7–14 × 1.5–2.5 µm. Intercalary and terminal conidia aseptate, (5–)6–7(–8) × (1.5–)2(–2.5) µm.

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.

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

*Synanamorph: Cladophialophora* sp.

*Exophialae bergeri* morphologicae similis, sed conidis majoribus, (2–3–5(–6) × (2–3)–3.5(–4) µm.

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

**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** 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.

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**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* morphologic similis, sed conidis 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 ellipsoidal, 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.


**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* morphologic similis, sed conidis 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 ellipsoidal, 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.

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**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.


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 *Catenulostrum* 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


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.


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](image-url) **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.


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

*Toxicocladosporium veloxum* similis, 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, macroconidematous, subcylindrical, straight to geniculate-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 ellipsoidal to subcylindrical, 8–15 × 2.5–4 µm; intermediate and terminal conidia ellipsoidal, 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.

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

Phacidiellaceae morphologi similis, sed conidiomata pycnidialibus, conidios pigmentis, haud hyalinos, 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, multicellular, 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. **Conidia** medium brown, verruculose, cylindrical conidia that disarticulate at the median septum. — Scale bars = 10 µm.

**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 *Penidiella protea* (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 *Pheotheccidea 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, Pirzynski 1972, Pascoe 1990, Hawksworth 1991, 1998, 2001, 2004, Dreyfuss & Chapelaa 1994, Rossman 1994, Hyde et al. 1997, Fröhlich & Hyde 1999, Crous et al. 2006b). In this number, 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.
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 & Chapel 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 leucaderi (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 Penicillum, namely P. crocicola (= P. thornii) (DTO 132D6 = DTO 133G5) and P. ramulosum (DTO 133G6 = DTO 133G7) (Visage et al. 2009), and three species of Cladosporium, namely C. cladosporioides complex (CPC 18230), C. perangustum (CPC 18228, 18229), and C. ramosidentum (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 Peniella, Teratosphaeria and Xenophachiella 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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REFERENCES


