Why everlasting don’t last
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Key words
Batcheloromyces
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Toxicocladosporium
Xenophacidiella

Abstract The Cape Floral Region represents one of the world’s biodiversity hot spots, with a high level of plant, animal and insect endemicity. 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 phaeoconae, Devriesia tardicrescens, Exophiala capensis, Penidella 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.

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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 endemicity, 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 (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 bract. The latter eventually fade, becoming white with age (Jackson 1990, Koekoemoor 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...
## Species Strain no.1 Substrate Country Collector(s) GenBank Accession number

| Species                      | Strain | Collection number | Substrate | Country | Collector(s) | GenBank Accession number |
|------------------------------|--------|-------------------|-----------|---------|--------------|--------------------------|
| Catenulostroma hermanusense  | CPC 18276 | = CBS 128768     | leaf bracts of | South Africa | K.L. Crous & P.W. Crous | JF499834 JF499854 JF499872 JF499878 |
| Cladosporium cladosporioides  | CPC 18230 | leaf bracts of | South Africa | K.L. Crous & P.W. Crous | JF499835 JF499855 JF499873 JF499879 |
| Cladosporium perangustum     | CPC 18277 | leaf bracts of | South Africa | South Africa | K.L. Crous & P.W. Crous | JF499836 JF499856 JF499874 JF499880 |
| Cladosporium phaenocomae     | CPC 18278 | leaf bracts of | South Africa | South Africa | K.L. Crous & P.W. Crous | JF499837 JF499857 JF499875 JF499881 |
| Cladosporium ramotenellum     | CPC 18279 | leaf bracts of | South Africa | South Africa | K.L. Crous & P.W. Crous | JF499838 JF499858 JF499876 JF499882 |
| Devriesia tardicrescens      | CBS 128770 | = CPC 18259     | leaf bracts of | Phaenocoma prolifera | South Africa | JF499841 JF499861 – – |
| Exophiala capensis           | CBS 128771 | = CPC 18473     | leaf bracts of | Phaenocoma prolifera | South Africa | JF499842 JF499862 – – |
| Penidiella ellipsoidea       | CPC 18280 | leaf bracts of | South Africa | South Africa | K.L. Crous & P.W. Crous | JF499843 JF499863 – – |
| Teratosphaeria cf. bellula   | CPC 18281 | leaf bracts of | South Africa | Phaenocoma prolifera | South Africa | JF499845 JF499865 – – |
| Teratosphaeria karinae       | CBS 128774 | = CPC 18255     | leaf bracts of | Phaenocoma prolifera | South Africa | JF499847 JF499867 – – |
| Toxicocladosporium pseudoveloxum | CBS 128775 | = CPC 18257   | leaf bracts of | Phaenocoma prolifera | South Africa | JF499848 JF499868 – – |
| Toxocarospora pseudocatenata | CPC 18283 | leaf bracts of | South Africa | South Africa | K.L. Crous & P.W. Crous | JF499849 JF499869 – – |
| Xerophacidiella pseudocatenata | CPC 18284 | leaf bracts of | South Africa | South Africa | K.L. Crous & P.W. Crous | JF499850 JF499870 – – |

**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

**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 4.0b10; Swfford 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).
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 obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 10 changes from a heuristic search with 100 random taxon additions of the 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 obtained 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 Capnodiales, 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 1000 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 1102 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 protearum* simile, sed conidiis minoribus, 10–25 × 5–10 µm.

**Etymology.** Named after the locality where it was collected, Hermanus, South Africa.
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 ellipsoidal, 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 submerging, iron-grey; reaching 15 mm diam after 2 wk. On MEA similar in colour, also reaching 15 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-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 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 phaenocomae** Crous, sp. nov. — MycoBank MB560018; Fig. 5

*Cladosporio australiensis* phylogenetic 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. Microco-
nidiophores 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. Ramoconid
ia 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-
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; forming additional septa with age, becoming irregular, microsclerotal, 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.

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* morphologicis similis, sed conidios 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 and reverse iron-grey; reaching 8 mm diam after 2 wk. On MEA surface and reverse iron-grey; reaching 12 mm diam after 2 wk.

*Specimen examined.* SOUTH AFRICA, Western Cape Province, Hermanus, Fernkloof Nature Reserve, S 34°23'38" E 19°16'7.7", 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* morphologicis similis, sed conidios 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.

*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 vaginae mucoide, hau Judegerminanti et tubis germinationis paralleli ad axem sporae.*

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

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![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.

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

Toxicocladosporium veloxum similis, sed rochoconidiis 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, macroseptate, 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; rochoconidia 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.

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 *Phaeocoma prollifera*, 2 May 2010, K.L. Crous & P.W. Crous, CBS H-20536 holotype, cultures ex-type CPC 18278 = 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 ramosconidia (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 morphologic simili, sed conidiatomus pycnidialibus, conidiosis 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, multicellular, with several ostioles, erumpent, 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 *Phaeocoma prollifera*, 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 *Pheaothecoides 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, Bissy & Ainsworth 1943, Pirozynski 1972, Pascoe 1990, Hawsworth 1991, 1998, 2001, 2004, Dreyfuss & Chapela 1994, Rossman 1994, Hyde et al. 1997, Fröhlich & Hyde 1999, Crous et al. 2006b). In this study, the number that is commonly used to argue for fungal biodiversity is the 1.5 M estimate by Hawsworth (1991), though the fungal biodiversity in the Southern Hemisphere seems to greatly exceed this estimate (Crous et al. 2006b, Marinowitz 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. (2006b) 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. In contrast to the statement of Hawksworth (1991) that each plant species can be expected to have 5–6 novel fungal species, 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 Peniidiella, 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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