Systematic reappraisal of Coniella and Pilidiella, with specific reference to species occurring on Eucalyptus and Vitis in South Africa

Jan M. VAN NIEKERK1, J. Z. ‘Ewald’ GROENEWALD2, Gerard J. M. VERKLEY2, Paul H. FOURIE2, Michael J. WINGFIELD2 and Pedro W. CROUS1,2*

1Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa.
2Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalaalan 8, 3584 CT Utrecht, The Netherlands.
3Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa.
E-mail: crous@CBS.knaw.nl

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The genus Pilidiella, including its teleomorphs in Schizoparme, has a cosmopolitan distribution and is associated with disease symptoms on many plants. In the past, conidial pigmentation has been used as a character to separate Pilidiella (hyaline to pale brown conidia) from Coniella (dark brown conidia). In recent years, however, the two genera have been regarded as synonymous, the older name Coniella having priority. To address the generic question, sequences of the internal transcribed spacer region (ITS1, ITS2), 5.8S gene, large subunit (LSU) and elongation factor 1-α (EF 1-α) were analysed to compare the type species of Pilidiella and Coniella. All three gene regions supported the separation of Coniella from Pilidiella, with the majority of taxa residing in Pilidiella. Pilidiella is characterised by having species with hyaline to pale brown conidia (avg. length:width >1.5), in contrast to the dark brown conidia of Coniella (avg. length:width <1.5). Pilidiella diplodiella, which is a pathogen associated with white rot of grapevines, was shown to be an older name for C. petrakii. To delineate species in the P. diplodiella species complex, isolates were also compared based on histone (H3) gene sequences. Analyses derived from these sequence data separated P. diplodiella from a newly described species, P. diplodiopsis. The new species P. eucalyptorum sp. nov. is proposed for isolates formerly treated as C. fragariae and associated with leaf spots of Eucalyptus spp. This species clustered basal to Pilidiella, and may represent yet a third genus within this complex. Pilidiella destruens sp. nov. is newly described as anamorph of Schizoparme destruens, which is associated with twig dieback of Eucalyptus spp. in Hawaii. A key based on morphological characteristics is provided to separate the taxa treated in this study.

INTRODUCTION

The anamorph genera Coniella and Pilidiella have a cosmopolitan distribution and include plant pathogens that cause leaf, stem and root diseases on a wide variety of hosts. Pilidiella has been linked to teleomorphs in Schizoparme (Maas, Pollack & Uecker 1979). Van der Aa (in von Arx 1973) and von Arx (1981) treated Coniella and Pilidiella as separate genera with Coniella having dark brown conidia and Pilidiella hyaline to medium brown conidia. Sutton (1980) and Nag Raj (1993), however, treated the two genera as synonyms. Samuels, Barr & Lowen (1993) linked several Coniella anamorphs to species of Schizoparme, which the authors regarded as a member of the Diaporthales (Melanconidaceae). Recent DNA-based studies suggest that the Schizoparme-complex is representative of an undescribed family, and that the anamorph genera, Coniella and Pilidiella, should be retained as separate entities in the Diaporthales (Castlebury et al. 2002).

Schizoparme was originally described for a single species, S. straminea, which was found on a wide variety of woody and herbaceous hosts (Shear 1923). In 1979, the anamorph-teleomorph relationship for S. straminea was established when Maas et al. (1979) recognised that Pilidiella castaneicola (as P. quercicola), a hitherto unknown coelomycete isolated from strawberry, was the anamorph of S. straminea.

The most important species belonging to the Coniella/Pilidiella complex on grapevines is C. diplodiella, the causal agent of white rot (Sutton & Waterston 1966). This disease is especially severe in cases where hailstorms have damaged vineyards, and many wounds are available for infection. Severe infections can
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\^2 Sequences deposited in GenBank in February 2006.
\^3 Sequences deposited in GenBank in December 2006.

Areas sequenced\^b

LSU  ITS  EF1-α  H3
reportedly lead to crop losses of between 20–80% (Bisiach 1988). Sutton (1980) was unable to distinguish *C. diplodiella* from *C. fragariae*, a species that is known to commonly occur in soil, and also to cause leaf diseases of strawberries (Jarvis & Hargreaves 1972) and *Eucalyptus* (Sharma, Mohanan & Maria Florence 1985).

Both *C. diplodiella* and *C. fragariae* have previously been reported from South Africa (Crous, Phillips & Baxter 2000). Of these fungi, *C. diplodiella* is listed as an organism of quarantine significance. During 2000, several shipments of grapevine cuttings imported into South Africa from Europe and Australia were found to be contaminated with *C. diplodiella*, leading to their rejection. For this reason, Winetech, the body funding grapevine research in South Africa, requested a clarification of records of *Coniella* species from South Africa.

The aims of this study were to clarify the taxonomic status of *C. diplodiella*, to compare it with other species in the genus, and to determine whether this species occurs in South Africa.

**MATERIALS AND METHODS**

**DNA isolation, amplification and phylogeny**

34 isolates of *Coniella* from South Africa, Europe, North and South America, Asia and Australasia were studied (Table 1). The methods of Lee & Taylor (1990) were used to isolate genomic DNA from fungal mycelium grown on potato dextrose agar (PDA; Biolab, Midrand, South Africa).

The primers ITS1 and ITS4 (White et al. 1990) were used to amplify part of the nuclear rRNA operon spanning the 3′ end of the 18S (small subunit) rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS (ITS2) region and the 5′ end of the 28S (large subunit) of the rRNA gene. The PCR reaction mixture consisted of 1.5 units Biotaq (Bioline, London), 1× PCR buffer, 2.5 mM MgCl₂, 0.2 mM of each dNTP, 4 pmoles of each primer, approximately 10 to 30 ng of fungal genomic DNA and was made up to a total volume of 25 µl with sterile water. Reactions were performed on a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA) and the cycling conditions comprised denaturation for 5 min at 96 °C, followed by 30 cycles at 96 °C (30 s), 55 °C (30 s), 72 °C (45 s) and a final 7 min extension step at 72 °C to complete the reaction. Part of the elongation factor 1-alpha (EF-1α) gene was amplified with primers EF1-728F and EF1-986R (Carbone & Kohn 1999) for a selected subset of representative isolates. Part of the histone 3 (H3) gene (H3), elongation factor 1-alpha (EF-1α), histone gene (H3), elongation factor 1-α (EF-1α), and large subunit (LSU) was amplified with primers LR0R (Rehner & Samuels 1994) and LR7 (Vilgalys & Hester 1990) using

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* ATCC, American Type Culture Collection, VA; CBS, Centraalbureau voor Schimmelcultures, Utrecht; IMI, CABI-Bioscience, Egham; PPRL, Plant Protection Research Institute, Pretoria; and STE-U, Department of Plant Pathology, University of Stellenbosch.
* a ATCC, American Type Culture Collection, VA; CBS, Centraalbureau voor Schimmelcultures, Utrecht; IMI, CABI-Bioscience, Egham; PPRL, Plant Protection Research Institute, Pretoria; and STE-U, Department of Plant Pathology, University of Stellenbosch.
* b Ex-type cultures.
the same conditions as described here but with 4 mM MgCl₂. PCR products were separated by electrophoresis at 80 V for 1 h in a 0.8% (w/v) agarose gel in 0.5× TAE running buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK) following ethidium bromide staining.

The amplification products were purified using NucleoSpin® Extract 2 in 1 kit (Macherey-Nagel, Germany). The purified products were sequenced in both directions using the PCR primers and the cycle sequencing reaction was carried out as recommended by the manufacturer with an ABI PRISM Big Dye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) containing AmpliTaq DNA Polymerase. The reaction was set up as denaturing at 94 °C for 5 min, followed by 25 cycles of 96 °C for 10 s, 55 °C for 10 s, and 60 °C for 4 min, with a final incubation of 30 s at 60 °C. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, CN).

The ITS nucleotide sequences generated in this study were added to other ITS sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov). The large subunit sequences were added to sequences obtained from the alignment of Castlebury et al. (2002). The alignments were assembled using Sequence Alignment Editor v2.0a11 (Rambaut 2002) and manual adjustments for improvements were made visually where necessary. The phylogenetic analyses of sequence data were done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2000). Alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed for all data sets using the heuristic search option with 100 random taxa additions and tree bisection and reconstruction (TBR) as the branch swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Hillis & Bull 1993). Other measures including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC) were also calculated. The resulting trees were printed with TreeView Version 1.6.6 (Page 1996). A partition homogeneity test (Farris et al. 1994) was conducted in PAUP (Swofford 2000) to consider the feasibility of combining the various sequence data sets.

**RESULTS**

**Phylogenetic analyses**

Partition homogeneity tests (where \( P < 0.05 \) was taken as significantly incongruent) of the different datasets indicated that they were combinable. However, since different questions at generic and species level are addressed using different datasets, the phylogenetic data are presented as separate rather than combined trees. The large subunit alignment was used for inference of the higher order taxonomic relationship between Pilidiella and Coniella, while the ITS alignment was used to discriminate species and species complexes. Species relationships between *P. diplodiella*, *P. diplodiopsis*, *P. eucalyptorum* and *C. fragariae* were established with the elongation factor 1-α alignment. The division between *P. diplodiella* and *P. diplodiopsis* was further investigated and confirmed using alignment of the histone gene sequences. New sequences were deposited in GenBank (Table 1), and the alignments in TreeBASE (SN 1525).

The large subunit sequence alignment contained 25 taxa and spanned 1255 characters including the gaps (in TreeBASE). Of the aligned nucleotide sites for the data set, 138 characters were parsimony-informative, six variable characters were parsimony-uninformative and 1111 were constant. Maximum parsimony analysis of the sequence data resulted in 47 equally most parsimonious trees (TL = 178 steps, CI = 0.848, RI = 0.904, RC = 0.767), one of which is shown in Fig. 1. Most of the isolates grouped with the type strain of *Schizoparme straminea* (CBS 149.22) (58% support). The *P. diplodiella/diplodiopsis* isolates grouped together (56% bootstrap support) and three of these isolates (CBS 111857, 111858, 166.84) formed a separate cluster (60% bootstrap support) within this group. Two isolates of *Pilidiella* occurring on *Eucalyptus* (STE-U...
3905 and CBS 112640) formed a well-supported cluster (100% support) within the Pilidiella clade, as did isolates of *P. granati* (97% support). The *C. fragariae* isolates formed a separate clade with a bootstrap support value of 100%.

The manually adjusted alignment of the ITS nucleotide sequences contained 36 taxa and 504 characters including alignment gaps (in TreeBASE). Of the aligned nucleotide sites, 90 characters were parsimony-informative, 37 variable characters were parsimony-uninformative and 377 were constant. Six equally most parsimonious trees (TL = 192 steps, CI = 0.859, RI = 0.965, RC = 0.829) were obtained from maximum parsimony analysis of the ITS sequence data, one of which is shown (Fig. 2). The Pilidiella isolates sequenced in this study grouped in a single Pilidiella clade (100% bootstrap support), with two well-supported subclades. The first subclade (92% bootstrap support), contained two isolates of *P. castaneicola*, an isolate of *P. granati*, *S. straminea*, unnamed Pilidiella spp., and isolates initially identified as *P. diplodiella* and *P. petrakii*. The correct names for the *P. diplodiella* and *P. petrakii* isolates are shown in the tree. All the isolates from symptomatic *Eucalyptus* leaves formed another well-supported subclade (100% bootstrap support) basal to the Pilidiella subclade. A further

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**Fig. 1.** One of 47 most parsimonious trees obtained from the large subunit rRNA gene sequence data (TL = 178 steps, CI = 0.848, RI = 0.904, RC = 0.767). The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. Ex-type cultures are indicated in bold. The sequences of *Magnaporthe grisea* AB026819 and *Pyricularia grisea* AF362554 were included as outgroups.
clade (bootstrap support of 100%) contained Coniella fragariae isolates from various countries and hosts, as well as an isolate of C. australiensis.

Part of the elongation factor 1-α gene was sequenced for a subset of isolates. The manually adjusted alignment of the nucleotide sequences contained eighteen taxa and 452 characters including alignment gaps (in TreeBASE). Of the aligned nucleotide sites 304 characters were parsimony-informative, 60 variable characters were parsimony-uninformative and 88 were constant. The elongation factor 1-α sequence data were also subjected to maximum parsimony analysis and resulted in a single most parsimonious tree (TL = 1056 steps, CI = 0.701, RI = 0.785, RC = 0.550). The phylogenetic tree (Fig. 3) delimited several clades that correlated with the ITS and LSU trees. As with the ITS and LSU trees, the clade containing the Pilidiella isolates (56% bootstrap support) contained two subclades. The first subclade (99% bootstrap support) contained Pilidiella macrospora, Pilidiella granati, a cluster (88% bootstrap support) containing the isolates S. straminea and Pilidiella castaneicola and a cluster (97% bootstrap support) containing an isolate of Pilidiella castaneicola and isolates originally identified as Pilidiella diplodiella. In the Pilidiella diplodiella cluster (98% bootstrap support), isolates were further divided into two clusters (100% bootstrap support, respectively) containing Pilidiella diplodiella and a previously undescribed species, Pilidiella diplodiopsis. Isolates from Eucalyptus grouped in a second (100% bootstrap support) subclade basal to Pilidiella while those of C. fragariae formed a separate well-supported cluster (100% bootstrap support).
To further evaluate the subdivision within the *P. diplodiella* isolates, approximately 500 bases of the histone gene were sequenced for the isolates and the manually adjusted alignment of the nucleotide sequences contained sixteen taxa and 505 characters including alignment gaps (in TreeBASE). Of the aligned nucleotide sites for the data set, 309 characters were parsimony-informative, 37 variable characters were parsimony-uninformative, and 159 were constant. Maximum parsimony analysis of the histone sequence data resulted in a single most parsimonious tree (Fig. 4; TL = 462 steps, CI = 0.718, RI = 0.785, RC = 0.550). Ex-type cultures are indicated in bold. The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values based on 1000 resamplings. The tree is rooted to two *Cryphonectria* species.

Fig. 3. Single most parsimonious tree obtained from elongation factor 1-α sequence data (TL = 1056 steps, CI = 0.701, RI = 0.785, RC = 0.550). Ex-type cultures are indicated in bold. The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values based on 1000 resamplings. The tree is rooted to two *Cryphonectria* species.

To further evaluate the subdivision within the *P. diplodiella* isolates, approximately 500 bases of the histone gene were sequenced for the isolates and the manually adjusted alignment of the nucleotide sequences contained sixteen taxa and 505 characters including alignment gaps (in TreeBASE). Of the aligned nucleotide sites for the data set, 309 characters were parsimony-informative, 37 variable characters were parsimony-uninformative, and 159 were constant. Maximum parsimony analysis of the histone sequence data resulted in a single most parsimonious tree (Fig. 4; TL = 462 steps, CI = 0.718, RI = 0.785, RC = 0.550). As with the elongation factor data, the isolates previously identified as *P. diplodiella* formed a well-supported clade (96% bootstrap support), which was further divided into two major clusters (each with 100% bootstrap support) containing isolates of *P. diplodiella* and *P. diplodiopsis*. A further species of *Pilidiella* (IMI 100482) formed a poorly supported group (67% bootstrap support value), with the *P. diplodiella* isolates. Isolate CBS 111021, which also represented a distinct species of *Pilidiella*, was placed outside the clade containing isolates of *P. diplodiella* and *P. diplodiopsis*.

**Morphology**


Pycnidia globose, 120–200 μm wide, initially appearing hyaline with a dark brown, internal conidial mass, becoming brown with age; ostiole central, 10–15 μm wide; wall 10–20 μm thick, consisting of 3–4 layers of medium brown *textura angularis*; pycnidia containing a basal, central cushion of hyaline cells that give rise to hyaline conidiophores. *Conidiophores* densely...
aggregated, slender, subulate, simple, frequently branched above, reduced to conidiogenous cells, or with 1–2 supporting cells, 12–25 × 4–5 μm. Conidiogenous cells simple, tapering, hyaline, smooth, 7–15 × 3–4 μm, 1.5–3 μm wide at apex, surrounded by a gelatinous coating, apex with visible periclinal thickening. Conidia broadly ellipsoidal, (9–)10–11 (–14) × (6–)7–8 (–10) μm, (l:w = 1.4), apices obtuse, base subtruncate, inequilateral, multiguttulate when young, bi-guttulate when mature, hyaline to pale brown, becoming dark brown at maturity, wall of medium thickness, smooth, germ slits absent; small, hyaline, mucoid basal appendage frequently present, 1–2 μm in length.

Description based on IMI 334797 in vitro.

Cultures: Colonies raised, olivaceous buff (21"d) on the surface, and greenish olivaceous (23°i) underneath, reaching 39 mm after 7 d at 25 °C. Cardinal temperature requirements for growth: min. 5 °C, max. 30 °C, opt. 25 °C.

Notes: The conidial shape and absence of a germ slit distinguishes this species from C. fragariae.


Pilidiella castaneicola CBS 149.22

Schizoparme straminea CBS 149.22

Pilidiella sp. CBS 111021

Pilidiella sp. IMI 100482

Pilidiella diplodiella CBS 166.84

Pilidiella diplodiella CBS 111858

Pilidiella diplodiella STE-U 3709

Pilidiella diplodiella CBS 111857

Pilidiella diplodiella CBS 111022

Pilidiella diplodiella STE-U 3768

Pilidiella diplodiella STE-U 3769

Pilidiella diplodiella STE-U 3775

Pilidiella diplodiella STE-U 3778

Pilidiella diplodiopsis CBS 109.23

Pilidiella diplodiopsis CBS 169.55

Pilidiella diplodiopsis CBS 590.84

Fig. 4. Single most parsimonious tree obtained from histone sequence data (TL = 462 steps, CI = 0.918, RI = 0.919, RC = 0.844). Ex-type cultures are indicated in bold. The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values based on 1000 resamplings. The tree is rooted to Fusarium proliferatum AF291059 and Fusarium subglutinans AF236781.


Synonyms are listed in Sutton (1980).

Pycnidia globose to depressed, 250–500 μm wide, initially appearing hyaline with a dark brown, internal conidial mass, becoming brown with age; ostiole
Figs 5–10. Coniella fragaria. Fig. 5. Vertical section through a pycnidium. Fig. 6. Ostiolar area. Figs 7–8. Conidiogenous cells covered in mucous. Figs 9–10. Conidia. Bars = 10 μm.
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Figs 11–13. *Coniella* spp. **Fig. 11.** Conidia, conidiophores and spermatia of *C. fragariae* (STE-U 3772). **Fig. 12.** Conidia and conidiophores of *C. fragariae* (CBS 766.31). **Fig. 13.** Conidia and conidiophores of *C. australiensis* (IMI 261318). Bar = 10 μm.
central, 10–50 μm wide; wall 20–30 μm thick, consisting of 3–6 layers of pale to medium brown textura angularis; pycnidia containing a basal, central cushion of hyaline cells that give rise to hyaline conidiophores. Conidiophores densely aggregated, slender, subulate, simple, frequently branched above, reduced to conidiogenous cells, or with 1–2 supporting cells, 15–30 × (2–)3–4 μm. Conidiogenous cells simple, tapering, hyaline, smooth, 10–20 × 2–3 μm, 1–1.5 μm wide at apex, surrounded by a gelatinous coating, apex with visible periclinal thickening, rarely with percurrent proliferation. Conidia ellipsoidal, apices tapering, narrowly obtusely rounded, tapering from middle towards a narrowly subtruncate base, medium brown, multiguttulate when young, mostly 1–2-guttulate when mature, wall of medium thickness, darker brown than medium brown body of conidiophium, frequently with a lighter band of pigment extending over conidia, with a germ slit visible in older conidia, and mucous appendages also visible in lactophenol; appendages mostly basal, but also lateral along the length of the conidium, (8–)9–10–(12.5) × (5–)6–7–(8) μm (l:w = 1.5). Microconidia also observed in some cultures, cylindrical, hyaline, straight with obtuse ends, 4–5 × 1–1.5 μm.

**Cultures:** Colonies flat, white on the surface, and pale luteous (17 l) in reverse, reaching 32 mm after 7 d at 25 °C. Cardinal temperature requirements for growth: min. 5 °C, max. 35 °C, opt. 30 °C.

**Notes:** Although analysis of sequence data did not distinguish *C. australiensis* from *C. fragariae*, the two species have distinct conidial shapes, with those of *C. australiensis* being wider, more broadly ellipsoidal, (6–)7–8–(10) μm, and having more obtusely rounded apices, than the more tapering apices of *C. fragariae*. *C. fragariae* is also characterized by forming copious amounts of mucous that encase the conidiophores. This forms a mucous sheath through which subsequent conidia extend. At dehiscence, the remains of this mucus are frequently visible as a basal conidial appendage, while in rare cases the sheath encases the whole conidium, and this can be seen as an appendage extending at either end of the conidiophium. This species was reported as *C. pulchella* by Marasas & van der Westhuizen (1971). The latter name has been reduced to synonymy with *C. fragariae* (Sutton 1980). The specimen of the South African record (PREM 44310) was examined, and confirmed to be the same as *C. fragariae*.

**Specimens examined:** **South Africa:** Western Cape Province: Paarl, Bienne Donne, on Fragaria sp., 8 Dec. 1986, C. Roux (PREM 48853; pycnidia of Septoria aciculosa and Gnomonia comari also present; both are new records for South Africa); Mpumalanga: Sabie, Tweekoentin nursery, Pinus elliottii seedlings, 19 Sept. 1986, N. J. Van Rensburg (PREM 48889=IMI 312146); Eastern Cape Province: East London, on roots of Ananas sp., Feb. 1968, M. Dalsdorf (PREM 44310).

*Pilidiella diplodiella* (Speg.) Crous & J. M. van Niekerk, comb. nov. (Figs 15–17, 22–23)

Basionym: *Phoma diplodiella* Speg., *Ampelomiceti Italici* no. 4 (1878).


Pycnidia globose and slightly depressed to subglobose, in some cases tapering slightly towards the ostiole, 200–350 μm wide, smooth, initially hyaline with a dark central conidial mass, becoming dark brown, ostiole central, up to 100 μm wide, with cells darker brown around the ostiole; wall 15–25 μm thick, consisting of 3–5 layers of medium brown textura angularis; pycnidia containing a basal, central cushion of hyaline cells that give rise to conidiophores. Conidiophores dense, slender, simple or branched below, 0–3-septate, 10–20 × 3–4 μm, surrounded by a mucous coating. Conidiogenous cells simple, slender, hyaline, smooth, 8–15 × 2–3 μm, 1 μm wide at the apex, with prominent periclinal thickening. Conidia hyaline when immature, becoming pale to medium brown, inequilateral, smooth, frequently with a hyaline, lateral appendage, narrowly ellipsoidal, apices tapering, subobtusely rounded, bases subtruncate, multiguttulate, straight to slightly curved, wall of medium thickness, multiguttulate, (10–)12–15–(19) × (4–)5–6 μm (l:w = 2.3).

**Cultures:** Colonies flat, buff (19 d) coloured on surface, and honey (21 b) in reverse, reaching 36 mm after 7 d at 25 °C. Cardinal temperature requirements for growth: min. 5 °C, max 35 °C, opt. 30 °C.

**Notes:** *Pilidiella diplodiella* (as *C. petrakii*) has previously been recorded from South Africa on *Eucalyptus* (Lundquist & Baxter 1985). An examination of this specimen showed that it was not *C. petrakii*, but *C. petrakioidea*.

*Pilidiella diplodiella* (as *Coniella*) was first reported from South Africa in 1977 by Verbeek in the Annual Report of the Secretary for Agricultural Services (Crous & Carstens 2000). A second report of the fungus was published by Matthee & Thomas (1981). However, the morphology and cultural characteristics were not described and no herbarium specimens were lodged, making confirmation of these records impossible (Crous & Carstens 2000). The present study has shown that *C. diplodiella* does occur on grapevines in South Africa, and that it should no longer be considered as an organism of quarantine significance.

**On the type specimen of Phoma diplodiella,** we found two fungi, namely *Pilidiella diplodiella* and *Coniothyrium olivaceum*. Sutton (1969) studied the same specimen, and described loose conidia on the surface similar to those of *P. diplodiella*, i.e. ‘medium brown, ellipsoid, having truncate bases and smooth walls, 10–10.5 × 5 μm’. As the type specimen of this fungus consists of two slide preparations that are in poor condition, Sutton (1969) regarded *Phoma diplodiella* as a nomen dubium. In our examination of the same specimen, conidia appeared thick-walled and finely
Figs 22–24. Conidia and conidiophores of *Pilidiella* spp. Fig. 22. *P. diplodiella* (WINF(M) 7526, holotype). Fig. 23. *P. diplodiella* (epitype). Fig. 24. *P. petrakioidea* (PREM 47146). Bar = 10 μm.
Conidia were 9–15 × 4–6 µm, ellipsoidal with subobtuse to obtusely rounded apices, thus fitting the general description of *P. diplodiella*. Sutton (1980) described a similar species, *C. petrakii*, which was later reduced to synonymy with *C. diplodiella* (IMI Distribution Map no. 335, 3rd edn, 1992). After examination of type material of both fungi, we agree that they are synonymous, and that the older name be used for this fungus. Given the fact that conidia are initially hyaline, becoming pale brown, this species is assigned to *Pilidiella*, and a new epitype specimen and culture are designated.


*Pilidiella diplodiopsis* Crous & J. M. van Niekerk, **sp. nov.**

(Figs 25–26)

*Pilidiellae diplodiellae* similis, sed conidiis anguste ellipsoideis, sursum magis angustatis distincta; conidia breviora, (8–)10–12(–13) × (5–)6–7(–7.5) µm.

**Typus**: Italy: on canes of *Vitis vinifera* (herb. CBS 6947 – holotypus; ex-type cultures CBS 590.84, STE-U 3940).

*Pycnidia* globose and slightly depressed to subglobose, in some cases tapering slightly towards the ostiole, 200–400 µm wide, smooth, initially hyaline with a dark central conidial mass, becoming dark brown, ostiole central, up to 150 µm wide; wall 10–25 µm thick, consisting of 3–6 layers of medium brown *textura angularis*; pycnidia containing a basal, central cushion of hyaline cells that give rise to conidiophores. Conidiophores dense, slender, simple or branched below, 0–3-septate, 10–35 × 3–4 µm, surrounded by a mucous coating. Conidiogenous cells simple, slender, hyaline, smooth, 10–15 × 2–3 µm, 1 µm wide at the apex, with prominent periclinal thickening. *Conidia* pale to medium brown, narrowly ellipsoidal with attenuating conidial apices that are acutely rounded, (8–)10–12(–13) × (5–)6–7(–7.5) µm (l:w = 1.7).

**Cultures**: Similar to that described for *P. diplodiella*.

**Notes**: Morphologically similar to *P. diplodiella*, but distinct in having conidia that are pale to medium brown, narrowly ellipsoidal, but with more attenuating conidial apices (less pronounced when mature), that are acutely rounded; conidia also shorter, (8–)10–12(–13) × (5–)6–7(–7.5) µm, with a lower l:w (1.7). Presently *P. diplodiopsis* is known from grapevines in Italy and Switzerland (Table 1).

*Pilidiella eucalyptorum* Crous & M. J. Wingf., **sp. nov.**

(Figs 18–21, 27)

*Coniellae fragariae* similis, sed conidiis maioribus, (9–)10–12(–14) × (6–)7–8 µm, late ellipsoideis vel limoniformibus, sursum magis angustatis distincta, sursum magis angustatis distincta; conidia breviora, (8–)10–12(–13) × (5–)6–7(–7.5) µm.

**Typus**: Australia: on leaves of *Eucalyptus* sp., P. Q. Thu & R. J. Gibbs (herb CBS 6946 – holotypus; ex-type culture CBS 112640).

Leaf spots large, irregular, pale brown with diffuse margins, frequently secondary, associated with primary pathogens or with insect or wind damage. *Pycnidia* subependimal, erumpent, exuding masses of black conidia that disperse with water over the leaf surface; pycnidia frequently forming in concentric circles from point of infection; pycnidia in culture with a red brown ostiolar area and base; pycnidia globose, up to 300 µm wide, smooth, medium to dark brown, with a central ostiole, up to 60 µm wide; wall up to 25 µm wide, 3–5 layers of medium brown *textura angularis*; pycnidia
containing a central basal cushion of hyaline cells that give rise to conidiophores. Conidiophores densely aggregated, hyaline, smooth, slender, branched, 1–3-septate, 15–25 × 4–5 μm; conidiophores similar to those of C. fragariae, but surrounded by less mucous, and more branched than in C. fragariae. Conidiogenous cells hyaline, smooth, with prominent periclinical thickening at the apex, rarely proliferating percurrently, 10–17 × 3–3.5 μm. Conidia medium to dark red–brown, broadly ellipsoidal or limoniform, widest in the middle, tapering to an acutely rounded apex and a subtruncate base, multiguttulate, with a longitudinal germ slit, wall of medium thickness as in C. fragariae, but basal mucoid appendage less common than in C. fragariae, (9–)10–12(–14) × (6–)7–8 μm (l:w = 1.6).

Cultures: Colonies olivaceous (21°i) on the surface and citrine green (23°b) in reverse, reaching 20 mm after 7 d at 25 °C. Cardinal temperature requirements for growth: min. 10 °C, max. 30 °C, opt. 30 °C.

Notes: This species has been regarded as C. fragariae (Sharma et al. 1985, Park et al. 2000), and is similar to it. It shares the same dark conidial pigmentation, and also has the same germ slits found in C. fragariae. Conidia of P. eucalyptorum are slightly larger, (9–)10–12(–14) × (6–)7–8 μm, than those of C. fragariae, (8–)9–10(–12.5) × (5–)6–7(–8) μm. The most

Fig. 27. Conidia and conidiophores of Pilidiella eucalyptorum. Bar = 10 μm.
obvious difference, however, lies in their conidial shape and colour. Conidia of *P. eucalyptorum* have acutely rounded apices, are red–brown, and frequently limoniform. In contrast those of *C. fragariae* are brown, and have tapering, narrow, obtusely rounded apices, and are never limoniform in shape.


For synonyms and description see Nag Raj (1993).

*Cultures*: Colonies straw coloured (21k) on the surface, and pale luteous (17f) in reverse, reaching 28 mm after 7 d at 25 °C. Cardinal temperature requirements for growth: min. 20 °C, max. 30 °C, opt. 30 °C.

*Notes*: Conidia of IMI 233050 were fusiform, 15–27 × 2.5–3.5 µm, thus longer than those of *C. granati* (CBS 252.38), and the description provided for this species by Nag Raj (1993). This suggests that the collection represents *C. castaneicola*, and that the record of *C. granati* from South Africa is incorrect.


*Pilidiella petrakioidea* (Nag Raj) Crous & J. M. van Niekerk, *comb. nov.* (Figs 24, 33–34)


For synonyms and description see Nag Raj (1993).

*Notes*: Conidia are pale brown to brown, (9–)10–12(–15) × 7–8 µm, and narrowly ellipsoidal with acutely rounded apices and a lateral mucous sheath, thus closely matching the description of *C. petrakioidea* provided by Nag Raj (1993). The hyaline to pale brown conidia, suggest that this species is more appropriately accommodated in *Pilidiella* than in *Coniella*. No cultures of *P. petrakioidea* were available for study.


Anamorph: Pilidiella destruens Crous & M. J. Wingf., sp. nov.

Pilidiellae diplodiellae similis, sed conidiis fusoideo-ellipsoideis, (10–)12–13(–15) × (3–)4–5(–6) μm (long.:lat. 2.7), sursum paene obtuse rotundatis distincta.


Perithecia cauliicolous, solitary, subepidermal, becoming erumpent, but not superficial as described for the type (Samuels et al. 1993), globose, up to 300 μm wide, apex short papillate, dark brown; wall up to 80 μm wide, consisting of three regions, namely an outer warty region visible near erumpent apical part of

Figs 30–32. Schizoparme destruens and its anamorph, Pilidiella destruens. Fig. 30. Asci. Fig. 31. Ascospores. Fig. 32. Conidia and conidiophores. Bar = 10 μm.
perithecium, 20–40 μm wide an intermediate layer of medium brown *textura angularis*, 15–20 μm wide, an inner layer of thin-walled, flattened, hyaline cells, 5–15 μm; ostiolum central, circular, up to 75 μm wide; ostiolar channel lined with slender, septate, hyaline, thin-walled periphysoids, 15–20 × 2–3 μm. *Asci* clavate,
requirements for growth: min. 15 × white on the surface and pale luteous (17f) in reverse,

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cally, it is most similar to minor importance as a foliar pathogen. Morphologi-

leaves, but is generally regarded to be of and narrower (13–29 × 2.5–4 μm), has fusiform to naviculate conidia which are longer

two fungi can easily be distinguished.

Gloeosporium castaneicola Ellis & Everh.,

Schizoparme straminea

anamorph occurs

P. diplodiella and P. destruens

have l:w of 2.7, and acutely rounded apices, whereas

P. destruens have l:w of 2.7, and subobtusely rounded apices.

Notes: The morphology of the teleomorph in our collection closely matches that of the type specimen, which is known from Eucalyptus globulus twigs collected in Hawaii (Samuels et al. 1993). Although the anamorph–teleomorph connection could not be verified in culture, pycnidia of the Pilidiella anamorph occurs intermingled with perithecia of the Schizoparme tele-

omorph. The conidia of P. diplodiella and P. destruens are similar, but differ in shape. Conidia of P. diplodiella have a l:w of 2.3, and acutely rounded apices, whereas those of P. destruens have l:w of 2.7, and subobtusely rounded apices.

Schizoparme straminea Shear, Mycologia 15: 121 (1923).


For synonyms and description see Nag Raj (1993).

Cultures: Colonies are raised with a concave edge, white on the surface and pale luteous (17f) in reverse, reaching 26 mm after 7 d at 25 °C. Cardinal temperature requirements for growth: min. 15 °C, max. 30 °C, opt. 20 °C.

Notes: This species is commonly encountered on Eucalyptus leaves, but is generally regarded to be of minor importance as a foliar pathogen. Morphologi-
cally, it is most similar to P. granati, although the two fungi can easily be distinguished. P. quercicola has fusiform to naviculate conidia which are longer and narrower (13–29 × 2.5–4 μm), than the ellipsoidal conidia of P. granati (9–16 × 3–4.5 μm) (Nag Raj 1993).


DISCUSSION

Conidial pigmentation has been used to separate Pilidiella from Coniella (von Arx 1981), but was rejected as a distinguishing characteristic by Sutton (1980) and Nag Raj (1993) who used the older name, Coniella. Results of a study by Castlebury et al. (2002) suggested, however, that Pilidiella with its telemorphs in Schizoparme represents a genus distinct from Coniella. In the present study, additional species were examined, and analysed based on their ITS, EF 1-α and LSU sequence data. All three data sets confirmed the separation of Pilidiella typified by P. castaneicola from Coniella typified by C. fragariae (Figs 1–3). Pilidiella is charac-
terised by having species with hyaline to pale brown conidia (l:w >1.5), in contrast to the dark brown conidia of Coniella (l:w <1.5).

Until now, isolates from Eucalyptus, herein recognized as P. eucalyptorum, have been treated as representative of C. fragariae (Sharma et al. 1985, Park et al. 2000). Other than conidial shape, length:width and colour, P. eucalyptorum is similar to C. fragariae. Based on its dark conidia, this species should be classified in Coniella sensu von Arx (1981). However, P. eucalyptorum clustered basal to the distinct Pilidiella clade in the LSU, ITS and EF 1-α analyses (Figs 1–3). Although treated as a species of Pilidiella, the possibility exists that P. eucalyptorum may represent yet a third discrete genus within this complex.

The link between Schizoparme and Pilidiella has been reconfirmed in this study. Other than reporting a Pilidiella anamorph for S. destruens, a possible link is also shown between P. macrospora and S. botrytidis (Fig. 1).

Although C. australiensis is distinguishable from C. fragariae based on morphology, these differences were not supported by analyses of LSU and ITS sequence data. Isolates of C. fragariae have commonly been obtained from soil, but were also associated with disease symptoms of Fragaria and Vitis. Isolate CBS 111021, from Fragaria in South Africa, clustered within Pilidiella based on ITS data (Fig. 2), and separated from it based on the histone sequence data (Fig. 4), which provided a better separation of closely related taxa than the ITS sequences. Morphologically, this isolate is distinct from C. fragariae, in having more ellipsoidal to limoniform conidia, (10–)11–13(–15) × 6–7(–7.5) μm (l:w = 1.8), with acutely rounded apices, and probably represents an undescribed species.

The taxonomic position of isolates residing in the P. petrakii/diplodiella complex on grapevines cannot be
resolved based on these data. A re-examination of type material revealed that ‘C. petrakii’ is the older name for the fungus treated as ‘C. petrakii’ (Sutton 1980). The type specimens of these two species closely resemble the morphology of isolates clustering in the main clade of P. diplodiella (Fig. 2), which represents isolates collected from grapevines in Australia, France, Germany, Italy, Switzerland and South Africa. Analyses of sequences of the elongation factor 1-α, histone and the LSU regions for a subset of isolates (Figs 1, 3 and 4), suggest that the P. diplodiella complex contains two species, P. diplodiella (with C. petrakii as synonym), P. diplodiopsis, as well as some undescribed species. Presently P. diplodiopsis is known from isolates collected in Switzerland and Italy. The isolate collected on Vitis in India (IMI 100482) is distinct, as its conidia are pale brown, and more narrowly ellipsoidal with acute rounded apices, (9–)10–12×(5–)6–7(–7.5) μm (l:w = 1.7) .

Artificial inoculations on grapevines by von Tiedemann (1985) showed that isolates of P. diplodiella (as C. petrakii), and to a lesser extent C. fragariae, could both cause white rot symptoms of grapevines. Our data suggest that both these species are widely distributed, and that P. diplodiella may occur in most countries where grapevines are cultivated.

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