Mycol. Res. **108** (3): 283–303 (March 2004). © The British Mycological Society
DOI: 10.1017/S0953756204009268 *Printed in the United Kingdom.*

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

Jan M. VAN NIEKERK[1], J. Z. 'Ewald' GROENEWALD[2], Gerard J. M. VERKLEY[2], Paul H. FOURIE[1], Michael J. WINGFIELD[3] and Pedro W. CROUS[1,2]*

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

Received 24 June 2003; accepted 12 December 2003.

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 gene (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

* Corresponding author.

Table 1. *Coniella* and *Pilidiella* isolates studied.

Species	Accession no.[a]	Substrate	Country	Collector	Areas sequenced[b]			
					LSU	ITS	EF1-α	H3
Coniella australiensis	IMI 261318, CBS 111025, STE-U 4196	Leaf litter	South Africa	*K. T. van Warmelo*		AY339313		
C. fragariae	CBS 172.49, STE-U 3930	*Fragaria* sp.	Belgium	*A. Jaarsveld*	AY339282	AY339317	AY339352	
C. fragariae	IMI 111019, STE-U 3772	*Fragaria* sp.	South Africa	*P. W. Crous*		AY339316	AY339351	
C. fragariae	CBS 167.84, STE-U 3934	*Vitis vinifera*	Germany	*A. von Tiedemann*		AY339318		
C. fragariae	IMI 253210, STE-U 4199	Unknown	USA	*B. C. Sutton*	AY339283	AY339320		
C. fragariae	IMI 081599, CBS 165.60, STE-U 4198	Soil	India	*P. N. Mathur et al.*		AY339319		
C. fragariae	CBS 766.71, STE-U 3713	Unknown	South Africa	*H. A. van der Aa*		AY339315	AY339350	
C. fragariae	STE-U 3327	*Fragaria* sp.	South Africa	*P. W. Crous*		AY339314	AY339349	
Pilidiella diplodiella	CBS 166.84, STE-U 3931	*V. vinifera*	Germany	*A. von Tiedemann*	AY339286	AY339331	AY339357	AY339305
P. diplodiella	CBS 111858[c], STE-U 3708	*V. vinifera*	France	*P. W. Crous*	AY339284	AY339323	AY339355	AY339297
P. diplodiella	STE-U 3709	*V. vinifera*	Australia	*P. W. Crous*		AY339324		AY339298
P. diplodiella	CBS 111857, STE-U 3735	*V. vinifera*	South Africa	*F. Halleen*	AY339285	AY339325	AY339356	AY339299
P. diplodiella	CBS 111022, STE-U 3736	*V. vinifera*	South Africa	*F. Halleen*		AY339326		AY339300
P. diplodiella	STE-U 3768	*V. vinifera*	France	*P. W. Crous*		AY339327		AY339301
P. diplodiella	STE-U 3769	*V. vinifera*	France	*P. W. Crous*		AY339328		AY339302
P. diplodiella	STE-U 3775	*V. vinifera*	France	*P. W. Crous*		AY339329		AY339303
P. diplodiella	STE-U 3778	*V. vinifera*	France	*P. W. Crous*		AY339330		AY339304
P. diplodiopsis	CBS 109.23, STE-U 3933	*V. vinifera*	Switzerland	*H. Faes*	AY339287	AY339332	AY339358	AY339306
P. diplodiopsis	CBS 169.55, STE-U 3938	*V. vinifera*	Switzerland	*M. Staehelin*		AY339333		AY339307
P. diplodiopsis	CBS 590.84[c], STE-U 3940	*V. vinifera*	Italy	Unknown	AY339288	AY339334	AY339359	AY339308
P. eucalyptorum	STE-U 610	*Eucalyptus*	Brazil	*P. W. Crous*		AY339335		
P. eucalyptorum	STE-U 1334	*Eucalyptus*	Indonesia	*M. J. Wingfield*		AY339336		
P. eucalyptorum	CBS 111023, STE-U 3843	*Eucalyptus*	Mexico	*M. J. Wingfield*		AY339337	AY339360	
P. eucalyptorum	CBS 112640[c], STE-U 3904	*Eucalyptus*	Australia	*P. Q. Thu & R. J. Gibbs*	AY339290	AY339338		
P. eucalyptorum	STE-U 3905	*Eucalyptus*	Vietnam	*M. J. Dudzinski & P. Q. Thu*	AY339289	AY339339	AY339361	
P. eucalyptorum	IMI 111024, STE-U 3906	*Eucalyptus*	Australia	*P. Q. Thu & R. J. Gibbs*		AY339340		
P. eucalyptorum	STE-U 3912	*Eucalyptus*	Brazil	*A. C. Alfenas*		AY339341		
P. granati	ATCC 12685, CBS 252.38, STE-U 3714	*Vitis vinifera*	Italy	*G. Goidànich*	AY339291	AY339342	AY339362	

Table 1. (*Cont.*)

Species	Accession no.[a]	Substrate	Country	Collector	Areas sequenced[b]			
					LSU	ITS	EF1-α	H3
P. macrospora	CBS 524.73[c], STE-U 3935	*Terminalia ivoriensis*	Ivory Coast	*H. A. van der Aa*	AY339292	AY339343	AY339363	
Pilidiella sp.	PPRI 3870, CBS 111021, STE-U 3828	*Fragaria* sp.	South Africa	*C. Roux*		AY339346		AY339310
Pilidiella sp.	IMI 100482, STE-U 4200	*V. vinifera*	India	*K. S. Bilgrami*	AY339295	AY339347		AY339311
Schizoparme straminea (*P. castaneicola*)	CBS 149.22[c], STE-U 3932	Unknown	USA	*C. L. Shear*	AY339296	AY339348	AY339366	AY339312
S. straminea (*P. castaneicola*)	PPRI 3871, STE-U 3829	Debris	South Africa	*C. Roux*	AY339294	AY339345	AY339365	
S. straminea (*P. castaneicola*)	STE-U 405	*Eucalyptus*	South Africa	*P. W. Crous*	AY339293	AY339344	AY339364	AY339309

[a] ATCC, American Type Culture Collection, VA; CBS, Centraalbureau voor Schimmelcultures, Utrecht; IMI, CABI-Bioscience, Egham; PPRI, Plant Protection Research Institute, Pretoria; and STE-U, Department of Plant Pathology, University of Stellenbosch.
[b] Sequence data of: internal transcribed spacer region (ITS), histone gene (H3), elongation factor 1-α (EF1-α); and large subunit (LSU).
[c] Ex-type cultures.

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) plates.

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 $MgCl_2$, 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 ° (30 s), 55 ° (30 s), 72 ° (90 s) and a final 7 min extension step at 72 ° 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 was amplified with primers H3-1a and H3-1b (Glass & Donaldson 1995). PCR conditions were the same for EF-1α and H3 as for ITS, except for the $MgCl_2$ concentration, which was lowered to 1.5 mM for elongation factor and 2.0 mM for histone, respectively. The 5′ end of the large subunit RNA (28S) (LSU) gene was amplified using primers LR0R (Rehner & Samuels 1994) and LR7 (Vilgalys & Hester 1990) using

the same conditions as described here but with 4 mM $MgCl_2$. 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 ° for 5 min, followed by 25 cycles of 96 ° for 10 s, 55 ° for 10 s, and 60 ° for 4 min, with a final incubation of 30 s at 60 °. 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 improvement 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.

Morphology

Cultures were grown on PDA and placed under mixed cool white fluorescent and nuv light at 25 ° to enhance sporulation. Morphological observations were made from structures mounted in lactic acid. The 95 % confidence intervals of conidial measurements were derived from at least 30 observations at ×1000 magnification. As certain species show overlapping conidial dimensions, but differ regarding spore volume, the average conidial length : width (l : w) is provided to further distinguish these taxa (Nag Raj 1993). Growth rates, cultural characteristics and cardinal temperature requirements for growth were determined for isolates plated onto PDA in 90 mm Petri dishes and incubated in the dark for 7 d at seven different temperatures, ranging from 5 to 35 ° in 5 ° intervals. Three plates were used for each isolate at each temperature. Radial mycelial growth was assessed using two perpendicular measurements for each plate, and the means calculated to determine the growth rates for each species. The colours of cultures were described from isolates incubated at 25 ° in the dark for 2 wk using the colour charts of Rayner (1970). Isolates are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U), and the Centraalbureau voor Schimmelcultures, Utrecht (CBS).

RESULTS

Phylogenetic analyses

Partition homogeneity tests (where $P \geqslant 0.05$ 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

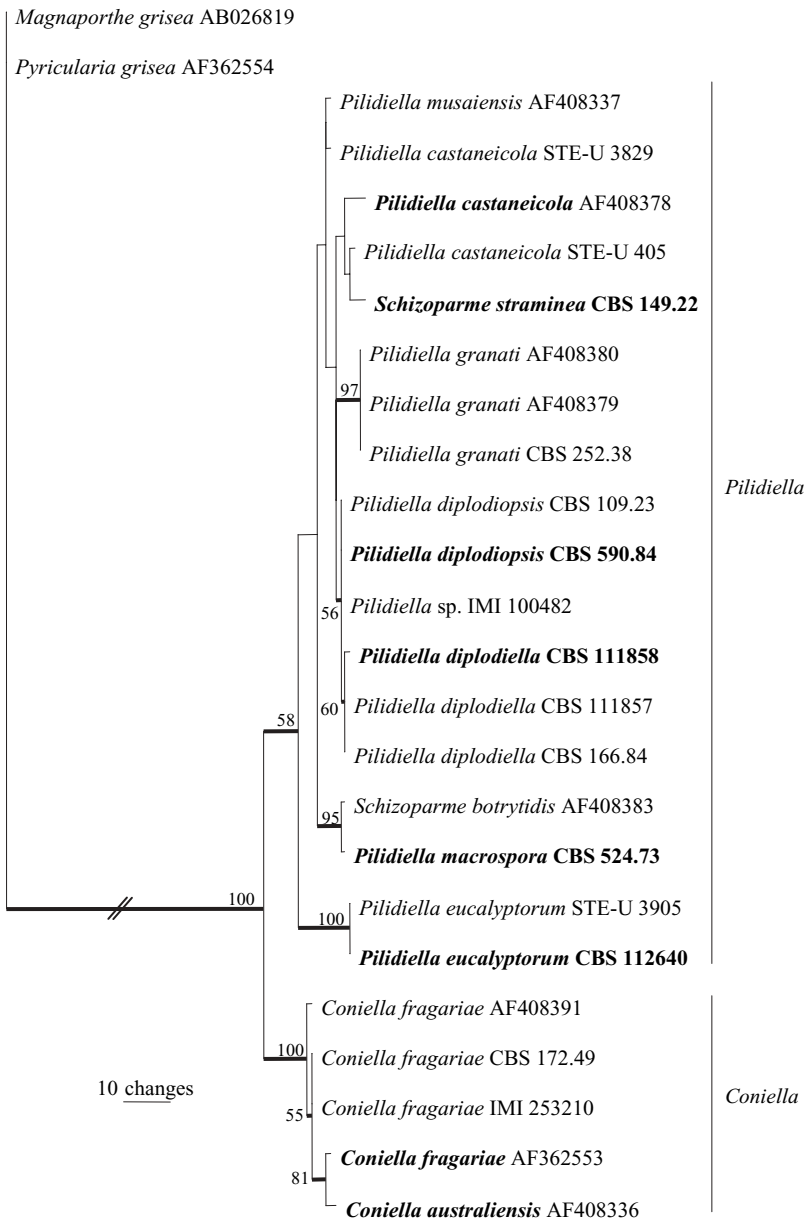


Fig. 1. One of 47 most parsimonious trees obtained from the large subunit rRNA gene sequence data (TL = 178 steps, CI = 0.848, RI = 0.904, RC = 0.767). The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict 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.

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

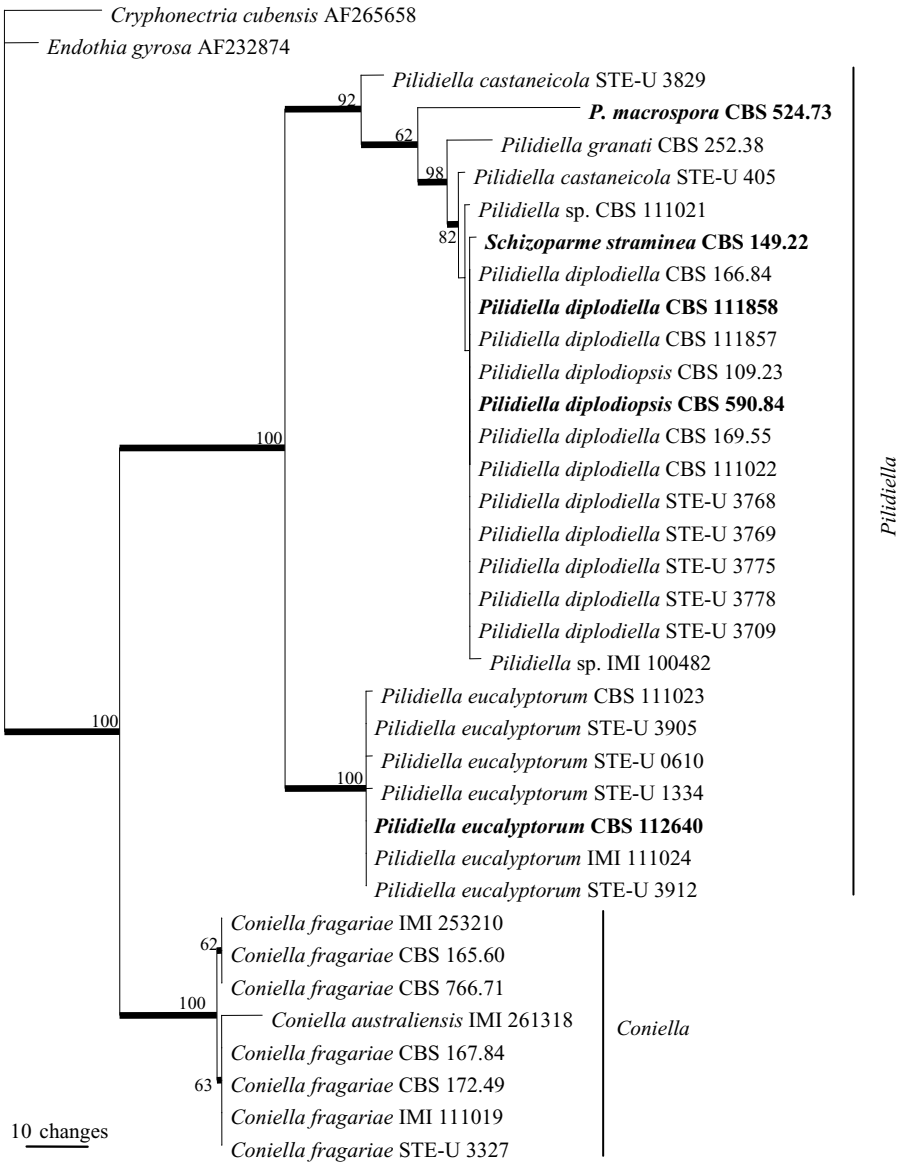


Fig. 2. One of six most parsimonious trees obtained from ITS sequence data (TL = 192 steps, CI = 0.859, RI = 0.965, RC = 0.829). The numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. Ex-type cultures are indicated in bold. The bar indicates 10 changes. The tree is rooted to *Cryphonectria cubensis* AF265658 and *Endothia gyrosa* AF232874.

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 *P. macrospora*, *P. granati*, a cluster (88 % bootstrap support) containing the isolates *S. straminea* and *P. castaneicola* and a cluster (97 % bootstrap support) containing an isolate of *P. castaneicola* and isolates originally identified as *P. diplodiella*. In the *P. diplodiella* cluster (98 % bootstrap support), isolates were further divided into two clusters (100 % bootstrap support, respectively) containing *P. diplodiella* and a previously undescribed species, *P. 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).

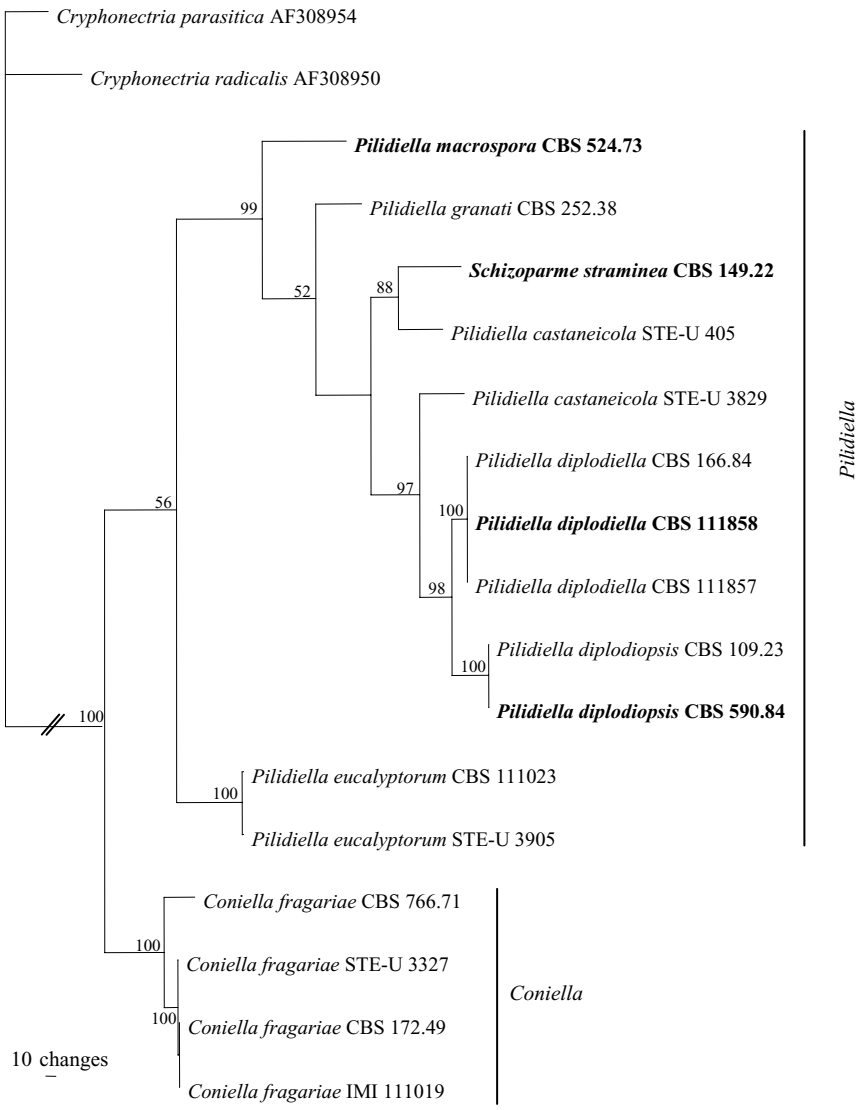


Fig. 3. Single most parsimonious tree obtained from elongation factor 1-α sequence data (TL = 1056 steps, CI = 0.701, RI = 0.785, RC = 0.550). Ex-type cultures are indicated in bold. The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values based on 1000 resamplings. The 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 = 0.918, RI = 0.919, RC = 0.844). 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

Coniella australiensis Petrak, *Sydowia* **9**: 567 (1955). (Figs 13–14)

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

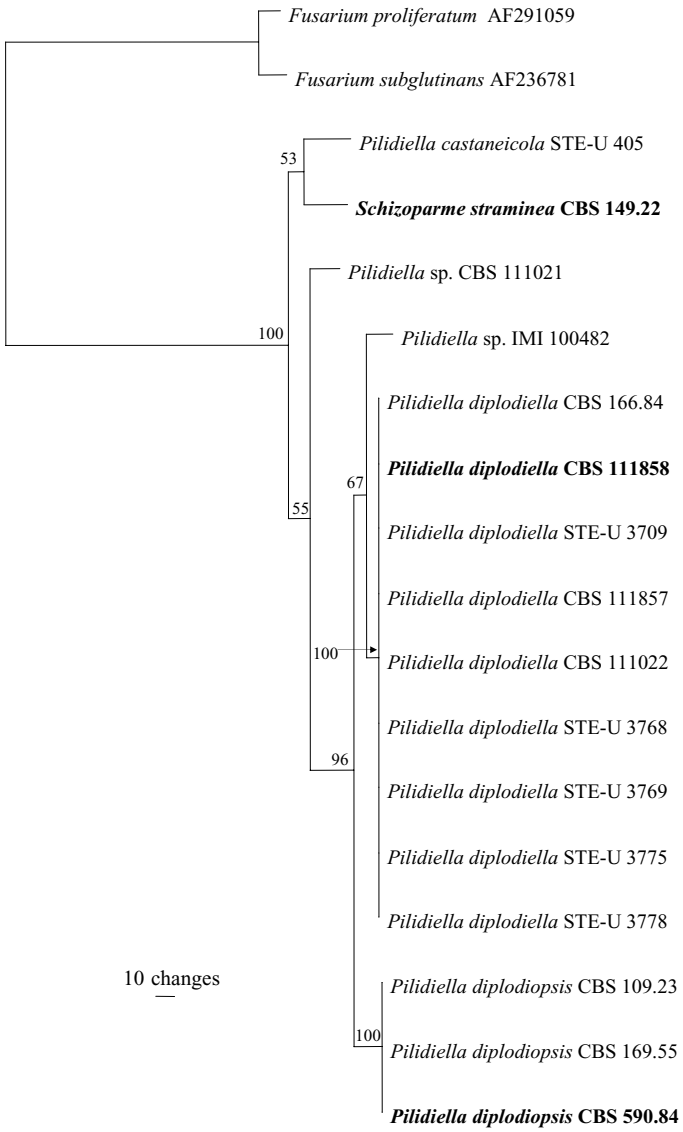


Fig. 4. Single most parsimonious tree obtained from histone sequence data (TL = 462 steps, CI = 0.918, RI = 0.919, RC = 0.844). Ex-type cultures are indicated in bold. The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values based on 1000 resamplings. The tree is rooted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

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 °. Cardinal temperature requirements for growth: min. 5 °, max. 30 °, opt. 25 °.

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

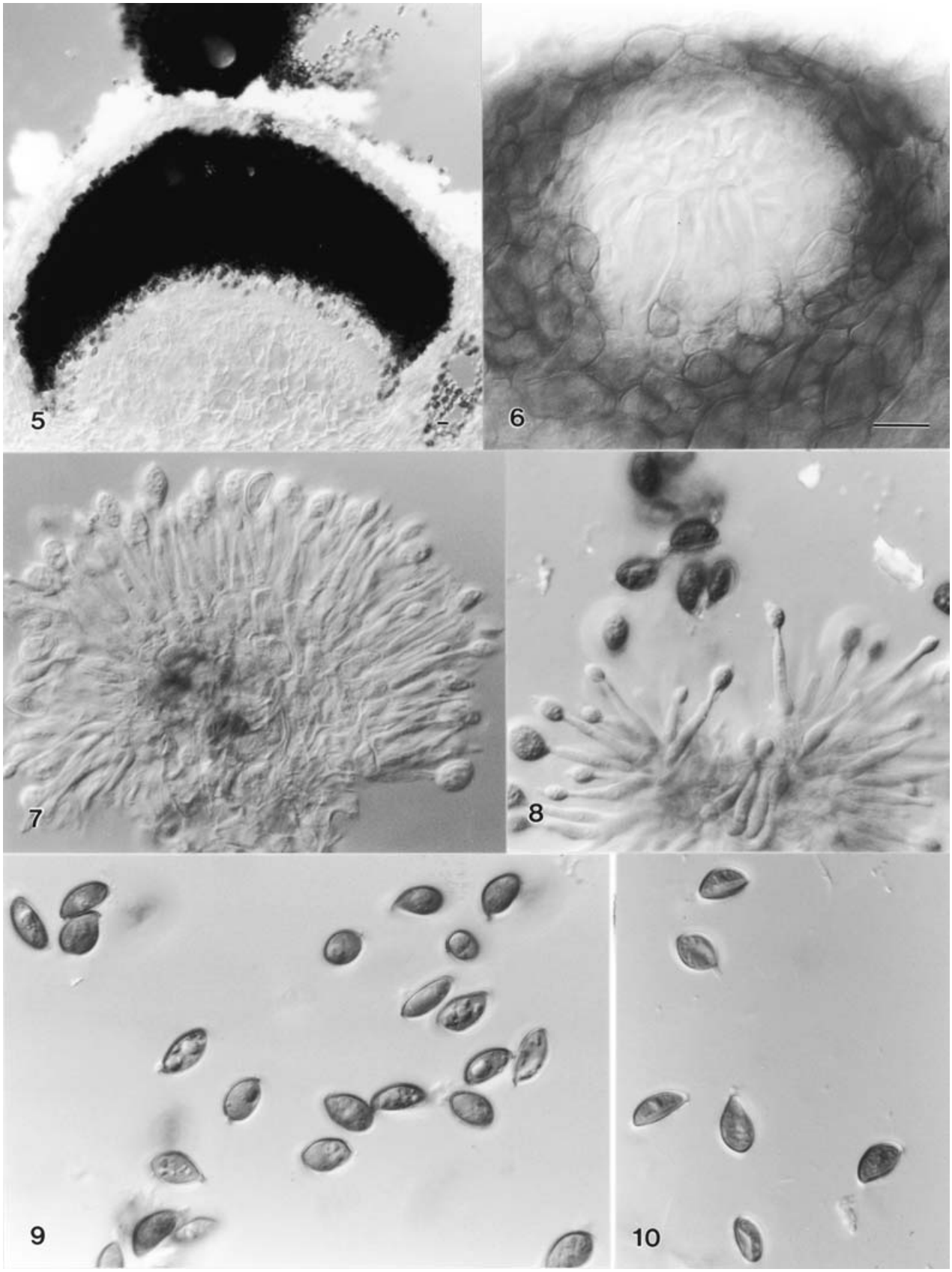
Specimens examined: **South Africa**: *Gauteng*: on leaf litter, 1981, *K. T. van Warmelo* (IMI 261318 = STE-U 4196); *Western Cape Province*: Cape Town, Kirstenbosch Botanical Gardens, on *Pelargonium centrilobum*, 11 Aug. 1989, *J. Isaacs* (PREM 50600, culture IMI 334797).

Coniella fragariae (Oudem.) B. Sutton, *Mycol. Pap.* **141**: 47 (1977). (Figs 5–12)

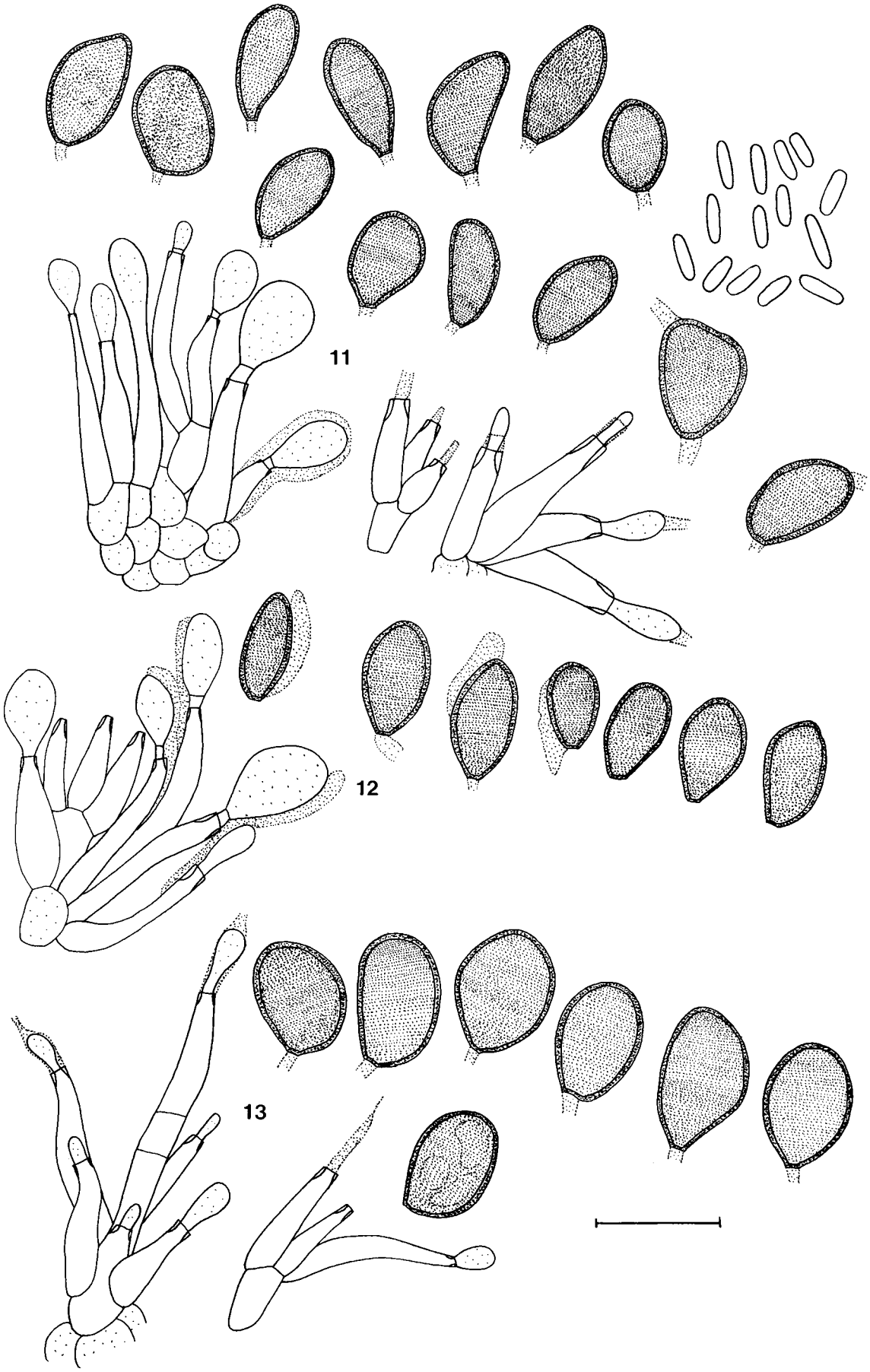
Coniothyrium fragariae Oudem., Verh. Kon. Ned. Akad. Wetensch., ser. 2 **18**: 37 (1883).

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.



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 conidium, frequently with a lighter band of pigment extending over conidium, 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 f) in reverse, reaching 32 mm after 7 d at 25 °. Cardinal temperature requirements for growth: min. 5 °, max. 35 °, opt. 30 °.

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 conidium. 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, Tweefontein nursery, *Pinus elliotii* 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. Dallsdorf* (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).

Coniothyrium diplodiella (Speg.) Sacc., *Syll. Fung.* **3**: 310 (1884).

Coniella diplodiella (Speg.) Petr. & Syd., *Feddes Repert., Beih.* **42**: 460 (1927).

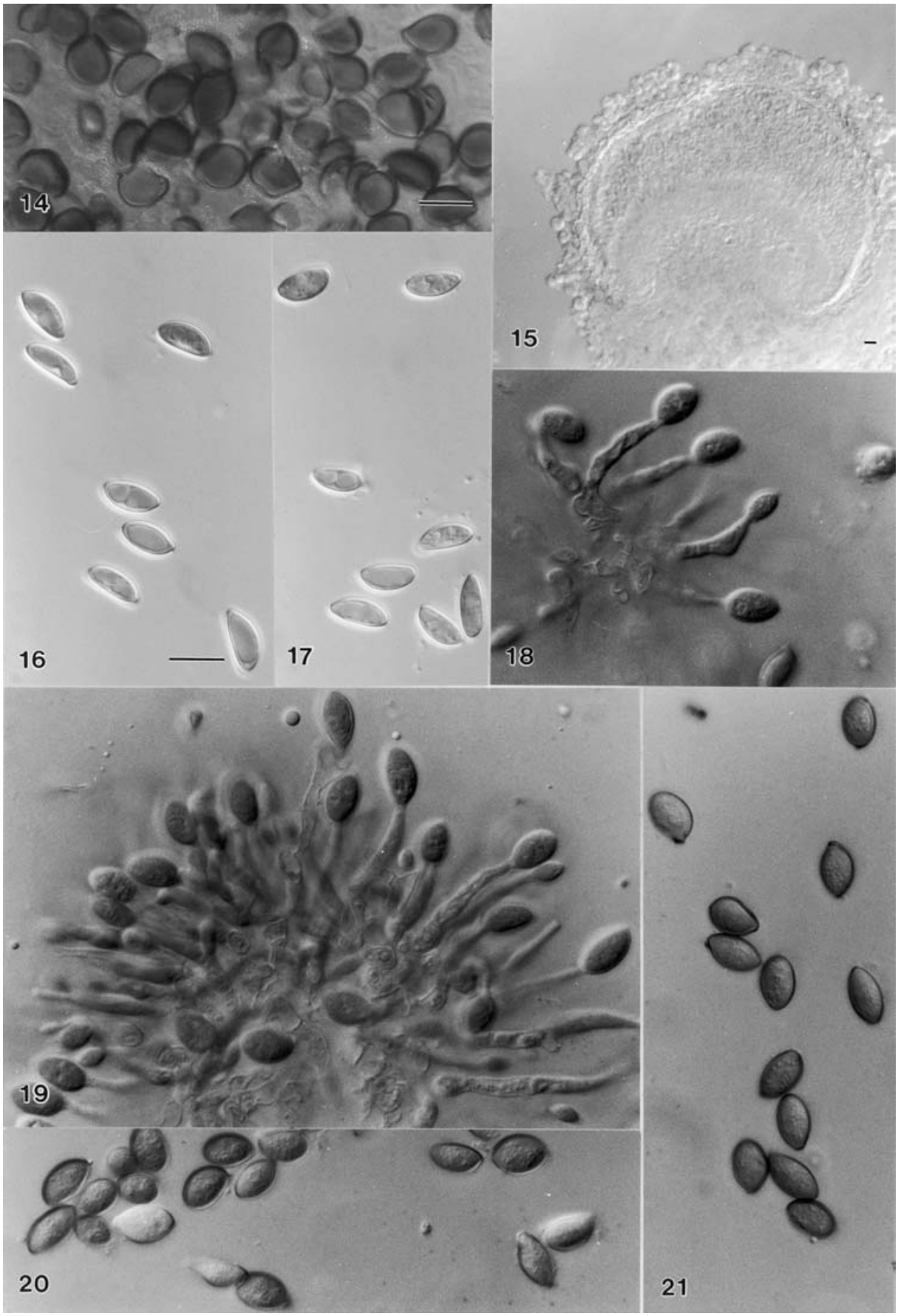
Coniella petrakii B. Sutton, *Coelomycetes*: 422 (1980).

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).

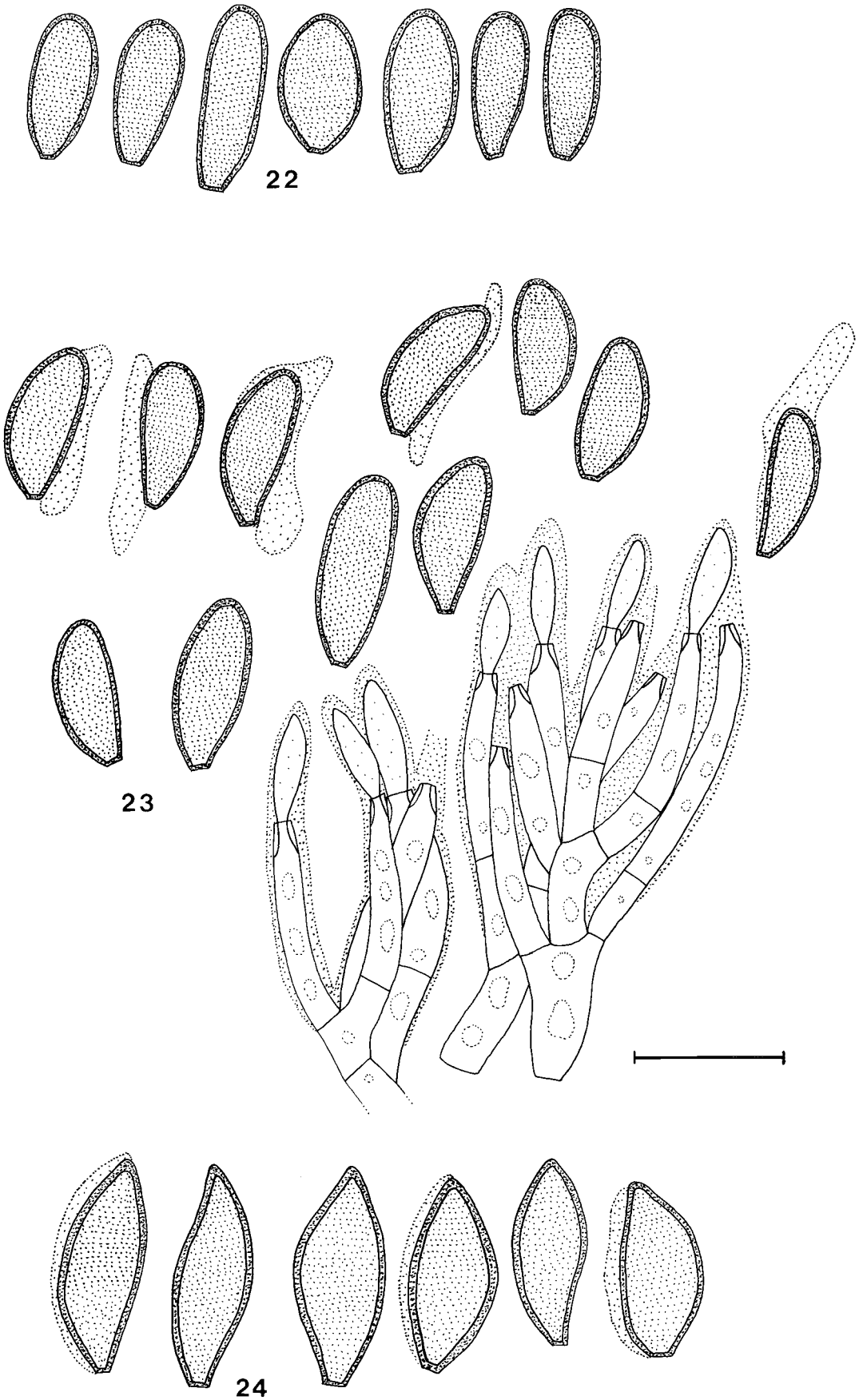
Cultures: Colonies flat, buff (19″d) coloured on surface, and honey (21″b) in reverse, reaching 36 mm after 7 d at 25 °. Cardinal temperature requirements for growth: min. 5 °, max 35 °, opt. 30 °.

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.

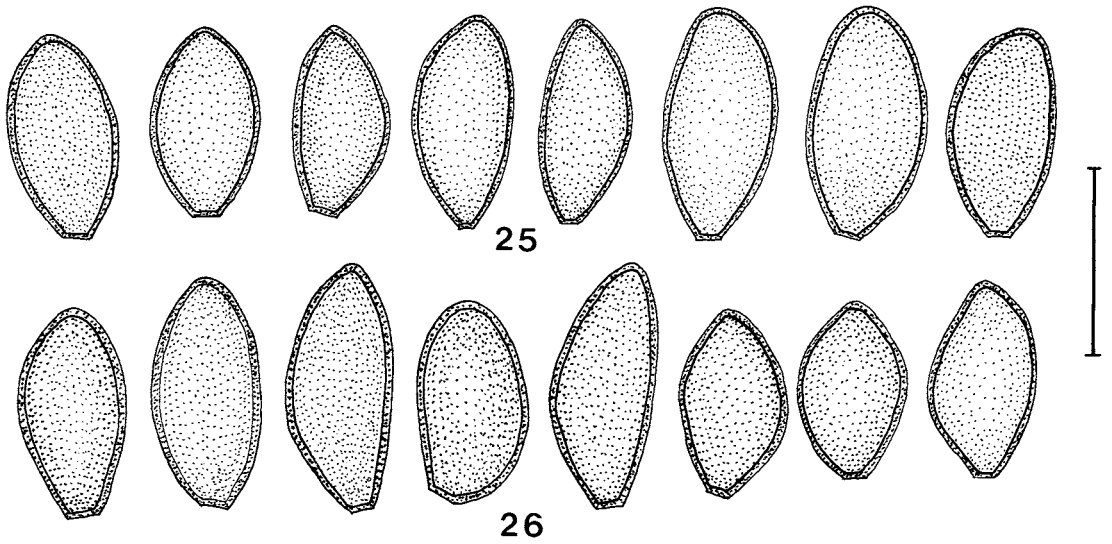
On the type specimen of *Phoma diplodiella*, we found two fungi, namely *Pilidiella diplodiella* and *Coniothyrium olivaceum*. Sutton (1969) studied the same specimen, and described loose conidia on the surface similar to those of *P. diplodiella*, i.e. 'medium brown, ellipsoidal, having truncate bases 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 14–21. Morphological structures of *Coniella* and *Pilidiella* spp. **Fig. 14.** Conidia of *C. australiensis*. **Fig. 15.** Vertical section through a pycnidium of *P. diplodiella*. **Figs 16–17.** Conidia of *P. diplodiella*. **Figs 18–19.** Conidiogenous cells of *P. eucalyptorum* showing mucous sheaths. **Figs 20–21.** Conidia of *P. eucalyptorum*. Bars = 10 μm.



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.



Figs 25–26. Conidia of *Pilidiella diplodiopsis*. **Fig. 25.** STE-U 3940 (ex-type). **Fig. 26.** STE-U 3938. Bar = 10 μm.

verruculose. 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.

Specimens examined: **France**: on stems of *V. vinifera*, 2000, *P. W. Crous* (herb. CBS 6948, ***epitypus e Pilidiella diplodiella, hic designatus***; culture ex-epitype, CBS 111858, STE-U 3708). – **India**: on *Vitis vinifera*, 29 Jan. 1965, *M. A. Salam* (IMI 112027 – holotype of *Pilidiella petrakii*). – **Italy**: on seed of *Vitis vinifera*, *Briosi & Cavara 48* (PREM 7870); Conegliano, on *Vitis vinifera*, 1877, *C. L. Spegazzini* (holotype of *Phoma diplodiella* in LPS-Spegazzini no. 11518 missing; slides ex-type WINF(M) 7526).

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 acute rotundatis distincta.

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* subepidermal, 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

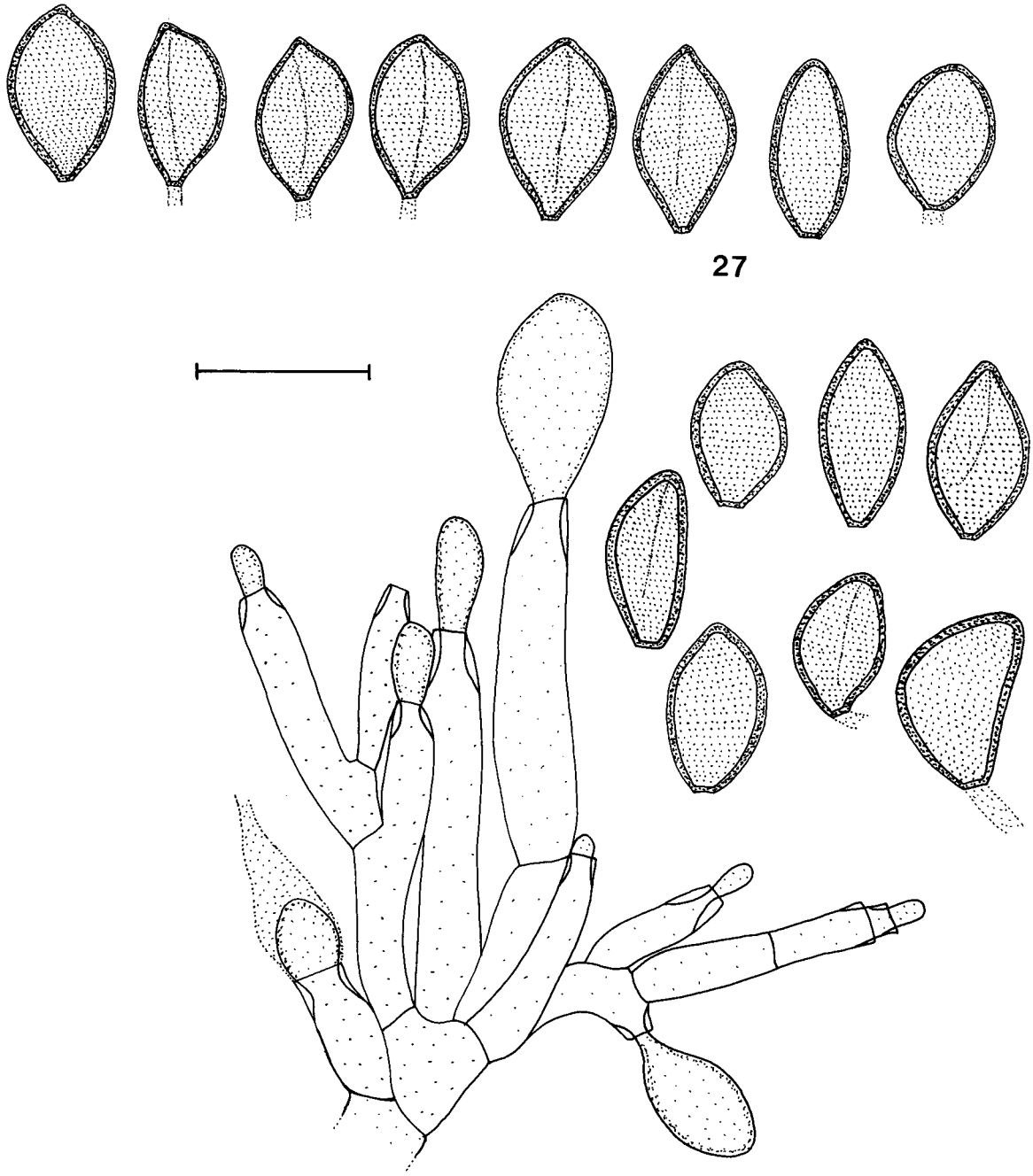
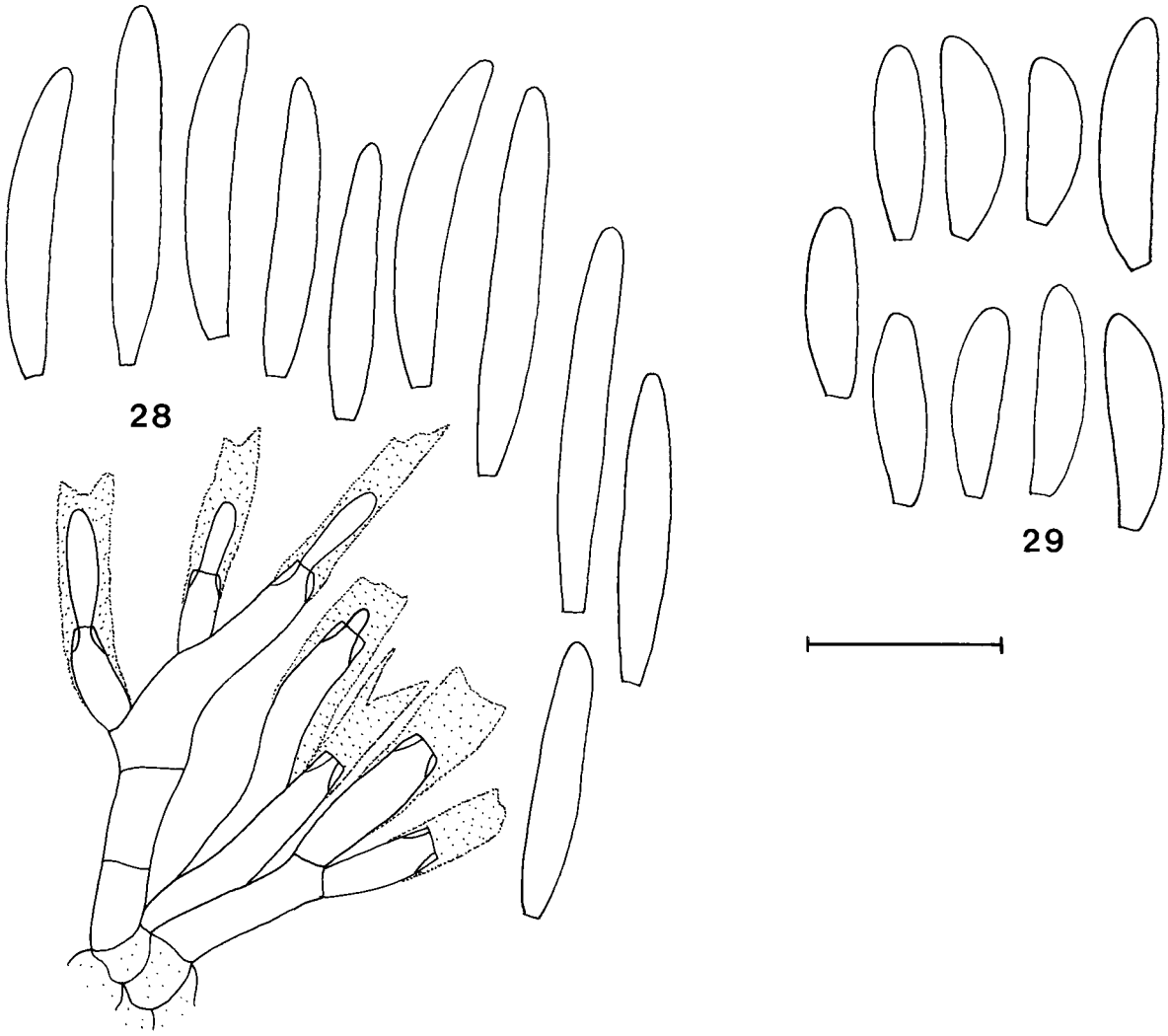


Fig. 27. Conidia and conidiophores of *Pilidiella eucalyptorum*. Bar = 10 μm.

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 periclinal 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 °. Cardinal temperature requirements for growth: min. 10 °, max. 30 °, opt. 30 °.

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



Figs 28–29. Conidia and conidiophores of *Pilidiella* spp. **Fig. 28.** *P. quercicola* (IMI 233050). **Fig. 29.** *P. granati* (CBS 252.38). 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.

Pilidiella granati (Sacc.) Aa, *Verh. Kon. Ned. Akad. Wetensch. Afd. Natuurk., ser.* 2 **61**: 51 (1972). (Fig. 29)

Phoma granati Sacc., *Nuovo G. Bot. Ital.* **8**: 200 (1876).

For synonyms and description see Nag Raj (1993).

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

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.

Specimen examined: **South Africa**: Gauteng: Pretoria, Fragaria sp., 1978, *G. C. A. van der Westhuizen & K. T. van Warmelo* (IMI 233050).

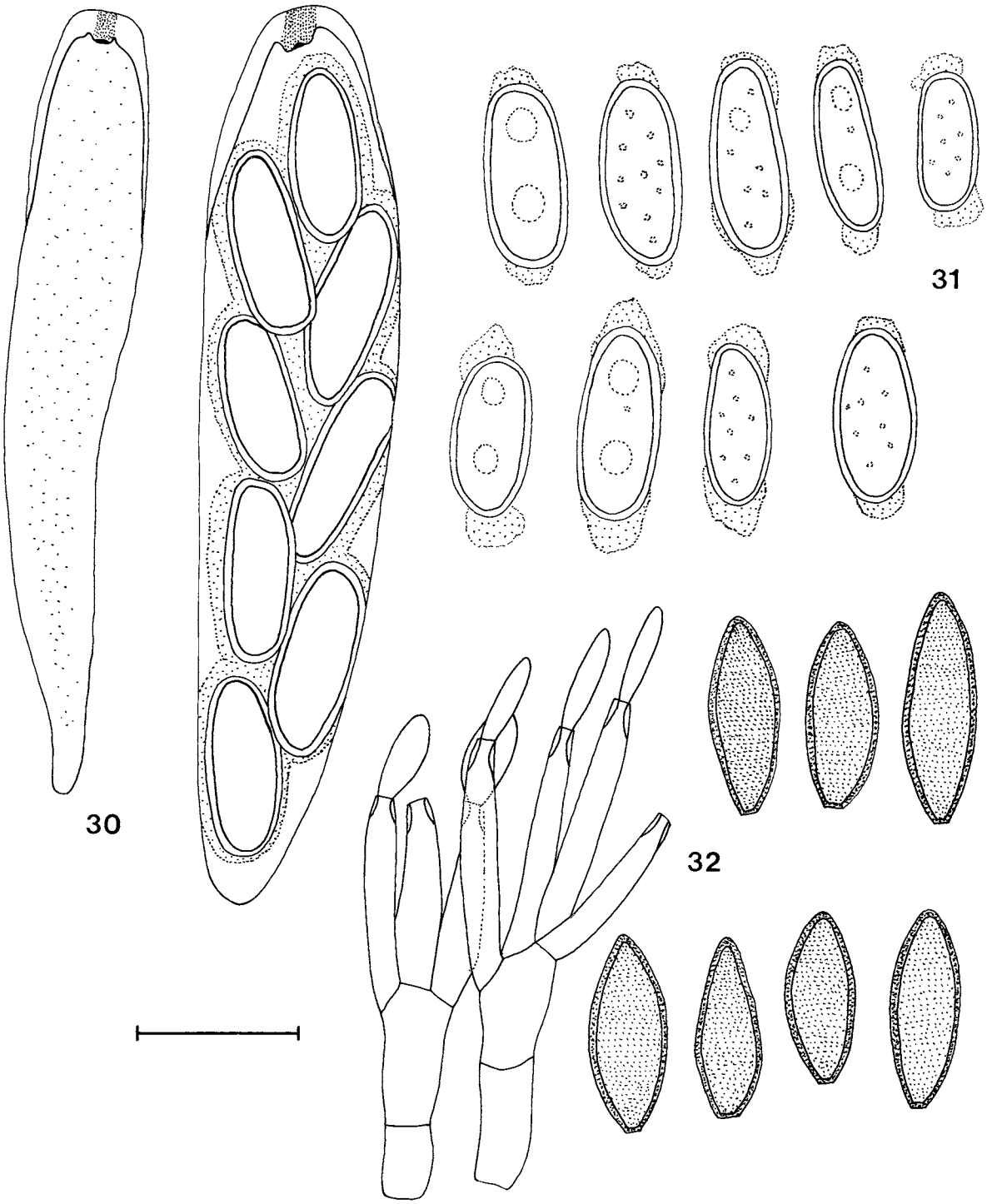
Pilidiella petrakioidea (Nag Raj) Crous & J. M. van Niekerk, **comb. nov.** (Figs 24, 33–34)

Coniella petrakioidea Nag Raj, *Coelomycetous Anamorphs*: 233 (1993).

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.

Specimen examined: **South Africa**: on *Eucalyptus* sp., 22 Jun. 1982, *J. Lundquist* (PREM 47146).



Figs 30–32. *Schizoparme destruens* and its anamorph, *Pilidiella destruens*. **Fig. 30.** Asci. **Fig. 31.** Ascospores. **Fig. 32.** Conidia and conidiophores. Bar = 10 μm.

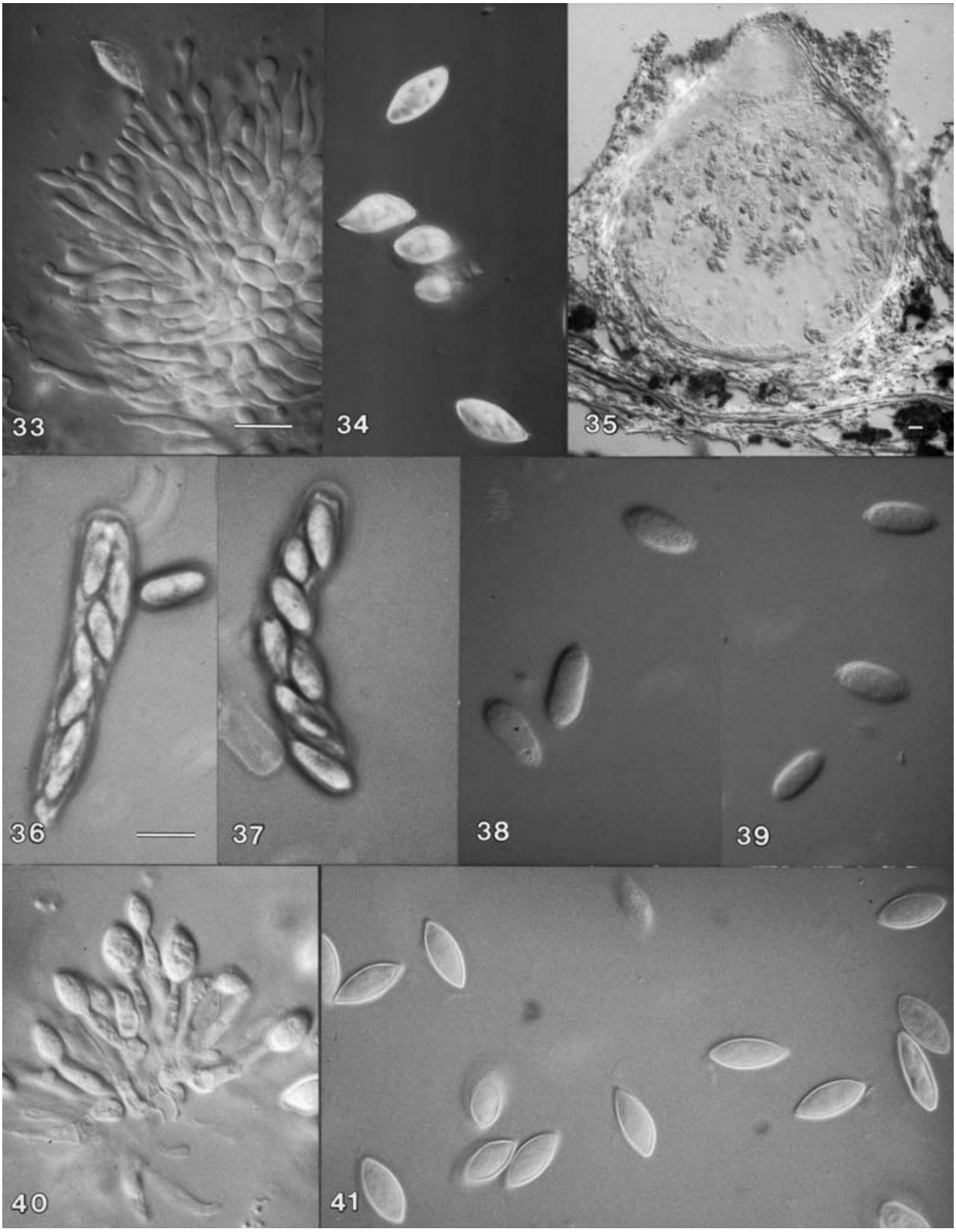
Schizoparme destruens (M. E. Barr & Hodges) Samuels, M. E. Barr & Lowen, *Mycotaxon* **46**: 470 (1993). (Figs 30–32, 35–41)

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.

Typus: **USA**: *Hawaii*: on twigs of *Eucalyptus grandis*, Oct. 2000, *M. J. Wingfield* (herb. CBS 6945 – holotypus).

Perithecia caulicolous, 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 33–41. Morphological structures of *Pilidiella petrakioidea* and *Schizoparme destruens*. **Fig. 33.** Conidiogenous cells of *P. petrakioidea*. **Fig. 34.** Conidia of *P. petrakioidea*. **Fig. 35.** Vertical section through a perithecium of *S. destruens*. **Figs 36–37.** Asci of *S. destruens*. **Figs 38–39.** Ascospores of *S. destruens*. **Fig. 40.** Conidiogenous cells of *C. destruens*. **Fig. 41.** Conidia of *C. destruens*. Bars = 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 chanal lined with slender, septate, hyaline, thin-walled periphysoids, 15–20 × 2–3 μm. *Asci* clavate,

35–50 × 7–14 μm; with inconspicuous apical apparatus, 8-spored, bi-seriate. *Ascospores* ellipsoidal, hyaline, thick-walled, granular, with terminal mucous caps, (9–)11–13 × (4.5–)5–6 μm.

Pycnidia intermingled between perithecia, globose, up to 200 μm wide; ostiole central, consisting of dark brown cells, up to 50 μm wide; wall 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, branched, hyaline, smooth, 15–25 × 3–5 μm. Conidiogenous cells slender, tapering towards a truncate apex, smooth, hyaline, 10–15 × 2–3 μm, 1 μm wide at the apex, with minute periclinal thickening, coated in mucous. Conidia long, fusoid–ellipsoidal, widest in the middle, tapering to an acutely rounded apex and subtruncate base with minute scar, pale to medium brown, granular, wall of medium thickness, (10–)12–13(–15) × (3–)4–5(–6) μm (l:w = 2.7).

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* teleomorph. 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). (Fig. 28)

Anamorph: *Pilidiella castaneicola* (Ellis & Everh.) Arx, *Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk*. **51**: 69 (1957).

Gloeosporium castaneicola Ellis & Everh., *Proc. Acad. Nat. Sci. Philadelphia* **1895**: 435 (1895).

Coniella castaneicola (Ellis & Everh.) B. Sutton, *The Coelomycetes*: 420 (1980).

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 . Cardinal temperature requirements for growth: min. 15 °, max. 30 °, opt. 20 °.

Notes: This species is commonly encountered on *Eucalyptus* leaves, but is generally regarded to be of minor importance as a foliar pathogen. Morphologically, 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).

Specimens examined: **South Africa**: *Gauteng*: Pretoria, Menlyn Shopping Centre, on leaves of *Eucalyptus camaldulensis*, Aug. 1989, *C. Roux* (PREM 50633, culture IMI 329098 = PPRI 3531); *Gauteng*: Pretoria, Menlyn Shopping Centre, on leaves of *Eucalyptus camaldulensis*, 2 Mar. 1990, *C. Roux* (PREM 49369); *Northern Province*: Pietersburg, Driedoornhoek, on plant debris, 1989, *E. van der Linde* (PREM 50631, culture IMI 334801); Tzaneen, on leaves of *Eucalyptus globulus* seedlings, 27 Mar. 1991, *P. W. Crous* (PREM 51101); *Mpumalanga*: Barberton, on leaves of *Eucalyptus* sp., 27 Mar. 1991, *P. W. Crous* (PREM 51098).

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 teleomorphs 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 characterised 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

Key to selected species

1 Conidia consistently hyaline, or with a greenish tinge 2
Conidia pale to dark brown when mature 3

2(1) Conidia ellipsoidal, 9–16 μm long (l:w = 1.9) . . . . . **Pilidiella granati**
Conidia fusiform to falcate or naviculate, 13–29 μm long (l:w = 7.1) **Schizoparme straminea (Pilidiella castaneicola)**

3(1) Conidial broadly ellipsoidal with obtuse apices, germ slits absent, (9–)10–11(–14) × (6–)7–8(–10) μm (l:w = 1.4) . . . . . **Coniella australiensis**
Conidia ellipsoidal, but tapering towards apices (l:w > 1.4) 4

4(3) Conidial mean l:w > 2 5
Conidial mean l:w < 2 6

5(4) Conidia fusoid–ellipsoidal, apices acutely rounded, (10–)12–13(–15) × (3–)4–5(–6) μm (l:w = 2.7) . . . . . **Schizoparme destruens (Pilidiella destruens)**
Conidia narrowly ellipsoidal, apices subobtusely rounded, (10–)12–15(–19) × (4–)5–6 μm (l:w = 2.3) . . . . . **Pilidiella diplodiella**

6(4) Conidia with germ slits; broadly ellipsoidal or limoniform (l:w = 1.5–1.6) 7
Conidia without germ slits; narrowly ellipsoidal (l:w = 1.7–1.8) 8

7(6) Conidia ellipsoidal, apices narrowly obtusely rounded, (8–)9–10(–12.5) × (5–)6–7(–8) μm (l:w = 1.5) . . . . . **Coniella fragariae**
Conidia broadly ellipsoidal or limoniform, apices acutely rounded, (9–)10–12(–14) × (6–)7–8 μm (l:w = 1.6) . . . . . **Pilidiella eucalyptorum**

8(6) Conidia (8–)10–12(–13) × (5–)6–7(–7.5) μm (l:w = 1.7) . . . . . **Pilidiella diplodiopsis**
Conidia (9–)10–12(–15) × 7–8 μm (l:w = 1.9) . . . . . **Pilidiella petrakioidea**

resolved based on these data. A re-examination of type material revealed that '*C.*' *diplodiella* 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, India, 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 acutely rounded apices, (9–)10–12(–15) × 4–5 μm.

Artificial inoculations on grapevines by von Tiedeman (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.

ACKNOWLEDGEMENTS

We thank Kenneth M. Old and Mark J. Dudzinski (CSIRO Forestry and Forest Products, Canberra), as well as the curators of the various herbaria cited for making cultures and specimens available for study.

REFERENCES

Arx, J. A. von (1973) Centraalbureau voor Schimmelcultures Baarn and Delft. Progress Reports 1972. *Verhandelingen der Koninklijke Nederlandsche Akademie van Wetenschappen, Afdeling Natuurkunde* **61**: 59–81.

Arx, J. A. von (1981) The Genera of Fungi Sporulating in Pure Culture, 3rd edn. J Cramer, Vaduz.

Bisiach, M. (1988) White Rot. In *Compendium of Grape Diseases* (R. C. Pearson & A. C. Goheen, eds): 22–23. American Phytopathological Society Press, St Paul, MN.

Carbone, I. & Kohn, L. M. (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553–556.

Castlebury, L. A., Rossman, A. Y., Jaklitsch, W. J. & Vasilyeva, L. N. (2002) A preliminary overview of the *Diaporthales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia* **94**: 1017–1031.

Crous, P. W. & Carstens, E. (2000) Confusion surrounding white rot disease of grapevines. *Winelands* **9**: 89–90.

Crous, P. W., Phillips, A. J. L. & Baxter, A. P. (2000) *Phytopathogenic Fungi from South Africa*. Department of Plant Pathology Press, Stellenbosch.

Farris, J. S., Kallersjo, M., Kluge, A. G. & Bult, C. (1994) Testing significance of incongruence. *Cladistics* **10**: 315–320.

Glass, N. L. & Donaldson, G. C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* **61**: 1323–1330.

Hillis, D. M. & Bull, J. J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**: 182–192.

Jarvis, W. R. & Hargreaves, A. J. (1972) *Coniothyrium fragariae* on strawberry in Scotland. *Plant Pathology* **21**: 47.

Lee, S. B. & Taylor, J. W. (1990) Isolation of DNA from fungal mycelia and single spores. In *PCR Protocols: A Guide to Methods and Applications* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 282–287. Academic Press, San Diego.

Lundquist, J. E. & Baxter, A. P. (1985) Fungi associated with *Eucalyptus* in South Africa. *South African Forestry Journal* **135**: 9–19.

Maas, J. L., Pollack, F. G. & Uecker, F. A. (1979) Morphology and development of *Pilidiella quercicola*. *Mycologia* **71**: 93–102.

Marasas, W. F. O. & Van Der Westhuizen, G. C. A. (1971) New and interesting records of South African fungi, part VII. *Bothalia* **10**: 411–416.

Matthee, F. N. & Thomas, A. C. (1981) Rot Blanc in vines: a new disease in South Africa. *Deciduous Fruit Grower* **31**: 268–273.

Nag Raj, T. R. (1993) *Coelomycetous Anamorphs with Appendage-bearing Conidia*. Mycologue Publications, Waterloo.

Page, R. D. M. (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**: 357–358.

Park, R. F., Keane, P. J., Wingfield, M. J. & Crous, P. W. (2000) Fungal diseases of eucalypt foliage. In *Diseases and Pathogens of Eucalypts* (P. J. Keane, G. A. Kile, F. D. Podger & B. N. Brown eds): 153–239. CSIRO Publishing, Victoria.

Rambaut, A. (2002) *Sequence Alignment Editor*. Version 2.0. Department of Zoology, University of Oxford, Oxford.

Rehner, S. A. & Samuels, G. J. (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98**: 625–634.

Rayner, R. W. (1970) *A Mycological Colour Chart*. Commonwealth Mycological Institute, Kew.

Samuels, G. J., Barr, M. E. & Lowen, R. (1993) Revision of *Schizoparme* (*Diaporthales*, *Melanconidaceae*). *Mycotaxon* **46**: 459–483.

Sharma, J. K., Mohanan, C. & Maria Florence, E. J. (1985) Disease survey in nurseries and plantations of forest tree species grown in Kerala. *Kerala Forest Research Institute Research Report* **36**: 1–268.

Shear, C. L. (1923) Life histories and undescribed genera and species of fungi. *Mycologia* **15**: 120–131.

Sutton, B. C. (1969) Type studies of *Coniella*, *Anthasthoopa*, and *Cyclodomella*. *Canadian Journal of Botany* **47**: 603–608.

Sutton, B. C. (1980) *The Coelomycetes*. Commonwealth Mycological Institute, Kew.

Sutton, B. C. & Waterston, J. M. (1966) *Coniella diplodiella*. *CMI Descriptions of Pathogenic Fungi and Bacteria* **82**: 1–2.

Swofford, D. L. (2000) *PAUP* 4.0: Phylogenetic Analysis Using Parsimony*. Sinauer Associates, Sunderland, MA.

Tiedeman, A. von (1985) *Untersuchungen zur Pathogenität des Erregers der Weissfäule* (*Coniella petrakii Sutt*.) *an Amerikaner- und Europäerreben und zur Verbreitung und Bedeutung des Pilzes in den Deutschen Weinbaugebieten*. PhD dissertation, Georg-August University, Göttingen.

Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. Academic Press, San Diego.

Corresponding Editor: S. Takamatsu