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# Co-occurring species of *Teratosphaeria* on *Eucalyptus*

P.W. Crous[1], J.Z. Groenewald[1], B.A. Summerell[2], B.D. Wingfield[3], M.J. Wingfield[3]

**Key words**

*Colletogloeopsis*
*Coniothyrium*
*Eucalyptus*
*Kirramyces*
*Mycosphaerella*
*Mycosphaerella* leaf disease
*Readeriella*
taxonomy

**Abstract** A common leaf spot disease occurring on *Eucalyptus cladocalyx* and *E. lehmannii* in the Western Cape Province of South Africa is known from literature to be caused by the fungus *Coniothyrium ovatum*, which is a pathogen native to several eucalypts in Australia. Recent collections have shown that Australian material identified as *C. ovatum* is morphologically and phylogenetically distinct from the South African specimens, and that all these taxa would be better accommodated in the genus *Teratosphaeria*. South African specimens previously identified as *C. ovatum* were found to represent two species that co-occur in the same leaves and even spots and are described here as *T. juvenalis* and *T. verrucosa*. Furthermore, a fresh collection of *T. ovata* from *E. phoenicea* in Australia, is distinguished morphologically and phylogenetically from similar, newly described taxa such as *T. veloci* on *E. miniata*, and *Readeriella dimorpha*, which is also placed in *Teratosphaeria*. Although these leaf pathogens appear to be of minor economic importance, they are morphologically similar to two serious eucalypt canker pathogens, namely *T. gauchensis* and *T. zuluensis*, which predominantly cause stem cankers, but could also be found occurring in leaf spots on their own, or in association with some of the other species treated here. Further research is, therefore, required to develop molecular detection techniques for these taxa to enable researchers to rapidly distinguish the minor pathogens from the more serious quarantine pathogens that co-occur on leaves.

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## INTRODUCTION

Prior to the application of culture-based and DNA sequence-based studies, host preference was the most important characteristic used to distinguish species of *Mycosphaerella* and their associated anamorphs (Crous & Braun 2003, Aptroot 2006). When detailed studies of ascospore germination patterns, anamorph states and DNA phylogenies emerged, it became clear that many species in the Mycosphaerella leaf disease complex could co-occur on the same lesions on a single host (Crous & Wingfield 1996, Crous 1998). In this regard, species in the *Teratosphaeria* complex are no different from those in *Mycosphaerella*, with many different taxa frequently co-occurring in the same leaf lesion (Crous et al. 2004a, b, 2007, 2008a, b, Crous & Groenewald 2005, Burgess et al. 2007, Arzanlou et al. 2008, Cheewangkoon et al. 2008) or substrate (Ruibal et al. 2008).

In the 1950s, an unidentified coniothyrium-like fungus was collected on leaf spots of *E. cladocalyx* and *E. lehmannii* in the coastal regions of the Western Cape Province of South Africa. A specimen of the fungus (PREM 49001), was later identified by Dr H.J. Swart as *C. ovatum* (Wingfield 1987). In a subsequent study, Crous et al. (1989) determined its ability to infect *Eucalyptus* leaves. A re-examination of the type material of *C. ovatum* and *C. parvum* led Crous (1998) to reduce *C. parvum* to synonymy with *C. ovatum* due to their similar morphology. Furthermore, based on the differences in morphology and symptomatology between Australian and South African collections Crous (1998) concluded that the South African collections probably represented an undescribed species, which could only be treated subsequent to clarifying the substantially confused phylogeny of the genus *Coniothyrium*.

*Coniothyrium* is typified by *C. palmarum* (*Pleosporales*) and is polyphyletic (Lennox et al. 2004, Verkley et al. 2004, Crous et al. 2006a, Damm et al. 2008, Marincowitz et al. 2008a). Species that resemble members of the *C. ovatum* complex belong to the *Teratosphaeriaceae*, and they have teleomorphs in *Teratosphaeria* (Crous et al. 2007).

The first potential link between *C. ovatum* and a teleomorph was made by Milgate et al. (2001), who regarded *C. ovatum* as the anamorph of *Teratosphaeria molleriana* (as *Mycosphaerella vespa*) (Hunter et al. 2006, Crous et al. 2007). In further studies, Cortinas et al. (2006a, b) showed that these *Teratosphaeria* anamorphs would be more appropriately accommodated in *Colletogloeopsis*, a genus that was subsequently emended to accommodate taxa with acervular to pycnidial conidiomata and 0–1-septate conidia, formerly in *Coniothyrium*, *Kirramyces* or *Phaeophleospora*. Contrary to Cortinas et al. (2006a, b), Andjic et al. (2007) chose to place all these anamorphs in *Kirramyces*, while Crous et al. (2007) used the older name, *Readeriella*.

Although uncertainty existed regarding the species status of the South African collections of *Coniothyrium ovatum* (Crous 1998), recent papers have failed to provide any consensus regarding an appropriate anamorph genus for this complex on *Eucalyptus*. The aim of this study, was thus to re-evaluate the taxonomic position of *C. ovatum* and similar taxa, based on their morphology and a phylogenetic analysis of DNA sequence data for the internal transcribed spacer region (ITS1, 5.8S, ITS2), and the large subunit (28S) of the nuclear rDNA operon.

## MATERIAL AND METHODS

### *Isolates*

*Eucalyptus* leaves infected with the fungus previously treated as *C. ovatum* were collected in the Western Cape Province of South Africa, as well as in the Northern Territory and the Australian Capital Territory in Australia. Single-conidial isolates were established

[1] CBS Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; corresponding author e-mail: p.crous@cbs.knaw.nl.
[2] Royal Botanic Gardens and Domain Trust, Mrs. Macquaries Road, Sydney, NSW 2000, Australia.
[3] Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa.

**Table 1** Isolates included in this study for sequence analysis and morphological comparison. Sequences in round parentheses were not included in the phylogenetic analyses.

| Species | Accession no.[1] | Substrate | Country | Collector | GenBank Accession number[2] |
|---|---|---|---|---|---|
| *Catenulostroma macowanii* | CBS 111029; CPC 1488 | *Protea* sp. | South Africa | P.W. Crous | (AY260096), FJ493199 |
| *Cibiessia dimorphospora* | CPC 13627 | *Eucalyptus canobolensis* | Australia | B.A. Summerell | (FJ493182), FJ493200 |
| *Cibiessia nontingens* | CPC 14508 | *Eucalyptus oblonga* | Australia | B.A. Summerell | (FJ493183), FJ493201 |
| *Colletogloeopsis* sp. | CPC 13675 | *Eucalyptus canobolensis* | Australia | B.A. Summerell | (FJ493184), FJ493202 |
| *Kirramyces corymbiae* | CPC 13125 | *Eucalyptus henryii* | Australia | A.J. Carnegie | (FJ493185), FJ493203 |
| *Kirramyces viscidus* | CPC 13306 | *Eucalyptus* sp. | Australia | P.W. Crous | (FJ493186), FJ493204 |
| '*Phaeophleospora*' *concentrica* | CPC 3615 | *Protea caffra* | Kenya | P.F. Cannon & P.M. Kirk | (FJ493187), FJ493205 |
| *Phaeophleospora eugeniae* | CPC 15143 | *Eugenia uniflora* | Brazil | A.C. Alfenas | (FJ493188), FJ493206 |
| | CPC 15159 | *Eugenia uniflora* | Brazil | A.C. Alfenas | (FJ493189), FJ493207 |
| *Phaeophleospora* sp. | CPC 2557 | *Eugenia uniflora* | Brazil | A.C. Alfenas | (FJ493190), FJ493208 |
| | CPC 2558 | *Eugenia uniflora* | Brazil | A.C. Alfenas | (FJ493191), FJ493209 |
| '*Phaeophleospora*' *stonei* | CPC 13330 | *Eucalyptus* sp. | Australia | P.W. Crous | (EF394856), FJ493210 |
| '*Readeriella*' *guyanensis* | CBS 117550; MUCL 46082 | Leaf litter | French Guiana | C. Decock | (EU707900), FJ493211 |
| *Readeriella mirabilis* | CPC 12370 | *Eucalyptus globulus* | Australia | I. Smith | (FJ493192), FJ493212 |
| | CPC 13633 | *Eucalyptus delegatensis* | Tasmania | B.A. Summerell | (FJ493193), FJ493213 |
| *Readeriella novaezelandiae* | CPC 10895 | *Eucalyptus botryoides* | New Zealand | M. Dick | (AY725578), FJ493214 |
| *Teratosphaeria dimorpha* | CPC 14132 | *Eucalyptus caesia* | Australia | B.A. Summerell | FJ023537, FJ493215 |
| *Teratosphaeria flexuosa* | CBS 111048; CPC 1199 | *Eucalyptus grandis* | Colombia | M.J. Wingfield | (FJ493194), FJ493216 |
| *Teratosphaeria juvenalis* | CBS 110906; CPC 40; CRY 13347 | *Eucalyptus cladocalyx* | South Africa | P.W. Crous | AY725513, FJ493217 |
| | CBS 111149; CPC 23 | *Eucalyptus cladocalyx* | South Africa | P.W. Crous | AY725514, EU019294 |
| | CBS 116427; CPC 10941 | *Eucalyptus* sp. | South Africa | P.W. Crous | AY725516, — |
| *Teratosphaeria ovata* | CPC 14632 | *Eucalyptus phoenicea* | Australia | B.A. Summerell | FJ023538, FJ493218 |
| *Teratosphaeria readeriellophora* | CPC 12920 | *Eucalyptus* sp. | Australia | A.J. Carnegie | (FJ493195), FJ493219 |
| *Teratosphaeria* sp. | CPC 12821 | *Eucalyptus nitens* | Australia | A.J. Carnegie | (FJ493196), FJ493220 |
| | CPC 14597 | *Eucalyptus miniata* | Australia | B.A. Summerell | (FJ493197), FJ493221 |
| *Teratosphaeria suttonii* | CPC 11279 | *Eucalyptus tereticornis* | Bolivia | M.J. Wingfield | (DQ303055), FJ493222 |
| *Teratosphaeria toledana* | CBS 115513 | *Eucalyptus* sp. | Spain | P.W. Crous & G. Bills | (FJ493198), FJ493225 |
| *Teratosphaeria veloci* | CPC 14600 | *Eucalyptus miniata* | Australia | B.A. Summerell | FJ023539, FJ493223 |
| *Teratosphaeria verrucosa* | CBS 113621; CPC 42 | *Eucalyptus cladocalyx* | South Africa | P.W. Crous | AY725515, — |
| | CPC 12949 | *Eucalyptus* sp. | South Africa | P.W. Crous | FJ023540, FJ493224 |
| | CPC 18 | *Eucalyptus cladocalyx* | South Africa | P.W. Crous | AY725517, EU019293 |

[1] CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; MUCL: Université Catholique de Louvain, Louvain-la-Neuve, Belgium; CMW & CRY: Culture collection of Mike Wingfield, housed at FABI, Pretoria, South Africa.
[2] ITS: Internal transcribed spacers 1 and 2 together with 5.8S nrDNA; LSU: 28S nrDNA.

on malt extract agar (MEA; 20 g/L Biolab malt extract, 15 g/L Biolab agar) using the technique of Crous (1998). Cultures were plated onto fresh MEA and oatmeal agar (OA; Gams et al. 2007), and subsequently incubated at 25 °C under near-ultraviolet light to promote sporulation. Reference strains are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands and in the collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa (Table 1). Descriptions, nomenclature, and illustrations were deposited in MycoBank.

### *DNA isolation, amplification and analyses*

Genomic DNA was extracted from mycelia taken from fungal colonies on MEA using the UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA, USA). The Primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White et al. 1990) and LR0R (Rehner & Samuels 1994) were used as internal sequence primers to ensure high quality sequences over the entire length of the amplicon. The PCR amplifications were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) in a total volume of 12.5 µL solution containing 10–20 ng of template DNA, 1 × PCR buffer, 2.5 mM $MgCl_2$, 15 pmol for each primer, 60 µM of each dNTP and 0.75 U *Taq* DNA polymerase (Bioline GmbH, Luckenwalde, Germany). PCR amplification conditions were set as follows: an initial denaturation temperature of 94 °C for 5 min, followed by 40 cycles of denaturation temperature of 94 °C for 45 s, primer annealing at 48 °C for 30 s, primer extension at 72 °C for 90 s and a final extension step at 72 °C for 7 min. The resulting fragments were sequenced using the PCR primers together with a BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, Foster City, CA) and analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, CN).

Separate alignments were made for the ITS and LSU regions. The generated sequences were compared with other fungal DNA sequences from NCBI's GenBank sequence database using a blastn search; sequences with high similarity were added to the alignments. Additional GenBank sequences were manually aligned using Sequence Alignment Editor v. 2.0a11 (Rambaut 2002). Phylogenetic analyses of the aligned sequence data were performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) and consisted of neighbour-joining analyses with the uncorrected ('p'), the Kimura 2-parameter and the HKY85 substitution models. Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Any ties were broken randomly when encountered. For parsimony analyses, alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed using the heuristic search option with 100 random (ITS) or simple (LSU) 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 trees obtained was evaluated by 1 000 bootstrap replications (Hillis & Bull 1993). Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated and the resulting trees were printed with TreeView v. 1.6.6 (Page 1996). New sequences were lodged in GenBank and the alignments and phylogenetic trees in TreeBASE (www.treebase.org).

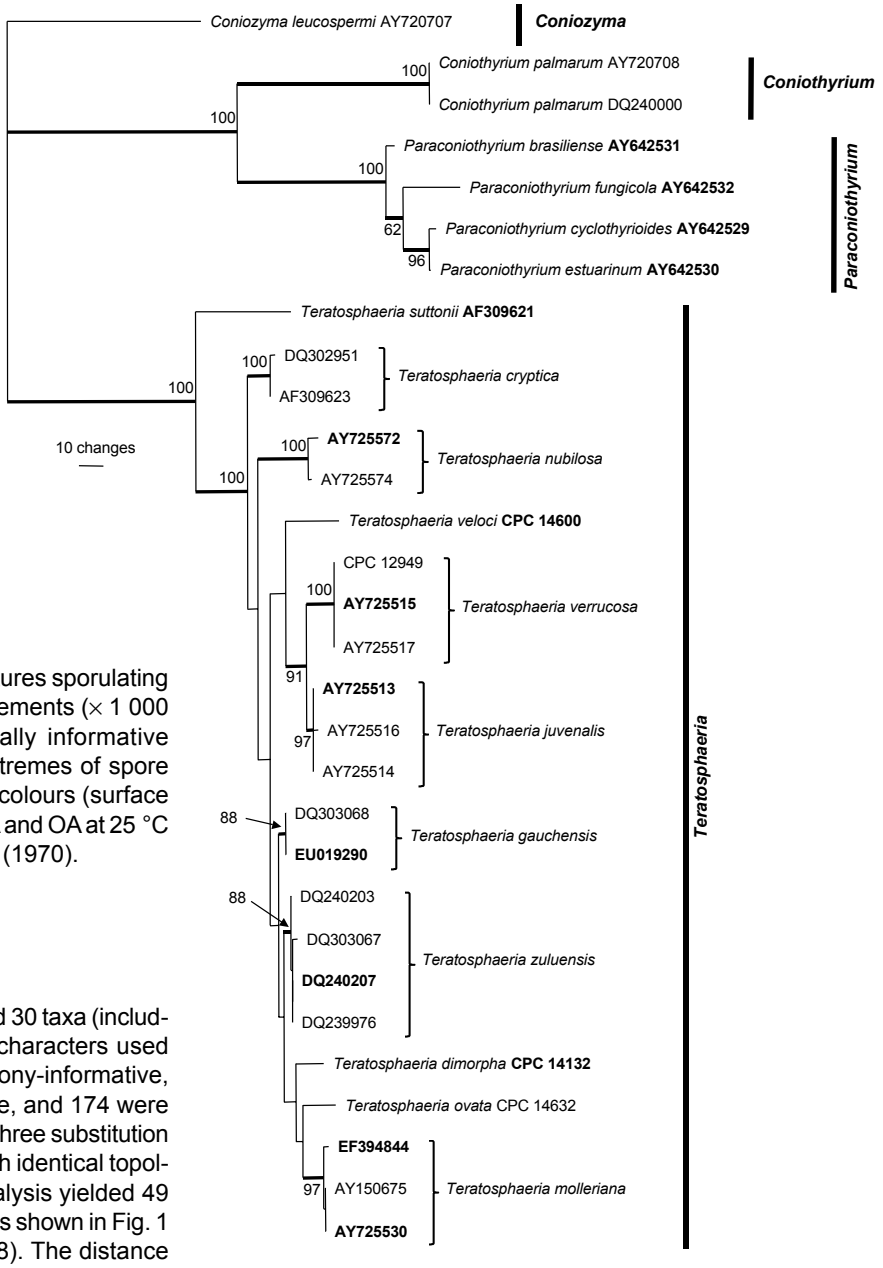


**Fig. 1** One of 49 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the ITS sequence alignment using PAUP v. 4.0b10. The scale bar shows 10 changes, and bootstrap support values 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and ex-type sequences are printed in bold face. The tree was rooted to *Coniozyma leucospermi* (GenBank accession AY720707).

### *Morphology*

Morphological descriptions were based on cultures sporulating on OA in vivo. Wherever possible, 30 measurements (× 1 000 magnification) were made of all taxonomically informative structures mounted in lactic acid, with the extremes of spore measurements given in parentheses. Colony colours (surface and reverse) were assessed after 1 mo on MEA and OA at 25 °C in the dark, using the colour charts of Rayner (1970).

## RESULTS

### *Phylogenetic analysis*

The manually adjusted ITS alignment contained 30 taxa (including the outgroup sequence) and, of the 464 characters used in the phylogenetic analysis, 256 were parsimony-informative, 34 were variable and parsimony-uninformative, and 174 were constant. Neighbour-joining analysis using the three substitution models on the sequence data yielded trees with identical topology and bootstrap values. The parsimony analysis yielded 49 equally most parsimonious trees, one of which is shown in Fig. 1 (TL = 637, CI = 0.776, RI = 0.887, RC = 0.688). The distance and parsimony trees differed only with regard to the arrangement of species within *Paraconiothyrium* and *Teratosphaeria* (data not shown; position of strict consensus branches within the *Teratosphaeria* clade in Fig. 1).

The manually adjusted LSU alignment contained 87 taxa (including the outgroup sequence) and, of the 796 characters used in the phylogenetic analysis, 191 were parsimony-informative, 57 were variable and parsimony-uninformative, and 548 were constant. Neighbour-joining analysis using the three substitution models on the sequence data yielded trees with identical topology and bootstrap values. For the parsimony analysis, only the first 1 000 equally most parsimonious trees were saved, one of which is shown in Fig. 2 (TL = 610, CI = 0.557, RI = 0.863, RC = 0.481). The distance and parsimony trees differed only with regard to the arrangement of species within the clades (data not shown; position of strict consensus branches in Fig. 2).

### *Taxonomy*

Phylogenetic analyses based on the LSU sequence data showed that four clades could be distinguished in the *Teratosphaeriaceae*. Clade 1 (*Readeriella*) also had *Cibiessia* and *Nothostrasseria* anamorphs, while Clade 2 (*Batcheloromyces*) also had phaeophleospora-like anamorphs. Clade 3 contained only *Teratosphaeria* teleomorphs. Clade 4 (*Teratosphaeria* s.str.) had *Kirramyces* (incl. *Colletogloeopsis*), batcheloromyces-like and *Catenulostroma* anamorphs. Crous et al. (2007) used a wider concept for *Readeriella* and recognised it as being polyphyletic within the *Teratosphaeriaceae*. Furthermore, based on its similar conidiogenesis to *Kirramyces*, with conidiogenous cells ranging from mono- to polyphialides with periclinal thickening, to phialides with percurrent proliferation, the two genera were seen as synonymous. As can be seen in the present analysis (Fig. 2), however, there are at least four well-defined clades within the *Teratosphaeriaceae*. Although they are morphologically similar, *Readeriella* species have conidia that tend to have tapering subtruncate bases and frequently form *Cibiessia* synanamorphs. In contrast, *Kirramyces* (incl. *Colletogloeopsis*) anamorphs have truncate conidial bases and are never found associated with *Cibiessia* synanamorphs. *Nothostrasseria* (1983) has a similar conidiogenesis to *Readeriella* and forms conidia with a basal appendage, which can also occur in *Readeriella* (1908) (Crous et al. 2007). For the present it seems best to retain *Nothostrasseria* until more taxa have been collected, but the basal appendage does not appear to be a generic feature in the *Teratosphaeriaceae*. *Batcheloromyces*, which clusters intermediate between these two larger clades,

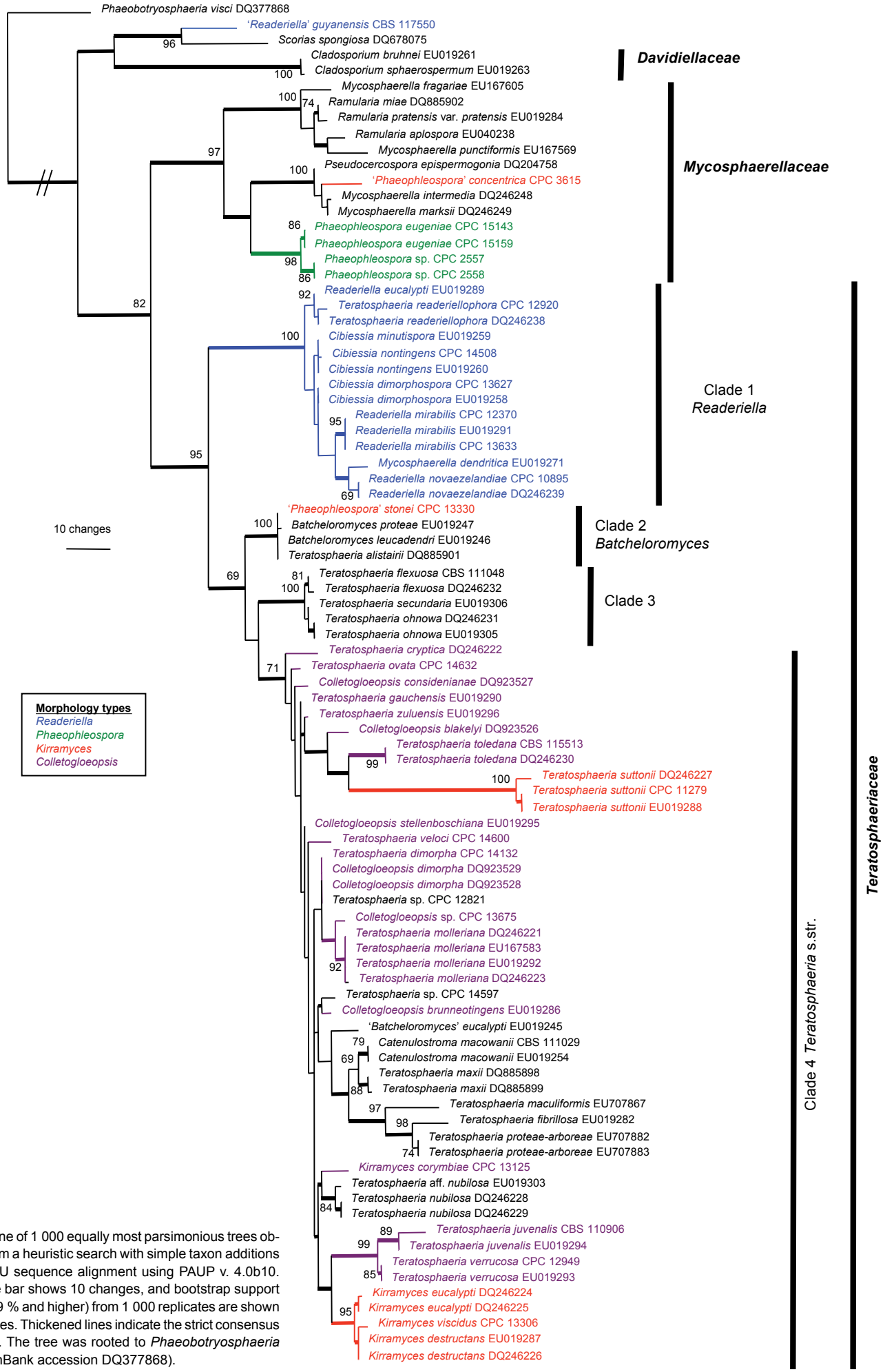


**Fig. 2** One of 1 000 equally most parsimonious trees obtained from a heuristic search with simple taxon additions of the LSU sequence alignment using PAUP v. 4.0b10. The scale bar shows 10 changes, and bootstrap support values (69 % and higher) from 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches. The tree was rooted to *Phaeobotryosphaeria visci* (GenBank accession DQ377868).

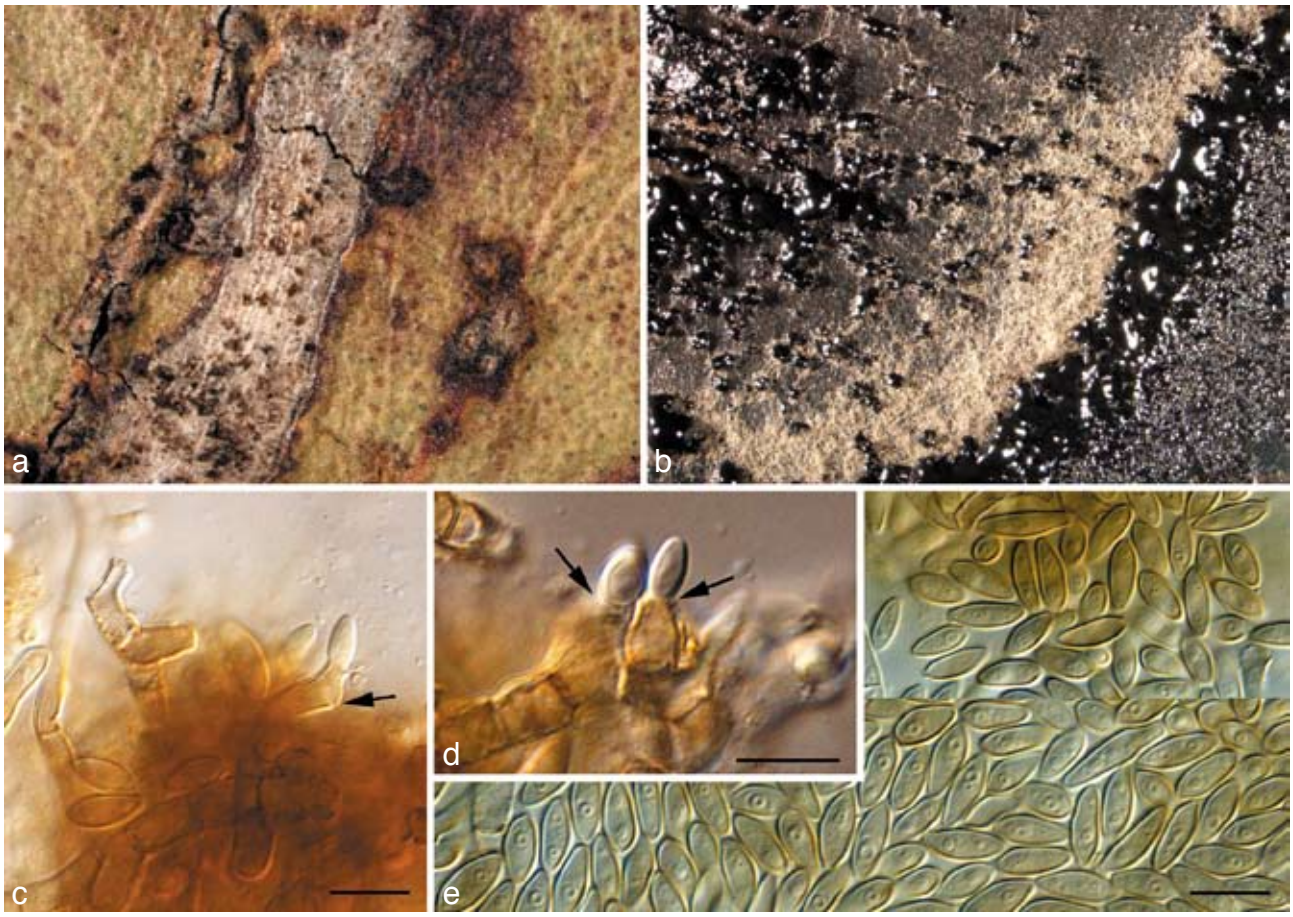


**Fig. 3** *Teratosphaeria dimorpha* (CPC 14132). a. Leaf spot; b. colony on OA; c, d. conidiogenous cells with sympodial and percurrent proliferation (arrows); e. conidia. — Scale bars = 10 µm.

is readily distinguishable, but also poorly understood. *Teratosphaeria* as defined by Crous et al. (2007) is thus heterogeneous, and will be separated into more natural units or genera as more taxa and collected are added to the analysis.

South African isolates of '*Coniothyrium' ovatum* represented two distinct species (Fig. 1). These taxa could also be distinguished from '*Coniothyrium' ovatum* s.str. and each other based on their morphological and cultural characteristics. Furthermore, during the course of this study several additional species, morphologically similar to '*C.' ovatum*, were collected from *Eucalyptus* hosts in Australia, which are also treated here as members of *Teratosphaeria* s.str., clustering in Clade 4 (Fig. 2).

***Teratosphaeria dimorpha*** (Crous & Carnegie) Crous & Summerell, *comb. nov*. — MycoBank MB508347; Fig. 3

*Basionym*. *Colletogloeopsis dimorpha* Crous & Carnegie, Fungal Diversity 23: 345. 2006.

≡ *Readeriella dimorpha* (Crous & Carnegie) Crous & U. Braun, Stud. Mycol. 58: 26. 2007.

*Leaf spots* amphigenous, irregular blotches 2–5 mm diam, pale brown with a thin, raised border, and red-purple margin. *Conidiomata* amphigenous, predominantly hypophyllous, pycnidial, substomatal, brown, globose, up to 90 µm diam; wall consisting of 3–4 layers of brown *textura angularis*; becoming large and acervular when cultivated on agar. Description on OA (CPC 14132). *Conidiogenous cells* brown, verruculose, aseptate, dolliform to ampulliform, proliferating percurrently near apex, 5–10 × 3–5 µm; sympodial proliferation observed in culture. *Conidia* solitary, brown, aseptate, verruculose, guttulate, ellipsoidal to subcylindrical, apex subobtuse, tapering to a subtruncate or truncate base (1–2 µm wide), with inconspicuous marginal frill, (7–)8–10(–13) × (2.5–)3(–3.5) µm.

Cultural characteristics — *Colonies* on MEA flat, spreading, without aerial mycelium, and with feathery margin, iron-grey, reverse greenish black, reaching 30 mm diam after 1 mo; on OA flat, spreading, without aerial mycelium and uneven feathery margin, iron-grey to greenish black, reaching 30 mm diam after 1 mo.

*Specimens examined*. Australia, New South Wales, Rosewood, on leaves of *Eucalyptus* sp., native regeneration within *Pinus radiata* plantation, Carabost State Forest, Downfall Road, about 3 km north-west of Rosewood, Southern Highlands, Jan. 2006, *A.J. Carnegie*, CBS H-19739 holotype, DAR 77443 isotype, culture ex-type CPC 12919 = CBS 120086; New South Wales, Laurel Hill, on *E. nitens*, in eucalypt species trial established within *P. radiata* plantation, Bago State Forest, 20 km north of Tumbarumba, Southern Highlands, Jan. 2006, *A.J. Carnegie*, DAR 77444, culture CPC 12798 = CBS 120085; Australian Capital Territory, Canberra, Australian National Botanical Garden, adjacent to Crosbie Morrison Building, on leaves of *E. caesia*, Mar. 2007, *B.A. Summerell*, CBS H-20179, cultures CPC 14132 = CBS 124051, CPC 14133, 14134.

Notes — The present collection (CPC 14132–CPC 14134) was initially assumed to be a distinct species, as cultures did not form the dimorphic conidia typical of *T. dimorpha* (Summerell et al. 2006). The dominant conidial morphology, symptomatology, and ITS sequence data, however, proved to be identical with that of the type strain of *T. dimorpha* (CPC 12919).

Although phylogenetically distinct, *T. dimorpha* is morphologically very similar to *T. ovata*. It can be distinguished from the latter species by its somewhat narrower conidia 2.5–3.5 µm (*T. ovata* 2.5–4 µm) and faster growth in culture.

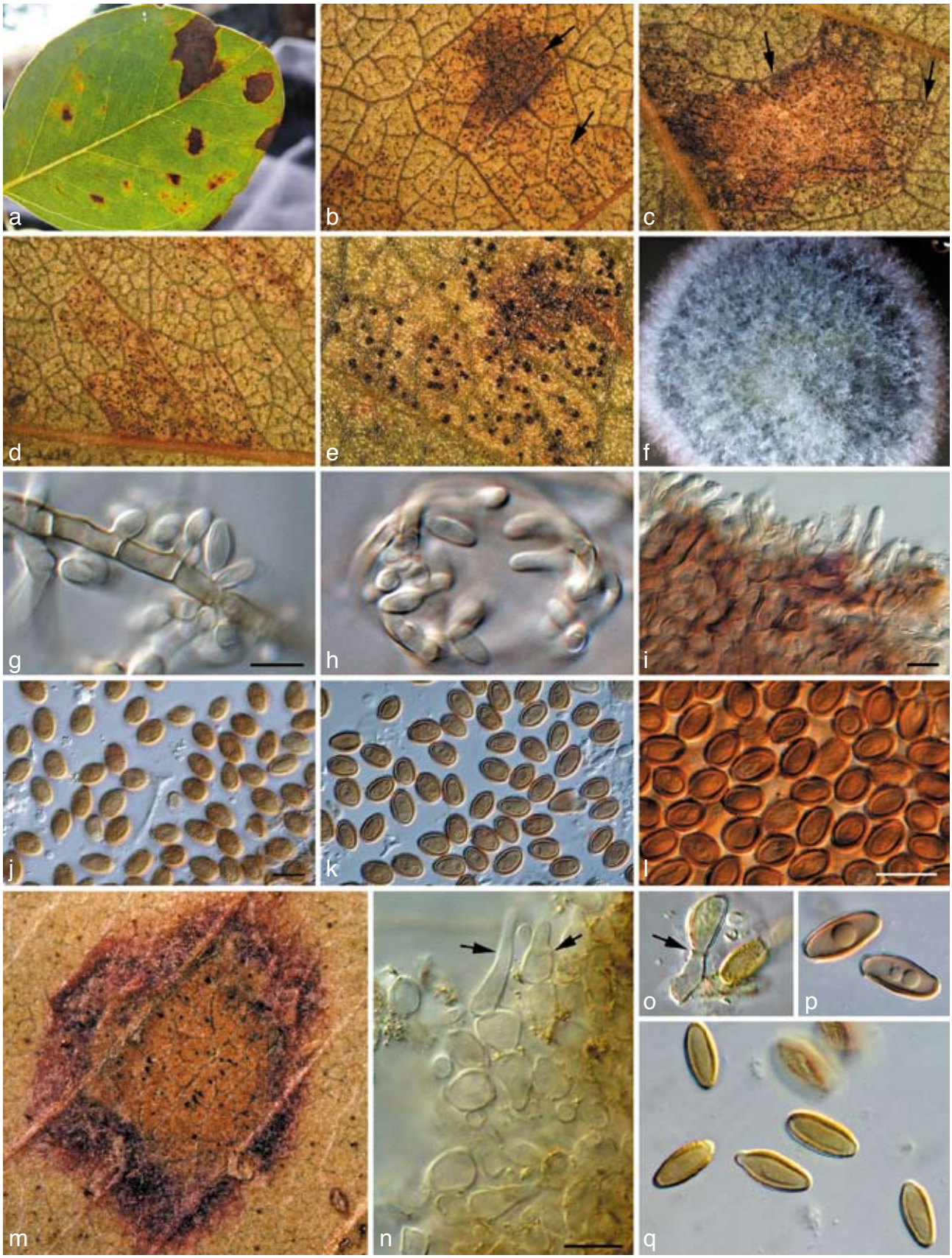


**Fig. 4** *Teratosphaeria juvenalis* and *T. verrucosa*. a. Leaf spot with mixed infection of both species; b–e. pycnidia of *T. verrucosa* on apparently healthy tissue, while those of *T. juvenalis* occur primarily in leaf spots (arrows). — f–l. *Teratosphaeria verrucosa* (CBS 113621): colony on PDA; g, h. sporulation on aerial hyphae; i. pycnidial wall with conidiogenous cells in vivo; j–l. conidia in vivo; m–q. *Teratosphaeria juvenalis* (CBS 110906); m. leaf spot associated with pycnidia; n, o. conidiogenous cells; p, q. conidia. — Scale bars = 10 µm.

***Teratosphaeria juvenalis*** Crous & M.J. Wingf., *sp. nov.* — MycoBank MB508348; Fig. 4, 5

*Teratosphaeriae ovatae* similis, sed conidiis longioribus et plus minusve subcylindraceis.

*Etymology.* Name refers to the fact that this fungus occurs on juvenile *Eucalyptus* leaves often associated with coppice regrowth.

*Leaf spots* raised, medium brown, circular, up to 7 mm diam; border medium to dark brown, raised, with a red-purple margin. Description based on material in vivo: *Conidiomata* substomatal, pycnidial, amphigenous, separate or aggregated, globose, up to 80 µm diam; wall of 2–3 layers of dark brown *textura angularis*. *Conidiogenous cells* ampulliform to doliiform, hyaline to medium brown, smooth to verruculose, proliferating percurrently, with irregular annulations, 3–12 × 5–6 µm. *Conidia* ellipsoidal to subcylindrical, apex subobtuse, base truncate to subtruncate, (1–)2–3(–4) µm wide, generally widest at the median, thin-walled, guttulate, verruculose, (10–)11–13(–15) × (4–)5(–6) µm; basal marginal frill present; cultures sterile.

Cultural characteristics — Colonies on MEA erumpent, with moderate aerial mycelium and feathery margins; surface fuscous-black with olivaceous-grey aerial mycelium and submerged greyish sepia margin; reverse fuscous-sepia; colonies reaching up to 25 mm after 1 mo. On PDA erumpent, spreading with moderate aerial mycelium and feathery margins; surface olivaceous-grey with patches of iron-grey; iron-grey in reverse; colonies reaching up to 30 mm diam after 1 mo. On OA erumpent, spreading, with moderate aerial mycelium and feathery margin; surface olivaceous-grey with patches of iron-grey and pale olivaceous-grey aerial mycelium; reaching up to 30 mm diam after 1 mo.

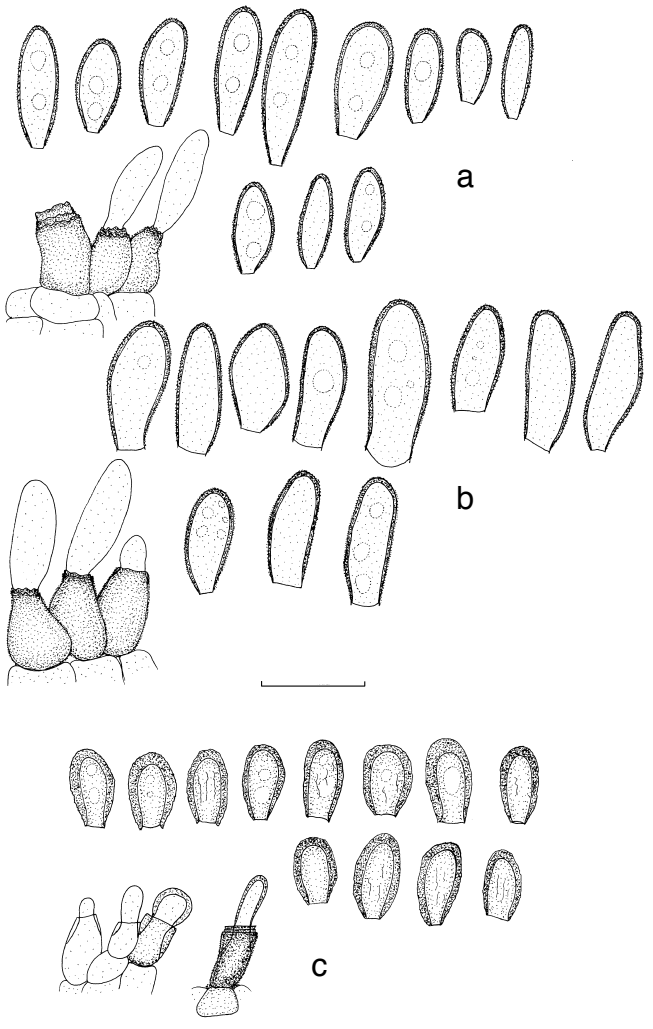


**Fig. 5** Conidia and conidiogenous cells of *Teratosphaeria* spp. a. *T. juvenalis* (CBS 110906); b. *T. ovata* (DAR 49461); c. *T. verrucosa* (CBS 113621). — Scale bar = 10 µm.

*Specimen examined*. South Africa, Western Cape Province, Stellenbosch, Stellenbosch Mountain, living leaves of *E. cladocalyx*, Apr. 1988, *P.W. Crous*, holotype CBS H-20180, culture ex-type CPC 40 = CMW 13347 = CBS 110906; Western Cape Province, Stellenbosch, Stellenbosch Mountain, living leaves of *E. cladocalyx*, 1 Dec. 2003, *P.W. Crous*, CPC 10941 = CBS 116427.

Notes — On average, conidia of *T. juvenalis* are longer than those of *T. ovata* and more subcylindrical in shape, with wider conidial hila. Conidia are thus more similar to those of *T. molleriana*, but distinct in being wider and having wider conidial hila than those observed in *T. molleriana*.

***Teratosphaeria ovata*** (H.J. Swart) Crous & Summerell, *comb. nov*. — MycoBank MB508349; Fig. 5–7

*Basionym*. *Coniothyrium ovatum* H.J. Swart, Trans. Brit. Mycol. Soc. 86: 495. 1986.

≡ *Coniothyrium parvum* H.J. Swart, Trans. Brit. Mycol. Soc. 86: 495. 1986.

*Specimens examined*. Australia, Victoria, Calder Highway, living leaves of *E. dives*, 17 May 1983, *H.J. Swart*, DAR 49461, holotype of *C. ovatum*; Victoria, Kinglake West, living leaves of *E. melliodora*, 2 June 1984, *H.J. Swart*, DAR 49462, holotype of *C. parvum*; Northern Territory, Edith Falls, on leaves of *E. phoenicea*, Sept. 2007, *B.A. Summerell*, CBS H-20181, cultures CPC 14632 = CBS 124052, CPC 14633.

Description based on DAR 49461: *Leaf spots* amphigenous, raised, medium brown, circular, 1–2 mm diam; border medium to dark brown, raised. *Conidiomata* substomatal, pycnidial, amphigenous, separate or aggregated, globose, up to 80 µm diam; wall of 2–3 layers of dark brown *textura angularis*. *Conidiogenous cells* ampulliform to doliiform or subcylindrical, proliferating percurrently and enteroblastically, 3–6(–14) × 4–6 µm. *Conidia* ellipsoidal, apex subobtuse, base truncate, generally widest at or below the median, finely verruculose, (6–)7–10(–14) × 3–3.5(–6) µm; basal marginal frill present.

Description based on CBS H-20181: *Leaf spots* amphigenous, irregular 1–5 mm diam, medium brown with a thin, raised border. *Conidiomata* amphigenous, pycnidial, substomatal, brown, globose, up to 150 µm diam; wall consisting of 3–4 layers of brown *textura angularis*; becoming large and acervular when cultivated on agar. Description of CPC 14632 on OA. *Conidiogenous cells* brown, verruculose, aseptate, dolliform to ampulliform, proliferating percurrently near apex, 5–15 × 3–4 µm; sympodial proliferation observed in culture. *Conidia* solitary, brown, aseptate, verruculose, guttulate, ellipsoidal to subcylindrical, apex subobtuse, tapering to a subtruncate or truncate base (1–2 µm wide), with inconspicuous marginal frill, (6–)7–10(–12) × (2.5–)3(–4) µm.

Cultural characteristics — *Colonies* on MEA erumpent, uneven, moderate to sparse aerial mycelium, with patches of white, smoke-grey to olivaceous-grey, margins uneven; reverse iron-grey, reaching 20 mm diam after 1 mo; on OA spreading with fluffy white-pink aerial mycelium; outer region pale olivaceous-grey, margin olivaceous-grey, uneven; reaching 30 mm diam after 1 mo.

Notes — In the description of *C. ovatum* and *C. parvum*, Swart (1986) noted that the main difference between these species lay in their conidial dimensions. A re-examination of these specimens by Crous (1998) showed that conidia of *C. parvum* were (7–)8–10(–12) × 3–3.5(–4) µm, thus somewhat longer than those of *C. ovatum* (6–)7–9(–11) × 3–3.5(–4) µm. Re-examination of the type of *C. ovatum* in this study revealed some conidia of up to 14 µm long and 6 µm wide.

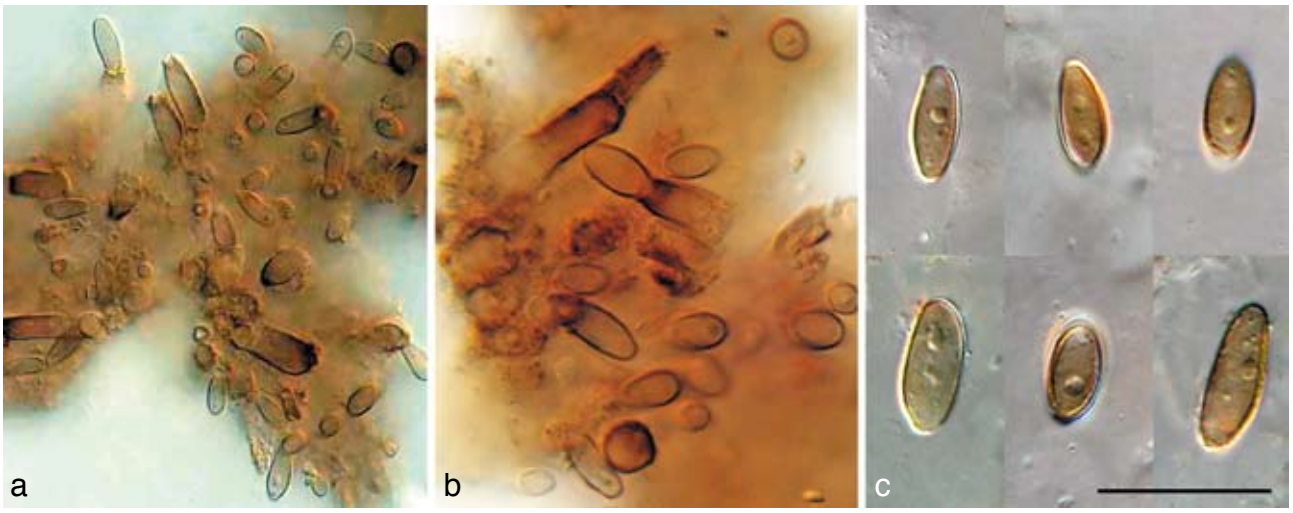


**Fig. 6** *Teratosphaeria ovata* (DAR 49461). a, b. Conidiogenous cells; c. conidia. — Scale bar = 10 µm.

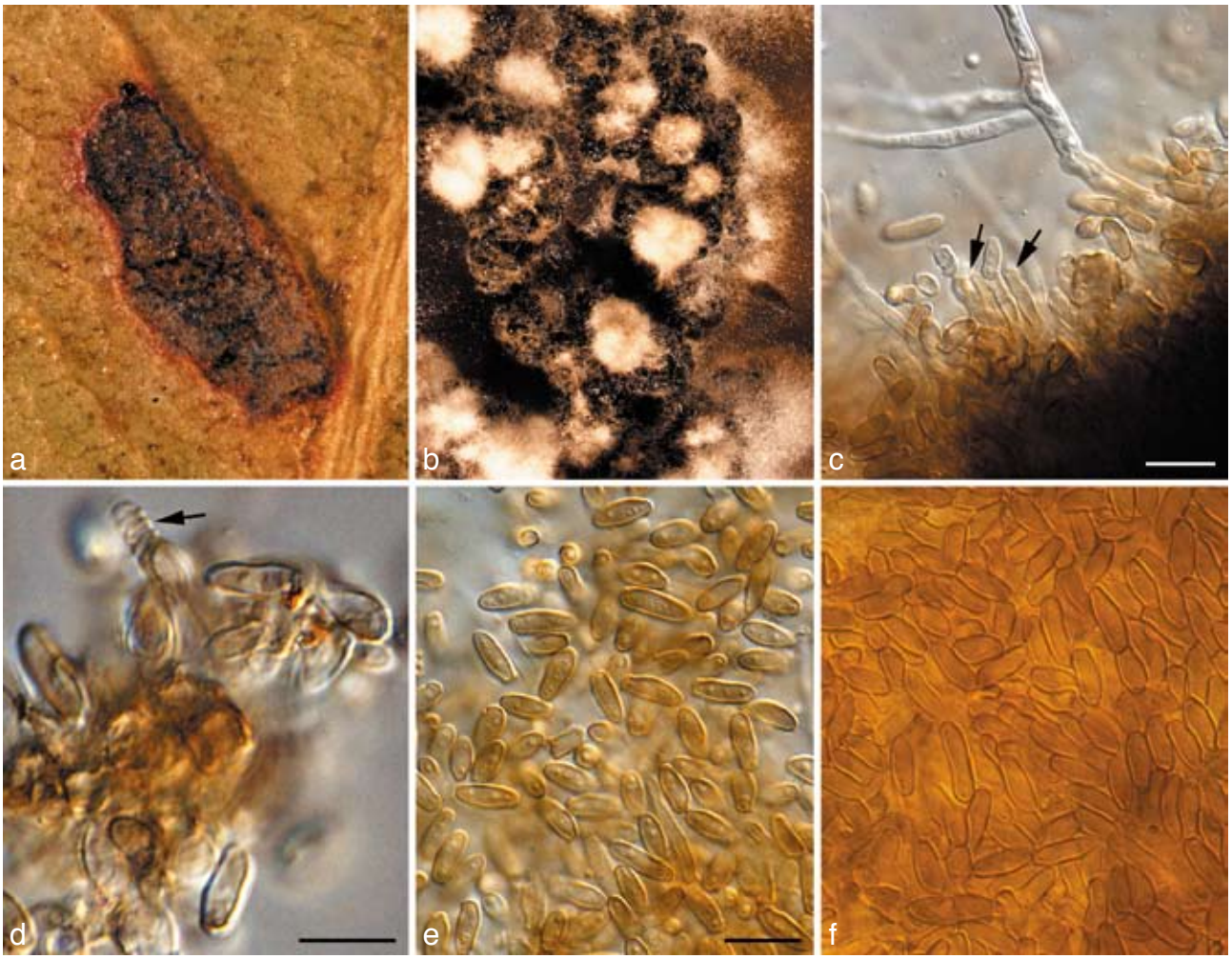


**Fig. 7** *Teratosphaeria ovata* (CPC 14632). a. Leaf spot; b. colony on OA; c, d. conidiogenous cells (arrows); e, f. conidia. — Scale bar = 10 µm.

Based on phylogenetic comparisons of multi-allelic data, Hunter et al. (2006) reduced *M. vespa* and *M. ambiphylla* to synonymy under *M. molleriana* (= *Teratosphaeria*). *Teratosphaeria molleriana* has conidia that are (7–)9–12(–13) × (2.5–)3–3.5(–4) µm (Crous 1998), while those of *M. ambiphylla* are (5–)10–15(–20) × (3–)3.5–4.5(–5) µm (Maxwell et al. 2003). No ex-type culture is available of *M. vespa*, but cultures collected and identified as *M. vespa* produced conidia which were (7.5–)9(–12) × 2.5–3(–5) µm (Milgate et al. 2001). Although these anamorphs show considerable overlap with *T. ovata* in conidial dimensions, they differ by having more subcylindrical to ellipsoidal conidia, and generally tend to have wider conidial bases (2 µm), than the narrower conidial bases of *T. ovata* (1–2 µm). The present collection (CPC 14632) closely matches *T. ovata* in host symptoms and morphology. Although *T. ovata* was reported from several hosts by Swart (1986), none of these were *Eucalyptus phoenicea*, which grows in extremely different ecosystems than *E. dives* or *E. molleriana*, and thus we refrain from designating this collection as epitype, pending further collections.

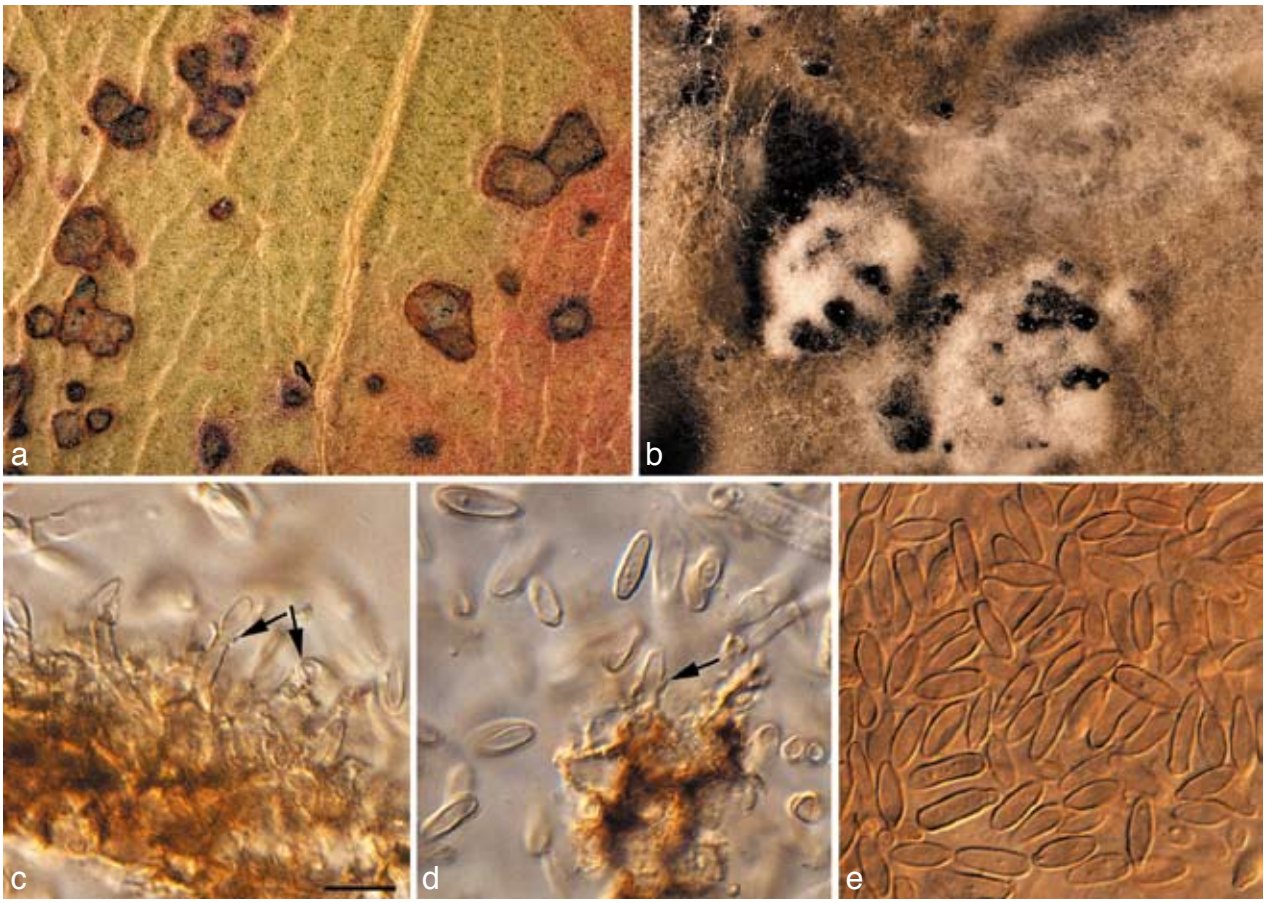


**Fig. 8** *Teratosphaeria veloci* (CPC 14600). a. Leaf spots; b. colony on OA; c, d. conidiogenous cells (arrows); e. conidia. — Scale bar = 10 µm.

***Teratosphaeria veloci*** Crous & Summerell, *sp. nov*. — MycoBank MB508350; Fig. 8

*Teratosphaeriae ovatae* similis, sed cellulis conidiogenis angustioribus et coloniis olivaceo-griseis, incremento in vitro (OA) citiore.

*Etymology*. Named after its fast growth rate in culture.

*Leaf spots* amphigenous, subcircular, 1–5 mm diam, pale brown with a thin, raised border and red-purple margin. *Conidiomata* amphigenous, pycnidial, substomatal, brown, globose, up to 120 µm diam; wall consisting of 3–4 layers of brown *textura angularis*; becoming large and acervular when cultivated on agar. Description on OA. *Conidiogenous cells* brown, verruculose, aseptate, dolliform to ampulliform, proliferating percurrently near apex, 4–8 × 2–3 µm; sympodial proliferation observed in culture. *Conidia* solitary, brown, aseptate, verruculose, guttulate, ellipsoidal to subcylindrical, apex subobtuse, tapering to a subtruncate or truncate base (1–2 µm wide), with inconspicuous marginal frill, (6–)8–10(–11) × (2.5–)3(–3.5) µm.

Cultural characteristics — *Colonies* on MEA flat, spreading, with sparse aerial mycelium, folded on surface, and smooth margins; pale olivaceous-grey with patches of white and olivaceous-grey; margin smoke-grey; reverse iron-grey; colonies reaching 30 mm diam after 1 mo; on OA flat, spreading with sparse aerial mycelium, olivaceous-grey, with patches of white and smoke-grey; margin smooth, regular; reaching 45 mm diam after 1 mo.

*Specimen examined*. Australia, Northern Territory, ENE Pine Creek, 13°40'49.0"S, 131°57'04.9"E, on leaves of *E. miniata*, 23 Sept. 2007, *B.A. Summerell*, CBS H-20182, cultures ex-type CPC 14600, 14601, 14602 = CBS 124061.

Notes — *Teratosphaeria veloci* is morphologically similar to *T. dimorpha* and *T. ovata*, but can be distinguished from those species based on its narrower conidiogenous cells and olivaceous-grey colonies that grow faster on OA than those of the other two species.

***Teratosphaeria verrucosa*** Crous & M.J. Wingf., *sp. nov*. — MycoBank MB508351; Fig. 4, 5

*Teratosphaeriae ovatae* similis, sed conidiis latioribus, parietibus crassioribus et verrucosis.

*Etymology.* Name refers to the rough-walled conidia in this species.

*Leaf spots* absent, sporulating with long black cirri from submerged pycnidia in apparently healthy, green tissue, or occurring in lesions that are amphigenous, raised, medium brown, circular, up to 7 mm diam; border medium to dark brown, raised, with a red-purple margin, occurring in association with *T. juvenalis*. *Conidiomata* chiefly hypophyllous, substomatal, pycnidial, separate or aggregated, globose, up to 80 µm diam; wall of 2–3 layers of dark brown *textura angularis*. *Conidiogenous cells* ampulliform to doliiform, hyaline to pale brown, finely verruculose, proliferating percurrently, with irregular annulations, 5–10 × 4–6 µm. *Conidia* ellipsoidal, apex subobtuse, base truncate to subtruncate, generally widest at or below the median, thick-walled, verrucose, (7–)8–10(–15) × (4–)5(–6) µm in vitro, (6–)7–9(–10) × (4–)5(–6) µm in vivo.

Cultural characteristics — Colonies on MEA erumpent, spreading, with moderate aerial mycelium and feathery margins; surface olivaceous-grey with patches of iron-grey and pale olivaceous-grey; reverse iron-grey; reaching up to 30 mm diam after 1 mo. On PDA erumpent, spreading with moderate aerial mycelium and feathery margins; surface iron-grey with patches of olivaceous-grey; reverse iron-grey; colonies reaching up to 35 mm diam after 1 mo. On OA erumpent, spreading with moderate aerial mycelium and lobate, smooth to feathery margins; surface olivaceous-grey with patches of iron-grey and pale olivaceous-grey; colonies reaching up to 35 mm diam after 1 mo.

*Specimen examined*. South Africa, Western Cape Province, Stellenbosch, Stellenbosch Mountain, living leaves of *E. cladocalyx*, Apr. 1988,

*P.W. Crous*, holotype CBS H-20183, culture ex-type CPC 42 = CBS 113621; Western Cape Province, Malmesbury, living leaves of *Eucalyptus* sp., 1 Jan. 2006, *P.W. Crous*, CPC 12949.

Notes — *Teratosphaeria verrucosa* can be distinguished from *T. ovata* by its slightly wider conidia that have thicker walls, and that are more verrucose than the thinner-walled, ellipsoid to subcylindrical, verruculose conidia of the other species treated here. Pycnidia and conidiogenous cells of *T. verrucosa* also form in the aerial mycelium, a fact which has not been observed in cultures of *T. juvenalis*.

### *Coniothyrium canker of Eucalyptus*

Two species of coelomycetes are known to cause the serious disease of *Eucalyptus* known as *Coniothyrium* canker (Cortinas et al. 2006a, b). *Coniothyrium zuluense* was originally described from South Africa and has since been found in other African countries, as well as South and Central America, and South-East Asia (Roux et al. 2002, Alemu et al. 2003, 2004, 2005, Old et al. 2003, Cortinas et al. 2006a, b). The second species, *Colletogloeopsis gauchensis*, was described from eucalypt cankers in Argentina and Uruguay (Cortinas et al. 2006b). Subsequent to their description, their taxonomy has been confused and largely reflected the vibrant debates regarding the anamorphs of *Mycosphaerella* s.l.

Although it became clear that these species did not belong in *Coniothyrium* (Verkley et al. 2004) but were rather allied to the *Mycosphaerella* complex (Crous et al. 2004a, Alemu et al. 2005), taxonomists that have treated these fungi have disagreed regarding the most appropriate anamorph genus in the complex in which to accommodate them. Opinions have included *Colletogloeopsis* (Cortinas et al. 2006a), *Kirramyces* (Andijc et al. 2007) and *Readeriella* (Crous et al. 2007). Because the taxonomy of the anamorphs in this complex is confused and does not reflect any obvious patterns regarding morphological forms, we have chosen to provide a more stable solution to the nomenclature of these species by placing them in *Teratosphaeria*, their teleomorph genus. The following new combinations are thus proposed:

***Teratosphaeria gauchensis*** (M.-N. Cortinas, Crous & M.J. Wingf.) M.J. Wingf. & Crous, *comb. nov*. — MycoBank MB508352

*Basionym*. *Colletogloeopsis gauchensis* M.-N. Cortinas, Crous & M.J. Wingf., Stud. Mycol. 55: 143. 2006.

≡ *Readeriella gauchensis* (M.-N. Cortinas, Crous & M.J. Wingf.) Crous & U. Braun, Stud. Mycol. 58: 26. 2007.

≡ *Kirramyces gauchensis* (M.-N. Cortinas, Crous & M.J. Wingf.) Andjic, M.-N. Cortinas & M.J. Wingf., Mycol. Res. 111: 1192. 2007.

***Teratosphaeria zuluensis*** (M.J. Wingf., Crous & T.A. Cout.) M.J. Wingf. & Crous, *comb. nov*. — MycoBank MB508353

*Basionym*. *Coniothyrium zuluense* M.J. Wingf., Crous & T.A. Cout., Mycopathologia 136: 142. 1996.

≡ *Colletogloeopsis zuluensis* (M.J. Wingf., Crous & T.A. Cout.) M.-N. Cortinas, M.J. Wingf. & Crous (*zuluense*), Mycol. Res. 110: 235. 2006.

≡ *Readeriella zuluensis* (M.J. Wingf., Crous & T.A. Cout.) Crous & U. Braun, Stud. Mycol. 58: 26. 2007.

≡ *Kirramyces zuluensis* (M.J. Wingf., Crous & T.A. Cout.) Andjic & M.J. Wingf., Mycol. Res. 111: 1192. 2007.

## DISCUSSION

The taxa treated in this paper represent asexual states of *Teratosphaeria* (Crous et al. 2007). Considerable controversy exists in recent literature regarding the anamorph generic names used for these species. For example, Cortinas et al. (2006a, b) treated them in *Colletogloeopsis*, Andjic et al. (2007) argued for *Kirramyces* and Crous et al. (2007) utilised *Readeriella*. There are reasonable arguments in all of these cases but none presents a clear harmony between morphological forms and phylogenetic relationships. Andijc et al. (2007), for example, show that there is a range in conidial septation from *Colletogloeopsis* (0–1-septate) to *Kirramyces* (> 1-septate). A problem here is that a single monophyletic clade on *Eucalyptus* is treated, while there are many other anamorphs in the group that show not such monophyly, and the kirramyces-like morphology on other hosts also clusters elsewhere. In contrast, Crous et al. (2007) provide a strong argument that most of these anamorph form genera have evolved more than once within the family *Teratosphaeriaceae*, and hence the oldest generic name *Readeriella* should be used. The latter approach lumps a large number of very different morphological forms under one name, and data obtained in the present study suggests that the *Readeriella* clade should be recognised as a separate genus within the *Teratosphaeriaceae*. The logical solution to this problem and one that will likely satisfy all taxonomists working with these fungi is to apply the approach used to resolve a similar problem in the *Botryosphaeriaceae* (Crous et al. 2006b, Marincowitz et al. 2008b, Phillips et al. 2008). In that case, a single generic name, irrespective of whether it has been applied to an anamorph or teleomorph in the past, is used for each well-resolved phylogenetic clade. Crous et al. (2007) demonstrated that the taxa treated in the present study are anamorphs of the *Teratosphaeriaceae*, of which clade 4 applies to *Teratosphaeria* s.str. (1912), which is the oldest name available for these species. Further studies are underway to resolve the status of other anamorphs of *Teratosphaeria* and *Mycosphaerella*, and to determine what names are available for them (Crous et al. In prep.).

*Teratosphaeria ovata* (as *Coniothyrium ovatum*) has long been clouded in controversy. In South Africa, a disease associated with this fungus was first noticed in 1956, on material collected by Peter S. Knox-Davies, at Uniepark, Stellenbosch. From these various collections, it is apparent that *T. verrucosa* and not *T. juvenalis* was, and still remains, the dominant species on these leaf spots. Interestingly, *T. verrucosa* can occur on healthy tissue apparently in a biotropic state, while *T. juvenalis* is associated with necrotic leaf spots. However, on older leaves both species co-occur on these necrotic spots, obscuring their separate identities.

It is surprising that mixed infections of *T. verrucosa* and *T. juvenalis* on the same lesions of *Eucalyptus* leaves in South Africa only became obvious after independent studies (at FABI in Pretoria, South Africa, and at CBS in the Netherlands). Until recently, however, we have been unable to address the taxonomy of these species, as uncertainty surrounded the generic and species status of *T. ovata*. Although we regard the present Australian collection from *E. phoenicea* as typical for *T. ovata*, we have refrained from designating it as epitype. This must await future collections on the original host, *E. dives*.

Little is known regarding the importance of *T. ovata* and the other taxa described in the present study to commercial forestry operations. In South Africa, however, *T. juvenalis* and *T. verrucosa* appear to be of minor importance, and largely restricted to non-commercial species of eucalypts that are planted as ornamental trees in the Western Cape Province. This is in contrast to two other similar *Teratosphaeria* species, namely *T. gauchensis* and *T. zuluensis* that are major canker pathogens of *Eucalyptus* in many parts of the world where these trees are grown as non-natives in plantations (Cortinas et al. 2006a, b). Although their origin is currently not known, they are regarded as important quarantine organisms in many countries. Thus research is required to develop molecular techniques that can

rapidly distinguish these morphologically similar pathogens, especially when they occur in mixed infections with other *Teratosphaeria* spp. on *Eucalyptus* leaves.

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