Phylogenetic lineages in the *Botryosphaeriaceae*

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Abstract: *Botryosphaeria* is a species-rich genus with a cosmopolitan distribution, commonly associated with dieback and cankers of woody plants. As many as 18 anamorph genera have been associated with *Botryosphaeria*, most of which have been reduced to synonymy under *Diplodia* (conidia mostly ovoid, pigmented, thick-walled), or *Fusicoccum* (conidia mostly fusoid, hyaline, thin-walled). However, there are numerous conidial anamorphs having morphological characteristics intermediate between *Diplodia* and *Fusicoccum*, and there are several records of species outside the *Botryosphaeriaceae* that have anamorphs apparently typical of *Botryosphaeria s.str*. Recent studies have also linked *Botryosphaeria* to species with pigmented, septate ascospores, and *Dothiorella* anamorphs, or *Fusicoccum* anamorphs with *Dichomera* synanamorphs. The aim of this study was to employ DNA sequence data of the 28S rDNA to resolve apparent lineages within the *Botryosphaeriaceae*. From these data, 12 clades are recognised. Two of these lineages clustered outside the *Botryosphaeriaceae*, namely *Diplodia*-like anamorphs occurring on maize, which are best accommodated in *Stenocarpella* (*Diaporthales*), as well as an unresolved clade including species of *Camarosporium/Microdiplodia*. We recognise 10 lineages within the *Botryosphaeriaceae*, including an unresolved clade (*Diplodia/Lasiodiplodia/Tiarosporella*), *Botryosphaeria s.str*. (*Fusicoccum* anamorphs), *Macrophomina*, *Neoscytalidium* gen. nov., *Dothidotthia* (*Dothiorella* anamorphs), *Neofusicoccum* gen. nov. (*Botryosphaeria*-like teleomorphs, *Dichomera*-like synanamorphs), *Pseudofusicoccum* gen. nov., *Saccharata* (*Fusicoccum*- and *Diplodia*-like synanamorphs), "*Botryosphaeria*" *quercuum* (*Diplodia*-like anamorph), and *Guignardia* (*Phyllosticta* anamorphs). Separate teleomorph and anamorph names are not provided for newly introduced genera, even where both morphs are known. The taxonomy of some clades and isolates (e.g. *B. mamane*) remains unresolved due to the absence of ex-type cultures.

INTRODUCTION

The genus *Botryosphaeria* Ces. & De Not. was introduced in 1863 (Cesati & De Notaris 1863), emended by Saccardo (1877), and is based on the type species *Botryosphaeria dothidea* (Moug. : Fr.) Ces. & De Not. (Barr 1972, Slippers *et al*. 2004). *Botryosphaeria* is a species-rich genus with a cosmopolitan distribution (Denman *et al*. 2000). Species occur on a wide range of monocotyledonous, dicotyledonous and gymnosperm hosts, on woody branches, herbaceous leaves, stems and haulms of grasses, on twigs and in the thalli of lichens (Barr 1987). Taxa range in habit from being saprobic, parasitic and endophytic (Smith *et al*. 1996, Denman *et al*. 2000), and can cause die-back and canker diseases of numerous woody hosts (von Arx 1987).

Botryosphaeria has been well circumscribed, and can be defined as forming uni- to multilocular ascomata with multi-layered walls, occurring singly or in clusters, often intermixed with conidiomata, which are pycnidial. Asci are bitunicate, with a thick endotunica, stalked or sessile, clavate, with a well-developed apical chamber, forming in a basal hymenial layer, intermixed among hyaline pseudoparaphyses that are frequently constricted at the septa. Ascospores are hyaline, aseptate, fusoid to ellipsoid or ovoid, bi- to triseriate, mostly without a mucoid sheath or appendages; ascospores turn brown and become septate and even slightly verruculose upon germination (von Arx & Müller 1954, Shoemaker 1964, Eriksson 1981, Sivanesan 1984, Denman *et al*. 2000, Alves *et al*. 2004).

Theissen & Sydow (1915) placed *Botryosphaeria* in the *Botryosphaerieae,* a sub-family of the *Pseudosphaeriaceae*, which was not assigned to any specific order. The *Pseudosphaeriaceae* was later placed in the *Myriangiales* (Theissen 1916), and in 1917 Theissen & Sydow were of the opinion

that the *Pseudosphaeriaceae* should be united with the *Dothideaceae* (Luttrell 1951). The *Dothideales* were characterised by the formation of asci in locules embedded in stromata, and contained the *Dothideaceae*, a family established to accommodate multiloculate forms like *Botryosphaeria*. Petrak (1923) placed *Botryosphaeria* in the sub-family *Pseudosphaerieae,* which was placed in the *Pleosporaceae* (*Sphaeriales*).

Miller (1928) placed *Botryosphaeria* in the *Dothideales* because true perithecial walls were absent. He later recognised three orders, namely the *Sphaeriales* (with perithecia and paraphyses), the *Dothideales* (ascostromatic forms without paraphyses), and the *Pseudosphaeriales* (ascostromatic forms with interthecial threads) and assigned *Botryosphaeria* to the *Pseudosphaeriales*.

Luttrell (1955) identified eight types of centrum development, and highlighted the taxonomic value of sterile, interthecial tissues in the taxonomy of the *Ascomycetes*. He furthermore replaced the name *Pseudosphaeriales* with *Pleosporales,* and assigned *Botryosphaeria* to this order. Luttrell's views were supported by Eriksson (1981) and Barr (1987). The orders proposed by Luttrell and Barr were not accepted by von Arx & Müller (1975) and von Arx (1987), as they comprised a mixture of unrelated genera (von Arx 1987). Von Arx & Müller (1975) only delimited the *Dothideales*, with two sub-orders and 24 families. Their view was that this was a more appropriate means of dealing with the taxonomy of this very large heterogeneous group, at least until a more natural method of classification could be developed. Thus, *Botryosphaeria* was maintained in the *Botryosphaeriaceae*, but retained in the *Dothideales*. This delimitation is widely accepted and the *Dictionary of Fungi* accommodates *Botryosphaeria* in the *Botryosphaeriaceae*, and the *Dothideales* (Kirk *et al.* 2001). Although the *Botryosphaeriaceae* is treated in the present study, its ordinal position in the *Dothideomycetes* will be treated elsewhere as part of the AToL (Assembling the Tree of Life) project (Schoch *et al.*, in prep.).

Anamorphs of *Botryosphaeria* have been assigned to 18 coelomycete genera, of which only two were recognised by Denman *et al.* (2000). This taxonomic sub-division was supported by comparisons of ITS sequence data, which separated the examined *Botryosphaeria* spp. into two groups, correlating to those species with *Diplodia*-like anamorphs and those with *Fusicoccum*-like anamorphs (Jacobs & Rehner 1998, Denman *et al.* 2000). Later studies including additional species and a larger suite of DNA-based markers supported this view (Zhou & Stanosz 2001, Alves *et al.* 2004, Slippers *et al.* 2004). However, this apparently clear sub-division is questioned by *Saccharata proteae* Denman & Crous (as *Botryosphaeria proteae* (Wakef.) Denman & Crous with *Fusicoccum* and *Diplodia* synanamorphs), which is morphologically and phylogenetically distinct from representatives of the *Diplodia*- and *Fusicoccum*-like groups (Crous *et al.* 2004). Some authors have continued to use *Lasiodiplodia* Ellis & Everh. as a genus distinct from *Diplodia* Fr., because of its distinct phylogenetic (usually ITS or EF-1α) and morphological

(striate conidia and paraphyses) characteristics (Pavlic *et al.* 2004). Recently, the name *Dothiorella* Sacc. has also been re-introduced as a distinct *Botryosphaeria* anamorph (conidia brown, septate while still attached to the conidiogenous cells) (Phillips *et al.* 2005a) and *Dichomera* Cooke has been linked to *Botryosphaeria* species with *Fusicoccum* anamorphs (Barber *et al.* 2005). Many of the other 18 coelomycete genera linked to *Botryosphaeria* remain untested in terms of phylogenetic association to the above groups.

The representation and phylogenetic understanding of major groups within *Botryosphaeria* remains poor. Previous analyses based on DNA sequence comparisons have included limited numbers of species, not representing the full anamorph diversity associated with *Botryosphaeria*. The value of the intron-dominated sequences of the ITS, β-tubulin and EF 1-α loci (on which most previous studies were based) to infer phylogenetic relationships across the diversity of the genus, is also unclear. The more conserved mtSSU data have, for example, suggested that *B. dothidea* and *B. corticis* (Demaree & Wilcox) Arx & E. Müll. are unrelated to *Fusicoccum s.str.* (Zhou & Stanosz 2001) even though they are typically assigned to this genus.

Botryosphaeria as a single genus is clearly unaligned with evolutionary radiations in the group, as exemplified by the morphologically and phylogenetically distinct anamorph genera linked to it. A preferable approach would be natural unit classification, also referred to as the "genus for genus concept" (Seifert *et al.* 2000). Here, morphologically distinct anamorph genera are linked to unique teleomorphs on a one for one basis, correlating with phylogenetic DNA data. This is an approach that has been applied to genera such as *Calonectria* De Not. (Crous 2002), *Cryphonectria* (Sacc.) Sacc. & D. Sacc. (Gryzenhout *et al.* 2006 – this volume), *Ophiostoma* Syd. & P. Syd. (Zipfel *et al.* 2006 – this volume) and *Botryosphaeria* (Rossman & Samuels 2005). The primary aim of the present study is to delineate the phylogenetic lineages of the *Botryosphaeriaceae*, and to discuss the morphological differences and generic concepts that can be ascribed to them. For this purpose, we have chosen comparisons of sequences for the 28S rRNA gene (LSU) because of its favourable size (approx. 900–1000 bp. used), and its relatively conserved (medium–high level) nature, suitable to consider taxonomic sub-divisions at the generic level.

MATERIALS AND METHODS

Isolates

Single-conidial or ascospore isolates were made from ascomata or pycnidia on dead or dying twigs of various hosts as explained in Slippers *et al.* (2004). Other isolates of representative *Botryosphaeria* spp. were obtained from the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands and the Culture Collection of the Tree Protection Co-operative Programme (CMW), FABI, University of Pretoria, South Africa (Table 1).

Cultural characteristics were determined on plates containing 2 % malt extract agar (MEA), 2 % potato-dextrose agar (PDA), and oatmeal agar (OA) (Gams *et al*. 1998).

DNA phylogeny
The isolation protocol of Lee & Taylor (1990) was used to extract genomic DNA from fungal mycelia grown on MEA. The primers ITS1 (White *et al.* 1990) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rRNA operon using the PCR conditions recommended by the authors and spanning the 3' end of the 18S rRNA gene, the internal spacers, the 5.8S rRNA gene and a part of the 5' end of the 28S rRNA gene. 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, U.K.) following ethidium bromide staining. The amplification products were purified using a GFX PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech Europe GmbH, Germany). The purified products were sequenced in both directions using an ABI PRISM Big Dye Terminator v. 3.1 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA) containing AmpliTaq DNA Polymerase as recommended by the manufacturer. The primers LR0R (Rehner & Samuels 1994), LR3R (http://www.biology.duke.edu/fungi/mycolab/primers.htm), LR16 (Moncalvo *et al.* 1993), and LR5 (Vilgalys & Hester 1990) were used to ensure good quality sequences over the entire length of the amplicon. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, CN).

DNA sequences were assembled and added to the outgroups and additional GenBank sequences using Sequence Alignment Editor v. 2.0a11 (Rambaut 2002), and manual adjustments for improvement were made by eye where necessary. The phylogenetic analyses of sequence data were done in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002) and consisted of neighbour-joining analysis with the uncorrected ("p"), the Kimura 2-parameter and the HKY85 substitution model in PAUP. 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 analysis, alignment gaps were treated as both missing and 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 simple 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 1000 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).

Bayesian analysis was conducted on the same aligned LSU dataset as the distance analysis. First MrModeltest v. 2.2 (Nylander 2004) was used to determine the best nucleotide substitution model. Phylogenetic analyses were performed with MrBayes v. 3 (Ronquist & Huelsenbeck 2003) applying a general time-reversible (GTR) substitution model with gamma (G) and proportion of invariable site (I) parameters to accommodate variable rates across sites. The Markov Chain Monte Carlo (MCMC) analysis of 4 chains started from random tree topology and lasted 10 000 000 generations. Trees were saved each 100 000 generations, resulting in 1000 saved trees. Burn-in was set at 500 000 generations after which the likelihood values were stationary, leaving 950 trees from which the consensus trees and posterior probabilities were calculated. PAUP 4.0b10 was used to reconstruct the consensus tree, and maximum posterior probabilities were assigned to branches after a 50 % majority rule consensus tree was constructed from the 950 sampled trees.

Taxonomy
Morphological descriptions were made for isolates sporulating on 2 % water agar (WA) with sterilised pine needles as substratum, at 25 °C under near-UV light, to induce sporulation. Structures were mounted in lactic acid, and 30 measurements at × 1000 magnification were made of each structure where possible. The 95 % confidence levels were determined, and the extremes of spore measurements given in parentheses. All cultures used in this study are maintained in the CBS culture collection.

RESULTS

DNA phylogeny
For the LSU gene, approximately 1000 bases were determined for the isolates listed in Table 1. Additional sequences, some of which were shorter, were also obtained from GenBank and added to the alignment. The manually adjusted alignment contained 115 taxa (including the two outgroups) and 576 characters including alignment gaps in TreeBASE (S1505, M2707). Of the 576 characters used in the phylogenetic analysis, 190 were parsimony-informative, 19 were variable and parsimony-uninformative, and 367 were constant when gaps were treated as missing characters. When gaps were treated as a new character state, seven more parsimony informative characters were added. Neighbour-joining analysis using the three substitution models on the sequence data yielded trees with similar topology and bootstrap values.

Twelve clades could be identified in the distance tree obtained using the HKY85 substitution model (Fig 1). These are discussed in the Taxonomy and Discussion sections. Parsimony analysis with gaps treated as missing characters yielded 79604 equally parsimonious trees (TL = 582 steps; CI = 0.509; RI = 0.905; RC = 0.460). Treating gaps as new states resulted in 79775 equally parsimonious trees (TL = 603 steps; CI = 0.524; RI = 0.910; RC = 0.477). The
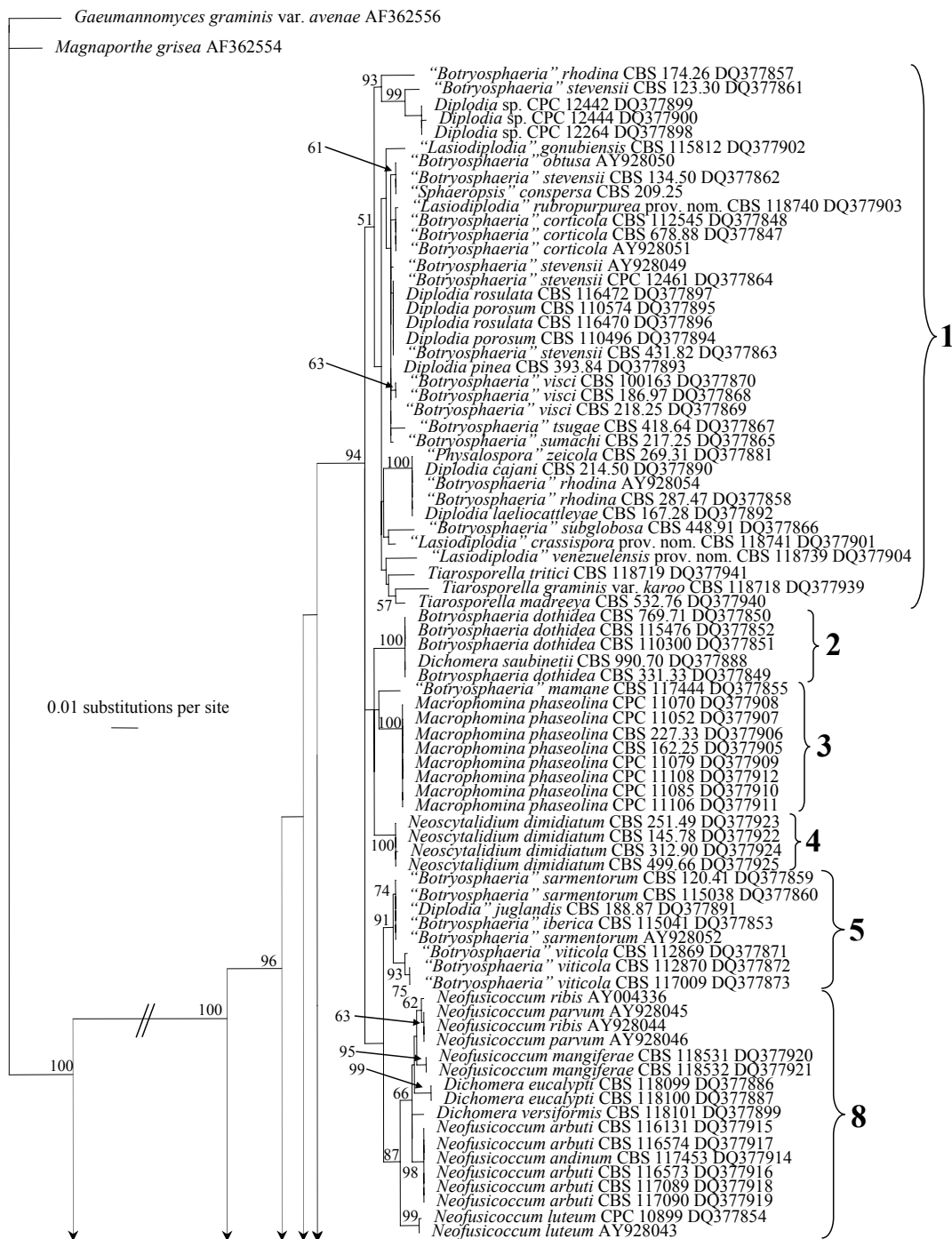
Fig. 1. Distance tree obtained using the HKY85 substitution model on the LSU sequence alignment. The scale bar shows 0.01 substitutions per site and bootstrap support values from 1000 replicates are shown at the nodes. Clades are numbered from 1 to 12 next to the brackets. The tree was rooted to *Gaeumannomyces graminis* var. *avenae* (AF362556) and *Magnaporthe grisea* (AF362554).

strict consensus trees calculated from the equally parsimonious trees were identical to each other and are shown in TreeBASE. Between the neighbour-joining and parsimony analyses, the same clades were supported with two exceptions. The first exception is *Botryosphaeria mamane* D.E. Gardner (CBS 117444), which resides in Clade 3 (Fig. 1), but is basal to Clades 1 to 6 in the strict consensus trees. Also, Clade 7 groups with Clade 10 in the strict consensus trees, but this is not supported with a bootstrap analysis (data not shown).

Bayesian analysis resulted in a tree with largely the same topology and clades (Fig. 2). Differences observed

were related to the position of *B. mamane*, which clustered close to Clade 3 in the distance analysis, but clustered in Clade 4 in the Bayesian analysis. A further difference was that in the Bayesian analysis Clades 8–9 clustered basal to the *Botryosphaeriaceae*.

Taxonomy
A total of 113 isolates representing most of the morphological variation presently recognised in the *Botryosphaeriaceae* were subjected to DNA sequence analysis. These analyses revealed 11 clades in the family. These phylogenetic clades can also be correlated with distinct morphological features.
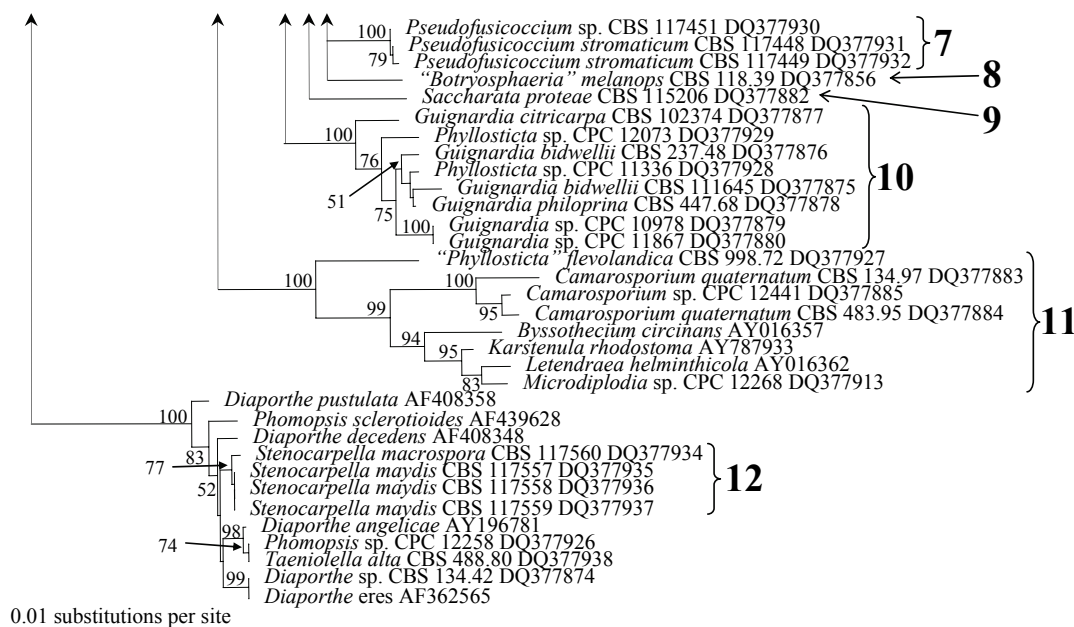
Fig. 1. (Continued).

Clade 1 includes species with *Diplodia*, *Sphaeropsis* Sacc. and *Lasiodiplodia* anamorphs clustering together. Although sequences of gene regions such as ITS, EF-1α and β-tubulin, support the synonymy of *Sphaeropsis* under *Diplodia*, in various cases they separate *Lasiodiplodia* from the *Diplodia* clade (Pavlic *et al.* 2004, Phillips *et al.* 2005a). This is in contrast to the LSU dataset (Fig. 1) in which species having *Diplodia* anamorphs could not be separated from those in *Lasiodiplodia*. Inclusion of additional strains resulted in a basal polytomy with low bootstrap support. Furthermore, uncertainty remains as to which teleomorph name is best suited for this clade, as the form genus *Diplodia* is known to be polyphyletic (Sutton 1980, Sivanesan 1984). The clustering of three species of *Tiarosporella* Höhn. in this clade was also unexpected.

Clade 2 is represented by the type species of the genus *Botryosphaeria*, namely *B. dothidea*, and its anamorph *Fusicoccum aesculi* Corda. The genus *Macrophomopsis* N.E. Stevens & Baechler, represented by a strain identified as *M. coronillae* (Desm.) Petr. (type species) (CBS 769.71), clusters in this clade, as does the genus *Dichomera* Cooke, represented by a strain identified as *D. saubinetii* (Mont.) Cooke (type species) (CBS 990.70).

Clade 3 is represented by isolates of *Macrophomina phaseolina* (Tassi) Goid. This fungus is the coelomycete synanamorph of "*Rhizoctonia*" *bataticola* (Taubenh.) E.J. Butler, which is shown to be a member of the *Botryosphaeriaceae*. Conidia also have apical mucous appendages early in their development, which has in the past led to confusion, and the allocation of this species to the genus *Tiarosporella* (von Arx 1981). With age, conidia lose their apical appendages, and become brown and slightly roughened, appearing more *Diplodia*-like in morphology.

Clade 4 represents *Fusicoccum dimidiatum* (Penz.) D.F. Farr. This species, which has a large number of synonyms (Farr *et al.* 2005), is unusual in having a

Fusicoccum-like coelomycete anamorph (with mucoid apical appendages). It also has a powdery *Scytalidium*-like hyphomycete synanamorph that is lacking in other species in the *Botryosphaeriaceae*. *Scytalidium*, typified by *S. lignicola* Pesante (CBS 233.57) clusters outside of the *Botryosphaeriaceae*. However, DNA sequence data derived from the *Scytalidium* species present in the CBS collection lead us to conclude that this genus is also polyphyletic (results not given).

Botryosphaeria mamane has a *Fusicoccum* anamorph and clusters most closely with species in either Clade 3 or Clade 4 in the various analyses. The *Fusicoccum* anamorph is morphologically most similar to species residing in Clade 2 (*Fusicoccum s.str.*). It does not have the apical appendages or discoloration found in species residing in Clade 3, nor does it have a *Scytalidium*-like synanamorph occurring in species residing in Clade 4. Consequenctly, its taxomic position remains unresolved.

Clade 5 represents *Botryosphaeria*-like teleomorphs with pigmented, septate ascospores for which the genus *Dothidotthia* Höhn. is available (Barr 1987, 1989). Anamorphs of this genus reside in *Dothiorella* Sacc. (Phillips *et al.* 2005a).

Clade 6 represents *Botryosphaeria*-like species with *Fusicoccum*-like anamorphs and *Dichomera*-like synanamorphs, for which the name *Neofusicoccum* gen. nov. is introduced. The *Dichomera*-like synanamorphs in this clade are characterised by globose to pyriform conidia. The older, brown conidia in Clade 2 (*Botryosphaeria s.str.*) are obovoid, ellipsoid or fusiform, never globose or subglobose (Phillips *et al.* 2005b, Barber *et al.* 2005).

Clade 7 represents isolates of "*Fusicoccum*" *stromaticum* Mohali, Slippers & M.J. Wingf. (Mohali *et al.* 2006). This taxon is distinguished from *Fusicoccum aesculi* and other *Fusicoccum*-like genera by having conidia that are enclosed in a persistent mucous sheath, for which the genus *Pseudofusicoccum* is proposed.

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Clade 8 is represented by a single species, *Botryosphaeria quercuum* (Schwein.) Sacc., for which the genus *Melanops* Nitschke ex Fuckel is available. *Saccharata* Denman & Crous (anamorph *Fusicoccum*-like; synanamorph *Diplodia*-like) (Clade 9) is morphologically distinguished from *Botryosphaeria s. str.* by having unilocular ascomata that develop under a clypeus.

Clade 10 includes species of *Guignardia* Viala & Ravaz with *Phyllosticta* Pers. anamorphs (Van der Aa & Vanev 2002). Clade 11 contains several distinct genera, namely *Camarosporium* Schulzer [type = *C. quaternatum* (Hazsl.) Sacc.], "*Phyllosticta*" *flevolandica* Aa, and other morphologically distinct taxa. "*Diplodia*" *macrospora* Earle and "*Diplodia*" *maydis* (Berk.) Sacc. (Clade 12) are shown to be distinct from the *Botryosphaeriaceae*. *Stenocarpella* Syd. & P. Syd. is appropriate for them, as they cluster apart from the *Botryosphaeriaceae*. Surprisingly, they cluster in the *Diaporthales* although no teleomorph connections are currently known for species of *Stenocarpella*.
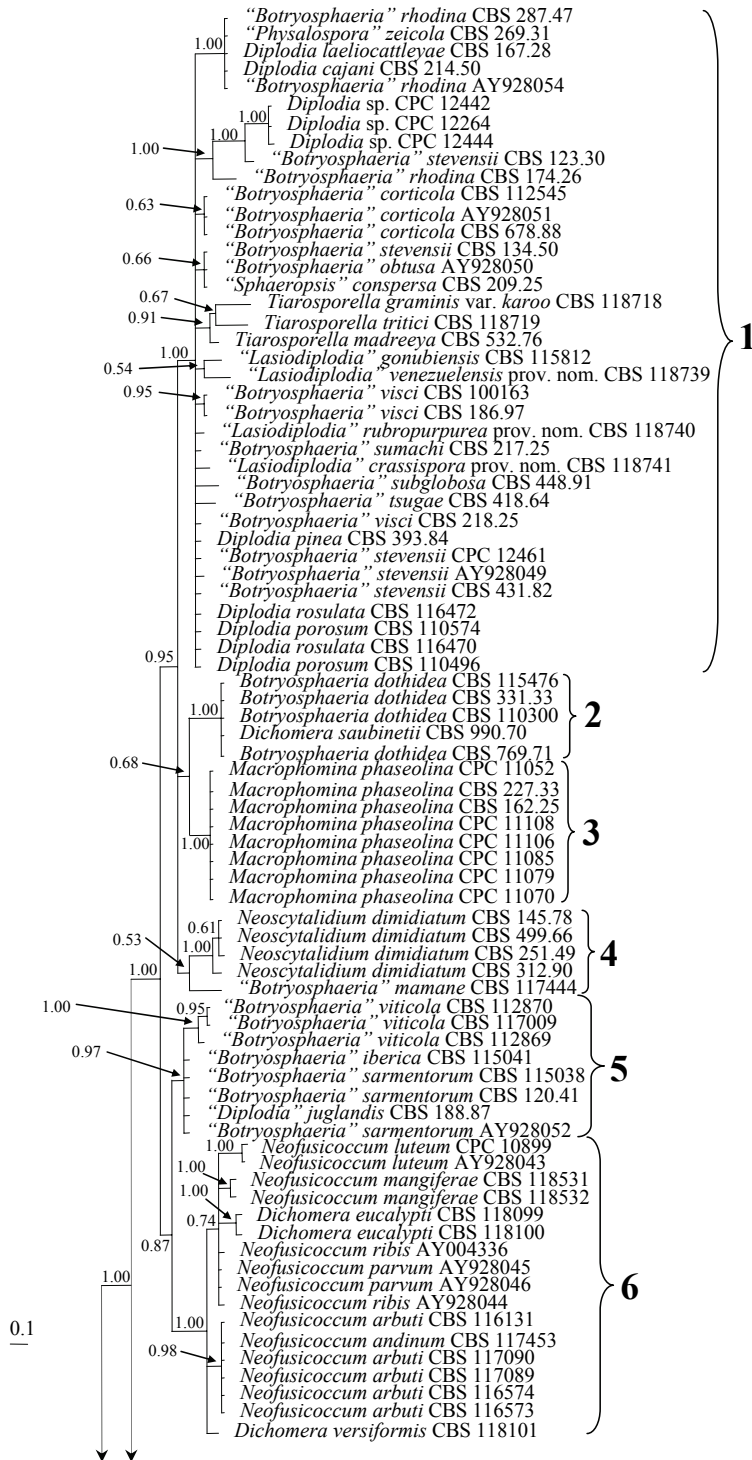
Fig. 2. Consensus phylogram of 950 trees resulting from a Bayesian analysis of 115 LSU sequences. Bayesian posterior probabilities are given at the nodes. Clades are numbered from 1–12 next to the brackets, following the number-to-clade assignment presented in Fig. 1. The tree was rooted to *Gaeumannomyces graminis* var. *avenae* (AF362556) and *Magnaporthe grisea* (AF362554) (*Diaporthales, Magnaporthaceae*).
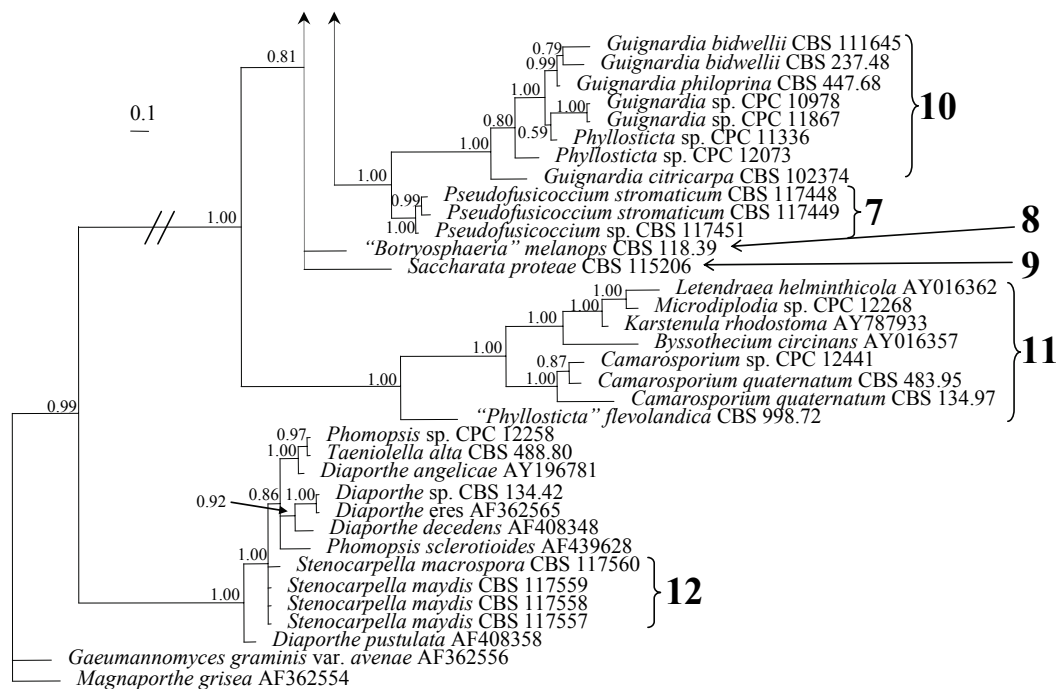
Fig. 2. (Continued).

DISCUSSION

Based on the LSU phylogeny obtained in the present study (Fig. 1), 11 clades could be recognised for species that have been applied to or that have anamorphs allied to the *Botryosphaeriaceae*. An additional 12th Clade included two species that have been treated in *Diplodia* and that reside in the *Diaporthales*. These 12 clades are discussed individually as follows:

Clade 1: *Diplodia/Lasiodiplodia* (several teleomorph genera available)

Species in Clade 1 are poorly resolved in both distance and Bayesian analyses. This is due to the few informative sites in this clade (19) in the section of LSU selected for this study. A larger segment of the LSU or additional gene regions will be required to resolve the phylogenetic relationships of species residing in this clade.

In the past, anamorphs of *Botryosphaeria* have been described in up to 18 different genera (Denman *et al*. 2000), many of which were not clearly defined and contain dark conidia typical or similar to those typical of *Diplodia*. Sutton (1980) reduced *Macrophoma* (Sacc.) Berl. & Voglino to synonymy with *Sphaeropsis*. Pennycook & Samuels (1985) reduced *Macrophomopsis* to synonymy with *Fusicoccum*. Crous & Palm (1999) showed that *Botryodiplodia* (Sacc.) Sacc. was a *nomen dubium*, and reduced *Dothiorella* to synonymy with *Diplodia*. Denman *et al*. (2000) also regarded *Sphaeropsis* and *Lasiodiplodia* as synonyms of *Diplodia*. Phillips *et al*. (2005a) again separated *Dothiorella* from *Diplodia*, and also provided evidence to show that the teleomorphs were different.

Recent treatments of *Botryosphaeria* anamorphs have revealed that they cluster in two clades, namely *Diplodia* (dark, mostly >10 μm broad, thick-walled conidia), and *Fusicoccum* (hyaline, mostly <10 μm broad, thin-walled conidia) (Jacobs & Rehner 1998, Denman *et al*. 2000, Zhou & Stanosz 2001, Alves *et al*. 2004). With age, however, conidia in species of *Fusicoccum* become dark, and frequently also septate. This complicates identification, especially where this is attempted based on structures occurring on natural substrates and in the absence of fresh cultures.

Pavlic *et al*. (2004) described a new species of *Lasiodiplodia* based on its characteristic conidial ornamentation typical of the genus (von Arx 1987). Xiao & Rogers (2004) described a new species of *Diplodia*, and placed it in *Sphaeropsis*. The correct generic names to be used for the dark-spored anamorphs of *Botryosphaeria* thus remains uncertain.

The genus *Sphaeropsis* is based on *S. visci* (Fr.) Sacc., while the genus *Lasiodiplodia* is based on *L. theobromae* (Pat.) Griff. & Maubl. In the present study we included cultures of both species. Based on the LSU phylogeny, it is clear that strains of *Sphaeropsis visci* reside in this clade (CBS 186.97, 100163). Furthermore, our data also reveal that the strains deposited in CBS under the name *L. theobromae* represent several distinct species with this typical conidial ornamentation; the LSU phylogenetic data could not resolve the *Lasiodiplodia/Diplodia* clade. *Lasiodiplodia gonubiensis* and three new species of *Lasiodiplodia* recently described (Burgess *et al.*, unpubl. data), were interspersed among species of *Diplodia*. The rather atypical *Diplodia* species recently described by Van Niekerk *et al*. (2004) as *D. porosum* Van Niekerk & Crous, also clustered in this clade. *Botryosphaeria subglobosa* (C. Booth) Arx & E. Müll. is another interesting example, as it has an anamorph described as *Sphaeropsis subglobosa* C. Booth. Although the latter species is illustrated to have what appears to be a germ slit in its aseptate conidia

(Punithalingam 1969, De Hoog *et al.* 2000), conidia of CBS 448.91 were found to be hyaline, thick-walled, and to become pigmented with age. Mature conidia were observed to have more than one "germ slit", actually appearing more like striations (Fig. 3). This observation suggests that if *Diplodia* and *Lasiodiplodia* are seen as separate genera, *S. subglobosa* would be better accommodated in the latter genus.

The choice of the correct teleomorph name to use for species residing in Clade 1 is not clear. Denman *et al.* (2000) listed several synonyms of *Botryosphaeria*, many of which have *Diplodia* or *Diplodia*-like anamorphs, and could thus potentially be available for this clade. This can be resolved only once appropriate type specimens have been examined, epitypes recollected and designated, and ex-epitype sequences generated. To avoid adding to the confusion, we refrain

from designating a teleomorph name for this clade, in anticipation of the additional research that is needed to elucidate the status of these older names. A further possibility is that the taxa in Clade 1 still represent more than one genus.

Clade 2: *Botryosphaeria* (anamorph *Fusicoccum*)
Barber *et al.* (2005) have recently shown that species of *Fusicoccum* can have *Dichomera* Cooke synanamorphs. Furthermore, two species of *Dichomera*, *D. versiformis* Z.Q. Yuan, Wardlaw & C. Mohammed and *D. eucalypti* (G. Winter) B. Sutton also cluster in Clade 6 with the majority of the "*Fusicoccum*" species. Phillips *et al.* (2005b) also reported that conidia of *Fusicoccum aesculi* from olives can become pigmented, ovoid, ellipsoid or fusiform, 1–2-septate, similar to those observed by Barber *et al.* (2005) for *F. aesculi*. Phillips *et al.* (2005b) also revealed *Fusicoccum dalmaticum* (Thüm.) Vanev is the same as *Camarosporium dalmaticum* (Thüm.) Zachos & Tzav.-Klon., both being later synonyms of *F. aesculi*. Sutton (1980) stated that *Camarosporium* Schulzer was the pycnidial analogue of *Dichomera*, which had more stromatic conidiomata. Given our current knowledge of the phylogenetic value of conidiomatal structure in the *Dothideomycetes*, it seems redundant to separate these anamorph genera based on this character alone (also see illustrations in Sutton 1980). The generic name *Camarosporium* (1870) is older, and thus has preference over *Dichomera* (1876). When a strain identified as *D. saubinetii* (Mont.) Cooke (CBS 990.70; sterile, morphology unconfirmed) was subjected to sequence analysis, it clustered in Clade 2, while strains identified as *C. quaternatum* (CBS 134.97, 483.95; fertile, matching the original description) clustered outside of the *Botryosphaeriaceae*. In *Fusicoccum s.str.* (*F. aesculi*) conidia are fusiform to ellipsoid, and with age become septate and brown, to some extent appearing *Dichomera*-like. Whether strains matching *D. saubinetii* in morphology will cluster in Clade 2 remains to be determined.

Sutton (1980) stated that *Macrophomopsis coronillae* was closely related to *F. aesculi*, but distinguishable by its annellidic conidiogenous cells. Crous & Palm (1999) showed that percurrent proliferation occurred in the type of *F. aesculi*, and concluded that Pennycook & Samuels (1985) were probably correct to reduce *M. coronillae* to synonymy with *F. aesculi*. As shown in the current study, an isolate of *M. coronillae* (CBS 769.71) was indistinguishable from *F. aesculi* based on sequence data. However, this isolate produced pycnidial paraphyses in culture, which had not been observed on the type specimen of *F. aesculi* (Sutton 1980). Phillips (2000) used this feature to distinguish *F. populi* A.J.L. Phillips from *F. aesculi*. DNA sequence comparisons revealed, that this feature is uninformative at the species level (*F. aesculi* = *F. populi*), and that not all strains of *F. aesculi* form paraphyses. Furthermore, some pycnidia in the culture CBS 769.71 produced numerous paraphyses, while they were almost completely absent in others. A note by H.A. van der Aa in the CBS database mentions the fact that this
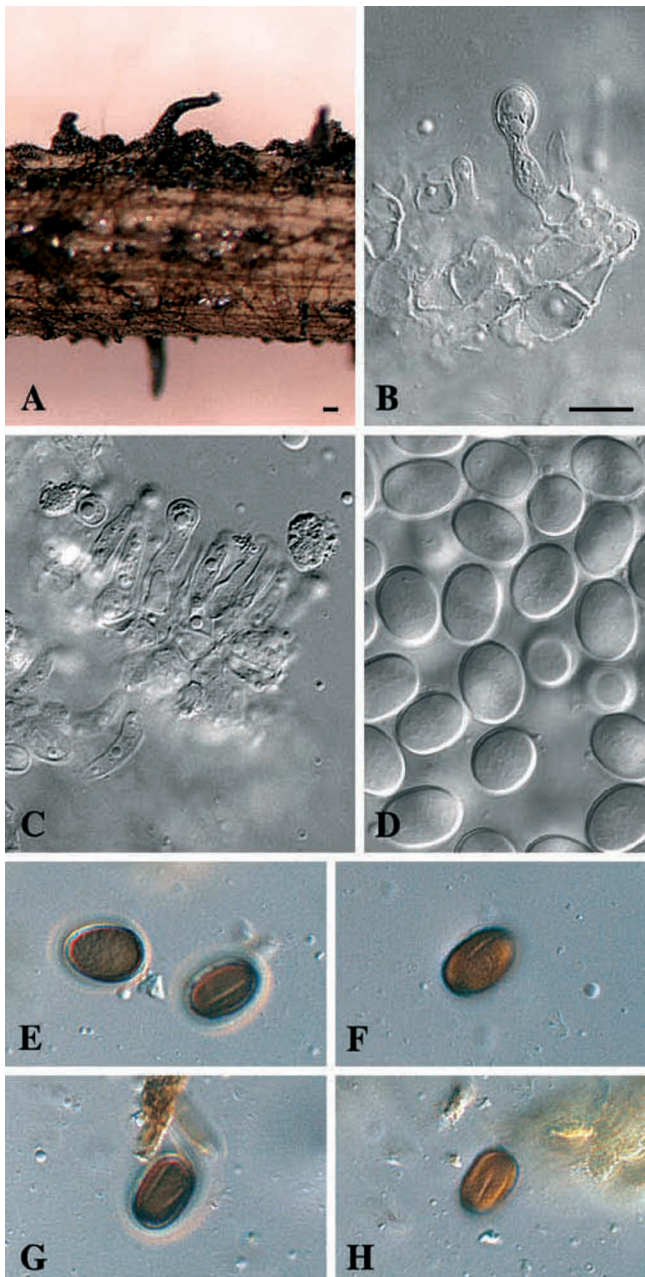

Fig. 3. "*Botryosphaeria*" *subglobosa* (CBS 448.91). A. Pycnidia on pine needles. B–C. Conidiogenous cells. D. Young conidia. E–H. Mature conidia with striations. Scale bars: A = 150 μm, B = 9 μm.
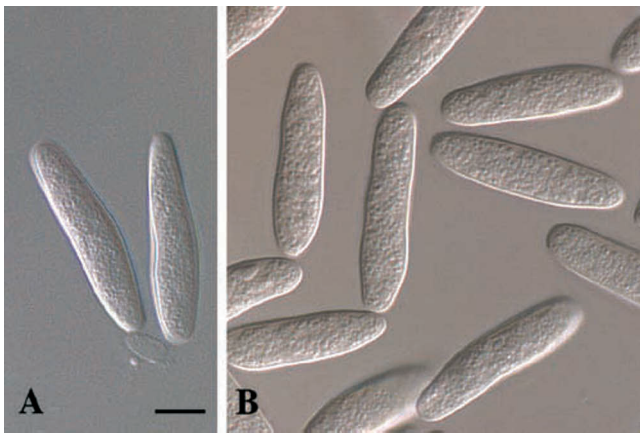
Fig. 4. *Botryosphaeria mamane* (CBS 117444). A–B. Conidia in culture. Scale bar = 7 µm.

isolate had also formed microconidia when it was first collected.

Botryosphaeria mamane was described for a fungus occurring on *Sophora chrysophylla* in Hawaii, where it is associated with witches' brooms of this host (Gardner 1997). By employing simple sequence repeat fingerprinting, Zhou *et al*. (2001) were the first to show that *B. mamane* clustered apart from other species of *Botryosphaeria*. Presently there is no authentic culture of this species, and subsequent collections from the type locality so far were unsuccessful. The culture included in the present study (Table 1) (Fig. 4) has the same ITS sequence as that of the original ex-type strain. However, its exact position in our trees remains unresolved, as it clustered differently when distance and Bayesian analyses were compared (Figs 1–2). Morphologically its anamorph is most similar to *Fusicoccum s.str.* in Clade 2.

Clade 3: *Macrophomina* (teleomorph unknown)
The asexual, sterile basidiomycete genus *Rhizoctonia* DC. has been linked to a number of teleomorphs in different families, namely the *Platygloeaceae*, *Exidiaceae*, *Tulasnellaceae*, *Ceratobasidiaceae*, etc. (Stalpers & Andersen 1996). The reason these species are treated in the form-genus *Rhizoctonia*, is that they are mostly sterile, and share the same vegetative features typical of *Rhizoctonia* spp. Species have thus been distinguished based on features such as sclerotium production, mycelial colour, wide hyphae, all with two or several nuclei, length of cells, shape and size of monilioid cells, and sclerotial size (Parmeter & Whitney 1970).

Macrophomina phaseolina is the type species of the genus *Macrophomina* Petr., and is also the name given to the coelomycete synanamorph of *Rhizoctonia bataticola* (Taubenh.) E.J. Butler. The latter fungus is a root inhabitant, and has been implicated in numerous root rot diseases of a wide range of crops, commonly causing charcoal rot and ashy stem blight (Holliday & Punithalingam 1998). The name of the synanamorph, and its taxonomic placement, has been the topic of much controversy. In their IMI description sheet of the fungus, Holliday & Punithalingam (1988) mention that the conidiophores are hyaline phialides, short

obpyriform to cylindrical, 5–13 × 4–6 µm. Conidia are described as being hyaline, ellipsoid to obovoid, 14–30 × 5–10 µm. Von Arx (1981) introduced the name *Tiarosporella phaseolina* (Tassi) van der Aa for this fungus, and also reduced the genus *Macrophomina* to synonymy under *Tiarosporella* Höhn. As no explanation was given, this treatment has largely been ignored by the plant pathological and mycological community, though von Arx (1987) retained it. The genus *Tiarosporella* [based on *T. paludosa* (Sacc. & Fiori ex P. Syd.) Höhn.] is characterised by having conidia formed from smooth, hyaline conidiogenous cells that lack periclinal thickenings and percurrent proliferations, and hyaline, subcylindrical to fusiform conidia that have irregular apical mucoid appendages (Nag Raj 1993). H.A. van der Aa (pers. comm.) informed the first author that the decision was based upon the fact that he had succeeded to induce sporulation of *M. phaseolina* in culture, and observed that the conidia have apical mucoid appendages, which prompted von Arx to place this fungus in *Tiarosporella*. In the present study we have been able to induce numerous strains of *M. phaseolina* to sporulate on sterile pine needles, and found that conidia form apical mucoid appendages (Figs 5–6) as previously suggested by van der Aa. As this is inconsistent with the description provided by Holliday & Punithaligham (1988), we provide an emended description below:

Macrophomina phaseolina (Tassi) Goid., Annali Sper. agr. N.S. 1: 457. 1947. Figs 5–6.
Basionym: *Macrophoma phaseolina* Tassi, Bull. Lab. Ort bot. Siena IV: 9. 1901.
   = *Tiarosporella phaseoli* (Maubl.) Aa, Verh. Kon. Ned. Akad. Wetensch., Sectie 2, 68: 4. 1977.
Additional synonyms listed by Holliday & Punithalingam (1988).

Sclerotia occurring in host tissue or in soil, black, smooth, hard, 100–1000 µm diam. *Conidiomata* pycnidial, dark brown to black, solitary or gregarious, up to 200 µm diam, opening by a central ostiole; wall multilayered, cells dark brown, thick-walled. *Conidiogenous cells* lining the inner surface of the conidioma, hyaline, short obpyriform to subcylindrical, proliferating several times percurrently near the apex, 6–12 × 4–6 µm; young condiogenous cells covered by a mucous layer that extends over the apex of the developing conidium. *Conidia* ellipsoid to obovoid, (16–)20–24(–32) × (6–)7–9(–11) µm; immature conidia hyaline, enclosed in a mucous sheath, that upon dehiscence encloses the top half of the conidium, transformed into two lateral tentaculiform, apical mucoid appendages (type C, Nag Raj 1993); mature conidia becoming medium to dark brown, with a granular outer layer that in some cases appears pitted, without any mucoid appendages; conidial hilum frequently with a marginal frill.

Notes: Although *Macrophomina phaseolina* can have conidia with apical mucoid appendages as found in *Tiarosporella* (Sutton & Marasas 1976), it is distinguished by having percurrently proliferating conidiogenous cells [not seen in any species of *Tiarosporella sensu* Nag
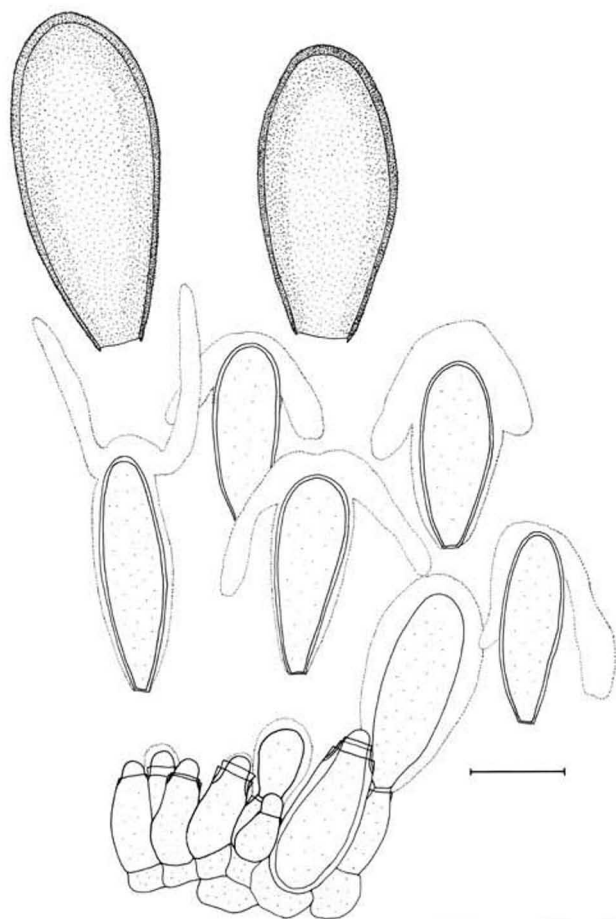
Fig. 5. *Macrophomina phaseolina* (CPC 11052). Conidiogenous cells, young conidia with apical mucoid appendages, and mature, brown conidia devoid of appendages. Scale bar = 10 µm.

Rag (1993), nor in those investigated here], and conidia that become dark brown at maturity. Based on these differences (and in the absence of authentic cultures of *T. paludosa*), the genus *Macrophomina* and the name *M. phaseolina* is retained. The three species of *Tiarosporella* that were available for this study clustered in Clade 1 (Figs 7–8), suggesting that the latter clade is still unresolved.

Clade 4: *Neoscytalidium dimidiatum* (teleomorph unknown)

Several distinct *Fusicoccum*-like fungi with conidia that become septate with a darker central cell have been treated under the epithet "*mangiferae*". Some isolates, however, formed a *Scytalidium*-like synanamorph, while others did not.

Dothiorella mangiferae Syd. & P. Syd. was originally described from mango (Sydow *et al.* 1916). Nattrass (1933) described a similar fungus from pome and stone fruit trees, but noticed a pigmented conidial state, which led him to describe *Hendersonula toruloidea* Nattrass. Sutton & Dyko (1989) revised the genus *Hendersonula* Speg. and synonymised both *D. mangiferae* and *H. toruloidea* under the redescribed *Nattrassia mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko. Furthermore, the mycelial synanamorph was described as *Scytalidium dimidiatum* (Penz.) B. Sutton & Dyko. Farr *et al.* (2005) made the point that the oldest name for the fungus was *Torula dimidiata* Penz., and hence introduced the

combination *Fusicoccum dimidiatum* (Penz.) D.F. Farr, stating that the type species of the genera *Nattrassia* and *Scytalidium* were synonyms of *F. dimidiatum*. As seen in the present study, this fungus, with its powdery disarticulating aerial mycelium, is a genus in its own right within the *Botryosphaeriaceae*. Furthermore, the ex-type strain of *Scytalidium*, *S. lignicola* Pesante, (CBS 233.57) clusters outside the *Botryosphaeriaceae* (results not given), and hence *Scytalidium* is unavailable for this fungus.

Fusicoccum mangiferae (Syd. & P. Syd.) Johnson, Slippers & M.J. Wingf. (≡ *D. mangiferae*, ≡ *N. mangiferae*) is a distinct taxon (see Clade 6, *Neofusicoccum*) that should not be confused with *F. dimidiatum*. When Sutton & Dyko (1989) and Johnson (1992) re-examined the type of *D. mangiferae*, they did not observe the *Scytalidium*-like anamorph on the type specimen, in accordance with Sydow *et al.* (1916). Slippers *et al.* (2005) studied isolates identified as *D. mangiferae* (= *N. mangiferae*) from mango in Australia, and found them to belong to *Fusicoccum*, for which they introduced the name *F. mangiferae* (now *Neofusicoccum*, Clade 6). They also did not observe the *Scytalidium*-like synanamorph as described by Sutton & Dyko (1989). The synonymy of *H. toruloidea* (which has a *Scytalidium*-like synanamorph) with *F. mangiferae* (which does not appear to have a *Scytalidium*-like synanamorph), is thus rejected here. A new genus is proposed to accommodate this fungus.

Neoscytalidium Crous & Slippers, **gen. nov.** MycoBank MB500868.

Genus anamorphosis hyphomyceticum. Arthroconidia catenata in mycelio aerio, pulverulenta, disarticulantia, cylindrica-truncata, oblongo-obtusa vel doliiformia, fusca, crassitunicata, 0–2-septata.

Conidia occurring in arthric chains in aerial mycelium, powdery to the touch, disarticulating, cylindrical–truncate, oblong–obtuse to doliiform, dark brown, thick-walled, 0–2-septate.

Type species: *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers, comb. nov.

Neoscytalidium dimidiatum (Penz.) Crous & Slippers, **comb. nov.** MycoBank MB500869. Fig. 9.
Basionym: *Torula dimidiata* Penz., Michelia 2: 466. 1882 (basionym; hyphomycete synanamorph).
    ≡ *Scytalidium dimidiatum* (Penz.) B. Sutton & Dyko, Mycol. Res. 93: 484. 1989.
   ≡ *Fusicoccum dimidiatum* (Penz.) D.F. Farr, Mycologia 97: 740. 2005.
    = *Hendersonula toruloidea* Nattrass, Trans. Brit. Mycol. Soc. 18: 197. 1933 (coelomycete synanamorph).

Clade 5: *Dothidotthia* (anamorph *Dothiorella*)

Concepts defining morphological features of *Botryosphaeria* (ascomata, asci and hamathecium) have developed slowly (Denman *et al.* 2000). This has resulted in confusion between *Botryosphaeria* and superficially similar genera such as *Physalospora* Niessl. and *Guignardia* (von Arx & Müller 1954, Hanlin 1990), *Auerswaldiella* Theiss. & Syd., *Discochora* Höhn., *Dothidotthia* Höhn., *Neodeightonia* C. Booth, *Homostegia* Fuckel and *Otthia* Nitschke. Subsequent
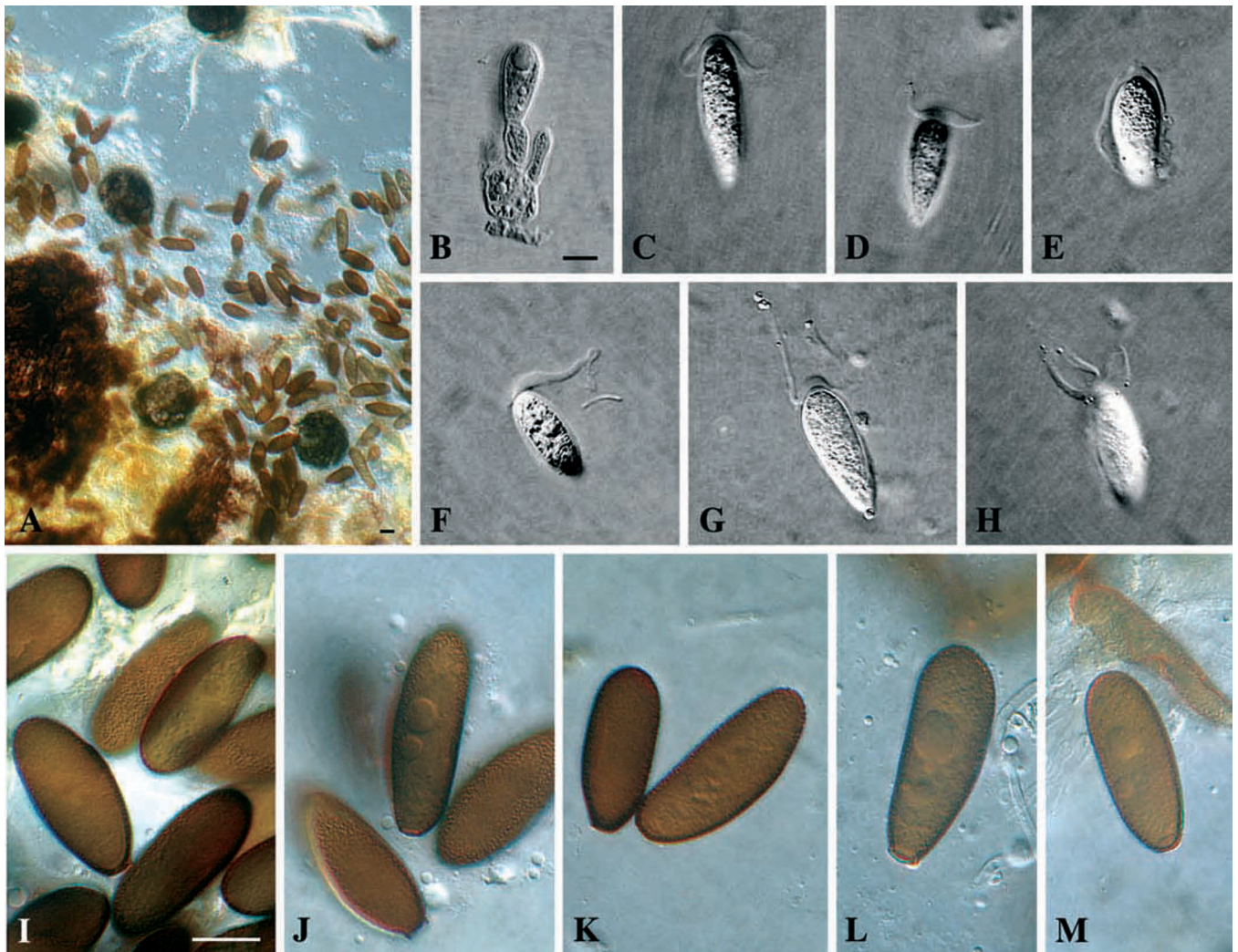
Fig. 6. *Macrophomina phaseolina* (CPC 11052). A. Conidia and sclerotia formed on pine needles. B. Conidiogenous cells. C–H. Hyaline conidia with apical, mucoid appendages. I–M. Brown, mature conidia with verrucose walls. Scale bars = 8 µm.
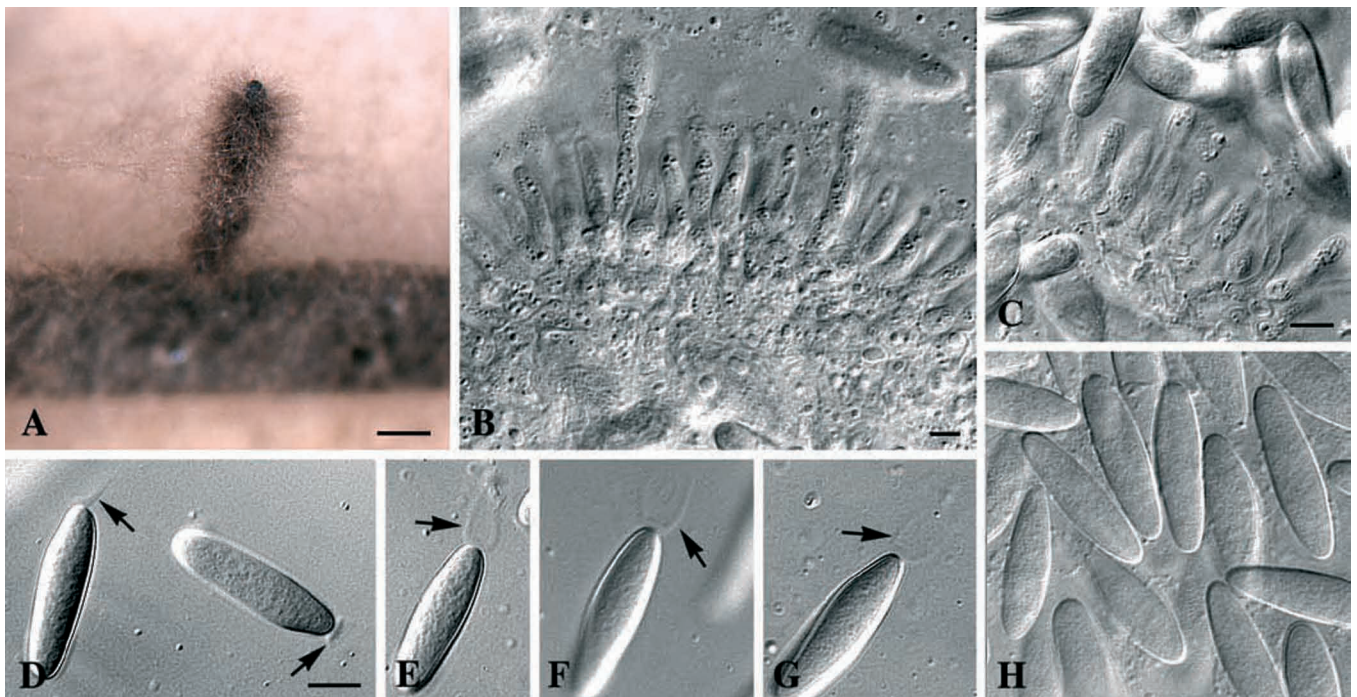
Fig. 7. *Tiarosporella graminis* var. *karoo* (CBS 118718). A. Pycnidium on a pine needle. B–C. Conidiogenous cells. D–G. Conidia (arrows denote apical, mucoid appendages). H. Conidia. Scale bars: A = 200 µm, B–D = 7 µm.

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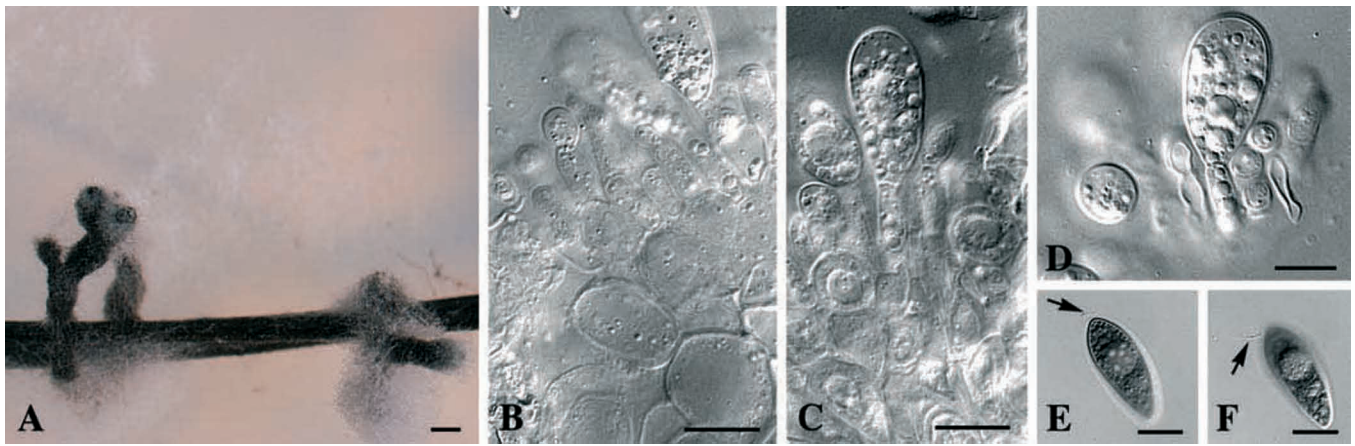
Fig. 8. *Tiarosporella tritici* (CBS 118719). A. Pycnidia on a pine needle. B–D. Conidiogenous cells. E–F. Conidia (arrows denote apical, mucoid appendages). Scale bars: A = 300 µm, B–F = 10 µm.
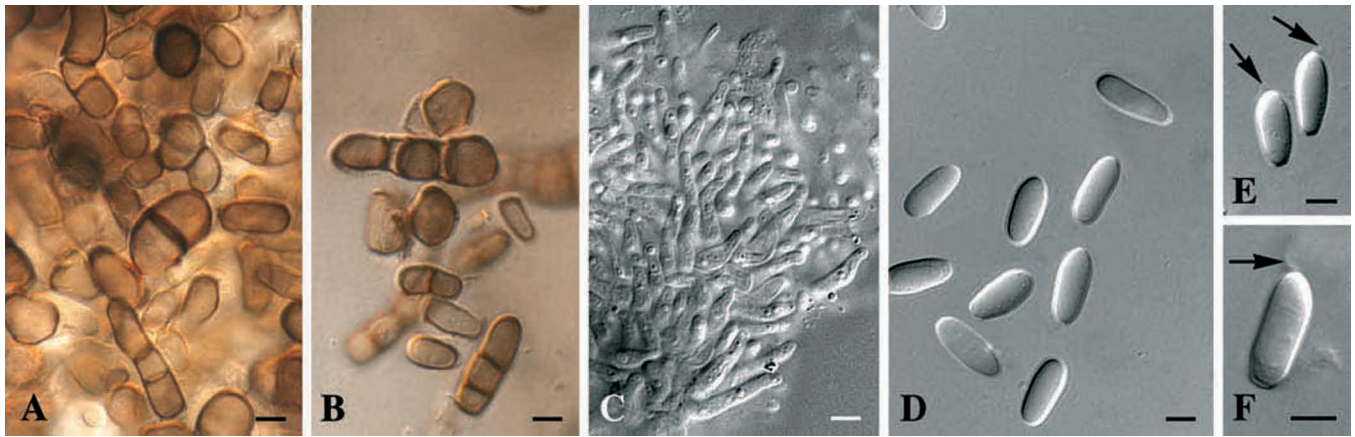
Fig. 9. *Neoscytalidium dimidiatum* (CBS 312.90). A–B. *Neoscytalidium* conidia. C. Conidiogenous cells of coelomycete synanamorph. D–F. Conidia of coelomycete synanamorph (arrows denote sheath). Scale bars = 4 µm.
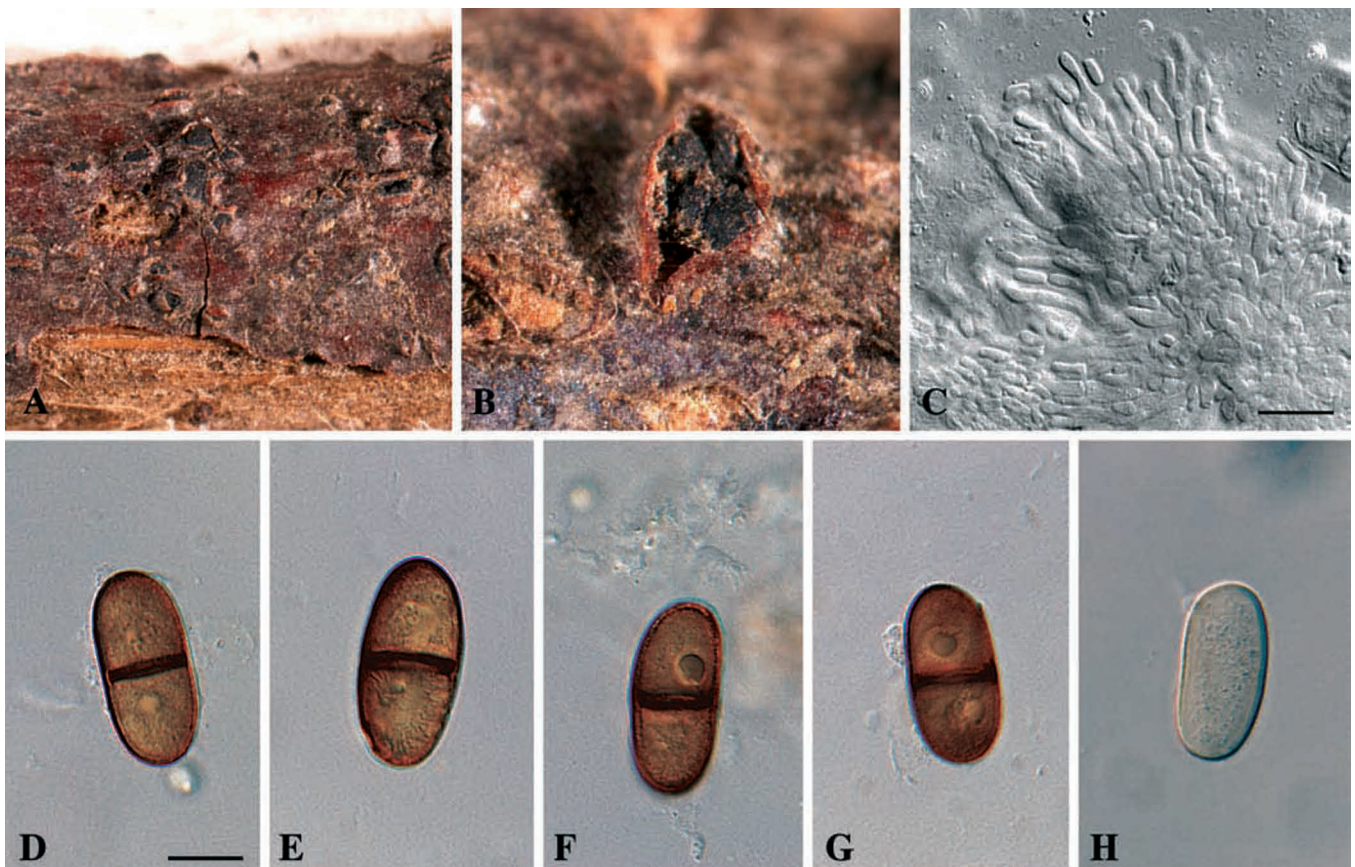
Fig. 10. *Dothiorella pyrenophora* (K 54912). A–B. Pycnidia on stems. B. Spermatia and spematogenous cells. D–H. Conidia. Scale bars = 10 µm.

to the review of Denman *et al*. (2000), two of these genera, namely *Otthia* and *Dothidotthia,* have been variously treated, and they are discussed here.

The genus *Otthia* was described as having short-stalked, cylindrical, bitunicate asci containing hyaline ascospores that become brown and 1-septate at maturity (Dennis 1981, Sivanesan 1984). Booth (1958) designated *Otthia spiraeae* (Fuckel) Fuckel as lectotype of the genus, citing *Diplodia sarmentorum* (Fr.) Fr. as anamorph. Von Arx (1974) listed *Otthia* as the teleomorph of *Aplosporella* Speg., which is indistinguishable from *Sphaeropsis* Sacc., and thus similar to *Diplodia sarmentorum*. Denman *et al*. (2000) were thus of the opinion that *Otthia* should be reduced to synonymy with *Botryosphaeria*, but stated that further morphological and DNA sequence comparisions were first required. Van Niekerk *et al*. (2004) showed that the Wollenweber isolate of *Diplodia sarmentorum* (CBS 120.43) is identical to the Booth isolate of *Otthia spiraeae* (IMI 63581b). Recently, Phillips *et al*. (2005a) re-examined the lectotype of *O. spiraeae* (K 104853), and found that it represents a fungus distinct from that collected and treated by Booth (1958) as "*O. spiraeae*". These authors also showed that the anamorph-teleomorph connection reported by Booth (1958), was in fact incorrect. Consequently, the new species, *Botryosphaeria sarmentorum* A.J.L. Phillips, Alves & Luque (anamorph: *Dothiorella sarmentorum* (Fr.) A.J.L. Phillips, Alves & Luque) was introduced for the fungus treated by Booth (1958) and Wollenweber (1941). The incorrect link between *Otthia* and "*Diplodia*" *sarmentorum* as reported by Booth (1958) was thus resolved. *Otthia* was retained as a distinct, but poorly known genus, characterised by cylindrical asci, brown, 1-septate ascospores that are obliquely uniseriate, and thin, sparingly septate pseudoparaphyses. Due to the lack of cultures, the correct placement of *Otthia* remains unknown.

In the phylogenetic analysis of DNA sequence data of the ITS region and EF 1-α gene, Phillips *et al*. (2005a) demonstrated that the "*Botryosphaeria*" species with pigmented, septate ascospores and *Diplodia*-like anamorphs formed a separate clade. *Dothiorella* had been reduced to synonymy under *Diplodia* by Crous & Palm (1999), who did not have access to cultures and DNA sequence data and thus used a wider morphological concept for *Diplodia*. Phillips *et al*. (2005a) re-examined the type of *Dothiorella pyrenophora* Sacc. (K 54912) (Fig. 10), and stated that it differs from *Diplodia* by having conidia that are brown and 1-septate early in their development, while they are still attached to the conidiogenous cells. In *Diplodia* (*D. mutila*), conidial darkening and septation takes place after discharge. We have re-examined the types of both genera in the present study, and concur with Phillips *et al.* (2005a) that the genera have distinct conidial characeristics. Teleomorphs of *Dothiorella* have pigmented, septate ascospores, for which the genus *Dothidotthia* is available. The latter genus had been recognised as a member of the *Botryosphaeriaceae* by Barr (1987, 1989), and is known to have *Diplodia*-like anamorphs, which are now accommodated in *Dothiorella*. The new taxa described by Phillips *et al*. (2005a) must thus

be placed in *Dothidotthia*. These taxa will be treated elsewhere (A.J.L. Phillips, in prep.).

Clade 6: *Neofusicoccum* (teleomorph *Botryosphaeria-like*)

An interesting issue to resolve is the morphological distinction between the two larger *Fusicoccum* clades, namely *Fusicoccum s.str.* (based on *F. aesculi*, and linked to the name *Botryosphaeria*), and the larger *Fusicoccum*-like clade (linked to *Botryosphaeria*-like teleomorphs), which includes most of the species that are currently known from DNA sequence data. Although the teleomorphs are similar in both clades, their anamorphs provide some clues for a possible separation. There is little to choose in their *Fusicoccum* anamorphs, but as seen in *Saccharata*, the distinguishing feature is to be found in their synanamorphs. In *Fusicoccum s. str*. (*F. aesculi*) conidia are fusiform to ellipsoid, and with age turn septate and brown. In the *Fusicoccum*-like clade, two distinct conidial types are seen, namely the first with a *Fusicoccum*-like morphology, which can turn brown and septate with age (as seen in Clade 2). The second conidial form has globose to pyriform conidia, which are brown, slightly verruculose, and muriformly septate. It is debatable if *Fusicoccum s.str.* (Clade 2) forms a distinct synanamorph. The synanamorph observed in the *Fusicoccum*-like clade (Clade 6) cannot be accommodated in *Camarosporium* (the stromatic analogue of *Dichomera*), as the type species of *Camarosporium*, *C. quaternatum* (CBS 134.97, 483.95) clusters outside the *Botryosphaeriaceae*. As far as we could establish, however, no genus is presently available for this clade, and thus a new one is proposed below. We introduce a single generic name, namely for the anamorph (which occurs with a *Dichomera*-like synanamorph), which is the more informative morphological state:

Neofusicoccum Crous, Slippers & A.J.L. Phillips, **gen. nov.** MycoBank MB500870.
Teleomorph: *Botryosphaeria*-like
Synanamorph: *Dichomera*-like

Genus anamorphosis coelomyceticum. Fusicocco simile sed synanamorphe Dichomerae simili et conidiis brunneis, globosis vel pyriformibus, distinguendum.

Resembling species of *Fusicoccum*, but distinct in forming a *Dichomera*-like synanamorph with brown, globose to pyriform conidia.

Type species: *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, comb. nov.

Most species that have thus far been described in *Fusicoccum* appear to reside in this clade, as can be seen in the present, as well as other recent studies. To facilitate clarity, new combinations are proposed for those known to us from culture:

Neofusicoccum andinum **(**Mohali, Slippers & M.J. Wingf.) Mohali, Slippers & M.J. Wingf. **comb. nov.** MycoBank MB500871.
Basionym: *Fusicoccum andinum* Mohali, Slippers & M.J. Wingf., Mycol. Res. 10: 408. 2006.

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Neofusicoccum arbuti (D.F. Farr & M. Elliott) Crous, Slippers & A.J.L. Phillips, **comb. nov.** MycoBank MB500872.
Basionym: *Fusicoccum arbuti* D.F. Farr & M. Elliott, Mycologia 97: 731. 2005.

Neofusicoccum australe (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, **comb. nov.** MycoBank MB500873.
Basionym: *Fusicoccum australe* Slippers, Crous & M.J. Wingf., Mycologia 96: 1035. 2004.
Teleomorph: "*Botryosphaeria" australis* Slippers, Crous & M.J. Wingf., Mycologia 96: 1035. 2004.

Neofusicoccum eucalypticola (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, **comb. nov.** MycoBank MB500874.
Basionym: *Fusicoccum eucalypticola* Slippers, Crous & M.J. Wingf., Stud. Mycol. 50: 351. 2005.
Teleomorph: "*Botryosphaeria" eucalypticola* Slippers, Crous & M.J. Wingf., Stud. Mycol. 50: 351. 2005.

Neofusicoccum eucalyptorum (Crous, H. Sm. ter & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, **comb. nov.** MycoBank MB500875.
Basionym: *Fusicoccum eucalyptorum* Crous, H. Sm. ter & M.J. Wingf., Mycologia 93: 280. 2001.
Teleomorph: "*Botryosphaeria" eucalyptorum* Crous, H. Sm. ter & M.J. Wingf., Mycologia 93: 280. 1998.

Neofusicoccum luteum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, **comb. nov.** MycoBank MB500876.
Basionym: *Fusicoccum luteum* Pennycook & Samuels, Mycotaxon 24: 456. 1985.
Teleomorph: "*Botryosphaeria" lutea* Phillips, Sydowia 54: 59. 2002.

Neofusicoccum macroclavatum (Burgess, Barber & Hardy) Burgess, Barber & Hardy, **comb. nov.** MycoBank MB500877.
Basionym: *Fusicoccum macroclavatum* Burgess, Barber & Hardy, Austral. Pl. Pathol. 34: 562. 2005.
Note: For further information about this recently published species, see Burgess *et al.* (2005).

Neofusicoccum mangiferae (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips, **comb. nov.** MycoBank MB500878.
Basionym: *Dothiorella mangiferae* Syd. & P. Syd., Ann. Mycol. 14: 192. 1916.
   ≡ *Nattrassia mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko, Mycol. Res. 93: 484. 1989.
   ≡ *Fusicoccum mangiferae* (Syd. & P. Syd.) Johnson, Slippers & M.J. Wingf., Mycologia 97: 106. 2005.
Teleomorph: "*Botryosphaeria*" sp.

Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, **comb. nov.** MycoBank MB500879.
Basionym: *Fusicoccum parvum* Pennycook & Samuels, Mycotaxon 24: 455. 1985.
Teleomorph: "*Botryosphaeria*" *parva* Pennycook & Samuels, Mycotaxon 24: 455. 1985.
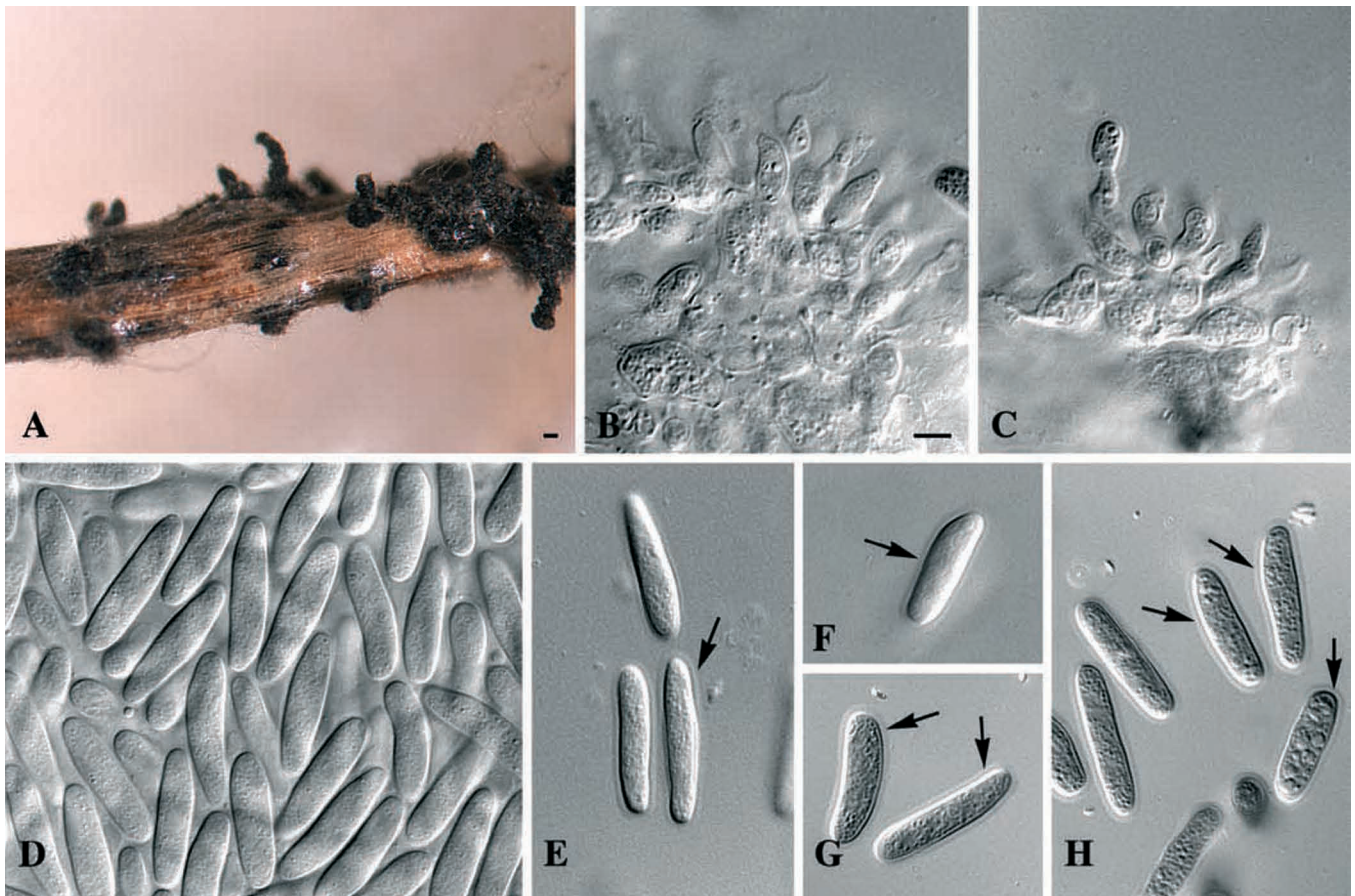
Fig. 11. *Pseudofusicoccum stromaticum* (CBS 117448). A. Pycnidia on pine needles. B–C. Conidiogenous cells. D–H. Conidia (arrows denote mucoid sheaths). Scale bars = A = 90 μm, B = 6 μm.

Neofusicoccum protearum (Denman & Crous) Crous, Slippers & A.J.L. Phillips, **comb. nov.** MycoBank MB500880.
Basionym: *Fusicoccum protearum* Denman & Crous, Mycologia 95: 301. 2003.
Teleomorph: "*Botryosphaeria*" *protearum* Denman & Crous, Mycologia 95: 301. 2003.

***Neofusicoccum ribis* (**Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, **comb. nov.** MycoBank MB500881.
Basionym: *Fusicoccum ribis* Slippers, Crous & M.J. Wingf., Mycologia 96: 96. 2004.
Teleomorph: "*Botryosphaeria*" *ribis* Grossenb. & Duggar, Tech. Bull. N.Y. Agric. Exp. St. 18: 128. 1911.

Neofusicoccum viticlavatum (Niekerk & Crous) Crous, Slippers & A.J.L. Phillips, **comb. nov.** MycoBank MB500882.
Basionym: *Fusicoccum viticlavatum* Niekerk & Crous, Mycologia 96: 792. 2004.

Neofusicoccum vitifusiforme (Niekerk & Crous) Crous, Slippers & A.J.L. Phillips, **comb. nov.** MycoBank MB500883.
Basionym: *Fusicoccum vitifusiforme* Niekerk & Crous, Mycologia 96: 793. 2004.

Clade 7: "*Fusicoccum*" *stromaticum* (teleomorph unknown)
"*Fusicoccum*" *stromaticum* Mohali, Slippers & M. J. Wingf. (Mohali *et al*. 2006) was described for a new *Fusicoccum*-like species occurring on *Eucalyptus* and *Acacia* spp. in Venezuela. The taxon was distinguished from other species of *Fusicoccum* based on its unusually large conidiomata, the ability to grow at 35 ºC, and thick-walled conidia. Strains of this species have conidia that are encased in a persistent mucous sheath, which is absent in other species of *Fusicoccum*, and is the character that distinguishes it as genus from *Fusicoccum s.str*.

Pseudofusicoccum Mohali, Slippers & M.J. Wingf. **gen. nov.** MycoBank MB500884.

Genus anamorphosis coelomyceticum. Fusicocco simile, sed conidiis strato mucido persistente circumdatis distinguendum.

Resembling species of *Fusicoccum*, but distinct in having conidia encased in a persistent mucous sheath.

Type species: *Pseudofusicoccum stromaticum* (Mohali, Slippers & M.J. Wingf.) Mohali, Slippers & M. J. Wingf., comb. nov.

Pseudofusicoccum stromaticum (Mohali, Slippers & M.J. Wingf.) Mohali, Slippers & M.J. Wingf., **comb. nov.** MycoBank MB500885. Fig. 11.
*Basionym*: *Fusicoccum stromaticum* Mohali, Slippers & M.J. Wingf., Mycol. Res. 110: 408. 2006.

### Clade 8: "*Botryosphaeria*" *quercuum* (anamorph *Diplodia*-like)
Von Arx & Müller (1954) placed *B. melanops* (Tul. & C. Tul.) G. Winter under *B. quercuum* (Schwein.) Sacc., which is a complex in need of revision, including *Melanops tulasnei* (Tul. & C. Tul.) Fuckel, the oldest generic name available for this clade. To resolve the status of *B. quercuum*, however, authentic cultures of all these names need to be studied, and linked to existing names. The strain in Clade 8 closely matches the morphology of the type specimen (Phillips *et al*., unpubl. data). No name is, however, proposed for this genus pending the outcome of studies based on authentic isolates.

Clade 9: *Saccharata* (anamorph *Fusicoccum*-like)
Wakefield (1922) described an ascomycete associated with leaf spots and stem cankers of *Protea* and *Leucospermum* species as *Phyllachora proteae* Wakef. This latter fungus is characterised by having unilocular ascomata that develop under a very small epidermal clypeus, cylindrical asci, pseudoparaphyses, and hyaline, aseptate, ellipsoidal ascospores. Doidge (1942) found the ascomatal walls are continuous with, and similar in structure to the clypeus, and stated that the fungus should be allocated elsewhere, possibly in *Botryosphaeria*. Denman *et al*. (1999) recollected this species, placed it in *Botryosphaeria*, and also established a cultural link with its anamorph, "*Fusicoccum*" *proteae* Denman & Crous, which also
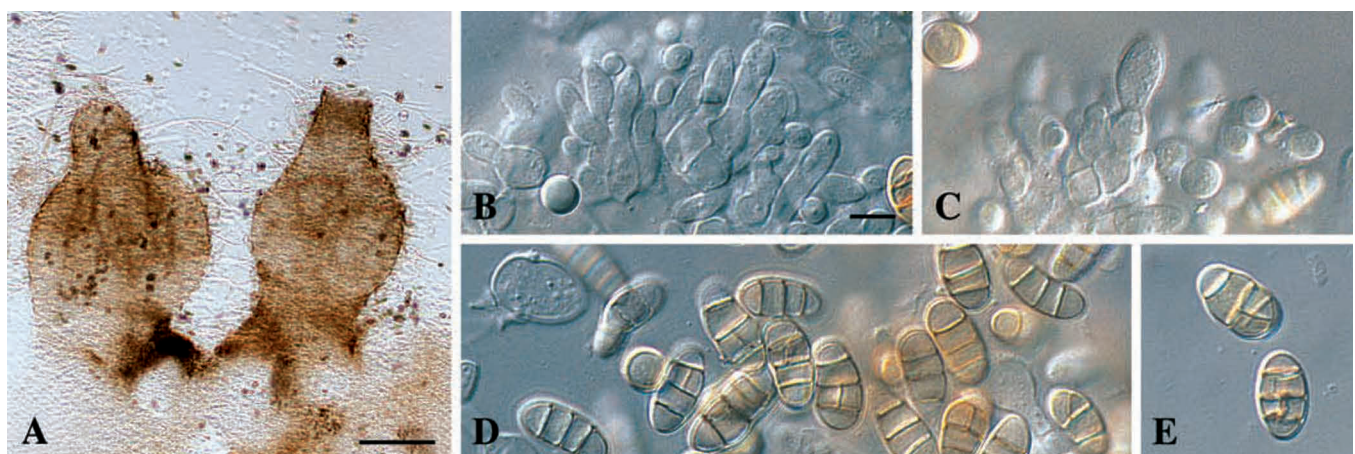
Fig. 12. *Camarosporium quaternatum* (CBS 134.97). A. Pycnidia in agar. B–C Conidiogenous cells. D–E. Conidia. Scale bars: A = 100 µm, B = 6 µm.
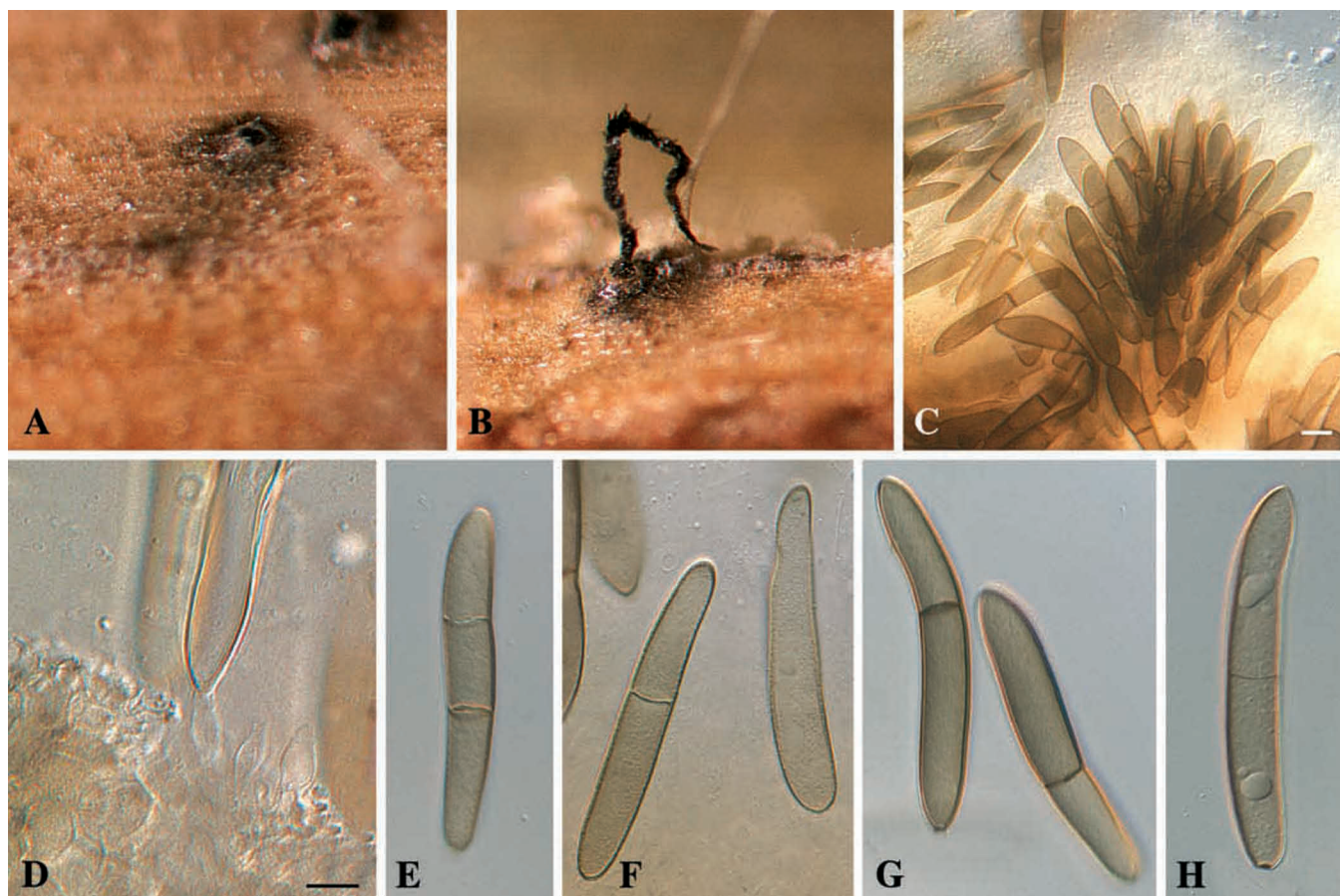
Fig. 13. *Stenocarpella maydis* on *Zea mays*. A. Pycnidium on upper leaf surface. B–C. Conidial cirrhi. D. Conidiogenous cells giving rise to conidia. E–H. Conidia. Scale bars = 10 µm.

forms a *Diplodia*-like synanamorph in culture. On the basis of ITS DNA sequence comparisons, Denman *et al*. (2000) later showed that the fungus clustered outside the *Botryosphaeria* clades that accommodated *Fusicoccum* and *Diplodia* anamorphs. Given its unilocular ascomata, the presence of a clypeus, and its unusual *Fusicoccum*- and *Diplodia*-like synanamorphs, Crous *et al*. (2004) established a new genus, *Saccharata* Denman & Crous to accommodate *S. proteae* (Wakef.) Denman & Crous. The LSU phylogenies in this study supports *Saccharata* as a distinct genus that is basal to, but probably outside the *Botryosphaeriaceae*.

Clade 10: *Guignardia* (anamorph *Phyllosticta*)
The genus *Phyllosticta* was revised by Van der Aa & Vanev (2002), who treated 2936 taxa, accepting 143 species in *Phyllosticta*, many of which have teleomorphs in *Guignardia* (*Botryosphaeriaceae*), and *Leptodothiorella* spermatial states. As seen with "*Phyllosticta*" *flevolandica* Aa, it is to be expected that more "*Phyllosticta*" taxa will be allocated elsewhere once subjected to DNA analysis (Crous *et al*. in prep.).

Clade 11: *Camarosporium* and relatives
Several morphologically discordant taxa group in this clade, including *C. quaternatum*, the type species of *Camarosporium* (Fig. 12), but also an unidentified *Dothiorella*-like strain (1-septate, brown conidia), and another seen as an atypical *Phyllosticta*, namely *P. flevolandica* (hyaline, 0–1-septate conidia) (Van

der Aa 1973). Other taxa include *Karstenula* Speg. (anamorph *Microdiplodia* Tassi), *Letendraea* Sacc. (*Tubeufiaceae*?), and *Byssothecium* Fuckel (anamorph *Chaetophoma*-like). It appears that the *Dothiorella*-like strain (CPC 12268) is, in fact, a species of *Microdiplodia*. Sutton (1977) reported that the genus was introduced for stem- or branch-inhabiting species with small, brown, 1-septate conidia. This is consistent with the results of this study where the strain examined was isolated from branches of *Sophora chrysophylla* collected in Hawaii. As we have shown here that the genus *Microdiplodia* Tassi could be used for these small-spored *Dothiorella*-like species, the genus needs to be lectotypified according to Tassi's original concept (see Sutton 1977).

Clade 12: *Stenocarpella* (teleomorph unknown)
The genus *Stenocarpella* Syd. & P. Syd. is based on *S. macrospora* (Earle) B. Sutton. The genus contains two species that cause Diplodia ear rot of maize, namely *S. macrospora* and *S. maydis* (Berk.) B. Sutton. These taxa were formerly treated in *Diplodia* by Sutton (1964), and *Macrodiplodia* Sacc. by Petrak & Sydow (1927). Sutton (1964, 1980) was of the opinion that these species should be accommodated in a genus other than *Diplodia*, and because the status of *Macrodiplodia* was unknown, he placed them in *Stenocarpella* (Sutton 1977). This treatment has not been widely accepted, and plant pathologists refer to "Diplodia ear rot", and continue to use the *Diplodia* names.

Because cultures were not available for *S. maydis* and *S. macrospora*, these two species were recollected as part of the present study, and subjected to DNA sequence comparisions. Interestingly, they clustered in the *Diaporthales*, clearly supporting the decision by Sutton (1964, 1980) to move them to their own genus, *Stenocarpella*. Species of *Diplodia* are quite variable in morphology, and hence it is difficult to see immediately which morphological features separate *Stenocarpella* from *Diplodia*. Species of *Stenocarpella* tend to have unilocular, thick-walled pycnidia with walls of brown *textura angularis*. Under moist conditions, they exude a long cirrhus of brown conidia via a central ostiole (reminiscent of *Phaeophleospora* Rangel). Conidiogenous cells are phialidic, thin-walled and hyaline, but also proliferating percurrently. Conidia are septate, brown, smooth, thin-walled, subcylindrical to narrowly obclavate (Fig. 13), thus different from typical fusoid to ellipsoid, thick-walled conidia of *Diplodia*. To facilitate future research with these pathogens, epitype specimens and cultures are designated below:

Stenocarpella maydis (Berk.) B. Sutton, in Sutton, *The coelomycetes, fungi imperfecti with pycnidia, acervuli and stromata*: 432. 1980.

Basionym: *Sphaeria maydis* Berk., Hooker's J. Bot. London 6: 15. 1847.

Additional synonyms listed in Sutton (1980).

Types: **U.S.A.**, Salem, Bethlehem, *Zea mays*, Schweinitz, 1832, IMI 96546 **isotype**. **South Africa**, KwaZulu-Natal, Simdlangentsha, Bt *Zea mays* hybrid from 2003-04 season, J. Rheeder, **epitype designated here**, CBS 117558, preserved as freeze-dried inactive strain (= MRC 8613); ibid. CBS 117557 = MRC 8612; Hlabisa, commercial hybrid PAN-6043, MRC 8614 = CBS 117559.

Stenocarpella macrospora (Earle) B. Sutton, Mycol. Pap. 141: 202. 1977. Fig. 13.

Basionym: *Diplodia macrospora* Earle, Bull. Torrey Bot. Cl. 24: 29. 1897.

Additional synonyms in Sutton (1980).

Types: **U.S.A.**, Alabama, Auburn, Lee Co., F.S. Earle, 1896, IMI 12790, **isotype**. **South Africa**, KwaZulu-Natal, Hlabisa, rain damaged Bt *Zea mays* hybrid, 2003-04 season, J. Rheeder, **epitype designated here**, CBS 117560, preserved as freeze-dried inactive strain (= MRC 8615).

This study provides a framework to align the taxonomy of the *Botryosphaeriaceae* with the phylogenetic lineages within the group. It also highlights the previously unrealised morphological and evolutionary complexity of the group. Specific studies are now needed to clarify some remaining, and arising, taxonomic and phylogenetic questions within this family. To resolve remaining taxonomic uncertainties, epitypes of key species, representing the oldest names in the respective groups, will need to be collected, studied and designated. To resolve the phylogenetic uncertainties (e.g. Clade 1 and *B. mamane*) sequences for additional gene regions (to add more informative sites, and from unlinked loci) will have to be added. Specific studies focussing on the phylogenies within the clades, and expanding on the current set of available cultures (e.g. Clades 1, 10 and 11), will add valuable information on the evolution within these groups, and also help identify definitive morphological characters.

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