

The genus *Mycosphaerella* and its anamorphs

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Abstract: More than 2000 names have been described in *Mycosphaerella* and *Sphaerella* (*Dothideales*). Based on various morphological features depicted by earlier workers, six sections are recognized in the genus. About 27 anamorph genera have been linked to *Mycosphaerella*, 23 of which are recognized here. Based on phylogenetic analyses of the ITS-1, 5.8S and ITS-2 DNA sequence data of 46 species of *Mycosphaerella* species (58 strains), one large cluster was distinguished containing primarily taxa with cercosporoid anamorphs, as well as another smaller cluster for species with *Dissoconium* anamorphs.

Key words: *Cercospora*, *Mycosphaerella*, *Dissoconium*, ITS rDNA, phylogeny.

Introduction

Mycosphaerella Johanson (*Mycosphaerellaceae*, *Dothideales*) (including species described in *Sphaerella* Ces. & De Not.) is one of the largest genera of ascomycetes. Many species are economically important plant pathogens, causing leaf spots and stem cankers (Park & Keane, 1984). Some saprophytic species grow as endophytes, and fruit only later on decaying parts of their hosts. The genus includes about 2000 described species, most of which have been distinguished based on the host (Corlett, 1991, 1995). The host has also played a dominant role in distinguishing anamorph species linked to *Mycosphaerella* (Chupp, 1954).

Kendrick & DiCosmo (1979) calculated that *Mycosphaerella* had been linked to more than 27 different anamorph genera. Von Arx (1983) treated several generic names that he regarded as anamorphs, or potential anamorphs, of *Mycosphaerella*, many based on the different morphs colonizing the same substratum. Sutton & Hennebert (1994) and Braun (1995a), who studied the different types of conidogenesis occurring in these genera, found that many

had been prematurely reduced to synonymy by von Arx (1983), or had teleomorph affinities elsewhere. Twenty-three genera were eventually accepted as anamorphs of *Mycosphaerella* by Sutton & Hennebert (1994). *Asteromella* Pass. & Thüm. is now commonly accepted as a spermatial anamorph that occurs in most species of *Mycosphaerella* (Crous & Wingfield, 1996); these may also have been described as anamorphs in genera such as *Ascochyta* Lib., *Asteroma* DC. and *Phoma* Sacc.

In contrast, genera such as *Stigmia* Sacc. *sensu* Sutton & Pascoe (1989), *Polythrincium* Kunze and *Lecanosticta* Syd., though sometimes cited as anamorphs of *Mycosphaerella*, have teleomorphs in other genera (Sivanesan, 1984; Barr, 1996; Crous, 1998, 1999; Crous & Corlett, 1998), while new genera such as *Cercostigmia* and *Xenostigmia* have been erected for stigmia-like species with *Mycosphaerella* teleomorphs (Braun, 1993; Crous, 1998; Crous & Corlett, 1998). In addition, *Mycovellosiella* and *Thegonia* have recently been linked to *Mycosphaerella* (Crous *et al.*, 1998; Kaiser & Crous, 1998), as well as *Phaeophleospora* (= *Kirramyces* J.

Walker, B. Sutton & Pascoe) (Crous & Wingfield, 1997b; Crous *et al.*, 1997; Crous, 1998), *Colletogloeopsis* (Crous & Wingfield, 1997a), *Uwebraunia* (Crous & Wingfield, 1996), *Clypeispora* (Ramaley, 1991) and *Sonderhenia* (Park & Keane, 1984; Swart & Walker, 1988). Some of the genera reported as possible anamorphs of *Mycosphaerella* by Sutton & Hennebert (1994) are based on older literature citations reporting a species of *Mycosphaerella* occurring in close proximity to an anamorph [e.g. *Toxosporium camptospermum* Maubl. and *Mycosphaerella abietis* (Rostr.) Lindau *fide* Grove (1937) (= *Delphinella abietis* (Rostr.) E. Müll.) *fide* Müller & von Arx, 1962]; these remain to be confirmed.

A significant problem in the taxonomy of *Mycosphaerella* is the degree of host specificity of the various species. Most anamorph species are still defined based on the host and thus assumed to be host-specific, restricted at least to a family of phanerogamic plants (Chupp, 1954; Braun, 1995a). However, the tenability of many species is called into question because some taxa, including *M. punctiformis*, the type species of *Mycosphaerella*, have been shown to be polyphagous (von Arx, 1949). The co-occurrence of morphologically different anamorphs on the same host adds valuable information to support arguments against conspecificity of morphologically similar teleomorphs. Morphologically identical species on different hosts, which also conform in ecology and anamorphs, might be regarded as conspecific. However, this would still have to be confirmed by cross inoculation trials and molecular studies.

Because of their simple ascomatal morphology, it is unclear whether *Mycosphaerella* forms a monophyletic group or whether the simple morphology is derived, in which case *Mycosphaerella* would be paraphyletic or even polyphyletic. If the group turns out to be para- or polyphyletic, then monophyletic groups could possibly be characterized by the anamorphs.

In the past, several segregates from *Mycosphaerella* have been proposed, based on anamorphs. Examples include: *Cercosphaerella* Klebahn (with *Cercospora* anamorphs), *Ovosphaerella* Laibach (with *Ovularia*), *Ramisphaerella* Laibach (with *Ramularia*), *Septisphaerella* Laibach (with *Septoria*), *Ramularisphaerella* Klebahn (with *Ramularia*) and *Septorisphaerella* Klebahn (with *Septoria*). These genera, described by Klebahn (1918) and Laibach (1922) respectively, have never been widely used. Moreover, most of these generic names were not validly published, and are therefore unavailable for potential future segregation of *Mycosphaerella*.

It also remains to be seen whether the anamorph genera as currently circumscribed are monophyletic; preliminary studies on the ultrastructure of conidiogenesis of *Septoria sensu lato* (Verkley, 1997; Verkley & Priest, this volume) have shown that percurrently and sympodially proliferating cells can occur within a single strain, as is the case with other, unrelated fungi. Some *Mycosphaerella* species produce a *Septoria* anamorph in the spring (under humid conditions) that develops into a *Phloeospora* Wallr. conidioma in the summer on the same leaves. This suggests that the supposed differences between pycnidial and acervular conidiomata may be taxonomically insignificant. *Septoria heraclei* (Lib.) Desm. is a typical example. Potebnia (1908) was the first author to describe and discuss this phenomenon in detail.

Recent molecular data support the hypothesis that convergent evolution could have occurred in *Mycosphaerella* (Crous *et al.*, 1999). Thus, although *Uwebraunia* and *Dissoconium* are two morphologically similar anamorphs, they differ in ecology, and may not both be allied with *Mycosphaerella*. Based on these data (Crous *et al.*, 1999; Stewart *et al.*, 1999) and the preceding discussion, 23 anamorph genera are presently accepted to have *Mycosphaerella* teleomorphs (Table 1). Using rDNA sequence analysis of the ITS-1 and ITS-2 regions, representatives of some of these genera have been compared in the present study (Table 2). The objectives were to determine whether *Mycosphaerella* is para- or polyphyletic, and whether the morphological characters used to separate the various anamorph genera are phylogenetically informative, or whether some generic concepts require revision.

Materials and methods

DNA AMPLIFICATION AND SEQUENCING

Genomic DNA was isolated from fungal mycelium (Table 1) collected directly from malt extract agar plates (MEA) (Biolab, Midrand, South Africa), using a modification of the isolation protocol of Lee & Taylor (1990). DNA quantification was done by UV spectroscopy using a Beckman Du Series 7500 Spectrophotometer. Template DNA was amplified in a 25 µl PCR reaction mix containing 2 units of *Taq* polymerase (Boehringer Mannheim, Germany), PCR reaction buffer supplied by the manufacturer, an additional 1.25 mM MgCl₂, and 500 µM each of dATP, dCTP, dGTP, and dTTP, with 60 pmol ITS-1 and ITS-4 primers (White *et al.*, 1990). The reaction profile was as follows: initial denaturation at 96°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 75°C for 2 min, and final extension at 75°C for 7 min in a Rapidcycler (Idaho Technology Idaho, U.S.A.). A negative control using water instead of template

Table 1. Anamorph genera linked to *Mycosphaerella*¹.

Conidia								
Anamorph genus	Conidiomata ²	Proliferation ³	Colour ⁴	Septation	Locii ⁵	Arrangement ⁶	Mycelium ⁷	Reference
<i>Cercospora</i> Fres.	F	S	H	0–multi	T,D,R	S	I	Pons & Sutton (1988)
<i>Cercosporella</i> Sacc.	F	S	H	multi	T,R	S	I	Deighton (1973)
<i>Cercostigmina</i> U. Braun	F,S	P	P	multi	I	S	I	Braun (1993)
<i>Cladosporium</i> Link	F,Sol	S	P	0–5	T,D,R, P	S,C	I	von Arx (1949); David (1997)
<i>Clypeispora</i> Ramaley	P	M	H	0	I	S	I	Ramaley (1991)
<i>Colletogloeopsis</i> Crous & M.J. Wingf.	A	P,S	P	0–1	I	S	I,E	Crous & Wingfield (1997a)
<i>Dissoconium</i> de Hoog, Oorschot & Hijwegen	F/Sol	S	P	1	I	S	I,E	Crous <i>et al.</i> (1999)
<i>Fusicladiella</i> Höhn.	F/S	M	P	1	D	S	I	von Arx (1983)
<i>Miuraea</i> Hara	F,Sol	S	H,P	muriform, multi	I	S	I,E	von Arx (1983)
<i>Mycovellosiella</i> Rangel	F,Sol	S	P	0–multi	T,D,R	S,C	I,E	Deighton (1973); Crous <i>et al.</i> (1998)
<i>Passalora</i> Fr.	F,S	S	P	0–multi	T,D,R	S	I	Braun (1995b)
<i>Phaeoisariopsis</i> Ferraris	F,Syn	S	P	1–multi	T,D,R	S	I	von Arx (1983)
<i>Phaeophleospora</i> Rangel	P	P	P	0–multi	I	S	I	Crous & Wingfield (1997b)
<i>Phloeospora</i> Wallr.	A	S	H	multi	I	S	I	Sivanesan (1984)
<i>Pseudocercospora</i> Speg.	F,S,Sol,Syn	S	P	1–multi	I	S,C	I,E	Deighton (1976)
<i>Pseudocercosporella</i> Deighton	F,S,Sol	S	H	1–multi	I	S,C	I,E	Braun (1998)
<i>Ramularia</i> Unger	F,S,Sol	S	H	0–5	T,D,R	S,C	I,E	von Arx (1983); Braun (1998)
<i>Septoria</i> Sacc.	P	S	H	1–multi	I	S	I	von Arx (1983)
<i>Sonderhenia</i> H.J. Swart & J. Walker	P	P	P	0–5	I	S	I	Swart & Walker (1988)
<i>Stenella</i> Syd.	F,Sol	S	P	0–multi	T,D,R	S,C	I,E	Sivanesan (1984)
<i>Thedgonia</i> B. Sutton	F,Sol	S,P	H	1–multi	I	C	I,E	Braun (1995a); Kaiser & Crous (1998)
<i>Uwebraunia</i> Crous & M.J. Wingf.	F/Sol	S	P	1	I	S	I,E	Crous & Wingfield (1996)
<i>Xenostigmina</i> Crous	F,S,Sol	S,P	P	muriform, multi	I	S	I,E	Crous & Corlett (1998)

¹ *Asteromella* Pass. & Thüm. spermatial states have also been described in *Ascochyta* Lib., *Asteroma* DC., *Phyllosticta* Pers. and *Phoma* Sacc.² Fasciculate (F), sporodochial (S), solitary (Sol), pycnidial (P), acervular (A) or synnematosus (Syn).³ Sympodial (S), percurrent (P), monoblastic, determinate (M).⁴ Hyaline (H), pigmented (P).⁵ Thickened (T), darkened (D), refractive (R), protruding (P), inconspicuous (I).⁶ Solitary (S), chains (C).⁷ Internal (I), external (E).

DNA was included in each experiment. PCR products were separated on a 0.8 % (wt/v) agarose gels (Promega, Madison, Wisconsin) stained with ethidium bromide and visualized under UV illumination. PCR products were purified by using a QIAquick PCR Purification Kit (Qiagen GmbH, Germany). The purified PCR products were sequenced using an ABI Prism 377 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut), with an ABI PRISM™ Dye Terminator Cycle sequencing Ready Reaction Kit (Perkin Elmer, Warrington, UK).

PHYLOGENETIC ANALYSIS

The nucleotide sequences of the 5.8S rRNA gene and the flanking internal transcribed spacers (ITS-1 and ITS-2) were assembled using Sequence Navigator™ version 1.0.1. (Perkin Elmer, Applied Biosystems, Inc., Foster City, CA). Alignments of the sequence files were made using CLUSTAL W (Thompson *et al.*, 1994) and improved by eye where necessary. Alignment gaps were coded as missing data. Phylogenetic analyses were performed with PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0b2a (Swofford, 1999). Maximum parsimony analysis was conducted for 284 parsimony-informative characters in the alignment using heuristic search option with 1000 random sequence input orders for exact solution. A strict consensus dendrogram was constructed with the equally most parsimonious trees. Clade stability was assessed by 1000 parsimony bootstrap replications. Tree scores, including tree length, consistency index, retention index, rescaled consistency index and homoplasy index (CI, RI, RC and HI), were also calculated. *Botryosphaeria dothidea* (GenBank accession no. AF195774) was used as outgroup. Two species of *Ramulispora* Miura were also included. The latter genus has been linked to teleomorphs in *Tapesia* (Pers.) Fuckel (Dyer *et al.*, 1996). Sequence data of isolates were deposited in GenBank (AF222826–222850).

Results

MORPHOLOGY OF TELEOMORPHS

Mycosphaerella is well-defined by erumpent to superficial, small, simple, globose, ostiolate ascomata, bitunicate, fasciculate asci, the complete absence of hamathecial filaments, and 1-septate, hyaline ascospores that lack appendages, but can have a mucous sheath (Taylor & Crous, 1998).

In contrast to the vast number of *Mycosphaerella* species described, only a few taxa can be distinguished among the saprobic species. Based on a preliminary examination of 500 type specimens by A. Aptroot, many synonyms of polyphagous saprobic species were identified, e.g. 121 for *M. punctiformis*, 140 for *M. tassiana*, 65 for *M. subradians* (Fr.) J. Schröter and 43 for *M. longissima* (Fuckel) Lindau. These species were often described merely on the basis of the host. These synonymies still have to be

confirmed with cultural studies and molecular techniques.

Based on the monographic revision of *Mycosphaerella* to date (A. Aptroot, in prep.), the following sections (modified from Barr, 1972) are recognized:

(i) Section *Mycosphaerella*, characterized by cylindrical asci and mostly uniseriate, thin-walled, often small, inequilateral ascospores that are constricted at the septum, with rounded apices. Anamorphs: Typically *Ramularia* with *Asteromella* spermatial anamorphs. Representative species: The common polyphagous *M. punctiformis* (Pers. : Fr.) Starb.

(ii) Section *Tassiana* M.E. Barr, characterized by pyriform asci and irregularly arranged, thick-walled, nearly equilateral ascospores that are often large and constricted at the septum, relatively broad with rounded ends. Anamorph: Typically *Cladosporium*. Representative species: The common polyphagous species *M. tassiana* (De Not.) Johanson (with large ascospores) and *M. longissima* (Fuckel) Lindau (with small ascospores). Further research is required to determine if the teleomorphs of *Cladosporium* subgen. *Heterosporium* (David, 1997) could be accommodated here.

(iii) Section *Caterva* M.E. Barr, characterized by cylindrical asci and irregularly arranged, thin-walled, inequilateral, often medium-sized ascospores that are rarely constricted at the septum, with more or less pointed ends. *Asteroma* and *Asteromella* spermatial anamorphs are typical. Representative species: The common polyphagous *M. subradians* (Fr.) J. Schröter.

(iv) Section *Longispora* M.E. Barr, characterized by cylindrical asci with aggregated, thin-walled, long, slender, mostly equilateral ascospores that are rarely constricted at the septum, characteristically with rounded apices and pointed bases. Anamorphs: *Phloeospora* or *Septoria sensu lato*. Representative species: *M. eryngii* (Fr.) Oudem. (with short spores), *M. latebrosa* (Cooke) J. Schröt. (with longer spores) and *M. populi* (Auersw.) J. Schröt. (with the longest spores in the genus). *Sphaerulina* Sacc., which differs only by additional ascospore septa, may be a synonym.

(v) Section *Fusispora* M.E. Barr, characterized by pyriform asci and irregularly arranged, thin-walled, mostly equilateral, fusiform ascospores that are rarely constricted at the septum, pointed at both ends. Anamorphs have not been proven. Representative species: The common *M. lineolata* (Roberge) J. Schröt. on members of the *Poaceae*.

(vi) Section *Plaga* M.E. Barr (including Section *Macula* M.E. Barr) including endophytic species sporulating on leaf spots, many of which are descri-

bed as plant pathogens. This section is characterized by obovoid to ellipsoidal or cylindrical asci, small to medium sized, fusiform to obovoid ascospores, with rounded ends. The majority of the described species originate from warm-temperate and tropical areas. Anamorphs: *Colletogloeopsis*, *Mycovellosiella*, *Phaeophleospora*, *Pseudocercospora*, *Pseudocercospora*, *Sonderhenia*, *Stenella* Syd., *Uwebraunia* and possibly others. Representative species: *M. suttoniae* Crous & M.J. Wingf. and others listed by Crous (1998) on *Eucalyptus* species.

ANAMORPH RELATIONSHIPS

Of the 27 anamorph genera linked to *Mycosphaerella* by Kendrick & DiCosmo (1979), 23 are recognized in the present study (Table 1). These anamorph genera have been separated into more 'natural' or recognizable units based on features such as mycelium (presence or absence of superficial mycelium, and texture thereof), conidiophores (arrangement, branching, pigmentation), conidiogenous cells (placement, proliferation, scar type) and conidia (formation, shape, septation, wall and pigmentation).

MOLECULAR PHYLOGENY

Phylogenetic analysis of the 5.8S, ITS-1 and ITS-2 rDNA of 46 teleomorph and/or anamorph species in the *Mycosphaerella* complex, as well as two species of *Ramulispora* (*Tapesia* teleomorphs) revealed three clades. The major *Mycosphaerella* clade included most of the anamorphs studied, except *Dissoconium* (clade 2). However, this clade was only weakly supported by bootstrap values (53%), and should still be considered as part of the major *Mycosphaerella* clade. As expected, species of *Ramulispora* (*Tapesia* clade 3) grouped outside *Mycosphaerella*. Contrary to expectations, *Mycosphaerella* proved to be monophyletic. In most cases, isolates representing the same anamorph genus clustered together. In several instances, however, representatives of the same anamorph genus were found in more than one clade, suggesting that some forms may have evolved more than once or be plesiomorphic in the complex (Fig. 1).

Discussion

Species of *Mycosphaerella* are either saprobes or plant pathogens, usually causing leaf spots. In addition, some species have been isolated as endophytes (Crous, 1998), and such taxa might sporulate either before or after shedding of leaves. Crous (1998) reported that as many as four species could occur in the same lesion on leaves of *Eucalyptus* species, further suggesting that some may be primary and

others secondary pathogens. There may be a gradient of parasitism or succession of *Mycosphaerella* spp. on some hosts, i.e. lesions caused by primary pathogens could later be colonized by secondary pathogens, and possibly even later by saprobic taxa or endophytes. However, no species of *Mycosphaerella* has yet been recovered in detailed studies of foliicolous fungi occurring as endophytes in hosts in the *Proteaceae* known to have *Mycosphaerella* pathogens (Swart *et al.*, 2000; J. E. Taylor *et al.*, unpublished). It can be assumed that many plant pathogenic species have a latent phase, and that during this phase they could be isolated together with true endophytes. More research needs to be focused on the different sections of *Mycosphaerella*, however, to further clarify these issues, and determine if the species included differ in general ecology.

Field observations suggest that a major division of *Mycosphaerella* into two groups with different life styles (parasitic vs. saprophytic) would be artificial. There appears to be a strong correlation between the life styles of *Mycosphaerella* spp. and different climatic zones. In arctic and boreal regions, species appear to be saprobic, fruiting on overwintered leaves and stems (with species of the section *Tassiana* dominant). In the tropics, species of *Mycosphaerella* tend to be predominantly plant pathogens, sporulating on leaf spots on leaves that are often still attached to the host (with species of the section *Plaga* dominant). In temperate and subtropical regions, both groups occur, often sporulating on leaf spots or whole leaves that may or may not be dead (with species of the sections *Caterva* and *Mycosphaerella* dominant).

ANAMORPH RELATIONSHIPS

In *Mycosphaerella*, the morphology of the teleomorphs is conserved, and the morphologically informative morph tends to be the anamorph. A single teleomorph morphology can be linked to more than one anamorph morphology. This may reflect our inability to distinguish the teleomorphs of two biological species with distinct anamorphs, or a failure to recognize a species of *Mycosphaerella* with synanamorphs. Although the latter has rarely been considered in the literature on *Mycosphaerella*, it has been noted in cultures of *Mycosphaerella suttoniae* Crous & M.J. Wingf. (Crous & Wingfield, 1997b). Conflicting reports of different anamorphs for a single teleomorph may also be based on uncorroborated links observed in nature. Given the fact that several species regularly occur on the same lesion, any links based on association only must be treated with suspicion. Taxonomists working with *Mycosphaerella* have not typically included cultural experiments, a situation that

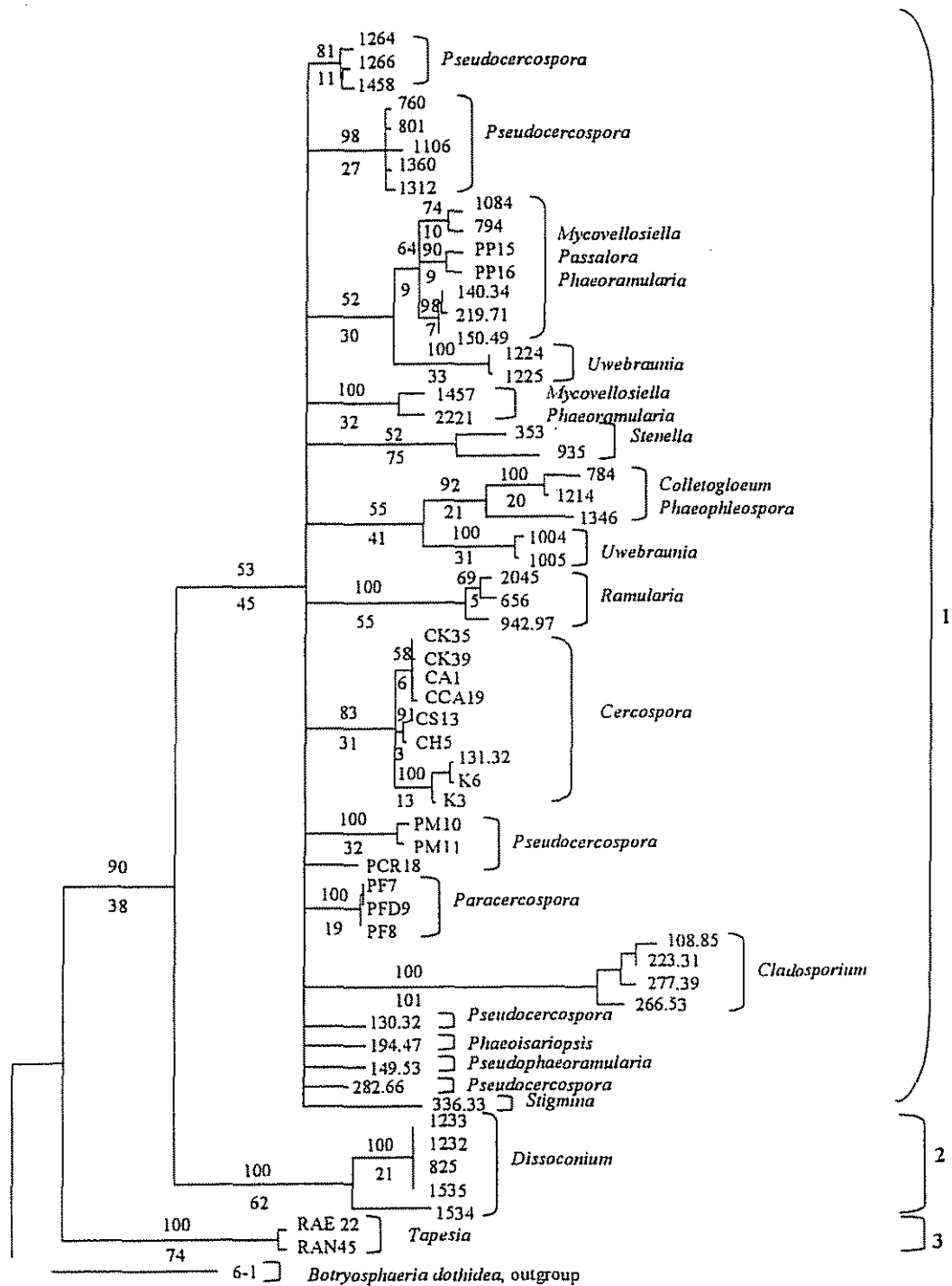


Fig. 1. The majority rule consensus tree of 80 equally most parsimonious trees derived from the alignment of the 5.8S rDNA and ITS-1 and ITS-2 regions of 58 *Mycosphaerella* strains using parsimony analysis with heuristic search and 1000 random sequence input orders. The robustness of the tree was assessed with 1000 bootstrap replicates. The tree was rooted with the outgroup *Botryosphaeria dothidea* (GenBank Accession No. AF195774). Tree length = 1367, CI = 0.407, RI = 0.680, RC = 0.277, HI = 0.593. The bootstrap values and lengths are indicated above and below the tree branches, respectively.

should be remedied in order to prove the links between species of *Mycosphaerella* and their anamorphs.

Considerable attention has been given to conidiomatal structure and mode of conidiogenesis (von Arx, 1983; Sutton & Hennebert, 1994) in this group of fungi, and significant overlap has been observed

between pycnidia, acervuli and subepidermal sporodochia (Braun, 1995a). Nag Raj (1993) discussed the lack of distinguishing characters between pycnidia and acervuli, while Crous (1998) commented on sporodochia that appeared acervular when immature, which were noted by Braun (1995a) as subepidermal sporodochia. Sutton *et al.* (1996) examining immature

material of *Cylindrosporium ribis* J.J. Davis, concluded that the conidiomata were acervular, and established the name *Phloeosporrella ribis* (J.J. Davis) B. Sutton, Crous & Shamoun. Braun (1998), however, interpreted the conidiomata as subepidermal sporodochia, and established the name *Pseudocercosporrella ribis* (J.J. Davis) U. Braun. This single criterion, and how it is interpreted, is all that distinguishes these two genera. It is clear that distinctions between some of the coelomycete and hyphomycete anamorph genera associated with *Mycosphaerella* are debatable.

Deighton recognized genera of cercosporoid fungi based on conidiogenesis and conidium release, as it is inferred from the dehiscence scar (Deighton, 1973, 1976, 1987, 1990). David (1993) used SEM to show differences in conidial scar structures between *Mycosphaerella* anamorphs such as *Cladosporium*, *Cercospora* and *Stenella*. The stability of this feature in some genera, however, is questionable. Genera that have been distinguished by different modes of proliferation of the conidiogenous locus sometimes include species that are variable for this character, as reported in *Septoria/Stagonospora* (Sacc.) Sacc. by Verkley (1997; see also Verkley & Priest, this volume). Crous (1998) also reported that marginal thickening along the conidial rim, the character that separates *Paracercospora* from *Pseudocercospora*, disappears in culture, while conidiogenous cells on older herbarium specimens of *Pseudocercospora* species sometimes again develop such marginal thickening. For the majority of genera, however, scar features appear to be a usable, stable feature (Braun, 1995a).

MOLECULAR PHYLOGENY

Based on the ITS-1 and ITS-2 DNA phylogeny obtained here for the various *Mycosphaerella* spp. and their anamorphs (Fig. 1), three clusters were identified. These include a major monophyletic *Mycosphaerella* cluster (with subclusters for cercosporoid and coelomycete anamorphs), and a second cluster with *Dissoconium* anamorphs. A third cluster represents *Ramulispora* spp. with *Mollisia* (Fr.) P. Karst. (= *Tapesia*) teleomorphs. The *Ramulispora* spp. were included in this study because they are still often incorrectly treated as anamorphs of *Mycosphaerella* (under the name *Pseudocercosporrella*). Although not related to *Mycosphaerella*, their taxonomy must still be resolved, and for the time being they will be referred to as the *Tapesia* cluster.

Based on the ITS data, it is still too early to say whether *Mycosphaerella* is mono- or polyphyletic. Although the *Dissoconium* anamorphs cluster outside the main monophyletic *Mycosphaerella* cluster, this smaller cluster is not strongly supported by bootstrap

values. This situation will be reassessed in future studies incorporating more isolates (those included here being primarily cercosporoid), and also by analyzing other areas of the genome. Of note, however, are the subclusters coinciding with the different anamorph genera, which will be discussed below.

Clade 1. *Mycosphaerella* species with cercosporoid and coelomycete anamorphs

Pseudocercospora, *Paracercospora* and aggregates

Pseudocercospora is morphologically variable, and accommodates all cercosporoid anamorphs with pigmented conidiophores and conidia with inconspicuous conidial scars. Braun (1995a) showed that all the other characters are variable. These include the presence or absence of secondary mycelium, conidiophores that can be solitary to fasciculate, arranged in loose or rich fascicles or dense sporodochial conidiomata, which can also be superficial or subepidermal. Conidia are primarily formed singly, rarely in chains; they are scolecosporous, multiseptate, subhyaline to brown, mostly have transverse eusepta, and rarely have longitudinal or oblique septa. Conidiogenous cells are polyblastic, proliferating sympodially, or rarely percurrently by inconspicuous, regular annellations. Scars are mostly inconspicuous and flat, or can occur on small outgrowths that appear as reduced denticles.

Several genera are morphologically similar to *Pseudocercospora*. *Semipseudocercospora* J.-M. Yen includes species with conidiogenous cells that are conspicuously denticulate, non-geniculate, and give rise to transversely euseptate conidia with basal cells that constrict to narrow hila. Similarly, species of *Denticularia* Deighton also have denticulate conidiogenous cells that give rise to 0–1(–3)-septate, fusiform–ellipsoidal, catenate conidia. *Pantospora* Cif. species have dense, synnematus fascicles, and produce dictyospores or scolecospores.

Deighton (1976) originally distinguished *Cercoseptoria* Deighton from *Pseudocercospora* based on the narrowly acicular conidia that taper only slightly at the truncate base in species of *Cercoseptoria*. This separation was later found to be impractical, and *Cercoseptoria* was accepted as a synonym of *Pseudocercospora* (Deighton, 1987). *Paracercospora* Deighton (1979) was distinguished from *Pseudocercospora* based on the narrow thickening along the rim of the scars on the conidiogenous cells and the conidial hila. Crous (1998), in a study of *Pseudocercospora basiramifera* in culture, found that this thickening was not a stable feature. Furthermore, based on ITS-1 & ITS-2 rDNA sequence analysis, Stewart *et al.* (1999) were

unable to distinguish species of *Pseudocercospora* from those of *Paracercospora*, and therefore reduced *Paracercospora* to synonymy under *Pseudocercospora*. Following the concepts of Deighton (1976, 1979), *Pseudocercospora natalensis* would have been placed in *Cercoseptoria*, and *P. basiramifera* and *Paracercospora fijiensis* in *Paracercospora*.

Pseudophaeoramularia U. Braun represents species intermediate between *Pseudocercospora* and *Phaeoramularia* Muntaniola, with unthickened, but slightly darkened, refractive hila, and catenate conidia. Although separate in the final analysis, in some trees *Pseudophaeoramularia angolensis* clustered with *Pseudocercospora*, suggesting that the concept of *Pseudocercospora* could be further expanded to incorporate taxa with catenate conidia, and unthickened, but slightly darkened, refractive hila. *Cercospora angolensis* was placed in *Phaeoramularia* by Kirk (1986), who observed conidia to be catenate. Braun (1999b) found conidial hila to be unthickened, and he therefore placed the species in *Pseudophaeoramularia*. Analysis of additional species of the former genus would help clarify this subgroup. For the present, however, it seems best to retain *Pseudophaeoramularia* separate from *Pseudocercospora*.

Many different groups of cercosporoid fungi are now accommodated in *Pseudocercospora*. In 1991 R.K. Verma (pers. comm.), who studied over 300 different species of *Pseudocercospora*, suggested that seven groups could be recognized in the genus. Three groups were characterized by having well-developed stromata and branched conidiophores, that could be (i) arranged in dense fascicles, (ii) be longer and in loose fascicles, or (iii) in dense, synnematosous conidiomata, with sympodially, or rarely percurrently proliferating conidiogenous cells. A further two groups were distinguished based on their poorly developed or absent stromata, and (iv) denticulate conidiogenous cells, or (v) percurrent proliferation of the conidiogenous cells. The final two groups were distinguished by having no or poorly developed stromata, with conidia that could be catenate, with slightly thickened hila, and (vi) not forming rope-like structures on leaf surfaces, or (vii) forming them in abundance. Based on current taxonomic concepts, groups 6 and 7 can be accommodated in *Mycovellosiella*, because of the smooth superficial hyphae and thickened conidial loci. Group 5 was described as *Cercostigmina* U. Braun (1993), although some species have well-developed stromata, and rough conidia. Braun *et al.* (1999) recently introduced *Scolecostigmina* U. Braun to accommodate species with sporodochial conidiomata, firm stromata, verruculose conidiophores, coarse annellations, and multi-septate scolecospores that are

smooth to rough, and thick-walled. Group 4 could possibly be accommodated in *Denticularia* or *Semipseudocercospora*, as discussed above, which essentially leaves three groups that are distinguished based on whether their conidiomata consist of short, densely arranged conidiophores, longer, loosely arranged conidiophores, or loosely synnematosous conidiophores. The type species of *Pseudocercospora*, *P. vitis* (Lév.) Speg., frequently forms loosely synnematosous conidiomata, and it is therefore debatable whether conidiophore arrangement can provide useful characteristics for further subdivision of the genus.

Braun (1998) proposed a formal division of *Pseudocercospora* into five sections, viz. sect. *Pseudocercospora* (conidiophores very long, in dense, loosely synnematosous conidiomata), section *Cercocladospora* (G.P. Agarwal & S.M. Singh) U. Braun (conidiophores solitary or in loose fascicles, non-sporodochial, conidia scolecosporous, pluriseptate, superficial mycelium formed or lacking), section *Cercoseptoria* (Petr.) U. Braun (conidiophores in dense sporodochial conidiomata, conidia scolecosporous, pluriseptate, superficial mycelium usually absent, rarely present), section *Helicomina* (L.S. Olive) U. Braun (conidia strongly curved to helicoid), and section *Brachypseudocercospora* U. Braun (conidia solitary, rarely in chains, non-scolecosporous, (0-)1-3(-4)-septate), but he emphasized that these 'units' should only be regarded as morphological groups, which are formally treated as sections, independent of their taxonomic value.

Based on ITS-1 and ITS-2 sequence data obtained for species of *Pseudocercospora* in the present study (STE-U 760, 801, 1106, 1264, 1266, 1312, 1360, 1458, PCR 18, PM 10, 11, CBS 130.32, 282.66), several subclades could be distinguished within the cercosporoid clade (Fig. 1). Isolates from *Eucalyptus* species occurred in two subclades, while those from banana clustered separately, suggesting that coevolution with the host may play a role, or species on the same host are simply more closely related. In earlier analyses (Crous *et al.*, 1999; Stewart *et al.*, 1999), species of *Paracercospora* grouped with *Pseudocercospora*, although in our final analysis in this study, they again grouped separately. However, *Ps. basiramifera* (STE-U 1266), which has a marginal thickening along its conidial rim, clustered with species of *Pseudocercospora*.

In some trees, *Phaeoisariopsis griseola* (synnematosous conidiophores with slightly thickened conidial loci) (CBS 194.47), and *Stigmina platani* (muriformly distoseptate conidia, and conidiogenous cells with irregular percurrent proliferations) (CBS 336.33) also clustered with species of *Pseudocercospora*, although

the final analysis showed them to be distinct, but closely related. Braun (1993) and Crous (1998) speculated that species of *Stigmina* would not have *Mycosphaerella* teleomorphs, and that those species with reported *Mycosphaerella* teleomorphs would be better accommodated in *Cercostigmina* or *Xenostigmina*. The fact that *S. platani* groups in the *Mycosphaerella* cluster refutes these arguments. Additional genera in this complex that could possibly also have teleomorphs in *Mycosphaerella* include *Scolecostigmia* (Braun *et al.*, 1999) and *Dictyorostrella* U. Braun (Braun, 1999a).

Another *Pseudocercospora* subgroup that contains species from *Eucalyptus* leaves includes *P. crystallina*, *P. colombiensis*, *P. heimii*, *P. irregulariramosa*, and *P. heimioides* (STE-U 801, 760, 1360, 1312). All species in this cluster (except *P. colombiensis*) form red crystals in culture. Although the ascospore germination patterns differ among these species (Crous, 1998), the DNA data obtained here suggest that these taxa are closely related, and with the exception of *P. colombiensis*, may represent different varieties of *P. heimii*. Isolates representing the *P. heimii*-complex have also recently been obtained from eucalypts collected in Hawaii and Brazil, and this species may thus have a very wide geographical distribution. Further collections and pathogenicity trials are required to clarify this issue. Species in this clade have light brown, smooth to finely verruculose, obclavate to subcylindrical conidia, with internal and external mycelium, small, dense fascicles situated on brown stromata, and conidiophores that are frequently reduced to conidiogenous cells.

Presently no single character or set of morphological characters separates the subgroups of *Pseudocercospora* that have emerged from DNA sequence data. Analysis of more species is required to provide an expanded view of these groupings. What these data have shown is that several of the morphological features discussed above, used to help group species of *Pseudocercospora*, are not indicative of phylogeny.

Cercospora and *Passalora*

Species of *Cercospora* have conspicuously thickened, darkened conidial scars and hyaline to subhyaline, acicular, cylindrical-filiform, multiseptate conidia. These species formed a distinct subclade in *Mycosphaerella* (Fig. 1). Species with slightly thickened conidial scars and subglobose, ellipsoidal-ovoid, broadly obclavate-fusiform, sparsely septate, usually pigmented conidia are accommodated in *Passalora*. Several taxa that have been traditionally treated in *Cercospora* have subsequently been reallocated to *Passalora*, while *Phaeoisariopsis* is seen as the syn-nematous analogue of *Passalora* (Braun, 1995b).

Passalora, *Mycovellosiella* and *Phaeoramularia*

Phaeoramularia comprises *Ramularia*-like species with pigmented conidia, thickened, darkened and refractive loci, and lacking superficial mycelium. *Mycovellosiella* is similar, but differs in having superficial mycelium that frequently climbs leaf hairs.

Three species of *Mycovellosiella*, namely *M. bellynckii* (CBS 150.49), *M. vaginae* (CBS 140.34) and *M. eucalypti* (STE-U 1457), were included in the present study. The former two species clustered with *Phaeoramularia dissiliens* (CBS 219.77), *Passalora arachidicola* (PP16) and *Passalora personata* (PP15), suggesting that these genera are very similar, while *M. eucalypti* clustered with *Phaeoramularia saururi* (STE-U 2221). The type specimen of *Phaeoramularia* was recently examined by U. Braun, who found it to represent a *Mycovellosiella* species.

Braun (in Braun & Melnik, 1997) assigned *Fusicladium levieri* Magnus (= *F. kaki* Hori & Yoshino) to *Phaeoramularia* but monographic studies of *Fusicladium* and allied genera recently completed suggest that *F. levieri* has to be retained in *Fusicladium* (U. Braun *et al.*, unpublished). Braun (in Braun & Melnik, 1997) reduced *Hormocladium* Höhn., based on *F. levieri*, to synonymy with *Phaeoramularia*. However, *Hormocladium* is an older name and would have priority unless the name *Phaeoramularia* is conserved. This species has catenate conidia similar to *Phaeoramularia* species, but SEM studies reveal that scars and conidial hila are fusicladium-like. Some species of *Fusicladium* produce conidia singly, in chains, or both, depending on the specimen examined. Catenation of conidia can thus not be used to separate these species into different genera. Hence, *Hormocladium*, which has catenate conidia, has to be considered a synonym of *Fusicladium* and is no longer considered a synonym of *Phaeoramularia*. This complex will be fully addressed in a separate molecular study that is presently underway.

Mycosphaerella africana (STE-U 794) and *M. keniensis* (STE-U 1084) have no known anamorphs, but cluster together in a clade with *Mycovellosiella*, *Passalora* and *Phaeoramularia* anamorphs. This suggests that they would probably have the same type of anamorph if they exist, or that they may have lost the ability to form anamorphs, and are holomorphic teleomorphs.

Stenella

Mycosphaerella parkii (anamorph: *Stenella parkii*) (STE-U 353) clustered with *M. marksii* (STE-U 935), for which no anamorph has yet been described. A recent examination of several cultures of *M. marksii*

has shown, however, that hyphae can be verruculose, suggesting that the anamorph, if it exists, will probably be a species of *Stenella*. *Stenella* species are characterized by solitary or catenate conidia with thickened, darkened, refractive conidial hila and verrucose, superficial hyphae. The formation of verrucose, superficial hyphae separates this genus from *Mycovellosiella* and *Phaeoramularia*. The relationships among these anamorph genera also deserve further investigation.

Ramularia

Three species with *Ramularia* anamorphs were included in this study, namely *Mycosphaerella fragariae* (anamorph *R. grevilleana*) (STE-U 656), *M. punctiformis* (*Ramularia* sp.) (CBS 942.97) and *R. collo-cygni* (STE-U 2428). While the first two species have typical *Mycosphaerella* teleomorphs, *R. collo-cygni* was considered an unusual species of *Ramularia* (Braun, 1998) because of its curled conidiophores, and conidia with somewhat eccentrically positioned scars. *R. collo-cygni* was initially considered distinct from *Ramularia*, and typified the genus *Ophiocladium* Cavara (as *O. hordei* Cavara) (Sutton & Waller, 1988). Results of our study show that not only does *R. collo-cygni* cluster closely with the other two typical *Ramularia* species, but the teleomorph of *R. collo-cygni*, if it exists, would likely be a species of *Mycosphaerella*. *Mycosphaerella punctiformis* is also the type of the genus *Mycosphaerella*, and is appropriately included in the main *Mycosphaerella* clade. The conidia of *M. fragariae* and *M. punctiformis* are catenate, but *R. collo-cygni* belongs to a group of *Ramularia* species with solitary conidia. The present molecular data support Braun's (1998) taxonomic treatment of *Ramularia*, based on the premise that separation into two genera based on conidial catenation is untenable.

Colletogloeum and *Phaeophleospora*

The genera *Colletogloeum* and *Phaeophleospora* respectively represent acervular and pycnidial anamorphs of *Mycosphaerella*. Both genera include species with verruculose, brown conidia situated on brown conidiogenous cells that proliferate percurrently in both genera, but also sympodially in *Colletogloeum*. Furthermore, *Colletogloeum* species mostly form 0(-1)-septate conidia, whereas species of *Phaeophleospora* form aseptate to multiseptate scolecospores. These two genera form one subclade within the cercosporoid clade, together with *Uwebraunia*. As more species of *Phaeophleospora* are added in future analyses, this grouping may begin to diverge from

Uwebraunia. For the present, however, *Phaeophleospora* and *Colletogloeum* remain easily recognizable anamorph genera.

Clade 2. *Mycosphaerella* species with *Dissoconium* anamorphs

The genus *Dissoconium* was originally described for *D. aciculare*, a hyperparasite of an *Erysiphe* species (de Hoog *et al.*, 1983). A further species, *D. dekkeri*, was later described (de Hoog *et al.*, 1991). Crous & Wingfield (1996) erected the genus *Uwebraunia* for species thought to be pathogenic to *Eucalyptus*. However, later comparisons showed that one species, *U. lateralis* (STE-U 825, 1232, 1233), was morphologically similar and had identical ITS sequences to isolates of *D. dekkeri* (STE-U 1535) (Crous *et al.*, 1999). This was the first report of a *Mycosphaerella* teleomorph for a *Dissoconium* species. Based on sequence data (Crous *et al.*, 1999), it also appeared that *Dissoconium* represented a separate group in *Mycosphaerella*. Presently, *Dissoconium* is accepted for species thought to be hyperparasitic, with wide host ranges. This contrasts with the more host-specific, plant-pathogenic species that are accommodated in *Uwebraunia*. This also suggests that convergent evolution of morphology has occurred, and thus *U. ellipsoidea* (STE-U 1224, 1225) and *U. juvenis* (STE-U 1004, 1005) with similar morphologies reside in separate subclades in the larger *Mycosphaerella* clade.

Clade 3. *Mycosphaerella* species with *Cladosporium* anamorphs

Cladosporium is distinguished from other genera in this complex by its species with pigmented, catenate conidia with thickened, darkened, characteristically protuberant conidial scars and hila. Furthermore, the species usually lack abundant superficial mycelium, and conidia are usually in branched chains, being subglobose, ellipsoidal-ovoid to subcylindrical, and sparsely septate.

Two species of *Mycosphaerella* are found in this clade, namely *M. tassiana* (CBS 108.85, 223.31) (anamorph: *Cladosporium herbarum*) and *M. asteroma* (CBS 266.53), which has no known anamorph. In some analyses, *Cladosporium* clustered outside the larger *Mycosphaerella* clade, although this separation was never strongly supported by bootstrap values. In the final analysis, however, the *Cladosporium* clade grouped within the main *Mycosphaerella* clade. These data are preliminary, however, and many more taxa must be added to determine whether all species of *Cladosporium* are allied with *Mycosphaerella*, or only the saprobes.

Clade 4: *Tapesia* species with *Ramulispora* anamorphs

Several *Pseudocercospora*-like species occur on roots and basal parts of small-grain crops. These species are presently included in the anamorph genus *Ramulispora*, which is confined to species occurring on grasses and cereals (von Arx, 1983) and have discomycetous, *Tapesia* teleomorphs (Robbertse *et al.*, 1995; Dyer *et al.*, 1996). Although morphologically similar to *Pseudocercospora*, species of this genus typically have teleomorphs in *Mycosphaerella* (Braun, 1995a). In the present analysis, *Ramulispora* clusters well outside the *Mycosphaerella* cluster.

Future research

The observations presented in this study suggest that the ITS-1, 5.8S and ITS-2 regions, which contain approximately 300 informative sites in the alignment (data not shown), are suitable for distinguishing species and the various anamorph genera in *Mycosphaerella*. However, other more conserved areas of the genome such as 18S and 28S rDNA should also be analyzed to see if they reveal additional distinct groups within *Mycosphaerella*, or provide better bootstrap support for otherwise weakly supported clades. Other questions are whether saprobic and pathogenic taxa might emerge as distinct genera, and whether any tendency towards coevolution with hosts might exist.

Thoughts and observations presented here represent only a preliminary step towards solving questions of host specificity and determining whether *Mycosphaerella* is monophyletic. Presently, the majority of the anamorph genera discussed appear to be correctly allied with *Mycosphaerella*. The distinctions between the anamorph genera in this complex are currently based on small and frequently inconspicuous differences and these need clarification. For the present, the classification system followed by most taxonomists interested in anamorphs of *Mycosphaerella* remains valid. Delimitation of some anamorph genera in the major *Mycosphaerella* clade must now be studied in more detail. An unexpected result of this study is that the very large genus *Mycosphaerella* appears to be an essentially monophyletic group. This holds true despite the many anamorph genera that occur in the genus.

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