The value of ascospore septation in separating *Mycosphaerella* from *Sphaerulina* in the Dothideales: a Saccardoan myth?

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Ascospore septation was used by Saccardo as a primary character to separate genera in the Dothideales (Ascomycetes). The genus *Sphaerulina* (3- to multi-septate ascospores) was thus distinguished from *Mycosphaerella* (1-septate ascospores). Several species in *Sphaerulina* were found to have similar anamorphs as *Mycosphaerella*. Sequence data derived from the rDNA (ITS 1, 5.8S and ITS 2) gene suggest that *Sphaerulina* is heterogeneous, and that species with *Mycosphaerella*-like anamorphs belong in *Mycosphaerella*, while those with yeast-like anamorphs belong to the Dothioraceae. *Sphaerulina eucalypti*, which occurs on *Eucalyptus* leaves in South Africa, is transferred to *Sydowia*. This species is also newly linked to its anamorph, *Selenophoma eucalypti*. *Mycosphaerella sphaerulinae* and its *Pseudocercospora sphaerulinae* anamorph are newly described from leaf spots of *Eucalyptus globulus* and *E. nitens* in Chile. This study contributes to growing phylogenetic evidence that ascospore septation is a poor indicator of generic status in the Ascomycetes.

Keywords: *Eucalyptus*, leaf spot, *Mycosphaerella*, *Pseudocercospora*, *Sphaerulina*, *Sydowia*, systematics.

Ascospore septation was originally proposed as a character of considerable value to separate genera (Saccardo, 1882). This character continues to be employed as pivotal and convenient in the classification of various ascomycetes. Results obtained from recent phylogenetic studies have, however, shown that ascospore septation is an unreliable feature to define genera, e.g. in the Hypocreales (Rossman & al., 1999), the Valsaceae (Venter & al., 2002), the Lasiosphaeriaceae (Miller & Huhndorf, 2002) and the Sordariaceae (Dettman & al., 2001).

In the Dothideales, ascomatal morphology and type of centrum development are routinely used to separate families, whereas habit, ascomatal arrangement, stromatal tissue anatomy, ascus and ascos-

pore morphology are used to separate genera (von Arx & Müller, 1975; Dennis, 1981; Sivanesan, 1984; Barr, 1987; Hanlin, 1990). The Dothidealean genera *Mycosphaerella* Johanson and *Sphaerulina* Sacc. are chiefly distinguished based on species having 1-septate ascospores in the former, and three- to pluriseptate ascospores in the latter genus. The present study emerged from the discovery of a specimen with 3-septate ascospores, matching the generic description of *Sphaerulina*. This specimen was collected in Chile, where it was associated with severe leaf spots on species of *Eucalyptus* L'Herit. A review of records of fungi associated with *Eucalyptus* revealed an additional species of *Sphaerulina, S. eucalypti* Verwoerd & Du Plessis (Verwoerd & Du Plessis, 1931). The aims of this investigation were, therefore, to compare the Chilean *Sphaerulina* sp. with *S. eucalypti*. In addition, we considered the phylogenetic relationship of *Sphaerulina* to *Mycosphaerella*, a genus that is commonly associated with leaf spots on *Eucalyptus*, and with which *Sphaerulina* is morphologically similar.

Materials and methods

Morphology

Samples were processed by soaking leaf tissue pieces bearing ascomata in water for 2 h, and inducing ascospore discharge on 2% malt extract agar plates (MEA; Biolab, Midrand, South Africa), supplemented with 0.1 g/L streptomycin sulphate, as explained in Crous (1998). Colonies derived from single ascospores were subsequently transferred to divided plates containing MEA and carnation leaf agar (CLA; Fisher & al., 1982) to promote sporulation. Cultures were incubated at 25 °C under continuous near-ultraviolet light. Cultural characteristics were described and colony colours (Rayner, 1970) were assessed. For microscopic examination structures were mounted in lactic acid and measurements made at 1000 × magnification. The 95% confidence intervals for spore sizes were determined from 30 measurements and the minimum and maximum ranges given in parentheses, rounded to the nearest 0.5 µm. For other structures, minimum and maximum values are given. Herbarium specimens have been lodged and reference cultures linked to these specimens are maintained at the Centraalbureau voor Schimmelcultures in Utrecht, the Netherlands (CBS).

DNA amplification and sequence determination

Genomic DNA was isolated from fungal mycelium grown on MEA plates as described by Crous & al. (2000). Approximately 550 bases were amplified for each isolate (spanning the 3' end of 18S,

ITS1, the 5.8S rRNA gene, ITS2 and the 5' end of the 28S rRNA gene) using the primers ITS1 and ITS4 (White & al., 1990). The protocol and conditions followed were as explained in Braun & al. (2003).

Phylogenetic analysis

The nucleotide sequences generated in this study were added to the outgroup, *Mycocentrospora acerina* AY266155 and other sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov). The alignments were assembled using Sequence Alignment Editor version 2.0a11 (Rambaut, 2002). Adjustments for improvement were made by eye where necessary. Phylogenetic analyses with neighbour joining (Saitou & Nei, 1987, using the Kimura-2-parameter substitution model) were done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford, 2000). Alignment gaps were treated as missing data and all characters were unordered and of equal weight. The robustness of the trees was evaluated by 1,000 bootstrap replications (Hillis & Bull, 1993). Resulting trees were printed with TreeView Version 1.6.6 (Page, 1996). Isolates included in the phylogenetic analysis are listed in Tab. 1.

Results

Taxonomy and morphology

The genus *Sphaerulina* is typified by *S. myriadea*. Although pseudothecia are anatomically similar to those of *Mycosphaerella*, the genus is separated from the latter based on ascospore septation. To clearly illustrate the similarities between these two genera, a full description of *S. myriadea* is provided below. Although attempts have been made to recollect and culture this fungus, these have thus far proven unsuccessful, and hence no cultures were available for study.

Sphaerulina Sacc., Michelia 1: 339. 1878.

Sphaerulina myriadea (DC. : Fr.) Sacc., Syll. Fung. 2: 186. 1883. – Figs. 1–4.

≡ *Sphaeria myriadea* DC., Fl. Française 6: 145. 1815: sanctioned by Fries, Systema Mycol. 2: 524. 1823.

≡ *Sphaerella myriadea* (DC. : Fr.) Rabenh., Fungi Europaei Exs., ed. nov., ser. 2, no. 149. 1860.

Type. – FRANCE: On upper and lower surface of dead leaves of *Quercus* L. (Fagaceae). *S. myriadea* designated as lectotype of *Sphaerulina* by Höhnel in Ann. Mycol. 18: 95, 1920.

Tab. 1. – Isolates sequenced for phylogenetic analysis

Species	Isolate	Host	Geographic origin	GenBank accession no.
Dothistroma sp.	STE-U 3779	*Pinus radiata*	Ecuador	AY293062
Passalora ampelopsis	CBS 249.67	*Parthenocissus tricuspidata*	Simeria, Romania	AY293063
	STE-U 3630			
Passalora sp.	STE-U 3951	*Tilia americana*	Canada	AY293064
Mycosphaerella sphaerulinae[1]	STE-U 4314	*Eucalyptus* sp.	Chile	AY293066
(*Pseudocercospora sphaerulinae*)[2]				
Sphaerulina musae	STE-U 5380	*Musa* sp.	Costa Rica	AY293061
Sphaerulina polyspora	CBS 354.29	Unknown	Unknown	AY293067
	STE-U 4301			
Sphaerulina rehmiana	CBS 355.58	*Rosa* sp.	Unknown	AY293065
(*Septoria rosae*)	STE-U 4302			
Sydowia eucalypti	STE-U 5247	*Eucalyptus* sp.	Tulbagh, South Africa	AY293060
(*Selenophoma eucalypti*)				
Sydowia eucalypti	STE-U 659	*Eucalyptus* sp.	Robben Island, South Africa	AY293059
(*Selenophoma eucalypti*[1])				
Sydowia polyspora	CBS 544.95	*Larix decidua*	Netherlands	AY293068

[1] Ex-type cultures.
[2] Anamorphs with known teleomorphs are listed in brackets.

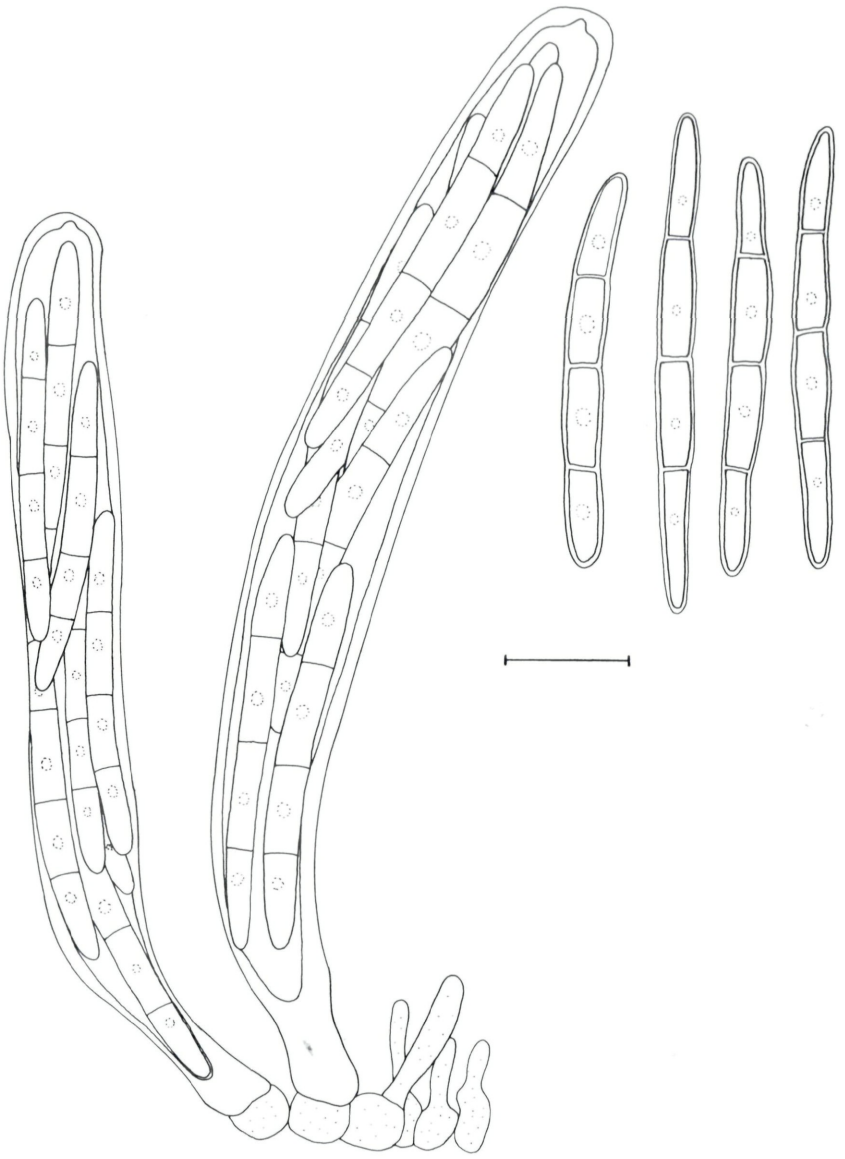
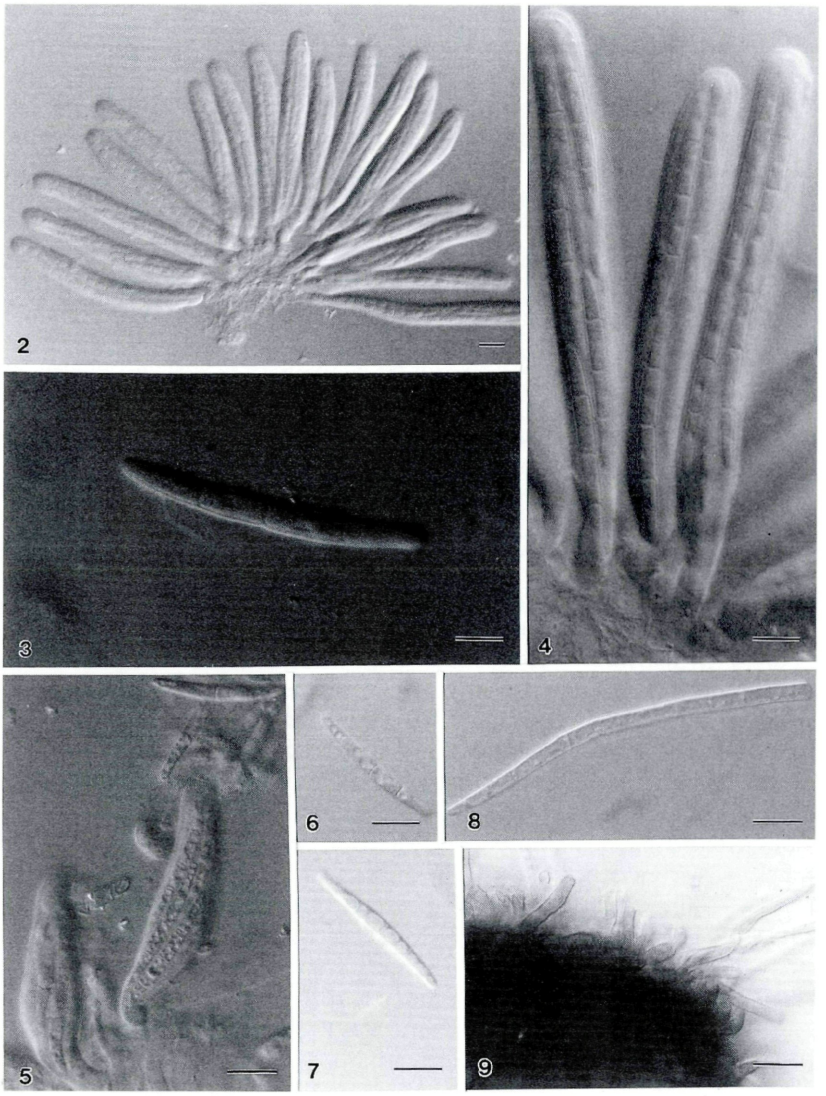


Fig. 1. – Asci and ascospores of *Sphaerulina myriadea*. – From type. – Bar = 10 µm.

Leaf spots amphigenous, but more prominent on upper surface, subcircular to irregular, separate or coalescing, from 1 mm diam to large lesions covering the whole width of the leaf, light brown. – Pseudothecia predominantly epiphyllous, black, globose, subepidermal and evenly dispersed on *Quercus* L. and *Fagus* L., erumpent



Figs. 2–9. *Sphaerulina myriadea* and *Mycosphaerella sphaerulinae*. – 2–4. *S. myriadea*. – 2. Asci. – 3. Three-septate ascospore. – 4. Asci. – 5–9. *M. sphaerulinae* and its anamorph *Pseudocercospora sphaerulinae*. – 5. Asci. – 6, 7. Ascospores. – 8. Conidium. – 9. Conidiogenous cells. – Figs. 2–4 from BPI 623686, Figs. 3–9 from holotype. – Bars = 10 µm.

and superficial on *Castanopsis* (D. Don) Spach, aggregated in groups of 3–9, up to 300 µm diam; wall up to 30 µm thick, consisting of 2–6 layers of medium to dark brown *textura angularis*. – Asci aparaphysate, fasciculate, bitunicate, subsessile, cylindrical to subcylin-

drical, straight to slightly curved, 8-spored, 80–240 × 12–15 µm. – Ascospores multiseriate, overlapping, hyaline, smooth, guttulate, thick-walled, straight, fusiform with obtuse ends, 3(–4)-septate, widest in two middle cells, not or slightly constricted at septa, tapering towards both ends, (30–)42–48(–50) × 4(–5) µm. (Description based on BPI 623686).

Geographic distribution. – Temperate regions of the Northern Hemisphere.

Hosts. – *Carpinus* L., *Castanopsis* Spach, *Fagus* L., *Quercus* L. (Fagaceae).

Specimens examined. – GERMANY: Driesen, Lasch, distributed in Rabenhorst, Fungi Europaei no. 149 (L). – USA: California, Sequoia National Park. alt. 2590 m, on leaves of *Castanopsis sempervirens* Dudley, H. E. Parks, 18 Jun. 1931, (BPI 623686). – USA: California, Hoberg's Resort, Lake Co., on leaves of *Quercus kelloggii* Newberry, V. Miller, 15 May 1943, (BPI 623707). – USA: Maryland, Marlboro, on leaves of *Quercus alba* L., C. L. Shear, 26 Apr. 1929, BPI 623705. – USA: Texas, Houston, on leaves of *Quercus alba*, H. W. Ravenel, 8 Apr. 1869, BPI 623704.

Preliminary examinations of specimens identified as *S. myriadea* that have been lodged at BPI, lead us to conclude that this taxon represents a species complex. On *Quercus*, for instance, the pseudothecia are more evenly distributed, are smaller and more subepidermal, and the asci (50–70 × 8–11 µm), and ascospores (30–38 × 2.5–3 µm) are smaller than specimens from *Castanopsis*. On one specimen from *Quercus kelloggii* Newberry (BPI 623707), however, pseudothecia also occurred on the abaxial leaf surface, were more closely spaced, larger, and more erumpent than observed on specimens from other *Quercus* species.

Sphaerulina spp. occurring on *Eucalyptus*

Sphaerulina eucalypti was described from leaf spots on a *Eucalyptus* sp. collected in the Western Cape Province by Verwoerd & Du Plessis (1931). The authors also mentioned a species of *Phyllosticta* Pers. to be associated with the teleomorph. We recently recollected this fungus from its type location. The teleomorph was rarely encountered, and found to only be present on leaf litter. Leaf spots were typical of *Selenophoma eucalypti* Crous, C. L. Lennox & B. Sutton (Crous & al., 1995). The '*Phyllosticta*' state on the type specimen was found to be *Selenophoma eucalypti*, which Verwoerd & Du Plessis (1931) correctly suspected to be the anamorph of *Sphaerulina eucalypti*.

In culture, ascospores of *Sphaerulina eucalypti* initially produced a *Hormonema* Lagerb. & Melin synanamorph, and the coelomycetous state developed only later on CLA. The original description

of *Selenophoma eucalypti* was based on thin-walled pycnidia, and polyblastic conidiogenous cells. In the present study the pycnidia on the incubated leaves became thick-walled and stromatic, and developed well-defined conidiophores. On older leaves some of these conidiomata developed into morphologically similar ascomata. Although the anamorph was originally placed in *Selenophoma* Maire, the matured structures on litter also resemble *Sclerophoma* Höhn., characterised by thick-walled, stromatic conidiomata, phialidic conidiogenous cells, and fusiform, aseptate, hyaline conidia. The fact that incubation of leaf tissue can mask differences between these two form genera suggests that the characters circumscribing these genera should be reconsidered.

Sydowia Bres. has anamorphs in *Sclerophoma,* and *Hormonema* synanamorphs (Sivanesan, 1984). Thus the presence of these two anamorphs, and the thick-walled ascostromata dictate that *Sphaerulina eucalypti* would best be accommodated in *Sydowia.*

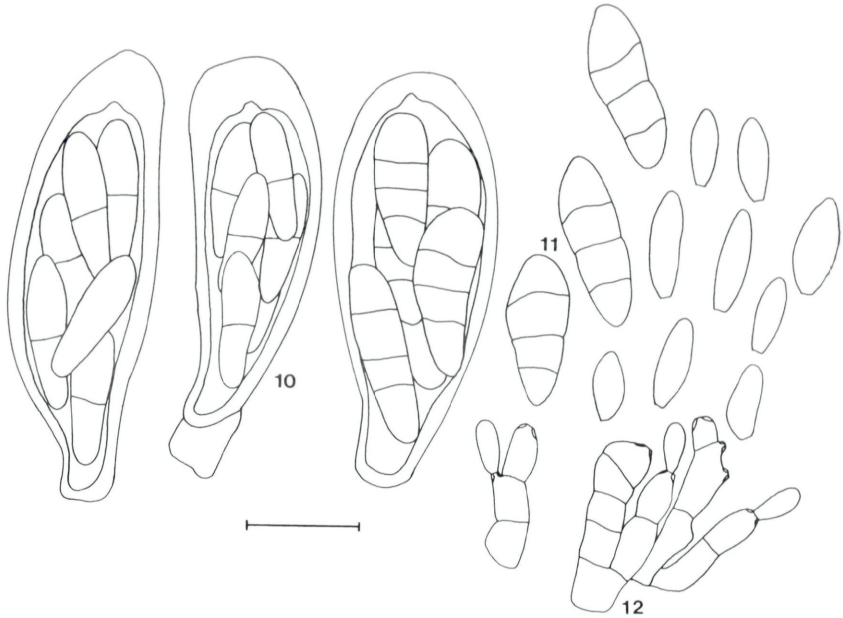
Sydowia eucalypti (Verwoerd & du Plessis) Crous, **comb. nov. –** Figs. 10–12.

Bas.: *Sphaerulina eucalypti* Verwoerd & du Plessis, S. Afr. J. Sci. 28: 296. 1931.

Anamorph: *Selenophoma eucalypti* Crous, C. L. Lennox & B. Sutton, Mycol. Res. 99: 648. 1995.

Synanamorph: *Hormonema* sp.

Leaf spots amphigenous, subcircular, becoming confluent, covering large areas of the leaf, pale brown, surrounded by a narrow, raised, dark brown margin. – Mycelium internal, medium brown, consisting of septate, branched, smooth hyphae, 3–6 µm wide. – Pseudothecia amphigenous, black, subepidermal, becoming erumpent, separate or aggregated in clusters of up to 8, globose, up to 200 µm diam; apical ostiole 10–25 µm wide; wall consisting of several layers of dark brown *textura angularis.* – Asci aparaphysate, fasciculate, bitunicate, subsessile, broadly ellipsoid to clavate, straight to slightly curved, 8-spored, 25–50 × 8–18 µm, with a well-developed apical chamber. – Ascospores bi- to multiseriate, overlapping, hyaline, non-guttulate, thin-walled, straight to slightly curved, obovoid to ellipsoid with obtuse ends, widest at the apical septum or middle of apical cell if 1-septate, (1–)3(–4)-septate at maturity, constricted at the median septum, tapering towards both ends, but more prominently towards the lower end, 10–19 × 5–7 µm. – Conidiomata pycnidial, amphigenous, subepidermal, concolorous with the lesion, with a thin-walled basal layer consisting of 2–3 layers of *textura angularis*, up to 120 µm diam; dehiscence by irregular



Figs. 10–12. *Sydowia eucalypti* and its anamorph *Selenophoma eucalypti*. – 10. Asci. – 11. Ascospores. – 12. Conidiophores and conidia. – From holotype. – Bar = 10 µm.

rupture of the upper wall; becoming stromatic on leaf litter, wall developing 3–6 layers of thick-walled *textura angularis*, black, separate or aggregated, intermingled between pseudothecia, up to 200 µm thick. – Conidiophores absent on living leaves, but becoming prominent once leaves are incubated in moist chambers, hyaline, smooth, branched, 1–7-septate, constricted at the septa, 15–70 × 6–8 µm. – Conidiogenous cells predominantly terminal, hyaline, smooth, phialidic with prominent periclinal thickening, ampulliform to cylindrical, 5–20 × 3.5–7 µm. – Conidia aseptate, hyaline, smooth, fusoid with an acutely rounded apex and subtruncate base, straight to falcate, 8–13(–15) × 2–4(–5) µm *in vivo* (description based on incubated host material).

Geographic distribution. – South Africa (Western Cape, Gauteng), Robben Island.

Hosts. – *Eucalyptus* spp. (Myrtaceae).

Specimens examined. – SOUTH AFRICA: Western Cape Province, Tulbach, on living leaves and leaf litter of *Eucalyptus* sp., B. J. Dippenaar, Holotype PREM 46423, ex Herb. L. Verwoerd, 472. – SOUTH AFRICA: Western Cape, Tulbach, on living leaves and leaf litter of *Eucalyptus* sp., P. W. Crous & J. Stone, 15 Mar. 2002, living culture STE-U 5247.

Cultural characteristics. – As described in Crous & al. (1995)

Colonies obtained from single ascospores of *Sydowia eucalypti* gave rise to *Selenophoma eucalypti* in culture. Sequence data (ITS1, 5.8S and ITS2) derived from the ex-type strain of *Selenophoma eucalypti* (STE-U 659) exhibited seven changes over 565 bases (98.7% homology) with an ascospore isolate of *Sydowia eucalypti* (STE-U 5247).

Mycosphaerella sphaerulinae Crous & M. J. Wingf., **sp. nov.** – Figs. 5–9, 13–17.

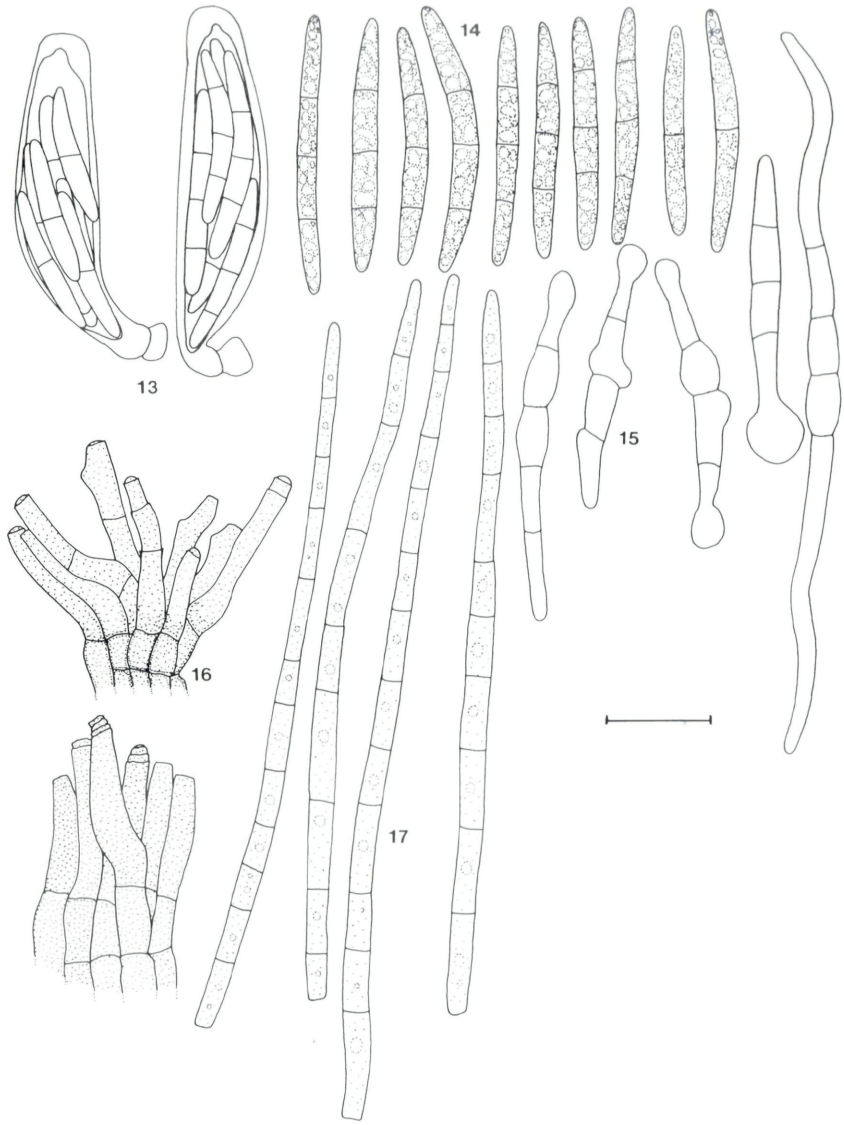
Anamorph: ***Pseudocercospora sphaerulinae*** Crous & M. J. Wingf., **sp. nov.**

Maculae foliorum amphigenae, sed praecipue adaxiales, dilute brunneae, zona fusciore diffusa circumdatae. Mycelium praecipue internum, dilute brunneum, hyphis 3–4 µm latis. Pseudothecia praecipue epiphylla, fusca, subepidermalia, deinde erumpentia, globosa, 55–90 µm diam; ostiolum apicale 10–20 µm diam; paries e 2–3 stratis cellularum brunnearum, textura angulari constans. Asci aparaphysati, fasciculati, bitunicati, subsessiles, obovoidei vel late ellipsoidei, recti vel curvati, 8-spori, 30–50 × 8–10 µm, camera apicali distincta. Ascosporae trivel multiseriatae, hyalinae, guttulatae, tenuitunicatae, modice curvatae, fusoideae-ellipsoideae, utrinque obtusatae, latissimae in media spora, immaturae 1-septatae, maturae 3-septatae, haud constrictae, utrinque aequaliter angustatae, (20–)25–30(–35) × (3–)3.5–4 µm; post 24 horas in agaro maltoso utrinque germinantes, tubis secundum axim sporae extendentibus.

Caespituli fasciculati vel sporodochiales, plerumque epiphylli, brunnei, ad 110 µm diam, 70 µm alti. Conidiophora in fasciculis densis aggregata, e cellulis stromatis brunnei orientia, brunnea, levia, 1–3-septata, subcylindrica, recta vel geniculata-sinuosa, 25–30 × 3–5 µm. Cellulae conidiogenae integratae terminales, dilute brunneae, leves, sursum angustatae in locos conidiogenos truncatos vel hebeter rotundatos, sursum semel vel bis percurrenter proliferantes, 10–20 × 3–4 µm. Conidia singula, dilute brunnea, levia, guttulata, subcylindrica, sursum subobtusa, ad basim truncata, recta vel modice curvata, 5–10-septata, 70–100 × 2–3 µm, hilo inconspicuo praedita.

Etymology.– in reference to *Sphaerulina*, the genus with 3-septate ascospores.

Leaf spots amphigenous, subcircular, coalescing to form larger blotches, mostly along leaf margins, up to 30 mm diam, more prominent on adaxial than abaxial leaf surface, pale brown, surrounded by a dark brown, diffuse border on adaxial surface; spots pale brown with indistinct border on abaxial surface. – Mycelium predominantly internal, pale to medium brown, consisting of septate, branched, smooth-walled hyphae, 3–4 µm wide. – Pseudothecia predominantly epiphyllous, dark brown to black, subepidermal, becoming erumpent, globose, 55–90 µm diam; apical ostiole 10–20 µm wide; wall consisting of 2–3 layers of medium brown textura an-



Figs. 13–17. *Mycosphaerella sphaerulinae* and its anamorph *Pseudocercospora sphaerulinae*. – 13. Asci. – 14. Ascospores. – 15. Ascospores germinating on malt extract agar. – 16. Conidiophores. – 17. Conidia. – From holotype. – Bar = 10 µm.

gularis. – Asci aparaphysate, fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid, straight to curved, 8-spored, 30–50 × 8–10 µm, with a well-developed apical chamber. – Ascospores tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, slightly curved, fusoid-ellipsoidal with obtuse ends, widest in middle of the

spore, 1-septate when immature, 3-septate at maturity within the ascus, not constricted at the septa, tapering equally towards both ends, (20–)25–30(–35) × (3–)3.5–4 µm; ascospores germinating after 24 h on MEA from both ends, with germ tubes extending parallel to the long axis of the spore; original spore becoming constricted and distorted, but remaining hyaline. Several germ tubes form swollen ends, which could indicate the onset of appressorium formation. – Caespituli fasciculate to sporodochial, predominantly epiphyllous, medium brown on leaves, up to 110 µm diam and 70 µm high. Conidiophores aggregated in dense fascicles arising from the upper cells of a brown stroma up to 100 µm wide and 40 µm high; conidiophores medium brown, smooth, 1–3-septate, subcylindrical, straight to geniculate-sinuous, 25–50 × 3–5 µm. – Conidiogenous cells integrated, terminal, unbranched, pale brown, smooth, tapering to flat-tipped or bluntly rounded apical loci, proliferating sympodially or 1–2 times percurrently near the apex, 10–20 × 3–4 µm. – Conidia solitary, pale brown, smooth, guttulate, subcylindrical, apex subobtuse, base truncate, straight to slightly curved, 5–10-septate, 70–100 × 2–3 µm; hila inconspicuous (teleomorph and anamorph described from host material).

Geographic distribution. – Chile.

Hosts. – *Eucalyptus globulus* Labill., *E. nitens* Maiden (Myrtaceae).

Specimens examined. – CHILE: on living leaves of *Eucalyptus nitens*, M. J. Wingfield, May 2001, Holotype herb. CBS 6597, Living culture STE-U 4314. – CHILE: on living leaves of *Eucalyptus globulus*, M. J. Wingfield, May 2001, herb. CBS 6580, 6581.

Cultural characteristics. – Colonies circular, erumpent with entire margins, surface and reverse brown (13k), with sparse to no aerial mycelium, reaching up to 15 mm diam after 2 mo at 25 °C in the dark, producing *Pseudocercospora sphaerulinae* in culture.

Sivanesan (1984) illustrated species of *Sphaerulina* that have typical *Mycosphaerella* anamorphs, namely *Cercospora* Fresen. and *Septoria* Sacc. Our results show *Sphaerulina* species with *Pseudocercospora* Speg. and *Septoria* anamorphs cluster in *Mycosphaerella*. These findings suggest that species of *Sphaerulina* having *Mycosphaerella* anamorphs, may be better accommodated in *Mycosphaerella* than *Sphaerulina*.

Phylogeny

The manually adjusted alignment of the ITS nucleotide sequences contained 68 taxa and 626 characters including alignment gaps

(data not shown). Sequence data of the various species were deposited in GenBank (Tab. 1), and the alignment in TreeBase (SN1451).

The analysis of data revealed four large clades (Fig. 18). These included a major *Mycosphaerella* clade (91% bootstrap support), as well as clades containing isolates of the Dothioraceae (100% bootstrap support), and possibly the Xylariales (100% bootstrap support) and a second clade from the Mycosphaerellaceae (*Davidiella* Crous & U. Braun; 100% bootstrap support). Different anamorphs of *Mycosphaerella* represented subgroups within the larger *Mycosphaerella* clade, for example *Passalora* Fr., *Stigmina* Sacc., *Pseudocercospora*, *Cercospora* Fresen. and *Septoria* in group 1 (60% bootstrap support), *Passalora* in group 2 (86% bootstrap support), *Stenella* Syd. in groups 3, 4 and 6 (94, 96 and 100% bootstrap support, respectively) and finally group 7, which contains species of *Ramularia* Unger (100% bootstrap support). Isolates of '*Sphaerulina*' with *Mycosphaerella*-like anamorphs (*M. sphaerulinae, M. rehmiana*) clustered in the Mycosphaerellaceae, while those with yeast-like anamorphs clustered in the Dothioraceae (*Sydowia eucalypti*) and what is assumed to be the Xylariales (*Sphaerulina musae*). *Sphaerulina polyspora* (CBS 354.29) clustered among *Cladosporium* Link anamorphs in *Davidiella*. This placing could not be confirmed based on morphology, as the culture proved to be sterile.

Discussion

To date, the phragmosporous ascospores of *Sphaerulina* have distinguished it from *Mycosphaerella*. This was done in spite of the fact that these species may have had typical *Mycosphaerella* anamorphs such as *Cercospora*, *Cercosporella* Sacc. or *Septoria* (Sivanesan, 1984; Hanlin, 1990). Results of the present study have clearly demonstrated that the number of ascospore septa, although a valuable morphological characteristic in many complexes, should be restricted to a character at species level in the Dothideales. In contrast, anamorph morphology appears to provide a better indication of generic affinity in this order.

As already noted by Kuijpers & Aptroot (2002), the type species of *Sphaerulina*, *S. myriadea,* is very similar to species of *Mycosphaerella* Sect. *Longispora* M. E. Barr, especially *M. latebrosa* (Cooke) J. Schröt., one of the species of *Mycosphaerella* sect. *Longispora*, but has 3(–4)-septate ascospores. As no cultures of *S. myriadea* were available for inclusion in this study, no firm conclusions can yet be made regarding the taxonomic fate of the genus *Sphaerulina*. However, if *S. myriadea* should turn out to be a species of *Mycosphaerella* (1884), the name *Sphaerulina* (1878), being older, would have preference. This would call for a special proposal to

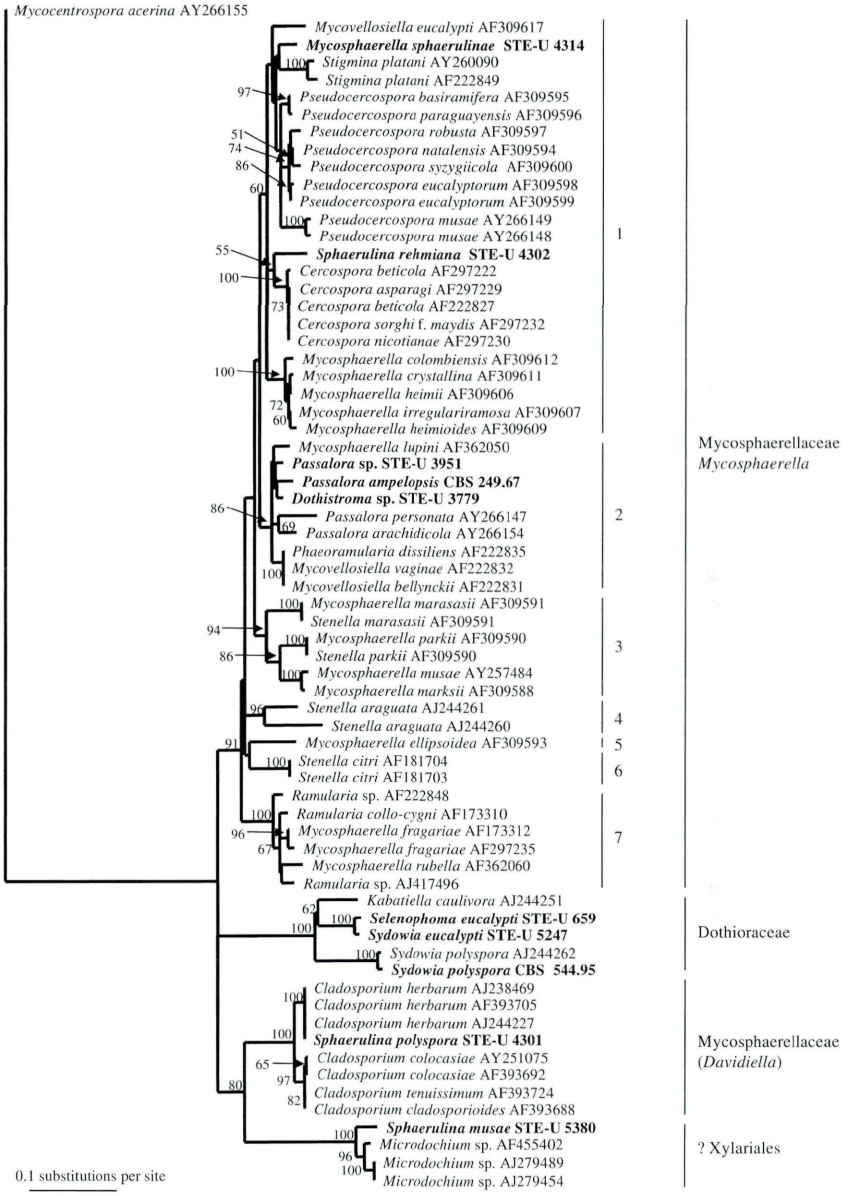


Fig. 18. – Neighbour joining tree obtained from a search of the ITS data set using the Kimura-2-parameter substitution model. The tree was rooted to *Mycocentrospora acerina* AY266155. Bootstrap support values from 1000 replicates are shown at the nodes. Names listed in bold were sequenced during the present study.

conserve the name *Mycosphaerella* over that of the less well-known *Sphaerulina*.

In the present study *M. sphaerulinae* clustered in group 1 of the *Mycosphaerella* clade (Mycosphaerellaceae). This is not totally surprising, however, given its *Pseudocercospora* anamorph. For this species, therefore, the anamorph proved more informative of its phylogenetic position than its ascospore septation. Likewise, a recollection and subsequent cultural study of *Sphaerulina eucalypti* revealed it to be a species of *Sydowia* (Dothioraceae). As in the former example, ascospore septation did not prove indicative of its phylogeny. A preliminary survey of the genus *Sphaerulina* has revealed that some species belong to different families in the Dothideales, or to the Verrucariales (Aptroot 1998), as was confirmed by our study.

From these results we can conclude that the generic definition of the genus *Mycosphaerella* may be inordinately narrow. Although the taxonomic validity of *Sphaerulina* may require revision, some species with 3-septate ascospores assigned to this genus might be better placed in *Mycosphaerella*. Similarily, *S. rehmiana* Jaap, which has a *Septoria* anamorph, clustered together with *Cercospora*, as has been shown for *Septoria* in previous studies (Crous & al., 2001; Verkley & al., 2002). *Sphaerulina polyspora* F. A. Wolf, however, clustered close to the *Cladosporium* Link clade, which is not seen as part of *Mycosphaerella s.str.* (Braun & al., 2003). These findings suggest that species of *Sphaerulina* that have been observed to have *Mycosphaerella* anamorphs (i.e. *Cercospora*, *Cercosporella* Sacc., *Septoria*; Sivanesan, 1984), could be true species of *Mycosphaerella*. Several other species of *Sphaerulina* have also been cultured that have black yeast anamorphs. *Sphaerulina musae* T. Y. Lin & J. M. Yen, which also produced a yeast-like stage in culture, clustered distant from the other *Sphaerulina* species investigated. Species of *Sphaerulina* with *Mycopshaerella* anamorphs, however, appear to be better accommodated in *Mycosphaerella*.

The diversity of anamorph genera associated with *Mycosphaerella* has led to questions as to whether the genus should not be subdivided (Crous, 1998). Although many changes have been proposed pertaining to the circumscription of these anamorph genera (Crous & al., 2000, 2001), few have been shown to reside outside *Mycosphaerella s. str.* (Braun & al., 2003). Some of these anamorphs have clear and well-defined relationships with other teleomorph genera such as *Guignardia* Viala & Ravaz (*Phyllosticta*), while the spermatial *Asteromella* Pass. & Thüm. states have again been shown to be common for many diverse genera. Although recent molecular studies have resulted in the exclusion of *Davidiella* (*Cladosporium* anamorphs) from *Mycosphaerella*, other studies have again led to the

inclusion of *Teratosphaeria* Syd. & P. Syd. (Taylor & al., 2003), elements of *Sphaerulina*, as well as anamorph genera such as *Batcheloromyces* Marasas, P.S. van Wyk & Knox-Dav. (Taylor & al., 2003) and *Ramulispora* Miura (Crous & al., 2003), indicating that the circumsciption of *Mycosphaerella* is still not fully resolved.

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