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# Eyespot of cereals revisited: ITS phylogeny reveals new species relationships

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## Abstract

Four species so far classified in *Pseudocercosporella* or *Ramulispora* (hyphomycetes) are associated with eyespot disease symptoms of cereals. Two of these have been linked to teleomorphs that were described in *Tapesia*. Sequence data derived from the Internal Transcribed Spacer region (ITS1, 5.8S and ITS2) of the rDNA operon showed, however, that the eyespot fungi associated with *Tapesia* are not congeneric with *Ramulispora sorghi*, the type of *Ramulispora*. The genus name *Tapesia* is now rejected in favour of the conserved name *Mollisia*, which appears to comprise heterogeneous fungi. *Tapesia yallundae* is not closely related to the type of *Mollisia*, *M. cinerea*, but clusters separately, being more closely allied to species with *Cadophora* anamorphs. A new holomorph genus, *Oculimacula,* is therefore proposed for teleomorphs of the eyespot fungi, while the anamorphs are accommodated in *Helgardia* gen. nov.

## Introduction

Eyespot disease of cereals is widespread throughout the temperate regions of the world, and causes a damaging stem-base infection of these hosts (Fitt et al., 1990). Severe eyespot lesions girdle the stem and soften the stem-base, resulting in lodging and heavy crop losses (Scott and Hollins, 1974). Four cercosporoid species are known to be associated with eyespot disease of cereals (Nirenberg, 1981; Robbertse et al., 1995), while a sexual state is known for two of these species (Robbertse et al., 1995). The cercosporoid species associated with eyespot disease are rather unusual in resembling leaf spot pathogens of *Pseudocercosporella* Deighton.

The eyespot fungus was originally described as *Cercosporella herpotrichoides* Fron (Fron, 1912). Deighton (1973) established the new genus *Pseudocercosporella* for anamorphs of *Mycosphaerella* Johanson that were *Cercosporella*-like, but had unthickened and inconspicuous conidial scars. He included *C. herpotrichoides* in this genus. Nirenberg (1981) found that the best-known eyespot fungus on wheat, *Pseudocercosporella herpotrichoides*, includes two varieties, *P. herpotrichoides* (Fron) Deighton var. *herpotrichoides* and var. *acuformis* Nirenberg. These varieties were initially thought to correlate with two pathotypes, respectively known as the wheat-type (W-type) and the rye-type (R-type) (Priestley et al., 1992), though an examination of more strains found this to not always be the case (Lucas et al., 2000). In her treatment of this complex, Nirenberg (1981) followed Deighton (1973), and chose *Pseudocercosporella* in which to place *C. herpotrichoides* together with the new variety, as well as two new species which she described from eyespot lesions on cereals in Germany, namely *P. anguioides* Nirenberg and *P. aestiva* Nirenberg.

Nirenberg's treatment received wide recognition and was the first to highlight the fact that several taxa are involved in this disease complex. Von Arx

(1983), however, recognized that the eyespot fungi are unrelated to the *Mycosphaerella* anamorphs included in *Pseudocercosporella*. He observed them to have a mode of conidiogenesis similar to that of *Ramulispora sorghi* (Ellis & Everh.) Olive & Lefebvre, the type of *Ramulispora* Miura. He also found that conidia in all these species developed lateral branches. Robbertse et al. (1995) later demonstrated that the lateral conidial branches were, in most cases, the result of microcyclic conidiation, which is not uncommon among the cercosporoid taxa (Fernandez et al., 1991).

Von Arx (1983) expanded the genus *Ramulispora* to include those species that are indeed *Pseudocercosporella*-like, with or without lateral branches in the conidia that are formed in slimy masses, and parasitize the culm base of gramineous hosts. He transferred *P. herpotrichoides* to *Ramulispora* and indicated that the other species treated by Nirenberg (1981) also had to be allocated in this genus. This recommendation was followed by Boerema et al. (1992), in their treatment of the two varieties of *R. herpotrichoides*. In a later revision of this species complex, Robbertse et al. (1995) found that the two varieties shared a very low percentage RAPD similarity, exhibited differences in spore and colony morphology, infection pathway, fungicide sensitivity, virulence to specific hosts (Scott and Hollins, 1980) and distinct mating populations (Daniels et al., 1991; Dyer et al., 1994; Robbertse et al., 1994). These taxa were therefore recognized as separate species of *Ramulispora* (Robbertse et al., 1995), a genus known to represent pathogens of gramineous plants (Von Arx, 1983; Braun, 1995).

The discovery that the teleomorphs of the eyespot pathogens were actually discomycetes belonging to the genus *Tapesia* (Pers.) Fuckel (Wallwork and Spooner, 1988; Boerema et al., 1992) seemed to support the position taken by Von Arx (1983), namely to remove these pathogens from the *Mycosphaerella* anamorphs in *Pseudocercosporella*. *Tapesia* resides well outside *Mycosphaerella* (Stewart et al., 1999) in the *Helotiales*. But *Tapesia* is now recognized to be congeneric with species of the younger but better-known genus *Mollisia* (Fr.) P. Karst. (Dennis, 1968; Baral, 1985), and the name was therefore rejected in favour of the conserved name *Mollisia* (Hawksworth and David, 1989). Species of *Tapesia* thus require transfer to the recognized generic name *Mollisia*.

*Ramulispora* is typified by *R. sorghi*, a pathogen that causes prominent leaf spots on sorghum called sooty stripe, due to the abundant production of microsclerotia on the leaf surface (Olive et al., 1946; Braun, 1995). The latter pathogen was recently encountered on sorghum in the KwaZulu-Natal Province of South Africa, where it was associated with a severe outbreak of sooty leaf stripe (Mchau et al., 1996). In an attempt to clarify the taxonomic position of *R. sorghi*, as well as the eyespot pathogens of cereals, the present study was undertaken to infer a phylogeny for these fungi in comparison with other members representing their respective anamorph (*Ramulispora*) and teleomorph (*Tapesia*) genera. This was achieved by sequencing the Internal Transcribed Spacer region (ITS1, 5.8S and ITS2) of the rDNA operon, and comparing sequence data from the eyespot and *Ramulispora* isolates with those of known *Mycosphaerella* species (Crous et al., 2001).

## Materials and methods

### *Isolates and DNA amplification*

Isolates studied were obtained from the culture collections of the Centraalbureau voor Schimmelcultures (CBS), and the Department of Plant Pathology at the University of Stellenbosch (STE-U) (Table 1). Single-conidium subcultures were grown on malt extract agar (Biolab, Midrand, Johannesburg) (MEA) plates for 7 days. The isolation protocol of Crous et al. (2000) was used to isolate genomic DNA from fungal mycelia grown on MEA plates. The primers ITS1 (5′TTT CCG TAG GTG AAC CTG C3′) and ITS4 (5′TCC TCC GCT TAT TGA TAT GC′) (White et al., 1990) were used to amplify part of the nuclear rRNA operon using polymerase chain reaction (PCR). The amplified region included the 3′ end of the 18S (small subunit) rRNA gene, the first ITS (ITS1), the 5.8S rRNA gene, the second ITS (ITS2) region and the 5′ end of the 26S (large subunit) of the rRNA gene. The reaction mixture contained 5 μl of diluted sample, 1 × buffer, 8 mM $MgCl_2$, 500 μM of each of the dNTPs, 2.5 U (Bioline) Taq polymerase and 10 pM of each primer and made up to a total volume of 25 μl with sterile water. The cycling conditions comprised denaturing at 96 °C for 5 min, followed by 30 cycles of denaturation at 96 °C (30 s), annealing 55 °C (30 s) and elongation at 72 °C (90 s). A final elongation step at 72 °C for 7 min was included. PCR products were separated by electrophoresis at 75 V for 1 h in a 0.8% (w/v) agarose gel in 0.5 × TAE buffer (0.4 M Tris, 0.05 M NaAc and 0.01 M EDTA, pH 7.85) and visualized under UV light

*Table 1*. Strains sequenced in the present study

| Teleomorph | Anamorph | Accession no. | Collector | Substrate | Origin | GenBank no. (ITS) |
|---|---|---|---|---|---|---|
| *M. cinerea* | Unknown | STE-U 5092 = CBS 412.81 | O. Petrini | *Juniperus communis* | Switzerland | AY259135 |
| *M. dextrinospora* | Unknown | STE-U 5093 = CBS 401.78 | R.P. Korf | Decaying wood | Spain | AY259134 |
| *M. fusca* | *T. fusca* | CBS 234.71 | B. Aebi | *Fagus sylvatica* | Switzerland | AY259138 |
| *M. fusca* | *T. fusca* | CBS 486.48 | Unknown | *Azalea* sp. | Netherlands | AY259137 |
| *M. melaleuca* | Unknown | STE-U 5094 = CBS 89.84 | H. Butin | *Picea abies* needle | Germany | AY259136 |
| *Mycosphaerella capsellae* | *P. capsellae* | CBS 112032, 112033 | R. Evans | *Pisum sativum* | UK | AY259139, AY259140 |
| Unknown | *R. sorghi* | STE-U 905 = CBS 110578 | D. Nowell | *Sorghum bicolor* | South Africa | AY259131 |
| Unknown | *R. sorghi* | STE-U 906 = CBS 110579 | D. Nowell | *Sorghum bicolor* | South Africa | AY259132 |
| Unknown | *R. sorghi* | STE-U 908 = CBS 110580 | D. Nowell | *Sorghum bicolor* | South Africa | AY259133 |

using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK) following ethidium bromide staining.

Polymerase chain reaction products were purified using a NucleoSpin Extract 2 in 1 Purification Kit (Macherey-Nagel GmbH, Germany). The cycle sequencing reaction with 20–40 ng of purified PCR products and 10 pmol primer in a total volume of 10 µl was carried out with an ABI PRISM BigDye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA, USA) containing AmpliTaq DNA Polymerase. The reaction was set up as denaturing at 94 °C for 5 min, followed by 25 cycles of 96 °C for 10 s, 55 °C for 10 s and 60 °C for 4 min, with a final incubation of 30 s at 60 °C. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut).

*Phylogenetic analysis*

The nucleotide sequences of the rDNA gene generated in this study were added to the outgroup, *Botryosphaeria dothidea* (Moug.) Ces. & De Not. (AF027741) and other sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov/) and TreeBASE (http://www.treebase.org/), which were assembled using Sequence Alignment Editor v2.0 (Rambaut, 2002). The sequences were aligned using CLUSTAL W software (Thompson et al., 1994). Adjustments for improvement were made by eye where necessary. Phylogenetic analyses were undertaken using PAUP Version 4.0b10 (Swofford, 2000). Alignment gaps were treated as missing characters and all characters were unordered and of equal weight. Heuristic searches were conducted using 1000 replicates of random addition sequences and tree bisection and reconstruction (TBR) as the branch-swapping algorithm to find maximum parsimony trees. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the trees was evaluated by 1000 bootstrap replications (Hillis and Bull, 1993). Other measures including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC) were also calculated. Resulting trees were printed with TreeView Version 1.6.6 (Page, 1996) and decay indices were calculated with AutoDecay Version 4.0.2 (Eriksson, 1998).

## Results

*Phylogenetic analysis*

Approximately 520–560 bases were determined for each isolate, of which approximately 450–490 bases per sequence (spanning ITS1, 5.8S rRNA gene, ITS2 and the first part of the small subunit gene) were added to the alignment. The manually adjusted alignments of the nucleotide sequences contained 601 characters including alignment gaps (data not shown). Of the aligned nucleotide sites for the data set, 245 characters were parsimony-informative, 61 variable characters were parsimony-uninformative and 295 were constant. Sequences were deposited in GenBank (Table 1), and the alignment in TreeBASE (SN 1392).

Aligned sequences of 39 isolates and an outgroup were subjected to maximum parsimony analysis using the heuristic search option with 1000 random taxon-additions in PAUP (Swofford, 2000). The 14th most parsimonious tree obtained from the heuristic search was evaluated with 1000 bootstrap replications. The

three *R. sorghi* isolates (STE-U 905, 906 and 908) grouped in a strongly supported clade (100%), sharing 55% support with a subclade containing *P. capsellae* (Ellis & Everh.) Deighton (*M. capsellae* A.J. Inman & Sivan.) within *Mycosphaerella* (Figure 1). Species of *Mollisia* and *Tapesia* grouped in a large clade (100% bootstrap support), consisting of three well-defined subclades outside of the *Mycosphaerellaceae*

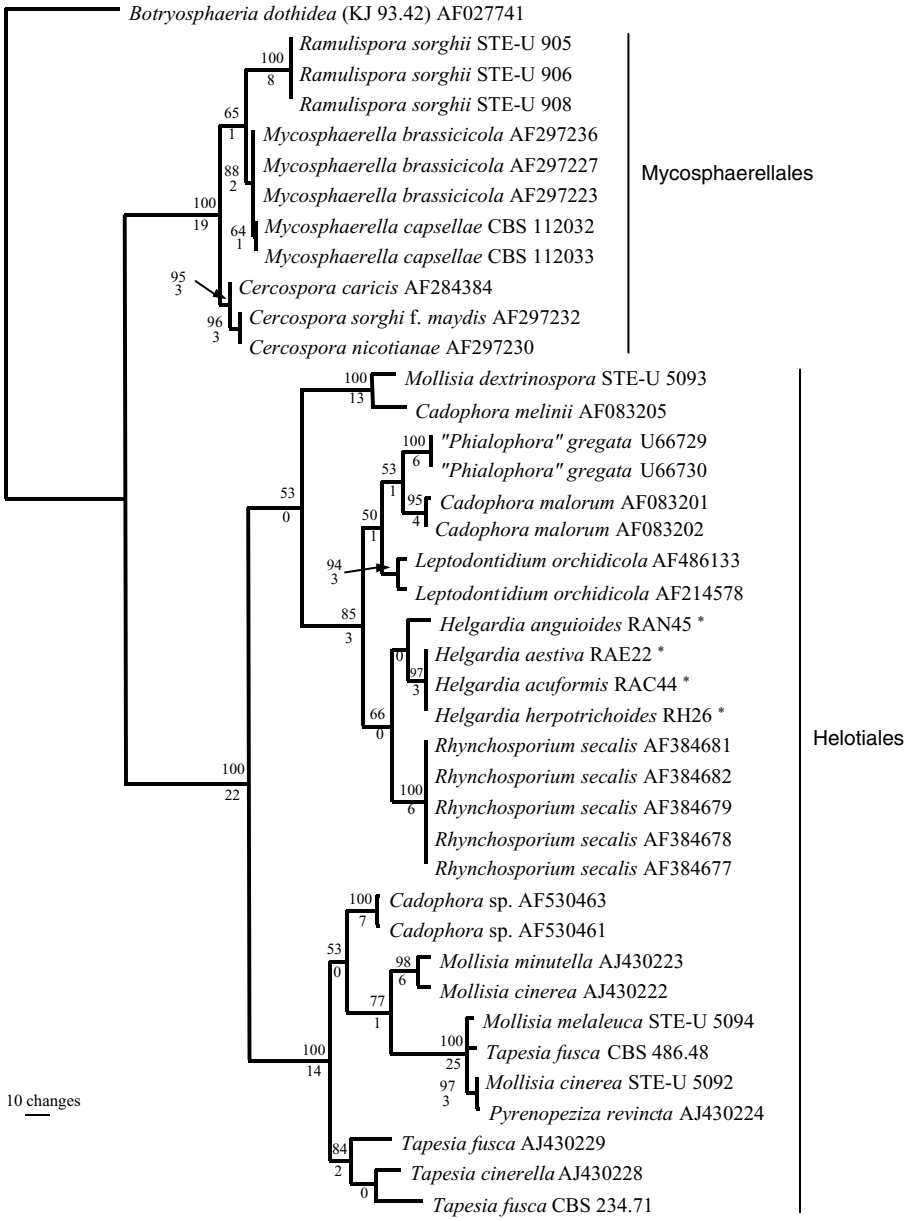



*Figure 1.* One of 14 most parsimonious trees (length = 606 steps, CI = 0.738, RI = 0.919, RC = 0.678) obtained from a heuristic search with 1000 random taxon-additions using a 601 bp alignment of ITS1, the 5.8S rRNA gene and ITS2. Bootstrap support values from 1000 replicates are shown above and decay values below the nodes. *B. dothidea* was used as outgroup (*Sequences from TreeBASE matrix M691).

(*Mycosphaerellales*), comprising species of *Mollisia*, *Tapesia* and *Pyrenopeziza* Fuckel of the *Dermateaceae* (*Helotiales*). *Mollisia dextrinospora* Korf and *Cadophora melinii* Nannf. clustered apart from the main clade. *M. cinerea* (Batsch) P. Karst. and *M. melaleuca* (Fr.) Sacc. grouped in a clade (100% bootstrap support) together with *M. minutella* (Sacc.) Rehm, *Pyrenopeziza revincta* (P. Karst.) Gremmen, *Tapesia fusca*, *T. cinerella* Rehm and *Cadophora* sp. The eyespot '*Ramulispora*' spp. clustered in a clade containing *Phialophora* Medlar (or rather *Cadophora* Lagerb. & Melin *sensu* Gams, 2000), *M. dextrinospora* Korf (STE-U 5093), *Leptodontidium* de Hoog, and *Rhynchosporium* Heinsen ex A.B. Frank isolates (97% bootstrap support). Within this clade, the four species of '*Ramulispora*' together with *Rhynchosporium secalis* (Oudem.) Davis formed a subclade with 88% bootstrap support.

*Taxonomy*

The four species associated with cereal eyespot are obviously not congeneric with *R. sorghi*. For the teleomorphs of these cereal pathogens, the genus *Tapesia* is not available being a rejected name in favour of the conserved name *Mollisia* (Hawksworth and David, 1989), with which it is considered as being synonymous. Furthermore, *Mollisia* also appears to be morphologically and ecologically heterogeneous, and is linked to several different anamorph genera.

Species of *Mollisia* in a broad sense, including the eyespot pathogens, grouped in a large clade containing two well-defined subclades. The first subclade includes the type of *Mollisia*, *M. cinerea* (CBS 412.81, STE-U 5092), with a phialidic anamorph suggestive of a moderately branched *Cystodendron* Bubák, and *Pyrenopeziza revincta*. Species of *Pyrenopeziza* have in the past been linked to *Cystodendron*/*Cadophora*-like anamorphs (Hütter, 1958). *T. fusca* (Pers.) Fuckel, the type of *Tapesia*, has also been linked to a *Cystodendron* anamorph (Aebi, 1972), and is thus distinct from the eyespot pathogens. Isolates identified as *T. fusca*, clustered with *M. cinerea*, apart from the eyespot pathogens.

Species of the second subclade have *Cadophora* (incl. several taxa presently still in *Phialophora*), *Leptodontidium* and *Rhynchosporium* anamorphs. The *Ramulispora*-like anamorphs of the eyespot pathogens of cereals are quite distinct from all these anamorphs of the Dermateaceae, though phylogenetically appear closely related to *Rhynchosporium* (Figure 1). *Ramulispora*, as typified by *R. sorghi*, is a member of the Mycosphaerellaceae. Therefore, it cannot be congeneric with a fungus having a Helotialean teleomorph (viz. the eyespot complex). The latter fungi do therefore not belong in *Ramulispora*, but require a new anamorph genus. *Mollisia*, as typified by *M. cinerea*, occurs in a separate cluster to the eyespot fungi, and has a different anamorph. Likewise, *Tapesia*, typified by *T. fusca*, has a different anamorph, and clusters with *Mollisia*, separate from the eyespot fungi. A new teleomorph genus thus needs to be described for the eyespot fungi.

Oculimacula *Crous & W. Gams, gen. nov.*
Apothecia sessilia, gregaria, 0.5–2.5 mm diam., circularia vel lobata, subiculo hypharum plus minusve brunnearum persistentium insidentia, texto superficiali hypharum pallide brunnearum, angustarum substrato affixa. Discus levis, griseus, marginem *versus* pallide griseus, maturus emarginatus, applanatus ad convexus. Receptaculum pallide brunneum ad griseo-brunneum, crateriforme. Asci 8-spori, unitunicati, clavati vel subcylindrici vel fusoidei, breviter stipitati, poro apicali iodi ope caerulescente. Ascosporae biseriatae ad multiseriatae, hyalinae, leves, unicellulares, fusoideae vel subcylindricae-fusoideae vel clavatae, utrinque rotundatae, plerumque rectae. Paraphyses filiformes, sursum obtusatae, ascis longitudine similes. Excipulum medullare ex hyphis multiseptatis, hyalinis compositum, excipulum ectale e cellulis tenuitunicatis, fuscis, angularibus, marginem versus magis elongatis, constans.

*Anamorphe:* Helgardia Crous & W. Gams.

*Type*: AUSTRALIA. Yallunda Flat, on wheat stubble, 18 Nov. 1986, *H. Wallwork* and *B. Spooner*, K (holotype), ADW (isotype), of *Oculimacula yallundae* (Wallwork & Spooner) Crous & W. Gams.

*Etymology: Oculimacula* = Latin for eyespot, named after the characteristic lesions induced on stems of cereals.

*Apothecia* sessile, gregarious, 0.5–2.5 mm diam., circular to lobate, situated on a subiculum consisting of white to dark brown persistent hyphae, attached to the substrate via a superficial mat of pale brown, thin

hyphae. *Disk* smooth, grey with a pale grey margin, becoming emarginate and flattened to convex at maturity. *Receptacle* pale brown to grey-brown, cup-shaped. *Asci* 8-spored, unitunicate, clavate to subcylindrical or fusoid, with a short stalk, and an apical pore staining blue in Melzer's reagent. *Ascospores* bi- to multiseriate, hyaline, smooth, aseptate, fusoid to subcylindrical-fusoid or clavate with rounded ends, mostly straight. *Paraphyses* filiform with obtuse ends, similar in length to the asci. *Medullary excipulum* consisting of multiseptate, hyaline hyphae. *Ectal excipulum* consisting of thin-walled, dark brown, angular cells, becoming more elongated towards the margin. Anamorph *Helgardia* Crous & W. Gams.

Helgardia *Crous & W. Gams, gen. nov.*
Conidiophora fasciculata vel solitaria in hyphis superficialibus, vel e stromate pallide brunneo oriunda, subcylindrica vel geniculato-sinuosa, raro ramosa, hyalina ad pallide olivacea, levia, seu tantum e cellulis conidiogenis constantia seu uno vel duobus septis divisa, paulo distincta; cellulae conidiogenae integratae, ad apicem dense sympodialiter elongascentes; loci conidiogeni haud inspissati, inconspicui nec fuscescentes. Conidia solitaria, hyalina, levia, in acervis mucidis aggregata, acicularia-filiformia, recta vel curvata, uni- vel multiseptata, saepe conidia secundaria statim proferentia.

Type: FRANCE, holotype of *Helgardia herpotrichoides* (could not be traced in herb. PC); SOUTH AFRICA. Western Cape Province, Moorreesburg, on wheat stubble, 1991, *F. Bester*, CBS 110665 (Dried culture in herb. CBS designated here as *neotype*) of *Helgardia* (isolate genetically identical and sexually compatible with European isolates).

Etymology: *Helgardia*, named after the German mycologist and phytopathologist, Dr. Helgard I. Nirenberg, who first recognized the distinctiveness of these anamorphs on cereals.

*Conidiophores* fasciculate or solitary on the superficial mycelium, or arising from pale brown stromata, subcylindrical to geniculate-sinuous, rarely branching, hyaline to pale olivaceous, smooth, consisting of conidiogenous cells only, or slightly differentiated with up to 2 septa, conidiogenous cells integrated, proliferating sympodially at the apex, with inconspicuous, dense geniculations; loci unthickened, inconspicuous, not darkened. Conidia solitary, hyaline, smooth, arranged in slimy packets, acicular-filiform, straight to curved, one- to multiseptate, forming smaller, secondary conidia via microcyclic conidiation.

Oculimacula yallundae *(Wallwork & Spooner) Crous & W. Gams, comb. nov. Figures 2–6*

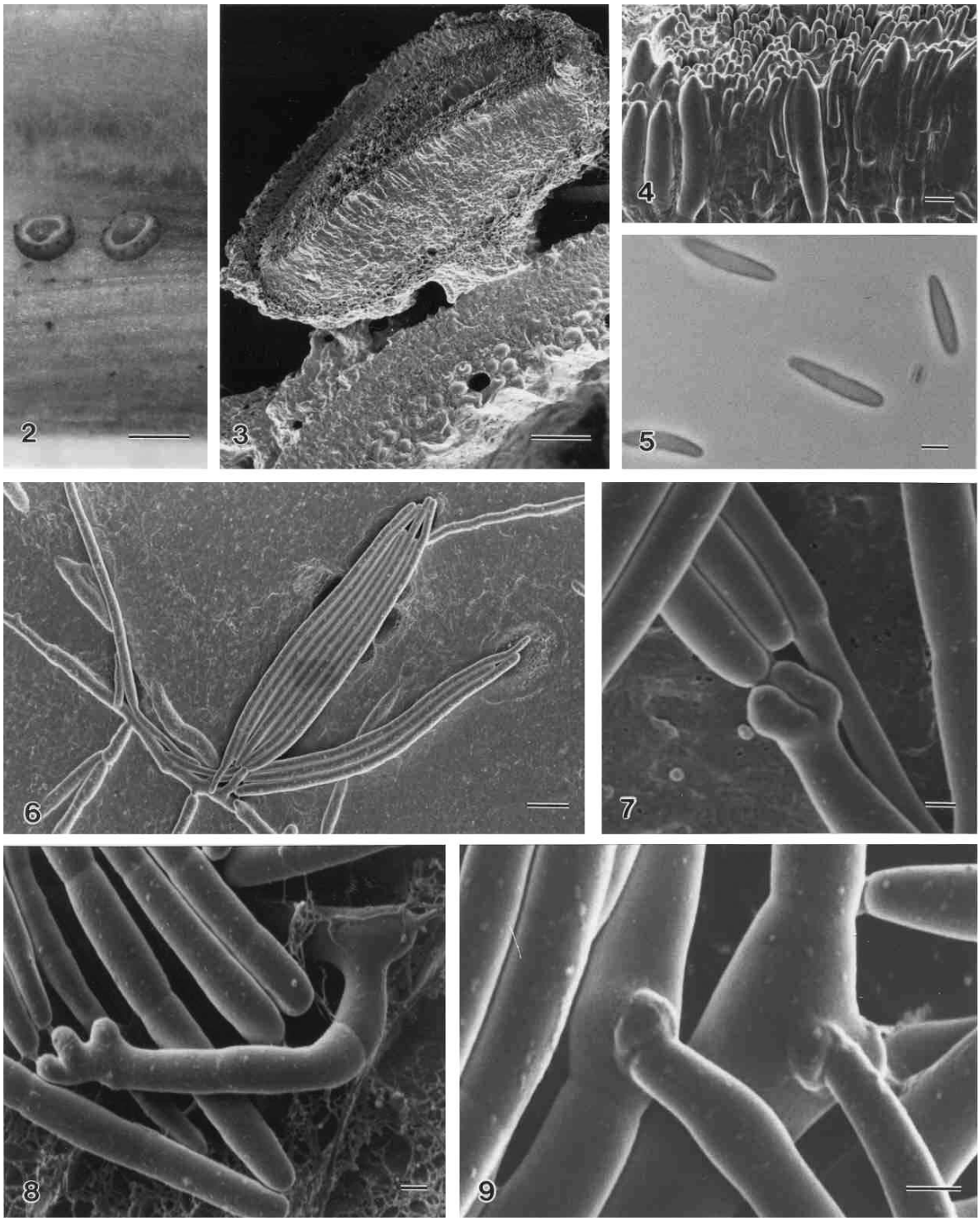
≡ *Tapesia yallundae* Wallwork & Spooner, Trans. Brit. Mycol. Soc. 71: 703 (1988). Anamorph: *Helgardia herpotrichoides* (Fron) Crous & W. Gams, comb. nov.
≡ *Cercosporella herpotrichoides* Fron, Ann. Sci. Agron. Franç. Étrangère, Sér. 4, 1: 11 (1912).
≡ *Pseudocercosporella herpotrichoides* (Fron) Deighton, Mycol. Pap. 133: 46 (1973).
≡ *Ramulispora herpotrichoides* (Fron) Arx, Proc. K. Ned. Akad. Wet. C 86(1): 36 (1983).

Oculimacula acuformis *(Boerema, R. Pieters & Hamers) Crous & W. Gams, comb. nov. Figure 7*

≡ *Tapesia yallundae Wallwork & Spooner var. acuformis Boerema, R. Pieters & Hamers, Neth. J. Pl. Pathol. 98(Suppl 1): 22 (1992).*
≡ *Tapesia acuformis (Boerema, R. Pieters & Hamers Crous, S. Afr. J. Bot. 61: 46 (1995)*. Anamorph: *Helgardia acuformis* (Nirenberg) Crous & W. Gams, comb. nov.
≡ *Pseudocercosporella herpotrichoides* var. *acuformis* Nirenberg, Z. Pflanzenkr. Pflanzensch. 88: 244 (1981).
≡ *Ramulispora herpotrichoides* var. *acuformis* (Nirenberg) Boerema, R. Pieters & Hamers, Neth. J. Pl. Pathol. 98(Suppl 1): 22 (1992) (combination also made by U. Braun, Nova Hedwigia 56: 423, 1993).
≡ *Ramulispora acuformis* (Nirenberg) Crous, S. Afr. J. Bot. 61: 46 (1995).

Helgardia anguioides *(Nirenberg) Crous & W. Gams, comb. nov. Figure 8*

≡ *Pseudocercosporella anguioides* Nirenberg, Z. Pflanzenkr. Pflanzensch. 88: 244 (1981).
≡ *Ramulispora herpotrichoides* var. *anguioides* (Nirenberg) U. Braun, Nova Hedwigia 56: 423 (1993).




*Figures 2–9.* Apothecia of *Oculimacula*, with *Helgardia* anamorphs. (2) Apothecia of *O. yallundae* on wheat stubble. (3) Vertical section through an apothecium of *O. yallundae*. (4) Section through an apothecium of *O. yallundae,* showing ascal layer. (5) Ascospores of *O. yallundae*. (6) Conidia and conidiogenous cells of *H. herpotrichoides*. (7) Conidial hila and conidiogenous cell of *H. acuformis*. (8) Conidial hila and conidiogenous cell of *H. anguioides*. (9) Conidia of *H. aestiva* giving rise to secondary conidia via microcyclic conidiation. Bars = 2 mm, 100, 5, 2, 10 μm in (a)–(e), and 1 μm in (f)–(h).

Helgardia aestiva *(Nirenberg) Crous & W. Gams, comb. nov. Figure 9*

≡ *Pseudocercosporella aestiva* Nirenberg, Z. Pflanzenkr. Pflanzensch. 88: 246 (1981).
≡ *Ramulispora aestiva* (Nirenberg) E.L. Stewart & Crous, Mycol. Res. 103: 1497 (1999).

## Discussion

A recent reclassification of the eyespot pathogens in *Ramulispora* seemed to correct the inadequacy of their placement in *Pseudocercosporella*, which comprises anamorphs of *Mycosphaerella*. The present study has revealed that these assumptions about the phylogenetic position and affinity of the genus *Ramulispora* were incorrect, as was the placement of the sexual state of the eyespot fungi in the genus *Tapesia*. To address this issue, a new teleomorph genus, *Oculimacula*, with its associated anamorph genus *Helgardia*, are proposed. Although it can be argued that a teleomorph genus alone would suffice for these organisms, two related species, namely *H. anguioides* and *H. aestiva*, have not yet been linked to teleomorphs, and thus they require anamorph names for the present. Our data suggest, however, that their teleomorphs, if found, would reside in *Oculimacula*.

The genus *Mollisia* is known to have anamorphs that reside in the *Phialophora* complex, particularly *Cadophora* (Gams, 2000). As shown in the present study, and reported elsewhere (Webster et al., 1993; Nauta and Spooner, 2000), *Mollisia* is heterogeneous. The eyespot taxa reside in one clade together with some species of *Mollisia* that have *Cadophora* or *Cystodendron* or other anamorphs such as *Leptodontidium* and *Rhynchosporium*. The type species of *Mollisia*, *M. cinerea*, has an inconspicuously phialidic, unnamed anamorph, which is distinct from *Cadophora*. The molecular divergence also suggests that *Mollisia* species with *Cadophora* anamorphs will require a new teleomorph genus, while *Tapesia* might possibly be available for species with *Cystodendron* anamorphs (Aebi, 1972). The eyespot pathogens are sufficiently distinct ecologically and in their anamorphs from these two groups to warrant the introduction of a new holomorph. However, the ascomata offer relatively few criteria for this distinction.

The presence or absence of a subiculum has in the past been regarded as significant to separate genera such as *Tapesia* from *Mollisia* (Boudier, 1885; Saccardo, 1889; Rehm, 1891). In later years, less weight was placed in this feature, which appeared insignificant at the generic level (Dennis, 1968; Aebi, 1972; Baral, 1985; 1994), and hence Aebi (1972) reduced *Mollisia* (1871) to synonymy under *Tapesia* (1870). The genus *Mollisia* encompasses more than 100 species, and is better known than *Tapesia* (20 spp.) (Hawksworth and David, 1989). Therefore, Hawksworth and David (1989) proposed conservation of *Mollisia* over *Tapesia*, a proposal that was accepted by the Committee for Fungi and Lichens (Gams, 1992), and the conservation is now listed in the Code. *Mollisia*, however, consists of several different groups that can be distinguished primarily on the basis of their anamorph associations.

Deighton (1973) introduced the genus *Pseudocercosporella* to accommodate taxa with unthickened, not darkened or refractive conidial hila that were formerly placed in *Cercosporella* Sacc. He did not, however, consider the morphological similarity of *Pseudocercosporella* with *Ramulispora*, and hence placed *C. herpotrichoides* in *Pseudocercosporella*. Braun (1995) stated that if *R. sorghi*, the type of *Ramulispora*, had a teleomorph other than *Tapesia*, a new anamorph genus would have to be introduced to accommodate *R. herpotrichoides* and related taxa. We have shown here that *R. sorghi* (and hence *Ramulispora*) represents an anamorph of *Mycosphaerella*, as does *Pseudocercosporella*. *Ramulispora* is distinct from *Helgardia* in that *R. sorghi* induces characteristically sooty leaf spots, which is due to the abundant sclerotia that form on the leaf surface. The latter are, however, not produced in culture. Colonies of *R. sorghi* grow more slowly than those of *Helgardia*. They are compact, grey to black, and sporulate by forming masses of pink, slimy conidia. Slimy conidial masses are known to also occur in *Pseudocercosporella* and *Helgardia*.

A further issue not addressed in the present paper concerns the distinction between and priority of the genera *Pseudocercosporella* (1973) and *Ramulispora* (1920). Although Deighton (1973) did not compare these two genera when he introduced *Pseudocercosporella*, Von Arx (1983) chose to retain *Ramulispora* for taxa occurring on gramineous hosts. Morphologically, these two genera are similar, and also cluster closely together (Figure 1). With *Ramulispora* being the older name, the International Code for Botanical Nomenclature determines that all names in *Pseudocercosporella* actually would have to

be transferred to *Ramulispora*. To reach a final conclusion, however, more species of *Pseudocercosporella* and *Ramulispora* need to be compared in a larger morphological and molecular study. If these two genera were indeed shown to be synonymous, it is evident that the name *Pseudocercosporella* would deserve conservation over the lesser-known *Ramulispora*. A further 13 species of *Ramulispora* are known (www.speciesfungorum.org), but without cultures and molecular analyses, their correct phylogenetic affinities remain unclear. *Pseudocercosporella* has recently been monographed (Braun, 1995). It contains more than 100 species that are well known to plant pathologists and mycologists, and the genus should thus be retained. The erection of new generic names for the eyespot pathogens of cereals was necessary, however, as neither *Pseudocercosporella* nor *Ramulispora* is available for the anamorphs, nor are *Tapesia* or *Mollisia* for the teleomorphs.

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