Uwebraunia and *Dissoconium*, two morphologically similar anamorph genera with different teleomorph affinity

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More than 20 anamorph form genera, including *Uwebraunia*, have been linked to *Mycosphaerella*. The diversity of anamorphs associated with *Mycosphaerella* has led to hypotheses that the genus is either polyphyletic, or that the features used to separate anamorphs are indicative of natural lineages within *Mycosphaerella*. *Uwebraunia* is morphologically similar to *Dissoconium*, a genus suspected to include species hyperparasitic on other fungi. Phylogenetic analysis of 5.8S, ITS1 and ITS2 rDNA operon of several *Mycosphaerella* spp. with *Uwebraunia*, *Dissoconium* and other anamorphs, as well as species of *Ramulispora* (*Tapesia* teleomorph), using *Mycocentrospora* (teleomorph unknown) as the outgroup taxon, identified three clades. One major clade represented species of *Mycosphaerella sensu stricto*, comprising several subclades that correlate with the various anamorph form-genera. The other two clades were distinct from the larger *Mycosphaerella* clade, and represented a smaller clade with *Dissoconium* anamorphs, and a *Tapesia* clade with *Ramulispora* anamorphs. *Uwebraunia* was shown to include two species representing *Mycosphaerella* anamorphs. A third species was synonymous with *Dissoconium dekkeri*, forming a clade separate from *Mycosphaerella sensu stricto*. Species of *Dissoconium* are thought to be hyperparasitic on other fungi, whereas species of *Uwebraunia* are apparent plant pathogens. These findings suggest that divergence occurred in the *Mycosphaerella* lineage, followed by convergent evolution of morphological form, which accounts for the similar anamorphs.

Keywords: systematics, cladistics, molecular taxonomy

Mycosphaerella Johanson (Dothideales: Mycosphaerellaceae) is one of the largest ascomycete genera. It includes more than 2,000 names (Corlett, 1991, 1995), most of which are distinguished by their host taxa. Kendrick & DiCosmo (1979) reported that *Mycosphaerella* spp. are linked to more than 27 different anamorph genera. Von Arx (1983) treated 23 generic names that he regarded as anamorphs, or potential anamorphs of *Mycosphaerella*. Sutton & Hennebert (1994) studied the different forms of conidiogenesis that occur in these anamorph genera, and found that many had been incorrectly synonymised by von Arx (1983). Nevertheless, they named 23 anamorph form-genera for *Mycosphaerella*.

In addition to the list of *Mycosphaerella* anamorphs produced by Sutton & Hennebert (1994), several other anamorph genera are recognised (Park & Keane, 1984; Swart & Walker, 1988; Braun, 1993, 1995; Crous & Wingfield, 1996; Crous & Wingfield, 1997a, 1997b; Crous & al., 1997; Crous, 1998; Crous & al., 1998; Kaiser & Crous, 1998). G. S. de Hoog (CBS, The Netherlands, pers. comm.) has suggested that one of these genera, *Uwebraunia* Crous & M. J. Wingf. (1996), resembles another anamorph genus, *Dissoconium* de Hoog, van Oorschot & Hijwegen (1983).

No teleomorph has been reported for any of the three known *Dissoconium* species. Furthermore, *Uwebraunia* appears to represent plant pathogens, with narrow host ranges in *Eucalyptus* (Myrtaceae). In contrast, *Dissoconium* has wide host ranges, and is antagonistic to, or mycoparasitic on other phyllosphere fungi (de Hoog & al., 1991; de Hoog & Takeo, 1991).

Although morphologically similar, *Uwebraunia* and *Dissoconium* occupy distinctly different ecological niches. The aim of this study, therefore, was to utilise DNA sequence comparisons to elucidate the taxonomic importance of morphological similarities between *Uwebraunia* and *Dissoconium*, and determine their phylogenetic position in *Mycosphaerella*. Several molecular techniques have been successfully employed to answer similar phylogenetic questions in various groups of fungi (Witthuhn & al., 1998; Myburg & al., 1999; Schoch & al., 1999; Stewart & al., 1999; Viljoen & al., 1999). The internal transcribed spacers (ITS1 & ITS2) of the rDNA operon represent a phylogenetically informative region of the gene, indicating inter- and intraspecies differences (Lee & Taylor, 1991; Witthuhn & al., 1998; Schoch & al., 1999). As this region has already proven useful in distinguishing between species and anamorph genera of *Mycosphaerella* (Stewart & al., 1999), it was decided to also sequence this region to elucidate the phylogenetic relationship of *Uwebraunia* and *Dissoconium* to other species of *Mycosphaerella*.

Materials and methods

Isolates studied

Data pertaining to the various isolates studied are given in Tab. 1. Several of these formed the basis of a previous study (Stewart & al., 1999), in which a different protocol for DNA sequencing was followed. For the present study, single conidium or ascospore isolates

Tab. 1. – Fungal isolates included for ITS sequence analysis

Accession no.	Teleomorph species	Anamorph state	Origin
CA1	*Mycosphaerella* state unknown	*Cercospora apii* Fresen.	ATCC 12246*
CCA19	*Mycosphaerella* state unknown	*Cercospora canescens* Ellis & G. Martin	ATCC 32779 (Mungbean, Taiwan)*
CH5	*Mycosphaerella* state unknown	*Cercospora hayi* Calpouzos	ATCC 12234 (Banana, Cuba)*
CK35	*Mycosphaerella* state unknown	*Cercospora kikuchii* (T. Matsumoto & Tomoy) M. W. Gardner	Soybean, Illinois, U.S.A.*
CK39	*Mycosphaerella* state unknown	*Cercospora kikuchii* (T. Matsumotoa & Tomoy) M. W. Gardner	Soybean, Illinois, U.S.A.*
CS13	*Mycosphaerella* state unknown	*Cercospora sojina* Hara	ATCC 44531 (Soybean, Illinois, U.S.A.)*
STE-U 784	*M. molleriana* (Thüm.) Lindau	*Colletogloeum molleriana* Crous & M. J. Wingf.	*Eucalyptus*, California, U.S.A.
STE-U 1214	*M. molleriana* (Thüm.) Lindau	*Colletogloeum molleriana* Crous & M. J. Wingf.	*Eucalyptus*, Portugal
STE-U 1534	Unknown	*Dissoconium aciculare* de Hoog, van Oorschot & Hijwegen	CBS 342.82 (ex *Erysiphe* sp. on *Medicago*, Germany
STE-U 1233	*M. lateralis* Crous & M. J. Wingf.	*Dissoconium dekkeri* de Hoog & Hijwegen	*Eucalyptus*, Zambia
STE-U 1232	*M. lateralis* Crous & M. J. Wingf.	*Dissoconium dekkeri* de Hoog & Hijwegen	*Eucalyptus*, Zambia
STE-U 825	*M. lateralis* Crous & M. J. Wingf.	*Dissoconium dekkeri* de Hoog & Hijwegen	*Eucalyptus*, South Africa
STE-U 1535	*M. lateralis* Crous & M. J. Wingf.	*Dissoconium dekkeri* de Hoog & Hijwegen	CBS 567.89 (*Juniperus*, The Netherlands)
MA12	Unknown	*Mycocentrospora acerina* (Hartig) Deighton	ATCC 34539 (Carrot, Norway)*
STE-U 1457	*Mycosphaerella* state unknown	*Mycovellosiella eucalypti* Crous & A. C. Alfenas	*Eucalyptus*, Brazil
PP15	*M. berkeleyi* W. A. Jenkins	*Passalora personata* (Berk. & M. A. Curt.) S. H. Khan & M. Kamal	MPPD L2121 (Peanut, Oklahoma, U.S.A.)*

* Sequence data from Stewart & al. (1999)

STE-U = Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa

Tab. 1. Cont. – Fungal isolates included for ITS sequence analysis

Accession no.	**Teleomorph species**	**Anamorph state**	**Origin**
PP16	*Mycosphaerella arachidis* Deighton	*Passalora arachidicola* (Hori) U. Braun	MPPD L2122 (Peanut, Oklahoma, U.S.A.)*
PF7	*M. fijiensis* M. Morelet	*Paracercospora fijiensis* (M. Morelet) Deighton	ATCC 22116 (Banana, Philippines)*
PF8	*M. fijiensis* M. Morelet	*Paracercospora fijiensis* (Morelet) Deighton	ATCC 22117 (Banana, Hawaii)*
PFD9	*M. fijiensis* var. *difformis* J. L. Mulder & R. H. Stover	*Paracercospora fijiensis* var. *difformis* (J. L. Mulder & R. H. Stover) Deighton	ATCC 36054 (*Musa*, Honduras)*
STE-U 1346	*M. suttoniae* Crous & M. J. Wingf.	*Phaeophleospora epicoccoides* (Cooke & Massee) Crous,	*Eucalyptus*, Indonesia, F. A. Ferreira & B. Sutton
PCR18	*M. cruenta* Latham	*Pseudocercospora cruenta* (Sacc.) Deighton	ATCC 262271 (Cowpea, Puerto Rico)*
PM10	*M. musicola* J. L. Mulder	*Pseudocercospora musae* (Zimm.) Deighton	ATCC 22115 (Banana, Philippines)*
PM11	*M. musicola* J. L. Mulder	*Pseudocercospora musae* (Zimm.) Deighton	ATCC 36143 (*Musa*, Honduras)*
STE-U 2428	*Mycosphaerella* state unknown	*Ramularia collo-cygni* B. Sutton & J. M. Waller	Barley, Germany
STE-U 656	*M. fragariae* (Tul.) Lindau	*Ramularia grevilleana* (Tul. & C. Tul.) Jørst.	Strawberry, South Africa
RAE22	*Tapesia* state unknown	*Ramulispora aestiva* Nirenberg	Wheat, Washington, U.S.A.*
RAN45	*Tapesia* state unknown	*Ramulispora anguioides* (Nirenberg) Crous	ATCC 60971 (Wheat, Germany)*
STE-U 353	*M. parkii* Crous & M. J. Wingf.	*Stenella parkii* Crous & Alfenas	*Eucalyptus*, Brazil
STE-U 1084	*M. keniensis* Crous & T. Coutinho	Unknown	*Eucalyptus*, Kenya
STE-U 794	*M. africana* Crous & M. J. Wingf.	Unknown	*Eucalyptus*, South Africa
STE-U 935	*M. marksii* Carnegie & Keane	Unknown	*Eucalyptus*, Australia
STE-U 1224	*M. ellipsoidea* Crous & M. J. Wingf.	*Uwebraunia ellipsoidea* Crous & M. J. Wingf.	*Eucalyptus*, South Africa
STE-U 1225	*M. ellipsoidea* Crous & M. J. Wingf.	*Uwebraunia ellipsoidea* Crous & M. J. Wingf.	*Eucalyptus*, South Africa
STE-U 1004	*M. juvenis* Crous & M. J. Wingf.	*Uwebraunia juvenis* Crous & M. J. Wingf.	*Eucalyptus*, South Africa
STE-U 1005	*M. juvenis* Crous & M. J. Wingf.	*Uwebraunia juvenis* Crous & M. J. Wingf.	*Eucalyptus*, South Africa

* Sequence data from Stewart & al. (1999)

STE-U = Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa were grown on 2% malt extract agar plates (MEA; Biolab, Midrand, South Africa) for 7–14 d in the dark at 25 C to provide mycelium for DNA isolation. Morphological comparisons with original descriptions were made from isolates incubated on divided plates containing MEA and carnation-leaf agar (CLA; Fisher & al., 1982) as explained in Crous (1998).

DNA amplification and sequence determination

Genomic DNA was isolated from fungal mycelium collected directly from MEA plates using the isolation protocol of Raeder & Broda (1985). DNA quantification was done by UV spectroscopy using a Beckman Du Series 7500 Spectrophotometer. Template DNA was amplified in a 50 µl PCR reaction by using reagents and primers described in White & al. (1990). The reaction was set up as follows: initial denaturation at 96 C for 2 min, followed by 40 cycles of denaturation at 94 C for 30 sec, annealing at 53 C for 30 sec, extension at 75 C for 2 min, and final extension at 75 C for 7 min in a Hybaid Omnigene Temperature Cycler (Hybaid, Middlesex, U.K.). A negative control using water instead of template DNA was set up for each experiment. The PCR products were separated on a 0.8% (wt/v) agarose (Promega, Madison, Wisconsin) gel stained with ethidium bromide and visualised under UV illumination. PCR products were purified by using a QIAquick PCR Purification Kit (Qiagen GmbH, Germany) and sequenced using the ABI Prism 377 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut), with an ABI PRISM^{TM} 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 (ITS1 and ITS2) were assembled using Sequence Navigator^{TM} version 1.0.1. (Perkin Elmer, Applied Biosystems, Inc. Foster City, CA). Alignments of the sequence files were conducted using the CLUSTAL W software (Thompson & al., 1994). Adjustments were made by eye where necessary. Alignment gaps were coded as missing data in the analysis. Phylogenetic analysis of aligned DNA sequences was performed using PAUP Version 4.0b1 (Swofford, 1998). The most parsimonious trees were inferred from the original sequence data set using the heuristic search option with 1000 randomizations of sequence input orders. The clade stability was evaluated by 1000 bootstrap replications. Other measures including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC) were also calculated. The data set was also subjected to Neighbor-joining (Saitou & Nei, 1987) analysis in the PHYLIP package version 3.572c (Felsenstein, 1993). Sequence data of the various species were deposited in Genbank (Accession No. AF 173298–AF 173316).

Results

A single most parsimonious tree with the length of 1045 steps (CI = 0.547, RI = 0.719, RC = 0.394) was obtained using the heuristic search option with 1000 randomizations of sequence input orders. Subsequently 1000 bootstrap replicates with 1000 random addition sequences were applied. The majority consensus tree (Fig. 1) showed the same topology of the major clades to the most parsimonious tree. A similar clustering was also observed in the neighbor-joining tree.

The analysis of data identified three clades. These included a major *Mycosphaerella* clade, as well as clades of *Dissoconium* (teleomorph *Mycosphaerella*) and *Ramulispora* (teleomorph *Tapesia*) isolates. The different anamorphs of *Mycosphaerella sensu stricto* represented subgroups within the larger *Mycosphaerella* clade. The three species of *Uwebraunia* clustered separately. These results suggest that the morphological characteristics that typify *Uwebraunia* evolved more than once. Although *M. juvenis* and *M. ellipsoidea* represent distinct species of *Mycosphaerella*, *M. lateralis*, which falls outside the larger *Mycosphaerella* clade, has a *Dissoconium*, and not *Uwebraunia* anamorph. Furthermore, from the aligned DNA sequence (data not shown, available on request), a number of informative sites were observed in 5.8S rRNA gene, which suggest that the *Dissoconium* and *Tapesia* clades are only distantly related to *Mycosphaerella sensu stricto*.

Discussion

Data provided in this study suggest that *Mycosphaerella* could be polyphyletic as hypothesized by Crous (1998). Contrary to expected results, species of *Mycosphaerella* with anamorphs in the form genera *Cercospora* Fresen., *Pseudocercospora* (= *Paracercospora* Deighton; Stewart & al., 1999), *Passalora* Fr., *Mycovellosiella* Rangel, *Stenella* Syd., *Ramularia* Sacc., *Colletogloeum* Crous & M. J. Wingf. and *Phaeophleospora* Rangel clustered together within the major, monophyletic *Mycosphaerella* clade (Fig. 1).

Different morphological species, occurring on diverse hosts, generally grouped according to their anamorph form genera within the larger *Mycosphaerella* clade. The only exception was *Uwebraunia*, with teleomorphs *M. juvenis* and *M. ellipsoidea* clustering separately within the larger *Mycosphaerella* clade. This suggests that

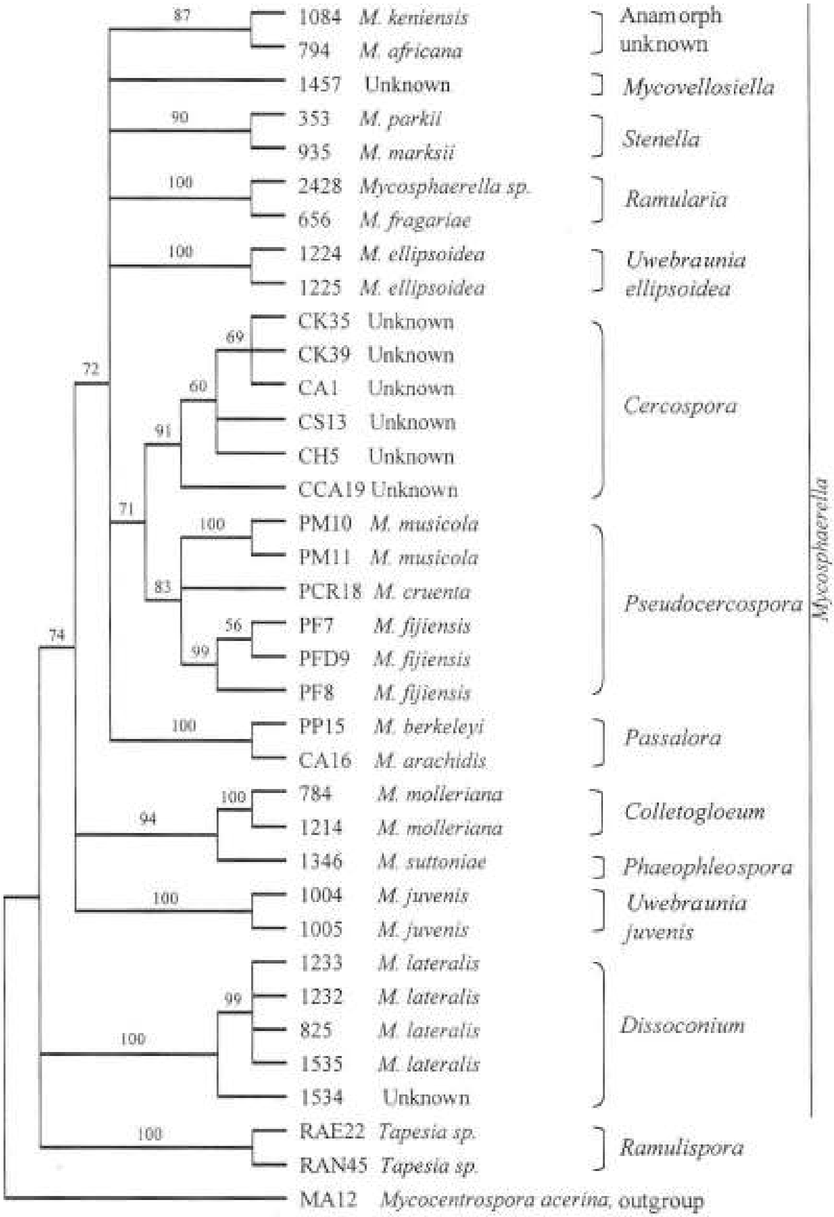

Fig. 1. The majority consensus tree with major clades resembling the single most parsimonious tree (length = 1045, CI = 0.547, RI = 0.719, RC = 0.394) derived from a heuristic search option in PAUP version 4.0b1 (Swofford, 1998) with 1000 randomizations of sequence input orders and 1000 bootstrap replications using the data set of 5.8S, ITS1 and ITS2.

the *Uwebraunia* anamorph evolved more than once within *Mycosphaerella*. Furthermore, *M. lateralis* clustered with fungi having *Dissoconium* anamorphs outside the larger *Mycosphaerella* clade.

Genera shown to have teleomorph affinities other than *Mycosphaerella* include *Mycocentrospora* Deighton (teleomorph unknown), *Ramulispora* Miura (teleomorph *Tapesia* [Pers.] Fuckel). Morphologically, the teleomorph of *Dissoconium* resembles other species of *Mycosphaerella*, having a thin-walled pseudothecium with sessile asci, no pseudoparaphyses, and 1-septate, hyaline, smooth ascospores. Additional data sets would be required, however, to confirm that this genus is not part of *Mycosphaerella sensu stricto*, and that the former is in fact polyphyletic as suggested here.

Dissoconium, originally thought to have affinities with the *Dactylaria*-complex, was described for *D. aciculare*, which is thought to be a hyperparasite on *Erysiphe* (de Hoog & al., 1983). *D. aciculare* has two conidal types. Macroconidia are 1-septate, olivaceous, obclavate and constricted at the septa, while microconidia are obovoid and aseptate. Another species, *D. dekkeri* (de Hoog & al., 1991) is morphologically similar to the three species of *Uwebraunia*, and also forms small pear-shaped microconidia, such as those observed in *U. lateralis* and *U. juvenis* (Crous, 1998). *D. subuliphorum* (Mats.) Castañeda, has macroconidia resembling those of the other species in this complex, but was illustrated by Matsushima (1975) to have strongly sympodial conidiophores, which are less obvious in the other species of *Dissoconium*.

Uwebraunia was established as the anamorph form genus for three species of *Mycosphaerella* associated with leaf spot diseases of *Eucalyptus* spp. cultivated in Africa (Crous & Wingfield, 1996). The three species are all characterized by smooth, olivaceous, obclavate, 1-septate, constricted conidia with unthickened hila, produced on light to medium brown conidiogenous cells with several percurrent proliferations. These features suggest a similarity with *Pseudocercospora* Speg. and *Cercostigmina* U. Braun, although the genera are characterized by scolecospores that are absent in *Uwebraunia*. In *Pseudocercospora*, conidiogenous cells generally proliferate sympodially, and rarely percurrently, while in *Cercostigmina*, conidiogenous cells usually proliferate percurrently, and have unthickened conidiogenous walls.

Uwebraunia was described as having percurrently proliferating conidiogenous cells, while both sympodial and percurrent proliferation was noted for *D. aciculare* and *D. dekkeri*, respectively (de Hoog & al., 1983; de Hoog & al., 1991). De Hoog & Takeo (1991) used SEM to illustrate the percurrent proliferation of conidiogenous cells of *D. dekkeri*. This study showed that the indistinct annellations observed on conidiogenous cells of *D. dekkeri* using light microscopy were not remnants of percurrent proliferation, but two sympodial conidia formed at the same level on conidiogenous cells, with apical proliferation.

Active conidium discharge is known for species of *Dissoconium* (de Hoog & al., 1983, 1991). In *D. aciculare*, a single micro- and macroconidium form at the same level on the conidiogenous cell, become enclosed in a small droplet of liquid, and are then discharged together. Both conidia germinate, but are frequently found to become attached and to subsequently anastomose. *D. dekkeri*, which more closely resembles species of *Uwebraunia*, also has a simultaneous discharge of micro- and macroconidia. Although the formation of macro- and microconidia at the same level on conidiophores was not observed in *Uwebraunia*, the general similarities in conidia and conidiophores suggest that the same discharge mechanisms could occur in both genera.

Based on the morphological similarities between *Dissoconium* and *Uwebraunia*, we hypothesized that the sequence data generated in this study would support their synonymy. Phylogenetic analysis of the sequence data (Fig. 1), however, showed that *M. juvenis* and *M. ellipsoidea* fall in the larger *Mycosphaerella* clade, but that *M. lateralis* groups with *Dissoconium*. Based on similarities in general morphology, as well as their sequence data, *Uwebraunia lateralis* should therefore be reduced to synonymy under *D. dekkeri*.

Mycosphaerella*(?) *lateralis Crous & M. J. Wingf., Mycologia 88: 454. 1996.

Anamorph: *Dissoconium dekkeri* de Hoog & Hijwegen, Mycol. Res. 95: 679. 1991.
= *Uwebraunia lateralis* Crous & M. J. Wingf., Mycologia 88: 454. 1996.

Mycosphaerella lateralis is known only from leaves of *Eucalyptus* spp. in South Africa and Zambia. The *Dissoconium* anamorph has never been observed on this host, but only induced in culture. In contrast, *D. dekkeri* has been reported from The Netherlands, Germany, New Zealand and Malaysia as an apparent antagonist or mycoparasite on Erysiphaceae, on hosts such as *Abies, Picea, Tsuga*, (Pinaceae), *Berberis* (Berberidaceae), *Buxus* (Buxaceae), *Calocedrus, Chamaecyparis, Juniperus, Thuja* (Cupressaceae), *Pieris* (Ericaceae), *Skimmia* (Rutaceae), *Taxus* (Taxaceae), and *Brassica* (Brassicaceae).

In contrast to *M. lateralis*, *M. juvenis* and *M. ellipsoidea* are highly host specific on *Eucalyptus* spp. *M. juvenis* is also regarded as one of the most serious pathogens of certain provenances of *E. nitens* in South Africa, and has caused the forestry industry to abandon the propagation of similar species such as *E. globulus* (Crous & Wingfield, 1996). Of the three *Mycosphaerella* spp. originally described to have *Uwebraunia* anamorphs, *M. lateralis* is least well known. This species most frequently occurs on lesions in association with other leaf pathogens, and thus far no evidence of its pathogenicity has been produced. It is possible, therefore, that the hypothesis by de Hoog & al. (1991) that it is a hyperparasite is correct, also explaining its unusually wide host range. Further collections and inoculation studies of *M. lateralis* are required to determine its ecological role, host range, and the function of its two conidial forms.

Key to species of *Dissoconium* and *Uwebraunia*

1. Species apparently hyperparasitic on other fungi 2
1. Species occurring singly on leaf spots, apparently as plant pathogens. 5

2. Macroconidia 12–25 × 5–8 µm, microconidia 7.5–12 × 3.5–6 µm. . . . . *D. aciculare*
2. Macroconidia less than 6 µm wide, microconidia 3–4 × 2–3 µm. . 3

3. Macroconidia (15–)20–30(–35) × (2–)3–4(–4.5) µm . . . . *D. dekkeri*
3. Macroconidia shorter than 15 µm or wider than 4.5 µm. 4

4. Macroconidia 11–16 × 3.8–4.5(–5) µm . . . . *D. subuliphorum*
4. Macroconidia longer than 16 µm 5

5. Macroconidia (25–)26–30(–40) × (4–)4.5–5.5(–6) µm . . . . *U. juvenis*
5. Macroconidia (16–)17–21(–22) × (3–)4–5(–6) µm. . . . *U. ellipsoidea*

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