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RESEARCH ARTICLE

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Dissoconiaceae associated with sooty blotch and flyspeck on fruits in China and the United States

H.Y. Li[1], G.Y. Sun[1], X.R. Zhai[1], J.C. Batzer[2], D.A. Mayfield[2], P.W. Crous[3, 4, 5], J.Z. Groenewald[3], M.L. Gleason[2]

Key words

hyphomycetes
Malus
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SBFS
taxonomy

Abstract *Zasmidium angulare*, a novel species of *Mycosphaerellaceae*, and several novel taxa that reside in *Dissoconiaceae*, were identified from a collection of apples and *Cucurbita maxima* (cv. Blue Hubbard) from China and the USA that exhibited sooty blotch and flyspeck (SBFS) signs on their host substrata. Morphology on fruit surfaces and in culture, and phylogenetic analyses of the nuclear ribosomal DNAs 28S and internal transcribed spacer regions, as well as partial translation elongation factor 1-alpha gene sequences in some cases, were used to delineate seven previously unidentified species and three known species. *Pseudoveronaea* was established as a new genus of *Dissoconiaceae*, represented by two species, *P. ellipsoidea* and *P. obclavata*. Although *Pseudoveronaea* was morphologically similar to *Veronaea*, these fungi clustered with *Dissoconiaceae* (*Capnodiales*) rather than *Chaetothyriales* (*Herpotrichiellaceae*). *Ramichloridium mali* comb. nov., and three novel species, *R. cucurbitae*, *R. luteum* and *R. punctatum* were closely related with *R. apiculatum*, which together formed a distinct subclade in *Dissoconiaceae*. Species of *Dissoconium* s.lat. clustered in two well-supported clades supported by distinct morphological and cultural features. Subsequently *Uwebraunia*, a former synonym of *Dissoconium*, was resurrected for the one clade, with new combinations proposed for *U. australiensis*, *U. commune*, *U. dekkeri* and *U. musae*. Furthermore, we also reported that *D. aciculare*, *Dissoconium* sp., *U. commune* and *U. dekkeri* were associated with SBFS on apples.

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INTRODUCTION

Dissoconium, an anamorphic genus based on the type species *Dissoconium aciculare* (de Hoog et al. 1983), is known to have different ecological niches. Two species, *D. aciculare* and *D. dekkeri*, were reported as mycoparasitic fungi on *Erysiphaceae* (de Hoog et al. 1983, 1991). However, recent studies showed that *D. dekkeri* acts as a foliar pathogen of *Eucalyptus* (Crous 1998, Jackson et al. 2004). Other *Dissoconium* species were mostly reported from or associated with leaf spots. For example, *D. australiensis*, *D. commune* and *D. eucalypti* co-occurred with plant pathogenic species of *Capnodiales* on leaf spots of *Eucalyptus* spp. (Crous et al. 2004b, 2007a, b, 2009a, d). Two other species, *D. protea* and *D. musae*, colonised leaves of *Protea* sp. and *Musa* sp., respectively (Arzanlou et al. 2008, Crous et al. 2008). Although some species of '*Dissoconium*' have a *Mycosphaerella*-like teleomorph, they were shown to cluster between *Teratosphaeriaceae* and *Schizothyriaceae* (Crous et al. 2004b). Species of *Dissoconium*, together with *Ramichloridium apiculatum*, formed a distinct clade, indicating they represented a group different from other members of *Capnodiales* (Crous et al. 2009b). A new family, *Dissoconiaceae*, was therefore established to accommodate this group (Crous et al. 2009b).

[1] State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi Province, 712100, China; corresponding author e-mail: sgy@nwsuaf.edu.cn.
[2] Department of Plant Pathology and Microbiology, Iowa State University, Ames Iowa 50011, USA.
[3] CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.
[4] Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.
[5] Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

Several *Dissoconium* species contribute to a disease complex known as sooty blotch and flyspeck (SBFS) on the surface of several types of fruit (Batzer et al. 2005, Díaz Arias et al. 2010, Gleason et al. 2011). On apples, blemishes caused by SBFS result in substantial economic losses to growers in humid production areas worldwide (Colby 1920, Williamson & Sutton 2000, Batzer et al. 2005, Díaz Arias et al. 2010, Gleason et al. 2011). The SBFS fungal complex is highly diverse, comprising more than 80 species (Batzer et al. 2008, Frank et al. 2010, Yang et al. 2010, Gleason et al. 2011, Li et al. 2011). Several putative species of *Dissoconium* were first reported as SBFS fungi on apple in the USA (Batzer et al. 2005, Díaz Arias et al. 2010), and *D. aciculare* was also isolated from SBFS colonies on the fruit of pawpaw (*Asimina triloba*) (Hemnani et al. 2008). Additionally, *D. mali* was found to cause SBFS on apple and persimmon in China (Zhang et al. 2007, Sun et al. 2008).

Based on an extensive culture collection obtained from apples and winter squash in the United States and China, we aimed to resolve the taxonomy of this epiphytic fungal group, using both DNA sequence analyses and morphological comparisons.

MATERIAL AND METHODS

Isolates and scanning electron microscopy

Sixteen isolates were included in the present study. Two were obtained from apple fruit collected from Weifang City, Shandong Province, China in October 2006, following the method of Sun et al. (2003). Twelve isolates were obtained from surveys conducted in apple orchards in the eastern and midwestern USA in 2000 and 2005 (Batzer et al. 2005, Díaz Arias et al. 2010). The other two isolates were obtained from winter squash (*Cucurbita maxima* cv. Blue Hubbard) harvested from

Table 1 Collection details and GenBank accession numbers of isolates for which novel sequences were generated in this study.

Species	Strain number			Substrate	Country	Collector	GenBank Accession no.		
	CBS[a]	CPC[b]	CMG[c]				ITS[d]	LSU[e]	TEF[f]
Dissoconium aciculare	132079	18965	MA1 10B1a	*Malus domestica* fruit surface	Massachusetts, USA	D. Cooley	JQ622082	JQ622090	JQ622107
	132080	18966	PEB1a	*Malus domestica* fruit surface	Iowa, USA	M. Gleason	JQ622083	JQ622091	JQ622108
	132081	18967	CUB2c	*Malus domestica* fruit surface	Illinois, USA	M. Gleason	AY598874	JQ622097	JQ622114
	132082	18968	MSTB4b	*Malus domestica* fruit surface	Wisconsin, USA	P. McManus	JQ622081	JQ622089	JQ622106
	132083	18973	UMB4b	*Malus domestica* fruit surface	Missouri, USA	M. Gleason	AY598875	JQ622098	JQ622115
Dissoconium sp.	132084	18969	KY4 19.1B2	*Malus domestica* fruit surface	Kentucky, USA	J. Hartman	JQ622084	JQ622092	JQ622109
Pseudoveronaea ellipsoidea	132085*	18970*	MI3 34F1a*	*Malus domestica* fruit surface	Michigan, USA	G. Sundin	FJ425205	JQ622103	JQ622120
Pseudoveronaea obclavata	132086*	18972*	UIF3a*	*Malus domestica* fruit surface	Illinois, USA	M. Gleason	AY598877	JQ622102	JQ622119
Ramichloridium cucurbitae	132087*	19423*	BHF50a3*	*Cucurbita maxima* fruit surface	Gilbert, Iowa, USA	D. Mayfield	JQ622087	JQ622095	JQ622112
Ramichloridium luteum	132088*	18961*	ZXRSD2*	*Malus domestica* fruit surface	Weifang, Shandong Province, China	G.Y. Sun	EU329730	JQ622099	JQ622116
	132089	18962	ZXRSD5	*Malus domestica* fruit surface	Weifang, Shandong Province, China	G.Y. Sun	EU329731	JQ622100	JQ622117
Ramichloridium punctatum	132090*	18974*	BHE35b1*	*Cucurbita maxima* fruit surface	Gilbert, Iowa, USA	D. Mayfield	JQ622086	JQ622094	JQ622111
Uwebraunia commune	132091	18963	NC1 32C1d	*Malus domestica* fruit surface	North Carolina, USA	T. Sutton	JQ622085	JQ622093	JQ622110
	132092	18971	MSTF3b	*Malus domestica* fruit surface	Wisconsin, USA	P. McManus	AY598876	JQ622101	JQ622118
Uwebraunia dekkeri	132093	18964	OH3 37E1d	*Malus domestica* fruit surface	Ohio, USA	M. Ellis	FJ425204	JQ622104	JQ622121
Zasmidium angulare	132094*	19042*	GA2 27B1a*	*Malus domestica* fruit surface	Georgia, USA	M. Wheeler	JQ622088	JQ622096	JQ622113

* Ex-type strains are indicated with an asterisk.
[a] CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.
[b] CPC: Culture collection of P.W. Crous, housed at CBS.
[c] CMG: Culture collection of M. Gleason, housed at Iowa State University, Ames, Iowa, the USA.
[d] ITS: Internal transcribed spacers 1 and 2 together with 5.8S nrDNA.
[e] LSU: 28S nrDNA.
[f] TEF: partial translation elongation factor 1-alpha gene sequence.

an Iowa State University's Horticulture Research Farm near Gilbert, Iowa, USA, in 2009 (Mayfield et al. 2011). Colonies on fruit cuticles from which isolates were obtained were pressed between paper towels and photographed under a dissecting microscope. To further clarify the sporulation of a novel species of *Ramichloridium* (CPC 18961), the protocol of Zhang et al. (2009) was used to obtain scanning electron micrographs (SEM) of this isolate from China.

DNA isolation, amplification and phylogenetic analysis

Genomic DNA was isolated from fungal mycelium grown on potato-dextrose agar (PDA), using the PrepMan™ Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocols. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify a part of the nuclear rDNA operon spanning the 3' end of the 18S nrRNA gene (SSU), the internal transcribed spacer 1, the 5.8S nrRNA gene, the internal transcribed spacer 2 (ITS) and the first 900 bases at the 5' end of the 28S nrRNA gene (LSU). Besides V9G and LR5, primers ITS4 (White et al. 1990) and LR0R (Rehner & Samuels 1994) were also used as additional internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The PCR reaction mixture and amplification conditions were the same as those described by Cheewangkoon et al. (2008). Additionally, part of the translation elongation factor 1-alpha gene (TEF) was amplified and sequenced as described by Bensch et al. (2010) to better resolve taxa showing identical or near identical ITS sequences.

Sequences generated from the present study were used as queries for a Blastn search in NCBIs GenBank nucleotide (nr) database, and those with high nucleotide identities were downloaded. The sequence alignment was performed in ClustalX v. 2.1 (Thompson et al. 1994, Larkin et al. 2007), followed by manual adjustment in BioEdit v. 7.0.5 (Hall 1999).

Phylogenetic analyses were conducted on the LSU and ITS alignments in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) by employing distance and maximum parsimony algorithms. Distance analyses employed the uncorrected ('p'), the Kimura 2-parameter and the HKY85 substitution models, with the neighbour-joining search. Alignment gaps were treated as missing data, and ties were broken randomly when encountered. The neighbour-joining bootstrap support values (NJBP) equal or greater than 50 % are shown after the slash at nodes (generated from the HKY85 substitution model analysis). Parsimony analyses were performed using the heuristic search option with 100 replicates of random stepwise additions and tree bisection reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and given equal weight and all equally most parsimonious trees were saved. The robustness of obtained trees was evaluated by 1 000 bootstrap replications. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were also calculated. Phylogenetic trees were drawn in TreeView v. 1.6.6 (Page 1996) and Geneious v. 5.5.4 (Drummond et al. 2011) and layout was done in Adobe Illustrator CS 5.1. New sequences from this study were deposited in GenBank (Table 1) and the alignment and trees in TreeBASE (www.treebase.org). The results of the TEF sequences are discussed under the species notes where applicable.

Morphology

Isolates were established on PDA, synthetic nutrient-poor agar (SNA) and oatmeal agar (OA; Crous et al. 2009e), and subsequently incubated at 25 °C under near-ultraviolet light (300–400 nm) to promote sporulation. Preparations from cultured fungal colonies on SNA were mounted on glass slides with

Shear's solution using transparent adhesive tape (Titan Ultra Clear Tape, Conglom Inc., Toronto, Canada) as explained by Schubert et al. (2007) for microscopic examination after 1 mo of incubation. Thirty measurements per relevant microscopic structure were made where possible. Colony colours on PDA and OA (surface and reverse) were determined using the colour charts of Rayner (1970) after 1 mo at 25 °C in the dark. Reference strains are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS-KNAW), Utrecht, The Netherlands, the working collection of P.W. Crous (CPC), and at Iowa State University (Table 1). Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004a).

RESULTS

Phylogenetic analysis

The LSU alignment consisted of 74 taxa (including two outgroups) and 753 characters including alignment gaps. Of these characters, 478 were constant, 28 were variable and parsimony-uninformative, and 247 were parsimony-informative. The first of 48 equally most parsimonious trees generated from maximum parsimony analysis is shown in Fig. 1 (TL = 862 steps; CI = 0.491; RI = 0.855; RC = 0.420). The neighbour-joining analyses using three substitution models yielded trees with topologies and bootstrap support values similar to those of MP analysis (data not shown). From the LSU phylogenetic

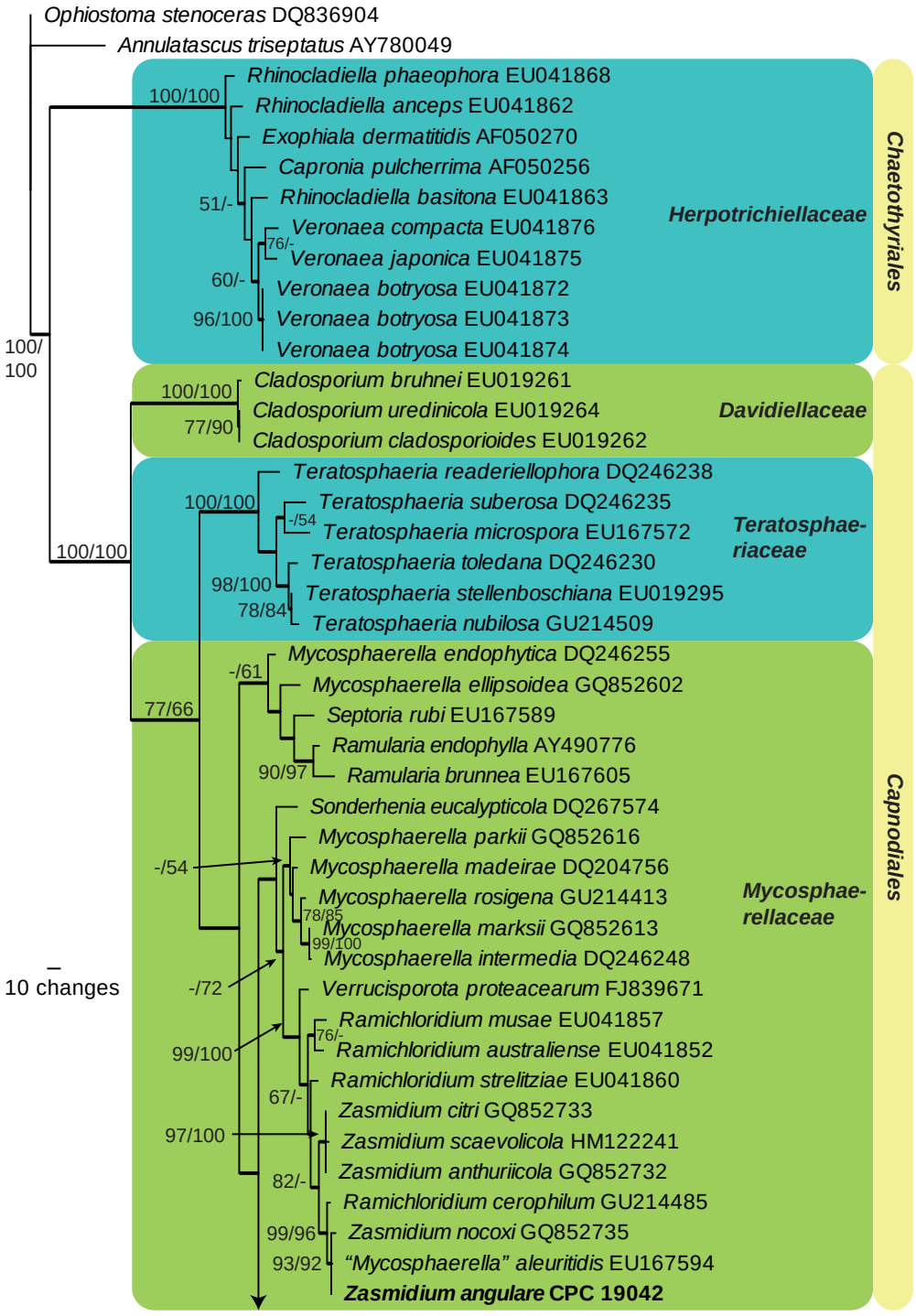
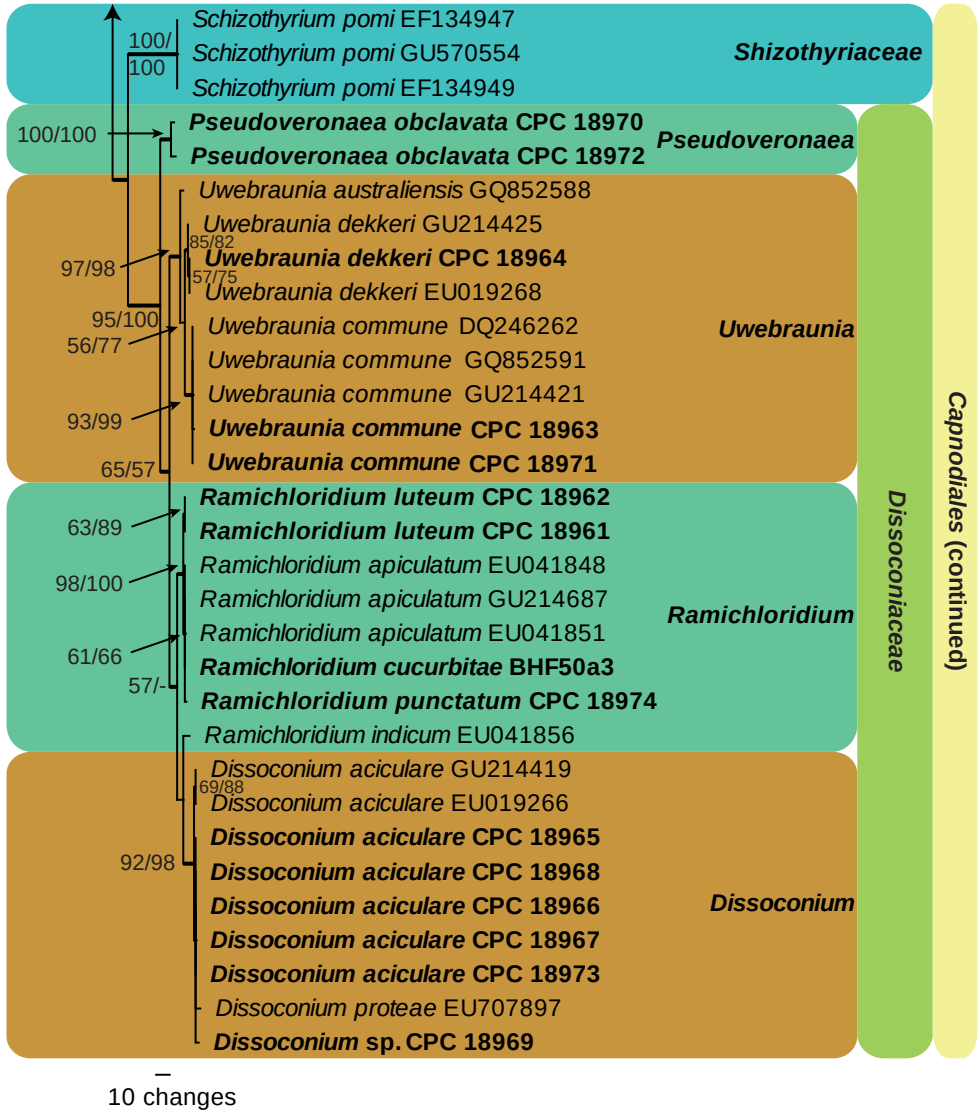


Fig. 1 The first of 48 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. Thickened lines indicate branches present in the strict consensus tree. The scale bar indicates 10 changes and bootstrap support values from 1 000 replicates are shown at the nodes, followed by bootstrap support values from 1 000 replicates obtained by a neighbour-joining analysis using the HKY85 substitution model on the same alignment. Our SBFS isolates treated in this study were indicated in **bold**. The tree was rooted to *Annulatascus triseptatus* (GenBank AY780049) (*Annulatascaceae*, *Sordariomycetes*) and *Ophiostoma stenoceras* (GenBank DQ836904) (*Ophiostomataceae*, *Ophiostomatales*, *Sordariomycetes*).

Fig. 1 (cont.)



inference, the strain CPC 19042 clustered together with *Zasmidium nocoxi* and *Mycosphaerella aleuritidis* (93 % MPBP, 92 % NJBP), indicating it is a member of *Mycosphaerellaceae*. The other 15 strains all clustered in *Dissoconiaceae* (95 % MPBP, 100 % NJBP) and cluster in four subclades. The first subclade consisted of two strains, CPC 18970 and CPC 18972, with 100 % bootstrap support from both MP and NJ analyses, suggesting that they represent a novel genus of *Dissoconiaceae*. Four strains including BHF50a3, CPC 18974, CPC 18961 and CPC 18962 grouped with *Ramichloridium apiculatum* (98 % MPBP, 100 % NJBP), indicating they are closely related with *R. apiculatum*. All *Dissoconium* species grouped in two subclades. One was composed of the former *Dissoconium* species *D. australiensis*, *D. dekkeri* and *D. commune* (97 % MPBP, 98 % NJBP; here combined into *Uwebraunia*). The other subclade (92 % MPBP, 98 % NJBP) included *D. aciculare*, *D. eucalypti*, *Dissoconium* sp. and *D. proteae* (Fig. 1).

The ITS alignment contained 39 taxa (including two outgroups) and 519 characters including alignment gaps. Of these characters, 271 were constant, 27 were variable and parsimony-uninformative, and 221 were parsimony-informative. Five equally most parsimonious trees were saved from the maximum parsimony analysis, the first of which shown in Fig. 2 (TL = 576 steps; CI = 0.727; RI = 0.927; RC = 0.674). The neighbour-joining analysis of the ITS sequence alignment also yielded similar tree topologies and bootstrap support with the MP algorithm. Most species were strongly supported in the ITS tree (Fig. 2), including *Zasmidium angulare*, *Pseudoveronaea* spp., *Ramichloridium* spp. and *D. commune* (as *Uwebraunia commune* below). Several other species had moderate or no bootstrap support in the ITS analysis, including *D. aciculare*, *D. eucalypti*, *Dissoconium* sp. and *D. dekkeri* (as *Uwebraunia dekkeri* below) (Fig. 2). The clustering of isolate CPC 18969 with *D. eucalypti* was not supported in any of the analyses. Isolates of *D. aciculare*, together with *D. eucalypti*, had moderate bootstrap support (72 % MPBP, 66 % NJBP). *Dissoconium dekkeri* (as *Uwebraunia dekkeri* below) grouped with *D. australiensis* (as *Uwebraunia australiensis* below), *U. commune* and *D. musae* (as *Uwebraunia musae* below) (100 % MPBP, 100 % NJBP). *Uwebraunia dekkeri* was better supported in the distance analysis (69 % MPBP, 90 % NJBP), whereas *U. commune* was well-supported in both analyses (99 % MPBP, 100 % NJBP).

Taxonomy

Several species of *Dissoconium*, *Ramichloridium*, *Uwebraunia*, one species of *Zasmidium*, and members of an undescribed genus were found to be associated with SBFS blemishes on fruit surfaces of apple and winter squash (Fig. 3). These taxa are treated below.

Dissoconium and *Uwebraunia*

The genus *Dissoconium* was established based on *D. aciculare*, a suspected hyperparasite on *Erysiphe* (de Hoog et al. 1983). In contrast, *Uwebraunia* was described as an anamorph genus for three species with *Mycosphaerella*-like teleomorphs associated

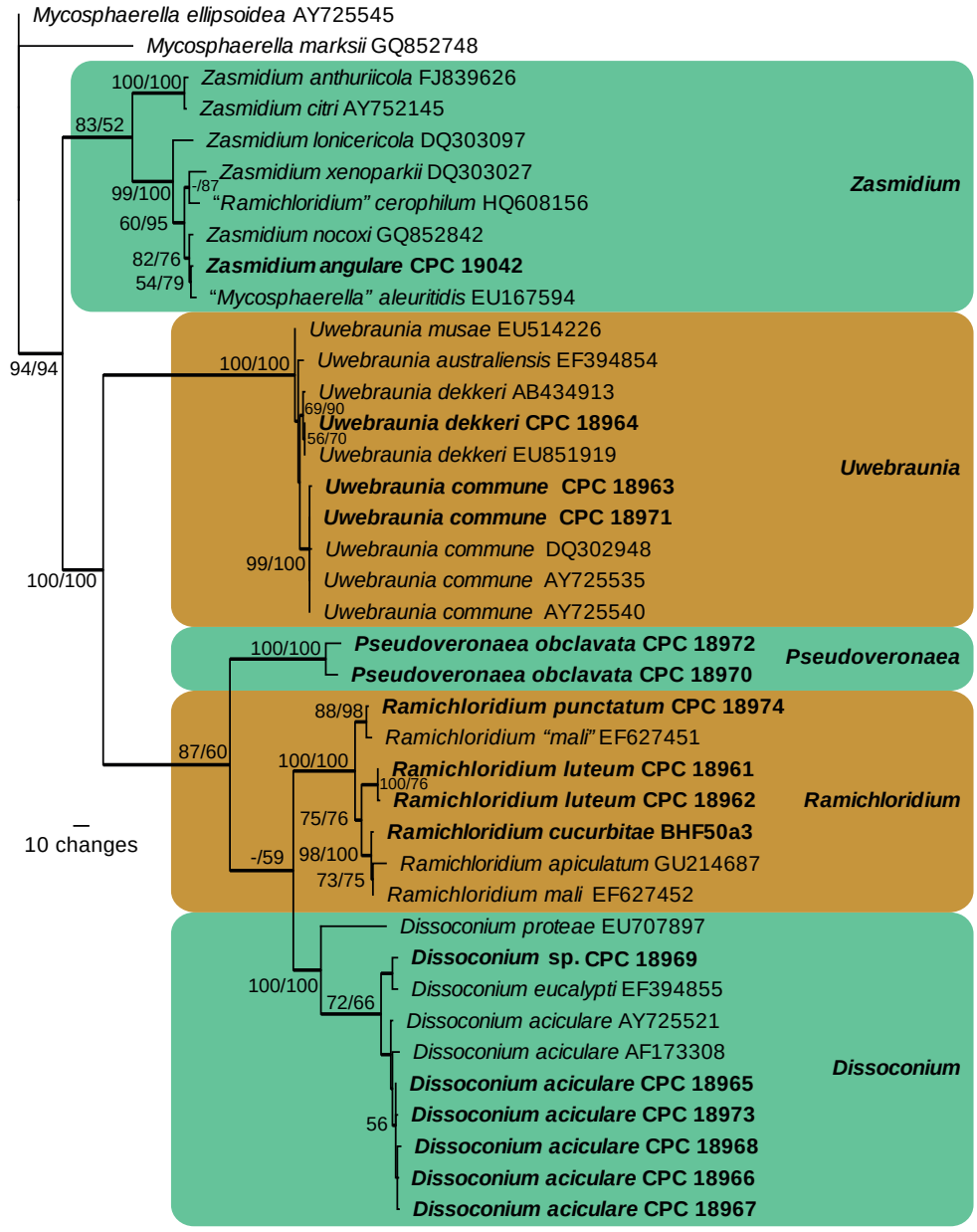


Fig. 2 The first of five equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of ITS sequence alignment. Thickened lines indicated branches present in the strict consensus tree. The scale bar indicates 10 changes and bootstrap support values from 1 000 replicates are shown at the nodes, followed by bootstrap support values from 1 000 replicates obtained by a neighbour-joining analysis using the HKY85 substitution model on the same alignment. Our SBFS isolates treated in this study were indicated in **bold**. The tree was rooted to *Mycosphaerella ellipsoidea* (GenBank AY725545) and *Mycosphaerella marksii* (GenBank GQ852748).

with leaf spot diseases of *Eucalyptus* spp. (Crous & Wingfield 1996, Crous 1998). *Uwebraunia* was based on *U. juvenis*, a species incorrectly linked to '*Mycosphaerella*' *juvenis* (Crous et al. 2004b). Based on DNA sequence data and type studies, strains of the latter taxon were later shown to be heterogeneous, '*Mycosphaerella*' *juvenis* was considered as a synonym of *Teratosphaeria nubilosa*, a major foliar pathogen of *Eucalyptus* (Crous 2009, Hunter et al. 2009, 2011), and segregated from *T. ohnowa* (Crous et al. 2004b, 2007a). Furthermore, molecular data revealed a second species, *U. lateralis*, to be synonymous with the earlier described *D. dekkeri* (Crous et al. 1999).

Although *Uwebraunia* was seen as representing a genus of plant pathogens (Crous & Wingfield 1996, Crous et al. 1999, Jackson et al. 2004), compared to the hyperparasitic *Dissoconium* (de Hoog et al. 1983), there was no robust support for retaining them as separate genera in *Dissoconiaceae* (Crous et al. 2009b, Seifert et al. 2011). Preference was thus given to *Dissoconium*, which was the older name.

Since these initial studies several species have been added to the *Dissoconium* complex (Crous et al. 2004b, 2006, 2007b, 2008, Arzanlou et al. 2008), which is shown to cluster in two well-supported clades, separated by *Ramichloridium* s.str. (Fig. 1, 2). Although little is known about their ecology, several morphological differences support generic segregation of *Uwebraunia* from *Dissoconium*. The genus *Dissoconium* (*D. aciculare*, *D. eucalypti* and *D. protea*) has large, obclavate to ellipsoid microconidia, and produces sclerotia as well as a yellow pigment in culture. In contrast, the genus *Uwebraunia* (*U. australiensis*, *U. commune*, *U. dekkeri* and *U. musae*) has small, pyriform microconidia, does not form sclerotia or any yellow pigment in culture, and is associated with a *Mycosphaerella*-like teleomorph. Based on these differences and the robust phylogenetic support for separating these genera (Fig. 1, 2), we choose to resurrect the generic name *Uwebraunia* for the second clade resolved in this study.

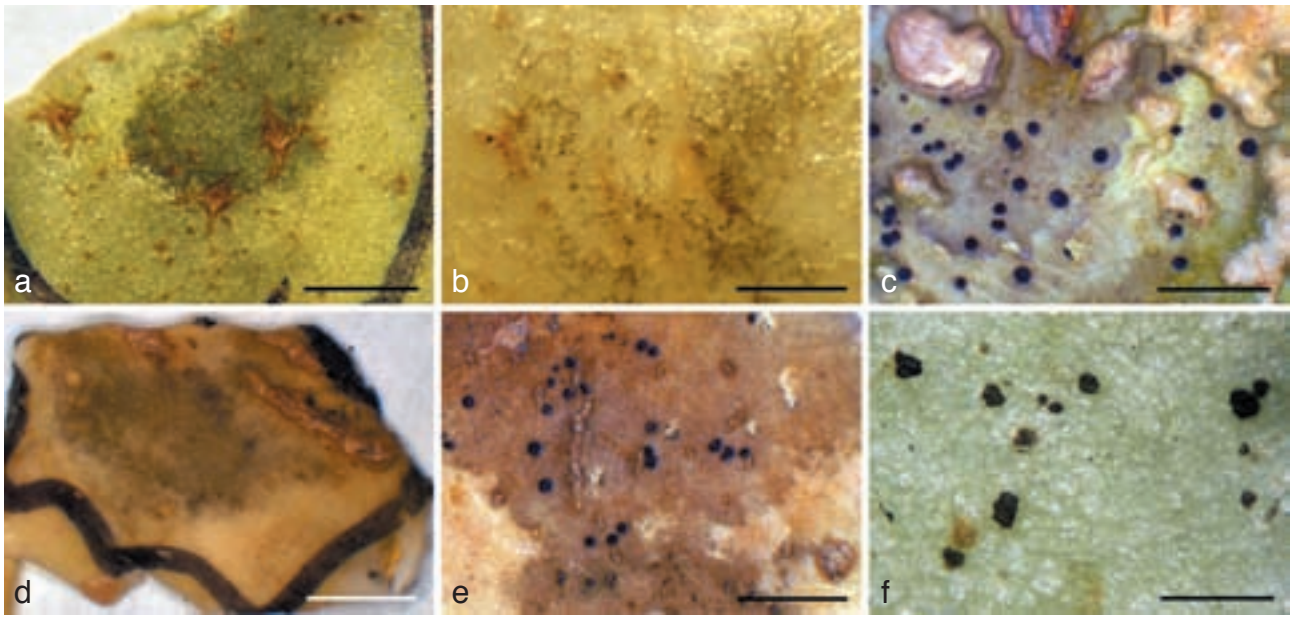


Fig. 3 SBFS signs on fruit surface. a. *Pseudoveronaea obclavata* on apple; b. *Pseudoveronaea ellipsoidea* on apple; c. *Ramichloridium punctatum* on winter squash; d. *Ramichloridium cucurbitae* on winter squash; e. *Ramichloridium luteum* on apple; f. *Zasmidium angulare* on apple. — Scale bars: a, d = 5 mm, b, c, e, f = 2 mm.

Dissoconium de Hoog, Oorschot & Hijwegen, Proc. Kon. Ned. Akad. Wetensch. C 86, 2: 198. 1983.

Type species. *Dissoconium aciculare* de Hoog, Oorschot & Hijwegen.

Mycelium internal and external, consisting of branched, septate, smooth, hyaline to pale brown hyphae, that anastomose, forming nets in culture. *Conidiophores* separate, arising from hyphae, subcylindrical, subulate or lageniform to cylindrical, tapering to a bluntly rounded or truncate apex, straight to once geniculate, smooth, medium brown, 0–2-septate; loci terminal and lateral, visible as slightly thickened, darkened scars on a rachis; proliferation sympodial but also appearing to be percurrent. *Primary conidia* solitary, pale olivaceous-brown, smooth, ellipsoid to obclavate, 1-septate; hila somewhat darkened. *Secondary conidia* developing adjacent to primary conidia, pale olivaceous to subhyaline, aseptate, smooth, obclavate to ellipsoid; conidium discharge active, usually with both conidial types being discharged simultaneously. One or more secondary conidia anastomosing with primary conidium once discharged. Sexual state unknown, producing yellow pigment and black sclerotia in culture.

Notes — Three species are presently accepted in *Dissoconium* based on DNA data and morphology, which are listed here along with their ex-type strains.

Dissoconium aciculare de Hoog, Oorschot & Hijwegen, Proc. Kon. Ned. Akad. Wetensch. C 86, 2: 198. 1983.

Specimens examined. Germany, on *Medicago lupulina*, CBS 342.82 ex-type. – USA, Illinois, on fruit surface of *Malus domestica*, Oct. 2000, *M. Gleason*, CPC 18967 = CUB2c = CBS 132081; Iowa, on fruit surface of *Malus domestica*, Oct. 2000, *M. Gleason*, CPC 18966 = PEB1a = CBS 132080; Massachusetts, on fruit surface of *Malus domestica*, Oct. 2005, *D. Cooley*, CPC 18965 = MA110B1a = CBS 132079; Missouri, on fruit surface of *Malus domestica*, Oct. 2000, *M. Gleason*, CPC 18973 = UMB4b = CBS 132083; Wisconsin, on fruit surface of *Malus domestica*, Oct. 2000, *P. McManus*, CPC 18968 = MSTB4b = CBS 132082.

Notes — TEF sequence data support the taxon resolution suggested on the basis of ITS sequences (Fig. 2).

Dissoconium eucalypti Crous & Carnegie, Fung. Diversity 26: 157. 2007.

Specimen examined. Australia, New South Wales, Morpeth Park, Plantation, Bonalbo, E152°36'47", S28°46'3", on leaves of *Eucalyptus tereticornis*, 8 Feb. 2006, *A. Carnegie*, holotype CBS-H 19770, cultures ex-type CPC 13004 = CBS 120039, CPC 13005–13006.

Dissoconium protea Crous, Persoonia 20: 68. 2008.

Specimen examined. Canary Islands, Tenerife, on leaves of *Protea* sp., 1 Mar. 2007, *P.W. Crous*, holotype CBS H-20091, culture ex-type CPC 13853 = CBS 122900.

***Dissoconium* sp.**

Specimen examined. USA, Kentucky, on fruit surface of *Malus domestica*, Sept. 2005, *J. Hartman*, CPC 18969 = KY4 19.1B2 = CBS 132084.

Notes — Both ITS (Identities = 484/489 (99 %), Gaps = 3/489 (1 %)) and TEF (Identities = 168/225 (75 %), Gaps = 26/225 (12 %)) sequences suggested a degree of similarity between CBS 132084 and *D. eucalypti* (CBS 120039). However, these two isolates were not identical and it is possible that the isolates from *Eucalyptus* and *Malus* represent distinct species (Fig. 2). More isolates from these hosts should be collected to test this hypothesis.

Pseudoveronaea Crous & Batzer, *gen. nov*. — MycoBank MB564667

Type species. Pseudoveronaea obclavata Batzer & Crous.

Etymology. Named after its morphological similarity to *Veronaea*.

Colonies flat, spreading, olivaceous grey, either slow or moderately fast growing. *Submerged hyphae* hyaline to pale olivaceous, smooth; *aerial hyphae* hyaline to brown, smooth to warty. *Conidiophores* erect, straight or flexuose, unbranched or occasionally branched at apex, smooth-walled, medium-brown to brown. *Conidiogenous cells* terminal or lateral, integrated, pale brown to brown, smooth, subcylindrical, but with apical taper, terminal and intercalary, proliferating sympodially, forming a rachis with crowded, flat to slightly prominent, somewhat

darkened, unthickened scars, 0.5–1 µm diam. *Conidia* solitary, finely verruculose to verruculose, obclavate to ellipsoid, 0–2-septate, apex with or without mucoid appendage; base truncate, darkened, somewhat thickened, not refractive, 1–1.5 µm diam; conidial secession schizolytic.

Notes — Using the key of Arzanlou et al. (2007) *Pseudoveronaea* has conidiophores similar to those of both *Veronaea* (up to 200 µm in length) or *Veronaeopsis* (up to 60 µm in length); its conidiogenous cells lack denticles, and are more *Veronaea*-like than those of *Veronaeopsis* (*Venturiaceae*). Morphologically it is similar to *Veronaea*, except that the latter is a genus of *Chaetothyriales* (*Herpotrichiellaceae*), whereas *Pseudoveronaea* represents a new genus in *Dissoconiaceae* (*Capnodiales*), characterised by having septate conidia.

Pseudoveronaea ellipsoidea Batzer & Crous, *sp. nov*. — MycoBank MB564668; Fig. 4

Etymology. Named after the shape of its conidia, which are ellipsoid.

Mycelium consisting of septate, branched, hyaline, smooth, or brown and warty, 2–4 µm diam hyphae. *Conidiophores* erect, solitary, arising as lateral branches on superficial hyphae, 2–15-septate, straight to flexuous, subcylindrical, brown, smooth, thick-walled, unbranched or with fertile lateral branches close to apex, 35–130 × 3–5 µm. *Conidiogenous cells* terminal or lateral, integrated, pale brown to brown, smooth, subcylindrical, but with apical taper to acutely rounded pale brown apex, terminal and intercalary, 6–20 × 3–5 µm; proliferating sympodially, forming a rachis with crowded, flat to slightly prominent, somewhat darkened, unthickened scars, 0.5–1 µm diam. *Conidia* (6–)8–10(–15) × (3–)3.5–4(–5) µm, 0–1-septate, solitary, finely verruculose, pale brown, granular, ellipsoid to obclavate (in larger conidia), apex subobtuse, base truncate, slightly darkened and thickened, 1–1.5 µm diam.

Culture characteristics — Colonies after 1 mo at 25 °C in the dark flat, spreading, with sparse aerial mycelium and lobate, feathery margin; reaching 15–25 mm diam. On SNA olivaceous grey; on PDA iron-grey on surface and reverse; on OA olivaceous grey.

Appearance on natural substratum — On apple surfaces forming a fuliginous appearance (Gleason et al. 2011) characterised by olive-grey, smooth mycelial mats with distinct, but uneven edges.

Specimen examined. USA, Michigan, on fruit surface of *Malus domestica*, Oct. 2005, *G. Sundin*, holotype CBS H-20926, ex-type cultures CBS 132085 = CPC 18970 = MI3 34F1a.

Notes — *Pseudoveronaea ellipsoidea* is distinct from *P. obclavata* (described below) by having smaller, 0–1-septate conidia that lack apical appendages.

Pseudoveronaea obclavata Batzer & Crous, *sp. nov*. — MycoBank MB564669; Fig. 5

Etymology. Named after the shape of its conidia, which are obclavate.

Mycelium consisting of subhyaline, smooth, septate, branched hyphae, 1.5–2.5 µm diam. *Conidiophores* erect, solitary, arising as lateral branches on superficial hyphae, 1–6-septate, unbranched, mostly straight to flexuous to geniculate-sinuous, subcylindrical, brown, smooth, thick-walled, 10–70 × 3–4 µm. *Conidiogenous cells* terminal, integrated, brown, subcylindrical, even in width or with slight apical taper, 10–25 × 2–4 µm; proliferating sympodially, forming a rachis with crowded, flat to slightly prominent, somewhat darkened, unthickened scars, 0.5 µm diam. *Conidia* (10–)13–18(–27) × (2.5–)3(–3.5) µm, solitary, finely verruculose to verruculose, obclavate, 0–2-septate, apex subobtuse with globose mucoid apical appendage (2–4 µm diam); base truncate, darkened, somewhat thickened, not refractive, 1 µm diam.

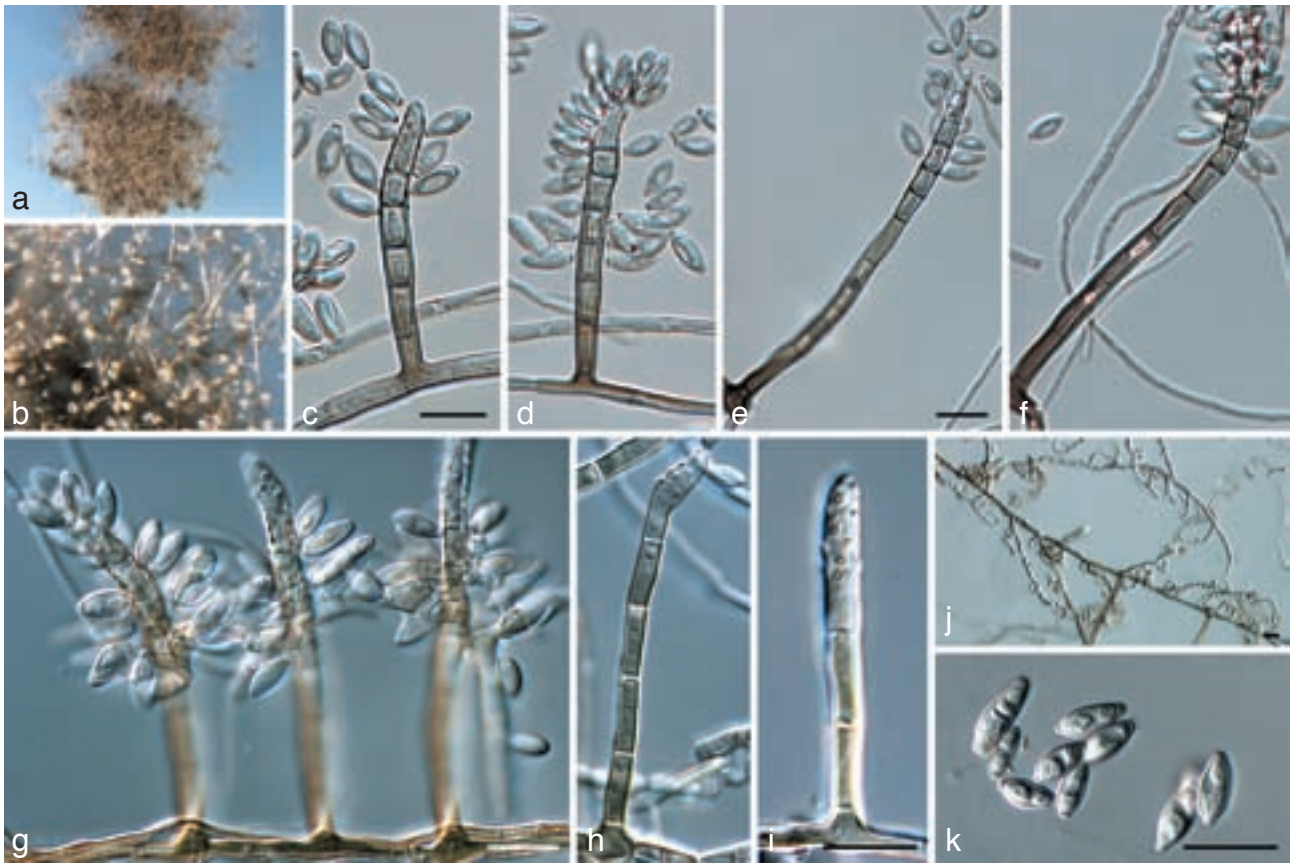


Fig. 4 *Pseudoveronaea ellipsoidea* (CPC 18970). a. Colony on synthetic nutrient poor agar (SNA); b. aerial mycelium on SNA; c–i. macroconidiophores showing rachis; j. curling lateral hyphae on SNA; k. conidia. — Scale bars = 10 µm.

Fig. 5 *Pseudoveronaea obclavata* (CPC 18972). a. Colony on oatmeal agar; b. aerial mycelium of colony on malt extract agar; c–i. macroconidiophores showing rachis; j. conidiophore reduced to conidiogenous cell; k. conidia. — Scale bars = 10 µm.

Culture characteristics — Colonies after 1 mo at 25 °C in the dark flat, spreading, with sparse aerial mycelium and lobate, smooth margin; reaching 3–6 mm diam. On SNA olivaceous grey; on PDA olivaceous grey, iron-grey in reverse; on OA olivaceous grey.

Appearance on natural substratum — On apple surfaces forming a fuliginous appearance (Gleason et al. 2011) characterised by olive-grey smooth mycelial mats with distinct, but uneven edges.

Specimen examined. USA, Illinois, on fruit surface of *Malus domestica*, Oct. 2000, *M. Gleason*, holotype CBS H-20927, ex-type cultures CBS 132086 = CPC 18972 = UIF3a.

Notes — *Pseudoveronaea obclavata* is characterised by having conidia that are obclavate, 0–2-septate, and have a globose, apical mucoid appendage.

Ramichloridium Stahel ex de Hoog, Stud. Mycol. 15: 59. 1977.

Type species. Ramichloridium apiculatum (J.H. Mill., Giddens & A.A. Foster) de Hoog.

Ramichloridium cucurbitae Mayfield, Batzer & Crous, *sp. nov*. — MycoBank MB564670; Fig. 6

Etymology. Named after the host genus from which it was collected, *Cucurbita*.

Mycelium consisting of smooth, subhyaline to pale brown, septate, branched, 1.5–2 µm diam hyphae. *Conidiophores* erect, arising as lateral branches on superficial hyphae, 0–3-septate or reduced to intercalary conidiogenous cells, unbranched, straight to gently curved, subcylindrical, with apical taper in upper fertile part, brown, smooth, 3–90 × 2–3 µm. *Conidiogenous cells* terminal, integrated, tapering towards an acutely rounded

Fig. 6 *Ramichloridium cucurbitae* (BHF50a3). a–d. Macroconidiophores showing rachis; e. conidiophores reduced to conidiogenous cells; f. conidia. — Scale bars = 10 µm.

apex, pale to medium brown, subcylindrical, 3–50 × 1.5–2.5 µm; proliferating sympodially, forming a rachis with slightly thickened and darkened, circular, somewhat protruding scars, ± 0.5 µm diam. *Conidia* (4–)5–6(–7) × (2–)2.5–3(–3.5) µm, solitary, aseptate, pale brown, smooth, clavate, apex obtuse, base truncate, slightly darkened and thickened, not refractive, 0.5 µm diam.

Culture characteristics — Colonies after 1 mo at 25 °C in the dark flat, spreading, with sparse to moderate aerial mycelium and smooth, even margin; reaching 25–30 mm diam. On SNA smoke-grey; on PDA smoke-grey to pale olivaceous grey, olivaceous grey in reverse; on OA pale olivaceous grey.

Appearance on natural substratum — On squash surfaces (Fig 3d) and re-inoculated apple; fuliginous signs (Gleason et al. 2011) characterised by uniform mats of mycelia with abrupt to feathered edges. As described in Batzer et al. (2005), a modified version of Koch's postulates was performed to verify the signs observed on squash are also observed on apple.

Specimen examined. USA, Iowa, Gilbert, on fruit surface of *Cucurbita maxima* (cv. Blue Hubbard), Oct. 2009, *D. Mayfield*, holotype CBS H-20928, ex-type cultures CBS 132087 = CPC 19423 = BHF50a3.

Notes — *Ramichloridium cucurbitae* is phylogenetically closely related to *R. apiculatum*, but distinct in having conidiophores that can be reduced to conidiogenous cells. Its conidiogenous cells are slightly narrower than those of *R. apiculatum* (2–3.5 µm; Arzanlou et al. 2007). Generally colonies of *R. apiculatum* are also fast-growing, reaching 35 mm after 2 wk, which is not the case for the slower growing *R. cucurbitae*, which only reaches this diameter after 1 mo of incubation.

Ramichloridium luteum G.Y. Sun, H.Y. Li & Crous, *sp. nov*. — MycoBank MB564671; Fig. 7, 8

Etymology. Named after the luteous pigment produced when cultivated on artificial medium.

Mycelium consisting of subhyaline to pale brown, smooth, septate, branched, 1.5–3 µm diam hyphae. *Conidiophores* erect, arising as lateral branches on superficial hyphae, 1–3-septate, unbranched, mostly straight to gently curved, subcylindrical, brown, smooth, 25–80 × 2–3 µm. *Conidiogenous cells* terminal, integrated, tapering towards an acutely rounded apex, pale to medium brown, subcylindrical, 15–30 × 2–3 µm; fertile part as wide as lower part of conidiogenous cell, with taper towards apex; proliferating sympodially, forming a rachis with slightly thickened and darkened, circular, somewhat protruding scars, ± 0.5 µm diam. *Conidia* (6–)7–10(–13) × (2–)3–4(–4.5) µm, solitary, aseptate, pale brown, finely verruculose, oblong to ellipsoid, apex subobtuse, base truncate, slightly darkened and thickened, not refractive, 0.5 µm diam.

Culture characteristics — Colonies after 1 mo at 25 °C in the dark flat, spreading, with sparse aerial mycelium and lobate, feathery margin; reaching 20–30 mm diam. On SNA grey olivaceous; on PDA grey olivaceous, iron-grey in reverse; on OA

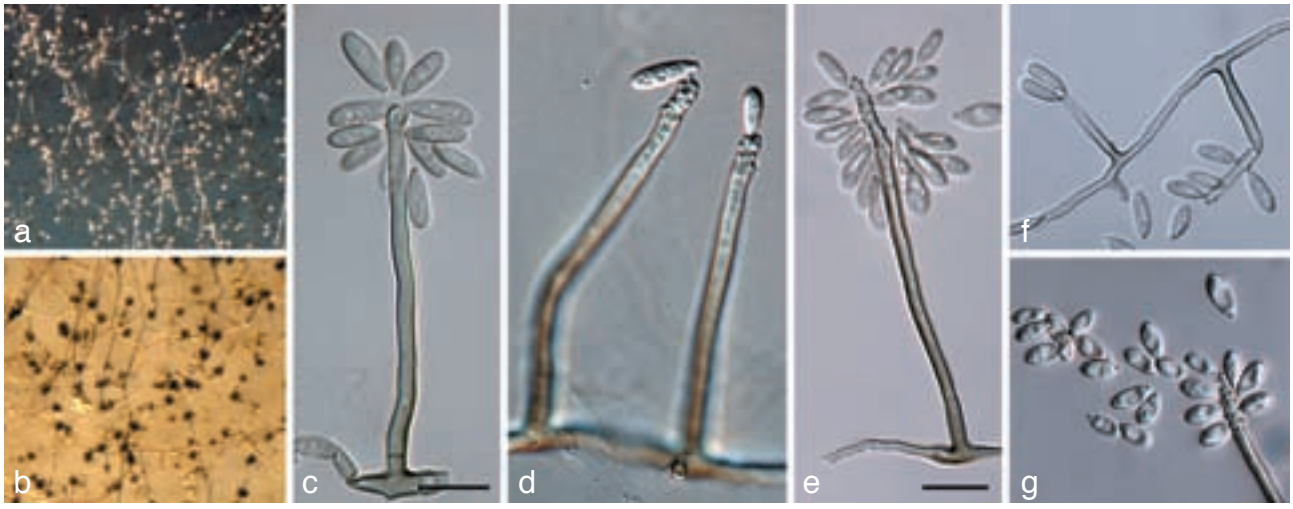


Fig. 7 *Ramichloridium luteum* (CPC 18961). a, b. Sporulating colonies on potato-dextrose agar; c–e. macroconidiophores showing rachis; f. conidiophores reduced to conidiogenous cells; g. conidia. — Scale bars = 10 µm.

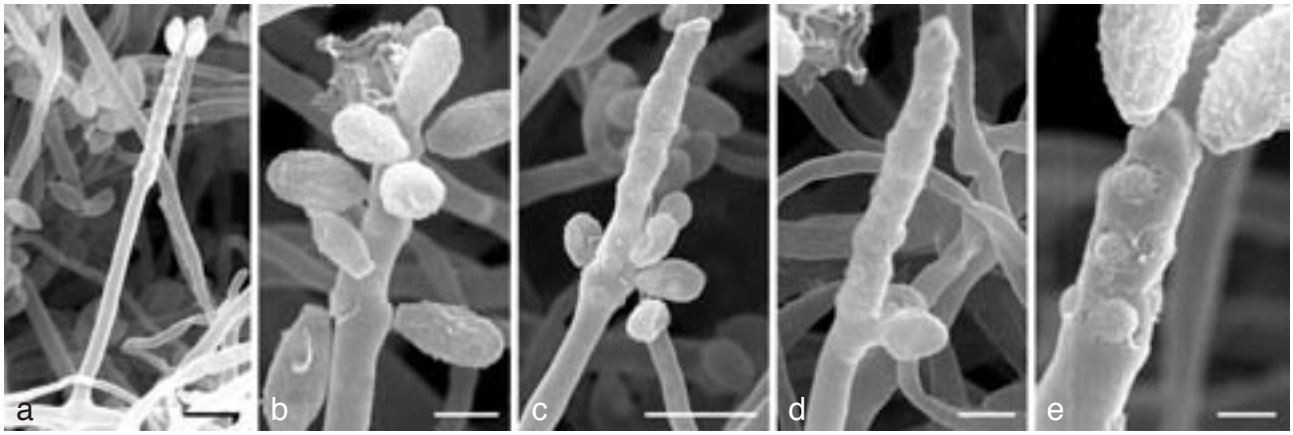


Fig. 8 *Ramichloridium luteum* (CPC 18961). a–e. Scanning electron micrographs showing sympodial proliferation with scars on conidiogenous cells. — Scale bars: a, c = 5 µm; b, d = 2 µm; d = 1 µm.

grey olivaceous, reverse iron-grey, with pale luteous pigment diffusing into agar.

Specimens examined. China, Shandong Province, Weifang City, on fruit surface of *Malus domestica*, Oct. 2006, *G.Y. Sun*, holotype CBS H-20929, ex-type cultures CBS 132088 = CPC 18961 = ZXRSD2; Weifang City, on fruit surface of *M. domestica*, Oct. 2006, *G.Y. Sun*, CPC 18962 = ZXRSD5 = CBS 132089.

Notes — Morphologically *R. luteum* resembles *R. apiculatum* in conidiophore and conidium morphology, though conidia are larger than those of *R. apiculatum* (3–7.5 × 2–4 µm; Arzanlou et al. 2007).

Ramichloridium mali (G.Y. Sun, Z. Zhang & Rong Zhang) G.Y. Sun, H.Y. Li & Crous, *comb. nov*. — MycoBank MB564672

Basionym. *Dissoconium mali* G.Y. Sun, Z. Zhang & Rong Zhang, Mycotaxon 101: 167. 2007.

Specimen examined. China, Shaanxi, Liquan, on fruit surface of *Malus pumila*, holotype HMUABO 822500, culture ex-type LQ73.

Notes — Based on its well-developed rachis, mode of conidiogenesis (Zhang et al. 2007) and phylogeny, this species is better accommodated in *Ramichloridium*. A second isolate identified as *D. mali* (strain LQ45, GenBank EF627451) appears to be allied with *R. punctatum* rather than *R. mali* (Fig. 2; see *R. punctatum* species notes below).

Ramichloridium punctatum Mayfield, Batzer & Crous, *sp. nov*. — MycoBank MB564673; Fig. 9

Etymology. Named after the speckled appearance of colonies on squash cuticles.

Mycelium consisting of smooth, brown, septate, branched, pale to medium brown, 1.5–2 µm diam hyphae. *Conidiophores* erect, arising as lateral branches on superficial hyphae, straight, unbranched, subcylindrical, 1–3-septate, thin-walled, brown, smooth, 20–90 × 2–2.5 µm. *Conidiogenous cells* terminal, subcylindrical or somewhat clavate, uniformly subcylindrical with subobtuse apex, or with a series of irregular, nodulose swellings along the length of the conidiogenous cell, 20–50 × 2–5 µm, brown, becoming paler brown towards apex; fertile part wider than basal part, proliferating sympodially, forming a short rachis with slightly thickened and darkened, circular, somewhat protruding scars, 1 µm diam. *Conidia* solitary, aseptate, guttulate, finely verruculose, pale brown, clavate to ellipsoid, apex obtuse, base truncate, 1 µm diam, slightly darkened and thickened, not refractive, (5–)8–10(–12) × (3–)3.5–4.5(–5) µm.

Culture characteristics — Colonies after 1 mo at 25 °C in the dark flat, spreading, with sparse aerial mycelium and lobate, feathery margin; reaching 20–30 mm diam. On SNA pale mouse-grey; on PDA olivaceous grey in centre, iron-grey in outer margin and underneath; on OA iron-grey.

Appearance on natural substratum — Appearance on squash (Fig. 3c) and re-inoculated apple; ramose signs (Gleason et al. 2011) characterized by olive-grey, smooth mycelial mat with uneven edges consisting of shiny, black, flattened, round sclerotium-like bodies (30–500 µm diam), densely (52–68/cm^2) to sparsely (6–38/cm^2) distributed on apple and squash, respectively. As described in Batzer et al. (2005), a modified version of Koch's postulates was performed to verify the signs observed on squash are also observed on apple.

Specimen examined. USA, Iowa, Gilbert, on fruit surface of *Cucurbita maxima* (cv. Blue Hubbard), Oct. 2009, *D. Mayfield*, holotype CBS H-20930, ex-type cultures CBS 132090 = CPC 18974 = BHE35b1.

Notes — Phylogenetically, *R. punctatum* is closely related to an isolate previously identified as *R. mali* (strain LQ45, GenBank EF627451) (Fig. 2). However, conidia of *R. mali* were reported as being 3.4–6.2 × 1.6–3 µm (aseptate) and 6–12.6 × 2.1–2.8 µm (1-septate) (Zhang et al. 2007), thus being morphologically quite distinct from those of *R. punctatum*. The ex-type culture of *R. mali* (LQ73) does not appear to be conspecific to the second strain identified as *R. mali* (LQ45), which clusters closer to *R. apiculatum*. Unfortunately, neither LQ45 nor LQ73 are

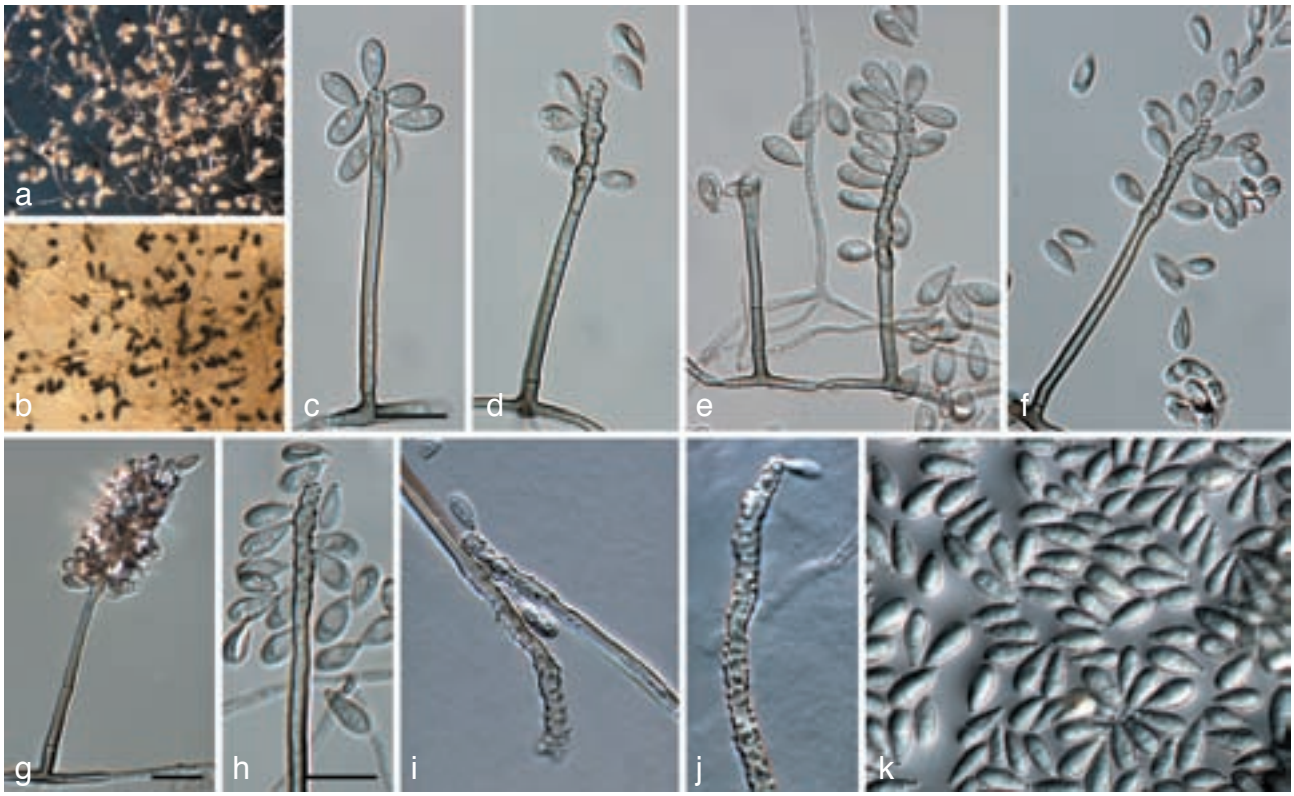


Fig. 9 *Ramichloridium punctatum* (CPC 18974). a, b. Sporulating colonies on potato-dextrose agar; c–h. macroconidiophores showing rachis; i, j. rachis showing pimple-like denticles; k. conidia. — Scale bars = 10 µm.

available for morphological examination, and thus we describe *R. punctatum* as new, noting that it may be the same species represented by LQ45.

Uwebraunia Crous & M.J. Wingf., Mycologia 88: 446. 1996.

Type species. Uwebraunia juvenis Crous & M.J. Wingf.

Ascomata pseudothecial, immersed, globose, unilocular, papillate, ostiolate, canal periphysate; wall consisting of 3–4 layers of brown *textura angularis*; inner layer of flattened, hyaline cells. *Pseudoparaphyses* absent. *Asci* fasciculate, 8-spored, bitunicate. *Ascospores* ellipsoid-fusoid, 1-septate, hyaline, with or without mucoid sheath. *Mycelium* internal and external, consisting of branched, septate, smooth, hyaline to pale brown hyphae, that anastomose, forming nets in culture. *Conidiophores* separate, arising from hyphae, subcylindrical, subulate or lageniform to cylindrical, tapering to a bluntly rounded or truncate apex, straight to once geniculate, smooth, medium brown, 0–2-septate; loci terminal and lateral, visible as slightly thickened, darkened scars on a rachis; proliferation sympodial but also appearing to be percurrent. *Primary conidia* solitary, pale olivaceous-brown, smooth, ellipsoid to obclavate, 1-septate; hila somewhat darkened. *Secondary conidia* developing adjacent to primary conidia, pale olivaceous to subhyaline, aseptate, smooth, pyriform; conidium discharge active, usually with both conidial types being discharged simultaneously. One or more secondary conidia anastomosing with primary conidium once discharged. Colonies not forming yellow pigment, nor sclerotia in culture.

Specimen examined. South Africa, KwaZulu-Natal, Pietermaritzburg, leaves of *Eucalyptus nitens*, Jan. 1995, *M.J. Wingfield*, PREM 51910.

Notes — The proposed link between *U. juvenis* and *Tetatosphaeria nubilosa* (as *Mycosphaerella juvenis*) was incorrect (Crous et al. 2004b, 2007a), and all ex-type strains of *M. juvenis* retained (CPC 932–934) were representatives of *T. nubilosa*, and none formed *U. juvenis* in culture. Presently there are no living strains of *U. juvenis*.

Uwebraunia australiensis (Crous & Summerell) Crous, *comb. nov.* — MycoBank MB564674

Basionym. *Dissoconium australiensis* Crous & Summerell, Fung. Diversity 26: 156. 2007.

Specimen examined. Australia, Queensland, Cairns, nr Kuranda, S16°56'23.3", E145°32'34.6", on leaves of *Eucalyptus platyphylla*, 26 Aug. 2006, *P.W. Crous*, holotype CBS H-19837, culture ex-type CPC 13282 = CBS 120729.

Uwebraunia commune (Crous & Mansilla) Crous, *comb. nov*. — MycoBank MB564675

Basionym. *Dissoconium commune* Crous & Mansilla, Stud. Mycol 50: 203. 2004.

= *Mycosphaerella communis* Crous & Mansilla, Stud. Mycol 50: 203. 2004.

Specimens examined. Spain, Pontevedra, Lourizán, Areeiro, on leaves of *E. globulus*, Dec. 2002, *J.P. Mansilla*, CBS H-9900, holotype of *M. communis* and *D. commune*, culture ex-type CBS 114238 = CPC 10440. – USA, North Carolina, on fruit surface of *Malus domestica*, Aug. 2005, *T. Sutton*, CPC 18963 = NC132C1d = CBS 132091; Wisconsin, on fruit surface of *M. domestica*, Oct. 2000, *P. McManus*, CPC 18971 = MSTF3b = CBS 132092.

Uwebraunia dekkeri (de Hoog & Hijwegen) Crous, *comb. nov.* — MycoBank MB564676

Basionym. *Dissoconium dekkeri* de Hoog & Hijwegen, Mycol. Res. 95: 679. 1991.

= *Uwebraunia lateralis* Crous & M.J. Wingf., Mycologia 88: 454. 1996.

= *Mycosphaerella lateralis* Crous & M.J. Wingf., Mycologia 88: 454. 1996.

= *Mycosphaerella shimabarensis* H.C. Evans & P.F. Cannon, Mycoscience 50: 187. 2009.

Specimens examined. Netherlands, Maarssen, on *Juniperus chinensis*, Nov. 1989, *T. Hijwegen*, ex-type of *D. dekkeri*, CBS 567.89. – South Africa, Northern Province, Tzaneen, Magoebaskloof, on leaves of *E. grandis* × *saligna*, Oct. 1994, *G. Kemp*, ex-type of *U. lateralis*, CPC 825 = CBS 110748. – USA, Ohio, on fruit surface of *Malus domestica*, Oct. 2005, *M. Ellis*, CPC 18964 = OH3 37E1d = CBS 132093.

Uwebraunia musae (Arzanlou & Crous) Crous, *comb. nov.* — MycoBank MB564677

Basionym. *Dissoconium musae* Arzanlou & Crous, Persoonia 20: 24. 2008.

Specimen examined. India, Tamil Nadu, Tiruchirapally, *Musa* cv. Nendran (Plantain) AAB, 2005, *I. Buddenhagen*, holotype CBS H-20036, culture ex-type X1021 = CBS 122453.

Zasmidium Fr., Summa Veg. Scand., Sect. Post (Stockholm): 407. 1849.

Type species. *Zasmidium cellare* (Pers.) Fr., Summa Veg. Scand., Sect. Post (Stockholm): 407. 1849.

Zasmidium angulare Batzer & Crous, *sp. nov*. — MycoBank MB564678; Fig. 10

Etymology. Named after the angular shaped sclerotium-like bodies observed on apple host.

Mycelium consisting of septate, branched, brown, verruculose, 1.5–2.5 µm diam hyphae. *Conidiophores* erect, solitary, arising as lateral branches on superficial hyphae, 0–2-septate, mostly straight to flexuous, subcylindrical, brown, smooth to verruculose, unbranched, 20–40 × 2–3 µm. *Conidiogenous cells* terminal, integrated, brown, smooth to finely verruculose, subcylindrical, straight, 10–15 × 1.5–2.5 µm; proliferating sympodially, with scars aggregated at clavate apex; truncate, somewhat darkened, thickened, 0.5–1 µm diam. *Conidia* (7–)17–50(–130) × 2–3 µm, (0–)3–6(–20)-septate, solitary, verruculose, brown, occurring in branched chains, subcylindrical to narrowly obclavate, apex subobtuse, base long obconically truncate; hilum darkened and thickened, somewhat refractive, 0.5–1 µm diam.

Culture characteristics — Colonies after 1 mo at 25 °C in the dark flat, spreading, somewhat erumpent, with moderate aerial mycelium and lobate, even margin; reaching 20–25 mm diam. On SNA olivaceous; on PDA olivaceous grey on surface, iron-grey in reverse; on OA olivaceous grey, but iron-grey in areas where aerial mycelium has collapsed.

Appearance on natural substratum — On apple surfaces forming a discrete speck appearance (Gleason et al. 2011) consisting of shiny, black, irregular, sclerotium-like bodies, rounded to angular (30–630 µm diam), sparsely or densely distributed (6–41/cm^2).

Specimen examined. USA, Georgia, on fruit surface of *Malus domestica*, Aug. 2005, *M. Wheeler*, holotype CBS H-20931, ex-type cultures CBS 132094 = CPC 19042 = GA227B1a.

Notes — Of the species of *Zasmidium* presently known in *Mycosphaerellaceae* (Arzanlou et al. 2007, Crous et al. 2007a, Braun et al. 2010, Kamal 2010), none have thus far been described from SBFS signs on apple. Phylogenetically, *Z. angulare* appears to be distinct from other species of *Zasmidium* presently deposited in GenBank, being closest to *Z. nocoxi* and '*Mycosphaerella*' *aleuritidis* (Crous et al. 2009b). Morphologically, *Z. angulare* is different from *Z. nocoxi* by having shorter

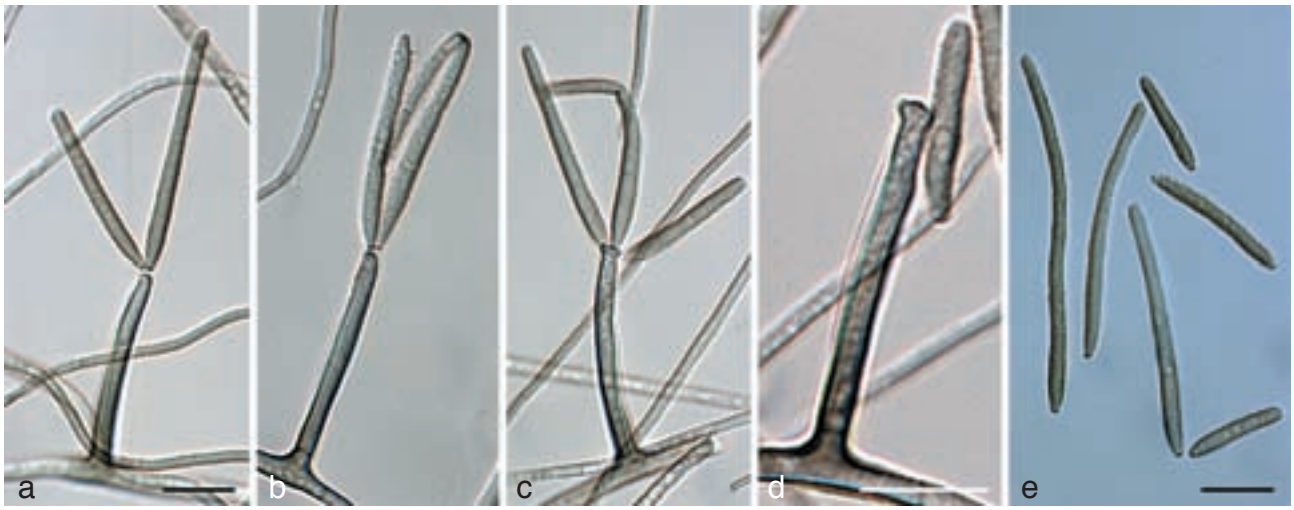


Fig. 10 *Zasmidium angulare* (CPC 18942). a–d. Macroconidiophores showing apical conidiogenous loci; e. conidia. — Scale bars = 10 µm.

conidiophores and a slower growth rate in culture. *Zasmidium nocoxi* reaches 40 mm diam after 1 mo on MEA, with conidiophores up to 100 µm (Crous et al. 2009c). *Zasmidium angulare* only reaches 20–25 mm diam after 1 mo and conidiophores up to 40 µm tall. Furthermore, *Z. nocoxi* produces a synanamorph state in its aerial mycelium (Crous et al. 2009c), which is absent in *Z. angulare*.

DISCUSSION

We resolved the taxonomy of five new taxa that reside in *Dissoconiaceae*, as well as a novel species of *Zasmidium*, *Z. angulare*, based on a collection of apples and cv. Blue Hubbard squash with SBFS signs from China and the USA. The novel genus and species described here expanded *Dissoconiaceae* to include species of four genera. *Pseudoveronaea*, which is morphologically similar but phylogenetically distinct from *Veronaea*, was introduced as a new genus of *Dissoconiaceae*, and two species, *P. ellipsoidea* and *P. obclavata*, were described.

We proposed three novel species of *Ramichloridium* in this study, *R. cucurbitae*, *R. luteum* and *R. punctatum*, as well as a new combination, *R. mali*. The originally described *Ramichloridium* comprised a heterogeneous group of taxa with diverse life styles. Arzanlou et al. (2007) demonstrated that only species clustering in *Capnodiales* were true *Ramichloridium*, whereas other '*Ramichloridium*' species segregated into different genera, including *Rhinocladiella* (*Herpotrichiellaceae*, *Chaetothyriales*), *Pleurothecium* (*Chaetothyriales*), *Myrmecridium* (*Sordariomycetes*) and *Radulidium* (*incertae sedis*). However, the phylogenetic inference of LSU sequence data from both Arzanlou et al. (2007) and the present study showed that *R. indicum* and *R. apiculatum* (*Ramichloridium* s.str.) clustered with *Dissoconium* species in *Dissoconiaceae*. By means of contrast, *Pseudoramichloridium* clustered in *Teratosphaeriaceae* (Cheewangkoon et al. 2009), and other *Ramichloridium*-like species grouped in *Mycosphaerellaceae*. The fact that *Ramichloridium*-like species clustered in both *Dissoconiaceae* and *Mycosphaerellaceae* suggests that it is still a heterogeneous genus and more molecular and morphological data are necessary to clarify the taxonomy of several of these species. The three novel *Ramichloridium* species described in our study are closely related to *R. apiculatum* in *Dissoconiaceae*, providing more taxa to further help delineate *Ramichloridium* s.str.

Our study further revealed that *Dissoconium* species clustered in two clades based on phylogenetic analyses of LSU and ITS sequences, indicating that *Dissoconium* is paraphyletic. Based on morphological differences and their distinct phylogeny, the name *Uwebraunia* was resurrected to accommodate taxa closely related to *U. dekkeri*. Furthermore, four species, namely *D. aciculare*, *Dissoconium* sp., *U. dekkeri* and *U. commune* are shown to be members of SBFS fungal complex, although they were originally described from different ecological niches, being either mycoparasitic or plant pathogenic (de Hoog et al. 1983, 1991, Crous et al. 2004b, 2007b). Batzer et al. (2005) and Díaz Arias et al. (2010) showed that these species caused characteristic signs on inoculated apples using a modified version of Koch's postulates. SBFS fungal species within *Dissoconiaceae* may cause distinct mycelial types on the fruit surface. For example, *D. aciculare* causes discrete speck and *U. commune* causes a fuliginous mycelial type on apple. However, a single species will consistently express a single mycelial type on a fruit host. In addition, *D. aciculare* is tolerant to lower temperatures and grows considerably faster on the media than other SBFS fungal species (Batzer et al. 2010). Further collections are required to determine if the distinction between *Dissoconium* and *Uwebraunia* can also be correlated with differences in their ecology.

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