Metulocladosporiella gen. nov. for the causal organism of Cladosporium speckle disease of banana

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ABSTRACT

Cladosporium musae, a widespread leaf-spotting hyphomycete on Musa spp., is genetically and morphologically distinct from Cladosporium s. str. (Davidiella anamorphs, Mycosphaerellaceae, Dothideales). DNA sequence data derived from the ITS and LSU gene regions of C. musae isolates show that this species is part of a large group of hyphomycetes in the Chaetothyriales with dematiaceous blastoconidia in acropetal chains. Cladosporium adianticola, a foliicolous hyphomycete known from leaf litter in Cuba is also a member of this clade and is closely related to C. musae. A comparison with other genera in the Cladosporium complex revealed that C. musae belongs to a lineage for which no generic name is currently available, and for which the genus Metulocladosporiella gen. nov. is proposed. Two species of Metulocladosporiella are currently known, namely M. musae, which is widely distributed, and M. musicola sp. nov., which is currently known from Africa.

Introduction

The name Cladosporium musae was introduced by Mason (in Martyn 1945) for a leaf-spotting hyphomycete causing Cladosporium speckle disease of banana. This disease occurs in most countries where banana is cultivated (Jones 2000). Although the disease is generally regarded as insignificant, it can be serious depending on the cultivar and location. Symptoms initially appear as pale green flecks that elongate into brown streaks of about 2 cm or longer. Leaf specks frequently turn orange in colour, with sparse grey–green blotching becoming evident on the adaxial surface of older leaves. Lesions eventually become dark brown, coalesce, and occupy large areas of the photosynthetic leaf surface (Surridge et al. 2003).

C. musae was described in Cladosporium because of its pigmented conidiophores and conidia that are formed in acropetal chains. Cladosporium s. lat. is heterogeneous, composed of many kinds of superficially similar, but unrelated dematiaceous hyphomycetes with acroblastic conidial formation. A total of 772 names have thus far been assigned to this genus (Dugan et al. 2004). Roquebert (1981) and David (1997) examined the conidiogenesis and structure of the conidiogenous loci of Cladosporium species in detail and demonstrated that Cladosporium s. str. is well-characterised by having a unique 'coronate' scar type (scars more or less protuberant, with a central dome surrounded by a raised periclinal rim). Braun et al. (2003) published a phylogenetic study of cladosporioid hyphomycetes (i.e. Cladosporium s. lat.), based on sequences of the ITS (ITS-1, 5.8 S, ITS-2) and 18 S rRNA genes. This study supported David's (1997) narrow circumscription of Cladosporium s. str. Braun et al. (2003) also proposed the new genus Davidiella for teleomorphs of Cladosporium s. str.

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Numerous other groups of cladosporioid hyphomycetes (*Cladosporium s. lat.*) have already been excluded on the basis of morphological reassessments and molecular data. For example, human pathogenic 'Cladosporium' species belonging to the *Herpotrichiellaceae* are presently placed in *Cladophialophora* (Masclaux *et al.* 1995; Untereiner 1997; de Hoog *et al.* 2000) and cladosporioid *Venturia* anamorphs are accommodated in *Fusicladium* (Schubert *et al.* 2003). Other species, originally placed in *Cladosporium*, proved to be *Mycosphaerella* anamorphs belonging in *Passalora*, *Pseudocercospora* and *Stenella* (Crous & Braun 2003; Schubert & Braun 2005). On account of morphological, molecular and ecological features, Seifert *et al.* (2004) recently separated *Cladosporium staurophorum* from *Cladosporium s. str.* and introduced the new genus *Devriesia* to accommodate a group of five heat-resistant species that also appeared *Cladosporium*-like in their general morphology.

In this study, morphological characters and DNA sequence data of the ITS and 28 S nrDNA were used to taxonomically and phylogenetically characterise *C. musae*. Preliminary morphological examinations suggested that the conidiogenesis and structure of the conidiogenous loci differ from *Cladosporium s. str.*

Materials and methods

Isolates

Isolates used in this study were retrieved from the Centraalbureau voor Schimmelcultures (CBS; Utrecht), and CABI Bioscience (IMI; Egham). Freshly isolated strains included in this study were obtained from symptomatic *Musa* leaves collected in South Africa and Mozambique (Table 1).

Leaves were incubated in a moist chamber for 3 d and observed under a dissecting microscope. Conidia were removed from *Cladosporium*-like conidiophores with the help of a sterile glass needle, and streaked out on 2 % malt extract agar (MEA) (Sigma-Aldrich Chemie, Zwijndrecht) containing streptomycin and penicillin (Gams *et al.* 1998).

DNA isolation, amplification and sequencing

General methods used for DNA isolation, amplification, and sequencing, as well as for phylogenetic analyses are those used by Halleen *et al.* (2004). Amplification of the rDNA was performed using the primers V9G/LR5 (de Hoog & Gerrits van den Ende 1998; Vilgalys & Hester 1990) or ITS1/ITS4 (White *et al.* 1990). The amplicons were sequenced with the BigDye terminator cycle (Applied Biosystems, Foster City, CA) or DYEnamicET dye terminator (Amersham Biosciences, Freiburg) sequencing kits and analysed on an ABI Prism 3700 (Applied Biosystems) by using the standard conditions recommended by the vendor. The PCR primers were used as sequence primers for both genes. To ensure a good-quality sequences across the length of the LSU sequence, primers LR0R (Rehner & Samuels 1994) and LR16 (Moncalvo *et al.* 1993) were used as additional, internal sequence primers. Newly generated sequences were compared with published sequences of a broad range of taxa downloaded from GenBank. The selection of the sequences partly followed results obtained by BLAST-searches, in which sequences similar to those of *C. musae* strains were retrieved. Obtained LSU trees were rooted using a sequence of *Peziza natrophila* as outgroup, and a sequence of *Mycosphaerella punctiformis* was used as outgroup for the ITS tree. A gap caused by the longer ITS2 region of the *Metulocladosporiella musae* sequences in the ITS alignment was coded as a single indel (characters 585–617 of the alignment). Tree topologies were obtained from the aligned sequences by the maximum parsimony and neighbour-joining criteria as implemented in PAUP 4.0b10 (Swofford 2003). For parsimony analyses, heuristic searches with 100 random taxon additions were performed using parsimony-informative, unordered, and equally weighted characters. Gaps were treated as both new character states and missing characters and a maximum of 1000 trees was allowed. For neighbour-joining analyses, the uncorrected "p", Kimura 2-parameter and F84 substitution models were tested and ties were broken randomly if encountered. Branch robustness in the analyses was tested by 1000 bootstrap replicates. Newly generated sequences and the alignments were deposited in GenBank (DQ008125–DQ008163) and TreeBASE (SN2290), respectively (Table 1).

Morphology

Isolates were inoculated onto potato–dextrose agar (PDA), synthetic nutrient-poor agar (SNA), and oatmeal agar (OA) (Gams *et al.* 1998), and incubated under continuous near-ultra-violet light at 25 °C for 6 d. Microscopic observations were made from colonies cultivated on SNA, and preparations mounted in lactic acid. Conidial branching patterns were studied by placing squares of transparent adhesive tape (1 cm^2) on conidiophores at colony margins, and mounting these between two drops of clear lactic acid under a glass coverslip. Cultural characteristics were determined from colonies cultivated on PDA and OA using the colour charts of Rayner (1970).

Results

Phylogeny

Neighbour-joining analyses on the LSU and ITS datasets resulted in the same tree topology irrespective of the substitution model tested (data not shown). Some rearrangements of the deep nodes were observed when the most parsimonious trees were compared with the trees obtained from the neighbour-joining analyses (data not shown). Parsimony analysis of the datasets with gaps coded as missing data or as new states did not alter the consensus tree topologies obtained.

Approximately 975 nucleotides were sequenced for the LSU gene for the isolates studied (Table 1). The manually adjusted alignment contained 60 taxa (including the outgroup) and 611 characters including alignment gaps. Of the 611 characters used in the phylogenetic analysis, 245 were parsimony-informative, 79 were variable and parsimony-uninformative and 287 were constant. Twenty equally most parsimonious trees, one of which is shown in Fig 1, were obtained from the parsimony analysis. Two classes are represented in this

Table 1 – Isolates subjected to DNA analysis and morphological examination				
Species	Accession No.[b]	Source	Origin	GenBank accession no. (ITS, LSU)
Alternaria malorum	CBS 216.65, NRRL A-13702	*Triticum aestivum*	USA	—, DQ008142
'Cladosporium' adianticola	CBS 582.92	*Adiantum tenerum*	Cuba	—, DQ008143
	CBS 735.87[a]	*Adiantum* sp.	Cuba	DQ008125, DQ008144
C. cladosporioides	CBS 574.78A	Mycophilic	USSR	—, DQ008145
	CBS 109501	Deep mycosis of human patient	Turkey	—, DQ008146
C. uredinicola	CBS 306.84	*Puccinia allii*	UK	—, DQ008147
Davidiella macrospora	CBS 138.40	*Iris* sp.	The Netherlands	—, DQ008148
D. tassiana	CBS 813.71	*Polygonatum odoratum*	Czech Republic	—, DQ008149
Devriesia staurophora	CBS 374.81B	Páramo soil	Colombia	—, DQ008150
	CBS 375.81	Páramo soil	Colombia	—, DQ008151
Metulocladosporiella musicola	CBS 194.63, ATCC 36952	*Musa* sp.	France	DQ008126, DQ008152
	CBS 110959, CPC 4628	*Musa* sp.	South Africa	—
	CBS 110960, CPC 4629[a]	*Musa* sp.	South Africa	DQ008127, DQ008153
	CBS 110963, CPC 4632	*Musa* sp.	South Africa	DQ008128, —
	CBS 110964, CPC 4633	*Musa* sp.	South Africa	—
	CBS 110966, CPC 4635	*Musa* sp.	South Africa	—, DQ008154
	CBS 110965, CPC 4634	*Musa* sp.	South Africa	DQ008129, —
	CBS 113860, IMI 380629	*M. sapientum*	Uganda	DQ008130, DQ008155
	CBS 113861, IMI 295939	*M. sapientum*	Zimbabwe	DQ008131, DQ008156
	CBS 113862, IMI 380793	*M. sapientum*	Kenya	DQ008132, —
	CBS 113864, IMI 374551	*Musa* sp.	Uganda	DQ008133, DQ008157
	CBS 113865, IMI 380626	*M. sapientum*	Uganda	DQ008134, DQ008158
	CBS 113873	*M. sapientum*	Mozambique	DQ008135, DQ008159
	IMI 327290	*M. paradisiaca*	Uganda	DQ008136, DQ008160
M. musae	CBS 161.74, ATCC 36973[a]	*Musa* sp.	Honduras	DQ008137, DQ008161
	CBS 113863, IMI 380798	*M. sapientum*	Cameroon	DQ008138, DQ008162
Passalora fulva	CBS 119.46, CPC 3688	*Lycopersicon esculentum*	The Netherlands	AY251069, DQ008163
'Pseudocladosporium' sp.	CBS 115142, FRR 5582, CPC 11044	Fruit-based drink	Australia	DQ008139, —
	CBS 115143, FRR 5599, CPC 11047	Bottled spring water	Australia	DQ008140, —
	CBS 115144, FRR 3318, CPC 11048	Apple juice drink	Australia	DQ008141, —

a Ex-type strain.
b CBS, Centraalbureau voor Schimmelcultures, (Utrecht); C.P.C., culture collection of Pedro Crous (at CBS); ATCC, American Type Culture Collection (Manassas); F.R.R., CSIRO Division of Food Science & Technology (Sydney); IMI, CABI Bioscience UK Centre (Egham); NRRL, ARS Culture Collection, Northern Regional Research Laboratory, USA, (Peoria).

tree, namely the *Chaetothyriomycetes* (100 % bootstrap support) and the *Dothideomycetes* (57 % bootstrap support). In the *Chaetothyriomycetes*, a representative of the *Rhynchostomataceae* and several taxa from the *Herpotrichiellaceae* are present. In the *Herpotrichiellaceae*, a clade supported by a bootstrap support value of 92 % contains two sequences of two species of *Phaeococcomyces* and three well-supported clades containing strains of *Cladosporium adianticola* (99 % bootstrap support), *Metulocladosporiella musicola* (91 % bootstrap support) and *Metulocladosporiella musae* (99 % bootstrap support). The two

Metulocladosporiella species are joined with a bootstrap support value of 100 %. Sequences of 'Ramichloridium' mackenziei and 'Ramichloridium' anceps are found in the *Herpotrichiellaceae* clade. Representatives of the *Pleosporales*, *Dothideaceae* and *Mycosphaerellaceae* are present in the *Dothideomycetes* clade. A sequence of *Ramichloridium cerophilum* clustered with *Mycosphaerella* species in the *Mycosphaerellaceae* clade.

Approximately 600 bases were determined for the ITS region for the isolates studied (Table 1). The manually adjusted alignments contained 23 taxa (including the outgroup) and
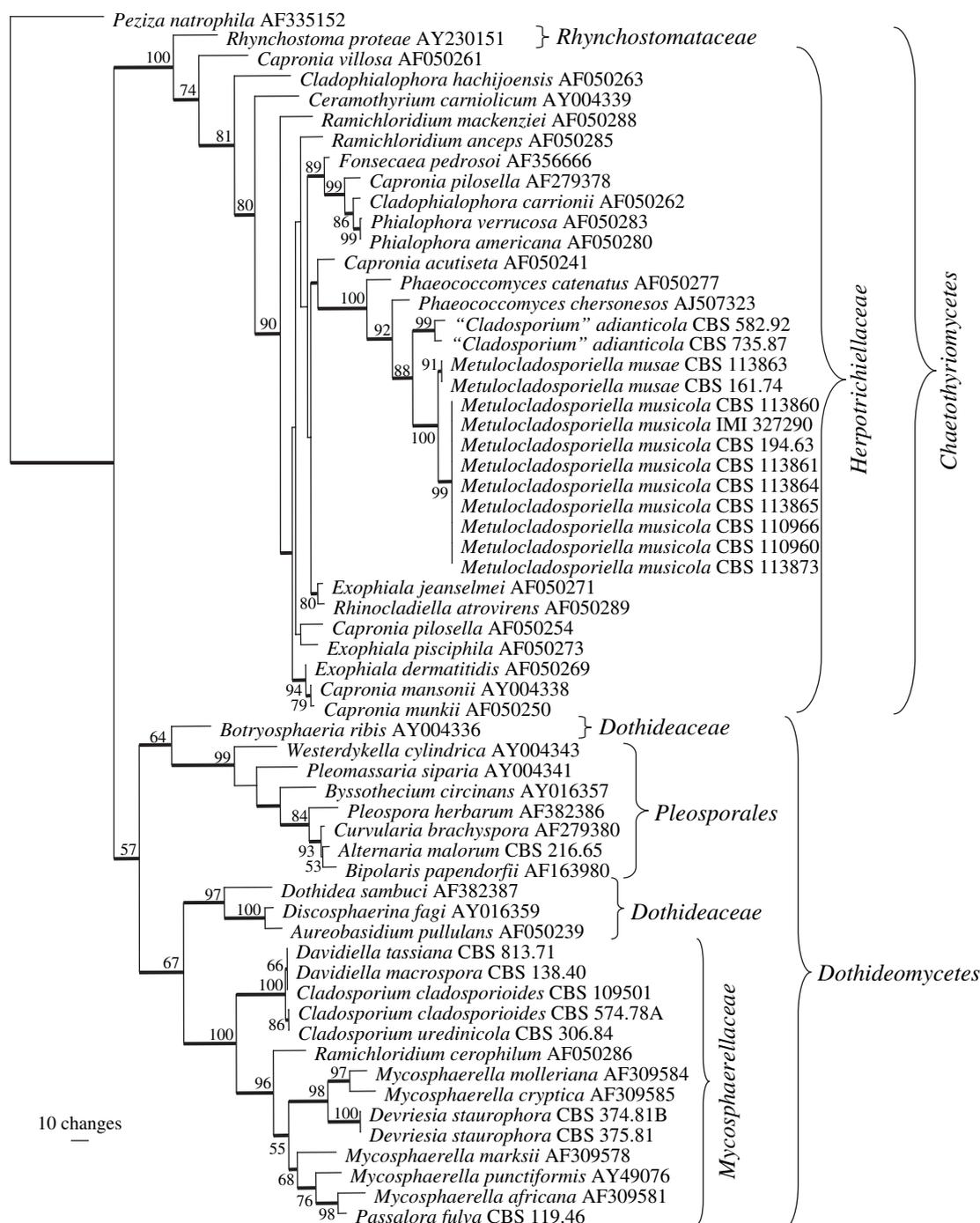
Fig 1 – One of 20 equally most parsimonious trees obtained from large subunit sequence data (TL = 1247 steps, CI = 0.460, RI = 0.820, RC = 0.377). The scale bar indicates a 10 changes and the numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. The GenBank sequence of *Peziza natrophila* (AF335152) was included as outgroup.

612 characters including alignment gaps. Of the 612 characters used in the phylogenetic analysis, 296 were parsimony-informative, 83 were variable and parsimony-uninformative and 233 were constant. Two equally most parsimonious trees, one of which is shown in Fig 2, were obtained from the parsimony analysis. As with the LSU tree, strains of 'Cladosporium' adianticola, Metulocladosporiella musicola (100 % bootstrap

support) and *Metulocladosporiella musae* (100 % bootstrap support) cluster together with a bootstrap support value of 100 %. Two sequences of 'Ramichloridium' anceps obtained from GenBank formed a distant, highly supported sister clade (bootstrap support = 100 %) to the clade containing the *Metulocladosporiella* species and *C. adianticola*. The *Ramichloridium*–*Metulocladosporiella*–*C. adianticola* clade is weakly supported (58 %).
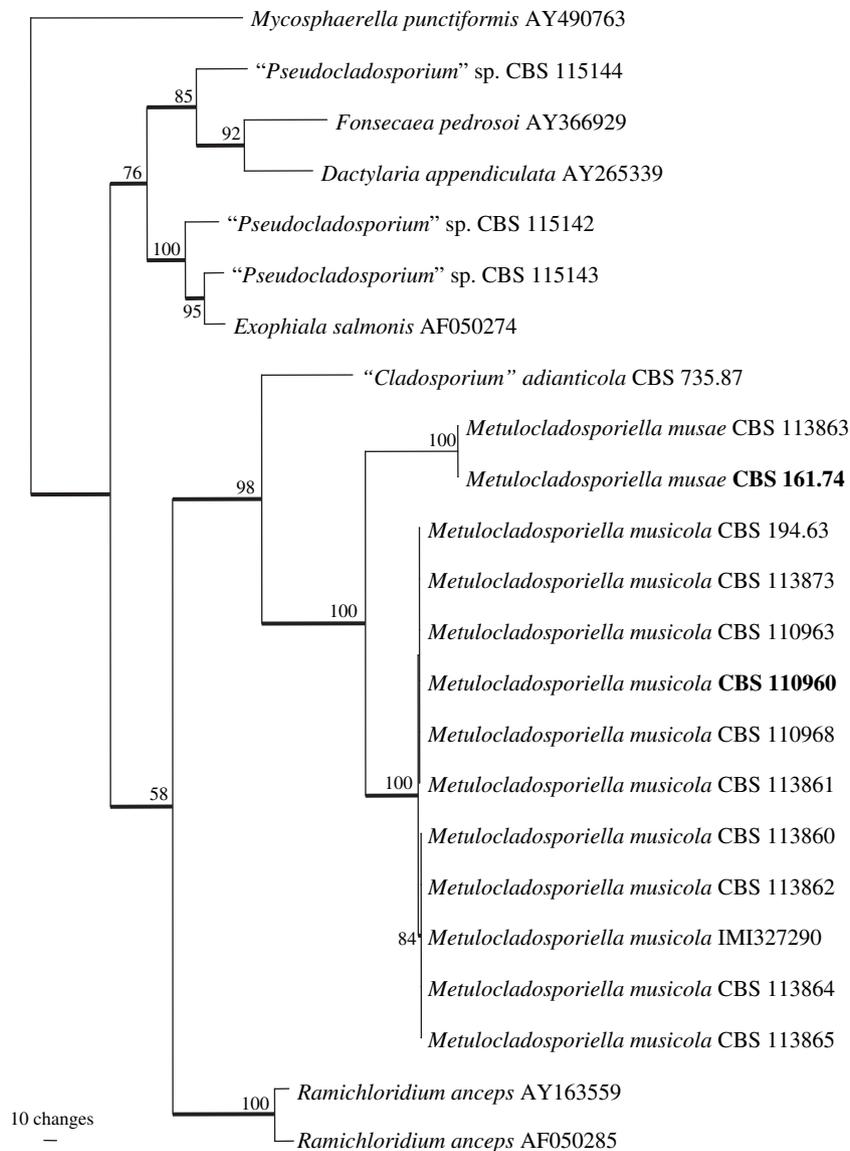
Fig 2 – One of two equally most parsimonious trees obtained from ITS sequence data (TL = 1008 steps, CI = 0.718, RI = 0.819, RC = 0.588). The scale bar indicates 10 changes and the numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. The GenBank sequence of *Mycosphaerella punctiformis* (AY490763) was included as outgroup. Ex-type strains are in bold.

Taxonomy

Conidia in *Cladosporium musae* are formed holoblastically in acropetal, often branched chains. This pattern is similar as in *Cladosporium s. str.* The examination of cultures and herbarium specimens of *C. musae* revealed clear differences in the conidiogenesis and structure of the conidiogenous loci and conidial hila in comparison with species of *Cladosporium s. str.*, typified by *C. herbarum*. A septum separating the maturing conidia is formed, which is cleft in the middle. The structure of the walls of the conidiogenous loci and the conidial hila is uniform and remains unchanged. The conidiogenous loci are subdenticulate, apically truncate, unthickened to slightly so, and somewhat darkened-refractive. A convex central dome

surrounded by a raised periclinal rim, as in *Cladosporium s. str.*, is not formed. On account of the quite distinct conidiogenous loci and conidial hila, supported by molecular analyses of DNA sequences (see below), *C. musae* has to be excluded from *Cladosporium s. str.* Based on its peculiar features, *C. musae* belongs to a group of hyphomycetes that have been classified by Kiffer and Morelet (1999) as 'Acroblastosporae', i.e. hyphomycetes with holoblastic conidiogenesis and conidia formed in acropetal, often branched chains. Most genera in this group are phaeoacroblastic, i.e. they are pigmented, and they are morphologically, ecologically and, as far as known, genetically clearly distinct (Fig 3).

A comparison with phaeoblastosporic hyphomycetous genera (see below) revealed that *C. musae* does not fit into the concepts of any of the genera concerned. The present
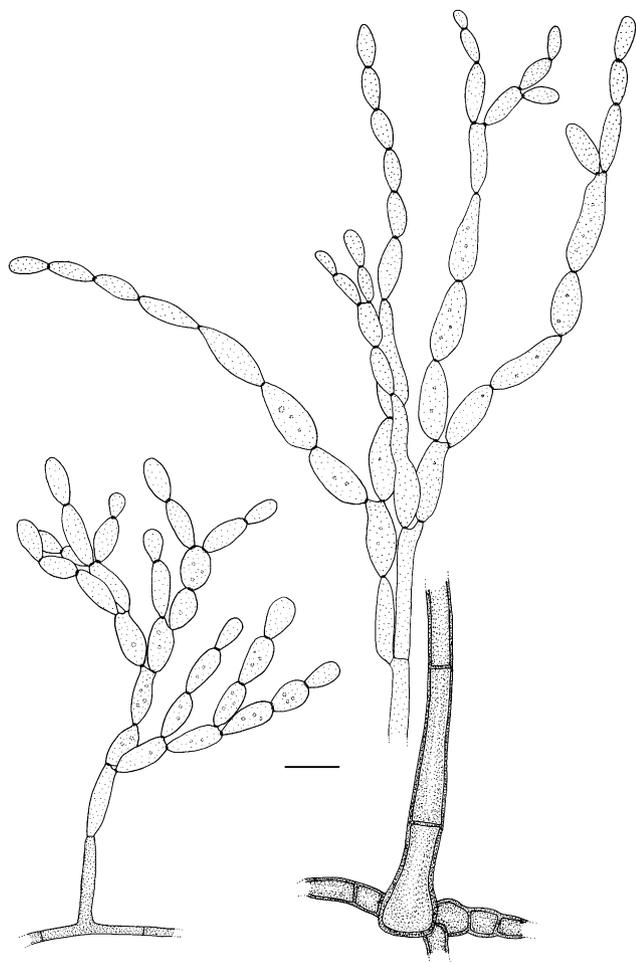
with some intermixed micronematous conidiophores), solitary or in loose groups, arising from hyphae, erect, composed of a long, subcylindrical, simple stipe and a branched terminal part; stipe septate, pigmented, smooth or almost so, usually swollen at the very base; branched part loose to dense, metuloid, composed of short to long branchlets and ramoconidia, tips paler than the stipes, subhyaline to very pale olivaceous; conidiogenous cells integrated, terminal, occasionally intercalary, polyblastic, sympodial, conidiogenous loci (conidial scars) subconspicuous to conspicuous, subdenticulate, truncate, unthickened to slightly thickened, and somewhat darkened-refractive. *Conidia* and ramoconidia in simple and branched chains, ellipsoid, ovoid, subcylindrical, fusiform, 0–1-septate, subhyaline to very pale olivaceous, thin-walled, smooth, hila truncate, unthickened to slightly thickened and slightly darkened-refractive, secession schizolytic.

Metulocladosporiella musae (E.W. Mason) Crous, Schroers, Groenewald, U. Braun & K. Schubert, **comb. nov.**

MycoBank MB500185

Basionym: *Cladosporium musae* E.W. Mason, *in* Martyn, *Mycol. Pap.* **13**: 2 (1945).

Synonym: *Periconiella sapientumicola* Siboe, *African J. Mycol. Biotechnol.* **2**: 4 (1994); non *Periconiella musae* M.B. Ellis 1967.

(Figs 3–4)

Leaf spots amphigenous, at first visible as pale greenish flecks, ellipsoid to oblong, forming streaks up to 2 cm or even longer, pale to blackish brown, occasionally somewhat zonate, with age turning orange in colour, later often dark brown, finally often confluent, forming large patches, in severe infections entire leaves occasionally becoming necrotic, often with dark, sunken, water-soaked lesions along the midrib, 10–20 mm wide. *Mycelium* internal and external, superficial; external hyphae branched, 1–3(–4) μm wide, septate, occasionally slightly constricted at the septa, with small swellings, hyaline, subhyaline to very pale olivaceous, thin-walled, smooth, hyphae occasionally aggregated, forming ropes; sometimes with some intermixed micronematous conidiophores, erect from the vegetative mycelium, intercalary, straight to flexuous, unbranched, subhyaline, usually with simple terminal conidial chains. *Macronematous conidiophores* arising from superficial hyphae, erect, solitary to loosely aggregated, 45–500(–600) μm long, composed of a subcylindrical stipe, 3–8 μm wide, 2–12-septate, swollen or lobed at the base, 10–17 μm diam, with short rhizoid hyphae growing from the base, medium to dark brown in the lower half, paler towards the apex, tips pale olivaceous or even subhyaline, thick-walled below, thin-walled towards the apex, smooth; apex persistently branched, branched part composed of usually fairly compact, closely arranged subcylindrical branchlets; primary branches aseptate, 15–30 × 3.5–5 μm, giving rise to 1–2 secondary branches, or to conidiogenous cells; secondary branches 0(–1)-septate, 30–50 × 3–4.5 μm, giving rise to 1(–3) conidiogenous cells; conidiogenous cells subcylindrical, 10–45 × 3–4 μm, terminal or occasionally intercalary, sympodial, polyblastic, conidiogenous loci subconspicuous to conspicuous, subdenticulate, somewhat protuberant, truncate, wall unthickened, but somewhat darkened-refractive, 1–2 μm wide. *Conidia* in simple and branched acropetal chains, ellipsoid-ovoid, fusiform, subcylindrical, (6–)8–11(–16) × (3–)4(–5) μm [ramoconidia (10–)15–19(–25) × (3.5–)5(–6) μm)], 0(–1)-septate,

Fig 3 – *Metulocladosporiella musae* (CBS 161.74, ex-epitype). Micro- and macronematous conidiophores and conidia. Bar = 10 μm.

fungus is well-characterised and distinguished by frequently branched, metuloid, pigmented conidiophores with paler tips. The ultimate branchlets are composed of conidiogenous cells and ramoconidia, giving rise to pale, mostly subhyaline conidia. Therefore, the new genus is proposed below for *C. musae* and another newly described species. Fig 4.

Metulocladosporiella Crous, Schroers, Groenewald, U. Braun & K. Schubert, **gen. nov.**

MycoBank MB500224.

Hyphae ramosae, septatae, hyalinae, subhyalinae vel pallide olivaceae, tenuitunicatae. Conidiophora solitaria vel laxe aggregata, erecta, subcylindrico, septato, brunneo, levi; ramuli terminales ex cellulis conidiogenis et ramoconidiis compositi; cellulae conidiogenae integratae, terminales, polyblasticae; cicatrices conidiales subconspicuae vel conspicuae. Conidia et ramoconidia catenata vel rami-catenata, ellipsoidea, ovoidea, subcylindrica vel fusiformia septata, subhyalina vel pallide olivacea, hila non-incrassata, leviter fuscata-refractiva, secessio schizolytica.

Typus: *Metulocladosporiella musae* (E.W. Mason) Crous, Schroers, Groenewald, U. Braun & K. Schubert 2006.

Hyphomycetes. Acroblastosporae. On living leaves. *Mycelium* internal and external, hyphae branched, septate, hyaline, subhyaline to pale olivaceous, thin-walled. *Stromata* lacking. *Conidiophores* macronematous, mononematous (occasionally
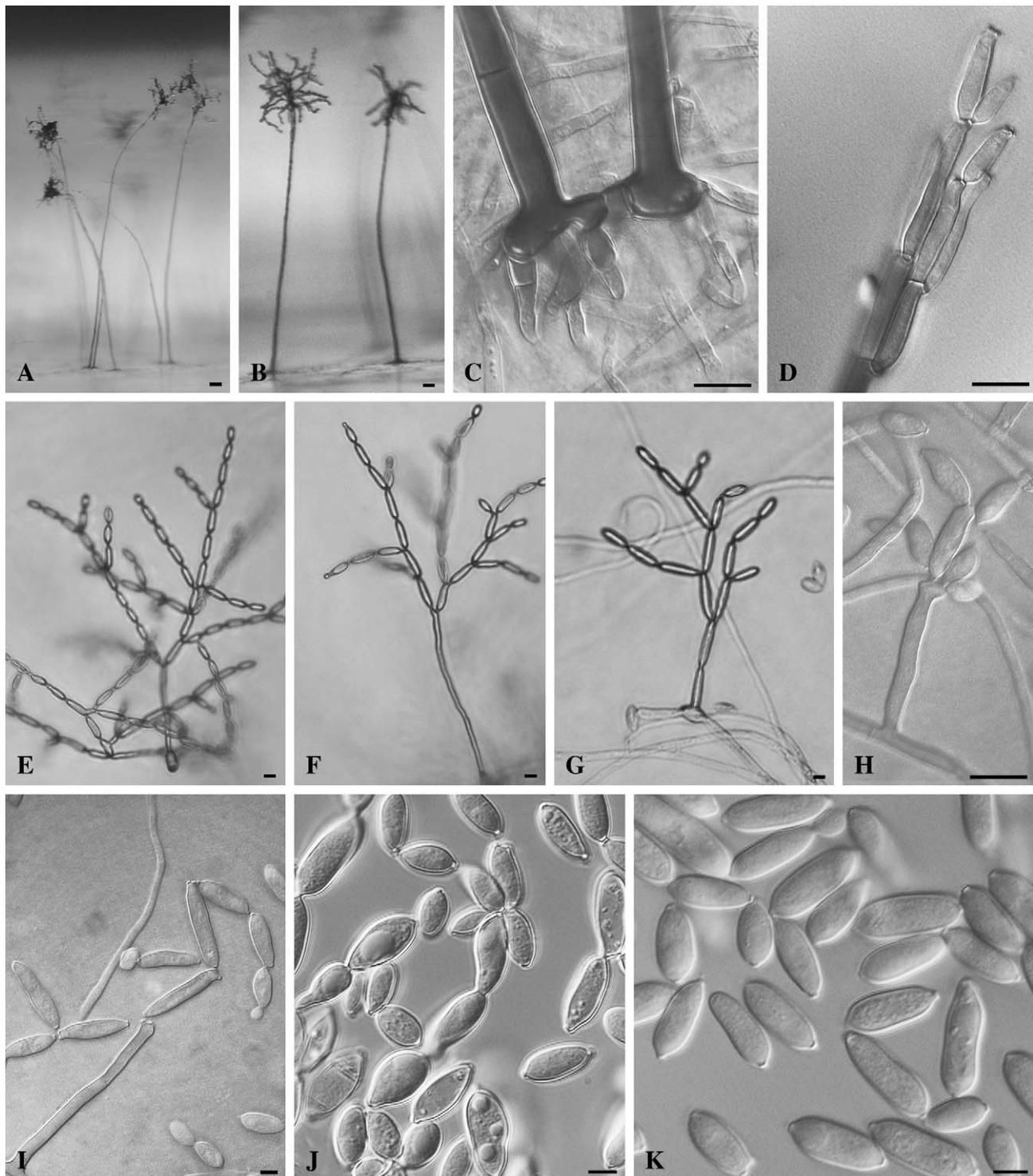
Fig 4 – A–K – *Metulocladosporiella musae* (CBS 161.74, ex-epitype). Figs A–B – Macronematous conidiophores. Fig C – Lobed to swollen bases of conidiophores. Fig D – Conidiogenous apparatus. Figs E–I – Micronematous conidiophores. Figs J–K – Conidia. Bars: (A) = 16 μm, (B) = 20 μm, (C–D), (H) = 10 μm, (E–G) = 8 μm, (I–K) = 4 μm.

hyaline, subhyaline to very pale olivaceous, thin-walled, smooth, with 1–3 hila, truncate, 1–2 μm wide (up to 3 μm wide at the base of ramoconidia), unthickened or almost so, but somewhat darkened-refractive, secession schizolytic.

Cultures: Colonies 37–50 mm diam on PDA after 14 d under NUV at 25 °C. Colonies on PDA and OA spreading, with smooth, regular margins and sparse aerial mycelium; surface on PDA pale mouse-grey to mouse-grey due to profuse sporulation; margins of sub-merged mycelium, mouse-grey; reverse on PDA greenish black.

Host range and distribution: On *Musa* spp., incl. *M.×paradisiaca* (incl. var. *sapientum*) and *M. schweinfurthii* (syn. *Ensete gillesii*); Africa (Burundi, Cameroon, Côte d'Ivoire,

Democratic Republic of Congo, Egypt, Ethiopia, Ghana, Guinea, Kenya, Mozambique, Rwanda, Sierra Leone, South Africa, Sudan, Togo, Uganda, Zimbabwe), Asia (Bangladesh, Hong Kong, Indonesia, Malaysia, Nepal, Sabah, Sri Lanka, Thailand, Vietnam), Australasia and Oceania (Solomon Islands, W. Samoa), Central America (Mexico), Latin America, Caribbean (Cuba, Ecuador, Honduras, Jamaica) (Jones 2000).

Literature: Ellis (1971), Stover (1972), David (1988), Siboe (1994), Jones (1994, 2000), Ho *et al.* (1999), Surridge *et al.* (2003).

Specimens examined: All on *Musa* sp.: **Jamaica**: 7 Sept. 1942, *E. B. Martyn* [slide ex type coll.] (IMI 7521)-**lectotypus hic designatus. Honduras**: *R.H. Stover* (CBS herb. 14788-**epitypus hic designatus**; culture ex-epitype CBS 161.74 = ATCC 36973).-**Mexico**: 12 Feb. 1983, *J. M. van Valkenburgh* (BPI 427272).

Notes: Mason (*in* Martyn 1945) described long conidiophores, 60–500 × 3.5–6 μm, and aseptate conidia, 6–22 × 2.5–4 μm. In the type material from IMI (slide only), the conidiophores are much shorter, 45–150 × 3–6 μm, but the conidia agree well with the original description [5–16 × 3–5 μm, ramo-conidia 11–17(–22) μm long].

Cladosporium pannosum (Cooke 1883) is an additional *Cladosporium* species described from banana leaves. Type material of this species has been re-examined (**USA**: *South Carolina*, on *Musa* sp., *Ravenel*, K 121564) and proved to be a true species of *Cladosporium s. str.* It was introduced in connection with the ascomycete *Chaetophoma musae*. However, this species is undoubtedly not hyperparasitic but probably saprobic on the *Musa* leaves (Heuchert *et al.* 2005).

Mason (*in* Martyn 1945) cited three collections, viz. from Jamaica (on *Musa* sp., Sept. 1942, *E.B. Martyn*), from Sierra Leone (on *Musa schweinfurthii*) and Ghana. The collection from Jamaica was marked as type material. Herbarium material of this collection could not be traced and is probably not preserved, but a slide based on the type collection has been found at IMI. This sample has thus been selected as lectotype. Morphologically this material closely resembles a culture obtained from Honduras, which is selected as ex-epitype strain, with a dried down specimen as epitype.

Metulocladosporiella musicola Crous, Schroers & Groenewald, **sp. nov.**

MycoBank MB500186   (Figs 5–6)

Differt a *M. musae* conidiophoris ad apicem valde ramosis, conidiis (9–)11–13(–16) μm longis, locis conidiogenis latioribus, (1–)2 (–4) μm, saepe distinctioribus.

Typus: **South Africa**: *Northern Province*: Levubu, on *Musa acuminata* subg. "Cavendish 'Grand Nain'", Mar. 2000, *A. Viljoen* (CBS herb. 14787-**holotypus**; culture ex-type CBS 110960 = CPC 4629).

Leaf spots similar to those of *M. musae*. *Mycelium* internal and external, superficial; hyphae branched, 1–3(–4) μm wide, septate, occasionally slightly constricted at the septa, with small swellings, hyaline, subhyaline to very pale olivaceous, thin-walled, smooth, hyphae occasionally aggregated, forming ropes; sometimes with some intermixed micronematous conidiophores (but less common than in *M. musae*), erect from the vegetative mycelium, intercalary, straight to flexuous, unbranched, subhyaline, usually with simple terminal conidial chains. *Macronematous conidiophores* arising from superficial hyphae, erect, solitary to loosely aggregated, 80–600(–700) μm long, composed of a subcylindrical stipe, 3–8 μm wide, 2–18 septate, swollen or lobed at the base, 10–15 μm diam, with short rhizoid hyphae growing from the base,
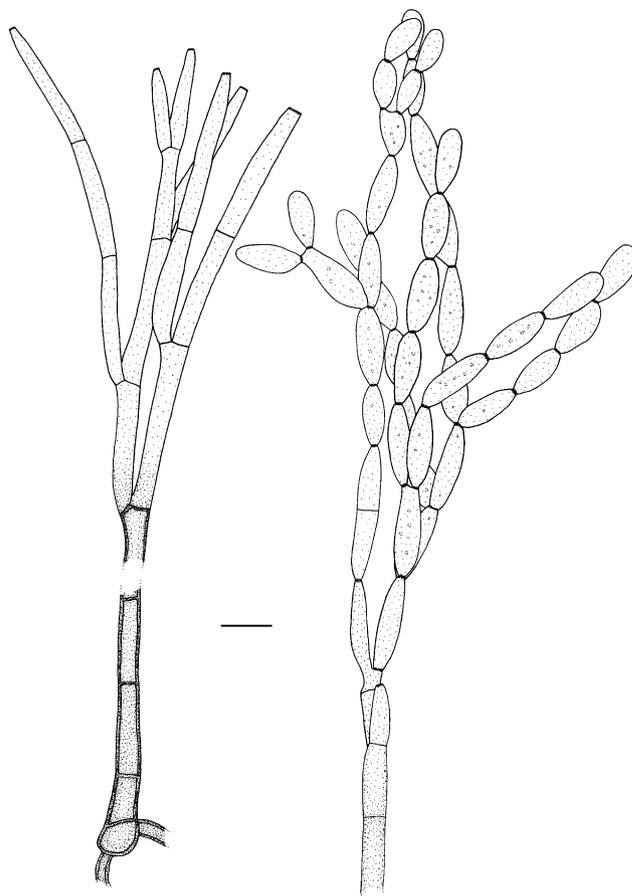

Fig 5 – *Metulocladosporiella musicola* **(CBS 113865, ex-holotype). Conidiophores and conidia. Bar = 10 μm.**

medium to dark brown in the lower half, paler towards the apex, tips pale olivaceous or even subhyaline, thick-walled below, thin-walled towards the apex, smooth; apex persistently branched, branched part composed of usually fairly compact, closely arranged subcylindrical branchlets; primary branches 0(–2)-septate, 15–85 × 3.5–6 μm, giving rise to 1–3 secondary branches, or to conidiogenous cells; secondary branches 0(–1)-septate, 20–40 × 3–4 μm, giving rise to (1–)2–3 conidiogenous cells; conidiogenous cells subcylindrical, 20–30 × 3–5 μm, terminal or occasionally intercalary, sympodial, polyblastic, conidiogenous loci subconspicuous to conspicuous, subdenticulate, somewhat protuberant, truncate, wall unthickened to somewhat so, darkened-refractive, 1–2 μm wide. *Conidia* in simple and branched acropetal chains, ellipsoid–ovoid, fusiform, subcylindrical, (9–)11–13(–16) × (3.5–)4 (–5) μm [ramoconidia (12–)15–20(–25) × (3.5–)5(–6) μm)], 0(–1)-septate, hyaline, subhyaline to very pale olivaceous, thin-walled, smooth, with 1–3 hila, truncate, (1–)2 μm diam (up to 4 μm diam at the base of ramoconidia), unthickened or almost so, and somewhat darkened-refractive (more prominent than in *M. musae*), secession schizolytic.

Cultures: Colonies 20–30 mm diam on PDA after 14 d under NUV at 25 °C. Colonies on PDA and OA spreading, with smooth, regular margins and sparse aerial mycelium; centres of colonies darker than margins due to grey–white aerial mycelium;
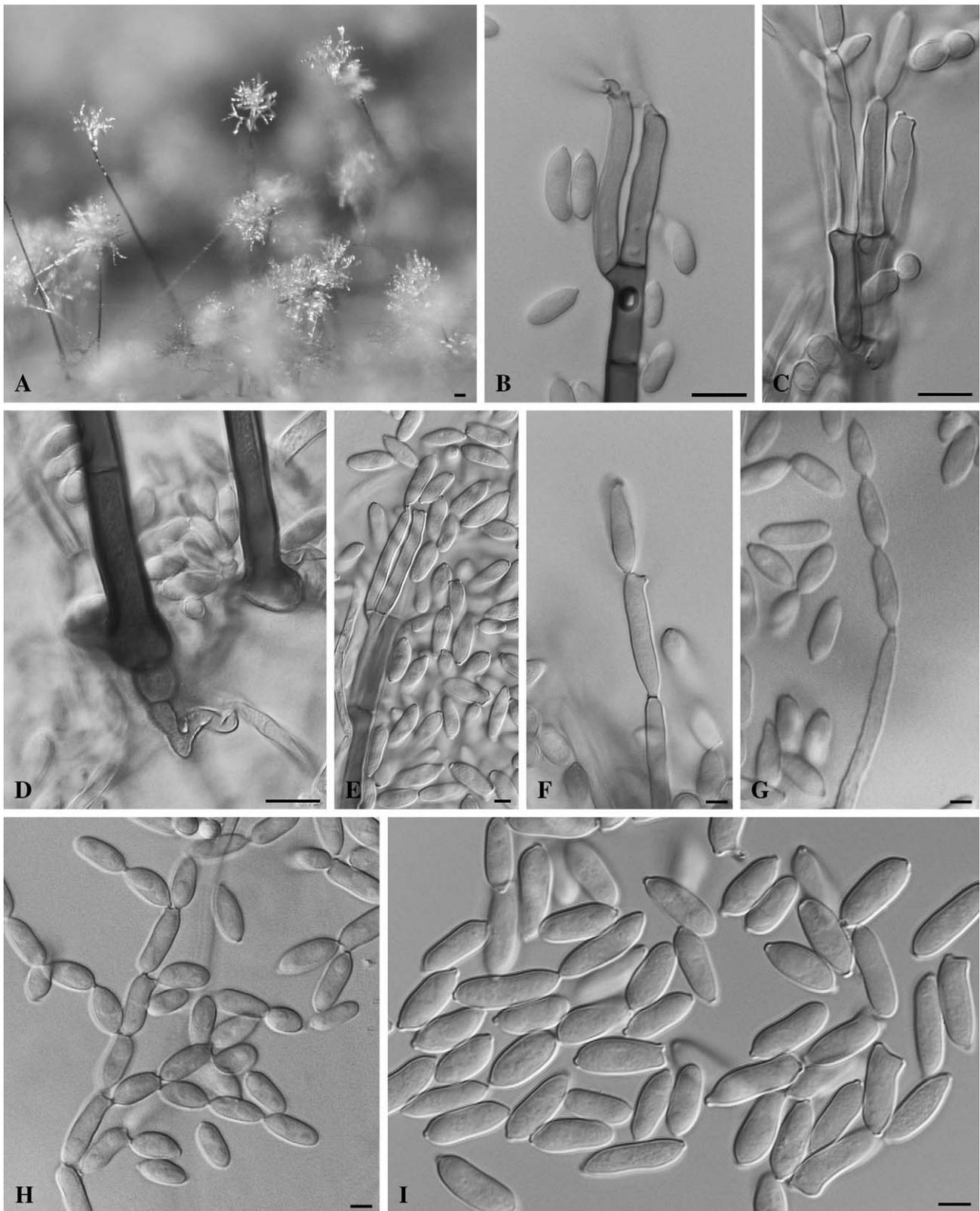
Fig 6 – *Metulocladosporiella musicola* (CBS 113865, ex-holotype). Fig A – Macronematous conidiophores. Figs B–C,
E – Conidiogenous apparatus. Fig D – Lobed bases of conidiophores. Figs F–G – Micronematous conidiophores. Figs H–I –
Conidia. Bars: (A) = 20 μm, (B–D) = 10 μm, (E–I) = 4 μm.

surface on PDA pale mouse-grey to dirty white–grey (centre); margins leaden-black to olivaceous–grey.

Host range and distribution: on *Musa* spp.; Africa (Kenya, Mozambique, South Africa, Uganda, Zimbabwe; one record, CBS 194.63 = ATCC 36952, is incorrectly cited in the literature as a distribution record for France, but in fact represents the French territories outside Europe, the exact location being unknown).

Notes: *Metulocladosporiella musicola* is morphologically distinguishable from *M. musae* in having: (1) conidiophores that are more frequently branched in their apical region (more secondary branches and conidiogenous cells); (2) longer conidia, (9–)11–13(16) *vs* (6–)8–11(–16) μm; and (3) wider loci, (1–)2(–4) *vs* 1–2(–3) μm. *M. musae* is also more prone to form micronematous conidiophores in culture than *M. musicola*, and has conidial scars which are barely thickened, and only somewhat refractive, while those of *M. musicola* are more prominently visible.

Comparison of Metulocladosporiella *and other genera*

The following genera are easily distinguishable from *Metulocladosporiella* by having little differentiated, micronematous or semi-micronematous to semi-macronematous conidiophores: *Bispora* (saprobic), *Cladophialophora* (human pathogenic), *Devriesia* (heat-resistant soil fungi), *Dimorphospora* (saprobic on submerged leaves), *Pseudocladosporium* (saprobic), *Torula* (mostly saprobic), *Xylohypha*, *Xylohyphopsis* (saprobic, human pathogenic) (Ellis 1971, 1976; Carmichael *et al.* 1980; Braun 1998; Kiffer & Morelet 1999; Partridge *et al.* 2000; Seifert *et al.* 2004).

Polyscytalum (Ellis 1971) and *Websteromyces* (Partridge *et al.* 2000) are two comparable genera that also have branched conidiophores. They differ, however, in having semi-micronematous to semi-macronematous conidiophores. Furthermore, the lignicolous genus *Websteromyces* is easily distinguishable by inconspicuous conidiogenous loci. *Polyscytalum* species are leaf litter fungi, differing in having little differentiated, rather inconspicuous conidiogenous loci (Ellis 1971).

Periconia species are characterised by basipetal conidial maturation; *Haplobasidion* and *Haplographium* possess conidiogenous cells arranged in terminal penicilli. Species of *Cladosporium*, *Passalora* emend. (incl. *Mycovellosiella*, *Phaeoramularia*), and *Stenella* (all anamorphs of either *Davidiella* or *Mycosphaerella*, *Mycosphaerellaceae*) as well as species of the hyperparasitic genus *Cladosporiella* are easily distinguishable by having conspicuously thickened and darkened conidiogenous loci (Kiffer & Morelet 1999; Partridge & Morgan-Jones 2003). Species of the genus *Fusicladium* are anamorphs of the *Venturiaceae*. The conidiophores are usually unbranched and the conidia are more or less concolorous with the conidiophores (Schubert *et al.* 2003). *Fusicladosporium* (2003) was introduced for *Fusicladium* species with catenate conidia, although two older generic names were available for this taxon, viz., *Hormocladium* and *Ramalia*. Based on a re-assessment of the conidial formation, conidiogensis and molecular data, *Fusicladosporium* has recently been reduced to synonymy with *Fusicladium* (Beck *et al.* 2005).

The conidiophores and conidial chains in *Anungitea*, *Castanedaea*, *Hormiactella*, *Lobatopedis*, *Pleurotheciopsis* and *Parapleurotheciopsis* are unbranched (Ellis 1971, 1976; Carmichael *et al.* 1980; Kiffer & Morelet 1999; Partridge *et al.* 2001c). The conidiogenous cells in *Anungitea* are raduliform. The conidiophores in *Castanedaea* are inflated at the very base, as in *Metulocladosporiella*, but they proliferate percurrently and the conidiogenous cells are verruculose and non-cicatrised. The conidia in *Parapleurotheciopsis* are pale, subhyaline as in *Metulocladosporiella*, but the conidiophores arise from lobed basal cells. Species of *Pleurotheciopsis* are also close to *Metulocladosporiella* as the conidiophores may arise from a swollen basal cell and the conidiogenous cells and conidia are hyaline or subhyaline. However, the latter genus is easily separable by its unbranched, percurrently proliferating conidiophores and conidia formed in simple chains. Several other genera are characterised by conidiophores arising from enlarged basal cells, e.g. *Beltrania*, *Beltraniopsis*, *Beltraniella*, *Hemibeltrania* and *Pseudobeltrania*, but these genera belong to the 'Sympodulosporae' (*sensu* Kiffer & Morelet 1999, i.e., conidia formed singly). Within the 'Sympodulosporae' they form a group of genera with more or less rhombic, biconic to turbinate conidia ('Rhombospores' *sensu* Kiffer & Morelet 1999; obovoid in *Hemibeltrania*). *Cordana*, *Parapyricularia* and *Sterigmatobotrys* are additional genera in which the conidiophores arise from inflated bases, but they form solitary conidia.

Species of *Septonema* have inconspicuous conidiogenous loci, pigmented conidia and they are ecologically distinct, and those of *Heteroconium* possess monoblastic, determinate to percurrent conidiogenous cells (Ellis 1971, 1976).

Siboe (1994) assigned '*Cladosporium*' *musae* to *Periconiella*. However, this treatment is not tenable as *Periconiella* species are characterised by having conspicuously thickened and darkened conidiogenous loci and conidial hila. The conidia are usually formed singly.

Haplotrichum (syn. *Acladium*, *Alysidium*; Partridge *et al.* 2001a), *Sorocybe resinae* (Partridge & Morgan-Jones 2002), *Parahaplotrichum* (Partridge *et al.* 2001b), *Phaeoblastophora* and *Subramaniomyces* are some morphologically comparable genera with branched conidiophores. *Haplotrichum*, comprising wood-inhabiting hyphomycetes, differs in having quite distinct, denticulate conidiogenous cells and pigmented unicellular conidia. *Parahaplotrichum* species are also lignicolous, denticulate, amerosporous and pigmented throughout. *Sorocybe resinae* is morphologically very close to *Metulocladosporiella*, but ecologically, genetically and also morphologically distinguishable. *S. resinae* occurs on resinous wood, does not cluster within the *Chaetothyriales* (Braun *et al.* 2003), and differs morphologically from *Metulocladosporiella* in having unthickened, non-pigmented conidiogenous loci and conidial hila as well as pigmented conidia. The wood-inhabiting *Phaeoblastophora* species have often inflated conidiogenous cells with inconspicuous conidiogenous loci and pigmented, amerosporous conidia with relatively broad, truncate, unthickened, non-pigmented hila. *Subramaniomyces* species are saprobic and possess conidiophores with lobed bases as well as aseptate conidia (amerospores).

Cladosporium adianticola, a foliicolous fungus described from Cuba (Castañeda 1987), clustered close to *Metulocladosporiella*. Type material of *C. adianticola* has been examined (**Cuba**: *Prov. Matanzas*: San Miguel de los Baños, on *Adiantum* sp., 23 Jan. 1987, *R.F. Castañeda*, INIFAT C87/44-holotype; permanent slide at HAL; culture ex-type CBS 735.87). In some

basic features, such as the branched conidiophores, paler conidiogenous cells and subhyaline conidia, this species resembles *Metulocladosporiella*, but, *C. adianticola* is distinguished from the latter species by having loosely branched, non-metuloid conidiophores and strongly dimorphic conidia [ramo-conidia narrowly subclavate, subcylindrical, filiform, 15–25 × 1.5 μm, 0(–1)-septate; conidia broadly ellipsoid–ovoid, subglobose, 7–18 × 4–10 μm, 0–1-septate, subhyaline to very pale olivaceous]. Furthermore, conspicuous basal swellings of the conidiophores are lacking, and the conidiogenous loci and conidial hila are rather inconspicuous, unthickened, neither darkened nor refractive. *C. adianticola* must be excluded from *Cladosporium s. str.*, but a final conclusion about its generic affinity is not yet possible, and awaits the recollection of fertile cultures. It seems to be close to *Metulocladosporiella*, but it is not yet clear if it is congeneric.

Key to *Metulocladosporiella* and morphologically similar genera (bearing branched acropetal chains of dematiaceous blastoconidia)

1 Conidiophores micronematous to semi-macronematous, little-differentiated 2
Conidiophores macronematous 3

2(1) Conidiophores little branched, with short lateral branchlets; conidia broadly ellipsoid-ovoid to somewhat clavate, 4–5 μm wide, verruculose, with broadly rounded ends, with inconspicuous hila; on dead wood ....**Websteromyces**
Conidiophores often branched, branches short to long; conidia narrowly cylindrical, 1–3 μm wide, smooth, ends attenuated to a more or less pointed hilum; on leaf and stem litter or parasitic on *Solanum tuberosum* tubers ....**Polyscytalum**

3(1) Conidiophores composed of a long stipe and a complex, mostly dense head of branches; conidia aseptate to septate 4
Conidiophores without branched head, irregularly branched, sometimes deeply cleft; conidia consistently aseptate 5

4(3) Branched head of the conidiophores loose to dense, but not typically metuloid; conidiogenous loci conspicuous, thickened and darkened, non-denticulate ....**Periconiella**
Branched head of the conidiophores dense, often metuloid; conidiogenous loci more or less inconspicuous, unthickened or slightly thickened, slightly darkened-refractive, subdenticulate ....**Metulocladosporiella**

5(3) Conidiophores arising from an inflated, more or less lobed base; saprobic, mostly on leaf-litter ....**Subramaniomyces**
Without inflated, lobed base; wood-inhabiting 6

6(5) Colonies effuse, dark, blackish; conidiophores simple or occasionally branched; conidiogenous cells often inflated, ampulliform, doliiform or clavate, non-denticulate; conidia at least partly subglobose, dark brown when mature ....**Phaeoblastophora**
Conidiogenous cells not inflated or, if somewhat inflated, conidiogenous cells distinctly denticulate 7

7(6) Conidiogenous cells distinctly denticulate; conidia broad, about 7–13 μm wide ....**Haplotrichum**
Conidiogenous cells non-denticulate or at most subdenticulate; conidia narrower, 3–6 μm wide 8

8(7) Colonies effuse, dense, resupinate, hypochnoid, powdery, chocolate brown; conidiophores mononematous, densely caespitose; conidiogenous cells terminal and intercalary; conidia subhyaline to very pale yellowish ....**Parahaplotrichum**
Colonies effuse, dense, but felted, black, brittle and appearing carbonaceous when dry; conidiophores solitary, mononematous and arranged in synnemata; conidiogenous cells terminal and pleurogenous; conidia pale brown to brown ....**Sorocybe**

Discussion

Metulocladosporiella is an additional segregate of *Cladosporium s. lat.*, which demonstrates that a combination of morphological re-examination, molecular analyses and ecological data are useful approaches to find and define more natural anamorph genera reflecting monophyletic fungal groups. The new genus belongs to a large assemblage of dematiaceous hyphomycetes with holoblastic conidia formed in acropetal, often branched chains (*sensu* Kiffer & Morelet 1999). However, it differs from morphologically allied genera in having frequently branched, pigmented conidiophores with much paler tips and paler, often subhyaline conidia. The conidiogenous loci are subconspicuous to conspicuous, i.e. unthickened or almost so, but somewhat darkened-refractive. The phylogenetic analyses showed that *Metulocladosporiella* belongs to the *Chaetothyriales*. The conidiogenous loci and conidial hila in *Cladophialophora* (anamorphs of *Capronia*, *Herpotrichiellaceae*, *Chaetothyriales*) resemble those of *Metulocladosporiella*, but the conidiophores are unbranched, micronematous to semimacronematous, the conidia are concolorous with the conidiophores, and the species of this genus are human pathogenic.

Acknowledgements

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