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RESEARCH ARTICLE

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Phylogeny of *Sarocladium* (*Hypocreales*)

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Key words

Acremonium
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phylogeny
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taxonomy

Abstract The circumscription of the genus *Acremonium* (*Hypocreales*) was recently reviewed on the basis of a DNA phylogenetic study. Several species were subsequently transferred to *Sarocladium*, but the relationships between both genera remained unresolved. Based on multilocus phylogenetic inferences combined with phenotypic data, we have revised the species concepts within *Sarocladium* and some genetically related species of *Acremonium*. As a result of these studies, six species are described as new, viz. *S. bifurcatum*, *S. gamsii*, *S. hominis*, *S. pseudostrictum*, *S. subulatum* and *S. summerbellii.* In addition, the new combinations *S. implicatum* and *S. terricola* are proposed for *A. implicatum* and *A. terricola*, respectively. *Sarocladium attenuatum* is confirmed as synonym of the type species of the genus, *S. oryzae*. An epitype and neotype are also introduced for *S. oryzae* and *S. implicatum*, respectively. Although *Sarocladium* species have traditionally been considered as important phytopathogens, the genus also contains opportunistic human pathogens. This study extends the spectrum of clinical species that could be diagnosed as causal agents of human infections.

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INTRODUCTION

Acremonium is a complex and large polyphyletic genus of *Ascomycota* with species scattered in diverse orders of *Sordariomycetes* (Glenn et al. 1996, Perdomo et al. 2011, Summerbell et al. 2011). Based on a recent molecular phylogenetic study the taxonomy of *Acremonium* was reviewed and some important animal and plant pathogenic species transferred to *Sarocladium*. Although both genera are morphologically similar and members of the order *Hypocreales*, they are phylogenetically distant: the type species of *Acremonium* is related to *Bionectriaceae* while that of *Sarocladium* is still considered as *incertae sedis* (Summerbell et al. 2011). According to Summerbell et al. (2011), *Sarocladium* can be morphologically differentiated from *Acremonium* by its elongated phialides rising solitary on vegetative hyphae or on conidiophores that are sparsely or repeatedly branched, the production of abundant adelophialides and elongated conidia. In contrast, in *Acremonium* the conidiophores are mainly unbranched or poorly basitonously branched, the conidia are more variable in shape (subglobose, obovate, ellipsoidal) and adelophialides are usually absent.

Sarocladium presently encompasses 10 species. *Sarocladium oryzae*, the type species of the genus, is an important plant pathogen causing sheath-rot of rice (*Oryza sativa*) (Ayyadurai et al. 2005). It is also known to produce antimicrobial secondary metabolites, such as helvolic acid and cerulenin (Tschen et al. 1997, Ghosh et al. 2002, Bills et al. 2004). *Sarocladium attenuatum* and *S. sinense* are also pathogens of rice (Gams & Hawksworth 1975, Chen et al. 1986), although the former was considered conspecific with *S. oryzae* (Bills et al. 2004). *Sarocladium mycophilum*, which was found on *Cortinarius subsertipes*, is the only mycoparasitic species of the genus (Helfer 1991). More recently, in a comprehensive phylogenetic study of *Acremonium* and related genera based on rDNA sequences, Summerbell et al. (2011) re-allocated some hypocrealean species of *Acremonium* to the genus *Sarocladium*, including the clinically important species *A. kiliense* and *A. strictum* and the maize endophyte, *A. zeae*. Other *Acremonium* species transferred to *Sarocladium* by Summerbell et al. (2011) were *A. bacillisporum*, *A. bactrocephalum*, *A. glaucum* and *A. ochraceum*.

Acremonium implicatum is a confusing species, which is morphologically reminiscent of *Sarocladium*, but presently lacks an ex-type culture. In the study of Summerbell et al. (2011) this species was represented by two strains, i.e., CBS 397.70B phylogenetically close to the ex-type strain of *A. exiguum*, and CBS 243.59 (the ex-type strain of *Acremonium terricola*, considered a heterotypic synonym of *A. implicatum* (Gams 1975)); nested in the *Sarocladium* clade. However, the lack of an ex-type culture of *A. implicatum* remains problematic.

In a recent phylogenetic study that included numerous clinical isolates of *Acremonium* (Perdomo et al. 2011), isolates previously identified as *A. implicatum* clustered in different clades. While most of these isolates nested with the ex-type strain of *A. terricola*, some were phylogenetically distant from this species (Perdomo et al. 2011). In the same study, isolates morphologically comparable with *A. strictum* or *A. bactrocephalum* were also shown to be phylogenetically distant from the ex-type strains of the respective species, and therefore could not be reliably identified. These data demonstrated that the correct delimitation of some species of *Acremonium* and their relationships with members of *Sarocladium* were still unresolved. Here, we have revised the taxonomy of *Sarocladium* and clarified its phylogenetic relationship with *Acremonium implicatum* by integrating multilocus DNA sequence and phenotypic data from numerous strains available from different international culture collections.

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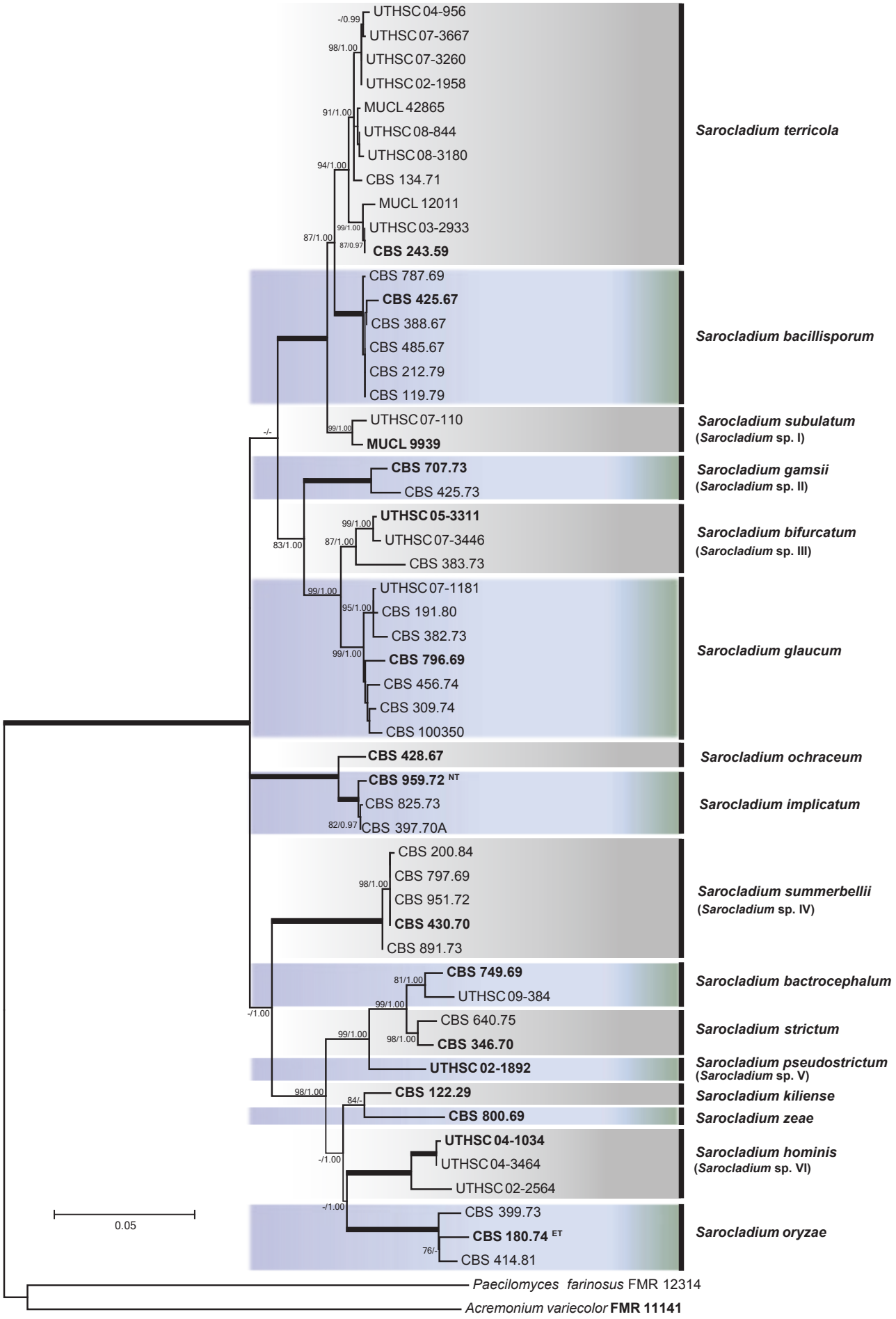

Fig. 1 Maximum-likelihood (ML) tree obtained from the combined DNA sequence data from three loci (D1/D2, ITS and *ACT1*). Bootstrap support values above 70 % / Bayesian posterior probability values above 0.95, are shown at the nodes (BS/PP). Branches supported by BS = 100 % and PP = 1.00 are depicted as black thickened lines. [ET] Epitype. [NT] Neotype. Ex-type strains are indicated in **bold**.

Table 1 Strains included in this study.

Species	Strain[1] (original identification)	Origin	GenBank accession no.[2]		
			LSU/D1D2	ITS	*ACT1*
Sarocladium bacillisporum	CBS 119.79 (*S. bacillisporum*)	Smoked sliced meat, Sweden	**HG965050**	**HG965001**	**HG964951**
	CBS 212.79 (*S. bacillisporum*)	Insect, Romania	**HG965051**	**HG965002**	**HG964952**
	CBS 388.67 (*S. bacillisporum*)	Soil, The Netherlands	**HG965052**	**HG965003**	**HG964953**
	CBS 425.67^{T} (*S. bacillisporum*)	Soil, Ontario, Canada	HE608658	HE608639	HE608633
	CBS 485.67 (*S. bacillisporum*)	Unknown	**HG965053**	**HG965004**	**HG964954**
	CBS 787.69 (*A. implicatum*)	Teleutosorus of *Puccinia graminis* on *Lolium temulentum*, Italy	**HG965054**	**HG965005**	**HG964955**
Sarocladium bactrocephalum	CBS 749.69^{T} (*S. bactrocephalum*)	*Ustilago* sp., Canada	HQ231994	**HG965006**	**HG964956**
	UTHSC 09-384 (*A. strictum*)	Eye, USA	**HG965055**	**HG965007**	**HG964957**
Sarocladium bifurcatum (*Sarocladium* sp. III)	CBS 383.73 (*S. ochraceum*)	Dead stem of bamboo, India	**HG965056**	**HG965008**	**HG964958**
	UTHSC 05-3311^{T} (*Acremonium* sp.)	Bronchoalveolar lavage fluid, USA	**HG965057**	**HG965009**	**HG964959**
	UTHSC 07-3446 (*Acremonium* sp.)	Bronchial wash fluid, USA	**HG965058**	**HG965010**	**HG964960**
Sarocladium gamsii (*Sarocladium* sp. II)	CBS 425.73 (*S. glaucum*)	Dead petiole of *Pandanus lerum*, Sri Lanka	**HG965062**	**HG965014**	**HG964964**
	CBS 707.73^{T} (*A. implicatum*)	Dead stem of *Pandanus lerum*, Sri Lanka	**HG965063**	**HG965015**	**HG964965**
Sarocladium glaucum	CBS 191.80 (*S. glaucum*)	Dead stem of bamboo, Colombia	**HG965064**	**HG965016**	**HG964966**
	CBS 309.74 (*S. glaucum*)	Air, above sugarcane field, India	**HG965065**	**HG965017**	**HG964967**
	CBS 382.73 (*S. glaucum*)	Dead stem of bamboo, India	**HG965066**	**HG965018**	**HG964968**
	CBS 456.74 (*S. glaucum*)	Sugar, Mauritius	**HG965067**	**HG965019**	**HG964969**
	CBS 796.69^{T} (*S. glaucum*)	Woolen overcoat, Solomon Islands	HE608657	FN691454	HE608631
	CBS 100350 (*S. glaucum*)	Dead stem of bamboo, Japan	**HG965068**	**HG965020**	**HG964970**
	UTHSC 07-1181 (*A. glaucum*)	Sputum, USA	**HG965069**	FN691445	**HG964971**
Sarocladium hominis (*Sarocladium* sp. VI)	UTHSC 02-2564 (*Acremonium* sp.)	Leg, USA	**HG965059**	**HG965011**	**HG964961**
	UTHSC 04-1034^{T} (*Acremonium* sp.)	Right calf tissue, USA	**HG965060**	**HG965012**	**HG964962**
	UTHSC 04-3464 (*Acremonium* sp.)	Sputum, USA	**HG965061**	**HG965013**	**HG964963**
Sarocladium implicatum	CBS 397.70A (*A. implicatum*)	*Saccharum officinarum*, Jamaica	**HG965070**	**HG965021**	**HG964972**
	CBS 825.73 (*A. implicatum*)	*Saccharum officinarum*, India	**HG965071**	**HG965022**	**HG964973**
	CBS 959.72^{NT} (*A. implicatum*)	Desert soil, Egypt	**HG965072**	**HG965023**	**HG964974**
Sarocladium kiliense	CBS 122.29^{T} (*S. kiliense*)	Skin, Germany	HQ232052	FN691446	**HG964975**
Sarocladium mycophilum	CBS 166.92^{T} (*S. mycophilum*)	*Cortinarius subsertipes*, Germany	**HG965046**	**HG965024**	**HG964976**
Sarocladium ochraceum	CBS 428.67^{T} (*S. ochraceum*)	*Zea mays*, Kenya	HQ232070	**HG965025**	**HG964977**
Sarocladium oryzae	CBS 180.74^{ET} (*S. oryzae*)	*Oryza sativa*, India	**HG965047**	**HG965026**	**HG964978**
	CBS 399.73 (*S. attenuatum*)	*Oryza sativa*, India	**HG965048**	**HG965027**	**HG964979**
	CBS 414.81 (*S. attenuatum*)	*Oryza sativa*, Nigeria	**HG965049**	**HG965028**	**HG964980**
Sarocladium pseudostrictum (*Sarocladium* sp. V)	UTHSC 02-1892^{T} (*Acremonium* sp.)	Sputum, USA	**HG965073**	**HG965029**	**HG964981**
Sarocladium strictum	CBS 346.70^{T} (*S. strictum*)	*Triticum aestivum*, Germany	HQ232141	FN691453	**HG964982**
	CBS 640.75 (*S. bacillisporum*)	Decaying wood, The Netherlands	**HG965074**	**HG965030**	**HG964983**
Sarocladium subulatum (*Sarocladium* sp. I)	MUCL 9939^{T} (*A. implicatum*)	Soil, Egypt	**HG965075**	**HG965031**	**HG964984**
	UTHSC 07-110 (*Acremonium* sp.)	Bone, USA	**HG965076**	**HG965032**	**HG964985**
Sarocladium summerbellii (*Sarocladium* sp. IV)	CBS 200.84 (*S. ochraceum*)	Water in air moistener, The Netherlands	**HG965077**	**HG965033**	**HG964986**
	CBS 430.70^{T} (*S. ochraceum*)	Soil from greenhouse, The Netherlands	**HG965078**	**HG965034**	**HG964987**
	CBS 797.69 (*S. ochraceum*)	Decaying leaf of *Canna indica*, The Netherlands	**HG965079**	**HG965035**	**KP057619**

	CBS 891.73 (*S. ochraceum*)	Dead leaf, Sri Lanka	**HG965080**	**HG965036**	**HG964988**
	CBS 951.72 (*S. ochraceum*)	Agricultural soil, The Netherlands	**HG965081**	**HG965037**	**HG964989**
Sarocladium terricola	CBS 134.71 (*A. implicatum*)	*Arundo donax*, Italy	**HG965082**	**HG965038**	**HG964990**
	CBS 243.59[T] (*A. terricola*)	Forest soil, USA	HE608659	FN706553	HE608632
	MUCL 12011 (*A. implicatum*)	Decaying leaf of *Milleta laurentii*, Democratic Republic of Congo	**HG965083**	**HG965039**	**HG964991**
	MUCL 42865 (*A. implicatum*)	Infected palm grove, Morocco	**HG965084**	**HG965040**	**HG964992**
	UTHSC 02-1958 (*A. implicatum*)	Sputum, USA	**HG965085**	FN706540	**HG964993**
	UTHSC 03-2933 (*A. implicatum*)	Bronchial wash fluid, USA	**HG965086**	**HG965041**	**HG964994**
	UTHSC 04-956 (*A. implicatum*)	Sinus, USA	**HG965087**	**HG965042**	**HG964995**
	UTHSC 07-3260 (*A. implicatum*)	Bone, USA	**HG965088**	**HG965043**	**HG964996**
	UTHSC 07-3667 (*A. implicatum*)	Bronchial wash fluid, USA	**HG965089**	**HG965044**	**HG964997**
	UTHSC 08-844 (*A. implicatum*)	Bronchoalveolar lavage fluid, USA	**HG965091**	**HG965045**	**HG964999**
	UTHSC 08-3180 (*A. implicatum*)	Bronchoalveolar lavage fluid, USA	**HG965090**	FN706541	**HG964998**
Sarocladium zeae	CBS 800.69[T] (*S. zeae*)	*Zea mays* stalk, USA	HQ232152	FN691451	**HG965000**
Acremonium roseolum	CBS 381.73 (*A. implicatum*)	Dead stem of bamboo, India			
Acremonium sp.	CBS 397.70B (*A. implicatum*)	Dying leaf (*Cladium mariscus*), The Netherlands			
	CBS 114748 (*A. implicatum*)	Tropical green seaweed, USA			
	MUCL 8122 (*A. implicatum*)	Seed from *Triticum* sp., France			
	MUCL 8123 (*A. implicatum*)	Seed from *Triticum* sp., France			
	MUCL 34528 (*A. ochraceum*)	Banana fruit (*Musa* sp.), Unknown			

[1] CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; MUCL: Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; UTHSC: Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio TX; [ET] Epitype strain; [NT] Neotype strain; [T] Type strain.

[2] Accession numbers of sequences newly determined in this study are indicated in **bold**. ITS: internal transcribed spacer regions of the nrDNA and intervening 5.8S nrDNA; LSU/D1D2 large subunit of the nrDNA; *ACT1* partial actin gene.

MATERIALS AND METHODS

Fungal isolates

Fungal isolates included in this study are shown in Table 1. Sixteen clinical isolates were provided by the Fungus Testing Laboratory at the University of Texas Health Science Center (UTHSC), which were previously identified as *A. implicatum* or *Acremonium* spp. and were included in the informal 'clade E' by Perdomo et al. (2011), and which agree with the *Sarocladium*-clade sensu Summerbell et al. (2011). In addition, 44 ex-type or reference strains provided by different international culture collections were also included in this study. The ex-type strains from the new species described here were deposited in the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands.

DNA extraction, amplification and sequencing

Isolates were grown on yeast extract sucrose agar (YES; yeast extract, 20 g; sucrose, 150 g; agar, 20 g; distilled water to final volume of 1 000 mL) for 10 d at 25 °C and DNA extracted using PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol. The DNA was quantified using a NanoDrop 3000 (ThermoScientific, Asheville, NC, USA). The internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) and D1/D2 domains of the large-subunit nrRNA were amplified with the primer pairs ITS5/ITS4 and NL1/NL4b, respectively (White et al. 1990, O'Donnell 1993). The D1/D2 domain was amplified in all isolates with the primers mentioned above, except in *S. oryzae* (CBS 180.74, CBS 399.73 and CBS 414.84) and *S. mycophilum*, for which the primers LR0R/LR5 were used (Vilgalys & Hester 1990). A fragment of the actin gene (*ACT1*) was amplified with the primer pairs Act1/Act4 (Voigt & Wöstemeyer 2000). PCR products were purified using a GFXTM PCR DNA (Pharmacia Biotech, Cerdanyola, Spain) or Diffinity RapidTip® (Sigma-Aldrich, St. Louis, USA) and were stored at -20 °C until sequencing. PCR products were sequenced with the same primers used for amplification to ensure good quality sequences over the total length of the amplicon and following the *Taq* DyeDeoxy Terminator cycle sequencing kit protocol (Applied Biosystems, Gouda, The Netherlands). DNA sequencing reaction mixtures were analysed on a 310 DNA sequencer (Applied Biosystems). In addition, some amplified fragments were purified and sequenced at Macrogen Corp. Europe (Amsterdam, The Netherlands) with a 3730XL DNA analyser (Applied Biosystems). The program SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA) was used to obtain consensus sequences of each isolate. Some ITS, D1/D2 and *ACT1* sequences, corresponding to several species of *Acremonium* or *Sarocladium*, were retrieved from GenBank and included in the phylogenetic study (Table 1). These sequences were published in different studies (Perdomo et al. 2011, Summerbell et al. 2011, Giraldo et al. 2012).

Alignment and phylogenetic analysis

Multiple sequence alignments were performed with Clustal W using MEGA v. 5.05 (Tamura et al. 2011) and manually corrected where necessary. The ambiguous parts from the alignment were removed using the Gblocks server v. 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) with less stringent selection parameters (Castresana 2000). Selection of the best-fit nucleotide substitution models for each locus and for the combined dataset (Tamura-Nei with Gamma distribution) and Maximum Composite Likelihood (ML) phylogenetic analyses were performed with MEGA v. 5.05 (Tamura et al. 2011). Gaps or missing data were treated as partial deletion with a site coverage cut-off of 95 % and Nearest-Neighbour-Interchange (NNI)

used as Heuristic method. The internal branch support was assessed by a search of 1 000 bootstrapped sets of data. A bootstrap support (BS) ≥ 70 was considered significant. A second phylogenetic analysis using a Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) algorithm was done using MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003) with two simultaneous runs for 1 M generations. Bayesian posterior probabilities (PP) were obtained from the 50 % majority-rule consensus of trees sampled every 100 generations after removing the first 25 % of the resulting trees. A PP value ≥ 0.95 was considered significant. The selection of the best nucleotide substitution model for each gene in the Bayesian analysis (GTR+G+I) was made using MrModelTest v. 2.3 (Nylander 2004). Congruency of the sequence datasets for the separate loci were determined using tree topologies of 70 % reciprocal Neighbour-Joining (NJ) bootstrap trees with Maximum Likelihood distances, which were compared visually to identify conflicts between partitions (Gueidan et al. 2007). *Acremonium variecolor* (FMR 11141) and *Paecilomyces farinosus* (FMR 12314) were used as outgroup taxa in both ML and Bayesian analyses. All novel DNA sequences were deposited in GenBank (Table 1), the alignment and the resulting tree in TreeBASE (http://www.treebase.org), and taxonomic novelties in MycoBank (http://www.MycoBank.org; Crous et al. 2004).

Phenotypic studies

Morphological characterisation of the fungal isolates was carried out based on cultures grown on oatmeal agar (OA; filtered oat flakes after 1 h of simmering, 30 g; agar, 20 g; distilled water to final volume of 1 000 mL) and 2 % potato dextrose agar (PDA; Pronadisa, Madrid, Spain). Cultures were incubated at 25 ± 1 °C in the dark and periodically examined each 7 d up to 4 wk. Colony diameters were measured after 14 d of growth, and colony colours determined using the colour charts of Kornerup & Wanscher (1978). In addition, the ability of the isolates to grow at 15, 20, 25, 30, 35, 37 and 40 °C was determined on PDA. Microscopic features were examined and measured by making direct wet mounts with 85 % lactic acid or lactophenol cotton blue or by slide cultures on OA, using an Olympus CH-2 light microscope (Olympus Corporation, Tokyo, Japan). Photomicrographs were made with a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany), using phase contrast and Nomarski differential interference. Scanning electron microscope (SEM) micrographs were obtained with a Jeol JSM-6400 scanning electron microscope (JEOL, Peabody, MA, USA) using techniques described previously (Figueras & Guarro 1988).

RESULTS

Phylogenetic analysis

Of the 21 isolates morphologically identified as *A. implicatum*, five were shown to be unrelated to *Sarocladium* on the basis of their D1/D2 and the ITS regions (data not shown) and were therefore not included in the multilocus analysis. Comparisons of the 70 % reciprocal bootstrap NJ tree topologies of the individual genes showed no contradiction (data not shown) and therefore the three sequence datasets were combined. The combined analysis from ITS, D1/D2 and the *ACT1* partial gene consisted of 1 667 characters including alignment gaps. The tree topology was similar via the Bayesian and ML analyses. The phylogenetic analysis allowed distributing the isolates included in this study into 16 lineages (Fig. 1). These lineages were phylogenetically distant enough to be considered different species.

The first lineage included the ex-type strain of *A. terricola* CBS 243.59, seven clinical isolates previously identified as *A. implicatum* (Perdomo et al. 2011) and three reference strains of environmental origin of the latter species. The second lineage contained six strains of *S. bacillisporum*, among them the ex-type of the species (CBS 425.67), and one reference strain of *A. implicatum* (CBS 787.69). The third lineage consisted of two isolates of an unnamed species (*Sarocladium* sp. I) isolated from soil (MUCL 9939) and human bone (UTHSC 07-110). The fourth lineage (*Sarocladium* sp. II) was represented by two reference strains (CBS 707.73 and CBS 425.73) of *A. implicatum* and *S. glaucum*, respectively, both obtained from *Pandanus lerum*. The fifth lineage, which represented another *Sarocladium* species (*Sarocladium* sp. III), grouped two unidentified clinical isolates (UTHSC 05-3311 and UTHSC 07-3446) and a strain from bamboo (CBS 383.73), received as *S. ochraceum*. The ex-type strain of *S. glaucum* (CBS 796.69) together with five reference strains and one clinical isolate (UTHSC 07-1181) of that species clustered in the sixth lineage. The seventh lineage included the ex-type strain of *S. ochraceum* (CBS 428.67). The eighth lineage comprised three environmental reference strains received as *A. implicatum* obtained from sugar cane (CBS 397.70A, CBS 825.73) and soil (CBS 959.72). The ninth lineage (*Sarocladium* sp. IV) was represented by a well-supported group (BS 100, PP 1.00) that included five reference strains from environmental origin, all previously identified as *S. ochraceum*. The remaining species were distributed in the other seven lineages (10–16), five of which represent known *Sarocladium* species (*S. bactrocephalum*, *S. kiliense*, *S. oryzae*, *S. strictum* and *S. zeae*), and two corresponding to putative new species (i.e., *Sarocladium* sp. V and *Sarocladium* sp. VI). These two undescribed *Sarocladium* species were represented exclusively by clinical isolates previously included in the study of Perdomo et al. (2011).

Taxonomy

On the basis of the phylogenetic analysis we conclude that the species resolved here as *Sarocladium* sp. I–VI represent undescribed taxa. In addition, *A. terricola*, for a long time left in the limb of synonymy is re-considered as a distinct species, better accommodated in *Sarocladium*, hence the new combination *S. terricola* is proposed; in addition, the new combination *S. implicatum* is also proposed for *A. implicatum*.

Sarocladium bifurcatum Giraldo, Gené & Deanna A. Sutton, *sp. nov*. — MycoBank MB807943; Fig. 2

Etymology. Refers to the presence of phialides with a bifurcate apex.

Colonies on OA at 25 °C attaining 14–18 mm in 14 d, greyish orange (5–6B3) at the centre and brownish orange (7C4) toward the margin, flat, powdery. On PDA at 25 °C attaining 13–14 mm in 14 d, orange white (5A2), rugose, slimy. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 1–1.5 µm wide. *Conidiophores* erect, usually simple, straight or slightly bent, up to 75 µm long, hyaline, smooth-walled. *Phialides* subulate, 17–43 µm long, 1–2 µm wide at the base, with distinct apical periclinal thickening, hyaline, thin- and smooth-walled; adelophialides sometimes present; schizophialides commonly present. *Conidia* unicellular, fusiform, 4–6 × 1–2 µm, with slightly truncate ends, initially hyaline and smooth-walled, becoming subhyaline and apparently rough-walled due to the production of a mucilaginous exudate, arranged in chains. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 20–25 °C, maximum 30 °C, minimum 15 °C, no growth at 35 °C.

Specimens examined. India, Bangalore, Hortus Lal Bagh, on a dead stem of bamboo, Jan. 1973, *W. Gams*, CBS 383.73 = FMR 12316. – USA, Texas, from bronchoalveolar lavage fluid, 2005, *D.A. Sutton* (holotype CBS H-21627, culture ex-type CBS 137658 = FMR 10405 = UTHSC 05-3311); from bronchial wash fluid, 2007, *D.A. Sutton*, UTHSC 07-3446 = FMR 10451.

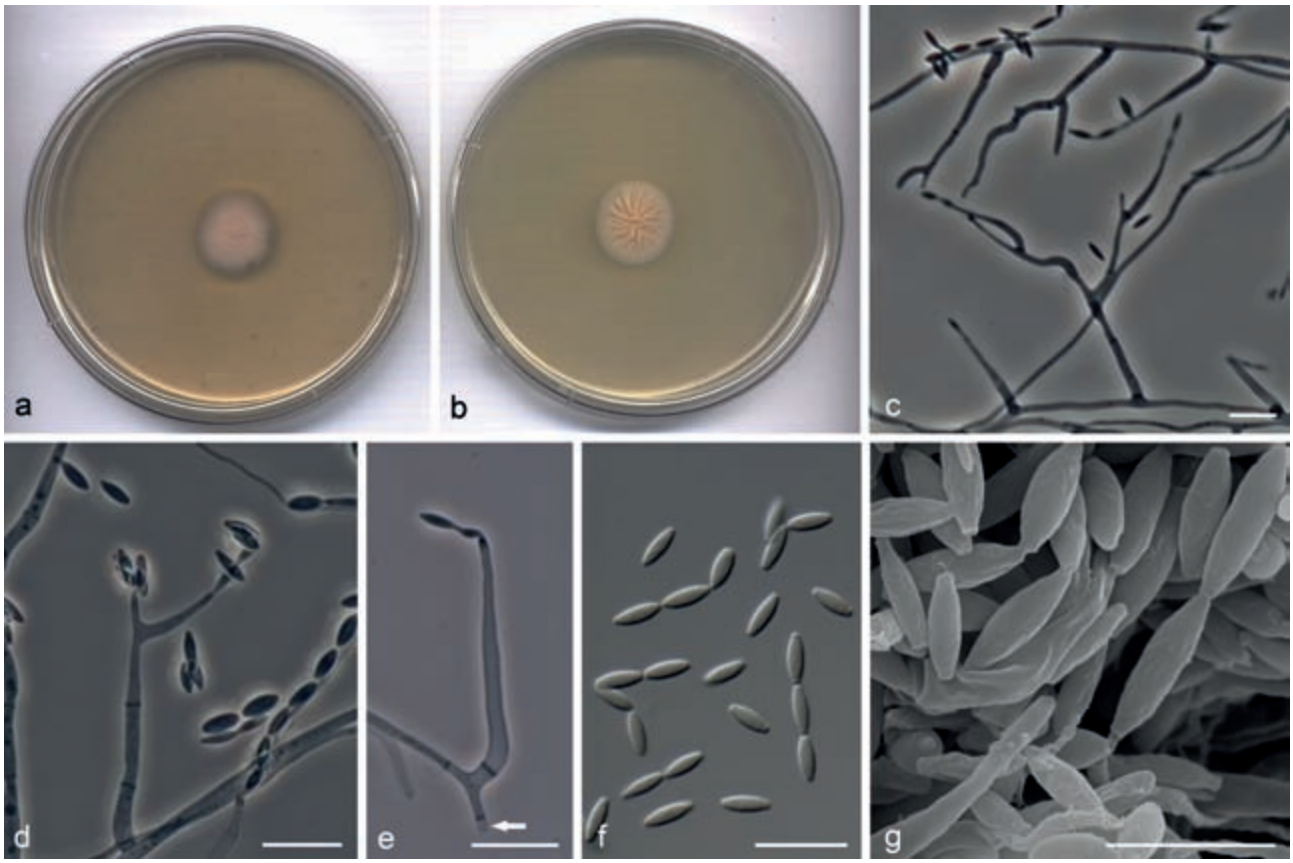

Fig. 2 *Sarocladium bifurcatum* (sp. III) UTHSC 05-3311. a, b. Colonies on OA and PDA, respectively, after 14 d at 25 °C; c. simple and branched conidiophores; d. schizophialides; e. phialide with periclinal thickening at the apex producing conidia in chains and adelophialide (arrow); f, g. fusiform conidia with slightly truncate ends. — Scale bars: c–f = 10 µm; g = 5 µm.

Notes — No phenotypic differences were observed among the three isolates of *S. bifurcatum* studied here; however, the two clinical specimens showed some genetic distance in the three regions analysed with respect to that isolated from bamboo (2–2.2 %), suggesting that two different species could be represented by this clade. The isolate CBS 383.73 was originally identified as *Paecilomyces ochraceus* (currently *S. ochraceum*), but it can be clearly differentiated from this latter species by its growth rate and the colony colour on OA after 14 d (14–18 mm and greyish to brownish orange vs 30 mm and ochraceus in *S. ochraceum*), by the abundance of schizophialides and by the inability to grow at 37 °C. Although in the phylogenetic analysis (Fig. 1) *S. bifurcatum* constituted a sister clade of *S. glaucum*, both species can be clearly differentiated as mentioned above by the colour of the colony, which is intensely grey-green to bluish green in the latter species and greyish orange in the former.

Sarocladium gamsii Giraldo, Gené & Guarro, *sp. nov*. — MycoBank MB807944; Fig. 3

Etymology. Named in honour of the eminent Austrian mycologist Walter Gams.

Colonies on OA at 25 °C attaining 12–20 mm diam in 14 d, white (1A1), flat, at first glabrous becoming powdery at centre. On PDA at 25 °C reaching 13–21 mm diam in 14 d, yellowish white (4A2), radially folded, umbonated, powdery. Diffusible pigment absent. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 1.5–2 µm wide. *Conidiophores* erect, arising directly from vegetative hyphae or ropes of hyphae, straight or slightly bent, simple or poorly branched, up to 55 µm long, hyaline, smooth-walled. *Phialides* acicular, 18–45 µm long, 1–1.5 µm wide at the base, with distinct apical periclinal thickening, hyaline, thin- and smooth-walled; adelophialides and schizophialides not observed. *Conidia* unicellular, fusiform, 3–5 × 1–2 µm, hyaline to subhyaline, thin- and smooth-walled, arranged in both slimy heads and chains. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 20–25 °C, maximum 30 °C, minimum 15 °C. No growth at 35 °C.

Specimens examined. Sri Lanka, Paradeniya, Royal Botanical Garden, from dead stem of *Pandanus lerum*, Jan. 1973, *W. Gams* (holotype CBS H-8197), culture ex-type CBS 707.73 = FMR 11419; from a dead petiole of *Pandanus lerum*, Jan. 1973, *W. Gams*, CBS 425.73 = FMR 12432.

Notes — Although the two isolates of *S. gamsii* were obtained from the tropical palm *P. lerum* at the same time and place, they were originally identified as *S. glaucum* (CBS 425.73) and *S. implicatum* (CBS 707.73A), respectively. We did not find any phenotypic differences between these isolates. Genetically, they showed an overall similarity of 98.6 % for the three loci analysed. *Sarocladium gamsii* can be differentiated from *S. glaucum* and *S. implicatum* mainly by their colony colour, yellowish white in the former, intensely grey-green to bluish green in *S. glaucum* and pinkish white in *S. implicatum*; and by the conidial arrangement, which is in chains and slimy heads in *S. gamsii*, and exclusively in chains in *S. glaucum* and *S. implicatum*.

Sarocladium hominis Giraldo, Gené & Deanna A. Sutton, *sp. nov*. — MycoBank MB807945; Fig. 4

Etymology. Refers to the origin of the isolates, namely from human specimens.

Colonies on OA at 25 °C attaining 41–50 mm in 14 d, yellowish white (1A2), flat, usually fasciculate at the center and glabrous

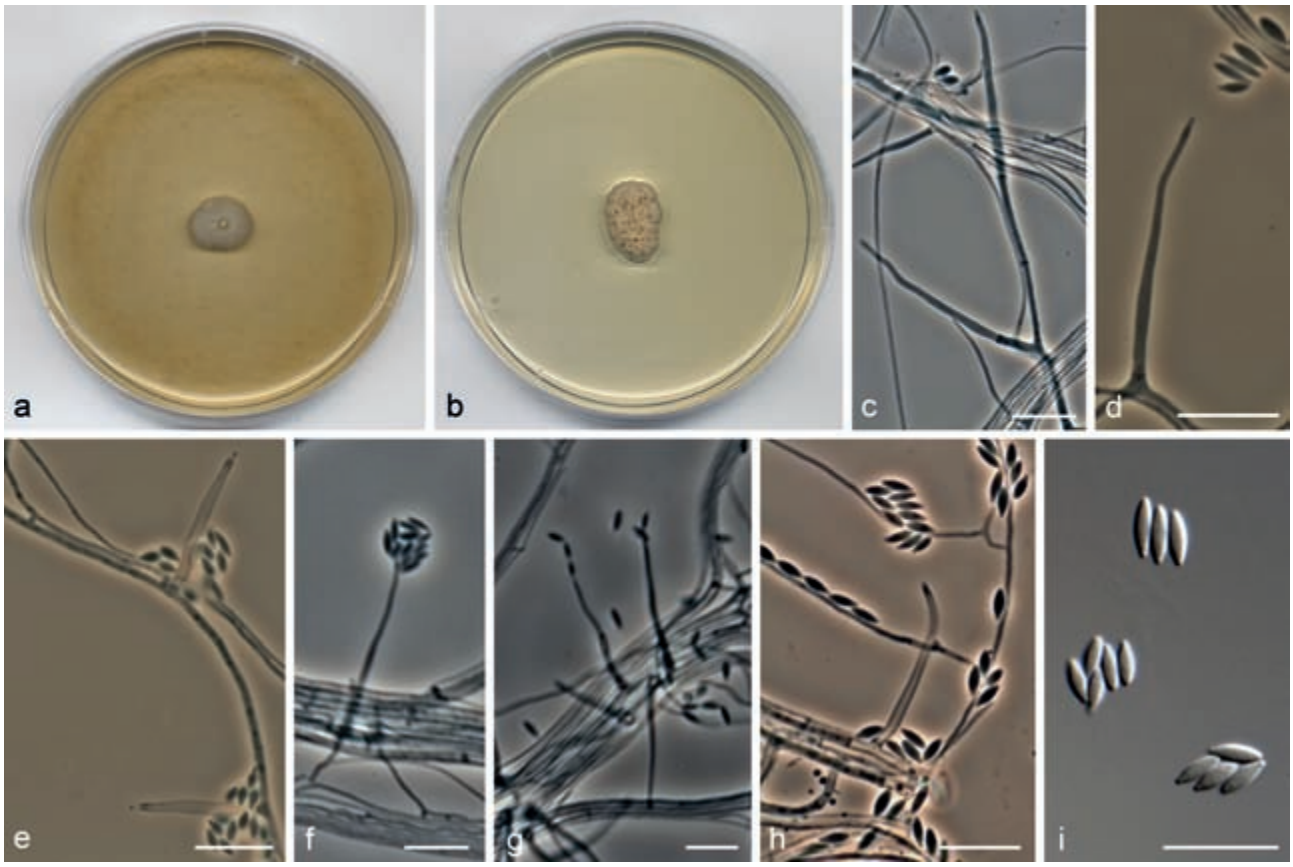

Fig. 3 *Sarocladium gamsii* (sp. II) CBS 703.73. a, b. Colonies on OA and PDA, respectively, after 14 d at 25 °C; c, d. simple conidiophores; e. phialide with periclinal thickening at the apex; f. conidia arranged in slimy heads; g. conidia arranged in chains; h, i. fusiform conidia. — Scale bars = 10 µm.

toward the periphery. On PDA at 25 °C attaining 22–30 mm in 14 d, orange white (5A2), slightly wrinkled or cerebriform, glabrous or fasciculate. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 1–1.5 µm wide. *Conidiophores* erect, arising directly from vegetative hyphae or from ropes of hyphae, simple or poorly branched, straight, hyaline, smooth-walled, up to 45 µm long. *Phialides* acicular, 22–37 µm long, 1–2 µm wide at the base, with distinct periclinal thickening on the conidiogenous locus, thin- and smooth-walled, hyaline; adelophialides and schizophialides not observed. *Conidia* unicellular, cylindrical with rounded ends, 3–4(–7) × 1–1.5 µm, hyaline to subhyaline, thin- and smooth-walled, arranged in slimy heads. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 20–25 °C, maximum 35 °C (UTHSC 04-1034 and UTHSC 02-2564) or 37 °C (UTHSC 04-3464), minimum 15 °C. No growth at 40 °C.

Specimens examined. USA, Florida, from right calf tissue, 2004, *D.A. Sutton* (holotype CBS H-21628, culture ex-type CBS 137659 = FMR 10418 = UTHSC 04-1034); Alaska, isolated from leg, 2002, *D.A. Sutton*, FMR 10352 = UTHSC 02-2564; Texas, from sputum, 2004, *D.A. Sutton*, FMR 10425 = UTHSC 04-3464.

Notes — *Sarocladium hominis* together with *S. kiliense*, *S. oryzae* and *S. zeae* formed a clade morphologically characterised by cylindrical or ellipsoidal conidia arranged in slimy heads. *Sarocladium kiliense* differs in the formation of chlamydospores, adelophialides and appears as dirty orange to pale ochraceous colonies on OA; *S. zeae* has longer (up to 80 µm) and branched conidiophores with basitonous whorls of phialides; and *S. oryzae* produces white and cottony colonies, gnarled hyphae and longer (up to 82 µm) and repeatedly branched conidiophores. Although the three isolates of *S. hominis* are from clinical origin, the pathogenicity of such isolates remains to be proven. However, this species could be considered as a potential agent of human infections because of its ability to grow at 35–37 °C, and the deep tissue origin of the isolates.

Sarocladium implicatum (J.C. Gilman & E.V. Abbott) Giraldo, Gené & Guarro, *comb. nov*. — MycoBank MB807946

Basionym. Monilia implicata J.C. Gilman & E.V. Abbott, Iowa State Coll. J. Sci. 1: 269. 1927.

≡ *Acremonium implicatum* (J.C. Gilman & E.V. Abbott) W. Gams, Trans. Brit. Mycol. Soc. 64: 394. 1975.

≡ *Sagrahamala implicata* (J.C. Gilman & E.V. Abbott) Subram., Kavaka, 5: 98. 1977.

Colonies on OA at 25 °C attaining 38–45 mm in 14 d, yellowish white (4A2), flat, powdery. On PDA at 25 °C attaining 18–30 mm in 14 d, pinkish white (7A2) to salmon (6A4), raised, woolly or downy, reverse pale orange (6A5). *Conidiophores* erect, simple, hyaline, smooth-walled. *Phialides* solitary, straight or slightly flexuous, subulate, 15–30 µm long, 1–2 µm wide at the base, with distinct periclinal thickening of the conidiogenous locus, hyaline, thin- and smooth-walled. Adelophialides and schizophialides not observed. *Conidia* unicellular, fusiform, 5–8 × 1–2 µm, hyaline, smooth- and thin-walled, arranged in long dry chains. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 20–25 °C, maximum 37 °C, minimum 15 °C. No growth at 40 °C.

Specimens examined. Egypt, desert soil, Nov. 1972, *J. Mouchacca* (neotype designated here CBS H-21634, MBT177687), culture ex-neotype CBS 959.72 = ATCC 32210 = FMR 12360 = IHEM 3713. – India, from *Saccharum officinarum*, Nov. 1973, *V.P. Agnihotri*, CBS 825.73 = FMR 11418. – Jamaica, from *S. officinarum*, Mar. 1970, *W. Gams*, CBS 397.70A = FMR 11422 = IMI 131645.

Notes — The three isolates of *S. implicatum* showed the same morphological features that Gilman & Abbott (1927) described in the protologue of *Monilia implicata*. This is the main

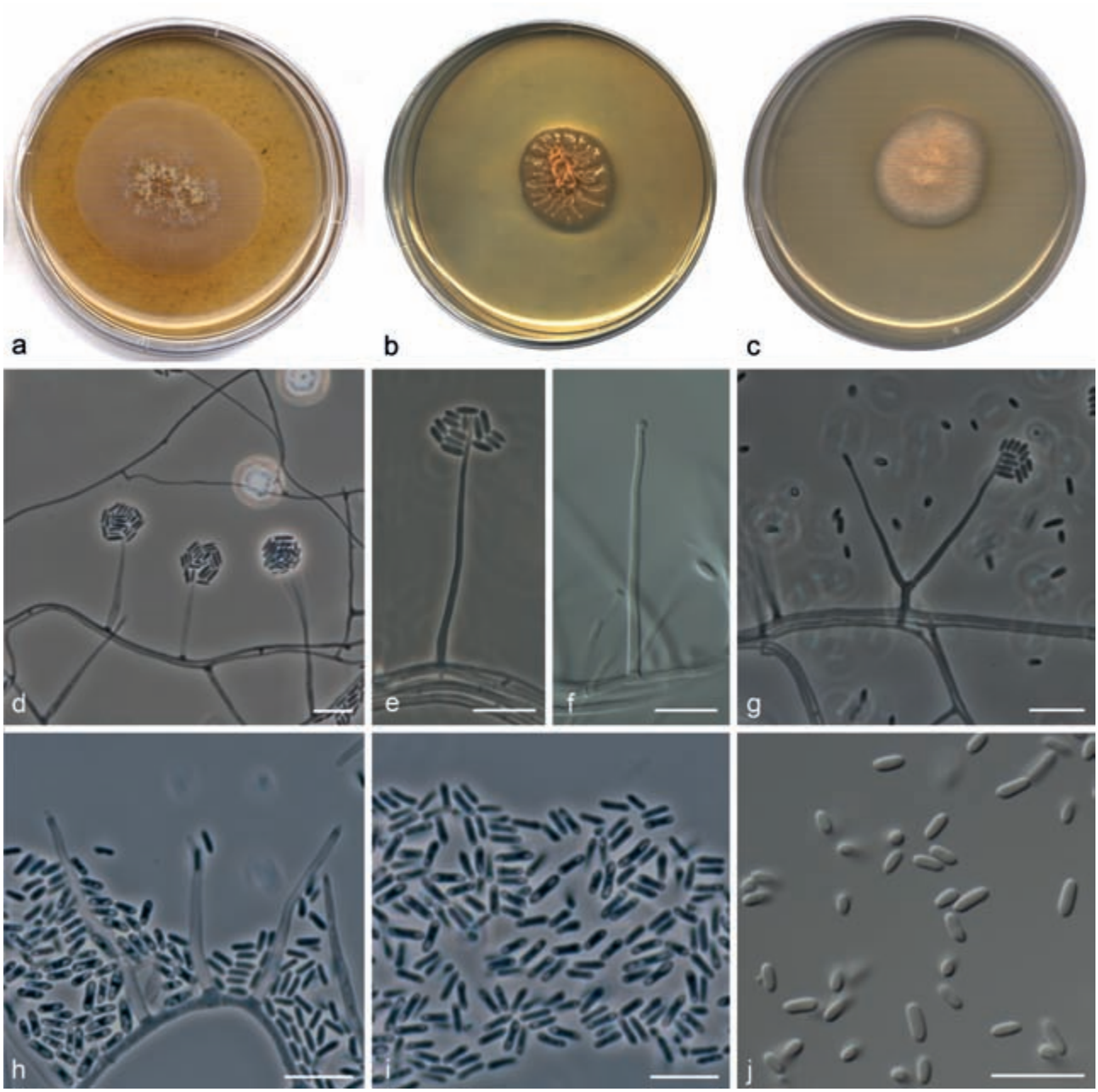

Fig. 4 *Sarocladium hominis* (sp. VI). a, c. UTHSC 04-1034; b, d, h, i. UTHSC 04-3464; e–g, j. UTHSC 02-2564. a. Colonies on OA after 14 d at 25 °C; b, c. colonies on PDA after 14 d at 25 °C; d–g. simple and branched conidiophores with conidia arranged in slimy heads; h. phialides with periclinal thickening at the apex; i, j. cylindrical conidia. — Scale bars = 10 µm.

reason why we prefer maintaining the epithet of the species rather than to introduce a new one. Although Gams (1975) examined a possible holotype of *M. implicata* (BPI 1769), presently it has been impossible to trace that material in the U.S. National Fungus Collections (Herbarium BPI, Farr & Rossman 2014). Therefore, designation of a neotype would stabilize the species concept. We have selected CBS 959.72 as neotype because, despite the fact that it does not originate from the same country than the type specimen of *M. implicata*, the CBS strain was isolated from the same substratum. *Monilia implicata* was originally described from soil in the USA (Gilman & Abbott 1927).

Monilia implicata and *A. terricola* were for a long time considered as conspecific (Gams 1975) and synonyms. However, our study showed that isolates morphologically identified as *A. implicatum* are dispersed into several clades, some of them distant from the ex-type strain of *A. terricola* (CBS 243.59). It is clear that *A. implicatum* and *A. terricola* represent different species within *Sarocladium.* Both species are morphologically similar, but they can be differentiated by the colour and the texture of the colonies on PDA, being white and cottony in *S. terricola* and pinkish to salmon and woolly or downy in *S. implicatum;* the lower limits of conidial and phialide length, which are slightly shorter in *S. terricola* (4 µm and 12 µm, respectively) than in *S. implicatum* (5 µm and 15 µm, respectively), and the maximum temperature for growth, which is 35 °C in *S. terricola* and 37 °C in *S. implicatum*. In addition, adelophialides and schizophialides are sometimes present in *S. terricola* but absent in *S. implicatum*.

In our phylogenetic tree *S. ochraceum* clustered as sister to *S. implicatum*, but the former species can easily be distinguished based on the production of ochraceous-yellow colonies on OA, usually branched conidiophores, and smaller phialides (15–26 µm long) and conidia (4.5–5 µm long). In contrast, *S. implicatum* produces yellowish white colonies on OA, and longer solitary phialides (up to 30 µm long) and conidia (up to 8 µm long).

Table 2 Distinctive features of *Sarocladium* species, based on PDA (colony characteristics and growth temperature) and OA (microscopic characteristics) after 14 d.

Species	Colonies		Conidia shape & size (µm)	Adelophialides	Schizophialides	Growth (°C)		
	Diameter (mm)	Color obverse/reverse				30	35	37
Species producing conidia in chains								
S. bacillisporum	20–24	White/uncoloured	Rod-shaped 4–6 × 1–1.2	Not observed	Not observed	+	–	–
S. bifurcatum	13–14	Orange white/uncoloured	Fusiform with slightly truncate ends 4–6 × 1–2	Present	Present	+	–	–
S. glaucum	12–21	Bluish green/uncoloured	Fusiform 3.9–5 × 1.1–1.4	Not observed	Not observed	+	–	–
S. implicatum	18–30	Pinkish white to salmon/pale orange	Fusiform 5–8 × 1–2	Not observed	Not observed	+	+	+
S. ochraceum	17–18	Ochraceous or yellow/uncoloured	Fusiform 4.5–5 × 1.3–1.5	Not observed	Not observed	+	+	+
S. subulatum	17–20	Yellowish white/uncoloured	Fusiform 5–8(–9) × 1–2	Present	Not observed	+	–	–
S. terricola	27–37	White/light orange	Fusiform 4–7(–8) × 1–2	Present	Present	+	+	–
Species producing conidia in chains and slimy heads								
S. gamsii	13–21	Yellowish white/uncoloured	Fusiform 3–5 × 1–2	Not observed	Not observed	+	–	–
S. summerbellii	15–21	Pale yellow, light orange/uncoloured	Fusiform, swelling with age 3.5–8 × 1.5–2.6	Present	Present	+	–	–

Species	Colonies		Conidia shape & size (µm)	Adelophialides	Chlamydospores	Growth (°C)		
	Diameter (mm)	Color obverse/reverse				30	35	37
Species producing conidia in slimy heads and lacking schyzophialides								
S. bactrocephalum	21–25	White/uncoloured	Cylindrical 4.1–5.3 × 0.5–1	Not observed	Not observed	+	–	–
S. chinense[a]	Unknown	Light grey/light cinnamon	Cylindrical 3–6(–7) × 0.7–1.2	Not reported	Not reported	+	+	Unknown
S. hominis	22–30	Orange white/uncoloured	Cylindrical 3–4(–7) × 1–1.5	Not observed	Not observed	+	+	V
S. kiliense	36–46	Dirty white to pale orange/uncoloured	Ellipsoidal to cylindrical 3–6 × 1–1.5	Present	Usually present	+	+	+
S. mycophilum[b]	30–31	White/uncoloured	Cylindrical 3–8(–11) × 1.5–2.5	Not reported	Not reported	–	–	–
S. oryzae	23–34	White to pinkish white/apricot	Cylindrical 4–7 × 1–2	Present	Not observed	+	+	+
S. pseudostrictum	19–23	Salmon/uncoloured	Ellipsoidal to cylindrical 3–5 × 1.5–2	Not observed	Not observed	+	–	–
S. strictum	30–45	White or pale orange/uncoloured	Cylindrical or ellipsoidal 3.3–5.5(–7) × 0.9–1.8	Present	Not observed	+	+	+
S. zeae	19–24	White to pale pink/uncoloured	Cylindrical 3.5–6 × 1.2–1.8	Not observed	Not observed	+	+	+

[a] After the protologue (Chen et al. 1986).
[b] Due to lack of sporulation, the microscopic features included here are based on the protologue of the species (Helfer 1991).
+ Growth; – no growth; V variable growth.

Sarocladium oryzae (Sawada) W. Gams & D. Hawksw., Kavaka 3: 57. 1976 ('1975') — MycoBank MB323106

Basionym. Acrocylindrium oryzae Sawada, Rep. Gov. Res. Inst. Dept. Agric. Formosa 2: 135. 1922.

= *Sarocladium attenuatum* W. Gams & D. Hawksw., Kavaka 3: 59. 1976 ('1975').

= *Cephalosporium caerulens* Matsumae, Kamio & Hata, J. Antibiotics 16: 236. 1963, nom. inval. (Art. 36, 37).

Specimens examined. India, Hyderabad, from *O. sativa*, Apr. 1973, *K.S Amin* (epitype designated here CBS H-466, MBT177330, culture ex-epitype CBS 180.74 = IMI 176759); Bangalore, from *O. sativa*, 1973, *V. Agnihothrudu*, CBS H-467, CBS 399.73 = IMI 184922. – Nigeria, Ibadan, from *O. sativa*, July 1981, *G.N. Ngala*, CBS 414.81. – Taiwan, Taipei, Taiwan National University, from *O. sativa*, *K. Sawad* (TAI, holotype of *Acrocylindrium oryzae;* IMI 189860, slide ex-holotype).

Notes — *Sarocladium oryzae*, previously described as *Acrocylindrium oryzae* (Sawada 1922), is a common pathogen of rice (*O. sativa*) and different species of bamboo (*Bambusa balcooa*, *B. tulda*, *B. vulgaris*) (Gams & Hawksworth 1975, Boa & Brady 1987, Bridge et al. 1989, Pearce et al. 2001, Ayyadurai et al. 2005). It has been reported to cause sheath-rot of rice in many countries (Sakthivel et al. 2002). The species has been extensively treated by Gams & Hawksworth (1975), Bridge et al. (1989) and Bills et al. (2004).

The isolates included in the present study exhibit the morphological features described for the species, which briefly consist of white to orange-white colonies on PDA at 25 °C, simple and branched conidiophores, cylindrical phialides of up to 60 µm long and cylindrical conidia, 4–7 × 1–2 µm arranged in slimy heads. Since no living culture of the type material of *S. oryzae* was preserved, we selected three isolates representative of the species to be included in the study i.e., CBS 180.74, considered an authentic strain of *S. oryzae* (Agnihothrudu 1974, Gams & Hawksworth 1975, Summerbell et al. 2011); CBS 399.73 ex-type strain of *S. attenuatum*, a synonym of *S. oryzae* (Bills et al. 2004), and an isolate of *S. attenuatum* (CBS 414.81) genetically different from the other two mentioned isolates (Bills et al. 2004). Our phylogenetic study showed that the three isolates had a similarity of 98.4–98.8 % with the three loci compared. In addition, the phenotypic characteristics observed were quite similar between them, which is why we preferred to maintain these isolates as a single species. *Sarocladium oryzae* is the type species of the genus and since living type material does not exist, we considered it important to design an epitype. The holotype of the species consists of a slide preserved in the Laboratory of Plant Pathology of the Taiwan National University. This material was studied and compared with CBS 180.74 by Gams & Hawksworth (1975) and Bridge et al. (1989). According to Gams, the structures observed in the type slide are identical

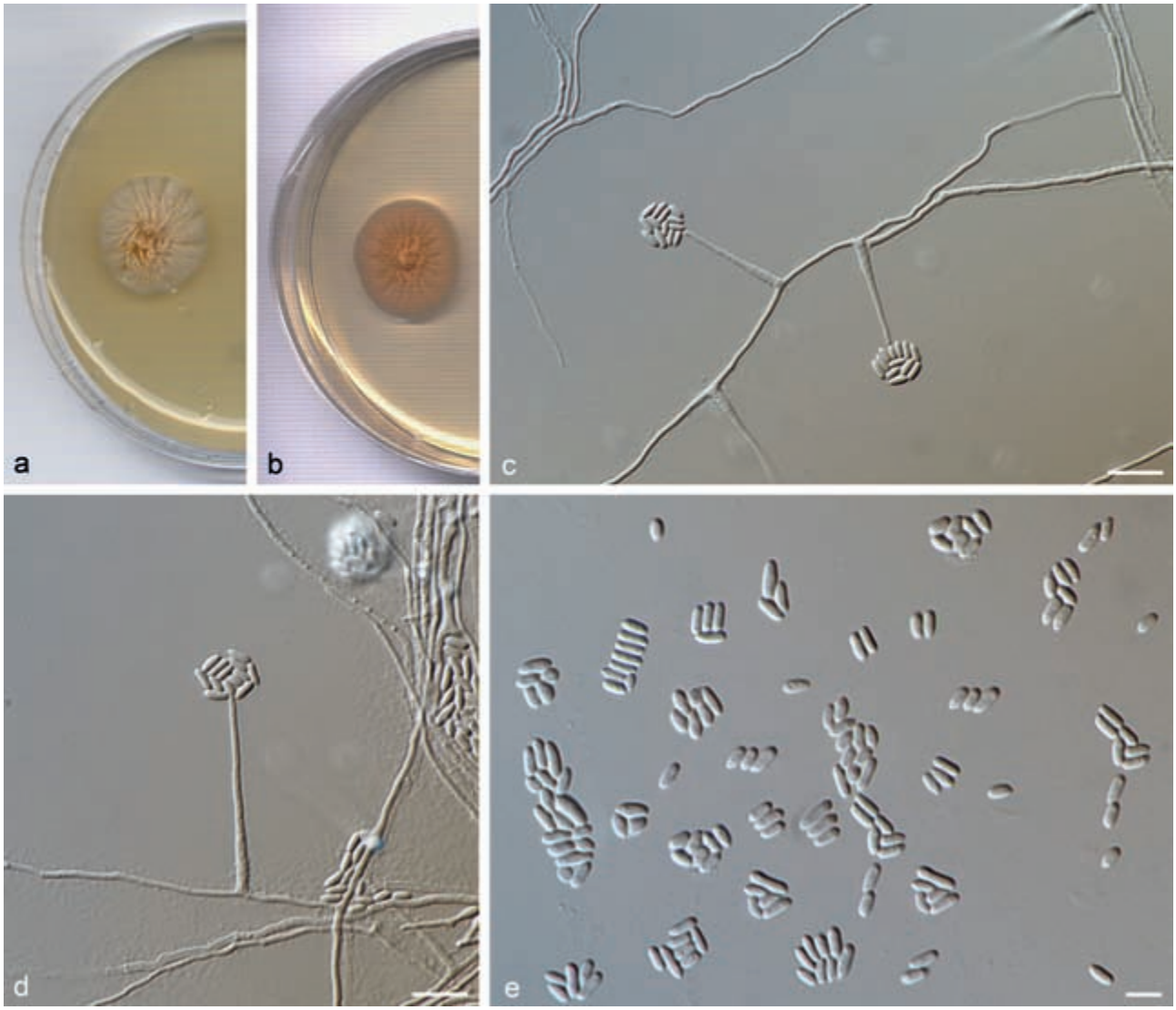

Fig. 5 *Sarocladium pseudostrictum* (sp. V) UTHSC 02-1892. a, b. Colonies on PDA after 14 d at 25 °C; c. acicular phialides arising laterally on the vegetative hyphae; d. conidia grouped in slimy heads; e. conidia. — Scale bars = 10 µm.

to those observed in CBS 180.74. We agree that the morphological features of this strain fit with the protologue of *S. oryzae* (Gams & Hawksworth 1975), and hence we here designate it as epitype. The morphological differences between *S. oryzae* and the other species of the genus are summarised in Table 2.

Sarocladium pseudostrictum Giraldo, Gené & Deanna A. Sutton, *sp. nov*. — MycoBank MB807947; Fig. 5

Etymology. Refers to the morphological similarity and the close phylogenetic relationship with *Sarocladium strictum.*

Colonies on OA at 25 °C attaining 20–31 mm diam in 14 d, yellowish white (1A2), flat, slightly powdery. On PDA at 25 °C reaching 19–23 mm diam in 14 d, orange white (6A2) to salmon (6A4), radially folded, membranous. Diffusible pigment absent. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 1.5–2 µm wide. *Conidiophores* erect, simple, hyaline, smooth-walled. *Phialides* arising directly from vegetative hyphae, acicular, 20–47 µm long, 1–1.5 µm wide at the base, with a distinct periclinal thickening at the conidiogenous locus, thin- and smooth-walled, hyaline; adelophialides and schizophialides not observed. *Conidia* unicellular, ellipsoidal to cylindrical with rounded ends, occasionally slightly apiculate at the base, 3–5 × 1.5–2 µm, hyaline to subhyaline, smooth- and thin-walled, arranged in slimy heads. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 20–25 °C, maximum 30 °C, minimum 15 °C. No growth at 35 °C.

Specimen examined. USA, Wisconsin, from sputum, 2002, *D.A. Sutton* (holotype CBS H-21635, culture ex-type CBS 137660 = FMR 10347 = UTHSC 02-1892).

Notes — *Sarocladium pseudostrictum* nested together with *S. strictum* and *S. bactrocephalum* in a well-supported clade (BS = 99; PP = 1.00), which correlates with the morphological similarity of the three species; however, subtle differences among them can be observed. In contrast to *S. pseudostrictum*, *S. strictum* has a faster growth rate on PDA, larger phialides (up to 65 × 2.5 µm), the conidiophores are usually branched, produce adelophialides, and its conidia are longer (up to 7 µm). In contrast, *S. bactrocephalum* has a slower growth rate, white colonies on PDA, the conidia are narrower (0.5–1 µm), and the phialides shorter (up to 35 µm) than *S. pseudostrictum.*

Sarocladium subulatum Giraldo, Gené & Guarro, *sp. nov*. — MycoBank MB807948; Fig. 6

Etymology. Refers to the phialide shape.

Colonies on OA at 25 °C attaining 26–30 mm diam in 14 d, yellowish white (4A2), flat with diffuse margin, powdery. On PDA at 25 °C reaching 17–20 mm diam in 14 d, yellowish white (4A2), flat, radially striated or crateriform with a lobulate margin, at first membranous becoming velvety. The isolate UTHSC 07-110 produces a diffusible deep yellow (4A8) pigment on PDA at 25 °C. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 1.5–2 µm wide. *Conidiophores* erect, simple, hyaline, smooth. *Phialides* arising directly from vegetative hyphae or ropes of hyphae, straight or slightly flexuous, subulate, 14–24(–32) µm long, 2–2.5 µm wide at the base, with a distinct periclinal thickening at the conidiogenous locus, hyaline, thin- and smooth-walled; adelophialides sometimes present on OA, 8–12(–15) µm long, 1.5 µm wide at the base. *Conidia* unicellular, fusiform, 5–8(–9) × 1–2 µm, hyaline, thin- and smooth-walled, arranged in chains. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 20–25 °C, maximum 30 °C, minimum 15 °C. No growth at 35 °C.

Specimens examined. Egypt, from soil, Apr. 1935, *Sabet* (holotype CBS H-21636), culture ex-type MUCL 9939 = CBS 217.35 = FMR 11044. – USA, California, from bone, July 2007, *D.A. Sutton*, CBS 137661 = FMR 10441 = UTHSC 07-110.

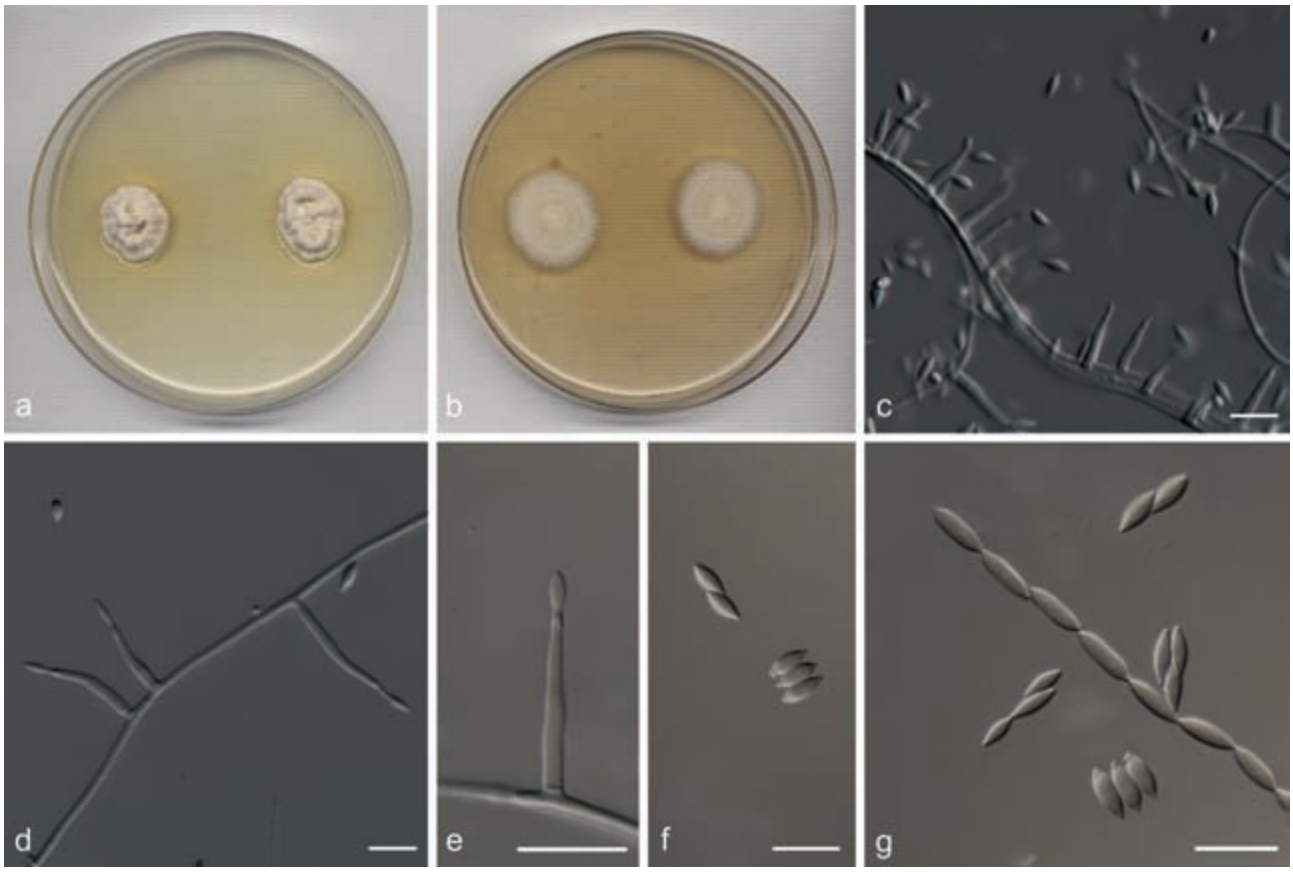

Fig. 6 *Sarocladium subulatum* (sp. I) UTHSC 07-110. a, b. Colonies on PDA and OA, respectively, after 14 d at 25 °C; c, d. phialides arising directly from ropes of hyphae or on vegetative hyphae; e. phialide with periclinal thickening at the apex; f, g. conidia. — Scale bars = 10 µm.

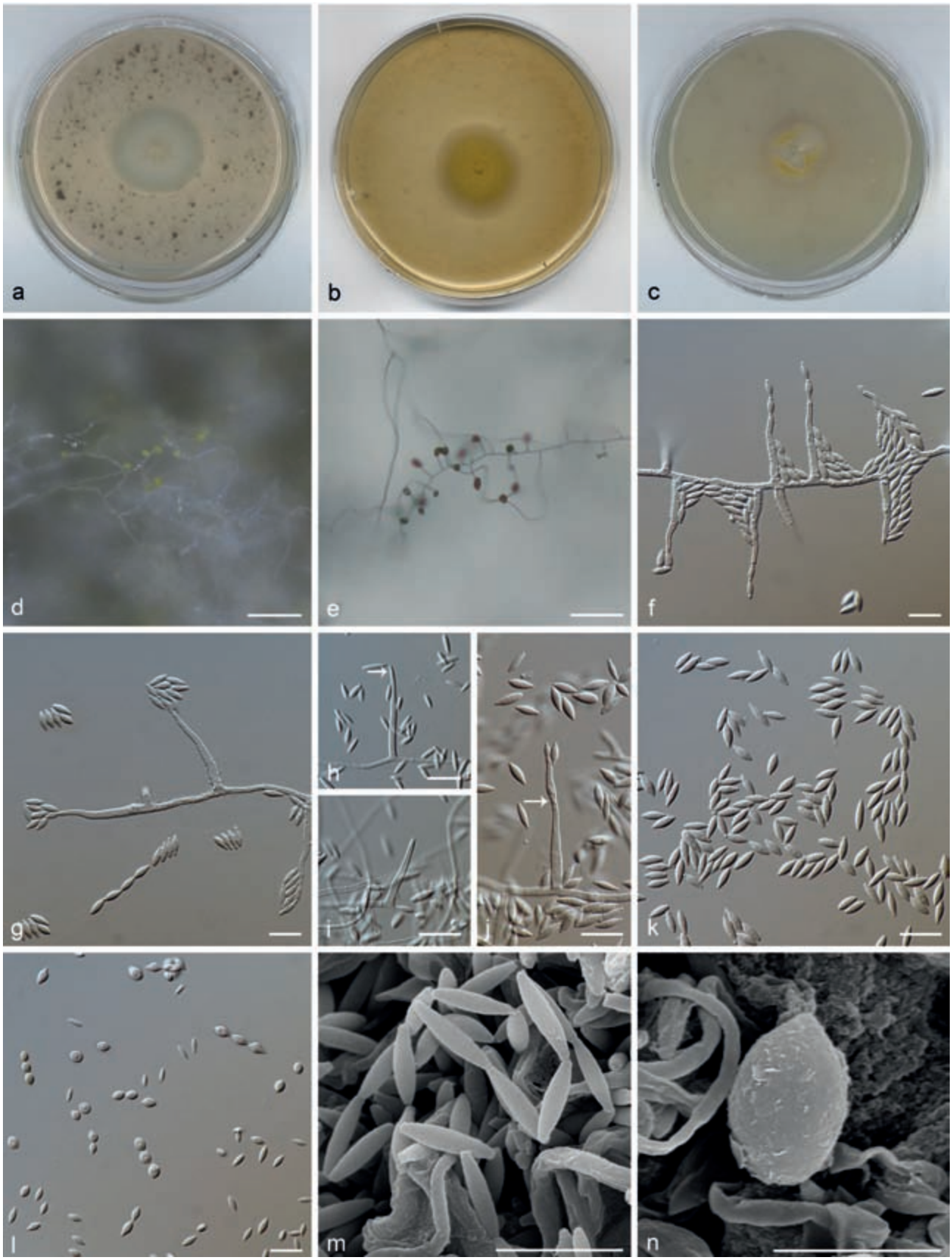

Fig. 7 *Sarocladium summerbellii* (sp. IV). a, b, d–g. CBS 891.73; c, j–n. CBS 430.70; h, i. CBS 951.72. a. Colonies on PDA after 14 d at 25 °C; b, c. colonies on OA after 14 d at 25 °C; d, e. pigmented conidia collapsing in heads on PDA; f. phialide bearing conidia in chains; g. lateral and terminal phialides; h. phialide with distinct periclinal thickening at the apex (arrow); i. adelophialide; j. phialide with percurrently proliferation (arrow); k, m. fusiform conidia; l. conidia in different maturation phases; n. swollen conidia. — Scale bars: d, e = 20 µm; f–l = 10 µm; m, n = 5 µm.

Notes — This species is closely related to *S. bacillisporum* and *S. terricola. Sarocladium subulatum* can be differentiated by its slower growth rate on OA and PDA at 25 °C, its inability to grow at 35 °C, and its conidial size (Table 2).

Sarocladium summerbellii Giraldo, Gené & Guarro, *sp. nov*. — MycoBank MB807949; Fig. 7

Etymology. Named in honour of the eminent Canadian mycologist Richard Summerbell.

Colonies on OA at 25 °C attaining 26–30 mm diam in 14 d, waxy yellow (3B5), sunflower yellow (4C7–8), flat, powdery. On PDA at 25 °C reaching 15–21 mm diam in 14 d, pale yellow (4A3–4), light orange (5A4–5), crateriform, radially folded with a lobulate margin, velvety. Diffusible pigment absent. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 1.5–2 µm wide. *Conidiophores* erect, usually simple, up to 42 µm long, straight or slightly bent, hyaline to subhyaline, smooth-walled. *Phialides* subulate, 15–40 µm long, 2–3.5 µm wide at the base, with a distinct periclinal thickening at the conidiogenous locus, thin- and smooth-walled, hyaline, sometimes with golden pigment accumulation at the base, often proliferating percurrently; adelophialides and schizophialides sometimes present. *Conidia* unicellular, fusiform with pointed ends, 3.5–8 × 1.5–2.5 µm, becoming limoniform to subglobose with age, up to 4 µm wide, hyaline, sometimes covered by a golden mucilaginous exudate, thin-walled, forming chains that soon collapse in slimy heads. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 20–25 °C, maximum 30 °C, minimum 15 °C. No growth at 35 °C.

Specimens examined. Netherlands, Bleiswijk, isolated from soil of a greenhouse, Mar. 1970, *G.J. Bollen* (holotype CBS H-8266, culture ex-type CBS 430.70 = FMR 12318); from water in air moistener, June 1984, *M.H.F. Luykx*, CBS 200.84 = FMR 11761; Baarn, from decaying leaf of *Canna indica*, 1968, *W. Gams*, CBS 797.69 = FMR 12319; Wageningen, from agricultural soil, Nov. 1972, *J.W. Veenbaas-Rijks*, CBS 951.72 = FMR 12317. – Sri Lanka, Pidurutalagala, dead leaf, Jan. 1973, *W. Gams*, CBS 891.73 = FMR 12315.

Notes — All the isolates included in the *A. summerbellii* clade were originally identified as *A. ochraceum*. With the exception of CBS 891.73 that was collected in the tropics (Sri Lanka), the other isolates originate from temperate areas (viz. The Netherlands). Although the Dutch isolates clustered together into a well-supported subclade within the *A. summerbellii* clade, they are morphologically and genetically almost identical (> 99.8 % identity) to the Sri Lanka isolate. *Sarocladium summerbellii* differs from *S. ochraceum* by the presence of percurrently proliferating phialides producing conidia both in chains and slimy heads that are limoniform to subglobose with age, and its inability to grow at 35 °C.

Sarocladium terricola (J.H. Mill., Giddens & A.A. Foster) Giraldo, Gené & Guarro, *comb. nov.* — MycoBank MB807950

Basionym. Fusidium terricola J.H. Mill., Giddens & A.A. Foster, Mycologia 49: 796. 1957.

≡ *Paecilomyces terricola* (J.H. Mill., Giddens & A.A. Foster) Onions & G.L. Barron, Mycol. Pap. 107: 10. 1967.

≡ *Acremonium terricola* (J.H. Mill., Giddens & A.A. Foster) W. Gams, Cephalosporium-artige Schimmelpilze: 67. 1971.

≡ *Sagrahamala terricola* (J.H. Mill., Giddens & A.A. Foster) Subram. & Pushkaran, Kavaka 3: 89. 1975.

Colonies on OA at 25 °C attaining 44–50 mm in 14 d, white (1A1), flat, floccose, reverse light orange (5A4). On PDA at 25 °C attaining 24–37 mm in 14 d, white (1A1), raised, radially folded, cottony, reverse light orange (5A4). *Conidiophores* erect, simple or poorly branched, hyaline to subhyaline, smooth-walled. *Phialides* subulate, 12–30(–35) µm long, 1–2 µm wide at the base, with distinct periclinal thickening at the conidiogenous locus, hyaline, thin- and smooth-walled; adelophialides and schizophialides sometimes present. *Conidia* unicellular fusiform with sharply pointed ends, 4–7(–8) × 1–2 µm, hyaline, smooth- and thin-walled, arranged in long dry chains. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 20–25 °C, maximum 35 °C, minimum 15 °C. No growth at 37 °C.

Specimens examined. Democratic Republic of Congo, Kinshasa, from decaying leaf of *Milleta laurentii*, Apr. 1967, *G. Hennebert*, FMR 11045 = MUCL 12011. – Italy, Sardegnia, from *Arundo donax*, Aug. 1970, *W. Gams*, CBS 134.71 = FMR 11421. – Morocco, from infected palm grove, FSSM, FMR 11047 = MUCL 42865. – USA, Georgia, from mixed forest soil, May 1956, *J.H. Miller* (holotype J.H. Miller No. 1679, personal collection, culture ex-type CBS 243.59 = ATCC 13215 = FMR 10460 = IAM 14651 = IMI 100712 = MUCL 4112); Texas, from sputum, 2002, *D.A. Sutton*, FMR 10348 = UTHSC 02-1958; Michigan, from bronchial wash fluid, 2003, *D.A. Sutton*, FMR 10388 = UTHSC 03-2933; Minnesota, from sinus, 2004, *D.A. Sutton*, FMR 10561 = UTHSC 04-956; Illinois, from bone, 2007, *D.A. Sutton*, FMR 10450 = UTHSC 07-3260; Minnesota, from bronchial wash fluid, 2007, *D.A. Sutton*, FMR 10571 = UTHSC 07-3667; Texas, from bronchoalveolar lavage fluid, 2008, *D.A. Sutton*, FMR 10369 = UTHSC 08-3180; Texas, from bronchoalveolar lavage fluid, 2008, *D.A. Sutton*, FMR 10356 = UTHSC 08-844.

Notes — *Sarocladium terricola* is a species commonly found in soil and on plant material in tropical and subtropical countries (Onions & Barron 1967, Gams 1971). However, in our case, most of our strains are from clinical origin. Despite the fact that *S. terricola* has never been described as the etiological agent of any human disease, its repeated isolation from human samples, mainly from the respiratory tract, would suggest a possible pathogenic role.

This species nests in a well-supported clade together with *S. bacillisporum* and *S. subulatum.* These species are morphologically very similar, but can be differentiated in the following features: *S. terricola* has a fast growth rate on all the media tested and it is able to grow at 35 °C; *S. bacillisporum* produces small rod-shaped conidia (4–6 × 1 µm) and *S. subulatum* has large conidia (5–8(–9) × 1–2 µm) and its phialides are wider at the base (2–2.5 µm) than the other two species.

DISCUSSION

In this study we clarified the taxonomy of *Sarocladium* and an important group of *Acremonium* sensu lato species based on the analyses of three DNA loci obtained from several reference strains and some fresh isolates from different origins. This study allowed the re-identification not only of numerous strains of *A. implicatum* sensu lato, recognised as a species complex in previous studies (Perdomo et al. 2011, Summerbell et al. 2011), but also other misidentified strains of *A. glaucum* and *A. ochraceous.* In spite of the high morphological similarity among the strains investigated, we were able to find subtle, but suitable features to phenotypically differentiate the novel phylogenetic species (Table 2).

Traditionally, species of *Sarocladium* have been reported as plant pathogens or as saprobes (Gams & Hawksworth 1975, Chen et al. 1986, Helfer 1991). However, numerous recent studies have demonstrated that some might also be involved in human infections (Das et al. 2010, de Hoog et al. 2011, Khan et al. 2011, Perdomo et al. 2011, Summerbell et al. 2011, Fernández-Silva et al. 2013, Júnior et al. 2013, Sharma et al. 2013). Specifically, the new species described here, i.e., *S. bifurcatum*, *S. hominis*, *S. pseudostrictum* and *S. subulatum*, were isolated from human samples. Despite the fact that these species have not been demonstrated to be etiological agents of human infections, their ability to grow at 35–37 °C and their

repeated occurrence from clinical specimens, sometimes from deep tissues, could indicate a possible role as human pathogens.

Our study also showed that all the *Sarocladium* species producing cylindrical conidia arranged in slimy heads including those clinically relevant grouped in the same lineage, while those species with fusiform conidia arranged in chains, or/and slimy heads were distributed in other clades. This distribution suggests that such conidial features could have a phylogenetic signal in this group of fungi.

Sarocladium mycophilum is the only mycoparasitic species of the genus. It is characterised by the presence of verticillate conidiophores and acicular phialides with conspicuous cylindrical collarettes, and the production of cylindrical conidia grouped in slimy heads (Helfer 1991). The ex-type strain of this species was included in our study but, unfortunately, the fungus did not sporulate on any of the culture media tested and therefore the morphological characteristics mentioned previously could not be verified. It is noteworthy that *S. mycophilum* was the only species unable to grow at 30 °C, showing growth well below 15 °C. Additionally, sequence analysis of the LSU and ITS of this strain showed that *S. mycophilum* is phylogenetically distant from the type of *Sarocladium*. A Megablast search performed with the rDNA sequences revealed a close relationship of *S. mycophilum* with members of the *Leotiomycetes* (98–99 % identity with: *Gorgomyces honrubiae* GenBank KC834028, *Flagellospora curvula* GenBank KC834023, *Alatospora constricta* GenBank KC834017 and *A. pulchella* GenBank KC834019), which excludes this species from *Sarocladium* s.str.

Sarocladium sinense was described by Chen et al. (1986) as the causal agent of the rice purple sheath disease in China. There is presently no strain available to infer its affinities with other species of *Acremonium*/*Sarocladium*. However, considering its morphology, the isolation source and symptomatology, this species could be a member of *Sarocladium.*

Sarocladium attenuatum is also responsible of sheath-rot of rice. This species was originally identified as *S. oryzae* (Agnihothrudu 1974). Gams & Hawksworth (1975) distinguished *S. attenuatum* from that species by the presence of more regularly verticillate conidiophores, somewhat less frequent solitary phialides, and longer and slightly narrower conidia, tapering gradually and having truncate ends. Nevertheless, the status of this species remained debated; several authors considered *S. oryzae* and *S. attenuatum* to be synonymous on the basis of conidial size, production of secondary metabolites and the use of molecular and physiological tests (Bridge et al. 1989, Pearce et al. 2001, Bills et al. 2004). Summerbell et al. (2011) sequenced the ITS region of the ex-type strain of *S. attenuatum* (CBS 399.73), and showed it to differ from the sequence of the same strain obtained by Bills et al. (2004). We have sequenced the LSU and the ITS regions of the strain CBS 399.73 on two different occasions using different DNA extractions methods. The LSU sequence proved to be identical to that published by Bills et al. (2004), while the ITS sequence differed by 8 nucleotides and 1 gap. Unfortunately, the ITS sequence obtained by Summerbell et al. (2011) was not available for comparison. In addition, we sequenced another strain of *S. attenuatum* (CBS 414.81) and a strain of *S. oryzae* (CBS 180.74). The combined analysis of the three loci showed that all the strains grouped in a single well-supported clade (Fig. 1), and lacked significant genetic differences to be considered as two different species, which correlated with the morphological similarity observed among the strains.

As mentioned above, in the previous phylogenetic study on *Acremonium* (Summerbell et al. 2011), the taxonomic position of *A. implicatum* was not resolved due to the lack of an ex-type or authentic strain and because the strains supposedly belonging to *A. implicatum* clustered in different lineages. One of these strains, CBS 243.59, the ex-type strain of *Fusidium terricola* (Miller et al. 1957), considered conspecific with *A. implicatum* (Gams 1975), nested in the *Sarocladium* clade (Perdomo et al. 2011, Summerbell et al. 2011, Giraldo et al. 2012). Therefore, we retained both species in *Sarocladium* as *S. implicatum* and *S. terricola*, respectively. To promote taxonomic stability, we have chosen CBS 959.72 as ex-neotype of the former species.

In summary, all the strains included in our study identified previously as *A. implicatum* and obtained from the CBS-KNAW Fungal Biodiversity Centre and MUCL culture collections (Table 1), have been re-identified as follows: *S. bacillisporum* (CBS 787.69), *S. gamsii* (CBS 707.73), *S. implicatum* (CBS 397.70A, CBS 825.73, CBS 959.72), *S. terricola* (CBS 134.71, MUCL 12011, MUCL 42865) and *S. subulatum* (MUCL 9939). Apart from the reference strains identified as different *Sarocladium* species, five other reference strains did not cluster within the *Sarocladium* clade. The strain CBS 381.73 (from bamboo stems) is morphologically similar to *A. roseolum* (Gams 1971) and the D1/D2 sequence showed a similarity of 99.8 % with the ex-type strain of this species. Comparisons of the D1/D2 region of the strain CBS 397.70B showed that it is closely related to the ex-type species of *A. exiguum* (CBS 587.73; 97.6 % similarity). The strain CBS 114748 was related to a strain of *A. longisporum* (CBS 669.73), but this species lacks a living ex-type culture, which would enable a more accurate identification. Finally, two strains isolated from wheat seeds, MUCL 8122 and MUCL 8123, were related to *A. egyptiacum* (CBS 303.64), but are phylogenetically distant from the ex-type strain of this species.

In conclusion, these results show that a lot of research still needs to be conducted on isolates identified as species of *Acremonium*. Many of these species still lack a clear taxonomy, and only by including them in modern phylogenetic studies we will be able to advance our knowledge of this heterogeneous group of apparently asexual fungi, that all share simple morphological structures on the one hand, but display a great genetic diversity on the other.

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