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Phylogeny of Sarocladium (Hypocreales)

A. Giraldo1, J. Gené1, D.A. Sutton2, H. Madrid3,4, G.S. de Hoog3, J. Cano1, C. Decock5, P.W. Crous3, J. Guarro1

Key words

Acremonium
Hypocreales
phylogeny
Sarocladium
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 Ascomycetes with species scattered in diverse orders of Sordariomycetes (Glen 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 adelophiliades 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 adelophiliades 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) (Ayadurai et al. 2005). It is also known to produce antimicrobial secondaries.

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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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>(original identification)</th>
<th>Origin</th>
<th>GenBank accession no.</th>
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<td></td>
<td>CBS 787.69</td>
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<td>Teleutosorus of Puccinia graminis on Lolium temulentum, Italy</td>
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<td>(A. strictum)</td>
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<td>CBS 100350</td>
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<td>Dead stem of bamboo, Japan</td>
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<td>(Acremonium sp.)</td>
<td>Leg, USA</td>
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<td>(Acremonium sp.)</td>
<td>Right calf tissue, USA</td>
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<td>Sputum, USA</td>
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<td>Skin, Germany</td>
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<td>Cortinarius subtrepipes, Germany</td>
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<td>Zea mays, Kenya</td>
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<td>Sarocladium oryzae</td>
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<td>(S. oryzae)</td>
<td>Oryza sativa, India</td>
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<td>(S. attenuatum)</td>
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<td>(S. attenuatum)</td>
<td>Oryza sativa, Nigeria</td>
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<td>(Acremonium sp.)</td>
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<td>(S. strictum)</td>
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<td>Soil, Egypt</td>
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<td>(Acremonium sp.)</td>
<td>Bone, USA</td>
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<td>Sarocladium summerbellii</td>
<td>CBS 200.84</td>
<td>(S. ochraceum)</td>
<td>Water in air moistener, The Netherlands</td>
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<td>(Sarocladium sp. IV)</td>
<td>CBS 430.70</td>
<td>(S. ochraceum)</td>
<td>Soil from greenhouse, The Netherlands</td>
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<td></td>
<td>CBS 797.69</td>
<td>(S. ochraceum)</td>
<td>Decaying leaf of Cannas Indica, The Netherlands</td>
<td>HG965079</td>
</tr>
</tbody>
</table>
### 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 rRNA genes 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 & Wösthe et al. 1990, O’Donnell 1993). The D1/D2 domain was amplified in all isolates with the primers mentioned above, except in

#### 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 (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 as-
essed by a search of 1 000 bootstrapped sets of data. A boot-
strap support (BS) ≥ 70 was considered significant. A second
phylogenetic analysis using a Metropolis-coupled Markov Chain
Monte Carlo (MCMC) algorithm was done using MrBayes v.
3.2.1 (Ronquist & Huelsenbeck 2003) with two simultane-
ous 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 as Heuristic method. The internal branch support was as-
essed by a search of 1 000 bootstrapped sets of data. A boot-
strap support (BS) ≥ 70 was considered significant. A second

Phenotypic studies
Morphological characterisation of the fungal isolates was car-
rried out based on cultures grown on oatmeal agar (OA; fil-
tered 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 incu-
bated 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 Komerup & 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 Axios-Imager
M1 light microscope (Zeiss, Oberkochen, Germany), using
phase contrast and Nomarski differential interference. Scan-
ning 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 in-
dividual genes showed no contradiction (data not shown) and
therefore the three sequence datasets were combined. The
combined analysis from ITS, D1/D2 and the ACTf1 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 differ-
ent species. The first lineage included the ex-type strain of A. terricola CBS 243.59, seven clinical isolates previously identified as A. im-

plicatum (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 re-
ference strains (CBS 707.73 and CBS 425.73) of A. implicatum
and S. glaucum, respectively, both obtained from Pandanus
liferum. 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 625.73) 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. kilienise, 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
underscribed taxa. In addition, A. terricola, for a long time left
in the limbo of synonymy is re-considered as a distinct species,
better accommodated in Sarocladium, hence the new combina-
S. implicatum 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 or-
ange (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; adelo-
phialides sometimes present; schizophilides commonly pre-
ent. 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, maxi-
mum 30 °C, minimum 15 °C, no growth at 35 °C.

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


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...
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; adelo-philialides and schizophialides not observed. Conidia unicellular, cylindrical with rounded ends, 3–4(–7) x 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, adelo-philialides and appears as dirty orange to pale ochraceous colonies on OA; S. zeae has longer (up to 80 µm) and branched conidiophores with basitonic 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


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. Adelo-philialides and schizophialides not observed. Conidia unicellular, fusiform, 5–8 x 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.


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
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).

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

<table>
<thead>
<tr>
<th>Species producing conidia in chains</th>
<th>Color</th>
<th>Conidial shape &amp; size (µm)</th>
<th>Adelophialides</th>
<th>Schizophialides</th>
<th>Growth (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. bacillisporum</td>
<td>20–24</td>
<td>White/uncoloured</td>
<td>Rod-shaped</td>
<td>Not observed</td>
<td>+</td>
</tr>
<tr>
<td>S. bifurcatum</td>
<td>13–14</td>
<td>Orange white/uncoloured</td>
<td>Fusiform</td>
<td>Present</td>
<td>+</td>
</tr>
<tr>
<td>S. glaucum</td>
<td>12–21</td>
<td>Bluish green/uncoloured</td>
<td>Not observed</td>
<td>Not observed</td>
<td>+</td>
</tr>
<tr>
<td>S. implicatum</td>
<td>18–30</td>
<td>Pinkish white to salmon/pale orange</td>
<td>Fusiform</td>
<td>Not observed</td>
<td>+</td>
</tr>
<tr>
<td>S. ochraceum</td>
<td>17–18</td>
<td>Ochaceous or yellow/uncoloured</td>
<td>Fusiform</td>
<td>Not observed</td>
<td>+</td>
</tr>
<tr>
<td>S. subulatum</td>
<td>17–20</td>
<td>Yellowish white/uncoloured</td>
<td>Fusiform</td>
<td>Present</td>
<td>+</td>
</tr>
<tr>
<td>S. terricola</td>
<td>27–37</td>
<td>White/light orange</td>
<td>Fusiform</td>
<td>Present</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species producing conidia in chains and slimy heads</th>
<th>Color</th>
<th>Conidial shape &amp; size (µm)</th>
<th>Adelophialides</th>
<th>Schizophialides</th>
<th>Growth (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. gamsii</td>
<td>13–21</td>
<td>Yellowish white/uncoloured</td>
<td>Fusiform</td>
<td>Not observed</td>
<td>+</td>
</tr>
<tr>
<td>S. summerbellii</td>
<td>15–21</td>
<td>Pale yellow, light orange/uncoloured</td>
<td>Fusiform, swelling with age</td>
<td>Present</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species producing conidia in slimy heads and lacking schizophialides</th>
<th>Color</th>
<th>Conidial shape &amp; size (µm)</th>
<th>Adelophialides</th>
<th>Chlamydospores</th>
<th>Growth (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. bactrocephalum</td>
<td>21–25</td>
<td>White/uncoloured</td>
<td>Cylindrical</td>
<td>Not observed</td>
<td>+</td>
</tr>
<tr>
<td>S. chinense*</td>
<td>Unknown</td>
<td>Light grey/light cinnamon</td>
<td>Cylindrical</td>
<td>Not reported</td>
<td>+</td>
</tr>
<tr>
<td>S. hominis</td>
<td>22–30</td>
<td>Orange white/uncoloured</td>
<td>Cylindrical</td>
<td>Not observed</td>
<td>+</td>
</tr>
<tr>
<td>S. kiliense</td>
<td>36–46</td>
<td>Dirty white to pale orange/uncoloured</td>
<td>Ellipsoidal to cylindrical</td>
<td>Present</td>
<td>+</td>
</tr>
<tr>
<td>S. mycophilum*</td>
<td>30–31</td>
<td>White/uncoloured</td>
<td>Cylindrical</td>
<td>Not reported</td>
<td>Unknown</td>
</tr>
<tr>
<td>S. oryzae</td>
<td>23–34</td>
<td>White to pinkish white/apricot</td>
<td>Cylindrical</td>
<td>Present</td>
<td>+</td>
</tr>
<tr>
<td>S. pseudostrictum</td>
<td>19–23</td>
<td>Salmon/uncoloured</td>
<td>Ellipsoidal to cylindrical</td>
<td>Not observed</td>
<td>+</td>
</tr>
<tr>
<td>S. strictum</td>
<td>30–45</td>
<td>White or pale orange/uncoloured</td>
<td>Cylindrical or ellipsoidal</td>
<td>Present</td>
<td>+</td>
</tr>
<tr>
<td>S. zeae</td>
<td>19–24</td>
<td>White to pale pink/uncoloured</td>
<td>Cylindrical</td>
<td>Not observed</td>
<td>+</td>
</tr>
</tbody>
</table>

* After the protologue (Chen et al., 1986).
* Due to lack of sporulation, the microscopic features included here are based on the protologue of the species (Helfer 1991).
* Growth: + growth; – no growth; V variable growth.
**Sarocladium oryzae** (Sawada) W. Gams & D. Hawksw., Kavaka 3: 57. 1976 (‘1975’) — MycoBank MB323106


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


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.
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 Cuban 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 erect, usually simple, up to 42 µm long, straight or slightly bent, hyaline to subhyaline, smooth-walled. 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 conidigenous 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 30 °C, minimum 15 °C. No growth at 35 °C.


Notes — Sarocladium terricola is a species commonly found in soil and 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. ochraceus. 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. pseudoostriatum 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 aciculate 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 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 (Agnihotruh 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 extraction 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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