TAXONOMIC STUDY OF CLINICAL AND ENVIRONMENTAL ISOLATES OF ARTHROCONIDIAL, ACREMONIUM-LIKE AND OCHROCONIS-LIKE FUNGI

Dixie Alejandra Giraldo López

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Universitat Rovira i Virgili

Taxonomic study of clinical and environmental isolates of acremonium-like, arthroconidial and ochroconis-like fungi

Dixie Alejandra Giraldo López

Doctoral Thesis

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Doctoral Thesis

Dixie Alejandra Giraldo López

Directed by Drs. Josepa Gené Díaz, Josep Guarro Artigas and José Francisco Cano Lira

Departament de Ciències Mèdiques Bàsiques
Facultat de Medicina i Ciències de la Salut

Reus

2015

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I STATE that the present study, entitled "**Taxonomic study of clinical and environmental isolates of acremonium-like, arthroconidial and ochroconis-like fungi**", presented by **Dixie Alejandra Giraldo López** for the award of the degree of Doctor, has been carried out under our supervision at the Department Ciències Mèdiques Bàsiques of this university, and it fulfils the requirements to obtain the International Doctorate Mention.

Reus, 10 de Enero de 2015

Doctoral Thesis Supervisors

Josepa Gené Díaz   Josep Guarro Artigas   José F. Cano Lira

In memory of my father, Leonel

To my mother and my little princesses Sofía and Ana

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°C	Degrees Celsius
ACT	Actin
AFG	Anidulafungin
AIDS	Acquired Immune Deficiency Syndrome
AMB	Amphotericin B
ATCC	American Type Culture Collection
BAL	Bronchoalveolar Lavage
BHI	Brain Heart Infusion
BI	Bayesian Inference
BLAST	Basic Local Alignment Search Tool
bp	Base Pairs
bs	Bootstrap support
BSC	Biological Species Concept
BT2	A fragment of the β-tubulin gene
Bx	Biopsy
CAPD	Continuous Ambulatory Peritoneal Dialysis
CBS	CBS-KNAW Fungal Biodiversity Centre
CFG	Caspofungin
CHS1	Chitin Synthase
CI	Consistency Index
CLSI	Clinical and Laboratory Standards Institute
cm	Centimeter
cm^2	Square centimeter
Cmd	Calmodulin
comb. nov.	*combinatio nova*, Latin expression meaning "new combination"
CSC	Consolidated Species Concept
d	Days
diam	Diameter
D1/D2	Domains of the 28S rRNA gene
DNA	Deoxyribonucleic Acid
e. g.	*exempli gratia*, Latin expression meaning "for example"
EMBL	European Molecular Biology Laboratory
ENA	European Nucleotide Archive
ESC	Ecological Species Concept
et	Latin word meaning “and”
et al.	*et alii* (Latin expression meaning "and others")
etc.	*et cetera*, Latin expression meaning "and the rest (of such things)"

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Fig.	Figure
FMR	Faculty of Medicine, Reus
g	Gram
gen. nov.	*genus novus*, Latin expression meaning “new genus”
GCPSR	Genealogical Concordance Phylogenetic Species Recognition
HI	Homoplasy Index
HIS	Histone H3
HIV	Human Immunodeficiency Virus
ICBN	International Code of Botanical Nomenclature
ICN	International Code of Nomenclature for Algae, Fungi and Plants
i. e.	*id est*, Latin expression meaning "that is" or “namely”
IGS	Intergenic Spacer
ILD	Incongruence length difference test
ITC	Itraconazole
ITS	Internal Transcribed Spacer
L	Liter
LSU	Large Subunit of the rRNA
M	Molar
MAT	Mating Type Genes
MCMC	Markov Chain Monte Carlo Algorithm
MEA	Malt Extract Agar
MEC	Minimal Effective Concentration
MFG	Micafungin
mg	Milligram
MIC	Minimal Inhibitory Concentration
min	Minute
ML	Maximum Likelihood
mL	Milliliter
mm	Millimeter
MP	Maximum Parsimony
MSC	Morphological or Phenotypic Species Concept
MUCL	Mycothèque de l’Université Catholique de Louvain
NCBI	National Center for Biotechnology Information
NJ	Neighbour-Joining
NNI	Nearest-Neighbour-Interchange
NRBC	NITE Biological Resource Center
nrDNA	Nuclear Ribosomal DNA

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OA	Oatmeal Agar
PAUP	Phylogenetic Analysis Using Parsimony
PCA	Potato Carrot Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PHT	Partition Homogeneity Test
PP	Bayesian Posterior Probabilities
PSC	Phylogenetic Species Concept
PSC	Posaconazole
RFLP	Restriction Fragments Length Polymorphism
RI	Retention Index
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
RPB2	RNA polymerase II second largest subunit
SEM	Scanning Electron Microscopy
sp. nov.	*species nova*, Latin expression meaning "new species"
SSU	Small Subunit of the rRNA
TBF	Terbinafine
TEF1-α	Translation Elongation Factor 1-alpha
UAMH	University of Alberta Microfungus Collection and Herbarium
µg	Microgram
µL	Microliter
µm	Micrometer
UPGMA	Unweighted Pair-Group Method with Arithmetic means
URV	Universitat Rovira i Virgili
UTHSC	University of Texas Health Science Center
v.	Version
VRC	Voriconazole
YES	Yeast Extract Sucrose Agar

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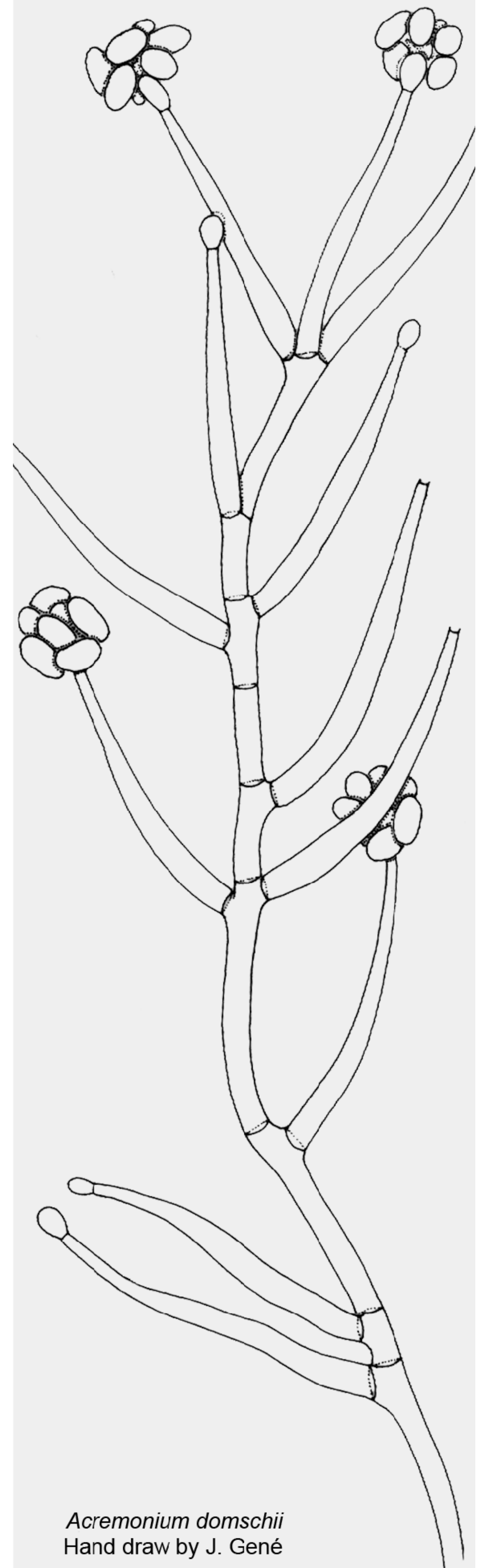
1. INTRODUCTION



Acremonium domschii
Hand draw by J. Gené

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1.1. General features of fungi

Fungi constitute a group of heterotrophic eukaryotic organisms with unicellular or multicellular thalli, absorptive nutrition (osmotrophic), cellular walls rich in chitin and β-glucan, devoid of plastids and chlorophyll, and reproducing by means of spores (Kirk et al. 2008, Seifert et al. 2011). Fungi with an unicellular thallus and the ability to reproduce by budding are called **yeasts**; while those with a multicellular thallus, which is composed by individual microscopic filaments (hyphae), constitute the group called **filamentous fungi**. Hyphae can be divided into compartments or cells by cross walls (septa) and generally referred to as septate, or can be coenocytic (aseptate – without cross wall) (Alexopoulos et al. 1996). The fungi that can express either a filamentous or yeast form usually are called **dimorphic** fungi; this switch in the form can be influenced by physical or chemical conditions. An example of this behaviour can be observed in *Histoplasma capsulatum* Darling, the causal agent of histoplasmosis in humans. This fungus presents a yeast form when it is inside the host (temperature around 37 °C), and a filamentous phase in its natural habitat (nitrogen-rich soils) (de Hoog et al. 2011).

Most of the fungi are saprobic, colonizing rotting wood and dead organic matter present in the soil (Watanabe 2002), but they can also be found in different and diverse substrates, such as water (Webster & Descals 1981, Shearer et al. 2007), dung (Kendrick 2001), food (Samson et al. 2010), indoor and outdoor environments (Nobuo & Niichiro 2008, Lian & de Hoog 2010), cave rocks and Paleolithic paintings (Nováková 2009, Kiyuna et al. 2011, Martin-Sanchez et al. 2012) or hot springs effluents (Yarita et al. 2010), among others. Some species can establish mutualistic relationships with root plants or algae to form mycorrhizae and lichens, respectively (Seifert et al. 2011). However, a considerable number of fungi live as parasites on animals, plants or even other fungi (Alexopoulos et al. 1996).

Two different ways of reproduction are described in fungi, i.e. sexual and asexual modes, which produce meiospores and mitospores, respectively. Meiospores usually result from interbreeding and produce descendants with different genotypes. In contrast, mitospores generate a progeny genetically identical to the progenitor (Seifert & Samuels 2000). The mechanism of sexual reproduction is controlled by **mating type (*MAT*) genes**. Heterothallic fungi can only reproduce sexually when two individuals of opposite mating type are present. In contrast, homothallic fungi can self-fertilise because of the presence of both mating type genes in one individual (Crous et al. 2009).

Individuals with sexual reproduction are called **teleomorph** (meiosporic fungus, perfect state or sexual morph) and those with asexual reproduction **anamorph** (mitosporic fungus or asexual morph) (Alexopoulos et al. 1996, Kirk et al. 2008). Anamorphic fungi are widely distributed throughout the fungal system, and are cited in older literature as

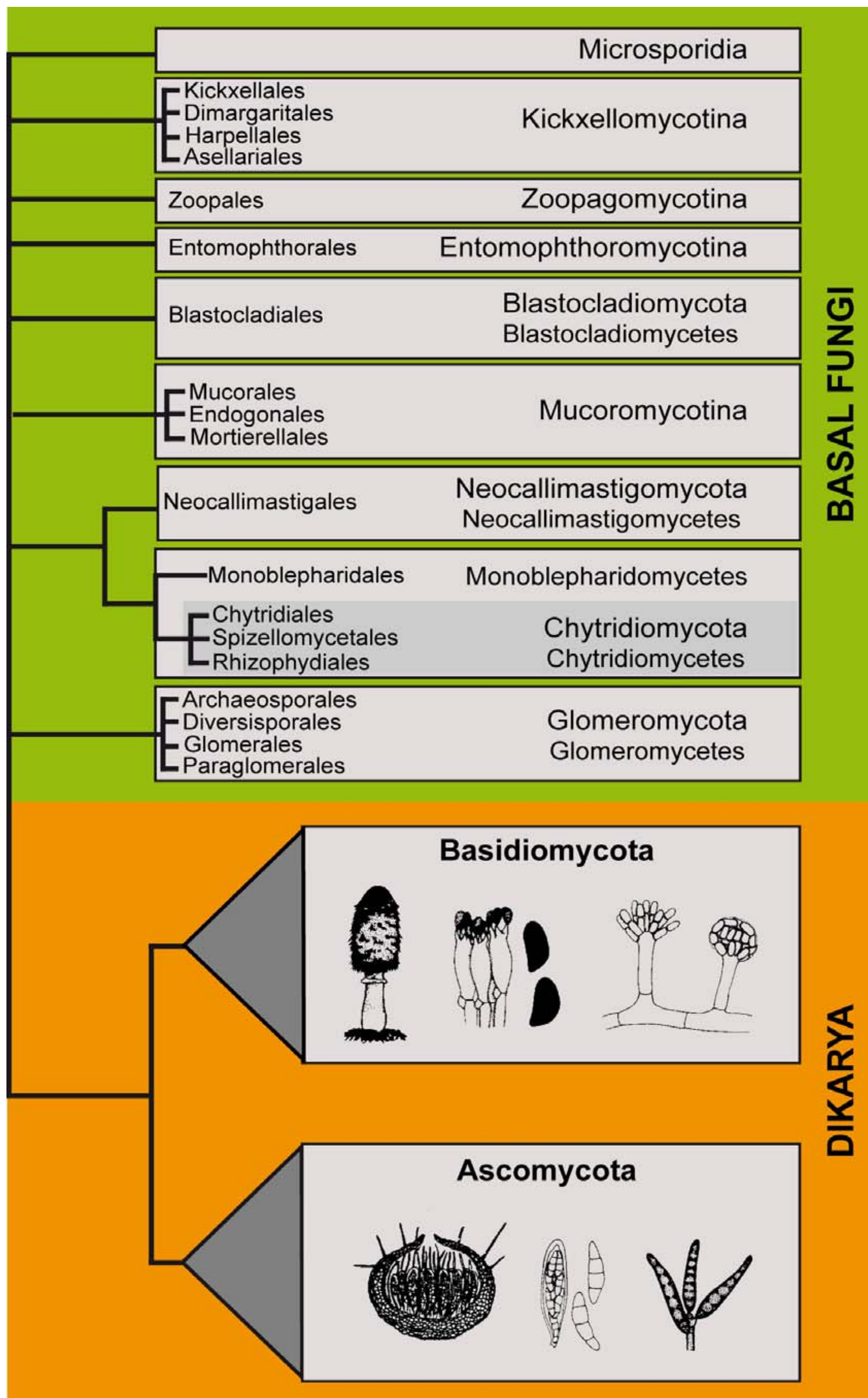

Fig. 1 Arrangement of the major fungal taxa (Basal Fungi and Subkingdom *Dikarya*) within the Kingdom *Fungi.* The scheme is based on Hibbett et al. (2007) and Guarro et al. (1999).

deuteromycetes" or “fungi imperfecti", but presently these terms are no longer used as formal classes (Seifert et al. 2011). A fungus in all its forms of sporulation is called **holomorph**, and when it has more than one independent form or spore stage in its life cycle, the term used is **pleomorphism** (Kirk et al. 2008). Some fungal species produce different asexual morphs which are known as **synanamorphs** (synasexual morph). In some cases, sexual and asexual morphs develop side-by-side, but in general they are produced at different times or on different substrates. Although numerous sexual-asexual connections are presently established, a great number of fungal species are only known by their asexual morph. They seem to have lost the potential to mate or develop a sexual morph (Seifert et al. 2011).

In the past, fungi were classified within the Kingdom *Plantae* (Briquet 1912), but nowadays they have their own kingdom, the Kingdom *Fungi,* which is more related to the animals than to the plants (Whittaker 1969, Shenoy et al. 2007). This kingdom contains the Subkingdom ***Dykaria*** that includes fungi with dikaryotic hyphae, belonging to either ***Ascomycota*** or ***Basidiomycota*** phyla (Fig. 1) (Hibbett et al. 2007). Fungi traditionally placed in the *Zygomycota* and *Chytridiomycota* are treated as "basal fungi" and are scattered in different paraphyletic lineages, outside of the *Dykaria* (Fig. 1) (James et al. 2006, Hibbett et al. 2007, McLaughlin et al. 2009).

1.1.1. Soil-borne fungi

Soil is one of the main reservoirs of fungal species. The variety of microhabitats present in the soil favors the diversity of fungal communities. These communities represent the most abundant component of the soil microbiota in terms of biomass, although their numbers in dilution plates are lower than those of bacteria (Anderson & Domsch 1978). The fungi play an important role in the decomposition of organic compounds, such as cellulose, chitin and lignin present on the upper soil layers, producing humus and maintaining soil fertility (Watanabe 2002, Bills et al. 2004a).

The fungi from underground parts, and especially associated with soil-borne diseases, seem to be typical soil fungi; however, some of them are also often recovered from seed and are called "seed fungi" (Watanabe 2002). Most of the soil-borne fungi are saprotrophs and can live associated with decaying vegetative material. Other species may be parasitic or semiparasitic on plant roots (root-inhabiting fungi).

It is common in soil to find mucorales species, sexual and asexual morphs of ascomycetes, and particularly basidiomycetes. They can be present in soil as mycelium, sexual or/and asexual spores, chlamydospores or sclerotial bodies. The latter stages are dormant survival structures, having little activity and limited importance in soil metabolism;

only mycelial states may have considerable metabolic activity (Domsch et al. 2007, Kirk et al. 2008, Guarro et al. 2012).

In the last decades the interest in the study of soil fungi has increased. This substratum contains a pool of interesting and undescribed species, especially when samples from diverse places are examined (Blackwell 2011, Tedersoo et al. 2014). In addition, soil contains numerous species which may be used as unique sources of enzymes or secondary metabolites of biotechnological or pharmaceutical potential, such as antimicrobials, vitamins or cofactors (Hujslová et al. 2010).

Previous studies originating from our research unit (Cano 1989, Ulfig 1992, Gené 1994, Ulfig et al. 1997, 1998; Stchigel 2000, García 2005, Madrid 2011, Hernández 2013), specifically in the research line "biodiversity of soil microfungi", have demonstrated the richness of novel fungal species from this substratum. In the case of the studies by Ulfig (1992), Ulfig et al. (1998) and Gené (1996), numerous acremonium-like and arthroconidial fungi were isolated, some of those described as new to science (Ulfig et al. 1995, Gené et al. 1996, Cano et al. 1997, Vidal et al. 1999) and others only recognized as potentially new, but not formally proposed as a novelty because of the limited morphological differences found with respect to the known species.

1.1.2. Fungi and infection

Most fungi are found in the nature as saprobes, but some of them can live as animal (including human) **commensals,** forming part of the microbiota [e.g., *Malassezia furfur* (C.P. Robin) Baill]. In this sense, they use excreted compounds produced by the host (e.g., excretions of the skin or keratinous materials), but without causing damage on the living animal itself (de Hoog et al. 2011). In some occasions, commensals may invade adjacent tissues but in a coincidental way. Other fungi can be found as **contaminant** on the skin or other surface, but unlike commensal fungi, the contaminants do not use the host products as nutrients source (de Hoog et al. 2011). However, some fungi have developed the ability to evade the host's first unspecific line defense, causing infections in healthy individuals. These organisms are considered to be **pathogenic fungi,** and the infection caused by them is called **mycosis.** According to the localization, the mycoses can be grouped in four main categories, i.e., superficial, cutaneous, subcutaneous and deep. When only the outermost skin layers and its dermal appendages (hair and nails) are affected, and no immune response from the host is involved, the mycosis is considered as **superficial** (e.g., onychomycosis), whereas, if the infection is produced in deeper layers of the skin, generating an inflammatory response, it is considered as a **cutaneous mycosis** or **dermatomycosis**. Cutaneous mycoses, such as ringworm (tinea) or keratitis can be produced by dermatophytic species [e.g., *Trichophyton rubrum* (Castell.) Sabour. or

Microsporum canis E. Bodin ex Guég.], or by other unrelated genera such as *Fusarium* Link or *Aspergillus* P. Micheli (Mellado et al. 2013). **Subcutaneous mycoses** are local, primary infections of the subcutaneous tissue (including dermis and hypodermis), which produce an inflammatory response that leads to cysts or granuloma (de Hoog et al. 2011). These infections are acquired after traumatic inoculation of fungal structures and are produced by a heterogeneous group of fungi (Queiroz-Telles et al. 2003). Subcutaneous mycoses include non-ulcerative infections, eumycetoma (white- and black-grain mycetoma), sporotrichosis, chromoblastomycosis, lobomycosis, etc. According to the appearance of the causal agent in tissues, the non-ulcerative infections are classified in two groups, i.e., **hyalohyphomycosis** and **phaeohyphomycosis**. If colourless fungal structures are observed on the host tissue, it is called hyalohyphomycosis, where *Fusarium* spp., *Acremonium* spp., *Penicillium* spp., *Scedosporium* spp. and *Paecilomyces* spp. are common representatives (Nucci & Anaissie 2009). On the other hand, if dematiaceous yeast-like cells, pseudohyphae, hyphae or any combination of these forms are observed in tissue, the infection is known as phaeohyphomycosis. In this case, several agents are involved, e.g., *Alternaria* spp., *Exophiala* spp., *Ochroconis* spp., *Phaeoacremonium* spp., among others (Crous et al. 1996, Schell 2003, Revankar & Sutton 2010). Finally, **deep mycoses** encompass localized infections in deep tissues and internal organs, or disseminated infections via blood or the lymphatic system. In immunocompetent patients, these are produced by primary pathogens, such as *Paracoccidioides brasiliensis* (Splend.) F.P. Almeida and *H. capsulatum*, which are characterized by a thermal dimorphism. However, deep infections are most common in immunocompromised individuals, being acquired after the inhalation of conidia, by enteric invasion, or by implantation of the fungus during abrasion or penetrating injury to the host. In this case, the organism involved is named **opportunist** and the resulting infection is globally described under the term of **opportunistic infection**. Opportunistic fungi are those traditionally considered as saprobes or innocuous, or with a slow pathogenicity degree, which are able to cause serious and even fatal infections, when the host immune response is decreased or absent. Different fungi have been described as causal agents of opportunistic infections, e.g., *Candida* spp., *Cryptococcus* spp., species of zygomycetes, and most of the previously mentioned filamentous fungi such as *Aspergillus* or *Fusarium* (Sigler 2003, Summerbell 2003, de Hoog et al. 2011, Guarro 2012). Some of the genera treated in the present thesis have been also reported as opportunistic pathogens.

Opportunistic infections have gradually increased in recent decades, showing high morbidity and mortality rates, especially in immunocompromised patients. The management and treatment of these infections are difficult to establish for several reasons: i) different degree of virulence among the causal agents; ii) variable or poor response to most of the

currently available antifungal drugs; iii) the limited knowledge of the genera and species of the implicated pathogens; iv) the limited accessibility and high cost of the antifungal therapies; v) and the slow availability of fast diagnostic tools that allow a quick and exact diagnosis (Nucci & Marr 2005, Richardson & Lass-Flörl 2008, Guarro 2012, Athanasakis et al. 2013, Heimann et al. 2014).

1.2. Taxonomy and nomenclature

The taxonomy of fungi follows a Linnaean hierarchy, which organizes the fungi in different taxonomic ranks. All ranks are defined with a Latin name that must be written in italics (at least genus and species). In general, each category above genus has a particular ending (e.g., -*mycota*, -*mycetes, -ales*) which indicates its hierarchical position (phylum, class and order, respectively), except the categories, Kingdom and Subkingdom, and the two lowest categories, genus and species. For example, the taxonomic position of *Bionectria tonduzii* Speg. is: **Kingdom** *Fungi,* **Subkingdom** *Dikarya,* **Phylum** *Ascomycota,* **Subphylum** *Pezizomycotina,* **Class** *Sordariomycetes,* **Subclass** *Hypocreomycetidae*, **Order** *Hypocreales,* **Family** *Bionectriaceae,* **Genus** *Bionectria,* **Species** *B. tonduzii.* Since Linnaeus (1753), the correct formulation for species names is the Latin binomial or binary nomenclature, *Genus* (capitalized) *species* (non-capitalized) Authority, e.g. *Emericellopsis terricola* J.F.H. Beyma. The "authority" part of the name attributes the responsibility for the original description of the species epithet and its classification in the genus in question (Seifert et al. 2011).

For many years, the formal activities about scientific naming of fungi, and many of those involving their classification, have been governed under the rules of the *International Code of Botanical Nomenclature* (ICBN), which since 1867 has been published with updates every five years at the International Botanical Congress (McNeill et al. 2006). A special provision of the ICBN (Article 59) has allowed pleomorphic fungi to have separate names ("dual nomenclature") for asexual and sexual stages [e.g., *Cryptococcus neoformans* (San Felice) Vuill. for the asexual morph, and *Filobasidiella neoformans* Kwon-Chung for the sexual morph]; when referring to the whole fungus (holomorph), the teleomorph name has taken precedence (McNeill et al. 2006). However, the fungal community (Hawksworth 2004, Hawksworth et al. 2011, Taylor 2011) has expressed the need to abolish the dual nomenclature and to use a single-name nomenclatural system for all fungi (**one fungus = one name** or **1F=1N**), through "The Amsterdam declaration on fungal nomenclature" (Hawksworth et al. 2011). In the last meeting of the International Botanical Congress held in Melbourne in July 2011, several important decisions were taken concerning fungal taxonomy; among them, the acceptance of the Amsterdam Declaration. The latest version of the Code is now called *International Code of Nomenclature for Algae, Fungi and Plants*

(ICN), and in its new edition, the concept of dual nomenclature was removed, giving anamorph names the same priority as teleomorph names, thus leading to a new era in fungal taxonomy, where one name will be applied to every fungal taxon (McNeill et al. 2011, Norvell 2011, Wingfield et al. 2012). In addition, the ICN established that all nomenclatural details of fungal novelties should be registered in a public database, such as MycoBank (Crous et al. 2004) or Index Fungorum (www.indexfungorum.org), Latin diagnoses are not required anymore, and the English diagnoses are accepted by the new Code (Hawksworth 2011). In this thesis, we have followed the new rules established in the new Code. The biggest question now is which name to retain (**one fungus = which name or 1F = ?N**) for a holomorphic species. In this sense, a preliminary list of protected generic names for fungi has been recently published (Kirk et al. 2013) in order to be ratified by the next International Botanical Congress (IBCXIX, China, 2017) (Hawksworth 2012). The choice of these names is crucially important and will impact strongly on different fields of mycology, such as plant pathology and clinical mycology.

1.2.1. Species concepts in fungi

A species is the basic rank of biological taxonomy, but there is not a single universally accepted definition for species delimitation (Guarro et al. 1999, de Queiroz 2007, Seifert et al. 2011, Costello et al. 2013). Different concepts have been used in mycology to distinguish fungal species. The **Morphological or Phenotypic Species Concept (MSC)** defines a species based exclusively on its phenotypic features (morphology and physiology), and the discontinuity in these characters is used to differentiate among them (Guarro et al. 1999). This concept has been widely used by mycologists for a long time and applied to establish most of the fungal species known to date (Alexopoulos et al. 1996, Kirk et al. 2008). However, the phenotypic features are strongly affected by environmental conditions, and species diagnosed under this approach often comprise more than one biological-, ecological- or phylogenetic species (Taylor et al. 2000, Crous et al. 2009). The **Biological Species Concept** (BSC) emphasizes gene exchange within species (sexual and parasexual reproduction) (Davis 1995), and delimits species as groups of individuals that are able or potentially capable to mate and are reproductively isolated from other such populations (Mayr 1942). However, this concept cannot be applied to asexually-reproducing fungi (Seifert et al. 1995), which is a serious problem because approximately 20 % of fungi are morphologically asexual and do not produce sexual spores (Reynolds 1993). The **Ecological Species Concept** (ESC) emphasizes adaptation to a particular ecological niche (van Valen 1976) rather than on reproductive isolation and it is often used for plant-pathogenic fungi (Guarro et al. 1999). With the development of molecular techniques, especially DNA sequencing, the **Phylogenetic Species Concept** (PSC), that emphasizes

nucleotide (non-) divergence, has been introduced into systematics and has been useful to define fungal groups with asexual reproduction. Under this concept a species is defined as the smallest diagnosable monophyletic group of individual organisms, within which there is a pattern of ancestry and descent (Cracraft 1983). Phylogenetic species share an exclusive combination of primitive and derived characters, which is diagnosable from all other species (Cracraft 1983). Although using the PSC makes it easy to define individual groups, depending on the gene used the decision about where to place the limit of the species is subjective (Taylor et al. 2000). An adaptation of the PSC was proposed by Taylor et al. (2000) to delimit fungal species, namely the **Genealogical Concordance Phylogenetic Species Recognition** (GCPSR). In this approach, DNA sequences from multiple independent loci are analyzed individually and in combination, and the results are compared to delimit fungal species. The limit between species is established where the groupings suggested by the different analyses are discordant. This methodology has been accepted by many molecular taxonomists as an objective method and a benchmark for phylogenetic species delimitation in mycology (Giraud et al. 2008, Seifert et al. 2011, Crous et al. 2014a). In addition, it has been useful to describe cryptic species, i.e., those genetically distinct phylogenetic species that cannot or can scarcely be differentiated by morphological characters (Cruse et al. 2002, Crous et al. 2014a). This concept has been used in different genera such as *Fusarium* (O'Donnell 2000, O'Donnell et al. 2004), *Trichoderma* Pers. (Druzhinina et al. 2006, Sandoval-Denis et al. 2014), *Cladosporium* Link (Bensch et al. 2010), *Pseudallescheria* Negr. & I. Fisch. (Gilgado et al. 2005), and *Sporothrix* Hektoen & C.F. Perkins (Marimon et al. 2006, 2007), *Diaporthe* Nitschke (Gomes et al. 2013) among others. During the last decade the **polyphasic approach** or **polyphasic taxonomy** has been widely used for species delimitation within the fungal kingdom, by integrating multilocus sequence data or GCPSR, morphological and physiological characteristics, and ecological data (Samson et al. 2007, Samson & Varga 2009). This approach has been recently proposed under the formal name **Consolidated Species Concept** (CSC) (Quaedvlieg et al. 2014) and the underlying principle is generally accepted within the mycological community, being applied in different fungal genera such as *Aspergillus* (Hong et al. 2005, Samson et al. 2007), *Penicillium* Link (Frisvad & Samson 2004), *Saksenaea* S.B. Saksena (Alvarez et al. 2010), *Ramularia* Unger (Videira et al. 2015), *Cercospora* Fresen (Groenewald et al. 2013, Bakhshi et al. 2015), *Alternaria* Nees (Woudenberg et al. 2013), and members of *Teratosphaeriaceae* (Quaedvlieg et al. 2014), among others.

1.2.2. Traditional and modern criteria for fungal species delimitation

In the past, the classification and taxonomic position of fungi were based on morphological features, considered priority, and which formed the basis for ranks such as

species, genera, classes, etc. In the case of the sexual morphs of *Basidiomycota* and *Ascomycota*, meiospores are called **basidiospores** and **ascospores**, respectively. Basidiospores are produced externally on structures called **basidia**, which are developed in the cavity of fruiting bodies called **basidiomata** or **basidiocarps** (Alexopoulos et al. 1996). In contrast, ascospores are produced within sac-like structures called the **ascus** that are usually enclosed in fruit bodies, the **ascomata** or **ascocarps**. The latter structure can be composed of a network of hyphae with asci at the centre (**gymnothecium**), completely closed (**cleistothecium**), open and cupulate (**apothecium**), globose or flask-shaped (**perithecium**) and mostly with an apical opening (**ostiole**), or asci develop in cavities (loculi) of an **ascostroma**, where they are arranged in a hymenium or clustered on cushion-like structures (Kendrick 2001, Guarro et al. 2012). In the sexual morphs, the morphological features of the fruiting body, the basidium/asci and basidiospores/ascospores have been used for identification purposes (Guarro et al. 1999). However, in the case of the asexual morphs, the morphological study of their fertile structures, including conidia, conidiophores, and conidiogenous cells have been useful criteria to define morphospecies (Guarro 2012).

Based on conidia formation, the asexual fungi were traditionally grouped in three major groups: **blastomycetes**, **coelomycetes** and **hyphomycetes**. Although these terms do not represent taxonomic categories or natural groups, they are still used in order to facilitate the morphological identification of these organisms (Seifert et al. 2011). The former group included asexually reproducing yeast. Coelomycetes was formed by anamorphs with septate hypha, conidia originating inside of fruiting bodies (**conidiomata**), that can be flattened or cup-shaped (**acervuli**), or spherical with an apical pore (**pycnidia**). Hyphomycetes included organisms with a filamentous thallus, forming conidia exogenously on open structures (never enclosed within a covered conidioma), and directly on simple or in aggregated hyphae (Kendrick 2001, Kirk et al. 2008). Phenotypic features, including macro- and microscopic characters and conidial ontogeny (**conidiogenesis**) have been considered as primary characters for identification of hyphomycetes (Dixon & Salkin 1986, de Hoog et al. 2011). Other characteristics such as temperatures for growth and in some cases production of metabolites are also used, but mainly to distinguish species.

In hyphomycetes, the **colonies** can have different colours, textures (slimy, membranous woolly, velvety, fasciculate, etc), morphologies (flat, raised, umbonate, crateriform, etc), exudates and diffusible pigments, which are useful taxonomic features but strongly affected by medium composition and environmental conditions (Dixon & Salkin 1986, de Hoog et al. 2011).

Hyphae are divided into compartments or cells by cross walls called **septa**, which are distributed at regular intervals. In basidiomycetes, hyphae produce an outgrowth (**clamp connection**) that develops at a septum, grows backward and fuses with the preceding cell

(Kirk et al. 2008). According to the degree of melanization, hyphae can be described as hyaline (without pigment) or dematiaceous (darkly pigmented). Hyphal walls can be thin or thick, smooth or sometimes they can be ornamented with different kind projections, ranging from minute irregularities to coarse warts (Kiffer & Morelet 2000). Commonly, hyphal cells have a constant width, but in some occasions they are swollen with a constriction at the septum and are referred to as "moniliform hyphae" (de Hoog & Hermanides-Nijhof 1977).

Conidiophores are fertile hyphae on which one or several conidiogenous cells are borne. They can be single or form columnar aggregations called **synnemata** (e.g. *Stilbella* Lindau), or cushion-shaped masses named **sporodochia** (e.g. *Epicoccum* Link) (Alexopoulos et al. 1996). The single conidiophores can be with or without lateral branches (**branched** and **simple**/**unbranched,** respectively). Branches can arise near the base, the middle or the tip of the conidiophore, which is described as **basitonous**, **mesotonous** and **acrotonous** branching, respectively (Gams 1971). When the branches are disposed forming whorls or penicilli (broom- or brush-like) the conidiophore is described as **verticillate** or **penicillate**, respectively (Seifert et al. 2011). When the conidiophore ends its growth after the production of a conidium, the conidiophore and the conidiogenous cells are called **determinate**; but if it continues growing after the formation of the first conidium, the conidiophore and conidiogenous cells are called **percurrent** (e.g. *Phaeoacremonium* W. Gams, Crous & M.J. Wingf.). Proliferation may also be produced in a sympodial way, i.e., by a succession of apices that develop behind and to one side of the previous apex, as it occurs in the genus *Ochroconis* de Hoog & Arx (Ellis 1971, de Hoog & von Arx 1973).

The **conidiogenous cell** is the cell from which one or more conidia are formed (Kirk et al. 2008). It can be borne directly from a vegetative hypha or on differentiated supporting structures such as stipes and branches, and it has one or many conidiogenous loci (areas on the conidiogenous cell where conidia are produced and attached) (Samson et al. 2010). The kind of conidiogenous cell depends of the type of **conidiogenesis** (Fig. 2). This process is described as progressive or percurrent (explained above), and **retrogressive** when the conidiogenous cell shortens after each conidium is produced (e.g. *Trichothecium* Link), or **stationary** when no changes take place (Alexopoulos et al. 1996).

There are two basic types of conidiogenesis, blastic (Fig. 2A) and thallic (Fig. 2B). In **blastic conidiogenesis**, the wall of the cell bulges out to form the wall of the conidium, whose initial shape is recognizable before the basal septum is formed. Conidiogenous cells and conidia usually have two wall layers. When both conidial layers are continuous with those from the conidiogenous cell, the term **holoblastic** is used (Fig. 2C); if the conidia are produced from a single point it is called **monoblastic**, but if there are more conidiogenous loci it is called **polyblastic** (Ellis 1971). When several holoblastic conidia are produced simultaneously on the surface of the conidiogenous cell (e.g. *Cephaliophora* Thaxt. or

Botrytis P. Micheli), the process is described as **synchroneous** (Fig. 2D). When a single holoblastic conidium is formed and the conidiogenous cell grows out laterally to produce a new one, the mechanism is known as **sympodial** conidiogenesis (Fig. 2E). This process can be repeated many times, so that a long outgrowth is formed, which, after conidial secession, bears denticles or flat scars (Ellis 1971); as such it occurs in *Ochroconis* and *Curvularia* Boedijn, respectively.

On the other hand, when the outer wall of the conidium is only continuous with the inner part of the cell wall or is completely independent from it, the conidiogenesis is described as **enteroblastic** (Fig. 2F). When in this type of conidiogenesis the cells give rise to conidia by protrusion of the inner wall through one or more channels or "pores" in the outer wall, it is described as **tretic**, e.g. *Alternaria* (Kirk et al. 2008). One or more pores can be involved in the process, being described as **monotretic** or **polytretic**, respectively (Mercado-Sierra et al. 1997). Conidia can be arranged in branched or unbranched chains; if the youngest conidium is at the tip of the chain (e.g. *Alternaria*, *Cladosporium*), it is called an **acropetal chain** (blastocatenate); if the youngest conidium is at the base of the chain, it is called a **basipetal chain** (e.g. *Acremonium* Link, *Acrophialophora* Edward) (Kiffer & Morelet 2000). When enteroblastic conidia are produced from an opening of a conidiogenous cell, the process is described as **phialidic** (Fig. 2G) and such conidiogenous cell is called a **phialide**, (e.g. *Fusarium*, *Aspergillus*). The phialide may have different shapes (awl, flask, cylindrical), and sometimes shows a collarette (cup-shaped structure at the apex), such as *Phialophora* Medlar. They can be located in a lateral or terminal position on the hyphae, as well as between two septa from this (**intercalary** phialide). Phialides with one conidiogenous opening are called **monophialidic**, and with more openings not delimited by a septum, are called **polyphialidic** (Ellis 1971).

Another kind of enteroblastic conidiogenesis is the **annellidic** (Fig. 2H), where the conidia are formed from a series of short percurrent proliferations (annellations) on a conidiogenous cell called the **annellide** (Ellis 1971). A typical example of the annellidic conidiogenesis is observed in the genera *Scopulariopsis* Bainier and *Cephalotrichum* Link.

In **thallic conidiogenesis** (Fig. 2B), numerous septa are formed and the entire conidiogenous cell (in this case, the hypha) breaks up to produce one or more conidia. This kind of conidiogenesis may occur in two different ways: **holothallic** (Fig. 2I) or **thallic-arthric** (Fig. 2J). In the former, a portion of the hyphae converts into a single conidium (e.g. *Microsporum* Gruby or *Trichophyton* Malmsten); whereas, in thallic-arthric conidiogenesis the hyphae are transformed into a series of conidia (de Hoog et al. 2011). The later mechanism is subdivided into four types: i) **holoarthric** (Fig. 2K), when the cells of the hyphae are disarticulated to form chains of conidia, called **arthroconidia** (e.g. *Geotrichum*

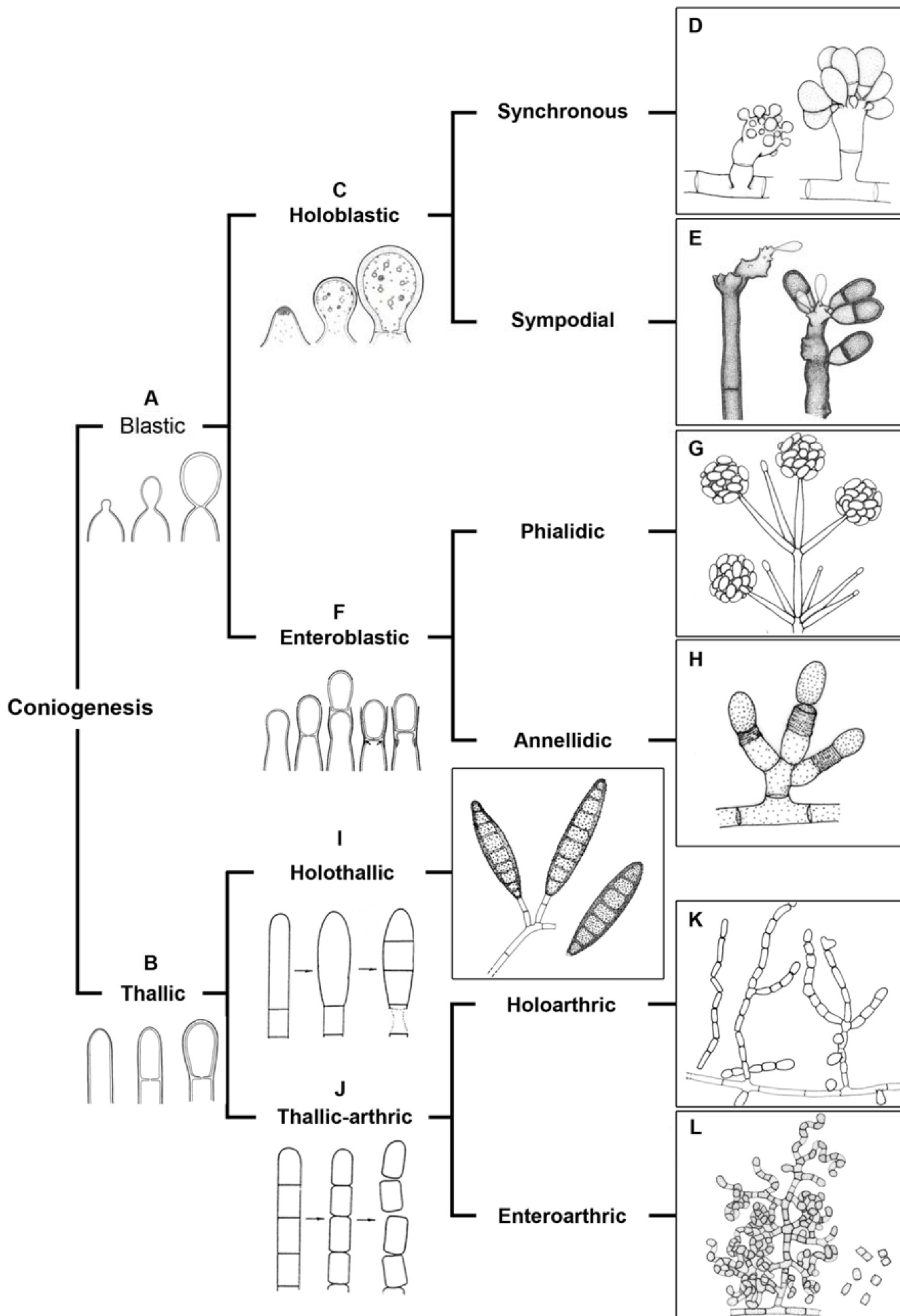

Fig. 2 Basic types of conidiogenesis. Blastic (A, C–H) and thallic (B, I–J); synchronous (D, *Cephaliophora);* sympodial (E, *Anungitopsis*); phialidic (G, *Verticillium*); annellidic (H, *Cephalotrichum*); holothallic (I, *Microsporum*); holoarthric (K, *Arthrographis*); enteroarthric (L, *Malbranchea*). Images modified from Cole & Samson (1979), Gené (1996), de Hoog et al. (2011).

Link, *Arthrographis* G. Cochet ex Sigler & J.W. Carmich); ii) **enteroarthric** (Fig. 2L), when some hyphal cells degenerate to release the intervening cells as alternate arthroconidia (e.g. *Malbranchea* Sacc., *Coremiella* Bubák & Krieg); iii) **endogenous**, when the fertile hyphae produce internal wall layers that surround the nuclei, each becoming an individual conidium (endoconidium), which are released after the rupture of the mother cell (e.g. old cultures of *Aureobasidium* Viala & G. Boyer); iv) **sarcinic**, when fertile hyphae increase in size and develop transversal and longitudinal septa, producing multiseptate conidia (e.g. *Botryomyces caespitosus* de Hoog & C. Rubio) (de Hoog et al. 2011).

One of the most important criteria used for fungal identification is the conidium morphology (Samson et al. 2010). Hyphomycetes produce immobile **conidia**, ranging from unicellular (e.g. *Aspergillus),* bicellular (e.g. microconidia of *Fusarium*) and multicellular (e.g. *Cylindrocarpon* Wollenw.). According to Saccardo's spore terminology those unicellular conidia are called amerospores, two-celled conidia are didymospores, and multicellular conidia are called phragmospores (transversely septate) or dictyospores (transversely and longitudinally septate) (Alexopoulos et al. 1996). Presence or absence of pigmentation in the conidia is an important character, as well as the shape, which may be globose, elliptical, fusiform, cylindrical, branched or spirally coiled (helicospores) or star-shaped (staurospores), among others (Kirk et al. 2008). As well as the hyphae, the wall of the conidia can show different textures, i.e., smooth, rough, echinulate, warty, etc., which are better appreciated under electron microscopy than conventional microscopy. Conidia are produced at the apex or side of a conidiogenous cell, either arranged **singly**, in **chains** or in **slimy heads**, and are liberated by cleavage at a separating septum (**schizolytic** mechanism) or by fracture of the lateral wall between two septa (**rhexolytic** mechanism). In rhexolytic dehiscence, conidia usually show a conspicuous frill at their base, consisting of cell wall remnants from the lysed cell (Kiffer & Morelet 2000).

Not all fungal species are able to produce conidia. They can grow only as mycelium (***Mycelia sterile*** or **Agonomycetes**) or produce structures that allow the fungi to survive for long periods of scarcity of nutrients and hard environmental conditions. These structures include chlamydospores, sclerotia, stromata and bulbils, which are also produced by some fungal species, in addition to conidia (Alexopoulos et al. 1996, Kiffer & Morelet 2000). **Chlamydospores** are distinguished from ordinary conidia by a thicker wall and tighter connection with the hyphae (not seceding easily). Their main function is to ensure long-time survival rather than massive distribution. Its shape, number of cells, grouping (solitary, chains, clusters) and position (terminal, lateral and intercalary) are criteria also used in morphological identification, and can be useful to distinguish among similar morphologically species, such as *Sarocladium kiliense* (Grütz) Summerb., produces chlamydospores, and *S. strictum* (W. Gams) Summerb., does not produce chlamydospores (Summerbell et al.

2011). **Sclerotia** are masses of hyphae that form a hard, frequently rounded structure with a differentiated rind in or on a host, but are not associated with spore production at any time. **Stromata** are similar to sclerotia as they also are hard masses of fungal tissue that may include host tissue, but they usually are irregular in shape and not rounded (Alexopoulos et al. 1996, Kendrick 2001). Sclerotia and stromata are more rarely produced and in sometimes are used as generic or specific morphological criteria (Crous et al. 2009). These structures resistance can be observed in *Acremonium sclerotigenum* (Moreau & R. Moreau ex Valenta) W. Gams and *Acremonium stromaticum* W. Gams & R.H. Stover, respectively (Gams 1971, 1975).

Another structure not associated with fungal survival is represented by the **setae**, which are sterile hyphae, stiff, erect, often pointed which protrude from a fertile layer or fruiting body and may have a protective function (Kendrick 2001). They can be produced by fungal species with sexual (e.g., *Hymenochaete* Lév.) or asexual morphology (e.g., *Monocillium* S.B. Saksena), and although setae are mostly sterile, sometimes they are capable of producing conidia at their tips (e.g., *Colletotrichum* Corda or *Cylindrotrichum* Bonord.) (Alexopoulos et al. 1996).

The examination of those morphological features, depending on the reproductive structures developed by the fungus studied, has allowed during many years the description of diverse fungal genera and species. However, their study involves some limitations, such as: slow or absence of growth *in vitro* of some fungi, difficulties to sporulate on artificial media, overlapping of structures in related taxa, among others. These reasons hinder a proper identification of many fungi or lead to a subjective interpretation of the taxonomical value of some morphological features for delineation of fungal species. Due to these problems, morphological identification of fungi is a difficult task, mainly for non-specialist personnel such as the clinical microbiologist, who usually has little training in traditional mycology (Shenoy et al. 2007, Balajee et al. 2009). In this sense, the use of molecular tools, mainly those based on **DNA sequencing**, shows several advantages in fungal species delimitation or identification: i) it is not necessary to have live material (e.g., in the case of a biopsy), ii) results can be obtained rapidly after the DNA extraction, iii) they generate objective results and the results are reproducible in different laboratories, iv) they can be performed by “non-specialized personnel”, and v) they can be cheaper on the long run (Balajee et al. 2009, Samson et al. 2010).

Currently the sequence-based identification can be considered the new "gold standard" for fungal species delimitation (Summerbell et al. 2005). With this purpose as well as for phylogenetic studies, the **nuclear ribosomal DNA operon** (nrDNA) has been used extensively (White et al. 1990, Mitchell & Xu 2003, Begerow et al. 2010). This operon is tandemly repeated and codes for the nuclear RNA component of the ribosomes, and

consists of the small subunit (SSU) 18S rRNA gene, the 5.8S rRNA gene and the large subunit (LSU) 28S rRNA gene. Between SSU and the 5.8S rRNA genes, and between the 5.8S rRNA gene and LSU, the internal transcribed spacer regions (ITS), ITS 1 and ITS 2, respectively, are found. These two spacers, including the 5.8S rRNA gene, are usually referred as the ITS region. The region located between the LSU and SSU genes is known as the intergenic spacer region (IGS), and is sometimes used for inter- or intra-species variation studies (O'Donnell et al. 2009, Migheli et al. 2010). The 18S, 28S and 5.8S rRNA genes evolve relatively slowly and are frequently used to study higher-order relationships, for example between families, orders and genera (Réblová et al. 2004, Shenoy et al. 2007, Summerbell et al. 2011). However, the initial portion (5' end) of the 28S rRNA gene shows a variable region, the D1/D2 domains, which are used to discriminate species in yeast (Kurtzman & Robnett 1998, Fell et al. 2000, Samson et al. 2010, Schoch et al. 2012), but also proved useful to distinguish species in some groups of filamentous fungi, such as *Phialemonium* W. Gams & McGinnis, *Lecythophora* Nannf., *Phialemoniopsis* Perdomo, Dania García, Gené, Cano & Guarro, and *Ochroconis*, among others (Yarita et al. 2007, Perdomo et al. 2011a, 2013; Samerpitak et al. 2014).

The ITS region is the most common target used for species-level identification, for several reasons, i.e., i) because of the high evolutionary rate of the internal transcribed spacers, ii) it is present in multiple copies in the fungal genome, which makes it easy to amplify for most fungi, iii) the presence of flanking conserved regions allow its amplification with universal primer sets, iv) and finally, it has the advantage that several public databases such as the NCBI's GenBank (http://www.ncbi.nlm.nih.gov/), the European Molecular Biology Laboratory nucleotide sequence database (EMBL, http://www.ebi.ac.uk), the Biological Resource Center of the National Institute of Technology and Evaluation (NITE, NRBC, http://www.nbrc.nite.go.jp/e/), and the CBS-KNAW Fungal Biodiversity Centre (http://www.cbs.knaw.nl/), among others, contain a large number of sequences from this region, enabling a ready comparison of the sequence from an unknown isolate (Balajee et al. 2009, Samson et al. 2010). However, in some genera (e.g. *Aspergillus*, *Trichoderma*, *Ramularia*, *Fusarium*, *Phaeoacremonium*, *Cladosporium* and *Alternaria*), the ITS region alone does not have enough resolution to separate between closely related species and can only be used as a starting point for identification. As a consequence it is necessary to use other gene sequences (mainly from protein-coding regions) to resolve species delineation within a species complex (O'Donnell et al. 2004, Druzhinina et al. 2005, Mostert et al. 2005, Geiser et al. 2007, Bensch et al. 2010, Woudenberg et al. 2013). Although, the **ITS region** has been proposed as the standard **barcode** (a fragment of 500–800 bp DNA sequence used for species identification) for fungal species identification (Schoch et al. 2012), additional studies carried out by different international working groups to propose alternative

or secondary barcodes that will distinguish species with better resolution are already well-advanced (Quaedvlieg et al. 2012, Gryganskyi et al. 2013, Tretter et al. 2013). Among the protein-coding genes commonly used to resolve species we can cite β-tubulin (*BT2*), translation elongation factor 1-alpha (*TEF1-α*), RNA polymerase II second largest subunit (*RPB2),* actin *(ACT),* calmodulin (*Cmd*), and histone H3 (*HIS*). These genes often contain introns, which are highly variable, making them useful targets for species identification. However, the choice of which protein-coding gene to use depends on the organism, because the level of differentiation and time of evolution differs widely among fungal groups, as well as the availability of a validated sequence database for query and comparison (Mitchell & Xu 2003, Balajee et al. 2007, Crous et al. 2009, Samson et al. 2010).

Once the appropriate DNA region has been sequenced, it is necessary to compare with DNA sequences in a database (such as those previously mentioned). This is usually performed using the BLAST (basic local alignment search tool) algorithm to find regions of homology between two sequences (Altschul et al. 1990) and establishes a percentage of identity between the unknown sequence(s) and the closest reference sequences in the database. This percentage is referred to as the number of identical nucleotides between two compared sequences. In general, identity values of 99–100 % with one or more reliable sequences (ideally from ex-type cultures) of a known species in the used database are accepted for species identification (Balajee et al. 2007). However, due to the fact that error rates in fungal identifications within GenBank (perhaps the most commonly used database) have been found to be as high as 20 % (Nilsson et al. 2006), and since there is no consensus about breakpoint values for species identification (Balajee et al. 2009, Samson et al. 2010), the identification based on BLAST results need to be interpreted carefully.

In recent years, there is a trend to delimit fungal species through the **phylogenetic analysis** of one or more genes. When several genes are used, the approach is called multilocus sequence analysis that is linked to the GCPSR concept proposed by Taylor et al. (2000). Phylogenetic analyses are conducted in order to establish an evolutionary hypothesis, and classify species in a way that, as closely as possible, reflects their evolution (Wiley et al. 1991). Thereby, when a DNA fragment of a given locus is compared among several species, two closely related individuals are more likely to share the same polymorphisms than two distant ones. The ideal is that all taxonomic categories are natural or **monophyletic** groups, i.e., comprising one ancestor and all of its descendants only (Alexopoulos et al. 1996). In the case of genera, two individuals can be maintained in the same genus if they show similar phenotypic characteristics and belong to the same family or at least to the same order, according to phylogenetic analyses (Arzanlou et al. 2007, Crous et al. 2007). However, in the last decades, several genera and higher taxonomic ranks, proposed on the basis of phenotypic characters, are identified as being either **paraphyletic**,

i.e., comprising an ancestor but not all its descendants, or **polyphyletic**, i.e., comprising descendants from different ancestors (Seifert et al. 2011). The evolutionary relationships among different taxa are graphically represented through a **phylogenetic tree**, where i) the topology shows the phylogenetic relationships, ii) the length of the branches is proportional to the distance between them, and, iii) each node or branching point represents the common ancestor of the branches arising from that point (Kendrick 2001, Chun & Hong 2010). Different methods or algorithms can be used for phylogenetic reconstruction. They can be classified into four groups, i.e., **distance**, **maximum parsimony** (MP), **maximum likelihood** (ML) and **Bayesian inference** (BI). Distance-based methods (e.g., Neighbour-Joining, NJ and Unweighted Pair-Group Method with Arithmetic means, UPGMA) attempt to produce a tree that fits to a matrix of pairwise evolutionary distances, thus, they do not use sequence information directly (non-character-based). On the other hand, MP, ML and BI utilize direct sequence information (character-based), applying an explicit model of evolution in the latter two algorithms (Chun & Hong 2010). The robustness of the phylogenetic analysis is tested through statistical methods such as bootstrapping and jackknifing (being the former the most commonly used in biological sciences), which generate a **support value** (Efron 1981, 1982). Thereby, a value of 100 % means that the taxa connected to that node were clustered in the same clade in all bootstrap replicates. Lower bootstrap support values represent less confidence in the clustering of the isolates. In order to compare the resulting trees, different phylogenetic methods can be applied to the same data set. If the topology and bootstrap support values are similar or identical, independently of the algorithm used, confidence that the best possible topology based on the underlying data was achieved is increased.

1.3. Acremonium-like fungi

Acremonium-like fungi are defined as those asexual morphs with a very simple conidiogenous apparatus, usually consisting of solitary orthotropic phialides (i.e., conidiogenous cell borne perpendicularly from the vegetative hyphae with a basal septum in the side branch) to conidiophores with one or a few branches, and unicellular conidia arranged in slimy heads or chains. Numerous ascomycetous genera produce an acremonium-like asexual morph (Gams 1971, Guarro et at. 2012). Some examples are: *Emericellopsis* J.F.H. Beyma, *Nigrosabulum* Malloch & Cain, *Mycoarachis* Malloch & Cain, *Hapsidospora* Malloch & Cain, *Bulbithecium* Udagawa & T. Muroi, *Nectriopsis* Maire, *Heleococcum* C.A. Jørg, *Nectria* (Fr.) Fr, *Nectriella* Nitschke ex Fuckel, *Pronectria* Clem. and *Leucosphaerina* Arx. However, the representative genus of these fungi is *Acremonium.*

The genus *Acremonium* currently contains more than 100 species (Domsch et al. 2007, Summerbell et al. 2011). Most of these are saprobic and are commonly isolated from

dead plant material and soil (Gams 1971, Gené 1994, Domsch et al. 2007). Some species are opportunistic pathogens of human and animals (Guarro et al. 1997, Summerbell 2003, Guarro 2012), while other species have been recognized as plant pathogens (Alfaro-García et al. 1996, Ko & Kunimoto 1999, Lin et al. 2004). In humans, *Acremonium* species can produce localized infections, mainly associated with traumatic inoculation in immunocompetent patients, such as keratitis, mycetoma, onychomycosis, otomycosis or complications in burn patients (Guarro et al. 1997, Gupta et al. 2000, 2012; Kan et al. 2004, Das et al. 2010, de Hoog et al. 2011, Kim et al. 2014). More rarely, the species produce disseminated infections involving multiple organs following fungemia (Khan et al. 2011), generally in immunocompromised individuals such as those undergoing allogeneic haematopoietic stem cell transplant or acute leukaemia patients, receiving corticoid therapy or with AIDS (Guarro et al. 1997, Das et al. 2010, de Hoog et al. 2011, Guitard et al. 2014). However, other species, like *A. chrysogenum* (Thirum. & Sukapure) W. Gams or *A. fusidioides* (Nicot) W. Gams, are important for the pharmaceutical industry because of the production of the β-lactam antibiotic cephalosporin C (Bloemendal et al. 2014) and fusidic acid, respectively (Domsch et al. 2007).

Morphologically, the species of this genus are characterized by producing colonies with slow or moderate growth (Fig. 3A–C), thin hyphae, simple or poorly basitonously branched conidiophores (Fig. 3D, E), orthotropic phialides (Fig. 3D, F, G), usually gradually tapering toward the tip, and unicellular conidia, hyaline or pigmented, arranged in slimy heads, chains or both (Fig. 3F, G) (Gams 1971, Domsch et al. 2007). *Acremonium* species are morphologically similar to one another and can only be differentiated on the basis of subtle features (Perdomo et al. 2011b). These morphological characters are also present in other genera, such as *Phialemonium*, *Lecanicillium* W. Gams & Zare, *Simplicillium* W. Gams & Zare, *Lecythophora*, *Cylindrocarpon* and *Fusarium*, especially in the latter two when the isolates do not produce their distinctive macroconidia. Therefore, the identification at the species level and, to a lesser extent, at the genus level, based exclusively on morphological characters is difficult and the possibility of a misidentification is high (Guarro et al. 1999, Perdomo et al. 2011b).

Acremonium was erected by Link (1809) with *A. alternatum* Link as the type species. Later, Gams (1971) made an extensive revision of the genus and transferred several species of *Cephalosporium* Corda, *Gliomastix* Guég and the monophialidic species of *Paecilomyces* Bainier (Onions & Barron 1967) to the genus *Acremonium*. Based on morphological features, the eighty-two species treated in his monograph were distributed into three major sections: *Simplex*, a name later updated as the type section *Acremonium* (Gams 1975), *Gliomastix,* and *Nectrioidea.* At the same time, each section was divided into series. The section *Simplex* or *Acremonium* included species with mostly simple

conidiophores, without conspicuous periclinal wall thickening (a thickening of the wall around the apex of the phialide) and undulate outline; this section included the type species of the genus, *A. alternatum.* The section *Nectrioidea* encompassed species with branched conidiophores, phialides with periclinal thickening and usually with cylindrical collarettes, including many *Nectria sensu lato* asexual morphs. The species of the section *Gliomastix* were characterized by pigmented or hyaline conidia, and chondroid hyphae, which could be seen under the microscope as thick-walled hyphae and which render the colonies tough. Two new sections, *Albo-lanosa* and *Chaetomioides*, were introduced by Morgan-Jones and Gams (1982). The former included endophytic species belonging to the *Clavicipitaceae* family and the latter was formed by all the acremonium-like asexual morphs of *Chaetomium* Kunze and *Thielavia* Zopf species. Later, Lowen (1995) proposed the section *Lichenoidea* to accommodate lichenicolous species, such as *A. rhabdosporum* W. Gams and *A. spegazzinii* D. Hawksw.

Although Gams' monograph and the subsequent works have been a great contribution to the taxonomy of *Acremonium*, several molecular studies have demonstrated that this classification is artificial, because sections and series include fungi that are

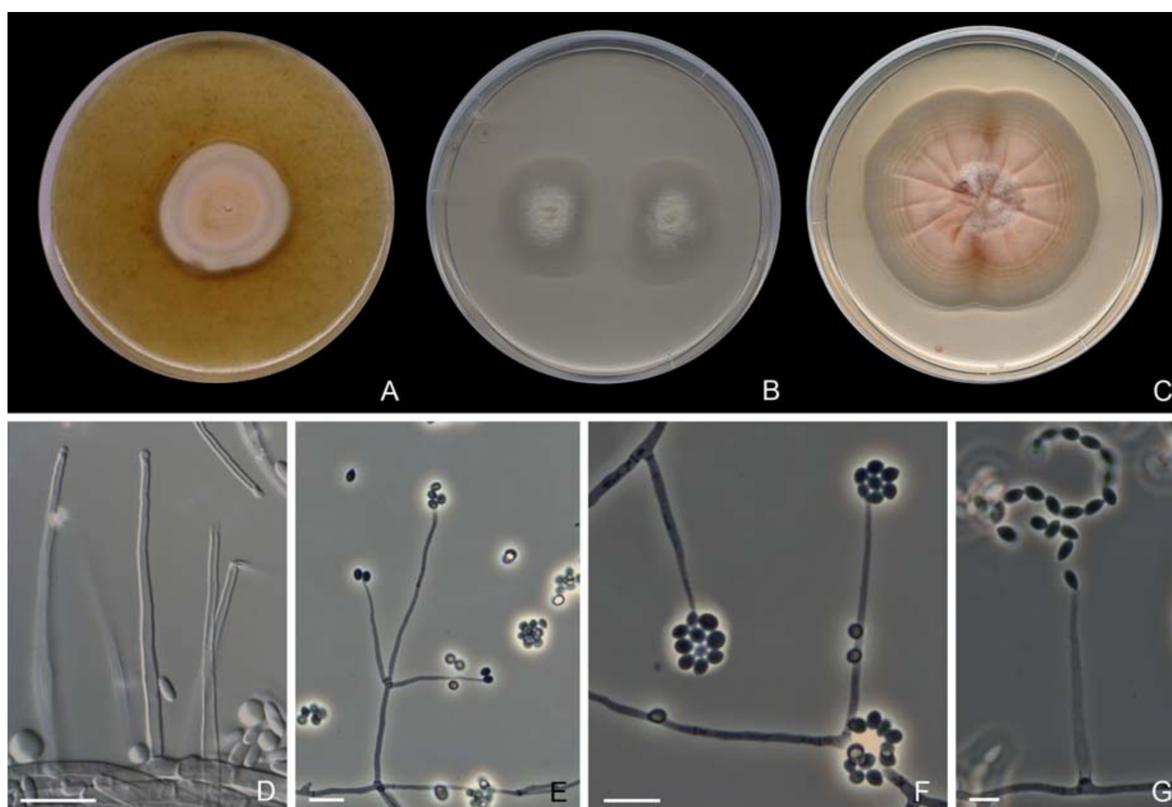

Fig. 3 Morphological features of *Acremonium* species. Colonies with slow (A, B) or moderate growth (C); simple (D) and poorly basitonously branched conidiophores (E); orthotropic phialides (D, F, G); conidia arranged in slimy heads (F) and chains (G). Scale bars = 10 µm.

phylogenetically divergent, scattered into different orders of *Sordariomycetes*. Therefore, *Acremonium* is nowadays considered a complex and large polyphyletic genus of *Ascomycota* with most of the species belonging to *Hypocreales* (Glenn et al. 1996, Gräfenhan et al. 2011, Kiyuna et al. 2011, Perdomo et al. 2011b, Summerbell et al. 2011, Grum-Grzhimaylo et al. 2013b). These molecular studies have revealed that species included within the section *Nectrioidea* (Gams 1971, 1975), such as *A. furcatum* Moreau & R. Moreau ex Gams, *A. stromaticum*, *A. restrictum* (J.F.H. Beyma) W. Gams, *A. brunnescens* W. Gams, *A. alcalophilum* G. Okada and *A. nepalense* W. Gams are members of *Plectosphaerellaceae* (Zare et al. 2007, Summerbell et al. 2011, Carlucci et al. 2012, Grum-Grzhimaylo et al. 2013a), a family outside of *Hypocreales* and basal to the *Glomerellales* (Réblová et al. 2011). Other species such as *A. kiliense* Grütz, *A. strictum* W. Gams, *A. zeae* W. Gams & D.R. Sumner have been recently transferred to the genus *Sarocladium* W. Gams & D. Hawksw. (Summerbell et al. 2011); *A. arxii* W. Gams, *A. berkeleyanum* (P. Karst.) W. Gams species complex and *A. cymosum* W. Gams are now placed in the genus *Cosmospora* Rabenh. (Gräfenhan et al. 2011); *A. falciforme* (Carrion) W. Gams is already recognized as a member of the *Fusarium solani* species complex (Summerbell & Schroers 2002), while *A. diospyri* (Crand.) W. Gams was transferred into *Nalanthamala* Subram. along with other nectriaceous species (Schroers et al. 2005). Based on sequences of the 18S rDNA, Glenn et al. (1996) relocated the endophytic species included in the section *Albo-lanosa* into the new genus *Neotyphodium* Glenn, C.W. Bacon & Hanlin (*Clavicipitaceae, Hypocreales*), while Liang et al. (2009) transferred several *Acremonium* species of the section *Chaetomioides* into the new genus *Taifanglania* Z.Q. Liang, Y.F. Han, H.L. Chu & R.T.V. Fox (*Chaetomiaceae, Sordariales*). In order to give nomenclatural stability to *Acremonium* and to clarify its taxonomy, Summerbell et al. (2011) redefined the type species of the genus based on the analysis of ribosomal gene sequences of numerous species of *Acremonium* and related genera. The strain CBS 407.66 was designated as the epitype of *A. alternatum,* which was included into a large phylogenetic clade with other *Acremonium* species and some ascomycetes of the family *Bionectriaceae*. Therefore, all those acremonium-like fungi that are phylogenetically close to that strain should be considered as an *Acremonium sensu stricto* (Summerbell et al. 2011). In the same study, some hypocrealean species were transferred to the genera *Sarocladium* and *Trichothecium* Link, and the genus *Gliomastix* was reinstated. Although this study has been an important step in clarifying the taxonomy of this complex genus, more studies are needed to define the taxonomic position of all those *Acremonium* species and acremonium-like fungi placed outside of the *Bionectriaceae.*

Perdomo et al. (2011b) carried out a morphological and molecular study of numerous clinical isolates of *Acremonium* from the USA, in which *A. sclerotigenum* and

A. egyptiacum (J.F.H. Beyma) W. Gams were shown to be a species complex, indistinguishable by their ITS sequences. In that study, this species complex was the most common, followed by the species *A. kiliense*, *A. implicatum* (J.C. Gilman & E.V. Abbott) W. Gams, *A. persicinum* (Nicot) W. Gams and *A. atrogriseum* (Panas.) W. Gams. The latter species has been recently relocated in *Phialemonium* as *P. atrogriseum* (Panas.) Dania García, Perdomo, Gené, Cano & Guarro (Perdomo et al. 2013). Even though most of the isolates included in the Perdomo's study were identified at the species level, some isolates did not match with any known *Acremonium* species at that time, and therefore could not be reliably identified.

Another hypocrealean genus with acremonium-like morphology is *Sarocladium*. This genus currently comprises ten species (Summerbell et al. 2011), including plant and human pathogens, as well as endophytes and fungiculous species (Gams & Hawksworth 1975, Chen et al. 1986, Helfer 1991). The species of the genus are common in soil, decaying plant residues, dung and sediments (Gams 1971, Gené 1994). The genus was recently reviewed by Summerbell et al. (2011), who transferred *Acremonium* species forming conidial chains to the genus *Sarocladium*, i.e., *A. bacillisporum* (Onions & G.L. Barron) W. Gams, *A. glaucum* W. Gams and *A. ochraceum* (Onions & G.L. Barron) W. Gams as well as the clinically important species *A. kiliense* and *A. strictum,* and the maize endophyte *A. zeae* (Summerbell et al. 2011). Although *Acremonium* and *Sarocladium* species produce morphological structures that overlap one another, such as colonies with slow or moderate growth (Fig. 4A–C), solitary phialides with a basal septum and hyaline conidia arranged in slimy heads or chains, in *Sarocladium* it is common to find repeatedly branched conidiophores (Fig. 4D), elongated phialides (Fig. 4E–G), fusiform or cylindrical conidia (Fig. 4H, I) and adelophialides (Fig. 4J). In contrast, in *Acremonium* species the latter character is usually absent, the simple or scarcely branched conidiophores are predominant and the conidia have other shapes apart from cylindrical and fusiform. Although both genera belong to the *Hypocreales*, they are phylogenetically distant. The type species of *Acremonium* is included within the *Bionectriaceae,* while that of *Sarocladium* is still regarded as *incertae sedis* at family level (Summerbell et al. 2011).

Sarocladium was erected by Gams & Hawksworth (1975) based on *Acrocylindrium oryzae* Sawada, a fungus previously described by Sawada (1922) from *Oryza sativa* in Taiwan. *Sarocladium oryzae* (Sawada) W. Gams & D. Hawksw., the type species of the genus, is an important and common pathogen of rice (*O. sativa*) and bamboo species (*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 different countries, producing yield losses of 3 to 85 % depending on disease severity (Sakthivel et al. 2002). This species is also known for the production of

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Dixie Alejandra Giraldo López
Dipòsit Legal: T 1677-2015

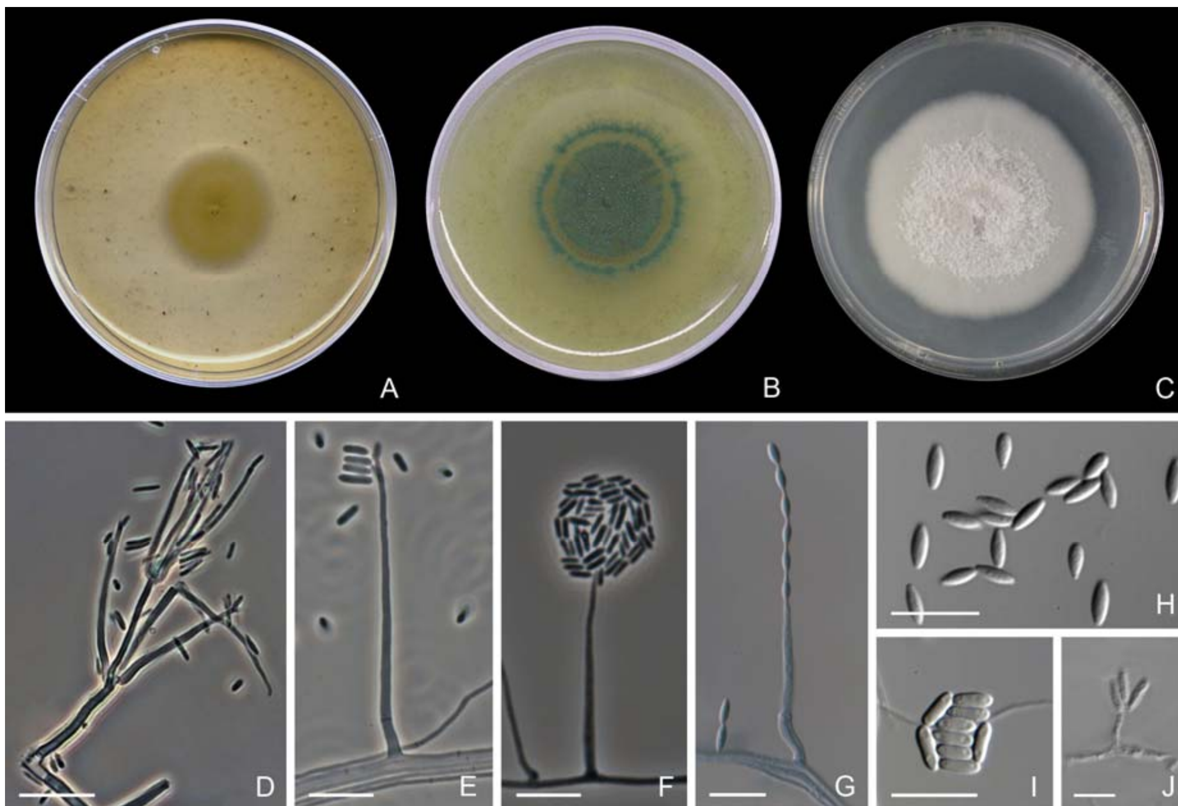

Fig. 4 Morphological features of *Sarocladium* species. Coloured (A, B) and whitish (C) colonies, with slow (A) or moderate growth (B, C); branched conidiophore (D); elongated phialides (E–G); fusiform (H) and cylindrical (I) conidia; adelophialide (J). Scale bars D–I = 10 µm, J = 5 µm. (Images G and J are modified from Perdomo et al. 2011b).

antimicrobial secondary metabolites, such as helvolic acid and cerulenin (Tschen et al. 1997, Ghosh et al. 2002, Bills et al. 2004b). *Sarocladium attenuatum* W. Gams & D. Hawksw. and *S. sinense* J.D. Chen, Guo C. Zhang & X.H. Fu 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 zeae* (W. Gams & D.R. Sumner) Summerb. is a protective endophyte of maize and an economically important species because it produces secondary metabolites (i.e., Pyrrocidines A and B, and Dihydroresorcylide) with antifungal activity against pathogenic fungi such as *Aspergillus flavus* Link and *Fusarium verticillioides* (Sacc.) Nirenberg or other pathogens (Poling et al. 2008, Wicklow et al. 2005, 2008, Wicklow & Poling 2009). *Sarocladium zeae* also produces hemicellulases enzymes with industrial application for the enzymatic hydrolysis of recalcitrant lignocellulosic feedstocks, such as corn fibre and wheat straw (Bischoff et al. 2011). After the inclusion of *A. kiliense* and *A. strictum* into *Sarocladium*, the genus has become relevant in the clinical setting (Fernández-Silva et al. 2013, 2014a, b) because these species are commonly reported as important opportunistic human and animal pathogens (Guarro et al. 1997, Summerbell 2003, de Hoog et al. 2011). These species have been reported to cause mycetoma (Guarro et al. 1997, Das et al. 2010, de Hoog et al.

2011), often occurring in immunocompetent patients in tropical areas (Summerbell 2003), keratitis, endophthalmitis (Fridkin et al. 1996, Weissgold et al. 1996, 1998), endocarditis (Lacaz et al.1981), continuous ambulatory peritoneal dialysis (CAPD)-associated peritonitis (Khan et al. 2011), and catheter-related fungaemia (Ioakimidou et al. 2013). *Sarocladium kiliense* shows a poor response to antifungal treatment (Khan et al. 2011, Fernández-Silva et al. 2014a) and appears to be the predominant species associated with invasive infections (Summerbell 2003, Khan et al. 2011). In a murine model of disseminated infection by *S. kiliense* and some *Acremonium* spp., *S. kiliense* was the most virulent species compared with *A. sclerotigenum*-*A. egyptiacum* complex and *A. implicatum,* producing severe infection in immunocompromised mice and affecting mainly the spleen (Fernández-Silva et al. 2014b). Morphologically, *S. strictum* is easily confused with *S. kiliense* (Khan et al. 2011, Perdomo et al. 2011b) and *A. sclerotigenum*, especially when the latter species does not produce its characteristic sclerotia (Perdomo et al. 2011b). Therefore, an unknown number of clinical reports attributed to *S. strictum,* such as Novicki et al. (2003) and Guarro et al. (2009), are in fact based on *A. sclerotigenum* isolates. The clinical isolates in those cases were re-identified later by other authors (Perdomo et al. 2011b, Summerbell et al. 2011). Since *Acremonium* and *Sarocladium* are two members of *Hypocreales* with species producing simple and similar morphological characters, the species delimitation between the genera is difficult and more studies are required to establish their phylogenetic boundaries.

1.4. Arthroconidial fungi

The arthroconidia are a type of asexual spore produced by both basidiomycetous and ascomycetous fungi; those studied in the present thesis belong to the *Ascomycota* and correspond to the following genera: *Arthrographis*, *Arthropsis* Sigler, M.T. Dunn & J.W. Carmich and *Scytalidium* Pesante. These three arthroconidial genera include species of worldwide distribution, commonly found in soil (Gené et al. 1996, Sigler 2003), decaying plant material (Sigler & Carmichael 1983, Sigler et al. 1990, Kang et al. 2010, de Hoog et al. 2011), compost and sediments (Ulfig et al. 1995). In the case of *Arthrographis* and *Scytalidium,* they are occasionally reported as human opportunistic pathogens (Sigler 2003, Nucci & Anaissie 2009, de Hoog et al. 2011).

Arthrographis species are morphologically characterized by white or pale coloured colonies with a slow growth rate (Fig. 5A), and hyaline, smooth-walled, cylindrical arthroconidia that are released schizolytically from dendritic (tree-like) conidiophores (Fig. 5D) (Sigler & Carmichael 1976). A trichosporiella-like synasexual morph, characterized by solitary globose to subglobose conidia growing laterally and sessile on undifferentiated vegetative hyphae (Fig. 5E), is produced by the type species of the genus, *A. kalrae* (R.P. Tewari & Macph.) Sigler & J.W. Carmich. (Sigler & Carmichael 1976, 1983). In contrast,

Arthropsis and *Scytalidium* show pale or dark pigmented colonies (Fig. 5B, C) and arthroconidia produced from undifferentiated conidiophores (Fig. 5F, G) and released respectively by rhexolytic and schizolytic secession (Sigler et al. 1982, Gené et al. 1996, de Hoog et al. 2011). The typical feature of *Arthropsis* is the presence of adjacent connectives between conidia (Fig. 5F) and occasionally a humicola-like synasexual morph is observed in some species (Sigler et al. 1982, van Oorschot & de Hoog 1984), while the typical features of *Scytalidium* are its expanding colonies (Fig. 5C), and pigmented or hyaline cylindrical arthroconidia with truncated ends (Fig. 5G) (de Hoog et al. 2011).

The genus *Arthrographis* was proposed by Cochet (1939) with *A. langeronii* G. Cochet as the type species, which was described as the causal agent of human onychomycosis. However, the publication of the genus was not valid because the author did not include a Latin diagnosis, which at that time was still required by the International Code of Botanical Nomenclature for the formal proposal of new taxa as a prerequisite. Later, Sigler and Carmichael (1976) revised the type material of *Oidiodendron kalrae* R.P. Tewari & Macph., a dimorphic fungus previously described by Tewari & Macpherson (1971) and isolated from human sputum. This species was found to be conspecific with Cochet's fungus and, therefore, the genus *Arthrographis* was validated with *A. kalrae* as the type species (Sigler & Carmichael 1976). In the same study, *Oospora cuboidea* Sacc. & Ellis, a cellulolytic species described from rotting oak by Saccardo (1882), was also accommodated in *Arthrographis* as *A. cuboidea* (Sacc. & Ellis) Sigler. This species, however, has been recently transferred to the genus *Scytalidium* (Kang et al. 2010).

Arthrographis sulphurea (Grev.) Stalpers & Oorschot, initially described as *Sporotrichum sulphureum* Grev., was considered by Stalpers (1984) to be a synonym of *A. kalrae.* Von Arx (1985) considered the same fungus as a possible synonym of *Pachysolen tannophilus* Boidin & Adzet (*Saccharomycetes*), yeast that produces mucoid colonies and ellipsoidal or short cylindrical cells, which were morphologically similar to the type material of *S. sulphureum*. Other species currently accepted in *Arthrographis* are *A. lignicola* Sigler (Sigler & Carmichael 1983), *A. pinicola* Sigler & Yamaoka (Sigler et al. 1990), and *A. alba* Gené, Ulfig & Guarro (Gené et al. 1996). *Arthrographis lignicola* and *A. pinicola* have cellulolytic capacity (Sigler & Carmichael 1983, Sigler et al. 1990). The latter species has been described as a producer of active secondary metabolites, such as arthrographol (Ayer & Nozawa 1990). This metabolite has an inhibitory activity against *Ophiostoma clavigerum* (Rob.-Jeffr. & R.W. Davidson) T.C. Harr., currently named *Grosmannia clavigera* (Rob.-Jeffr. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf, a fungus considered to be the most aggressive blue-stain species among the fungi associated with mountain pine beetle attacks (Yamaoka et al. 1990).

Pithoascus langeronii Arx was described by von Arx (1978) as the sexual morph of

A. kalrae, later transferred to the genus *Pithoascina* (Arx) Valmaseda, A.T. Martínez & Barrasa (Valmaseda et al. 1986), and finally relocated in the genus *Eremomyces* Malloch & Cain (Malloch & Sigler 1988). Malloch & Sigler (1988) established the family *Eremomycetaceae* to accommodate the following ascomycetes: *E. langeronii* (Arx) Malloch & Sigler, *E. bilateralis* Malloch & Cain and *Rhexothecium globosum* Samson & Mouch. The genera *Eremomyces* and *Rhexothecium* are characterized by producing pseudoparenchymatous ascomatal initials, cleistothecia, clavate to ovoid evanescent asci, one-celled hyaline to pale brown ascospores and arthrographis-like or trichosporiella-like asexual morphs (Malloch & Sigler 1988). Other ascomycetes previously described with arthrographis-like morphs are *Leucothecium coprophilum* Valldos & Guarro, *L. emdenii* Arx & Samson and *Faurelina indica* Arx, Mukerji & N. Singh (von Arx & Samson 1973, von Arx et al. 1981, Valldosera et al. 1991).

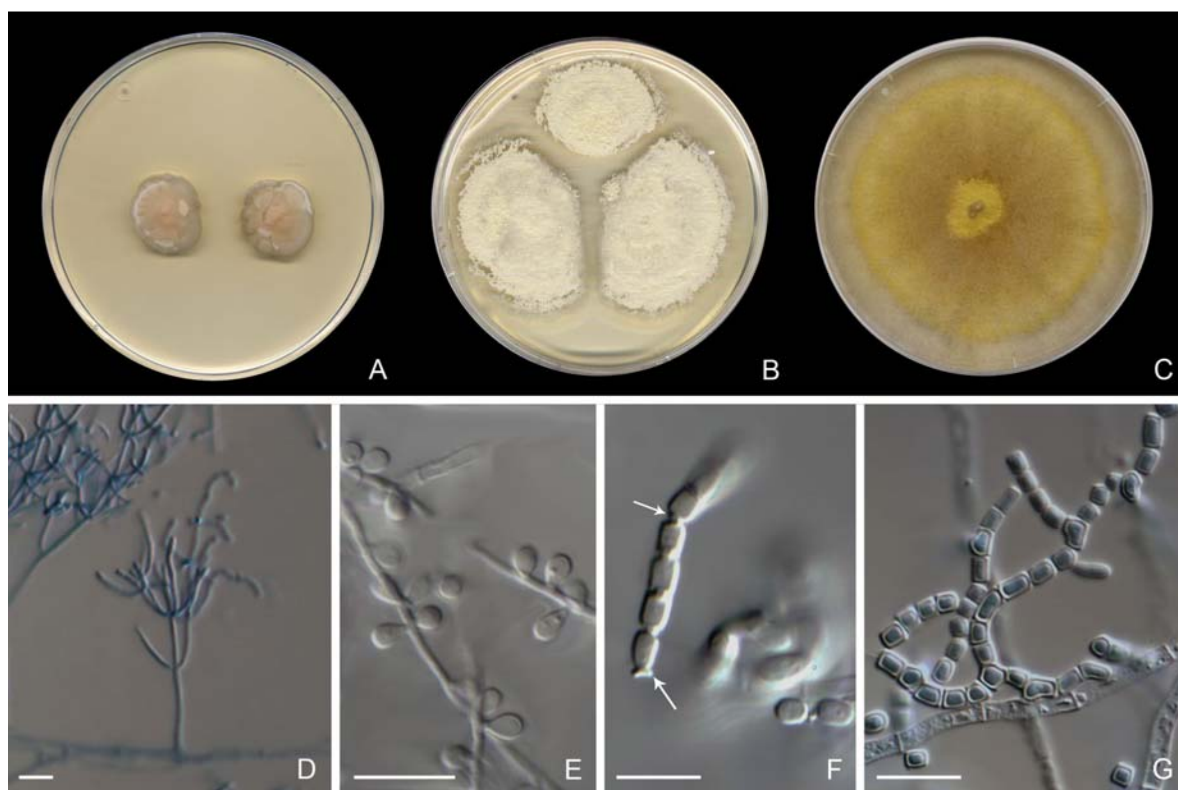

Fig. 5 Morphological features of the athroconidial genera *Arthrographis* (A, D, E)*, Arthropsis* (B, F) and *Scytalidium* (C, G). Colonies with slow (A), moderate (B) and rapid growth (C); differentiated conidiophores with tree-like aspect (D); trichosporiella-like synasexual morph (E); arthroconidia joined by narrow connectives (arrows) (F); conidiogenous hyphae fragmenting schizolytically and producing cylindrical and cuboid arthroconidia (G). Scale bars = 10 µm.

Molecular studies on *Arthrographis* are scarce. Gené et al. (1996), through the RFLP technique, demonstrated that *A. kalrae* and *E. langeronii* produced different restriction patterns, questioning the validity of the holomorphic concept of the species, in agreement with Sigler & Carmichael (1983). Recently, in a study on ascomycetous fungi producing

yellow rot disease on crops of the mushroom *Ganoderma lucidum* (Curtis) P. Karst*,* Kang et al. (2010) demonstrated by sequence analysis of SSU, ITS and *RPB2* that *A. cuboidea* is related to *Scytalidium* and that *Arthographis* must be considered a polyphyletic genus, since three of its species, *A. kalrae*, *E. langeronii* and *A. lignicola*, did not form a natural group. However, the rest of the species of *Arthrographis* were not included in this work. An extensive taxonomic study of all these species has never been done.

Until now, *A. kalrae* is the only species of the genus involved as an opportunistic pathogen in humans that causes onychomycosis (Cochet 1939, Sigler & Carmichael 1983, Volleková et al. 2008, Sugiura & Hironaga 2010), mycetoma (Degavre et al. 1997), sinusitis, meningitis (Chin-Hong et al. 2001, Xi et al. 2004), cerebral vasculitis (Pichon et al. 2008), keratitis (Perlman & Binns 1997, Biser et al. 2004, Thomas et al. 2011, Ramli et al. 2013), pulmonary infections (Vos et al. 2012) and endocarditis (de Diego Candela et al. 2010). Most cases occur in immunocompromised patients and occasionally in immunocompetent patients (Thomas et al. 2011). Due to *A. kalrae* being a common habitant of soil and compost (Sugiura & Hironaga 2010), direct contact with these materials through the respiratory tract or by inoculation by trauma seems to be the most probable source of infection (Pichon et al. 2008, Vos et al. 2012). *Arthrographis kalrae* is a thermotolerant dimorphic fungus (Sigler et al. 1990, Gené et al. 1996) that in young cultures usually shows a yeast-like appearance, exhibiting elongated oval budding yeast cells. However, this feature is not present in all the isolates of the species and may be influenced by the culture conditions (Sigler 2003). This characteristic is common in fresh isolates from soils or human origin, which can be easily confused with yeasts and subsequently submitted to methods normally used for yeast identification (Chin-Hong et al. 2001, Sigler 2003) and this leads to a misidentification of some isolates. Additionally, the hyphal growth and arthroconidial development could be confused with other arthroconidial genera such as *Geotrichum, Oidiodendron* Robak or hyaline species of *Arthropsis*. However, the former genus and *Arthropsis* do not produce differentiated conidiophores. *Oidiodendron* produces pigmented conidiophores and its arthroconidia, as well as those of *Arthropsis*, are joined by prominent connectives and are released rhexolytically from the conidiogenous hyphae (Sigler & Carmichael 1976, Sigler et al. 1982).

Arthropsis was proposed by Sigler et al. (1982), with *A. truncata* Sigler, M.T. Dunn & J.W. Carmich. as the type species, to accommodate anamorphic fungi with dark arthroconidia joined by adjacent connectives (Fig. 5F) and developed from undifferentiated conidiogenous hyphae (Sigler et al. 1982). However, after the inclusion of unpigmented species such as *A. hispanica* Gené, Ulfig & Guarro*,* the generic description was extended to include species with either dark or hyaline arthroconidia (Ulfig et al. 1995). In addition to *A. truncata* and *A. hispanica*, the genus encompasses two other species, *A. microsperma*

(Berk. & Broome) Sigler and *A. cirrhata* Oorschot & de Hoog (Sigler & Carmichael 1983, van Oorschot & de Hoog 1984). At present, no sexual morph has been linked with *Arthropsis* and according to the MycoBank (Crous et al. 2004) and Index Fungorum databases its taxonomic position within *Ascomycota* is in the *Onygenales* (*Eurotiomycetes*). Although the morphological distinction between *Arthropsis* and *Arthrographis* seems to be clear, van Oorschot & de Hoog (1984) considered the presence of arthroconidial connectives of the former genus to be an unstable character, finding the distinction between the two genera to be vague. Therefore, they suggested transferring all the *Arthrographis* species, except *A. kalrae*, to the genus *Arthropsis* (van Oorschot & de Hoog 1984). However, this proposal was rejected by other authors (Malloch & Sigler 1988, Sigler et al. 1990). At present, molecular studies involving *Arthropsis* have not been carried out and the relationship between both genera remains uncertain.

Scytalidium was established by Pesante (1957) for a single species, *S. lignicola* Pesante, growing on decaying *Platanus* wood. The genus currently encompasses around 20 species (Index Fungorum database) which are usually described as saprobic on different substrates (de Hoog et al. 2011). However, some species, such as *S. japonicum* Udagawa, K. Tominaga & Hamaokaand and *S. infestans* Iwatsu, Udagawa & Hatai, have been reported as causal agents of cattle bronchiolitis (Udagawa et al. 1986) and systemic mycosis in fish (Iwatsu et al. 1990). The sexual morphs linked with *Scytalidium* are described in *Xylogone* Arx & T. Nilsson, such as *X. ganodermophthora* Kang, Sigler, Y.W. Lee & S.H. Yun and *X. sphaerospora* Arx & T. Nilsson, which together with *S. lignicola* and *S. cuboideum* are reported as yellow root pathogens (Kang et al. 2010). Currently, the taxonomic position of the type species is in *Helotiales* (*Leotiomycetes*) (Crous et al. 2006).

Scytalidium dimidiatum and its pycnidial synasexual morph *Nattrassia mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko are commonly associated as causal agents of dermatomycosis and onychomycosis in patients living in or migrating from tropical areas (Sigler 2003, Madrid et al. 2009, Sutton et al. 2009), but they are rarely reported to cause invasive disease in immunocompromised hosts (Sigler et al. 1997). Similar infections are reported by the albino variant of this species, *S. hyalinulum*, but with less virulence and better prognosis for the patient (Madrid et al. 2009). A phylogenetic study carried out with 28S rDNA sequences demonstrated that *S. dimidiatum* and *N. mangiferae* were not conspecific (Crous et al. 2006). They belonged to two different lineages in the family *Botryosphaeriaceae* (*Botryosphaeriales, Dothideomycetes*) and placed phylogenetically distant from the type species of the genus *Scytalidium*, *S. lignicola*. Consequently, *S. dimidiatum* and *N. mangiferae* were re-accommodated into two new genera, *Neoscytalidium* Crous & Slippers and *Neofusicoccum* Crous, Slippers & A.J.L. Phillips, respectively, which morphologically differed in the presence of a dichomera-like synasexual morph in the latter

genus (Crous et al. 2006, Phillips et al. 2013). Arthric chains of dry, powdery conidia are a prominent feature of *Neoscytalidium* species (Phillips et al. 2013).

1.5. Ochroconis-like fungi

This type of fungi include dematiaceous filamentous species morphologically characterized by having slow to moderate growth, brown to olivaceous colonies (Fig. 6A–D), brownish conidiophores, and septate dark pigmented rough-walled conidia (Fig. 6E, F, H, K), which are produced by sympodial conidiogenesis (Fig. 6E–G, I, J) and liberated by rhexolytic dehiscence (Fig. 6I, J). This group of fungi includes the genera *Ochroconis*, *Scolecobasidium* E. V. Abbott and the recently described *Verruconis* Samerpitak & de Hoog (Ellis 1971, 1976, de Hoog & von Arx 1973, Samerpitak et al. 2014).

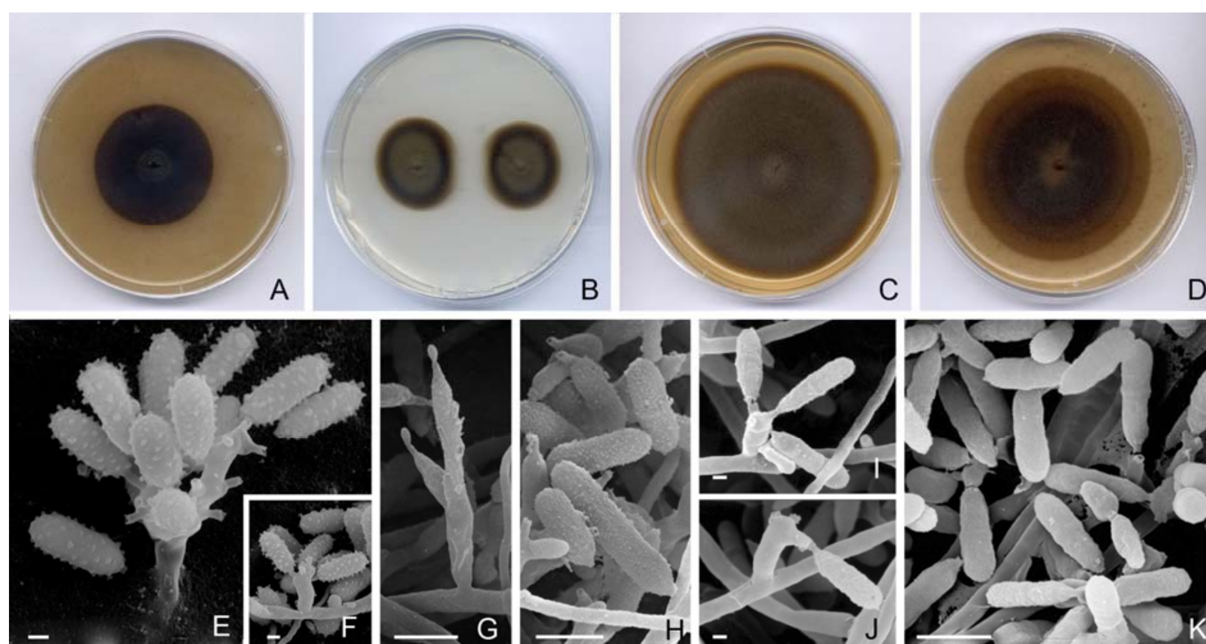

Fig. 6 Morphological features of *Ochroconis* (A, B, E–H) and *Verruconis* species (C, D, I–K). Brown colonies with slow (A, B) and moderate growth (C, D); conidiophores with sympodial proliferation bearing conidia on long, open denticles (E–G, I–J); ellipsoidal (F), cylindrical (H) and clavate conidia (K). Scale bars E, F, I, J = 10 µm; G, H, K = 5 µm. (Electron microcopy images are modified from de Hoog et al. 2011).

These genera include mainly soil-borne and plant litter species with worldwide distribution (Dwivedi 1959, Barron & Busch 1962, Domsch et al. 2007, Samerpitak et al. 2014). In addition, a wide variety of habitats can contain ochrochonis-like fungi, such as air (Lugauskas et al. 2003), indoor and outdoor environments (Nobuo & Niichiro 2008, Lian & de Hoog 2010), cave rocks and Paleolithic paintings (Nováková 2009, Martin-Sanchez et al. 2012), water plants (Orłowska et al. 2004), foods (Comerio et al. 2005), and washing machines (Nobuo 2005a,b). Because of the scarcity of nutrients of some of these

environments, oligotrophism is an ecological trend in this group of fungi (Samerpitak et al. 2014). Due to the thermotolerance of some species, it is common to find them in extreme environments with low pH and high temperature, such as thermal soil (Tansey & Brock 1973, Redman et al. 1999), hot springs effluents (Tansey & Brock 1973, Yarita et al. 2007, 2010), sewage from nuclear power plants, coal waste piles (Tansey & Brock 1973, Tansey et al. 1979, Rippon et al. 1980, Odell et al. 2000, Ohori et al. 2006) and broiler-house litters (Waldrip et al. 1974, Randall et al. 1981). Some species have been described as opportunistic pathogens in humans and animals (Revankar & Sutton 2010, de Hoog et al. 2011). While *Ochroconis* species affect mostly cold-blooded vertebrates, those in *Verruconis* have a special tropism towards warm-blooded animals (Yarita et al. 2007, de Hoog et al. 2011, Samerpitak et al. 2014). *Ochroconis tshawytschae* (Doty & D.W. Slater) Kiril. & Al-Achmed and *O. humicola* (G.L. Barron & Lév. Busch) de Hoog & Arx have been established as agents of phaeohyphomycosis, producing systemic infections mainly in fish (Doty & Slater 1946, Ross & Yasutake 1973, Schaumann & Priebe 1994). However, the first human subcutaneous mycosis by *O. tshawytschae* in an immunocompetent patient has been recently described (Ge et al. 2012). *Ochroconis humicola* has also been recovered from a superficial lesion of a frog (Domsch et al. 2007), a tortoise (Weitzman et al. 1983) and from granulomatous lesions of a cat (VanSteenhouse et al. 1988). Several isolates of *O. mirabilis* Samerpitak & de Hoog have been recovered from superficial tissues of humans and fish, producing mild cutaneous infections (Samerpitak et al. 2014). The most common species in soils, *O. constricta* (E.V. Abbott) de Hoog & Arx, was reported as causal agent of pulmonary abscess in a heart transplant recipient (Mancini & McGinnis 1992). *Verruconis gallopava* (W.B. Cooke) Samerpitak & de Hoog is the most clinically relevant species (Revankar & Sutton 2010, de Hoog et al. 2011), mainly being reported as a causal agent of brain abscesses in immunocompromised humans, as well as pulmonary infections in immunocompetent hosts (Revankar & Sutton 2010), and occasionally, subcutaneous phaeohyphomycosis, endocarditis and systemic infections, sometimes with fatal outcome (Yarita et al. 2007, Wong et al. 2010, de Hoog et al. 2011, Meriden et al. 2012, Qureshi et al. 2012). In recent years, clinical reports of *V. gallopava* as a human opportunist have increased, mainly in the immunocompromised population, with organ transplantation as the most common underlying condition (Yarita et al. 2007, Shoham et al. 2008, Meriden et al. 2012, Qureshi et al. 2012). However, some cases in healthy individuals have also been described, with pulmonary infections being the most common manifestation (Odell et al. 2000, Bravo & Ngamy 2004, Hollingsworth et al. 2007, Revankar & Sutton 2010). In warm-blooded animals, *V. gallopava* shows a special neurotropism (Meriden et al. 2012, Qureshi et al. 2012), being an important etiological agent of encephalitis in poultry and wild birds (Connole 1967, Blalock et al. 1973, Randall et al. 1981, Shane et al. 1985, Karesh et al.

1987, Salkin et al. 1990). Several epidemic outbreaks by this pathogen have occurred in poultry and birds reared in zoos in the USA (Yarita et al. 2007, Samerpitak et al. 2014). Occasionally, *V. gallopava* has been linked to lung and systematic infections in cats (Dixon & Salkin 1986, Padhye et al. 1994, Ohori et al. 2006) and dogs (Singh et al. 2006).

Abbott (1927) erected the genus *Scolecobasidium* to accommodate fungi with small, brownish to black colonies and pale brown, two-celled conidia liberated by rhexolytic secession. The genus included two soil-borne species: *Scolecobasidium terreum* E. V. Abbott, as the type species, with T- or Y-shaped conidia and lateral chlamydospores, which were described by Fassatiová (1967) as a humicola-like synasexual morph, and *S. constrictum* E.V. Abbott with oblong two-celled conidia, constricted at the septum (Abbott 1927). Since Atkinson (1952) described *Heterosporium terrestre* R.G. Atk. as a new fungus from soil of Canada with the same morphological features as *S. constrictum,* the former has been considered a heterotypic synonym of the latter (Barron & Busch 1962). Several species have been described under the name *Scolecobasidium* and, according to Seifert et al. (2011) and the Index Fungorum and MycoBank (Crous et al. 2004) databases, the genus comprises around 50 species. De Hoog & von Arx (1973) published a detailed review of *Scolecobasidium* species and established the new genus *Ochroconis,* with *O. constricta* (E.V. Abbott) de Hoog & Arx as the type for those species with ellipsoidal, clavate and fusiform conidia, usually provided with a distinct hilum. *Scolecobasidium sensu stricto* was restricted to species with more or less Y-shaped conidia and ampulliform, often seriate, conidiogenous cells. Despite this division being practical, this did not reflect the natural relationships among those taxa (de Hoog 1985) and therefore it was not accepted by several authors who considered *Ochroconis* as a synonym of *Scolecobasidium* (Matsushima 1971, 1975, 1983, 1993; Ellis 1976, Domsch et al. 2007). In addition to *S. constrictum*, other species re-accommodated by de Hoog & von Arx (1973) in *Ochroconis* were *S. anelli* Graniti, *S. humicola* G.L. Barron & Lév. Busch, *S. crasshumicola* Matsush, *S. variable* G.L. Barron & L.V. Busch and *S. verruculosum* R.Y. Roy, R.S. Dwivedi & R.R. Mishra. By contrast, some species were transferred to other genera, such as *Dactylaria* Sacc (de Hoog & von Arx 1973), *Veronaea* Cif. & Montemart. (de Hoog et al. 1983), *Trichoconis* Clem (de Hoog & van Oorschot 1985) and *Neta* Shearer & J.L. Crane 1971 (de Hoog 1985).

Verruconis gallopava was originally described as *Diplorhinotrichum gallopavum* W. B. Cooke, an etiological agent of fungal meningitis in turkey (Georg et al. 1964). Bhatt & Kendrick (1968) considered the genus *Diplorhinotrichum* as synonym of *Dactylaria,* and after an emended description of the latter genus, the new combination *Dactylaria gallopava* (W.B. Cooke) G.C. Bhatt & W.B. Kendr. was proposed. Subsequently, de Hoog (1983) included this species within *Ochroconis* under the name *O. gallopava* (W. B. Cooke) de

Hoog. Dixon & Salkin (1986), rejecting or being unaware of the taxonomic changes proposed by de Hoog (1983), considered that *D. gallopava* was morphologically indistinguishable from *S. constrictum* and proposed a new combination *D. constricta* (E.V. Abbott) D.M. Dixon & Salkin. However, differences in physiological characters and virulence studies carried out with the type specimens of both species lead them to propose two varieties for *D. constricta*, i.e., *D. constricta* var. *gallopava* (W.B. Cooke) Salkin & D.M. Dixon and *D. constricta* var. *constricta* (Abbott) Salkin & D.M. Dixon (Salkin & Dixon 1987). While the former variety grew at 42 °C, it was unable to grow with cycloheximide and was pathogenic in experimentally infected mice; the second showed opposite results and was suspected to be a different taxon (Salkin & Dixon 1987), although not proven by molecular methods.

Recently, in an overview involving ochroconis-like fungi, Samerpitak et al. (2014) reviewed all the currently available type material from the species included in *Ochroconis* and *Scolecobasidium,* and suggested the latter genus must be abandoned since the original material of the type species, *S. terreum* (CBS 203.27), is now sterile (Horré et al. 1999) and phylogenetically distant from the other species with identical Y-shaped conidia; these findings led them to regard *S. terreum,* based on CBS 203.27 as a dubious species (Samerpitak et al. 2014). In Samerpitak et al. (2014), the species studied were distributed into two distinct lineages within the family *Sympoventuriaceae*, which clearly represented two different genera. Therefore, based on multilocus sequencing of six genes (SSU, ITS, LSU, *ACT*1*, BT*2 and *TEF*-1*α*) and phenotypic data, the new genus *Verruconis* was proposed to accommodate thermophilic species with light to dark-brown, verrucose to coarsely ornamented conidia, such as *O. gallopava*, *O. calidifluminalis* Yarita, A. Sano, de Hoog & Nishim. and *O. verruculosa* (R.Y. Roy, R.S. Dwivedi & R.R. Mishra) de Hoog & Arx. In contrast, those mesophilic species with subhyaline, smooth-walled to verruculose conidia and commonly associated with infections in cold-blooded animals were retained in *Ochroconis* (Samerpitak et al. 2014). Thirteen species were accepted in *Ochroconis*, including three new ones, i.e., *O. cordanae* Samerpitak, Crous & de Hoog, *O. mirabilis* and the first sexual morph described for the genus, *O. sexualis* Samerpitak, van der Linde & de Hoog. That study showed the division between *Ochroconis* and *Scolecobasidium* previously proposed by de Hoog & von Arx (1973) to be artificial because species with trilobate and ellipsoidal conidia were phylogenetically related and included within the *Ochroconis* group (Samerpitak et al. 2014). In parallel, Machouart et al. (2014) located *Verruconis* and *Ochroconis* within the family *Sympoventuriaceae*, in the recently described order *Venturiales* (*Dothideomycetes*).

The recent proposals highlight that morphological features alone are not enough for distinguishing species in ochroconis-like fungi, and that fresh isolates belonging to such

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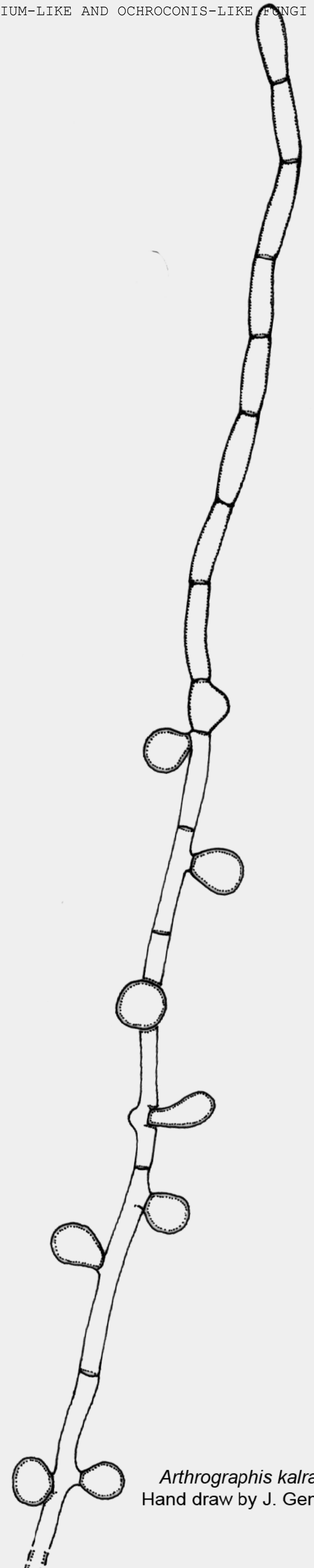
genera must be studied with both modern and classical techniques to confirm their identity and taxonomic position.

2. INTEREST AND OBJECTIVES



Arthrographis kalrae
Hand draw by J. Gené

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In the last three decades the number of fungal species pathogenic for humans, especially those producing opportunistic infections, has increased dramatically. This is linked to the increase of the immunocompromised population, including organ transplant recipients, HIV-infected patients or under corticoid therapy, among others. The classical opportunistic pathogens, such as *Candida* Berkhout and *Cryptococcus* Kütz within the yeast group, and *Aspergillus*, *Fusarium* or *Scedosporium* Sacc. ex Castell. & Chalm. in the filamentous fungal group are being isolated ever more frequently from clinical samples. However, other species have either never been reported before in the clinical setting, or have been considered laboratory contaminants. They are not usually identified due to their complex taxonomy, which shows many genera of filamentous fungi and the limited training of the clinical microbiologists in fungal identification. Consequently, the pathogenic role of many filamentous fungi in human infections, as well as their epidemiology and their antifungal susceptibility patterns are still unknown.

In this thesis, we have chosen several genera of asexual fungi, viz, *Acremonium*, *Arthrographis*, *Arthropsis, Ochroconis, Sarocladium, Scytalidium* and *Verruconis*, because isolates of some of them are relatively common in clinical specimens and the diversity of their species in the clinical setting is poorly known. Some of these genera encompass a large number of species, all of which have a complex taxonomy, thus making their identification difficult by conventional methods. Nowadays, the precise identification of the species involved in fungal infections is essential for better treatment of patients. On many occasions those species show different degrees of virulence and different responses to the antifungal treatments currently available.

Acremonium is a complex genus formed by numerous species, some of them considered emerging opportunistic pathogens and causative agents of hyalohyphomycoses in humans, with a high mortality rate among neutropenic patients in particular (Guarro et al. 1997, Das et al. 2010, Tortorano et al. 2014). These fungi have a simple morphology, sharing similarities with species of other genera such as *Fusarium*, *Phialemonium*, *Lecythophora* or *Cylindrocarpon*, among others, with which they can be easily confused morphologically (Guarro et al. 1997, Perdomo et al. 2011b). Morphological differences among *Acremonium* species are often very subtle, making their identification very difficult (Perdomo et al. 2011b). In a previous study carried out in our unit (Perdomo et al. 2011b), a large panel of *Acremonium* clinical isolates from the USA was identified using morphology and DNA sequencing. However, numerous isolates could not be reliably identified at the species level and some of them revealed might represent new species. Some species reported in that study, such as *A. kiliense* and *A. strictum*, were recently transferred to the genus *Sarocladium*, but the relationship among other *Acremonium* species and the latter genus is still unresolved. Several studies have demonstrated the polyphyly of *Acremonium*

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(Glenn et al. 1996, Zare et al. 2007, Gräfenhan et al. 2011, Perdomo et al. 2011b, Summerbell et al. 2011, Grum-Grzhimaylo et al. 2013b), and even though these studies have helped to clarify the taxonomy of some species, there are still numerous species waiting to be resolved.

The selected arthroconidial fungi, *Arthrographis* and, less frequently, *Scytalidium* are occasionally reported as human pathogens, although the real range of the species involved in clinical infections and their response to the antifungal drugs have not been well studied. In addition, the phylogenetic relationship between *Arthrographis* and other morphologically similar genera, such as *Arthropsis,* has never been investigated.

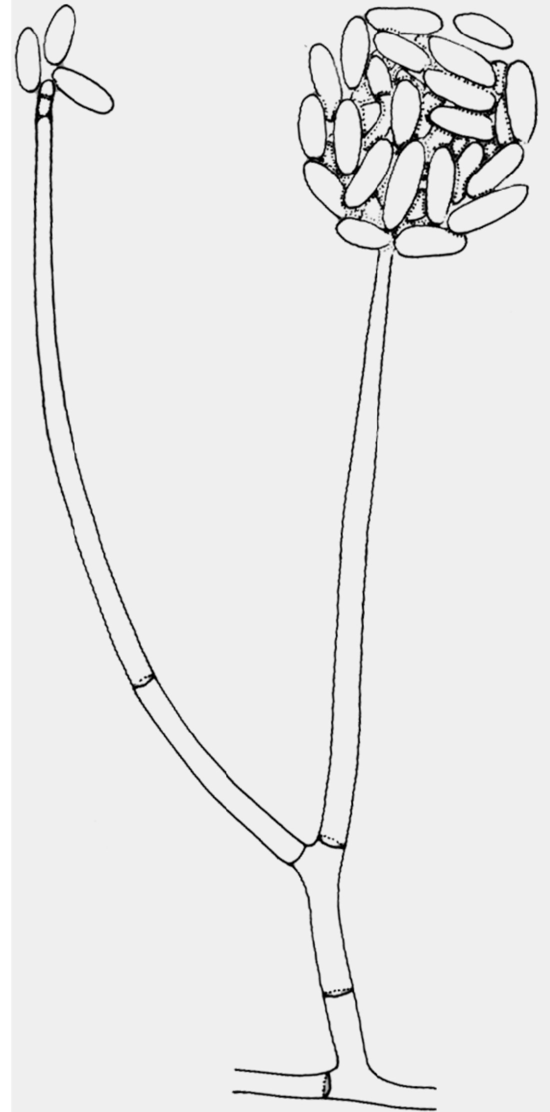
Ochroconis and *Verruconis* are two dematiaceous genera, which have undergone important taxonomic changes and several new species have been recently described (Hao et al. 2013, Crous et al. 2014b, Machouart et al. 2014, Samerpitak et al. 2014). Therefore, the diversity of these fungi in clinical samples is probably different from that so far reported and studies on the antifungal susceptibility of their species are practically nonexistent.

Therefore, the main objective of this thesis is: **to study morphologically and molecularly clinical and environmental isolates of genera with clinically relevant species, such as *Acremonium, Arthrographis, Ochroconis, Sarocladium, Scytalidium* and *Verruconis*, to clarify their taxonomy and to provide key features for species recognition.**

To reach this objective, specific goals are proposed:

1. To obtain clinical and environmental isolates with acremonium-, arthroconidial and ochroconis-like morphology and confirm their identity at the genus and species level through phenotypic studies and DNA sequencing.
2. To establish the phylogenetic relationships among the species of the genera *Acremonium*, *Arthrographis, Arthropsis, Sarocladium*, *Scytalidium, Ochroconis* and *Verruconis,* using multilocus sequence analysis.
3. To describe putative new species within the studied genera, when encountered.
4. To determine the *in vitro* antifungal activity of different drugs against clinical isolates of species with poorly known or unknown antifungal susceptibility patterns of the genera under study.

3. MATERIALS AND METHODS



Acremonium tubakii
Hand draw by J. Gené

3.1. Origin of the isolates

A total of 260 isolates were included in the different studies of this thesis. Among them, 142 were obtained from clinical or soil samples (Table 1), and the rest (n=118) were ex-type or reference strains obtained from different international culture collections (Table 2) and used mainly for comparison. The clinical isolates were provided by the Fungus Testing Laboratory, University of Texas Health Science Center (UTHSC, San Antonio, USA). The type and reference strains used in each study were obtained from the CBS-KNAW Fungal Biodiversity Centre (CBS-KNAW, Utrecht, The Netherlands), the Mycothèque de l'Université Catholique de Louvain (MUCL, Louvain-la-Neuve, Belgium) and the University of Alberta Microfungus Collection and Herbarium (UAMH, Edmonton, Canada). Since part of the work was related to the National Project CGL2011-2785 to study the asexual fungi from Spain, all the fresh isolates from soil were from samples collected in different Natural Parks in this country. The collection procedure is explained below.

3.2. Technique for the isolation of soil fungi

Samples were taken from the surface layer of soil and from river sediments and placed in sterilized polyethylene bags, closed with rubber bands. In the laboratory, samples were stored at 4–7 °C until they were processed. One gram of each soil sample was washed ten times with 10 mL of sterilized water to reduce excessive microbial growth. After the final wash, excess water was decanted and the remaining soil was distributed across three Petri dishes. Potato dextrose agar (PDA, Pronadisa, Madrid, Spain), supplemented with chloramphenicol (200 mg/L) and cycloheximide at a final concentration of 2 g/L, at 45 °C was mixed with the soil and, once solidified, the cultures were incubated at 25 °C in the dark. Cultures were examined weekly with a stereomicroscope for up to 1 mo. To purify the isolates, conidia were transferred using a sterile dissection needle from colonies on PDA to Petri dishes containing potato carrot agar (PCA; potatoes, 20 g; carrots, 20 g; agar, 20 g; distilled water to final volume of 1000 mL), which we prepared following the procedure described in Onions & Pitt (1988), and incubated at 25 °C in the dark.

3.3. Phenotypic characterization

The phenotypic characterization of isolates was made by the study of macroscopic and microscopic morphology, testing the ability to grow at different temperatures. In addition, we evaluated the resistance to cycloheximide and the ability to convert to the yeast phase in isolates of *Arthrographis* spp.

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Table 1 Clinical and environmental isolates included in this study.

Species	FMR number[a]	Other codes[b]	Origin[c]	Received as
Acremoniopsis suttonii	11780 [T]	CBS 138708	Forest soil, Spain	Isolated by ourself
Acremonium acutatum	10368	UTHSC 08-3115	BAL, USA	*Acremonium* sp.
Acremonium asperulatum	11065 [T]	CBS 130362, MUCL 5781	Forest soil, Spain	Isolated by ourself
	11135	-	Forest soil, Spain	Isolated by ourself
	11136	-	Forest soil, Spain	Isolated by ourself
	11137	-	Forest soil, Spain	Isolated by ourself
	11138	-	Forest soil, Spain	Isolated by ourself
	11139	-	Forest soil, Spain	Isolated by ourself
	11783	CBS 130363, MUCL 53782	Sediments, Spain	Isolated by ourself
Acremonium dimorphosporum	10548 [T]	CBS 139050, UTHSC 08-3639	BAL, USA	*Acremonium* sp.
Acremonium fusidioides	10360	UTHSC 08-1188	BAL, USA	*Acremonium fusidioides*
	10362	UTHSC 08-1455	Bronchial wash fluid, USA	*Acremonium fusidioides*
	10444	UTHSC 07-646	Bronchial wash fluid, USA	*Acremonium fusidioides*
Acremonium moniliforme	10363	CBS 139052, UTHSC 08-2284	Toe nail, USA	*Acremonium* sp.
	11785 [T]	CBS 139051	Soil, Spain	Isolated by ourself.
Acremonium pteridii	11786	-	Forest soil, Spain	Isolated by ourself
	12085	-	Forest soil, Spain	Isolated by ourself
	13209	-	Forest soil, Spain	Isolated by ourself
Acremonium roseolum	10351	UTHSC 02-2482	BAL, USA	*Acremonium hyalinulum*
Acremonium sclerotigenum	12086	-	Forest soil, Spain	Isolated by ourself
	12087	-	Forest soil, Spain	Isolated by ourself
Acremonium variecolor	11140 [T]	CBS 130360	Forest soil, Spain	Isolated by ourself
	11141	CBS 130361, MUCL 53780	Forest soil, Spain	Isolated by ourself
Arthrographis alba	4031	-	Marine sediments, Spain	*Arthrographis alba*
Arthrographis chlamydospora	12129 [T]	CBS 135396, UTHSC 06-1053	Urine, USA	*Scytalidium cuboideum*
Arthrographis curvata	4032	CBS 135934	Ebro river sediments, Spain	*Arthrographis kalrae*
	12125 [T]	CBS 135933, UTHSC 11-1163	Nails, USA	*Arthrographis* sp.
Arthrographis globosa	12124 [T]	CBS 135397, UTHSC 11-757	Bronchial wash, USA	*Arthrographis alba*

Table 1 (continued)

Species	FMR number[a]	Other codes[b]	Origin[c]	Received as
Arthrographis kalrae	4034	-	River sediments, Spain	*Arthrographis kalrae*
	4036	-	River sediments, Spain	*Arthrographis kalrae*
	12094	UTHSC 01-2742	Artificial pulmonary valve, USA	*Arthrographis kalrae*
	12097	UTHSC 04-2580	Blood, USA	*Arthrographis kalrae*
	12098	UTHSC 04-3423	Toe nail, USA	*Arthrographis kalrae*
	12099	UTHSC 05-17	Blood, USA	*Arthrographis kalrae*
	12102	UTHSC 06-982	Pleural fluid, USA	*Arthrographis kalrae*
	12103	UTHSC 06-3158	Toe nail, USA	*Arthrographis kalrae*
	12105	UTHSC 07-2450	Eye, USA	*Arthrographis* sp.
	12108	UTHSC 08-527	Lung tissue, USA	*Arthrographis kalrae*
	12109	UTHSC 08-786	Lung biopsy, USA	*Arthrographis kalrae*
	12110	UTHSC 08-1699	Bronchial wash, USA	*Arthrographis kalrae*
	12111	UTHSC 08-1804	Nails, USA	*Arthrographis kalrae*
	12112	UTHSC 08-2107	Leg, USA	*Arthrographis kalrae*
	12114	UTHSC 08-3547	Sputum , USA	*Arthrographis kalrae*
	12115	UTHSC 09-141	Lung biopsy, USA	*Arthrographis kalrae*
	12116	UTHSC 09-2903	Bronchial wash, USA	*Arthrographis kalrae*
	12118	UTHSC 10-1652	Cornea, USA	*Arthrographis kalrae*
	12119	UTHSC 10-1719	Cornea, USA	*Arthrographis kalrae*
	12120	UTHSC 10-2021	Catheter tip, USA	*Arthrographis* sp.
	12121	UTHSC 10-2583	Urine, USA	*Arthrographis kalrae*
	12122	UTHSC 10-2729	Nasal sinus, USA	*Arthrographis kalrae*
	12123	UTHSC 11-302	Eye, USA	*Arthrographis kalrae*
	12126	UTHSC 11-1256	Bronchial wash, USA	*Arthrographis* sp.
Arthrographis longispora	12101 [T]	CBS 135935, UTHSC 05-3220	Foot, USA	*Arthrographis* sp.
Arthropsis hispanica	12093	UTHSC 01-2199	Bronchial wash, USA	*Arthrographis* sp.
	12095	UTHSC 02-1022	Sputum, USA	*Arthrographis* sp.
	12113	UTHSC 08-2158	Nails, USA	*Arthrographis* sp.
	12117	UTHSC 09-3174	Bronchial wash, USA	*Arthrographis* sp.
Bjerkandera adusta	12107	UTHSC 07-3306	Bone marrow, USA	*Arthrographis* sp.

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Table 1 (continued)

Species	FMR number[a]	Other codes[b]	Origin[c]	Received as
Brunneomyces hominis	10429 [T]	CBS 139053, UTHSC 06-415	Sputum, USA	*Acremonium hyalinulum*
	10437	CBS 139054, UTHSC R-3853	Sputum, USA	*Acremonium hyalinulum*
Cervusimilis alba	10433	UTHSC 06-874	Sputum, USA	*Acremonium* sp.
	10549	CBS 139055, UTHSC 08-3693	Nails, USA	*Acremonium* sp.
Collarina aurantiaca	11134	CBS 138273	Forest soil, Spain	Isolated by ourself
	11784 [T]	CBS 138274	Sediments, Spain	Isolated by ourself
Cosmospora berkeleyana	11777	-	Forest soil, Spain	Isolated by ourself
Geotrichum sp.	12100	UTHSC 05-1497	Stool, USA	*Arthrographis* sp.
	12106	UTHSC 07-3034	Not available, USA	*Arthrographis* sp.
Gliomastix murorum	11781	-	Forest soil, Spain	Isolated by ourself
Ochroconis constricta	3906	-	Marine sediments, Spain	*Ochroconis constricta*
Ochroconis cordanae	12508	UTHSC 10-1875	Tissue left thigh, USA	*Ochroconis humicola*
Ochroconis mirabilis	12498	UTHSC 01-1570	Nail, USA	*Ochroconis constricta*
	12499	UTHSC 03-1114	BAL, USA	*Ochroconis constricta*
	12501	UTHSC 04-2378	BAL, USA	*Ochroconis constricta*
	12502	UTHSC 02-232	BAL, USA	*Ochroconis humicola*
	12503	UTHSC 03-3089	Skin, USA	*Ochroconis humicola*
	12504	UTHSC 05-1500	BAL, USA	*Ochroconis humicola*
	12505	UTHSC 07-3073	Toe nail, USA	*Ochroconis humicola*
	12506	UTHSC 08-1958	Toe nail, USA	*Ochroconis humicola*
	12507	UTHSC 10-1519	Skin, USA	*Ochroconis humicola*
	12510	UTHSC 11-2020	BAL, USA	*Ochroconis humicola*
	12511	UTHSC 11-3523	BAL, USA	*Ochroconis humicola*
Ochroconis olivacea	12509 [T]	CBS 137170, UTHSC 10-2009	BAL, USA	*Ochroconis humicola*
Ochroconis ramosa	12512	CBS 137171, UTHSC 03-3677	Skin, USA	*Scolecobasidium terreum*
	12513	CBS 137172, UTHSC 04-2729	BAL, USA	*Scolecobasidium terreum*
	12514 [T]	CBS 137173, UTHSC 12-1082	Nail, USA	*Scolecobasidium terreum*
Sarocladium bactrocephalum	10552	UTHSC 09-384	Eye, USA	*Acremonium strictum*
Sarocladium bifurcatum	10405 [T]	CBS 137658, UTHSC 05-3311	BAL, USA	*Acremonium* sp.
	10451	UTHSC 07-3446	Bronchial wash fluid, USA	*Acremonium* sp.

Table 1 (continued)

Species	FMR number[a]	Other codes[b]	Origin[c]	Received as
Sarocladium hominis	10352	UTHSC 02-2564	Leg, USA	*Acremonium* sp.
	10418 [T]	CBS 137659, UTHSC 04-1034	Right calf tissue, USA	*Acremonium* sp.
	10425	UTHSC 04-3464	Sputum, USA	*Acremonium* sp.
Sarocladium glaucum	10447	UTHSC 07-1181	Sputum, USA	*Acremonium glaucum*
Sarocladium pseudostrictum	10347 [T]	CBS 137660, UTHSC 02-1892	Sputum, USA	*Acremonium* sp.
Sarocladium subulatum	10441	CBS 137661, UTHSC 07-110	Bone, USA	*Acremonium* sp.
Sarocladium terricola	10348	UTHSC 02-1958	Sputum, USA	*Acremonium implicatum*
	10356	UTHSC 08-844	BAL, USA	*Acremonium implicatum*
	10369	UTHSC 08-3180	BAL, USA	*Acremonium implicatum*
	10388	UTHSC 03-2933	Bronchial wash fluid, USA	*Acremonium implicatum*
	10450	UTHSC 07-3260	Bone, USA	*Acremonium implicatum*
	10561	UTHSC 04-956	Sinus, USA	*Acremonium implicatum*
	10571	UTHSC 07-3667	Bronchial wash fluid, USA	*Acremonium implicatum*
Scytalidium cuboideum	12127	UTHSC 03-2193	Bronchial wash, USA	*Scytalidium cuboideum*
	12128	UTHSC 05-1821	Bronchial wash, USA	*Scytalidium cuboideum*
	12130	UTHSC 09-3329	Nasal sinus, USA	*Scytalidium cuboideum*
	12131	UTHSC 10-2389	Lung mass, USA	*Scytalidium cuboideum*
	12132	UTHSC 11-510	Bronchial wash, USA	*Scytalidium cuboideum*
Trichothecium crotocinigenum	11782	-	Forest soil, Spain	Isolated by ourself
Verruconis gallopava	12500	UTHSC 04-1355	Sputum, USA	*Ochroconis constricta*
	12515	UTHSC 03-447	Sputum, USA	*Ochroconis gallopava*
	12516	UTHSC 04-43	Bronchial wash, USA	*Ochroconis gallopava*
	12517	UTHSC 04-236	Brain, USA	*Ochroconis gallopava*
	12518	UTHSC 04-539	Canine liver, USA	*Ochroconis gallopava*
	12519	UTHSC 04-2693	Brain abscess, USA	*Ochroconis gallopava*
	12520	UTHSC 05-1018	Brain abscess, USA	*Ochroconis gallopava*
	12521	UTHSC 06-513	Sputum, USA	*Ochroconis gallopava*
	12522	UTHSC 06-541	Sputum, USA	*Ochroconis gallopava*
	12523	UTHSC 06-3565	Canine abdominal fluid, USA	*Ochroconis gallopava*
	12524	UTHSC 06-4445	Right hand abscess, USA	*Ochroconis gallopava*

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Table 1 (continued)

Species	FMR number[a]	Other codes[b]	Origin[c]	Received as
Verruconis gallopava	12525	UTHSC 07-153	Left lower lobe lung Bx, USA	*Ochroconis gallopava*
	12526	UTHSC 07-212	Induced sputum, USA	*Ochroconis gallopava*
	12527	UTHSC 07-623	Canine L2-3 disc, USA	*Ochroconis gallopava*
	12528	UTHSC 07-2994	Sputum, USA	*Ochroconis gallopava*
	12529	UTHSC 08-40	Bronchial wash, USA	*Ochroconis gallopava*
	12530	UTHSC 08-112	Sputum, USA	*Ochroconis gallopava*
	12531	UTHSC 08-657	Feline wound, USA	*Ochroconis gallopava*
	12532	UTHSC 08-810	Sputum, USA	*Ochroconis gallopava*
	12533	UTHSC 08-1340	Bronchial wash, USA	*Ochroconis gallopava*
	12534	UTHSC 08-1625	Right hip, USA	*Ochroconis gallopava*
	12535	UTHSC 08-1756	Sputum, USA	*Ochroconis gallopava*
	12536	UTHSC 08-3158	Bronchial wash, USA	*Ochroconis gallopava*
	12537	UTHSC 09-1229	Ear/Mastoid, USA	*Ochroconis gallopava*
	12538	UTHSC 09-3111	Lung, USA	*Ochroconis gallopava*
	12539	UTHSC 10-510	Brain abscess, USA	*Ochroconis gallopava*
	12540	UTHSC 10-1509	Bronchial wash, USA	*Ochroconis gallopava*
	12541	UTHSC 10-1541	Bronchial wash, USA	*Ochroconis gallopava*
	12542	UTHSC 10-3013	Sputum, USA	*Ochroconis gallopava*
	12543	UTHSC 11-315	Sputum, USA	*Ochroconis gallopava*
	12544	UTHSC 11-509	Bronchial wash, USA	*Ochroconis gallopava*
	12545	UTHSC 11-2401	Bronchial wash, USA	*Ochroconis gallopava*
	12546	UTHSC 11-2569	Bronchial wash, USA	*Ochroconis gallopava*
	12547	UTHSC 12-69	Bronchial wash, USA	*Ochroconis gallopava*
	12548	UTHSC 12-549	Bronchial wash, USA	*Ochroconis gallopava*

[a] FMR: Faculty of Medicine, Reus.

[b] 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, Texas, USA.

[c] BAL: Bronchoalveolar lavage fluid. [T] Type strain.

Table 2 Type and reference strains from international culture collections used for comparison.

Species[a]	Strain number[b]	FMR number[c]	Origin	Received as
Acremonium acutatum	CBS 682.71 [T]	11766	On *Solanum nigrum*	*Acremonium acutatum*
Acremonium alternatum	CBS 407.66 [T]	10489	*Hypoxylon deustum,* Austria	*Acremonium alternatum*
Acremonium blochii	CBS 993.69	5330	Skin, The Netherlands	*Acremonium blochii*
Acremonium borodinense	CBS 101148 [T]	10655	Soil in sugarcane field, Japan	*Acremonium borodinense*
Acremonium cavaraeanum	CBS 101149 [T]	12306	Outer wall of wooden house, Japan	*Acremonium cavaraeanum*
	CBS 111656	12305	Coconut, Brazil	*Acremonium cavaraeanum*
Acremonium citrinum	CBS 758.69	11764	Sputum, Unknown	*Acremonium cavaraeanum*
	CBS 384.96 [T]	11427	On decaying fruit, Papua New Guinea	*Acremonium hansfordii*
Acremonium curvulum	CBS 430.66 [T]	10483	Wheat field soil, Germany	*Acremonium curvulum*
Acremonium domschii	CBS 764.69 [T]	11596	*Inonotus obliquus*, Germany	*Acremonium domschii*
Acremonium egyptiacum	CBS 114785 [T]	10656	Ground, Egypt	*Acremonium egyptiacum*
"*Acremonium egyptiacum*"	CBS 303.64	12359	Seed from *Triticum aestivum,* France	*Acremonium egyptiacum*
Acremonium furcatum	MUCL 9745 [T]	10603	Sand, France	*Acremonium furcatum*
Acremonium fusidioides	CBS 266.89	11411	Agricultural soil, Germany	*Acremonium fusidioides*
	CBS 673.82	11412	Unknown, Denmark	*Acremonium fusidioides*
	CBS 705.86	11428	Unknown, France	*Acremonium hansfordii*
	CBS 840.68 [T]	10480	Dung of antelope, Central African Republic	*Acremonium fusidioides*
	CBS 991.69	11417	Sputum, The Netherlands	*Acremonium fusidioides*
	CBS 109069	11409	Barley seeds, Ethiopia	*Acremonium fusidioides*
	MUCL 9579	11048	Monkey dung, Japan	*Acremonium fusidioides*
	MUCL 9580	11050	Unknown, Italy	*Acremonium fusidioides*
"*Acremonium hansfordii*"	CBS 110390	11426	Stucco with ring of moisture, Germany	*Acremonium hansfordii*
Acremonium hennebertii	CBS 768.694 [T]	11904	*Elaeis guineensis*, Zaire	*Acremonium hennebertii*
"*Acremonium implicatum*"	CBS 397.70B	11423	Dying leaf of *Cladium mariscus*, The Netherlands	*Acremonium implicatum*
	CBS 114748	11425	Tropical green seaweed, USA	*Acremonium implicatum*
	MUCL 8122	11046	Seed from *Triticum* sp., France	*Acremonium implicatum*
	MUCL 8123	11043	Seed from *Triticum* sp., France	*Acremonium implicatum*

Table 2 (continued)

Species[a]	Strain number[b]	FMR number[c]	Origin	Received as
Acremonium incoloratum	CBS 146.62 [T]	11320	Soil, Poona, Maharashtra	*Acremonium incoloratum*
Acremonium incrustatum	CBS 159.70 [T]	12361	Rhizomorph of *Armillaria mellea,* The Netherlands	*Acremonium incrustatum*
Acremonium inflatum	CBS 212.69 [T]	11332	Iintertidal salt marsh mud, UK	*Acremonium inflatum*
	CBS 305.74	11335	Cereal stem, UK	*Acremonium inflatum*
	CBS 439.70	11334	Sandy soil under permanent wheat, The Netherlands	*Acremonium inflatum*
	CBS 497.82	11336	Agricultural soil, The Netherlands	*Acremonium inflatum*
	CBS 604.68	11333	Soil, Germany	*Acremonium inflatum*
Acremonium longisporum	CBS 993.87	5331	Ulcer on arm and back, The Netherlands	*Acremonium longisporum*
"*Acremonium ochraceum*"	MUCL 34528	11051	Fruit of *Musa* sp., Unknown	*Acremonium ochraceum*
Acremonium parvum	CBS 381.70A [T]	12358	*Tubercularia vulgaris*, The Netherlands	*Acremonium alternatum*
	CBS 831.97	12357	Old stem, The Netherlands	*Acremonium alternatum*
Acremonium persicinum	CBS 310.59 [T]	10487	Coastal sand under *Ammophila arenaria*, France	*Acremonium persicinum*
Acremonium pilosum	CBS 124.70	11415	Agricultural soil, The Netherlands	*Acremonium fusidioides*
	CBS 125.70	11416	Agricultural soil, The Netherlands	*Acremonium fusidioides*
	CBS 390.73	11429	On *Periconia cookei* on dead bamboo, India	*Acremonium hansfordii*
	CBS 410.70	11414	Agricultural soil, The Netherlands	*Acremonium fusidioides*
	CBS 511.82	11413	Agricultural soil, The Netherlands	*Acremonium fusidioides*
Acremonium pinkertoniae	CBS 157.70 [T]	11597	Soil from tropical greenhouse, the Netherlands	*Acremonium pinkertoniae*
Acremonium pteridii	CBS 782.69 [T]	12362	Dead petiole of *Pteridium aquilinum*, England	*Acremonium pteridii*
Acremonium roseolum	CBS 289.62 [T]	11359	Dead stems, England	*Acremonium roseolum*
	CBS 381.73	11420	Dead stem of bamboo, India	*Acremonium implicatum*
	MUCL 30020	10606	Soil, France	*Acremonium hyalinulum*
Acremonium rutilum	MUCL 6094	11315	Soil, Belgium	*Acremonium persicinum*
Acremonium sordidulum	CBS 385.73 [T]	11762	Old stem, India	*Acremonium sordidulum*
	CBS 629.73	11763	Toe nail, UK	*Acremonium sordidulum*
Acremonium stromaticum	CBS 863.73 [T]	13583	Root and rhizome of *Musa sapientum*	*Acremonium stromaticum*

Table 2 continued

Species[a]	Strain number[b]	FMR number[c]	Origin	Received as
Acremonium vitellinum	CBS 622.76	11759	Gymnosperm forest litter, Belgium	*Acremonium vitellinum*
	CBS 792.69 [T]	11758	Dead leaf, The Netherlands	*Acremonium vitellinum*
Acremonium zonatum	CBS 211.74	12296	*Eichhornia crassipes*, USA	*Acremonium zonatum*
	CBS 232.75B	12297	Skin, Sweden	*Acremonium zonatum*
	CBS 400.70	12298	Leaf and pericarp, Zaire	*Acremonium zonatum*
Arthrographis alba	CBS 370.92 [T]	4030	Marine sediments, Spain	*Arthrographis alba*
Arthrographis arxii	CBS 203.78 [T]	5262	Dung of herbivore, India	*Eremomyces langeronii*
Arthrographis kalrae	CBS 693.77 [T]	5264	Sputum, India	*Arthrographis kalrae*
Arthrographis lignicola	CBS 689.83 [T]	-	Gymnosperm wood chips and bark, Canada	Arthrographis lignicola
Arthrographis pinicola	CBS 653.89 [T]	-	Gallery of *Ips latidens* in *Pinus contorta*, Canada	*Arthrographis pinicola*
Arthropsis cirrhata	CBS 628.83 [T]	-	Wall, The Netherlands	*Arthropsis cirrhata*
Arthropsis hispanica	CBS 351.92 [T]	4058	Marine sediments, Spain	*Arthropsis hispanica*
	CBS 352.92	4059	Bottom water deposit, Spain	*Arthropsis hispanica*
Arthropsis microsperma	UAMH 4290	12430	Grass, England	*Arthropsis microsperma*
Arthropsis truncata	CBS 584.82 [T]	-	Leaf litter, Peru	*Arthropsis truncata*
Brunneomyces brunnescens	CBS 559.73 [T]	13582	Dead stem of *Dendrocalamus giganteus*, Sri Lanka	*Acremonium brunnescens*
Brunneomyces europaeus	CBS 560.86 [T]	3406	Leaf of bamboo, France	*Acremonium hyalinulum*
	CBS 652.96	3962	River sediment, Spain	*Acremonium hyalinulum*
Bulbithecium hyalosporum	CBS 318.91 [T]	11598	Dung of horse, Peru	*Bulbithecium hyalosporum*
Cervusimilis alba	CBS 987.87 [T]	10886	*Hypogymnia physodes*, Luxembourg	*Acremonium antarcticum*
Eremomyces bilateralis	CBS 781.70 [T]	-	Dung of pack rat, USA	*Eremomyces bilateralis*
Leucosphaerina arxii	CBS 737.84 [T]	11599	Dung of horse, USA	*Leucosphaerina arxii*
Paecilomyces farinosus	CBS 117534	12314	Polished rice, unknown	*Sarocladium bacillisporum*
Rhexothecium globosum	CBS 955.73 [T]	-	Desert soil, Egypt	*Rhexothecium globosum*
Sarocladium bacillisporum	CBS 119.79	12311	Smoked sliced meat, Sweden	*Sarocladium bacillisporum*
	CBS 212.79	12309	Insect, Romania	*Sarocladium bacillisporum*
	CBS 388.67	12307	Soil, The Netherlands	*Sarocladium bacillisporum*
	CBS 425.67 [T]	10660	Soil, Canada	*Sarocladium bacillisporum*

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Dipòsit Legal: T 1161-2015

Table 2 continued

Species[a]	Strain number[b]	FMR number[c]	Origin	Received as
Sarocladium bacillisporum	CBS 485.67	12308	Unknown	*Sarocladium bacillisporum*
	CBS 787.69	11424	Teleutosorus of *Puccinia graminis* on *Lolium temulentum,* Italy	*Acremonium implicatum*
Sarocladium bactrocephalum	CBS 749.69 [T]	10479	*Ustilago* sp., Canada	*Sarocladium bactrocephalum*
Sarocladium bifurcatum	CBS 383.73	12316	Dead stem of bamboo, India	*Acremonium ochraceum*
Sarocladium gamsii	CBS 425.73	12432	Dead petiole of *Pandanus lerum*, Sri Lanka	*Sarocladium glaucum*
	CBS 707.73 [T]	11419	Dead stem of *Pandanus lerum*, Sri Lanka	*Acremonium implicatum*
Sarocladium glaucum	CBS 191.80	12300	Dead stem of bamboo, Colombia	*Sarocladium glaucum*
	CBS 309.74	12301	Air, above sugarcane field, India	*Sarocladium glaucum*
	CBS 382.73	12299	Dead stem of bamboo, India	*Sarocladium glaucum*
	CBS 425.73a	12303	Dead petiole of *Pandanus lerum*, Sri Lanka	*Sarocladium glaucum*
	CBS 456.74	12302	Sugar, Mauritius	*Sarocladium glaucum*
	CBS 796.69 [T]	10657	Woolen overcoat, Solomon Islands	*Sarocladium glaucum*
	CBS 100350	12304	Dead stem of bamboo, Japan	*Sarocladium glaucum*
Sarocladium implicatum	CBS 397.70A	11422	*Saccharum officinarum*, Jamaica	*Acremonium implicatum*
	CBS 825.73	11418	*Saccharum officinarum*, India	*Acremonium implicatum*
	CBS 959.72 [NT]	12360	Desert soil, Egypt	*Acremonium implicatum*
Sarocladium kiliense	CBS 122.29 [T]	10604	Skin, Germany	*Sarocladium kiliense*
Sarocladium mycophilum	CBS 166.92 [T]	-	*Cortinarius subsertipes*, Germany	*Sarocladium mycophilum*
Sarocladium ochraceum	CBS 428.67 [T]	11760	*Zea mays*, Kenya	*Sarocladium ochraceum*
Sarocladium oryzae	CBS 180.74 [ET]	-	*Oryza sativa*, India	*Sarocladium oryzae*
	CBS 399.73	12077	*Oryza sativa*, India	*Sarocladium attenuatum*
	CBS 414.81	-	*Oryza sativa*, Nigeria	*Sarocladium attenuatum*
Sarocladium strictum	CBS 346.70 [T]	10659	*Triticum aestivum*, Germany	*Sarocladium strictum*
	CBS 565.67	11767	Leaf, unknown	*Acremonium zonatum*
	CBS 640.75	12310	Decaying wood, The Netherlands	*Sarocladium bacillisporum*
Sarocladium subulatum	MUCL 9939 [T]	11044	Soil, Egypt	*Acremonium. implicatum*
Sarocladium summerbellii	CBS 200.84	11761	Water in air moistener, The Netherlands	*Sarocladium ochraceum*
	CBS 430.70 [T]	12318	Soil from greenhouse, The Netherlands	*Sarocladium ochraceum*

Table 2 continued

Species[a]	Strain number[b]	FMR number[c]	Origin	Received as
Sarocladium summerbellii	CBS 797.69	12319	Decaying leaf, The Netherlands	*Sarocladium ochraceum*
	CBS 891.73	12315	Dead leaf, Sri Lanka	*Sarocladium ochraceum*
	CBS 951.72	12317	Agricultural soil, The Netherlands	*Sarocladium ochraceum*
Sarocladium terricola	CBS 134.71	11421	*Arundo donax*, Italy	*Acremonium. implicatum*
	CBS 243.59 [T]	10460	Forest soil, USA	*Acremonium terricola*
	MUCL 12011	11045	Decaying leaf, Democratic Republic of Congo	*Acremonium. implicatum*
	MUCL 42865	11047	Infected palm grove, Morocco	*Acremonium. implicatum*
Sarocladium zeae	CBS 800.69 [T]	10661	*Zea mays* stalk, USA	*Sarocladium zeae*
Trichothecium crotocinigenum	MUCL 8085 [T]	11321	Basidiome, Hungary	*Trichothecium crotocinigenum*

[a] Species names with quotes correspond to isolates that could not be assigned to a phylogenetically confirmed name and are listed with the name and code under which they are currently held in the CBS and MUCL collections.
[b] CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; MUCL: Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; UAMH: University of Alberta Microfungus Collection and Herbarium, Edmonton, Canada; [ET] Epitype strain; [NT] Neotype strain; [T] Type strain.
[c] FMR: Faculty of Medicine, Reus.

3.3.1. Macroscopic and microscopic morphology

The macroscopic and microscopic features of the isolates were studied on different culture media, i.e., PDA, PCA, 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 % malt extract agar (MEA 2 %; BD Difco™, Franklin Lakes, NJ, USA), depending on the fungal species investigated and mostly following the recommendations of different authors (Gams 1971, Gams & Hawksworth 1975, Sigler & Carmichael 1976, 1983, Sigler et al. 1982, Gené 1994). Cultures were incubated at 25 ± 1 °C in the dark and periodically examined each 7 days up to 4 weeks. Colony diameters were measured after 7, 14 and/or 21 days of incubation and rated according to the colour chart of Kornerup & Wanscher (1978). The texture, topography and production of diffusible pigment and exudate drops were also determined in these media. Microscopic features (i.e., shape, size, colour and ornamentation of the vegetative hyphae, the conidiogenous apparatus and conidia, and of those structures associated to the sexual morph and chlamydospores, when present) were examined and measured from slide cultures and from direct wet mounts in either 85 % lactic acid or lactophenol cotton blue under a light microscope Olympus CH-2 (Olympus Corporation, Tokyo, Japan). Photomicrographs were obtained with a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany) using phase contrast and Nomarski differential interference.

For a more detailed description of the microscopic features, such as conidial ornamentation of the species belonging to the genera *Acremonium* and *Sarocladium*, several isolates were examined under scanning electron microscopy (SEM). The isolates were cultured on OA and incubated at 25 °C in the dark for up to 14 days. Pieces of agar of 1 cm^2 were cut and transferred to vials containing a 2 % glutaraldehyde solution in 0.1 M phosphate buffer; later, the vials were sent to the *Servei de Recursos Científics i Tècnics* of the *Universitat Rovira i Virgili* (URV) and processed following the protocol described previously by Figueras & Guarro (1988). Images were obtained with a Jeol JSM-6400 scanning electron microscope (JEOL, Tokyo, Japan).

3.3.2. Physiological tests

To establish cardinal temperatures for growth of the different fungal species investigated, the isolates were cultured on PDA in Petri dishes of 9 cm diam and incubated at temperatures ranging from 4 to 45 °C. The colony diameters were measured after 7, 14 or 21 days depending on the species tested.

To determine the resistance to cycloheximide, isolates were transferred to Petri dishes containing PCA supplemented with cycloheximide at a final concentration of 2 g/L, and incubated at 25 °C for two weeks.

To evaluate the ability of isolates to convert to the yeast phase, a portion from a fresh culture on PDA was transferred to tubes with Brain Heart Infusion broth (BHI; Becton Dickinson & Company, Franklin Lakes, NJ, USA) and incubated at 37 °C for two weeks. Subsequently, several transfers to BHI broth were made until yeast-like budding cells were observed on direct mounts of a drop of this medium. All physiological tests were carried out in duplicate.

3.4. Molecular studies

Molecular studies were performed in different steps and included: DNA extraction, amplification and sequencing, obtaining of consensus sequences, BLAST searches (in some cases), sequence alignment and finally, construction of phylogenetic trees.

Prior to the **DNA extraction**, all the isolates included in this thesis were grown on yeast extract sucrose agar (YES; yeast extract, 20 g; sucrose, 150 g; agar, 20 g; distilled water to final volume of 1000 mL) for one or two weeks at 25 °C. Genomic DNA was extracted from fungal colonies with a PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. For *Ochroconis* and *Verruconis* isolates, DNA extraction was carried out using a FastDNA® kit (MP Biomedicals, Solon, OH, USA) following the manufacturer's protocol, with the homogenization step done using a FastPrep® FP120 cell disrupter (Thermo Savant, Holbrook, NY, USA). DNA was quantified with a GeneQuant *pro* (Amersham Pharmacia Biotech, Cambridge, UK) or using a NanoDrop 3000 (ThermoScientific, Asheville, NC, USA).

For the **amplification and sequencing** , an initial screening using the internal transcribed spacer regions (ITS) and intervening 5.8S nrRNA gene and a portion of the 28S nrRNA gene (LSU) was carried out for all the isolates included in the thesis. The ITS region was amplified and sequenced using primer pairs ITS5/ITS4 (White et al. 1990). The amplification and sequencing of the domains D1/D2 and D1/D3 of the 5' end of the LSU was carried out with the primer set NL1/NL4b (O'Donnell 1993) and LR0R/LR5 (Vilgalys & Hester 1990), respectively. On some occasions, the ITS and the 5' end of the LSU regions were amplified in a single PCR reaction using the primer set ITS5/NL4 (for the ITS+D1/D2 fragment) or ITS5/LR5 (for the ITS+D1/D3 fragment). The generated PCR product was sequenced with the same primers used for the amplification, as well as with the internal primers ITS4 and NL1 or LROR. Additionally, different loci were sequenced for inclusion in the multilocus analyses for each particular study.

For the studies involving acremonium-like fungi, the multilocus analysis included the ITS region, the D1/D2 domains and a fragment of the actin (*ACT1*) or β-tubulin (*BT2*) genes. The latter two genes were amplified and sequenced with the primer set Act1/Act4 (Voigt &

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Wöstemeyer 2000) and Tub-F/Tub-R (Cruse et al. 2002), respectively. In the particular case of those *Acremonium* isolates related to *Plectosphaerellaceae* or the genus *Emericellopsis*, the multilocus analysis included the ITS region, the D1/D3 domains, and fragments of the translation elongation factor 1-alpha (*TEF1-α*) and the RNA polymerase II second largest subunit gene (*RPB2*) genes, the latter two being amplified and sequenced with the primer sets EF 983F/EF 2218R (Rehner & Buckley 2005) and Rpb2-5F/Rpb2-7R (Liu et al. 1999).

In the study on arthroconidial fungi, the ITS region and the D1/D2 domains were used to identify at the species level, and then portions of the *ACT1* and the chitin synthase 1 genes (*CHS1*) were added to the multilocus sequences analysis. The primer set CHS-79F/CHS-354R (Carbone & Kohn 1999) was used to amplify and sequence the latter gene.

Finally, the D1/D2 domains and ITS region were used to identify the ochroconis-like isolates at the species level. A multilocus sequences analysis including the ITS region, the D1/D3 domains, the small subunit of nrDNA (SSU) and fragments of the *ACT1* and *BT2* genes was used to resolve the new taxa. Amplification and sequencing of the SSU and the *ACT1* and *BT2* genes were carried out with the primer pairs NS1/NS4 (White et al. 1990), ACT-512F/ACT-783R (Carbone & Kohn 1999) and Bt1a/Bt1b (Glass & Donaldson 1995), respectively.

PCR conditions were set as follows: an initial denaturation temperature of 94 °C for 5 min, followed by 35 cycles of denaturation of 95 °C for 30 s, primer annealing of 51-60 °C (depending of the primers set used) for 1 min, extension at 72 °C for 1 min and a final extension step at 72 °C for 7 min. Amplicons were purified using a GFX™ PCR DNA and Gel Band Purification kit (Pharmacia Biotech, Cerdanyola, Spain) or a Diffinity RapidTip® kit (Sigma-Aldrich, St Louis, MO, USA) and 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 BigDye Terminator v. 3.1 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 Inc. (Seoul, South Korea) or Macrogen Corp. Europe (Amsterdam, The Netherlands) with a 3730xl DNA analyser (Applied Biosystems).

To obtain the **consensus sequences** from the complementary strand of each isolate, the program SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA) was used. In the studies on acremonium-like fungi isolated from soil samples and some arthroconidial fungi, a **BLAST** sequence identity search (Altschul et al. 1990) was made to compare the sequence of the each isolate studied with those of other fungi deposited in the NCBI's GenBank and NITE Biological Resource Center (NBRC) nucleotide databases. Sequences of the closest hits were downloaded and included in an alignment. Multiple **sequence**

alignments were made with Clustal X v. 1.8 (Thompson et al. 1997) with default parameters, followed by manual adjustments with a text editor, or with Clustal W using MEGA v. 5.05 (Tamura et al. 2011) and manually corrected where necessary. For some studies involving acremonium-like fungi, ambiguous (unalignable) parts from the alignments from the ITS region and actin gene, were removed with Gblocks v. 0.91b software, using relaxing selection parameters (Castresana 2000, Talavera & Castresana 2007).

For the construction of the **phylogenetic trees**, different methods were used, either for the analysis of a single gene or for the concatenated dataset.

The **Neighbor-Joining (NJ)** method (Saitou & Nei 1987) was carried out with MEGA v. 4.0 (Tamura et al. 2007) or MEGA v. 5.05 (Tamura et al. 2011) with the algorithm Kimura 2-parameter as the nucleotide substitution model, and treating the gaps as pairwise deletions. This algorithm was applied to analysis of the ITS region in one of the studies on acremonium-like fungi.

In the **Maximum Composite Likelihood (ML)** method, distance trees were constructed with MEGA v. 5.05 (Tamura et al. 2011) with Nearest-Neighbour-Interchange (NNI) used as the Heuristic method. The nucleotide substitution model for each locus and for the combined datasets was determined using the best-fit nucleotide model function under the same software. Gaps and missing data were treated as partial deletion with a site coverage cut-off of 95 %. Congruency of the sequence datasets for the separate loci were determined using tree topologies of 70 % reciprocal NJ bootstrap trees with Maximum Likelihood distances, which were compared visually to identify conflicts between partitions (Gueidan et al. 2007). This method was used in most of the studies carried out in this thesis, and applied for the analysis of a single gene and for the concatenated dataset.

For the **Maximum Parsimony (MP)** analysis, phylogenetic inference to find the most parsimonious tree was carried out by using PAUP* v. 4.0b10 software (Swofford 2003). One hundred heuristic searches were made with random sequence addition and tree bisection-reconnection branch-swapping algorithms, collapsing zero-length branches and saving all minimal-length trees (MULTREES). Gaps were treated as missing data. The analysis of the combined dataset in the MP method was tested for incongruence with the partition homogeneity test (PHT), as implemented in the same software. For NJ, ML and MP methods the internal branch support was assessed by a search of 1000 bootstrapped sets of data, and bootstrap support (bs) values of ≥70 were considered significant. The MP method was used in multilocus sequence analyses in some studies on acremonium- and ochroconis-like fungi.

The **Bayesian Inference (BI)** method was carried out by using MrBayes v. 3.2.1 software (Ronquist & Huelsenbeck 2003) with two simultaneous runs for 1–3 million 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 best nucleotide substitution model for each gene was selected using MrModelTest v. 2.3 (Nylander 2004). This method was used in studies of acremonium- and ochroconis-like fungi.

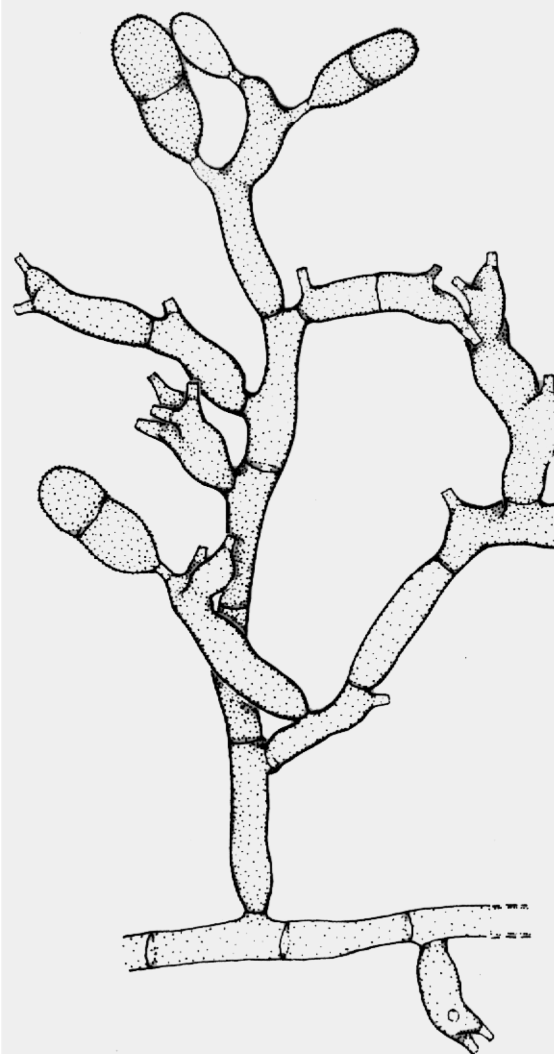
DNA sequence data generated in this study were deposited in GenBank or ENA databases, the alignments and trees in TreeBASE (http://www.treebase.org) and taxonomic novelties in MycoBank (http://www.MycoBank.org; Crous et al. 2004).

3.5. Antifungal susceptibility

The antifungal susceptibility testing was carried out for isolates of the species belonging to the genera *Arthrographis, Arthropsis, Scytalidium* and *Ochroconis.* The isolates of the first three genera were tested at the Fungal Testing Laboratory (UTHSC, San Antonio, USA) and those of the latter at the *Unitat de Micologia* of the *Facultat de Medicina i Ciències de la Salut* (URV, Reus, Spain).

The antifungal drugs evaluated were amphotericin B (AMB), anidulafungin (AFG), caspofungin (CFG), itraconazole (ITC), micafungin (MFG), posaconazole (PSC), terbinafine (TBF) and voriconazole (VRC) at final concentrations ranging from 0.016 to 16 µg/ml, following the methods outlined in the Clinical and Laboratory Standards Institute (CLSI, 2008) document M38-A2 for filamentous fungi. The minimal effective concentration (MEC) was determined at 24 or 48 h for the echinocandins, and the minimal inhibitory concentration (MIC) was determined at 48 h and 72 h for the remaining drugs. The MIC was defined as the lowest concentration exhibiting 100% visual inhibition of growth for AMB, VRC, ITC and PSC and an 80 % reduction in growth for TBF. *Paecilomyces variotii* ATCC MYA-3630 and *Aspergillus fumigatus* ATCC MYA-3626 were used as quality control strains. The CLSI protocol was slightly modified for the *Ochroconis* species. Due to the inability of most *Ochroconis* isolates to grow at 35 °C and their slow growth rate, the microplates were incubated at 30 °C and the MEC values were determined at 48 h.

4. RESULTS



Ochroconis constricta
Hand draw by J. Gené

4.1. Studies on acremonium-like fungi

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Final identification and taxonomic placement of the acremonium-like isolates studied in this thesis are shown in Table 3. Twenty-one fresh isolates obtained from soil samples were collected in different areas from Spain. Isolates from clinical origin (n = 21) are mostly unidentified fungi derived from a previous study on *Acremonium* isolates carried out by Perdomo et al. (2011b). The remaining isolates are reference strains from different international culture collections used for comparison, and some of them re-identified as a result of our polyphasic taxonomic study.

Table 3 Acremonium-like fungi from environmental or clinical origin identified in this thesis.

Species[a]	**Taxonomy** (Family, Order)	FMR[b]	Origin[c]	Section
Acremoniopsis suttonii	*Incertae sedis, Hypocreales*	11780	Env.	4.1.5
Acremonium acutatum	*Incertae sedis, Hypocreales*	10368	Clin.	
Acremonium asperulatum	*Bionectriaceae, Hypocreales*	11065	Env.	4.1.1
		11135	Env.	4.1.1
		11136	Env.	4.1.1
		11137	Env.	4.1.1
		11138	Env.	4.1.1
		11139	Env.	4.1.1
		11783	Env.	4.1.1
Acremonium citrinum	*Bionectriaceae, Hypocreales*	11427	Env.	4.1.2
		11764	Clin.	4.1.2
Acremonium dimorphosporum	*Bionectriaceae, Hypocreales*	10548	Clin.	4.1.3
Acremonium fusidioides	*Bionectriaceae, Hypocreales*	11048	Env.	4.1.2
		11050	Unk.	4.1.2
		11409	Env.	4.1.2
		11411	Env.	4.1.2
		11412	Unk.	4.1.2
		11417	Clin.	4.1.2
		11428	Unk.	4.1.2
Acremonium moniliforme	*Bionectriaceae, Hypocreales*	10363	Clin.	4.1.3
		11785	Env.	4.1.3
Acremonium parvum	*Bionectriaceae, Hypocreales*	12357	Env.	4.1.2
		12358	Env.	4.1.2
Acremonium pilosum	*Bionectriaceae, Hypocreales*	11413	Env.	4.1.2
		11414	Env.	4.1.2
		11415	Env.	4.1.2
		11416	Env.	4.1.2
		11429	Env.	4.1.2
Acremonium pteridii	*Incertae sedis, Hypocreales*	11786	Env.	
		12085	Env.	
		13209	Env.	
Acremonium roseolum	*Incertae sedis, Hypocreales*	10351	Clin.	
		11420	Env.	
		10606	Env.	
Acremonium rutilum	*Incertae sedis, Hypocreales*	11315	Env.	
Acremonium sclerotigenum	*Bionectriaceae, Hypocreales*	12086	Env.	
		12087	Env.	
Acremonium sp.		11043	Env.	4.1.4
		11046	Env.	4.1.4
		12359	Env.	
		11426	Env.	4.1.2
		11423	Env.	4.1.4

Table 3 (continued)

Species[a]	**Taxonomy** (Family, Order)	**FMR**[b]	**Origin**[c]	**Section**
Acremonium sp.		11425	Env.	4.1.4
		11051	Env.	4.1.4
Acremonium variecolor	*Bionectriaceae, Hypocreales*	11140	Env.	4.1.1
		11141	Env.	4.1.1
Brunneomyces brunnescens	*Plectosphaerellaceae, Incertae sedis*	13582	Env.	4.1.3
Brunneomyces europaeus	*Plectosphaerellaceae, Incertae sedis*	3406	Env.	4.1.3
		3962	Env.	4.1.3
Brunneomyces hominis	*Plectosphaerellaceae, Incertae sedis*	10429	Clin.	4.1.3
		10437	Clin.	4.1.3
Cervusimilis alba	*Plectosphaerellaceae, Incertae sedis*	10433	Clin.	4.1.3
		10886	Env.	4.1.3
		10549	Clin.	4.1.3
Collarina aurantiaca	*Clavicipitaceae, Hypocreales*	11134	Env.	4.1.6
		11784	Env.	4.1.6
Cosmospora berkeleyana	*Nectriaceae, Hypocreales*	11777	Env.	
Gliomastix murorum	*Incertae sedis, Hypocreales*	11781	Env.	
Paecilomyces farinosus	*Trichocomaceae, Eurotiales*	12314	Env.	4.1.4
Sarocladium bacillisporum	*Incertae sedis, Hypocreales*	11424	Env.	4.1.4
		12307	Env.	4.1.4
		12308	Unk.	4.1.4
		12309	Env.	4.1.4
		12311	Env.	4.1.4
Sarocladium bactrocephalum	*Incertae sedis, Hypocreales*	10552	Clin.	4.1.4
Sarocladium bifurcatum	*Incertae sedis, Hypocreales*	10405	Clin.	4.1.4
		10451	Clin.	4.1.4
		12316	Env.	4.1.4
Sarocladium gamsii	*Incertae sedis, Hypocreales*	11419	Env.	4.1.4
		12432	Env.	4.1.4
Sarocladium glaucum	*Incertae sedis, Hypocreales*	12299	Env.	4.1.4
		12300	Env.	4.1.4
		12301	Env.	4.1.4
		12302	Env.	4.1.4
		12303	Env.	
		12304	Env.	4.1.4
Sarocladium hominis	*Incertae sedis, Hypocreales*	10352	Clin.	4.1.4
		10418	Clin.	4.1.4
		10425	Clin.	4.1.4
Sarocladium implicatum	*Incertae sedis, Hypocreales*	11418	Env.	4.1.4
		11422	Env.	4.1.4
		12360	Env.	4.1.4
Sarocladium mycophilum	*Incertae sedis, Leotiomycetes*	(CBS 166.92)	Env.	4.1.4
Sarocladium oryzae	*Incertae sedis, Hypocreales*	12077	Env.	4.1.4
		(CBS 180.74)	Env.	4.1.4
		(CBS 414.81)	Env.	4.1.4
Sarocladium pseudostrictum	*Incertae sedis, Hypocreales*	10347	Clin.	4.1.4
Sarocladium strictum		11767	Env.	
		12310	Env.	4.1.4
Sarocladium subulatum	*Incertae sedis, Hypocreales*	10441	Clin.	4.1.4
		11044	Env.	4.1.4
Sarocladium summerbellii	*Incertae sedis, Hypocreales*	11761	Env.	4.1.4
		12315	Env.	4.1.4
		12317	Env.	4.1.4
		12318	Env.	4.1.4
		12319	Env.	4.1.4
Sarocladium terricola	*Incertae sedis, Hypocreales*	10348	Clin.	4.1.4
		10356	Clin.	4.1.4

Table 3 (continued)

Species[a]	**Taxonomy** (Family, Order)	**FMR**[b]	**Origin**[c]	**Section**
Sarocladium terricola		10369	Clin.	4.1.4
		10388	Clin.	4.1.4
		10450	Clin.	4.1.4
		10460	Env.	4.1.4
		10561	Clin.	4.1.4
		10571	Clin.	4.1.4
		11045	Env.	4.1.4
		11047	Env.	4.1.4
		11421	Env.	4.1.4
Trichothecium crotocinigenum	*Incertae sedis, Hypocreales*	11782	Env.	

[a] New taxa proposed from our study are shown in bold face.

[b] Isolates from the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

[c] Clin.: Clinical; Env.: Environmental.

Publications derived from the study on acremonium-like fungi are shown in sections 4.1.1–4.1.6 of the thesis.

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4.1.1. Two new species of *Acremonium* from Spanish soils

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Two new species of *Acremonium* from Spanish soils

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Abstract: In a survey on the diversity of microfungi in Spanish soils, two new species of *Acremonium* were found. Both species were characterized as having more or less erect, mostly branched conidiophores bearing whorls of acicular phialides. In addition, one of these species, *Acremonium asperulatum,* produced abundant chlamydospores and globose rough-walled conidia. The other species, *Acremonium variecolor,* produced a brownish diffusible pigment and smooth-walled, subglobose conidia with apiculate base; sessile conidia inserted directly on vegetative hyphae also were present. The analysis of the sequences of the ITS region, the D1/D2 domains of the 28S rRNA gene and a fragment of the actin gene revealed relationships of both species with members of the Bionectriaceae (Hypocreales). Genetic differences were observed with morphologically similar species.

Key words: Hypocreales, phylogeny, soil fungi, taxonomy

INTRODUCTION

Acremonium Link is an anamorph genus that currently contains more than 100 species. Most of these are saprobic and are isolated mainly from dead plant material and soil (Gams 1971, Domsch et al. 2007). Some taxa can cause opportunistic infections in humans and animals (de Hoog et al. 2000, Guarro et al. 2009, Das et al. 2010). Cultures of *Acremonium* species generally grow slowly and form narrow, tapered phialides on creeping hyphae, although differentiated conidiophores with or without verticillate branching may be observed in some species. The conidia are usually small, unicellular and aggregate in slimy heads or chains; sometimes heads and chains are present in a single culture. Characteristic chlamydospores and sclerotia are produced in some species (Gams 1971, 1975; Domsch et al. 2007; Perdomo et al. 2011).

Recent molecular studies have demonstrated that *Acremonium* is polyphyletic, with species belonging to different orders of Sordariomycetes. Most species, including the type species *A. alternatum* Link: Fr., belong to the Hypocreales, some to the Sordariales and a small group to the family Plectosphaerellaceae in the Glomerellales (Glenn et al. 1996, Zare et al. 2007, Schoch et al. 2009, Perdomo et al. 2011, Summerbell et al. 2011). In a recent phylogenetic study, which included many species of *Acremonium* and related genera, Summerbell et al. (2011) re-accommodated some hypocrealean species of *Acremonium* in *Sarocladium* W. Gams & D. Hawksw. and in the reinstated genus *Gliomastix* Guég.

In a survey on the diversity of microfungi from Spanish soils, several strains morphologically as attributed to the genus *Acremonium,* denoted as species I and species II, were isolated. Because the relevant features of these isolates did not match any described species, we carried out a detailed study to characterize them, with sequences of several genes combined with phenotypic characteristics.

MATERIALS AND METHODS

Site, sampling and fungal isolation.—Soil samples were collected in two areas in northern Spain, in the provinces of Huesca (Aragon) and Lugo (Galicia). Aragonese samples were collected in the Ordesa valley, the Añisclo Canyon and in the valleys of Escuaín and Bujaruelo. The first three sites belong to the Ordesa y Monte Perdido National Park. The abundant forest is 750–2100 m, and its vegetation comprises mainly oaks, pines, hazel and beech trees. Average temperature is 0.4–0.7 C in the coldest months (January, February) to 13 C in the warmest months (July, August), with an average annual rainfall of approximately 1735 mm. Galician samples were from the Sierra de Os Ancares Natural Reserve, an area formed by high mountains, valleys, and influenced by some rivers. The area covers 53 664 ha and its altitude is 300 m in the valley and 1925 m at the highest peak. Its average temperature is 7–8 C in the coldest months to 18 C in the warmest, and the average annual rainfall is approximately 2042 mm. The tree vegetation comprises mainly poplar, birch, chestnut, walnut, oak, ash and alder.

Samples were taken from the superficial layer of soil and from river sediments with sterilized polyethylene bags

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closed with rubber bands. In the laboratory samples were stored at 4–7 C until they were processed. One gram of each soil sample was washed repeatedly with 10 mL sterilized water to reduce excessive microbial growth. After the final wash, excess water was decanted and the remaining soil was distributed among three Petri dishes. Potato dextrose agar (PDA, Difco Laboratories, Detroit, Michigan), supplemented with chloramphenicol (200 mg/L) and cycloheximide at a final concentration of 2 g/L at 45 C, was mixed with the soil, and once solidified the cultures were incubated at 25 C in the dark. All cultures were examined weekly with a stereomicroscope up to 1 mo. To purify isolates conidia were transferred with a sterile dissection needle from isolation plate cultures to Petri dishes containing potato carrot agar (PCA; 20 g potatoes, 20 g carrot; 20 g agar, 1000 mL distilled water) prepared by ourselves following the procedure in Onions and Pitt (1988) and incubated at 25 C in the dark.

Fungal isolates.—In addition to the isolates obtained with the above procedure, numerous reference strains of morphologically similar species were included in the study (TABLE I). They were provided mainly by the CBS-KNAW Fungal Biodiversity Centre (CBS) (Utrecht, the Netherlands) and the Mycothèque de l'Université Catholique de Louvain (MUCL) (Louvain-la-Neuve, Belgium).

DNA extraction, amplification and sequencing.—Isolates were grown on yeast extract sucrose agar (YES; yeast extract 2%, sucrose 15%, agar 2%, 1000 mL water) 5 d at 25 C, and DNA was extracted with a PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, California), according to the manufacturer's protocol. DNA was quantified with GeneQuant pro (Amersham Pharmacia Biotech, Cambridge, UK). The internal transcribed spacer (ITS) region and D1/D2 domains of the 28S of the nuclear rRNA gene were amplified respectively with the primer pairs ITS5/ITS4 and NL1/NL4, following the protocols of Cano et al. (2004) and Gilgado et al. (2005). A fragment of the actin gene was amplified with the primer pairs Act1/Act4 (Voigt and Wöstermeyer 2000). PCR products were purified with a GFXTM PCR DNA kit (Pharmacia Biotech, Cerdanyola, Spain) and were stored at −20 C until sequencing. PCR products were sequenced with the same primers used for amplification and following the *Taq* DyeDeoxy Terminator cycle sequencing kit protocol (Applied Biosystems, Gouda, the Netherlands). DNA sequencing reaction mixtures were analyzed on a 310 DNA sequencer (Applied Biosystems). In addition, some amplified fragments were purified and sequenced at Macrogen Inc. (Seoul, South Korea) with a 3730XL DNA analyzer (Applied Biosystems). The program SeqMan (7.0.0 DNASTAR, Madison, Wisconsin) was used to obtain consensus sequences of each isolate. Some ITS sequences, corresponding to several species of *Acremonium* or other genera phylogenetically related in Summerbell et al. (2011) or that were morphologically similar to our isolates, were retrieved from GenBank and included in the phylogenetic analysis (TABLE I).

Aligment and phylogenetic analysis.—BLAST sequence identity queries (Altschul et al. 1990) were carried out to compare the soil isolates with other fungi deposited in the NITE Biological Resource Center (NBRC) and GenBank databases. Sequences were aligned with ClustalX 1.8 (Thompson et al. 1997) with default parameters, followed by manual adjustments with a text editor. The phylogenetic relationships were predetermined with ITS sequences. A multilocus sequence analysis of a selected group subsequently was carried out to confirm the results obtained from ITS data. For the first analysis we used Gblocks 0.91b software with relaxing selection parameters (Castresana 2000, Talavera and Castresana 2007) to remove ambiguous (unalignable) parts. The phylogenetic analysis was carried out with MEGA 4.0 (Tamura et al. 2007), with neighbor joining (NJ) (Saitou and Nei 1987) and the algorithm Kimura 2-parameter to obtain the distance tree. Gaps were treated as pairwise deletion. Support for internal branches was assessed by a search of 1000 bootstrapped sets of data. The multilocus sequence analysis comprised 19 of the 51 isolates, including the soil isolates and phylogenetically related type and reference strains of *Acremonium* and other genera. Other randomly selected type or reference strains were included in the analysis. The most parsimonious trees of the combined dataset, including ITS, D1/D2 and actin gene, were performed with PAUP* 4.0b10 (Swofford 2002). One hundred heuristic searches were conducted with random sequence addition and tree bisection-reconnection branch-swapping algorithms, collapsing zero-length branches and saving all minimal-length trees (MULTREES). Gaps were treated as missing data. The internal branch support was assessed with a heuristic parsimony search of 1000 bootstrapped datasets. Tree length, consistency, homoplasy and retention indexes (CI, HI, RI respectively) were recorded. The combined dataset was tested for incongruence with the partition homogeneity test (PHT), as implemented in PAUP*.

Phenotypic studies.—Macro- and microscopic features of the soil isolates were studied on oatmeal agar (OA; 30 g filtered oat flakes after 1 h simmering, 20 g agar, 1000 mL distilled water) prepared by ourselves following the procedure of Onions and Pitt (1988), malt extract agar 2% (MEA, Difco Laboratories) and PDA, incubated at 25 C in the dark 7–14 d up to 1 mo. The isolates' ability to grow at 5, 15, 20, 25, 30, 32, 35 and 37 C was tested on PDA. Color notations in parentheses were taken from Kornerup and Wanscher (1978). Microscopic features were examined by making direct wet mounts with 85% lactic acid or lactophenol cotton blue or by slide cultures on OA, with an Olympus CH-2 light microscope. Photomicrographs were obtained with a Zeiss Axio-Imager M1 light microscope with phase contrast and Nomarski differential interference.

Nucleotide sequence accession numbers.—The new DNA sequences generated in this study were deposited in GenBank (TABLE I). The alignment used in the phylogenetic analysis was deposited in TreeBASE (www.treebase.org, submission number 12146).

RESULTS

Phylogenetic analysis.—The BLAST query revealed that ITS sequences of our unidentified *Acremonium*

TABLE I. Species and strains included in the study, their origin and GenBank accession numbers

Species	Strains	Source	GenBank accession number		
			ITS region	D1/D2 domains of 28S rRNA gene	Actin
Acremonium alternatum	CBS 407.66^T	*Hypoxylon deustum,* Austria	**HE798150**		
Acremonium antarcticum	CBS 987.87	*Hypogymnia physodes,* Luxembourg	DQ825970		
Acremonium blochii	CBS 993.69	Skin, the Netherlands	**HE608636**	**HE608654**	**HE608628**
Acremonium borodinense	CBS 101148^T	Soil in sugarcane field, Japan	**HE608635**	**HE608653**	**HE608624**
Acremonium sp. I (= *A. asperulatum* sp. nov.)	CBS 130362^T	Forest soil, refuge San Nicolas, Bujaruelo Valley, Huesca, Spain	**HE608641**	**HE608649**	**HE608620**
	FMR 11135	Forest soil, Añisclo canyon, Huesca, Spain	**HE608642**		
	FMR 11136	Forest soil, Broto, Ordesa Valley, Huesca, Spain	**HE608643**		
	FMR 11137	Forest soil, Añisclo canyon, Huesca, Spain	**HE608644**		
	FMR 11138	Forest soil, Ordesa Valley, Huesca, Spain	**HE608645**		
	FMR 11139	Forest soil, Torla, Ordesa Valley, Huesca, Spain	**HE608646**		
	CBS 130363	Sediments, Ara River, Bujaruelo Valley, Huesca, Spain		**HE608650**	**HE608621**
Acremonium sp. II (= *A. variecolor* sp. nov.)	CBS 130360^T	Forest soil, Garganta de Escuaín, Huesca, Spain	**HE608647**	**HE608651**	**HE608622**
	CBS 130361	Forest soil, Lugo, Spain	**HE608648**	**HE608652**	**HE608623**
Acremonium curvulum	CBS 430.66^T	Wheat field soil, Germany	**HE608638**	**HE608656**	**HE608630**
Acremonium domschii	CBS 764.69^T	*Inonotus obliquus*, Germany			
Acremonium egyptiacum	CBS 114785^T	Ground, Egypt	FN706550		
Acremonium exuviarum	CBS 113360^T	*Corucia zebrata*, California	AY882946		
Acremonium fuci	CBS 112868^T	Blade of *Fucus serratus*, Germany	AY632653		
Acremonium furcatum	MUCL 9745^T	Sand, France			
Acremonium fusidioides	CBS 840.68^T	Dung of antelope, Central African Republic	FN706542		
Acremonium hansfordii	CBS 390.73	*Periconia cookei* on *Dendrocalamus* sp., India	AB540578		
Acremonium implicatum	MUCL 4112	Soil, Georgia, USA	FN706553	**HE608659**	**HE608632**
Acremonium incoloratum	CBS 146.62^T	Soil, Poona, Maharashtra			
Acremonium inflatum	CBS 439.70	Sandy soil under permanent wheat, the Netherlands			
Acremonium longisporum	CBS 993.87	Ulcer on arm and back, the Netherlands			
Acremonium persicinum	CBS 310.59^T	Coastal sand under *Ammophila arenaria*, France	FN706554		
Acremonium pinkertoniae	CBS 157.70^T	Soil from tropical greenhouse, the Netherlands		**HE608660**	**HE608625**
Acremonium potronii	CBS 379.70F	Skin lesion in dolphin, Belgium			
Acremonium recifei	MUCL 9696^T	Mycetoma, Brazil			
Acremonium roseolum	CBS 289.62^T	Dead stems, England			
Acremonium sclerotigenum	CBS 124.42^T	Dune sand under *Ammophila* and *Convolvulus*, France	FN706552		
Acremonium spinosum	CBS 136.33^T	Toe nail, Argentina	**HE608637**	**HE608655**	**HE608629**
Bulbithecium hyalosporum	CBS 318.91^T	Dung of horse, Cuzco, Perú	**HE608634**	**HE608661**	**HE608626**
Cosmospora butyri	CBS 301.38^T	Butter, Denmark	DQ286652		

TABLE I. Continued

Species	Strains	Source	GenBank accession number ITS region	D1/D2 domains of 28S rRNA gene	Actin
Cosmospora coccinea	CBS 114050	Dead crust, on fallen branch of *Fagus sylvatica*, Bavaria, Germany	FJ474072		
Geosmithia morbida	CBS 124663	*Pityophthorus juglandis*, Colorado, USA	FN434082		
Gibellulopsis nigrescens	CBS 120949	Soil under lawn, Baarn, the Netherlands	EF543857		
Gibellulopsis piscis	CBS 892.70[T]	Granuloma in gold- fish (*Carassius auratus*), Brazil	DQ825985		
Gliomastix polychroma	MUCL 9834[T]	Bark of *Hevea brasiliensis*, Sumatra, Indonesia	FN706547		
Haematonectria haematococca	—	*Grevillea robusta*, Kenya	HQ651171		
Leucosphaerina arxii	CBS 737.84[T]	Dung of horse, North Carolina, USA	**HE608640**	**HE608662**	**HE608627**
Nalanthamala diospyri	CBS 430.89	*Diospyros virginiana*, Mississippi, USA	AY554209		
Neonectria fuckeliana	—	*Picea abies*, Austria	AJ557573		
Pochonia suchlasporia var. catenata	CBS 248.83[T]	Egg of *Heterodera avenae*, Sweden	AJ292406		
Pochonia suchlasporia var. suchlasporia	CBS 251.83[T]	Egg of *Heterodera avenae*, Sweden	AJ292402		
Sarocladium bacillisporum	CBS 425.67[T]	Soil, Ontario, Canada	**HE608639**	**HE608658**	**HE608633**
Sarocladium bactrocephalum	CBS 749.69[T]	*Ustilago* sp., Manitoba, Canada			
Sarocladium glaucum	CBS 796.69[T]	Woollen overcoat, Solomon Islands	FN691454	**HE608657**	**HE608631**
Sarocladium kiliense	MUCL 9724[T]	Skin, Germany	FN691446		
Sarocladium strictum	CBS 346.70[T]	Old leaf on *Triticum aestivum*, Germany			
Sarocladium zeae	CBS 800.69[T]	Stalk of *Zea mays*, Nebraska, USA	FN691451		

FMR Faculty of Medicine Reus, Spain; CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; MUCL, Mycothèque de l'Université Catholique de Louvain, Louvain-la Neuve, Belgium; [T] type strain; Accession numbers of sequences newly determined in this study are indicated in boldface.

isolates did not match significantly any sequence deposited in GenBank or NBRC. The phylogenetic tree inferred from neighbor joining analysis of the ITS sequences revealed that the seven isolates of *Acremonium* sp. I and the two isolates of *Acremonium* sp. II were clustered with different members of the Bionectriaceae, where the ex-epitype strain of *A. alternatum* (CBS 407.66) was included. *Acremonium* sp I and II grouped into a highly supported terminal clade (100% bootstrap support [bs]) located far from remaining species included in this study (FIG.1). The clade was split into two well supported sister clades (92% and 100% bs, respectively), each of them including one of the two mentioned species. A highly supported (94% bs) sister clade of I and II, although phylogenetically distant, also included two subclades. One of them comprised the ex-type strains of the ascomycetes *Bulbithecium hyalosporum* Udagawa & T. Muroi and *Leucosphaerina arxii* Malloch and the other the ex-type strains of *Acremonium borodinense* Tad. Ito, Okane, Nagak. & W. Gams and *A. pinkertoniae* W. Gams and a reference strain of *A. blochii* W. Gams. In the phylogenetic study, numerous species were included that showed morphological similarities with the two possible new species but all were distributed distantly from *Acremonium* species I and II in the ITS tree.

For the multilocus sequence analysis, we selected strains that were closely related to the soil isolates in the first analysis, supplemented with reference strains of *Acremonium* and related taxa (TABLE I). With our primer set we were able to amplify and sequence 434–550 bp, 435–584 bp and 712–835 bp of the ITS regions, D1/D2 of 28S rRNA and the partial actin gene respectively. Of the 1668 characters from the three loci used in this analysis, 303 were parsimony informative. The lowest number was 47 for D1/D2 and the highest was 133 for ITS. The result of the

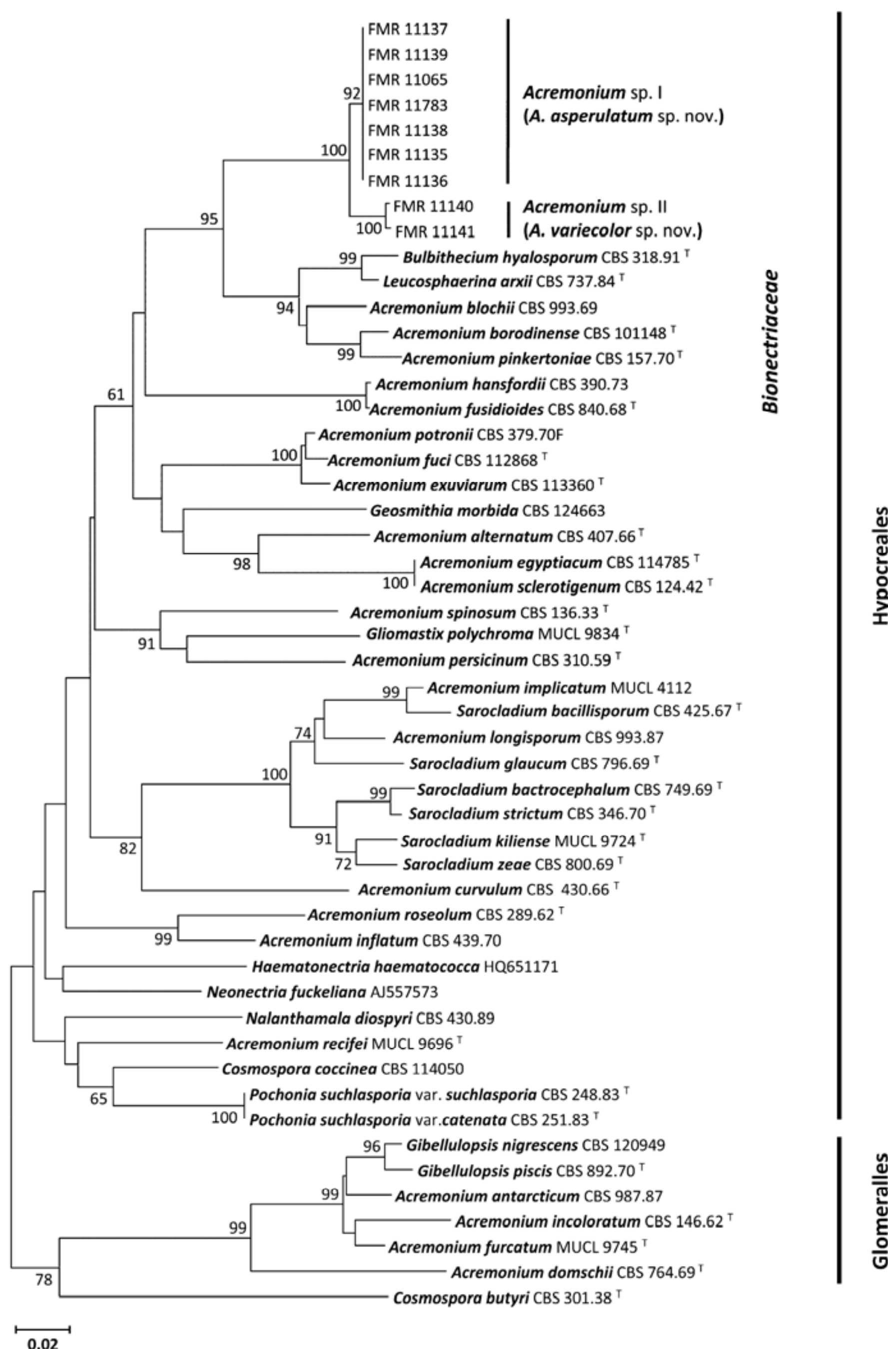

FIG. 1. Neighbor joining tree constructed with sequences of the ribosomal internal transcribed spacer (ITS) regions and 5.8S rRNA gene. Branch lengths are proportional to distance. Bootstrap support values above 60% are indicated at the nodes. T = type strain.

partition homogeneity test showed that the datasets for the three loci were congruent ($P = 0.16$) and could be combined. A total of 1142 most parsimonious trees, 465 steps long, with a CI of 0.6589, an HI of 0.3411 and a RI of 0.8046, were produced from heuristic searches with the combined dataset from the three loci (FIG. 2). The isolates identified as *Acremonium* sp. I and II clustered in a well supported clade

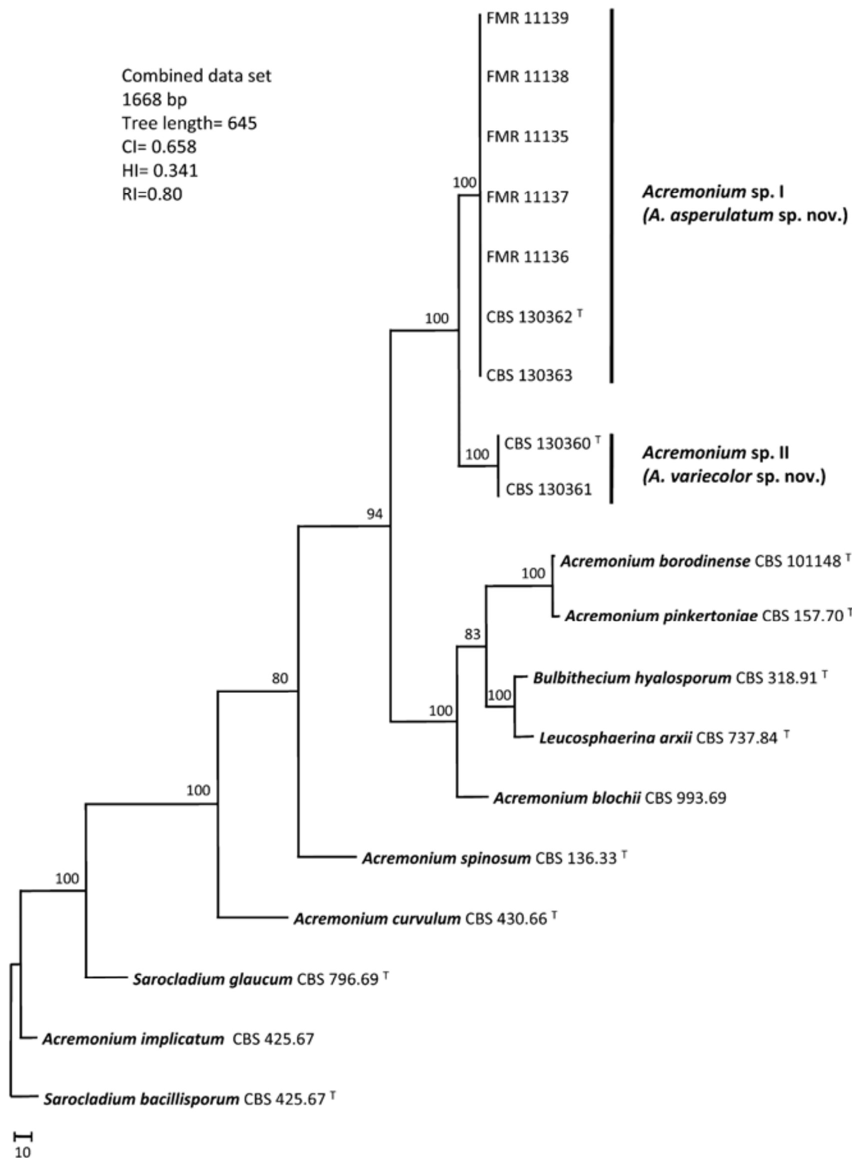

FIG. 2. One of 1142 most parsimonious trees obtained from heuristic searches based on analysis produced from the combined ITS, D1/D2 and actin datasets. Bootstrap support values are indicated at the nodes. CI = consistency index; HI = homoplasy index; RI = retention index. Bootstrap support values above 70% are indicated at the nodes. T = type strain.

divided in two subclades representing each of the two species. The combined tree topology was similar to the one observed in the trees of individual genes analyzed with NJ (data not shown). The sequences of the seven strains of *Acremonium* sp. I had 100% homology in each of the three loci studied. Those of the two strains of *Acremonium* sp. II showed 99.8% homology for the ITS region and 100% for the D1/D2 domains and the actin fragment. The differences between the sequences of the two species were 3–4.6% for the three loci.

Phenotypic studies.—Cardinal temperatures after 14 d in the dark were similar for *Acremonium* sp. I and *Acremonium* sp. II, that is the minimum, optimum and maximum growth temperatures were respectively 5 C (2–7 mm diam), 20–25 C (26–44 mm diam) and 32 C (2–4 mm diam). No isolate of either species grew

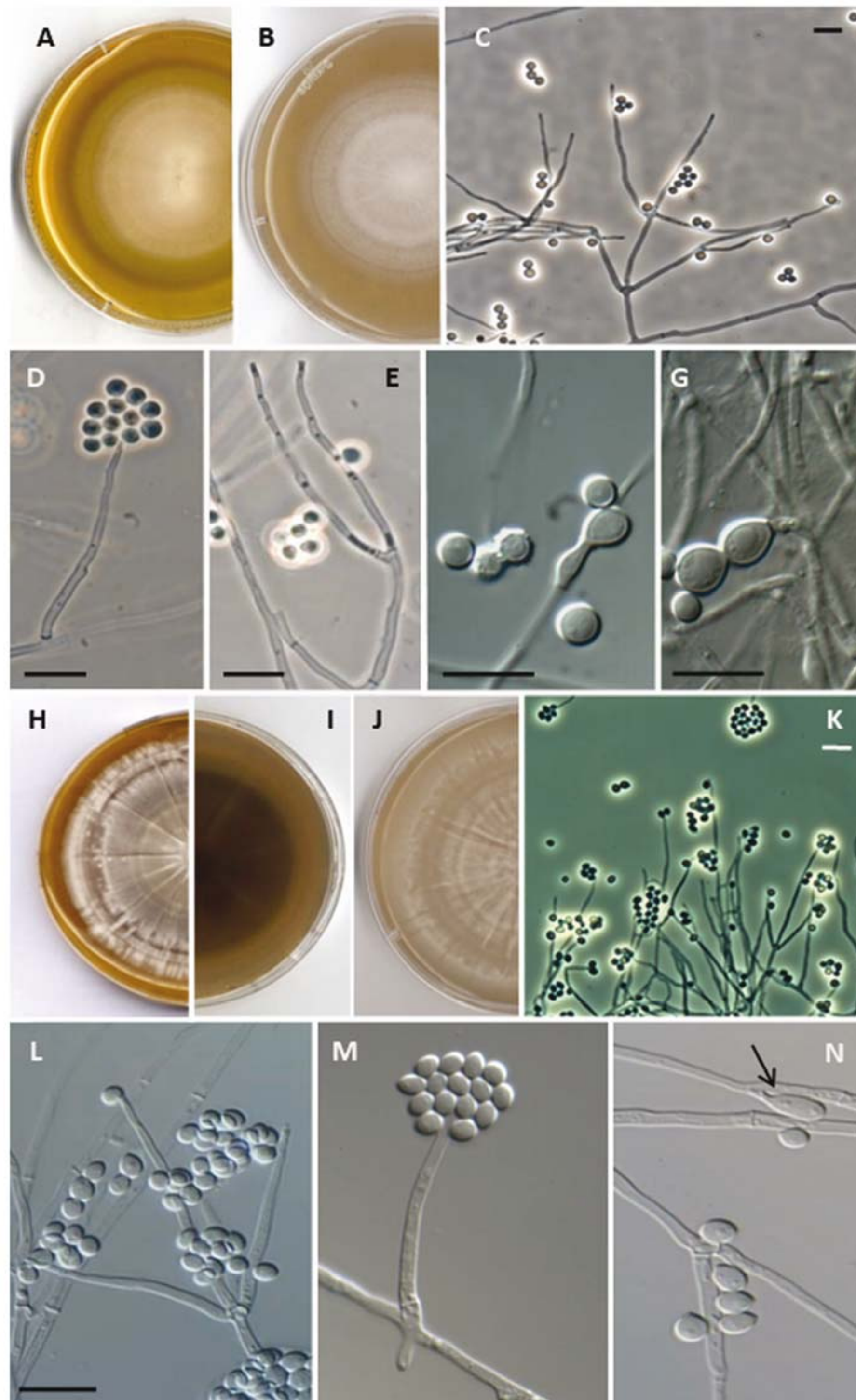

FIG. 3. *Acremonium asperulatum* FMR 11136 (A, B), CBS 130362 (C–F), FMR 11137 (G) and *A. variecolor* CBS 130360 (H–N). A, B. Colonies on PDA and OA respectively at 25 C after 22 d. C. Branched conidiophores. D. Phialide with a basal septum and conidia arranged in slimy heads. E. Phialides with collarettes. F, G. Globose rough- and thick-walled conidia and chlamydospores. H–J. Colonies on PDA (obverse and reverse) and OA respectively at 25 C after 22 d. K, L. Branched conidiophores and conidia arranged in slimy heads. M. Phialide with conidia arranged in slimy heads. N. Subglobose conidia with apiculate base and a sessile conidium on vegetative hyphae (arrow). Bars: C–G, K–N = 10 μm.

at 35 C. Colonies at 20–30 C were flat, slightly cottony and whitish. They were characterized microscopically by more or less erect conidiophores with whorls of acicular phialides. *Acremonium* sp. I produced abundant chlamydospores, and its conidia were rough-walled and globose. *Acremonium* sp. II produced a brownish diffusible pigment on PDA, and its conidia were smooth-walled, subglobose with an apiculate base. In addition, sessile conidia growing directly from vegetative hyphae were observed in the latter species on all media tested.

TAXONOMY

On the basis of the phylogenetic analysis and phenotypic features, we conclude that the species *Acremonium* sp. I and *Acremonium* sp. II are different from any previously described species in this genus and therefore are proposed as new.

Acremonium asperulatum A. Giraldo, Guarro, Gené & Cano, sp. nov. FIG. 3A–G
MycoBank MB563319

Etymology. Refers to the wall ornamentation of the conidia.

Colonies on OA and MEA at 25 C, attaining 44–60 mm and 31–63 mm diam respectively after 14 d in the dark, white (1A1) to yellowish white (3–4A2), often zonate, flat, velvety to slightly cottony; reverse brownish orange (5C4–6, 6C5) or yellowish white (3A2). On PDA at 25 C, attaining 31–39 mm diam after 14 d in the dark, white (1A1) or yellowish white (4A2), radially folded or rugose, at first glabrous becoming velvety to slightly cottony; reverse yellowish (4A2–5) or amber yellow (4B6). Vegetative hyphae septate, hyaline, smooth- and thin-walled, 1.5–3 μm wide. Sporulation abundant, phalacrogenous to nematogenous. Conidiophores erect, simple or mostly branched, bearing whorls of 2–4 phialides, septate, up to 105 μm long, hyaline, smooth, with cell walls usually thicker than those of the vegetative hyphae. Phialides terminal or lateral, straight or slightly bent, acicular, 28–68 μm long, 1–2 μm wide at the base, with minute collarette and distinct periclinal thickening at the apex, thin- and smooth-walled, hyaline. Conidia unicellular, globose, 3–4(–5) μm diam, hyaline to subhyaline, rough- and thick-walled, chromophilic, arranged in slimy heads. Chlamydospores abundant on PDA and OA, few on MEA, terminal or intercalary, single or in chains, unicellular, subglobose or oval, 5–10 × 5–9 μm, hyaline to subhyaline, smooth- and thick-walled, strongly chromophilic. Teleomorph not observed.

Specimens examined: SPAIN. ARAGÓN REGION, HUESCA PROVINCE: Bujaruelo Valley: refuge San Nicolas de Bujaruelo, isolated from forest soil, Jun 2009, *M. Hernández, J. Mena & J. Cano* (HOLOTYPE, IMI 500816; culture ex-type, CBS 130362 = MUCL 53781 = FMR 11065); isolated from sediments of Ara River, Mar 2011, *M. Hernández, A. Giraldo & X. Capilla* (CBS 130363 = MUCL 53782 = FMR 11783). Ordesa y Monte Perdido National Park: Añisclo canyon, isolated from forest soil, Jun 2009, *M. Hernández, J. Mena & J. Cano* (FMR 11135, FMR 11137); Ordesa Valley, Broto, isolated from forest soil, Jun 2009, *M. Hernández, J. Mena & J. Cano* (FMR 11136 and FMR 11138); Ordesa Valley, Torla, isolated from forest soil, Jun 2009, *M. Hernández, J. Mena & J. Cano* (FMR 11139).

Acremonium variecolor A. Giraldo, Guarro, Gené & Cano, sp. nov. FIG. 3H–N
MycoBank MB563320

Etymology. Refers to the variable colony color, ranging from olive to brown.

Colonies on OA and MEA at 25 C, attaining 43–45 mm diam after 14 d in the dark, white (1A1) to yellowish white (4A2), flat, glabrous to slightly cottony; reverse on OA brownish orange (5C4), on MEA pinkish white (7A2–3) or olive gray (3E2) at the center, yellowish white (4A2) toward the periphery. On PDA reaching 41–44 mm diam after 14 d in the dark at 25 C, yellowish white (3A2) to grayish yellow (4B4), often zonate, radially folded, velvety at the center, slightly cottony toward the periphery; diffusible pigment brownish gray (5–8F1) or brown (6E5) in the center, olive (1F4–5) toward the periphery was produced on PDA at 20, 25 and 30 C but not at ≤15 C. Vegetative hyphae septate, hyaline, smooth- and thin-walled, 2 μm wide. Sporulation abundant, phalacrogenous to nematogenous. Conidiophores erect, mostly branched, bearing whorls of 2–5 phialides, septate, up to 290 μm long, hyaline, smooth, with walls usually thicker than those of the vegetative hyphae. Phialides terminal or lateral, straight, acicular, 18–95 μm long, 1–2 μm wide at the base, with periclinal thickening at the apex, collarette inconspicuous, thin- and smooth-walled, hyaline; some phialidic conidiogenous cells without a basal septum (adelophialides) were observed on OA. Conidia unicellular, subglobose or ovoid, 3–4(–5) × 2–4 μm, slightly apiculate base, hyaline to subhyaline, thick- and smooth-walled, chromophilic, arranged in slimy heads. Sessile conidia growing directly on vegetative hyphae, solitary, blastic, unicellular, cylindrical or ellipsoidal, 5–7(–9) × 2–3(–4) μm, hyaline and smooth-walled. Chlamydospores and teleomorph not observed.

Specimens examined: SPAIN. ARAGÓN REGION, HUESCA PROVINCE: Ordesa y Monte Perdido National Park, Garganta de Escuaín, isolated from forest soil, Jun 2009, *M. Hernández, J. Mena & J. Cano* (HOLOTYPE, IMI 500815; ex-type culture, CBS 130360 = MUCL 53779 = FMR 11140). GALICIA REGION, LUGO PROVINCE:

Nature Reserve Sierra de Os Ancares, isolated from forest soil, May 2010, *M. Hernández, J. Mena & J. Guarro* (CBS 130361 = MUCL 53780 = FMR 11141).

DISCUSSION

Based on the combination of a multilocus analysis and phenotypic features, we propose and describe two new species of *Acremonium*, *A. asperulatum* and *A. variecolor*. Apart from morphological features, the reason for including these fungi in *Acremonium* was based mainly on the recent phylogenetic study carried out by Summerbell et al. (2011). After discussing several alternatives concerning the epitypification of *Acremonium*, Summerbell et al. (2011) designated CBS 407.66 as epitype of *A. alternatum*, the type species of the genus. The phylogentic D1/D2 tree given in that study shows that the strain belonged to the family Bionectriaceae, where a large group of species currently accepted in *Acremonium* also were placed. Therefore, all those *Acremonium*-like fungi that are phylogenetically close to CBS 407.66 should be recognized as *Acremonium* sensu stricto. Our ITS sequences analyses showed that the two new species make up a well supported clade, with some members of the Bionectriaceae being related at the same time to the epitype of *A. alternatum*.

These novel *Acremonium* species are characterized mainly by the production of verticillately branched conidiophores, long phialides and by the production of globose or ovoid, thick-walled conidia. Key morphological features that differentiate the two fungi are that *A. asperulatum* produces chlamydospores and has globose conidia with rough walls arranged in slimy heads, while *A. variecolor* produces a characteristic diffusible brownish pigment on PDA and has two types of conidia, subglobose with an apiculate base grouped in slimy heads on the phialides and solitary and sessile elongated conidia, arising directly on vegetative hyphae. Chlamydospores were not observed in the latter species. The conjunction of such characteristics with their phylogenetic distances from the other species of *Acremonium* and related genera makes these two species clearly different from all the known fungal species. In the phylogenetic trees, the clade with the Bionectriaceae species *Bulbithecium hyalosporum*, *Leucosphaerina arxii*, *Acremonium borodinense*, *A. pinkertoniae* and *A. blochii* was the closest to the new taxa. The former two species develop *Acremonium* anamorphs, which can be distinguished from the novel species by their simple and much smaller conidiophores (12–40 μm long) and ellipsoidal conidia (Malloch 1989, Udagawa and Muroi 1990). The other two species, *A. borodinense* and *A. pinkertoniae*, also differ morphologically by having smaller and simple conidiophores (25–30 and 17–27 μm long respectively), by absence of chlamydospores, and by colonies with a colorless reverse (Gams 1971, Ito et al. 2000). In addition, *A. borodinense* produces two types of phialidic conidia, both with morphological features different from the species described here (Gams 1971, Ito et al. 2000). The two types of conidia observed in *A. variecolor* differ in their ontogeny: One is phialidic and the other is blastic. The latter kind of conidia also was described in *Pochonia suchlasporia* W. Gams & Dackman (Gams 1988, Zare et al. 2001), but this species is phylogenetically distant from *A. variecolor* (FIG. 1). *Acremonium blochii*, which was nested in a clade close to that of *A. variecolor*, has morphologically similar phialidic conidia, but these are arranged in long chains or slimy heads and it has only single phialides.

Other *Acremonium* species included in Bionectriaceae, with some morphological features similar to our new taxa, are *Acremonium fusidioides* (Nicot) W. Gams and *A. hansfordii* (Deighton) W. Gams. Both produce a characteristic brownish diffusible pigment (Gams 1971, 1975), but they usually have simple conidiophores; *A. hansfordii* has fusiform and more or less brown-pigmented conidia with truncate ends; *A. fusidioides* produces two types of phialidic conidia, the most abundant ones being slightly pigmented and fusiform and the less common ones globose and hyaline (Gams 1971, 1975). In our ITS analysis both species formed a well supported lineage distant from our new species.

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4.1.2. *Acremonium* with catenate elongate conidia: phylogeny of *Acremonium fusidioides* and related species

Giraldo A, Gené J, Cano J, de Hoog S, Decock C, Guarro J.

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TAXONOMIC STUDY OF CLINICAL AND ENVIRONMENTAL ISOLATES OF ARTHROCONIDIAL, ACREMONIUM-LIKE AND OCHROCONIS-LIKE FUNGI
Dixie Alejandra Giraldo López
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Acremonium with catenate elongate conidia: phylogeny of *Acremonium fusidioides* and related species

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Abstract: *Acremonium* is one of the largest and most complex genera of hyphomycetes. Its taxonomy is not yet resolved and the phylogenetic value of the most distinctive morphological features is unknown. The species of this genus produce conidia in chains or form slimy heads or both. We have studied a set of clinical and environmental *Acremonium* strains morphologically characterized by producing mostly catenate, elongate, more or less fusiform conidia. Based on phenotypic data and in the analysis of the sequences of the ITS region, the D1/D2 domains of the 28S rRNA gene and a fragment of the β-tubulin gene, three new species morphologically similar to *Acremonium fusidioides* and belonging to the family Bionectriaceae (Hypocreales) are described and illustrated; they are *Acremonium pilosum, Acremonium parvum* and *Acremonium citrinum.* The first species produces two kinds of conidia: clavate with smooth walls and globose with abundant filiform projections. *Acremonium parvum* is characterized by slow growth and pyriform or slightly lanceolate conidia with an elongate and truncate base. *Acremonium citrinum* produces a diffusible yellow pigment and obovoid conidia.

Key words: *Acremonium*, anamorphic fungi, phylogeny, taxonomy

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Introduction

Acremonium is so far a large and polyphyletic anamorph genus with species belonging to different orders of Sordariomycetes but mainly to the Hypocreales (Glenn et al. 1996, Guarro et al. 2011, Perdomo et al. 2011, Summerbell et al. 2011). The genus was reviewed morphologically by Gams (1971, 1975) and characterized to include fungi with usually slow-growing colonies, thin hyphae, simple or poorly basitonously branched conidiophores and orthotropic phialides, gradually tapering toward the tip and with a basal septum; conidia generally unicellular, hyaline or pigmented, arranged in slimy heads or chains or both. Based on morphological features, the genus was divided into sections and series. *Acremonium alternatum*, the type species of the genus, characterized by simple conidiophores that produced at least partly catenate conidia, was included in the ''series *Terricola*'' of the ''section *Acremonium*'' (Gams 1971, 1975). However, several molecular studies have demonstrated that this classification did not represent natural groupings because sections and series included fungi that are phylogenetically divergent. Therefore, numerous species of *Acremonium* have been transferred to different genera (Glenn et al. 1996; Zare and Gams 2001a,b; Zare et al. 2007; Schoch et al. 2009; Gräfenhan et al. 2011; Summerbell et al. 2011; Perdomo et al. 2013). In an attempt to give nomenclatural stability to the genus and to clarify its taxonomy, based on the analysis of ribosomal gene sequences of numerous species of *Acremonium* and related genera, Summerbell et al. (2011) epitypified *Acremonium* with the strain CBS 407.66 of *A. alternatum.* It was included into a large clade with other *Acremonium* species of the family Bionectriaceae. That study revealed that several species of the series *Terricola* sensu Gams were phylogenetically distant from the epitype, apart from *A. fusidioides, A. cavaraeanum, A. hansfordii, A. hennebertii* and two isolates that were morphologically compatible with *A. alternatum* (FIG. 1). These latter fungi, characterized by producing fusiform conidia arranged in dry chains, were grouped in an unsupported clade named ''*fusidioides* clade'' because the type strain of *A. fusidioides* was included (Summerbell et al. 2011). Perdomo et al. (2011) identified, by morphological and molecular methods, several isolates of *A. fusidioides*, recovered from clinical specimens from USA, mainly from a respiratory origin. However, when

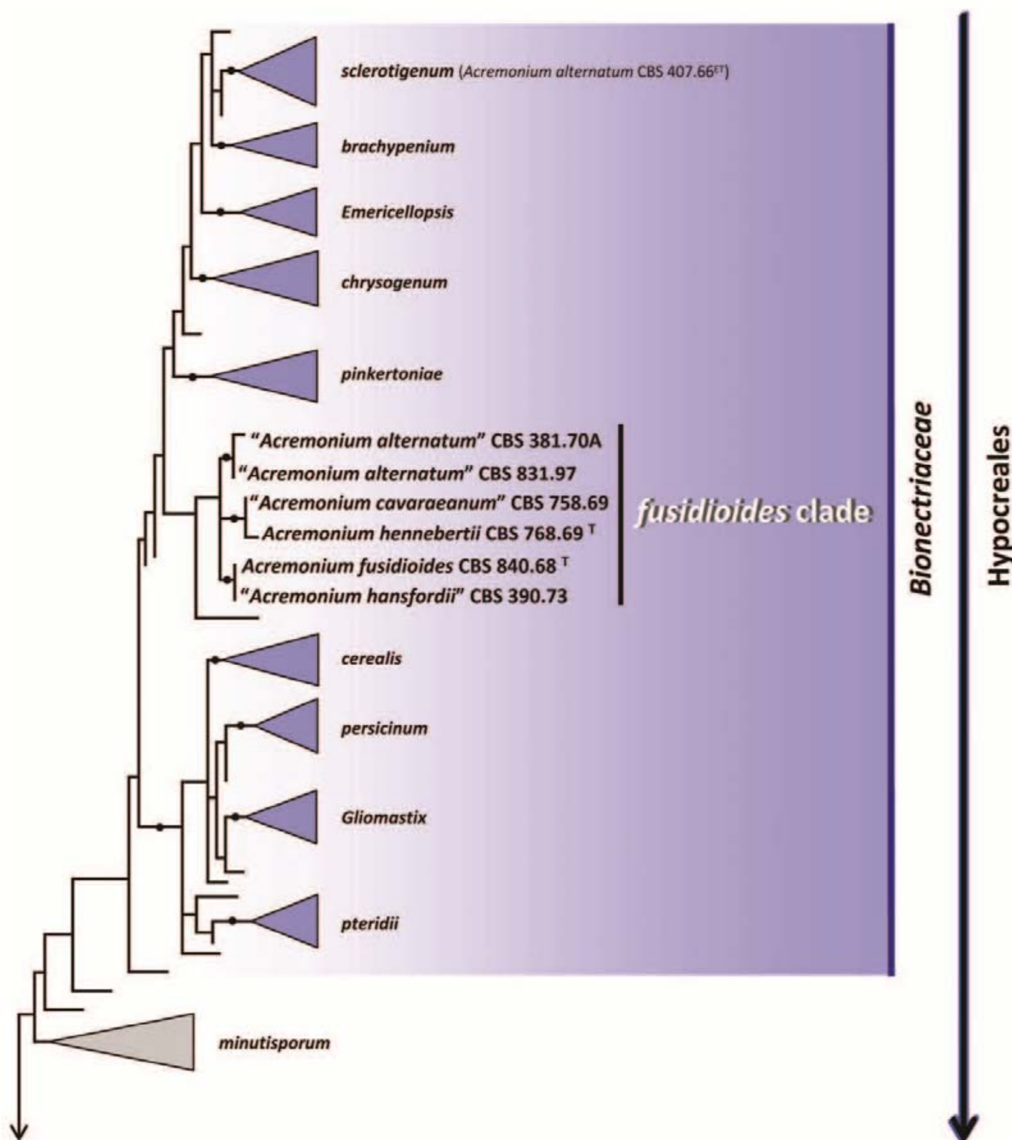

FIG. 1. Scheme representing part of Summerbell's LSU tree (Summerbell et al. 2011), in which dark gray triangles are the clades of *Acremonium* species and related fungi within the Bionectriaceae family (shading); the gray triangle is the basal clade of *Acremonium* species in Hypocreales but outside Bionectriaceae. Arrows represent the continuation of the phylogenetic tree of the Hypocreales. Isolates labeled with quotation marks indicate a provisional identification. Bootstrap support values above 70% are indicated by a black dot on the relevant internode. ^{ET} = ex-epitype strain; ^{T} = ex-type strain.

sequences of those isolates were compared with the ex-type strain and other reference strains of that species, significant differences were found, suggesting that the former concept of *A. fusidioides* could comprise more than a single species.

To clarify the taxonomy and the phylogeny of *A. fusidioides* and related species, we have studied phenotypically and molecularly a set of clinical and environmental strains that were morphologically compatible with *A. fusidioides* and several reference strains retrieved from different culture collections.

MATERIALS AND METHODS

Fungal isolates.—A total of 23 isolates were included in this study (TABLE I). Among them, 13 were clinical or environ-

TABLE I. *Acremonium* species and strains included in the study, their origin and GenBank accession nos.

Species	Strain (original identification)	Origin	GenBank accession nos. D1/D2	ITS	β-tubulin
Acremonium cavaraeanum	CBS 101149^T (*A. cavaraeanum*)	outer wall of wooden house, Japan	**HF680202**	**HF680220**	**HF680240**
	CBS 111656 (*A. cavaraeanum*)	coconut, Brazil	**HF680203**	**HF680221**	**HF680241**
Acremonium citrinum	CBS 384.96^T (*A. hansfordii*)	on decaying fruit, Papua New Guinea	**HF680217**	**HF680236**	**HF680257**
	CBS 758.69 (*A. cavaraeanum*)	sputum, the Netherlands	HQ232012	**HF680222**	**HF680242**
Acremonium fusidioides	CBS 840.68^T (*A. fusidioides*)	dung of antelope, Central African Republic	HQ232039	FN706542	**HF680243**
	CBS 991.69 (*A. fusidioides*)	sputum, the Netherlands	**HF680211**	**HF680230**	**HF680251**
	CBS 673.82 (*A. fusidioides*)	unknown, Denmark	**HF680206**	**HF680225**	**HF680246**
	CBS 705.86 (*A. hansfordii*)	unknown, France	**HF680218**	**HF680237**	**HF680259**
	CBS 266.89 (*A. fusidioides*)	agricultural soil, Germany	**HF680205**	**HF680224**	**HF680245**
	CBS 109069 (*A. fusidioides*)	barley seeds, Ethiopia	**HF680204**	**HF680223**	**HF680244**
	MUCL 9579 (*A. fusidioides*)	monkey dung, Japan	**HF680212**	**HF680231**	**HF680252**
	MUCL 9580 (*A. fusidioides*)	unknown, Italy	**HF680213**	**HF680232**	**HF680253**
	UTHSC 07-646 (*A. fusidioides*)	bronch wash, Florida, USA	**HF680214**	**HF680233**	**HF680254**
	UTHSC 08-1188 (*A. fusidioides*)	bronch wash, Texas, USA	**HF680215**	**HF680234**	**HF680255**
	UTHSC 08-1455 (*A. fusidioides*)	bronch wash, Texas, USA	**HF680216**	**HF680235**	**HF680256**
Acremonium parvum	CBS 381.70A^T (*A. alternatum*)	*Tubercularia vulgaris*, the Netherlands	HQ231986	**HF680219**	**HF680239**
Acremonium pilosum	CBS 124.70^T (*A. fusidioides*)	agricultural soil, the Netherlands	**HF680209**	**HF680228**	**HF680249**
	CBS 410.70 (*A. fusidioides*)	agricultural soil, the Netherlands	**HF680208**	**HF680227**	**HF680248**
	CBS 125.70 (*A. fusidioides*)	agricultural soil, the Netherlands	**HF680210**	**HF680229**	**HF680250**
	CBS 390.73 (*A. hansfordii*)	on *Periconia cookei* on dead bamboo, India	HQ232043	AB540578	**HF680258**
	CBS 511.82 (*A. fusidioides*)	agricultural soil, the Netherlands	**HF680207**	**HF680226**	**HF680247**
Acremonium hennebertii	CBS 768.69^T (*A. hennebertii*)	*Elaeis guineensis*, Zaire	HQ232044	**HF680238**	**HF680260**
Acremonium sp.	CBS 110390 (*A. hansfordii*)	stucco with ring of moisture, Germany			

Note: UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX; CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; MUCL, Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium. ^{T} = ex-type strain; accession numbers of sequences newly determined in this study are indicated in boldface.

mental isolates of *A. fusidioides* and the rest were ex-type or reference strains of the "*fusidioides* clade" sensu Summerbell et al. available in culture collections.

DNA extraction, amplification and sequencing.—Isolates were grown on yeast extract sucrose agar (YES; 20 g yeast extract, 15 g sucrose, 20 g agar, 1000 mL distilled water) for 7 d at 25 C, and DNA was extracted with a PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, California) according to the manufacturer's protocol. The DNA was quantified with NanoDrop 3000 (Thermo-Scientific, Asheville, North Carolina). The internal transcribed spacer (ITS) regions and D1/D2 domains of the 28S of the nuclear rRNA gene were amplified with the primer pairs ITS5/ITS4 and NL1/NL4b respectively (White et al. 1990, O'Donnell 1993). A fragment of the β-tubulin gene was amplified with the primer pairs Tub-F/Tub-R (Cruse et al. 2002). PCR products were purified with a Diffinity RapidTip® kit (Sigma-Aldrich, St Louis, Missouri) and were stored at −20 C until sequencing. PCR products were sequenced with the same primers used for amplification and following the *Taq* DyeDeoxy Terminator cycle sequencing kit protocol (Applied Biosystems, Gouda, the Netherlands). DNA sequencing reaction mixtures were analyzed on a 310 DNA sequencer (Applied Biosystems). In addition, some amplified fragments were purified and sequenced at Macrogen Europe (Amsterdam, the Netherlands) with a 3730XL DNA analyzer (Applied Biosystems). The SeqMan program 7.0.0 (DNASTAR, Madison, Wisconsin) was used to obtain consensus sequences of each isolate. Some D1/D2 and ITS sequences, from several species of *Acremonium*, were retrieved from GenBank and included in the phylogenetic study. All sequences generated in this study and the alignments were deposited respectively in GenBank (TABLE I) and TreeBASE (www.treebase.org, submission number 14232).

Alignment and phylogenetic analysis.—The sequences were aligned with the Clustal X 1.8 computer program (Thompson et al. 1997) with default parameters, followed by manual

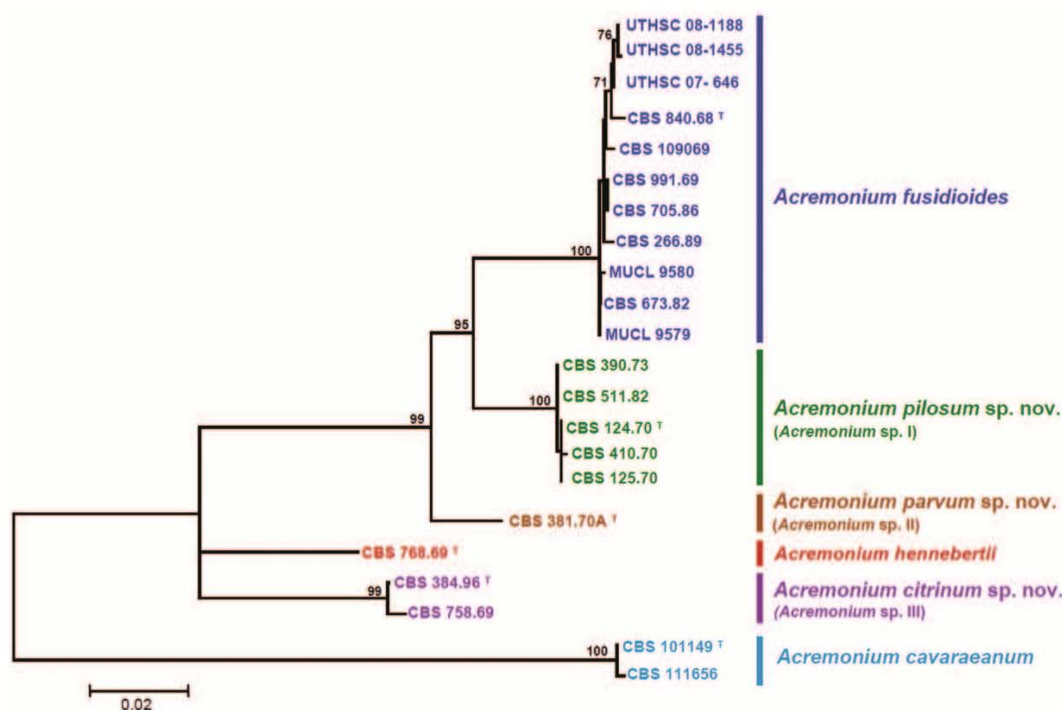

FIG. 2. A maximum composite likelihood tree based on analysis produced from the combined ITS, D1/D2 and β-tubulin datasets of the *Acremonium* species included in the study. Bootstrap support values above 70% are indicated at the nodes. T = ex-type strain.

adjustments with a text editor. The phylogenetic analysis was carried out with the MEGA 5.05 software (Tamura et al. 2011), with the maximum composite likelihood (ML) with Tamura-Nei substitution model with gamma distribution to obtain the distance tree. Gaps or missed data were treated as partial deletion with a site coverage cut-off of 95% and nearest-neighbor-iterchange (NNI) was used as heuristic method. The internal branch support was assessed by a search of 1000 bootstrapped sets of data.

Phenotypic study.—Macro- and microscopic features of the isolates were studied on oatmeal agar (OA; 30 g filtered oat flakes after 1 h simmering, 20 g agar, 1000 mL distilled water) and potato carrot agar (PCA; 20 g potatoes, 20 g carrot, 20 g agar, 1000 mL distilled water), both media prepared by ourselves according to the procedure described in Onions and Pitt (1988), and on potato dextrose agar (PDA; Pronadisa, Madrid, Spain), incubated at 25 C in the dark. The isolates' ability to grow at 10, 15, 20, 25, 30, 32, 35 and 37 C was tested on PDA. In the descriptions, color colony codes in parenthesis refer to Kornerup and Wanscher (1978). Microscopic features were examined by making direct wet mounts with 85% lactic acid or lactophenol cotton blue or by slide cultures on OA. Photomicrographs were obtained with a Zeiss Axio-Imager M1 light microscope. Scanning electron microscope (SEM) micrographs were obtained with a Jeol JSM- 6400 with techniques described by Figueras and Guarro (1988).

RESULTS

Phylogenetic analysis and phenotypic features.—With the primers used we were able to amplify and sequence 450–500 bp, 460–550 bp and 550–620 bp of the D1/D2 domains, the ITS region and the β-tubulin gene respectively. The topology of the combined tree was similar to those observed in the trees of individual genes analyzed by ML (data not shown).

The combined tree showed four unrelated clades (FIG. 2). The first and the biggest included three well supported subclades. The first subclade (100% bootstrap support, bs) was formed by 11 strains from environmental (dung, soil, seeds) and clinical (sputum, bronchial wash) origins, including the ex-type strain of *A. fusidioides*. These strains showed the typical characters described for *A. fusidioides*, which are: colonies at 20–25 C with moderate growth (up to 45 mm diam in 14 d on OA), flat to elevated, dusty or velvety, whitish to brownish gray (8D2, 11E2); a maximum growth temperature of 37 C; phialides mainly solitary, acicular, hyaline, thin- and smooth-walled; conidia of two types, (i) fusiform with slightly truncate ends, subhyaline, smooth-walled, arranged in long chains and (ii) globose, hyaline, warted- and

thick-walled, in short chains. The second subclade (*Acremonium* sp. I, 100% bs) grouped five reference strains from environmental origin, mainly from soil and bamboo, with an intraspecific similarity of 99–100% for the three loci studied. The strains included in this group had morphological characteristics similar to those of the previously mentioned subclade but with some particular characteristics such as colonies up to 25 mm diam in 14 d on OA, absence of growth at 37 C, and dimorphic catenate conidia, which are (i) subhyaline, pyrifom or clavate with a truncate base and (ii) pale brown, globose and with abundant filiform projections (under SEM) (FIG. 3). The last subclade (*Acremonium* sp. II) included only the strain CBS 381.70A characterized by a restricted growth in all media tested, absence of growth at 32 C and pyriform or slightly lanceolate conidia with rounded apex and an elongated and truncate base (FIG. 4).

The second clade included only the type strain of *A. hennebertii*, characterized by whitish colonies and the production of long chains of fusiform conidia with pointed ends. The third clade (*Acremonium* sp. III) grouped two strains of an unnamed species, mainly characterized by the production of a diffusible yellow pigment in all media tested and obovoid conidia arranged in chains (FIG. 5). Finally, the fourth clade comprised two strains of *A. cavaraeanum*, including the ex-type of this species, which produced whitish or yellow colonies with a strong diffusible vinaceous pigment in all media tested.

TAXONOMY

On the basis of the morphological features observed, which correlated with the phylogenetic analysis, we concluded that *Acremonium* sp. I, *Acremonium* sp. II and *Acremonium* sp. III represent three species different from any previously described in this genus and are therefore proposed as new.

Acremonium pilosum Giraldo, Guarro, Cano & Gené, sp. nov. FIG. 3

MycoBank MB804371

Etymology: From Latin *pilosus*, hairy, referring to the woolly or hairy appearance of the globose conidia.

Diagnosis: A. fusidioides and *A. pilosum* produce two types of conidia, but the former grows at 37 C and its globose conidia are hyaline and have a warty wall. *A. pilosum* has pale brown globose conidia with filiform projections and does not grow at this temperature. *A. hansfordii* mainly differs in having livid vinaceous to brown vinaceous colonies, shorter phialides and the production of only fusiform conidia with truncate ends and more or less brown pigmented.

Colonies on OA at 25 C attaining 20–25 mm diam after 14 d, brownish orange (7C3) to grayish brown (8E3) at the center, whitish toward the periphery, flat, dusty. On PCA reaching 15–21 mm diam after 14 d at 25 C, white, flat, slightly fasciculate at the center, velvety or felt-like toward the periphery. On PDA at 25 C, reaching 7–12 mm diam after 14 d, yellowish white (4A2), elevated and sometimes slightly sulcate, membranous and embedded in the agar. The optimum temperature for growth was 25 C, the minimum 15 C and the maximum 32 C. No growth at 35 C. Vegetative hyphae septate, hyaline, smooth- and thin-walled, 2 μm wide. Conidiophores erect, simple or poorly branched, septate at the base, up to 40 μm long, hyaline, smooth. Phialides terminal or lateral, straight, acicular, 10–40 μm long, 1–2 μm wide at the base, thin- and smooth-walled, hyaline; schizophialides occasionally present. Conidia unicellular, of two types: pyrifom or clavate, 4–5(–6) × 1.5–3 μm, distinctly truncate at the base, subhyaline, thin- and smooth-walled, adhering in long chains; globose, 3–4(–5) μm diam, at first hyaline and then subhyaline or pale brown, with abundant filiform projections that give a woolly appearance in SEM, arranged in short chains (two or three conidia). Chlamydospores and teleomorph not observed.

Specimens examined: THE NETHERLANDS, OOSTELIJK FLEVOLAND. From agricultural soil, Sep 1969, *J.H. van Emden No. 03.625* (HOLOTYPE: CBS H-8173; ex-type cultures: CBS 124.70, FMR 11415). From agricultural soil, Sep 1969, *J.H. van Emden No. 543* (CBS 125.70). From agricultural soil, Mar 1970, *J.H. van Emden No. 690312/122* (CBS 410.70). Nagele. From agricultural soil, Sep 1982, *H. Nylander No. 771* (CBS 511.82). INDIA, BANGALORE, Mysore. From *Periconia cookei* on *Dendrocalamus* sp., Mar 1973, *W. Gams* (CBS 390.73, IMI 185375).

Acremonium parvum Giraldo, Guarro, Cano & Gené, sp. nov. FIG. 4

MycoBank MB804372

Etymology: From Latin *parvus*, small, referring to the size of the colonies.

Diagnosis: It differs from *A. fusidioides* by the production of one type of conidia and from *A. alternatum* mainly by having slower growth, phialides with two septa and pyriform or lanceolate conidia with a conspicuously elongated and truncate base.

Colonies on OA and PCA at 25 C attaining 2–3 mm diam after 14 d, white (1A1), flat, dusty. On PDA at 25 C, reaching 4–5 mm diam after 14 d, yellowish white (4A2), elevated, cerebriform, embedded in the agar. The optimum temperature for growth was 25 C, the minimum 10 C and the maximum 30 C. No growth at 32 C. Vegetative hyphae septate, hyaline, smooth- and thin-walled, 1–2 μm wide. Conidiophores

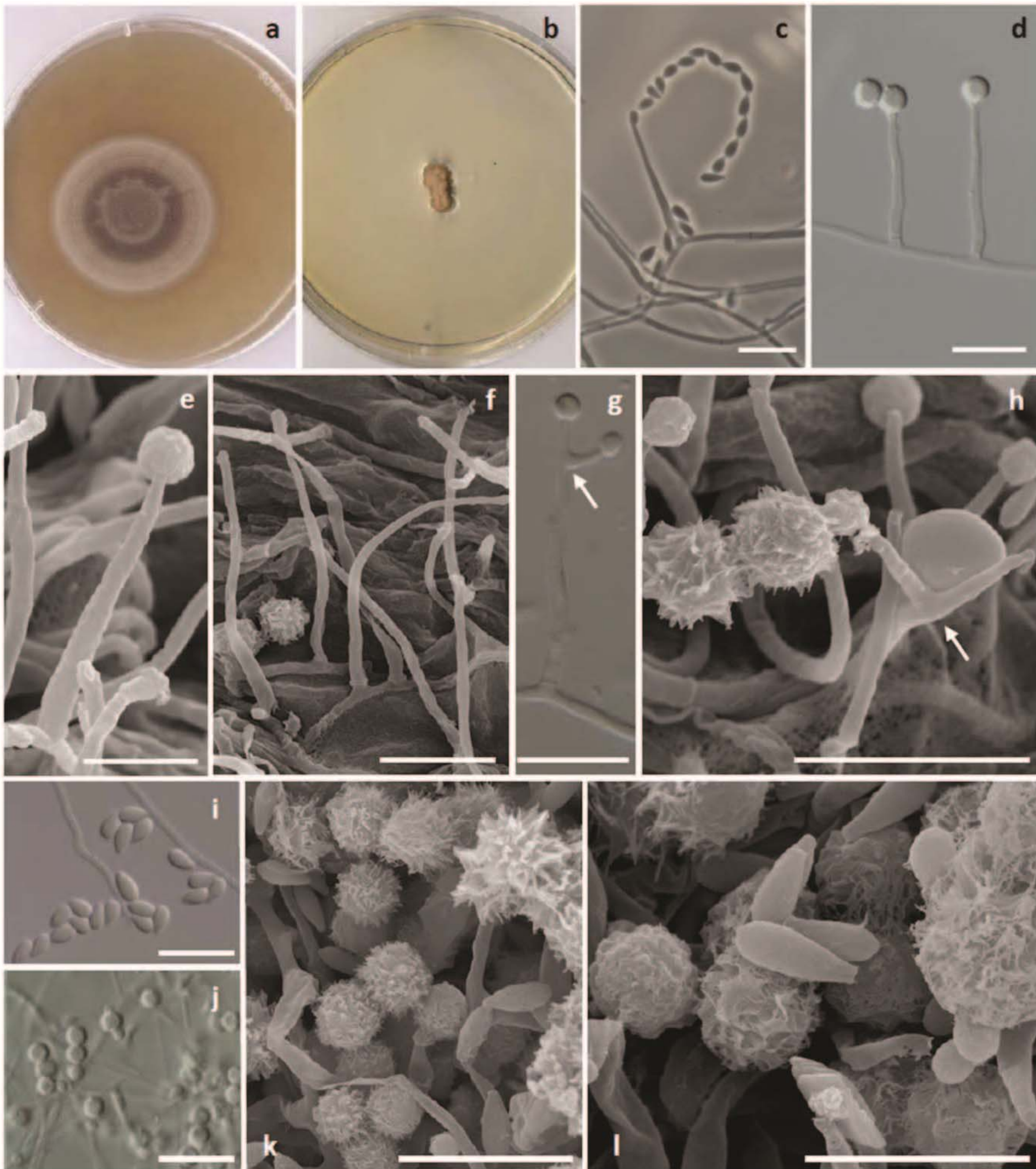

FIG. 3. *Acremonium pilosum* CBS 511.82 (a, b, d, f–j, l), CBS 124.70 (c, e, k). a, b. Colonies on OA and PDA at 25 C after 20 d and 14 d, respectively. c–f. Phialides producing two types of conidia. g, h. Detail of schizophialides (arrows) producing globose conidia. i–l. Clavate and globose conidia. Bars: c–g, i–k = 10 μm; h = 9 μm; l = 7 μm.

erect, simple or poorly branched, septate at the base, up to 35 μm long, hyaline, smooth. Phialides terminal or lateral, straight, acicular, 13–30(–33) μm long, 1–2 μm wide at the base, thin- and smooth-walled, hyaline; phialides with a secondary septum near to the apex occasionally present. Conidia unicellular, pyriform or slightly lanceolate, with rounded apex and conspicuously elongated and truncate base, 3–5(–5.5)

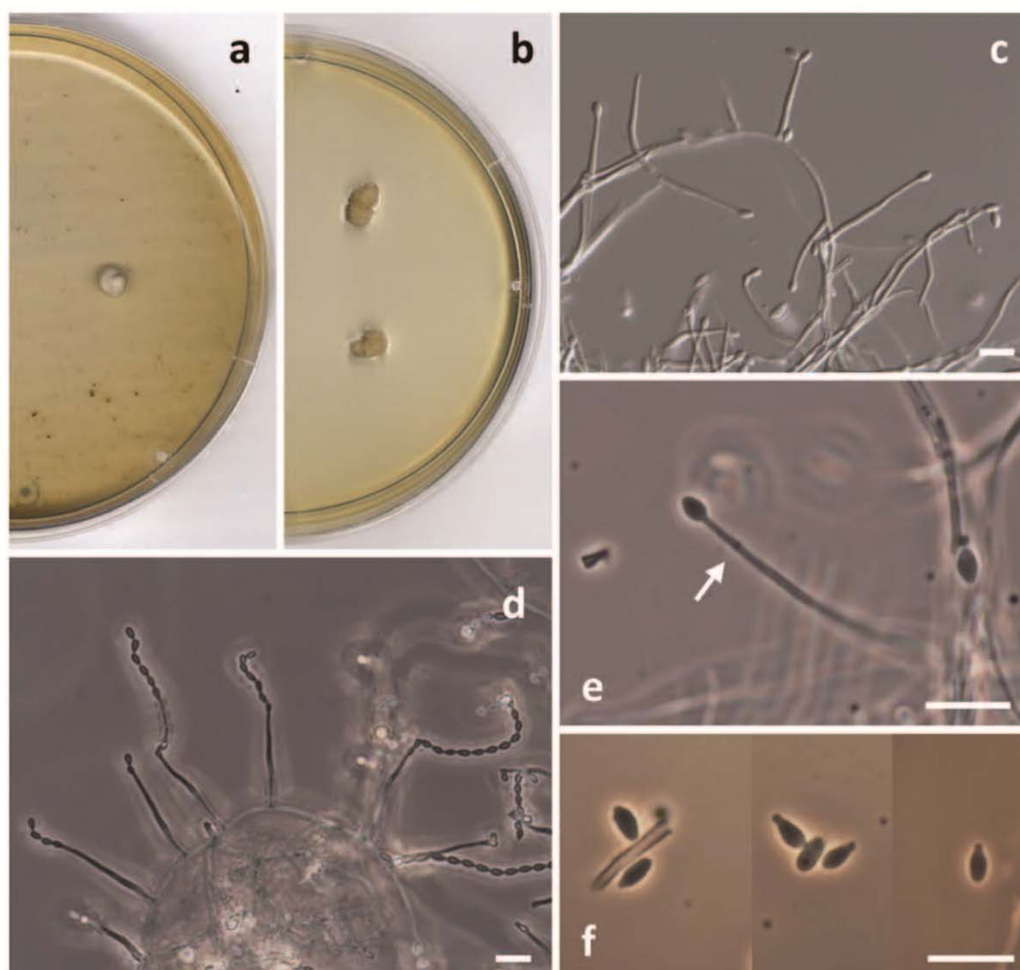

FIG. 4. *Acremonium parvum* CBS 381.70A. a, b. colonies on OA and PDA respectively at 25 C after 28 d. c, d. Phialides and conidia in chains. e. Phialide with a septum near the apex (arrow). f. Pyriform or slightly lanceolate conidia with elongated and truncate base. Bar = 10 μm.

× 1.5–2 μm, hyaline to subhyaline, thin- and smooth-walled, arranged in chains, often collapsing in heads. Chlamydospores and teleomorph not observed.

Specimens examined: THE NETHERLANDS, TEXEL. Isolated from *Tubercularia vulgaris*, Mar 1970, *W. Gams No. 1452* (HOLOTYPE: CBS H-21329; ex-type cultures: CBS 381.70A, VKM F-2845, FMR 12358).

Acremonium citrinum Giraldo, Guarro, Cano & Gené, sp. nov. FIG. 5

MycoBank MB804373

Etymology: From Latin *citrinus*, citrine or lemon-yellow, referring to the diffusible yellow pigment produced in all media tested.

Diagnosis: It differs from *A. hansfordii* and *A. cavaraeanum* by the production of a yellow diffusible pigment and having longer phialides and hyaline obovoid conidia.

Colonies on OA at 25 C attaining 28–30 mm diam after 14 d, whitish (1A1) to grayish brown (7D3), flat, at first membranous becoming dusty. On PCA at 25 C, reaching 17–23 mm diam after 14 d, white (1A1), flat, fasciculate at the center, glabrous or dusty toward the periphery. On PDA at 25 C, attaining 4–5 mm diam after 14 d, light yellow (2A5), elevated, rugose or cerebriform, embedded in the agar. Diffusible light yellow pigment (2A5) produced on all media tested 15–30 C. The optimum temperature for growth was 25 C, the minimum 15 C and the maximum 35 C. No growth at 37 C. Vegetative hyphae septate, hyaline, smooth- and thin-walled, 1.5–2 μm wide. Conidiophores erect, mostly simple, hyaline, smooth. Phialides arising laterally from vegetative hyphae, straight or flexuous, acicular or subulate, 18–40(–50) μm

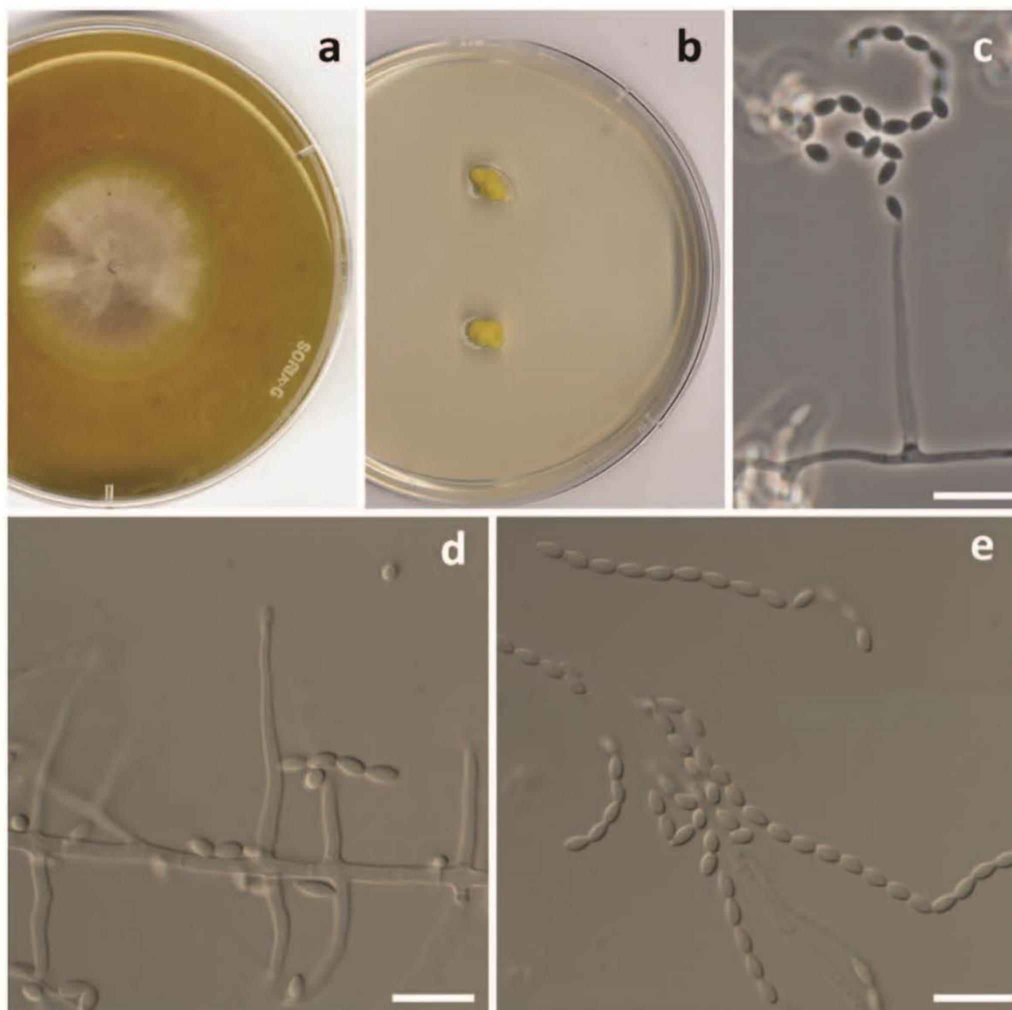

FIG. 5. *Acremonium citrinum* CBS 384.96. a, b. Colonies on OA and PDA at 25 C after 20 d and 14 d, respectively. c, d. Phialides and obovoid conidia in chains. e. Conidial chains. Bar = 10 μm.

long, 1–2 μm wide at the base, thin- and smooth-walled, hyaline; sometimes with a secondary septum in the middle zone. Conidia unicellular, obovoid, sometimes ellipsoidal, 3–4(–5) × 1–1.5 μm, hyaline, thin- and smooth-walled, arranged in chains, sometimes collapsing in heads. Chlamydospores and teleomorph not observed.

Specimens examined: PAPUA NEW GUINEA, MADANG, Jais Aben. From decaying fruit, Apr 1996, *A. Aptroot* (HOLOTYPE: CBS H-21330; ex-type cultures: CBS 384.96, FMR 11427). THE NETHERLANDS, GRONINGEN. From sputum, Sep 1969, *A. Kikstra* (CBS 758.69).

DISCUSSION

We have conducted a detailed study of the morphology of the available reference strains and some fresh isolates from different origins of the five species included in the "*fusidioides* clade" sensu Summerbell et al. (2011) to delineate the relationship among the species that form this clade. All strains studied share the presence of elongate, more or less fusiform conidia arranged in chains; however, the study revealed the presence of some relevant features, not reported before. These features were useful for discriminating species (TABLE II) and correlated with the data provided by the phylogenetic analysis. Such results lead us to propose and describe three new species of the family Bionectriaceae and restrict the "*fusidioides* clade" to the species *A. fusidioides*, *A. parvum* and *A. pilosum*.

The clade representing the new species *A. pilosum* comprised four strains previously identified as *A.*

TABLE II. Relevant phenotypic features to distinguish the *Acremonium* species included in study

Species	Growth 32 C	Growth 37 C	Diffusible pigment on OA at 25 C	Conidial features: Arrangement	Dimorphic conidia	Morphology
A. cavaraeanum	+	−	+ (vinaceous)	chains	−	fusiform to ellipsoidal
A. citrinum	+	−	+ (yellow)	chains/heads	−	obovoid to ellipsoidal
A. fusidioides	+	+	−	chains	+	i) globose, warty ii) fusiform with slightly truncate ends
A. hennebertii	+	−	−	chains	−	fusiform with pointed ends
A. parvum	−	−	−	chains/heads	−	pyriform to slightly lanceolate
A. pilosum	+	−	−	chains	+	i) globose with filiform projections ii) pyrifom to clavate

fusidioides and one strain as *A. hansfordii. Acremonium pilosum* and *A. fusidioides* share the ability to produce two types of conidia. In the former species they are globose or clavate to pyriform, and in the latter they are globose or fusiform with slightly truncated ends. In addition, the wall ornamentation of the globose conidia of *A. pilosum* is hairy, while that of *A. fusidioides* is wart-like. In addition, *A. fusidioides* is the only species among those included in the study able to grow at 37 C. Other species of *Acremonium* that also produce two kinds of conidia are *Acremonium borodinense* and the recently described *Acremonium variecolor*, although they are phylogenetically distant from the *A. fusidioides* clade (Giraldo et al. 2012). In *A. borodinense*, the conidia are cylindrical and smooth or ovoid with rough wall; both are arranged in slimy heads (Ito et al. 2000). In *A. variecolor* one type of conidia are subglobose with an apiculate base and grouped in slimy heads at the tip of the phialides, and the other cylindrical or ellipsoidal and sessile on vegetative hyphae (Giraldo et al. 2012).

Initially some of the fresh isolates included in this study were identified as *A. hansfordii.* This species was described with vivid vinaceous to brown vinaceous colonies, short phialides (up to 27 µm long) and more or less brown-pigmented fusiform conidia with truncate ends forming long chains (Deighton 1969; Gams 1971, 1975). Because ex-type cultures of the species do not exist, we compared our isolates with the four available reference strains of *A. hansfordii* (CBS 390.73, CBS 705.86, CBS 384.96, CBS 110390). However, those strains have important phenotypic features different from the protolog of *A. hansfordii* (Deighton 1969). Strains CBS 390.73 and CBS 705.86 produced two types of conidia, strain CBS 384.96 had hyaline conidia and produced a diffusible light yellow pigment in all media tested and strain CBS 110390 had light brown cylindrical conidia with truncate ends. Based on morphological and molecular data, we accommodated those strains in *A. fusidioides* (CBS 705.86), *A. pilosum* (CBS 390.73) and *A. citrinum* (CBS 384.96). The conidial morphology of the fourth strain (CBS 110390) was very different from those observed in the *A. fusidioides* group. Comparison of LSU sequences demonstrated that this strain was phylogenetically distant from that group and related to "*pseudozeylanicum* clade" sensu Summerbell et al. (2011) (data not shown). The taxonomy of *A. hansfordii* remains uncertain because the holotype of *A. hansfordii* (IMI 115.823) is not currently available for examination, and considering the morphological discrepancies shown by the existing supported reference strains the neotypification of the species was not possible.

The new species *A. citrinum* was represented by two strains previously identified as *A. hansfordii* (CBS 384.96) and *A. cavaraeanum* (CBS 758.69). Both were characterized by the production of a diffusible light yellow pigment, phialides up 50 µm long and hyaline obovoid conidia, features that did not match with the respective descriptions of *A. hansfordii* and *A. cavaraeanum* (Gams 1971, 1975; Ito et al. 2000). *Acremonium* species similar to *A. citrinum* with colonies producing a yellowish diffusible pigment or with yellow colonies are *A. vitellinum, A. chrysogenum, A. flavum* and *A. guillematii.* With exception of the first species, the main difference between the last three and *A. citrinum* is the production of conidia in slimy heads. In addition, *A. chrysogenum* has colonies with a yeast-like growth and ellipsoidal conidia; *A. flavum* produces ellipsoidal conidia, abundant chlamydospores and is thermotolerant and *A. guillematii* has numerous octahedral crystals in culture (Gams 1971). *Acremonium vitellinum* differs from *A. citrinum* by its conidia slightly truncate at the base and also by the production of abundant crystals in culture (Gams 1971). In the phylogenetic analysis of Summerbell et al. (2011), *A. chrysogenum* and *A. flavum* were grouped in the "*chrysogenum* clade" together with other species of the Bionectriaceae, such as *Hapsi-*

dospora irregularis, *Nigrosabulum globosum* and *Mycoarachis inversa*, while *A. vitellinum* and *A. guillemati* formed a weakly supported clade close to the Clavicipitaceae.

In our phylogenetic analysis the new species *A. parvum*, represented in this study by a single strain (CBS 381.70A), formed a well supported clade with *A. fusidioides* and *A. pilosum*. In the study of Summerbell et al. (2011) that strain clustered in the LSU tree with the strain CBS 831.97, both strains having previously been identified as *A. alternatum*. We were not able to obtain viable cultures of this latter strain, but LSU sequences of both strains showed 99.5% similarity. *Acremonium parvum* differs from the original description of *A. alternatum* by having slower growth, phialides with an additional septum near to the apex and by the production of pyriform or lanceolate conidia rounded at the tip and with an elongated and truncate base. Other *Acremonium* species characterized by a restricted growth are *A. potronii*, *A. pinkertoniae*, *A. guillematii* and *A. incrustatum*. However, apart from producing conidia in slimy heads, they differ in other important phenotypic features: *A. potronii* has shorter phialides (11–27 μm long) and wider conidia (up to 3 μm wide); *A. pinkertoniae* has ellipsoidal conidia with rough walls; *A. guillematii* has yellow colonies and produces crystals in culture; and *A. incrustatum* has shorter phialides (12–20 μm long), smaller dacryoid conidia (2–2.3 × 1.4–1.5 μm) and chondroid hyphae. Species with catenate conidia morphologically similar to *A. parvum* are *A. egyptiacum* and *A. persicinum*, but they grow faster, have pigmented colonies and longer phialides. In previous molecular studies, these two species were related to other *Acremonium* species of the Bionectriaceae but phylogenetically placed far away and grouped with species different from the *A. fusidioides* clade (Perdomo et al. 2011, Summerbell et al. 2011).

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4.1.3. New species of *Acremonium* from clinical samples and recircumscription of some acremonioid species in *Plectosphaerellaceae*

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(In preparation)

UNIVERSITAT ROVIRA I VIRGILI
TAXONOMIC STUDY OF CLINICAL AND ENVIRONMENTAL ISOLATES OF ARTHROCONIDIAL, ACREMONIUM-LIKE AND OCHROCONIS-LIKE FUNGI
Dixie Alejandra Giraldo López
Dipòsit Legal: T 767-2015

New species of *Acremonium* from clinical samples and recircumscription of some acremonioid species in *Plectosphaerellaceae*

Key words: *Emericellopsis, Hypocreales, Plectosphaerellaceae,* Phylogeny, Taxonomy

ABSTRACT

Acremonium is one of the largest and complex genera of ascomycetes. Several molecular studies have demonstrated that this genus is polyphyletic and its taxonomy and phylogeny are under revision. *Acremonium sensu stricto* is restricted to the family *Bionectriaceae* of the *Hypocreales*. Based on the phenotypic and molecular study of fresh material and strains from different culture collections, we describe two new *Acremonium* species, *A. moniliforme* and *A. dimorphosporum*. The former is related to *Emericellopsis* and is characterized by cylindrical conidia, elongated phialides and moniliform hyphae. *Acremonium dimorphosporum,* which shows some morphological similarities with *Acremonium borodinense,* produces two types of conidia, i.e. cylindrical with smooth walls and ellipsoidal with rough walls. Two new acremonium-like genera are proposed within the family *Plectosphaerellaceae*, i.e., *Brunneomyces* and *Cervusimilis*. The former genus is established to accommodate *Acremonium brunnescens* as the type species, and the new species *Brunneomyces hominis* and *B. europaeus*, all of which are morphologically characterized by brown, thick-walled hyphae, sympodial conidiophores, polyphialides and pyriform conidia arranged in chains. The monotypic genus *Cervusimilis,* based on *C. alba,* is distinguished by its light coloured colonies, simple or branched conidiophores, phialides with cylindrical collarettes, and ellipsoidal or subglobose conidia. The combined analysis of the LSU, ITS, *Rpb*2 and *TEF1-α* loci*,* supports their inclusion within this family.

INTRODUCTION

Acremonium is one of the largest and most complex genera of ascomycetous hyphomycetes, encompassing common saprobic species with worldwide distribution, most of them able of colonizing very diverse substrates (Gams 1971, 1975, Domsch et al. 2007). Some species are recognized as important plant pathogens (Alfaro-García et al. 1996, Lin et al. 2004) or causing opportunistic infections in humans (Summerbell 2003, de Hoog et al. 2011, Guarro 2012).

The morphological identification of *Acremonium* species is difficult because its asexual structures are poorly differentiated and their recognition requires expert training. DNA sequencing techniques facilitate the species recognition, but otherwise have demonstrated a clear polyphyly of the genus and complicate its taxonomy. Its members are scattered in different lineages throughout the *Ascomycota* (Glenn et al. 1996, Zare et al.

2007, Schoch et al. 2009, Gräfenhan et al. 2011, Perdomo et al. 2011, Summerbell et al. 2011, Giraldo et al. 2012). Regarding the available phylogenetic data, it seems reasonable to circumscribe the genus *Acremonium sensu stricto* to the species of *Bionectriaceae* (*Hypocreales*), which accommodates the recently epitypified type species of the genus, *A. alternatum*. Several sexual morph genera traditionally associated to *Acremonium*, such as *Emericellopsis*, *Hapsidospora*, *Nigrosabulum*, *Bulbithecium* or *Mycoarachis* (Gams 1971, Summerbell et al. 2011), also belong to this family. By contrast, molecular evidence has demonstrated that other traditional species of *Acremonium* are phylogenetically distant from the type species of the genus, remaining *incertae sedis* within *Hypocreales* or some of them close to the members of *Plectosphaerellaceae*, a family outside of *Hypocreales* and basal to the *Glomerellalles* (Zare et al. 2007, Réblová et al. 2011). The *Plectosphaerellaceae* was proposed by Zare et al. (2007) to accommodate the genera *Acrostalagmus*, *Gibellulopsis*, *Musicillium*, *Plectosphaerella* (as *Plectosporium*) and *Verticillium sensu stricto*, as well as some *Acremonium* species such as *A. restrictum, A. furcatum, A. brunnescens, A. alcalophilum, A. stromaticum* and *A. antarcticum* (Zare et al. 2007).

In a recent study on *Acremonium* species from clinical samples in USA (Perdomo et al. 2011), some of the isolates distributed in different groups within the *Hypocreales* (informally named groups J and N) and the *Plectosphaerellaceae* (groups Q and R), could not be identified and they were considered undescribed species. In the present study, by using multilocus sequence analyses and phenotypic methods, we have clarified the taxonomy of those unidentified isolates, as well as some conflictive *Acremonium* species members of *Plectosphaerellaceae*.

MATERIALS AND METHODS

Fungal isolates and sequences

The fungi included in the study are shown in Table 1. Six clinical isolates were provided by the Fungus Testing Laboratory at the University of Texas Health Science Center (UTHSC), which were previously identified as *Acremonium* sp. or *Acremonium hyalinulum.* Those isolates were studied in Perdomo et al. (2011) and included in the “groups J and N”, related to hypocrealean *Acremonium* species, and "groups Q and R", related to members of *Plectosphaerellaceae*. In addition, one *Acremonium* isolate (FMR 11785) obtained from soil with the procedure described in Giraldo et al. (2012), and five ex-type or reference strains provided by the CBS-KNAW Fungal Biodiversity Centre (CBS) were also included in our study. Numerous sequences of *Acremonium* species and related genera reported in different studies (Sigler et al. 2004, Zuccaro et al. 2004, Zare et al. 2007, Summerbell et al. 2011, Carlucci et al. 2012, Grum-Grzhimaylo et al. 2013a,b, Giraldo et al.

2014) were retrieved from GenBank database (Table 2) and included in the phylogenetic analyses.

Phenotypic studies

Morphological features were examined on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) and 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). Cultures were incubated at 25 °C in the dark for 4 wk. Colony diameters were measured after 14 d of incubation and the colony colour rated after Kornerup & Wanscher (1978). Microscopic features were examined and measured under a light microscope Olympus CH-2 (Olympus Corporation, Tokyo, Japan) by making direct wet mounts with either 85 % lactic acid or Shear's solution, or by slide cultures on OA. Photomicrographs were obtained with a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany), using phase contrast and Nomarski differential interference. The ability of the fungi to grow at 4, 12, 15, 20, 25, 30, 32, 35, 37 and 40 °C was determined on PDA in duplicate.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from fresh colonies using PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA, USA), following the manufacturer’s protocol. The DNA was quantified using a NanoDrop 3000 (ThermoScientific, Asheville, NC, USA). The internal transcribed spacer (ITS) regions and the 5’ end of the 28S nrDNA gene (LSU) were amplified and sequenced with the primer pairs ITS5/ITS4 (White et al. 1990) and LR0R/LR5 (Vilgalys & Hester 1990), respectively. Fragments of the translation elongation factor 1-alpha (*TEF1-α*), RNA polymerase II second largest subunit (*Rpb2*) and β-tubulin (*BT2*) genes were amplified and sequenced with the primer set EF 983F/EF 2218R (Rehner & Buckley 2005), Rpb2-5F/Rpb2-7R (Liu et al. 1999) and Bt1a/Bt1b (Glass & Donaldson 1995), respectively. PCR products were purified and sequenced at Macrogen Europe (Amsterdam, The Netherlands). The program SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA) was used to obtain consensus sequences of each isolate.

Phylogenetic analysis

The phylogenetic relationships between the soil isolate and clinical isolates belonging to the groups J and N with all the *Acremonium* species and related genera currently accepted in *Hypocreales* were pre-established through the analysis of their LSU sequences. The same approach was applied for the isolates of the groups Q and R, but in this case the LSU sequences from the isolates were compared with all the *Acremonium*

species and other genera accommodated in *Plectosphaerellaceae*. Subsequently, several multilocus sequence analyses were performed for each particular clade to confirm the results obtained from LSU data. ITS, *BT2, Rpb2* and *TEF1-α* loci were used for the isolate of the group N; ITS and LSU for the group J; and ITS, LSU, *Rpb2* and *TEF1-α* for the isolates included in the groups Q and R. Multiple sequence alignments for each locus were made in MEGA v. 5.05 (Tamura et al. 2011) using the Clustal W and MUSCLE applications (Thompson et al. 1994, Edgar 2004), and manually corrected under the same software platform. Selection of the best-fit nucleotide substitution models for each locus and for the combined dataset and Maximum Composite Likelihood (ML) phylogenetic analyses were performed with the same software. 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 1000 bootstrapped sets of data. A bootstrap support (bs) ≥ 70 was considered significant. Phylogenetic distance values among isolates were estimated with Kimura 2-parameter as nucleotide substitution model under MEGA v. 5.05. A second phylogenetic reconstruction via Bayesian inference (BI) was done using MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003). Markov chain Monte Carlo (MCMC) sampling was performed with two simultaneous runs for 3 million generations, with samples taken every 100 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 best nucleotide substitution model for each gene in the Bayesian analysis (GTR+G+I) was determined 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). Because no incongruence was observed, the different matrices were combined for the final phylogenetic 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).

RESULTS

The phylogenetic analysis based on LSU sequences of the isolates UTHSC 08-2284 (group N), UTHSC 08-3639 (group J) and FMR 11785 together with all the hypocrealean *Acremonium* species and related genera reported by Summerbell et al. (2011) is shown in the Figure 1. The final alignment included 75 taxa and consisted of 847 characters including gaps (conserved 580, variable 267, parsimony informative 195), and Tamura-Nei with

gamma distribution (TN+G) and general time reversible with gamma distribution and a portion of invariable sites (GTR+G+I) were found as the best-fit nucleotide substitution models for ML and BI, respectively. The phylogenetic tree revealed that the isolates were distributed into two strongly supported Bionectriaceous clades, namely *Emericellopsis* and *fusidioides* in Summerbell et al. (2011). The isolates UTHSC 08-2284 and FMR 11785 fell into *Emericellopsis*-clade (98 % bs, 1.00 PP), where the type species of *Emericellopsis*, *E. terricola* (CBS 120.40), together with some *Acremonium* species, viz. *A. exuviarum* (UAMH 9995), *A. fuci* (CBS 113889) and *A. salmoneum* (CBS 721.71) were also included. Within the *Emericellopsis*-clade the mentioned unidentified isolates UTHSC 08-2284 and FMR 11785 were grouped in a highly supported subclade (*Acremonium* sp. I, 89 % bs, 0.99 PP). The sequences of both isolates were 100 % identical. The isolate UTHSC 08-3639 (*Acremonium* sp. II) was placed in a single branch, phylogenetically related (83 % bs, 1.00 PP) with the members of the *fusidioides*-clade (Fig. 1), i.e., *A. fusidioides, A. hennebertii* and the recently described species *A. citrinum, A. parvum* and *A. pilosum* (Giraldo el al. 2014).

To resolve with better resolution the results obtained in the LSU phylogeny of the hypocrealean species *Acremonium* sp. I (UTHSC 08-2284 and FMR 11785) and *Acremonium* sp. II (UTHSC 08-3639), two multilocus sequence analyses were performed separately for each species and their closely related species. The multilocus sequences analysis of the isolates of *Acremonium* sp. I and the members of the *Emericellopsis* clade included the ITS, *BT2, Rpb2* and *TEF1-α* regions (Fig. 2). The data set matrix included 48 taxa and 2807 characters including alignment gaps (conserved 2004, variable 803, parsimony informative 604), *Verrucostoma freycinetiae* and *Selinia pulchra* were used as outgroup. In addition to the species showed in the LSU analysis, other species previously reported to be related with members of *Emericellopsis* (Sigler et al. 2004, Zuccaro et al. 2004, Grum-Grzhimaylo et al. 2013b), such as *Stanjemonium grisellum*, *S. ochroroseum* and *Acremonium potronii*, were included in this analysis. Tamura 3-parameter with gamma distribution (T92+G) and GTR+G+I were found to be the best nucleotide substitution models for ML and BI, respectively. Although the trees generated by using ML and BI had a similar topology, the statistical support for several clades were higher with BI than with ML. The phylogenetic tree (Fig. 2) was consistent with the phylogenies previously reported (Sigler et al. 2004, Zuccaro et al. 2004, Grum-Grzhimaylo et al. 2013b). The two isolates included in *Acremonium* sp. I (UTHSC 08-2284 and FMR 11785) clustered in a strong supported basal clade (84 % bs, 1.00 PP), phylogenetically distant from the species of *Acremonium*, *Emericellopsis* and *Stanjemonium*. *Acremonium* sp. I is described below as a new species, named *Acremonium moniliforme*.

The third phylogenetic analysis (Fig. 3) included a combination of the ITS and LSU

sequences of *Acremonium* sp. II (UTHSC 08-3639) and the type and reference strains of the *Acremonium* species included in the *fusidioides* clade. The data set consisted of 11 taxa and 915 characters, including alignment gaps (conserved 731, variable 184, parsimony informative 135). ML analysis was done with Kimura-two parameter with gamma distribution (K2+G) as the best-fit nucleotide substitution model. The type strains of *Acremonium pinkertoniae* CBS 157.70 and *A. borodinense* CBS 101148 were used as outgroup. In this analysis, *Acremonium* sp. II was placed in a single lineage, phylogenetically distant from those *Acremonium* species with dimorphic conidia such as *A. fusidioides, A. pilosum* and *A. borodinense*, and other species with elongate conidia in chains, such as *A. hennebertii, A. parvum* and *A. citrinum*. *Acremonium* sp. II is proposed here as a new species named *Acremonium dimorphosporum*.

The phylogenetic reconstruction using the LSU, ITS, *TEF1-α* and *Rpb2* loci from the isolates of the groups Q (UTHSC 06-415 and UTHSC R-3853) and R (UTHSC 06-874 and UTHSC 08-3693) and representatives of the *Plectosphaerellaceae* is shown in Figure 4. The combined dataset consisted of 3271 characters from which 805 sites were phylogenetically informative (LSU 135, ITS 151, *TEF1-α* 158 and *Rpb2* 361), and 33 strains including the outgroup species *Colletotrichum orbiculare* and *C. lagerarium.* The best-fit nucleotide substitution model for ML analysis was K2+G. The phylogenetic tree showed that the clinical isolates UTHSC 06-874 and UTHSC 08-3693 together with the reference strain of *Acremonium antarcticum* (CBS 987.87) were clustered in a well supported clade (*Acremonium* sp. III, 98 % bs). Genetic similarity among the sequences of the isolates included in *Acremonium* sp. III ranged from 94.6–98.3 % for *Rpb2* and 99.6–100 % for LSU. *Acremonium* sp. III is proposed here as a new species named *Cervusimilis alba*, which belongs to an unnamed lineage in the mentioned family and is therefore described below as the new monotypic genus *Cervusimilis.*

A strongly supported monophyletic group (82 % bs) included two subclades and a single branch. The first subclade (*Acremonium* sp. IV, 95 % bs) consisted of the clinical isolates UTHSC 06-415 and UTHSC R-3853, showing sequences genetically almost identical in the loci analyzed (99.1 % for *TEF1-α,* and 100 % for LSU and ITS). The type strain of *A. brunnescens* (CBS 559.73) was placed in a well-supported single branch, while two environmental reference strains (CBS 560.86 and CBS 652.96), received as *A. hyalinulum,* were clustered in the second subclade (97 % bs). Because the type strain of *A. brunnescens* and other reference strains of acremonium-like fungi encompassed a monophyletic lineage within the *Plectosphaerellacae*, which is outside of the *Hypocreales*, the new genus *Brunneomyces* is proposed to accommodate three new taxa, named *B. hominis* (UTHSC 06-415 and UTHSC R-3853), *B. brunnescens* (CBS 559.73) and *B. europaeus* (CBS 560.86 and CBS 652.96).

Taxonomy

Acremonium dimorphosporum Giraldo, Deanna A. Sutton & Gené, *sp. nov.* —— MycoBank MB811461; Fig. 5

Etymology. Refers to the presence of two kinds of conidia.

Colonies on OA at 25 °C reaching 10–11 mm in 14 d, white (1A1), flat, with scarce aerial mycelium; reverse colourless. On PDA at 25 °C attaining 14–15 mm in 14 d, pinkish white (7A2), flat, cottony; reverse orange (6A6). *Mycelium* consisting of hyaline, smooth- and thin-walled hyphae, 1.5–2 µm wide. *Conidiophores* erect, usually simple, consisting of single phialides growing directly on vegetative hyphae, occasionally basitonously branched, bearing 2–4 phialides, straight, up to 60 µm long, hyaline, smooth, with cell walls usually thicker than those of the vegetative hyphae. *Phialides* subulate, 17–30(–45) µm long, 1–1.5 µm wide at the base, thick- and smooth-walled, hyaline; phialides with a secondary septum near to middle or proliferating percurrently are occasionally present. *Conidia* unicellular, hyaline, of two types: i) cylindrical with more or less rounded ends, 3–7 × 1–1.5 µm, thin- and smooth-walled; ii) ellipsoidal, 3–4 × 2–3 µm, thick- and rough-walled, arranged in slimy heads. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 20–25 ºC, maximum 30 ºC, minimum 15 ºC.

Specimen examined. USA, Texas, from bronchoalveolar lavage fluid, 2008, *D.A. Sutton* (holotype CBS H-22021; culture ex-type CBS 139050 = FMR 10548 = UTHSC 08-3639).

Notes — Although *A. dimorphosporum* is phylogenetically distant, it is morphologically similar to *A. borodinense* (Ito et al. 2000) in producing both ellipsoidal rough-walled and cylindrical smooth-walled conidia. However, *A. borodinense* grows more rapidly at 25 ºC (27–29 mm diam after 10 d), is able to grow at 37 ºC, the cylindrical conidia are slightly curved and smaller (4.5–5.5 µm long), and the ellipsoidal conidia are larger (4.2–5.5 × 3–4 µm) than those of *A. dimorphosporum.*

Acremonium moniliforme Giraldo, Deanna A. Sutton & Guarro, *sp. nov.* —— MycoBank MB811462; Fig. 6

Etymology. Refers to the presence of monilifom hyphae.

Colonies on OA at 25 ºC reaching 45–60 mm in 14 d, yellowish white (4A2), flat, glabrous; reverse colourless. On PDA at 25 ºC attaining 36–50 mm in 14 d, pinkish white (7A2), radially folded, zonate at the border, felty, grayish red (7B4); reverse salmon (6A4).

Mycelium consisting of branched, septate, hyaline, smooth- and thick-walled hyphae, initially 2–2.5 µm wide, with cells gradually swelling, becomes barrel-shaped, up to 7 µm wide. *Conidiophores* erect, simple, consisting of single conidiogenous cells growing directly on vegetative hyphae. *Phialides* cylindrical with apex slightly flexuose, 30–50 µm long, 1.5–2 µm wide at the base, with a distinct periclinal thickening, thick- and smooth-walled, hyaline. *Conidia* unicellular, cylindrical with rounded ends, 3–5(–6) × 1–2 µm, hyaline, thick- and smooth- walled, arranged in slimy heads. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 25–30 ºC, maximum 37 ºC, minimum 4 ºC.

Specimens examined: SPAIN, Aragon region, Huesca province, Ordesa y Monte Perdido National Park, from forest soil, 2011, coll. *A. Giraldo, M. Hernández, & J. Capilla,* isol. *A. Giraldo* (holotype CBS H-22022, culture ex-type CBS 139051 = FMR 11785). USA, Utah, from toe nail, 2008, *D.A. Sutton* (FMR 10363 = UTHSC 08-2284).

Notes — *Acremonium monilifome* is phylogenetically distant from the species of the *Emericellopsis*-clade, and it can be morphologically differentiated from the current *Acremoniun* species, including those unnamed asexual morphs of *Emericellopsis*, by the production of moniliform hyphae. *Acremonium fuci* occasionally produces small rounded hyphal swellings, similar to the moniliform hyphae of *A. moniliforme*. However, both species can be distinguished by the conidial shape, which is obovoid or broadly ellipsoidal in the former species, and cylindrical in the latter. Additionally, the maximum temperature for growth in *A. fuci* is 33 ºC (Zuccaro et al. 2004) while in *A. moniliforme* is 37 ºC.

Brunneomyces Giraldo, Gené & Guarro, *gen. nov.* —– MycoBank MB811471;
Type species. *Brunneomyces brunnescens* (W. Gams) Giraldo, Gené & Guarro
Etymology. Referring to presence of brown pigmented hyphae.

Mycelium consisting of branched, septate, hyaline and thin-walled hyphae, often becoming dark brown, verrucose and thick-walled with age. Strong mushroom-like odour. *Conidiophores* erect, simple or poorly branched and often proliferating sympodially showing conidiogenous cells as short lateral cylindrical projections. *Conidiogenous cells* enteroblastic, mono- and polyphialidic, hyaline, terminal, lateral or intercalary (adelophialides), subulate, lageniform or cylindrical, usually with short cylindrical collarettes, often subhyaline or pale brown, and with a distinct periclinal thickening at the conidiogenous locus. *Conidia* unicellular, pyriform or ellipsoidal, hyaline or brown, arranged in chains. Sexual morph unknown.

Brunneomyces brunnescens (W. Gams) Giraldo, Gené & Guarro, *comb. nov*. —— MycoBank MB811472; Fig. 7

Basionym. Acremonium brunnescens W. Gams, Trans. Br. mycol. Soc., 64: 398. 1975.

Specimen examined. SRI LANKA, Hakgala Bot. Gardens, from dead stem of *Dendrocalamus giganteus*, Jan. 1973, *W. Gams* (holotype CBS H-6641, culture ex-type CBS 559.73 = ATCC 32180 = IMI 185378).

Notes — Although the three species included in the novel genus show a similar conidiogenous apparatus to that of the genus *Acremonium*, they can be distinguished by the presence of sympodial conidiophores and dark brown, verrucose, thick-walled hyphae. The combination of these morphological features are usually absent in the species of *Acremonium* and other genera of plectosphaerellaceous fungi. In addition, *Brunneomyces* is the only genus of the *Plectosphaerellaceae* with conidial chains.

A detailed description of *B. brunescens* was given in Gams (1975). According to our observation, the type strain of this species was characterized by slow growing colonies, which reached 6–8 mm and 21–22 mm diam after 14 d on PDA and OA, respectively; the pigmented verrucose hyphae and dark brown conidia appeared after 21 d; the phialides showed short cylindrical and slightly pigmented collarettes, adelophialides were present and measured 6–10 × 1.5–2.5 µm, and the conidial chains often collapsed soon in slimy heads. In addition, this fungus was unable to growth at 32 ºC.

Brunneomyces hominis Giraldo, Deanna A. Sutton & Gené*, sp. nov* —— MycoBank MB811473; Fig. 8

Etymology: Refers to the human origin of the isolates.

Colonies on OA at 25 °C reaching 26–28 mm in 14 d, orange white (6A2), flat, dusty; reverse colourless. On PDA at 25 °C attaining 17–18 mm in 14 d, grey (5F1) at centre, yellowish white (4A2) at periphery, crateriform and radially folded, felty; reverse grey (5F1). Strong mushroom-like (moist soil) odour. *Mycelium* consisting of septate, hyaline, smooth- and thin-walled hyphae, 1.5–2 µm wide at the beginning, becomes dark brown, verrucose and thick-walled, up to 3 µm wide with age. *Conidiophores* erect, mostly simple, occasionally few branched and proliferating sympodially, straight or slightly bent, up to 35 µm long, hyaline, smooth-walled. *Phialides* subulate, 12–20(–30) µm long, 1.5–2 µm wide at the base, hyaline at first, turning dark brown in old cultures, thick- and smooth-walled, with conspicuous periclinal thickening and cylindrical collarettes; adelophialides sometimes present, up to 10 µm long; polyphialides with up to two conidiogenous locus commonly present. *Conidia* unicellular, pyrifom or ellipsoidal, 4–5(–6) × 2–2.5 µm, with truncate base,

subhyaline, thin- and smooth-walled, arranged in long dry chains. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 25–30 ºC, maximum 35 ºC, minimum 4 ºC.

Specimens examined. USA, Minnesota, from human sputum, 2006, *D.A. Sutton* (holotype CBS H-22023, culture ex-type CBS 139053 = FMR 10429 = UTHSC 06-415); California, from human sputum, *D.A. Sutton,* CBS 139054 = FMR 10437 = UTHSC R-3853.

Notes — *Brunneomyces hominis* differs from the other two species of the genus, *B. brunnescens* and *B. europaeus*, by the following features: the colony colour on OA was orange-white and yellowish white in *B. hominis* and *B. europaeus,* respectively, and grey with dark grey reverse in *B. brunnescens;* the conidial arrangement was in long dry chains in *B. hominis,* while in *B. europaeus* and *B. brunnescens* the chains collapsed soon in slimy heads; and *B. hominis* was the only species that grew at 35 ºC (7 mm diam in 14 d).

Brunneomyces europaeus Giraldo, Gené & Guarro*, sp. nov* —– MycoBank MB811474; Fig. 9

Etymology: Refers to the geographic origin of the isolates, Europe.

Colonies on OA at 25 °C reaching 31–50 mm in 14 d, yellowish white (4A2), flat, dusty; reverse colourless. On PDA at 25 °C attaining 25–36 mm in 14 d, grayish brown (6E2) at centre, white (1A1) to orange-white (6A2) at periphery, radially folded, felty; reverse brown (6E2). Slight mushroom-like (moist soil) odour. *Mycelium* consisting of septate, hyaline, smooth- and thin-walled hyphae, 2–2.5 µm wide, becomes brownish, verrucose and thick-walled with age. *Conidiophores* erect, usually simple, some proliferating sympodially, up to 45 µm long, straight or slightly bent, hyaline to subhyaline, smooth-walled. *Phialides* subulate or more or less cylindrical, 15–35(–40) µm long, 2–3 µm wide at the base, thick- and smooth-walled, hyaline, with a distinct periclinal thickening at the conidiogenous locus and short cylindrical collarettes; adelophialides sometimes present, up to 15 µm long; polyphialides with up to three conidiogenous locus commonly present. *Conidia* unicellular, pyrifom or ellipsoidal, 5–6(–7) × 2–3 µm, with distinct truncate base, subhyaline, thin- and smooth-walled, forming chains that soon collapse in slimy heads. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 20–25 ºC, maximum 32 ºC, minimum 4 ºC.

Specimens examined. SPAIN, Riumar, from sediments of Ebro River, 1991, coll. *K. Ulfig*, isol. *J. Gené* (holotype CBS H-22024, culture ex-type CBS 652.96 = FMR 3962).

FRANCE, Provence, from leaf of *Bambusa* sp., Dec. 1986, *O. Petrini*, CBS 560.86 = FMR 3406.

Notes — The two isolates of *B. europaeus* were previously identified as *Acremonium hyalinulum.* However, this species does not have type strain for a reliable comparison and, according to different studies it seems to be a polyphyletic species (Perdomo et al. 2011, Summerbell et al. 2011). Since we have observed some morphological discrepancy with respect to the protologue of *A. hyalinulum* (Gams 1971), we preferred to describe both isolates as a new species. *Acremonium hyalinulum* differs from *B. europaeus* mainly in having hyaline smooth-walled hyphae, and in the absence of sympodial conidiophores and adelophialides.

Cervusimilis Giraldo, Gené & Guarro, *gen. nov.* —– MycoBank MB811475
Type species. *Cervusimilis alba* Giraldo, Deanna A. Sutton & Guarro
Etymology. Referring to the branched conidiophores that resemble the antlers of a deer.

Mycelium consisting of branched, septate, hyaline and smooth-walled hyphae. *Conidiophores* erect, simple, some with percurrent proliferations, or branched repeatedly. *Conidiogenous cells* enteroblastic, monophialidic and polyphialidic, cylindrical or subulate, with conspicuous cylindrical collarettes and distinct periclinal thickening at the conidiogenous locus, subhyaline. *Conidia* unicellular, subglobose, ellipsoidal or cylindrical, hyaline, arranged in chains. Sexual morph unknown.

Cervusimilis alba Giraldo, Deanna A. Sutton & Guarro***,*** *sp. nov* —– MycoBank MB811476; Fig. 10
Etymology: Referring to its light coloured colonies.

Colonies on OA at 25 °C reaching 40–41 mm in 14 d, yellowish white (4A2), flat, dusty; reverse colourless. On PDA at 25 °C attaining 10–11 mm in 14 d, pale yellow (4A2–3), raised, membranous, with lobulate margin; reverse colourless. *Mycelium* with septate, hyaline, smooth- and thin-walled hyphae, 1.5–2 µm wide. *Conidiophores* erect, simple, occasionally with 1 percurrent proliferation and sometimes with a second septum near the base, or branched usually at the middle part, with branches bearing 2–4 phialides, up to 75 µm long, straight or slightly curved, hyaline to subhyaline, smooth-walled. *Phialides* cylindrical or subulate, 12–22(–50) µm long, 2–2.5 µm wide at the base, with a distinct periclinal thickening at the conidiogenous locus and cylindrical collarettes, thick- and smooth-walled, hyaline; polyphialides with two conidiogenous locus commonly present, one of them usually placed near the basal septum as a short cylindrical lateral projection, up to 5

µm long. *Conidia* unicellular, subglobose to limoniform (in UTHSC 08-3693) or ellipsoidal to near cylindrical (in CBS 987.87 and UTHSC 06-874), 2–4 × 2–2.5 µm, subhyaline, thick- and smooth-walled, in slimy heads. Chlamydospores and sexual morph not observed.

Cardinal temperature for growth — Optimum 20–25 ºC, maximum 32–35 ºC, minimum 4 ºC.

Specimens examined. LUXEMBOURG, Hautecharage, on *Hypogymnia physodes,* Dec. 1987, coll. *G. Marson*, isol. *W. Gams* (holotype CBS H-8083, culture ex-type CBS 987.87 = FMR 10886). USA, Hawaii, from human sputum, 2006, *D.A. Sutton* FMR 10433 = UTHSC 06-874; Missouri, from human nail, 2008, *D.A. Sutton* CBS 139055 = FMR 10549 = UTHSC 08-3693.

Notes — The three isolates of *Cervusimilis alba* studied showed some morphological differences, i.e. while the conidia of CBS 987.87 and UTHSC 06-874 were ellipsoidal to near cylindrical, those of the isolate UTHSC 08-3693 were subglobose to limoniform. However, their sequences were practically identical, with the exception of the *Rpb2* that showed a variability ranging from 1.7–5.4 %. The analysis of the combined dataset showed a high genetic identity (99.6–100 %).

The isolate CBS 987.87 was originally deposited at the CBS collection as *Acremonium antarcticum*, but the lack of type material, makes difficult a reliable identification of this species. However, morphological features such as the presence of mono- and polyphialides with conspicuous cylindrical collarettes and percurrent conidiophores, observed in *C. alba*, were not described in the protologue of *A. antarcticum* (Hawksworth 1979).

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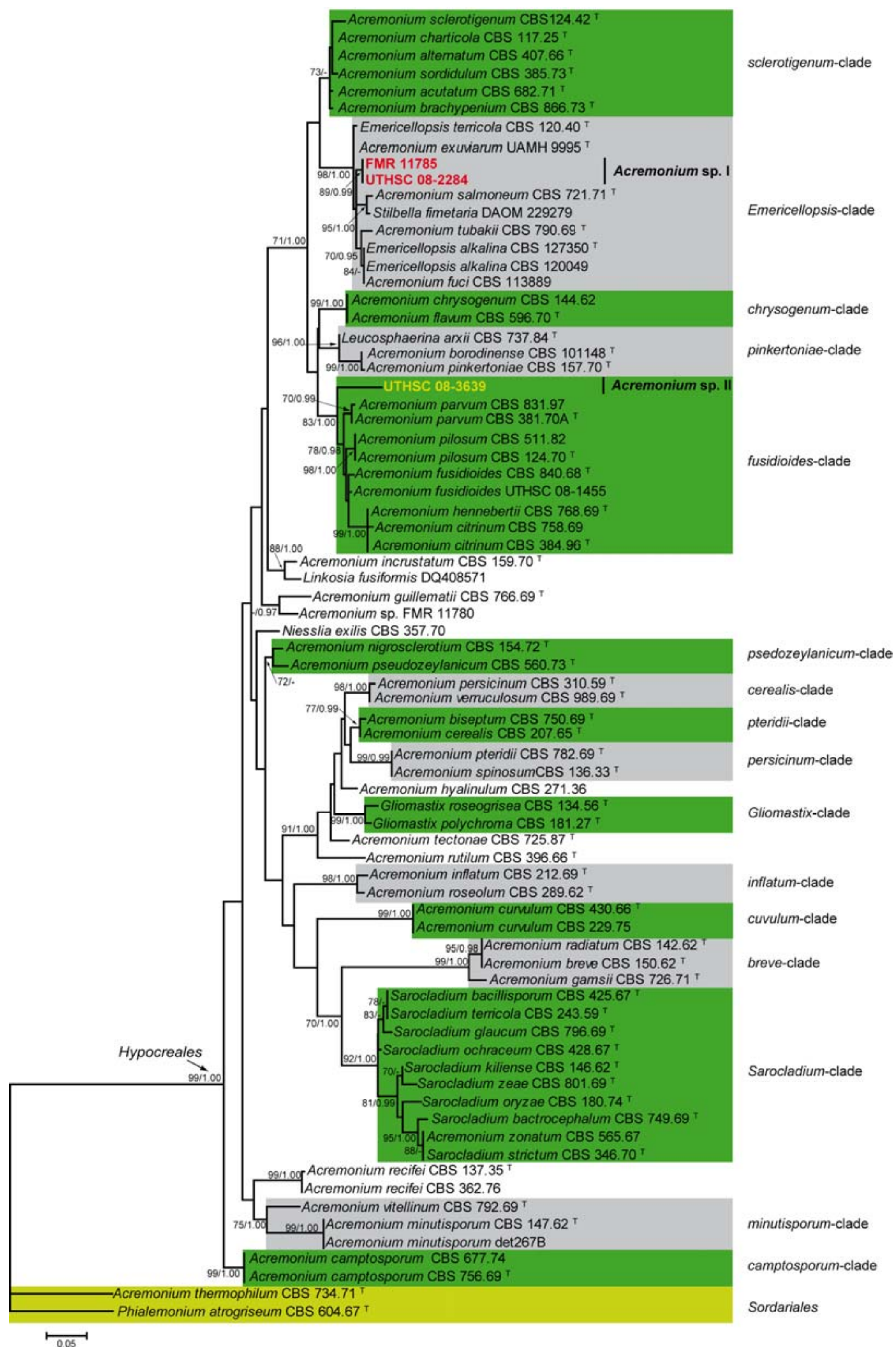

Fig. 1. Maximum composite likelihood tree based on analysis from the partial LSU sequences of *Acremonium* species and related genera in *Hypocreales*. Clade names are based on Summerbell et al. (2011). Bootstrap support values above 70 % / Bayesian posterior probability values above 0.95, are shown at the nodes. ^{T}, Type strain.

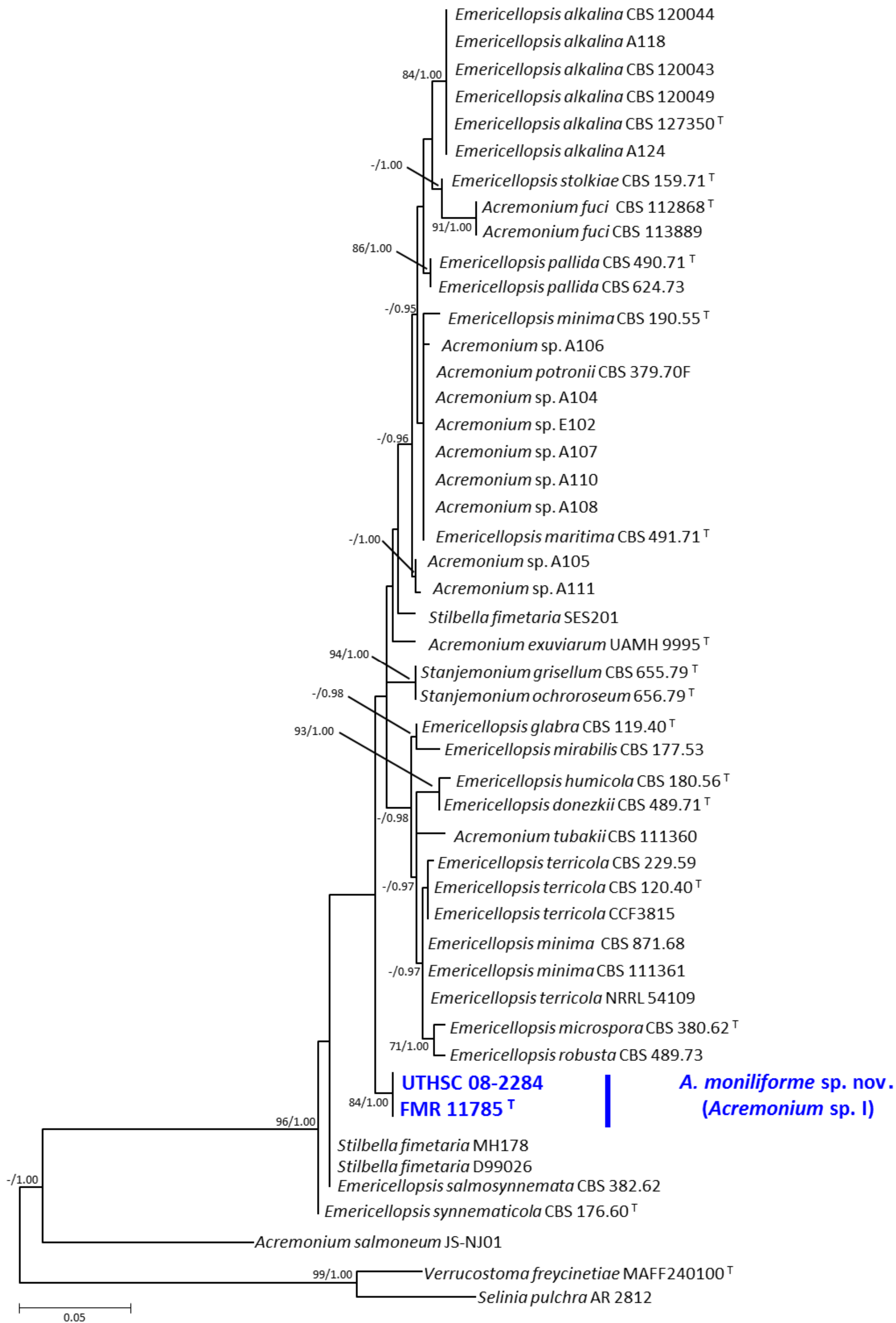

Fig. 2 Maximum composite likelihood tree based on partial sequences of ITS, *BT2, Rpb*2 and *TEF1-α* genes from *Acremonium* sp. I and different members of the *Emericellopsis*-clade. Bootstrap support values above 70 % / Bayesian posterior probability values above 0.95, are shown at the nodes. T, Type strain.

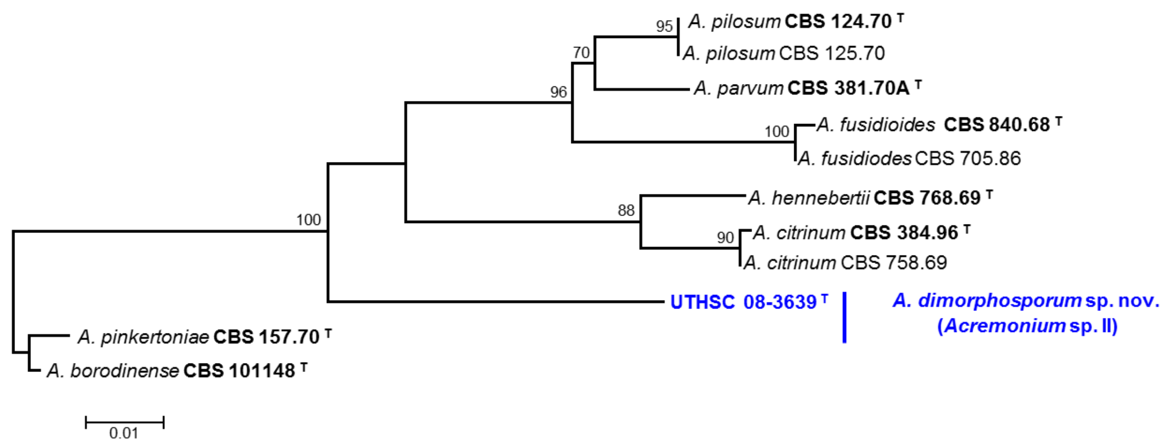

Fig. 3 Maximum composite likelihood tree based on partial sequences of ITS and LSU genes from *Acremonium* sp. II and *Acremonium* species members of the *fusidioides*-clade. Bootstrap support values above 70 % are shown at the nodes. T, Type strain.

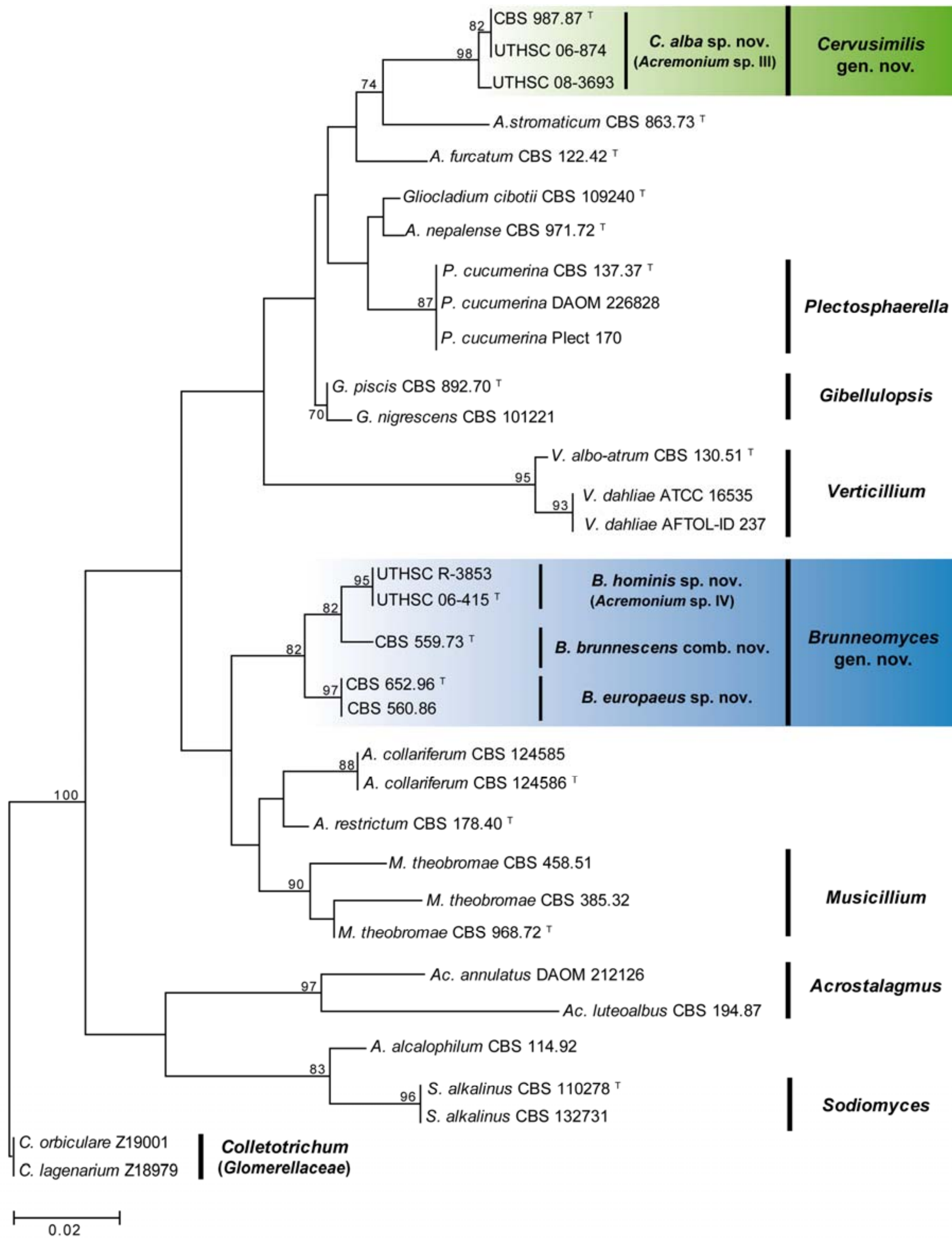

Fig. 4. Maximum composite likelihood tree based on analysis from the partial LSU, ITS, *Rpb2* and *TEF1-α* sequences of *Acremonium* sp. III, *Acremonium* sp. IV and different genera of the *Plectosphaerellaceae* family. Bootstrap support values above 70 % are shown at the nodes. T, Type strain.

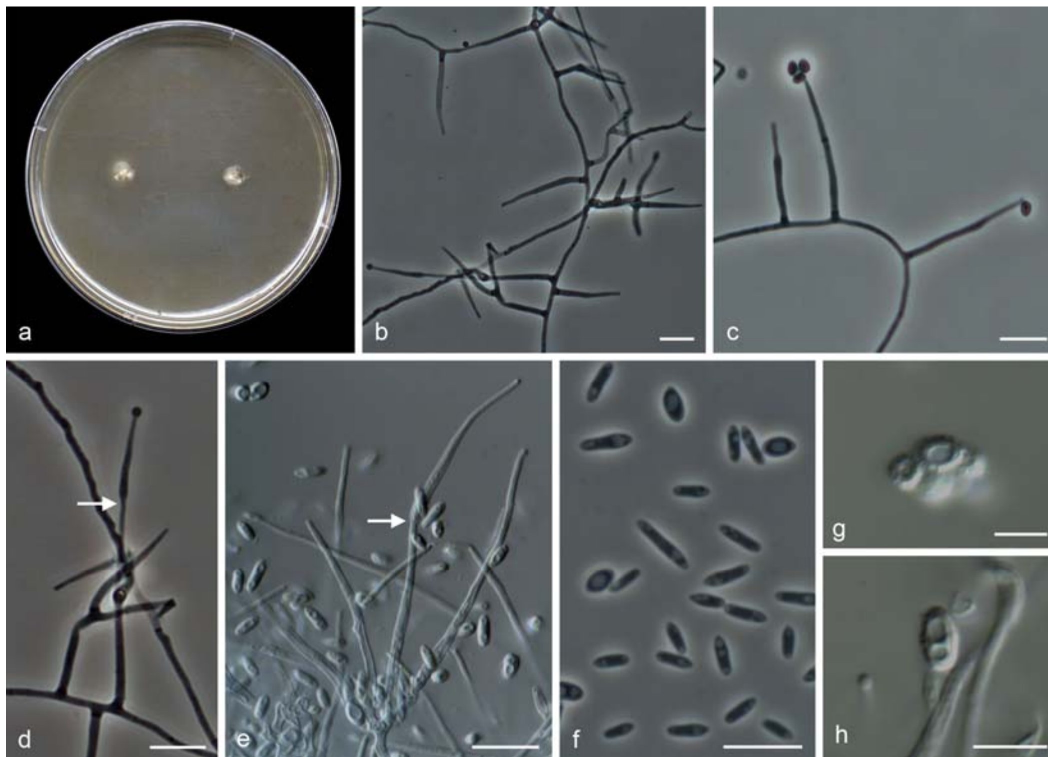

Fig. 5 *Acremonium dimorphosporum* (= *Acremonium* sp. II) UTHSC 08-3639. a. Colonies on OA, after 21 d at 25 ºC; b, c. simple conidiophores and conidia forming heads; d, e. phialides with percurrent proliferation (arrow); f–h. conidia. — Scale bars b–f = 10 μm, g, h = 5 μm.

Fig. 6 *Acremonium moniliforme* (= *Acremonium* sp. I) FMR 11785. a. Colonies on PDA after 14 d at 25 ºC; b, c. simple conidiophores arising directly from ropes of hyphae; d, monilifom hyphae; e, phialide with periclinal thickening at the apex; f, g. conidia. — Scale bars = 10 μm.

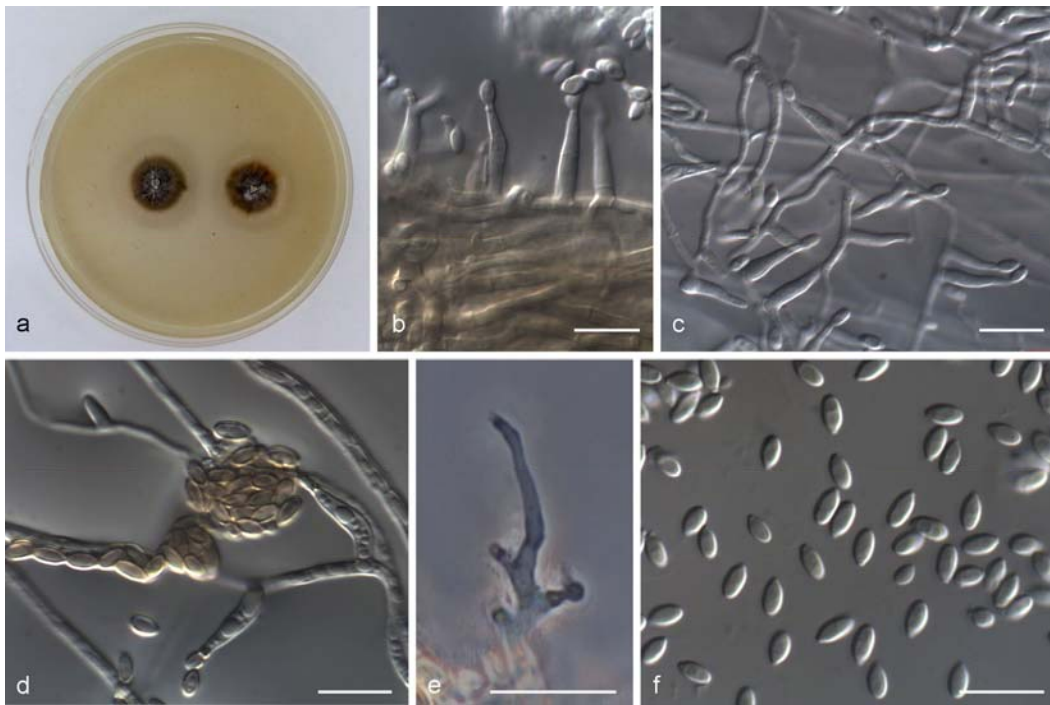

Fig. 7 *Brunneomyces brunnescens* CBS 559.73. a. Colonies on OA after 14 d at 25 ºC; b. brown pigmented hyphae; c. discrete phialides; d. phialides with slightly pigmented collarettes and conidial chains collapsing in slimy heads; e. sympodial conidiophore; f. conidia. — Scale bars = 10 µm.

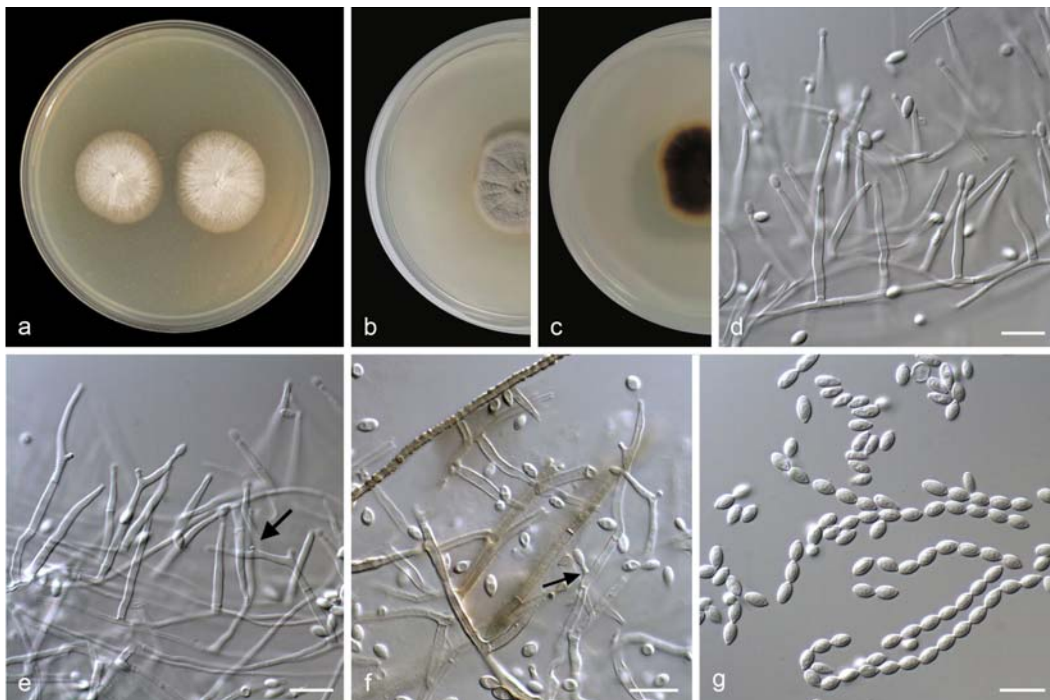

Fig. 8. *Brunneomyces hominis* (= *Acremonium* sp. IV) a–c, f, g UTHSC 06-415; d, e UTHSC R-3853. a. colonies on OA after 14 d at 25 ºC; b, c. colonies on PDA after 21 d at 25 ºC obverse and reverse, respectively; d. simple conidiophores; e. simple conidiophores with terminal polyphialides and a sympodial conidiophore (arrow); f. pigmented verrucose hyphae and intercalary phialide (arrow); g. conidia. — Scale bars = 10 µm.

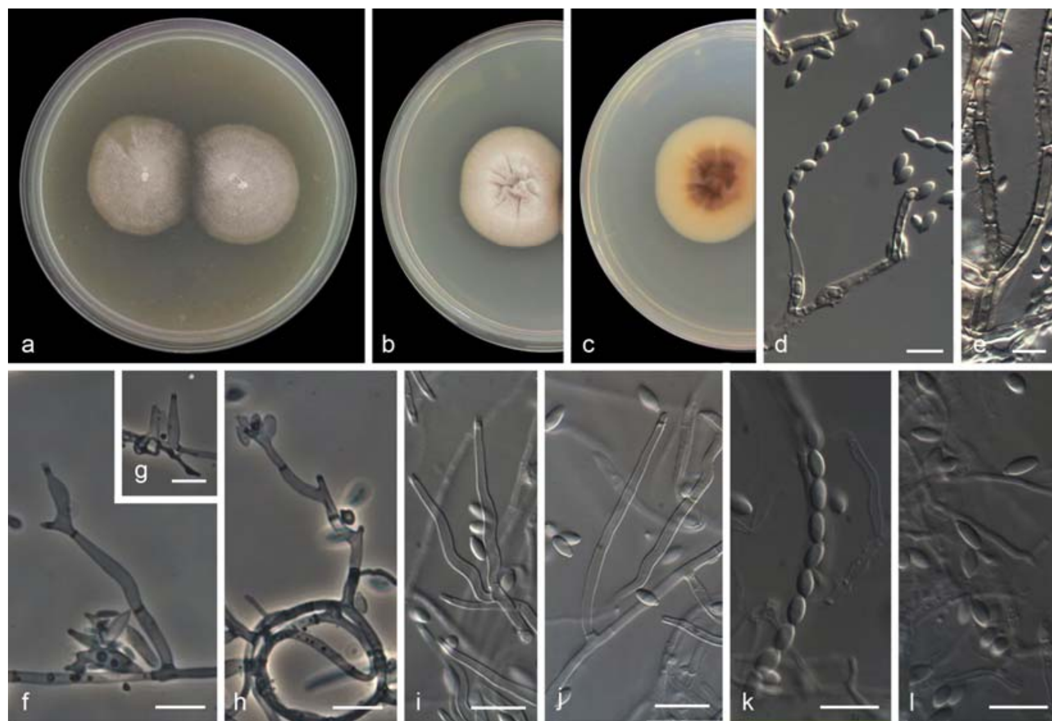

Fig. 9 *Brunneomyces europaeus* a–c, f–h, k, l CBS 560.86; d, e, i, j CBS 652.96. a. colonies on OA after 14 d at 25 ºC; b, c. colonies on PDA after 14 d at 25 ºC obverse and reverse, respectively; d. phialide producing a long conidial chain; e. pigmented verrucose hyphae; f, polyphialide; g, adelophialide; h. sympodial conidiophore; i, j. phialides with short cylindrical collarettes; k, l. conidia. — Scale bars = 10 µm.

Fig. 10. *Cervusimilis alba* (= *Acremonium* sp. III). a–f, h UTHSC 08-3693; g, i CBS 987.87. a. colonies on PDA after 7 d at 25 ºC; b. phialide with a cylindrical collarette (arrow); c, d. branched conidiophores; e, f, i. polyphialides and conidia; g. unbranched conidiophore with percurrent proliferations; h, i. conidia. — Scale bars =10 µm.

Table 1 Isolates included in this study.

Species	Strain [a]	Origin [b]	Previous identification (Perdomo et al. 2011)	GenBank accession number [c]				
				LSU	ITS	*BT2*	*TEF1-α*	*Rpb2*
Acremonium dimorphosporum (=*Acremonium* sp. II)	UTHSC 08-3639 [T]	BAL, USA	*Acremonium* sp. (Group J)	**LN810506**	**LN810515**	–	–	–
Acremonium moniliforme (=*Acremonium* sp. I)	FMR 11785 [I]	Soil, Spain	*Acremonium* sp.	**LN810507**	**LN810516**	**LN810523**	LN810531	**LN810525**
	UTHSC 08-2284	Toe nail, USA	*Acremonium* sp. (Group N)	**LN810508**	**LN810517**	**LN810524**	**LN810532**	**LN810526**
Acremonium stromaticum	CBS 863.73 [I]	Root and rhizome of *Musa sapientum,* Honduras	*A. stromaticum*	HQ232143	DQ825969	–	**LN810533**	–
Brunneomyces brunnescens	CBS 559.73 [T]	On dead stem of *Dendrocalamus giganteus,* Sri Lanka	*A. brunnescens*	HQ231966	**LN810520**	–	**LN810534**	–
Brunneomyces hominis	UTHSC 06-415 [I]	Sputum, USA	*A. hyalinulum* (Group Q)	**LN810509**	KP131517	–	**LN810535**	–
	UTHSC R-3853	Sputum, USA	*A. hyalinulum* (Group Q)	**LN810510**	KP131518	–	**LN810536**	–
Brunneomyces europaeus	CBS 560.86	Leaf of *Bambusa* sp., France	*A. hyalinulum* (Group Q)	**LN810511**	**LN810518**	–	**LN810537**	**LN810527**
	CBS 652.96 [T]	River sediment, Spain	*A. hyalinulum*	**LN810512**	**LN810519**	–	**LN810538**	**LN810528**
Cervusimilis alba	CBS 987.87 [I]	On *Hypogymnia physodes,* Luxembourg	*A. antarcticum*	JX158444	DQ825970	–	JX158400	JX158466
	UTHSC 06-874	Sputum, USA	*Acremonium* sp. (Group R)	**LN810513**	**LN810521**	–	**LN810539**	**LN810529**
	UTHSC 08-3693	Nail, USA	*Acremonium* sp. (Group R)	**LN810514**	**LN810522**	–	**LN810540**	**LN810530**

[a] CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; FMR, Faculty of Medicine Reus, Spain; UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas, USA; [T] Type strain. [b] BAL, Bronchoalveolar lavage fluid. [c] Accession numbers of sequences newly generated in this study are indicated in boldface. LSU large subunit of the nrDNA; ITS internal transcribed spacer regions of the nrDNA and intervening 5.8S nrDNA; *BT2* beta-tubulin gene; *TEF1*-α transcription elongation factor 1-alpha; *Rpb2* RNA polymerase II second largest subunit.

Table 2 Taxa used in the phylogenetic analyses with their GenBank accession numbers.

Species	Strain [a]	GenBank accession number [b]				
		LSU	ITS	*BT2*	*TEF1-α*	*Rpb2*
Acremonium acutatum	CBS 682.71 [T]	HQ231965	–	–	–	–
Acremonium alcalophilum	CBS 114.92	JX158443	DQ825967	–	JX158399	JX158465
Acremonium alternatum	CBS 407.66 [T]	HQ231988	–	–	–	–
Acremonium biseptum	CBS 750.69 [T]	HQ231998	–	–	–	–
Acremonium borodinense	CBS 101148 [T]	HQ232003	–	–	–	–
Acremonium brachypenium	CBS 866.73 [T]	HQ232004	–	–	–	–
Acremonium breve	CBS 150.62 [T]	HQ232005	–	–	–	–
Acremonium camptosporum	CBS 677.74	HQ232007	–	–	–	–
	CBS 756.69 [T]	HQ232008	–	–	–	–
Acremonium cerealis	CBS 207.65	HQ232013	–	–	–	–
Acremonium charticola	CBS 117.25 [T]	HQ232016	–	–	–	–
Acremonium citrinum	CBS 384.96 [T]	HF680217	HF680236	–	–	–
	CBS 758.69	HQ232012	HF680222	–	–	–
Acremonium collariferum	CBS 124585	FJ765364	–	–	–	–
	CBS 124586 [T]	FJ765366	–	–	–	–
Acremonium chrysogenum	CBS 144.62 [T]	HQ232017	–	–	–	–
Acremonium curvulum	CBS 430.66 [T]	HQ232026	–	–	–	–
	CBS 229.75	HQ232021	–		–	–
Acremonium exuviarum	UAMH 9995 [T]	HQ232036	AY882946	AY882947	–	–
Acremonium flavum	CBS 596.70 [T]	HQ232037	–	–	–	–
Acremonium fuci	CBS 113889	HQ232038	AY632652	–	–	–
	CBS 112868 [T]	–	AY632653	AY632690	–	–
Acremonium furcatum	CBS 122.42 [T]	EF543831	AY378154	–	–	–
Acremonium fusidioides	CBS 705.86	HF680218	HF680237	–	–	–
	CBS 840.68 [T]	HQ232039	FN706542	–	–	–
	UTHSC 08-1455	HF680216	HF680235	–	–	–
Acremonium gamsii	CBS 726.71 [T]	HQ232040	–	–	–	–
Acremonium guillematii	CBS 766.69 [T]	HQ232042	–	–	–	–
Acremonium hennebertii	CBS 768.69 [T]	HQ232044	HF680238	–	–	–
"Acremonium hyalinulum"	CBS 271.36	HQ232045	–	–	–	–
Acremonium incrustatum	CBS 159.70 [T]	HQ232049	–	–	–	–
Acremonium inflatum	CBS 212.69 [T]	HQ232050	–	–	–	–
Acremonium minutisporum	CBS 147.62 [T]	HQ232061	–	–	–	–
	det267B*	HQ232062	–	–	–	–
Acremonium nepalense	CBS 971.72 [T]	HQ231970	DQ825971	–	–	–

Table 2 (continued)

Species	Strain [a]	GenBank accession number [b]				
		LSU	ITS	*BT2*	*TEF1-α*	*Rpb2*
Acremonium nigrosclerotium	CBS 154.72 [T]	HQ232069	–	–	–	–
Acremonium parvum	CBS 381.70A [T]	HQ231986	HF680219	–	–	–
	CBS 831.97	HQ231989	–	–	–	–
Acremonium persicinum	CBS 310.59 [T]	HQ232077	–	–	–	–
Acremonium pilosum	CBS 124.70 [T]	HF680209	HF680228	–	–	–
	CBS 125.70	HF680210	HF680229	–	–	–
	CBS 511.82	HF680207	HF680226	–	–	–
Acremonium pinkertoniae	CBS 157.70 [T]	HQ232089	–	–	–	–
Acremonium potronii	CBS 379.70F	–	AY632655	AY632691	–	–
Acremonium pseudozeylanicum	CBS 560.73 [T]	HQ232101	–	–	–	–
Acremonium pteridii	CBS 782.69 [T]	HQ232102	–	–	–	–
Acremonium radiatum	CBS 142.62 [T]	HQ232104	–	–	–	–
Acremonium recifei	CBS 137.35 [T]	HQ232106	–	–	–	–
	CBS 362.76	HQ232108	–	–	–	–
Acremonium restrictum	CBS 178.40 [T]	HQ232119	–	–	–	–
Acremonium roseolum	CBS 289.62 [T]	HQ232123	–	–	–	–
Acremonium rutilum	CBS 396.66 [T]	HQ232124	–	–	–	–
Acremonium salmoneum	CBS 721.71 [T]	HQ232125	–	–	–	–
	JS-NJ01 *	–	HM747162	–	–	–
Acremonium sclerotigenum	CBS 124.42 [T]	HQ232126	–	–	–	–
Acremonium sordidulum	CBS 385.73 [T]	HQ232136	–	–	–	–
Acremonium spinosum	CBS 136.33 [T]	HQ232137	–	–	–	–
Acremonium tectonae	CBS 725.87 [T]	HQ232144	–	–	–	–
Acremonium thermophilum	CBS 734.71 [T]	HQ232145	–	–	–	–
Acremonium tubakii	CBS 790.69 [T]	HQ232148	–	–	–	–
	CBS 111360	–	AY632654	AY632689	–	–
Acremonium verruculosum	CBS 989.69 [T]	HQ232150	–	–	–	–
Acremonium vitellinum	CBS 792.69 [T]	HQ232151	–	–	–	–
Acremonium zonatum	CBS 565.67	HQ232155	–	–	–	–
Acremonium sp.	A104 *	–	–	–	–	–
	A105 *	–	KC987142	KC987104	KC998964	KC999002
	A106 *	–	KC987143	KC987105	KC998965	KC999003
	A107 *	–	KC987144	KC987106	KC998966	KC999004
	A108 *	–	KC987145	KC987107	KC998967	KC999005
	A110 *	–	KC987147	KC987109	KC998969	KC999007
	A111 *	–	KC987148	KC987110	KC998970	KC999008

Table 2 (continued)

Species	Strain [a]	GenBank accession number [b]				
		LSU	ITS	*BT2*	*TEF1-α*	*Rpb2*
Acremonium sp.	E102*	–	KC987172	KC987134	KC998994	KC999030
	FMR 11780	KJ807179	–	–	–	–
Acrostalagmus annulatus	DAOM 212126	GU180646	GU180632	–	–	GU180662
Acrostalagmus luteoalbus	CBS 194.87	EF543826	–	–	–	–
Bionectria aureofulvella	CBS 195.93	–	–	AF358181		
Bionectria oblongispora	CBS 100285 [T]	–	AF358248	AF358169		
Bionectria samuelsii	CBS 699.97 [T]	–	AF358236	AF358190		
Emericellopsis alkalina	A124 *	–	KC987161	KC987123	KC998983	KC999020
	A118 *	–	KC987155	KC987117	KC998977	KC999014
	CBS 127350 [T]	KC987247	KC987171	KC987133	KC998993	KC999029
	CBS 120049	KC987246	KC987170	KC987132	KC998992	KC999028
	CBS 120043	–	KC987168	KC987130	KC998990	KC999026
	CBS 120044	–	KC987169	KC987131	KC998991	KC999027
Emericellopsis donezkii	CBS 489.71 [T]	–	AY632658	AY632674		
Emericellopsis glabra	CBS 119.40 [T]	–	AY632657	AY632673	–	–
Emericellopsis humicola	CBS 180.56 [T]	–	AY632659	AY632675	–	–
Emericellopsis maritima	CBS 491.71 [T]	–	AY632670	AY632686	FJ238393	KC999033
Emericellopsis microspora	CBS 380.62 [T]		AY632663	AY632679		
Emericellopsis minima	CBS 190.55 [T]	–	AY632669	AY632685	KC998995	KC999031
	CBS 871.68	–	AY632660	AY632676	KC998996	KC999032
	CBS 111361	–	AY632661	AY632677	–	–
Emericellopsis mirabilis	CBS 177.53	–	AY632656	–	–	–
Emericellopsis pallida	CBS 490.71 [T]		KC987176	KC987138	KC998998	KC999034
	CBS 624.73	–	AY632667	AY632683		
Emericellopsis robusta	CBS 489.73		AY632664	AY632680		
Emericellopsis salmosynnemata	CBS 382.62	–	AY632666	AY632682		
Emericellopsis stolkiae	CBS 159.71 [T]	–	AY632668	AY632684		
Emericellopsis synnematicola	CBS 176.60 [T]	–	AY632665	AY632681		
Emericellopsis terricola	CBS 120.40 [T]	U57082	U57676	–	–	–
	CBS 229.59	–	AY632662	AY632678		
	CCF 3815	–	FJ430737	–	–	–
	NRRL 54109	–	HQ698592	–	–	
Gibellulopsis piscis	CBS 892.70 [T]	EF543835	DQ825985	–		–
Gibellulopsis nigrescens	CBS 101221	EF543840	EF543848	–	EF543797	–
Gliocladium ciboti	CBS 109240 [T]	EF543842	DQ825980	–	–	–
Gliomastix polychromum	CBS 181.27 [T]	HQ232091	–			
Gliomastix roseogrisea	CBS 134.56 [T]	HQ232121	–			

Table 2 (continued)

Species	Strain [a]	GenBank accession number [b]				
		LSU	ITS	*BT2*	*TEF1-α*	*Rpb2*
Leucosphaerina arxii	CBS 737.84 [T]	HQ232159	–			
Linkosia fusiformis	HKUCC 10824	DQ408571				
Musicillium theobromae	CBS 385.32	EF543836	–	–	–	–
	CBS 458.51	EF543837	EF543858	–	–	–
	CBS 968.72 [T]	EF543838	EF543859	–	–	–
Niesslia exilis	CBS 357.70	AY489718				
Phialemonium atrogriseum	CBS 604.67 [T]	HQ231981				
Plectosphaerella cucumerina	CBS 137.37 [T]	JF780520	JF780522	–	–	–
	DAOM 226828	GU180647	GU180630	–	–	GU180663
	Plect 170 *	HQ239032	HQ238991	–	–	–
Sarocladium bacillisporum	CBS 425.67 [T]	HE608658				
Sarocladium bactrocephalum	CBS 749.69 [T]	HQ231994	–	–	–	
Sarocladium glaucum	CBS 796.69 [T]	HE608657				
Sarocladium kiliense	CBS 122.29 [T]	HQ232052				
Sarocladium ochraceum	CBS 428.67 [T]	HQ232070				
Sarocladium oryzae	CBS 180.74 [T]	HQ232166				
Sarocladium strictum	CBS 346.70 [T]	HQ232141				
Sarocladium terricola	CBS 243.59 [T]	HQ232046				
Sarocladium zeae	CBS 801.69 [T]	HQ232152				
Selinia pulchra	AR 2812 *		HM484859	HM484884	HM484841	
Sodiomyces alkalinus	CBS 110278 [T]	JX158427	JX158405	–	JX158383	JX158449
	F12 *	JX158428	JX158406	–	JX158384	JX158450
Stanjemonium grisellum	CBS 655.79 [T]	–	AY632671	AY632687		
Stanjemonium ochroroseum	CBS 656.79 [T]	–	AY632672	AY632688		
Stilbella fimetaria	DAOM 229279	HQ232176				
	D99026 *	–	AY952467	–	–	–
	MH178 *	–	FJ430712	–	–	–
	SES201 *	–	FJ939394	–	–	–
Verrucostoma freycinetiae	MAFF 240100		HM484866	HM484885	HM484853	
Verticillium albo-atrum	CBS 130.51 [T]	HQ231976	DQ825977	–	–	–
Verticillium dahliae	AFTOL-ID 237	DQ470945	–	–	–	–
	ATCC 16535	U17425	–	–	AY489632	DQ522468

[a] AFTOL, Assembling the Fungal Tree of Life project; ATCC, American Type Culture Collection, Manassas, Virginia, USA; CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; CCF, Culture Collection of Fungi, Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic; DAOM, Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; FMR, Faculty of Medicine Reus, Spain; HKUCC, Hong Kong University Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, US Department of Agriculture, Peoria, Illinois, USA; MAFF, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan; UAMH, University of Alberta Microfungus Collection and Herbarium; Edmonton, Canada; UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas, USA; [T] Type strain; * from GenBank. [b] LSU large subunit of the nrDNA; ITS internal transcribed spacer regions of the nrDNA and intervening 5.8S nrDNA; *BT2* beta-tubulin gene; *TEF1*-α transcription elongation factor 1-alpha; *Rpb2* RNA polymerase II second largest subunit.

4.1.4. Phylogeny of *Sarocladium* (*Hypocreales*)

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Phylogeny of *Sarocladium* (*Hypocreales*)

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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 *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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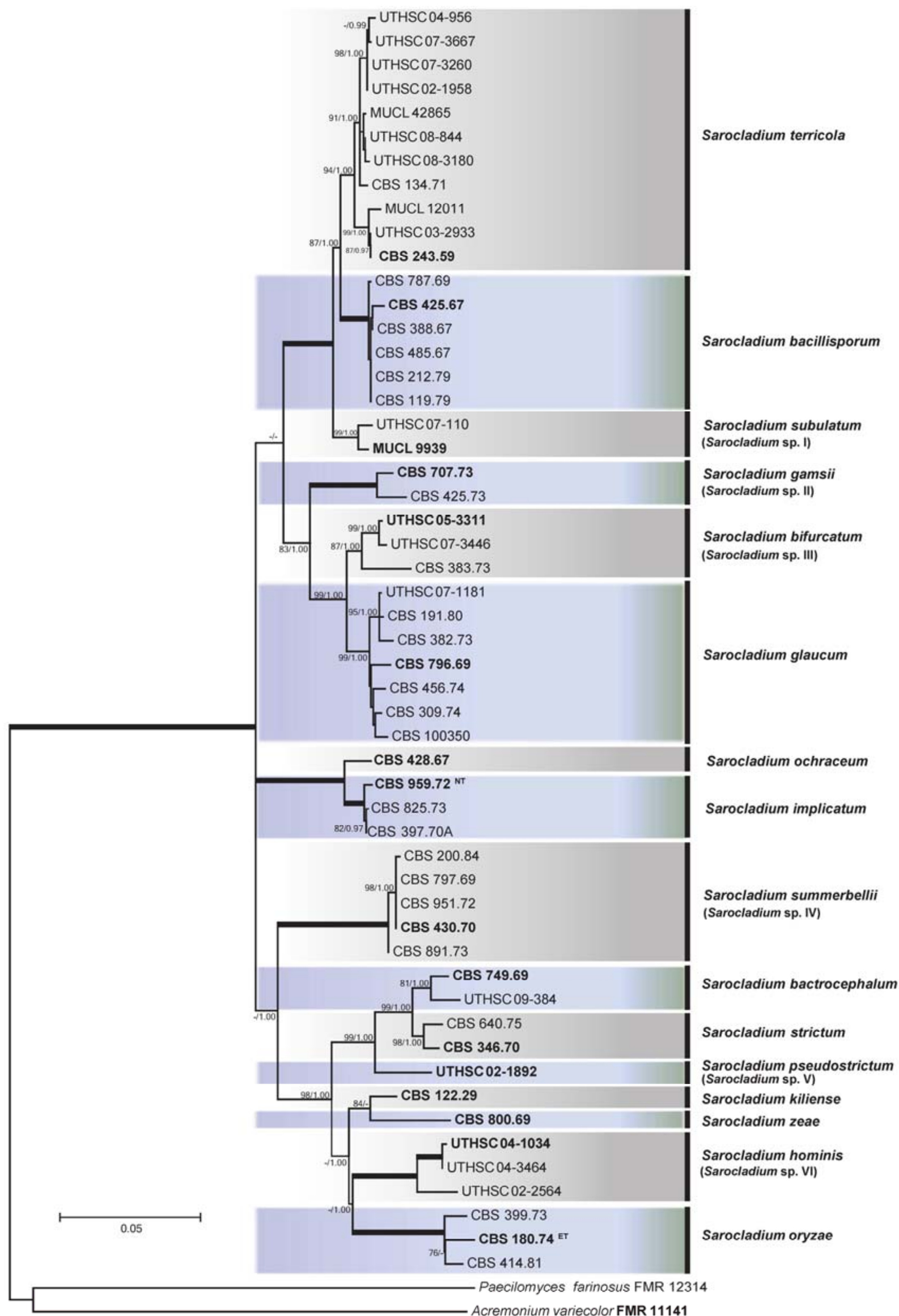

Fig. 1 Maximum-likelihood (ML) tree obtained from the combined DNA sequence data from three loci (D1/D2, ITS and *ACT1*). Bootstrap support values above 70 % / Bayesian posterior probability values above 0.95, are shown at the nodes (BS/PP). Branches supported by BS = 100 % and PP = 1.00 are depicted 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**

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	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.

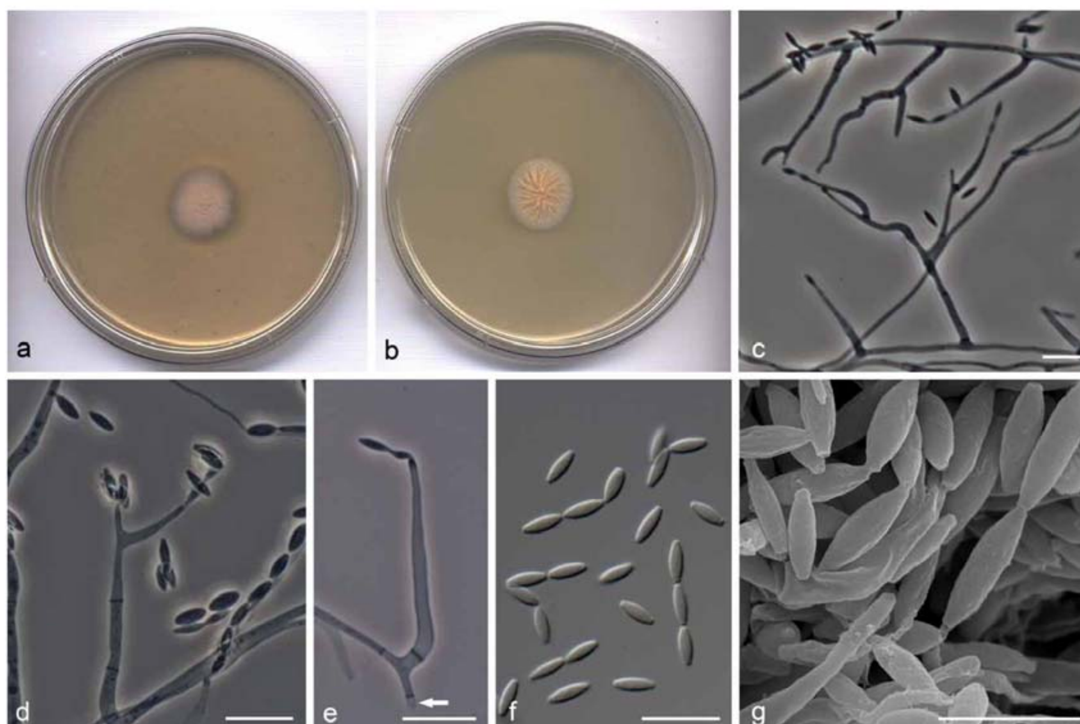

Fig. 2 *Sarocladium bifurcatum* (sp. III) UTHSC 05-3311. a, b. Colonies on OA and PDA, respectively, after 14 d at 25 °C; c. simple and branched conidiophores; d. schizophialides; e. phialide with periclinal thickening at the apex producing conidia in chains and adelophialide (arrow); f, g. fusiform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

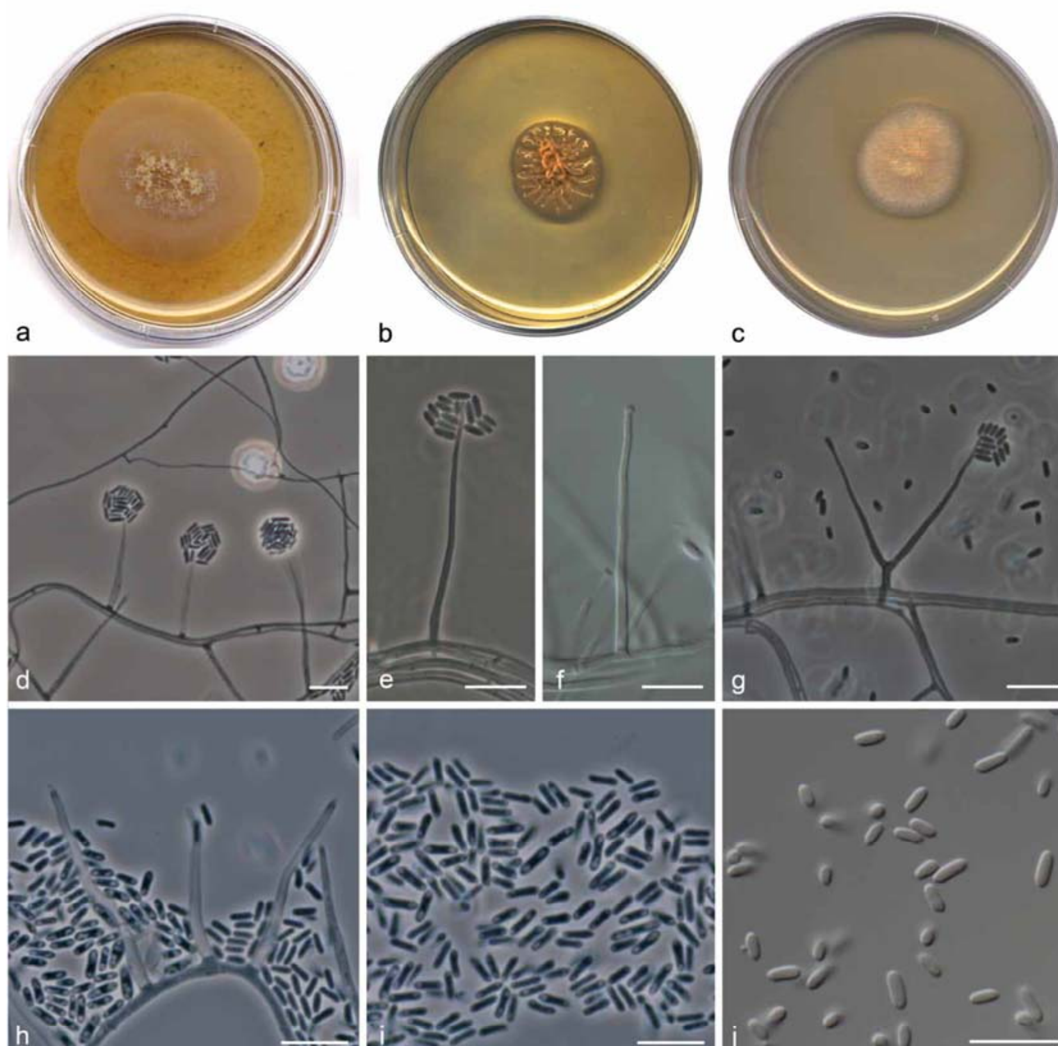

Fig. 4 *Sarocladium hominis* (sp. VI). a, c. UTHSC 04-1034; b, d, h, i. UTHSC 04-3464; e–g, j. UTHSC 02-2564. a. Colonies on OA after 14 d at 25 °C; b, c. colonies on PDA after 14 d at 25 °C; d–g. simple and branched conidiophores with conidia arranged in slimy heads; h. phialides with periclinal thickening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

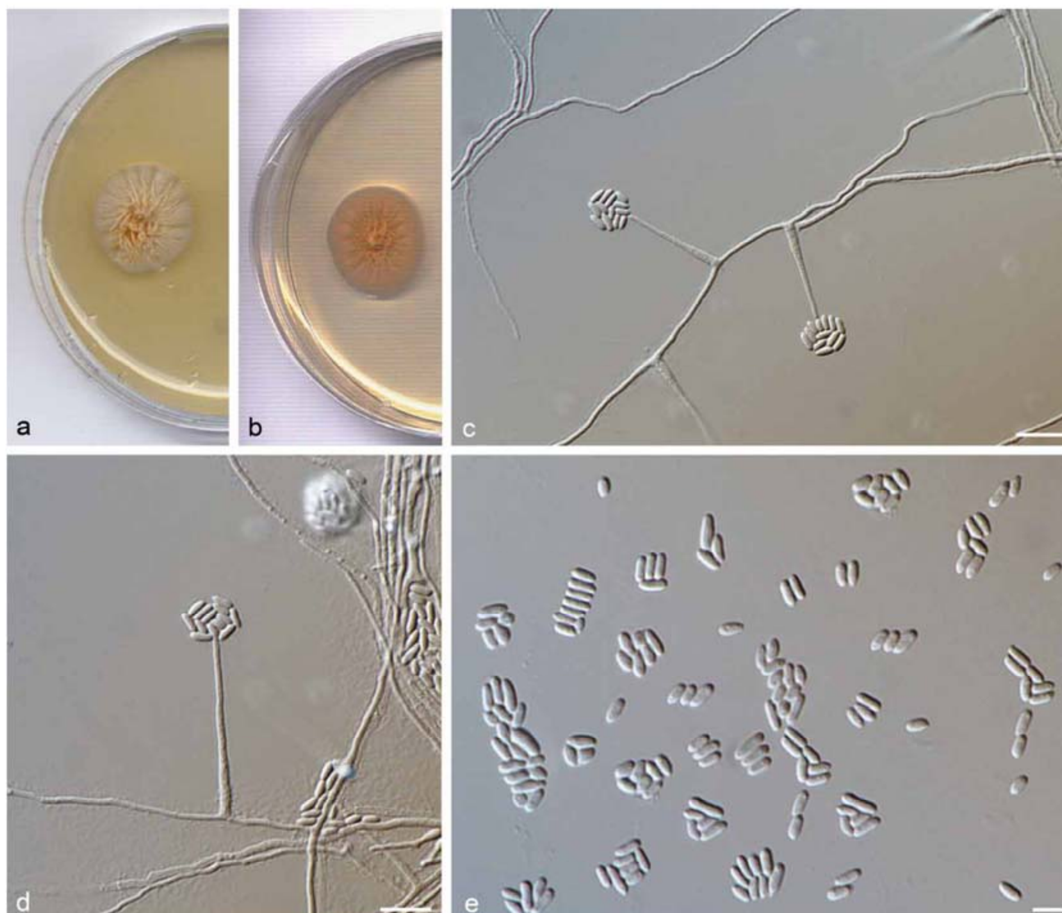

Fig. 5 *Sarocladium pseudostrictum* (sp. V) UTHSC 02-1892. a, b. Colonies on PDA after 14 d at 25 °C; c. acicular phialides arising laterally on the vegetative hyphae; d. conidia grouped in slimy heads; e. conidia. — Scale bars = 10 μm.

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.

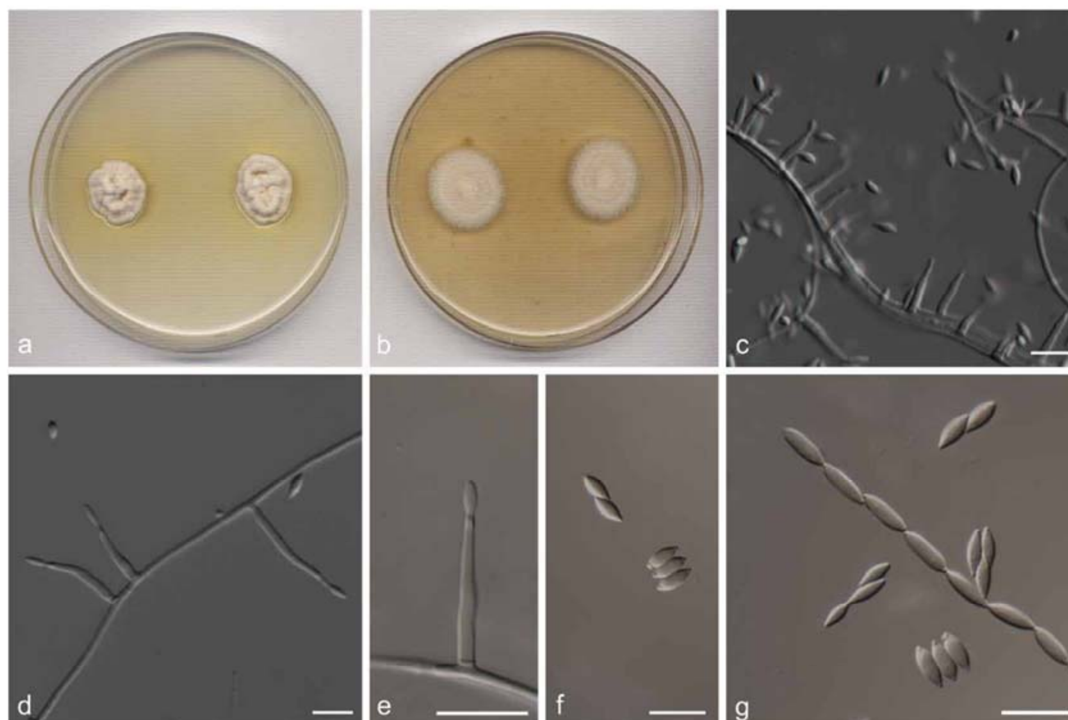

Fig. 6 *Sarocladium subulatum* (sp. I) UTHSC 07-110. a, b. Colonies on PDA and OA, respectively, after 14 d at 25 °C; c, d. phialides arising directly from ropes of hyphae or on vegetative hyphae; e. phialide with periclinal thickening at the apex; f, g. conidia. — Scale bars = 10 µm.

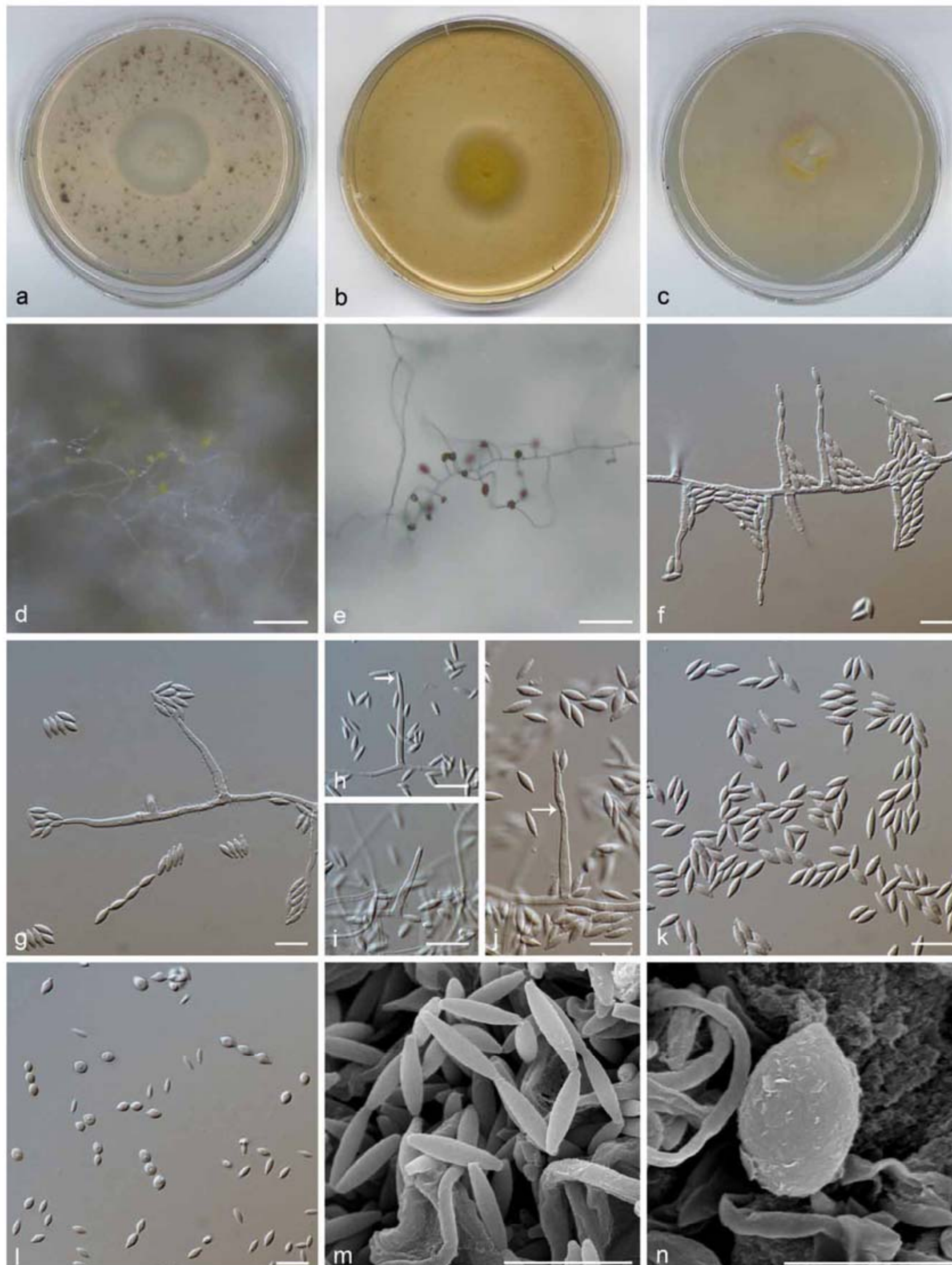

Fig. 7 *Sarocladium summerbellii* (sp. IV). a, b, d–g. CBS 891.73; c, j–n. CBS 430.70; h, i. CBS 951.72. a. Colonies on PDA after 14 d at 25 °C; b, c. colonies on OA after 14 d at 25 °C; d, e. pigmented conidia collapsing in heads on PDA; f. phialide bearing conidia in chains; g. lateral and terminal 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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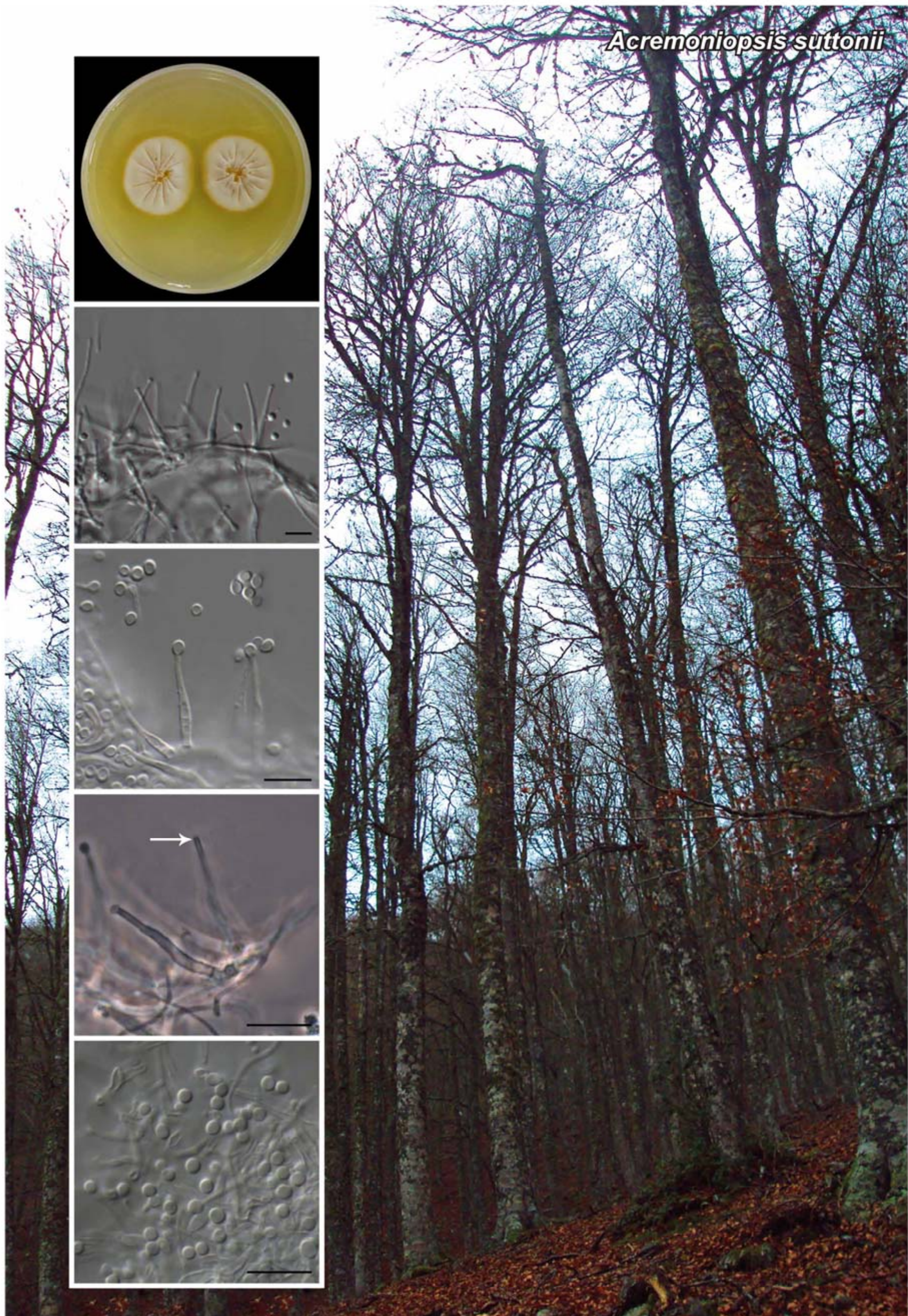
4.1.5. *Acremoniopsis suttonii* gen. nov. et sp. nov.

Giraldo A, Gené J, Guarro J.

Fungal Planet 308, Persoonia 2014; 33: 264–265


Acremoniopsis suttonii

Fungal Planet 308 – 1 December 2014

Acremoniopsis Giraldo, Gené & Guarro, *gen. nov.*

Etymology. Referring to the similarity with the genus *Acremonium*.

Mycelium consisting of branched, septate, smooth-walled hyphae. *Conidiophores* erect, simple or poorly branched. *Conidiogenous cells* enteroblastic, monophialidic, discrete, cylindrical to subulate, subhyaline. *Conidia* unicellular, globose or subglobose, hyaline, arranged in slimy heads.

Type species. Acremoniopsis suttonii.
MycoBank MB809883.

Acremoniopsis suttonii Giraldo, Gené & Guarro, *sp. nov.*

Etymology. Named in honour of the American mycologist Deanna A. Sutton.

Mycelium consisting of septate, hyaline, smooth- and thin-walled hyphae, 1–1.5 µm wide. *Conidiophores* consisting of single phialides arising orthotropically and directly from vegetative hyphae or ropes of hyphae. *Phialides* cylindrical to subulate, (12–)16–30(–40) µm long, 1.5–2 µm wide at the base, with distinct periclinal thickening at the conidiogenous locus, hyaline, thin-walled and rugose towards the base. *Conidia* unicellular, globose or subglobose, 2–3 × 2 µm, hyaline, smooth- and thick-walled, in slimy heads. Chlamydospores and sexual morph not observed.

Culture characteristics — Colonies on OA at 25 °C attaining 8–9 mm diam after 2 wk, yellowish white (2A2) (Kornerup & Wanscher 1978), flat, membranous; reverse pastel yellow (1A4). On PDA at 25 °C reaching 15–21 mm diam after 2 wk, white (1A1), radially folded, dusty; reverse pastel yellow (2A4); exudate and diffusible pale yellow pigment (2A5). Optimum growth temperature 25 °C, minimum 12 °C, maximum 35 °C.

Typus. SPAIN, Burgos, natural area of Sierra de la Demanda, isolated from forest soil, Nov. 2010, coll. *J. Gené & M. Hernández*, isol. *A. Giraldo* (holotype CBS H-21936, cultures ex-type CBS 138708 = FMR 11780; ITS sequence GenBank KJ807182, LSU sequence GenBank KJ807179, MycoBank MB809884).

Notes — *Acremonium* is a polyphyletic genus with species spread across different orders of the *Sordariomycetes*, but are mainly placed in the *Hypocreales* (Glenn et al. 1996, Perdomo et al. 2011, Summerbell et al. 2011, Giraldo et al. 2012). *Acremonium altematum*, the type species of the genus, was recently epitypified with the strain CBS 407.66, which was placed in the family *Bionectriaceae* (Summerbell et al. 2011). In *Hypocreales*, there are still some traditional species of *Acremonium* phylogenetically distant from *A. alternatum* and *Bionectriaceae* that could represent novel genera. Although *Acremoniopsis* shows the typical *Acremonium* morphology, it is phylogenetically closer to nectriaceous species such as *Pleonectria pyrrhochlora* and *P. virens*, rather than bionectriaceous species, so we preferred to accommodate the species described here within a new genus. *Acremoniopsis suttonii* produces a diffusible pale yellow pigment similar to *Acremonium citrinum*, *A. vitellinum*, *A. chrysogenum* and *A. flavum.* The main difference between the first two species and *Acremoniopsis suttonii* is that they produce conidia arranged in chains (Gams 1971, Giraldo et al. 2014). Additionally, *A. chrysogenum* has colonies with a yeast-like appearance and ellipsoidal conidia, and *A. flavum* produces ellipsoidal conidia, abundant chlamydospores and is thermotolerant. *Acremonium guillematii* exhibits yellow colonies, but does not produce diffusible pigment into the agar (Gams 1971). Previous phylogenetic studies have demonstrated that *Acremonium citrinum*, *A. chrysogenum* and *A. flavum* are members of *Bionectriaceae* (Summerbell et al. 2011, Giraldo et al. 2014) while *A. vitellinum* and *A. guillematii* form a weakly supported clade near to the *Clavicipitaceae* (Summerbell et al. 2011). *Acremonium pteridii* produces similar subglobose conidia to those of *A. suttonii*, but shows chondroid hyphae, abundant crystals and partially branched conidiophores (Gams 1971) nested in the *Gliomastix*/*Bionectria* clade (Summerbell et al. 2011).

Based on a megablast search of NCBIs GenBank nucleotide database, the closest hits using the LSU sequence are *Parasarcopodium ceratocary* CBS 110664 (GenBank AY425026; Identities = 772/796 (97 %), Gaps = 1/796 (0 %)), *Pleonectria pyrrhochlora* CBS 125131 (GenBank HM484570; Identities = 774/799 (97 %), Gaps = 3/799 (0 %)), *P. virens* A.R. 4558 (GenBank JF832754; Identities = 770/795 (97 %), Gaps = 3/795 (0 %)) and '*Acremonium persicinum*' CBS 110646 (GenBank HQ232088; Identities = 773/800 (97 %), Gaps = 4/800 (0 %)). *Parasarcopodium ceratocary* (incertae sedis, *Hypocreales*) has verruculose conidiophores with rows or whorls of phialides and cylindrical conidia with amorphous mucoid appendages at both ends (Mel'nik et al. 2004); while *Pleonectria* species (*Nectriaceae*, *Hypocreales*) produce a pycnidial asexual morph (zythiostroma-like) on the natural substratum, with verticillated conidiophores, intercalary phialides and ellipsoidal conidia; and sporodochial conidiophores, densely branched with cylindrical phialides and allantoid conidia in culture (Hirooka et al. 2012).

Colour illustrations. Forest from the natural area of Sierra de la Demanda (Burgos, Spain), where the soil sample was collected. Colony on PDA after 21 d at 25 °C, phialides and globose or subglobose conidia. Scale bars = 10 µm.

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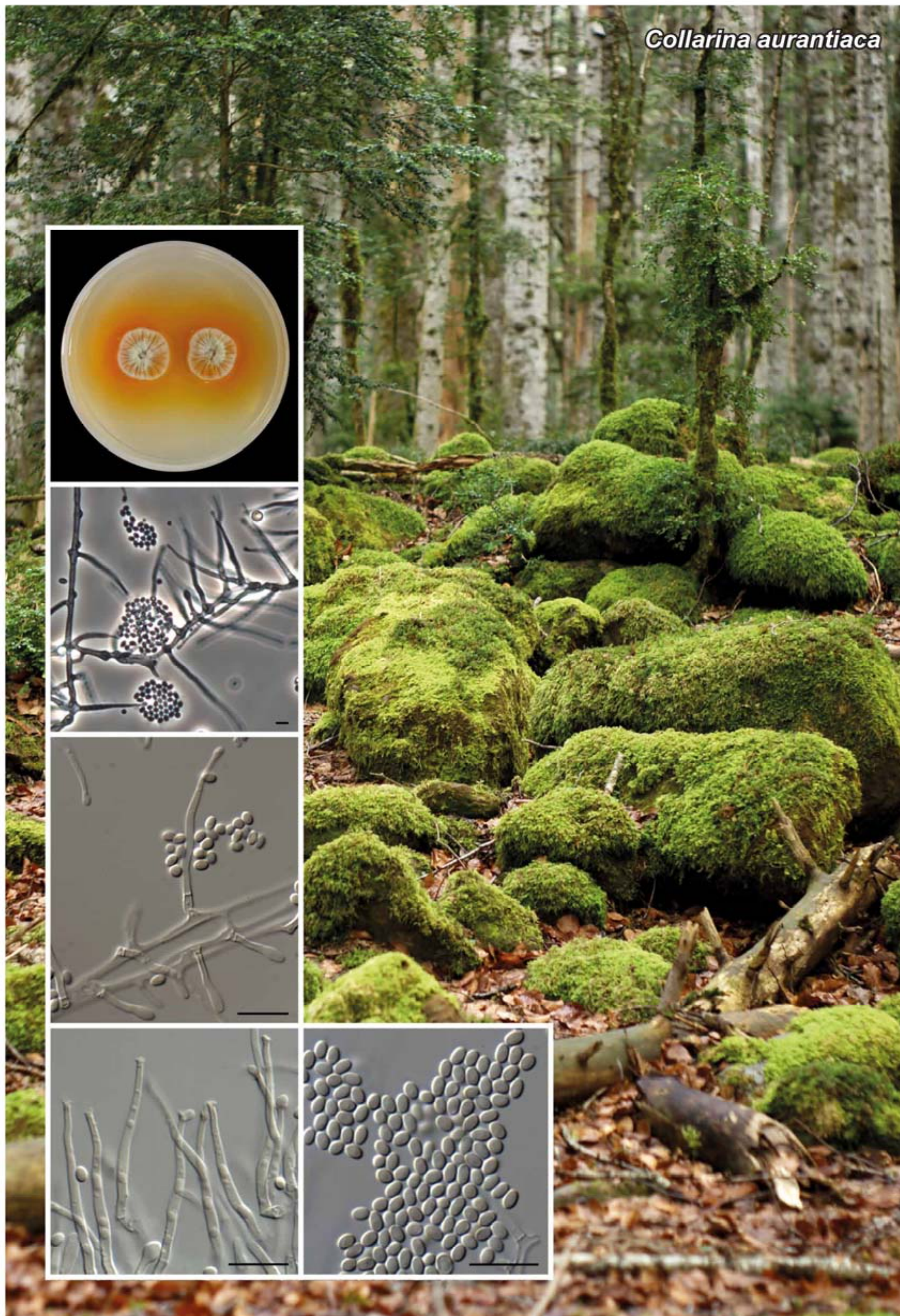
4.1.6. *Collarina aurantiaca* gen. nov. et sp. nov.

Giraldo A, Gené J, Guarro J.

Fungal Planet 311, Persoonia 2014; 33: 270–271

UNIVERSITAT ROVIRA I VIRGILI
TAXONOMIC STUDY OF CLINICAL AND ENVIRONMENTAL ISOLATES OF ARTHROCONIDIAL, ACREMONIUM-LIKE AND OCHROCONIS-LIKE FUNGI
Dixie Alejandra Giraldo López
Dipòsit Legal: T 767-2015


Collarina aurantiaca

Fungal Planet 311 – 1 December 2014

Collarina Giraldo, Gené & Guarro, *gen. nov.*

Etymology. Referring to the presence of conspicuous collarettes.

Mycelium consisting of branched, septate, smooth-walled hyphae. *Setae* interspersed with conidiophores, erect, unbranched, septate at the base, swollen at the apex, hyaline. *Conidiophores* erect, simple or poorly branched. *Conidiogenous cells* enteroblastic, monophialidic, discrete, cylindrical to acicular, with conspicuous collarettes, subhyaline or pale brown. *Conidia* unicellular, ellipsoidal or subglobose, hyaline to brownish in mass, arranged in slimy heads.

Type species. Collarina aurantiaca.
MycoBank MB809407.

Collarina aurantiaca Giraldo, Gené & Guarro, *sp. nov.*

Etymology. Referring to the diffusible orange pigment produced on PDA medium.

Mycelium consisting of septate, hyaline, smooth- and thin-walled hyphae, 1.5–2 µm wide. *Setae* arising directly from vegetative hyphae and interspersed with conidiophores, erect, unbranched, with a basal septum, straight to slightly flexuose, cylindrical and thick-walled towards the base, swollen and thin-walled at the apex, up to 20 µm long, 2 µm wide at the base, 3–4 µm at the apex, hyaline, smooth-walled. *Conidiophores* erect, simple or poorly branched, up to 40 µm long, hyaline or pale brown, smooth-walled. *Phialides* arising directly from vegetative hyphae or ropes of hyphae, cylindrical, slightly tapering at the apex, straight or slightly bent, 10–40 × 1–1.5 µm, with a brownish funnel-shaped collarette, subhyaline to pale brown with age, thick- and smooth-walled. *Conidia* ellipsoidal or subglobose, 2.5–3(–4) × 1–2 µm, hyaline to brownish in mass, smooth- and thin-walled. Chlamydospores and sexual morph not observed.

Culture characteristics — Colonies on OA and PCA at 25 °C attaining 8–14 mm diam in 14 d, brownish grey (6E2) (Kornerup & Wanscher 1978), flat, dusty. On PDA at 25 °C reaching 14–18 mm diam in 14 d, greyish white (1B1), radially folded, felt-like or fasciculate, reverse brownish grey (6D3) with a diffusible orange pigment. Optimum temperature for growth 25 °C, minimum 12 °C, maximum 30 °C.

Typus. Spain, Aragón, Huesca province, Ordesa y Monte Perdido National Park, isolated from sediments of Ara River, 23 Mar. 2011, coll. *A. Giraldo, M. Hernández & J. Capilla*, isol. *A. Giraldo* (holotype CBS H-21781, cultures ex-type CBS 138274 = FMR 11784; ITS sequence GenBank KJ807180, LSU sequence GenBank KJ807177, MycoBank MB809408).

Additional specimen examined. Spain, Aragón, Huesca province, Torla to direction Bujaruelos, from forest soil, 19 June 2009, coll. *M. Hernández, J. Mena-Portales, J. Cano*, isol. *A. Giraldo* (CBS 138273 = FMR 11134; ITS sequence GenBank KJ807181, LSU sequence GenBank KJ807178).

Colour illustrations. Forest from Ordesa y Monte Perdido National Park (Aragón, Spain), where the sample was collected (photo: Javier Capilla). Colony on PDA after 21 d at 25 °C, conidiophores simple with conidia arranged in slimy heads, phialides with brownish funnel-shaped collarettes and setae, ellipsoidal conidia. Scale bars = 10 µm.

Notes — The SSU sequence of *Collarina aurantiaca* revealed that it belongs to the *Clavicipitaceae* s.str. (*Hypocreales*, *Sordariomycetes*), with *Chamaeleomyces viridis*, *C. granulomatis*, *Pochonia bulbillosa*, *P. rubescens* and *Nomuraea rileyi* being the closest species. *Chamaeleomyces* differs from *Collarina* by pale green to greenish grey colonies, and a yeast-like growth, phialides basally swollen with narrow necks and conidia in fragile chains; *Pochonia* has yellowish white colonies, slender acicular phialides commonly arranged in whorls, and some species produce dictyochlamydospores and conidia in chains; *N. rileyi* has pale green slow-growing colonies, cylindrical phialides with short necks and greenish coloured conidia (Zare et al. 2001, Sung et al. 2007, Sigler et al. 2010). Although members of *Clavicipitaceae* s.str. have been reported as important entomopathogens of *Lepidoptera*, *Homoptera* and *Coleoptera*, they are common soil fungi.

Based on a megablast search of NCBIs GenBank nucleotide database, the closest hits using the LSU sequence are *Cordyceps* sp. (GenBank AB027378, Identities = 975/1029 (95 %), Gaps = 12/1029 (1 %)), *Eucasphaeria capensis* CBS 120027 (GenBank EF110619, Identities = 973/1029 (95 %), Gaps = 5/1029 (0 %)) and *Ascopolyporus philodendrus* (GenBank AY886545, Identities = 977/1037 (94 %), Gaps = 14/1037 (1 %)). The closest hits using the ITS sequence had the highest similarity to '*Acremonium psammosporum*' H28 (GenBank GU566287, Identities = 593/593 (100 %), no gaps) and with an unidentified hypocrealean fungus (GenBank KC007264, Identities = 544/550 (99 %), no gaps). *Acremonium psammosporum* was described by Gams (1971) and is characterised by slow-growing colonies with an orange-ochraceous reverse; conidiophores sometimes branched, up to 50 µm long, straight phialides with short collarettes; subglobose conidia, slightly apiculated at base, hyaline, 1.8–3.3 × 1.2–1.6 µm. *Collarina aurantiaca* differs morphologically from *A. psammosporum* by the presence of setae, conidiophores simple, shorter phialides (up to 40 µm), funnel-shaped collarettes and bigger and brownish conidia. In addition, the LSU sequence of the type strain of *A. psammosporum* (CBS 590.63) was 6.9 % different.

Collarina resembles *Monocillium* (asexual morphs of *Niesslia*) with the presence of thickened walls at the base in both phialides and setae. However, *Monocillium* species produce fast-growing colonies, phialides without collarettes and hyaline conidia that can be elongated and septate in several species (Gams 1971, Gams & Turham 1996, Girlanda & Luppi-Mosca 1998, Ramaley 2001).

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4.2. Studies on arthroconidial fungi

Final identification and taxonomic placement of the arthroconidial fungi included in this thesis are shown in Table 4. Thirty-eight of the isolates are from clinical samples received from the Fungus Testing Laboratory (UTHSC), San Antonio TX, US. The remaining isolates are reference strains from different international culture collections or fresh isolates obtained in a previous study carried out in our group (Gené 1994).

Table 4 Arthroconidial fungi from soil or clinical samples identified in this thesis.

Species[a]	**Taxonomy** (Family, Order)	**FMR**[b]	**Origin**[c]	**Section**
Arthrographis alba (*Leucothecium emdenii*)	*Incertae sedis, Onygenales*	4030	Env.	4.2.3
Arthrographis arxii	*Eremomycetaceae, incertae sedis*	5262	Env.	4.2.3
Arthrographis chlamydospora	*Eremomycetaceae, incertae sedis*	12129	Clin.	4.2.1, 4.2.3
Arthrographis curvata	*Eremomycetaceae, incertae sedis*	4032	Env.	4.2.1, 4.2.3
		12125	Clin.	4.2.1, 4.2.3
Arthrographis globosa	*Eremomycetaceae, incertae sedis*	12124	Clin.	4.2.1, 4.2.3
Arthrographis kalrae	*Eremomycetaceae, incertae sedis*	4034	Env.	
		4036	Env.	
		12094	Clin.	4.2.1, 4.2.2, 4.2.3
		12097	Clin.	4.2.1, 4.2.2, 4.2.3
		12098	Clin.	4.2.1, 4.2.2, 4.2.3
		12099	Clin.	4.2.1, 4.2.2, 4.2.3
		12102	Clin.	4.2.1, 4.2.2, 4.2.3
		12103	Clin.	4.2.1, 4.2.2, 4.2.3
		12105	Clin.	4.2.1, 4.2.2, 4.2.3
		12108	Clin.	4.2.1, 4.2.2, 4.2.3
		12109	Clin.	4.2.1, 4.2.2, 4.2.3
		12110	Clin.	4.2.1, 4.2.2, 4.2.3
		12111	Clin.	4.2.1, 4.2.2, 4.2.3
		12112	Clin.	4.2.1, 4.2.2, 4.2.3
		12114	Clin.	4.2.1, 4.2.2, 4.2.3
		12115	Clin.	4.2.1, 4.2.2, 4.2.3
		12116	Clin.	4.2.1, 4.2.2, 4.2.3
		12118	Clin.	4.2.1, 4.2.2, 4.2.3
		12119	Clin.	4.2.1, 4.2.2, 4.2.3
		12120	Clin.	4.2.1, 4.2.2, 4.2.3
		12121	Clin.	4.2.1, 4.2.2, 4.2.3
		12122	Clin.	4.2.1, 4.2.2, 4.2.3
		12123	Clin.	4.2.1, 4.2.2, 4.2.3
		12126	Clin.	4.2.1, 4.2.2, 4.2.3
Arthrographis lignicola	*Incertae sedis, Lecanoromycetes*	(CBS 689.83)	Env.	4.2.3
Arthrographis longispora	*Eremomycetaceae, incertae sedis*	12101	Clin.	4.2.1, 4.2.3
Arthrographis pinicola	*Eremascaceae, Onygenales*	(CBS 653.89)	Env.	4.2.3
Arthropsis cirrhata	*Incertae sedis, Onygenales*	(CBS 628.83)	Env.	4.2.3
Arthropsis hispanica	*Incertae sedis, Onygenales*	12093	Clin.	4.2.1
		12095	Clin.	4.2.1
		12113	Clin.	4.2.1

Table 4 (continued)

Species[a]	**Taxonomy** (Family, Order)	**FMR**[b]	**Origin**[c]	**Section**
Arthropsis hispanica	*Incertae sedis, Onygenales*	12117	Clin.	4.2.1, 4.2.3
Arthropsis microsperma	*Incertae sedis, Helotiales*	(UAMH 4290)	Env.	4.2.3
Arthropsis truncata	*Incertae sedis, Sordariomycetes*	CBS 584.82	Env.	4.2.3
Bjerkandera adusta	*Meruliaceae, Polyporales*	12107	Clin.	4.2.1
Eremomyces bilateralis	*Eremomycetaceae, incertae sedis*	CBS 781.70	Env.	4.2.3
Geotrichum sp.	*Dipodascaceae, Saccharomycetales*	12100	Clin.	4.2.1
		12106	Clin.	4.2.1
Rhexothecium globosum	*Eremomycetaceae, incertae sedis*	(CBS 955.73)	Env.	4.2.3
Scytalidium cuboideum	*Incertae sedis, Helotiales*	12127	Clin.	4.2.1
		12128	Clin.	4.2.1
		12130	Clin.	4.2.1
		12131	Clin.	4.2.1
		12132	Clin.	4.2.1

[a] New taxa proposed from our study are shown in bold face.

[b] Isolates from other collections (CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; UAMH, University of Alberta Microfungus Collection and Herbarium, Edmonton, Canada).

[c] Clin.: Clinical; Env.: Environmental.

Publications derived from the study on arthroconidial fungi are shown in sections 4.2.1–4.2.3 of the thesis.

4.2.1. Rare arthroconidial fungi in clinical samples: *Scytalidium cuboideum* and *Arthropsis hispanica*.

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UNIVERSITAT ROVIRA I VIRGILI
TAXONOMIC STUDY OF CLINICAL AND ENVIRONMENTAL ISOLATES OF ARTHROCONIDIAL, ACREMONIUM-LIKE AND OCHROCONIS-LIKE FUNGI
Dixie Alejandra Giraldo López
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Rare Arthroconidial Fungi in Clinical Samples: *Scytalidium cuboideum* and *Arthropsis hispanica*

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Abstract We report the presence of the two arthroconidial anamorphic fungi, *Scytalidium cuboideum* and *Arthropsis hispanica,* in clinical samples from the USA. Both fungi were morphologically and molecularly identified. The antifungal susceptibility of four isolates of *A. hispanica* and five of *S. cuboideum* to eight antifungal drugs is provided.

Keywords *Arthropsis hispanica* · DNA analysis · Clinical specimens · Morphological identification · *Scytalidium cuboideum*

Introduction

In the last few decades, the dramatic increase of immunocompromised patients has seen a parallel increase in the incidence of opportunist fungal infections [1]. Apart from the classic opportunists, *Aspergillus, Candida* and *Cryptococcus*, many other fungi traditionally considered saprobes have also been reported as etiologic agents in human diseases. In the last edition of the Atlas of Clinical Fungi, the number of fungal species of clinical interest was reported to be approximately 500 [2]. Additionally, in the international culture collections and in sequence databases, the entries corresponding to fungal isolates from clinical samples continue to grow. Although it has not been demonstrated that all these isolates are etiologic agents of the infection, the fact that some are being isolated repeatedly suggests their potential pathological role. The characterization of these fungi and the deposit of DNA sequences in public databases are important in order to increase the knowledge of the opportunistic fungi and to allow their rapid identification if, in the future, they result in human mycoses. The current use of molecular tools in numerous clinical microbiology laboratories allows the detection of some rare or cryptic species not easily identified if only the traditional phenotypic methods are used. At the same time, such studies have demonstrated that some important pathogens, traditionally considered a single species, are actually complexes of species with varying degrees of pathogenicity and antifungal responses, for example, *Aspergillus fumigatus* [3, 4], *Candida parapsilosis* [5], *Sporothrix schenckii* [7, 8] or *Fusarium* spp. [9–12] among many others.

Recently, we have characterized some interesting arthroconidial fungi from the United States of America, both phenotypically and at the molecular level,

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and here describe two of them, isolated on several occasions, but until now not reported from clinical specimens.

Materials and Methods

Fungal Isolates

Included in this study were a total of 38 isolates of arthroconidial fungi from different clinical origins submitted to the Fungus Testing Laboratory at the University of Texas Health Science Center (UTHSC) at San Antonio (Texas, USA) for identification and/or antifungal susceptibility, as well as type strains of *Arthrographis kalrae*, *Arthropsis hispanica* and *Scytalidium cuboideum* (Table 1).

Morphological Study

The morphological features of the isolates were examined on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) and oatmeal agar (OA; 30 g filtered oat flakes, 20 g agar, 1 l distilled water). Cultures were incubated at 25 and 37 °C for 2 weeks. The isolates were identified using the criteria given by Sigler and Carmichael [13, 14] and Ulfig et al. [15]. Microscopic morphological features were examined by making direct wet mounts with 85 % lactic acid or lactophenol cotton blue, using light microscopy.

Sequencing and Phylogenetic Analysis

Isolates were grown on yeast extract sucrose (YES; yeast extract, 2 %; sucrose, 15 %; agar, 2 %; 1 l water) for 7–14 days at 25 °C, and DNA was extracted using PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA), according to the manufacturer's protocol. The DNA was quantified using GeneQuant *pro* (Amersham Pharmacia Biotech, Cambridge, United Kingdom). The internal transcribed spacer (ITS) regions and D1/D2 domains of the 28S rDNA were amplified with the primer pairs ITS5/ITS4 and NL1/NL4, respectively [6, 16]. PCR products were purified and sequenced at Macrogen Europe (Amsterdam, The Netherlands) with a 3730XL DNA analyzer (Applied Biosystems). The program SeqMan (version 7.0.0; DNASTAR, Madison, WI, USA) was used to obtain consensus sequences of each isolate. BLAST sequence identity searches were carried out to compare data of the isolates with those of other fungi deposited in the NITE Biological Resource Center (NBRC) and GenBank databases. The sequences of clinical isolates were aligned using the Clustal X (version 1.8) computer program [17] with default parameters, followed by manual adjustments with a text editor and finally were compared

Table 1 Strains included in the study

Species	Strains	Source	Location	GenBank accession n°	
				ITS	D1/D2
Arthropsis hispanica	UTHSC 01-2199	Bronchial wash	USA		
	UTHSC 02-1022	Sputum	USA		
	UTHSC 08-2158	Nails	USA		
	UTHSC 09-3174	Bronchial wash	USA	**HE965756**	**HE965757**
	CBS 351.92 (Type)	Marine sediments	Spain	**HE965758**	**HE965759**
Arthrographis kalrae	CBS 693.77 (Type)	Sputum	India	AB116533	AB116541.1
Scytalidium cuboideum	UTHSC 03-2193	Bronchial wash	USA		
	UTHSC 05-1821	Bronchial wash	USA		
	UTHSC 09-3329	Nasal sinus	USA	**HE965760**	**HE965761**
	UTHSC 10-2389	Lung mass	USA	**HE965762**	**HE965763**
	UTHSC 11-510	Bronchial wash	USA		
	CBS 241.62 (Type)	Rotten timber	South Africa	GQ272628	AB213435.1

UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX; CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; The accession numbers of sequences generated in this study are indicated in bold

with those of type strains using the GeneDoc (version 2.6) program [18].

Antifungal Susceptibility

The in vitro activity of amphotericin B (AMB), itraconazole (ITC), posaconazole (PSC), voriconazole (VRC), anidulafungin (ANID), caspofungin (CAS), micafungin (MICA) and terbinafine (TRB) was determined according to the methods outlined in the CLSI document M38-A2 [19].

Nucleotide Sequence Accession Numbers

The DNA sequences generated in this study were deposited in GenBank (Table 1).

Results

Morphological and Molecular Analysis

Four of the 38 strains included in this study were morphologically identified as *Arthrographis* spp., twenty-two as *Arthrographis kalrae,* two as *Geotrichum* spp., one as *Bjerkandera adusta,* five as *S. cuboideum* and four as *A. hispanica*. Due to the rarity of the last two species and their lack of having ever been reported in clinical samples, the morphological identification was confirmed by sequencing the ITS region and the D1/D2 domains of the rDNA, and their sequences were compared with those of the ex type strains of both species.

With respect to the five strains of *S. cuboideum,* we were able to amplify and sequence 527–534 bp and 545–558 bp of the ITS regions and the D1/D2 domains, respectively. The ITS and D1/D2 sequences of the five strains showed a 99–100 % and 99.4–100 % of similarity, respectively. The ITS sequences from these five isolates showed a homology of 97.7–98.5 % with the type strain of *S. cuboideum* (CBS 241.62). One insertion 19 bp long located between position 314 and 334 of the D1/D2 domain of the type strain of *S. cuboideum* was detected and removed before analysis. The D1/D2 sequences from the five isolates showed a similarity of 99.4–99.9 % with the type strain of *S. cuboideum*.

The size of the sequences of *A. hispanica* was from 598 to 603 bp for the ITS regions and from 544 to 550 bp for the D1/D2 domains. The sequences of the isolates were practically identical between strains and also with the type strain of *A. hispanica* (CBS 351.92), showing similarities of 99.7–100 % for the ITS region and 100 % for the D1/D2 domains.

Description of the Species

Scytalidium cuboideum (Sacc. and Ellis) Sigler and Kang (Fig. 1)

Colonies on PDA at 25 °C attaining 80–90 mm diameter after 7 days in the dark, yellowish to brownish orange, flat to slightly folded, velvety at slightly cottony. On OA at 25 °C reaching 30–40 mm diameter after 7 days in the dark, yellowish granulose at center and cottony toward the periphery. At 37 °C, the colonies attaining 25–30 mm diameter after 7 days in the dark. *Conidiophores* undifferentiated. *Conidiogenous hyphae* produced mainly in tufts, hyaline, smooth- and thin-walled, 2–5 μm wide, usually unbranched, initially sparingly septate and then with septa at regular intervals in more or less basipetal succession to form arthroconidia released by schizolytic secession. *Arthroconidia* unicellular, hyaline to yellow, thick- and smooth-walled, cylindrical or cuboid with truncated ends, often broader than long, 1.5–2.5 × 2–3.5 μm. *Type strain*: CBS 241.62 (ex type cultures as UAMH 3101, KACC 41223, IFM 52649).

Arthropsis hispanica Gené, Ulfig and Guarro (Fig. 2)

Colonies on PDA at 25 °C attaining 17–23 mm diameter after 7 days in the dark. At first, white and floccose, becoming yellowish, powdery to granulose. At 25 °C, on OA attaining 16–18 mm diameter after 7 days in the dark, yellowish, flat, granulose. There is no growth at 37 °C. *Conidiophores* undifferentiated. *Conidiogenous hyphae* produced mainly in tufts, hyaline, smooth- and thin-walled, main hyphae straight to flexuose, 2–4 μm wide, lateral branches arising at right angles, often recurved, septating basipetally to form arthroconidia joined by narrow connectives (0.2–0.8 μm diameter) and released by rhexolytic secession. *Arthroconidia* unicellular, hyaline to subhyaline, thin- and smooth-walled, cylindrical or barrel-shaped, often broader than long, straight or curved, 1.5–7 (–10) × 2–3 (–4) μm. *Type strain*: CBS 351.92 (ex type culture as FMR 4058).

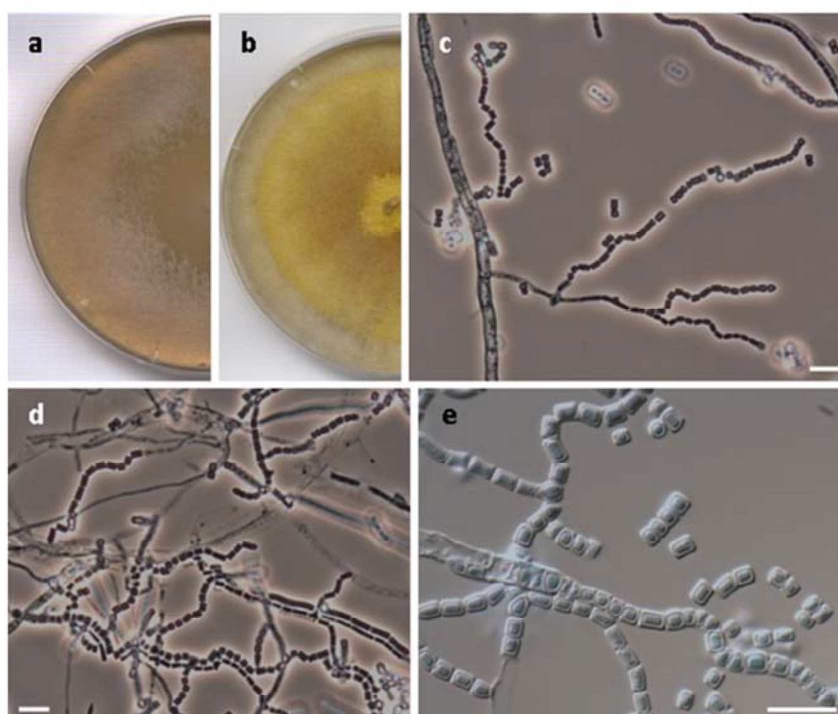

Fig. 1 *Scytalidium cuboideum* UTHSC 05-1821 (**a**), UTHSC 03-2193 (**b–e**). **a**, **b** Colonies on PDA at 25 °C after 10 days. **c** Arthroconidia from scarcely differentiated conidiophores. **d** Conidiogenous hyphae fragmenting schizolytically. **e** Cylindrical and cuboid with both truncated ends arthroconidia. *Bars* **c–e** = 10 µm

Antifungal Susceptibility

The results of the in vitro activity of the antifungal drugs tested are shown in Table 2. *S. cuboideum* was resistant to the three echinocandins and terbinafine; however, the other drugs showed generally good activity with posaconazole displaying the lowest MICs. In general, all the drugs tested showed good activity against *A. hispanica* although itraconazole, caspofungin and amphotericin B showed variable strain-dependent activity.

Discussion

The genus *Scytalidium* was created by Pesante (1957), based on *S. lignicola*, to accommodate dematiaceous arthroconidial fungi. Currently, the genus comprises more than 20 species usually saprobic on different substrates, although some of them, such as *S. japonicum* and *S. infestans*, have been reported occasionally producing infections in animals [2, 20, 21]. *Scytalidium dimidiatum* is a human opportunistic fungus causing mainly onychomycosis and superficial infections, and less frequently mycetomata, subcutaneous lesions and deep-seated infections in immunocompromised patients [22–24]. Crous et al. [25] on the basis of DNA sequences analysis, demonstrated that *S. lignicola*, the type species of *Scytalidium*, and *S. dimidiatum* belonged to different orders, that is, Leotiomycetes (Helotiales) and Dothideomycetes (Botryosphaeriales), respectively, the latter species being transferred to the genus *Neoscytalidium*.

Scytalidium cuboideum was described by Saccardo and Ellis [26] as *Oospora cuboidea*, being later transferred to *Arthrographis* by Sigler and Carmichael [13]. Recent phylogenetic analyses, using SSU, ITS and *RPB2* sequences revealed that *Scytalidium lignicola* and *Arthrographis cuboidea* grouped together in a strongly supported clade, while the type strain of the genus *Arthrographis*, *A. kalrae* grouped with members of the Pezizales and far from that clade. Accordingly, the new combination *S. cuboideum* was proposed [27].

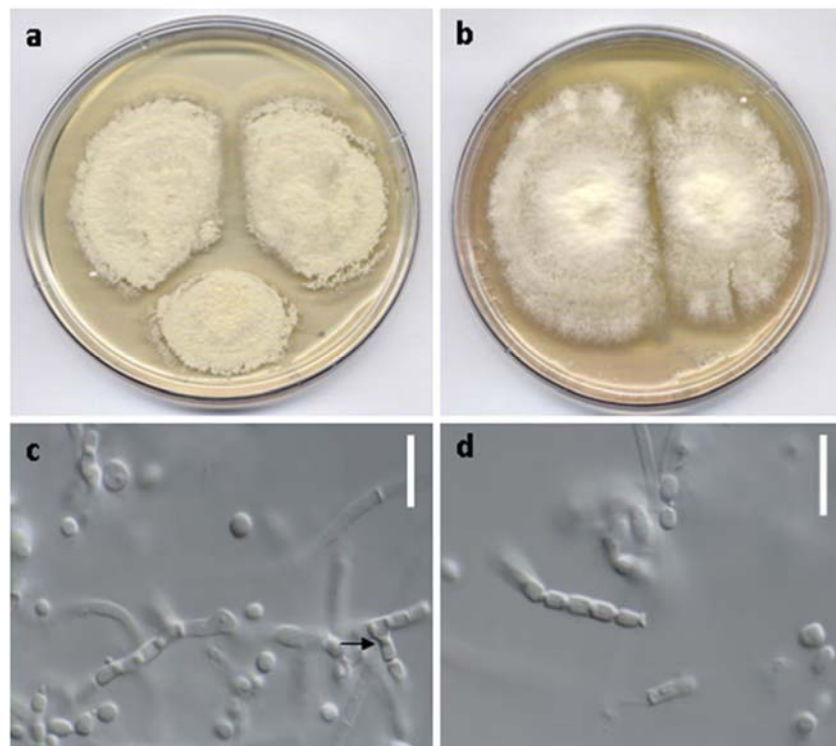

Fig. 2 *Arthropsis hispanica* UTHSC 01-2199 (**a**, **c**, **d**), CBS 351.92 (**b**). **a**, **b** Colonies on PDA at 25 °C after 21 days. **c** Conidiogenous hyphae fragmenting rhexolytically (*arrow*). **d** Arthroconidia with narrow connectives. *Bars* **c**–**d** = 10 µm

Table 2 Results of the in vitro antifungal susceptibility testing

Species	Strains	MIC (µg/ml)							
		AMB	ANID	CAS	MICA	ITC	PSC	VRC	TRB
Arthropsis hispanica	UTHSC 01-2199	0.25	0.06	1	0.03	0.5	0.5	0.125	0.125
	UTHSC 02-1022	1	0.125	1	0.06	1	0.5	0.5	0.03
	UTHSC 08-2158	1	0.03	1	0.03	1	0.5	0.25	0.03
	UTHSC 09-3174	0.5	0.03	0.5	0.06	4	1	0.5	0.06
Scytalidium cuboideum	UTHSC 03-2193	1	8	4	>8	0.5	0.25	0.5	8
	UTHSC 05-1821	0.5	8	8	8	0.5	0.125	0.25	8
	UTHSC 09-3329	1	8	2	8	1	0.5	1	4
	UTHSC 10-2389	1	4	4	>8	0.25	0.25	0.5	8
	UTHSC 11-510	0.5	8	8	8	0.25	0.25	0.5	8

UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX

AMB amphotericin B, *ANID* anidulafungin, *CAS* caspofungin, *MICA* micafungin, *ITC* itraconazole, *PSC* posaconazole, *VRC* voriconazole, *TRB* terbinafine

S. cuboideum is morphologically differentiated from the other species of the genus by the production of yellowish to brownish orange colonies, conidiogenous hyphae mainly produced in tufts, and yellow cuboid arthroconidia usually broader than long and a strong cellulolytic activity. *S. cuboideum* is a soft rot fungus

associated with pink- or blue-stained wood including oak [13, 14], and to date, not related to any clinical samples. In this study, this species was represented by five isolates from different samples of respiratory origin (bronchial wash, nasal sinus and lung mass). Although it was not proven that this fungus produced any type of infection, its repeated isolation and its ability to grow at 37 °C could indicate its potential to infect humans, as do other species of the genus, or at least to colonize the human respiratory tract.

The anamorphic genus *Arthropsis,* of the order Onygenales, was described by Sigler et al. [28] to include species with dark arthroconidia, joined by adjacent connectives and developed from undifferentiated conidiogenous hyphae. The genus includes four species, that is, *A. truncata* as the type species, *A. microsperma, A. cirrhata* and *A. hispanica,* which so far have not been associated with any teleomorph. These species are usually found on vegetable material or marine sediments [14, 15, 28, 29]. In the present study, we have identified four isolates of *A. hispanica* from respiratory samples and nails. We can expect that the clinical relevance of this species is limited since it does not grow at 37 °C in vitro. Morphologically, *A. hispanica* can be differentiated from the other species of the genus by its relatively fast growing colonies and hyaline to subhyaline, variably sized arthroconidia. The genus *Arthropsis* shows some morphological similarities with the genus *Arthrographis;* however, the former can be differentiated by its relatively fast growth, the granulose aspect of the colonies, undifferentiated conidiophores and the presence of connectives between the arthroconidia [14, 15, 28, 29]. Our comparison of the ITS and D1/D2 sequences of the type strains of *A. hispanica* and *A. kalrae* shows a low similarity in both regions (59 and 80.4 %, respectively), which confirms the long phylogenetic distance between both genera.

To our knowledge, this is the first report of the presence of *S. cuboideum* and *A. hispanica* in clinical samples. These types of studies with the morphological and molecular characterization of unusual species found in clinical samples and the deposit of their sequences in public databases are useful for comparison of future isolates in a clinical setting.

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4.2.2. *In vitro* antifungal susceptibility of clinical isolates of *Arthrographis kalrae*, a poorly known opportunistic fungus.

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In vitro antifungal susceptibility of clinical isolates of *Arthrographis kalrae*, a poorly known opportunistic fungus

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Summary

The *in vitro* antifungal activity of amphotericin B (AMB), itraconazole, voriconazole, posaconazole, terbinafine (TRB), caspofungin, anidulafungin and micafungin were evaluated by a broth microdilution technique against 22 isolates of *Arthrographis kalrae* of clinical origin. TRB showed the highest activity, followed by the azoles, particularly posaconazole. AMB exerted low activity whereas the echinocandins showed almost no antifungal activity.

Key words: *Arthrographis*, susceptibility testing, antifungal agents, fungal infections, fungi.

Introduction

Arthrographis is an arthroconidial anamorphic genus that comprises four species, *i.e.*, *A. kalrae, A. lignicola, A. pinicola* and *A. alba.* These fungi are commonly found in environmental samples (soil, wood, air and water),[1] but less frequently in clinical samples. It is likely that due to the abundant production of arthroconidia, species in this genus have been misidentified as belonging to other more clinically relevant fungi such as *Geotrichum* or *Trichosporon*. However, since the year 2000, the clinical cases attributed to *Arthrographis* have increased, most likely due to the more common use of molecular techniques for identification. Currently, only *A. kalrae* has been reported as a human opportunistic pathogen, inciting onychomycoses,[2] mycetoma,[3] sinusitis,[4,5] meningitis,[4] cerebral vasculitis,[6] keratomycosis,[7] pulmonary infections[8] and endocarditis,[9] among others. The most appropriate treatment of these infections is not known and there is a paucity of data on the *in vitro* antifungal susceptibility for this fungus. To increase the available knowledge on the susceptibility patterns of this fungal species, we have determined the *in vitro* activity of the main drugs used for the treatment of fungal infections against a set of clinical isolates of *A. kalrae.*

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Material and methods

A total of 22 strains of *A. kalrae* from different clinical sources were tested. The isolates were subcultured onto Potato Dextrose Agar and incubated at 25 °C for 14 days. All the isolates were identified on the basis of morphological characteristics and by sequencing the internal transcribed spacer (ITS) region of the rRNA.

Antifungal susceptibility testing was carried out according to the procedures described in the CLSI document M38-A2 for filamentous fungi.[10] The minimal effective concentration (MEC) was determined at 24 h for the echinocandins, and the minimal inhibitory concentration (MIC) was determined at 48 h for the remaining drugs. The MIC was defined as the lowest concentration exhibiting 100% visual inhibition of growth for amphotericin B (AMB), voriconazole (VRC), itraconazole (ITC) and posaconazole (PSC) and an 80% reduction in growth for terbinafine (TRB).

Results and discussion

The results are summarised in Table 1. AMB showed very little activity against most of the isolates tested with a geometric mean MIC (GM) and MIC_{90} of 2.64

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Table 1 *In vitro* susceptibility results of 22 clinical isolates of *A. kalrae*.

	MIC or MEC (µg ml^{-1})			
Antifungal agent	Range	GM	MIC_{50}	MIC_{90}
AMB	1–4	2.64	2	4
VRC	0.06–1	0.55	0.5	1
PSC	<0.03–0.5	0.28	0.25	0.5
ITC	<0.03–2	0.55	0.5	1
CSF	0.5 to >8	15.29	>8	>8
ANF	<0.015 to >8	14.54	>8	>8
MCF	<0.015 to >8	15.27	>8	>8
TRB	<0.004 to 0.06	0.04	0.03	0.06

AMB, amphotericin B; VRC, voriconazole; PSC, posaconazole; ITC, itraconazole; CSF, caspofungin; ANF, anidulafungin; MCF, micafungin; TRB, terbinafine; GM, geometric mean.

and 4 µg ml^{-1}, respectively. Similarly, the three echinocandins showed almost no activity, with elevated MEC values. In contrast, the azoles showed high *in vitro* activity, with PSC exerting the highest activity, followed by ITC and VRC. TRB demonstrated the lowest MICs against all isolates tested with GM and MIC_{90} values of 0.04 and 0.06 µg ml^{-1}, respectively.

To date, only a few clinical cases of *A. kalrae* infection have included *in vitro* antifungal susceptibility data. In our study, we observed high MICs for AMB, and, although our results showed good *in vitro* activity for the azoles, the results are variable in the clinical setting. VRC and ITC have been the most commonly used drugs against *A. kalrae* infections, although mostly with negative results even when high doses were employed.[7,9] In contrast, a mycetoma case was successfully treated using long-term ITC therapy,[3] whereas a case of panophthalmitis and invasive sinusitis required a combination of ITC, fluconazole and a drastic surgical debridement.[5] There are no reports on the use of echinocandins against *A. kalrae* infections and we observed high rates of *in vitro* resistance to these drugs. As in a previous *in vitro* study,[11] we observed that the highest activity against *A. kalrae* was by TRB. This drug has been used only in a few clinical cases with different outcomes; an onychomycosis case was successfully cured with oral TRB plus miconazole[2] whereas a knee-joint infection appeared to be refractory to TRB monotherapy.[12]

We provide data on the *in vitro* antifungal susceptibility patterns of a reasonable number of clinical isolates of *A. kalrae*, a rare opportunistic pathogen often associated with complicated human infections. Our *in vitro* results showed that TRB appears to be the best therapeutic option, followed by the azoles. Our results seem to discourage the use of echinocandins; however, further clinical data is needed to support these conclusions.

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4.2.3. Phylogenetic circumscription of *Arthrographis* (*Eremomycetaceae, Dothideomycetes*)

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Phylogenetic circumscription of *Arthrographis* (*Eremomycetaceae*, *Dothideomycetes*)

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Key words

arthroconidial fungi
Arthrographis
Arthropsis
Eremomyces
phylogeny
taxonomy

Abstract Numerous members of *Ascomycota* and *Basidiomycota* produce only poorly differentiated arthroconidial asexual morphs in culture. These arthroconidial fungi are grouped in genera where the asexual-sexual connections and their taxonomic circumscription are poorly known. In the present study we explored the phylogenetic relationships of two of these ascomycetous genera, *Arthrographis* and *Arthropsis*. Analysis of D1/D2 sequences of all species of both genera revealed that both are polyphyletic, with species being accommodated in different orders and classes. Because genetic variability was detected among reference strains and fresh isolates resembling the genus *Arthrographis*, we carried out a detailed phenotypic and phylogenetic analysis based on sequence data of the ITS region, actin and chitin synthase genes. Based on these results, four new species are recognised, namely *Arthrographis chlamydospora*, *A. curvata*, *A. globosa* and *A. longispora*. *Arthrographis chlamydospora* is distinguished by its cerebriform colonies, branched conidiophores, cuboid arthroconidia and terminal or intercalary globose to subglobose chlamydospores. *Arthrographis curvata* produced both sexual and asexual morphs, and is characterised by navicular ascospores and dimorphic conidia, namely cylindrical arthroconidia and curved, cashew-nut-shaped conidia formed laterally on vegetative hyphae. *Arthrographis globosa* produced membranous colonies, but is mainly characterised by doliiform to globose arthroconidia. *Arthrographis longispora* also produces membranous colonies, but has poorly differentiated conidiophores and long arthroconidia. Morphological variants are described for *A. kalrae* and our results also revealed that *Eremomyces langeronii* and *A. kalrae*, traditionally considered the sexual and asexual morphs of the same species, are not conspecific.

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INTRODUCTION

The arthroconidial genus *Arthrographis* was proposed by Cochet (1939) with *A. langeronii* as the type species, but it was invalid because it lacked a Latin diagnosis, which was at that time still required as prerequisite by the International Code of Botanical Nomenclature. Sigler & Carmichael (1976) subsequently validated the genus name based on *Oidiodendron kalrae.* In addition to the type species, *A. kalrae*, the genus currently includes three other taxa, *A. alba*, *A. lignicola* and *A. pinicola* (Sigler & Carmichael 1976, 1983, Sigler et al. 1990, Gené et al. 1996). Other species previously included in the genus were *A. cuboidea* and *A. sulphurea*. While the former species was transferred to the genus *Scytalidum* (Kang et al. 2010), *A. sulphurea* was considered a possible synonym of *Pachysolen tannophilus* (*Saccharomycetes*) (von Arx 1985).

Apart from *A. kalrae*, which was traditionally associated with the sexual morph *Eremomyces langeronii*, other ascomycetes have been described with unnamed *Arthrographis* morphs, i.e., *Leucothecium coprophilum*, *L. emdenii* and *Faurelina indica* (von Arx & Samson 1973, von Arx 1978, von Arx et al. 1981, Malloch & Sigler 1988, Valldosera et al. 1991).

Arthrographis species have been isolated from air, compost, marine sediments, soil, wood and, occasionally, from opportunistic infections in humans (de Hoog et al. 2011). Morphologically, they are recognised by a slow growth rate and by the presence of 1-celled, hyaline, smooth-walled, cylindrical arthroconidia released schizolytically from dendritic conidiophores (Sigler & Carmichael 1976). A particular feature of *A. kalrae* is the presence of a trichosporiella-like synasexual morph characterised by solitary, globose to subglobose conidia, which grow laterally and sessile on undifferentiated vegetative hyphae (Sigler & Carmichael 1983). Recently, a phylogenetic study based on sequences analysis of SSU, ITS and *RPB2*, revealed the polyphyly of *Arthrographis* (Kang et al. 2010).

Another arthroconidial genus morphologically similar to *Arthrographis* is *Arthropsis.* The genus comprises four species, i.e., *Arthropsis cirrhata*, *A. hispanica*, *A. microsperma* and *A. truncata* (Sigler et al. 1982, Sigler & Carmichael 1983, van Oorschot & de Hoog 1984, Ulfig et al. 1995). These fungi are usually reported from plant material, but *A. hispanica*, which was only known from marine sediments, has recently been isolated from clinical specimens (Giraldo et al. 2013). *Arthropsis* shows pigmented or non-pigmented arthroconidia, joined by adjacent connectives, released rhexolytically from undifferentiated conidiophores and occasionally has a *Humicola* synasexual morph (Sigler et al. 1982, van Oorschot & de Hoog 1984). Van Oorschot & de Hoog (1984) questioned the distinction between *Arthrographis* and *Arthropsis*, and suggested transferring *Arthrographis* species, excluding the type species *A. kalrae*, to the genus *Arthropsis.* Other authors, however, rejected this proposal (Malloch & Sigler 1988, Sigler et al. 1990).

In the present study we compared the D1/D2 sequences of the available types of *Arthrographis* and *Arthropsis* spp. with those of taxa retrieved from GenBank to clarify their taxonomy, and to determine their phylogenetic relationships. By combining morphological observations with multilocus DNA sequence

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analysis, several novel cryptic species of *Arthrographis* were delineated, which are newly described in this study.

MATERIALS AND METHODS

Isolates

The fungal isolates and DNA sequences included in the study are shown in Table 1. Twenty-six clinical *Arthrographis* isolates were provided by the Fungus Testing Laboratory at the University of Texas Health Science Center (UTHSC), the majority previously identified as *A. kalrae* by Giraldo et al. (2013). Because these isolates varied in morphology and their DNA sequence data, all isolates were re-examined in the present study. The type strains from the new species described here were deposited in the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

Phenotypic studies

Isolates were studied following the criteria of Sigler & Carmichael (1976, 1983) and Ulfig et al. (1995). Morphological features were examined on potato dextrose agar (PDA; Pronadisa, Madrid, Spain), 2 % malt extract agar (MEA; BD Difco™, Franklin Lakes, NJ, USA), potato carrot agar (PCA; potatoes, 20 g; carrot, 20 g; agar, 20 g; distilled water to final volume of 1 000 mL) and 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). Cultures were incubated at 25 °C in the dark for 4 wk. Colony diameters were measured after 14 d of incubation and rated according to the colour charts of Kornerup & Wanscher (1978). Microscopic features were examined and measured in either 85 % lactic acid or lactophenol cotton blue under a light microscope Olympus CH-2 (Olympus Corporation, Tokyo, Japan). Photomicrographs were obtained with a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany), using phase contrast and Nomarski differential interference.

The ability of the fungi to grow at 15, 20, 25, 30, 35, 37, 40, 42 and 45 °C was determined on PDA. To determine the resistance to cycloheximide, isolates were transferred to Petri dishes containing PCA supplemented with chloramphenicol (200 mg/L) and cycloheximide at a final concentration of 2 g/L, and incubated at 25 °C for 2 wk. All tests were performed in duplicate. To evaluate the ability of isolates to convert to the yeast phase, a portion from a fresh culture on PDA was transferred to tubes with Brain Heart Infusion broth (BHI; Becton Dickinson & Company, Franklin Lakes, NJ, USA) and incubated at 37 °C for 2 wk. Subsequently, several transfers to BHI broth were performed.

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 NanoDrop 3000 (ThermoScientific, Asheville, NC, USA). The internal transcribed spacer (ITS) regions and D1/D2 domains of the 28S rDNA were amplified with the primer pairs ITS5/ITS4, NL1/NL4b and LR0R/LR5 (Vilgalys & Hester 1990, White et al. 1990, O'Donnell 1993). A portion of the actin gene (*ACT1*) was amplified using the primer set Act1/Act4 (Voigt & Wöstemeyer 2000) and a chitin synthase gene (*CHS1*) using the primers CHS-79F/CHS-354R (Carbone & Kohn 1999). PCR products were purified and sequenced at Macrogen Europe (Amsterdam, The Netherlands). The program SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA) was used to obtain consensus sequences of each isolate. In addition, numerous D1/D2 sequences, corresponding to different classes, orders and families of ascomycetes retrieved from GenBank or NITE/NRBC databases were included in the phylogenetic study (Table 1). Most of these sequences were published by different authors (Sugiyama et al. 1999, Sugiyama & Mikawa 2001, Untereiner et al. 2002, Reeb et al. 2004, Xi et al. 2004, Murata et al. 2005, Wang et al. 2005, Wedin et al. 2005, Kodsueb et al. 2006, Réblová & Seifert 2007, Tsui et al. 2007, Gueidan et al. 2008, Boehm et al. 2009, Sugiyama et al. 2002, Boonmee et al. 2011, Pettersson et al. 2011, Réblová et al. 2011, Giraldo et al. 2013). The selection of these sequences was based on the results of a BLAST search using the D1/D2 and ITS sequences from each of the ex-type strains of the different species of *Arthrographis* and *Arthropsis*.

Phylogenetic analysis

Sequences were aligned using Clustal X v. 1.8 (Thompson et al. 1997) with default parameters, followed by manual adjustments with a text editor. The phylogenetic relationship between *Arthrographis* and *Arthropsis* species with other genera was determined through the analysis of D1/D2 sequences. Since genetic and also morphological variability was detected among isolates of *Arthrographis*, a multi-locus sequence analysis was carried out to confirm the results obtained from D1/D2 data. This analysis included a fragment of the *ACT1* gene, the *CHS1* gene and the ITS region. Phylogenetic analyses were performed with MEGA v. 5.05 (Tamura et al. 2011), using the Maximum Composite Likelihood (ML). The selection of the best nucleotide substitution model (Tamura-Nei with Gamma distribution) was made using the model selection analysis under MEGA v. 5.05. 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. DNA sequence data were deposited in GenBank (Table 1), the alignment and trees in TreeBASE (http://www.treebase.org) and taxonomic novelties in MycoBank (http://www.MycoBank.org; Crous et al. 2004).

RESULTS

Phylogenetic analyses

The D1/D2 phylogenetic tree that included the ex-type strains of the different species of *Arthrographis* and *Arthropsis* and representative members of different fungal classes and orders revealed that both genera are polyphyletic (Fig. 1). The type species of *Arthrographis*, *A. kalrae*, was included in a well-supported clade within the *Dothideomycetes* (85 % bootstrap, bs), forming together a highly supported subclade (99 % bs) with *Rhexothecium globosum*, *Eremomyces bilateralis*, *E. langeronii* and four unidentified species of *Arthrographis.* While the ex-type strain of *Arthrographis lignicola* was related to different genera of the *Lecanoromycetes*, such as *Sarea* and *Pycnora*, the ex-type strains of *Arthrographis pinicola* and *A. alba* were associated with the *Eurotiomycetes* (99 % bs). *Arthrographis pinicola* and *Eremascus albus* (*Eremascaceae*) formed a well-supported clade (93 % bs), while *A. alba* and *Leucothecium emdenii* formed a well-supported clade (99 % bs) within the *Onygenales*.

The type species of *Arthropsis*, *A. truncata*, clustered with *Porosphaerella borinquensis* and *Coniochaeta velutina* (99 % bs), both members of *Sordariomycetes*. *Arthropsis cirrhata* and *A. hispanica* were accommodated within the *Onygenales*. The only available reference strain of *A. microsperma* grouped with different members of the *Helotiales* (94 % bs).

The multilocus sequence analysis was carried out with the ex-type strains of *Arthrographis kalrae* (CBS 693.77) and *Eremomyces langeronii* (CBS 203.78), 12 isolates identified as *A. kalrae* and five isolates identified as an *Arthrographis* sp. Due

Table 1 Strains included in this study.

Species	Strains[1]	Origin	Previous identification	GenBank accession no.[2]			
				28S rDNA	ITS	*ACT1*	*CHS1*
Acarospora smaragdula	–	Unknown, Sweden	*Acarospora smaragdula*	AY853354	–	–	–
Ajellomyces dermatitidis	ATCC 18187 [T]	Human, unknown	*Ajellomyces dermatitidis*	AY176704	–	–	–
Amauroascus albicans	NRRL 5141 [T]	Soil, Honduras	*Amauroascus albicans*	–	–	–	–
Apinisia graminicola	CBS 156.77	Skin lesion in dog, USA	*Apinisia graminicola*	AB040696	–	–	–
	CBS 721.68 [T]	Grass, United Kingdom		AY176709	–	–	–
Aquaphila albicans	BCC 3520	On wooden test block (*Acacia oblonga*), Thailand	*Aquaphila albicans*	DQ341102	–	–	–
	BCC 3543	On wooden test block (*Dipterocarpus alatus*), Thailand		DQ341101	–	–	–
Arachnomyces minimus	CBS 324.70 [T]	Decayed wood, Canada	*Arachnomyces minimus*	FJ358274	–	–	–
Arachnotheca glomerata	CBS 348.71 [T]	Unknown, Central African Republic	*Arachnotheca glomerata*	AB075352	–	–	–
Arthroderma cajetanum	UAMH 2937	Single ascospore isolate from a gymnothecium on soil, unknown	*Arthroderma cajetanum*	AB075326	–	–	–
Arthroderma ciferrii	CBS 272.66 [T]	Soil, USA	*Arthroderma ciferrii*	AB040681	–	–	–
Arthrographis alba	CBS 370.92 [T]	Marine sediments, Spain	*Arthrographis alba*	**HG004546**	AB213434	–	–
Arthrographis arxii	CBS 203.78 [T]	Dung of herbivore, India	*Eremomyces langeronii*	AB213426	GQ272638	**HG316563**	**HG316582**
Arthrographis chlamydospora	UTHSC 06-1053 [T]	Urine, USA	*Arthrographis* sp. III	**HG004543**	**HG004554**	**HG316560**	**HG316580**
Arthrographis curvata	FMR 4032	Marine sediments, Spain	*Arthrographis* sp. I	**HG004539**	**HG004557**	**HG316557**	**HG316577**
	UTHSC 11-1163 [T]	Nails, USA	*Arthrographis* sp. I	**HG004542**	**HG004556**	**HG316558**	**HG316578**
Arthrographis globosa	UTHSC 11-757 [T]	Bronchial wash, USA	*Arthrographis* sp. IV	**HG004541**	**HG004553**	**HG316561**	**HG316581**
Arthrographis kalrae	CBS 693.77 [T]	Sputum, India	*Arthrographis kalrae*	AB116544	AB116536	**HG316544**	**HG316564**
	UTHSC 01-2742	Artificial pulmonary valve, USA		–	**HG004570**	–	–
	UTHSC 04-2580	Blood, USA		–	**HG004569**	**HG316545**	**HG316565**
	UTHSC 04-3423	Toe nail, USA		–	**HG004568**	**HG316546**	**HG316566**
	UTHSC 05-17	Blood, USA		–	**HG004567**	**HG316547**	**HG316567**
	UTHSC 06-982	Pleural fluid, USA		–	**HG004571**	–	–
	UTHSC 06-3158	Toe nail, USA		–	**HG004572**	–	–
	UTHSC 07-2450	Eye, USA		–	**HG004566**	**HG316548**	**HG316568**
	UTHSC 08-527	Lung tissue, USA		–	**HG004573**	–	–
	UTHSC 08-786	Lung biopsy, USA		–	**HG004565**	**HG316549**	**HG316569**
	UTHSC 08-1699	Bronchial wash, USA		–	**HG004574**	–	–
	UTHSC 08-1804	Nails, USA		–	**HG004564**	**HG316550**	**HG316570**
	UTHSC 08-2107	Leg, USA		–	–	–	–
	UTHSC 08-3547	Sputum , USA		–	**HG004575**	–	–
	UTHSC 09-141	Lung biopsy, USA		–	**HG004563**	**HG316551**	**HG316571**
	UTHSC 09-2903	Bronchial wash, USA		–	**HG004576**	–	–
	UTHSC 10-1652	Cornea, USA		–	**HG004562**	**HG316552**	**HG316572**
	UTHSC 10-1719	Cornea, USA		–	**HG004577**	–	–
	UTHSC 10-2021	Catheter tip, USA		–	**HG004561**	**HG316553**	**HG316573**
	UTHSC 10-2583	Urine, USA		–	**HG004560**	**HG316554**	**HG316574**
	UTHSC 10-2729	Nasal sinus, USA		–	**HG004559**	**HG316555**	**HG316575**
Arthrographis kalrae	UTHSC 11-1256	Bronchial wash, USA		–	**HG004558**	**HG316556**	**HG316576**
	UTHSC 11-302	Eye, USA		–	–	–	–
Arthrographis lignicola	CBS 689.83 [T]	Gymnosperm wood chips and bark, Canada	*Arthrographis lignicola*	**HG004547**	–	–	–
Arthrographis longispora	UTHSC 05-3220 [T]	Foot, USA	*Arthrographis* sp. II	**HG004540**	**HG004555**	**HG316559**	**HG316579**
Arthrographis pinicola	CBS 653.89 [T]	Gallery of *Ips latidens* in *Pinus contorta*, Canada	*Arthrographis pinicola*	**HG004548**	–	–	–
Arthropsis cirrhata	CBS 628.83 [T]	Wall, The Netherlands	*Arthropsis cirrhata*	**HG004549**	–	–	–
Arthropsis hispanica	CBS 351.92 [T]	Bottom of water deposit, Spain	*Arthropsis hispanica*	HE965759	–	–	–
	UTHSC 09-3174	Bronchial wash, USA		HE965757	–	–	–

Arthropsis microsperma	UAMH 4290	Grass, England	*Arthropsis microsperma*	**HG004551**	–	–	–
Arthropsis truncata	CBS 584.82 [T]	Leaf litter, Perú	*Arthropsis truncata*	**HG004550**	–	–	–
Chalara longipes	NBRC 100564	Decaying fir needles, Japan	*Chalara longipes*	–	–	–	–
Chlamydotubeufia huaikangplaensis	–	–	*Chlamydotubeufia huaikangplaensis*	JN865198	–	–	–
Coniochaeta velutina	UAMH 10912	Ex gametophytes of *Hylcomium splendens*, Canada	*Coniochaeta velutina*	EU999180	–	–	–
Ctenomyces serratus	CBS 187.61 [T]	Soil, Australia	*Ctenomyces serratus*	AB040683	–	–	–
Eremascus albus	CBS 975.69	Unknown, USA	*Eremascus albus*	FJ358283	–	–	–
Eremomyces bilateralis	CBS 781.70 [T]	Dung of pack rat, USA	*Eremomyces bilateralis*	**HG004545**	–	**HG316562**	–
Eurotium herbariorum	CBS 516.65	Unpainted board, USA	*Eurotium herbariorum*	JF922029	–	–	–
Faurelina indica	CBS 126.78	Dung of cow, India	*Faurelina indica*	GU180654	–	–	–
	CBS 301.78	Dung of goat, India		GU180653	–	–	–
Geomyces pannorum	UAMH 10473	Ex biofilm on soil, United Kingdom	*Geomyces pannorum*	GU951697	–	–	–
Gymnascella aurantiaca	CBS 655.71 [T]	Clay soil, USA	*Gymnascella aurantiaca*	AB040684	–	–	–
Gymnascella hyalinospora	CBS 548.72 [T]	Dung of Guinea pig, India	*Gymnascella hyalinospora*	AB040687		–	–
Gymnoascoideus petalosporus	UAMH 3593	*Tinea pedis*, human, USA	*Gymnoascoideus petalosporus*	AB359428		–	–
Gymnoascus reesii	CBS 410.72 [T]	Soil, USA	*Gymnoascus reesii*	JF922021	–	–	–
Helicomyces macrofilamentosus	HKUCC 10235	–	*Helicomyces macrofilamentosus*	AY849942	–	–	–
Hyalodendriella betulae	CBS 261.82	*Alnus glutinosa*, The Netherlands	*Hyalodendriella betulae*	EU040232	–	–	–
Hysterographium fraxini	CBS 109.43	Unknown, Switzerland	*Hysterographium fraxini*	FJ161171	–	–	–
	CBS 242.34	Unknown, Canada		FJ161189	–	–	–
Lambertella brunneola	NBRC 6894	*Aucuba japonica*, Japan	*Lambertella brunneola*	–	–	–	–
Leucothecium emdenii	CBS 576.73 [T]	Agricultural soil, The Netherlands	*Leucothecium emdenii*	FJ358286	–	–	–
Malbranchea aurantiaca	CBS 127.77 [T]	Culture contaminant, USA	*Malbranchea aurantiaca*	AB040704	–	–	–
Malbranchea cinnamomea	CBS 960.72	Unknown, France	*Malbranchea cinnamomea*	JF922020	–	–	–
Mallochia reticulata	CBS 392.61 [T]	Rhizosphere of *Musa sapientum*, Honduras	*Mallochia reticulata*	AB075320	–	–	–
Monascus lunisporas	CBS 113675	Soil, Brazil	*Monascus lunisporas*	JF922026	–	–	–
Monascus ruber	FRR 2447 [T]	Soil, India	*Monascus ruber*	JF922025	–	–	–
Onygena corvina	JCM 9546	Decaying bone, Japan	*Onygena corvina*	AB075355	–	–	–
Onygena equina	CBS 947.70	Cow hoof, Germany	*Onygena equina*	AB075356	–	–	–
Ostreichnion curtisii	CBS 198.34	On *Quercus* sp., USA	*Ostreichnion curtisii*	FJ161176	–	–	–
Polytolypa hystricis	UAMH 7299	Ex porcupine dung, Canada	*Polytolypa hystricis*	AY176718	–	–	–
Porosphaerella borinquensis	ICMP 15117	Wood, New Zealand	*Porosphaerella borinquensis*	EF063573	–	–	–
Pseudoarachniotus trochleosporus	CBS 591.71	Soil, USA	*Pseudoarachniotus trochleosporus*	AB075344	–	–	–
Pycnora xanthococca	–	Unknown, Sweden	*Pycnora xanthococca*	AY853388	–	–	–
Rhexothecium globosum	CBS 955.73 [T]	Desert soil, Egypt	*Rhexothecium globosum*	**HG004544**	–	–	–
Rutstroemia cuniculi	NBRC 9671	Dung of rabbit, England	*Rutstroemia cuniculi*	–	–	–	–
Rutstroemia paludosa	NBRC 9672	On *Symplocarpus foetidus*, USA	*Rutstroemia paludosa*	–	–	–	–
Sarcoleotia globosa	–	–	*Sarcoleotia globosa*	AY789409	–	–	–
Sarea resinae	–	–	*Sarea resinae*	AY640965	–	–	–
Scleromitrula shiraiana	NBRC 30255	On *Morus bombycis*, unknown	*Scleromitrula shiraiana*	–	–	–	–
Scytalidium cuboideum	UAMH 7144	Ex lingula specimen, USA	*Scytalidium cuboideum*	AB213427	–	–	–
	UAMH 8435	Bronchial washing, USA		AB213428	–	–	–
Shanorella spirotricha	CBS 304.56	Dung of rabbit, USA	*Shanorella spirotricha*	FJ358288	–	–	–
Stromatinia gladioli	NBRC 7169	–	*Stromatinia gladioli*	–	–	–	–
Trichophyton ajelloi var. *ajelloi*	–	–	*Trichophyton ajelloi* var. *ajelloi*	AB075329	–	–	–

[1] ATCC: American Type Culture Collection, Manassas, VA, USA; BCC: Biotec Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok, Thailand; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; FFR: culture collection of CSIRO, Australia; FMR: Faculty of Medicine Reus, Spain; HKUCC, Hong Kong University Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China; ICMP: International Collection of Microorganisms, Landcare Research, Auckland, New Zealand; JCM: Japanese collection of microorganism; NBRC: NITE Biological Resource Center, Japan; UAMH: University of Alberta Microfungus Collection and Herbarium; Edmonton, Canada; UTHSC: Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX, USA; [T] = Ex-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; *ACT1*: partial actin gene; *CHS1*: chitin synthase gene.

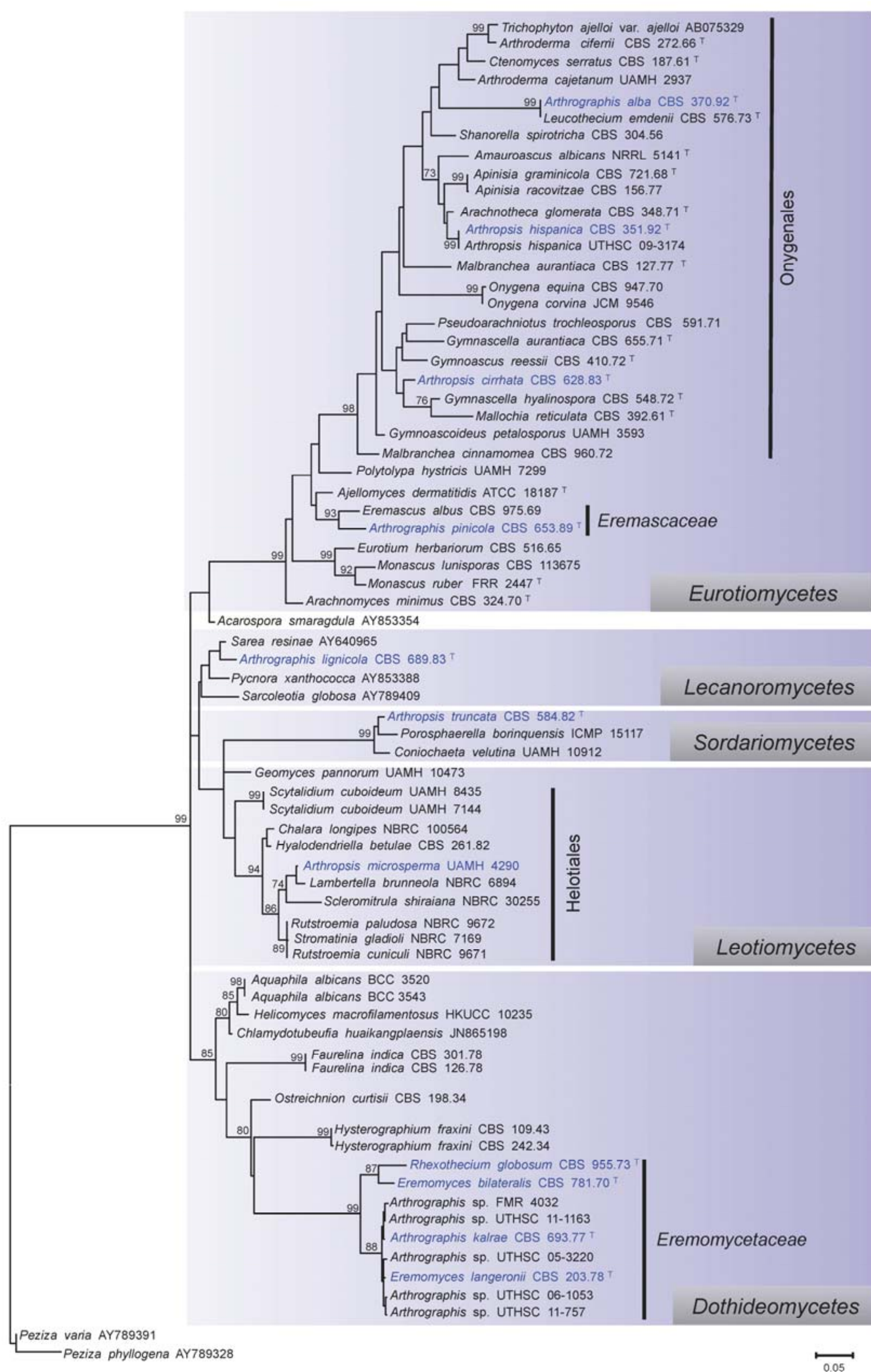

Fig. 1 Maximum-likelihood (ML) tree constructed with sequences of the D1/D2 domains of the 28S rRNA gene. Bootstrap support values above 70 % are indicated at the nodes. The phylogenetic tree was rooted to *Peziza varia* and *Peziza phyllogena*. [T] = Ex-type strain.

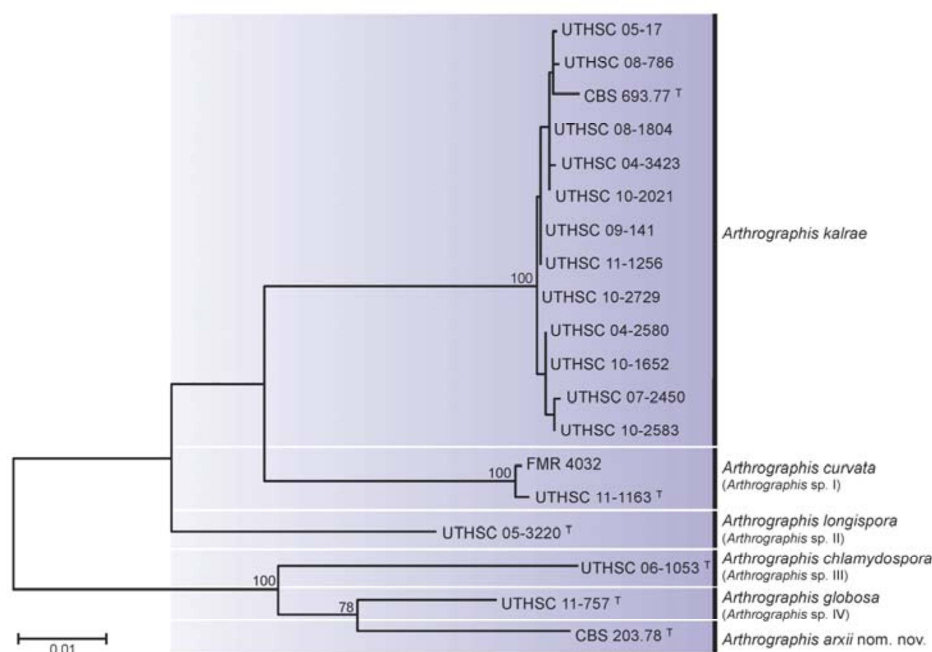

Fig. 2 Maximum-likelihood (ML) tree obtained from the combined DNA sequence data from three loci (ITS, *ACT1*, *CHS1*). Bootstrap support values above 70 % are indicated at the nodes. [T] = Ex-type strain.

to the low intra-specific variability detected in the ITS sequences among the 22 isolates identified as *A. kalrae* (98.5–100 % similarity), we selected 12 isolates that represented the most characteristic morphological variants observed.

With the primers used, we were able to amplify and sequence 300–350 bp, 450–500 bp and 750–820 bp of the *CHS1* gene, the ITS region and *ACT1* gene, respectively. The topology of the combined ML tree was similar to trees based on individual genes (data not shown). The combined tree included 1 544 bp and showed four main lineages (Fig. 2). The largest lineage was represented by a clade with 12 clinical strains of *A. kalrae*, including the ex-type strain. Sequences within the clade were practically identical, showing similarities of 98.5–100 % for each over the three loci. The second lineage (*Arthrographis* sp. I, 100 % bs) included one strain from marine sediments (FMR 4032) and another from nails (UTHSC 11-1163), with an intra-specific similarity of 99–100 %. The third lineage (*Arthrographis* sp. II) comprised only one strain (UTHSC 05-3220) from clinical origin (foot). Finally, the fourth lineage comprised a clade with three strains separated from each other by a considerable genetic distance. They were the clinical strains UTHSC 06-1053 (*Arthrographis* sp. III) and UTHSC 11-757 (*Arthrographis* sp. IV) and CBS 203.78, the ex-type strain of *E. langeronii* from herbivore dung. The latter two strains formed a well-supported subclade and showed genetic similarities that ranged from 93.8 % for ITS region to 96–96.7 % for *CHS1* and *ACT1* genes. Surprisingly, the ex-type strains of *A. kalrae* and *E. langeronii* were located in two different clades, showing genetic similarities of 94.1 % for ITS region and 92.8 % and 88.1 % for *ACT1* and *CHS1* genes, respectively.

Phenotypic studies

Most of the strains included in the *A. kalrae* clade (Fig. 2) showed the typical phenotypic characters described for the species; i.e., colonies at 25 °C with slow to moderate growth (up to 10–21 mm diam after 10 d on PDA), flat to slightly folded, initially beige and moist with a yeast-like appearance, becoming tan or yellowish and powdery to granular (Fig. 3a–f); conidiophores hyaline and usually branched (Fig. 3g); conidiogenous hyphae hyaline, simple or branched; arthroconidia 1-celled, hyaline, smooth-walled, cylindrical with truncate ends, 2.5–9 × 1–2 µm. All strains formed a trichosporiella-like synasexual morph with sessile, globose to subglobose, hyaline, thin and smooth-walled conidia, 2–4 × 2–3 µm (Fig. 3h). Several strains showed some atypical characters not previously described for this species. The strains UTHSC 02-1022, UTHSC 06-982, UTHSC 07-2450, UTHSC 08-1804, UTHSC 08-2107, UTHSC 10-1652, UTHSC 10-2583 and UTHSC 11-1256 produced intercalary or terminal chlamydospores with smooth or slightly rugose walls. While in most of these isolates the chlamydospores were hyaline to subhyaline, those of strain UTHSC 11-1256 turned brown on PDA and OA (Fig. 3i, j) giving a dark pigmentation to the colony. The UTHSC 05-17 strain showed a predominance of small conidiophores (up to 70 µm long) composed of a terminal whorl of numerous short chains of clavate or cylindrical arthroconidia with rounded ends (Fig. 3k, l); in old cultures (12 wk) this isolate developed immature ascomata submerged in the agar of all media tested. These ascomata were spherical, non-ostiolate, 37–70 µm diam, with a dark brown, pseudoparenchymatous peridium of *textura angularis*, surrounded by brown hyphae (Fig. 3m).

Arthrographis sp. I (FMR 4032 and UTHSC 11-1163) (Fig. 5a–j) showed similar morphological characteristics to those of the *A. kalrae* clade, but differed in the following features: the colonies on MEA 2 % were orange-yellow (4B8) and showed a very slow growth (6–7 mm diam in 14 d) (Fig. 5a); in addition to the trichosporiella-like synasexual morph (Fig. 5e), both strains produced on PDA at 25 °C and BHI at 37 °C curved and cashew-nut-shaped sessile conidia formed laterally on undifferentiated hyphae (Fig. 5f, g); and the strain UTHSC 11-1163 produced superficial spherical ascomata with evanescent asci and navicular ascospores (Fig. 5h–j).

The lineage representing *Arthrographis* sp. II (UTHSC 05-3220), produced membranous colonies in all the media tested (Fig. 7a, b),

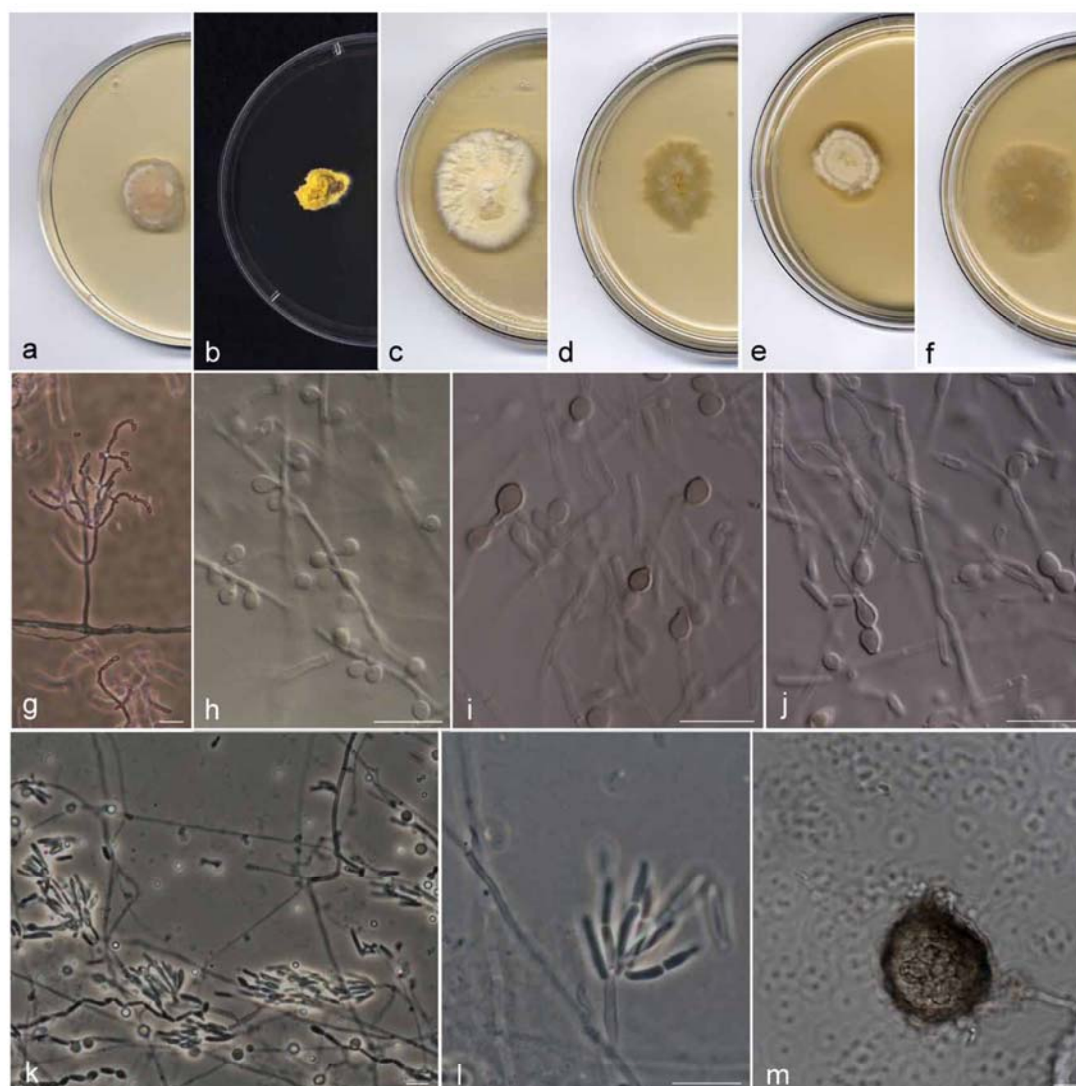

Fig. 3 *Arthrographis kalrae*. a–c. Colonies on PDA after 21 d at 25 °C; d–f. colonies on MEA 2 % at 25 °C after 21 d; g. branched conidiophores; h. lateral sessile conidia; i, j. pigmented chlamydospores and hyaline arthroconidia; k, l. whorls of short arthroconidial chains; m. sterile ascoma (a, d. CBS 693.77; b, e. UTHSC 09-141; c, f–h, k–m. UTHSC 05-17; i, j. UTHSC 11-1256). — Scale bars = 10 μm.

conidiophores poorly differentiated (Fig. 7c, d) and arthroconidia longer (5–10(–13) μm) than those of the members of *A. kalrae* clade (Fig. 7e, f). In this strain, as in *Arthrographis* sp. III and *Arthrographis* sp. IV, the production of a trichosporiella-like synasexual morph was not observed.

Arthrographis sp. III displayed umbonate, cerebriform and velvety colonies on PDA (Fig. 4a), branched conidiophores (Fig. 4c, d), cylindrical, cubic and doliiform arthroconidia (Fig. 4e–g) and terminal or intercalary globose chlamydospores (Fig. 4h).

Finally, the most representative morphological characters observed in *Arthrographis* sp. IV were the production of membranous colonies (Fig. 6a, b), poorly differentiated conidiophores (Fig. 6c) and doliiform, ellipsoidal, slightly fusiform or globose arthroconidia (Fig. 6d, e).

All strains grouped in the clade of *A. kalrae* were able to grow at all the temperatures tested, attaining up to 30 mm diam at 40 °C and 5–15 mm at 45 °C on PDA after 14 d. *Arthrographis* sp. I and *Arthrographis* sp. III grew well at 37 °C (13–16 mm diam after 14 d), but at 40 °C the growth of both species was restricted (6–7 mm diam after 14 d). Conversely, *Arthrographis* sp. II and sp. IV were not able to grow at 37 °C. All isolates tolerated high doses of cycloheximide (2 g/L). Only isolates of *A. kalrae* were able to convert to a yeast phase, producing oval to ellipsoidal (2.5 × 4 μm) yeast-like budding cells at 37 °C after several transfers in BHI broth.

Taxonomy

On the basis of the morphological features observed, which correlated with the phylogenetic analysis, we concluded that *Arthrographis* spp. I–IV are different from the taxa currently accepted in this genus and are therefore described here as new. These species are named *A. chlamydospora*, *A. curvata*, *A. globosa* and *A. longispora*. In addition, the new name *Arthrographis arxii* is proposed for the ascomycete *Eremomyces langeronii*.

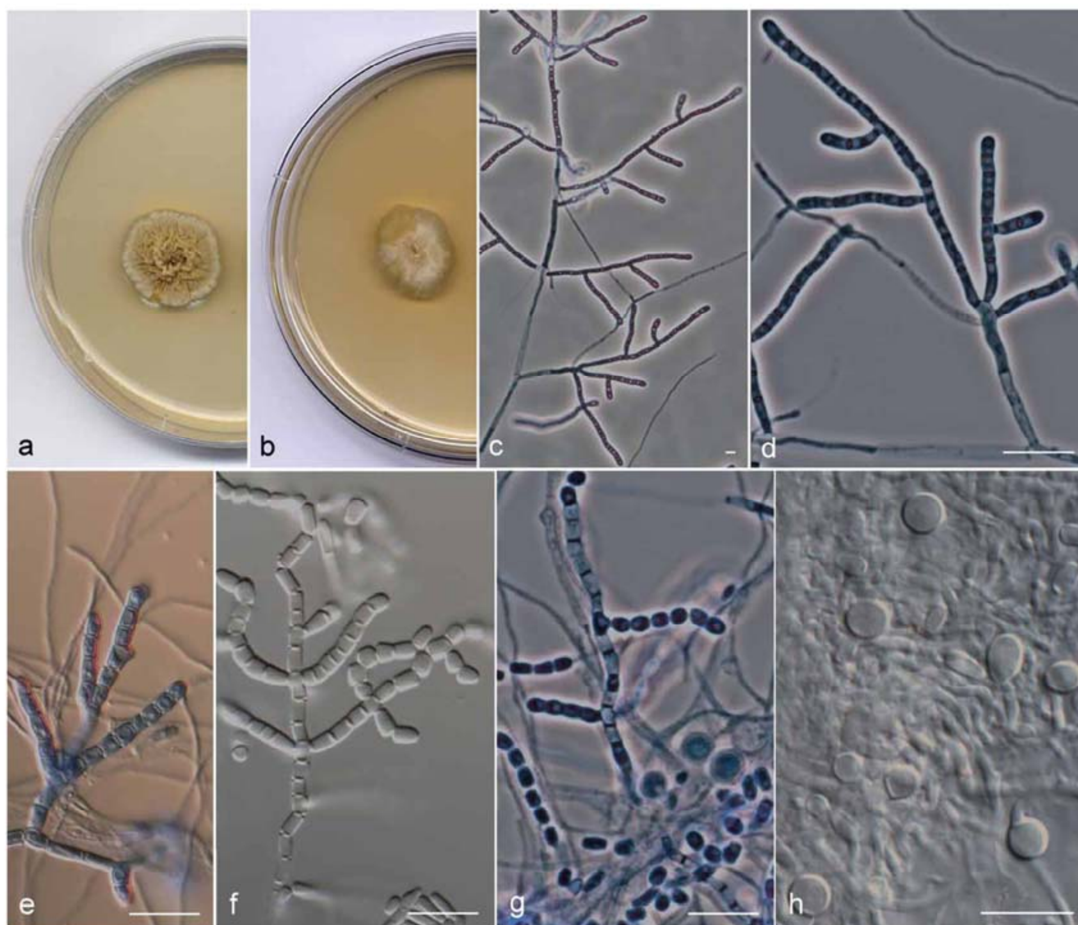

Fig. 4 *Arthrographis chlamydospora* UTHSC 06-1053. a, b. Colonies on PDA and MEA 2 %, respectively, after 21 d at 25 °C; c, d. branched conidiophores; e, f. conidiogenous hyphae fragmenting schizolytically; g. cylindrical and doliiform arthroconidia; h. chlamydospores. — Scale bars = 10 μm.

Arthrographis arxii Guarro, Giraldo, Gené & Cano, *nom. nov.* — MycoBank MB804634

Basionym. *Pithoascus langeronii* Arx, Persoonia 10: 24. 1978.
≡ *Pithoascina langeronii* (Arx) Valmaseda, T.A. Martínez & Barrasa, Canad. J. Bot. 65: 1805. 1987.
≡ *Eremomyces langeronii* (Arx) Malloch & Sigler, Canad. J. Bot. 66: 1931. 1988.

Etymology. The specific epithet is given in honour of the mycologist Josef Adolf von Arx (1922–1988), who actively published on this group of fungi.

Notes — Since our results demonstrated that *A. kalrae* and *E. langeronii* are not conspecific, and the name *A. langeronii* was occupied, a new name is proposed for *E. langeronii*.

Arthrographis chlamydospora Giraldo, Deanna A. Sutton, Gené & Madrid, *sp. nov.* — MycoBank MB804632; Fig. 4

Etymology. Referring to the presence of chlamydospores.

Colonies on PDA at 25 °C attaining 15–16 mm diam after 14 d, pale to greyish orange (5A–B3) with whitish margin, umbonate, cerebriform, velvety. On OA and PCA at 25 °C attaining 23–25 mm and 15–16 mm diam, respectively, after 14 d, orange-white (5A2), flat, powdery or granulose. On MEA 2 % at 25 °C attaining 14–15 mm diam in 14 d, orange-yellow (4B8), flat, radially striated, granulose. At 37 °C on PDA the colonies attaining 12–13 mm diam after 14 d, brownish orange (6C3–4), cerebriform, velvety. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 1.5–2 μm wide. *Conidiophores* mostly repeatedly branched, erect, up to 350 μm long, hyaline, smooth-walled. *Conidiogenous hyphae* simple or laterally branched, 1.5–2.5 μm wide, thick-walled, forming septa basipetally to form arthroconidia released via schizolythic secession. *Arthroconidia* unicellular, cylindrical, cuboid or doliiform, straight, 3–6(–7) × 1.5–2.5 μm, hyaline to subhyaline, thick- and smooth-walled. *Chlamydospores* terminal or intercalary, solitary, unicellular, globose or subglobose, 5–6 × 5–6 μm, hyaline, rough- and thick-walled, strongly chromophilic. Sexual morph and trichosporiella-like synasexual morph not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 42 °C, minimum 15 °C. The fungus was unable to grow at 45 °C.

Specimen examined. USA, Florida, from human urine, *D.A. Sutton* (holotype CBS H-21346, cultures ex-type CBS 135396, FMR 12129, UTHSC 06-1053).

Arthrographis curvata Giraldo, Gené, Deanna A. Sutton & Cano, *sp. nov.* — MycoBank MB804630; Fig. 5

Etymology. Referring to the presence of curved conidia.

Colonies on PDA at 25 °C attaining 17–19 mm diam in 14 d, pale to greyish orange (5A–B3) with whitish margin, umbonate at centre and flat toward the periphery, powdery. On OA and

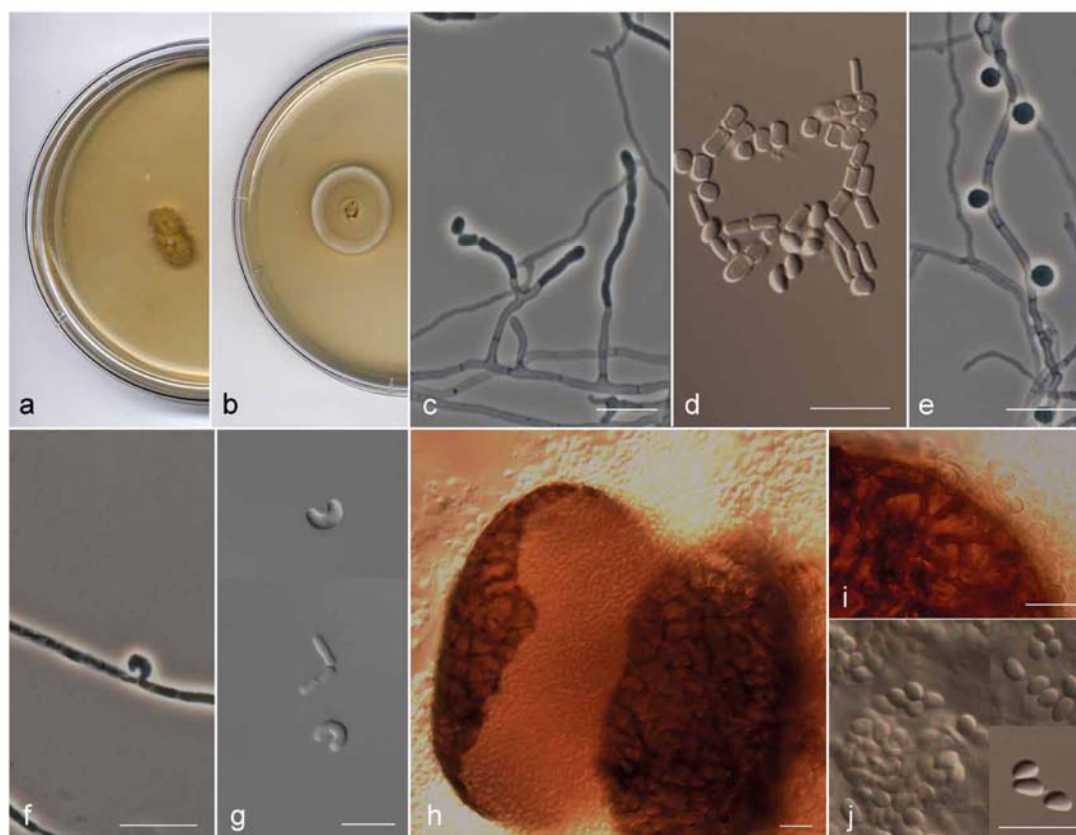

Fig. 5 *Arthrographis curvata*. a, b. Colonies on MEA 2 % and PDA, respectively, after 21 d at 25 °C. — c–g. Asexual morph: c. simple or poorly branched conidiophores; d. arthroconidia and ascospores; e. lateral sessile conidia. f, g. curved conidia produced in BHI at 37 °C. — h–j. Sexual morph: h. ascoma; i. dark brown hyphae on the peridium; j. ascospores (a–c, e, g. FMR 4032; d, f, h–j. UTHSC 11-1163). — Scale bars = 10 µm.

PCA at 25 °C attaining 19–20 mm and 24–26 mm diam, respectively, after 14 d, whitish, flat, dusty. On MEA 2 % at 25 °C attaining 6–7 mm diam in 14 d, orange-yellow (4B8), elevated, cerebriform, membranous. At 37 °C on PDA the colonies attaining 15–16 mm diam after 14 d, orange-grey (5B2), flat, powdery. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 1.5–2 µm wide. *Ascomata* cleistothecial, superficial, spherical, brown, 52–132 µm diam, peridium pseudoparenchymatous with *textura angularis*, surrounded by dark brown, thick-walled hyphae. *Asci* evanescent, globose, thin-walled. *Ascospores* unicellular, navicular in lateral view, ellipsoidal in front view, thin- and smooth-walled, without germ pores, 2.8–3.8 × 1.4–2 µm, hyaline to pale brown in mass. *Conidiophores* poorly differentiated, erect, simple or slightly branched, up to 35 µm long, hyaline, smooth-walled. *Conidiogenous hyphae* simple or branched, 1–2 µm wide, thin-walled, forming septa basipetally to form arthroconidia released by schizolythic secession. *Arthroconidia* unicellular, cylindrical or short-cylindrical, straight or slightly curved, 3–4.5(–7) × 1–2 µm, hyaline to subhyaline, thin- and smooth-walled. *Synasexual morph* trichosporiella-like with conidia growing directly on undifferentiated hyphae, lateral, sessile, globose, 2–3 µm diam, hyaline and smooth-walled. On PDA and BHI at 25 °C and 37 °C, respectively, conidia were occasionally observed to be unicellular, curved, cashew-nut-shaped, hyaline and smooth-walled, 3.5–6 × 1.5–2 µm, growing solitary and sessile on vegetative hyphae.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 42 °C, minimum 15 °C. The fungus was unable to grow at 45 °C.

Specimens examined. Spain, Amposta, Ebro river, from river bank, *K. Ulfig* (CBS 135934, FMR 4032). – USA, Colorado, from human nails, *D.A. Sutton* (holotype CBS H-21344, cultures ex-type CBS 135933, FMR 12125, UTHSC 11-1163).

Notes — The GenBank sequences AB128973.1 (ITS region) and AB128975 (28S rDNA), corresponding to the isolate *E. langeronii* UAMH 7600 from a fingernail, were 99.6 and 100 % (ITS region and 28S rDNA, respectively) similar to those of the type species of *A. curvata*.

Arthrographis globosa Giraldo, Deanna A. Sutton, Cano & Guarro, *sp. nov*. — MycoBank MB804633; Fig. 6

Etymology. Referring to the presence of globose conidia.

Colonies on PDA at 25 °C attaining 22–24 mm diam after 14 d, buttercup yellow (4A7), flat, membranous. On OA and PCA at 25 °C attaining 14–15 mm diam after 14 d, whitish, flat, at first glabrous becoming slightly powdery. On MEA 2 % at 25 °C attaining 4–5 mm diam in 14 d, orange-yellow (4A8), cerebriform, membranous. *Vegetative hyphae* septate, hyaline, with golden pigment accumulation inside, smooth- and thin-walled, 1.5 µm wide. *Conidiophores* absent or poorly differentiated, hyaline, smooth-walled. *Conidiogenous hyphae* simple or branched, 1–1.5 µm wide, thin-walled, forming septa basipetally to form arthroconidia released via schizolythic secession. *Arthroconidia* unicellular, doliiform, ellipsoidal, slightly fusiform or globose, 3–5(–6.5) × 2–4 µm, hyaline, thick- and smooth-walled. Sexual morph, trichosporiella-like synasexual morph and chlamydospores not observed.

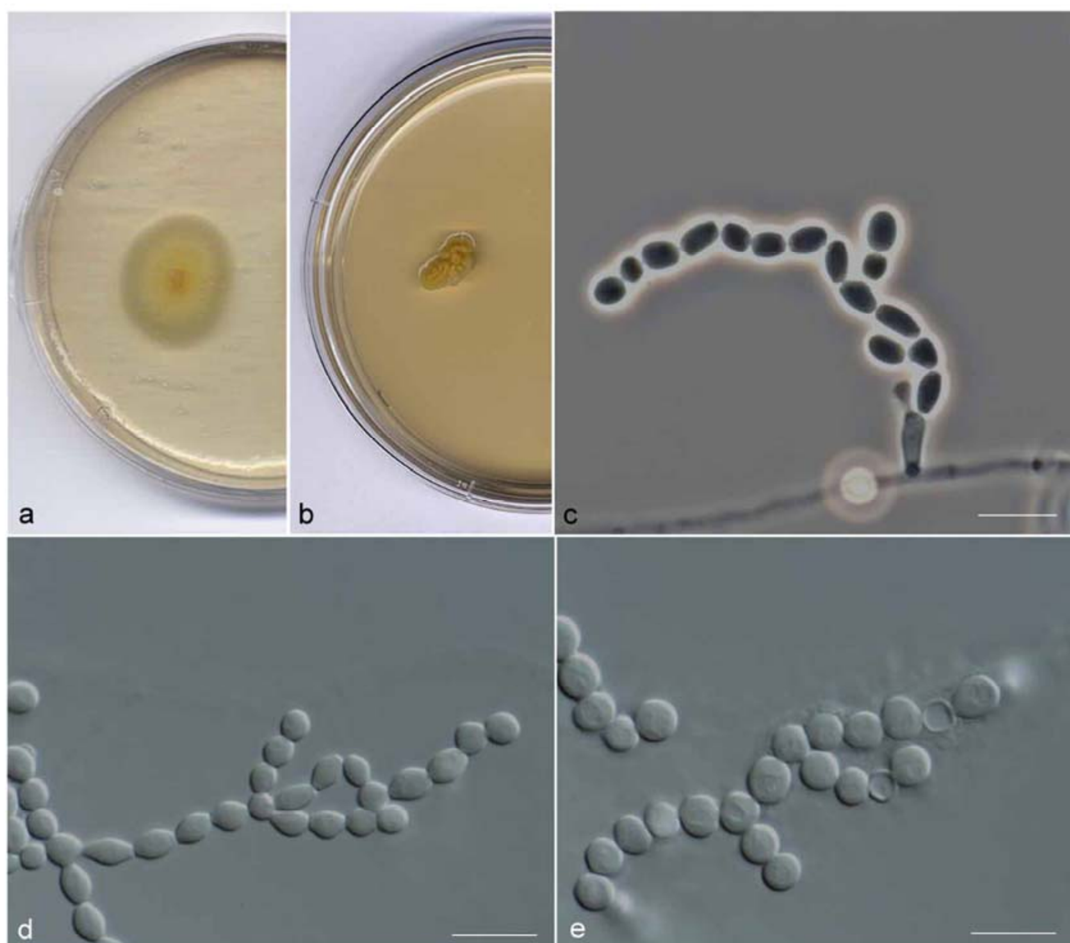

Fig. 6 *Arthrographis globosa* UTHSC 11-757. a, b. Colonies on PDA and MEA 2 %, respectively, after 21 d at 25 °C; c. poorly differentiated conidiophores; d. conidiogenous hyphae fragmenting schizolytically producing ellipsoidal, doliiform, slightly fusiform and globose arthroconidia; e. globose arthroconidia. — Scale bars = 10 µm.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 35 °C, minimum 15 °C. The fungus was unable to grow at 37 °C.

Specimen examined. USA, Texas, from human bronchial wash, *D.A. Sutton* (holotype CBS H-21347, cultures ex-type CBS 135397, FMR 12124, UTHSC 11-757).

Arthrographis longispora Giraldo, Deanna A. Sutton, Cano & Guarro, *sp. nov.* — MycoBank MB804631; Fig. 7

Etymology. Referring to the length of the arthroconidia.

Colonies on PDA at 25 °C attaining 18–21 mm diam after 14 d, yellowish orange (4A7), radially folded or rugose at centre and flat toward the periphery, membranous. On OA and PCA at 25 °C attaining 18–21 mm and 8–9 mm diam, respectively, after 14 d, whitish, flat, at first glabrous becoming slightly powdery. On MEA 2 % at 25 °C attaining 11–12 mm diam in 14 d, orange-yellow (4B8), cerebriform at centre and flat toward the periphery, membranous. *Vegetative hyphae* septate, hyaline, with golden pigment accumulation inside, smooth- and thin-walled, 1.5–2 µm wide. *Conidiophores* poorly differentiated, erect, up to 60 µm long, hyaline, smooth-walled. *Conidiogenous hyphae*, simple, occasionally slightly branched, 1–1.5 µm wide, thin-walled, septating basipetally to form arthroconidia released by schizolythic secession. *Arthroconidia* unicellular, cylindrical with truncated or rounded ends, straight or slightly curved, 5–10(–13) × 1–1.5 µm, hyaline, thin- and smooth-walled. Sexual morph, trichosporiella-like synasexual morph and chlamydospores not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 35 °C, minimum 15 °C. The fungus was unable to grow at 37 °C.

Specimen examined. USA, Utah, from human foot, *D.A. Sutton* (holotype CBS H-21345, cultures ex-type CBS 135935, FMR 12101, UTHSC 05-3220).

DISCUSSION

The genus *Arthrographis* was traditionally considered a member of the *Eremomycetaceae*, *Dothideomycetes* (Malloch & Sigler 1988). However, our D1/D2 analysis demonstrated that only the type species, *A. kalrae*, and the new taxa proposed here (i.e., *A. arxii*, *A. chlamydospora*, *A. curvata*, *A. globosa* and *A. longispora*) are members of the family, and that the name *Arthrographis* should be restricted to these species. The other species previously attributed to the genus are phylogenetically distant from the type. *Arthrographis lignicola* belongs to the *Lecanoromycetes*, forming a weakly supported clade with *Sarea resinae*, *Pycnora xanthococca* and *Sarcoletia globosa*. Although a BLAST search using D1/D2 and ITS sequences of *A. lignicola* showed close relationships with other members of

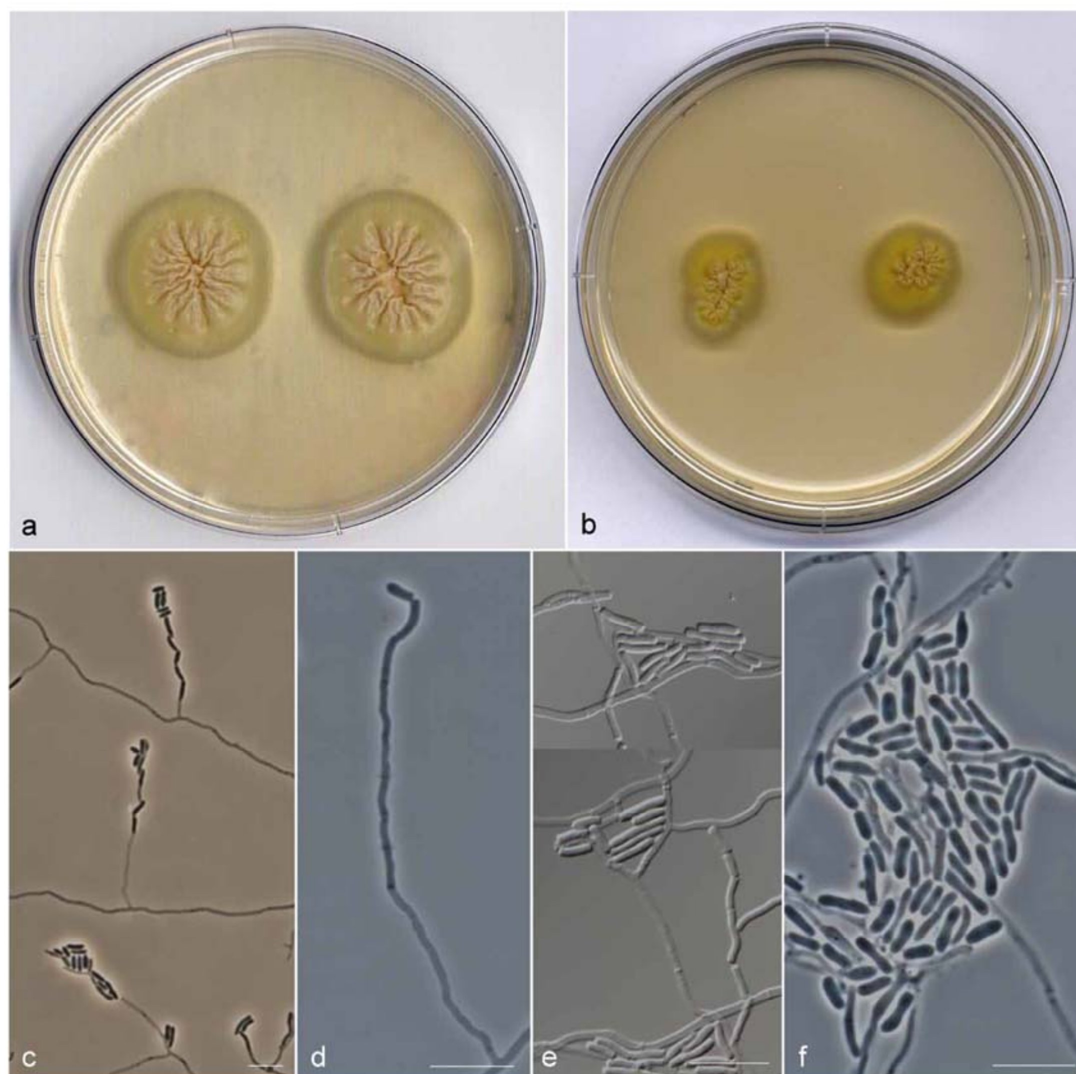

Fig. 7 *Arthrographis longispora* UTHSC 05-3220. a, b. Colonies on PDA and MEA 2 %, respectively, after 21 d at 25 °C; c, d. poorly differentiated conidiophores; e, f. cylindrical arthroconidia with truncate or rounded ends. — Scale bars = 10 µm.

that class, we could not include more sequences of *Lecanoromycetes* in our phylogenetic analysis due to the difficulties in performing a reliable alignment. *Arthrographis pinicola* and *A. alba* are accommodated in the *Eurotiomycetes*, more particularly, the former in *Eremascaceae*, closely related to *Eremascus albus*, and the latter in the *Gymnoascaceae*, closely related to *Leucothecium emdenii*. *Arthrographis alba* was described based on several isolates from different origins and, although some morphological similarity with the anamorph of *L. emdenii* was already mentioned, none of those isolates developed the sexual morph (Gené et al. 1996). The present study reveals that sequences from D1/D2 and ITS of both species are practically identical (data not shown), which indicates that *A. alba* must be considered the asexual morph of *L. emdenii*. The genus *Leucothecium* was described by von Arx & Samson (1973) to accommodate ascomycetes with yellowish globose ascomata, hyaline peridium, bivalve-lenticular ascospores and asexual morphs with hyaline arthroconidia. Currently, the genus comprises two species, *L. emdenii*, the type species, and *L. coprophilum*, both traditionally included in *Gymnoascaceae* on the basis of the morphology of the asexual morph and ascospores (von Arx & Samson 1973, Valldosera et al. 1991).

The ascomycete *Faurelina indica* also produces an arthrographis-like asexual morph similar to the *Arthrographis* anamorph of *E. langeronii* (von Arx 1978, von Arx et al. 1981). The genus *Faurelina*, with a coprophilous habitat, was originally included in the family *Chadefaudiellaceae*, *Microascales* (Locquin-Linard 1975, Cannon & Kirk 2007). This genus was characterised by pustulate or hemispherical ascomata, a peridium composed of vertical rows of dark cells, asci arranged in vertical chains and striate, and pale brown ascospores (Guarro et al. 2012). Our D1/D2 analysis revealed a well-supported relationship of *F. indica* with the *Dothideomycetes*, although distantly related to the genus *Arthrographis* and other members of *Eremomycetaceae*. Réblová et al. (2011), based on LSU sequences analysis, demonstrated the relationship of *F. indica* with the *Didymellaceae*, including it in the *Pleosporales*. The exclusion of *F. indica* from *Microascales* correlates with the morphological features of the asexual morph, since in that order the asexual morphs are characterised by percurrently proliferating conidiogenous cells (annellides) usually belonging to the genera *Scopulari-*

opsis, *Graphium*, *Scedosporium*, *Cephalotrichum* and *Wardomycopsis* (Valmaseda et al. 1986, Zhang et al. 2006, Réblová et al. 2011).

Eremomyces langeronii was traditionally considered to be the sexual morph of *A. kalrae* (von Arx 1978, Malloch & Sigler 1988). However, this connection was questioned by Sigler & Carmichael (1983), and later by Gené et al. (1996), arguing that both species produced different RFLP patterns. The present study confirms that *E. langeronii* and *A. kalrae* are not conspecific. *Eremomyces langeronii* was initially described as *Pithoascus langeronii* (von Arx 1978), later being transferred to the genus *Pithoascina* by Valmaseda et al. (1986). Malloch & Sigler (1988) accommodated this species in the genus *Eremomyces* (*Eremomycetaceae*) (Malloch & Cain 1971, Malloch & Sigler 1988) together with *E. bilateralis* and *Rhexothecium globosum*. Members of the *Eremomycetaceae* are characterised by cleistothecial ascomata, clavate to ovoid, evanescent asci, unicellular, hyaline to pale yellow-brown ascospores, arthrographis-like or trichosporiella-like asexual morphs and a coprophilous habitat (Malloch & Sigler 1988). Similar morphological features such as non-ostiolate dark ascomata and hyaline, unicellular ascospores can be also found in species of *Pseudeurotiaceae* (*incertae sedis*, Lumbsch & Huhndorf 2010), but the members of this family display pale-brown or olive-brown ascospores at maturity and asexual morphs with poorly differentiated conidiophores sympodially producing subspherical to ovoidal conidia. Our study demonstrated that the family *Eremomycetaceae* encompasses the genera *Arthrographis* s.str., *Rhexothecium* and *Eremomyces* (91.4–95.3 % intergeneric similarity in D1/D2 sequences). The latter now is restricted only to *E. bilateralis*, which is the type species of the genus. *Eremomyces bilateralis* is distinguished from *Arthrographis* s.str. and *Rhexothecium* by DNA sequence data (92.8 %, 89 % and 76.4 % similarity in D1/D2, *ACT1* and ITS sequences, respectively) and by its cephalothecoid peridium, dark coloured colonies and the absence of an asexual morph (Malloch & Cain 1971).

The multilocus sequence analysis revealed the existence of four new species in *Arthrographis*, *A. curvata* being the only one that showed both sexual and asexual morphs in culture. Its ascomata and ascospores are similar to those of *A. arxii*; however, in *A. arxii* the ascomata are immersed, and the ascomata and ascospores are larger (75–160 µm diam and 2.7–5 × 1.8–2.6 µm, respectively). The asexual morph of *A. curvata* differs from *A. arxii* and *A. kalrae* mainly by less differentiated and poorly branched conidiophores, the presence of curved, sessile conidia and a restricted growth at 40 °C. Another fungus that also produces curved, cashew-nut shaped conidia is the dermatophyte *Trichophyton phaseoliforme*, but this species is a member of *Eurotiomycetes*, produces pycnidium-like conidiomata and cigar-shaped macroconidia in clusters (de Hoog et al. 2011). *Arthrographis arxii* differs from *A. kalrae* in producing shorter and wider (3.5–5 × 2–2.5 µm) 1-septate arthroconidia.

Arthrographis chlamydospora is characterised by the production of repeatedly branched conidiophores, cuboid or doliiform arthroconidia and numerous chlamydospores. The species morphologically closest to *A. chlamydospora* is *A. kalrae*, but the latter differs by exhibiting yellowish to tan colonies, thin-walled arthroconidia, good growth at 40 °C and the presence of the trichosporiella-like synasexual morph. The other two new species, *A. globosa* and *A. longispora*, share several phenotypic features, i.e. absence of trichosporella-like synasexual morph, membranous colonies, inability to grow at 37 °C and resistance to high doses of cycloheximide. *Arthrographis globosa* can easily be distinguished by its globose to doliiform arthroconidia, and *A. longispora* by its poorly differentiated conidiophores producing large cylindrical arthroconidia. Other species of *Arthrographis* s.lat. unable to grow at 37 °C are *A. alba*, *A. lignicola* and *A. pinicola*. *Arthrographis alba* produces white colonies, pseudodichotomously branched conidiophores and, in our study, this species was susceptible to high doses of cycloheximide (2 g/L); *A. lignicola* can be distinguished by its lemon-yellow to olive-green colonies with a diffusible brown pigment, narrow branched conidiophores and yellow arthroconidia; and *A. pinicola* produces floccose conidiomata composed by repeatedly branched conidiophores and is susceptible to low doses of cycloheximide (Sigler & Carmichael 1983, Sigler et al. 1990, Gené et al. 1996).

In this study we observed some morphological variability in *A. kalrae*, with the presence of some characteristics not previously reported for this species. Such variations, however, did not correlate with genetic differences in any of the three loci sequenced. Several isolates showed chlamydospores that were terminal or intercalary, solitary or catenulate, hyaline or pigmented. In the protologue of *Oidiodendron kalrai*, based in the strain CBS 693.77, Tewari & Macpherson (1971) reported the occasional presence of oval to round, thick-walled chlamydospores; however, Sigler & Carmichael (1976) did not mention these structures and only reported the sessile conidia of the trichosporiella-like synasexual morph. The UTHSC 05-17 isolate produced infertile ascomata morphologically similar to the ascomata produced by *A. arxii* and *A. curvata*, but in that isolate these structures were smaller (37–70 µm diam). That isolate also produced abundant conidiophores with whorls of short chains of clavate or cylindrical arthroconidia. The clavate conidia was reported by von Arx (1978) in the description of the asexual morph of *E. langeronii*, but probably this description was based on a single strain of this species and not on the ex-type strain of *A. kalrae*.

The genus *Arthropsis* was established by Sigler et al. (1982) with *A. truncata* as the type species, to accommodate species with dark arthroconidia, joined by adjacent connectives and developed from undifferentiated conidiogenous hyphae. Until now the species of this genus have not been associated to any sexual morph. Our D1/D2 sequence analysis demonstrates that *Arthropsis* is polyphyletic and unrelated to *Arthrographis* s.str. *Arthropsis hispanica* and *A. cirrhata* fall into the *Onygenales*, as do other species previously included in *Arthrographis*. Other arthroconidial anamorphs of the *Onygenales* are included in the genus *Malbranchea*. However, *Malbranchea* is morphologically distinguished by its branched and arcuate fertile hyphae, straight in some species, that produce alternate arthroconidia (Sigler & Carmichael 1976). Our analysis placed the only available living strain of *A. microsperma* (UAMH 4290) in the *Helotiales* (*Leotiomycetes*). *Arthropsis microsperma* was originally described by Berkeley & Broome (1873) as *Oidium microspermum* and later transferred to *Arthropsis* by Sigler & Carmichael (1983) based on its arthroconidial ontogeny. Therefore, the name of this species should be reconsidered because *Oidium* anamorphs are currently associated with members of the *Leotiomycetes* (Braun & Cook 2012). Finally, *Arthropsis truncata* is related to members of the *Sordariomycetes*. Although such type of asexual morphs have not been described in that class, humicola-like asexual morphs similar to the *Humicola* synasexual morph of *A. truncata* are present in some species of *Chaetomium* (Gené & Guarro 1996, Seifert et al. 2011). Further studies with a greater number of taxa of *Sordariomycetes* are needed to ascertain a defined position for *A. truncata* within this class.

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4.3. Study on ochroconis-like fungi

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4.3.1. Occurrence of *Ochroconis* and *Verruconis* species in clinical specimens from the United States

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Occurrence of *Ochroconis* and *Verruconis* Species in Clinical Specimens from the United States

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***Ochroconis* is a dematiaceous fungus able to infect immunocompetent people. Recently, the taxonomy of the genus has been reevaluated, and the most relevant species, *Ochroconis gallopava*, was transferred to the new genus *Verruconis*. Due to the important clinical implications of these fungi and based on the recent classification, it was of interest to know the spectra of *Ochroconis* and *Verruconis* species in clinical samples received in a reference laboratory in the United States. A set of 51 isolates was identified morphologically and molecularly based on sequence analyses of the nuclear ribosomal RNA (nrRNA), actin, and β-tubulin genes. *Verruconis gallopava* was the most common species (68.6%), followed by *Ochroconis mirabilis* (21.5%). One isolate of *Ochroconis cordanae* was found, being reported for the first time in a clinical setting. The most common anatomical site of isolation was the lower respiratory tract (58.8%), followed by superficial and deep tissues at similar frequencies (21.6 and 19.6%, respectively). Interestingly, three new species were found, which are *Ochroconis olivacea* and *Ochroconis ramosa* from clinical specimens and *Ochroconis icarus* of an environmental origin. The *in vitro* antifungal susceptibilities of eight antifungal drugs against the *Ochroconis* isolates revealed that terbinafine and micafungin were the most active drugs.**

Ochroconis is a dematiaceous anamorphic genus described by de Hoog and von Arx (1) to accommodate species with slow to moderate growth, brown to olivaceous colonies, brownish conidiophores, and septate, dark-pigmented, and rough-walled conidia, which are produced by sympodial conidiogenesis and liberated rhexolytically (1–3). The species of the genus have a cosmopolitan distribution and are isolated from different sources, i.e., soil, decaying vegetable material (4, 5), indoor and outdoor environments (6), cave rocks, and Paleolithic paintings (7, 8). Due to the thermotolerance of some species, it is common to find them in thermal soils (9–12), hot spring effluents (11, 13), sewage from nuclear power plants, coal waste piles (14–16), and broiler-house litters (17, 18). Some species have been reported to be opportunistic pathogens in humans, producing localized infections in the brain and lungs, as well as subcutaneous and systemic infections, sometimes with fatal outcomes (13, 19–23). These organisms also cause infections in birds (18, 24–27), cats (28, 29), and dogs (30). The type species of the genus, *Ochroconis constricta*, was initially described by Abbott (31) as a species of *Scolecobasidium*. However, Samerpitak et al. (32) reviewed these fungi, and *Scolecobasidium* was abandoned, since the original material of the type species, *Scolecobasidium terreum* (strain CBS 203.27), was found to be of doubtful identity. In the same study, the thermophilic species with light to dark brown and verrucose to coarsely ornamented conidia, such as *Ochroconis gallopava*, *Ochroconis calidifluminalis*, and *Ochroconis verrucosa*, were transferred to the new genus *Verruconis*. The mesophilic species with subhyaline, smooth-walled to verruculose conidia and commonly associated with infections in cold-blooded animals were retained in *Ochroconis* (32). Both *Verruconis* and *Ochroconis* were located within the *Sympoventuriaceae* family in the recently described order *Venturiales* (*Dothideomycetes*) (33).

Due to the clinical relevance of these fungi and the recent taxonomical studies involving them, it was of interest to assess the spectra of the species of *Verruconis* and *Ochroconis* in clinical samples received by a reference center in the United States. Because little is known about the antifungal susceptibility of *Ochroconis*, we have determined the *in vitro* activity to the clinically available antifungal drugs against the *Ochroconis* species identified in the present study.

MATERIALS AND METHODS

Fungal isolates. Fifty-one clinical isolates (Table 1) received at the Fungus Testing Laboratory at the University of Texas Health Science Center (UTHSC) at San Antonio, TX, were investigated in this study. Several type and reference strains provided by the CBS-KNAW Fungal Biodiversity Centre (Utrecht, the Netherlands) were also included in the study.

Phenotypic studies. Morphological characterization of the isolates was done on oatmeal agar (OA) (30 g of filtered oat flakes after 1 h of simmering, 20 g of agar, distilled water to final volume of 1,000 ml), potato carrot agar (PCA) (20 g each of filtered potatoes and carrots, 20 g of agar, distilled water to final volume of 1,000 ml), 2% malt extract agar (MEA 2%) (10 g of malt extract, 20 g of agar, distilled water to final volume of 1,000 ml), and potato dextrose agar (PDA) (Pronadisa, Madrid, Spain). The cultures were incubated at 25°C in the dark and examined after 4 weeks. The colony diameters were measured after 14 days of growth, and colony colors were determined using the color charts of Kornerup and Wanscher (34). In addition, the ability of the isolates to grow at 4, 15, 30, 32, 33, 35, 37, 40, and 42°C was tested on PDA. Micro-

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TABLE 1 Strains included in the study

Species	Strain[a]	Origin[b]	GenBank accession no.[c]				
			ITS	LSU-D1/D2	*ACT1*	*BT2*	SSU
Ochroconis anellii	CBS 284.64[T]	Stalactite, Italy	FR832477	KF156138	KF155912	KF156184	KF156070
Ochroconis anomala	CBS 131816[T]	Lascaux Cave, France	HE575201	KF156137	KF155935	KF156194	KF156065
Ochroconis constricta	CBS 202.27[T]	Soil, USA	AB161063	KF156147	KF155942	KF156161	KF156072
	CBS 211.53	Soil, Canada	HQ667519	KF156148			
	FMR 3906	Goat dung, Spain	**LM644509**	**LM644552**			
Ochroconis cordanae	CBS 475.80[T]	Dead leaf, Colombia	KF156022	KF156122	HQ916976	KF156197	KF156058
	CBS 780.83	*Podocarpus* litter, Japan	HQ667539	KF156120			
	UTHSC 10-1875	Tissue of left thigh, USA	**LM644510**	**LM644553**			
Ochroconis gamsii	CBS 239.78[T]	Plant leaf, Sri Lanka	KF156019	KF156150	KF155936	KF156190	KF156088
Ochroconis humicola	CBS 116655[T]	Peat soil, Canada	HQ667521	KF156124	KF155904	KF156195	KF156068
Ochroconis icarus (= *Ochroconis* sp. III)	CBS 423.64	Rhizosphere of *Solanum tuberosum*, the Netherlands	HQ667523	KF156131	KF155943	KF156173	KF156085
	CBS 536.69[T]	Forest soil, Canada	HQ667524	KF156132	KF155944	KF156174	KF156084
	CBS 116645	Sandy soil, Canada	HQ667525		**LM644599**	**LM644604**	KF156083
Ochroconis lascauxensis	CBS 131815[T]	Stain in Lascaux Cave, France	FR832474	KF156136	KF155911	KF156183	KF156069
Ochroconis longiphorum	CBS 435.76	Soil, Canada	KF156038	KF156135	KF155908	KF156182	KF156060
Ochroconis minima	CBS 510.71[T]	Rhizosphere, Nigeria	HQ667522	KF156134	KF155945	KF156172	KF156087
	CBS 119792	Soil, India	KF156027	KF156133	KF155946	KF156175	KF156086
Ochroconis mirabilis	CBS 729.95[T]	Regulator of diver, the Netherlands	KF156029	KF156144	KF155948	KF156171	KF156082
	UTHSC 01-1570	Nail, USA	**LM644511**	**LM644554**			
	UTHSC 03-1114	BAL fluid, USA	**LM644512**	**LM644555**			
	UTHSC 04-2378	BAL fluid, USA	**LM644513**	**LM644556**			
	UTHSC 02-232	BAL fluid, USA	**LM644514**	**LM644557**			
	UTHSC 03-3089	Skin with impetigo, USA	**LM644515**	**LM644558**			
	UTHSC 05-1500	BAL fluid, USA	**LM644516**	**LM644559**			
	UTHSC 07-3073	Toenail, USA		**LM644560**			
	UTHSC 08-1958	Toenail, USA	**LM644517**	**LM644561**			
	UTHSC 10-1519	Skin, USA	**LM644518**				
	UTHSC 11-2020	BAL fluid, USA	**LM644519**	**LM644562**			
	UTHSC 11-3523	BAL fluid, USA	**LM644520**	**LM644563**			
Ochroconis olivacea (= *Ochroconis* sp. I)	UTHSC 10-2009[T]	BAL fluid, USA	**LM644521**	**LM644564**	**LM644600**	**LM644605**	**LM644548**
Ochroconis ramosa (= *Ochroconis* sp. II)	UTHSC 03-3677	Skin, USA	**LM644522**	**LM644565**	**LM644601**	**LM644606**	**LM644549**
	UTHSC 04-2729	BAL fluid, USA	**LM644523**	**LM644566**	**LM644602**	**LM644607**	**LM644550**
	UTHSC 12-1082[T]	Nail, USA	**LM644524**	**LM644567**	**LM644603**	**LM644608**	**LM644551**
Ochroconis sexualis	CBS 135765[T]	Domestic, South Africa	KF156018	KF156118	KF155902	KF156189	KF156089
Ochroconis tshawytschae	CBS 100438[T]	Fish, USA	HQ667562	KF156126	KF155918	KF156180	KF156062
Ochroconis verrucosa	CBS 225.77	Leaf, Burma	HQ667564	KF156130	KF155909	KF156186	KF156066
	CBS 383.81[T]	Soil, India	KF156015	KF156129	KF155910	KF156185	KF156067
Ochroconis sp.	CBS 119644	Indoor sample, Germany	KF961086	KF961097	KF956086	KF961065	KF961108
Scolecobasidium excentricum	CBS 469.95[T]	Leaf litter, Cuba	HQ667543	KF156105			
Veronaeopsis simplex	CBS 588.66	Leaf litter, South Africa	KF156041	KF156103			
Verruconis calidifluminalis	CBS 125817	Hot spring river, Japan	AB385699	KF156107			
	CBS 125818[T]	Hot spring river, Japan	AB385698	KF156108	KF155901	KF156202	KF156046

(Continued on following page)

TABLE 1 (Continued)

Species	Strain[a]	Origin[b]	GenBank accession no.[c]				
			ITS	LSU-D1/D2	*ACT1*	*BT2*	SSU
Verruconis gallopava	CBS 437.64[T]	Turkey brain, USA	HQ667553	KF156112	HQ916989	KF156203	KF156053
	UTHSC 04-1355	Sputum, USA					
	UTHSC 03-447	Sputum, USA	**LM644525**	**LM644568**			
	UTHSC 04-43	Bronchial wash, USA	**LM644526**	**LM644569**			
	UTHSC 04-236	Brain, USA	**LM644527**	**LM644570**			
	UTHSC 04-539	Canine liver, USA	**LM644528**	**LM644571**			
	UTHSC 04-2693	Brain abscess, USA		**LM644572**			
	UTHSC 05-1018	Brain abscess, USA		**LM644573**			
	UTHSC 06-513	Sputum, USA	**LM644529**	**LM644574**			
	UTHSC 06-541	Sputum, USA					
	UTHSC 06-3565	Canine abdominal fluid, USA	**LM644530**	**LM644575**			
	UTHSC 06-4445	Right hand abscess, USA	**LM644531**	**LM644576**			
	UTHSC 07-153	Left lower lobe lung Bx, USA		**LM644577**			
	UTHSC 07-212	Induced sputum, USA	**LM644532**	**LM644578**			
	UTHSC 07-623	Canine L2-L3 disc, USA	**LM644533**	**LM644579**			
	UTHSC 07-2994	Sputum, USA	**LM644534**	**LM644580**			
	UTHSC 08-40	Bronchial wash, USA	**LM644535**	**LM644581**			
	UTHSC 08-112	Sputum, USA	**LM644536**	**LM644582**			
	UTHSC 08-657	Feline wound, USA	**LM644537**	**LM644583**			
	UTHSC 08-810	Sputum, USA	**LM644538**	**LM644584**			
	UTHSC 08-1340	Bronchial wash, USA	**LM644539**	**LM644585**			
	UTHSC 08-1625	Right hip, USA		**LM644586**			
	UTHSC 08-1756	Sputum, USA					
	UTHSC 08-3158	Bronchial wash, USA		**LM644587**			
	UTHSC 09-1229	Ear/mastoid, USA	**LM644540**	**LM644588**			
	UTHSC 09-3111	Lung, USA		**LM644589**			
	UTHSC 10-510	Brain abscess, USA	**LM644541**	**LM644590**			
	UTHSC 10-1509	Bronchial wash, USA	**LM644542**	**LM644591**			
	UTHSC 10-1541	Bronchial wash, USA	**LM644543**	**LM644592**			
	UTHSC 10-3013	Sputum, USA	**LM644544**	**LM644593**			
	UTHSC 11-315	Sputum, USA		**LM644594**			
	UTHSC 11-509	Bronchial wash, USA	**LM644545**	**LM644595**			
	UTHSC 11-2401	Bronchial wash, USA					
	UTHSC 11-2569	Bronchial wash, USA		**LM644596**			
	UTHSC 12-69	Bronchial wash, USA	**LM644546**	**LM644597**			
	UTHSC 12-549	Bronchial wash, USA	**LM644547**	**LM644598**			
Verruconis verruculosa	CBS 119775	Plant root, *Hevea* sp., Malaysia	KF156041	KF156103	KF155919	KF156193	KF156055

[a] CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; FMR, Faculty of Medicine Reus, Spain; UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX; [T], type strain.
[b] BAL, bronchoalveolar lavage; Bx, biopsy.
[c] The accession numbers of sequences newly determined in this study are indicated in bold type. ITS, internal transcribed spacer regions of the nuclear ribosomal DNA (nrDNA) and intervening 5.8S nrDNA; LSU, large subunit of the nrDNA; *ACT1*, partial actin gene; *BT2*, β-tubulin gene; SSU, small subunit of the nrDNA.

scopic features were examined and measured by making direct wet mounts with 85% lactic acid or by slide cultures on OA and PCA using the light microscope Olympus CH-2 (Olympus Corporation, Tokyo, Japan). Photomicrographs were made with a Zeiss Axio Imager M1 light microscope (Zeiss, Oberkochen, Germany), using Nomarski differential interference contrast. The 95% confidence intervals were derived from 50 observations (×1,000 magnification), with the extremes given in parentheses. The ranges of the dimensions of other characters are given in the descriptions of new taxa.

DNA extraction, amplification, and sequencing. The isolates were grown on YES agar (20 g of yeast extract, 150 g of sucrose, 20 g of agar, distilled water to final volume of 1,000 ml) for 10 days at 25°C. DNA extraction was done by using FastDNA kit protocol (MP Biomedicals, Solon, OH), with the homogenization step done with a FastPrep FP120 cell disrupter (Thermo Savant, Holbrook, NY). The 18S nuclear small subunit (nuSSU), the internal transcribed spacer regions (ITS), including the 5.8S small subunit gene, and the D1/D2 domains of the 28S nuclear large subunit (nuLSU) were amplified with the primer pairs NS1/NS4, ITS5/ITS4, and NL1/NL4b or LR0R/LR5, respectively (35–37). The fragments of the actin (*ACT1*) and β-tubulin (*BT2*) genes were amplified using the primers ACT-512F/ACT-783R and Bt1a/Bt1b, respectively (38, 39). The amplified fragments were purified and sequenced at Macrogen Corp. Europe (Amsterdam, the Netherlands) with a 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). The sequencing was performed with the same primers used for amplification to ensure good-quality sequences over the total length of the amplicon. Consensus sequences were obtained using SeqMan version 7.0.0 (DNAStar, Madison, WI). Some ITS, D1/D2, *ACT1*, and *BT2* sequences corresponding to several species of *Ochroconis* or *Verruconis* were retrieved from GenBank (32, 33) and included in the phylogenetic study (Table 1).

Phylogenetic analysis. Multiple sequence alignments using the Clustal W and MUSCLE applications (40, 41) were made in MEGA version 5.05 (42), in which the best substitution models were searched for each locus and for the combined data set, and maximum likelihood (ML) and maximum parsimony (MP) analyses were also performed. For ML, gaps and missing data were treated as a partial deletion with a site coverage cutoff 95%, and nearest-neighbor interchange (NNI) was used as the heuristic method. Internal branch support was assessed by a search of 1,000 bootstrapped sets of data. A bootstrap support (BS) of ≥70 was considered significant. The phylogenetic distance values between the isolates were estimated with Kimura 2-parameter as a nucleotide substitution model under MEGA version 5.05. For the multilocus analysis, a phylogenetic analysis using a Markov chain Monte Carlo (MCMC) algorithm was done with MrBayes version 3.1.2 (43) on the CIPRES portal (http://www.phylo.org), with two simultaneous runs for 10 million generations, with a sampling frequency of 1,000 trees. A burn-in tree sample of 10% was discarded. Bayesian posterior probabilities (PP) were obtained from the 50% majority-rule consensus of trees. A PP value of ≥0.95 was considered significant. The congruencies of the sequence data sets for the separate loci were determined using tree topologies of 70% reciprocal neighbor-joining (NJ) bootstrap trees with maximum likelihood distances, which were compared visually to identify conflicts between the partitions (44). *Scolecobasidium excentricum* strain CBS 469.95 and *Veronaeopsis simplex* strain CBS 588.66 were used as outgroups.

Antifungal susceptibility. The *in vitro* activities of amphotericin B (AMB), itraconazole (ITC), posaconazole (PSC), voriconazole (VRC), anidulafungin (AFG), caspofungin (CFG), micafungin (MFG), and terbinafine (TBF) were determined for all the clinical isolates of *Ochroconis*, according to the methods outlined in the CLSI document M38-A2 (46) but with an incubation temperature of 30°C. The minimal effective concentration (MEC) was determined at 48 h for the echinocandins, and the MICs at 48 h and 72 h were determined for the remaining drugs. The MIC was defined as the lowest concentration exhibiting 100% visual inhibition of growth for AMB, ITC, PSC, and VRC and an 80% reduction in growth for TBF. *Paecilomyces variotii* strain ATCC MYA-3630 and *Aspergillus fumigatus* strain ATCC MYA-3626 were used as quality control strains. Statistical analyses of the data were done using the Kruskal-Wallis test in GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as a P value of ≤0.05 (two-tailed).

Nucleotide sequence accession numbers. All novel DNA sequences were deposited in GenBank under accession numbers shown in bold type in Table 1, and taxonomic novelties were deposited in MycoBank (http://www.MycoBank.org) (45).

RESULTS

Phylogenetic analysis. The D1/D2 analysis (Fig. 1) showed that 68.6% and 31.4% of the isolates studied were distributed in two main groups corresponding to *Ochroconis* and *Verruconis*, respectively. The *Verruconis* isolates grouped with high support in the same clade as the ex-type strain of *V. gallopava* (CBS 437.64). Eleven of the clinical isolates of *Ochroconis* were nested with the ex-type strain of *O. mirabilis* (CBS 729.95) and one with the ex-type strain of *O. cordanae* (CBS 475.80). Three clinical isolates (UTHSC 03-3677, UTHSC 12-1082, and UTHSC 04-2729), morphologically similar to *Ochroconis minima*, constituted a clade that was phylogenetically distant from the type strain of that species. Although the isolate UTHSC 10-2009 clearly showed the differential features of *Ochroconis*, it did not group with any of the species of that genus included in this study, including the reference strain CBS 536.69, which was received as *O. minima*.

With the aim of confirming and clarifying the data obtained in the D1/D2 analysis, a multilocus analysis of five concatenated gene regions (nuSSU, ITS, nuLSU, *ACT1*, and *BT2*) was performed, including the type and some reference strains of the currently accepted *Ochroconis* species. The concatenated sequence consisted of 4,188 bp (Fig. 2), of which 1,000 were parsimony informative (51 nuSSU, 127 LSU, 432 ITS, 175 *ACT1*, and 215 *BT2*) and revealed the formation of 17 lineages, 13 of them corresponding to known *Ochroconis* species and three representing putative new species (*Ochroconis* sp. I, *Ochroconis* sp. II, and *Ochroconis* sp. III). *Ochroconis* sp. I (strain UTHSC 10-2009) was located in the same clade as that of two reference strains of *Ochroconis verrucosa*, including the ex-type strain (CBS 383.81), although with significant phylogenetic distance. The clade corresponding to *Ochroconis* sp. II consisted of three clinical isolates (UTHSC 03-3677, UTHSC 12-1082, and UTHSC 04-2729), while *Ochroconis* sp. III included three environmental reference strains all previously identified as *O. minima* (CBS 423.64, CBS 536.69, and CBS 116645). The clades representing *Ochroconis* sp. II and *Ochroconis* sp. III were phylogenetically related (6.5% phylogenetic distance in the combined data set) although distinct from the sequences of *O. minima* type strain CBS 510.71 (5.7% and 7.0% phylogenetic distances with *Ochroconis* sp. III and *Ochroconis* sp. II, respectively).

Most of the clinical isolates included in this study were of respiratory origin (58.8%), mainly obtained from bronchoalveolar lavage (BAL) fluid and sputum samples, followed by superficial tissue samples (21.6%), principally from the nails and skin (Table 2). The remaining 19.6% of the isolates were from miscellaneous deep tissue or sterile fluid specimens, with most of them collected from the lungs and brain. *V. gallopava* was recovered from a wide range of clinical specimens, being isolated from all the deep tissues and in equal numbers from sputum and bronchial wash fluids. The isolates of *Ochroconis* spp. were exclusively recovered from respiratory samples (BAL fluid), skin, and nails.

Phenotypic studies. Most of the isolates belonging to the *V. gallopava* clade showed the typical phenotypic characteristics described for the species, i.e., colonies on PDA at 25°C with moderate growth (up to 58 mm diameter after 14 days), brownish gray (5E2) with a diffusible brown pigment, poorly differentiated conidiogenous cells, and pale brown clavate 1-septum conidia constricted at the septum that were (7)10 to 21 μm long by 2.5 to 4.5 μm wide. All the strains showed optimum growth at 42°C (up to 80 mm at 14 days). Some strains showed atypical features not previously reported for the species. Isolate UTHSC 07-623 produced yellowish white (4A2) colonies on all media tested and hyaline conidia. The isolates UTHSC 07-212 and UTHSC 12-549 showed slow growth at 25°C (17 to 20 mm and 7 to 10 mm in 14 days, respectively), and their colonies were radially striate, with a lobulate edge, and moist, with a cerebriform aspect, respectively. Several isolates, apart from the clavate conidia, produced some ellipsoidal conidia with an apiculated base (UTHSC 03-447, UTHSC 04-43, UTHSC 04-236, UTHSC 06-513, UTHSC 07-153, UTHSC 08-112, UTHSC 08-810, UTHSC 10-510, UTHSC 11-509, and UTHSC 12-549). The isolates that grouped in the *O. mirabilis* clade produced slow-growing (20 to 32 mm diameter in 14 days), olive brown (4D4), and velvety colonies on PDA and dark brown (5F8) and dry colonies on OA and PCA; the conidiophores were cylindrical, thick walled, and with denticles distributed sympodially along the conidiophore; the conidia were cylindrical or ellipsoidal 1-septum, slightly constricted at the septum, and pale brown with rugose walls. The isolate UTHSC 10-1875, clustered in the *O. cordanae* clade, displayed long (up to 40 μm), brown, and simple conidiophores, some of them with several

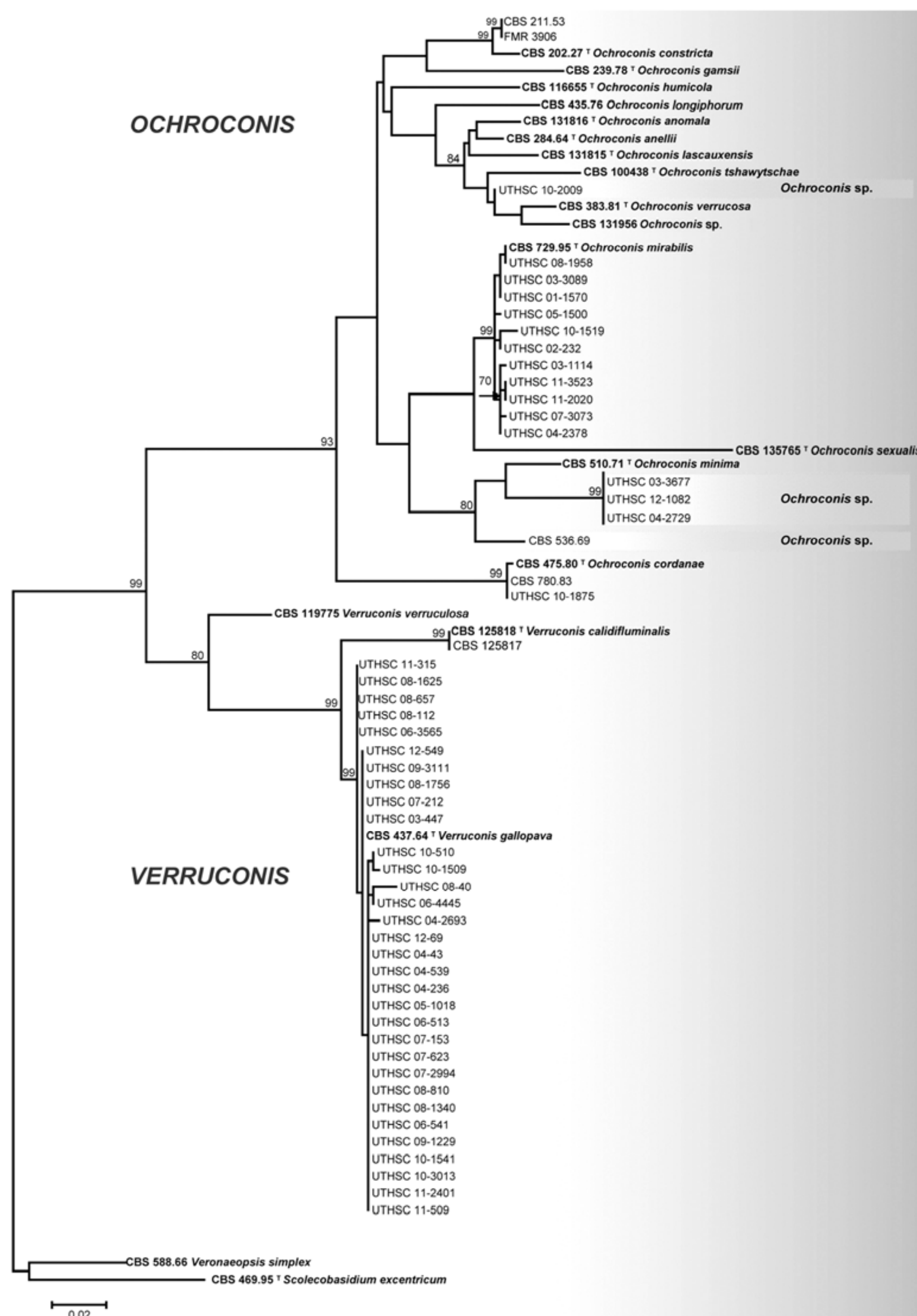

FIG 1 Maximum-likelihood (ML) tree constructed with sequences of the D1/D2 domains of the 28S rRNA gene. Bootstrap support (BS) values of >70% are shown at the nodes. *V. simplex* CBS 588.66 and *S. excentricum* CBS 469.95 were used as outgroup taxa. ^{T}, ex-type strains.

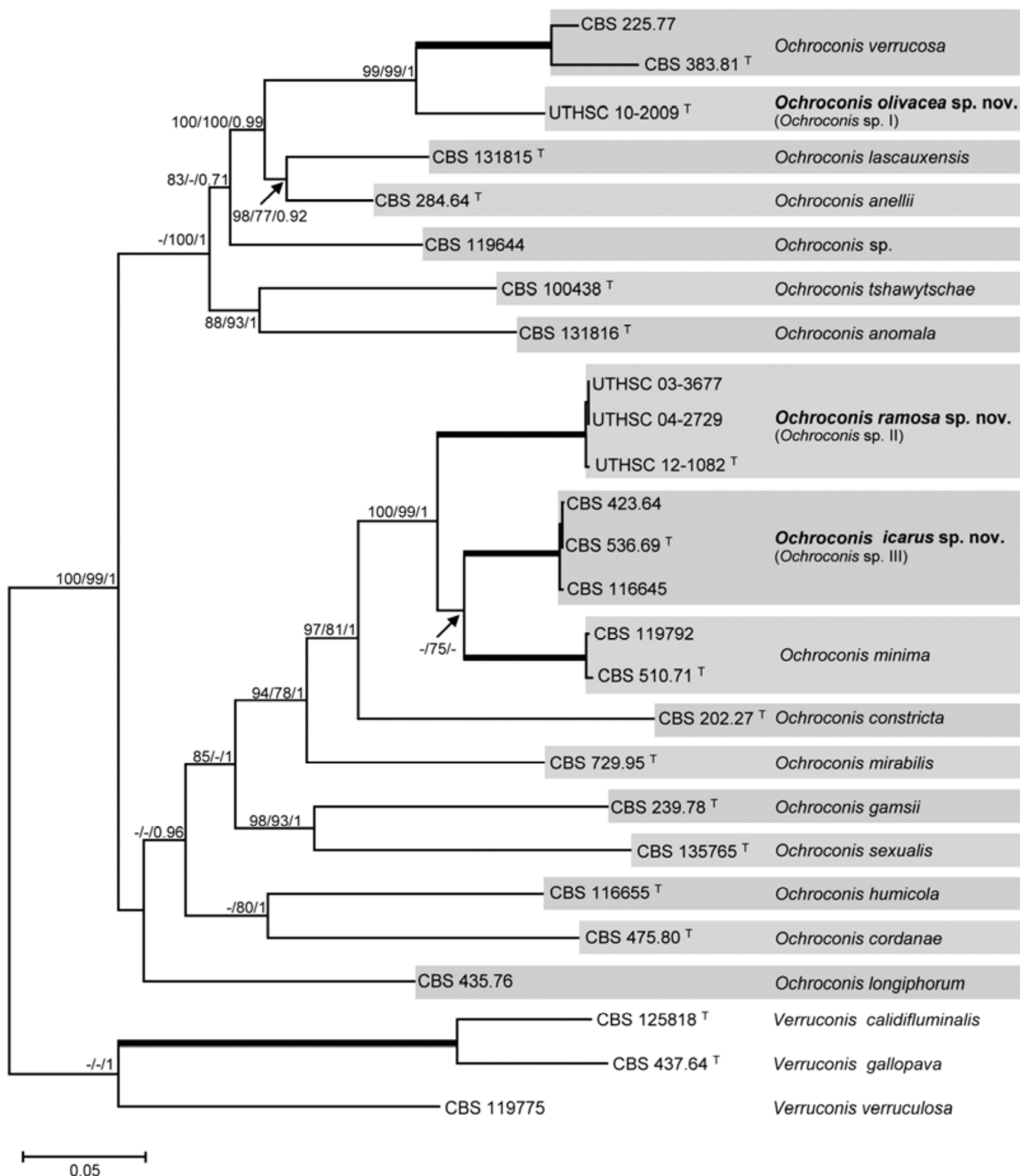

FIG 2 Bayesian tree from a concatenated data set, including the five regions nuSSU, ITS, nuLSU, *ACT1*, and *BT2*. Bootstrap support obtained with maximum-likelihood (left) and maximum parsimony (middle) of >70% and Bayesian posterior probability (right) values of >0.95 are shown at the nodes. ^{T}, ex-type strains. Full supported branches are depicted as thick lines.

septa, and were thick walled, with denticles on the apical region; the conidia were ellipsoidal, slightly constricted at the septum, smooth or finely verruculose, and 7 to 13 μm by 3 to 3.5 μm. *Ochroconis* sp. I (isolate UTHSC 10-2009) was mainly characterized by the production of long conidiophores (up to 60 μm) and verrucose broadly ellipsoidal conidia, sometimes slightly apiculated at the base (Fig. 3C and F). Both *Ochroconis* sp. II (Fig. 4) and *Ochroconis* sp. III (Fig. 5) showed phenotypic characteristics similar to those of *O. minima* but with some differences, i.e., in *Ochroconis* sp. II, the conidia had a rough surface (Fig. 4F and G), the conidiophores reached up to 20 μm long, the chlamydospores were smaller (3 to 3.5 μm by 3 to 3.5 μm), and the maximum and minimum temperatures for growth were 35°C and 15°C, respectively; in *Ochroconis* sp. III, the conidia were slightly smaller, with

a narrower lower cell (up to 2.5 μm wide), and the maximum and minimum temperatures for growth were 33°C and 4°C, respectively.

Antifungal susceptibility. The results of the *in vitro* activities of the antifungal drugs tested are summarized in Table 3. No statistical difference in antifungal activities were observed among the *Ochroconis* spp. studied. Terbinafine was the most active drug against all the species tested, followed by MFG, with MICs and MECs of 0.02 μg/ml and 0.25 μg/ml, respectively. AMB showed poor *in vitro* activity against all the isolates tested, with geometric mean (GM) MICs and MIC_{90}s of 24.5 μg/ml and 32 μg/ml, respectively. Similarly, the three azoles showed very little activity, with elevated MICs.

TAXONOMY

Based on the mentioned phylogenetic data, which correlated with the phenotypic features observed, we concluded that *Ochroconis* sp. I, *Ochroconis* sp. II, and *Ochroconis* sp. III are different from the taxa currently accepted in this genus and are therefore described here as new.

Ochroconis icarus Samerpitak, Giraldo, Guarro, & de Hoog, sp. nov., MycoBank accession no. MB809376 (Fig. 5). Etymology: the conidia look like the mythological figure Icarus, son of Daedalus, who made wings to reach the sun. Diagnosis: it differs from *O. minima* by the production of smaller conidia with a narrower lower cell and a maximum growth temperature of 33°C and from *O. ramosa* mainly by having longer denticles and smooth-walled conidia, with a wider upper cell.

Colonies on OA and PDA at 25°C attaining 16 to 20 mm and 17 to 24 mm diameter after 14 days, respectively; chocolate brown (6F4), flat, slightly curled, velvety at center, membranous at periphery. Colonies on PCA at 25°C attaining 21 to 24 mm diameter after 14 days, dark brown (7F4), flat, membranous becoming velvety. On MEA 2% at 25°C, reaching 13 to 17 mm diameter after 14 days, yellowish brown (5E5), flat, woolly at center. Vegetative hyphae septate, pale brown, smooth and thin walled, 1 to 2 μm wide; anastomosing and coiled hyphae usually present. Conidiophores poorly differentiated, arising laterally from vegetative hyphae, flexuose, clavate, or cylindrical, 15 to 20 μm by 1.5 to 2 μm, pale brown, thin and smooth walled, bearing one or more denticles in the apical region; denticles cylindrical, subhyaline to pale brown, up to 2 μm long. Conidia abundant on OA, moderate on MEA 2%, and scarce on PCA, mostly two celled, trilobate, T or Y shaped, 8 to 12 μm long, lower cell 1.5 to 2.5 μm wide, upper cell up to 4 to 8 μm wide, pale brown, smooth and thin walled, released by rhexolytic secession. Chlamydospores abundant on PCA, moderate on OA and MEA 2%, growing directly on vegetative hyphae, terminal or lateral, sessile, solitary, unicellular, globose to slightly subglobose, 4 to 5 μm in diameter, brown, smooth and thick walled. Sexual morph not observed. Cardinal temperatures for growth: optimum 24 to 27°C, maximum 33°C, minimum 4°C.

Specimens examined: Canada, Ontario, from forest soil, 1969, G. L. Barron (holotype CBS H-21643; cultures ex-type CBS 536.69 = MUCL 15054 = OAC 10212). From sandy soil, 1963, G. L. Barron (CBS 116645 = ATCC 16074 = MUCL 102118 = OAC 10094). The Netherlands, Wageningen, from rhizosphere of *Solanum tuberosum*, 1964, J. H. van Emden (CBS 423.64 = MUCL 10610).

Ochroconis olivacea Giraldo, Gené, Deanna A. Sutton, & Guarro, sp. nov., MycoBank accession no. MB809377 (Fig. 3).

TABLE 2 Anatomical sources of isolates of *Verruconis* and *Ochroconis* spp. from clinical samples

Species	No. of isolates obtained from samples from:															Total no. (%)
	Superficial tissue ($n = 11$)						Lower respiratory tract ($n = 30$)			Deep tissue ($n = 10$)						
	Nails	Skin	Tissue of left thigh	Hand abscess	Wound	Ear	Bronchial wash	BAL fluid[a]	Sputum	Brain	Liver	Lung biopsy sample	Intervertebral disc	Hip	Abdominal fluid	
V. gallopava	0	0	0	1	1	1	11	0	11	4	1	2	1	1	1	35 (68.6)
O. mirabilis	3	2	0	0	0	0	0	6	0	0	0	0	0	0	0	11 (21.5)
O. ramosa	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	3 (5.9)
O. olivacea	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1 (2)
O. cordanae	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1 (2)
Total	4	3	1	1	1	1	11	8	11	4	1	2	1	1	1	51 (100)

[a] BAL, bronchoalveolar lavage.

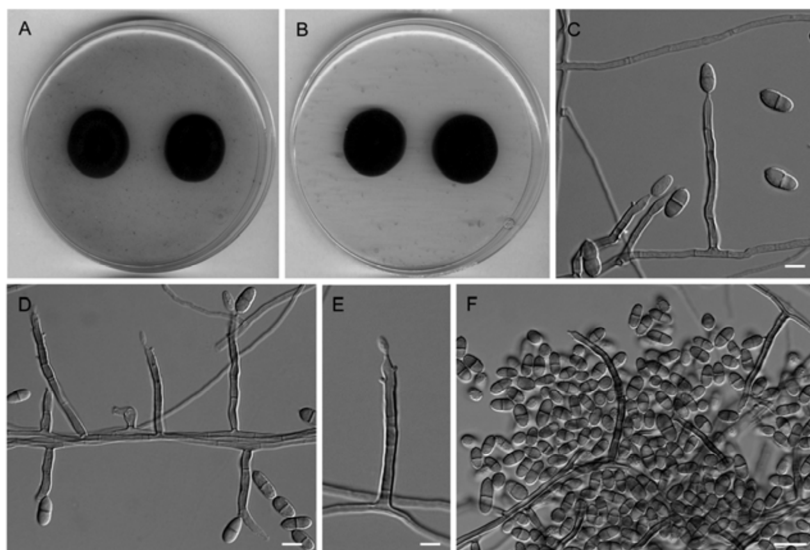

FIG 3 *Ochroconis olivacea* sp. nov. UTHSC 10-2009. (A and B) Colonies on OA and PCA, respectively, at 25°C after 14 days. (C, D, and F) Simple conidiophores arising directly from vegetative hyphae and conidia. (E) Young conidium growing on the apex of a conidiophore. Scale bars = 10 μm.

Etymology: referring to the colony color. Diagnosis: it differs from *Ochroconis humicola* mainly by having slower growth, shorter conidiophores, and verrucose conidia, and from *O. verrucosa* by the production of solitary two-celled conidia.

Colonies on OA at 25°C attaining 23 to 24 mm diameter after 14 days, from olive (1F4) to olive brown (4E5), flat, felty. Colonies on PCA at 25°C reaching 18 to 23 mm after 14 days, olive (2F8), flat, woolly at center, becoming felty toward the periphery. Colonies on MEA 2% and PDA attaining 15 to 17 mm and 22 to 23 mm diameter in 14 days, respectively, olive brown (4E6), radially folded, velvety. Vegetative hyphae septate, pale brown, smooth and thin walled, 1.5 to 2 μm wide. Conidiophores differentiated, arising directly from vegetative hyphae, erect, straight or slightly bent, simple, with 0 to 2 septa, cylindrical, (14)21 to 42(60) μm by

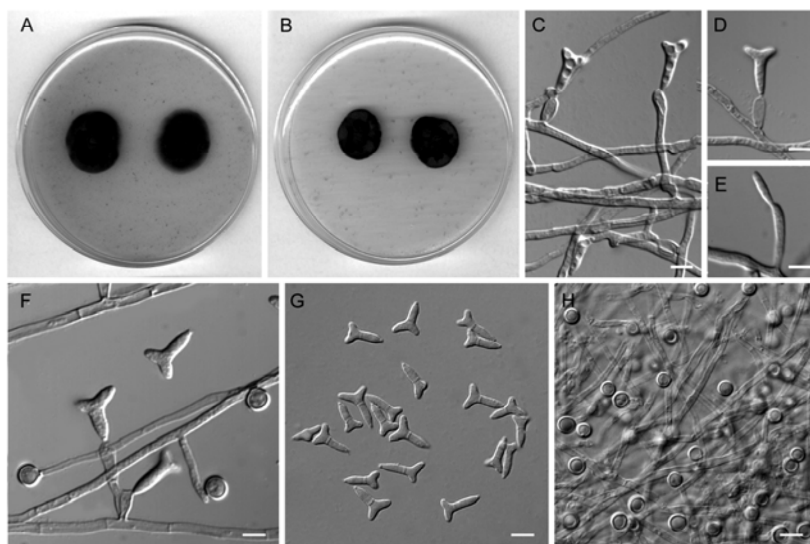

FIG 4 *Ochroconis ramosa* sp. nov. UTHSC 12-1082 (A and B), UTHSC 04-2729 (D and F to H), UTHSC 03-3677 (C and E). (A and B) Colonies on OA and PCA, respectively, at 25°C after 14 days. (C to F) Conidiophores producing trilobate conidia. (G) Trilobate conidia. (H) Chlamydospores growing directly on vegetative hyphae. Scale bars = 10 μm.

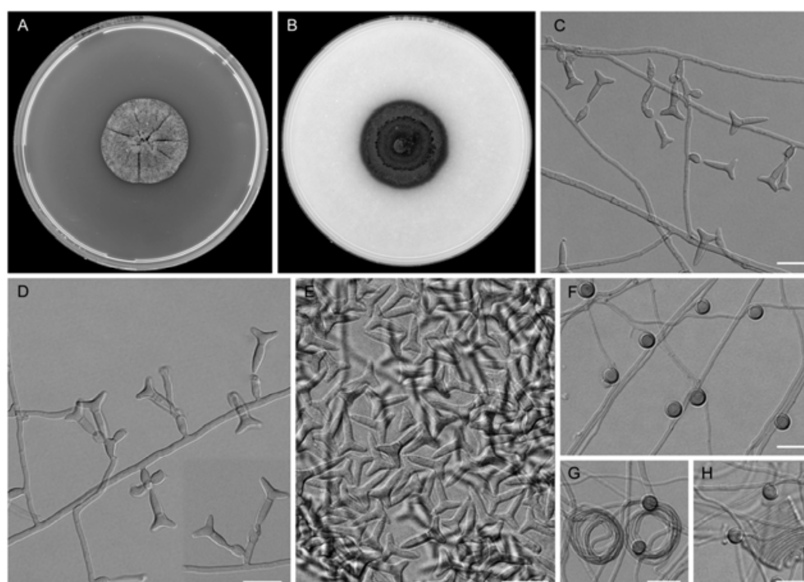

FIG 5 *Ochroconis icarus* sp. nov. CBS 536.69. (A and B) Colonies on MEA 2% and OA, respectively, at 25°C after 21 days. (C and D) Conidiophores bearing trilobate conidia. (E) Trilobate conidia. (F to H) Chlamydospores and coiled hyphae. Scale bars = 10 μm.

2 to 3 μm, brown, thick and smooth walled, producing conidia sympodially on long open denticles; denticles cylindrical, pale brown, up to 1 μm long. Conidia abundant on OA and PCA, absent on PDA and MEA 2%, mostly two celled, cylindrical or broadly ellipsoidal, 6 to 9.5 μm by 1.9 to 4.5 μm, sometimes slightly apiculate at the base and constricted at the septum, pale brown, verrucose and thick walled, released by rhexolytic secession. Chlamydospores and sexual morph not observed. Cardinal temperatures for growth: optimum 20 to 25°C, maximum 35°C, minimum 15°C.

Specimen examined: USA, Utah, from bronchoalveolar lavage fluid, 2010, D. A. Sutton (CBS H-21779 holotype; cultures ex-type CBS 137170 = FMR 12509 = UTHSC 10-2009).

Ochroconis ramosa Giraldo, Gené, Deanna A. Sutton & Guarro, sp. nov., MycoBank accession no. MB809378 (Fig. 4). Etymology: referring to branched conidia. Diagnosis: it differs from *O. minima* by the production of smaller and narrower conidia and a maximum growth temperature of 35°C and from *O. icarus* mainly by having shorter denticles and rough-walled conidia with a narrower upper cell.

Colonies on OA at 25°C attaining 22 to 24 mm diameter in 14 days, chocolate brown (6F4), flat, felty at center, membranous toward the periphery. Colonies on PCA at 25°C reaching 17 to 20 mm after 14 days, brownish black (6H8), flat, woolly at center, membranous toward the periphery. Colonies on MEA 2% and PDA attaining 13 to 16 mm and 20 to 24 mm diameter in 14 days,

TABLE 3 Results of *in vitro* antifungal susceptibility testing of the 16 clinical isolates of *Ochroconis* spp. included in the study

Species (no. of isolates tested)	GM or MIC data[a]	MIC or MEC (μg/ml) for[b]:							
		AMB	ITC	PSC	VRC	AFG	CFG	MFG	TBF
O. mirabilis (11)	GM	28.36	7.00	18.23	11.09	3.93	7.90	0.22	0.03
	MIC range	8–32	1–32	0.5–32	2–32	0.015–32	1–32	0.06–0.5	0.015–0.125
	MIC_{90}	32	32	32	32	4	4	0.25	0.02
O. cordanae (1)	GM	16	1	2	4	0.03	2	0.125	0.015
O. olivacea (1)	GM	32	1	1	4	0.03	1	0.125	0.015
O. ramosa (3)	GM	10.66	1	11.08	2.1	0.051	1	0.16	0.015
	MIC range	8–16	1	0.25–32	0.5–4	0.015–0.125	1	0.125–0.25	0.015
Overall (16)	GM	24.5	5.13	14.80	8.53	2.72	5.67	0.2	0.02
	MIC range	8–32	1–32	0.25–32	0.5–32	0.02–32	1–32	0.06–0.50	0.02–0.13
	MIC_{90}	32	2	32	16	4	8	0.25	0.02

[a] GM, geometric mean.
[b] MEC, minimal effective concentration; AMB, amphotericin B; ITC, itraconazole; PSC, posaconazole; VRC, voriconazole; AFG, anidulafungin; CFG, caspofungin; MFG, micafungin; TBF, terbinafine.

respectively, olive (3F5) to olive brown (4E4 to F4), slightly raised, velvety. Vegetative hyphae septate, pale brown, smooth and thin walled, 1.5 to 2 μm wide. Conidiophores differentiated arising directly from vegetative hyphae, erect, straight, simple, clavate or cylindrical with beaked apex, 15 to 20 μm by 1.5 to 2 μm, pale brown, thin and smooth walled, bearing one or more denticles in the apical region; denticles cylindrical, subhyaline to pale brown, up to 0.8 μm long. Conidia abundant on OA and PCA, absent on PDA and MEA 2%, mostly two celled, trilobate, T or Y shaped, (7)8 to 10(12) μm long, lower cell 1.5 to 2.5 μm wide, upper cell 3 to 6 μm wide, pale brown, rough and thin walled, released by rhexolytic secession. Chlamydospores abundant on PCA and OA, absent on MEA 2%, growing directly on vegetative hyphae, lateral, sessile, solitary, unicellular, globose or subglobose, 3 to 3.5 μm by 3 to 3.5 μm, brown, smooth and thick walled. Sexual morph not observed. Cardinal temperatures for growth: optimum 20 to 25°C, maximum 35°C, minimum 15°C.

Specimens examined: USA, California, from human nail, 2012, D. A. Sutton (CBS H-21780 holotype; cultures ex-type CBS 137173 = FMR 12514 = UTHSC 12-1082). Pennsylvania, from human skin, 2003, D. A. Sutton (CBS 137171 = FMR 12512 = UTHSC 03-3677). Utah, from bronchoalveolar lavage fluid, 2004, D. A. Sutton (CBS 137172 = FMR 12513 = UTHSC 04-2729).

DISCUSSION

In this study, we determined the distributions of *Verruconis* and *Ochroconis* species in a set of clinical specimens from human and animal origin from the United States, based on molecular and phenotypic analyses. *V. gallopava* was the most common species, found mainly on respiratory samples (BAL fluid and sputum), followed by deep-tissue samples (brain and others). These findings agree with those of previous studies, in which *V. gallopava* frequently involved the lung, producing either cavitary or noncavitary lung lesions. This fungus also shows special neurotropism in warm-blooded animals (21, 22). Although *V. gallopava* is commonly known as producing brain abscesses and encephalitis in birds and other animals, several reports document it as an etiologic agent in human disease. Most of these reports occur in immunocompromised patients, with organ transplantation being the most common underlying condition (13, 21, 22, 48). A few cases in immunocompetent patients with pulmonary manifestations have also been described. In these cases, *V. gallopava* was isolated from BAL fluid and lobectomy samples (49–51).

Some of our *V. gallopava* isolates showed atypical morphologies, such as yellowish white or moist colonies, slow growth at 25°C, and ellipsoidal conidia with an apiculate base. However, the molecular analysis demonstrated that they in fact belong to this species. The morphological variability in *V. gallopava* has also been reported in other studies (13, 28). Yarita et al. (13) analyzed four isolates from hot springs in Japan, which showed differences in the shape and size of the conidia, being slender or thicker and shorter than those of the type species; however, their D1/D2 regions were 99.7% identical with the those of the type strain of *V. gallopava*. The isolates studied by Dixon and Salkin (28) also produced a similar variation in conidial size.

V. gallopava has been described as a thermotolerant species (22, 32, 52), and all the isolates studied here grew well at 42°C. This explains its ability to survive in warm environments, like thermal soils or hot springs, and to infect warm-blooded animals, poultry, and other birds, in addition to humans. The *in vitro* thermotolerance is a useful physiological feature for identifying this species (13, 20).

In the present study, we report for the first time an albino isolate of *V. gallopava* (UTHSC 07-623), which was recovered from a canine intervertebral disc. This is an unusual phenomenon that occurs in a few other fungi, such as *Neoscytalidium hyalinum*, *Aspergillus flavus*, *Ophiostoma floccosum*, *Ophiostoma piceae*, and *Ophiostoma pluriannulatum* (53–55), which in general are involved almost exclusively in skin and nail disease (56, 57). The melanin plays an important role in fungal pathogenesis, and its absence often generates less virulent isolates than with pigmented ones (47, 58–60). Although we could not demonstrate that this isolate was the etiological agent of the infection, the presence of albino isolates recovered from deep tissues might suggest that this fungus has an additional mechanism of pathogenicity, apart from that of melanin. However, additional isolates should be studied to demonstrate this hypothesis.

O. mirabilis was the most common species recovered from superficial lesions, but it was also isolated from BAL fluid samples. This species is a waterborne fungus usually isolated from moist places in bathrooms and rarely from soil and plant material. However, several isolates of this species have also been recovered from clinical samples, producing mild cutaneous infections (skin, fingers, and toenails) in humans and fishes (32). It has been suggested that the bathroom-associated fungi can penetrate the skin and the nails during showering, when these barriers are weakened (6, 61).

The species *O. constricta* and *O. humicola*, occasionally reported from superficial infections in humans (20, 21, 61), were not represented in our set of isolates. However, most of the isolates identified here as *O. mirabilis* were received as either *O. constricta* or *O. humicola*. Only subtle morphological features allow the distinction between these three species: *O. constricta* has poorly differentiated conidiophores and markedly verruculose conidia, with a conspicuous constriction at the septum, *O. mirabilis* has differentiated cylindrical conidiophores (up to 20 μm) and conidia slightly constricted at the septum, and *O. humicola* has rapid growth, longer conidiophores than the other two species, and cylindrical conidia with a smooth or slightly rugose wall.

We obtained a single isolate of *O. cordanae* from the tissue of a left thigh, which was previously identified as *O. humicola*. *O. cordanae* can be differentiated by its more slowly growing colonies, shorter conidiophores, and smaller conidia, which are mostly ellipsoidal. *O. cordanae* was recently described by Samerpitak et al. (32) as a cosmopolitan species commonly inhabiting living leaves, sometimes found on decaying vegetal material, and less frequently from ant nests, but it has never been obtained from clinical samples. Therefore, this is the first report of this species in the clinical setting. Because only one isolate was obtained from a superficial tissue sample and because of its mesophilic abilities, the human-pathogenic role of this species is still doubtful.

Based on our multilocus sequence analysis and detailed phenotypic study, three new species are proposed here, i.e., *O. icarus* from environmental sources and *O. ramosa* and *O. olivacea* from clinical specimens. Both *O. icarus* and *O. ramosa* are morphologically similar and phylogenetically related to *O. minima* (Fig. 2). However, they can be easily differentiated by their maximum temperatures for growth (37°C for *O. minima*, 35°C for *O. ramosa*, and 33°C for *O. icarus*). Additionally, in *O. minima*, the conidia are longer than those of the other two species (up to 13.5 μm), in

O. ramosa, the conidia have a rugose wall, and in *O. icarus*, the lower cell of the conidia is narrower than that of *O. minima* (1.5- to 2.5-μm wide for *O. icarus* versus up to 4.5 μm for *O. minima*).

In the phylogenetic analyses, *O. olivacea* was placed close to *O. verrucosa* (Fig. 2). Both species produce verrucose conidia, but in *O. verrucosa*, they are cylindrical or fusiform, mostly four celled, and sometimes arranged in acropetal, branched, or unbranched chains (32). In contrast, in *O. olivacea*, the conidia are cylindrical or ellipsoidal, mostly two celled, and not arranged in chains. Morphologically, *O. olivacea* is similar to *Ochroconis gamsii* and *O. humicola* in its production of one-celled conidia and erected long cylindrical conidiophores. However, in *O. gamsii*, the conidia are broadly fusiform and unilaterally flattened, and the conidiophores are darker, while in *O. humicola*, the conidia are broadly cylindrical and finely echinulate and the conidiophores are longer (up to 300 μm) (2, 62). *Ochroconis macrozamiae*, a recently described species on *Macrozamia* leaf litter, also resembles *O. olivacea*, but, in addition to having broadly fusiform conidia, it is phylogenetically close to *O. gamsii* (63).

Few *in vitro* antifungal susceptibility studies are available for *Ochroconis* species, as members of this genus are rarely involved in human disease. The most relevant species in the clinical setting, *O. gallopava*, was recently transferred to *Verruconis*. Recently, Seyedmousavi et al. (64) evaluated the antifungal susceptibilities of numerous strains of *Verruconis* and *Ochroconis* spp. from clinical and environmental origins against eight antifungal drugs, using the broth microdilution test. In that study, the isolates of *V. gallopava* showed low MICs for AFG, PSC, ITC, AMB, CFG, and VRC, while 5-flucytosine and fluconazole showed no activity. In contrast, only AFG, PSC, and CFG showed good *in vitro* activity against *O. mirabilis*, the most frequently isolated *Ochroconis* species from clinical sources in that study (64). These results are in disagreement with our data, for which these drugs demonstrated high MICs. TBF and MFG, which were not tested by Seyedmousavi et al. (64), were the only drugs with some activity against *O. mirabilis*. These differences in the data between those of Seyedmousavi et al. (64) and our study might be explained by the different origins of the *Ochroconis* isolates tested (Europe versus the United States) but also by differences in the procedure, i.e., the incubation temperature (25°C versus 30°C) and the methodology or criteria used to read the endpoint.

There are few clinical cases reported in the literature regarding *Ochroconis* infections. Mancini and McGinnis (65) described a case of pulmonary abscess by *O. constricta* in a heart transplant recipient. The patient was successfully treated with systemic AMB, resulting in the resolution of clinical symptoms and a cavitary lesion. Recently, Ge et al. (23) reported a human case of subcutaneous phaeohyphomycosis in an immunocompetent patient due to *Ochroconis tshawytschae*. Several short courses of treatment with ITC or TBF were begun, but no cure was obtained. A subcutaneous infection by *O. humicola* in a cat was reported by VanSteenhouse et al. (66). The fungus was recovered from a granulomatous lesion, and although no antifungal test was performed, the cat was successfully treated with ketoconazole.

Despite the fact that *V. gallopava* is the species most frequently implicated in the clinical setting, the repeated isolation of several species of *Ochroconis* in the clinical setting never before reported suggests their potential pathogenic role and deserves further research.

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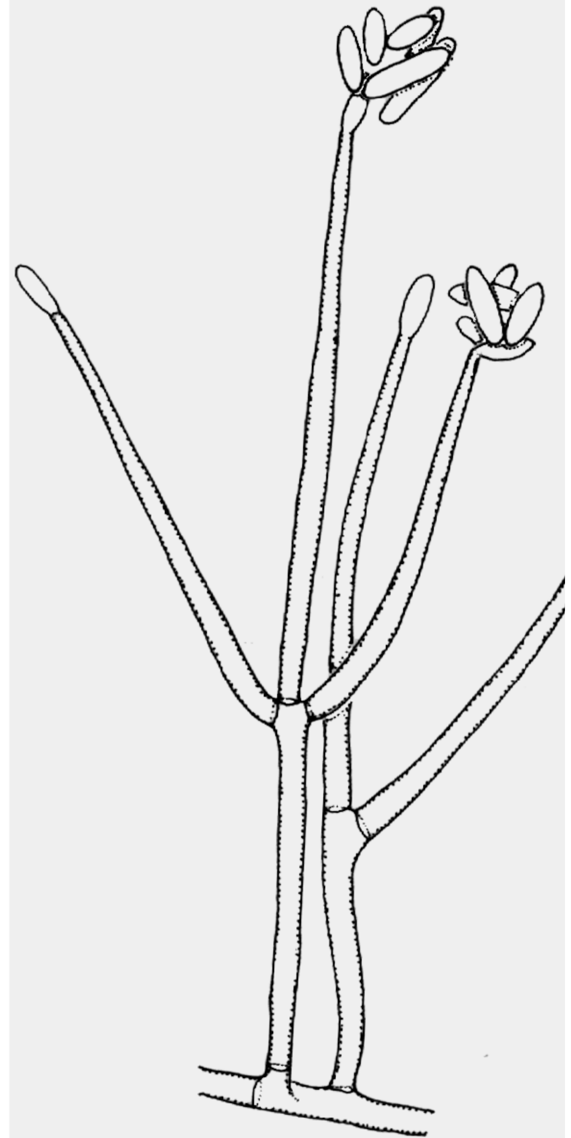
5. SUMMARIZING DISCUSSION



Sarocladium zeae
Hand draw by J. Gené

UNIVERSITAT ROVIRA I VIRGILI
TAXONOMIC STUDY OF CLINICAL AND ENVIRONMENTAL ISOLATES OF ARTHROCONIDIAL, ACREMONIUM-LIKE AND OCHROCONIS-LIKE FUNGI
Dixie Alejandra Giraldo López
Dipòsit Legal: T 767-2015

In the last decades, the number of fungal species reported as causal agents of opportunistic infections has considerably increased, mainly responding to the growing number of immunocompromised persons but also to the availability of new tools to detect fungi. With the use of methods such as the DNA sequencing, fungal species never before associated with human or animal infections are being identified and reported very often. Due to the fact that some of these fungi have been traditionally regarded as contaminants, as well as the fact of their non-demonstrated pathogenic role in all cases, the information about them and the diseases they can cause is scarce. This thesis is mainly focused on the study of different hyphomycetes genera, with a complex or confused taxonomy and with species occasionally reported as opportunistic pathogens. We have studied not only a large number of clinical isolates, but also numerous isolates from environmental sources, mainly soil, using morphological and molecular tools, which have allowed us to characterize and describe a considerable number of new species, contributing clearly to the knowledge of the fungal biodiversity, as well as to extend in many cases the spectrum of fungal species never before associated in the clinical setting.

As it was previously mentioned, the study of clinical isolates with modern techniques has allowed us to identify species never reported before from clinical specimens, i.e., *Arthropsis hispanica, Ochroconis cordanae, Sarocladium terricola* or *Scytalidium cuboideum;* as well as to describe several new species of *Acremonium, Arthrographis*, *Ochroconis,* and *Sarocladium* (Giraldo et al. 2013, 2014abc, 2015). Most of the animal and human infections involving these genera are attributed to *A. kalrae, S. kiliense* or *V. gallopava* (Guarro et al. 1997, Yarita et al. 2007, Das et al. 2010, Sugiura & Hironaga 2010, de Hoog et al. 2011, Boan et al. 2012, Meriden et al. 2012, Qureshi et al. 2012, Fernández-Silva et al. 2013, 2014a; Ramli et al. 2013). However, we have found that the range of species of these genera in samples from clinical origin is considerably wider than what was previously reported by the literature. This is because in most of the published studies, the identification of the causal agent was carried out only through morphological examination of the isolates (Doty & Slater 1946, Ross & Yasutake 1973, Weitzman et al. 1983, VanSteenhouse et al. 1988, Mancini & McGinnis 1992, Schaumann & Priebe 1994, Degavre et al. 1997, Perlman & Binns 1997, Chin-Hong et al. 2001, Biser et al. 2004, Domsch et al. 2007, Vollekovà et al. 2008, Thomas et al. 2011, Ramli et al. 2013), and in many occasions only performed at generic level (Gupta & Nakrieko et al. 2014, Sayyad et al. 2014). We know that the microbiological diagnostic of fungal species is really problematic, due to the difficulties in recognizing fungi of clinical relevance by morphological features, and also to the constant taxonomic changes that numerous taxa associated to human or animal infections suffer as a result of the study of fungi by molecular methods. For example, opportunistic species such as *Acremonium kiliense* and *A. strictum* are now

members of *Sarocladium*, a genus traditionally considered as a plant pathogen (Summerbell et al. 2011), or in the case of *Scytalidum dimidiatum*, *Natrassia mangifera* and *O. gallopava* are currently members of the recently created genera *Neoscytalidium*, *Neofusicoccum* and *Verruconis*, respectively (Crous et al. 2006, Phillips et al. 2013, Samerpitak et al. 2014). We hope that these changes that now appear to be chaos will be in a near future a reflection of monophyletic groups, and they contribute to a more stable and natural taxonomy.

The use of DNA sequencing has helped us to resolve the identity of previously unidentified clinical isolates of *Acremonium* (Perdomo et al. 2011b), as well as to re-identify numerous isolates from different culture collections received as representatives of different *Acremonium* species (Giraldo et al. 2014a, 2015). It is noteworthy that most of the species currently known in the genus *Acremonium* has been identified by different authors only on the basis of phenotypic features (Gams 1971, 1975; Hawksworth 1979, Okada et al. 1993, Alfaro-García et al. 1996, Lin et al. 2004). In *Acremonium*, like in other genera with simple conidiogenous structures such as *Sarocladium, Simplicillium, Verticillium, Fusarium*, *Cercospora*, *Ramularia,* or *Cladosporium*, their morphospecies usually include two or more phylogenetic species which can be recognized only by sequencing (O'Donnell 2000, Zare & Gams 2001, O'Donnell et al. 2004, Crous et al. 2007, Bensch et al. 2010, Inderbitzin et al. 2011, Summerbell et al. 2011, Groenewald et al. 2013, Videira et al. 2015). In our studies, apart from characterizing several new *Acremonium* species from clinical samples, we have detected numerous interesting acremonium-like fungi from environmental sources, mainly soil. In order to increase the number of isolates with acremonium-like morphology and to compare them with similar isolates but from clinical origin, and in the context of the project on "Hifomicetes de la Península Ibérica", we have examined numerous soil samples (including sediments) collected in different areas from Spain. Within those samples, three new *Acremonium* species have been found, i.e., *A. asperulatum, A. variecolor* and *A. moniliforme,* as well as two new genera *Acremoniopsis* and *Collarina*. These findings demonstrate once again, and support those of other authors (Gams 1971, 1975; Ulfig 1992, Gené 1994, Watanabe 2002, Cannon & Kirk 2007, Domsch et al. 2007, Seifert et al. 2011), that the soil is a good source to find and discover potential new species of this genus, or other acremonium-like fungi. These kind of studies, where different species are described from environmental sources and contribute to increase the number of sequences of new taxa in public databases, become truly useful to other researchers to identify the same species or similar fungi but recovered from other sources. An example is *A. variecolor,* a species described during our study (Giraldo et al. 2012), and recently found as an endophyte of *Pinus thunbergii* roots in Korea (Min et al. 2014).

In the case of the arthroconidial isolates studied, something similar has happened. The results of the fungal identification through DNA sequencing have been different in many

occasions to that obtained exclusively using morphological data. Several isolates received as *Arthrographis* sp. have been re-identified as *A. kalrae*, some recognized as new species of the genus (*A. chlamydospora, A. curvata, A. globosa* and *A. longispora*) and others belonging to other genera such as *Arthropsis* and *Scytalidium* (Giraldo et al. 2013, 2014b). Although the genus *Athrographis* encompasses several species, only *A. kalrae* has been reported as human pathogen (Volleková et al. 2008, Sugiura & Hironaga 2010, Boan et al. 2012, Ramli et al. 2013). It is worth mentioning that the morphological identification of *A. kalrae* is not easy. Occasionally, misidentification of this species can occur, particularly when young cultures with yeast-like appearance are examined, which can be confused with other clinically relevant genera such as *Candida* or another yeast-like fungus (Sigler 2003, Thomas et al. 2011). In addition, during our study we could observe some isolates with morphological features never attributed to *A. kalrae*, such as dark brown colonies, conidiophores forming whorls, or intercalary or terminal chlamydospores (Giraldo et al. 2014b). Only the sequencing of their ITS regions, and the actin and chitin synthase genes allowed us to confirm the identity of such atypical isolates as *A. kalrae*. By contrast, other isolates morphologically similar to *A. kalrae,* including its sexual morph *Eremomyces langeronii,* were phylogenetically distant from the type strain of such species, demonstrating therefore they are different taxa (Giraldo et al. 2014b). Regarding *Arthropsis,* it is a poorly known genus, up to date with species only described from environmental sources (leaf litter, grass and marine sediments). The few studies existing on the genus are those where new taxa have been proposed (Sigler et al. 1982, Sigler & Carmichael 1983, van Oorschot & de Hoog 1984, Ulfig et al. 1995). The fact that the clinical isolates of *A. hispanica* included in our studies were received as *Arthrographis* sp., suggests that probably this species has been under diagnosed in the clinical setting.

The infections caused by *Scytalidium* are mostly attributed to *S. dimidiatum,* now named *N. dimidiatum* (Xavier et al. 2010, Machouart et al. 2013, Dunlap et al. 2015), and in less proportion to *S. lignicola* (Dickinson et al. 1983, Potekaev et al. 1988, Costa et al. 1989), *S. infestans* and *S. japonicum* (Cambuim et al. 2011, de Hoog et al. 2011). We have identified other *Scytalidium* species from clinical samples, *S. cuboideum.* Up to date, the only clinical isolation of this species published in the literature was found in a sputum sample. Nevertheless, it was not considered a causal agent of infection due to the habitat associated with the species (rotten wood) and to the lack of published cases of human diseases caused by this species at that time (Pounder et al. 2007).

As in the other genera treated earlier in this thesis, the morphological features in ochroconis-like fungi are quite similar among their species, which commonly lead to misidentified isolates. In fact, many of the ochroconis-like fungi recently described, *O. cordanae, O. mirabilis, O. macrozamiae* and *Scolecobasidium musae,* were previously

identified as *O. humicola* or *O. constricta* (Hao et al. 2013, Samerpitak et al. 2014, Crous et al. 2014b), and recent reports attributed to these two latter species (Kaur et al. 2014, Yew et al. 2014) actually correspond to misidentified isolates of *O. mirabilis*. Similar results however were also observed in our study (Giraldo et al. 2014c), because most of the isolates received as *O. constricta* or *O. humicola* were reidentified as *O. mirabilis*, *O. cordanae* or described as new species, *O. ramosa*, *O. olivacea* or *O. icarus.* Additionally, like in *A. kalrae*, the use of different loci allowed us to confirm the identity of atypical isolates of *V. gallopava*, which would have been considered as a different morphospecies only using phenotypic features.

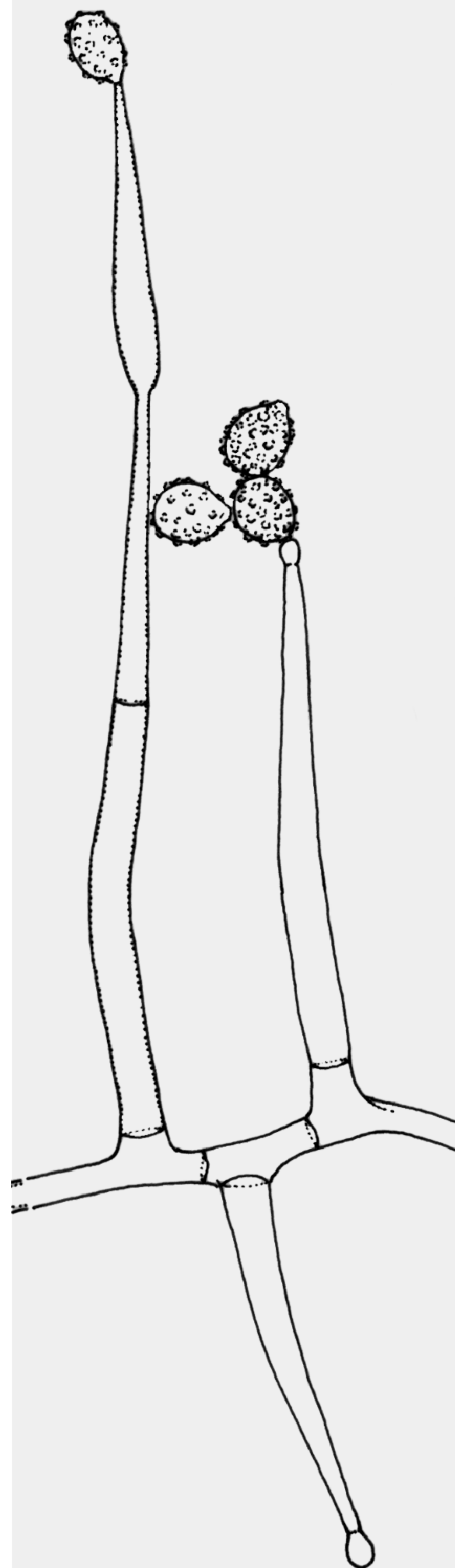
Due to the fact that the phenotypic features are strongly affected by environmental conditions, and that species diagnosed exclusively on the basis of phenotypic features can be phylogenetically diverse (Taylor et al. 2000, Seifert et al. 2011), our studies demonstrate once again that morphological data by itself are insufficient to separate closely related species, and therefore confirm the importance of DNA sequencing for fungal species delimitation. Although in our studies the sequencing of the ITS region and even more conserved regions as LSU were useful for species identification, the multilocus sequence analyses are suitable to explore with a deeper resolution the phylogenetic relationships among apparently related fungal genera. In our case, we have demonstrated that several *Acremonium* species are phylogenetically related with *Sarocladium,* or that they belong to genera quite distant such as those placed in *Plectosphaerellaceae*. In the case of *Arthrographis* and *Arthropsis,* despite the overlapped morphological characteristics among some of their species, we have demonstrated that both genera are phylogenetically unrelated and they are polyphyletic (Giraldo el al. 2014b). However, more studies are needed to accommodate their species in monophyletic genera, in order to contribute to a more natural taxonomy of these fungi, as it has occurred in other genera (Zare & Gams 2001, Zare et al. 2001, 2007; Crous et al. 2006, 2007; Gräfenhan et al. 2011, Cannon et al. 2012, Herrera et al. 2013, Perdomo et al. 2013, Phillips et al. 2013, Woudenberg et al. 2013, Samerpitak et al. 2014).

Because the infections caused by species of some genera treated in this thesis are rarely reported, data about *in vitro* antifungal susceptibility of these fungi are scarce and scattered in the published literature. Due to the antifungal activity of several drugs against acremonium-like fungi was previously determined (Perdomo et al. 2011b), in this thesis, we have focused on the species of *Ochroconis*, *Arthrographis*, *Arthropsis* and *Scytalidium*. In general, terbinafine was the most active drug against the evaluated species, except in *S. cuboideum* that showed a good response only to azoles, mainly posaconazol. Despite the fact that the optimal treatment for infections caused by these fungi is not well defined (Machouart et al. 2013), and the results of the *in vitro* activity do not always correlate with

the *in vivo* efficacy (Rodríguez et al. 2010, Calvo et al. 2012, Salas et al. 2012, Chowdhary et al. 2014, Fernández-Silva et al. 2014b), these kind of studies, where a number of isolates per species is tested against several antifungal drugs, are an important step to guide clinicians in the treatment of future infections caused by these fungi.

Although the real role of some species as a causal agent of infection was not proven in any of our studies, they could be considered as potential opportunistic pathogens due to their repeated recovering from several different anatomical sites, including deep tissue samples, or at least they must be taken into account at the moment of the species level identification of the genera treated here. Our results can be a starting point for future studies on epidemiology, pathogenicity and susceptibility of the novel species described here, as it has occurred in other genera such as *Scedosporium, Coccidioides* G.W. Stiles*, Trichoderma, Curvularia, Lecythophora, Phialemonium, Sporothrix, Alternaria* (Fisher et al. 2002, Gilgado et al. 2005, Marimon et al. 2007, 2008; Perdomo et al. 2013, Woudenberg et al. 2013, Madrid et al. 2014, Sandoval-Denis et al. 2014), where species newly described such as *Scedosporium aurantiacum* Gilgado, Cano, Gené & Guarro*, Sporothrix brasiliensis* Marimon, Gené, Cano & Guarro*,* S. *globosa* Marimon, Cano, Gené, Deanna A. Sutton, H. Kawas. & Guarro, *S. luriei* (Ajello & Kaplan) Marimon, Gené, Cano & Guarro*, Coccidioides posadasii* M.C. Fisher, G.L. Koenig, T.J. White & J.W. Taylor or *Phialemoniopsis ocularis* (Gené & Guarro) Perdomo, Dania García, Gené & Guarro, have been recently associated with human and animal infections and their role as pathogen have been already demonstrated. Also, they have been the subject of different epidemiological, pathogenicity and susceptibility studies (Kooijman et al. 2007, Ramani & Chaturvedi 2007, Fernández-Silva et al. 2012, Diab et al. 2013, Oliveira et al. 2013, Desoubeaux et al. 2014, Rodrigues et al. 2014).

6. CONCLUSIONS



Acremonium sp.
Hand draw by J. Gené

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The polyphasic approach on acremonium-like fungi from environmental and clinical sources has allowed us:

1. To propose four new genera: *Brunneomyces* and *Cervusimilis* for the family *Plectosphaerellaceae*, and *Acremoniopsis* and *Collarina* belonging to the order *Hypocreales.* In addition, the following new taxa are described in these genera: *Acremoniopsis suttonii, Brunneomyces brunnescens, B. hominis* and *B. europaeus*, *Cervusimilis alba*, and *Collarina aurantica.*

2. To propose seven new species in the genus *Acremonium*: *A. asperulatum, A. citrinum, A. dimorphosporum*, *A. moniliforme, A. parvum, A. pilosum* and *A. variecolor.*

3. To propose six new species in the genus *Sarocladium*: *S. bifurcatum, S. gamsii, S. hominis, S. pseudostrictum, S. subulatum* and *S. summerbellii.*

4. To demonstrate that *Acremonium implicatum* and *Acremonium terricola* are different species, being included in the genus *Sarocladium* as *S. implicatum* and *S. terricola,* respectively.

5. To designate an epitype for the type species of *Sarocladium, S. oryzae*, in order to stabilize the taxonomy of this genus, as well as to select a neotype for *S. implicatum*.

The polyphasic approach on the set of clinical isolates of arthroconidial fungi from USA has allowed us:

1. To identify most isolates as *Arthrographis kalrae,* confirming the relevance of this species in the clinical setting from that country.

2. To extend the list of potential opportunistic arthroconidial fungi with four new species of *Arthrographis sensu stricto*, namely *A. chlamydospora*, *A. curvata, A. globosa* and *A. longispora,* and also with *Arthropsis hispanica* and *Scytalidium cuboideum,* two species only known so far from environmental sources.

3. To determine that *Arthrographis* and *Arthropsis* are phylogenetically unrelated, and both are polyphyletic, with species scattered in different orders and classes within *Ascomycota*

4. To demonstrate that *Arthrographis kalrae* and *Eremomyces langeronii* previously considered the asexual and sexual morphs, respectively, of the same species are not conspecific. Therefore, in order to avoid nomenclatural confusion for the second taxon, the new name *Arthrographis arxii* was proposed.

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5. To conclude that the family *Eremomycetaceae* is circumscribed to the members of *Arthrographis sensu stricto*, *Eremomyces bilateralis* and *Rhexothecium globosum*.

The phenotypic studies and phylogenetic analyses on clinical isolates of ochroconis-like fungi from USA have showed that:

1. *Verruconis gallopava* is the most prevalent species among isolates, followed by *Ochroconis mirabilis*.

2. *Ochroconis cordanae* is described for the first time in the clinical setting.

3. Among the studied isolates of the genus *Ochroconis* there were three cryptic species which have been described as new to science, viz. *O. olivacea* and *O. ramosa* from clinical specimens and *O. icarus* from environmental origin.

From the studies on *in vitro* antifungal susceptibility, we conclude that:

1. For *Arthrographis kalrae,* terbinafine has the highest *in vitro* activity, followed by the azoles, especially posaconazole.

2. Among azoles, posaconazole showed the best *in vitro* activity against the five clinical isolates of *Scytalidium cuboideum*, in contrast to the echinocandines and terbinafine which have poor activity.

3. In general, all the antifungals tested against the four clinical isolates of *Arthropsis hispanica* show low MIC/MEC values, terbinafine being the most active drug, followed by the azoles.

4. Terbinafine and micafungin are the most active drugs against *Ochroconis* species.

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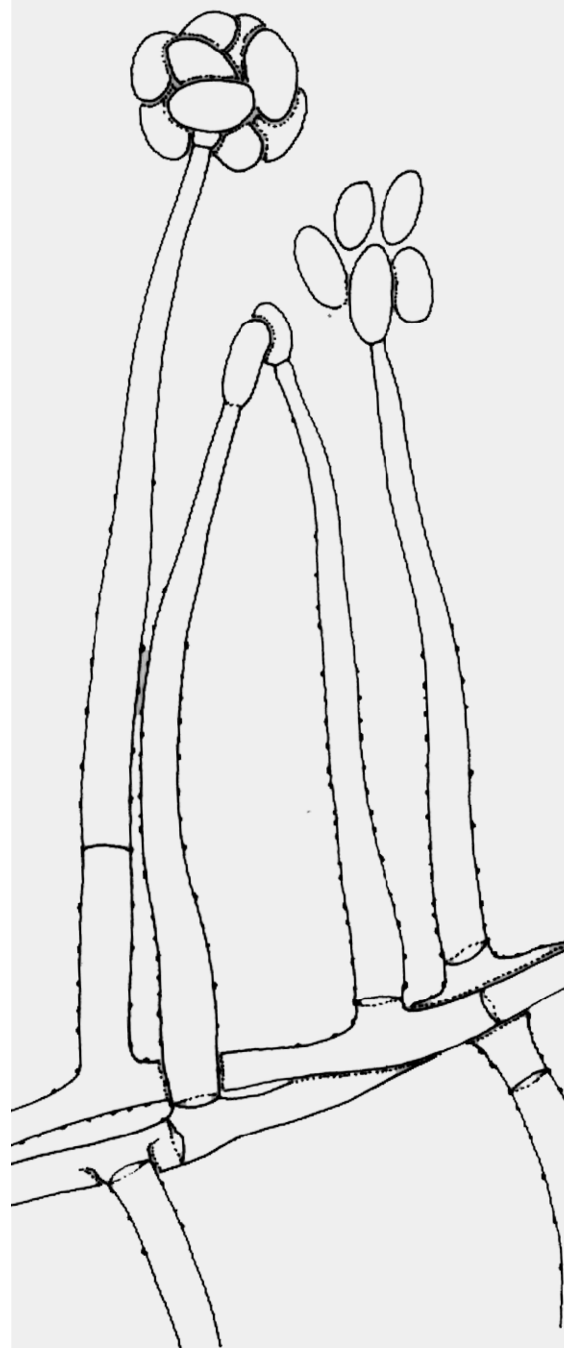

Acremonium domschii
Hand draw by J. Gené

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UNIVERSITAT ROVIRA I VIRGILI
TAXONOMIC STUDY OF CLINICAL AND ENVIRONMENTAL ISOLATES OF ARTHROCONIDIAL, ACREMONIUM-LIKE AND OCHROCONIS-LIKE FUNGI
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Front and back cover.
Acremonium pilosum dimorphic conidia: clavate with smooth walls and globose with abundant filiform projections