MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF ANAMORPHIC FUNGI

Hugo Madrid Lorca

Dipòsit Legal: T. 1355-2011

UNIVERSITAT ROVIRA I VIRGILI

Molecular and Phenotypic Characterization of Anamorphic Fungi

Hugo Madrid Lorca

Doctoral Thesis

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

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

Reus

2011

UNIVERSITAT ROVIRA I VIRGILI

Departament de Ciències Mèdiques Bàsiques

Carrer Sant Llorenç 21
43201. Reus
Tel: 977759341

Drs. Josep Guarro Artigas, Josep Cano Lira and Josepa Gené Díaz, professors of the *Departament de Ciències Mèdiques Bàsiques*, *Facultat de Medicina i Ciències de la Salut*, *Universitat Rovira i Virgili*

CERTIFY THAT:

The present work entitled "Molecular and Phenotypic Characterization of Anamorphic Fungi" presented by Hugo Madrid Lorca to obtain the degree of doctor by Universitat Rovira i Virgili, has been carried out under their supervision at the *Unitat de Micologia i Microbiologia Ambiental, Departament de Ciències Mèdiques Bàsiques*, and that it fulfils the requirements to obtain the European Doctorate Mention.

Reus, March 23, 2011

Josep Guarro Artigas Josep Cano Lira Josepa Gené Díaz

UNIVERSITAT ROVIRA I VIRGILI
MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF ANAMORPHIC FUNGI
Hugo Madrid Lorca
DL: T. 1355-2011

To those who live and breathe mycology

Acknowledgments

I would like to show my gratitude to Drs. Josep Guarro, Josepa Gené, Josep Cano and Alberto Stchigel for giving me the opportunity to work with them. I feel lucky to have worked in such a prestigious center. Thanks for the mycological lessons and for giving me the chance to work in fungal biodiversity!

I am heartily thankful to Drs. Victor Silva and Eduardo Piontelli for guidance and support years ago, when I started working in microbiology. Your enthusiasm breathes life into Chilean mycology!

I am grateful to Dr. Sybren de Hoog (CBS; Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands) for accepting me in his research group for 3 months and for his patience, encouragement and sense of humor. I also want to express my gratitude to other people who helped me during my stay at the CBS: Manon Verweij, Tineke van den Berg-Visser, Bert Gerrits van den Ende, Kasper Luijsterburg, Mohammad Javad Najafzadeh, Stephanie Miesch, and Qiaoyun Lu. I am thankful to Dr. Estibaliz Mateo Alesanco for her continuous support during my stay in Utrecht.

I appreciate the company and help of my colleagues at Universitat Rovira i Virgili: Eduardo Alvarez, Haybrig Perdomo, Margarita Hernandez, Fabiola Fernández, Valentina Salas and Carolina Silvera. Most of the micrographs used in the introduction of this thesis were kindly provided by M. Hernández and C. Silvera. I am also grateful to Cati Nuñez and Nuria Pilas for their technical assistance.

I owe my deepest gratitude to the people who have given me true love, respect, support and true friendship during these years. Like nature, life is cyclic: all the good you do will come back to you.

INDEX

Page

ATCC	American Type Culture Collection, Bethesda, USA
BAFC	Universidad de Buenos Aires Herbarium
BAYER	Bayer Healthcare, Wupertal, Germany
BLAST	Basic Local Alignment Search Tool
bp	base pair(s)
BPI	Systematic Botany and Mycology Laboratory, Beltsville, USA
BRIP	Queensland Department of Primary Industries Plant Pathology Herbarium, Brisbane, Australia
BT2	a fragment of the β-tubulin gene
Buck	William Buck, Jacksonville, Florida
ca.	*circa*, Latin word meaning "around","about" or "approximately"
CAL	calmodulin gene
CBS	Centraalbureau voor Schimmelcultures, Utrecht, Netherlands
CBSC	Carolina Biological Supply Company, Burlington, USA
CI	consistency index
CMA	corn meal agar
CMW	culture collection of the Forestry and Agricultural Biotechnology Institute, Pretoria, South Africa
comb. nov.	*combinatio nova*, Latin expression meaning "new combination"
d	day(s)
D1/D2	D1/D2 variable domain of the 28S rRNA gene
DAOM	National Mycological Herbarium, Ottawa, Canada
DNA	deoxyribonucleic acid
DRBC	dichloran rose Bengal chloramphenicol agar
e.g.	*exempli gratia,* Latin expression meaning "for example"

EMBL	European Molecular Biology Laboratory
et al.	*et alii* (Latin expression meaning "and others")
etc.	*et cetera*, Latin expression meaning "and the rest (of such things)"
F	Field Museum of Natural History, Chicago, USA
fam. nov.	*familia nova*, Latin expression meaning “new family”
Fig.	figure
FMR	Faculty of Medicine culture collection, Universitat Rovira i Virgili, Reus, Spain
g	gram(s)
gen. nov.	*genus novus*, Latin expression meaning “new genus”
HI	homoplasy index
HKI	Hans-Knöll Institute, Jena, Germany
ICBN	International Code of Botanical Nomenclature
i.e.	*id est*, Latin expression meaning "that is" or “namely”
IHEM	The Belgian Coordinated Collections of Microorganisms/ Institut d'Hygiène et d'Epidémiologie, section de Mycologie, Biomedical Fungi and Yeasts Collection, Brussels, Belgium
IISPV	Institut d`Investigació Sanitaria Pere Virgili
ILD	incongruence length difference test
IMI	International Mycological Institute, Kew, United Kingdom
IPEC	Instituto de Pesquisa Clínica Evandro Chagas, Rio de Janeiro, Brazil
ITS	internal transcribed spacer
J.F.	Jacques Fourier, Las Muros, France
kb	kilobase(s)

KMU	Kanazawa Medical University culture collection, Ishikawa, Japan
L	liter(s)
m	meter(s)
MCCL	Mycology Culture Collection Laboratory, Postgraduate Institute of Medical Education and Research, Chandigarh, India
MEA	malt extract agar
MEGA	Molecular Evolutionary Genetics Analysis
mL	mililiter(s)
mm	milimeter(s)
mo	month(s)
MP	maximum parsimony
MUCL	Mycothèque de L`Université Catholique de Louvain, Faculté des Sciences Agronomiques, Louvain-la-Neuve, Belgium
N	number
NA	not available
NBRC	Biological Resource Center, Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan
NITE	National Institute of Technology and Evaluation, Tsukuba, Japan
NJ	neighbor-joining
NT	neotype strain
OA	oatmeal agar
ºC	Celsius degrees
PAUP*	Phylogenetic Analysis Using Parsimony (*and other methods)
PCA	potato carrot agar
PCR	polymerase chain reaction

PDA	potato dextrose agar
rDNA	ribosomal DNA
RI	retention index
RNA	ribonucleic acid
rRNA	ribosomal RNA
s.l.	*sensu lato* (Latin expression meaning "in a broad sense")
S.M.H.	Sabine M. Huhndorf, Field Museum, Chicago, USA
s.n.	*sine nomine*, Latin expression meaning "without a name"
s.str.	*sensu stricto* (Latin expression meaning "in a strict sense")
sp. nov.	*species nova*, Latin expression meaning "new species"
sp., spp.	species
T	type strain
TENN	University of Tennessee, USA
URL	Uniform Resource Locator
UTHSC	Fungus Testing Laboratory, University of Texas Health Science Center, Texas, USA
vers.	version
vs	*versus*, Latin word meaning "against"
Wang	Yei Zei-Zeng Wang, Hong Kong University, China
wk	week(s)
y	year(s)
µg	microgram(s)
µL	microliter(s)

UNIVERSITAT ROVIRA I VIRGILI
MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF ANAMORPHIC FUNGI
Hugo Madrid Lorca
DL: T. 1355-2011

1. INTRODUCTION

1. INTRODUCTION

1.1 The kingdom *Fungi*: characteristics, ecology, and relevance to man

The kingdom *Fungi* includes an assemblage of heterotrophic eukaryotes characterized by cell walls rich in chitin and β-glucan, absorptive nutrition, and uni- to multicellular thalli (Kirk et al. 2008). Fungi with an unicellular thallus composed of budding elements are known as "yeasts", whereas those with a multicellular thallus formed by filaments called "hyphae" are generically termed "filamentous fungi" (Kwon-Chung and Bennett 1992). Fungi usually reproduce by means of spores and can be found in a variety of habitats that range from nutrient-rich substrata such as humus-enriched soil, spoiled food, plants and animals (Santamaría 1989, Calduch et al. 2004, Crous et al. 2007c), to harsh environments such as hypersaline water (Zalar et al. 1999), desert rocks (Staley et al. 1982, Selbmann et al. 2005) and hot springs (Yamazaki et al. 2010).

Fungi are fundamental for the maintenance of ecosystems due to: i) their ability to decompose organic material of plant and animal origin (van Oorschot 1980); ii) their role as mutualists of algae (Honegger 1991), plants (Quilambo 2003), and animals (Ho et al. 2000, Palma et al. 2005), and iii) their capacity to down-regulate populations of other organisms by parasitism (Zhang et al. 2004, Silva et al. 2007), depredation (Duddington 1951, Drechsler 1960), and by the production of toxic, inhibitory metabolites (Fleming 1929).

Fungi influence humans and human-related activities in many ways. Many cultivated and wild species of mushrooms are consumed by humans by their nutritional or medicinal properties (Singer 1964, Lo et al. 2004). The metabolic activities of fungi are employed in baking, brewing, cheesemaking, winemaking and in the preparation of other fermented foods (Andersen 1995, Kirk et al. 2008). In addition, fungal secondary

metabolites are used in the production of antibiotics such as cephalosporins and penicillins, as well as immunomodulatory and cholesterol-lowering drugs (Dreyfuss et al. 1976, Alberts 1988). Furthermore, fungi are widely used in the production of organic acids and enzymes with industrial applications (Pandey et al. 1999, Magnuson and Lasure 2004). Some studies also have demonstrated the ability of certain fungal species to degrade cyanides (Ezzi and Lynch 2005), and other inorganic and organic pollutants (Sanyal et al. 2005, D`Annibale et al. 2006).

More than 400 fungal species have been reported from human infections thus far, including a relatively small group of true pathogens and a long list of opportunists that increases every year (de Hoog et al. 2000, Guarro et al. 2002, Ferrer et al. 2009). In addition, several taxa are known to produce carcinogenic compounds (Marasas 1995), as well as toxic metabolites that can cause mild to serious intoxications in humans and other animals (Maresca and Fantini 2010). Fungi are also known to be powerful agents of biodeterioration of paper (Pinzari et al. 2006), wood (de Hoog 1974), stone (Cappitelli et al. 2007) and other substrata. Mycotic diseases of plants cause enormous economic losses every year and affect a variety of crops, including fruits, cereals, edible mushrooms, and trees of relevance in the wood industry (Gea 1993, Crous 2009).

1.2 The fungal life cycle

In *Fungi*, two morphologically different reproductive modes can be observed, i.e. sexual and asexual, which give rise to meiospores and mitospores, respectively (Fig. 1). Meiospores usually result from outcrossing and generate descendants with genes rearranged into new genotypes. On the other hand, mitospores propagate a progeny genetically identical to the parental (Seifert and Samuels 2000). The sexual and asexual stages of a fungus are called teleomorph and anamorph, respectively. The term holomorph refers to both stages, to “the whole fungus” (Kirk et al. 2008). Teleomorph

and anamorph of a taxon may appear together in nature or in culture (Stalpers et al. 1991), but depending on growth conditions, only one of them may be observed (Cáceres et al. 2006). In addition, certain fungal species are able to produce two or more different types of asexual stages which are called "synanamorphs" (von Arx 1985, de Hoog et al. 1995). Fungi which exhibit different reproductive stages are said to be "pleomorphic". Nevertheless, numerous fungal species seem to reproduce only sexually (Walther et al. 2005) or asexually (Gräser et al. 1999, 2006). Moreover, some fungi are apparently unable to produce spores in culture (Hambleton and Sigler 2005).

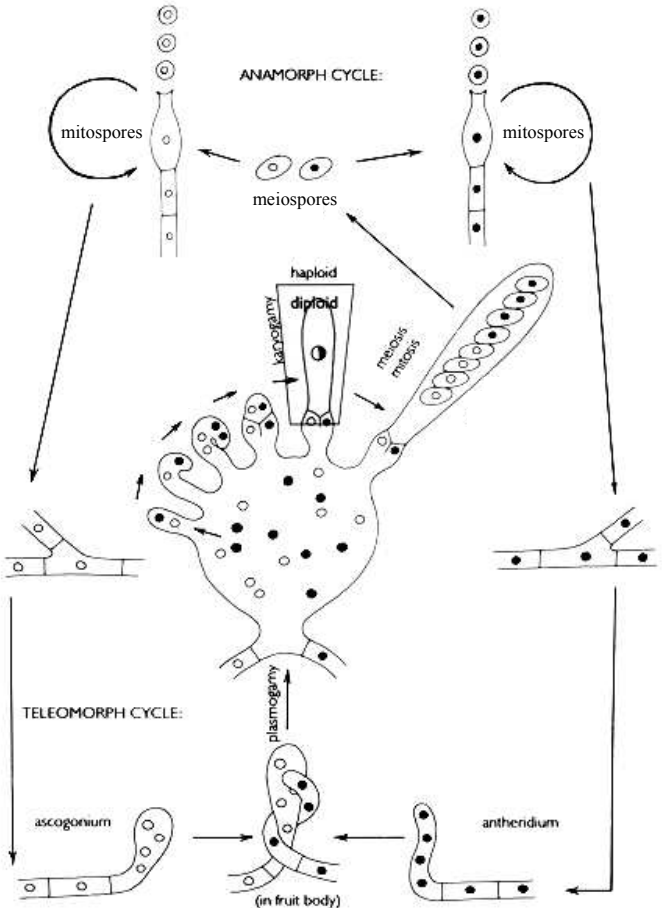

Fig. 1. Fungal life cycle (adapted from de Hoog et al. 2000). The sexual and asexual phases are shown in the lower and upper parts of the picture, respectively. In this example two compatible thalli give rise to specialized sexual organs called ascogonium and antheridium, which come into contact and undergo plasmogamy. Subsequently, karyogamy and meiosis occur and meiospores are produced, which give rise to new mycelia that are able to propagate by mitospores and to repeat the cycle.

1.3 Fungal taxonomy and nomenclature

Early naturalists classified fungi in kingdom *Plantae* (Philippi 1860, Briquet 1912). However, *Fungi* is currently considered a separate kingdom (Whittaker 1969, Kirk et al. 2008), which was proven to be evolutionarily closer to animals than to plants (Shenoy et al. 2007). The traditional taxonomy of fungi was based on morphological features and gave emphasis to the teleomorphs. For example, in the seventh edition of Ainsworth & Bisby`s Dictionary of the Fungi (Hawksworth et al. 1983), *Fungi* was divided into two divisions, i.e. 1) *Myxomycota* (slime molds, which include an amoeboid, phagocytic phase during their life cycle), and 2) *Eumycota* (true fungi, which lack an amoeboid stage and have absorptive nutrition). *Eumycota* was classified into five subdivisions, i.e. i) *Ascomycotina*, characterized by producing meiospores within a sac-like cell, called "ascus"; ii) *Basidiomycotina*, with meiospores produced externally on cells called "basidia"; iii) *Mastigomycotina*, recognized by the production of motile, flagellate meiospores; iv) *Zygomycotina*, an heterogeneous group with mostly non-septate hyphae and thick-walled meiospores called zygospores, and v) *Deuteromycotina*, which included fungi without a known sexual stage.

Over the last decades, molecular phylogenetic analyses have determined deep changes in fungal taxonomy. While *Myxomycota* was excluded from *Fungi* (Cavalier-Smith 1993), the *Microsporidia*, a group of obligate intracellular parasites, were included in this kingdom (Keeling et al. 2000, Hibbett et al. 2007). Currently, *Deuteromycotina* is not accepted as a valid taxon (Kirk et al. 2008) because many of its members are in fact either ascomycetes or basidiomycetes as revealed by ultrastructural (Saikawa et al. 1994) and molecular phylogenetic studies (de Beer et al. 2003, 2006). *Mastigomycotina* is also obsolete, with its members now included in the three segregate phyla of *Fungi*, i.e. *Chytridiomycota*, *Blastocladiomycota* and *Neocallimastigomycota*

(James et al. 2006, Hibbett et al. 2007), and in the kingdoms *Chromista* (Cooke et al. 2000) and *Protozoa* (Cavalier-Smith and Chao 2003). Important changes also occurred in the *Zygomycotina*, which was not accepted in a recent kingdom-wide phylogenetic assessment of *Fungi* by Hibbett et al. (2007). In that study, members of the *Zygomycotina* s.l. were dispersed in the segregate phylum *Glomeromycota*, and in four subphyla *incertae sedis*, i.e. *Entomophthoromycotina*, *Kickxellomycotina*, *Mucoromycotina* and *Zoopagomycotina*. In the phylogeny by Hibbett et al. (2007) the ascomycetes and basidiomycetes formed a well-supported group, for which the subkingdom *Dikarya* was erected, referring to their ability to form dikaryotic mycelia during a part of their life cycles.

Fungal taxonomy follows a Linnean hierarchy (Table 1) and is ruled by the International Code of Botanical Nomenclature (ICBN), published since 1867 with updates every International Botanical Congress (McNeill et al. 2006). The lowest main rank in the classification scheme is the "species", which is named using two italiziced words, i.e. the genus name (capitalized) and a species epithet (non-capitalized). Names of all higher taxonomic ranks consist of a single Latinized word. Most taxonomic categories above genus have a particular ending that defines their hierarchical position (Table 1). There is a consensus in using italics for genus and species names, but the names of higher ranks have been written in either italics or normal form indistinctly by different authors (Huhndorf et al. 2004, Baroni and Bocsusis 2008). Currently, in order to achieve uniformity, the ICBN recommends using italics for all taxonomic categories regardless of their hierarchical position (McNeill et al. 2006).

Table 1. Main ranks used in fungal classification[a]

Taxonomic rank	**Ending**	**Example**
Kingdom	undefined	*Fungi*
Subkingdom	undefined	*Dikarya*
Phylum	*-mycota*	*Ascomycota*
Subphylum	*-mycotina*	*Pezizomycotina*
Class	*-mycetes*	*Pezizomycetes*
Subclass	*-mycetidae*	*Pezizomycetidae*
Order	*-ales*	*Pezizales*
Family	*-aceae*	*Pezizaceae*
Genus	undefined	*Peziza*
Species	undefined	*Peziza domiciliana*

[a]Ranks according to McNeill et al. (2006) and Hibbett et al. (2007).

Each taxon is typified by an entity of a lower rank; e.g., an order by a family, a family by a genus, etc. A species is typified by a "holotype", an inactive, permanently preserved specimen usually deposited in a herbarium. In scientific publications, it is compulsory to designate a holotype and to specify its location for every new species proposed. It is usually difficult to find type material for old taxa, because it may not have been mentioned in the original description (the "protologue"). However, if only one collection was mentioned in the protologue it is automatically considered the holotype. If the holotype has been fractioned or duplicated, and deposited in different herbaria, all fragments are called "isotypes". Sometimes, different collections were mentioned in a protologue, they are called "syntypes". If the author did not specify a holotype, a subsequent author can designate a "lectotype" among the syntypes available. The remaining syntypes are known as "paratypes". If no collection was preserved of an old taxon proposed, or if the type is lost, a "neotype" can be chosen (Kirk et al 2008). When type material is available, but is inadequate for the precise application of the

name, an "epitype" can be designated (Simmons 2007, Crous et al. 2009b). In both neotypification and epitypification processes, care must be taken to choose new material that agrees with the protologue as closely as possible in morphology, substrate and locality (Hyde and Zhang 2008, Than et al. 2008).

Each taxon above species can be defined as a group of similar entities of the immediately lower rank: a genus is a group of similar species; a family is a group of similar genera, etc. However, there is much debate on how a species should be defined. Depending on the approach used, different species concepts have been proposed, i.e. morphological (phenotypic), biological and phylogenetic (Crous et al. 2009b). Morphological (phenotypic) species are recognized on the basis of similarities in anatomical (and commonly physiological) features (Ellis 1971, Kurtzman and Fell 1998). Biological species are defined as groups of actually or potentially interbreeding organisms reproductively isolated from other such groups (Mansuetus et al. 1997). Phylogenetic species are recognized on the basis of measurable differences in biochemical data, deoxyribonucleic acid (DNA) sequences, or other characters assessed using cladistic analysis (Kirk et al. 2008). These approaches are useful, but none of them works flawlessly in recognizing species because: i) phenotypic species usually comprise two or more biological (Mansuetus et al. 1997) and/or phylogenetic species (Taylor et al. 2000), ii) biological species cannot be recognized in fungi incapable of mating (Gräser et al. 2006), and iii) the delimitation of taxa based solely on the results of a cladistic analysis can be controversial (Simmons 2007). Therefore, nowadays there is a trend toward developing "polyphasic taxonomy" schemes, in which robust species delimitations are achieved using a combination of morphology, physiology (growth profilings, carbohydrate assimilation tests, etc.), and DNA sequence data (Samson and Varga 2009). These methodologies have been successfully applied in the systematics of

numerous fungal genera, e.g. *Aspergillus* P. Micheli ex Link (Hong et al. 2005, Samson and Varga 2009), *Fusarium* Link (Schmidt et al. 2004), *Penicillium* Link (Frisvad and Samson 2004), *Sporothrix* Hektoen & C.F. Perkins (Marimon et al. 2006, 2007) and *Pseudallescheria* Negr. & I. Fisch. (Gilgado et al. 2005).

The species name works as a systematic information-locating and predictive device. Once an organism has been adequately described and named, the scientific community can provide more information about its ecology, distribution, pathogenicity, evolutionary relationships, etc. The species name will work as a tag for retrieval in the published literature (Simmons 2007). Descriptions of new taxa must be published in print, and the rank of the taxonomic novelties must be indicated (fam. nov., gen. nov., sp. nov., etc.). A Latin diagnosis is also compulsory (Crous et al. 2009b). Traditionally, detailed Latin descriptions were provided (Singer 1960, Udagawa et al. 1986) but nowadays there is a trend toward writing very brief ones. In the case of several recently published new species, their brief Latin diagnoses focus on the difference(s) between the proposed taxon and a closely related one, but their morphology is described in detail in a modern language (Crous and Groenewald 2006, Crous et al. 2007a, Damm et al. 2008b). The protologue ideally should include illustrations of the new taxon, such as drawings and photographs (Seifert and Rossman 2010). Drawings may demand much time to be elaborated, but give the possibility to emphasize in diagnostic features. Morphological details which are difficult to capture in a photograph can be schematically represented, e.g. fragile chains of mitospores that disarticulate when mounts are prepared (Hugues and Sugiyama 1972, Taylor et al. 1999). However, the author may misinterpret important morphological features and these errors may be represented in the drawings (Hawksworth 1979). Therefore, it is strongly recommended to also include photographs, so that the readers can have additional information to

complement and critically evaluate species descriptions and drawings (Seifert and Rossman 2010).

A special classification system was developed for fungi in the International Botanical Congress held at Brussels in 1910 (Briquet 1912). It established a "dual nomenclature" which allows using different scientific names for the teleomorph and anamorph(s) of a given fungus. This system has been used by mycologists for decades (Hugues 1966, Ramaley 1999, Braun et al. 2003), but now has an increasing number of detractors, who argue that more than one name for the same organism creates confusion and hinders the development of a natural taxonomy (Rossman and Samuels 2005, Hawksworth 2010). The current version of the ICBN (McNeill 2006) still supports the dual system, but solves part of the problem by giving precedence to teleomorph names (art. 59.4). Furthermore, it recommends avoiding the publication and use of names for anamorphs when a teleomorph is known and there is no practical need for separate names (recommendation 59A.3). Deep changes in the ICBN are necessary and emendations to the dual system will be proposed in the next International Botanical Congress to be held in Melbourne in 2011. This topic was discussed in detail by Cline (2005), Gams (2005), Hawksworth (2010) and Rossman and Samuels (2005). In some recent papers, teleomorph genus names have been proposed for fungi known only in the asexual phase, based on their phylogenetic affinities (Damm et al. 2008a). This is a clever approach to avoid the proposal of anamorph names, but it can be used only in lineages which are clearly associated with one teleomorph genus (e.g. the *Cylindrocladium*/*Calonectria* clade, Lombard et al. 2010). However, it would be difficult to apply to numerous groups of fungi where teleomorph genera exhibit a high level of polyphyly, and where the association of teleomorph and anamorph genera is not 1:1 (Untereiner 1997, Pitt and Samson 2007, Mo et al. 2005, Zhang et al. 2010).

1.4 Taxonomy and morphology of anamorphic fungi

Traditionally, the taxonomy of anamorphs was based on morphological features which were considered prioritary, and which formed the basis for ranks such as species, genera and classes. The subdivision *Deuteromycotina* included three classes, i.e. *Blastomycetes*, *Hyphomycetes*, and *Coelomycetes*. *Blastomycetes* included fungi with a yeast thallus. The *Hyphomycetes* included species with a filamentous thallus divided into compartments by disc-like structures called "septa". These fungi can be sterile or produce specialized fertile structures called "conidiophores", and asexual spores known as "conidia". Finally, the *Coelomycetes* were characterized by the production of conidia within the cavity of specialized fruiting bodies generically called "conidiomata" (Kirk et al. 2008). This classification scheme is obviously artificial, as several species show morphological features that may place them into more than one of these classes, e.g. *Exophiala placitae* Crous & Summerell, a leaf inhabitant of *Eucalyptus placita* L.A.S. Johnson & K.D. Hill, produces conidiomata on the natural substrate, but shows mycelium, solitary conidiophores and yeast-like cells in culture (Crous et al. 2007b).

Early works on the taxonomy of hyphomycetes gave emphasis to the degree of aggregation of the conidiophores and to the colour of the vegetative hyphae, conidiophores, and conidia. Morphological features of some hyphomycetes are shown in Figs. 2–7. Saccardo (1886) divided the *Hyphomycetes* into four groups: the *Mucedineae*, the *Dematieae*, the *Tuberculariеae*, and the *Stilbeae*. The *Dematieae* and *Mucedineae* were characterized by solitary conidiophores (Figs. 2A,C–F). In the *Dematieae* the hyphae or conidia, or both were darkly pigmented (Figs. 2D–F; 3A–G), while in the *Mucedineae* they were colourless (Fig. 2A,C; 7A,B). The *Tuberculariеae* showed short conidiophores aggregated to form cushion-like structures called "sporodochia" (Figs. 5A,C). The *Stilbeae* produced special conidiogenous structures

called “synnemata”, which consisted of groups of more or less compacted, erect and sometimes fused conidiophores (Fig. 6A,B). The groups were then divided into sections based on conidial shape and septation: *Amerosporae* (conidia non-septate, globose or somewhat elongated, Figs 2A,C–F; 3A–C), *Didymosporae* (conidia two-celled, ovoid to oblong), *Phragmosporae* (conidia three- or more-celled with transverse septa, Figs. 3E,F; 5B), *Dictyosporae* (conidia divided by both transverse and longitudinal septa, Figs. 4A–D), *Scolecosporae* (conidia very slender, thread or worm-like, filiform, one- to several-celled), *Helicosporae* (conidia cylindric, curved or coiled, Fig. 6F), and *Staurosporae* (conidia star-shaped, radiate or trifurcate, one- to several-celled). Hugues (1953) proposed a new approach which gave emphasis to the method by which the conidia develop from conidiophores (conidiogenesis), and the way in which conidiophores and conidiogenous cells grow before, during and after the conidia are produced. Subsequent studies of the conidiogenesis provided new characters for the classification of anamorphs and enriched considerably the nomenclature used to describe their morphology (Subramanian 1962, Ellis 1971).

Colonies of hyphomycetes exhibit a range of colors, textures, and pigments which are useful taxonomic features (de Hoog et al. 2000). Some species also produce volatile metabolites that determine the presence of conspicuous odors (Marvanová and Stalpers 1987) or even toxic properties (Crous et al. 2007a). The **hyphae** of hyphomycetes generally have simple septa, but certain basidiomycetous anamorphs produce hyphal outgrowths called “clamp connections” (Fig. 7C) which, at cell division, make a connection between the resulting two cells (Gochenaur 1981, Kirschner and Chen 2004a). According to the degree of melanization, hyphae can be described as hyaline (colourless) (Fig. 2A,C) or dematiaceous (darkly pigmented, ranging from light brown to almost black, sometimes with olivaceous tinges) (Kirk et al.

2008) (Fig. 2D, 3A). Hyphae can have thin (Figs. 2A,C) or thick (Figs. 2D–F, 4C) cell walls and can appear quite straight or more or less wavy ("flexuous") (Mouchacca and Gams 1993). They can show a relatively regular width throughout or may appear as chains of inflated cells constricted at the septa, referred to as "moniliform hyphae" (Hugues 1970, de Hoog and Hermanides-Nijhof 1977). Hyphae can be smooth or ornamented with projections of different shape and size, ranging from minute irregularities to coarse warts (Chamuris and Wang 1990, Zalar et al. 2007). In culture, hyphae can be encrusted with colored granules (Muntañola-Cvetkovic et al. 2001) or may release mucilaginous exudates (Cheewangkoon et al. 2009) or crystal-forming metabolites (Gams 1971, Stalpers 2000). Hyphae can appear as individual branched filaments (McInnes 2003) or can be fused by the presence of anastomoses (Kirschner and Chen 2007). Sometimes hyphae form fascicles (Gams 1971), coils (Badali et al. 2008), and specialized organs called hyphopodia, which adhere to the host surface (Hawksworth 1979, Srivastava et al. 1995). In addition, modified hyphae or hyphal cells serve as trapping organs in a number of predacious microfungi that capture nematodes, amoebae and rotifers by means of "adhesive nets" (de Hoog 1985) and "adhesive knobs" (Thorn and Barron 1986, Meyer et al. 2005). A few fungal species produce specialized erect hyphae that capture wind-borne pollen grains, which are then invaded by penetration hyphae (Olivier 1978, 1983).

Conidiophores can be classified according to the degree of differentiation from the vegetative hyphae. Micronematous conidiophores are morphologically indistinguishable from the vegetative hyphae (Fig. 2A). Macronematous conidiophores are very different from vegetative hyphae and usually erect (Fig. 2E,F). Semimacronematous conidiophores differ only slightly from other hyphae; they are often ascending, but seldom erect (Ellis 1971). In most hyphomycetes the axis and

branches of conidiophores grow at the apical region: they are acroauxic (Kirk et al. 2008). When the growth of the conidiophore stops with the production of a terminal conidium, or a chain of conidia, the conidiophore and the conidiogenous cells are said to be "determinate". In some genera, however, growth continues after the production of the first conidium. The conidiophore and conidiogenous cells are then called "percurrent" (Fig. 2D). These proliferations sometimes occur at relatively long intervals, giving rise to nodose swellings at the point where the conidiophores proliferated, e.g. in *Cordana* Preuss (Ellis 1971) (Figs. 3A,B). Proliferation may also occur in a "sympodial" manner: by a succession of apices which develop behind and to one side of the previous apex as in the genera *Pleurophragmium* Constantin and *Rhinocladiella* Nannf. (Ellis 1971) (Figs. 3D,G). Some hyphomycetes have "basauxic" growth: their conidiophores consist of a mother cell and an extensible filament arising from within it. This filament elongates from the base and gives rise to conidia or sterile cells. The oldest conidia are usually found toward the apex and the youngest toward the base (Hugues 1953, Ellis 1971, Somrithipol 2007). A few genera of hyphomycetes have "meristematic" conidiophores: they elongate by the laying down of cross walls and elongation of cells behind the apex, e.g. *Coniosporium* Link (Ellis 1971, Sterflinger et al. 1999) (Fig. 4F).

Conidiogenous cells are anatomically diverse (Figs. 2–7) and their morphologies are determined in part by the conidial ontogeny concerned. Two main types of **conidiogenesis** can be distinguished: thallic and blastic. In thallic conidiogenesis there is no enlargement of the conidium initial or, when it occurs, it is observed after the initial has been delimited by a septum. Thallic conidiogenesis can occur by either a holothallic or a thallic-arthric mechanism. In holothallic conidiogenesis a segment of a hypha gives rise to a single conidium, e.g. the lateral and

terminal conidia of *Chrysosporium* Corda (de Hoog et al. 2000) (Fig. 7A). On the other hand, in thallic-arthric conidiogenesis a predetermined part of a hypha is converted into a series of conidia. Four types of thallic-arthric conidiogenesis are known: holoarthric, enteroarthric, endogenous and sarcinic. In holoarthric conidiogenesis the cells of a hypha disarticulate to form a chain of propagules called "arthroconidia" (de Hoog et al. 2000) (Fig. 7B). In the enteroarthric mode, two kinds of alternating cells are produced: one kind becomes thick-walled and serves as arthroconidium, while the other is sacrified to liberate the propagule (e.g. *Coccidioides immitis* G.W. Stiles, de Hoog et al. 2000). In endogenous conidiogenesis a mother cell forms internal wall layers that surround internal nuclei, producing several "endoconidia", which are released upon maturation by rupture of the mother cell, e.g. *Knufia* L.J. Hutchinson (Tsuneda and Currah 2004) (Figs. 7F,G). Finally, in the sarcinic mode a cell becomes larger and develops septa in different planes, becoming a multiseptate conidium (Sterflinger et al. 1999) (Fig. 4F,G). Most hyphomycetes have blastic conidiogenesis, i.e., the conidium initial undergoes enlargement before it is delimited by a septum. When both outer and inner walls of a blastic conidiogenous cell take part in the formation of conidia, such cell is called "holoblastic". When it produces conidia from a single point it is called "monoblastic", if there are more conidiogenous loci it is called "polyblastic". When only the inner wall of the conidiogenous cell or neither wall takes part in the production of conidia, such cell is called "enteroblastic". Enteroblastic conidiogenous cells are called "tretic" when they produce conidia by protrusion of the inner wall through one or more channels or "pores" in the outer wall (Kirk et al. 2008), e.g. *Pyrigemmula* D. Magyar & R. Shoemaker (Magyar et al. 2010) (Fig 5E,F). When there is only only one pore they are called "monotretic", if there are more pores they are called "polytretic" (Mercado-Sierra et al. 1997). Enteroblastic conidiogenous cells in which neither wall

contributes to the formation of conidia, and which release the conidia through one or more openings are called “phialidic” (Figs. 2A,C–F). If they have one conidiogenous opening they are “monophialidic”, if there are more openings they are “polyphialidic” (Ellis 1971). Phialidic conidiogenous cells may have a recurved tip called collarette (Kirk et al. 2008) (Figs. 2D–F), the shape of which is taxonomically informative in certain groups of hyphomycetes (de Hoog et al. 1999). In certain species with holoblastic conidiogenesis, the apex of the conidiogenous cells produce successive enteroblastic proliferations which give rise to darkened transverse bands called “annellations”. The latter kind of conidiogenous cells are known as “annellides” (Kirk et al. 2008) (Fig. 6B)

Conidia can be produced singly (Figs. 4A–D), in chains (Fig. 2D) or in mucilaginous heads (Fig. 2C). Their liberation occurs by a schizolytic or a rhexolytic mechanism. In schizolytic dehiscence, the septum delimiting the conidiogenous cell and the conidium splits so that one half of the crosswall becomes the base of the seceding conidium and the other half remains at the conidiogenous cell (Ellis 1971) (Figs. 4A,B; 7B). In rhexolytic dehiscence, an intermediate supporting cell is sacrified. Rhexolytically released conidia usually show a conspicuous frill at their base, consisting of cell wall remnants of the lysed cell (de Hoog et al. 2000) (Figs. 4C,D). Conidia can be of many different shapes: acicular, cylindrical, falcate, ellipsoid, globose, etc. (Figs. 2–7, for additional illustrations see Ellis 1971, Kirk et al. 2008 and de Hoog et al. 2000). They are usually simple (Figs 2A,D,F; 3E–G), but can be branched (Ellis 1971, Kirschner and Chen 2004b) (Fig. 5D) and vary from very small one-celled propagules (Gams 1971) to complex, multicellular structures (Goos 1970, Kirschner et al. 2010). Conidia sometimes bear appendages, which range from aseptate, delicate, hair-like, simple or branched outgrowths (Marvanová 1980, Udagawa and Toyazaki 1985,

Kuthubutheen 1987) to large, rigid or flexible multiseptate projections (Chen and Tzean 1993, Mercado-Sierra et al. 2005, Tanaka et al. 2009). Conidia can be smooth (Figs. 3E,F; 5A,B) or ornamented with different kinds of cell wall irregularities, such as fine to coarse warts (Sutton and Crous 1997, Schubert et al. 2007) (Figs. 4F–G), spines (Moustafa and Abdul-Wahid 1989) (Fig. 4E), ribs (Udagawa et al. 1986), grooves (Stolk 1969), and wrinkles (Madrid et al. 2010a) (Fig. 7D). Septa can occur in one (Figs. 3E–F, 5A–D) or several planes (Figs. 4A–G) and sometimes are marked by thick, darkened bands (Simmons 2007). Cells of a given conidium can be uniformly (Booth 1977) or differently colored (Castañeda and Heredia 2000) (Figs. 3E,F).

Certain species of hyphomycetes have the ability to produce **chlamydospores**, **sclerotia** and/or **stromata**. These specialized structures can remain dormant during periods of adverse environmental conditions, and reproduce the fungus when conditions improve. **Chlamydospores** are thick-walled, swollen cells of a variety of shapes and can appear at intercalary, lateral or terminal position (Stalpers 2000, Kirk et al. 2008) (Figs. 2B, 7E). Their presence/absence and morphology are useful characters to distinguish species in several genera (Gams 1971, Udagawa and Toyazaki 1985, Decock et al. 2006). **Stromata** are masses of tightly aggregated fungal cells, which can be "prosenchymatous" (i.e., composed of hyphae that can still be recognizable as such) or "pseudoparenchymatous" (elongated hyphal elements are not visible). Stromata commonly give rise to mycelium, conidiophores or fruiting bodies (Rawla 1973, Kirschner et al. 2009). **Sclerotia** are firm, usually more or less rounded structures composed of a melanized pseudoparenchymatous outer layer known as "rind", and an inner layer or "medulla" made up of interwoven hyphae (Willetts and Bullock 1992). Multicellular, compact propagules with a simpler structure, born from undifferentiated

hyphae or on sporodochia are often referred to as **bulbils** (Strobel and Stierle 1993, Diederich and Lawrey 2007, Kirschner et al. 2010).

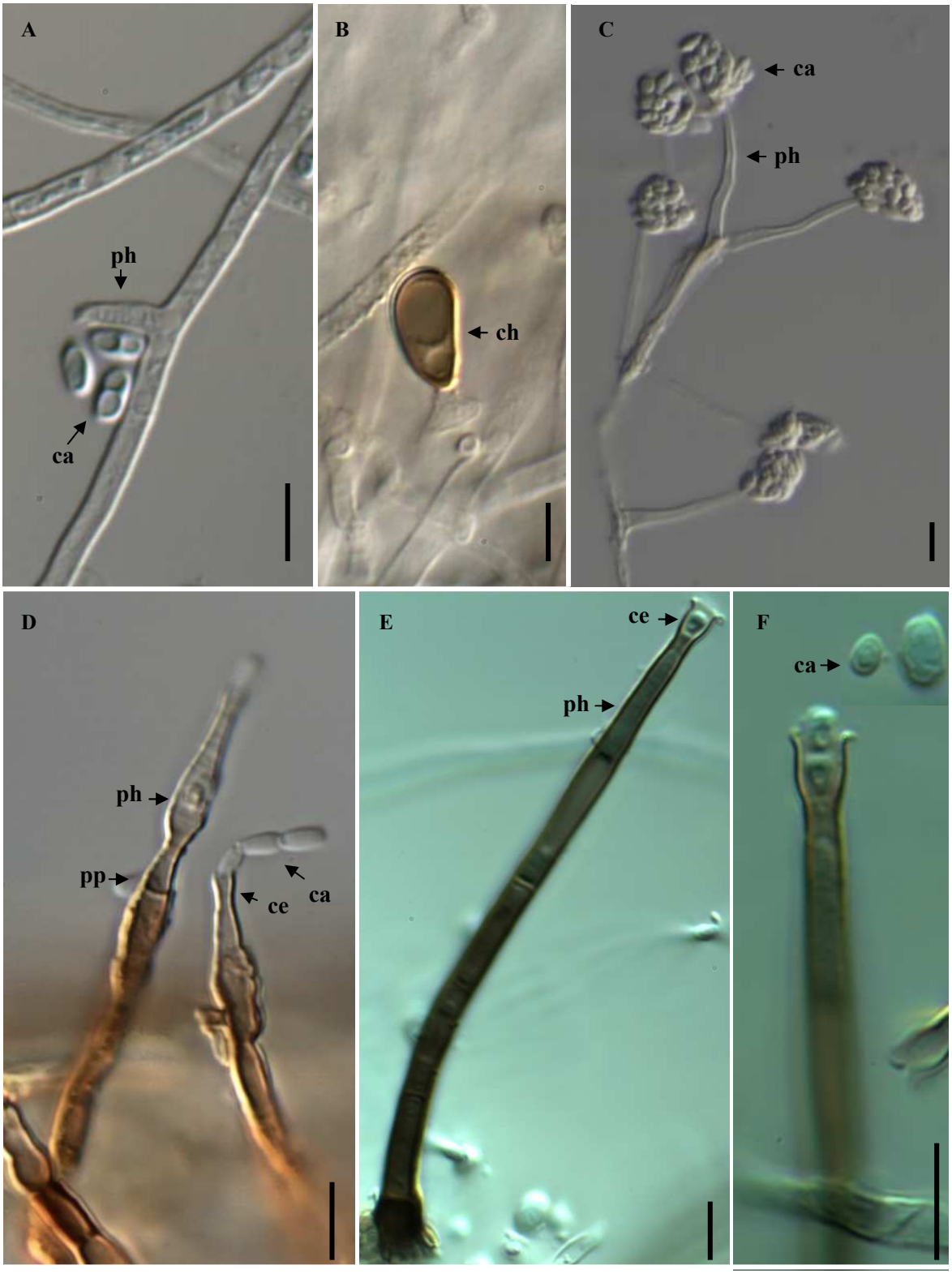

Fig. 2. Reproductive structures of hyphomycetes. A,B) *Lecythophora mutabilis* CBS 157.44; C) *Acremonium sclerotigenum* CBS 124.42; D) *Chalara* cf. *microchona* FMR 10751; E,F) *Chloridium* cf. *clavaeforme* FMR 9225. Abbreviations: ce = collarette, ca= conidia, ch= chlamydospore, ph= phialide, pp= percurrent proliferation. Bars= 5 µm.

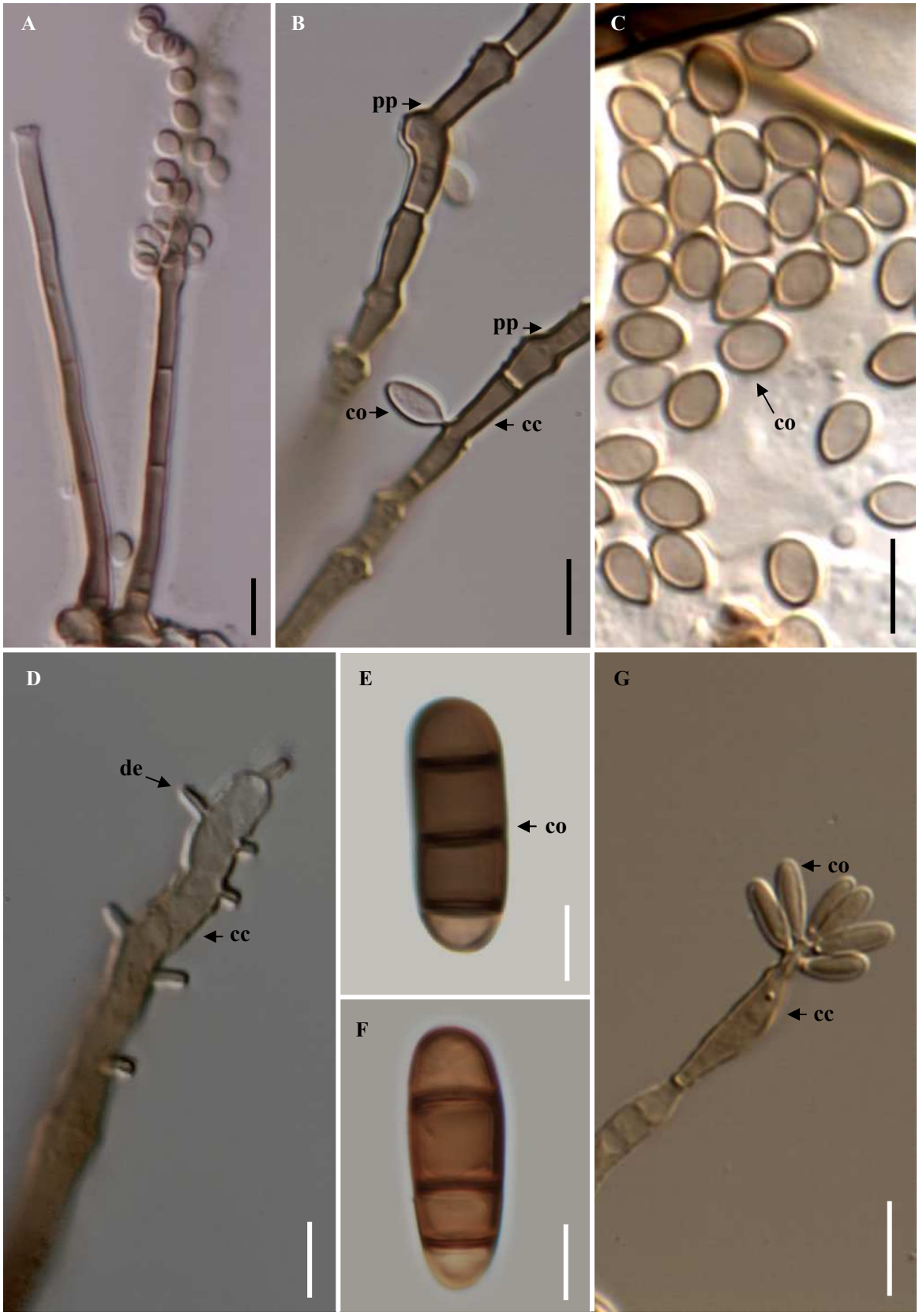

Fig. 3. Reproductive structures of hyphomycetes. A–C) *Cordana* sp. FMR 10754; D–F) *Pleurophragmium* sp. FMR 11490; G) *Rhinocladiella* sp. FMR 7699. Abbreviations: co= conidium, de= denticle, pp= percurrent proliferation. Bars= 5 µm.

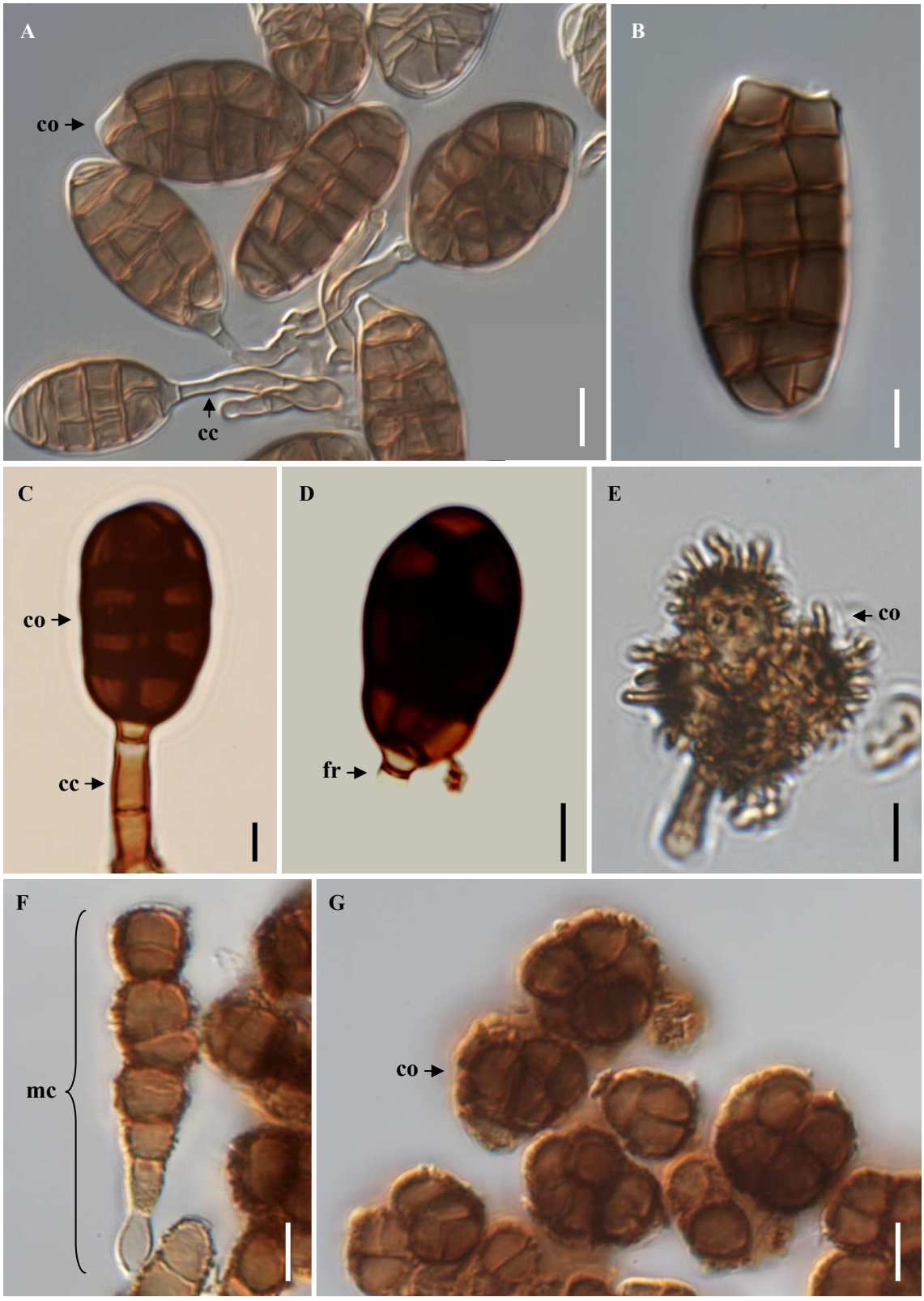

Fig. 4. Reproductive structures of hyphomycetes. A,B) *Oncopodiella hungarica* FMR 10746; C,D) *Rhexoacrodictys cf. erecta* FMR 10757; E) *Spegazzinia thessarthra* FMR 10900; F,G) *Coniosporium memorandum* FMR 10775a. Abbreviations: cc= conidiogenous cell, co= conidium, fr= frill, mc= meristematic conidiophore. Bars= 5 µm.

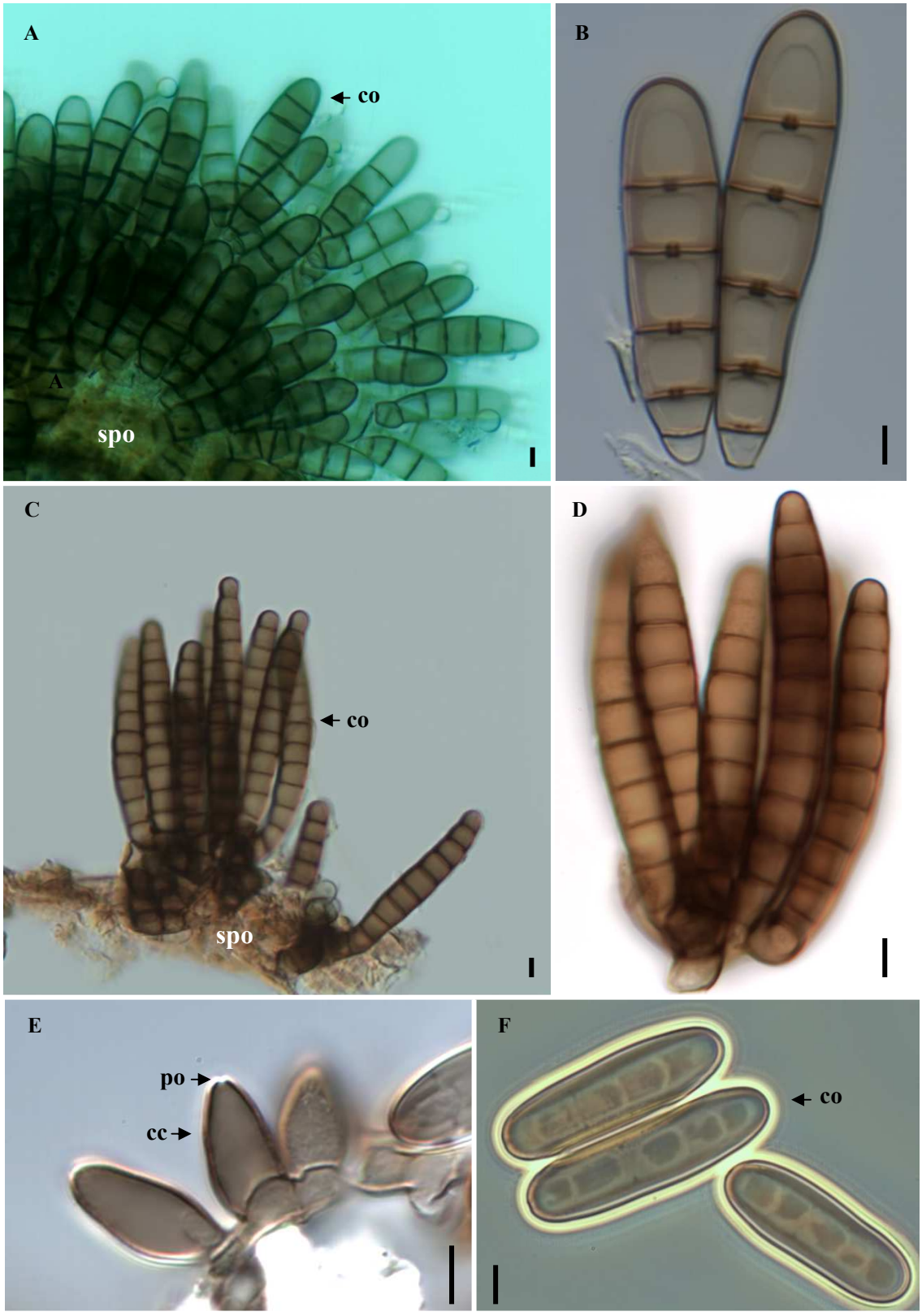

Fig. 5. Reproductive structures of hyphomycetes. A,B) *Bactrodesmium spilomeum* FMR 9216; C,D) *Digitodesmium intermedium* FMR 10088; E,F) *Pyrigemmula aurantiaca* FMR 10957. Abbreviations: cc= conidiogenous cell, co= conidia, po= pore. Bars= 5 µm.

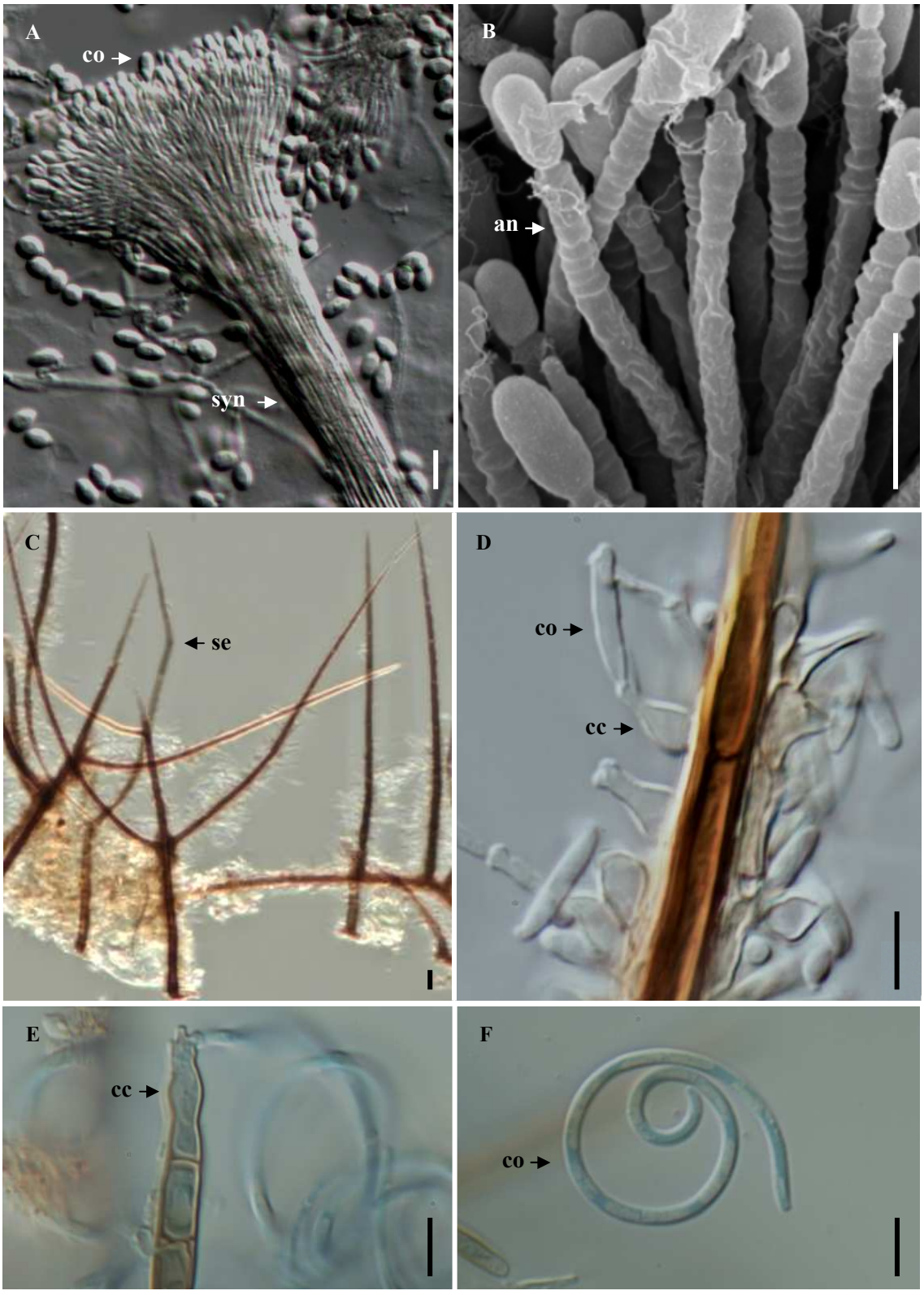

Fig. 6. Reproductive structures of hyphomycetes. A,B) *Parascedosporium tectonae* CBS 120338; C,D) *Ceratocladium polysetosum* IMI 398790; E,F) *Helicosporium griseum* FMR 11056. Abbreviations: an= annellide, cc= conidiogenous cell, co= conidium, se= setae. Bars= 5 µm.

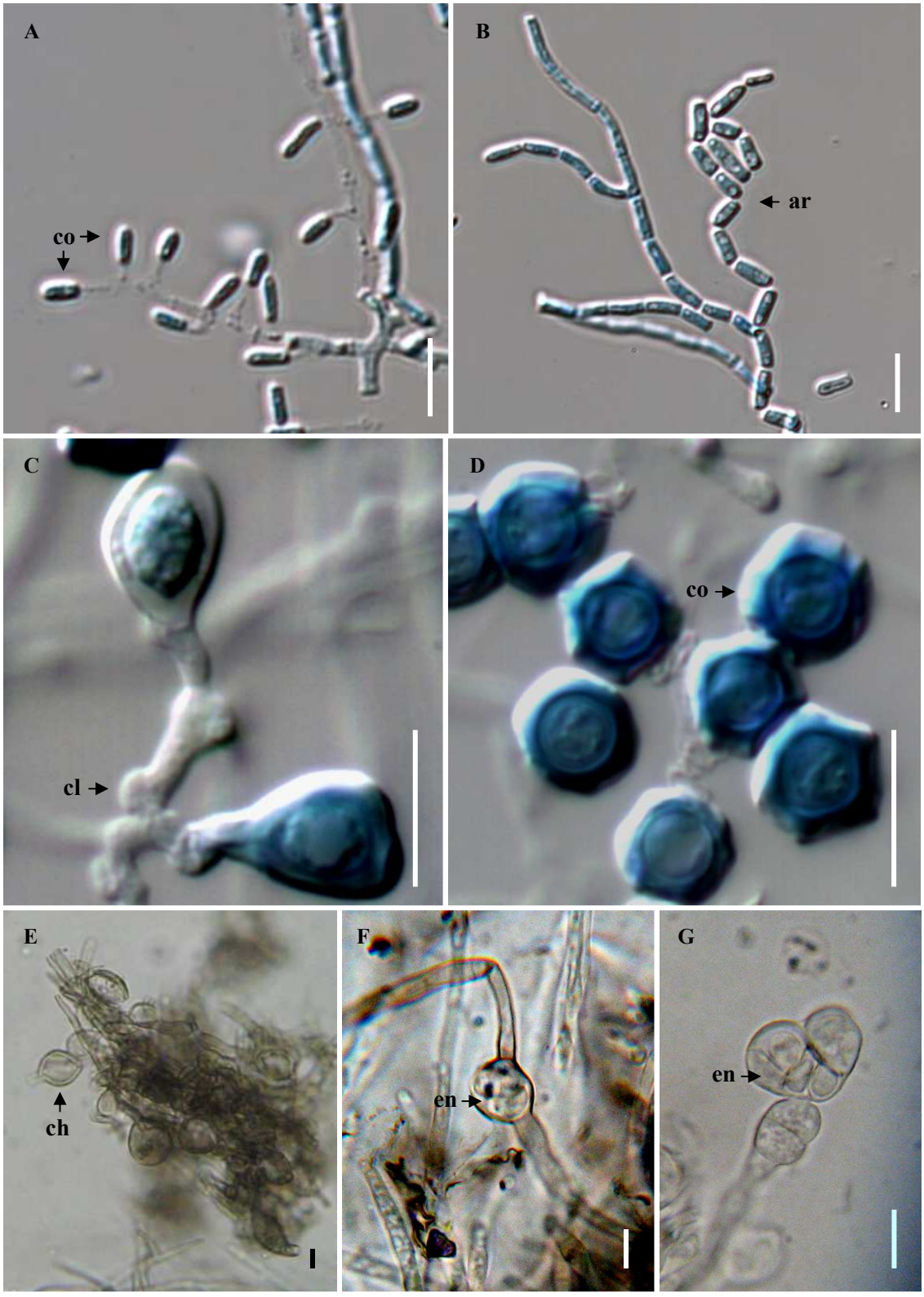

Fig. 7. Reproductive structures of hyphomycetes. A,B) *Chrysosporium ophiodiicola* FMR 9510; C,D) *Fibulochlamys chilensis* FMR 9694; E–G) *Knufia* sp. FMR 10621. Abbreviations: ar= arthroconidia, ch= chlamydospores, cl= clamp connection, co= conidium, en= endoconidia. Bars= 10 µm.

1.5 DNA sequence analyses of anamorphic fungi

The accurate morphological identification of a hyphomycete sometimes requires the opinion of an experienced mycologist, especially when the taxon under study belongs to a genus with poorly differentiated conidiogenous structures and conidia, or when its members show overlapping conidial sizes (Simmons 2007). Furthermore, some hyphomycetes may take months to produce mature colonies *in vitro* (Tsuneda and Currah 2004, Wang et al. 2009), and others tend to degenerate easily or rapidly lose the ability to sporulate on agar media (Simmons 2007). All these factors can delay the accurate identification, which in practice may severely influence critical decisions such as which treatment to apply in an infected plant or in a human patient. Therefore, rapid identification methods for fungi are necessary even when very little growth is obtained or when the fungus does not sporulate (Crous et al. 2009b). Molecular tools, especially DNA sequence analyses provide a good solution for this problem (Guarro et al. 1999).

DNA contains the information of the inherited treats of living organisms and can be considered a "blueprint" of them (Crous et al 2009b). The DNA molecule is a double-helix composed of units called "nucleotides", which consist of a phosphate group, a five-carbon sugar and a nitrogenous base. Nitrogenous bases are of two types: purines (adenine and guanine) and pyrimidines (cytosine and tymine). The genetic information is coded in the DNA as sequences of nucleotides, which, after a series of metabolic processes, will be ultimately expressed as proteins. Changes in the nucleotide sequences are called "mutations" and can result in changes of inherited traits of the organisms. These changes may be caused by a number of factors, i.e. natural synthesis errors made by the DNA polymerases, the insertion of foreign DNA (e.g. during a viral infection), the action of mutagenic compounds or UV radiation, etc. (Alberts et al. 2008). When sequences of the same gene of different organisms are compared,

homologous positions can show different nucleotides due to mutations. These differences are called "polymorphisms". The most common are transitions (interchanges of two purine or two pyrimidine bases), transversions (interchanges of a purine and a pyrimidine base) and insertions/deletions (indels), in which one or more nucleotides are acquired or lost, respectively. Different genes evolve at different rates and coding regions of a gene tend to show less inter- and intraspecific variation than non-coding regions (Alberts et al. 2008, Crous et al. 2009b).

The development of the polymerase chain reaction (PCR) method made possible the production of thousands of copies of specific fragments of DNA present at low concentrations (Mullis 1990). With the subsequent development of fast, relatively cheap techniques to sequence these fragments, scientists have generated much information for DNA sequence comparisons. Nowadays, important general databases such as GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and the EMBL nucleotide sequence database (http://www.ebi.ac.uk/embl/) contain entries representing genes of species in all kingdoms of nature, including thousands of fungi (Begerow et al. 2010). This information has been extensively used in phylogenetic studies (Cai et al. 2006, Tsui et al. 2006). Furthermore, ongoing projects are devoted to the development of DNA barcoding (i.e. the use of 500–800-bp long DNA sequences to recognize species) strategies for fungi (Begerow et al. 2010).

Fungal taxonomy is expected to reflect the evolutionary relationships of fungi. Such a natural classification system may have an impact in applied sciences because closely related species sometimes have similar properties regarding pathogenicity (de Hoog et al. 2000), metabolite-production (Bills et al. 1999), ecology (Untereiner et al. 2004), etc. Phylogenetic analyses aim to reconstruct the genealogical descent of organisms by means of objective, repeatable analyses. They are based on the

assumption that species arise from other species during the course of evolution. Therefore, when the DNA sequences of a given gene are compared among several species, two closely related taxa are more likely to share the same polymorphisms than two distant ones. Different genes have been used in phylogenetic studies of fungi, but perhaps the most widely used ones are those of the ribosomal RNA gene cluster (rDNA). These genes (Fig. 8), which code for the RNA component of the ribosomes, appear in tandem with numerous copies in the fungal genome (Maicas et al. 2000, Tsuchiya and Taga 2001). They contain two adjacent transcriptional units which are transcribed separately, i.e. one including the 5S rRNA gene (which is absent in certain fungal groups), and one including the 18S, 5.8S and 28S rRNA genes, separated by two internal transcribed spacers (ITS1 and ITS2) and flanked by two external transcribed spacers (ETS). These two transcriptional units are separated by intergenic spacers (IGS1 and IGS2).

Fig. 8. Structure of the ribosomal RNA gene cluster in fungi

The coding regions of the rDNA differ in length, i.e. the 18S, 5.8S, 28S, and 5S rRNA genes consist of about 1800, 160, 3400, and 120 bp, respectively (Iwen et al. 2002, Szymanski et al. 2002). Nevertheless, certain fungal species may have much longer 18S and 28S rDNA sequences due to the presence of introns (Roeijmans et al. 1997, Li et al. 2009). The non-coding ITS1 and ITS2 loci are highly variable in length, and often include numerous indels and duplications (Platas et al. 2004). The “ITS region”, i.e. the fragment composed by ITS1, 5.8S rDNA and ITS2, usually ranges between 450 and 700 bp (Bellemain et al. 2010), but it may be above 1 kb in certain

fungi (Gottlieb and Lichtwardt 2001, Platas et al. 2004). The different components of the rDNA evolve at varying rates. Coding regions such as the 18S, 5.8S and 28S rRNA genes evolve slowly, providing useful information to separate higher taxonomic ranks, i.e. genera (Berbee and Taylor 1992, Arzanlou et al. 2007), families (Reblová et al. 2004) and orders (Huhndorf et al. 2004, Rossman et al. 2010, Perdomo et al. 2011). Despite being a conserved gene, the 28S rDNA shows a variable region toward its 5' end called D1/D2 domains. This region has been widely used to identify yeasts (Scorzetti et al. 2002), but also proved useful to distinguish species in some groups of filamentous fungi (Rodríguez et al. 2004). The non-coding ITS1 and ITS2 are hypervariable and constitute a powerful tool to delimit species boundaries (Iwen et al. 2002, de Hoog et al. 2006, Davey and Currah 2007). Therefore, though not officially considered a barcode, the ITS region has played that role for a long time (Ahmed et al. 2003, Buzina et al. 2005, Pounder et al. 2007). However, which ITS percent identity should be considered as cut-off for species identification is controversial, because the interspecific variability of this region differs among fungal groups (Stchigel 2000, Crous et al. 2009b).

Protein-coding loci also have been used in fungal phylogenetic studies. In this thesis, two loci of this kind were used, the β-tubulin and the calmodulin genes. The β-tubulin gene codes for a polypeptide usually of 447 aminoacid residues which is one of the constituents of the microtubules (Msiska and Morton 2009). This gene was cloned and characterized in a strain of *Neurospora crassa* Shear & B.O. Dodge by Orbach et al. (1986). In this fungus, the β-tubulin gene (GenBank accession # M13630) consists of about 2.5 kb, with six introns which are mostly located toward the 5' end of the gene. The fungal calmodulin gene varies from 1 to 15 kb, usually has five introns and generally codes for a protein of 148–149 aminoacid residues (de Carvalho et al. 2003).

At resting calcium concentration, the protein exists in the cytoplasm in a Ca^{2+}-free form called apo-calmodulin. In response to a Ca^{2+} signal, the apo-calmodulin binds Ca^{2+} and undergoes conformational changes that allow it to activate a number of target enzymes which are involved in processes such as mitosis, endocytosis, and cellular growth (Cyert 2001).

For phylogenetic analyses, character matrices are constructed mostly from DNA sequence alignments generated with computer programs such as ClustalX (Thompson et al. 1997) or Muscle (Edgar 2004). The matrices are analyzed using software for statistical cluster analyses by one or more methods. The results are represented graphically by a branching diagram called "phylogenetic tree" (Fig. 9). The trees are composed of nodes and branches. External nodes are also called "operational taxonomic units" (OTUs), they represent extant taxa. Internal nodes are known as "hypothetical taxonomic units" (HTUs), and represent hypothetical ancestors of the OTUs. Branches define the relationships among the taxa in terms of descent and ancestry. A clade is a clustering of related OTUs that descend from the same HTU.

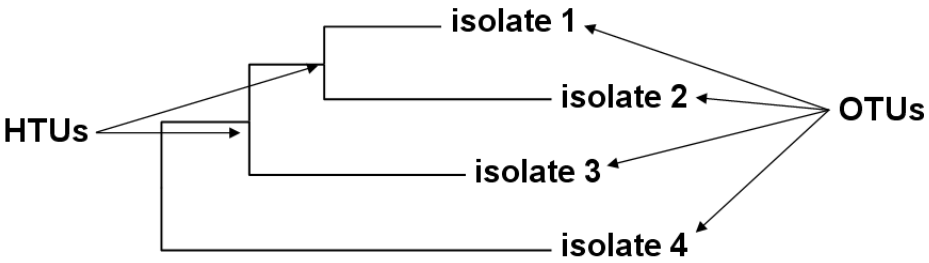

Fig. 9. Schematic representation of a phylogenetic tree.

The methods to infer phylogenetic relationships can be divided into two broad categories: those that operate on a matrix of discrete characters that assigns one or more attributes or character states to each taxon; and those that operate on a matrix of pairwise distances between taxa (Salemi and Vandamme 2004). In the present thesis, a

distance-based (neighbor-joining) and a character-based (maximum parsimony) method were used. A simple form to estimate genetic distances is to calculate the number of substitutions per site in the alignment between each pair of taxa. In the neighbor-joining method the genetic distance is corrected by means of models of evolution to cope with the presence of different substitution rates of transitions versus transversions and multiple subtitutions at one site. This method forms groupings with the taxa according to genetic distance, i.e. it finds the two closest taxa and connects them through an internal node, and then continues by successive clustering of the lineages, setting branch lengths as the lineages join (Saitou and Nei 1987). The maximum parsimony method is based on the philosophy that the most probable explanation for the evolution of a given set of data is the simplest one. This method searches all possible tree topologies for the optimal tree, i.e., the one that requires the minimum number of steps (mutations in the case of a DNA sequence matrix). However, for a given set of data more than one "optimal" or "most parsimonious tree" can be obtained. The robustness of the groupings obtained can be tested by means of statistical tests (e.g. bootstrapping, Felsenstein 1985). The higher the value obtained, the more robust is the grouping observed. A group that includes an ancestor and all its descendants is called "monophyletic". A group that includes an ancestor but not all its descendants is called "paraphyletic". A group that comprises descendants from different ancestors is called "polyphyletic". "Natural taxa" should be monophyletic. Nevertheless, numerous fungal genera, families and higher taxonomic ranks originally erected on the basis of morphological features, proved to be paraphyletic or polyphyletic when assessed using phylogenetic analyses (Huhndorf et al. 2004, Miller and Huhndorf 2004).

Several anamorphic genera, e.g. *Cladosporium* Link, *Phialophora* Medlar and *Ramichloridium* Stahel ex de Hoog have undergone important taxonomic changes due

to the application of phylogenetic analyses. These studies revealed that species traditionally placed in such form-genera appeared independently several times in the course of evolution, and were associated with different families, orders or classes of ascomycetes. Species which were too distantly related to their respective generic types had to be accommodated into new, segregate genera (Vijaykrishna et al. 2004, Arzanlou et al. 2007, Crous et al. 2007a). However, to avoid the excessive proliferation of genus names, most mycologists now accept certain level of polyphyly in anamorph genera. Two taxa can be retained in the same genus if they share similar morphological features and belong to the same family (Crous et al. 2007a) or at least to the same order (Arzanlou et al. 2007) according to DNA sequence comparisons.

Anamorphs should be understood simply as propagative organs, and hence it is not surprising that distantly related fungi may develop similar anamorphs due to evolutionary pressure (Seifert and Samuels 2000). Evident examples of this can be found among the microcolonial fungi which grow on rock surfaces. In this harsh environment, fungi must resist drought, direct exposure to sunlight and scarcity of nutrients (Scott et al. 2007, Ruibal et al. 2009). To survive under these conditions, these fungi form compact, meristematic, strongly melanised colonies (Staley et al. 1982). Melanin protects fungal cells against UV radiation (Dadachova and Casadevall 2008), and meristematic growth optimizes volume/surface ratio to avoid desiccation (Ruibal et al. 2009). These fungi lack well-developed conidiophores and often produce simple spores such as endospores and arthroconidia to ensure propagation with a minimal cost of energy (de Leo et al. 1999, 2003). Though genera in this group are phylogenetically very diverse, distributed in at least four orders of ascomycetes (Ruibal et al. 2008), they are morphologically so little differentiated that generic limits are difficult to establish from a morphological point of view (Selbmann et al. 2005). Thus, the correct

identification of a given isolate often requires DNA sequence analysis (Kurzai et al. 2003). Many other examples of morphological convergence of anamorphs have been reported in the *Ascomycota* (de Beer et al. 2006, Tsui and Berbee 2006, Perdomo et al. 2010) and *Basidiomycota* (Thorn and Barron 1986, Walther et al. 2005), and probably many more genera of hyphomycetes will be demonstrated to be polyphyletic in the future.

As stated above, genetically different individuals may show similar conidiogenesis modes and a similar morphology of both their vegetative and fertile structures (Crous et al. 2007a). Therefore, phylogenetically different entities may be identified as the same species if they have been identified solely on the basis of morphology. Each one of these entities hidden within a broad species concept is called a "cryptic species". When assessed by means of DNA sequence analyses, many fungi which were previously considered well-defined entities were revealed to be complexes of species (Koufopanou et al. 2001, Gilgado et al. 2007, Marimon et al. 2007). It is important to identify these cryptic species as they often differ in important traits such antifungal susceptibility, clinical spectrum, geographical distribution, and host range (de Hoog et al. 2006, Marimon 2007).

2. INTEREST OF THIS WORK AND OBJECTIVES

2. Interest of this work and objectives

The total biodiversity of fungi was estimated by Hawksworth (1991) in approximately 1.5 million species. However, the number of species described hitherto is in the range of 72.000–100.000 (Hawksworth 2001). If correct, this means that less than 7% of the extant fungi are known and thousands of species await discovery (Hawksworth and Rossman 1997). Despite centuries of taxonomic work by mycologists worldwide, it seems that no substratum has been studied so intensely that its mycobiota is completely known. So, undescribed species can still be discovered even in common substrata such as fruits (Batzer et al. 2008, Yang et al. 2010), fruit juices (Crous et al. 2007c) and garden soil (Rodríguez et al. 2002). Novel fungal species isolated and preserved in culture collections provide new material to be tested for their potential as biocontrol agents (Lewis and Larkin 1998, Holmes et al. 2004) or as producers of industrially valuable secondary metabolites (Bills et al. 1999, Zhao et al. 2010).

The general objective of this thesis was to assess the taxonomic position of a set of clinical and environmental isolates of anamorphic fungi using a polyphasic approach (i.e. DNA sequence data, morphology and physiology). We focused first on isolates identified morphologically as *Sporothrix schenckii* Hektoen & C.F. Perkins, the main etiological agent of sporotrichosis, a fungal disease with a clinical spectrum that ranges from localized skin lesions to systemic infections (Bustamante et al. 2009, de Hoog et al. 2000). *Sporothrix schenckii* was considered for more than a century the only species of *Sporothrix* pathogenic to humans (Hektoen and Perkins 1900, Sidrim and Rocha 2004). Nevertheless, the species was regarded as phenotypically variable (de Hoog 1974, Kwon-Chung and Bennett 1992). Image-based genotyping studies revealed a high level of intraspecific diversity (Lin et al. 1999, Ishizaki et al. 2004). Recently, molecular phylogenetic studies demonstrated that *S. schenckii* is actually a species

complex (Marimon et al. 2006). Using a polyphasic taxonomic approach, Marimon et al. (2007, 2008a) revealed that besides *S. schenckii*, three additional human-pathogenic *Sporothrix* species could be recognized. These species were proposed as new and named *S. brasiliensis* Marimon, Gené, Cano & Guarro, *S. globosa* Marimon, Gené, Cano & Guarro, and *S. luriei* (Ajello & Kaplan) Marimon, Gené, Cano & Guarro. An additional *S. schenckii*-like taxon, *S. mexicana* Marimon, Gené, Cano & Guarro, was proposed based on two environmental isolates (Marimon et al. 2007). The four clinically relevant *Sporothrix* species showed differences in antifungal susceptibility (Marimon et al. 2008b), in virulence (Marimon 2007, Arrillaga Moncrief et al. 2010), and apparently also in geographical distribution (Marimon et al. 2007).

The first specific objective of the present thesis was to identify molecularly a set of *Sporothrix* isolates, mostly of clinical origin, obtained from Colombia, Guatemala and Mexico. Sporotrichosis is endemic in these three countries (Mayorga et al. 1979, García Vargas et al. 2008, Rubio et al. 2010), but clinical strains from Guatemala and Mexico were not studied by Marimon et al. (2006, 2007, 2008a), and their works included only one strain from Colombia. Therefore, we wanted to assess whether a given species is related to sporotrichosis in such countries or, by contrast, a range of species could be identified.

The second specific objective of this thesis was to search for novel anamorphic fungi in soil. This substratum was chosen to continue one of the main lines of research of our institution, biodiversity of soil microfungi (Gené 1994, Stchigel 2000, García 2005). Some of the isolates studied had been originally identified as *Sporothrix inflata* de Hoog, a non-pathogenic fungus morphologically similar to *S. schenckii* (de Hoog 1974). Our isolates, despite matching well the morphological

description of *S. inflata*, showed a high level of phenotypic variability which suggested that possibly more than one taxon was present.

UNIVERSITAT ROVIRA I VIRGILI
MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF ANAMORPHIC FUNGI
Hugo Madrid Lorca
DL: T. 1355-2011

3. MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Origin of the isolates and methods for fungal isolation

The isolates studied during the present thesis were obtained from clinical samples of patients with sporotrichosis and from natural substrata, mainly soil.

3.1.1 Clinical isolates of *Sporothrix*

Clinical isolates from Mexico, Guatemala and Colombia, identified morphologically as *Sporothrix schenckii* s.l., were obtained from culture collections located at the *Servicio de Dermatología y Departamento de Micología*, *Hospital General de México*, *Mexico*, and at the *Departamento de Microbiología y Parasitología*, *Facultad de Medicina*, *Universidad Autónoma de México*, Mexico. These isolates, most of which were recovered in the period 1978–2007, are detailed in section Results 4.2, Table I.

3.1.2 Isolation of soil-borne anamorphic fungi

Soil samples were collected in Chile, Spain and U.S.A. mostly by the scientific staff of our institution in the period 1995–2009. The samples were placed in sterilized plastic bags and stored at 4–7 ºC until processed. Approximately 1 g of each soil sample was diluted in 9 mL sterilized water, and 500 µL of this mix were inoculated in duplicate onto dichloran rose-bengal chloramphenicol agar (Oxoid, United Kingdom) or potato dextrose agar (PDA, Difco) plates supplemented with cycloheximide at a final concentration of 0.1 mg/mL or with benomyl at a final concentration of 10 µg/mL (Gilgado et al. 2005). The mix was spread over the surface of the inhibitory media using a Digralsky spreader. In addition, a portion of approximately 20 g of each soil sample was placed in a sterile Petri dish, moistened with sterile distilled water and covered with

baits consisting of small pieces of sterilized wood (Calduch et al. 2004). The plates of the five methods were incubated in the dark at 25 ºC and examined weekly 1–2 months. The micromorphology of the fungi growing on the wood baits and on primary cultures was studied in lactic acid. We selected for further studies isolates which did not match previously described taxa, focusing mainly on dematiaceous species. To achieve pure cultures, conidia or small pieces of mycelium were transferred from primary cultures to Petri dishes with oatmeal agar (OA; 30 g filtered oat flakes, 20 g agar, 1 L distilled water), potato-carrot agar (PCA; 20 g potatoes, 20 g carrots, 20 g agar, 1 L distilled water) and potato dextrose agar (PDA, Difco, USA), which were incubated at 25 ºC for 14 days in the dark.

The environmental isolates generated in the course of this thesis, as well as type or reference strains used for comparison are shown in table I of each article in section Results 4.2, 4.3 and 4.4, and under "material examined" in Results 4.5.

3.2 Phenotypic characterization

The phenotypic characterization of the putative new taxa comprised the study of cultural features, growth profilings, and micromorphology. In addition, we assessed the ability to assimilate key carbon sources in environmental *Sporothrix* isolates (Marimon 2007).

3.2.1 Morphology

In order to achieve optimal growth and sporulation, we used different culture media (2% malt extract agar (MEA, Oxoid), OA, PCA or PDA) depending on the fungal species concerned and mostly following previous authors (Papendorf 1967, Mouchacca and Gams 1993, Calduch et al. 2004, Marimon et al. 2007). A standarized nomenclature for colony colour was used, according to Kornerup and Wanscher`s

(1978) manual. The micromorphology of environmental *Sporothrix* isolates was studied from slide cultures made on corn meal agar (CMA; 30 g corn, 15 g agar, 1 L distilled water) after 12 and 21 d at 25 ºC. Other microfungi were studied from colonies on sterilized wood or from OA or PCA after 14 d at 25 ºC. Structures were mounted in lactic acid, and studied using an Olympus CH-2 light microscope. Photographs of fungal colonies were obtained using a Cannon Ixus 60 digital camera. Photomicrographs were obtained mostly with a Zeiss Axio Imager M1 light microscope.

3.2.2 Physiological tests

Growth profilings were performed on Petri dishes with OA, PCA and/or PDA or 2% MEA at temperatures ranging from 6 to 42 ºC. Colony diameters were measured after 5, 14 or 21 d depending on the growth rate of the fungal especies tested. In environmental *Sporothrix* isolates, the ability to assimilate raffinose, ribitol and sucrose were tested in liquid medium according to Marimon et al. (2007). The tests were performed in 96-well microplates, with each well containing 150 µL of liquid nitrogen base medium (Beckton Dickinson Co., USA), with one test substrate, except those for the negative controls, which only had the base medium, and those for the positive controls, which had glucose. The inoculum was prepared in sterile saline solution from 7-day-old colonies on PDA and adjusted to a concentration of 2×10^5 to 2×10^6 UFC/mL as described by Marimon et al. (2007). Microplates were read after five and ten days at 25º C.

3.3 Molecular studies

DNA extraction was performed directly from fungal colonies on PDA after one or two weeks of incubation at 25 ºC. Young colonies were used to minimize the

presence of pigments which might inhibit PCR reactions later. We followed the Fast DNA kit protocol (Bio 101, Inc., Joshua Way, Vista, California, USA) as described by Gilgado (2007), but with the homogenization step repeated five times.

Amplification and sequencing of the 5.8S ribosomal RNA gene and the internal transcribed spacers 1 and 2 (hereafter referred to as "ITS region") were performed with the primer pair ITS5/ITS4 as described by White et al. (1990). The D1/D2 domains of the 28S rRNA gene were amplified and sequenced using primers NL1/NL4 following the protocol of O`Donnell (1993). Fragments of the calmodulin (CAL) and β-tubulin (BT2) genes were amplified with primer pairs CL1/CL2A (O`Donnell et al. 2000) and Bt2a/Bt2b (Glass and Donaldson 1995), respectively, following the Ready-To-Go bead protocol (Amersham Bioscience, Freiburg, Germany) and using the amplification programs described by Marimon et al. (2006). PCR products were purified using a GFXTM PCR DNA kit (Pharmacia Biotech, Cerdanyola del Vallès, Spain) and stored at -20 ºC until sequencing. PCR products were sequenced by using the same primers employed 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). The software Autoassember version 1.40 (Applied Biosystems, Perkin Elmer Corp., Norwalk, Connecticutt, USA) and SeqMan (Lasergene, Madison, Wisconsin) were used to obtain consensus sequences from the complementary sequences of each isolate. BLAST searches (Altschul et al. 1990**)** were performed to compare data of the isolates studied with those of other fungi deposited in the GenBank database. Nucleotide sequence alignments were performed with ClustalX version 1.81 (Thompson et al. 1997), followed by manual adjustments with a text editor. Distance trees were constructed with the neighbor-joining method (NJ, Saitou and Nei 1987)

excluding ambiguously aligned regions and using the Kimura-2-parameter substitution model with pairwise deletion of gaps, as implemented in the MEGA 4.0 computer program (Tamura et al. 2007). Maximum parsimony (MP) analyses were performed using the PAUP* version 4.9b10 software (Swofford 2002). Most parsimonious trees were obtained after 100 heuristic searches with random sequence addition and tree bisection-reconnection branch-swapping algorithms, collapsing zero-length branches and saving all minimal length trees. The robustness of branches obtained in the NJ and MP trees was assessed by bootstrap analysis of 1000 replicates. The sequences generated during our research and the alignments used in the cladistic analyses were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and TreeBASE (www.treebase.org), respectively.

UNIVERSITAT ROVIRA I VIRGILI
MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF ANAMORPHIC FUNGI
Hugo Madrid Lorca
DL: T. 1355-2011

4. RESULTS

UNIVERSITAT ROVIRA I VIRGILI
MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF ANAMORPHIC FUNGI
Hugo Madrid Lorca
DL: T. 1355-2011

4.1 *Sporothrix brunneoviolacea* and *Sporothrix dimorphospora*, two new members of the *Ophiostoma stenoceras-Sporothrix schenckii* complex.

Madrid H, Gené J, Cano J, Silvera C, Guarro J

Mycologia 2010; 102:1193–1203.

Mycologia, 102(5), 2010, pp. 1193–1203. DOI: 10.3852/09-320

Sporothrix brunneoviolacea and *Sporothrix dimorphospora*, two new members of the *Ophiostoma stenoceras-Sporothrix schenckii* complex

H. Madrid
J. Gené
J. Cano[1]
C. Silvera
J. Guarro

Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, C/ Sant Llorenc 21, 43201 Reus, Tarragona, Spain

Abstract: *Sporothrix inflata* is a saprobic member of the *Ophiostoma stenoceras-Sporothrix schenckii* species complex, reported mainly from soil. *Ophiostoma bragantinum*, an ascomycete described from Brazil, has been proposed as its possible teleomorph. Previous studies revealed that *Sporothrix inflata* is phenotypically and genetically variable, suggesting the existence of cryptic species. During a continued survey on the biodiversity of microfungi from different countries, seven isolates morphologically similar to *S. inflata* were obtained from soil samples collected in Spain and USA. In this study their phenotypic features and phylogenetic relationships were assessed. DNA sequence data of two nuclear loci revealed that these isolates correspond to two unnamed clades in *S. inflata* s.l., one of which also included the type strain of *Humicola dimorphospora*, a species that traditionally has been considered a synonym of *S. inflata*. These two groups are proposed herein as *Sporothrix brunneoviolacea* sp. nov. and *Sporothrix dimorphospora* comb. nov. *S. brunneoviolacea* is characterized phenotypically by the production of a diffusible violet-brown pigment in culture and mostly globose, pigmented, lateral blastoconidia. On the other hand *S. dimorphospora* lacks diffusible pigments and shows mostly subglobose to obovoid pigmented lateral blastoconidia. In contrast to the type strain of *S. inflata* *S. brunneoviolacea* and *S. dimorphospora* assimilate raffinose. The phylogenetic analysis suggested that the proposed anamorph-teleomorph connection between *S. inflata* and *O. bragantinum* might not be correct.

Key words: Ascomycetes, Ophiostomatales, phylogeny, taxonomy

Submitted 25 Dec 2009; accepted for publication 18 Feb 2010.
[1] Corresponding author. E-mail: josep.cano@urv.cat

INTRODUCTION

Sporothrix schenckii Hektoen & C.F. Perkins is a widespread anamorphic fungus living on soil, plant material and other substrata (Mackinnon et al. 1969, Kwon-Chung and Bennett 1992). Previous studies suggested that *Ophiostoma stenoceras* (Robak) Melin & Nannf., a well known ascomycete reported from conifers and hardwood trees, could be its teleomorph (Taylor 1970, de Hoog 1974). However subsequent DNA sequence analyses demonstrated that these taxa are different species (de Beer et al. 2003, Zhou et al. 2006). Although *S. schenckii* traditionally has been considered the only human pathogenic species of the genus (Kwon-Chung and Bennett 1992), recent phylogenetic studies have shown that this taxon contained several cryptic species (Marimon et al. 2006, Madrid et al. 2009). On the basis of phylogenetic and phenotypic differences Marimon et al. (2007, 2008) segregated three clinically relevant species from *S. schenckii* s. str. They are *Sporothrix brasiliensis* Marimon, Gené, Cano & Guarro, *Sporothrix globosa* Marimon, Gené, Cano & Guarro and *Sporothrix luriei* (Ajello & Kaplan) Marimon, Gené, Cano & Guarro. These pathogenic species, along with morphologically similar saprophytes such as *Sporothrix inflata* de Hoog, *Sporothrix pallida* (Tubaki) Matsush., *Sporothrix stylites* de Meyer, Z.W. de Beer & M.J. Wingf., *Sporothrix mexicana* Marimon, Gené, Cano & Guarro, and numerous *Ophiostoma* Syd. species, form the *O. stenoceras-S. schenckii* complex (de Meyer et al. 2008, Roets et al. 2008). Members of this species complex produce sympodial, hyaline to subhyaline conidia at the apex and along the sides of denticulate, more or less differentiated conidiophores. In addition a few species have been reported to produce darkly pigmented lateral blastoconidia on undifferentiated hyphae (de Hoog 1974, Pfenning and Oberwinkler 1993, Marimon et al. 2007).

S. inflata was erected by de Hoog in 1974 based on a set of isolates from soil of Europe and South America. The type strain, CBS 239.68, was isolated from soil of a wheat field in Germany. The species was described as being similar to *S. schenckii* but with more regular conidiogenous cells, on which the formation of sympodial conidia occurred on a well differentiated, inflated denticulate apex (de Hoog 1974). In the same year Roxon and Jong described a similar fungus from Canadian soil, *Humicola dimorphospora* Roxon & S.C. Jong. This taxon traditionally

has been considered a synonym of *S. inflata* (Aghayeva et al. 2005). *Ophiostoma bragantinum* Pfenning & Overw. a homothallic ascomycete described from Brazilian soil was proposed as the possible teleomorph of *S. inflata* (Pfenning and Oberwinkler 1993). Aghayeva et al. (2005) with DNA sequence data demonstrated that isolates identified morphologically as *S. inflata* belong to four different lineages, of which three represented putative cryptic species.

In a study on the biodiversity of soil microfungi from different countries seven isolates morphologically similar to *S. inflata* were obtained from soil samples from Spain and USA. This study aimed to (i) assess the phylogenetic relationships of these isolates to the different lineages of *S. inflata* s.l. found by Aghayeva et al. (2005) and other related species of *Sporothrix* Hektoen & C.F. Perkins and *Ophiostoma*, (ii) phenotypically characterize the putative cryptic species in *S. inflata* s.l. and (iii) elucidate with DNA sequence data whether *O. bragantinum* is the teleomorph of *S. inflata* as suggested by Pfenning and Oberwinkler (1993).

MATERIALS AND METHODS

Sampling areas, fungal isolation and isolates studied.—In 2006 soil samples were collected in Yosemite National Park, California (USA), and in three areas of Spain, Poblet (Tarragona Province), Lobeznos (Zamora Province) and Natural Park Señorío de Bertiz (Navarra Province). The sampling area in USA has a Mediterranean climate with hot summers and mild winters and precipitation of 915–1200 mm. The mean daily temperatures in this area are 8–32 C. The soil contains abundant organic material, and the vegetation is dominated by species of *Pinus* L., *Quercus* L. and *Pseudotsuga* Carr. The sampling areas in Spain exhibit a range of climates. Poblet has a Mediterranean climate with average annual temperature of 13.2 C and average annual precipitations about 600 mm. The soil is rich in plant debris, and the vegetation mainly consists of *Castanea sativa* Mill., *Tilia platyphyllos* Scop., *Pinus sylvestris* L., and species of *Acer* L and *Quercus*. Lobeznos has a continental climate, with average annual temperature of 13.6 C and average annual precipitation of about 400 mm. The soil is rich in humus, and the vegetation is dominated by *Cupressus arizonica* Greene and species of *Pinus*. Natural Park Señorío de Bertiz has an oceanic climate with average annual precipitation above 2000 mm and average annual temperature of 14–15 C. The sampling area has acidic soils and the predominant plant species are *Fagus sylvatica* L. and *Saxifraga hirsuta* L. Soil samples were placed in sterilized polyethylene bags. At the laboratory the material was stored approximately 1 y at 4–7 C until processed.

Approximately 1 g each soil sample was diluted in 9 mL sterilized water, and 500 µL of this mix were inoculated in duplicate onto dichloran rose-bengal chloramphenicol agar (Oxoid, United Kingdom) plates supplemented with cycloheximide at a final concentration of 0.1 mg/mL. The mix was spread over the surface of this selective medium with a Digralsky spreader. The plates were incubated at 25 C and examined weekly 1 mo. To achieve pure cultures a sterile dissection needle was used to transfer conidia from primary cultures to Petri dishes containing potato dextrose agar (PDA, Difco), which were incubated at 25 C for 14 d in the dark. In addition to the isolates generated in this study we studied eight reference isolates of *S. inflata* s.l., including its type strain and that of *H. dimorphospora*, and two isolates of *O. bragantinum* obtained from the Centraalbureau voor Schimmelcultures (CBS, Utrecht, the Netherlands) (TABLE I).

DNA extraction, amplification and sequencing.—DNA was extracted and purified directly from fungal colonies by following the Fast DNA kit protocol (Bio 101 Inc., Vista, California), with the homogenization step repeated five times with a FastPrep FP120 instrument (Thermo Savant, Holbrook, New York). Amplification and sequencing of the internal transcribed spacer region (ITS) and a fragment of the β-tubulin gene (BT2) were performed as described by Gilgado et al. (2005) and Marimon et al. (2006) respectively. The program Autoassembler 1.40 (Applied Biosystems) was used to obtain consensus sequences from the two complementary sequences of ITS and BT2 of each isolate.

DNA sequence analyses.—BLAST sequence identity queries were performed to compare the ITS and BT2 sequences of strains isolated in this study with those of species deposited in the GenBank database. For phylogenetic analyses sequences of both regions of species of *Ophiostoma* and *Sporothrix* generated here or obtained from GenBank (TABLE I) were aligned with those of our isolates using Clustal X 1.81. Only a limited number of taxa could be included in the phylogenetic analysis due to high interspecific sequence diversity in the ITS and BT2 loci. However all species of these genera morphologically similar to our isolates (i.e. those reported to produce pigmented lateral blastoconidia by de Hoog 1974, Pfenning and Oberwinkler 1993, Aghayeva et al. 2005 and Marimon et al. 2007), as well as other genetically related taxa, were included. Misalignments were corrected manually with a text editor, and ambiguous regions were excluded from the analysis. The combined ITS and BT2 dataset was tested for incongruence with the incongruence length difference test (ILD, Farris et al. 1994) as implemented in WinClada software (Nixon 2002). Phylogenetic analyses were performed with the neighbor joining method using the Kimura 2-parameter substitution model with pairwise deletion of gaps, as implemented in the MEGA 4 computer program (Tamura et al. 2007). The robustness of branches was assessed by bootstrap analysis of 1000 replicates.

Morphological and physiological studies.—Colony morphology and diameter at 15, 20, 25, 30, 35 and 37 C of all isolates were studied on PDA plates in duplicate, incubated 21 d in the dark. All isolates also were subcultured on potato carrot agar (PCA, 20 g potatoes, 20 g carrots, 20 g agar, 1 L distilled water) and oatmeal agar (OA, 30 g filtered oat

TABLE 1. Isolates included in the study, their origin and EMBL accession numbers

Taxon	Isolate numbers	Source	References	EMBL accession numbers	
				ITS	BT2
Ophiostoma bragantinum	CBS 430.92	Rhizosphere soil, Brazil	Pfenning and Oberwinkler 1993	**FN546964**	**FN547386**
	CBS 474.91[T]	Forest soil, Brazil	Pfenning and Oberwinkler 1993	**FN546965**	**FN547387**
Ophiostoma dentifundum	CBS 115865	*Quercus robur*, Poland	Aghayeva et al. 2005	AY495435	AY495446
	CBS 115790[T]	*Quercus* wood, Hungary	Aghayeva et al. 2005	AY495434	AY495445
Ophiostoma nigrocarpum	CBS 637.66[T]	*Abies* log infested with *Scolytus* sp.	Aghayeva et al. 2004	AY280489	AY280479
Ophiostoma palmiculminatum	CBS 119590[T]	*Protea repens*, South Africa	Roets et al. 2006	DQ316191	DQ316153
	CBS 119591	*Protea repens*, South Africa	Roets et al. 2006	DQ316192	DQ316154
Ophiostoma phasma	CBS 119721[T]	*Protea laurifolia*, South Africa	Roets et al. 2006	DQ316219	DQ316181
	CMW 20686	*Protea laurifolia*, South Africa	Roets et al. 2006	DQ316223	DQ316185
Ophiostoma stenoceras	CMW 2524	*Acacia mearnsii*, South Africa	de Beer et al. 2003, Aghayeva et al. 2004	AF484459	AY280473
Sporothrix brasiliensis	IPEC 15572	Human infection, Brazil	Marimon et al. 2007	**FN549903**	AM116955
	IPEC 17943	Human infection, Brazil	Marimon et al. 2007	**FN549902**	AM116935
Sporothrix globosa	FMR 8597	Human infection, Spain	Marimon et al. 2007	**FN549904**	AM116964
	CBS 120340	Human infection, Spain	Marimon et al. 2007	**FN549905**	AM116966
Sporothrix inflata	CBS 939.68[aT]	Wheat field soil, Germany	de Hoog 1974, Aghayeva et al. 2005	AY495426	AY495437
Sporothrix luriei	CBS 937.72	Human infection, South Africa	Marimon et al. 2008	AB128012	AM747289
Sporothrix mexicana	CBS 120341[T]	Soil, Mexico	Marimon et al. 2007	**FN549906**	AM498344
Sporothrix pallida	CBS 150.87	Sediment in water purification plant, Germany	de Meyer et al. 2008	EF127879	EF139109
Sporothrix schenckii	CBS 359.36[T]	Human infection, USA	Marimon et al. 2006	FJ545232	AM116911
Sporothrix sp.-1	CBS 156.72[a]	Greenhouse soil, the Netherlands	Aghayeva et al. 2005	AY495425	AY495436
	CBS 427.74[a]	*Lilium*, the Netherlands	Aghayeva et al. 2005	AY495427	AY495438
Sporothrix sp.-2	CBS 553.74[T a,b]	Soil, Canada	Roxon and Jong 1974, Aghayeva et al. 2005	AY495428	AY495439
(= *Sporothrix dimorphospora* comb. nov.)	CBS 792.73[a]	Soil, Chile	de Hoog 1974, Aghayeva et al. 2005	AY495429	AY495440
	CBS 125442 (= FMR 8977)	Soil, Spain		**FN546961**	**FN547379**
	CBS 125440 (= FMR 8979)	Soil, Spain		**FN546963**	**FN547380**
	CBS 125439 (= FMR 9033)	Soil, USA		**FN546962**	**FN547381**
Sporothrix sp.-3	CBS 793.73[a]	Meadow soil, Germany	de Hoog 1974, Aghayeva et al. 2005	AY495430	AY495441
(= *Sporothrix brunneoviolacea* sp. nov.)	CBS 110895[a]	Roots of *Quercus petraea*, Austria	Halmschlager and Kowalski 2003, Aghayeva et al. 2005	AY495432	AY495433
	CBS 110896[a]	Roots of *Quercus robur*, Austria	Halmschlager and Kowalski 2003, Aghayeva et al. 2005	AY495433	AY495444
	CBS 124560 (= FMR 9334)	Soil, Spain		**FN546960**	**FN547382**
	CBS 124564 (= FMR 9335)	Soil, Spain		**FN546958**	**FN547383**
	CBS 124562 (= FMR 9336)	Soil, Spain		**FN546958**	**FN547384**
	CBS 124561[T] (= FMR 9338)	Soil, Spain		**FN546959**	**FN547385**
Sporothrix stylites	CBS 118848[T]	Pine utility poles, South Africa	de Meyer et al. 2008	EF127883	EF139096

Isolates and sequences generated during this study appear in boldface type.

[T] Type strain.

[a] Obtained from the Centraalbureau voor Schimmelcultures (CBS; Utrecht, the Netherlands) as reference strains of *S. inflata*.

[b] Type strain of *Humicola dimorphospora*; CMW, culture collection, Forestry and Agricultural Biotechnology Institute, FABI, Pretoria, South Africa; FMR, Faculty of Medicine collection, Reus, Spain; IPEC, Instituto de Pesquisa Clínica Evandro Chagas, FIOCRUZ, Rio de Janeiro, Brazil.

flakes, 20 g agar, 1 L distilled water) plates, which were kept at room temperature with diffuse daylight 2 mo to assess the capacity of isolates to produce ascomata. Color notations in parentheses are from Kornerup and Wanscher (1978). The microscopic features were determined from slide cultures made on cornmeal agar (CMA; 30 g corn, 15 g agar, 1 L distilled water) after 12 and 21 d at 25 C. The anamorph of *O. bragantinum* also was studied on OA and PCA (Pfenning and Oberwinkler 1993). Fungal structures were mounted in lactic acid and examined under an Olympus CH-2 light microscope. At least 25 measurements were recorded as the maximum and minimum values for each taxonomically informative structure.

Conidial size is expressed as range. Mean and standard deviation are indicated in brackets in the diagnoses of new taxa. Photomicrographs were obtained with an Axio Imager M1 light microscope. Assimilation tests for sucrose, raffinose and ribitol were performed as described by Marimon et al. (2007).

RESULTS

Fungal isolates.—We obtained seven isolates morphologically similar to *S. inflata* from soil. (Data of these isolates are shown in TABLE I.)

DNA sequence analyses.—BLAST queries revealed that the ITS sequences of four isolates generated in this study (i.e. CBS 124560, CBS 124561, CBS 124562 and CBS 124564) had ≥ 97% similarities to those of isolates CBS 793.73, CBS 110895 and CBS 110896 (accession numbers AY495430, AY495432 and AY495433, respectively). The latter three isolates form clade IV, one of the putative cryptic species in *S. inflata* s.l., in the study by Aghayeva et al. (2005). All other sequences with high percent identities belonged to members of the *O. stenoceras-S. schenckii* complex, such as *Ophiostoma palmiculminatum* Roets, Z.W. de Beer, and M.J. Wing. (DQ316191, DQ316192, and others, ≈ 95%), *S. stylites* (EF127883 and others, ≈ 95%), and *S. pallida* (EF127879 and others, ≈ 95%). ITS sequences generated in this study for the remaining three isolates (CBS 125439, CBS 125440 and CBS 125442) exhibited similarities of ≥ 98% to those of isolates CBS 156.72, CBS 427.74, CBS 553.74 and CBS 792.73, which belong to two subclades of clade III in the study by Aghayeva et al. (2005) and represent additional putative cryptic species in *S. inflata* s.l. ITS sequences of *S. inflata* s. str. (AY495426, AY495431) and *Ophiostoma dentifundum* Aghayeva & M.J. Wingf. (AY495434, AY495435) exhibited 97% identity. Similar results were obtained when BLAST searches were performed with the β-tubulin sequences of our isolates.

With the primers we were able to amplify and sequence 432–561 bp and 386–481 bp of the ITS and BT2 loci respectively. After removing ambiguously aligned regions we obtained respectively ITS and BT2 alignments of 412 and 184 positions, which yielded similar main groups in the neighbor joining analyses. The result of the ILD test ($P = 0.56$) indicated that the ITS and BT2 sequence datasets were congruent and therefore could be combined. In the cladogram obtained from the combined dataset (FIG. 1) two isolates of *O. dentifundum* and the type strain of *S. inflata* formed a clade with low bootstrap support. The *S. inflata*-like isolates CBS 156.72 and CBS 427.74 formed a well supported clade, named herein *Sporothrix* sp.-1, distinct from the type strain of *S. inflata*. Isolates CBS 792.73, CBS 125439, CBS 125440, CBS 125442 and CBS 553.74, the type strain of *H. dimorphospora*, formed a clade with 87% bootstrap support (indicated in FIG. 1 as *Sporothrix* sp.-2, also distinct from *S. inflata*). Isolates CBS 793.73, CBS 110895, CBS 110896, CBS 124560, CBS 124561, CBS 124562 and CBS 124564 formed a group, with 99% bootstrap support, referred to as *Sporothrix* sp.-3, which was distantly related to the type strain of *S. inflata*. The two strains of *O. bragantinum* formed a clade with 100% bootstrap support, also distantly related to *S. inflata*.

Morphology and physiology.—All isolates grew and sporulated on PDA at 15, 20 and 25 C. On the same medium most isolates grew and sporulated at 30 C with only two exceptions, isolate CBS 427.74 (*Sporo-Sporothrix* sp.-1), which did not grow, and isolate CBS 239.68, the type strain of *S. inflata*, which produced sterile mycelium with abundant terminal and intercalary hyaline chlamydospore-like cells. At 15 and 20 C no clear differences in colony diameters and sporulation were observed among the isolates. At 25 C isolates of *O. bragantinum* grew faster than *S. inflata*, *Sporothrix* sp.-1 and *Sporothrix* sp.-2. On PDA at 30 C all species showed restricted growth and produced cerebriform or convoluted colonies with decreased sporulation, except *O. bragantinum*, which grew and sporulated well at this temperature. On the same medium at 35 C only isolates of the latter species grew, although very restrictedly and forming cerebriform, creamy colonies with abundant yeast-like budding cells. None of the isolates grew at 37 C.

Sporothrix sp.-1 and *Sporothrix* sp.-2 produced flat, often umbonate, pale yellowish colonies without diffusible pigments on PDA at 25 C, similar to those of *S. inflata*. Isolates of *Sporothrix* sp.-3 were variable in colony morphology, but all of them, except CBS 793.73, produced a diffusible violet-brown pigment on PDA (FIG. 2A), which was enhanced at 30 C. A weak pigment production was detected in the latter isolate only in old cultures on OA. All isolates showed denticulate, often apically inflated conidiogenous

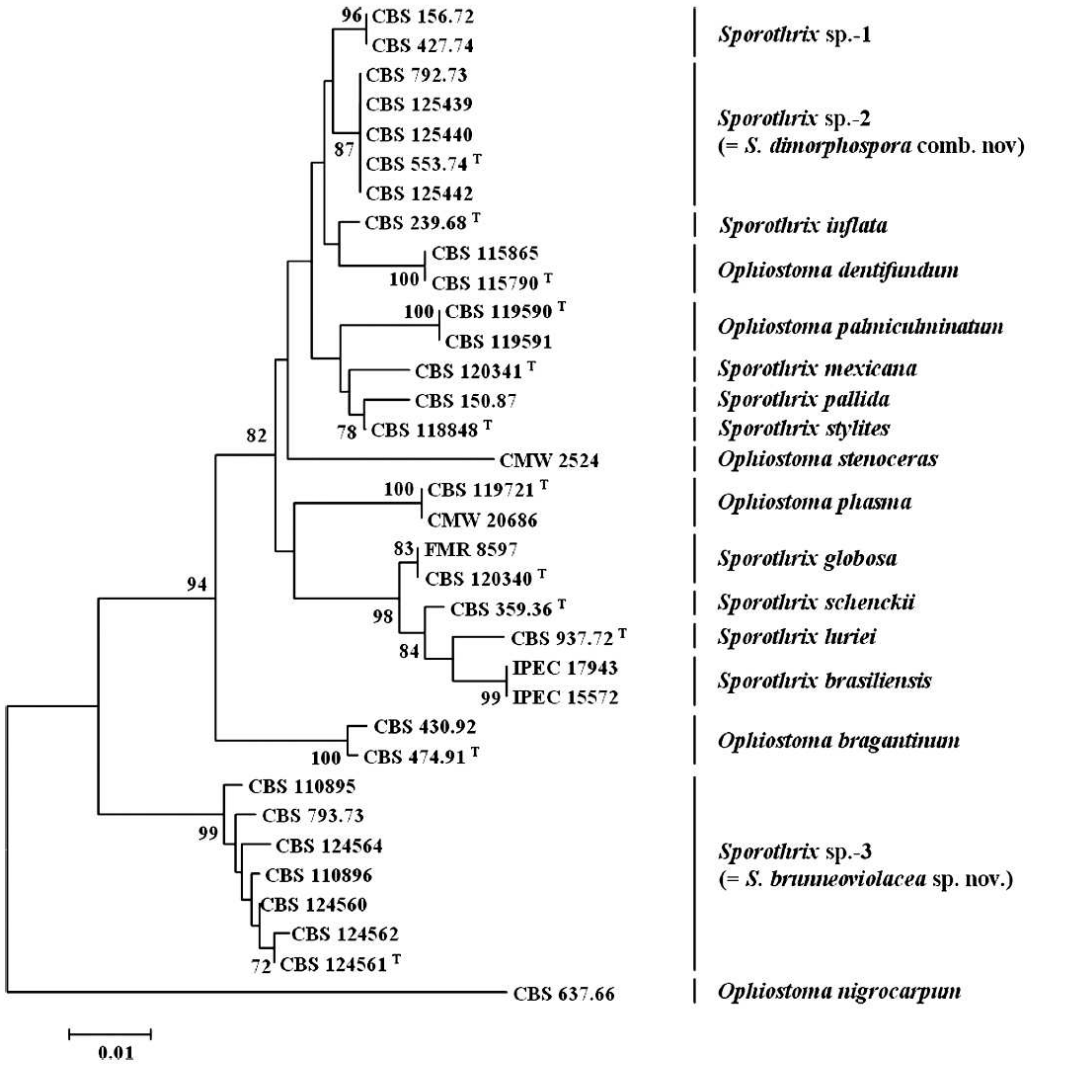

FIG. 1. Neighbor joining tree constructed with the combined ITS and β-tubulin dataset. Branch lengths are proportional to distance. Bootstrap values above 70% are indicated in the internodes. *Ophiostoma nigrocarpum* was used as outgroup. T, type strain.

cells that produced hyaline to subhyaline sympodial conidia with apiculate bases (FIGS. 2C; 3C, D). While the type strain of *S. inflata* and the isolate CBS 156.72 of *Sporothrix* sp.-1 produced mostly narrowly clavate sympodial conidia (3–9 × 1–1.5 µm and 3–9 × 1.5–2 µm respectively), these propagules were slightly shorter and wider, mostly guttuliform in isolate CBS 427.74 of *Sporothrix* sp.-1, *Sporothrix* sp.-2 (in both cases 3–8 × 1.5–3 µm) and *Sporothrix* sp.-3 (3–7 × 1.5–3 µm). The pigmented lateral blastoconidia of *S. inflata*, *Sporothrix* sp.-1 and *Sporothrix* sp.-2, were mostly subglobose to obovoid, about 3–5 × 3–5 µm and always acquired their dark pigmentation gradually, being hyaline in the early stages of development and brown at maturity. These blastoconidia were sessile, sometimes with a wide truncate base, or were supported by subhyaline or poorly pigmented pedicels. On the other hand *Sporothrix* sp.-3 produced mostly globose, 3.5–5 µm wide pigmented lateral blastoconidia, which often were strongly melanized from the initial stages of development (FIG. 2 B) and after 21 d sometimes showed thick deposits of a mucilaginous dark brown material (FIG. 2

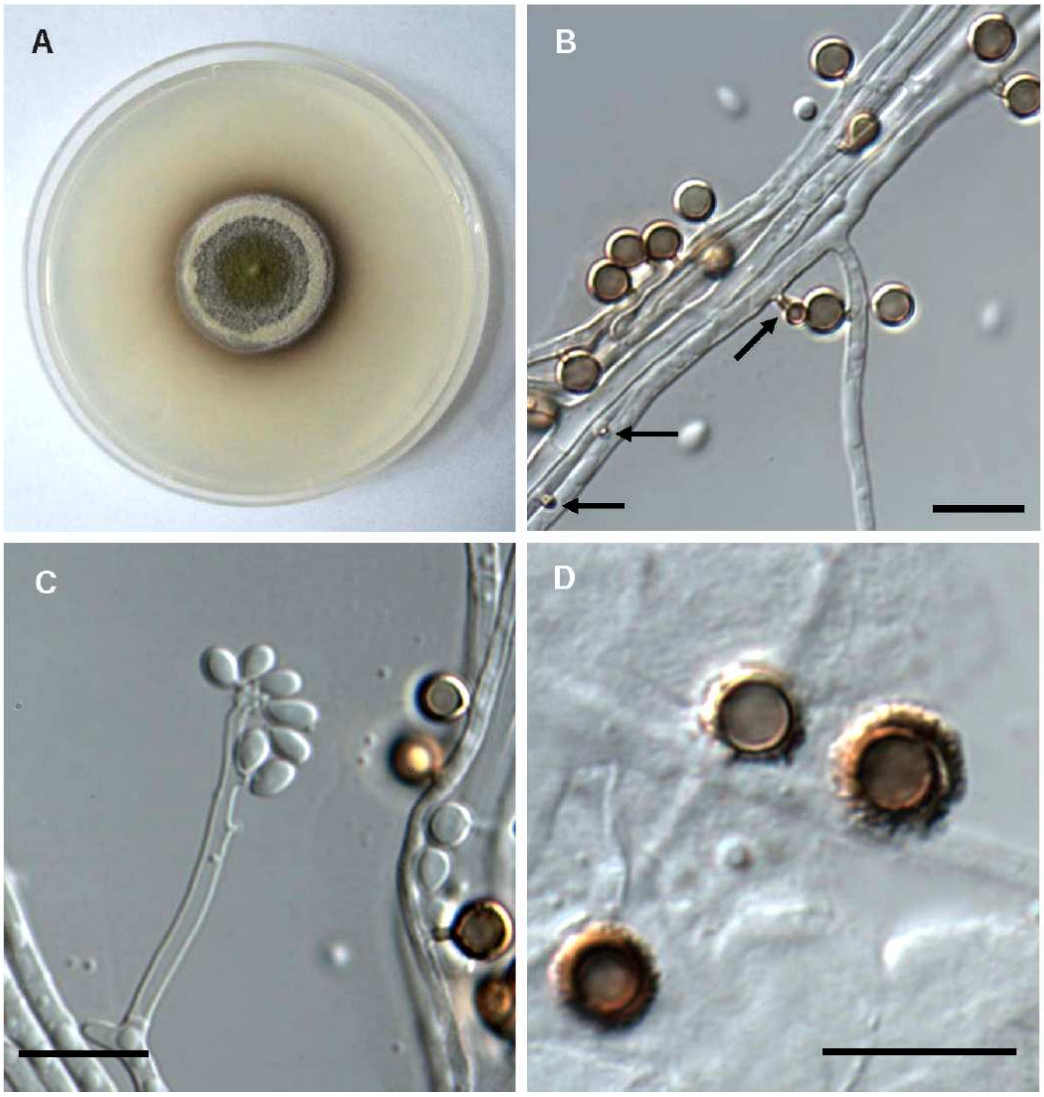

FIG. 2. *Sporothrix brunneoviolacea* (CBS 124561). A. Colony on PDA after 21 d at 25 C. B. Pigmented lateral blastoconidia, often strongly melanized from early stages of development (arrows). C. Denticulate conidiogenous cell producing conidia. D. Pigmented lateral blastoconidia covered by a mucilaginous dark brown material. Bars = 10 µm.

D), a feature not observed in *S. inflata*, *Sporothrix* sp.-1 or *Sporothrix* sp.-2. These blastoconidia were sessile or supported by pedicels that on average were darker than those of all other species studied. The anamorph of *O. bragantinum* on PCA and OA matched the description provided by Pfenning and Oberwinkler (1993), with pigmented lateral blastoconidia that appeared slightly rugose. This texture proved to be caused by the presence of thin deposits of a mucilaginous light brown material, possibly homologous to that observed in *Sporothrix* sp.-3. However these deposits were not produced on CMA, where the pigmented lateral blastoconidia appeared smooth. Only isolates of *O. bragantinum* produced ascomata in culture.

Sucrose was assimilated by all *Sporothrix* isolates, raffinose was assimilated by all isolates of *Sporothrix* sp.-2 and *Sporothrix* sp.-3, and by none of *Sporothrix* sp.-1 or by the type strain of *S. inflata*. Ribitol was assimilated or at least weakly assimilated by all *Sporothrix* isolates tested, except CBS 125439 (*Sporothrix* sp.-2) and CBS

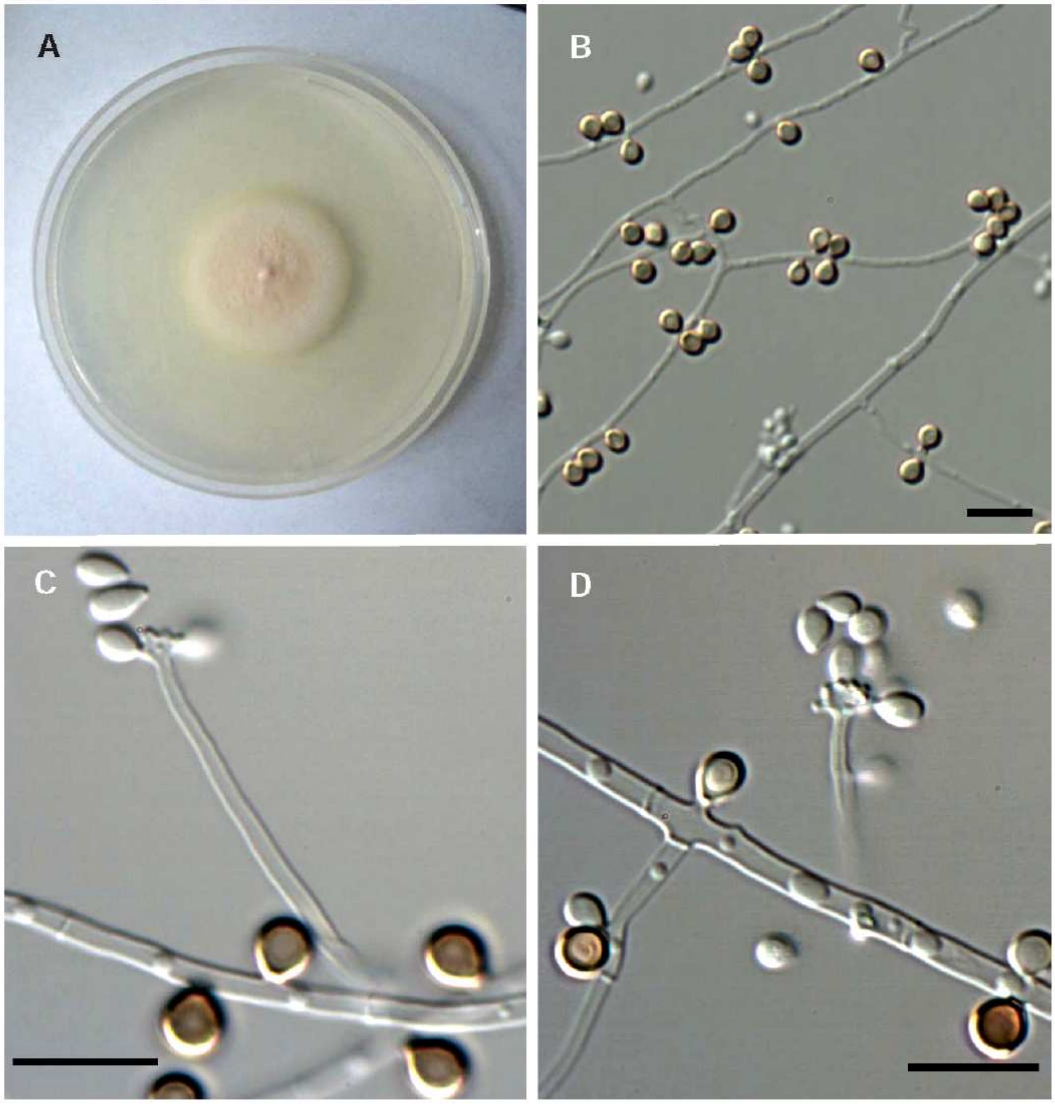

FIG. 3. *Sporothrix dimorphospora* (CBS 125440). A. Colony on PDA after 21 d at 25 C. B. Pigmented lateral blastoconidia. C, D. Pigmented lateral blastoconidia and conidiophores with terminal denticulate conidiogenous cells. Bars = 10 µm.

124564 (*Sporothrix* sp.-3). (Results of physiological tests are summarized in TABLE II.)

Sporothrix sp.-2 and *Sporothrix* sp.-3 are segregated from *S. inflata* s. str. because they are phylogenetically and phenotypically different from the type strain of this species and their combinations of morphological and physiological features do not match any of the currently accepted species of *Sporothrix* or *Ophiostoma*. We do not provide a Latin binomial for *Sporothrix* sp.-1 because this fungus could not be clearly distinguished phenotypically from *S. inflata*.

TAXONOMY

Sporothrix brunneoviolacea Madrid, Gené, Cano & Guarro sp. nov. FIGS. 1, 2
= Clade IV in Aghayeva et al. (2005)
MycoBank MB515559

Etymology. This name refers to the violet-brown pigment produced by the fungus in culture.

Coloniae in PDA ad 25 C post 21 d 30–57 mm diam, velutinae, interdum funiculosae. Pigmentum brunneo-violaceum plerumque formatur. Hyphae 0.5–3 µm latae. Conidiophora erecta, plerumque simplicia, usque ad 93 µm

TABLE II. Summary of the physiological features of the taxa studied

Taxon	Colony diameter (mm) on PDA after 21 d					Assimilation test result			Violet-brown pigment on PDA or OA
	15 C	20 C	25 C	30 C	35 C	Sucrose	Raffinose	Ribitol	
Ophiostoma bragantinum	20–25	35–40	62–64	50–64	1.5–7	NT	NT	NT	−
Sporothrix sp.-1	25–30	32–33	12–30	0–3	0	+	−	+ or W	−
Sporothrix sp.-2	24–35	38–45	25–44	3–6	0	+	+	V	−
Sporothrix sp.-3	18–32	23–40	30–57	5–17	0	+	+	V	+
Sporothrix inflata	24–29	30–32	32	2	0	+	−	W	−

Colony diameters are shown as minimum and maximum values obtained in duplicate plates for isolates belonging to each taxa. OA, oatmeal agar; PDA, potato dextrose agar; +, positive; −, negative; NT, not tested; V, variable; W, weak.

longa. Cellulae conidiogenae in conidiophoris terminaliter vel intercalariter incorporatae, sympodialiter proliferae, denticulatae, 16–43 µm longae, 1–2 µm latae ad partem mediam, plerumque inflatae ad apicem usque ad 4 µm latae. Conidia sympodialia plerumque guttuliformia, 3–7 × 1.5–3 µm, hyalina vel subhyalina, laevia, tenuitunicata. Blastoconidia lateralia plerumque globosa, 3.5–5 µm diam, brunnea vel atrobrunnea, laevia, crassitunicata. In agaro PDA ad 35 C non crescentes. Assimilantur raffinosum et sucrosum. Teleomorphosis ignota.

Colonies on PDA 30–57 mm diam after 21 d at 25 C, flat, velvety, variable in color, often zonate, yellowish white (2A2) or olive (2E3) and sometimes funiculose at the center, in shades of greenish gray (1B2), gray (4B1), olive (1E3), grayish green (27C4) or grayish violet (15D6) toward the periphery; reverse dull green (28D4) or grayish green (1C3, 27D4 or 28D6) at the center, grayish green (29B4) or yellowish white (1A2) toward the periphery, usually with a diffusible violet-brown (close to 11F8) pigment. Vegetative hyphae subhyaline to pale olive, rarely showing short brown segments, smooth and thin-walled, 0.5–3 µm wide, commonly forming mycelial strands. Conidiophores more or less differentiated, mostly unbranched, up to 93 µm long. Conidiogenous cells terminal or intercalary in the conidiophores, sympodial, denticulate, 16–43 µm long, 1–2 µm wide toward the center, often inflated at the apex up to 4 µm wide. Sympodial conidia mostly guttuliform, 3–7(4.1 ± 0.7) × 1.5–3(1.9 ± 0.2) µm, hyaline to subhyaline, smooth- and thin-walled. Lateral blastoconidia from undifferentiated hyphae mostly globose, 3.5–5(4 ± 0.3) µm wide, smooth- and thick-walled, brown to dark brown, often strongly melanized from early stages of development, frequently covered by a thick layer of mucilaginous dark brown material at maturity, borne sessile or on well developed, often strongly melanized pedicels. Optimum growth near 25 C, no growth observed at 35 C. Assimilation of raffinose and sucrose positive. Teleomorph not observed.

Specimens examined: SPAIN. NAVARRA PROVINCE: Señorío de Bertiz National Park. Isolated from forest soil, Feb 2006, J. Cano, J. Mena and C. Silvera (HOLOTYPE, IMI 397927; culture ex-holotype, CBS 124561 = FMR 9338). Same location, date and collectors (CBS 124560 = FMR 9334, CBS 124562 = FMR 9336, and CBS 124564 = FMR 9335). GERMANY. NORTH RHINE-WESTPHALIA: Solingen. Isolated from meadow soil, Oct 1973, A. von Klopotek (CBS 793.73). AUSTRIA. LOWER AUSTRIA: Patzmannsdorf. Isolated from roots of *Quercus petraea* (Matt.) Liebl., Jun 1993, E. Halmschlager (CBS 110895). AUSTRIA. LOWER AUSTRIA: Niederweiden. Isolated from roots of *Quercus robur* L., Jul 1993, E. Halmschlager (CBS 110896).

Sporothrix dimorphospora (Roxon & S.C. Jong) Madrid, Cano, Gené & Guarro comb. nov. FIGS. 1, 3
≡ *Humicola dimorphospora* Roxon & S.C. Jong, Can J Bot 52:517 (1974).

MycoBank MB515560

Colonies on PDA 25–44 mm diam after 21 d at 25 C, flat, smooth, umbonate, yellowish white (4A2). Vegetative hyphae subhyaline to pale olive, smooth- and thin-walled, 1–3 µm wide, commonly forming mycelial strands. Conidiophores more or less differentiated, mostly unbranched, up to 90 µm long. Conidiogenous cells terminal or intercalary, sympodial, denticulate, 22–43 long, 1–1.5 µm wide toward the center, often inflated at the apex up to 5 µm wide. Sympodial conidia mostly guttuliform, 3–8(4.3 ± 0.8) × 1.5–3(2.2 ± 0.3) µm, hyaline to subhyaline, smooth- and thin-walled. Lateral blastoconidia from undifferentiated hyphae mostly subglobose to obovoid, 3–5(4 ± 0.4) × 3–5(3.8 ± 0.4) µm, smooth- and thick-walled, brown, always becoming dark gradually, sessile or on subhyaline to weakly pigmented pedicels. Optimum growth near 20 C, no growth observed at 35 C. Assimilation of raffinose and sucrose positive. Teleomorph not observed.

Specimens examined: CANADA. SASKATCHEWAN PROVINCE: Stoughton. Isolated from soil, Oct 1974, R.A.A. Morrall (CBS 553.74, type strain of *H. dimorphospora*). CHILE. VALDIVIA PROVINCE: Valdivia area. Isolated from soil, 1972, J. Grinbergs (CBS 792.73). SPAIN. TARRAGONA PROVINCE: Poblet area. Isolated from soil, Feb 2006, C. Silvera (CBS 125440 = FMR 8979). SPAIN. ZAMORA PROVINCE: Lobeznos. Isolated from soil, Feb 2006, C.

Silvera (CBS 125442 = FMR 8977). USA. CALIFORNIA STATE: Yosemite National Park. Isolated from forest soil, Jan 2006, C. Silvera (CBS 125439 = FMR 9033).

DISCUSSION

Members of the *O. stenoceras-S. schenckii* complex have been associated with a wide range of habitats, including conifers and hardwood trees (de Hoog 1974), wood-destroying arthropods (Zhou et al. 2006), infructescens of *Protea* species (Roets et al. 2006), decaying plant material and vertebrate hosts (Kwon-Chung and Bennet 1992, Madrid et al. 2009). Most isolates of the new species proposed in this work were obtained from soil, which also is an important reservoir for species in this group. At least 18 species of the complex have been isolated directly from soil or from associated habitats (de Meyer et al. 2008). Nevertheless it is possible that many of the species currently known only from soil also occur in other substrata because several *Sporothrix* species are plurivorous (de Hoog 1974, Marimon et al. 2007). Of interest two strains of *S. brunneoviolacea* from Halmschlager and Kowalski (2003) were isolated from roots of *Quercus* spp. The fungus was reported to produce polyphenoloxidases and might be a common endophyte of oaks in Austria.

In the *O. stenoceras-S. schenckii* complex genetically divergent species often show similar asexual reproductive structures, possibly as a result of convergent evolution due to the adaptation to similar ecological niches (Upadhyay 1981, Wingfield et al. 1999). This phenomenon, which also occurs in other fungi (Mouchacca and Gams 1993, Thorn and Barron 1986), explains the proposal of incorrect synonymies (de Hoog et al. 1974, Marimon et al. 2007, de Meyer et al. 2008) and erroneous anamorph-teleomorph connections in the past, such as those of *S. schenckii* and *O. stenoceras* (Taylor 1970, Mariat 1971, de Hoog 1974) or *S. inflata* and *O. bragantinum* (Pfenning and Oberwinkler 1993). It also might explain the presence of only subtle morphological differences between the taxa proposed in this study and *S. inflata* (see RESULTS).

Recent studies have shown that morphologically similar species of this group of fungi may differ in physiological features. In a survey *Ophiostoma gemellus* Roets, Z.W. de Beer & Crous and *O. palmiculminatum*, two taxa genetically closely related, differed significantly in their abilities to tolerate different concentrations of cycloheximide (Roets et al. 2008). Another study demonstrated that carbohydrate assimilation profiles and growth rates at different temperatures differed among cryptic species detected in *S. schenckii* s.l. (Marimon et al. 2007). In our study the fast growth of *O. bragantinum* at 30 C distinguished this taxon from *Sporothrix* sp.-1, *S. inflata*, *S. brunneoviolacea* and *S. dimorphospora*. In addition the ability to assimilate raffinose distinguished the latter two species (both positive) from *S. inflata* and *Sporothrix* sp.-1 (both negative). Nevertheless more isolates of these taxa should be studied to corroborate whether these differences are stable. Additional physiological tests also should be performed to search for phenotypic traits to distinguish *Sporothrix* sp.-1 from *S. inflata*.

The production of a diffusible violet-brown pigment by *S. brunneoviolacea* also proved to be a valuable taxonomic character, which distinguishes this species from other members of the *O. stenoceras-S. schenckii* complex (de Hoog 1974, Upadhyay 1981, Pfenning and Oberwinkler 1993, Marimon et al. 2007). Diffusible pigments seldom have been reported in *Sporothrix* species. De Hoog and de Vries (1973) mentioned the production of a diffusible purple to violaceous pigment in *Sporothrix cyanescens* de Hoog & de Vries, a species originally isolated from human skin. However subsequent ultrastructural and phylogenetic studies revealed that this fungus is a basidiomycete (Smith and Batenburg-van der Vegte 1985, de Beer et al. 2006). Dixon et al. (1991), during the characterization of a large set of strains associated with an epidemic of sporotrichosis in USA, found environmental *Sporothrix* isolates that produced a brownish diffusible pigment in culture. In contrast to *S. brunneoviolacea* those isolates were able to grow at 35 C and lacked pigmented lateral blastoconidia. *S. brunneoviolacea* and *S. dimorphospora* also are morphologically similar to other *Sporothrix* species with pigmented lateral blastoconidia, such as *S. brasiliensis*, *S. globosa*, *S. mexicana* and *S. schenckii* (de Hoog 1974, Marimon et al. 2007). However the latter four species are able to grow at 35 C (Marimon et al. 2007). The anamorph of *O. stenoceras* also produces pigmented lateral blastoconidia, but this fungus is homothallic and produces perithecia on agar media (Aghayeva et al. 2004).

The four *Sporothrix* species known to infect vertebrates form a monophyletic group (Marimon et al. 2007, Roets et al. 2008), but other relatively distant Ophiostomatales also might act as opportunistic pathogens. *O. stenoceras* was mentioned as the possible etiological agent of cases of onychomycosis (Summerbell et al. 1993), and *Ophiostoma piceae* (Münch) Syd. & P. Syd., a species grouping outside the *O. stenoceras-S. schenckii* complex (Roets et al. 2008), has been reported from a case of disseminated human infection (Bommer et al. 2009). In our study *O. bragantinum* was able to grow at 35 C and exhibited phenotypic dimorphism, forming abundant yeast cells at this temperature, attributes that suggest

that it might be another potential opportunist (Kwon-Chung and Bennet 1992, Marimon et al. 2007). Experimental pathogenicity studies should be performed to determine whether this species also is able to infect animals.

Much confusion historically has surrounded the taxonomy of the genus *Sporothrix*. However recent studies solved part of this problem with DNA sequence analyses. The high discriminatory power of these techniques led to the discovery of numerous cryptic species (Marimon et al. 2007, de Meyer et al. 2008). It also clarified aspects of the anamorph-teleomorph connections (de Beer et al. 2003), distribution (Madrid et al. 2009) and ecology (Roets et al. 2006) of these fungi. Nevertheless the evolutionary relationships of many *Sporothrix* species are unknown and certain species of the genus remain genetically heterogeneous (Marimon et al. 2007). This highlights the need for further research, in which the combined analysis of DNA phylogenies and phenotypic features might provide a more natural classification of *Sporothrix* species.

ACKNOWLEDGMENTS

We are indebted to the curators of the Centraalbureau voor Schimmelcultures (Utrecht, the Netherlands) for supplying some of the isolates studied. This work was supported by the Spanish Ministerio de Educación y Ciencia grants CGL2007-65669, CGL 2008-04226 and CGL 2009-08698/BOS.

LITERATURE CITED

Aghayeva DN, Wingfield MJ, de Beer ZW, Kirisits T. 2004. Two new *Ophiostoma* species with *Sporothrix* anamorphs from Austria and Azerbaijan. Mycologia 96:866–878.

———, ———, Kirisits T, Wingfield BD. 2005. *Ophiostoma dentifundum* sp. nov. from oak in Europe, characterized using molecular phylogenetic data and morphology. Mycol Res 109:1127–1136.

Bommer M, Hütter ML, de Hoog GS, de Beer WZ, Wellinghausen N. 2009. Fatal *Ophiostoma piceae* infection in a patient with acute lymphoblastic leukaemia. J Med Microbiol 58:381–385.

de Beer ZW, Begerow D, Bauer R, Pegg GS, Crous PW, Wingfield MJ. 2006. Phylogeny of the *Quambalariaceae* fam. nov., including important *Eucalyptus* pathogens in South Africa and Australia. Stud Mycol 55:289–298.

———, Harrington TC, Vismer H, Wingfield BD, Wingfield MJ. 2003. Phylogeny of the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 95:434–441.

de Hoog GS. 1974. The genera *Blastobotrys*, *Sporothrix*, *Calcarisporium* and *Calcarisporiella* gen. nov. Stud Mycol 7:1–84.

de Meyer EM, de Beer ZW, Summerbell RC, Moharram AM, de Hoog GS, Vismer HF, Wingfield MJ. 2008. Taxonomy and phylogeny of new wood- and soil-inhabiting species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 100:647–661.

———, de Vries GA. 1973. Two new species of *Sporothrix* and their relation to *Blastobotrys nivea*. Antonie Van Leeuwenhoek 39:515–520.

Dixon DM, Salkin IF, Duncan RA, Hurd NJ, Haines JH, Kemna ME, Coles FB. 1991. Isolation and characterization of *Sporothrix schenckii* from clinical and environmental sources associated with the largest U.S. epidemic of sporotrichosis. J Clin Microbiol 29:1106–1113.

Farris JS, Källersjö M, Kluge AG, Bult C. 1994. Testing significance of incongruence. Cladistics 10:315–319.

Gilgado F, Cano J, Gené J, Guarro J. 2005. Molecular phylogeny of the *Pseudallescheria boydii* species complex: proposal of two new species. J Clin Microbiol 43: 4930–4942.

Halmschlager E, Kowalski T. 2003. *Sporothrix inflata*, a root-inhabiting fungus of *Quercus robur* and *Q. petraea*. Mycol Prog 2:259–266.

Kornerup A, Wanscher JH. 1978. Methuen handbook of color. 3rd ed. London: Eyre Methuen. 252 p.

Kwon-Chung KJ, Bennett JE. 1992. Medical mycology. Philadelphia: Lea & Febiger. 866 p.

Mackinnon JE, Conti-Díaz IA, Gezuele E, Civila E, da Luz S. 1969. Isolation of *Sporothrix schenckii* from nature and considerations on its pathogenicity and ecology. Sabouraudia 7:138–145.

Madrid H, Cano J, Gené J, Bonifaz A, Toriello C, Guarro J. 2009. *Sporothrix globosa*, a pathogenic fungus with widespread geographical distribution. Rev Iberoam Micol 26:218–222.

Mariat F. 1971. Adaptation de *Ceratocystis* a la vie parasitaire chez l'animal-etude de l'aquisition d'un pouvoir pathogene comparable a celui de *Sporothrix schenckii*. Sabouraudia 9:191–205.

Marimon R, Cano J, Gené J, Sutton DA, Kawasaki M, Guarro J. 2007. *Sporothrix brasiliensis*, *S. globosa* and *S. mexicana*, three new *Sporothrix* species of clinical interest. J Clin Microbiol 45:3198–3206.

———, Gené J, Cano J, Guarro J. 2008. *Sporothrix luriei*: a rare fungus from clinical origin. Med Mycol 46:621–625.

———, ———, ———, Trilles L, Dos Santos Lázera M, Guarro J. 2006. Molecular phylogeny of *Sporothrix schenckii*. J Clin Microbiol 44:3251–3256.

Mouchacca J, Gams W. 1993. The hyphomycete genus *Cladorrhinum* and its teleomorph connections. Mycotaxon 48:415–440.

Nixon KC. 2002. Winclada software. Version 1.00.08. Ithaca, New York: published by the author (available at: http://www.cladistics.com).

Pfenning L, Oberwinkler F. 1993. *Ophiostoma bragantinum* n. sp., a possible teleomorph of *Sporothrix inflata*, found in Brazil. Mycotaxon 46:381–385.

Roets F, de Beer ZW, Dreyer LL, Zipfel R, Crous PW, Wingfield MJ. 2006. Multi-gene phylogeny for *Ophiostoma* spp. reveals two new species from *Protea* infructescences. Stud Mycol 55:199–212.

———, ———, Wingfield MJ, Crous PW, Dreyer LL. 2008. *Ophiostoma gemellus* and *Sporothrix variecibatus* from

mites infesting *Protea* infructescences in South Africa. Mycologia 100:496–510.

Roxon JE, Jong SC. 1974. A new pleomorphic species of *Humicola* from Saskatchewan soil. Can J Bot 52:517–520.

Smith MT, Batenburg-van der Vegte WH. 1985. Ultrastructure of septa in *Blastobotrys* and *Sporothrix*. Antonie van Leeuwenhoek 51:121–128.

Summerbell RC, Kane J, Krajden S, Duke EE. 1993. Medically important *Sporothrix* species and related ophiostomatoid fungi. In: Wingfield MJ, Seifert KA, Webber JF, eds. Ceratocystis and *Ophiostoma*: taxonomy, ecology and pathogenicity. St Paul, Minnesota: American Phytopathological Society Press. p 185–192.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA 4: Molecular evolutionary genetics analysis (MEGA). Software version 4.0. Mol Biol Evol 24:1596–1599.

Taylor JJ. 1970. A comparison of some *Ceratocystis* species with *Sporothrix schenckii*. Mycopathol Mycol Appl 42: 233–240.

Thorn RG, Barron GL. 1986. *Nematoctonus* and the tribe *Resupinatae* in Ontario, Canada. Mycotaxon 25:321–453.

Upadhyay HP. 1981. A monograph of *Ceratocystis* and *Ceratocystiopsis*. Athens, Georgia: Univ. Georgia Press. 176 p.

Wingfield BD, Viljoen CD, Wingfield MJ. 1999. Phylogenetic relationships of ophiostomatoid fungi associated with *Protea* infructescences in South Africa. Mycol Res 103: 1616–1620.

Zhou X, de Beer ZW, Wingfield MJ. 2006. DNA sequence comparisons of *Ophiostoma* spp., including *Ophiostoma aurorae* sp. nov., associated with pine bark beetles in South Africa. Stud Mycol 55:269–277.

4.2 *Sporothrix globosa*, a pathogenic fungus with widespread geographical distribution

Madrid H, Cano J, Gené J, Bonifaz A, Toriello C, Guarro J

Revista Iberoamericana de Micología 2009; 26:218–222

Rev Iberoam Micol. 2009;26(3):218–222

Revista Iberoamericana de Micología

www.elsevier.es/reviberoammicol

Note

Sporothrix globosa, a pathogenic fungus with widespread geographical distribution

Hugo Madrid [a], Josep Cano [a,*], Josepa Gené [a], Alexandro Bonifaz [b], Conchita Toriello [c] and Josep Guarro [a]

[a] *Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, IISPV Reus, Tarragona, Spain*
[b] *Servicio de Dermatología y Departamento de Micología, Hospital General de México, Mexico City, Mexico*
[c] *Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, Mexico*

ARTICLE INFO

Article history:
Received 2 September 2008
Accepted 10 February 2009
Available online 23 de julio de 2009

Keywords:
Phylogeny
Calmodulin
Sporothrix globosa
Sporotrichosis
Distribution

ABSTRACT

Sporothrix globosa, reported from the USA, Europe, and Asia, is a recently described pathogenic species morphologically similar to *Sporothrix schenckii*. In this study, the phylogenetic affinities of 32 clinical and environmental isolates morphologically identified as *S. schenckii*, from Mexico, Guatemala, and Colombia, were assessed by cladistic analysis of partial sequences of the calmodulin gene using the maximum parsimony and neighbor-joining methods. The study revealed that one out of 25 isolates from Mexico (4%), one out of three isolates from Guatemala (33.3%), and two out of four isolates from Colombia (50%) belonged to *S. globosa*, while the other isolates belonged to *S. schenckii sensu stricto*. This is the first record of *S. globosa* from Mexico, and Central and South America.

Sporothrix globosa, un hongo patógeno con amplia distribución geográfica

Palabras clave:
Filogenia
Calmodulina
Sporothrix globosa
Esporotricosis
Distribución

RESUMEN

Sporothrix globosa es un hongo patógeno recientemente descrito. Esta especie, morfológicamente similar a *Sporothrix schenckii*, se ha descrito en EE.UU., Europa y Asia. En este trabajo se investigaron las relaciones filogenéticas de 32 aislamientos clínicos y ambientales, identificados morfológicamente como *S. schenckii*, procedentes de México, Guatemala y Colombia, mediante análisis cladístico de secuencias parciales del gen de la calmodulina usando los métodos de máxima parsimonia y *neighbor-joining*. El estudio reveló que uno de los 25 aislamientos de México (4%), uno de los tres aislamientos de Guatemala (33%) y dos de los cuatro aislamientos de Colombia (50%) correspondían a *S. globosa*, mientras que los demás aislamientos pertenecían a *S. schenckii sensu stricto*. La presencia de *S. globosa* en México, América Central y del Sur se describe por primera vez.

Sporotrichosis is a chronic infectious disease that typically involves the skin and subcutaneous tissue.[7,12] Cases of arthritis, meningitis, and other deep-seated forms have been reported less frequently.[1,3–5,10,25] Infection is acquired via traumatic implantation or less frequently by inhalation of propagules of the etiological agent living in soil, plant material, and other substrata.[14,19] Sporotrichosis is distributed worldwide, but most cases occur in temperate, warm, and tropical countries. The largest number of reports comes from North America, but it is also common in areas of Central and South America, Asia, and South Africa.[8,12]

For several decades, sporotrichosis has been attributed to a single pathogen, *Sporothrix schenckii* Hektoen & Perkins, an anamorphic fungus related to the ascomycetous genus *Ophiostoma* H. & P. Syd.[2] However, isolates identified as *S. schenckii* showed a great deal of phenotypic[6,12] and genetic[9,13,20] variability, suggesting that this taxon was a species complex. In a recent phylogenetic study based on the analysis of sequences of the chitin-synthase, ß-tubulin, and calmodulin (CAL) genes, numerous isolates phenotypically identified as *S. schenckii* were tested.[17] The strains were distributed into at least six distinct groups, which were considered as putative phylogenetic species. Later, the same authors found diagnostic features to separate phenotypically

* Corresponding author.
E-mail address: josep.cano@urv.cat (J. Cano).

1130-1406/$ - see front matter © 2008 Revista Iberoamericana de Micología. Publicado por Elsevier España, S.L. Todos los derechos reservados.
doi:10.1016/j.riam.2009.02.005

and genetically three of these clades, which were formally proposed as new species. They were *Sporothrix brasiliensis* Marimon, Gené, Cano & Guarro, *Sporothrix globosa* Marimon, Gené, Cano & Guarro, and *Sporothrix mexicana* Marimon, Gené, Cano & Guarro.[16]

Since variations in antifungal susceptibility have been demonstrated among the different species of the *S. schenckii* complex, their identification is clinically relevant.[18] Furthermore, considering that the taxonomy of the fungi causing sporotrichosis has been reevaluated, it becomes necessary to identify clinical isolates at the species level in order to study their epidemiology and geographical distribution, and to determine if different clinical patterns are associated with each of these taxa. Recently we have had the opportunity of studying numerous isolates from Mexico, Colombia, and Guatemala and our interest was to assess if a given species is predominantly implicated in cases of human infection in these countries, or, by contrast, a range of species can be present. To do this, we analyzed partial sequences of the CAL locus, which had previously proven to be the most informative gene.[16,17]

Materials and methods

Fungal isolates

Thirty-two clinical and environmental isolates morphologically identified as *S. schenckii*, from Colombia, Guatemala, and Mexico were included in this study (Table 1). These isolates were obtained from culture collections located at the *Servicio de Dermatología y Departamento de Micología*, in *Hospital General de México*, Mexico, and at the *Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Autónoma de México*, Mexico. The isolates were subcultured on potato dextrose agar (PDA; Difco Laboratories, USA) plates and incubated at 25 °C for 14 days in the dark. Isolates were stored at 4–7 °C and in slant cultures submerged in mineral oil at room temperature.

DNA extraction, amplification, and sequencing

DNA extraction, amplification, and sequencing of the CAL locus of the 32 isolates were performed as described previously,[16,17] using primers CL1 and CL2A.[21]

DNA sequence alignments

The program Autoassembler vers. 1.40 (Applied Biosystems) was used to obtain consensus sequences from the two complementary sequences of each isolate. The consensus sequences of the 32 isolates sequenced here were aligned with CAL sequences of 39 other isolates of *S. schenckii sensu stricto* and the related species *S. brasiliensis*, *S. globosa*, *S. mexicana*, and *Sporothrix albicans* S.B. Saksena determined in a previous study,[16] using ClustalX vers. 1.81,[24] followed by manual adjustments with a text editor.

Phylogenetic analysis

A phylogenetic analysis was performed by the maximum parsimony method using the PAUP* version 4.0b10 software.[23] Briefly, the most parsimonious trees were obtained after 100 heuristic searches with random sequence addition and tree

Table 1
Collection number, fungal species, source, origin, and EMBL accession numbers of the isolates studied.

Isolate no.	Species	Source	Origin	EMBL accession no.
FMR 9549	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9550	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9551	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9553	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9554	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9555	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9556	*S. globosa*	Human, lymphocutaneous sporotrichosis	Mexico	FM179331
FMR 9557	*S. schenckii*	Human, fixed cutaneous sporotrichosis	Mexico	–
FMR 9559	*S. schenckii*	Human, fixed cutaneous sporotrichosis	Mexico	–
FMR 9560	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9561	*S. schenckii*	Human, fungaemia	Mexico	–
FMR 9562	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	FM179332
FMR 9563	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9564	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9565	*S. schenckii*	Human, fixed cutaneous sporotrichosis	Mexico	–
FMR 9566	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9567	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9568	*S. schenckii*	Human, fixed cutaneous sporotrichosis	Mexico	–
FMR 9570	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9572	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9616	*S. schenckii*	Human, fixed cutaneous sporotrichosis, hand	Colombia	–
FMR 9617	*S. globosa*	Human, fixed cutaneous sporotrichosis, wrist	Colombia	FM179329
FMR 9619	*S. globosa*	Human, fixed cutaneous sporotrichosis, cheek	Colombia	FM179330
FMR 9620	*S. schenckii*	Human, lymphocutaneous sporotrichosis, arm	Colombia	–
FMR 9621	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9622	*S. schenckii*	Soil under *Coffea* sp.	Mexico	–
FMR 9624	*S. globosa*	Human, lymphocutaneous sporotrichosis, finger	Guatemala	FM207489
FMR 9625	*S. schenckii*	Human, fixed cutaneous sporotrichosis, forearm	Guatemala	–
FMR 9626	*S. schenckii*	Human, lymphocutaneous sporotrichosis foot	Guatemala	–
FMR 9629	*S. schenckii*	Soil	Mexico	–
FMR 9631	*S. schenckii*	Soil	Mexico	FM179333
FMR 9632	*S. schenckii*	Soil	Mexico	–

FMR, Facultat de Medicina i Ciències de la Salut, Reus, Spain.

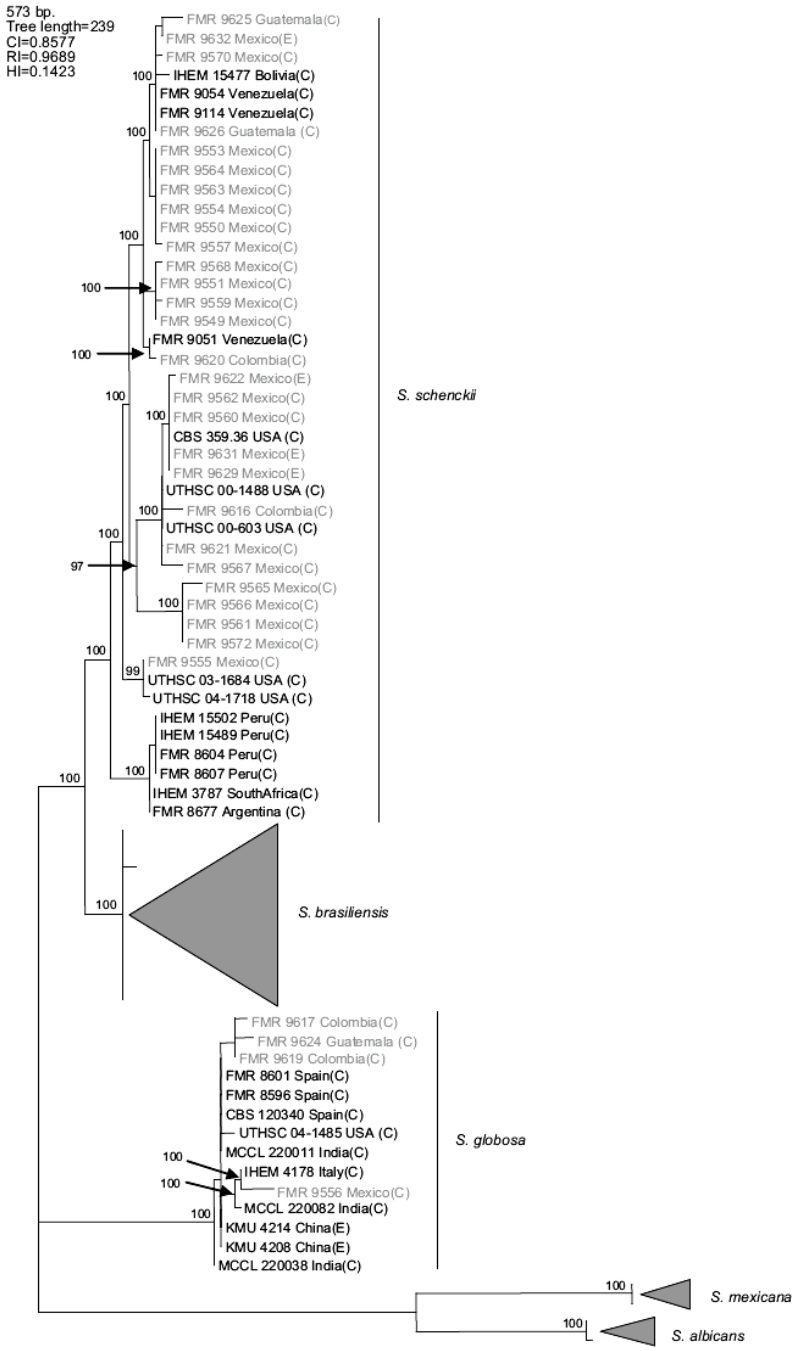

Figure 1. One of the 5000 most parsimonious trees obtained from heuristic searches based on an analysis of the CAL locus. Bootstrap values above 80% are indicated at the nodes. Type strains are indicated in bold type. Isolates for which new CAL sequences were generated during this study are highlighted in blue. CI, consistency index; RI, retention index; HI, homoplasy index; (E), environmental isolate; (C), clinical isolate; FMR, Facultat de Medicina i Ciències de la Salut, Reus, Spain; IHEM, The BCCM/IHEM Biomedical Fungi and Yeasts Collection, Brussels, Belgium; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center; MCCL, Mycology Culture Collection Laboratory, Postgraduate Institute of Medical Education and Research, Chandigarh, India; KMU, Kanazawa Medical University, Ishikawa, Japan.

bisection–reconnection branch-swapping algorithms, collapsing zero-length branches and saving all minimal-length trees (MulTrees). Also, a neighbor-joining phylogeny[22] was constructed using the Kimura-2-parameter substitution model with pairwise deletion of gaps, as implemented in the MEGA3 computer program.[11] The robustness of branches was assessed by bootstrap analysis of 1000 replicates.

Results and discussion

With the primers used, a fragment of 573 bp of the CAL locus was amplified and sequenced. The complete alignment included 71 sequences, 32 generated in this study and 39 retrieved from a previous study,[16] the latter belonging to *S. schenckii s. str.* (15), *S. brasiliensis* (10), *S. globosa* (10), *S. mexicana* (2), and *S. albicans* (2), resulting in a data set of 573 characters, including 403 constant, 160 variable parsimony informative (39.7%), and 10 variable parsimony noninformative sites. Cladistic analysis by the neighbor-joining and maximum parsimony methods generated trees with identical topology. Maximum parsimony analysis of the CAL data set yielded 5000 trees, 239 steps in length, in which 20 nodes received 100% bootstrap support. One of the most parsimonious trees is shown in figure 1. The 71 sequences were distributed into the five main groups detected in previous studies,[16,17] representing *S. brasiliensis*, *S. schenckii sensu stricto*, *S. globosa*, *S. mexicana*, and *S. albicans*. One out of 25 isolates from Mexico (4%), one out of three isolates from Guatemala (33.3%), and two out of four (50%) isolates from Colombia grouped into the *S. globosa* clade, which also included another 10 sequences belonging to isolates from India, China, Italy, USA, and Spain. The other isolates from Colombia, Mexico, and Guatemala grouped into the *S. schenckii s. str.* clade, which also included sequences of another 15 isolates from Bolivia, Venezuela, USA, Peru, South Africa, and Argentina. Since none of the sequences generated in this study grouped into the *S. mexicana*, *S. brasiliensis* or *S. albicans* clades, the isolates belonging to these clades are not detailed in figure 1. The 24 isolates from Mexico in the *S. schenckii* clade were distributed among 14 different haplotypes. Two clinical (FMR 9560 and FMR 9562) and two environmental isolates from Mexico (FMR 9629 and FMR 9631) exhibited the same haplotype as the type strain of *S. schenckii*, CBS 359.36. The *S. schenckii* isolates from Guatemala and Colombia were distributed among four different haplotypes. The *S. globosa* isolates from Mexico, Guatemala, and Colombia were distributed among four haplotypes different from that of the type strain.

A previous study revealed the existence of differences in the geographical distribution among the members of the *S. schenckii* complex, including widespread as well as geographically restricted species.[16] *S. brasiliensis* and *S. mexicana* occurred only in Brazil and Mexico, respectively, and these taxa grouped all the isolates from Brazil ($N = 29$) and Mexico ($N = 2$) tested in that study. Based on these observations, we first thought the 25 isolates from Mexico studied here could follow the same pattern of close phylogenetic affinity observed in isolates from Brazil. However, none of these isolates grouped into the *S. mexicana* clade, a species originally reported from soil and from carnation leaves. On the other hand, *S. schenckii* and *S. globosa* are widespread fungi showing transoceanic distributions.[16] Until now, 36 isolates of *S. globosa* have been reported from United Kingdom, Spain, Italy, China, Japan, USA, and India. This is the first record of this species from Mexico, and Central and South America.

An experimental model of disseminated infection by different *Sporothrix* species in immunocompetent mice showed that, while *S. schenckii s. str.* and *S. brasiliensis* were able to kill immunocompetent animals inoculated intravenously, *S. globosa* did not cause any apparent lesion, suggesting that it might be less virulent than the former species.[15] Interestingly, in contrast with *S. schenckii s. str.* and *S. brasiliensis*, which have been associated with both localized and invasive disease,[16,17] no cases of invasive infections have been attributed to *S. globosa*. This apparently lower virulence might be related to the inability of the fungus to grow at 37 °C.[16]

Marimon et al.[16] proposed as key features for the differentiation of the clinically relevant *Sporothrix* species the presence or absence of pigmented sessile conidia, growth rates at different temperatures, and carbohydrate assimilation test results. Although morphologically similar to other taxa within the *S. schenckii* complex, *S. globosa* is the only member of the complex unable to grow at 37 °C on PDA. Moreover, among the four *Sporothrix* species with pigmented sessile conidia treated, only *S. globosa* showed the combination of positive sucrose and negative raffinose assimilations. These easily diagnosed features allow the identification of *S. globosa* and related taxa by simple inexpensive laboratory procedures.

References

1. Al-Tawfiq JA, Wools KK. Disseminated sporotrichosis and *Sporothrix schenckii* fungemia as the initial presentation of human immunodeficiency virus infection. Clin Infect Dis. 1998;26:1403–6.
2. Berbee ML, Taylor JW. 18S ribosomal RNA gene sequence characters place the human pathogen *Sporothrix schenckii* in the genus *Ophiostoma*. Exp Mycol. 1992;16:87–91.
3. Carrada BT. Esporotricosis pulmonar cavitada: diagnóstico y tratamiento. Med Int Mex. 2006;22:457–61.
4. Cartwright MJ, Promersberger M, Stevens GA. *Sporothrix schenckii* endophthalmitis presenting as a granulomatous uveitis. Br J Ophtalmol. 1993;77:61–2.
5. Costa RO, de Mesquita KC, Damasco PS, Bernardes-Engemann AR, Dias CM, Silva IC, et al. Infectious arthritis as the single manifestation of sporotrichosis: serology from serum and synovial samples as an aid to diagnosis. Rev Iberoam Micol. 2008;25:54–6.
6. de Hoog GS. The genera *Blastobotrys*, *Sporothrix*, *Calcarisporium* and *Calcarisporiella* gen. nov.. Stud Mycol. 1974;7:1–84.
7. de Hoog GS, Guarro J, Gené J, Figueras MJ. Atlas of clinical fungi, 2nd ed. Utrecht and Reus, Centraalbureau voor Schimmelcultures/Universitat Rovira i Virgili, 2000
8. Ghosh A, Chakrabarti A, Sharma VK, Singh K, Singh A. Sporotrichosis in Himachal Pradesh (North India). Trans R Soc Trop Med Hyg. 1999;93:41–5.
9. Ishizaki H, Kawasaki M, Aoki M, Matsumoto T, Padhye AA, Mendoza M, et al. Mitochondrial DNA analysis of *Sporothrix schenckii* in North and South America. Mycopathologia. 1998;142:115–8.
10. Kosinski RM, Alexrod P, Rex JH, Burday M, Sivaprasad R, Wreiole A. *Sporothrix schenckii* fungemia without disseminated sporotrichosis. J Clin Microbiol. 1992;30:501–3.
11. Kumar S, Tamura K, Nei M. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 2004;5:150–63.
12. Kwon-Chung KJ, Bennett JE. Medical mycology. Philadelphia: Lea & Febiger; 1992.
13. Lin J, Kawasaki M, Aoki M, Ishizaki H, You G, Li R. Mitochondrial DNA analysis of *Sporothrix schenckii* clinical isolates from China. Mycopathologia. 1999;148:69–72.
14. Mackinnon JE, Conti-Díaz IA, Gezuele E, Civila E, Da Luz S. Isolation of *Sporothrix schenckii* from nature and considerations on its pathogenicity and ecology. Sabouraudia. 1969;7:138–45.
15. Marimon R. Filogènia molecular i caracterització fenotípica del complex d'espècies d'*Sporothrix schenckii*. PhD thesis. Universitat Rovira i Virgili, Reus, 2007.
16. Marimon R, Cano J, Gené J, Sutton DA, Kawasaki M, Guarro J. *Sporothrix brasiliensis*, S *globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. J Clin Microbiol. 2007;45:3198–206.
17. Marimon R, Gené J, Cano J, Trilles L, Dos Santos Lazéra M, Guarro J. Molecular phylogeny of *Sporothrix schenckii*. J Clin Microbiol. 2006;44:3251–6.
18. Marimon R, Serena C, Gené J, Cano J, Guarro J. *In vitro* antifungal susceptibilities of five species of *Sporothrix*. Antimicrob Agents Chemother. 2008;52:732–4.
19. Mendoza M, Diaz E, Alvarado P, Romero E, Bastardo M. Aislamiento de *Sporothrix schenckii* del medio ambiente en Venezuela. Rev Iberoam Micol. 2007;24:317–9.
20. Mesa-Arango AC, Reyes-Montes MR, Pérez-Mejía A, Navarro-Barranco H, Souza V, Zúñiga G, et al. Phenotyping and genotyping of *Sporothrix schenckii* isolates

according to geographic origin and clinical form of sporotrichosis. J Clin Microbiol. 2002;40:3004–11.
21. O'Donnell K, Niremberg HI, Aoki T, Cigelnik E. A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. Mycoscience. 2000;41:61–78.
22. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406–25.
23. Swofford DL. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Massachussets: Sunderland; 2002.
24. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;24:4876–82.
25. Vilela R, Souza GF, Fernandes Cota G, Mendoza L. Cutaneous and meningeal sporotrichosis in a HIV patient. Rev Iberoam Micol. 2007;24:161–3.

4.3 *Ramophialophora humicola* and *Fibulochlamys chilensis*, two new microfungi from soil

Madrid H, Cano J, Stchigel A, Gené J, Guarro J

Mycologia 2010; 102:605–612

Mycologia, 102(3), 2010, pp. 605–612. DOI: 10.3852/09-128

Ramophialophora humicola and *Fibulochlamys chilensis*, two new microfungi from soil

H. Madrid
J. Cano
A. Stchigel
J. Gené[1]
J. Guarro

Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, C/ Sant Llorenç 21, 43201 Reus, Tarragona, Spain

Abstract: In a study on soil microfungi from different countries two new hyphomycetes were found. The first one, *Ramophialophora humicola*, isolated from a soil sample collected in Ronda (Spain), is characterized by producing profusely branched conidiophores ending in sterile, sometimes swollen apices, and subhyaline, dacryoid conidia borne from both integrated and discrete phialides with conspicuous collaretes. ITS sequence data reveal its relationships with members of the Sordariales and its genetic differences with other fungi morphologically close, such as *Cladorrhinum* spp. The second species, *Fibulochlamys chilensis*, isolated from a soil sample collected in La Junta (Chile), is characterized by micronematous, clamped, mostly branched conidiophores producing thallic, one-celled, thick-walled conidia that exhibit strongly wrinkled surfaces in age. The analysis of partial sequences of the ITS region and 28S rRNA gene reveal that this fungus is close to members of the gilled Agaricales.

Key words: Agaricales, anamorphic fungi, phylogeny, Sordariales, taxonomy

INTRODUCTION

Two interesting taxa were found during a continued survey of filamentous microfungi from different geographic regions and habitats. The first one is a dematiaceous hyphomycete isolated from a Spanish soil sample whose morphological features fit into *Ramophialophora* Calduch, Stchigel, Gené & Guarro, a recently described anamorphic genus that until now included only the species *Ramophialophora vesiculosa* Calduch, Stchigel, Gené & Guarro (Calduch et al. 2004). The second interesting fungus, isolated from a Chilean soil sample, shows clamped hyaline mycelium with a conidiogenous apparatus similar to that of the only member of the basidiomycetous anamorphic genus *Fibulochlamys* Romero & Cabral, *Fibulochlamys ferruginosa* Romero & Cabral (Romero et al. 1989). Both fungi show distinctive morphological features that differentiate them from other species of the respective genera and therefore are proposed as new species. To confirm our proposal and to infer the phylogenetic relationships among the apparently new fungi and other related taxa we compared their sequences of different nuclear ribosomal DNA regions.

Submitted 3 Jun 2009; accepted for publication 27 Sep 2009.
[1] Corresponding author. E-mail: josepa.gene@urv.cat

MATERIALS AND METHODS

Sampling and fungal isolation.—Soil samples rich in organic material (humus) were collected in areas of Ronda, Málaga Province, Spain, and La Junta, Aisén Province, Chile. The vegetation of Ronda is composed mainly of different species of *Quercus* L., *Pinus* L., *Populus* L. and *Fraxinus* L. This area is characterized by a Mediterranean climate with average temperature of 15 C, and average annual rainfall above 607 mm. The soil is sandy and rich in dolomite and limestone. The vegetation of La Junta is composed mainly of dense rainforests, dominated by *Nothofagus dombeyi* (Mirb.) Oerst., *Saxegothea conspicua* Lindl. and *Laureliopsis philippiana* (Looser) Schodde. This area has a cold oceanic climate with average temperatures of 8–9 C and average annual rainfall of 3000–4000 mm. The soil contains volcanic material and has been enriched with fluvial, fluvoglacial and coluvial sediments.

Soil samples were placed in sterilized polyethylene bags. At the laboratory the material was stored at 4–7 C until processed. Fungi were isolated with a wood bait technique and by inoculating soil dilutions in sterile water onto dichloran rose-bengal chloramphenicol (DRBC) agar (Oxoid, United Kingdom) plates supplemented with benomyl at a final concentration of 10 µg/mL, as described respectively in Calduch et al. (2004) and Gilgado et al. (2005). The plates were incubated at 25 ± 1 C and examined weekly 2 mo. To achieve pure cultures a sterile dissection needle was used to transfer conidia from primary cultures to Petri dishes containing potato carrot agar (PCA; 20 g potatoes, 20 g carrots, 20 g agar, 1 L distilled water) and oatmeal agar (OA; 30 g filtered oat flakes, 20 g agar, 1 L distilled water), which were incubated at 25 ± 1 C for 21 d in the dark.

Morphological and physiological studies.—Colony morphology and growth rates were studied on OA, PCA and potato dextrose agar (PDA; Difco) incubated at 25 ± 1 C for 14 or 21 d in the dark. The ability of the isolates to grow at 30, 37 and 42 C was tested on PDA. Color notations in parentheses are from Kornerup and Wanscher (1978). In addition the

isolates were grown on sterilized wood 3 wk at 25 ± 1 C to study microscopic morphology. Microscopic features were studied in lactic acid. Photomicrographs were obtained with an Axio Imager M1 light microscope and a Cannon Ixus 60 digital camera.

Molecular study.—DNA extraction was performed directly from fungal colonies by following the Fast DNA Kit protocol (Bio 101 Inc., Vista, California), with the homogenization step repeated five times. Amplification of the ITS region and D1/D2 locus of the 28S rRNA gene was performed with primer pairs ITS5/ITS4 and NL1/NL4 respectively as described by Cano et al. (2004) and Gilgado et al. (2005). PCR products were purified with a GFXTM PCR DNA kit (Pharmacia Biotech, Cerdanyola, Spain) and were stored at −20 C until PCR products were sequenced with the same primers employed for amplification and following the *Taq* DyeDeoxy Terminator cycle sequencing kit protocol (Applied Biosystems, Gouda, Netherlands). DNA sequencing reaction mixtures were analyzed on a 310 DNA sequencer (Applied Biosystems). The program Autoassembler 1.40 (Applied Biosystems) was used to obtain consensus sequences from the complementary sequences of each isolate. BLAST sequence homology analyses were performed to compare data of our isolates with those of other fungi deposited in GenBank. Nucleotide sequence alignments were performed with Clustal X 1.81 (Thompson et al. 1997), followed by manual adjustments with a text editor. The ITS sequence of the *Ramophialophora* isolate was aligned with those of other morphologically similar or related fungi retrieved from GenBank or generated in this study (TABLE I). The D1/D2 region sequence of the *Fibulochlamys* isolate was aligned with those of other morphologically similar or related fungi available in GenBank (TABLE I). This locus, instead of the ITS region, was chosen for the phylogenetic study because the latter was excessively variable and generated numerous regions with ambiguous alignments. Phylogenetic trees were constructed with neighbor joining (Saitou and Nei 1987), excluding ambiguously aligned regions, and with the Kimura-2-parameter substitution model with pair-wise deletion of gaps, as implemented in the MEGA 3.1 program (Kumar et al. 2004). The robustness of branches was assessed by bootstrap analysis of 1000 replicates. The alignments used in both phylogenetic analyses were deposited in TreeBASE (www.treebase.org).

TAXONOMY

Ramophialophora humicola Madrid, Stchigel, Gené & Guarro, sp. nov. FIG. 1

MycoBank MB 513292

Coloniae in ligno sterili effusae, pilosae, cinerascentes virides. Hyphae 1–3 µm latae. Conidiophora cylindrica, septata, ramosa, pallide olivaceo-brunnea vel atrobrunnea, pallidiora ad apicem, longitudine indeterminata, 2–5 µm lata, laevia vel asperulata, saepe crassitunicata, ad apicem 1.5–4 µm lata in parte latissima. Cellulae conidiogenae discretae et integratae, pallide olivaceae vel brunneae, laeves. Cellulae conidiogenae discretae terminales et laterales, plerumque lageniformes, 5–13 µm longae, 2–4 µm latae in parte maxima. Cellulae conidiogenae integratae intercalares cylindricae, 9–14 µm longae, 2–3 µm latae, collulo phialidico laterali, 2.5–4 µm longae. Conidia unicellularia, subhyalina, laevia, dacryoidea, 2.5–4 µm longa, 2–3 µm lata, cum hilo basilari cylindrico, in capitulis mucidis aggregata. Teleomorphosis ignota.

Colonies on sterilized wood effuse, hairy, dull green (30D4). Vegetative hyphae hyaline to subhyaline, septate, branched, 1–3 µm wide. Conidiophores macronematous, mononematous, cylindrical, regularly septate, profusely branched, pale olivaceous brown to dark brown, becoming paler toward the apex, 2–5 µm wide, smooth to asperulate and thick-walled. Conidiophore axis and branches mostly ending in a sterile, subhyaline to brown, obtuse, sometimes slightly swollen apex, 1.5–4 µm wide at the broadest part. Conidiogenous cells monophialidic or polyphialidic, discrete and integrated, pale olive to brown, smooth-walled, with conspicuous collarettes up to 2 µm wide. Discrete phialides terminal or lateral, mostly lageniform, but also subcylindrical or centrally swollen, often sinuous, 5–13 µm long, 2–4 µm wide at the broadest part. Integrated phialides intercalary, cylindrical, 9–14 µm long, 2–3 µm wide, with a lateral neck 2.5–4 µm long. Conidia one-celled, subhyaline, smooth-walled, dacryoid, 2.5–4 × 2–3 µm, guttulate, often with a cylindrical hilum, accumulating in slimy masses. Teleomorph not observed.

Colonies on OA attaining 55–59 mm diam in 21 d at 25 ± 1 C, composed mostly of immersed mycelium, almost glabrous, yellowish green (30B8), with concentric dull green (30D4) conidial tufts and slightly irregular margin; reverse yellowish green (30B8). Colonies on PCA attaining 25–39 mm diam in 21 d at 25 ± 1 C, almost glabrous, cream (4A3) at the center, yellow (3A6) toward the periphery. Colonies on PDA at 25 ± 1 C attaining 27–28 mm diam in 21 d, glabrous, irregularly convoluted at the center, cream (4A3), with fimbriate margins. Best sporulation was obtained on OA. The fungus grew well on PDA at 30 C, attaining 19–25 mm diam in 21 d but did not sporulate. No growth was observed at 37 C.

Holotype. SPAIN. MÁLAGA PROVINCE: Ronda, ca. 36°44′14″N, 5°09′53″W, ca. 739 m, from forest soil, Nov 2006, *C. Silvera & A. Mercado* (HOLOTYPE: IMI 397094, ex-type cultures CBS 124563 and FMR 9523).

Comments. This fungus, isolated with a wood bait technique, resembles the type species of the genus, *R. vesiculosa*, in producing well developed, strongly pigmented, branched conidiophores ending in sterile, setiform apices. Conidial shape and size of the new taxon are also similar to those of *R. vesiculosa*. However the latter fungus possesses typically wider conidiophore apices (vesicles 3.5–6.5 µm wide), has

TABLE I. Strains, sources and sequences used in the analyses

	Species	Collection number	Origin	EMBL accession No.
Ascomycota				
Sordariales	*Cercophora caudata*	CBS 606.72	Soil, Netherlands	AY999135[a]
	Cercophora samala	CBS 109.93	Dung, Japan	AY999134 [a]
	Cercophora sparsa	JF00229	France	AY587912 [a]
	Cercophora sulphurella	SMH2531	USA	AY587913 [a]
	Cladorrhinum brunnescens	CBS 643.75A (T)	Fibers of *Cocos nucifera*, Netherlands	**FM955446** [a]
	Cladorrhinum bulbillosum	CBS 304.90 (T)	Desert sand, Egypt	**FM955448** [a]
	Cladorrhinum foecundissimum	CBS 180.66 (NT)	Agricultural soil, Netherlands	**FM955445** [a]
	Cladorrhinum phialophoroides	CBS 301.90 (T)	Desert soil, Egypt	**FM955444** [a]
	Cladorrhinum samala	CBS 303.90	Desert soil, Egypt	**FM955447** [a]
	Gelasinospora tetrasperma	CBS 178.33	Dung, Canada	AY681178 [a]
	Neurospora tetrasperma	NITE 32011	Burnt soil	AY681194 [a]
	Podospora appendiculata	NITE 8549	Dung, Japan	AY999126 [a]
	Podospora comata	NA	NA	AF443849 [a]
	Podospora didyma	CBS 232.78	Dung, Canada	AY999127 [a]
	Podospora fimiseda	Wang 9727	NA	EF197077 [a]
	Podospora pauciseta	B s. n.	Horse dung, France	AY525771 [a]
	Ramophialophora humicola	CBS 124563, FMR 9523	Forest soil, Spain	**FM955449** [a]
	Ramophialophora vesiculosa	CBS 110629	Forest soil, Spain	AJ579563 [a]
	Zopfiella erostrata	CBS 255.71	Dung, Africa	AY999133 [a]
	Zopfiella tabulata	CBS 230.78	Dung, Canada	AY999132 [a]
	Zopfiella tetraspora	NITE 32904	Soil	AY999130 [a]
Xylariales	*Pestalotiopsis versicolor*	BRIP 25468	*Garcinia mangostana*, Australia	AF409993 [a]
Basidiomycota				
Agaricales	*Asterophora lycoperdoides*	CBS 170.86	Germany	AF223190[b]
	Asterophora parasitica	CBS 683.82	NA	AF223191[b]
	Fibulochlamys chilensis	CBS 123018, FMR 9694	Soil, Chile	**FM955450**[a] **FM955451**[b]
	Flammulina velutipes	GLM 45921	NA	AY207200[b]
	Leucopaxillus gentianeus	TENN5616	USA	AF261394[b]
	Lyophyllum decastes	Lc42T5P	NA	AF357078[b]
	Lyophyllum fumosum	BAYER G 314	Germany	AY207229[b]
	Lyophyllum ulmarium	NA	NA	AF042584[b]
	Tephrocybe ambusta	CBS 451.87	France	AF223215[b]
	Tephrocybe tylicolor	BSI 92.245	NA	AF223195[b]
	Pholiota aurivella	HKI ST 27321	NA	AY207274[b]
	Squamanita odorata	DAOM225481	NA	AF261507[b]
	Xerula radicata	NA	Estonia	AM946475[b]
Polyporales	*Phanerochaete chrysosporium*	CBS 363.65	Soil, India	AB359433[b]

Sequences generated during this study appear in bold type. T, type strain; NT, neotype strain.

[a] ITS/5.8S rDNA.

[b] 28S rDNA; NA, not available; BAYER, Bayer Healthcare, Wupertal, Germany; BRIP, Queensland Department of Primary Industries Plant Pathology Herbarium, Australia; CBS, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; DAOM, National Mycological Herbarium, Ottawa, Canada; FMR, Faculty of Medicine collection, Reus, Spain; HKI, Hans-Knöll-Institute, Jena, Germany; JF, Jacques Fourier, Las Muros; NITE, Nacional Institute of Technology and Evaluation, Tsukuba, Japan; SMH, Sabine M. Huhndorf, Field Museum, Chicago, USA; TENN, University of Tennessee, USA; Wang, Yei Zei-Zeng Wang, Hong Kong University, China.

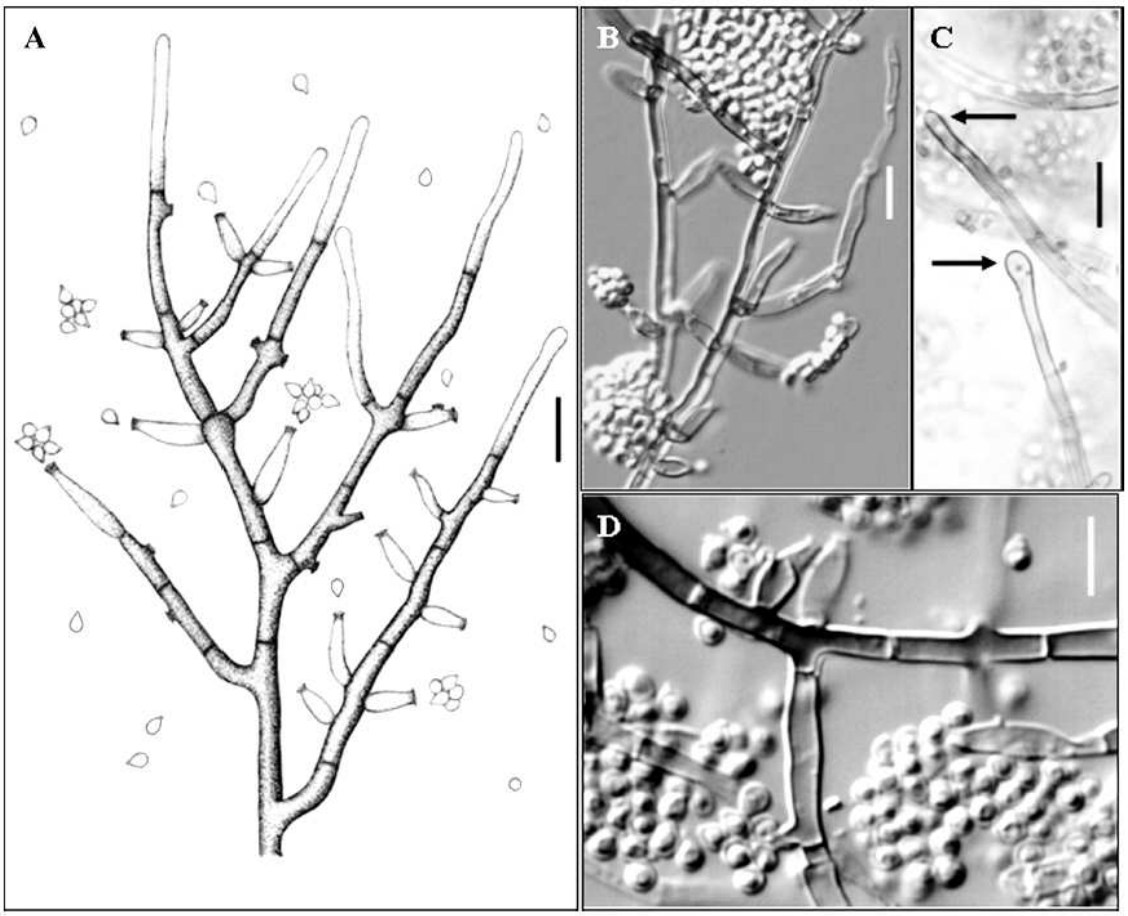

FIG. 1. *Ramophialophora humicola* (CBS 124563). A. Conidiophore sketch. B. Conidiophore detail. C. Detail of the sterile terminal cells of the conidiophores (upper arrow, noninflated; lower arrow, inflated). D. Detail of conidiogenous cells (discrete and integrated). Bars = 10 µm.

brown conidia and does not produce intercalary phialides (Calduch et al. 2004). *R. humicola* also is comparable to species of *Cladorrhinum* Sacc. & Marchal, an anamorphic genus of Sordariales related to *Cercophora* Fuckel, *Podospora* Ces. and *Apiosordaria* Arx & Gams teleomorphs. *Cladorrhinum* anamorphs are characterized by producing tufted aggregations of conidiophores, usually bearing intercalary phialides with lateral openings and collaretes, and one-celled conidia aggregated in slimy masses (Mouchacca and Gams 1993). In contrast to *Cladorrhinum* species our isolate shows predominantly discrete conidiogenous cells and produces well developed, branched, dark brown conidiophores on wood. Numerous *Podospora*, *Cercophora* and *Lasiosphaeria* Ces. & de Not. species produce *Phialophora*-like asexual states with conidia similar to those of *Ramophialophora*, but these anamorphs have undifferentiated or poorly differentiated conidiophores (Mirza and Cain 1969; Lundqvist 1972; Gams and Holubová-Jechová 1976; Udagawa and Muroi 1979; Bell and Mahoney 1995; Lundqvist et al. 1999; Gams 2000; Miller and Huhndorf 2001, 2004; Miller et al. 2007). A recently described ascomycete, *Jattaea prunicola* Damm & Crous (Calosphaeriales), has a phialidic anamorph with discrete and integrated phialides, and branched conidiophores that often end in sterile inflated cells. This fungus is easily distinguished from *Ramophialophora* species by the shape of its conidia, which are cylindrical to allantoid. Furthermore the sterile terminal cells of conidiophores differ from those of *Ramophialophora* in having an irregular shape, often

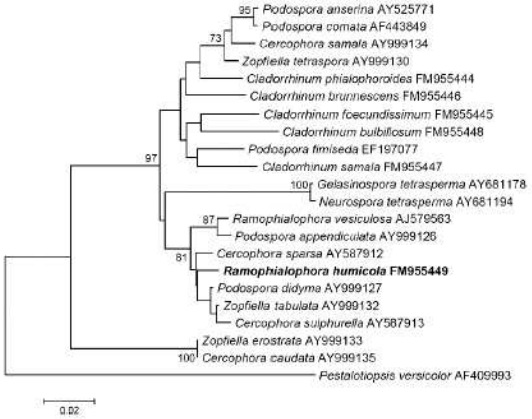

FIG. 2. Neighbor joining tree constructed with sequences of the 5.8S rRNA gene and partial ITS region. Branch lengths are proportional to distance. Bootstrap values above 70% are indicated in the internodes. *Pestalotiopsis versicolor* was used as outgroup.

appearing as phialides without openings (Damm et al. 2008).

A BLAST sequence homology analysis revealed that the ITS sequence of our isolate showed a high level of identity with different members of the Sordariales. However, the percentages of sequence identities to the closest taxa did not exceed 93%. In the phylogenetic study we included the ITS sequences of morphologically similar species of *Cladorrhinum* and *Ramophialophora* and those of some teleomorphic taxa belonging to different lineages of the Sordariales (FIG. 2). The new species grouped into a well supported clade that also included the type species of *Ramophialophora* as well as *Cercophora sparsa* (Sacc. & Fairm.) R. Hilber, *Podospora appendiculata* (Auersw. ex Niessl) Niessl, *Podospora didyma* J.H. Mirza & Cain, *Zopfiella tabulata* (Zopf) G. Winter and *Cercophora sulphurella* (Sacc.) R Hilber, five species that lack known anamorphs. In Cai et al. (2006) a phylogeny reconstruction of the Sordariales based on sequences of the ITS region, 28S rRNA and β-tubulin genes, placed the latter four species in a strongly supported clade distant from that grouping species with *Cladorrhinum* anamorphs. Our phylogeny also agrees with that study in revealing that *Podospora*, *Cercophora* and *Zopfiella* are polyphyletic genera that need to be recircumscribed.

The type strain of *R. vesiculosa* also was isolated from forest soil with a wood bait technique, but from a sample collected in the Asturias Province, Spain (Calduch et al. 2004). Because no additional reports of the fungus exist, the genus *Ramophialophora* is known thus far only from Spain.

Fibulochlamys chilensis Madrid, Stchigel, Gené & Guarro, sp. nov. FIG. 3

MycoBank MB 513296

Coloniae effusae, farinaceae, cremeae. Hyphae hyalinae, septatae, ramosae, fibulatae, 1–6 µm latae. Conidiophora micronematosa, mononematosa, cylindrica vel subcylindrica, hyalina, septata, plerumque ramosa, fibulata. Cellulae conidiogenae terminales et intercalares, plerumque cylindricae. Conidia holothallica, unicellularia, pallida flava vel flava, crassitunicata, globosa vel subglobosa vel obovoidea, 9–20 µm longa, 8–13 µm lata, interdum pedicellata, plerumque catenata. Conidia matura cum superficie corrugatissima. Teleomorphosis ignota.

Colonies on sterile wood effuse, farinose, cream (4A3). Hyphae hyaline, septate, branched, clamped, 1–6 µm wide. Conidiophores micronematous, mononematous, cylindrical to subcylindrical, hyaline, septate, mostly branched. Conidiogenous cells mostly cylindrical, terminal or intercalary, generally associated with a basal clamp. Conidia holothallic, one-celled, pale-yellow to yellow, globose, subglobose to obovoidal, 9–20 × 8–13 µm, sometimes pedicellate, commonly forming simple chains of up to five cells, with thick walls that become strongly wrinkled in age. Conidial secession rhexolytic. Teleomorph not observed.

Colonies on OA at 25 ± 1 C attaining 60–66 mm diam in 14 d, composed mostly of submerged hyphae, yellowish white (4A2). Colonies on PCA at 25 ± 1 C attaining 70–77 mm in 14 d, flat, powdery, cream (4A3) with light yellow (4A5) reverse. Colonies on PDA at 25 ± 1 C attaining 71–74 mm diam in 14 d, cerebriform, yellowish white (4A2), concolorous on reverse. Good sporulation was obtained in the three culture media used. The fungus attained 8 mm diam at 42 C in 14 d, but no growth was observed at 45 C.

Holotype. CHILE. AISÉN: La Junta, ca. 43°58′26″S, 72°24′17″W, ca. 904 m, from soil, Dec 1995, *L. Zaror* (HOLOTYPE: IMI 397095, ex-type cultures CBS 123018 and FMR 9694).

Comments. The fungus, isolated with DRBC agar and benomyl, was placed in genus *Fibulochlamys* because of the presence of hyaline, clamped mycelium and conidiophores producing holothallic, unicellular, thick-walled, yellowish conidia. We examined the type material of the only previously known species of the genus, *F. ferruginosa*, BAFC 30826, consisting of dry colonies on bark and wood of *Eucalyptus viminalis* Labill. This species differs from our isolate in producing mostly unbranched conidiophores and smooth conidia (Romero et al. 1989). Unfortunately no strain or DNA sequence of *F. ferruginosa* is available and no teleomorph has been described for this taxon. Attempts to culture the fungus from the holotype were unsuccessful, and DNA extraction could not be performed directly from the holotype due to contamination with other fungi.

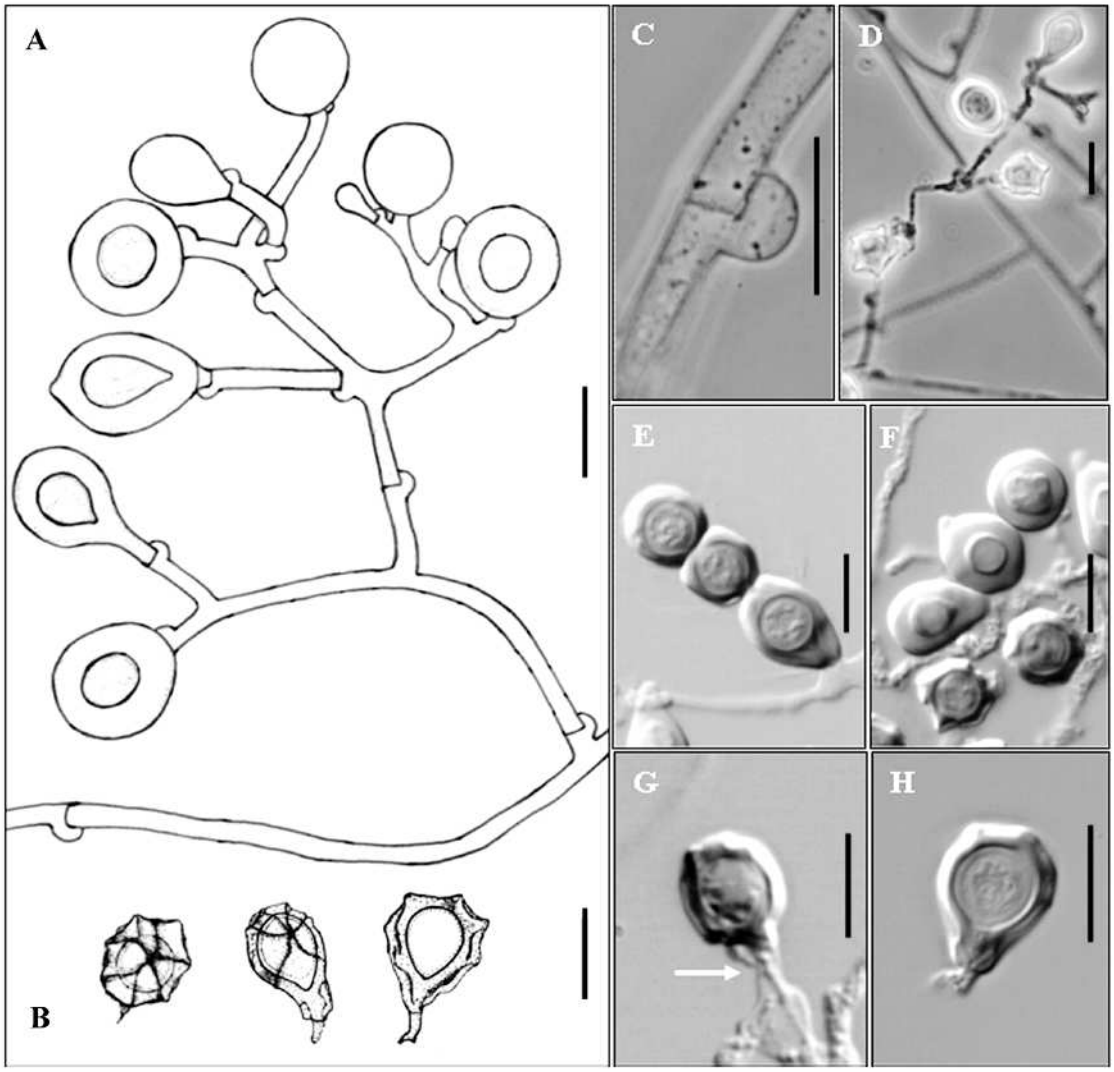

FIG. 3. *Fibulochlamys chilensis* (CBS 123018). A. Branched conidiophore and conidia. B. Released mature conidia. C. Detail of a hypha showing a clamp connection. D. Branched conidiophore. E. Conidia in a short chain. F. Group of young and mature conidia. G. Rhexolytic conidial secession (the arrow shows the subterminal cell in lytic process). H. Released conidium. Bars = 10 µm.

BLAST analyses with the ITS and D1/D2 sequences both revealed the genetic relationship of *F. chilensis* with different members of the Tricholomataceae (Agaricales), but only a maximum of 96% identity was obtained with the D1/D2 sequences of *Tephrocybe ambusta* (Fr.) Donk (AF223215), *Lyophyllum decastes* (AF357078), and *Tephrocybe atrata* (Fr.) Donk (AF223210) as closest matches. A cladogram built with the D1/D2 sequences of *F. chilensis*, some Tricholomataceae and basidiomycetes of other families producing anamorphs with thick-walled propagules, such as *Pholiota aurivella* (Batsch ex Fr.) Kumm. (Strophariaceae), *Flammulina velutipes* (Curtis) Singer, *Xerula radicata* (Relhan) Dörfelt (Physalacriaceae) and *Phanerochaete chrysosporium* Burds. (Phanerochaetaceae) (Kendrick and Watling, 1979), showed a clade with 100% bootstrap support that includes our isolate, *Lyophyllum fumosum* (Pers.) P.D. Orton, *T. ambusta* and *L. decastes* (FIG. 4). *F. chilensis* and *L. decastes* appeared as neighbors and grouped

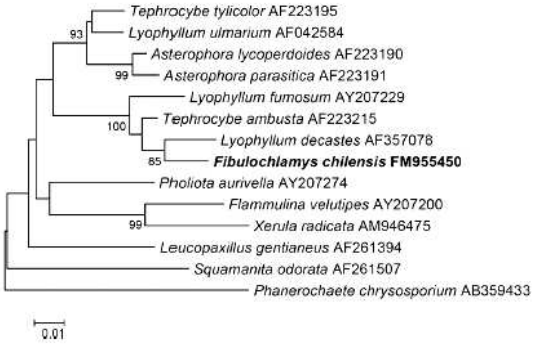

FIG. 4. Neighbor joining tree constructed with partial sequences of the 28S rRNA gene (D1–D2 domains). Branch lengths are proportional to distance. Bootstrap values above 70% are indicated in the internodes. *Phanerochaete chrysosporium* was used as outgroup.

into a statistically well supported subclade. Species of *Lyophyllum* P. Karst. and *Tephrocybe* Donk were included within two main clades with high bootstrap supports, one of them containing a subclade grouping two species of *Asterophora* Ditmar. Our phylogenetic analysis suggests that *Lyophyllum* and *Tephrocybe* are polyphyletic. Species genetically related to *F. chilensis*, such as *T. ambusta*, *L. decastes* and *L. fumosum*, do not produce conidia (Clémençon and Moncalvo 1990, Moncalvo et al. 1993, Walther et al. 2005). However anamorphs have been reported in other species of these genera, that is *Lyophyllum leucopaxilloides* (H.E. Bigelow & A.H. Smith) Clémençon, *Lyophyllum suburens* Clémençon, *Lyophyllum ulmarium* (Bull.) Kühner, *Tephrocybe palustris* (Peck) Donk and *Tephrocybe tylicolor* (Fr.) M.M. Moser. The anamorphs of the two former species differ from *F. chilensis* in producing smaller conidia and in forming synnema-like structures (Clémençon 1968). The anamorphs of *L. ulmarium* and *T. tylicolor* are characterized by forming both rhexolytically seceding "chlamydospores" and apparently schizolytic arthroconidia (Nagasawa and Arita 1988, Moncalvo et al. 1993). The anamorph of *T. palustris* forms schizolytic conidia by simple fragmentation of undifferentiated conidiogenous hyphae (Walther et al. 2005). Other members of the Tricholomataceae, such as *Calocybe fallax* (Sacc.) Redhead & Singer, *Leucopaxillus gentianeus* (Quél.) Kotl., *Leucopaxillus giganteus* (Sowerby) Singer, *Hypsizygus marmoreus* (Peck) H.E. Bigelow and *Asterophora* spp., produce anamorphs with rhexolytic conidial dehiscence in culture (Brefeld 1889, Pantidou et al. 1983, Buchalo 1988, Brunner and Miller 1988, Nagasawa and Arita 1988), but none of them shows the conidial ornamentation of *F. chilensis*. In addition, numerous other Agaricales and basidiomycetes in the orders Geastrales, Hymenochaetales and Polyporales produce thick-walled, one-celled conidia in culture or in nature (Lamoure 1954, Nobles 1964, Bas 1965, Lentz and McKay 1976, Kendrick and Watling 1979, Pantidou et al. 1983, Jacobsson 1989, Klán et al. 1989, Botha and Eicker 1991, Sede and Lopez 1999, Stalpers 2000, Stoytchev et al. 2001, Fausto-Guerra et al. 2002, Kobayashi and Yamada 2003, Baroni et al. 2007, Vizzini et al. 2007), but none of those anamorphs shows the combination of conidial ornamentation, secession mode and conidiophore structure observed in *F. chilensis*.

ACKNOWLEDGMENTS

Dr Andrea Romero and Ms Susana Pereira are thanked for providing the type material of *F. ferruginosa*. We are indebted to the curators of the Centraalbureau voor Schimmelcultures (Utrecht, the Netherlands) for supplying some of the isolates studied.
This work was supported by the Spanish Ministerio de Educación y Ciencia grant CGL2008-04226/BOS.

LITERATURE CITED

Baroni TJ, Franco-Moleano AE, Lodge DJ, Lindner DL, Horak E, Hofstetter V. 2007. *Arthromyces* and *Blastosporella*, two new genera of conidia-producing lyophylloid agarics (Agaricales, Basidiomycota) from the Neotropics. Mycol Res 111:572–580.

Bas C. 1965. The genus *Squamanita*. Persoonia 3:331–359.

Bell A, Mahoney DP. 1995. Coprophilous fungi in New Zealand I. *Podospora* species with swollen agglutinated perithecial hairs. Mycologia 87:375–396.

Botha WJ, Eicker A. 1991. Cultural studies on the genus *Termitomyces* in South Africa I. Macro- and microscopic characters of basidiome context cultures. Mycol Res 95: 435–443.

Brefeld O. 1889. Untersuchungen aus dem Gesammtgebiete der Mykologie VIII. Heft: Basidiomyceten III. Autobasidiomyceten. Leipzig: Arthur Felix. 305 p.

Brunner IL, Miller OK Jr. 1988. *Calocybe fallax*: its ecology and cultural behavior. Mycotaxon 33:41–50.

Buchalo AS. 1988. Higher edible basidiomycetes in pure culture. Kiev, Ukraine: Naukova Dumka. 144 p.

Cai L, Jeewon R, Hyde KD. 2006. Molecular systematics of *Zopfiella* and allied genera: evidence from multi-gene sequence analyses. Mycol Res 110:359–368.

Calduch M, Gené J, Stchigel AM, Cano J, Guarro J. 2004. *Ramophialophora*, a new anamorphic genus of *Sordariales*. Stud Mycol 50:83–88.

Cano J, Guarro J, Gené J. 2004. Molecular and morphological identification of *Colletotrichum* species of clinical interest. J Clin Microbiol 42:2450–2454.

Clémençon H. 1968. Contributions to the knowledge of the genera *Lyophyllum* and *Calocybe* (Agaricales, Basidiomycetes) III. Two chlamydospore forming *Lyophyllum* species. Nova Hedwig 16:417–427.

———, Moncalvo JM. 1990. Taxonomic analysis of cultural characters in the group *Lyophyllum shimeji* (Agaricales, Basidiomycetes) from Japan. Trans Mycol Soc Japan 31: 479–488.

Damm U, Crous PW, Fourie PH. 2008. A fissitunicate ascus mechanism in the Calosphaeriaceae, and novel species of *Jattaea* and *Calosphaeria* on Prunus wood. Persoonia 20:39–52.

Fausto-Guerra S, Guzmán-Dávalos L, Velásquez-Hueso JC. 2002. Cultural studies of *Gymnopilus* species (Cortinariaceae, Agaricales). Mycotaxon 84:429–444.

Gams W. 2000. *Phialophora* and some similar morphologically little-differentiated anamorphs of divergent ascomycetes. Stud Mycol 45:187–199.

———, Holubová-Jechová V. 1976. *Chloridium* and some other dematiaceous hyphomycetes growing on decaying wood. Stud Mycol 13:1–99.

Gilgado F, Cano J, Gené J, Guarro J. 2005. Molecular phylogeny of the *Pseudallescheria boydii* species complex: proposal of two new species. J Clin Microbiol 43: 4930–4942.

Jacobsson S. 1989. Studies on *Pholiota* in culture. Mycotaxon 36:95–145.

Kendrick B, Watling R. 1979. Mitospores in basidiomycetes. In: Kendrick B, ed. The Whole Fungus. Ottawa: National Museum of Natural Sciences. p 473–545.

Klán J, Baudišová D, Rulfová I. 1989. Cultural, enzymatic and cytological studies in the genus *Pholiota*. Mycotaxon 36:249–271.

Kobayashi H, Yamada A. 2003. Chlamydospore formation of *Entoloma clypeatum* f. *hybridum* on mycorrhizas and rhizomorphs associated with *Rosa multiflora*. Mycoscience 44:61–62.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd ed. London: Eyre Methuen. 252 p.

Kumar S, Tamura K, Nei M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163.

Lamoure D. 1954. Contribution à l'étude cytologique des germinations et du mycélium de quelques Agaricales, en particulier des Coprins de la Section Setulosi (Lange). Annal Univ Lyon, Sect C, Sci Nat 8:21–55.

Lentz PL, McKay HH. 1976. Basidiocarp and culture descriptions of *Hyphoderma* and *Hyphodontia* (Corticiaceae) in the Mississippi Delta region. Mem NY Bot Garden 28:141–162.

Lundqvist N. 1972. Nordic Sordariaceae s. lat. Symb Bot Upsal 20:1–374.

———, Mahoney DP, Bell A, Lorenzo LE. 1999. *Podospora austrohemisphaerica*, a new heterothallic ascomycete from dung. Mycologia 91:405–415.

Miller AN, Huhndorf SM. 2001. Neotropical ascomycetes 10. New and interesting *Cercophora* species. Sydowia 53: 211–226.

———, ———. 2004. A natural classification of *Lasiosphaeria* based on nuclear LSU rDNA sequences. Mycol Res 108:26–34.

———, Mugambi GK, Huhndorf SM. 2007. *Cercophora rubrotuberculata* sp. nov., a new pyrenomycete from the Great Smoky Mountains National Park. Mycologia 99: 488–491.

Mirza JH, Cain RF. 1969. Revision of the genus *Podospora*. Can J Bot 47:1999–2048.

Moncalvo JM, Rehner SA, Vilgalys R. 1993. Systematics of *Lyophyllum* section *Difformia* based on evidence from culture studies and ribosomal DNA sequences. Mycologia 85:788–794.

Mouchacca J, Gams W. 1993. The hyphomycete genus *Cladorrhinum* and its teleomorph connections. Mycotaxon 48:415–440.

Nagasawa E, Arita I. 1988. A note on *Hypsizygus ulmarius* and *Hypsizygus marmoreus* in Japan. Rep Tottori Mycol Inst 26:71–78.

Nobles MK. 1964. Identification of cultures of wood-inhabiting hymenomycetes. Can J Bot 43:1097–1139.

Pantidou M, Watling R, Gonou Z. 1983. Mycelial characters, anamorphs, and teleomorphs in genera and species of various families of Agaricales in culture. Mycotaxon 17: 409–432.

Romero AI, Cabral D, Lopez SE. 1989. Studies on xylophilous fungi from Argentina IV. Anamorphs of basidiomycetes on *Eucalyptus viminalis* (Myrtaceae). Mycotaxon 34:429–440.

Saitou N, Nei M. 1987. The neighbor joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Sede SM, Lopez SE. 1999. Cultural studies of *Agrocybe cylindrica*, *Gymnopilus pampeanus* and *Oudemansiella canarii* (Agaricales) isolated from urban trees. Mycotaxon 70:377–386.

Stalpers JA. 2000. The genus *Ptychogaster*. Karstenia 40:167–180.

Stoytchev I, Homolka L, Nerud F. 2001. *Geastrum pouzarii* Staněk in culture. Antonie van Leeuwenhoek 79:363–364.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24: 4876–4882.

Udagawa S, Muroi T. 1979. Coprophilous Pyrenomycetes from Japan V. Trans Mycol Soc Japan 20:453–468.

Vizzini A, Antonín V, Noorderloos M. 2007. *Crinipellis pedemontana* sp. nov. (Agaricomycetes), a new basidiomycete from Italy. Mycologia 99:786–791.

Walther G, Garnica S, Weiß M. 2005. The systematic relevance of conidiogenesis modes in the gilled Agaricales. Mycol Res 109:525–544.

4.4 Two new species of *Cladorrhinum*

Madrid H, Cano J, Gené J, Guarro J

Mycologia 2011 in press, DOI: 10.3852/10-150

Short title: Madrid et al: New species of *Cladorrhinum*

Two new species of *Cladorrhinum*

Hugo Madrid

Josep Cano

Josepa Gené[1]

Josep Guarro

Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, IISPV, Universitat Rovira i Virgili, C/ Sant Llorenc 21, 43201 Reus, Tarragona, Spain.

[1]Corresponding author, e-mail: josepa.gene@urv.cat, tel.: + 34 977759341, fax: +34 977759322.

Abstract: Two new species of *Cladorrhinum*, *C. flexuosum* and *C. microsclerotigenum*, are described and their sequences of the 5.8S and 28S ribosomal DNA and internal transcribed spacers 1 and 2 of the ribosomal RNA gene cluster analyzed. *Cladorrhinum flexuosum*, isolated from soil in Spain, forms fast-growing, dull-yellow colonies, flexuous conidiophores and globose to dacryoid conidia. *Cladorrhinum microsclerotigenum*, isolated from *Musa* sp. in Turkey, strongly resembles *Cladorrhinum phialophoroides* in the production of abundant terminal phialides, ellipsoid conidia, and microsclerotia in culture. It differs from *C. phialophoroides* in its ability to grow at 36 C and high numbers of intercalary phialides, which only infrequently occur in *C. phialophoroides*.

Key words: Ascomycota, Lasiosphaeriaceae, mitosporic fungi, Sordariales, taxonomy.

INTRODUCTION

The species of the anamorphic genus *Cladorrhinum* Sacc. & Marchal form slimy masses of one-celled conidia on intercalary conidiogenous cells with lateral phialidic openings. Fertile hyphae can accumulate into more or less branched conidiophores.

Sometimes, also terminal and lateral phialides and microsclerotia can be observed in culture (de Hoog et al 2000, Domsch et al 2007). In a revision of the genus by Mouchacca and Gams (1993), five named species were treated, i.e. *C. foecundissimum* Sacc. & Marchal (type species), *C. brunnescens* W. Gams, *C. bulbillosum* W. Gams & Mouch., *C. phialophoroides* Mouch. & W. Gams, and *C. samala* (Subram. & Lodha) W. Gams & Mouch. The unnamed anamorphs of *Cercophora samala* Udagawa & T. Muroi and certain species of *Apiosordaria* Arx & W. Gams were also ascribed to *Cladorrhinum* by those authors. *Cladorrhinum* species were distinguished mainly on the basis of the ability to produce microsclerotia, the relative abundance of intercalary v/s terminal phialides, growth rates at different temperatures, the pigmentation of the mycelium and conidial shape. In the concept of *Cladorrhinum*, Mouchacca and Gams (1993) gave importance to the tufted aggregation of the conidiophores. Therefore, the anamorph of *Apodospora viridis* Cain & J.H. Mirza and those of several species of *Podospora* Ces., which only produce scattered intercalary phialides in culture, were not included. Later, *Cladorrhinum* anamorphs matching Mouchacca and Gams` circumscription were described for *Podospora fimiseda* (Ces. & De Not) Niessl (Bell and Mahoney 1997) and *Cercophora striata* (Ellis & Everh.) N. Lundq. (Miller and Huhndorf 2001).

Cladorrhinum species are found as saprobes on dung and plant material and in soil, but at least one species of the genus, *C. bulbillosum*, is occasionally involved in opportunistic infections of mammals (Zapater and Scattini 1979, Chopin et al 1997). *Cladorrhinum foecundissimum* has been recently proposed as a potential biocontrol agent due to its strong antagonistic activity against *Rhizoctonia solani* J.G. Kühn and *Pythium ultimum* Trow (Lewis and Larkin 1998).

Little is known about the evolutionary relationships of *Cladorrhinum* species to other ascomycetes. The known associated teleomorphs belong to genera in the Lasiosphaeriaceae, Sordariales (Mouchacca and Gams 1993, Bell and Mahoney 1997, Miller and Huhndorf 2001). However, the family appeared as an artificial group in several phylogenetic studies (Huhndorf et al 2004, Miller and Huhndorf 2004, 2005). Cai et al (2006) demonstrated that four species with *Cladorrhinum* anamorphs, i.e. *Apiosordaria verruculosa* (C.N. Jensen) Arx & W. Gams, *Cercophora samala*, *C. striata*, and *Podospora fimiseda* belonged to a well supported lineage, which was named "clade A". This group also included other species that produce scattered intercalary phialides in culture, such as *Podospora austroamericana* (Speg.) J.H. Mirza & Cain and *Podospora pauciseta* (Ces.) Traverso, as well as other members of the Lasiosphaeriaceae without known anamorphs. Nevertheless, the phylogenetic placement of the anamorphic species of *Cladorrhinum* is unknown.

In the present study we address the taxonomic position of two *Cladorrhinum* isolates whose morphological features did not seem to match any of the currently described species of this genus. One of them, CBS 126090, was recently obtained from a soil sample collected in Spain, and the other, CBS 290.75, was isolated from *Musa* sp. in Turkey. The latter had been studied earlier by Mouchacca and Gams (1993) but considered an atypical isolate of *C. phialophoroides*.

MATERIALS AND METHODS

Sampling and fungal isolation. —The *Cladorrhinum* isolate CBS 126090 was obtained from a forest soil sample collected in Pola de Somiedo, Asturias Province, Spain, in 2006. The fungus was isolated by means of a wood bait technique as described previously (Calduch et al 2004). The isolate CBS 290.75 was obtained from *Musa* sp. in

Adana, Adana Province, Turkey, in 1975 (Mouchacca and Gams 1993). No information is available on the isolation technique used to obtain this isolate.

Phenotypic characterization.—Colony morphology and growth rates were studied on 85 mm wide Petri dishes with 2% malt extract agar (MEA, Oxoid) in duplicate. The plates were centrally inoculated with 2 mm wide plugs of mycelium and incubated in the dark for 5 days at temperatures ranging from 6 to 36 C at intervals of 6 C. Colony diameters were recorded as minimum and maximum values obtained for isolates belonging to each taxon. Color notations in parentheses are from Kornerup and Wanscher (1978). Microscopic morphology was studied in lactic acid from cultures on oatmeal agar (OA; 30 g filtered oat flakes, 20 g agar, 1 L distilled water), and sterilized wood after 14 d at 25 C, using an Olympus CH-2 light microscope. Photomicrographs were obtained using a Zeiss Axio Imager M1 light microscope.

DNA sequence analysis.—DNA extraction was performed directly from fungal colonies by following the Fast DNA kit protocol (Bio 101, Inc., Vista, California, USA), with the homogenization step repeated 5 times. Amplification of the 5.8S ribosomal DNA and the internal transcribed spacers 1 and 2 of the ribosomal RNA gene cluster (hereafter referred to as "ITS region") was performed with the primer pair ITS5/ITS4 as described by Gilgado et al (2005). The D1/D2 domains of the 28S rDNA were amplified and sequenced using primers NL1/NL4 as described by Cano et al (2004). PCR products were purified using a GFXTM PCR DNA kit (Pharmacia Biotech, Cerdanyola, Spain) and were stored at -20 C until sequencing. PCR products were sequenced by using the same primers employed 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). The program SeqMan (Lasergene, Madison, Wisconsin) was used to

obtain consensus sequences from the complementary sequences of each isolate. BLAST sequence homology searches (Altschul et al 1990**)** were performed to compare data of the isolates studied with those of other fungi deposited in the GenBank database. Nucleotide sequence alignments were performed with ClustalX version 1.81 (Thompson et al 1997), followed by manual adjustments with a text editor. Distance trees were constructed with the neighbor-joining method (Saitou and Nei 1987), excluding ambiguously aligned regions and using the Kimura-2-parameter substitution model with pairwise deletion of gaps, as implemented in the MEGA 4.0 computer program (Tamura et al 2007). The robustness of branches was assessed by bootstrap analysis of 1000 replicates. The sequences generated in this study and the alignments used in the cladistic analysis were deposited in GenBank and TreeBASE (www.treebase.org, submission number SN4946), respectively.

RESULTS

DNA sequence analyses.— BLAST searches revealed that the ITS sequence of isolate CBS 126090 (FN662474) showed a relatively high percent identity to that of *Cladorrhinum samala* (FM955447, 93%), while the sequence of the D1/D2 domains was closest to *Podospora fimiseda* and *Cercophora costaricensis* (G.C. Carroll & Munk) O. Hilber & R. Hilber (AY346296 and AY780059, both 98% identical). The ITS sequence of isolate CBS 290.75 (FN662475) had a relatively high percent identity to those of *Cercophora samala* and *Zopfiella tetraspora* (J.N. Rai, Mukerji & J.P. Tewari) S. Ahmad (AY999134 and AY999130, both 93%), while the closest matches for its D1/D2 sequence were *Cercophora samala* (AY999111, 98% identical) and *C. striata* (AY780065, 97% identical).

The ITS sequences of *Cladorrhinum* spp. and associated teleomorphs were too divergent to be aligned with confidence. Therefore, the cladistic analysis was performed

using the D1/D2 dataset. Isolates included in the analysis, their origins and EMBL accession numbers are shown in TABLE I. The manually edited alignment of the D1/D2 locus included 496 positions. The neighbor-joining tree (FIG. 1) showed five main groups, which corresponded to the clades named A, B, C, Chaetomiaceae and Sordariaceae by Cai et al (2006). In this study we used the same nomenclature for these groups. Clade A had 71% bootstrap support and included all the anamorphic species of *Cladorrhinum*, except *C. brunnescens*, which grouped with members of the Chaetomiaceae in a clade with low bootstrap support. In our opinion, it is not necessary to reallocate *C. brunnescens* to a different genus, because it belongs to the same order as the type species of *Cladorrhinum.* Similar taxonomic decisions have been made with other anamorphic genera such as *Cladophialophora* Borelli (Crous et al 2007, Badali et al 2008) and *Ramichloridium* Stahel ex de Hoog (Arzanlou et al 2007) to avoid the excessive proliferation of genus names. Clade A also included all the teleomorph species with *Cladorrhinum* anamorphs studied, and some taxa without known anamorphs. Nevertheless, it also included *Cercophora terricola* S. Ueda, which produces a *Chrysosporium*-like anamorph with lateral blastoconidia (Ueda 1994). This kind of anamorph, also produced by other members of the Lasiosphaeriaceae, was interpreted by Mouchacca & Gams (1993) as some kind of reduction of the intercalary phialides of *Cladorrhinum*, in which the conidiogenous opening becomes blocked after producing a single conidium.

Cladorrhinum samala proved to be distantly related to *Cercophora samala*, which was proposed by Udagawa and Muroi (1979) as its possible teleomorph. In addition, *C. foecundissimum* was at considerable genetic distance from *Apiosordaria verruculosa*, which was indicated by Cannon et al (1985) as its teleomorph. These molecular results support the conclusions by Mouchacca and Gams (1993), who

suspected on the basis of morphology and mating studies, that these anamorph-teleomorph connections had been erroneously suggested.

The D1/D2 neighbor-joining tree clearly distinguished isolates CBS 290.75 and CBS 126090 from the accepted species of *Cladorrhinum* and related teleomorphs. Isolate CBS 290.75 clustered with *Cercophora samala* and *C. striata* in a subclade of clade A with low bootstrap support. On the other hand, isolate CBS 126090 was placed in a well-supported subclade that also included *Podospora fimiseda*, *Cercophora costaricensis* and *Cladorrhinum samala*. Based on the results of the cladistic analysis and phenotypic differences, two new species of *Cladorrhinum* are proposed to accommodate the isolates CBS 290.75 and CBS 126090.

TAXONOMY

Cladorrhinum flexuosum H. Madrid, Cano, Gené & Guarro, sp. nov.

FIGS. 2, 3

MycoBank: MB 516710

Etymology. In reference to the flexuose conidiophores produced by the fungus.

Coloniae in agaro maltoso ad 24 C post 5 dies 63–65 mm diametro, griseo-luteae, velutinae vel floccosae. Hyphae septatae, ramosae, hyalinae vel pallide olivaceae, laeves, 1.5–5 µm latae. Microsclerotia absentia. Conidiophora semimacronemata, septata, dense ramosa, plerumque flexuosa, pallide olivacea vel pallide brunnea, laevia, saepe crassitunicata, longitudine indeterminata, 2.5–5 µm lata, in floccis aggregata. Phialides intercalares, laterales vel terminales; intercalares cylindricae vel subcylindricae, 5–21 × 2–4 µm, collulo laterali plerumque 2–5 µm longo; laterales et terminales subcylindricae vel lageniformes, laterales 4–11 × 2–3.5 µm, terminales 9–15 × 3–4 µm. Conidia unicellularia, subhyalina vel pallide olivaceo-

brunnea, laevia, tenuitunicata, plerumque globosa vel dacryoidea, 2–4 × 2–3 µm, guttulata, in capitulis mucidis aggregata. Teleomorphosis ignota.

Colonies on MEA attaining 63–65 mm diam in 5 d at 24 C, 6 mm at 36 C, dull yellow (3B4), radiating, flat, with scarce aerial mycelium, producing floccose conidial tufts at the center, velvety toward the periphery; reverse yellowish white (3A2) at the center, yellowish grey (3B2) toward the periphery. Vegetative hyphae septate, branched, hyaline to pale olive, smooth- and thin-walled, 1.5–5 µm wide. Microsclerotia not observed. Conidiophores semimacronematous, septate, profusely branched, branches usually flexuous and densely entangled, often ending in sterile cells with obtuse apices, pale olivaceous to pale brown, smooth, with cell walls usually thicker than those of the vegetative hyphae, length indeterminate, 2.5–5 µm wide, aggregated in tufts. Phialides mostly intercalary on the conidiophore axis and branches, but also terminal or lateral, commonly flexuous, light olive to light brown, with one or rarely two conidiogenous openings, bearing conspicuous collarettes; intercalary phialides cylindrical to subcylindrical, 5–21 × 2–4 µm, with a subcylindrical to lageniform lateral neck mostly 2–5 µm long, rarely longer, up to 9 µm; lateral and terminal phialides subcylindrical to lageniform, lateral phialides 4–11 × 2–3.5 µm, terminal phialides 9–15 × 3–4 µm. Conidia one-celled, subhyaline to pale olive-brown, smooth- and thin-walled, mostly globose to dacryoid, 2–4 × 2–3 µm, guttulate, often truncate at the base or bearing a subcylindrical hilum, aggregated in slimy masses. Teleomorph not observed.

Holotype. SPAIN. ASTURIAS PROVINCE: Pola de Somiedo, from soil, Oct 2006, *C. Silvera & A. Mercado* (IMI 397930, ex-type cultures CBS 126090 and FMR 10415).

This fungus is similar to other members of *Cladorrhinum* producing globose to dacryoid conidia, such as *Cladorrhinum samala*, *C. bulbillosum*, *C. brunnescens*, *C.*

foecundissimum, and the anamorphs of *Cercophora striata* and *Podospora fimiseda.* In contrast to *C. flexuosum*, *C. bulbillosum* and the anamorph of *Cercophora striata* produce blackish microsclerotia in culture (Mouchacca and Gams 1993, Miller and Huhndorf 2001). The dark, thick-walled setiform hyphae that characterize *Cladorrhinum samala* (Subramanian and Lodha 1964, Ellis 1971) were not observed in cultures of *C. flexuosum*; *C. samala* grew faster than *C. flexuosum* at all temperatures tested (TABLE II). While *C. flexuosum* produces fast-growing, flat colonies on MEA, *C. brunnescens* grows slowly (TABLE II) and its colonies show abundant aerial mycelium (Mouchacca and Gams 1993). Unlike *C. flexuosum*, *C. foecundissimum* has quite regular, not markedly flexuose conidiophores (Marchal 1885, Mouchacca and Gams 1993, Domsch et al 2007). *Podospora fimiseda* is a homothallic fungus that produces perithecia on agar media. Its anamorph seems to require media enriched with dung to achieve abundant sporulation (Bell and Mahoney 1997). In addition, the anamorphs of other species of *Podospora* and that of *Apodospora viridis* produce conidiogenous cells similar to those of *C. flexuosum*, but they appear scattered on the mycelium (Mouchacca and Gams 1993), not in dense clusters as in our isolate. Two species with this type of anamorph, *P. pauciseta* and *P. austroamericana* (Mirza and Cain 1969), were included in our cladistic analysis. In concordance with Cai et al (2006), these taxa clustered within clade A. The anamorph of *Arnium calymmatosporum* Jeng & J.C. Krug is also similar to *C. flexuosum*, but the former species produces light green colonies and the conidiophores illustrated in its protologue are not flexuous (Jeng and Krug 1976). We were not able to locate either strains or DNA sequences of *Arnium calymmatosporum* and *Apodospora viridis* for comparison. *Ramophialophora humicola* H. Madrid, Stchigel, Gené & Guarro is a morphologically similar anamorphic fungus which also belongs to the Sordariales, and shows terminal, lateral and intercalary phialides that

produce dacryoid conidia. Nevertheless, unlike *C. flexuosum*, *R. humicola* is a slow-growing taxon that produces conidiophores which often end in sterile, setiform, sometimes swollen apices (Madrid et al 2010). *Ramophialophora humicola* belongs to a different lineage of Sordariales (FIG. 1), which corresponds to clade B of the study by Cai et al (2006).

Cladorrhinum microsclerotigenum H. Madrid, Gené, Cano & Guarro, sp. nov.

FIGS. 4, 5

MycoBank: MB 516711

Etymology. In reference to the microsclerotia formed by the fungus.

Coloniae in agaro maltoso ad 24 C post 5 dies 26–29 mm diametro, brunneae vel luteo-griseae, pro partim albae, planae, velutinae, ad 36 C crescentes. Hyphae septatae, ramosae, hyalinae vel olivaceo brunneae, laeves, 1–9 µm latae. Microsclerotia numerosa, immersa vel paulo erumpentia, discreta vel confluentia, atrobrunnea, subglobosa vel forma irregularia, usque ad 55 µm diametro. Conidiophora semimacronemata, septata, simplicia vel ramosa, pallide olivacea vel pallide olivaceo-brunnea, laevia, saepe crassitunicata, longitudine indeterminata, 2–4 µm lata: phialides intercalares, laterales vel terminales. Phialides intercalares cylindricae vel subcylindricae, 4–8 × 3–4 µm, collulo laterali usque ad 6 µm longae; laterales et terminales plerumque lageniformes, 4–11 × 2.5–4 µm. Conidia unicellularia, hyalina vel pallide olivacea, laevia, plerumque ellipsoidea, 2–4 × 1.5–2.5 µm, guttulata, in capitulis mucidis aggregata. Teleomorphosis ignota.

Colonies on MEA attaining 26–29 mm diam in 5 d at 24 C, 21–23 mm at 36 C, brown (5F4) at the center, yellowish grey (3B2) toward the periphery, with patches of short, white mycelium, flat, velvety; reverse brownish grey (4F2) at the center, yellowish grey (3B2) toward the periphery. Vegetative hyphae septate, branched,

hyaline to olive-brown, smooth- and mostly thin-walled, 1–9 µm wide, giving rise to terminal, lateral or intercalary clumps of inflated, irregularly-shaped cells that differentiate into microsclerotia. Mature microsclerotia immersed to erumpent, discrete to confluent, subglobose or irregularly shaped, up to 55 µm wide, composed of dark brown, rounded to polygonal, thick-walled cells up to 17 µm diam. Conidiophores semimacronematous, septate, simple or branched, pale olive to pale olivaceous-brown, smooth, with cell walls usually thicker than those of the vegetative hyphae, length indeterminate, 2–4 µm wide. Phialides intercalary, lateral and terminal, pale olive to pale olivaceous-brown, with one or rarely two conidiogenous openings, bearing conspicuous collarettes; when intercalary, cylindrical to subcylindrical, 4–8 × 3–4 µm, with a subcylindrical to lageniform lateral neck up to 6 µm long; lateral and terminal phialides mostly lageniform, but also subcylindrical or centrally swollen, 4–11 × 2.5–4 µm. Conidia one-celled, hyaline to pale olive, smooth- and thin-walled, mostly ellipsoid, but also obovoid, clavate or cylindrical, 2–4 × 1.5–2.5 µm, guttulate, often truncate at the base, aggregated in slimy masses. Teleomorph not observed.

Holotype. TURKEY. ADANA PROVINCE: Adana, from *Musa* sp., May 1975, *A. Cengiz* (IMI 397929, ex-type cultures CBS 290.75 and FMR 10045).

The isolate CBS 290.75 was previously considered a deviating strain of *C. phialophoroides*, which differed from typical isolates by having conidiophores similar to those of *C. bulbillosum*, i.e. composed mainly of "short phialidic cells" (the size was not indicated, Mouchacca and Gams 1993). We compared *C. microsclerotigenum* with the type strain of *C. phialophoroides* and found evident length differences only in the terminal phialides, which were up to 19 µm in the latter species, but did not exceed 11 µm in *C. microsclerotigenum*. However, the phialides of *C. phialophoroides* were mostly terminal and lateral, and only a few of them were intercalary, while in *C.*

microsclerotigenum intercalary phialides were abundant. Furthermore, while *C. microsclerotigenum* grew at 36 C, *C. phialophoroides* did not grow at such temperature (TABLE II). We also examined the type strain of *C. bulbillosum*. This species could easily be distinguished from *C. microsclerotigenum* on the basis of conidial morphology, subglobose to dacryoid in *C. bulbillosum*, and mostly ellipsoidal in *C. microsclerotigenum.* The latter species is also similar to other *Cladorrhinum* anamorphs with ellipsoid conidia, such as those of *Cercophora samala* (Mouchacca and Gams 1993) and certain *Apiosordaria* species (Krug et al 1983). Nevertheless, in contrast to *C. microsclerotigenum*, these species do not produce microsclerotia in culture (Mouchacca and Gams 1993).

ACKNOWLEDGMENTS

We are indebted to the curators of the Centraalbureau voor Schimmelcultures (CBS; Utrecht, The Netherlands) for providing some of the isolates studied. The first author thanks Dr. Sybren de Hoog and Bert Gerrits van den Ende for guidance during a research stay at the CBS.

This work was supported by the Spanish Ministerio de Educación y Ciencia, grants CGL 2008-04226 and CGL 2009-08698/BOS.

LITERATURE CITED

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410.

Arzanlou M, Groenewald JZ, Gams W, Braun U, Shin H-D, Crous PW. 2007. Phylogenetic and morphotaxonomic revision of *Ramichloridium* and allied genera. Stud Mycol 58:57–93.

Badali H, Gueidan C, Najafzadeh MJ, Bonifaz A, Gerrits van den Ende AHG, de Hoog GS. 2008. Biodiversity of the genus *Cladophialophora*. Stud Mycol 61:175–191.

Bell A, Mahoney DP. 1997. Coprophilous fungi in New Zealand. II. *Podospora* species with coriaceous perithecia. Mycologia 89:908–915.

Cai L, Jeewon R, Hyde K. 2006. Molecular systematics of *Zopfiella* and allied genera: evidence from multi-gene sequence analyses. Mycol Res 110:359–368.

Calduch M, Gené J, Stchigel A, Cano J, Guarro J. 2004. *Ramophialophora*, a new anamorphic genus of Sordariales. Stud Mycol 50:83–88.

Cano J, Guarro J, Gené J. 2004. Molecular and morphological identification of *Colletotrichum* species of clinical interest. J Clin Microbiol 42:2450–2454.

Cannon PF, Hawksworth DL, Sherwood-Pike MA. 1985. The British Ascomycotina, an annotated checklist. Slough: Commonwealth Agricultural Bureaux. 302 p.

Chopin B, Sigler L, Connole MD, O`Boyle DA, Mackay B, Goldstein L. 1997. Keratomycosis in a percheron cross horse caused by *Cladorrhinum bulbillosum*. J Med Vet Mycol 35:53–55.

Crous PW, Schubert K, Braun U, de Hoog GS, Hocking AD, Shin D, Groenewald JZ. 2007. Opportunistic, human-pathogenic species in the Herpotrichiellaceae are

phenotypically similar to saprobic or phytopathogenic species in the Venturiaceae. Stud Mycol 58: 185–217.

de Hoog GS, Guarro J, Gené J, Figueras MJ. 2000. Atlas of clinical fungi. 2nd ed. Utrecht/Reus: Centraalbureau voor Schimmelcultures/Universitat Rovira i Virgili. 1126 p.

Domsch KH, Gams W, Anderson TH. 2007. Compendium of soil fungi. 2nd ed. Eching: IHW Verlag. 672 p.

Ellis MB. 1971. Dematiaceous Hyphomycetes. Kew: Commonwealth Mycological Institute. 608 p.

Gilgado F, Cano J, Gené J, Guarro J. 2005. Molecular phylogeny of the *Pseudallescheria boydii* species complex: proposal of two new species. J Clin Microbiol 43:4930–4942.

Huhndorf SM, Miller AN, Fernández FA. 2004. Molecular systematic of the Sordariales: the order and the family Lasiosphaeriaceae redefined. Mycologia 96:368–387.

Jeng RS, Krug JC. 1976. New records and new species of *Arnium*. Can J Bot 55:83–95.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd ed. London: Eyre Methuen. 252 p.

Krug JC, Udagawa S, Jeng RS. 1983. The genus *Apiosordaria*. Mycotaxon 17:533–549.

Lewis JA, Larkin RP. 1998. Formulation of the biocontrol fungus *Cladorrhinum foecundissimum* to reduce the damping-off disease caused by *Rhizoctonia solani* and *Pythium ultimum*. Biol Control 12:182–190.

Madrid H, Cano J, Stchigel A, Gené J, Guarro J. 2010. *Ramophialophora humicola* and *Fibulochlamys chilensis*, two new microfungi from soil. Mycologia 102:605–612.

Marchal E. 1885. Champignons coprophiles de la Belgique. Bull Soc R Bot Belg 24:57–77.

Miller AN, Huhndorf SM. 2001. Neotropical Ascomycetes 10. New and interesting *Cercophora* species. Sydowia 53:211–226.

———, ———. 2004. A natural classification of *Lasiosphaeria* based on nuclear LSU rDNA sequences. Mycol Res 108:26–34.

———, ———. 2005. Multi-gene phylogenies indicate ascomal wall morphology is a better predictor of phylogenetic relationships than ascospore morphology in the Sordariales (Ascomycota, Fungi). Mol Phylogenet Evol 35:60–75.

Mirza JH, Cain RF. 1969. Revision of the genus *Podospora*. Can J Bot 47:1999–2048.

Mouchacca J, Gams W. 1993. The hyphomycete genus *Cladorrhinum* and its teleomorph connections. Mycotaxon 48:415–440.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Subramanian CV, Lodha BC. 1964. Four new coprophilous hyphomycetes. Antonie van Leeuwenhoek 30:317–330.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA 4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882.

Udagawa S, Muroi T. 1979. Coprophilous Pyrenomycetes from Japan V. Trans Mycol Soc Japan 20:453–468.

Ueda S. 1994. A new *Cercophora* with a *Chrysosporium*-like anamorph. Mycoscience 35:287–290.

Zapater RC, Scattini F. 1979. Mycotic keratitis by *Cladorrhinum*. Sabouraudia 17:56–69.

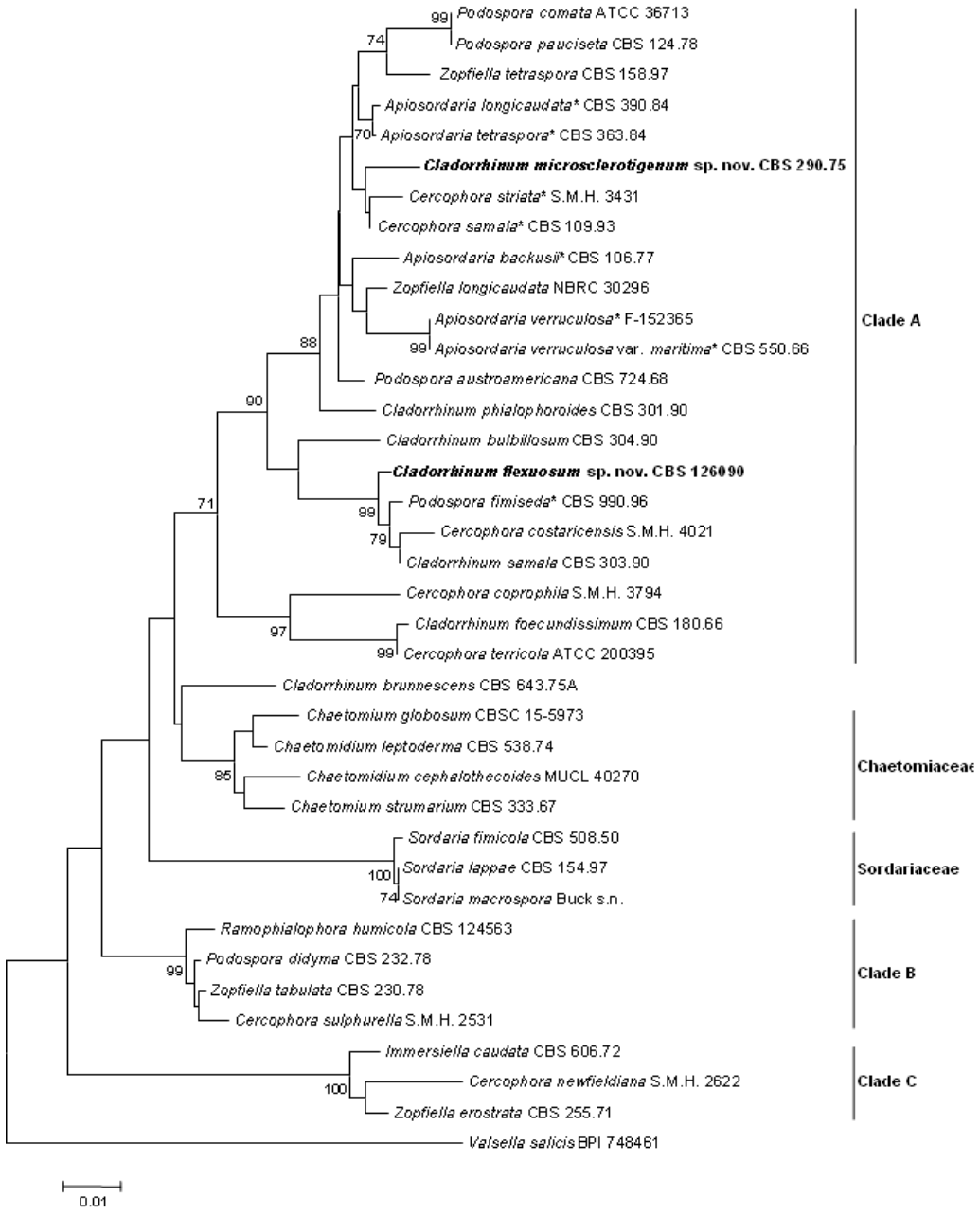

FIG. 1. Neighbor-joining tree constructed with sequences of the partial 28S rRNA gene. Branch lengths are proportional to distance. Bootstrap values above 70% are indicated near the internodes. *Valsella salicis* was used as outgroup. Teleomorph species known to produce *Cladorrhinum* anamorphs are indicated by an asterisk. Clades were labelled according to Cai et al (2006).

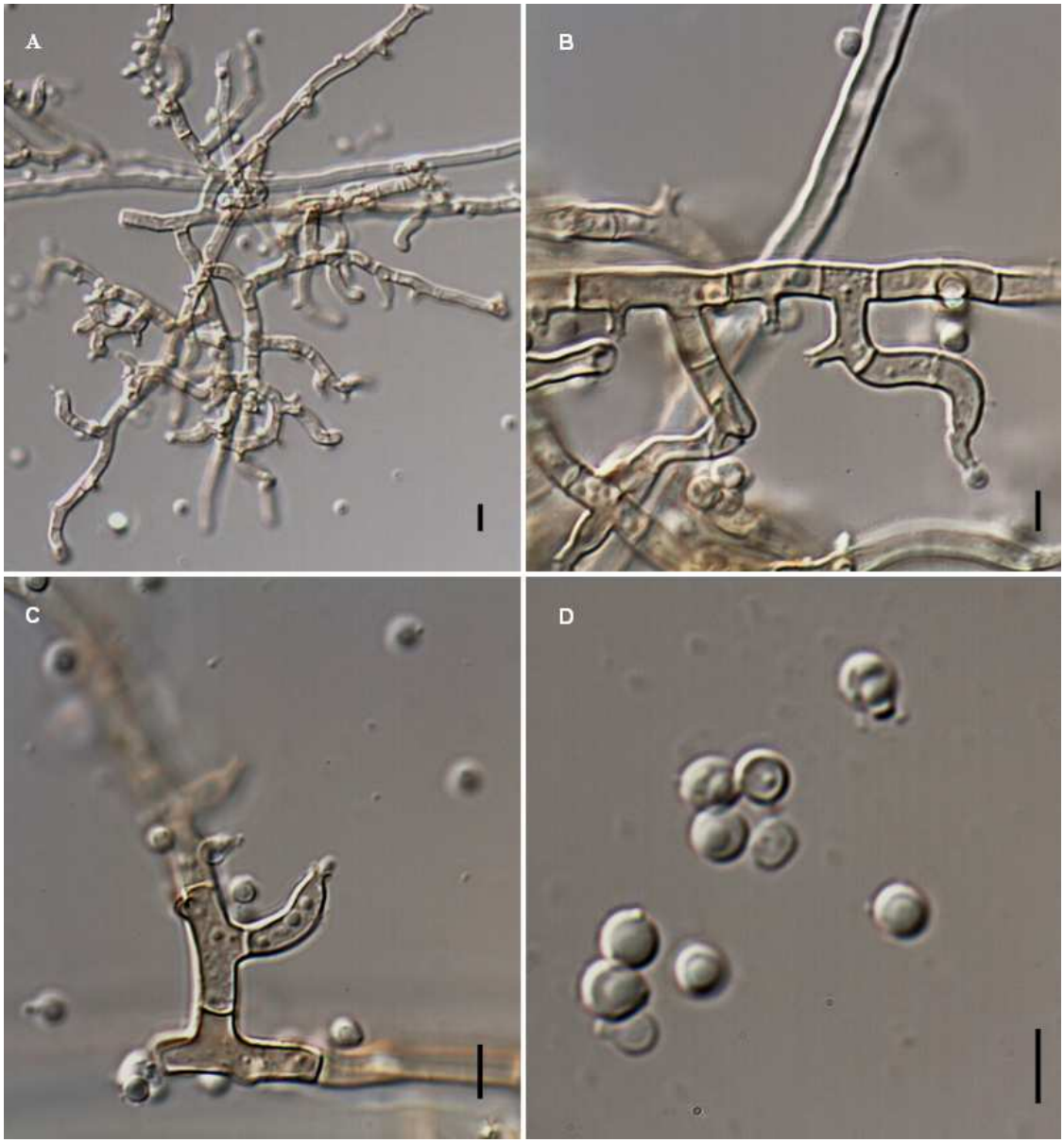

FIG. 2. *Cladorrhinum flexuosum* (CBS 126090): A. Densely branched, flexuous conidiophore. B. Intercalary and terminal phialides. C. Lateral phialide. D. Conidia. Scale bars = 5 µm.

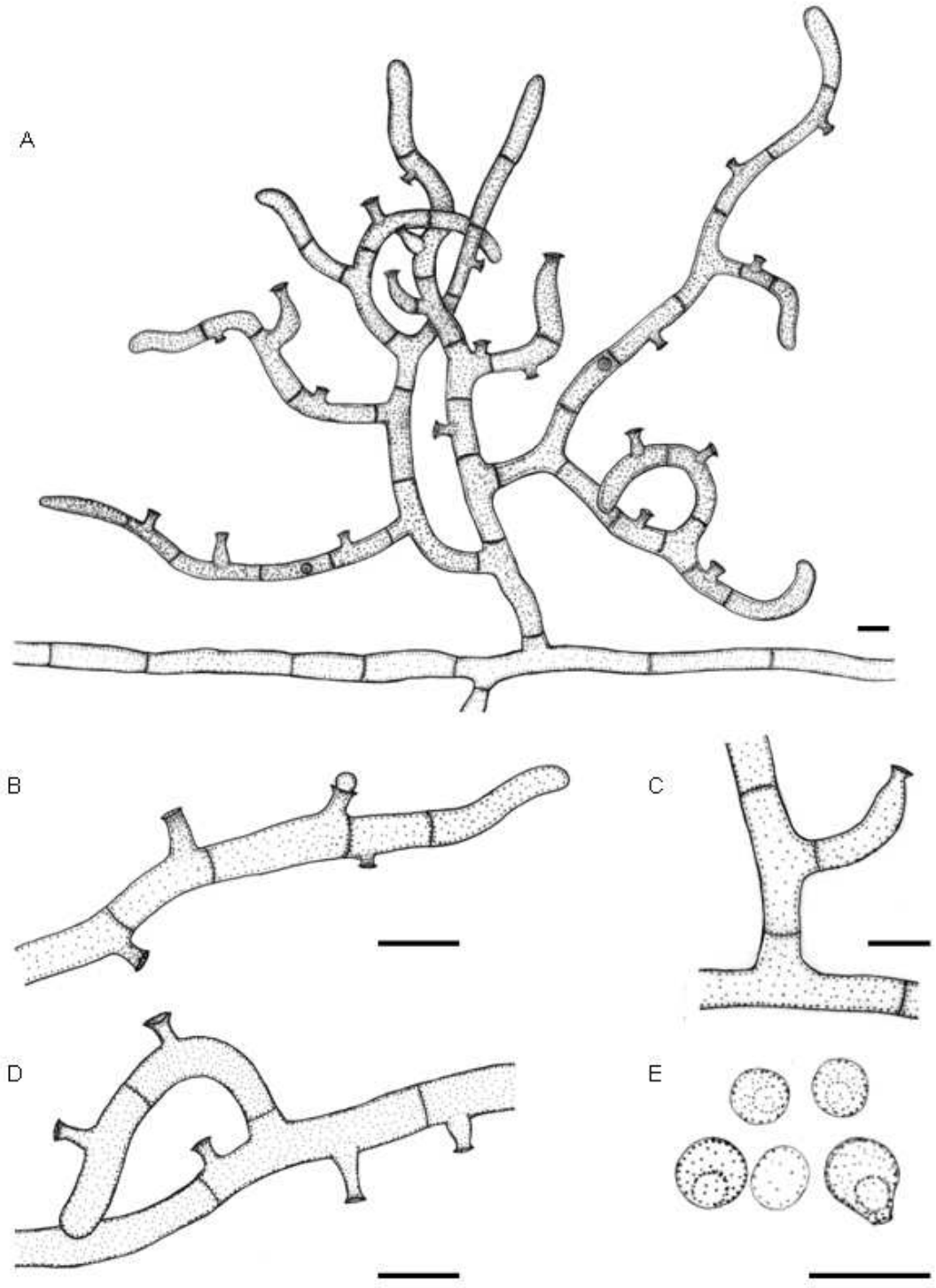

FIG. 3. *Cladorrhinum flexuosum* (CBS 126090). A. Conidiophore. B–D. Conidiogenous cells. E. Conidia. Scale bars = 5 μm.

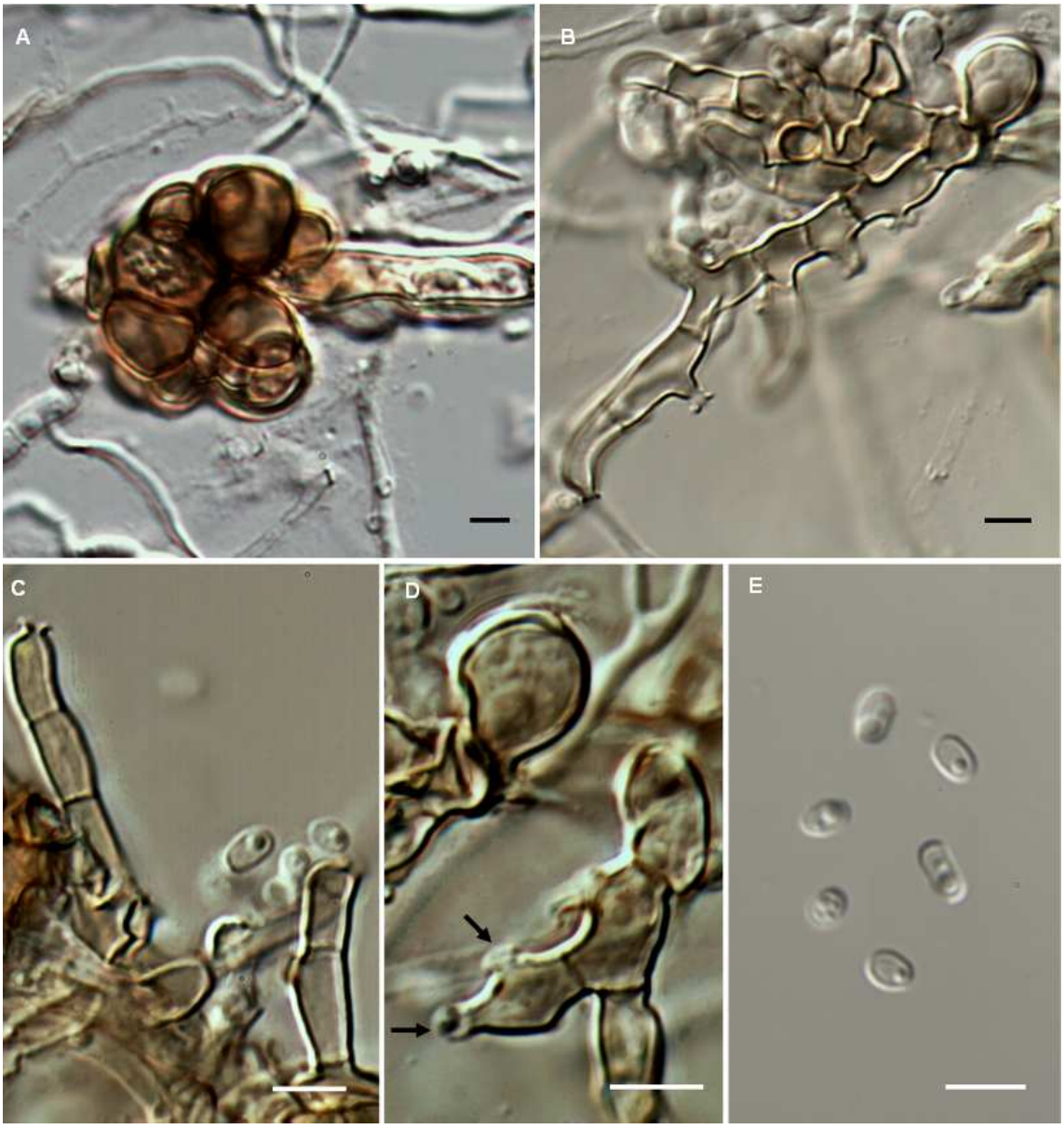

FIG.4. *Cladorrhinum microsclerotigenum* (CBS 290.75). A. Microsclerotium. B–C. Conidiophores and phialidic conidiogenous cells. D. Phialide with two conidiogenous openings (arrows). E. Conidia. Scale bars = 5 µm.

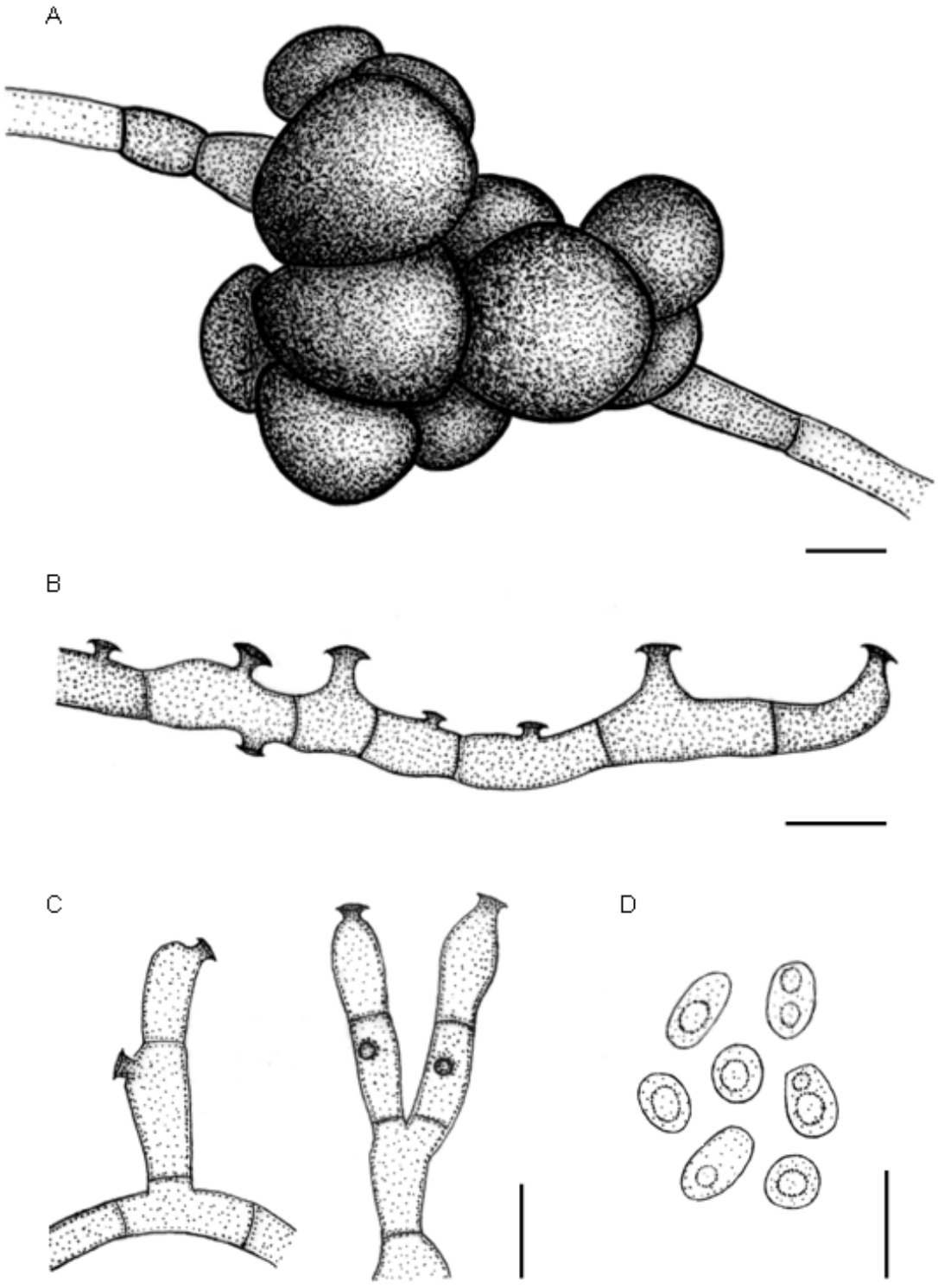

FIG. 5. *Cladorrhinum microsclerotigenum* (CBS 290.75). A. Microsclerotium. B,C. Conidiophores and conidiogenous cells. D. Conidia. Scale bars = 5 µm.

TABLE I. List of strains, sources, and sequences used in the phylogenetic analysis

Species	Collection number	Origin	EMBL accession # (28S rDNA)
Apiosordaria backusii	CBS 106.77	Sandy soil, Japan	AY780051
Apiosordaria longicaudata	CBS 390.84 (T)	Soil, Japan	**FR692340**
Apiosordaria tetraspora	CBS 363.84 (T)	Soil, Thailand	**FR692341**
Apiosordaria verruculosa	F-152365	Ethanol-pasteurized soil, Spain	AY346258
Apiosordaria verruculosa var. *maritima*	CBS 550.66	Salt-marsh soil, England	**FR692345**
Cercophora coprophila	S.M.H. 3794	Puerto Rico	AY780058
Cercophora costaricensis	S.M.H. 4021	Costa Rica	AY780059
Cercophora newfieldiana	S.M.H. 2622	USA	AF064642
Cercophora samala	CBS 109.93 (T)	Dung of cow, Japan	AY999111
Cercophora striata	S.M.H. 3431	Unidentified branch, Panama	AY780065
Cercophora sulphurella	S.M.H. 2531	Illinois, USA	AY587938
Cercophora terricola	ATCC 200395 (T)	River sediment, Japan	AY780067
Chaetomidium cephalothecoides	MUCL 40270 (T)	Mouse dung, USA	AF286413

TABLE I (cont.). List of strains, sources, and sequences used in the phylogenetic analyses

Chaetomidium leptoderma	CBS 538.74 (T)	Soil, England	AF096186
Chaetomium globosum	CBSC 15-5973	NA	AY545729
Chaetomium strumarium	CBS 333.67 (T)	Soil, India	AY681170
Cladorrhinum brunnescens	CBS 643.75A (T)	Fibres of *Cocos nucifera*, Netherlands	**FR692346**
Cladorrhinum bulbillosum	CBS 304.90 (T)	Desert sand, Egypt	**FR692339**
***Cladorrhinum flexuosum* sp. nov.**	CBS 126090 (T) (= FMR 10415)	Soil, Spain	**FN662477**
Cladorrhinum foecundissimum	CBS 180.66 (NT)	Agricultural soil, Netherlands	**FR692343**
***Cladorrhinum microsclerotigenum* sp. nov.**	CBS 290.75 (T) (= FMR 10045)	*Musa* sp., Turkey	**FN662476**
Cladorrhinum phialophoroides	CBS 301.90 (T)	Desert soil, Egypt	**FR692344**
Cladorrhinum samala	CBS 303.90	Desert soil, Egypt	**FR692338**
Immersiella caudata	CBS 606.72	Soil, The Netherlands	AY999113
Podospora austroamericana	CBS 724.68	Flower of *Carica papaya*, India	AY999101
Podospora comata	ATCC 36713	Cow dung, Venezuela	AY780072

TABLE I (cont.). List of strains, sources, and sequences used in the phylogenetic analyses

Podospora didyma	CBS 232.78	Rabbit dung, Canada	AY999100
Podospora fimiseda	CBS 990.96	Horse dung, New Zealand	AY346296
Podospora pauciseta	CBS 124.78	Dung of goat, India	EU999216
Ramophialophora humicola	CBS 124563 (T)	Forest soil, Spain	**FR692337**
Sordaria fimicola	CBS 508.50	Dung, Canada	AY681160
Sordaria lappae	CBS 154.97	Soil, Hungary	AY681137
Sordaria macrospora	Buck *s.n.*	Caribou dung, Canada	AY346301
Zopfiella erostrata	CBS 255.71	Dung, Central African Republic	AY999110
Zopfiella longicaudata	NBRC 30296	Soil, Japan	AY999109
Zopfiella tabulata	CBS 230.78	Porcupine dung, Canada	AY999105
Zopfiella tetraspora	CBS 158.97	Garden soil, India	AY999108
Valsella salicis	BPI 748461	*Salix fragilis*, Italy	AF408389

Sequences generated during this study appear in bold type. T, ex-type strain; NT, ex-neotype strain (Mouchacca and Gams 1993); NA, not available; ATCC, American Type Culture Collection, Bethesda, Maryland, USA; BPI, Systematic Botany and Mycology Laboratory, USDA, Beltsville, Maryland, USA; Buck, William Buck, Jacksonville, Florida, USA; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CBSC, Carolina Biological Supply Company, Burlington, North Carolina, USA; F, Field Museum of Natural History, Chicago, Illinois, USA; FMR, Faculty of Medicine collection, Reus, Spain; MUCL, Mycothèque de L`Université Catholique de Louvain, Faculté des Sciences Agronomiques, Louvain-la-Neuve, Belgium; NBRC, Biological Resource Center, Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan; S.M.H., Sabine M. Huhndorf, Field Museum, Chicago, USA; Wang, Yei Zei-Zeng Wang, Hong Kong University, China.

TABLE II. Colony diameters of *Cladorrhinum* spp. at different temperatures

Species	Colony diameter (mm) on MEA after 5 d at temperature:					
	6 C	12 C	18 C	24 C	30 C	36 C
Cladorrhinum brunnescens CBS 643.75A	3	5–6	7–9	9–11	6–7	0
Cladorrhinum bulbillosum CBS 304.90	4	34	71–78	> 85	> 85	> 85
***Cladorrhinum flexuosum* CBS 126090**	0	11–14	33–36	63–65	> 85	6
Cladorrhinum foecundissimum CBS 180.66	5	13–14	24–27	34–36	24	0
***Cladorrhinum microsclerotigenum* CBS 290.75**	0	9–10	19–21	26–29	54–55	21–23
Cladorrhinum phialophoroides CBS 301.90	3	6–7	13–16	21–23	17–21	0
Cladorrhinum samala CBS 303.90	4	26–27	63–66	> 85	> 85	15–18

MEA, malt extract agar. Colony diameters > 85 indicate full plates after 5 d of incubation.

4.5 A new species of *Leptodiscella* from Spanish soil

Madrid H, Gené J, Cano J, Guarro J

Submitted to Mycological Progress

A new species of *Leptodiscella* from Spanish soil

Hugo Madrid, Josepa Gené, Josep Cano*, Josep Guarro

Unitat de Micologia, Facultat de Medicina i Ciències de la Salut and Institut d`Investigació Sanitària Pere Virgili (IISPV), Universitat Rovira i Virgili, 43201 Reus, Spain.

*Corresponding author: josep.cano@urv.cat, tel +34 977759350, fax: +34 977759322

Abstract A new hyphomycete, *Leptodiscella brevicatenata*, is described based on morphology, growth profilings and DNA sequence comparisons. The new species, isolated from soil in Spain, is characterized by producing conidia with 3-9 µm long appendages and chlamydospores borne singly or in short, simple chains of 2–4 cells. Analyses of sequences of the 5.8S and 28S ribosomal DNA and internal transcribed spacers 1 and 2 of the ribosomal RNA gene cluster indicated that *L. brevicatenata* is close to *L. chlamydospora*. The latter species, however, grows faster than *L. brevicatenata* at 30ºC and produces conidia with appendages up to 4 µm long and chlamydospores which are often arranged in long, sometimes branched chains of 10–40 cells. The phylogenetic study revealed that *Leptodiscella* spp. represent a novel lineage of the Dothideomycetes relatively close to the order Acrospermales. A key to the accepted *Leptodiscella* species is provided.

Keywords Anamorphic fungi, appendaged conidia, Dothideomycetes, taxonomy

Introduction

Species of the anamorphic genus *Leptodiscella* Papendorf produce micro- to semimacronematous conidiophores and holoblastic, subcylindrical, mostly 1-septate conidia with two subpolar appendages (Papendorf 1969; Carmichael et al. 1980). The type species, *L. africana* (Papendorf) Papendorf, was isolated from leaf litter and soil of an *Acacia karroo* Heyne community in South Africa (Papendorf 1967). Subsequently, two additional species were described from Japan, i.e. *L. japonica* Matsush. from forest soil (Matsushima 1975) and *L. chlamydospora* Udagawa & Toyaz. from house dust (Udagawa and Toyazaki 1985). So far, no teleomorph connection has been established for *Leptodiscella*, and the molecular phylogenetic affinities of the genus remain unexplored.

During investigations on the biodiversity of soil microfungi in Spain, an interesting isolate of the genus *Leptodiscella* was recovered (CBS 129025). In the present study, its taxonomic position was assessed based on phenotypic features and DNA sequence comparisons.

Materials and methods

Sampling area, isolation technique and isolates studied

Soil samples were collected in Camarena de la Sierra area, Teruel Province, Spain, in 2009. The sampling area has a mountain Mediterranean climate with average annual temperature of 8.5ºC, and average annual rainfall of 635 mm. The altitude is 1600 m and the vegetation is dominated by *Pinus sylvestris* L. Soil samples were placed into sterilized plastic bags, closed and stored in a refrigerator at 4–7ºC until processed. Approximately 1 g of the soil sample was diluted in 9 mL sterilized water, and 300 µL of this mix were inoculated onto potato dextrose agar (PDA; Pronadisa, Madrid, Spain) plates supplemented with cycloheximide at a final concentration of 0.1 mg/mL. The mix

was spread over the surface of this medium using a Digralsky spreader. The plates were incubated at 25ºC and examined weekly during one month. To obtain pure cultures, small fragments of colonies growing in the primary cultures were inoculated onto potato-carrot agar (PCA; 20 g potatoes, 20 g carrots, 20g agar, 1L distilled water) and oatmeal agar (OA; 30 g filtered oat flakes, 20 g agar, 1L distilled water) plates, which were incubated at 25ºC for 14 days in the dark. The *Leptodiscella* isolate CBS 129025 was compared with type or reference strains of other species of the genus obtained from the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands) and the Mycothèque de L`Université Catholique de Louvain (MUCL, Louvain-la-Neuve, Belgium) (data of these isolates are given in section Taxonomy, material examined)

Phenotypic characterization

Colony morphology and growth rates were studied on PCA plates in duplicate after 14 d at 15, 25, 30 and 35ºC in the dark. This medium was considered by Papendorf (1967) the most suitable for the study cultural and micromorphological characters in *Leptodiscella*. Colony diameters were recorded as minimum and maximum values obtained for isolates belonging to each species Table 1. Color notations in parentheses are from Kornerup and Wanscher (1978). The microscopic features were studied in lactic acid from slide cultures made on PCA after 14 d at 25ºC, using an Olympus CH-2 light microscope. Photomicrographs were obtained using a Zeiss Axio Imager M1 light microscope.

DNA extraction, amplification and sequencing

DNA extraction was performed directly from fungal colonies by following the Fast DNA kit protocol (Bio 101, Inc., Vista, California, USA), with the homogenization step repeated 5 times. Amplification of the the internal transcribed spacer (ITS) region, comprising the 5.8S rRNA gene and ITS 1 and 2 of the ribosomal RNA (rRNA) gene

cluster, was performed with the primer pair ITS5/ITS4 as described by White et al. (1990). The D1/D2 domains of the 28S rRNA gene were amplified and sequenced using primers NL1/NL4 as described by O`Donnell (1993). PCR products were purified using a GFXTM PCR DNA kit (Pharmacia Biotech, Cerdanyola, Spain) and were stored at -20ºC until sequencing. PCR products were sequenced by using the same primers employed 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). The program SeqMan (Lasergene, Madison, Wisconsin) was used to obtain consensus sequences from the complementary sequences of each isolate. BLAST searches (Altschul et al. 1990) were performed to compare the sequences of the isolates studied with those of other fungi deposited in the GenBank database. Nucleotide sequence alignments were performed with ClustalX version 2.0 (Thompson et al. 1997), followed by manual adjustments with a text editor. Ambiguously aligned positions were excluded from the analyses. A phylogeny reconstruction was performed by the neighbor-joining method (Saitou and Nei 1987), using the Kimura-2-parameter substitution model with pairwise deletion of gaps, as implemented in the MEGA 4.0 computer program (Tamura et al. 2007). The statistical support of branches was assessed by bootstrap analysis of 1000 replicates. The sequences generated in this study and the alignment used in the phylogenetic analysis were deposited in GenBank and TreeBASE (www.treebase.org, accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S11239), respectively.

Results

Molecular study

A BLAST search revealed that the D1/D2 sequence of the *Leptodiscella* isolate CBS 129025 (FR821311) showed relatively high percent identities to those of *L. chlamydospora* (FN869567, 96%), *L. africana* (FN869566, 94%) and *L. japonica* (FN908212, 88%). Other significant matches were sequences of species in different orders and families of Dothideomycetes, but they showed percent identities ≤ 87%. The closest matches for the ITS sequence of our isolate (FR821312) were *L. chlamydospora* (FR745398, 90% identical) and *L. africana* (FR751089, 88 % identical).

The ITS sequences of *Leptodiscella* spp. and related fungi were too divergent to be aligned with confidence. Therefore, the cladistic analysis was based on sequences of the D1/D2 region (Fig. 1). After removing ambiguously aligned regions, we obtained a D1/D2 dataset of 389 characters. The groupings obtained in the neighbor-joining tree (Fig. 1) agreed with the main lineages revealed in previous phylogenetic assessments of the Dothideomycetes (Nelsen et al. 2009, Schoch et al. 2009), including 12 orders (i.e. Acrospermales, Botryosphaeriales, Capnodiales, Dothideales, Hysteriales, Jahnulales, Microthyriales, Myriangiales, Mytilinidiales, Patellariales, Pleosporales, and Trypetheliales) and eight families *incertae sedis* (i.e. Asterinaceae, Elsinoaceae, Gloniaceae, Monoblastiaceae, Phaeotrichaceae, Strigulaceae, Tubeufiaceae, and Venturiaceae). Most of these lineages showed high bootstrap support, but the backbone of the tree was poorly resolved. Therefore, the interrelationships of the orders and families were not clarified in most cases. *Leptodiscella* spp. formed a novel lineage with 96% bootstrap support, which appeared as the sister clade of the Acrospermales. The *Leptodiscella* isolate CBS 129025, *L. africana* and *L. chlamydospora* formed a well-supported subclade which can be considered the core of *Leptodiscella*. These three species are characterized by mono or polyblastic conidiogenous cells that mostly lack denticles and often produce conidia from rather flat conidiogenous loci (Papendorf

1967; Udagawa and Toyazaki 1985). The placement of *L. japonica* outside such subclade is consistent with its markedly different conidiogenous cells, i.e. with numerous narrow denticles (Matsushima 1975). The closest relative of *Leptodiscella* sp. CBS 129025 was *L. chlamydospora*, but the genetic distance observed and phenotypic differences indicate that they are distinct taxa.

Taxonomy

Leptodiscella brevicatenata H. Madrid, Cano, Gené & Guarro, sp. nov. (Fig. 2)

MB 561038

Etymology: referring to the short chains of chlamydospores produced by the fungus.

Coloniae in agaro PCA ad 25ºC post 14 dies 21 mm diametro, humidae, adpressae, cremeae vel brunneae. Hyphae septatae, ramosae, pallide olivaceae vel brunneae, laeves, 1–3 µm latae. Conidiophora micronematosa vel semimacronematosa, mononematosa, simplicia vel ramosa, pallide olivacea vel pallide olivaceo-brunnea, laevia, tenuitunicata, 5–37 × 1.5–6 µm. Celullae conidiogenae intercalares, laterales vel terminales, monoblasticae vel polyblasticae et sympodiales, plerumque discretae, subcylindricae vel globosae, 5–16 × 2–7 µm. Conidia primo pallide olivacea et aseptata, deinde pallide brunnea et plerumque 1-septata, subcylindrica, 9.5–25 × 4–6 µm, cum appendicibus simplicibus filiformibus subterminalibus 3–9 µm longa, usque ad 0.5 µm lata. Chlamydosporae intercalares, laterales vel terminales, singulae vel breviter catenulatae (2–4), plerumque globosae vel subglobosae, brunneae, crassitunicatae, 6–16 × 6–8 µm. Teleomorphosis ignota.

Colonies on PCA attaining 21 mm diam in 14 d at 25ºC, moist, appressed, with funiculose aerial mycelium and brown (6E4) at the center, nearly glabrous and yellowish white (4A2) towards the periphery; reverse concolorous. Vegetative hyphae

septate, branched, pale olivaceous to brown, smooth and thin walled, 1–3 µm wide, sometimes forming mycelial strands, with occasional anastomoses. Conidiophores micronematous or semimacronematous, mononematous, simple or branched, sometimes reduced to a conidiogenous cell, light olive to light olive-brown, smooth and thin-walled, 5–37 × 1.5–6 µm. Conidiogenous cells intercalary, lateral or terminal, monoblastic or polyblastic and sympodial, non-denticulate, usually discrete, subcylindrical to globose, often swollen, light olive to light olive-brown, smooth-walled, 5–16 × 2–7 µm. Conidia light olive and aseptate when young, becoming pale brown and mostly 1-septate in age, with cells often unequally melanized, subcylindrical and mostly rounded at both ends, but sometimes obconically truncate at the base, sometimes constricted at the septum, 9.5–25 × 4–6 µm, with a simple, filamentous subterminal appendage at each end, 3–9 µm long, up to 0.5 µm wide. Chlamydospores intercalary, lateral or terminal, borne singly or in chains of 2–4 cells, mostly globose to subglobose, brown, thick-walled, 6–16 × 6–8 µm. Teleomorph not observed.

Specimen examined: Spain, Teruel, Camarena de la Sierra area, isolated from forest soil, 08 October 2009, M. Hernandez, holotype (IMI 500299, culture ex-type: CBS 129025 = FMR 10885).

Additional specimens examined: *Leptodiscella africana*, South Africa, Transvaal, Potchefstroom, isolated from leaf litter of *Acacia karroo*, January/February 1964, M.C. Papendorf, type strain (CBS 400.65); *L. chlamydospora*, Japan, Osaka, Suita-city, isolated from house dust, 22 February 1984, N. Toyazaki, type strain (MUCL 28859); *L. japonica*, Cuba, Viñales, Pinar del Rio, isolated from twig of a forest tree, September 1988, G. Arnold (CBS 529.88).

Leptodiscella brevicatenata is morphologically similar to *L. africana* and *L. chlamydospora.* In contrast to *L. brevicatenata*, *L. africana* does not produce

chlamydospores in culture and its conidia are smaller (11–17.5 × 2–3 μm) (Papendorf 1967). Both *L. brevicatenata* and *L. chlamydospora* produce chlamydospores in culture, but in *L. brevicatenata* they appear singly or in short, simple chains (Fig. 2), whereas in *L. chlamydospora* they often form long, irregularly coiled and sometimes branched chains (Udagawa and Toyazaki 1985). The conidia of *L. chlamydospora* (8–14 × 3–5 μm) and its conidial appendages (2–4 μm long) are shorter than those of *L. brevicatenata*. At 15 and 25ºC, *L. africana*, *L. brevicatenata* and *L. chlamydospora* show similar growth rates, but these three species grow faster than *L. japonica*. At 30ºC, *L. brevicatenata* grows very restrictedly, but the other *Leptodiscella* spp. grow relatively well (Table 1). None of the *Leptodiscella* spp. tested grows at 35ºC.

Unnamed hyphomycetous anamorphs with sympodial conidiophores are produced by *Acrospermum compressum* Tode and *A. graminum* Lib., two species phylogenetically close to *Leptodiscella* spp. Nevertheless, the conidia of these *Acrospermum* spp. are not appendaged (Webster 1956). Certain species of the hyphomycetous genera *Dictyochaeta* Speg., *Hyphodiscosia* Lodha & K.R.C. Reddy, *Mycoleptodiscus* Ostaz. and *Stratiphoromyces* Goh & K.D. Hyde produce appendaged conidia similar to those of *Leptodiscella*. However, the conidiogenous cells in *Dictyochaeta* and *Mycoleptodiscus* are phialidic (Sutton 1973; Kuthubutheen and Nawawi 1991; Whitton et al. 2000), and the conidiophores of *Stratiphoromyces* and *Hyphodiscosia* are macronematous and strongly melanized (Lodha and Chandra Reddy 1974; Goh and Hyde 1998). Species of *Dictyochaeta* and *Mycoleptodiscus* included in previous phylogenetic studies were placed in the Sordariomycetes (Réblová and Seifert 2007; Thongkantha et al. 2009). Nevertheless, the phylogenetic affinities of *Hyphodiscosia* and *Stratiphoromyces* are unknown.

Leptodiscella seems to be an uncommon genus. The type species, *L. africana*, has been reported from Costa Rica (Bills and Polishook 1994), The Netherlands (Dugan et al. 1995), South Africa (Papendorf 1967, 1969) and USA (Dugan et al. 1995); *L. chlamydospora* is known only from Japan (Udagawa and Toyazaki 1985), and *L. japonica* is known from Japan (Matsushima 1975) and Cuba (R.F. Castañeda, pers. comm.). *Leptodiscella brevicatenata* is the only species of the genus known from Southern Europe.

The relevant features differentiating the four species of *Leptodiscella* are included in the following key.

Key to *Leptodiscella* species

1 Chlamydospores formed in culture

2 Chlamydospores often forming long chains of 10–40 cells, coiled in several planes; conidial appendages rudimentary, 2–4 µm long ***L. chlamydospora***

2* Chlamydospores formed singly or in short chains of 2–4 cells; conidial appendages well-developed, 3–9 µm long ***L. brevicatenata***

1* Chlamydospores not formed in culture

3 Conidiogenous cells subcylindrical, strongly denticulate ***L. japonica***

3* Conidiogenous cells rounded or irregularly-shaped, often non-denticulate ***L. africana***

Acknowledgments

We thank Dr. Cony Decock (Mycothèque de l`Université Catholique de Louvain, Louvain-la-Neuve, Belgium) and the curators of the Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands) for providing some of the isolates studied.

This study was supported by the Spanish Ministerio de Ciencia y Tecnología, grants CGL 2008-04226 and CGL 2009-08698/BOS.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Bills GF, Polishook JD (1994) Abundance and diversity of microfungi in leaf litter of a lowland rain forest in Costa Rica. Mycologia 86:187–198

Carmichael JW, Kendrick BW, Conners IL, Sigler L (1980) Genera of Hyphomycetes. University of Alberta Press, Edmonton

Dugan FM, Roberts RG, Hanlin RT (1995) New and rare fungi from cherry fruits. Mycologia 87:713–718

Goh TK, Hyde KD (1998) *Stratiphoromyces brunneisporus* gen. et sp. nov., an undescribed dematiaceous hyphomycete on *Licuala* palms. Mycol Res 102:1149–1152

Kornerup A, Wanscher JH (1978) Methuen handbook of colour. 3rd ed. Eyre Methuen, London

Kuthubutheen AJ, Nawawi A (1991) Key to *Dictyochaeta* and *Codinaea* species. Mycol Res 95:1224–1229

Lodha BC, Chandra Reddy KR (1974) *Hyphodiscosia* gen. nov. from India. Trans Br Mycol Soc 62:418–421

Matsushima T (1975) Icones Microfungorum a Matsushima Lectorum. Published by the author, Kobe

Nelsen MP, Lücking R, Grube M, Mbatchou JS, Muggia L, Rivas Plata E, Lumbsch HT (2009) Unravelling the phylogenetic relationships of lichenised fungi in Dothideomyceta. Stud Mycol 64:135–144

O`Donnell K (1993) *Fusarium* and its near relatives. In: Reynolds R, Taylor JW (eds.) The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. CAB International, Wallingford, pp 225–233

Papendorf MC (1967) *Leptodiscus africanus*, sp. nov. Trans Br Mycol Soc 50:687–690

Papendorf MC **(**1969) *Leptodiscella africana* gen. et comb. nov. Trans Br Mycol Soc 53:145–147

Réblová M, Seifert KA (2007) A new fungal genus, *Teracosphaeria*, with a *Phialophora*-like anamorph (Sordariomycetes, Ascomycota). Mycol Res 111:287–298

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Schoch GL, Crous PW, Groenewald JZ, Boehm EWA, Burgess TI, de Gruyter J, de Hoog GS, Dixon LJ, Grube M, Gueidan C, Harada Y, Hatakeyama S, Hirayama K, Hosoya T, Huhndorf SM, Hyde KD, Jones EBG, Kohlmeyer J, Kruys Å, Li YM, Lücking R, Lumbsch HT, Marvanová L, Mbatchou JS, McVay AH, Miller AN, Mugambi GK, Muggia L, Nelsen MP, Nelson P, Owensby CA, Phillips AJL, Phongpaichit S, Pointing SB, Pujade-Renaud V, Raja HA, Rivas Plata E, Robbertse B, Ruibal C, Sakayaroj J, Sano T, Selbmann L, Shearer CA, Shirouzu T, Slippers B, Suetrong S, Tanaka K, Volkmann-Kohlmeyer B, Wingfield MJ, Wood AR, Woudenberg JHC, Yonezawa H, Zhang Y, Spatafora JW (2009) A class-wide phylogenetic assessment of Dothideomycetes. Stud Mycol 64:1–15

Sutton BC (1973) *Pucciniopsis*, *Mycoleptodiscus* and *Amerodiscosiella*. Trans Br Mycol Soc 60:525–536

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Thongkantha S, Jeewon R, Vijaykrishna D, Lumyong S, Mckenzie EHC, Hyde KD (2009) Molecular phylogeny of Magnaporthaceae (Sordariomycetes) with a new species, *Ophioceras chiangdaoense* from *Dracaena loureiroi* in Thailand. Fungal Diversity 34:157–173

Udagawa S, Toyazaki N (1985) A new species of *Leptodiscella*. Mycotaxon 22:407–413

Webster J (1956) Conidia of *Acrospermum compressum* and *A. graminum*. Trans Br Mycol Soc 39:361–366

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Shinsky JJ, White TJ (eds) PCR Protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322

Whitton SR, McKenzie EHC, Hyde KD (2000) *Dictyochaeta* and *Dictyochaetopsis* species from the Pandanaceae. Fungal Diversity 4:133–158

Table 1. Colony diameters (mm) of *Leptodiscella* spp. on PCA[a] after 14 d at different temperatures

Species	15ºC	25ºC	30ºC
Leptodiscella africana CBS 400.65	10–11	23–24	12
Leptodiscella brevicatenata sp. nov. CBS 129025	14	21	1.5
Leptodiscella chlamydospora MUCL 28859	10–12	26	29
Leptodiscella japonica CBS 529.88	2–3	14–15	16

[a]PCA, potato carrot agar.

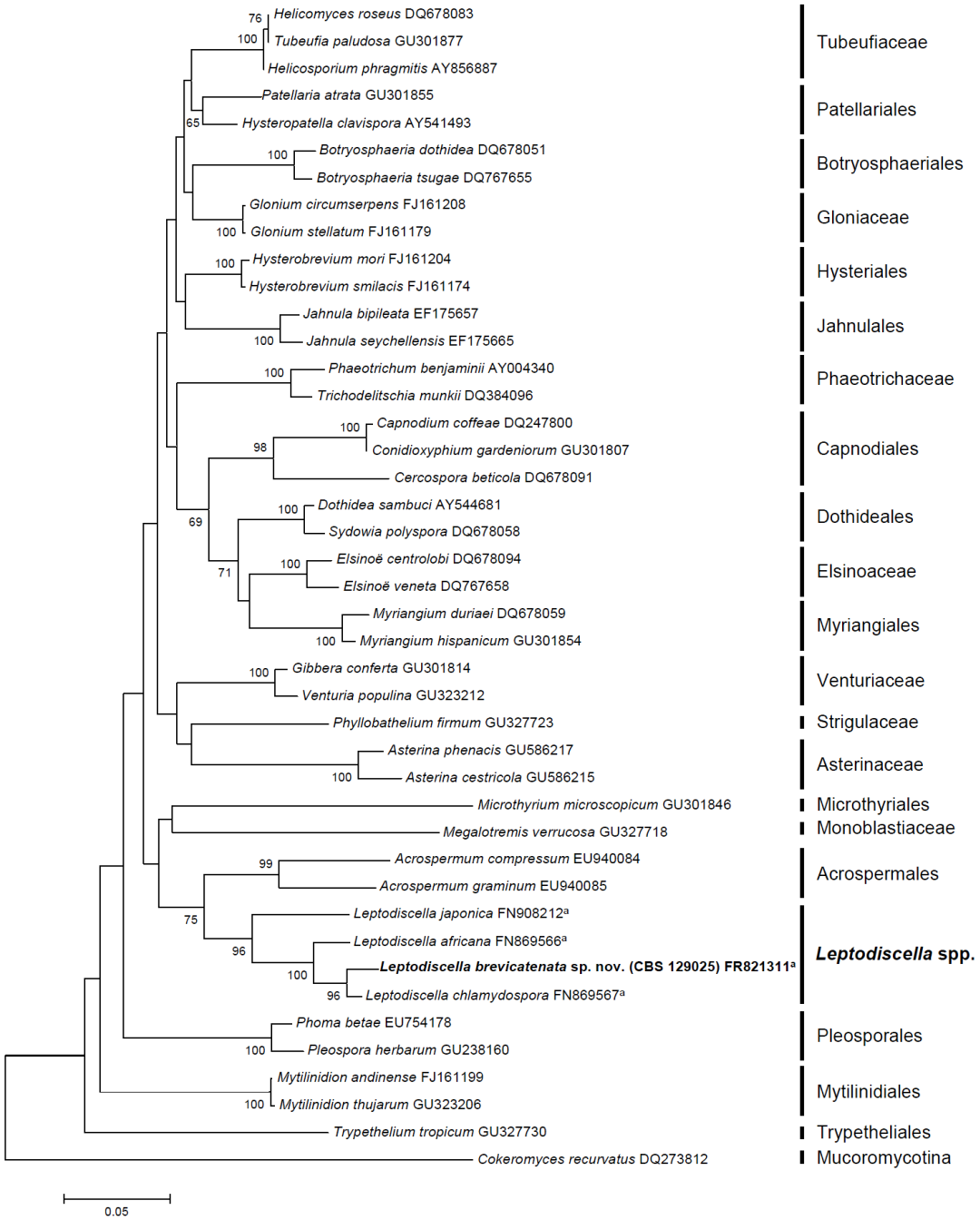

Fig. 1 Neighbor-joining tree constructed with partial sequences of the 28S rRNA gene. Branch lengths are proportional to distance. Bootstrap values above 65% are indicated near the internodes. *Cokeromyces recurvatus* was used as outgroup. [a]Sequences generated during this study.

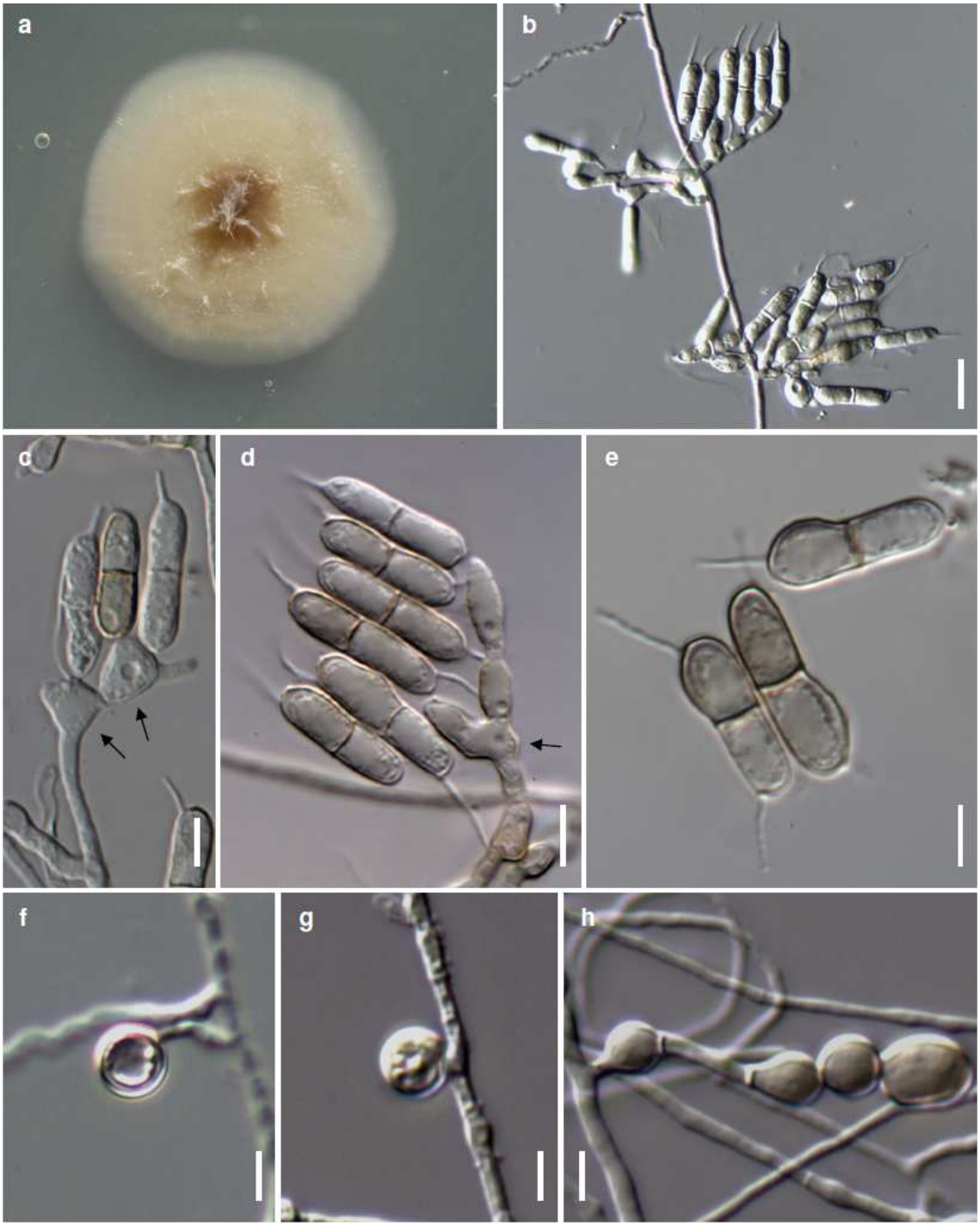

Fig. 2 *Leptodiscella brevicatenata*, CBS 129025. **a** Colony on PCA after 14 d at 25ºC, **b–d** conidiophores with terminal and intercalary (arrows), conidiogenous cells, producing conidia, **e** conidia, **f–h** chlamydospores. *Scale bar* **(b)** 10 µm, (**c–h**) 5 µm.

5. SUMMARIZING DISCUSSION

5. SUMMARIZING DISCUSSION

5.1 New members of the *Ophiostoma stenoceras-Sporothrix schenckii* complex and thermal dimorphism in *Ophiostoma bragantinum*

The vast majority of the fungal species currently known were described on the basis of phenotypic features. Most of them are routinely identified by morphology, assimilation profiles of carbon or nitrogen compounds, and other physiological tests (Kwon-Chung and Bennett 1992, Kurtzman and Fell 1998). These methods for species identification are relatively cheap and have been used so widely that comparisons can easily be made among existing species and between new and existing species (Taylor et al. 2000). Nevertheless, species defined solely on the basis of phenotypic features usually comprise two or more phylogenetic species (O`Donnell 2000, Fisher et al. 2002, Tavanti et al. 2005). This is especially frequent in species-rich anamorphic genera with simple conidiogenous structures, such as *Acremonium* (Guarro et al. 2009), *Fusarium* (Stewart et al. 2006), and *Sporothrix* (Marimon et al. 2007).

Many mycologists identify fungi based only on their morphology on the natural substratum (Castañeda et al. 1999, Rodrigues Barbosa et al. 2007). This *modus operandi* is preferred because some fungal species show poor or no sporulation on agar media (Guo et al. 1998) and some cannot even be cultivated (Fonseca and Lacaz 1971, Crous 2007). However, the identification of species in the *O. stenoceras-S. schenckii* complex may require further studies, because some of its members can only be distinguished by physiological and phylogenetic differences (Marimon et al. 2007, de Meyer et al. 2008). *Sporothrix inflata* s.l. is not an exception to this phenomenon. The main diagnostic feature stressed in its protologue was the production of inflated conidiophore apices (de Hoog 1974). However, this treat is also observed in other species described later, i.e. *S. brunneoviolacea*, *S. dimorphospora* (Madrid et al. 2010b),

S. globuligera K. Matsushima & Matsushima (Matsushima 1993), and in the anamorph of *Ophiostoma aurorae* X.D. Zhou & M.J. Wingf. (Zhou et al. 2006). Furthermore, apically swollen conidiophores occasionally occur in some isolates of *S. schenckii* and *S. globosa* (unpublished data). Therefore, the correct identification of a given isolate of this group relies on the use of a polyphasic study, which consists in a careful examination of its morphology, physiology and, whenever possible, DNA sequence data.

PDA proved to be a good medium for pigment production, being positive in most strains of *S. brunneoviolacea* (Madrid et al. 2010b). However, in future studies it would be interesting to test other media for this purpose, e.g. Borelli`s lactrimel agar, a medium routinely used to enhance pigment production in dermatophytes (Kaminsky 1985). Growth profiling at different temperatures is also highly informative, with human-pathogenic species such as *S. globosa* and *S. schenckii* being able to grow at temperatures ≥35 ºC, in contrast to *S. inflata*, *S. dimorphospora* and *S. brunneoviolacea.* The assimilation of key carbon sources also seems to be a valuable taxonomic tool. Marimon et al. (2007) reported that, among 35 carbon sources tested, only raffinose, ribitol and sucrose were useful to distinguish cryptic species in *S. schenckii* s.l. A recent paper by Brazilian researchers (de Oliveira et al. 2010) corroborated their results regarding the assimilation profile of *S. globosa*. That paper, which reported the first case of human infection by *S. globosa* in Brazil, highlights the usefulness and reproducibility of carbohydrate assimilation profiling for species-level identification in this difficult group of fungi. In our study, we tested the same three carbon sources in isolates of *S. inflata* s.l. Nevertheless, only raffinose seemed to be informative (positive in *S. brunneoviolacea* and *S. dimorphospora* and negative in *S. inflata* and the *S.*

inflata-like "clade 1"). However, we tested a small number of isolates and these apparent differences have to be corroborated by testing more isolates.

Most human-pathogenic fungi form a mycelial thallus in nature, but some of them have the ability to convert into yeast cells during infection, and in culture at temperatures near 37 ºC (de Hoog et al. 2000). Such morphological plasticity is known as "thermal dimorphism" and is observed in a number of ascomycetous pathogens belonging to the *Onygenales* (e.g. *Blastomyces dermatitidis* Gilchrist & W.R. Stokes, *Histoplasma capsulatum* Darling and *Paracoccidioides brasiliensis*, Untereiner et al. 2004), *Eurotiales* (e.g. *Penicillium marneffei*, Lobuglio and Taylor 1995) and *Ophiostomatales* (e.g. *Sporothrix schenckii*, *S. brasiliensis*, *S. globosa* and *S. luriei*, Marimon et al. 2007). Yeast cells express virulence factors not observed in the mycelial phase, which are involved in the adhesion to the host`s cells, in resistance to phagocytosis and in the modulation of the host`s response (Klein and Tebbets 2007). According to de Hoog et al. (2000), most fungi producing deep infections are able to grow at temperatures around 40 ºC. However, some fungi which have their maximum growth temperature around 35 ºC are able to produce cutaneous or lymphocutaneous disease (e.g. *Sporothrix globosa*, Marimon et al. 2007). In our study, *Ophiostoma bragantinum*, a soil-borne ascomycete reported as the possible teleomorph of *Sporothrix inflata* (Pfenning and Oberwinkler 1993), showed both thermotolerance and dimorphism, forming yeast cells at 35 ºC. These features suggested that the fungus might be a potential opportunist, though it has not been reported from human infections thus far. A recent paper reported the first case of human infection by another *Ophiostoma* species, *O. piceae* (Bommer et al. 2009). This fungus had the ability to produce yeast-cells during infection and in culture, suggesting that both thermal

dimorphism and the ability to infect mammals may be more common in the *Ophiostomatales* than previously suspected.

Phylogenetic analyses revealed that "true" *Sporothrix* species reside in the *Ophiostomatales* (Berbee and Taylor 1992), but *Sporothrix*-like anamorphs also occur in other groups of *Ascomycota* (Réblová and Štěpánek 2009) and even in *Basidiomycota* (Moore 1987, de Beer et al. 2006). The genus *Sporothrix* is quite heterogeneous and the phylogenetic placement of most of its members is unknown. Therefore, a phylogenetic assessment of the genus is necessary. Such study might reveal additional species complexes and possibly members with unexpected phylogenetic affinities.

5.2 Distribution of *Sporothrix globosa* and other pathogenic species of *Sporothrix*

Most of the eukaryotic microorganisms are so abundant that it is thought that their propagation is rarely (if ever) restricted by geographical barriers (Finlay 2002). This has been the general rule for most of the opportunistic fungi, such as *Aspergillus fumigatus* Fresen. and *Candida albicans* (C.P. Robin) Berkhout, which have worldwide distribution (Paoletti et al. 2005, Mokkadas et al. 2007). Nevertheless, certain fungal pathogens, especially the dimorphic fungi, are thought to occur in well-delimited endemic regions, e.g. *Coccidioides immitis* G.W. Stiles in California (Fisher et al. 2002, Laniado-Laborín 2007) and *Penicillium marneffei* Segretain in tropical Asia (Vanittanakom et al. 2006). Sporadic infections occurring outside the endemic areas are considered "imported mycoses" (Depraetere et al. 1998, Gino-Moor et al. 2003).

The high prevalence of a fungal disease in given area indicates the presence of the pathogen in the environment (Vismer and Hull 1997). However, the real distribution of a fungus may be much wider than its areas of endemicity. Recent studies provided surprising results on this subject, since fungi supposedly restricted to a given geographic area or climate have been found in unexpected regions, e.g., *Paracoccidioides*

brasiliensis (Splend) F.P. Almeida, previously thought to occur only (sub)tropical regions with moist climates in Central and South America (de Hoog et al. 2000, Kwon-Chung and Bennett 1992), was isolated once from penguin faeces collected in the Uruguayan Antarctic region (Franco et al. 2000). It seems that the "distribution of fungi" is strongly influenced with that of mycologists and the places they choose to take samples from. It should be taken into account that many of the pathogenic fungi are able to grow within a relatively wide range of temperatures, and on different substrata other than their potential human host. Therefore, a high level of adaptability can be expected for the fungal pathogens, and a real knowledge about their distribution can only be achieved by continued research.

Traditionally, sporotrichosis was thought to be caused by a single species, *S. schenckii*, with worldwide distribution (Kwon-Chung and Bennett 1992, Ghodsi et al. 2000). However, phylogenetic studies by Marimon et al. (2007, 2008a) revealed that isolates identified morphologically as *S. schenckii* encompassed at least five recognizable taxa, i.e. *S. schenckii* s.str., *S. brasiliensis*, *S. globosa*, *S. luriei*, and *S. mexicana.* Preliminary data (Marimon et al. 2007, 2008a and references therein) suggested that some of these species might have different geographical distributions, i.e. *S. brasiliensis* and *S. mexicana* were found only in Brazil and Mexico, respectively, and represented all the strains studied from such countries; *S. schenckii* s.str. was reported from Europe, North and South America, and South Africa; *S. globosa* was found in USA and in countries of Europe and Asia, and *S. luriei* was reported from South Africa, Italy, and India. In order to provide new data on the real distribution and habitat of these species our group has been investigating on this topic. Our studies expanded the known distribution of *S. globosa* to Mexico, and to Central (Guatemala) and South America (Colombia) (Madrid et al. 2009b). In addition, we recently reported that *S. mexicana*

was not restricted to Mexico but was also present in Australian soil (Madrid et al. 2009a). Interestingly, de Oliveira et al. (2010) reported a case of sporotrichosis caused by *S. globosa* in Brazil. Besides being a new report of *S. globosa*, they revealed that *S. brasiliensis* is not the only species causing human diseases in Brazil. Further research, ideally including DNA sequence analyses, is necessary to achieve a more complete understanding of the distribution of *Sporothrix schenckii* s.str. and its relatives. Furthermore, additional clinical isolates from different geographic regions should be tested to assess whether undescribed pathogenic lineages still await discovery within *S. schenckii* s.l.

5.3 Three new *Phialophora*-like fungi: *Cladorrhinum flexuosum*, *C. microsclerotigenes* and *Ramophialophora humicola*

The genus *Phialophora* was erected by Medlar in 1915 to accomodate a dematiaceous fungus causing verrucous skin lesions in man. The type species, *Phialophora verrucosa* Medlar, produces phialidic, elongate conidiogenous cells with conspicuous collarettes, and one-celled conidia aggregated in slimy masses (Medlar 1915, de Hoog 2000). The generic name means “small shallow cup bearer” in reference to the well-developed collarettes, and the species epithet referred to the appearance of the lesions caused by the fungus (Medlar 1915). Over the years, numerous species of *Phialophora* were described from soil, plant material, and clinical samples (Hughes 1958, Schol-Schwarz 1970, Jiang and Wang 2010). Currently, MycoBank (www.mycobank.org) lists 90 names in *Phialophora*.

The core of *Phialophora* resides in the *Herpotrichiellaceae*, *Chaetothyriales*, along with other clinically relevant genera such as *Cladophialophora* Borelli, *Exophiala* J.W. Carmich., and *Fonsecaea* Negroni (de Hoog et al. 1999, 2000). However, some species originally placed in *Phialophora* were proven to have different phylogenetic

affinities (Gams 2000). In recent years, some of these taxa were reallocated to other genera, e.g. *Cadophora* Lagerb. & Melin (*Helotiales*), *Harpophora* W. Gams (*Magnaporthales*), and *Pleurostomophora* D. Vijaykrishna, L. Mostert, R. Jeewon, W. Gams, K.D. Hyde & Crous (*Calosphaeriales*) (Gams 2000, Harrington and McNew 2003, Vijaykrishna et al. 2004). *Phialophora*-like anamorphs also exist in the *Sordariales*, e.g. the genera *Cladorrhinum* and *Ramophialophora*, and the unnamed anamorphs of numerous species of *Cercophora* (Udagawa and Muroi 1979, Miller et al. 2007), *Lasiosphaeria* Ces. & De Not (Schol-Schwarz 1970, Gams 2000) and *Podospora* (Mirza and Cain 1969, Lundqvist et al. 1999).

The genus *Cladorrhinum* is characterized by the production of tufted semimacronematous conidiophores, intercalary phialides with lateral openings and collarettes, and one-celled conidia (von Arx and Gams 1967, Domsch et al. 2007). The type species, *C. foecundissimum*, was described by Saccardo and Marchal (Marchal 1885) based on a fungus observed on dung in Belgium. Mouchacca and Gams (1993) added three new species, i.e. *C. bulbillosum*, *C. brunnescens* and *C. phialophoroides* and transferred *Bahupaathra samala* to *Cladorrhinum*, as *C. samala.* The unnamed anamorphs of several *Apiosordaria* spp., and those of *Cercophora samala* and *Podospora fimiseda* also belong in *Cladorrhinum* (Krug et al. 1983, Mouchacca and Gams 1993, Bell and Mahoney 1997). The anamorph of *Podospora obclavata* A.E. Bell was ascribed to *Cladorrhinum* by Bell (2006), but the protologue only shows terminal and lateral phialides delimited by a basal septum. A very similar anamorph was previously described for *Podospora austrohemisphaerica* N. Lundq., but classified in *Phialophora* s.l. (Lundqvist et al. 1999).

According to Mouchacca and Gams (1993), the regular formation of intercalary phialides by *Cladorrhinum* spp. distinguished the genus from *Phialophora*, where the

phialides were mostly discrete and delimited by a basal septum. The usefulness of this character to distinguish both genera was contradicted by later studies, which revealed that at least two members of *Phialophora* s.str. produce predominantly intercalary, *Cladorrhinum*-like conidiogenous cells, i.e. *P. reptans* de Hoog and *P. sessilis* de Hoog (de Hoog et al. 1999, Caretta et al. 2006). However, these species sometimes develop collarettes directly from conidia, indicating the ability to produce microcyclic conidiation (de Hoog et al. 1999). In *Cladorrhinum* spp., by contrast, microcyclic conidiation has never been reported. Furthermore, the conidia of several *Cladorrhinum* species do not even germinate in culture (Mouchacca and Gams 1993). *Cladorrhinum*-like phialides are also produced by *Calosphaeriophora* Réblová, L. Mostert, W. Gams & Crous, a recently proposed hyphomycete genus related to the *Calosphaeriales* (Réblová et al. 2004). In contrast to *Cladorrhinum*, *Calosphaeriophora* produces mostly unbranched conidiophores and conidia which are sometimes curved and finely ornamented (Réblová et al. 2004, Damm et al. 2008a). Considering the convergent morphology of *Phialophora*-like genera, the characterization of any new species of this group of fungi ideally should include phylogenetic analyses. The morphological delimitation of *Phialophora*-like genera should be based on a combination of characters.

Cladorrhinum has a few characters suited to distinguish species. The taxa accepted by Mouchacca and Gams (1993) can be roughly divided into two groups according to conidial shape: those producing mostly globose to dacryoid conidia (*C. brunnescens*, *C. bulbillosum*, *C. foecundissimum*, and the anamorph of *Cercophora samala*), and those with predominantly ellipsoid conidia (*C. phialophoroides* and the *Cladorrhinum* anamorphs of *Apiosordaria* spp.). The species within each group often cannot be distinguished by the size of their conidiogenous cells and conidia. However,

they are recognized by differences in other phenotypic features, such as the ability to produce microsclerotia, the proportion of intercalary vs terminal phialides, and growth profilings (Mouchacca and Gams 1993, Domsch et al. 2007). In our study, comparisons of DNA sequences and phenotypic features revealed two new *Cladorrhinum* spp., *C. flexuosum* and *C. microsclerotigenum* (Madrid et al. in press).The shape and structure of the conidiophores provided useful information to distinguish the new taxa from otherwise similar species. Morphologically, *Cladorrhinum flexuosum* and *C. foecundissimum* differed in producing flexuous vs regular conidiophores, respectively, and *C. microsclerotigenes* and *C. phialophoroides* differed in forming mostly intercalary vs mostly terminal and lateral phialides, respectively.

In their monograph of *Cladorrhinum*, Mouchacca and Gams (1993) listed several deviating isolates of *Cladorrhinum samala*, i.e. CBS 266.76, CBS 388.77, CBS 382.78 and CBS 295.79. They differed from typical isolates in producing chlamydospores or in the presence of a *Chrysosporium*-like synanamorph. Considering the little degree of morphological variation among *Cladorrhinum* species, possibly some undescribed taxa were concerned. A phylogenetic study might clarify this subject, but unfortunately the isolates are no longer available at the CBS or other recognized culture collecions.

The teleomorph genera associated with *Cladorrhinum*, i.e. *Apiosordaria* (Krug et al. 1983), *Cercophora* (Udagawa and Muroi 1979) and *Podospora* (Bell and Mahoney 1997), like other members of the *Lasiosphaeriaceae*, are distinguished mainly by differences in ascospore morphology (Lundqvist 1972, Barr 1990). However, recent phylogenetic studies proved that ascospore morphology is homoplastic in the family and hence most of its members are highly polyphyletic genera (Miller and Huhndorf 2005, Zhang et al. 2006). The *Lasiosphaeriaceae* itself is a heterogeneous group, paraphyletic

within the *Sordariales* (Huhndorf et al. 2004). Though ascospore morphology is not useful to delimit natural genera in the family, features of the ascomal wall proved to be informative in certain cases (Cai et al. 2005). Nevertheless, the delimitation of most lasiosphaeriaceous genera is still problematic and thereby other characters need to be evaluated. A phylogenetic study by Cai et al. (2006) showed that some species of *Apiosordaria*, *Cercophora* and *Podospora* with *Cladorrhinum* anamorphs clustered in a well-supported group called clade A. This clade also included taxa producing scattered intercalary phialides in culture (e.g. *Podospora pauciseta* and *P. austroamericana*), and species without known anamorphs. Members of this clade were quite divergent in aspects such as ascospore morphology, number of ascospores per ascus, peridial ornamentation, presence/absence of an ostiole, etc. Despite the heterogeneity of the telemorphs, the authors stated that the presence of a *Cladorrhinum* anamorph might be phylogenetically informative. In our studies, most *Cladorrhinum* species studied clustered in clade A. Nevertheless, *C. brunnescens* grouped outside this clade, in a clade with low support related to the *Chaetomiaceae*. Furthermore, *Cladorrhinum*-like phialides also occur in other relatively distant *Lasiosphaeriaceae*, e.g. *Ramophialophora humicola* (Madrid et al. 2010a) and *Schizothecium vesticola* (Berk. & Broome) N. Lundq. (Mirza and Cain 1969, Cai et al. 2005). At first glance, these structures seem not to provide useful information for the delimitation of natural taxa in the family.

The genus *Ramophialophora* was erected by Calduch et al. (2004) as a monotypic genus, typified by *R. vesiculosa*, which was isolated from soil. This fungus produced macronematous, dark brown, branched conidiophores ending in mostly sterile, setiform apices with terminal swellings. Its phialides were lageniform, terminal and lateral, delimited by a basal septum, and the conidia were brown, globose to dacryoid.

The new taxon proposed in our study, *R. humicola*, expands significantly the generic concept. It differs from the type species in the ability to produce some intercalary phialides, and in having non-swollen or only slightly swollen conidiophore apices and subhyaline conidia. The phylogenetic placement of *Ramophialophora* was not elucidated in the protologue. However, our analyses (Madrid et al. 2010a, 2011) placed the genus in a well supported clade of the *Lasiosphaeriaceae* previously named "clade B" in Cai et al. (2006). The presence of a phialidic genus with strongly pigmented, well-developed conidiophores in the *Lasiosphaeriaceae* is remarkable, because the *Phialophora*-like anamorphs in this family usually show micronematous conidiophores (Mirza and Cain 1969, Lundqvist et al. 1999). An exception is the anamorph of *Eosphaeria uliginosa* (Fr.) Höhn, but this fungus differs from *Ramophialophora* in having conidiophores that typically end in conidiogenous cells, and conidia forming chains, united by connectors (Petrini et al. 1984). We were not able to locate strains of this fungus for comparison, and no DNA sequence of it is available in the GenBank database.

5.4 *Fibulochlamys chilensis*, a new basidiomycetous anamorph from Chilean soil

The phylum *Basidiomycota* encompasses over 30.000 species, including numerous saprobes degrading plant debris, as well as important parasites of plants and animals (Singer 1986, Kirk et al. 2008). The sexual stages of most species in this phylum consist of macroscopic fruiting bodies rich in taxonomically informative treats. Therefore, the classification of this fungal group historically has focused mainly in the teleomorphs (Singer 1986, Ryvarden 1991, Calonge 1998). However, it is well-known that several basidiomycetes have the ability to produce mitospores (Kendrick and Watling 1979). They show a great morphological diversity, ranging from simple

arthroconidia born from undifferentiated hyphae (Walther et al. 2005) to richly ornamented propagules produced in complex fruiting bodies (Nag Raj et al. 1989). Basidiomycetous anamorphs occur in both terrestrial and aquatic environments, and many of them lack a known teleomorph (Hudson and Ingold 1960, Dyko and Sutton 1979, Kirschner et al. 2010).

The genus *Fibulochlamys* was erected by Romero and Cabral (in Romero et al. 1989) based on a fungus observed on bark and wood of *Eucalyptus* in Argentina. The type species, *F. ferruginosa*, had hyaline, clamped hyphae and produced micronematous, mostly unbranched conidiophores which gave rise to thallic, smooth- and very thick-walled conidia. The authors did not observe an associated teleomorph, and no DNA sequence data of the fungus was analyzed. Therefore, its affinities within the *Basidiomycota* remained unclear. A similar fungus was isolated from Chilean soil during the present thesis, but it differed from *F. ferruginosa* in producing mostly branched conidiophores and conidia which became strongly wrinkled in age. We tentatively placed this fungus in *Fibulochlamys*, as *F. chilensis*, based on morphology. Analysis of the ITS region and D1/D2 domains placed the taxon in the *Tricholomataceae*, *Agaricales* (Madrid et al. 2010a). No ex-type isolate of *F. ferruginosa* was preserved, and the holotype is contaminated with other fungi. Attempts to isolate the fungus from the holotype and to locate other isolates in major fungal collections were unsuccessful. Therefore, no material is available for an optimal DNA extraction. However, it is necessary to assess the phylogenetic affinities of *F. ferruginosa* to elucidate the definitive position of the genus. These analyses could be performed from an epitype. Of course, epitypification requires new material agreeing with the holotype as closely as possible in morphology, substrate and geographical origin (Hyde and Zhang 2008, Damm et al. 2009).

Fibulochlamys is morphologically similar to other basidiomycetous anamorph genera, such as *Allescheriella* Henn., *Confistulina* Stalpers, *Disporotrichum* Stalpers, *Glutinoagger* Sivanesan & Watling, *Ptychogaster* Corda, *Sporotrichum* Link and *Thermophymatospora* Udagawa, Awao & Abdullah. In contrast to *Fibulochlamys*, *Allescheriella* spp. often lack clamps and produce blastic, usually darkly pigmented conidia (Ellis 1971, Romero et al. 1989). *Confistulina* differs from *Fibulochlamys* in producing both conidia in dense racemose clusters and dendrohyphidium-like, encrusted hyphae (Stalpers and Vlug 1982). *Disporotrichum* and *Sporotrichum* are distinguished from *Fibulochlamys* by producing non-clamped conidiophores (von Arx 1971, Stalpers 1984). *Glutinoagger* differs from *Fibulochlamys* in producing conidia enveloped by a thick mucilaginous sheath (Sivanesan and Watling 1980). The conidia of *Ptychogaster* spp. have cell walls much thinner than those of *Fibulochlamys* (Stalpers 2000, Vizzini and Zotti 2008). *Thermophymatospora* is easily distinguished from *Fibulochlamys* by conidial ornamentation. While in *Fibulochlamys* the mature conidia are smooth (*F. ferruginosa*) or wrinkled (*F. chilensis*), those of *Thermophymatospora* are ornamented with both coarse warts and anastomosing ribs (Udagawa et al. 1986).

5.5 *Leptodiscella brevicatenata*, a new anamorphic member of the *Dothideomycetes* with appendaged conidia

Like many other anamorph genera (Kirk et al. 2008), the evolutionary affinities of *Leptodiscella* remained obscure for decades. In our study, DNA sequence data revealed its relationships with the *Dothideomycetes* (*Pezizomycotina*), the largest and phylogenetically most varied class of *Ascomycota* (Schoch et al. 2009). According to Kirk et al. (2008), over 19000 species belong in this class, including pathogens of plants (Crous et al. 2003, 2006) and animals (Revankar and Sutton 2010), saprobes ocurring

on dung and plant material and in soil (Eriksson and Winka 1997), and extremotolerant fungi inhabiting dry or acid environments (Selbmann et al. 2008).

Teleomorphs in the *Dothideomycetes* are amazingly diverse regarding shape and size of the ascomata (von Arx and Müller 1975, Eriksson and Winka 1997), but have in common an ascolocular development (i.e. the reproductive structures are derived from cells formed before fusion of sexually compatible mycelia) and the production of bitunicate (i.e. composed of two layers) asci which often release the ascospores by a fissitunicate mechanism (i.e. by rupture of the apex of the outer layer, allowing the inner layer to elongate and to release the ascospores forcefully) (Kirk et al. 2008, Schoch et al. 2009). Anamorphs of *Dothideomycetes* are also morphologically diverse and can be found in both terrestrial and aquatic environments (Tsui and Berbee 2006, Schoch et al. 2009). Their conidiogenous cells exhibit a wide range of morphologies and types of conidiogenesis, i.e. holoblastic (e.g. *Cladosporium* Link and *Penidiella* Crous & Braun, Crous et al. 2007a), phialidic (e.g. *Phoma* Sacc., Aveskamp et al. 2010), tretic (e.g. *Alternaria* Nees, Simmons 2007), thallic-arthric (e.g. *Neoscytalidium* Crous & Slippers, Crous et al. 2006), endogenous (*e.g. Phaeothecoidea* Crous, Crous et al. 2008), etc. Conidial morphology in the *Dothideomycetes* is also highly variable, including amero-, didymo-, phragmo-, dictyo-, scoleco-, stauro-, and helicosporous forms (Mantle et al. 2006, Tsui and Berbee 2006, Arzanlou et al. 2007, Cai et al. 2008, Frank et al. 2010). However, to the best of our knowledge, no anamorph with septate appendaged conidia similar to those of *Leptodiscella* has ever been reported in the *Dothideomycetes*.

Spore appendages are a variety of "outgrowths" (Kirk et al. 2008) of different nature which can be found in either the mitospores or meiospores (sometimes in both) of fungi. They are thought to play a role in dispersal and attachment of the spores to the substrate (Gareth Jones 2006), and are frequently observed in ecological groups such as

the *Harpellales* (fungi which inhabit the gut of some arthropods, Nelder et al. 2006), the coprophilous *Sordariales* (Lundqvist 1972), the coelomycetes (Nag Raj 1993), and the aquatic *Ascomycota* and *Basidiomycota* (Ingold 1975, Bauer et al. 2003). However, in the terrestrial hyphomycetes they are quite uncommon (Carmichael et al. 1980). According to Gareth Jones (2006), of 295 dematiaceous hyphomycete genera described by Ellis (1971), less than 1% produce appendages. Two main types of conidial appendages are recognized, i.e. i) cellular (tubular outgrows of the cell wall) and ii) extracellular (extruded and elaborated in different ways, often mucilaginous) (Nag Raj 1993, Gareth Jones 2006). The appendages of *Leptodiscella* spp. clearly belong to the former group. Some other members of the *Dothideomycetes* produce appendaged conidia, but they are predominantly extracellular, mucilaginous, e.g. *Guignardia podocarpi* Crous (Crous et al. 1996), *Macrophomina phaseolina* (Tassi) Goid. (Crous et al. 2006), *Tiarosporella tritici* B. Sutton & Marasas (Sutton and Marasas 1976) and *Dictyosporium alatum* Emden (Goh et al. 1999). Cellular appendages are produced by *Dothideomycetes* of the family *Tetraplosphaeriaceae* but, in contrast to the delicate appendages of *Leptodiscella* spp., they are multiseptate, rigid and thick-walled, appearing like setae (e.g. *Tetraplosphaeria nagasakiensis* Kaz., Tanaka & K. Hiray., Tanaka et al. 2009).

6. CONCLUSIONS

UNIVERSITAT ROVIRA I VIRGILI
MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF ANAMORPHIC FUNGI
Hugo Madrid Lorca
DL: T. 1355-2011

6. CONCLUSIONS

1. Two new anamorphic *Ophiostomatales*, *Sporothrix brunneoviolacea* and *S. dimorphospora* were proposed based on morphology, physiology and DNA sequence data.

2. The pathogenic fungus *Sporothrix globosa* was reported from Mexico, Central (Guatemala) and South America (Colombia) for the first time.

3. Thermal dimorphism was demonstrated for the first time in *Ophiostoma bragantinum*, a fungus phylogenetically related to *Sporothrix schenckii*, but not reported from human infections thus far.

4. Three new anamorphic *Sordariales* were described, i.e. *Cladorrhinum flexuosum*, *C. microsclerotigenum* and *Ramophialophora humicola*. Phylogenetic analyses placed them in the *Lasiosphaeriaceae*.

5. Molecular data indicated that three anamorph-teleomorph connections previously suggested were erroneous, i.e. those of *Cladorrhinum samala-Cercophora samala*, *Cladorrhinum foecundissimum-Apiosordaria verruculosa*, and *Sporothrix inflata-Ophiostoma bragantinum.*

6. A new basidiomycetous anamorph was discovered. It was tentatively placed in the genus *Fibulochlamys* based on morphology.

7. A new species of *Leptodiscella, L. brevicatenata*, was proposed. DNA sequence data suggested that the genus *Leptodiscella* is closely related to the order *Acrospermales* (*Dothideomycetes*).

7. REFERENCES

UNIVERSITAT ROVIRA I VIRGILI
MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF ANAMORPHIC FUNGI
Hugo Madrid Lorca
DL: T. 1355-2011

7. REFERENCES

Ahmed AOA, Desplaces N, Leonard P, Goldstein F, de Hoog S, Verbrugh H, van Belkum A. 2003. Molecular detection and identification of agents of eumycetoma. J Clin Microbiol 41:5813–5816.

Alberts AW. 1988. Discovery, biochemistry and biology of lovastatin. Am J Cardiol 62:10J–15J.

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. 2008. Molecular biology of the cell. 5th edition. New York: Garland Science.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410.

Andersen SJ. 1995. Taxonomy of *Penicillium nalgiovense* isolates from mould-fermented sausages. Antonie van Leewenhoek 68:165–171.

Arrillaga Moncrief I, Capilla J, Fernández AM, Fariñas F, Mayayo E. 2010. Diferencias en la patogenicidad del complejo de especies *Sporothrix* en un modelo animal. Patología 48:82–87.

Arzanlou M, Groenewald JZ, Gams W, Braun U, Shin H-D, Crous PW. 2007. Phylogenetic and morphotaxonomic revision of *Ramichloridium* and allied genera. Stud Mycol 58:57–93.

Aveskamp MM, de Gruyter J, Woudenberg JHC, Verkley GJM, Crous PW. 2010. Highlights of the *Didymellaceae*: a polyphasic approach to characterize *Phoma* and related pleosporalean genera. Stud Mycol 65:1–60.

Badali H, Gueidan C, Najafzadeh MJ, Bonifaz A, Gerrits van den Ende AHG, de Hoog GS. 2008. Biodiversity of the genus *Cladophialophora*. Stud Mycol 61:175–191.

Baroni TJ, Bocsusis N. 2008. A new species of *Pleurocollybia* (*Tricholomataceae*; *Agaricales*; *Basidiomycetes*) from Belize. Mycotaxon 13:353–363.

Barr ME. 1990. Prodromus to nonlichenized, pyrenomycetous members of class Hymenoascomycetes. Mycotaxon 39:43–184.

Batzer JC, Diaz Arias MM, Harrington TC, Gleason ML, Groenewald JZ, Crous PW. 2008. Four species of *Zygophiala* (Schizothyriaceae, Capnodiales) are associated with the sooty blotch and flyspeck complex on apple. Mycologia 100:246–258.

Bauer R, Begerow D, Oberwinkler F, Marvanová L. 2003. *Classicula*: the teleomorph of *Naiadella fluitans*. Mycologia 95:756–764.

Begerow D, Nilsson H, Unterseher M, Maier W. 2010. Current state and perspectives of fungal DNA barcoding and rapid identification procedures. Appl Microbiol Biotechnol 87:99–108.

Bell A. 2006. *Podospora obclavata* (*Lasiosphaeriaceae*), a new species from Australia. Australasian Mycologist 25:41–44.

Bell A, Mahoney DP. 1997. Coprophilous fungi in New Zealand. II. *Podospora* species with coriaceous perithecia. Mycologia 89:908–915.

Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H. 2010. ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiol 10:189.

Berbee ML, Taylor JW. 1992. 18S ribosomal RNA gene sequence characters place the human pathogen *Sporothrix schenckii* in the genus *Ophiostoma*. Exp Mycol 16:87–91.

Bills GF, Platas G, Peláez F, Masurekar P. 1999. Reclassification if a pneumocandin-producing anamorph, *Glarea lozoyensis* gen. et sp. nov., previously identified as *Zalerion arboricola*. Mycol Res 103:179–192.

Bommer M, Hütter ML, Stilgenbauer S, de Hoog GS, de Beer W, Wellinghausen N. 2009. Fatal *Ophiostoma piceae* infection in a patient with acute lymphoblastic leukaemia. J Med Microbiol 58:381–385.

Booth C. 1977. *Fusarium*: Laboratory guide to the identification of the major species. Kew: CAB International Mycological Institute.

Braun U, Crous PW, Dugan F, Groenewald JZ, de Hoog GS. 2003. Phylogeny and taxonomy of *Cladosporium*-like hyphomycetes, including *Davidiella* gen. nov., the teleomorph of *Cladosporium* s.str. Mycol Progress 2:3–18.

Briquet J. 1912. Règles internationals de la nomenclature botanique adoptees par le Congrès International de Botanique de Vienne 1905. Deuxième Édition mise au point

d`après les decisions du Congrès International de Botanique de Bruxelles 1910. Jena: Verlag Gustav Fischer.

Bustamante B, Lama JR, Mosquera C, Soto L. 2009. Sporotrichosis in human immunodeficiency virus infected Peruvian patients: two case reports and literature review. Infect Dis Clin Pract 17:78–83.

Buzina W, Lass-Flörl C, Kropshofer G, Freund MC, Marth E. 2005. The polypore mushroom *Irpex lacteus*, a new causative agent of fungal infections. J Clin Microbiol 43:2009–2011.

Cáceres O, Kirschner R, Piepenbring M, Schöfer H, Gené J. 2006. *Hormographiella verticillata* and an *Ozonium* stage as anamorphs of *Coprinellus domesticus*. Antonie van Leewenhoek 89:79–90.

Cai L, Jeewon R, Hyde KD. 2005. Phylogenetic evaluation and taxonomic revision of *Schizothecium* based on ribosomal DNA and protein coding genes. Fungal Diversity 19:1–21.

Cai L, Jeewon R, Hyde K. 2006. Molecular systematics of *Zopfiella* and allied genera: evidence from multi-gene sequence analyses. Mycol Res 110:359–368.

Cai L, Guo XY, Hyde KD. 2008. Morphological and molecular characterization of a new anamorphic genus *Cheirosporium*, from freshwater in China. Persoonia 20:53–58.

Calduch M, Gené J, Stchigel A, Cano J, Guarro J. 2004. *Ramophialophora*, a new anamorphic genus of *Sordariales*. Stud Mycol 50:83–88.

Calonge, F.D. 1998. Gasteromycetes I. Lycoperdales, Nidulariales, Phallales, Sclerodermatales, Tulostomatales. Flora Mycol Iberica 3:1-271.

Cappitelli F, Nosanchuk JD, Casadevall A, Toniolo L, Brusetti L, Florio S, Principi P, Borin S, Sorlini C. 2007. Synthetic consolidants attacked by melanin-producing fungi: case study of the biodeterioration of Milan (Italy) Cathedral marble treated with acrylics. Appl Environ Microbiol 73:271–277.

Caretta G, Tosi S, Piontelli E, de Hoog GS. 2006. *Phialophora sessilis*, a lithobiont fungus. Mycotaxon 95:281–284.

Carmichael JW, Kendrick BW, Conners IL, Sigler L. 1980. Genera of Hyphomycetes. Edmonton: University of Alberta Press.

Castañeda RF, Heredia G. 2000. Two new dematiaceous hyphomycetes from *Cyathea* from Mexico. Cryptog Mycol 21:221–228.

Castañeda RF, Heredia G, Reyes M, Arias RM, Decock C. 2001. A revision of the genus *Pseudospiropes* and some new taxa. Cryptog Mycol 22:1-18.

Castañeda RF, Saikawa M, Decock C, Guarro J, Calduch M. 1999. *Aenigmatospora*, an enigmatic new genus of fungi from tropical leaf litter. Cryptog Mycol 20:113–117.

Cavalier-smith T. 1993. Kingdom Protozoa and its 18 phyla. Microbiol Rev 57:953–994.

Cavalier-Smith T, Chao EEY. 2003. Phylogeny and classification of phylum Cercozoa (Protozoa). Protist 154:341–358.

Chamuris GP, Wang CJK. 1990. *Stenocephalum*, a new genus for *Rhinotrichum subalutaceum* (Hyphomycetes). Mycologia 82:528–532.

Cheewangkoon R, Groenewald JZ, Summerell BA, Hyde KD, To-anun C, Crous PW. 2009. *Myrtaceae*, a cache of fungal biodiversity. Persoonia 23:55–85.

Chen JL, Tzean SS. 1993. *Megacapitula villosa* gen. et sp. nov. from Taiwan. Mycol Res 97:347–350.

Cline E. 2005. Implications of changes to article 59 of the International Code of Botanical Nomenclature enacted at the Vienna Congress 2005. Inoculum 56:3–5.

Cooke DEL, Drenth A, Duncan JM, Wagels G, Brasier CM. 2000. A molecular phylogeny of *Phytophthora* and related Oomycetes. Fungal Genet Biol 30:17–32.

Crous PW. 2007. *Hoornsmania* Crous, gen. nov. *Hoornsmania pyrina* Crous, sp. nov. Fungal Planet 11.

Crous PW. 2009. Taxonomy and phylogeny of the genus *Mycosphaerella* and its anamorphs. Fungal Diversity 38:1–24.

Crous PW, Braun U. 2003. *Mycosphaerella* and its anamorphs: 1. Names published in *Cercospora* and *Passalora*. CBS Biodiversity Ser 1:1–571.

Crous PW, Braun U, Schubert K, Groenewald JZ. 2007a. Delimiting *Cladosporium* from morphologically similar genera. Stud Mycol 58:33–56.

Crous PW, Groenewald JZ. 2006. *Ramularia miae* Crous, sp. nov. Fungal Planet 3.

Crous PW, Groenewald JZ, Summerell B. 2007b. *Exophiala placitae* Crous & Summerell, sp. nov. Fungal Planet 17.

Crous PW, Schubert K, Braun U, de Hoog GS, Hocking AD, Shin D, Groenewald JZ. 2007c. Opportunistic, human-pathogenic species in the *Herpotrichiellaceae* are phenotypically similar to saprobic or phytopathogenic species in the *Venturiaceae*. Stud Mycol 58:185–217.

Crous PW, Seifert KA, Castañeda Ruiz RF. 1996. Microfungi associated with *Podocarpus* leaf litter in South Africa. S Afr J Bot 62:89–98.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Philips AJL, Alves A, Burgess T, Barber P, Groenewald JZ. 2006. Phylogenetic lineages in the *Botryosphaeriaceae*. Stud Mycol 55:235–253.

Crous PW, Summerell BA, Carnegie AJ, Mohammed C, Wingfield MJ, Himaman W, Groenewald JZ. 2007d. Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. III. Fungal Diversity 26:143–185.

Crous PW, Verkley GJM, Groenewald JZ, Samson RA (eds.). 2009b. Fungal biodiversity. CBS laboratory manual series 1. Utrecht: Centraalbureau voor Schimmelcultures.

Cyert MS. 2001. Genetic analysis of calmodulin and its targets in *Saccharomyces cerevisiae*. Annu Rev Genet 35:647–672.

Dadachova E, Casadevall A. 2008. Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. Curr Opin Microbiol 11:525–531.

Damm U, Crous PW, Fourie PH. 2008a. A fissitunicate ascus mechanism in the Calosphaeriaceae, and novel species of *Jattaea* and *Calosphaeria* on *Prunus* wood. Persoonia 20:39–52.

Damm U, Mostert L, Crous PW, Fourie PH. 2008b. Novel *Phaeoacremonium* species associated with necrotic wood of *Prunus* species. Persoonia 20:87–102.

Damm U, Woudenberg JHC, Cannon PF, Crous PW. 2009. *Colletotrichum* species with curved conidia from herbaceous hosts. Fungal Diversity 39:45–87.

Davey ML, Currah RS. 2007. A new species of *Cladophialophora* from boreal and montane bryophytes. Mycol Res 111:106–116.

D`Annibale, Rosetto F, Leonardi V, Federici F, Petruccioli. 2006. Role of authochthonous filamentous fungi in bioremediation of a soil historically contaminated with aromatic hydrocarbons. Appl Environ Microbiol 72:28–36.

de Beer ZW, Begerow D, Bauer R, Pegg GS, Crous PW, Wingfield MJ. 2006. Phylogeny of the *Quambalariaceae* fam. nov., including important *Eucalyptus* pathogens in South Africa and Australia. Stud Mycol 55:289–298.

de Beer ZW, Harrington TC, Vismer H, Wingfield BD, Wingfield MJ. 2003. Phylogeny of the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 95:434–441.

De Carvalho MJ, Amorim Jesuino RS, Daher BS, Silva-Pereira I, de Freitas SM, Soares CMA, Felipe MS. 2003. Functional and genetic characterization of calmodulin from the dimorphic and pathogenic fungus *Paracoccidioides brasiliensis*. Fungal Genet Biol 39:204–210.

de Hoog GS. 1974. The genera *Blastobotrys*, *Sporothrix*, *Calcarisporium* and *Calcarisporiella* gen. nov. Stud Mycol 7:1–84.

de Hoog GS. 1985. Taxonomy of the *Dactylaria* complex. Stud Mycol 26:1–124.

de Hoog GS, Guarro J, Gené J, Figueras MJ. 2000. Atlas of clinical fungi. 2nd Edition. Utrecht/Reus: Centraalbureau voor Schimmelcultures/Universitat Rovira i Virgili.

de Hoog GS, Guého E, Masclaux F, Gerrits van den Ende AHG, Kwon-Chung KJ, McGinnis MR. 1995. Nutritional physiology and taxonomy of human-pathogenic *Cladosporium-Xylohypha* species. J Med Vet Mycol 33:339–347.

de Hoog GS, Hermanides-Nijhof EJ. 1977. The black yeasts and allied Hyphomycetes. Stud Mycol 15:1–222.

de Hoog GS, Weenink XO, Gerrits van den Ende AHG. 1999. Taxonomy of the *Phialophora verrucosa* complex with the description of two new species. Stud Mycol 43:107–122.

de Hoog GS, Zeng JS, Harrak MJ, Sutton DA. 2006. *Exophiala xenobiotica* sp. nov., an opportunistic black yeast inhabiting environments rich in hydrocarbons. Antonie van Leewenhoek 90:257–268.

de Leo F, Urzi C, de Hoog. 1999. Two *Coniosporium* species from rock surfaces. Stud Mycol 43:70–79.

de Leo F, Urzi C, de Hoog GS. 2003. A new meristematic fungus, *Pseudotaeniolina globosa*. Antonie van Leewenhoek 83:351–360.

de Meyer EM, de Beer ZW, Summerbell RC, Moharram AM, de Hoog GS, Vismer HF, Wingfield MJ. 2008. Taxonomy and phylogeny of new wood- and soil-inhabiting species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 100:647–661.

de Oliveira MM, de Almeida-Paes R, de Medeiros Muniz M, de Lima Barros MB, Galhardo MC, Zancope-Oliveira RM. 2010. Sporotrichosis caused by *Sporothrix globosa* in Rio de Janeiro, Brazil: case report. Mycopathologia 169:359–363.

Decock C, Huret S, Charue P. 2006. Anamorphic fungi from French Guyana: two undescribed *Gliocephalotrichum* species. Mycologia 98:488–498.

Depraetere K, Colebunders R, Leven M, de Droogh E, Pelgrom Y, Hauben E, van Marck E, Devroey C. 1998. Two imported cases of *Penicillium marneffei* infection in Belgium. Acta Clin Belg 53:255–258.

Diedereich P, Lawrey JD. 2007. New lichenicolous, muscicolous, corticolous and lignicolous taxa of *Burgoa* s.l. and *Marchandiomyces* s.l. (anamorphic Basidiomycota), a new genus for *Omphalina foliacea*, and a catalogue and a key to the non-lichenized, bulbilliferous basidiomycetes. Mycol Progress 6:61–80.

Domsch KH, Gams W, Anderson TH. 2007. Compendium of soil fungi. 2nd ed. Eching: IHW Verlag.

Drechsler C. 1960. A clamp-bearing fungus using stalked adhesive young chlamydospores in capturing amoebae. Sydowia 14:246–257.

Dreyfuss M, Härri E, Hofmann H, Kobel H, Pache W, Tsecherter H. 1976. Cyclosporin A and C: new metabolites from *Trichoderma polysporum* (Link ex Pers.) Rifai. Eur J Appl Microbiol 3:125–133.

Duddington CL. 1951. The ecology of predacious fungi: I. Preliminary survey. Trans Br Mycol Soc 34:260–409.

Dyko BJ, Sutton BC. 1979. Two new and unusual deuteromycetes. Trans Br Mycol Soc 72:411–417.

Edgar RC. 2004. Muscle: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797.

Ellis MB. 1971. Dematiaceous Hyphomycetes. Kew: Commonwealth Mycological Institute.

Eriksson OE, Winka K. 1997. Supraordinal taxa of *Ascomycota*. Myconet 1:1–16.

Ezzi MI, Lynch JM. 2005. Biodegradation of cyanide by *Trichoderma* spp. and *Fusarium* spp. Enzyme Microb Technol 36:849–854.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Ferrer C, Pérez-Santonja JJ, Rodríguez AE, Colom MF, Gené J, Alio JL, Verkley GJM, Guarro J. 2009. New *Pyrenochaeta* species causing keratitis. J Clin Microbiol 47:1596–1598.

Finlay BJ. 2002. Global dispersal of free-living microbial eukaryote species. Science 296:1061–1063.

Fisher MC, Koenig GL, White TJ, Taylor JW. 2002. Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the non-California population of *Coccidioides immitis*. Mycologia 94:73–84.

Fleming A. 1929. On the antibacterial action of cultures of a *Penicillium* with special reference to their use in the isolation of *B. influenzae*. Br J Exp Pathol 70: 697–704.

Fonseca OJM, Lacaz CS. 1971. Estudo de culturas isoladas de blastomicose queloidiforme (doença de Jorge Lobo). Denominação de seu agente etiológico. Rev Inst Med Trop S Paulo 13:225–251.

Franco M, Bagagli E, Scapolio S, da Silva Lacaz C. 2000. A critical analysis of isolation of *Paracoccidioides brasiliensis* from soil. Med Mycol 38:185–191.

Frank J, Crous PW, Groenewald JZ, Oertel B, Hyde KD, Phengsintham P, Schroers HJ. 2010. *Microcyclospora* and *Microcyclosporella*: novel genera accommodating epiphytic fungi causing sooty blotch on apple. Persoonia 24:93–105.

Frisvad JC, Samson RA. 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate *Penicillia* and their mycotoxins. Stud Mycol 49:1–174.

Gams W. 1971. *Cephalosporium*-artige schimmelpilze (Hyphomycetes). Stuttgart: Gustav Fischer Verlag.

Gams W. 2005. Towards a single scientific name for species of fungi: a rebuttal. Inoculum 56:1–3.

Gams W. 2000. *Phialophora* and some similar morphologically little-differentiated anamorphs of divergent ascomycetes. Stud Mycol 45:187–199.

García D. 2005. Taxonomía de los hongos ascomicetos del suelo (Ph. D. thesis). Reus: Universitat Rovira i Virgili.

García Vargas A, Mayorga J, Soto Ortiz A, Barba Gómez JF. 2008. Esporotrichosis en niños. Estudio de 133 casos en el Instituto Dermatológico de Jalisco "Dr. José Barba Rubio". Med Cutan Iber Lat Am 36:18–22.

Gareth Jones EB. 2006. Form and function of fungal spore appendages. Mycoscience 47:167–183.

Gea FJ. 1993. Incidencia de *Verticillium fungicola* (Preuss) Hassebrauk en los cultivos de champiñón de Castilla-La Mancha. Bol San Veg. Plagas 19: 369–377.

Gené J. 1994. Estudio sobre la taxonomía y la actividad antimicrobiana de los deuteromicetos de origen coprófilo y geófilo de España. Ph. D. thesis. Reus: Universitat Rovira i Virgili.

Ghodsi SZ, Shams S, Naraghi Z, Daneshpazhooh M, Akhyani M, Arad S, Ataii L. 2000. An unusual case of cutaneous sporotrichosis and its response to weekly fluconazole. Mycoses 43:75–77.

Gilgado F. 2007. Caracterización molecular y fenotípica del género *Pseudallescheria* y géneros afines. Ph. D. thesis. Reus: Universitat Rovira i Virgili.

Gilgado F, Cano J, Gené J, Guarro J. 2005. Molecular phylogeny of the *Pseudallescheria boydii* species complex: proposal of two new species. J Clin Microbiol 43:4930–4942.

Gino-Moor S, Paz A, Pick N, Potasman I. 2003. Pulmonary coccidioidomycosis acquired during travel-a diagnostic and therapeutic challenge. Travel Med Infect Dis 1:53–57.

Glass N, Donaldson G. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330.

Gochenaur SE. 1981. *Cyrenella elegans* gen. et sp. nov. a dikaryotic anamorph. Mycotaxon 13:267–277.

Goh TK, Hyde KD, Ho WH, Yanna. 1999. A revision of the genus *Dictyosporium*, with descriptions of three new species. Fungal Diversity 2:65–100.

Goos RD. 1970. A new genus of the Hyphomycetes from Hawaii. Mycologia 62:171–175.

Gottlieb AM, Lichtwardt RW. 2001. Molecular variation within and among species of Harpellales. Mycologia 93:66–81.

Gräser Y, de Hoog GS, Summerbell RC. 2006. Dermatophytes: recognizing species of clonal fungi. Med Mycol 44:199–209.

Gräser Y, Kühnisch J, Presber W. 1999. Molecular markers reveal exclusively clonal reproduction in *Trichophyton rubrum*. J Clin Microbiol 37:3713–3717.

Guarro J, del Palacio A, Gené J, Cano J, Gómez Gonzalez C. 2009. A case of colonization of a prothethic mitral valve by *Acremonium strictum*. Rev Iberoam Micol 26:146–148.

Guarro J, Gené J, Stchigel AM. 1999. Developments in fungal taxonomy. Clin Microbiol Rev 12:454–500.

Guarro J, Höfling-Lima AL, Gené J, de Freitas D, Godoy P, Zorat-Yu ML, Zaror L, Fischman O. 2002. Corneal ulcer caused by the new fungal species *Sarcopodium oculorum*. J Clin Microbiol 40:3071–3075.

Guo LD, Hyde KD, Liew ECY. 1998. A method to promote sporulation in palm endophytic fungi. Fungal Diversity 1:109–113.

Hambleton S, Sigler L. 2005. *Meliniomyces*, a new anamorph genus for root-associated fungi with phylogenetic affinities to *Rhizoscyphus ericae* (≡ *Hymenoscyphus ericae*), *Leotiomycetes*. Stud Mycol 53:1–27.

Harrington TC, McNew DL. 2003. Phylogenetic analysis places the *Phialophora*-like anamorph genus *Cadophora* in the Helotiales. Mycotaxon 87:141–151.

Hawksworth DL. 1979. The lichenicolous hyphomycetes. Bull Brit Mus Nat Hist, Bot 6:183–300.

Hawksworth DL. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycol Res 95:641–655.

Hawksworth DL. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol Res 105:1422–1432.

Hawksworth DL. 2010. Naming *Aspergillus* species: progress towards one name for each species. Med Mycol (in press) DOI: 10.3109/13693786.2010.504753.

Hawksworth DL, Rosmann AY. 1997. Where are all the undescribed fungi? Phytopathology 87:888–891.

Hawksworth DL, Sutton BC, Ainsworth GC. 1983. Ainsworth & Bisby`s Dictionary of the Fungi. 7th ed. Kew: Commonwealth Mycological Institute.

Hektoen L, Perkins CF. 1900. Refractory subcutaneous abscesses caused by *Sporothrix schenckii*. A new pathogenic fungus. J Exp Med 5:77–89.

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin D, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Koljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadilowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schüßler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N. 2007. A higher-level phylogenetic classification of the *Fungi*. Mycol Res 111:509–547.

Ho YW, Abdullah N, Jalaludin S. 2000. The diversity and taxonomy of anaerobic gut fungi. Fungal Diversity 4:37–51.

Holmes KA, Schroers HJ, Thomas SE, Evans HC, Samuels GJ. 2004. Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin of South America. Mycol Progress 3:199–210.

Honegger R. 1991. Functional aspects of the lichen symbiosis. Annu Rev Plant Physiol Plant Mol Biol 42:553–578.

Hong SB, Go SJ, Shin HD, Frisvad JC, Samson RA. 2005. Polyphasic taxonomy of *Aspergillus fumigatus* and related species. Mycologia 97:1316–1329.

Hudson HJ, Ingold CT. 1960. Aquatic hyphomycetes from Jamaica. Trans Brit Mycol Soc 43:469–478.

Hughes SJ. 1953. Conidiophores, conidia, and classification. Can J Bot 31:577–659.

Hughes SJ. 1958. Revisiones Hyphomycetum aliquot cum appendice de nominibus rejiciendis. Can J Bot 36:727–836.

Hughes SJ. 1966. New Zealand fungi. 7. *Capnocybe* and *Capnophialophora*, new form genera of sooty moulds. N Z J Bot 4:333–353.

Hughes SJ. 1970. New Zealand fungi. *Antennaria*, *Antennularia*, *Antennatula*, *Hyphosoma*, *Hormisciella*, and *Capnobotrys* gen. nov. N. Z. J. Bot 8:153–209.

Hugues SJ, Sugiyama J. 1972. New Zealand Fungi 18. *Xylohypha* (Fr.) Mason N. Z. J. Bot 10:447–460.

Huhndorf SM, Miller AN, Fernandez FA. 2004. Molecular systematics of the Sordariales: the order and the family Lasiosphaeriaceae redefined. Mycologia 96:368–387.

Hyde KD, Zhang Y. 2008. Epitypification: should we epitypify? J Zhejiang Univ Sci B 9:842–846.

Ingold CT. 1975. Guide to aquatic hyphomycetes. Ambleside: Freshwater Biological Association.

Ishizaki H, Kwasaki M, Aoki M, Wu S, Lin J, Kim JA, Won YH, Calvo CR. 2004. Mitochondrial DNA analysis of *Sporothrix schenckii* from China, Korea and Spain. Nippon Ishinkin Gakkai Zasshi 45:23–25.

Iwen PC, Hinrichs SH, Rupp ME. 2002. Utilization of the internal transcribed spacer regions as molecular targets to detect and identify human pathogens. Med Mycol 40:87–109.

James TY, Letcher PM, Longcore JE, Mozley-Standridge SE, Porter D, Powell MJ, Griffith GW, Vilgalys R. 2006. A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia 98:860–871.

Jian YL, Wang Y. 2010. Two new species of *Phialophora* from Guizhou Province. Mycosystema 29:783–785.

Kaminsky GW. 1985. The routine use of modified Borelli`s lactrimel agar (MBLA). Mycopathologia 91:57–59.

Keeling PJ, Luker MA, Palmer JD. 2000. Evidence from beta-tubulin phylogeny that Microsporidia evolved from within the Fungi. Mol Biol Evol 17:23–31.

Kendrick B, Watling R. 1979. Mitospores in basidiomycetes. In: Kendrick B, ed. The Whole Fungus. Ottawa: National Museum of Natural Sciences. p 473–545.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Ainsworth & Bisby`s Dictionary of the Fungi. 10th ed. Wallingford: CAB International.

Kirschner R, Chen CJ. 2004a. *Helicomyxa everhartioides*, a new helicosporous sporodochial hyphomycete from Taiwan with relationships to the *Hyaloriaceae* (*Auriculariales*, *Basidiomycota*). Stud Mycol 50:337–342.

Kirschner R, Chen CJ. 2004b. Two new species of the staurosporous hyphomycetous genera *Ceratosporium* and *Diplocladiella* from Taiwan. Mycologia 96:917–924.

Kirschner R, Chen CJ. 2007. Foliicolous hyphomycetes from Taiwan. Fungal Diversity 26:219–239.

Kirschner R, Hou CL, Chen CJ. 2009. Co-ocurrence of *Pseudocercospora* species and rhytismatalean ascomycetes on maple and camellia in Taiwan. Mycol Progress 8:1–8.

Kirschner R, Yang ZL, Zhao Q, Feng B. 2010. *Ovipoculum album*, a new anamorph with gelatinous cupulate bulbilliferous conidiomata from China and with affinities to the Auriculariales (Basidiomycota). Fungal Diversity 43:55–65.

Klein BS, Tebbets B. 2007. Dimorphism and virulence in fungi. Curr Opin Microbiol 10:314–319.

Kornerup A, Wanscher JH (1978) Methuen handbook of colour. 3rd ed. Eyre Methuen, London.

Koufopanou V, Burt A, Szaro T, Taylor JW. 2001. Gene genealogies, cryptic species, and molecular evolution in the human pathogen *Coccidioides immitis* and relatives (Ascomycota, Onygenales). Mol Biol Evol 18:1246–1258.

Krug JC, Udagawa S, Jeng RS. 1983. The genus *Apiosordaria*. Mycotaxon 17:533–549.

Kumar S, Tamura K, Nei M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163.

Kurzai O, Keith P, de Hoog GS, Abele-Horn M, Frosch M. 2003. Postmortem isolation of *Pseudotaeniolina globosa* from a patient with aortic aneurysm. Mycoses 46:141–144.

Kurtzman CP, Fell JW. 1998. The yeasts, a taxonomic study. 4th Edition. New York: Elsevier.

Kuthubutheen AJ. 1987. Two new species of *Dictyochaeta* from Malaysia. Trans Br Mycol Soc 89:353–358.

Kwon-Chung JE, Bennett JE. 1992. Medical Mycology. 2nd edition. Philadelphia: Lea & Febiger.

Laniado-Laborín R. 2007. Coccidioidomycosis and other endemic mycoses in Mexico. Rev Iberoam Micol 24:249–258.

Lewis JA, Larkin RP. 1998. Formulation of the biocontrol fungus *Cladorrhinum foecundissimum* to reduce the damping-off disease caused by *Rhizoctonia solani* and *Pythium ultimum*. Biol Control 12:182–190.

Li DM, Li RY, de Hoog GS, Wang YX, Wang DL. 2009. *Exophiala asiatica*, a new species from a fatal case in China. Med Mycol 47:101–109.

Lin J, Kawasaki M, Aoki M, Ishizaki H, You G, Li R. 1999. Mitochondrial DNA analysis of *Sporothrix schenckii* clinical isolates from China. Mycopathologia 148:69–72.

Lo HC, Tu ST, Lin KC, Lin SC. 2004. The anti-hyperglycemic activity of the fruiting body of *Cordyceps* in diabetic rats induced by nicotinamide and streptozotocin. Life Sci 74:2897–2908.

Lobuglio KF, Taylor JW. 1995. Phylogeny and PCR identification of the human pathogenic fungus *Penicillium marneffei*. J Clin Microbiol 33:85–89.

Lombard L, Crous PW, Wingfield BD, Wingfield MJ. 2010. Multigene phylogeny and mating tests reveal three cryptic species related to *Calonectria pauciramosa*. Stud Mycol 66:15–30.

Lundqvist N. 1972. Nordic Sordariaceae s. lat. Symb Bot Upsal 20:1–374.

Lundqvist N, Mahoney DP, Bell A, Lorenzo LE. 1999. *Podospora austrohemisphaerica*, a new heterothallic ascomycete from dung. Mycologia 91:405–415.

Madrid H, Cano J, Gené J, Arthur I, Guarro J. 2009a. A putative new species in the *Sporothrix schenckii* complex and new records of *Sporothrix* species from Australia. Abstract volume XVII Congress of the International Society for Human and Animal Mycology. Tokyo.

Madrid H, Cano J, Gené J, Bonifaz A, Toriello C, Guarro J. 2009b. *Sporothrix globosa*, a pathogenic fungus with widespread geographical distribution. Rev Iberoam Micol 26:218–222.

Madrid H, Cano J, Stchigel A, Gené J, Guarro J. 2010a. *Ramophialophora humicola* and *Fibulochlamys chilensis*, two new microfungi from soil. Mycologia 102:605–612.

Madrid H, Gené J, Cano J, Silvera C, Guarro J. 2010b. *Sporothrix brunneoviolacea* and *Sporothrix dimorphospora*, two new members of the *Ophiostoma stenoceras*-*Sporothrix schenckii* complex. Mycologia 102:1193–1203.

Madrid H, Gené J, Cano J, Guarro J. 2011. Two new species of *Cladorrhinum*. Mycologia (in press, DOI: 10.3852/10-150).

Magnuson JK, Lasure LL. 2004. Organic acid production by filamentous fungi. In: Tkacz JS, Lange L, eds. Advances in fungal biotechnology for industry, agriculture, and medicine. New York: Kluwer Academic/Plenum Publishers. p. 307–340.

Magyar D, Shoemaker RA, Bobvos J, Crous PW, Groenewald JZ. 2010. *Pyrigemmula*, a novel hyphomycete genus on grapevine and tree bark. Mycol Progress (in press, DOI: 10.1007/s11557-010-0703-4).

Maicas S, Adam AC, Polaina J. 2000. The ribosomal DNA of the zygomycete *Mucor miehei*. Curr Genet 37:412–419.

Mansuetus ASB, Odvody GN, Frederiksen RA, Leslie JF. 1997. Biological species in the *Gibberella fujikuroi* species complex (*Fusarium* section *Liseola*) recovered from sorghum in Tanzania. Mycol Res 101:815–820.

Mantle PG, Hawksworth DL, Pazoutova S, Collinson LM, Rassing BR. 2006. *Amorosia littoralis* gen. sp. nov., a new genus and species name for the scorpinone and caffeine-producing hyphomycete from the littoral zone in The Bahamas. Mycol Res 110:1371–1378.

Marasas WFO. 1995. Fumonisins: their implications for human and animal health. Nat Toxins 3:193–198.

Marchal E. 1885. Champignons coprophiles de la Belgique. Bull Soc R Bot Belg 24:57–77.

Maresca M, Fantini J. 2010. Some food-associated mycotoxins as potential risk factors in humans predisposed to chronic intestinal inflammatory diseases. Toxicon (in press, DOI: 10.1016/j.toxicon.2010.04.016).

Marimon R. 2007. Filogènia molecular i caracterització fenotípica del complex d`espècies d`*Sporothrix schenckii*. Ph. D. thesis. Reus: Universitat Rovira i Virgili.

Marimon R, Cano J, Gené J, Sutton DA, Kawasaki M, Guarro J. 2007. *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. J Clin Microbiol 45:3198–3206.

Marimon R, Gené J, Cano J, Guarro J. 2008a. *Sporothrix luriei*: a rare fungus from clinical origin. Med Mycol 46:621–625.

Marimon R, Gené J, Cano J, Trilles L, Dos Santos Lázera, Guarro J. 2006. Molecular phylogeny of *Sporothrix schenckii*. J Clin Microbiol 44:3251–3256.

Marimon R, Serena C, Gené J, Cano J, Guarro J. 2008b. *In vitro* antifungal susceptibilities of five species of *Sporothrix*. Antimicrob Agents Chemother 52:732–734.

Marvanová L. 1980. New or noteworthy aquatic hyphomycetes. *Clavatospora*, *Heliscella*, *Nawawia*, and *Heliscina*. Trans Br Mycol Soc 75:221–231.

Marvanová L, Stalpers JA. 1987. The genus *Taeniospora* and its teleomorphs. Trans Br Mycol Soc 89:489–498.

Matsushima T. 1993. Matsushima mycological memoirs 8. Kobe: published by the author.

Mayorga R, Cáceres A, Toriello C, Gutiérrez G, Álvarez O, Ramírez ME, Mariat F. 1979. Investigación de una zona endémica de esporotricosis en la región de Ayarza, Guatemala. Bol Oficina Sanit Panam 87:20–34.

McInnes SJ. 2003. A predatory fungus (Hyphomycetes: *Lecophagus*) attacking Rotifera and Tardigrada in maritime Antarctic lakes. Polar Biol 26:79–82.

McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, Wiersema JH, Turland NJ (eds.). 2006. International Code of Botanical Nomenclature (Vienna Code) adopted by the Seventeenth International Botanical Congress, 2005. Regnum Vegetabile 146:i–xviii, 1–568.

Medlar EM. 1905. A new fungus, *Phialophora verrucosa*, pathogenic to man. Mycologia 7:200–203.

Mercado-Sierra A, Guarro J, Heredia G. 2005. The hyphomycete genus *Piricauda*, with the description of a new species. Mycol Res 109:723–728.

Mercado-Sierra A, Holubová-Jechová V, Mena-Portales J. 1997. Hifomicetes demaciáceos de Cuba. Enteroblásticos. Torino: Museo Regionale di Scienze Naturali. Monografie XXIII.

Meyer SLF, Carta LK, Rehner SA. 2005. Morphological variability and molecular phylogeny of the nematophagous fungus *Monacrosporium drechsleri*. Mycologia 97:405–415.

Miller AN, Huhndorf SM. 2004. A natural classification of *Lasiosphaeria* based on nuclear LSU rDNA sequences. Mycol Res 108:26–34.

Miller AN, Huhndorf SM. 2005. Multi-gene phylogenies indicate ascomal wall morphology is a better predictor of phylogenetic relationships than ascospore morphology in the Sordariales (Ascomycota, Fungi). Mol Phylogenet Evol 35:60–75.

Miller AN, Mugambi GK, Huhndorf SM. 2007. *Cercophora rubrotuberculata* sp. nov., a new pyrenomycete from the Great Smoky Mountains National Park. Mycologia 99:488–491.

Mirza JH, Cain RF. 1969. Revision of the genus *Podospora*. Can J Bot 47:1999–2048.

Mo MH, Huang XW, Zhou W, Huang Y, Hao YE, Zhang KQ. 2005. *Arthrobotrys yunnanensis* sp. nov., the fourth anamorph of *Orbilia auricolor*. Fungal Diversity 18:107–115.

Mokkadas EM, Al-Sweih NA, Khan ZU. 2007. Species distribution and antifungal susceptibility of *Candida* bloodstream isolates in Kuwait: a 10-year study. J Med Microbiol 56:255–259.

Moore RT. 1987. Micromorphology of yeasts and yeast-like fungi and its taxonomic implications. Stud Mycol 30:203–226.

Mouchacca J, Gams W. 1993. The hyphomycete genus *Cladorrhinum* and its teleomorph connections. Mycotaxon 48:415–440.

Moustafa AF, Abdul-Wahid OA. 1989. Setosporella, a new genus of Hyphomycetes from Egyptian soils. Mycol Res 93:227–229.

Msiska Z, Morton JB. 2009. Isolation and sequence analysis of a β-tubulin gene from arbuscular mycorrhizal fungi. Mycorrhiza 19:501–513.

Mullis KB. 1990. The unusual origin of the polymerase chain reaction. Sci Am 262:55–65.

Muntañola-Cvetkovic M, Hoyo P, Gómez-Bolea A. 2001. *Penicillium aureocephalum* anam sp nov. Fungal Diversity 7:71–79.

Nag Raj TR. 1993. Coelomycetous anamorphs with appendage-bearing conidia. Ontario: Mycologue Publications.

Nag Raj TR, Holubová-Jechová V, Castañeda Ruiz R. 1989. Genera coelomycetum. XXVII. *Pycnovellomyces* redescribed. Can J Bot 67:3386–3390.

Nelder MP, Beard CE, Adler PH, Kim S-K, McCreadie JW. 2006. *Harpellales* (*Trichomycota*: *Trichomycetes*) associated with black flies (*Diptera*: *Simuliidae*): world review and synthesis of their ecology and taxonomy. Fungal Diversity 22:121–169.

O`Donnell K. 1993. *Fusarium* and its near relatives. In: Reynolds R and Taylor JW (eds.) The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. CAB International, Wallingford, pp 225–233.

O`Donnell K. 2000. Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. Mycologia 92:919–938.

O`Donnell K, Niremberg HI, Aoki T, Cigelnik E. 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. Mycoscience 41:61–78.

Olivier DL. 1978. *Retiarius* gen. nov.: phyllosphere fungi which capture wind-borne pollen grains. Trans Br Mycol Soc 71:193–201.

Olivier DL. 1983. Phyllosphere fungi which capture wind-borne pollen grains. II. *Hexacladium corynephorum* gen et sp nov. Trans Br Mycol Soc 80: 237–245.

Orbach MJ, Porro EB, Yanofsky C. 1986. Cloning and characterization of the gene for β-tubulin from a benomyl-resistant mutant of *Neurospora crassa* and its use as a dominant selectable marker. Mol Cell Biol 6:2452–2461.

Palma MA, Valenzuela E, Parra P, Gutierrez M, Silva LT. 2005. *Cerrena unicolor* (Bull.) Murr (Basidiomycota) aislado de micangio de *Tremex fuscicornis* Fabr. (Hymenoptero, Siricidae) asociado a decaimiento y pudrición del álamo (*Populus* sp.) en Chile. Bol Micol 202:57–61.

Pandey A, Selvakumar P, Soccol CR, Nigam P. 1999. Solid-state fermentation for the production of industrial enzymes. Curr Sci 77:149–162.

Paoletti M, Rydholm C, Schwier EU, Anderson MJ, Szakacs G, Lutzoni F, Debeaupuis JP, Latgé JP, Denning DW, Dyer PS. 2005. Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. Curr Biol 15:1242–1248.

Papendorf MC (1967) *Leptodiscus africanus*, sp. nov. Trans Br Mycol Soc 50:687–690.

Perdomo H, Sutton DA, García D, Fothergill AW, Cano J, Gené J, Summerbell RC, Rinaldi MG, Guarro J. 2011. Spectrum of clinically relevant *Acremonium* species in the United States. J Clin Microbiol 49:243–256.

Petrini LE, Petrini O, Candoussau F. 1984. Anamorphs of ascomycetes: the *Phialophora*-like state of *Eosphaeria uliginosa* (syn. *Herminia dichroospora*) Trans Br Mycol Soc 82:554–556.

Pfenning L, Oberwinkler F. 1993. *Ophiostoma bragantinum* n. sp., a possible teleomorph of *Sporothrix inflata*, found in Brazil. Mycotaxon 46:381–385.

Philippi R. 1860. Viaje al desierto de Atacama, hecho de orden del gobierno de Chile en el verano 1853-54. Halle: Librería de Eduardo Anton.

Pinzari F, Pasquariello G, De Mico A. 2006. Biodeterioration of paper: a SEM study of fungal spoilage reproduced under controlled conditions. Macromol Symp 238:57–66.

Pitt JI, Samson RA. 2007. Nomenclatural considerations in naming species of *Aspergillus* and its teleomorphs. Stud Mycol 59:67–70.

Platas G, Ruibal C, Collado J. 2004. Size and sequence heterogeneity in the ITS1 of *Xylaria hypoxylon* isolates. Mycol Res 108:71–75.

Pounder JI, Simmon KE, Barton CA, Hohmann SL, Brandt ME, Petti CA. 2007. Discovering potential pathogens among fungi identified as nonsporulating molds. J Clin Microbiol 45:568–571.

Quilambo OA. 2003. The vesicular-arbuscular mycorrhizal symbiosis. Afr J Biotechnol 2:539–546.

Ramaley AW. 1999. Three species of *Microthyrium* from Nolina. Mycotaxon 70:7–16.

Rawla GS. 1973. *Gloeocercospora* and *Ramulispora* in India. Trans Br Mycol Soc 60:283–292.

Réblová M, Mostert L, Gams W, Crous PW. 2004. New genera in the *Calosphaeriales*: *Togniniella* and its anamorph *Phaeocrella*, and *Calosphaeriophora* as anamorph of *Calosphaeria*. Stud Mycol 50:533–550.

Réblová M, Štěpánek V. 2009. New fungal genera, *Tectonidula* gen. nov. for *Calosphaeria*-like fungi with holoblastic-denticulate conidiogenesis and *Natantiella* gen. nov. for three species segregated from *Ceratostomella*. Myc Res 113:991–1002.

Revankar SG, Sutton DA. 2010. Melanized fungi in human disease. Clin Microbiol Rev 23:884–928.

Rodrígues Barbosa F, Pascholati Gusmão LF, Castañeda Ruiz RF, Oliveira Marques MF, Costa Maia L. 2007. Conidial fungi from the semi-arid Caatinga biome in Brazil.

New species *Deightoniella rugosa* and *Diplocladiella cornitumida* with new records for the neotropics. Mycotaxon 102:39–49.

Rodríguez K, Stchigel A, Guarro J. 2002. Three new species of *Chaetomium* from soil. Mycologia 94:116–126.

Rodríguez K, Stchigel AM, Cano JF, Guarro J. 2004. A new species of *Achaetomium* from Indian soil. Stud Mycol 50:77–82.

Roeijmans HJ, de Hoog GS, Tan CS, Figge MJ. 1997. Molecular taxonomy and GC/MS of metabolites of *Scytalidium hyalinum* and *Nattrassia mangiferae* (*Hendersonula toruloidea*). J Med Vet Mycol 35:181–188.

Romero AI, Cabral D, Lopez SE. 1989. Studies on xylophilous fungi from Argentina. IV. Anamorphs of basidiomycetes on *Eucalyptus viminalis* (Myrtaceae). Mycotaxon 34:429–440.

Rossman AY, Samuels GJ. 2005. Towards a single scientific name for species of fungi. Inoculum 56:3–6.

Rossman AY, Schoch CL, Farr DF, Nishijima K, Keith L, Goenaga R. 2010. *Dolabra nepheliae* on rambutan and lychee represents a novel lineage of phytopathogenic Eurotiomycetes. Mycoscience 51:300–309.

Rubio G, Sánchez G, Porras L, Alvarado Z. 2010. Esporotricosis: Prevalencia, perfil clínico y epidemiológico en un centro de referencia en Colombia. Rev Iberoam Micol 27:75–79.

Ruibal C, Gueidan C, Selbmann L, Gorbushina AA, Crous PW, Groenewald JZ, Muggia L, Grube M, Isola D, Schoch CL, Staley JT, Lutzoni F, de Hoog GS. 2009. Phylogeny of rock-inhabiting fungi related to *Dothideomycetes*. Stud Mycol 64:123–133.

Ruibal C, Platas G, Bills GF. 2008. High diversity and morphological convergence among melanised fungi from rock formations in the Central Mountain System of Spain. Persoonia 21:93–110.

Ryvarden L. 1991. Genera of polypores. Nomenclature and taxonomy. Synopsis Fungorum 5:1–363.

Saccardo PA. 1886. Sylloge Fungorum. Vol IV. Pavia: published by the author.

Saikawa M, Baba N, Aoki Y. 1994. Electron microscopy of amoeba-capturing *Dactylella tylopaga*, showing the morphology of a basidiomycete. Mycologia 86:474–477.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Salemi M, Vandamme AM. 2004. The phylogenetic handbook: a practical approach to phylogenetic analysis and hypothesis testing. Cambridge: Cambridge university press.

Samson R, Varga J. 2009. What is a species in *Aspergillus*? Med Mycol 47 Suppl 1:S13–20.

Santamaría S. 1989. L'ordre Laboulbeniales (Fungi, Ascomycotina) a la Península Ibérica i Illes Balears. [doctoral dissertation]. Bellaterra: Universitat Autònoma de Barcelona.

Sanyal A, Rautaray D, Bansal V, Ahmad A, Sastry M. 2005. Heavy-metal remediation by a fungus as a means of production of lead and cadmium carbonate crystals. Langmuir 21:7220–7224.

Schmidt H, Adler A, Holst-Jensen A, Klemsdal SS, Logrieco A, Mach RL, Nirenberg HI, Thrane U, Torp M, Vogel RF, Yli-Matila T, Niessen L. 2004. An integrated taxonomic study of *Fusarium langsethiae*, *Fusarium poae* and *Fusarium sporotrichoides* based on the use of composite datasets. Int J Food Microbiol 95:341–349.

Schoch GL, Crous PW, Groenewald JZ, Boehm EWA, Burgess TI, de Gruyter J, de Hoog GS, Dixon LJ, Grube M, Gueidan C, Harada Y, Hatakeyama S, Hirayama K, Hosoya T, Huhndorf SM, Hyde KD, Jones EBG, Kohlmeyer J, Kruys Å, Li YM, Lücking R, Lumbsch HT, Marvanová L, Mbatchou JS, McVay AH, Miller AN, Mugambi GK, Muggia L, Nelsen MP, Nelson P, Owensby CA, Phillips AJL, Phongpaichit S, Pointing SB, Pujade-Renaud V, Raja HA, Rivas Plata E, Robbertse B, Ruibal C, Sakayaroj J, Sano T, Selbmann L, Shearer CA, Shirouzu T, Slippers B, Suetrong S, Tanaka K, Volkmann-Kohlmeyer B, Wingfield MJ, Wood AR, Woudenberg JHC, Yonezawa H, Zhang Y, Spatafora JW. 2009. A class-wide phylogenetic assessment of *Dothideomycetes*. Stud Mycol 64:1–15

Schol-Schwarz MB. 1970. Revision of the genus *Phialophora* (Moniliales). Persoonia 6:59–94.

Schubert K, Groenewald JZ, Braun U, Dijksterhuis J, Starink M, Hill CF, Zalar P, de Hoog GS, Crous PW. 2007. Biodiversity in the *Cladosporium herbarum* complex (*Davidiellaceae*, *Capnodiales*), with standardization of methods for *Cladosporium* taxonomy and diagnostics. Stud Mycol 58:105–156.

Scorzetti G, Fell JW, Fonseca A, Statzell-Tallman A. 2002. Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. FEMS Yeast Res 2:495–517.

Scott JA, Untereiner WA, Ewaze JO, Wong B, Doyle D. 2007. *Baudoinia*, a new genus to accommodate *Torula compniacensis*. Mycologia 99:592–601.

Seifert KA, Rossman AY. 2010. How to describe a new fungal species. IMA Fungus 1:109–116.

Seifert KA, Samuels GJ. 2000. How should we look at anamorphs? Stud Mycol 45:5–18.

Selbmann L, de Hoog GS, Mazzaglia A, Friedmann EI, Onofri S. 2005. Fungi at the edge of life: cryptoendolithic black fungi from Antartic desert. Stud Mycol 51:1–32.

Selbmann L, de Hoog GS, Zucconi L, Isola D, Ruisi S, Gerrits van den Ende AHG, Ruibal C, de Leo F, Urzi C, Onofri S. 2008. Drought meets acid: three new genera in a dothidealean clade of extremotolerant fungi. Stud Mycol 61:1–20.

Shenoy BD, Jeewon R, Hyde KD. 2007. Impact of DNA sequence data in the taxonomy of anamorphic fungi. Fungal Diversity 26:1–54.

Sidrim JJC, Rocha MFG. 2004. Micologia Médica à luz de autores contemporâneos. Rio de Janeiro: Guanabara Koogan.

Silva V, Madrid H, Anticevic S. 2007. Hallazgo de cuerpos escleróticos en un canino: sospecha de cromoblastomicosis cutánea. Arch Med Vet 39:159–162.

Simmons EG. 2007. *Alternaria*: an identification manual. Utrecht: Centraalbureau voor Schimmelcultures.

Singer R. 1964. Las setas y las trufas: La botánica, el cultivo y la utilización. Ciudad de México: Compañía Editorial Continental.

Singer R. 1969. Mycoflora Australis. Beih Nova Hedwigia 29:1–405.

Singer R. 1986. The Agaricales in modern taxonomy. 4th edition. Koeningstein: Koeltz.

Sivanesan A, Watling R. 1980. A new mitosporic basidiomycete. Trans Br Mycol Soc 74:424–43.

Somrithipol S. 2007. A synnematous species of *Dictyoarthrinium* from Thailand. Mycologia 99:792–796.

Srivastava N, Srivastava AK, Kamal. 1995. New hyphopodiate hyphomycetes from North-Eastern Uttar Pradesh, India. Mycol Res 99:395–396.

Staley JT, Palmer F, Adams JB. 1982. Microcolonial fungi: common inhabitants on desert rocks?. Science 215:1093–1095.

Stalpers JA. 1984. A revision of the genus *Sporotrichum* Stud Mycol 24:1–101.

Stalpers JA. 2000. The genus *Ptychogaster*. Karstenia 40:167–180.

Stalpers JA, Seifert KA, Samson RA. 1991. A revision of the genera *Antromycopsis*, *Sclerostilbum*, and *Tilachlidiopsis*. Can J Bot 69:6–15.

Stalpers JA, Vlug I.1982. *Confistulina*, the anamorph of *Fistulina hepatica*. Can J Bot 61:1660–1666.

Stchigel A. 2000. Estudio taxonómico de los ascomycetes del suelo (Ph. D. thesis). Reus: Universitat Rovira i Virgili.

Sterflinger K, de Hoog GS, Haase G. 1999. Phylogeny and ecology of meristematic ascomycetes. Stud Mycol 43:5–22.

Stewart JE, Mee-Sook K, James RL, Kasten Dumroese R, Klopfenstein NB. 2006. Molecular characterization of *Fusarium oxysporum* and *Fusarium commune* isolates from a conifer nursery. Phytopathology 96:1124–1133.

Stolk AC. 1969. Four new species of *Penicillium*. Antonie van Leewenhoek 35:261–274.

Strobel G, Stierle A. 1993. *Taxomyces andreanae*, a proposed new taxon for a bulbilliferous hyphomycete associated with pacific yew (*Taxus brevifolia*). Mycotaxon 47:71–80.

Subramanian CV. 1962. A classification of the Hyphomycetes. Curr Sci 31:409–411.

Sutton BC, Crous PW. 1997. *Lecanostictopsis* gen. nov. and related leaf-spotting fungi on *Syzygium* species. Mycol Res 101:215–225.

Sutton BC, Marasas WFO. 1976. Observations on *Neottiosporina* and *Tiarosporella*. Trans Br Mycol Soc 67:69–76.

Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Massachussets: Sunderland.

Szymanski M, Barciszewska MZ, Erdmann VA, Barciszewski J. 2002. 5S ribosomal RNA database. Nucl Acids Res 30:171–178.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA 4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599.

Tanaka K, Hirayama K, Yonezawa H, Hatakeyama S, Harada Y, Sano T, Shirouzu T, Hosoya T. 2009. Molecular taxonomy of bambusicolous fungi: *Tetraplosphaeriaceae*, a new pleosporalean family with *Tetraploa*-like anamorphs. Stud Mycol 64: 175–209.

Tavanti A, Davidson AD, Gow NAR, Maiden MCJ, Odds FC. 2005. *Candida orthopsilosis* and *Candida metapsilosis* spp. nov. to replace *Candida parapsilosis* groups II and III. J Clin Microbiol 43:284–292.

Taylor JE, Crous PW, Wingfield MJ. 1999. *Batcheloromyces* species occurring on Proteaceae in South Africa. Mycol Res 103:1478–1484.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genet Biol 31:21–32.

Than PP, Shivas RG, Jeewon R, Pongsupasamit S, Marney TS, Taylor PWJ, Hyde KD. 2008. Epitypification and phylogeny of *Colletotrichum acutatum* J.H. Simmonds. Fungal Diversity 28:97–108.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882.

Thorn RG, Barron GL. 1986. *Nematoctonus* and the tribe *Resupinatae* in Ontario, Canada. Mycotaxon 25:321–453.

Tsuchiya D, Taga M. 2001. Application of fibre-FISH (fluorescence in situ hybridization) to filamentous fungi: visualization of the rRNA gene cluster of the ascomycete *Cochliobolus heterostrophus*. Microbiology 147:1183–1187.

Tsui CK, Berbee ML. 2006. Phylogenetic relationships and convergence of helicosporous fungi inferred from ribosomal DNA sequences. Mol Phylogenet Evol 39:587–597.

Tsuneda A, Currah RS. 2004. *Knufia endospora*, a new dematiaceous hyphomycete from trembling aspen. Rep Tottori Mycol Inst 42:1–9.

Udagawa S, Awao T, Abdullah SK. 1986. *Thermophymatospora*, a new thermotolerant genus of basidiomycetous hyphomycete. Mycotaxon 27:99–106.

Udagawa S, Muroi T. 1979. Coprophilous Pyrenomycetes from Japan V. Trans Mycol Soc Japan 20:453–468.

Udagawa S, Toyazaki N. 1985. A new species of *Leptodiscella*. Mycotaxon 22:407–413.

Untereiner WA. 1997. Taxonomy of selected members of the ascomycete genus *Capronia* with notes on anamorph-teleomorph connections. Mycologia 89:120–131.

Untereiner WA, Scott JA, Naveau FA, Sigler L, Bachewich J, Angus A. 2004. The Ajellomycetaceae, a new family of vertebrate-associated Onygenales. Mycologia 96:812–821.

van Oorschot C.A.N. 1980. A revision of *Chrysosporium* and allied genera. Stud Mycol 20:1–89.

Vannitanakom N, Cooper CR, Fisher MC, Sirisanthana T. 2006. *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects. Clin Microbiol Rev 19:95–110.

Vijaykrishna D, Mostert L, Jeewon R, Gams W, Hyde KD, Crous PW. 2004. *Pleurostomophora*, an anamorph of *Pleurostoma* (Calosphaeriales), a new anamorph genus morphologically similar to *Phialophora*. Stud Mycol 50:387–395.

Vismer HF, Hull PR. 1997. Prevalence, epidemiology and geographical distribution of *Sporothrix schenckii* infections in Gauteng, South Africa. Mycopathologia 137:137–143.

Vizzini A, Zotti M. 2008. *Postia ptychogaster*, an unusual two-stage polypore new to Italian mycobiota. Mycotaxon 103:319–328.

von Arx JA. 1971. Über die typusart zwei neue und einige weitere arten der gattung *Sporotrichum*. Persoonia 6:179–184.

von Arx JA. 1985. Notes on *Triadelphia* and related genera (Moniliales). Trans Br Mycol Soc 85:564–566.

von Arx JA, Gams W. 1967. Über *Pleurage verruculosa* und die zugehörige *Cladorrhinum*-Konidienform. Nova Hedwigia 13:199–208.

von Arx JA, Müller E. 1975. A re-evaluation of the bitunicate ascomycetes with keys to families and genera. Stud Mycol 9:1–159.

Walther G, Garnica S, Weiss M. 2005. The systematic relevance of conidiogenesis modes in the gilled *Agaricales*. Mycol Res 109:525–544.

Wang W, McGhee D, Gibas CFC, Tsuneda A, Currah RS. 2009. *Phialocephala urceolata*, sp. nov. from a commercial, water-soluble heparin solution. Mycologia 101: 136–141.

Whittaker RH. 1969. New concepts of kingdoms of organisms. Science 163:150–160.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to the methods and applications. New York: Academic Press. p. 315–322.

Willetts HJ, Bullock S. 1992. Developmental biology of sclerotia. Mycol Res 96:801–816.

Yamazaki A, Toyama K, Nakagiri A. 2010. A new acidophilic fungus, *Teratosphaeria acidotherma* (Capnodiales, Ascomycota) from a hot spring. Mycoscience 51:443–455.

Yang HL, Sun GY, Batzer JC, Crous PW, Groenewald JZ, Gleason ML. 2010. Novel fungal genera and species associated with the sooty blotch and flyspeck complex on apple in China and the USA. Persoonia 24:29–37.

Zalar P, de Hoog GS, Gunde-Cimerman N. 1999. *Trimmatostroma salinum*, a new species from hypersaline water. Stud Mycol 43:57–62.

Zalar P, de Hoog GS, Schroers H-J, Crous PW, Groenewald JZ, Gunde-Cimerman N. 2007. Phylogeny and ecology of the ubiquitous saproble *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. Stud Mycol 58:157–183.

Zhang KQ, Yu ZF, Qiao M, Zhang Y, Qin L. 2010. *Pseudotripoconidium*, a new anamorph genus connected to *Orbilia*. Mycologia (in press, DOI: 10.3852/10-102).

Zhang N, Castlebury LA, Miller AN, Huhndorf SM, Schoch CL, Seifert KA, Rossman AY, Rogers JD, Kohlmeyer J, Volkmann-Kohlmeyer B, Sung GH. 2006. An overview

of the systematics of the Sordariomycetes based on a four gene phylogeny. Mycologia 98:1076–1087.

Zhang WM, Li TH, Chen YQ, Qu LH. 2004. *Cordyceps campsosterna*, a new pathogen of *Campsosternus auratus*. Fungal Diversity 17:239–242.

Zhao J, Mou Y, Shan T, Li Y, Zhou L, Wang M, Wang J. 2010. Antimicrobial metabolites from the endophytic fungus *Pichia guilliermondii* isolated from *Paris polyphylla* var *yunnanensis*. Molecules 15:7961–7970.

Zhou X, de Beer ZW, MJ Wingfield. 2006. DNA sequence comparisons of *Ophiostoma* spp., including *Ophiostoma aurorae* sp. nov. associated with pine bark beetles in South Africa. Stud Mycol 55:269–277.