FILOGENIA Y SENSIBILIDAD ANTIFÚNGICA DE AISLADOS CLÍNICOS DE ACROPHIALOPHORA, CLADOSPORIUM, MICROASCUS, SCOPULARIOPSIS Y TRICHODERMA.

Marcelo Patricio Sandoval Denis

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UNIVERSITAT ROVIRA I VIRGILI

Filogenia y sensibilidad antifúngica de aislados clínicos de *Acrophialophora*, *Cladosporium*, *Microascus*, *Scopulariopsis* y *Trichoderma*

Marcelo Patricio Sandoval Denis

TESIS DOCTORAL

Dirigida por los Doctores: Josepa Gené Díaz,
José Francisco Cano Lira y Josep Guarro Artigas

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HAGO CONSTAR que el presente trabajo, titulado "**Filogenia y sensibilidad antifúngica de aislados clínicos de *Acrophialophora*, *Cladosporium*, *Microascus*, *Scopulariopsis* y *Trichoderma***", que presenta **Marcelo Patricio Sandoval Denis** para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de Ciencias Médicas Básicas de esta universidad.

Reus, 02 de Septiembre de 2015

Los directores de la tesis doctoral

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ÍNDICE DE ABREVIATURAS

Act	Actina
***Act*1**	Actina
ADN	Ácido desoxirribonucleico
AFG	Anidulafungina
AMB	Anfotericina B
ARN	Ácido ribonucleico
ATCC	*American Type Culture Collection* (USA)
BAL	Lavado broncoalveolar
BCCM/MUCL	*Agro-Industrial Fungi and Yeasts Collection, Belgian Co-ordinated Collection of Micro-organisms* (Bélgica)
BI	Inferencia Bayesiana
BLAST	*Basic Local Alignment Tool*
bs	Soporte de bootstrap
Cal	Calmodulina
CBS	CBS-KNAW *Fungal Biodiversity Centre* (Holanda)
CFG	Caspofungina
***chi*18-5**	Endoquitinasa 18-5
CLSI	*Clinical Laboratory and Standards Institute* (USA)
cm	Centímetro
CMD	Agar harina de maíz dextrosa
CNRMAF	*Centre National de Réfeìrence Mycoses Invasives et Antifongiques, Institut Pasteur*, Paris (Francia).
comb. nov.	Nueva combinación
Cplx	Complejo de especies
CSF	Líquido cefalorraquídeo
D1/D2	Dominios D1 y D2 del gen 28S del rRNA
DAOM	*Agriculture and Agri-Food Canada National Mycological Culture Collection* (USA)
d	Días
diam	Diámetro
DIS	Colección de cultivos CABI-*Bioscience* mantenidos por Gary J. Samuels
DMSO	Dimetilsulfósido
dNTP	Desoxinucleótido trifosfato
dTTP	Desoxitimidina 5’ trifosfato
***Ech*42**	Endoquitinasa 18-5

EF1-α	Factor de elongación 1-alfa
EF-1α	Factor de elongación 1-alfa
ESCMID	*European Society of Clinical Microbiology and Infectious Diseases*
et al.	Y otros
ETS	Región espaciadora transcrita externa del rRNA
EUCAST	*European Committee on Antimicrobial Suceptibility Testing*
Fig.	Figura
FLC	Fluconazol
FMR	*Facultat de Medicina de Reus*
g	Gramo
GCPSR	*Genealogical Concordance Phylogenetic Species Recognition*
GJS	Colección de cultivos de Gary J. Samuels
GM	Media geométrica
GTR+G+I	Modelo general de tiempo reversible con categorías gamma y una porción de posiciones invariables.
HTU	Unidad taxonómica hipotética
ICBN	Código Internacional de Nomenclatura Botánica
ICTF	Comisión Internacional de Taxonomía Fúngica
IDSA	*Infectious Disease Society of America*
IGS	Región espaciadora intergénica
IHEM	*Biomedical Fungi and Yeast Collection, Institute of Hygiene and Epidemiology* (Bélgica)
ISBN	Número Internacional Normalizado de Libro
ISHAM	*International Society for Human and Animal Mycology*
ISSN	Número Internacional Normalizado de Publicaciones Seriadas
ISTH	Subcomisión Internacional sobre *Trichoderma* e *Hypocrea*
ITC	Itraconazol
ITS	Región espaciadora intergénica transcrita del rRNA
IUPAC	*International Union of Pure and Applied Chemistry*
KTC	Ketoconazol
LAMB	Anfotericina B liposomal
MCMC	*Markov chain Monte Carlo*
MCM-CMC	*Metrópolis-coupled Markov chain Monte Carlo*
MEA	Agar extracto de malta
MEC	Concentración mínima efectiva
MEGA	*Molecular Evolutionary Genetic Analysis*
MFG	Micafungina

MIC	Concentración mínima inhibitoria
min	Minuto
ML	Máxima verosimilitud
mL	Mililitro
MLI	Nivel máximo de identidad
mM	Mili molar
MP	Máxima parsimonia
mTorr	Mili Torr
MUCL	*Mycothèque de l'Université Catholique de Louvain* (Bélgica)
MUSCLE	*Multiple Sequence Comparison by Log-Expectation*
NBRC	*National Institute of Technology and Evaluation Biological Resource Center* (Japón)
NCBI	*National Center for Biotechnology Information* (USA)
NCF	Comité para la Nomenclatura de Hongos
NITE	*National Institute of Technology and Evolution* (Japón)
NJ	*Neighbor-Joining*
NNI	*Nearest-Neighbor-Interchange*
OA	Agar harina de avena
OTU	Unidad taxonómica operativa
PAUP*	*Phylogenetic Analysis Using Parsimony*
pb	Pares de bases
PC	Colección de cultivos de Priscilla Chaverri
PCA	Agar patata zanahoria
PCR	Reacción en cadena de la polimerasa
PDA	Agar patata dextrosa
pp	Probabilidad posterior
PSC	Posaconazol
RPB1	RNA polimerasa subunidad I
RPB2	RNA polimerasa subunidad II
RPMI	Medio de *Roswell Park Memorial Institute*
rRNA	RNA ribosomal
SNA	Agar bajo en nutrientes
sp. nov.	Nueva especie
stat. nov.	Nuevo estado
Tef	Factor de elongación 1-alfa
Tef1	Factor de elongación 1-alfa

Tr	Colección de cultivos de Earl Nelson mantenidas en el USDA-ARS (USA)
TRB	Terbinafina
TUB F	*Technical University of Budapest Microbial Culture Collection* (Hungría)
Tub	Tubulina
TUB	Tubulina
U	Unidades
UAMH	*University of Alberta Microfungus Collection and Herbarium* (Canadá)
UPGMA	*Unweighted Pair Group Method with Arithmetic Mean*
USDA-ARS	*United States Department of Agriculture - Agricultural Research Service*
UTHSC	*Fungus Testing Laboratory of the University of Texas Health Science Center* (USA)
UTHSCSA	*Fungus Testing Laboratory of the University of Texas Health Science Center in San Antonio* (USA)
var.	Variedad
VIH	Síndrome de inmunodeficiencia humana
VRC	Voriconazol
YES	Agar sacarosa extracto de levadura
µg	Microgramo
µL	Micro litro
µm	Micrómetro
µM	Micro molar
5FC	5-Fluorocitosina
ºC	Grados Celsius

1. INTRODUCCIÓN

1.1 Generalidades

El reino *Fungi* incluye un grupo heterogéneo de organismos con una gran riqueza, tanto en biodiversidad como en características morfológicas. Los hongos se caracterizan por ser organismos eucariotas, uni- o multinucleados, haploides o diploides, que se reproducen por medio de esporas; pueden vivir en condiciones aerobias o anaerobias, tanto en ambientes acuáticos como terrestres (Kirk *et al.* 2008, Griffith *et al.* 2010, Seifert *et al.* 2011). Originalmente fueron incluidos en el reino *Plantae* por ser organismos inmóviles y poseer una pared celular, por lo que durante mucho tiempo fueron estudiados como una disciplina de la botánica (Carlile & Watkinson 1994). Sin embargo, los hongos se diferencian de las plantas por ser incapaces de fijar energía luminosa directamente, siendo organismos heterótrofos, no fotosintéticos, y con nutrición de tipo absortiva, llevada a cabo mediante la liberación de enzimas hidrolíticas para la degradación de grandes polímeros, como proteínas o carbohidratos, que al convertirse en monómeros son transportados al interior de la célula a traves de su membrana citoplasmática (Brock 2006). La membrana plasmática de los hongos es rica en ergosterol y se encuentra rodeada por una pared celular compuesta principalmente por polisacáridos, especialmente quitina, β-glucanos, manano y galactomanano, además de glicoproteínas (Pontón 2008, Brandt & Warnock 2015). Y por último cabe destacar que, a diferencia de las plantas, los hongos son organismos no vasculares e incapaces de formar tejidos verdaderos.

Los hongos pueden ser organismos unicelulares o pluricelulares. Los primeros, referidos como hongos levaduriformes, presentan una estructura somática constituida por una célula solitaria, esférica a elipsoidal, la cual se reproduce asexualmente por un proceso denominado gemación, en el que la célula madre, a partir de una evaginación a nivel de su pared externa y membrana citoplasmática, forma una nueva célula denominada **blastoconidio** (Brock 2006). En algunos casos, los blastoconidios pueden quedar adheridos a la célula madre y alargarse formando estructuras casi tubulares, aunque bajo esta condición no existe conexión citoplasmática directa entre las células. Cuando esto ocurre en forma secuencial, la estructura formada recibe el nombre de **pseudohifa** (Brock 2006, Brandt & Warnock 2015).

Los hongos pluricelulares, comunmente denominados hongos filamentosos o mohos, están formados por una serie de células tubulares, la unión de las cuales recibe el nombre de **hifa**. Las células que componen la hifa pueden estar delimitadas entre sí, formando compartimentos internos definidos por la presencia de septos transversales (**hifas septadas**), aunque siempre existe una conexión directa entre los citoplasmas que permite el paso de nutrientes o material genético de una célula a otra mediante poros de complejidad estructural diversa. Aquellas hifas que no presentan septos a intervalos

regulares se denominan **hifas aseptadas** o **cenocíticas** (de Hoog *et al.* 2011). El conjunto de hifas recibe el nombre de **micelio**, el cual es por lo general ramificado y de crecimiento expansivo determinado por la elongación apical de las hifas que lo componen (Harris 2008).

En algunos hongos, un mismo individuo es capaz de presentarse en cualquiera de las dos formas morfológicas básicas (levaduriforme y/o filamentosa), dependiendo de las condiciones ambientales fundamentalmente de la temperatura, creciendo habitualmente en forma filamentosa a 25ºC y en forma levadurifome a 37ºC (de Hoog *et al.* 2011). Estos hongos se denominan **hongos dimórficos** y en zoopatología, este término, por lo general, se utiliza para delimitar la dualidad de formas observadas entre la forma saprobia y la forma patógena de un mismo hongo (Howard 2002). Ejemplos de hongos dimórficos son *Blastomyces dermatitidis* Gilchrist & W.R. Stokes, *Coccidioides immitis* G.W. Stiles, *Histoplasma capsulatum* Darling, *Paracoccidioides brasiliensis* (Splend.) F.P. Almeida y *Penicillium marneffei* Segretain, Capponi & Sureau.

Un buen número de hongos son organismos **pleomórficos**, es decir, muestran diferentes características morfológicas en función de sus diversas etapas reproductivas. Su ciclo vital está dividido en dos etapas fundamentales: una fase asexual o **anamorfo** (forma asexual u hongo imperfecto), en la cual el organismo se reproduce por medio de esporas mitóticas, y una fase sexual o **teleomorfo** (forma sexual u hongo perfecto) caracterizada por la formación de esporas de origen meiótico. Un hongo para el cual ambas fases y morfologías son conocidas se denomina **holomorfo**, mientras aquellos hongos que presentan dos anamorfos distintos para un mismo teleomorfo son denominados **sinanamorfos (Fig. 1)**. Sin embargo, para un elevado porcentaje de los hongos descritos hasta la fecha sólo se conoce su forma asexual, un hecho que puede ser debido a condicionantes ambientales o simplemente porque han perdido la capacidad de reproducirse sexualmente a lo largo de la evolución (Hambleton & Sigler 2005). Cabe destacar que la compatibilidad sexual en los hongos está regida genéticamente por el *locus MAT*, el cual define el tipo sexual o *mating type* y que puede presentarse en dos o más formas alternativas denominadas **idiomorfos** (Conde-Ferráez 2007, Crous *et al.* 2009a). Aquellos hongos que presentan un solo *locus MAT*, necesitan la participación de otro individuo compatible para poder llevar a cabo la reproducción sexual y se denominan **heterotálicos**, mientras que aquellos hongos que portan ambos idiomorfos, siendo por tanto capaces de autofertilizarse, se denominan **homotálicos** (Kirk *et al.* 2008).

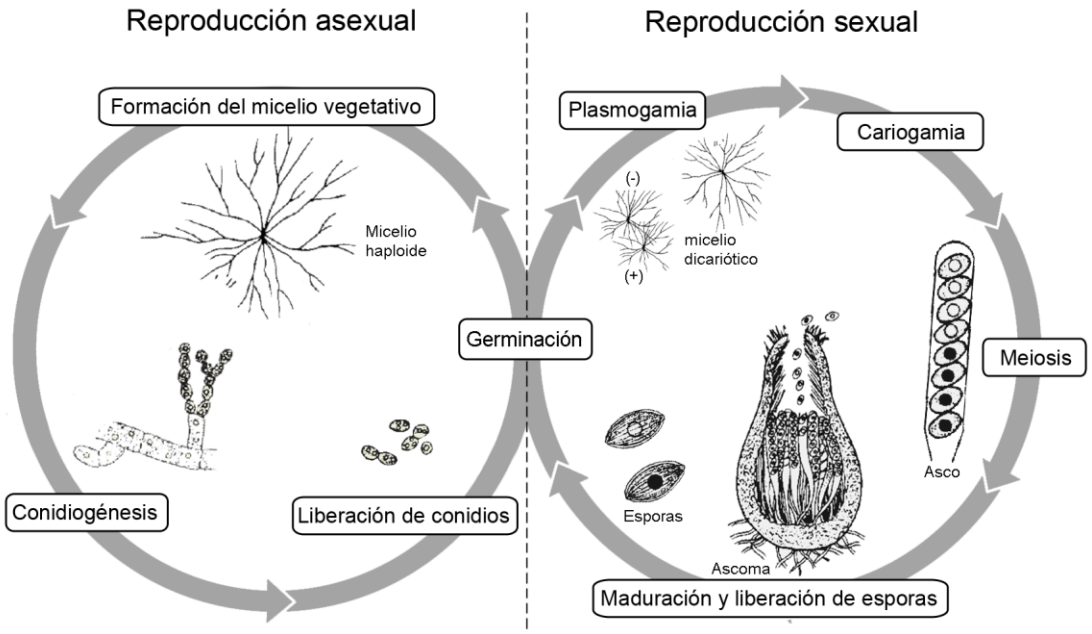

Figura 1 Representación esquemática del ciclo vital de los hongos. Adaptado de Esser (1985) y Crous *et al.* (2009a).

1.1.1 Ecología

El reino de los hongos es uno de los grupos de organismos con mayor diversidad del planeta (Taylor & Berbee 2006, Irinyi *et al.* 2015a). Los hongos están distribuidos en todos los ecosistemas terrestres. Mientras que la gran mayoría de especies son ubicuas, otras están muy especializadas para la supervivencia en ambientes extremos. Es así como se conocen especies fúngicas aisladas desde regiones con temperaturas extremas, como el frío polar (psicrófilos), suelo desértico (termófilos y termotolerantes), ambientes con muy baja actividad de agua (xerófilos) o de alto contenido osmótico (osmófilos) (Dix & Webster 1995).

Hasta la fecha se conocen unas 100.000 especies de hongos y, considerando que se describen de 1.000 a 1.500 nuevas especies por año (Perfect & Schell 1996, Brandt & Warnock 2015), se estima que aún quedan por descubrir entre uno a diez millones de especies (Webster & Weber 2007, Blackwell 2011, Brandt & Warnock 2015). Sin embargo, recientes estudios de diversidad fúngica basados en pirosecuenciación indican que esta cifra podría estar sobreestimada en 1.2 a 2.5 veces (Tedersoo *et al.* 2014). Esta discrepancia no hace más que demostrar y reforzar la necesidad de explorar la diversidad fúngica global.

La gran mayoría de los hongos son organismos saprobios, es decir, obtienen nutrientes a partir de la materia orgánica en descomposición y, en este sentido, están involucrados en una gran variedad de procesos biológicos (Carlile & Watkinson 1994 Brandt & Warnock 2015). Muchas especies juegan un papel fundamental en la

degradación de la materia orgánica (Brandt & Warnock 2015), otras son muy importantes en el ciclo del carbono y nitrógeno (Tedersoo *et al.* 2014), formando asociaciones simbióticas como los **líquenes** (asociación con cianobacterias o algas) o las **micorrizas** (asociación con plantas vasculares) consideradas de vital importancia al favorecer la nutrición de la planta (Dix & Webster 1995, Kendrick 2001). Muchas especies son biótrofas y crecen fundamentalmente como endófitos asintomáticos de plantas, mientras otras son capaces de colonizar superficies de plantas y animales alimentándose de secreciones y restos orgánicos (Crous *et al.* 2009a). Otros hongos han sido también de gran interés como organismos modelo para el estudio de procesos biológicos, como la generación de energía, el control del metabolismo y los mecanismos implicados en la herencia de caracteres (Carlile & Watkinson 1994). Sin embargo, los hongos también se destacan por tener un impacto negativo sobre el ser humano u otros organismos. Se conocen más de 8.000 especies de hongos fitopatógenos capaces de afectar tanto a plantas de cultivo como a granos almacenados, otras especies son reconocidos productores de toxinas en alimentos, otras ejercen una acción tóxica directa derivada de su ingestión, mientras que otros hongos son capaces de parasitar tanto al ser humano como a otros animales (Brock 2006, de Hoog *et al.* 2011).

1.1.2 Taxonomía y nomenclatura

La taxonomía de los hongos tradicionalmente se ha apoyado en la clasificación sistemática establecida por Linneo (1753), la cual se basa en la observación y comparación de estructuras morfológicas y establece que aquellos organismos que presentan similitudes estructurales deberían ser incluidos en un mismo grupo. Estos grupos se denominan **taxones** y están ordenados jerárquicamente. El taxón o categoría taxonómica inferior es la **especie** a la cual se le atribuye un binomio latinizado compuesto por el nombre del género (en letra cursiva y con la primera letra en mayúsculas) seguido del epíteto que determina la especie (en letra cursiva y minúsculas). Los taxones superiores reciben un único nombre en latín con una terminación diferente y única; y son sucesivamente tipificados por un miembro de la categoría inferior. Así, un conjunto de especies constituye un **género**, un conjunto de géneros forma una **familia** *(-ceae)*, un conjunto de familias, un **orden** (*-ales*); un conjunto de órdenes, una **clase** (*-etes*), y un conjunto de clases, un ***phylum*** (*-mycota*). Finalmente, todos los niveles se agrupan bajo el nivel superior, el **reino** (Crous *et al.* 2009a). Las reglas taxonómicas indican que para toda especie fúngica deberá designarse un tipo nomenclatural (**holotipo**), representado por material desecado o una ilustración proveniente de un solo espécimen, preservado a perpetuidad en una colección de acceso público (**herbario**), el cual debe ser citado expresamente en la

publicación original de la especie (**protólogo**). Existen además otros tipos nomenclaturales: un **isotipo** es cualquier duplicado del holotipo, es siempre un espécimen. Se denomina **lectotipo** a un espécimen o ilustración designado a partir del mismo material original u holotipo si este último no fue asignado en la publicación original, si el holotipo se ha perdido o si se demuestra que este pertenece a más de un taxón. Un **neotipo** es un espécimen o ilustración elegido como tipo nomenclatural cuando todo el material original en el cual se basó la descripción original se ha perdido. Un **sintipo** es cualquier material citado por el autor cuando no se ha designado expresamente o se ha designado más de un espécimen como tipo. Un **paratipo** es cualquier espécimen de un grupo designado como tipo y citado en el protólogo, pero que no corresponde ni al holotipo ni a ningún isotipo. Un **epitipo** es un espécimen o ilustración designado como tipo nomenclatural cuando el holotipo, lectotipo, neotipo, o todo el material original de un taxón se consideran ambiguos (Crous *et al.* 2009a, McNeill *et al.* 2012).

Desde sus inicios, el estudio, clasificación y nomenclatura de los hongos se ha regido por el Código Internacional de Nomenclatura Botánica (*ICBN*), vestigio de los orígenes de la micología como una rama de la botánica. En concreto, el Artículo 59 de dicho código permitía una nomenclatura dual para los hongos pleomórficos, es decir, la asignación de nombres específicos diferentes según si se trataba del anamorfo o del teleomorfo de un mismo hongo. En el caso de conocer ambas formas de reproducción, es decir el holomorfo, éste recibía el nombre específico atribuido al teleomorfo (McNeil *et al.* 2006). Sin embargo, en la actualidad, la filogenia molecular ha provocado grandes cambios en diversos aspectos de la taxonomía. Ello se debe a que los estudios moleculares además de permitir desvelar la posición taxonómica de muchos hongos, incluso de aquellos que nunca producen esporas, nos facilita el conocimiento de las conexiones entre anamorfo y teleomorfo de las especies y es en este sentido que la nomenclatura dual ha acabado siendo redundante o innecesaria (Hawksworth 2004, Hawksworth 2012). Por lo tanto, con la abolición de la nomenclatura dual surge la necesidad de reorganizar la nomenclatura fúngica, y además los estudios filogenéticos también han determinado la separación definitiva de la micología como una disciplina independiente de la botánica (Hawksworth 2011).

Recientemente, durante dos simposios internacionales de expertos en taxonomía y nomenclatura fúngica realizados en Ámsterdam, *1Fungus = 1Name* (1F=1N) en el 2011 y *1Fungus = What name?* (1F = ?N) en el 2012, se discutieron importantes temas relacionados con la nomenclatura dual (de Hoog *et al.* 2015). Como consecuencia de los mismos se dio a conocer la denominada “Declaración de Ámsterdam sobre Nomenclatura Fúngica”, llevada a cabo con el apoyo de la Comisión Internacional de

Taxonomía Fúngica (*ICTF*) (Hawksworth *et al.* 2011). Durante el Congreso Internacional de Botánica llevado a cabo en Melbourne (Julio del 2011), se realizaron importantes cambios relacionados con la taxonomía de hongos, lo cual derivó en la creación de un nuevo código nomenclatural, el **Código Internacional de Nomenclatura para Algas, Hongos y Plantas** (Código de Melbourne), el cual entró en vigencia en su totalidad el 1 de Enero de 2013 (McNeill *et al.* 2012). Los principales cambios incluidos en este nuevo código han sido: i) la abolición de la nomenclatura dual, desde ahora cada hongo debe de tener un solo nombre; ii) la preferencia del nombre más antiguo sobre todos los otros nombres publicados para un mismo taxón; iii) la desaparición de la obligatoriedad de describir los nuevos taxones en Latín; iv) la consideración como válidas de las descripciones publicadas en soporte electrónico (documentos disponibles en línea o archivos digitalizados) siempre y cuando estén asociadas a un Número Internacional Normalizado de Publicaciones Seriadas (*ISSN*) o un Número Internacional Normalizado de Libro (*ISBN*); y v) la entrada en vigencia del registro oficial de los nombres de los taxones fúngicos. Para ser aceptado como válido, todo nuevo taxón debe incluir en el protólogo, un número único de registro del nombre emitido por una base de datos consensuada de referencia (MycoBank: www.mycobank.org) (Crous *et al.* 2004).

Sin embargo, cabe destacar que la eliminación de la nomenclatura dual está creando mucha polémica entre los taxónomos, ya que la selección del nombre correcto no es tan fácil como en un principio se planteó. En realidad, se trata de un proceso complejo para el cual hay que analizar numerosos factores (número de cambios taxonómicos a realizar sobre el resto de hongos relacionados, antigüedad de cada nombre, nombre más usado, etc) y las consecuencias que esta decisión pueda ocasionar sobre las diversas áreas de estudio, especialmente en el campo de la micología clínica y la fitopatología (Hawksworth *et al.* 2013). Por este motivo, se han publicado listas de nombres protegidos para su conservación (Kirk *et al.* 2013, Rossman *et al.* 2015); la decisión del nombre final será posteriormente determinada por el Comité para la Nomenclatura de Hongos (*NCF*) (Hawksworth 2012).

Actualmente, el reino *Fungi* es uno de los seis reinos del árbol de la vida (Kendrick 2001, Brandt & Warnock 2015), el cual comprende un subreino (*Dikarya*) y siete *phyla* (*Ascomycota*, *Basidiomycota*, *Blastocladiomycota*, *Chytridiomycota*, *Glomeromycota*, *Microsporidia* y *Neocallimastigomycota*). El subreino *Dikarya* agrupa los *Ascomycota* y *Basidiomycota* que incluyen hongos formadores tanto de hifas como esporas dicarióticas (Hibbet *et al.* 2007, Brandt & Warnock 2015) **(Fig. 2)**. El antiguo *phylum Zygomycota,* que antaño agrupaba a los hongos evolutivamente más antiguos y productores de hifas cenocíticas, ya no es aceptado como tal debido a su naturaleza polifilética (Hibbet *et al.* 2007). Sus miembros se encuentran ahora distribuidos entre el

phylum Glomeromycota y cuatro linajes parafiléticos de agrupación incierta (Brandt & Warnock 2015), siendo en conjunto informalmente denominados “Hongos basales” (Hibbet *et al.* 2007).

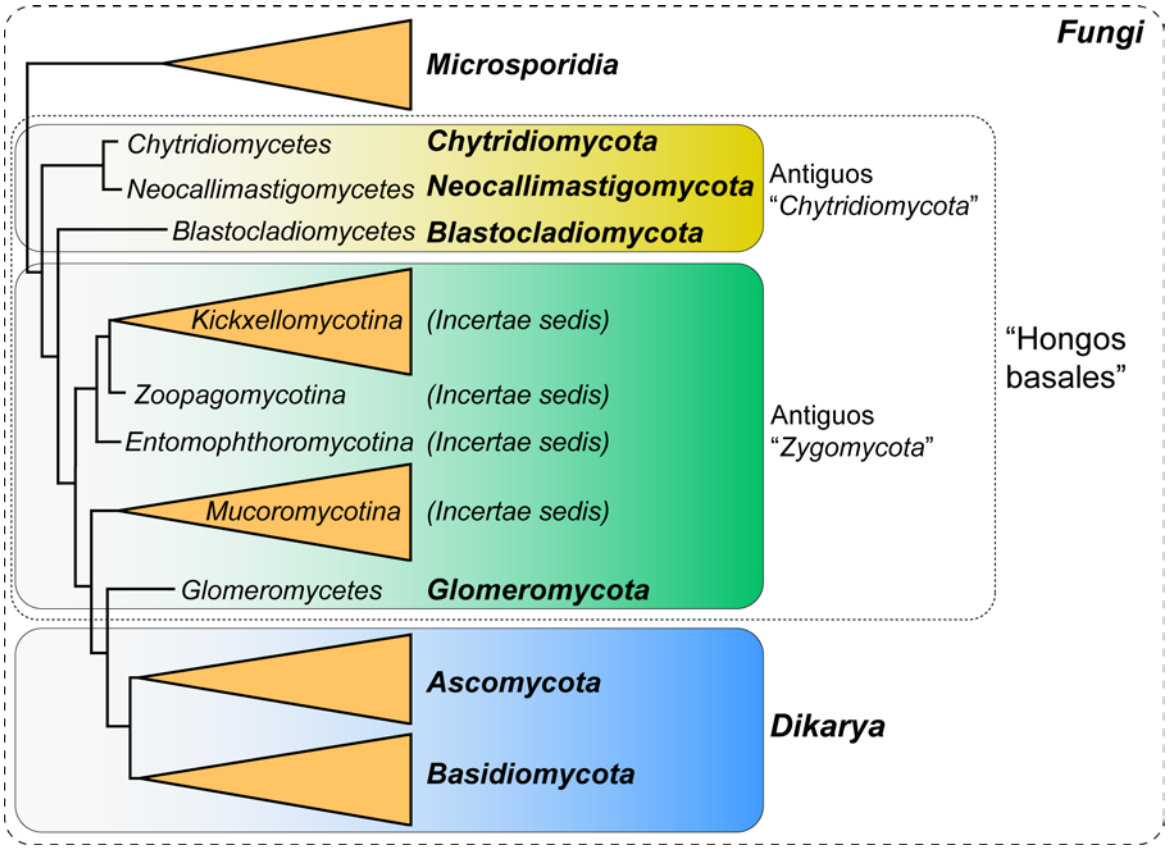

Figura 2 Representación esquemática de la clasificación taxonómica vigente del reino *Fungi*, se destacan los siete *phyla* actualmente aceptados (en negrita) y el subreino *Dikarya*, junto con otros grupos parafiléticos previamente incluidos en la anterior clasificación de *Chytridiomycota* y en el extinto *phylum Zygomycota*, informalmente denominados “hongos basales”. Adaptado de Hibbet *et al.* (2007), McLaughlin *et al.* (2009) y Stajich *et al.* (2009).

1.2 Identificación

1.2.1 Identificación morfológica

Historicamente, tanto la organización taxonómica de los hongos como su identificación se ha basado fundamentalmente en criterios morfológicos asociados principalmente al estudio de las estructuras de reproducción, tanto sexual como asexual. A partir de las características observadas en las formas sexuales se organizaron y establecieron los diferentes grupos taxonómicos, muchos de ellos todavía vigentes en la actualidad. Del mismo modo, también se estableció un sistema de clasificación para aquellos hongos que carecían o se desconocía su fase sexual, de manera que los que formaban únicamente esporas asexuales (conidios) comprendían el grupo de los Deuteromicetes u Hongos Imperfectos, los cuales se dividían a su vez en tres grandes categorías: Blastomicetes, Celomicetes e Hifomicetes. Sin embargo, dichas categorías

no correspondían a taxones formales, ni tampoco reflejaban relaciones naturales entre los individuos que las componían (Seifert *et al.* 2011). Los Blastomicetes comprendían los hongos con talo levadurifome, cuya identificación se basa fundamentalmente en la combinación de características fisiológicas y bioquímicas, con caracteres morfológicos; tales como la presencia de cápsula alrededor de las células, la producción de pseudohifas o hifas verdaderas durante alguna fase de su desarrollo, etc (de Hoog *et al.* 2011, Brandt & Warnock 2015). Dentro de los Celomicetes se incluían aquellos hongos con talo filamentoso y septado, produciendo conidios dentro de cuerpos fructíferos (**conidiomas**), normalmente globosos o piriformes y con una abertura apical (**picnidios, Fig. 3a**); o conidiomas abiertos y cóncavos, en forma de plato (**acérvulos**) en cuya superficie se localizan las **células conidiógenas** (productoras de conidios). Los Hifomicetes, en donde se incluían la mayoría de los hongos asexuales de importancia clínica, presentan también un talo filamentoso y septado, en donde los conidios se originan externamente a partir de células conidiógenas solitarias localizadas en el micelio, o por medio de hifas especializadas denominadas **conidióforos (Fig. 3b,c)**. Estas estructuras pueden ser solitarias, pueden estar formando acúmulos llamados **esporodoquios (Fig. 3d)**, o agrupadas en densas columnas denominadas **sinemas (Fig. 3e)**. A su vez, los conidióforos pueden ser simples o ramificados y estas ramificaciones pueden ser laterales o estar dispuestas en verticilos **(Fig. 3c)** (Seifert *et al.* 2011). Se denominan **determinados** cuando el crecimiento del conidióforo cesa antes de la producción del primer conidio, o **percurrentes** si el conidióforo continúa su crecimiento tras la formación del conidio.

Para la identificación a nivel de especie, además del estudio de los caracteres microscópicos del hongo ya mencionados creciendo sobre sustrato natural, también son de utilidad el estudio de los caracteres macroscópicos de los mismos creciendo en medios de cultivo de laboratorio. Entre los caracteres macroscópicos más destacados para tal finalidad, podemos citar la obtención de la tasa de crecimiento y diámetro de las colonias, datos sobre la textura, color, producción de pigmentos, etc. Sin embargo, estas características pueden estar muy influenciadas tanto por el medio como por las condiciones de cultivo empleadas (temperatura, humedad, presencia o ausencia de luz natural o ultra-violeta, en ciclos o continua, etc.), el inóculo, etc. En el caso de aislados clínicos, por lo general nos vemos limitados a estudiar los caracteres tanto macro como microscópicos en condiciones *in vitro,* por lo que si en tales condiciones el hongo es incapaz de esporular su identificación es imposible de realizar. Es por este motivo que resulta indispensable una selección adecuada de los medios y condiciones de cultivo para favorecer la formación de conidios y el estudio de sus estructuras asociadas.

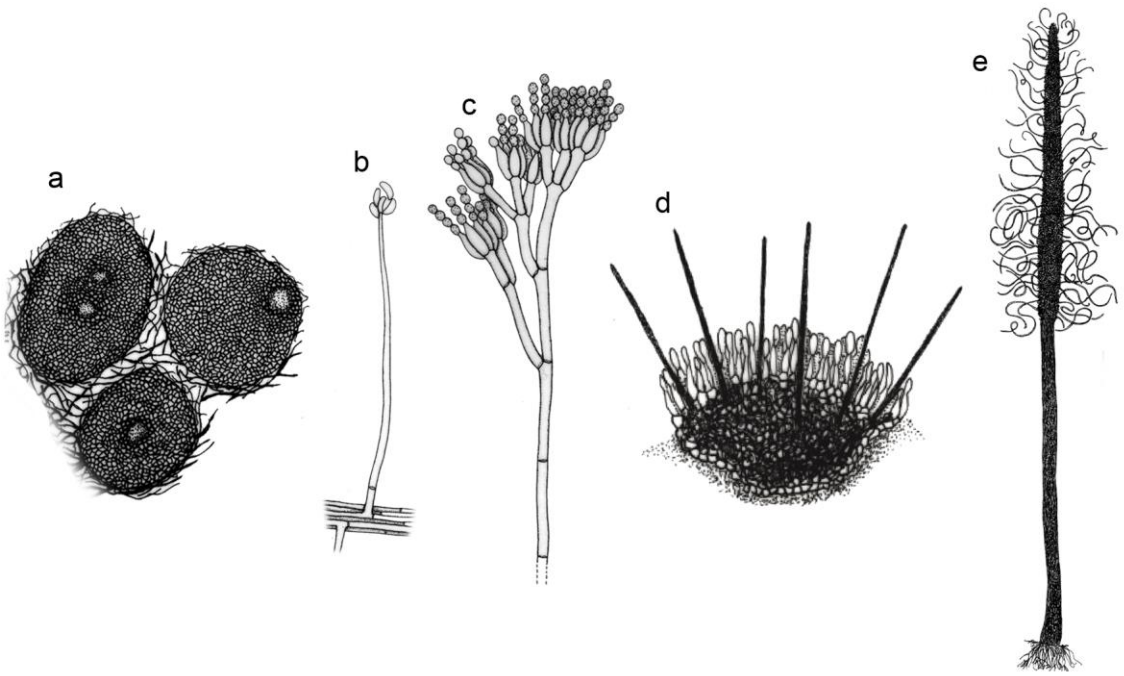

Figura 3 Estructuras reproductivas asexuales de Celomicetes (a) e Hifomicetes (b-e). a. Picnidio. b. Conidióforo simple. c. Conidióforo ramificado. d. Esporodoquio. e. Sinema. Adaptado de Gené (1994).

En este sentido, un criterio básico para la identificación morfológica de los hongos asexuales es el que involucra el estudio del proceso por el cual las hifas o células conidiógenas dan lugar a los conidios y cómo éstos se liberan de las mismas, nos referimos al proceso de **conidiogénesis (Fig. 4)** (Kirk *et al.* 2008).

La conidiogénesis puede ser de dos tipos: blástica y tálica. La **conidiogénesis blástica** involucra el crecimiento o hinchamiento evidente de un primordio de conidio a través de la pared celular y antes de ser delimitado por un septo; se distinguen dos mecanismos: enteroblástico y holoblástico. Se denomina **holobástico**, si las dos capas que componen la pared celular de la célula conidiógena participan en el hinchamiento del conidio. Una célula conidiógena puede presentar un *locus* conidiógeno (**monoblástica**) o más de uno (**poliblástica**). En algunos casos el conidio ya formado puede dar origen a otro conidio mediante el mismo proceso y así sucesivamente generando cadenas **acrópetas**, en éstas el conidio más joven se encuentra al final de la cadena. A su vez, es posible que cada conidio pueda tener más de un *locus* conidiogénico, por lo que en ciertos hongos se observan cadenas ramificadas de conidios (Brandt & Warnock 2015). Si una célula conidiógena poliblástica origina dos o más conidios simultáneamente se denomina **sincrónica**, o bien, si una vez formado el primer conidio, la célula conidiógena crece lateralmente para luego dar origen a un nuevo conidio se denomina **simpodial**. En la conidiogénesis **enteroblástica**, la capa

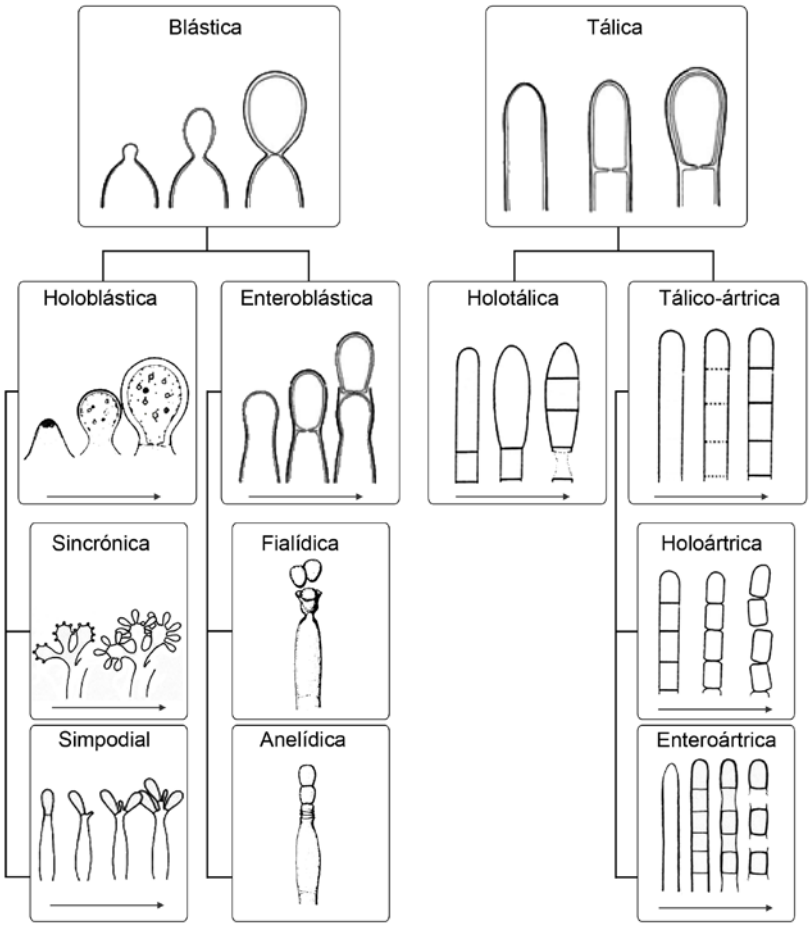

Figura 4 Representación esquemática de los modos básicos de conidiógenesis. Adaptado de Gené (1994), Gams *et al.* (1998), de Hoog *et al.* (2011).

externa de la pared se rompe formando una abertura, permitiendo el hinchamiento y protrusión de la capa interna y la producción de conidios en forma sucesiva a través de un mismo locus. Se denomina conidiogénesis **fialídica** cuando la pared externa abierta forma un **collarete** en el ápice de la célula conidiógena, la cual recibe el nombre de **fiálide**. El material remanente de la generación de los conidios puede permanecer en el interior del collarete y se observa como una zona más oscura denominada **anillo periclinal**. Los conidios que se producen en sucesión **basípeta** (el conidio más joven se desarrolla siempre desde la célula conidiógena) pueden formar cadenas (cadenas basípetas), o quedar adheridos entre sí formando cabezas mucosas. Se denomina conidiogénesis **anelídica** cuando después de la formación del primer conidio se forma una cicatriz a partir de la cual se genera el siguiente conidio y así sucesivamente. En este caso, la célula conidiógena se denomina **anélide** y presenta cicatrices anulares (**anelaciones**) originadas por la secesión de cada conidio. En este caso también es posible observar la formación de cadenas basípetas de conidios o de agregados mucosos.

En la **conidiogénesis tálica** el conidio se forma a partir de una hifa pre-existente después del desarrollo de un septo (Kirk *et al.* 2008, de Hoog *et al.* 2011). Se pueden distinguir dos variantes: holotálica y tálico-ártrica, en ambos casos la liberación del conidio se realiza por medio de la lisis de una célula intermedia entre el conidio y la hifa (**rexolisis**), o por la fisión a nivel de un doble septo (**esquizolisis**). En la conidiogénesis **holotálica**, de la diferenciación de la hifa resulta la formación de un único conidio. En la conidiogénesis **tálico-ártrica**, la hifa se convierte en una cadena de conidios, si estos son liberados por esquizolisis se denomina **holoártrica**, si son liberados por rexolisis se denomina **enteroártrica**. Cuando los conidios se desarrollan en el interior de la hifa y son liberados mediante la lisis de esta, se denomina conidiogénesis **endógena** y si los conidios son producidos por la fragmentación transversal y longitudinal de la hifa se denomina conidiogénesis **sarcínica** (Kirk *et al.* 2008, de Hoog *et al.* 2011).

Otras estructuras no especializadas, pero de gran utilidad para la identificación de los hongos, son las denominadas clamidosporas, bulbilos, esclerocios y estromas. Estas corresponden a estructuras de supervivencia, producidas bajo condiciones ambientales adversas. Las **clamidosporas** se originan en forma holotálica y son diferenciables por poseer una pared gruesa, generalmente encapsulada y estar firmemente conectadas a la hifa **(Fig. 5a)**. Los **bulbilos** son estructuras compactas, multicelulares, carentes de estructura interna, originadas en forma acrópeta desde una hifa, cuyas células posteriormente se hinchan y pigmentan **(Fig. 5b)** (Kirk *et al.* 2008). Los **esclerocios** son masas firmes y compactas de hifas organizadas, usualmente adoptando una forma esférica y pueden o no contener tejido del huésped o suelo entre ellas, pero no contienen conidios (Kirk *et al.* 2008). Los **estromas** son estructuras similares a los esclerocios, pero de forma irregular y sobre o dentro de las mismas pueden desarrollarse estructuras esporulantes (Kirk *et al.* 2008).

Otros caracteres microscópicos importantes para la identificación de los hongos son el tamaño, grosor, tipo de superficie y coloración de sus estructuras, ya sean vegetativas o reproductivas, las cuales pueden ser lisas o rugosas, **hialinas**, si carecen de pigmentación, o **dematiáceas** (pigmentadas) si contienen melanina en la pared celular. A su vez, una característica diferencial muy importante radica en la morfología de los conidios. El sistema propuesto por Saccardo (1882), estableció una nomenclatura propia para la clasificación de los diversos tipos de conidios, aún utilizada en la actualidad (Alexopoulos *et al.* 1996); según la misma, estos pueden ser unicelulares (amerosporas), bicelulares (didimosporas), multicelulares (fragmosporas si presentan múltiples septos transversales o dictiosporas si presentan septos transversales y longitudinales). Además pueden adoptar formas muy variadas desde globosos, ovales o piriformes hasta formas helicoidales o ramificadas muy complejas (Kirk *et al.* 2008).

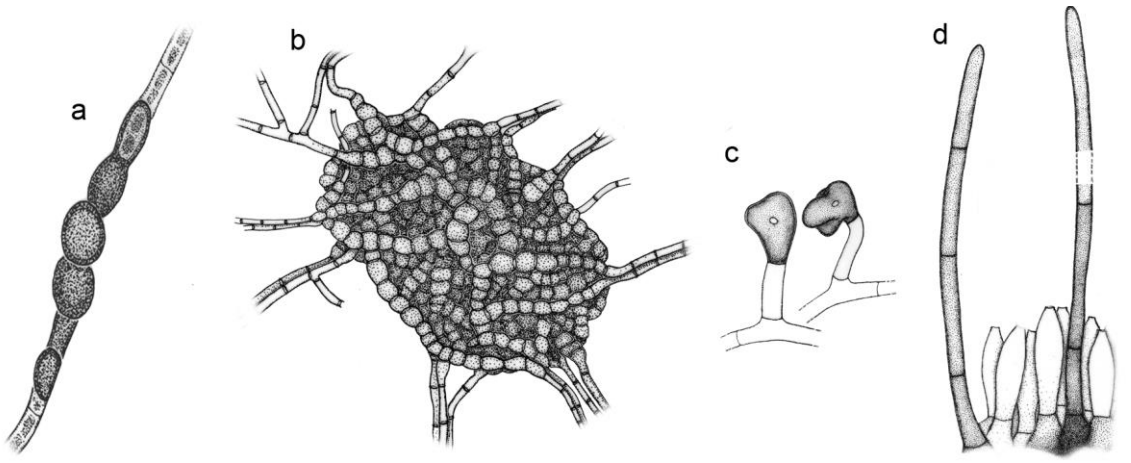

Figura 5 Otras estructuras de utilidad para la caracterización morfológica de los hongos. a. Clamidosporas. b. Bulbilo. c. Apresorios. d. Setas. Adaptado de Gené (1994)

Otras estructuras no reproductivas, pero que pueden ser de utilidad para la caracterización fúngica son los **apresorios** que se forman mediante el hinchamiento o modificación de una hifa y participan en la fijación del hongo al sustrato o al tejido del huésped **(Fig. 5c)** (Kirk *et al.* 2008). Las **setas**, es decir, hifas estériles, rectas, por lo general aguzadas, con paredes gruesas y pigmentadas, formadas habitualmente a partir de las paredes de un cuerpo fructífero y se les relaciona con una posible función protectora **(Fig. 5d)** (Kendrick 2001, Kirk *et al.* 2008).

Las características morfológicas más relevantes a estudiar para la identificación de las formas sexuales (teleomorfos) son las asociadas a las esporas sexuales y a las estructuras que las originan y protegen **(Fig. 6)**. En *Ascomycota*, las esporas sexuales, denominadas **ascosporas (Fig. 6a)**, pueden ser muy variables en morfología, número de células, color, ornamentación de su superficie, etc. Se desarrollan endógenamente en estructuras membranosas denominadas **ascos (Fig. 6b)**; los cuales pueden contener una o varias ascosporas, presentar una membrana simple (**unitunicados**) o doble (**bitunicados**), la cual puede ser persistente o ser lisada tempranamente y pueden o no liberar las ascosporas a través de una porción más delgada de la membrana en la región apical, denominada **opérculo**. Los ascos pueden desarrollarse directamente sobre hifas o a partir de cuerpos fructíferos denominados **ascocarpos** o **ascomas**, los cuales pueden presentar diversas formas: se denomina **gimnotecio** aquel ascoma formado por una agrupación laxa de hifas formando una red alrededor de un conjunto de ascos **(Fig. 6c)**; **apotecio**, si el ascoma es abierto en forma de disco con los ascos contenidos en el interior de una concavidad **(Fig. 6d)**; **peritecio**, si posee una forma piriforme con una abertura apical, el **ostiolo**, a través de la cual se liberan las ascosporas contenidas en su lumen **(Fig. 6e)**; **cleistotecio**, si es esférico, cerrado, sufriendo la ruptura o desintegración para liberar las ascosporas **(Fig. 6f)**, y **ascostroma**

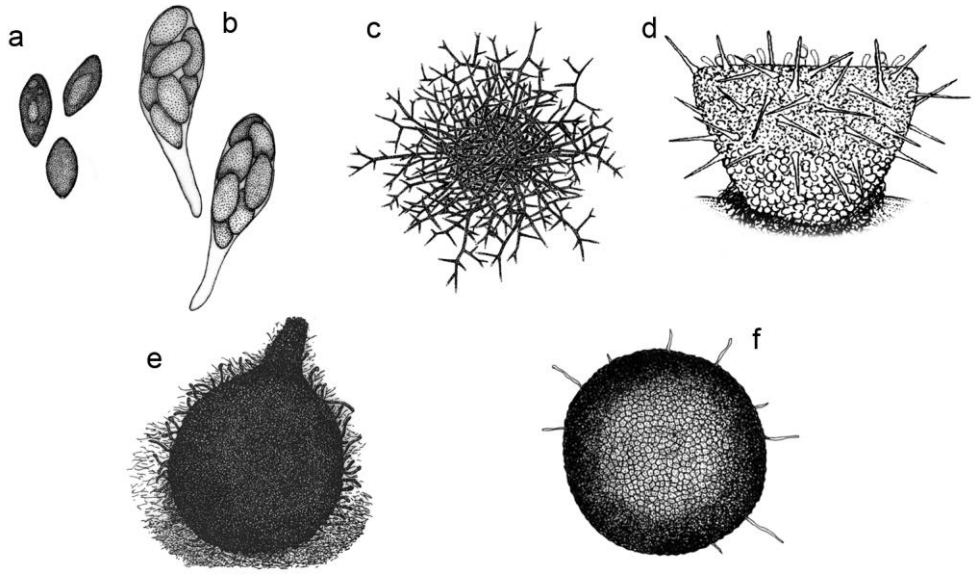

Figura 6 Estructuras reproductivas sexuales de *Ascomycota*. a. Ascosporas. b. ascos. c. Gymnotecio. d. Apotecio. e. Peritecio. f. Cleistotecio. Adaptado de Gené (1994) y Guarro *et al.* (2012).

o **pseudotecio** si los ascos se desarrollan directamente en la cavidad de un estroma, formado por el agrupamiento de hifas somáticas. En cuanto a la morfología del ascoma, tanto su forma, presencia o ausencia de cuello ostiolar y/o setas, tipo de agrupación celular que conforma su pared (**peridio**), color y textura, son características relevantes para la identificación. Los *Basidiomycota* se caracterizan por formar las esporas sexuales o **basidiosporas** externamente, sobre células especializadas denominadas **basidios**, formadas en la cavidad de cuerpos fructíferos denominados **basidiomas o basidiocarpos**. Otra característica diferencial de este grupo es la formación de conexiones citoplasmáticas intercelulares, denominadas **fíbulas**, proyecciones de la hifa que resultan en la fusión de dos células vecinas delimitadas por un septo.

Sin embargo, a pesar de la utilidad e importancia histórica de la caracterización morfológica en la identificación y taxonomía fúngica, la identificación de hongos patógenos sigue siendo una tarea difícil de realizar (Irinyi *et al.* 2015a), en parte por la falta en los laboratorios clínicos de profesionales especializados (Shenoy *et al.* 2007, Balajee *et al.* 2009), pero también, tal y como ya se indicó antes, debido a las propias limitaciones de las técnicas tradicionales basadas en el cultivo de las cepas. Muchos hongos pueden resultar difíciles o imposibles de cultivar *in vitro*. El aislamiento de un hongo en cultivo tampoco asegura su correcta identificación puesto que su morfología puede presentarse muy alterada o no expresar sus elementos reproductivos (pleomorfización). Además, mientras muchas especies diferentes presentan una alta similitud morfológica, otras pueden presentar diversas morfologías, incluyendo sinanamorfos o transiciones levadura-filamentoso en especies con ciclos vitales más

complejos (Irinyi *et al.* 2015a). Todo lo anterior dificulta enormemente su interpretación y puede determinar que la identificación sea incorrecta o sólo parcial (Guarro *et al.* 1999a). La correcta identificación del agente etiológico es fundamental dado que a menudo influye en la toma de decisiones clínicas así como en la instauración de un tratamiento antifúngico adecuado. El uso de otras técnicas más objetivas aplicadas a la identificación de los hongos, ha contribuido al agravamiento de esta problemática ya que ha permitido la detección y caracterización de nuevas especies patógenas, a menudo con escasas o nulas diferencias morfológicas, y por tanto muy difíciles o imposibles de identificar únicamente mediante técnicas tradicionales (Rodríguez-Tudela & Cuenca-Estrella 2001, Balajee *et al.* 2007a, Irinyi *et al.* 2015a).

1.2.2 Identificación molecular

El uso de herramientas de biología molecular ha significado un gran avance para la taxonomía, clasificación, delimitación de especies e identificación fúngica (Irinyi *et al.* 2015a). Sin embargo, en la actualidad aún no existe ningún método molecular ideal o de uso uso generalizado. Se pueden utilizar diferentes enfoques para cada uno de los objetivos que pretendamos abordar (identificación, evolucion, epidemiología, etc) y segun el hongo a investigar (Mitchell & Xu 2002). En la actualidad, la técnica más empleada es la **secuenciación del ADN**, principalmente del operón del ARN ribosómico. Este operón se compone de cuatro exones denominados 5.0S, 5.8S, 18S y 28S separados por espaciadores transcribibles, ETS e ITS (espaciador transcrito externo e interno), y un espaciador integénico (IGS). Dado sus bajas tasas de evolución, los genes 18S y 28S son comúnmente utilizados para establecer la posición taxonómica de un aislado a un nivel superior (orden, familia o género). En cambio, la **región ITS**, compuesta por dos espaciadores intergénicos ITS1 e ITS2 y el gen 5.8S, ha sido ampliamente utilizada tanto para la identificación de especies como para estudios filogenéticos (Schoch *et al.* 2012, Irinyi *et al.* 2015b), siendo recientemente propuesto como el marcador universal (código de barras o *barcode*) para la identificación de hongos (Schoch *et al.* 2012). Esta propuesta se basa fundamentalmente en su fácil amplificación y secuenciación, debido a sus múltiples copias en el genoma fúngico, su tamaño relativamente pequeño (alrededor de 500 pb), la disponibilidad de cebadores universales y su elevada variabilidad a nivel de secuencia, lo que permite obtener una buena resolución a nivel de especies, particularmente en la región ITS2 (Seifert 2009, Irinyi *et al.* 2015a). Sin embargo, aunque esta propuesta ha sido bien aceptada por la comunidad micológica, el marcador ideal aún no existe. A pesar de ser un muy buen marcador, la utilización de la región ITS como *barcode* ha sido criticada por su limitación para la diferenciación entre especies filogenéticamente muy cercanas o su falta de

resolución para la distinción a nivel de especie en determinados grupos fúngicos con mínima variabilidad interespecífica, como es el caso de *Aspergillus* spp. P. Micheli o *Colletotrichum* spp. Corda (Balajee *et al.* 2007a, Rojas *et al.* 2010, Irinyi *et al.* 2015b), o entre aquellos que presentan una importante variación intraespecífica como los miembros de los *Glomeromycota* o *Chytridiomycota* (Nilsson *et al.* 2008, Blaalid *et al.* 2013, Irinyi *et al.* 2015b). Además, la existencia de polimorfismos relacionados con la elevada cantidad de copias de la región ITS presentes en el genoma de un mismo microrganismo, como se ha observado en *Fusarium* Link (Irinyi *et al.* 2015b), sumado a la escasez de secuencias disponibles para comparación en bases de datos públicas o la escasa fiabilidad de alguna de ellas (Nilsson *et al.* 2006, Balajee *et al.* 2009) han llevado a la exploración de otros genes, principalmente entre aquellos codificantes de proteínas como la actina (*Act*), ARN polimerasa (*RPB*1 y *RPB*2), β-tubulina (*Tub*), calmodulina (*Cal*), factor de elongación (*Tef* o *EF*-1α), etc, los cuales, dada su presencia universal en todas las especies fúngicas y su alto contenido en intrones con tasas evolutivas más elevadas, constituyen buenas alternativas para su utilización como marcadores secundarios (Irinyi *et al.* 2015b).

Actualmente, la identificación de patógenos fúngicos mediante métodos moleculares se basa fundamentalmente en la secuenciación de uno o más genes y el establecimiento del grado de identidad de la secuencia problema o *query* comparada con secuencias nucleotídicas provenientes de material tipo o identificadas por otros métodos. Dicha comparación emplea algoritmos matemáticos disponibles en las bases de datos públicas como el NCBI (www.ncbi.nlm.nih.gov), CBS (www.cbs.knaw.nl), NITE (www.nbrc.nite.go.jp), ISHAM (http://its.mycologylab.org/) u otras bases de datos especializadas como Q-Bank (www.q-bank.eu), etc (Altschul *et al.* 1990). Los resultados son entregados en términos de porcentaje de identidad, complementados con una serie de parámetros como el *score*, puntuación que determina la calificación del alineamiento y es calculado en base al número y tipo de diferencias presentes entre la secuencia *query* y sus homólogas y el valor o parámetro E que determina la significancia estadística del alineamiento. Aunque no existe un punto de corte estandarizado, se estima que un grado de identidad igual o superior a 98% con una secuencia obtenida a partir de un cultivo tipo o de referencia de una especie determinada, es suficiente para una identificación a nivel de especie (Balajee *et al.* 2007a, 2009, Alvarez *et al.* 2009). Sin embargo, a pesar de ser una herramienta muy potente y de gran utilidad para la rápida identificación de prácticamente cualquier organismo, sus resultados deben ser interpretados con cautela (Balajee *et al.* 2009, Samson *et al.* 2010) debido a que éstos están condicionados por la disponibilidad de secuencias con las cuales comparar. Es necesario considerar tres factores importantes: i) para muchos géneros y especies

fúngicas no existen secuencias provenientes de material tipo o de referencia, ya sea, por la inexistencia de cultivos o por la falta de estudios previos; ii) una gran proporción (aproximadamente 20%) de las secuencias disponibles en las bases de datos públicas fueron identificadas de forma incorrecta, no hacen referencia a la cepa a partir del cual fueron generadas o poseen ambigüedades (Nilsson *et al.* 2006, 2008) y iii) los programas que determinán el porcentaje de identidad trabajan en función de algoritmos heurísticos, por lo tanto no es posible asegurar que esta es la solución (más) correcta (Meek *et al.* 2003).

Debido, en parte, a los factores antes mencionados, así como a la necesidad de contar con diagnósticos más certeros y rápidos, la tendencia actual va dirigida a la identificación por medio del análisis filogenético de uno o más genes ya sea en forma combinada o individual, comparando posteriormente los resultados obtenidos (Taylor *et al.* 2000). Ésto, permite mejorar la precisión de la identificación y la delimitación a nivel de especie, contribuyendo al descubrimiento de nuevos taxones al poner de manifiesto especies, hasta ese momento crípticas, entre grupos de individuos cuyas diferencias fenotípicas (morfológicas y fisiológicas) son prácticamente nulas (Roe *et al.* 2010, de Hoog *et al.* 2015, Irinyi *et al.* 2015b). La **filogenia** pone de manifiesto las relaciones evolutivas dentro un grupo determinado de organismos, las cuales por lo general se representan de forma gráfica mediante un árbol filogenético (Wiley *et al.* 1991). Un **árbol filogenético** o cladograma está compuesto por nodos y ramas que los conectan y es básicamente una representación hipotética de la historia evolutiva de un grupo de organismos (Wiley *et al.* 1991, Salemi & Vandamme 2004). Se denominan **nodos terminales** o unidades taxonómicas operacionales (OTUs) a los taxones existentes, conectados o provenientes de una sola rama, mientras que los **nodos internos** o unidades taxonómicas hipotéticas (HTUs) son aquellos conectados por más de una rama y que corresponden a ancestros teóricos a partir de los cuales derivan los OTUs. Un grupo de taxones que pertenecen a una misma rama tienen un origen **monofilético** y se denominan **clado** (Salemi & Vandamme 2004). Un grupo **parafilético** es aquel que incluye al ancestro común de sus miembros, pero no a todos los descendientes de este, en cambio se denomina grupo **polifilético** aquel que reúne artificialmente ramas no conectadas por un antepasado común. La **raíz** de un árbol filogenético corresponde al ancestro común de todo el grupo y representa su origen evolutivo (Wiley *et al.* 1991, Alexopoulos *et al.* 1996, Salemi & Vandamme 2004).

La **filogenia molecular** establece las relaciones evolutivas en base al análisis de secuencias de ADN (Whelan *et al.* 2001). El punto de partida es siempre una matriz o **alineamiento**, compuesta por fragmentos de ADN de diversos organismos, ordenados en función de su porcentaje de identidad. La formulación de la hipótesis en filogenia

molecular se realiza mediante el uso de algoritmos computacionales, existiendo varias alternativas, las cuales pueden ser clasificadas en dos grandes grupos: métodos basados en distancias y métodos basados en caracteres. Los **métodos basados en distancias** crean la hipótesis evolutiva en base a una matriz de distancias que puede ser calculada a partir de un alineamiento de secuencias, matrices de puntuación de alineamientos o del número de discordancias entre secuencias (distancia p) (Arnedo 1999, Heng 2006). Los algoritmos más conocidos basados en este enfoque son Neighbor-Joining (NJ) (Saitou & Nei 1987), el método de la media (UPGMA), BioNJ (Gascuel 1997), y el *Weighted* NJ o *Weighbor* (Bruno *et al.* 2000). Estos métodos poseen la ventaja de ser computacionalmente eficientes y permiten construir árboles rápidamente a partir de bases de datos extensas. Sin embargo, dado que se basan sólo en distancias, no utilizan toda la información contenida en el alineamiento. El árbol final sólo muestra el nivel de similitud entre los taxones y no refleja relaciones evolutivas, mientras que la posición que ocupan los taxones en el árbol está influenciada directamente por la posición de estos en el alineamiento (Farris *et al.* 1996). Los **métodos basados en caracteres**, en cambio, consideran la información contenida en el alineamiento y evalúan los cambios evolutivos que puedan explicar los datos presentes en la matriz y, con excepción de la máxima parsimonia, establecen su probabilidad de acuerdo a modelos de sustitución nucleotídica calculados para cada alineamiento (Chun & Hong 2010). La **máxima parsimonia (MP)** se basa en el principio de la "navaja de Ockham" (Audi 1999) o ley de parsimonia que explica que "en igualdad de condiciones, la explicación más sencilla suele ser la correcta", es decir, en un contexto filogenético la explicación más probable para la evolución de un grupo determinado de organismos es aquella que involucra el menor número de cambios. Para esto, MP busca entre el universo de árboles posible, aquel o aquellos que mejor expliquen los datos del alineamiento con un mínimo de sustituciones por sitio. Este método no necesita de complejos modelos matemáticos de sustitución nucleotídica, pero esto es considerado a su vez su principal desventaja ya que al asumir que la evolución es homogénea y constante en el tiempo y para todos los genes, puede no reflejar las relaciones evolutivas más correctas, estando especialmente influenciado cuando en el alineamiento se comparan taxones con tasas de sustitución muy diferentes. Estos últimos se reflejan en el árbol como "ramas largas", un fenómeno conocido como "atracción de las ramas largas" (*long branch attraction*) (Felsestein 1978). En la **Máxima Verosimilitud** o *Maximum Likelihood* (ML), se evalúa básicamente en términos probabilísticos, como cada uno de los árboles obtenidos explican los datos observados en el alineamiento y elige el más probable. Este método permite el uso de modelos evolutivos complejos para explicar las diferentes tasas de mutación y ha sido

considerado como el algoritmo más preciso para la reconstrucción de árboles filogenéticos (Kuhner & Felsenstein 1994; Hall 2005, Heng 2006). Sin embargo, tiene el inconveniente de ser muy exigente en términos de potencia computacional. A pesar de existir muchos modelos evolutivos, éstos no son combinables, lo que en cierta medida limita su utilidad para inferir filogenias complejas; es decir, a partir de particiones combinadas. Además, dado que la selección del árbol inicial se basa en una búsqueda heurística; en teoría, deberían realizarse varias réplicas del análisis para asegurarse que se está encontrando el árbol más adecuado. Por el contrario, en la **Inferencia Bayesiana** (BI) se evalúa el grado en que los datos observados en el alineamiento explican los diferentes árboles obtenidos, finalmente el algoritmo calcula el árbol consenso que mejor representa la totalidad de árboles observados. La principal ventaja de este método es su flexibilidad y la posibilidad de combinar diferentes modelos de sustitución para alineamientos consistentes en diferentes particiones. Al generar muchos árboles, éstos pueden en cierta medida ser considerados réplicas de un mismo análisis. Sin embargo, la BI es el método más exigente en términos computacionales.

Toda reconstrucción filogenética necesita de algún método que valide la robustez de la topología de los árboles obtenidos. Esto se realiza mediante tests estadísticos, siendo los más utilizados el análisis de *bootstrap* y la determinación de la probabilidad posterior. El **análisis de *bootstrap* (bs)** (Felsestein 1985), empleado tanto en NJ, ML y MP, consiste en la realización de réplicas al azar a partir del alineamiento original ejecutando muestreos aleatorios entre pares de taxones. Para cada réplica se genera un árbol filogenético y luego se evalúa el porcentaje de árboles en los cuales se presenta cada nodo. En general, se considera que un nodo con un valor bs ≥70% posee un buen soporte estadístico. El cálculo de la **probabilidad posterior (pp)** se emplea para evaluar la robustez del árbol consenso obtenido por BI y es una medida de la probabilidad de que el clado correspondiente sea el verdadero para el modelo escogido (Huelsenbeck *et al.* 2002). Se considera un clado bien soportado aquel que presente un valor de pp ≥0.95. Tanto para la determinación de valores de bs como de pp, cualquier clado no soportado indica que existe una alta probabilidad de que este se haya formado por efecto del azar (Erixon *et al.* 2003). Si bien ambos sistemas son equivalentes, éstos no son universalmente comparables entre sí y pueden entregar resultados muy variables según los datos disponibles, aunque por lo general dichos sistemas tienden a coincidir en función de la correcta selección/cálculo del modelo de sustitución (Erixon *et al.* 2003).

1.3 Hongos e infección

La gran mayoría de las especies de hongos conocidas hasta la fecha son organismos de bajo o nulo potencial patógeno para el ser humano u otros animales, incapaces de tolerar las condiciones fisiológicas en un huésped normal (Crous *et al.* 2009a), explicable además por el alto nivel de inmunidad natural frente a la gran mayoría de especies (Brock 2006). Sin embargo, ciertos hongos son capaces de superar estas condiciones adversas produciendo un cuadro infeccioso. La infección producida por un hongo recibe el nombre de **micosis** (Kirk *et al.* 2008).

Tradicionalmente, las micosis han sido clasificadas en base a criterios clínicos, principalmente teniendo en cuenta la localización anatómica de la infección, por lo que en este sentido se distinguen cuatro tipos: superficiales, cutáneas, subcutáneas y profundas. En las **micosis superficiales** se ven afectadas las capas más externas de la piel y/o sus anexos (pelos, uñas, pezuñas), sin invasión del tejido vivo y, por lo tanto, sin producir una respuesta inmunitaria del huésped (Walsh & Dixon 1996, de Hoog *et al.* 2011). Por el contrario, si la infección afecta a capas más profundas de la piel y membranas mucocutáneas, desencadenando una respuesta inflamatoria del huésped, ésta recibe el nombre de **micosis cutánea**, y pueden distinguirse dos tipos, dermatofitósis y dermatomicosis: las **dermatofitósis** son producidas por hongos queratinofílicos pertenecientes a los géneros *Epidermophyton* Sabour., *Microsporum* Gruby y *Trichophyton* Malmsten, mientras que las dermatomicosis son producidas por una amplia gama de hongos no relacionados como *Candida* Berkhout, *Cladosporium* Link o *Aspergillus* P. Micheli ex Haller (Walsh & Dixon 1996). Las **micosis subcutáneas** son infecciones crónicas resultado de la inoculación traumática del hongo en el tejido subcutáneo el cual provoca una fuerte reacción inmunitaria derivando en la formación de quistes o granulomas (de Hoog *et al.* 2011). Bajo esta denominación se incluyen lesiones no ulcerativas, eumicetomas, etc. A su vez, las lesiones no ulcerativas pueden clasificarse en hialohifomicosis y feohifomicosis según las características morfológicas de los agentes causales. En la **hialohifomicosis**, el análisis microscópico de las lesiones demuestra la presencia de elementos fúngicos hialinos (ejemplos: *Acremonium* Link, *Fusarium* Link, algunas especies de *Scopulariopsis* Bainier, *Trichoderma* Pers., etc), mientras que en la **feohifomicosis** se observan elementos fúngicos pigmentados o dematiáceos (ejemplos: algunas especies de *Scopulariopsis*, *Cladosporium*, etc) (de Hoog *et al.* 2011). Las **micosis profundas** implican la afectación de tejidos profundos y órganos internos. Son causadas exclusivamente por hongos dimórficos (micosis endémicas) u hongos oportunistas. Mientras las micosis endémicas tienen como vía de entrada el tracto respiratorio, los patógenos oportunistas pueden ingresar a través de la vía respiratoria, digestiva o afectar directamente el torrente circulatorio mediante

contaminación de sistemas de infusión intravascular (Walsh & Dixon 1996). La micosis profundas pueden ser localizadas o afectar tejidos contiguos o no contiguos (**micosis diseminada**) a través del sistema circulatorio o linfático (de Hoog *et al.* 2011).

Actualmente, se estima en alrededor de 500 especies capaces de actuar como patógenos en humanos (Brandt & Warnock 2015). Sólo un número muy limitado de especies fúngicas son considerados **patógenos primarios** (ej. *Blastomyces dermatitidis*, *Coccidioides* spp. *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*) es decir, que pueden provocar infección en individuos inmunocompetentes. Por lo general, los hongos patógenos primarios están confinados a determinadas regiones geográficas, por lo cual estas micosis reciben el nombre de **micosis endémicas**. Sin embargo, si las barreras inmunológicas primarias del hospedador son superadas, ciertos grupos de hongos, normalmente saprobios ambientales ubicuos o comensales, son capaces de colonizar, infectar o invadir tejidos y producir enfermedad (Crous *et al.* 2009a). Estos hongos se denominan **patógenos oportunistas** y la infección producida por los mismos, **micosis oportunista**. En la actualidad, las micosis oportunistas representan la principal infección secundaria en la población inmunocomprometida y los hongos una de las causas más importantes de infección nosocomial. Se considera que más del 40% de las muertes relacionadas con infecciones adquiridas en el ambiente hospitalario son resultado de una infección fúngica, y se estima que este porcentaje seguirá aumentando en la medida que aumente la población inmunocomprometida, particularmente aquellos individuos bajo terapias inmunosupresoras, VIH y edades extremas (Brock 2006).

Mientras la gran mayoría de micosis oportunistas son producidas por hongos levaduriformes, se ha visto una notable expansión del espectro de agentes causales, incluyendo un gran número de hongos filamentosos tradicionalmente considerados como simples contaminantes ambientales o colonizadores, los cuales, a pesar de su baja frecuencia se asocian comúnmente con desenlaces fatales o requieren de complejas debridaciones quirúrgicas, dada la incapacidad del huésped para iniciar una respuesta inmune eficaz y la tendencia de estos hongos a presentar una elevada resistencia a los antifúngicos actualmente disponibles en el mercado (Pontón & Quindós 2009, de Hoog *et al.* 2015, Köhler *et al.* 2015).

1.4 Generalidades sobre los géneros fúngicos incluidos en la presente tesis

En la presente tesis se han estudiado algunos géneros de hongos filamentosos ambientales: *Acrophialophora* Edward, *Cladosporium* Link, *Microascus* Zukal, *Scopulariopsis* Bainier y *Trichoderma* Pers., pertenecientes a diversas familias de los *Sordariomycetes* y *Dothidiomycetes* **(Fig. 7)** y considerados patógenos oportunistas. Los

organismos involucrados en estos cuadros infecciosos, por lo general, corresponden a hongos ubicuos de bajo potencial patogénico (Pfaller *et al.* 2006, Puebla 2012, Brandt & Warnock 2015). Debido a su baja incidencia en clínica se carece de datos sobre su epidemiología y tratamiento antifúngico. Pero a su vez son hongos poco estudiados a partir de herramientas modernas basadas en biología molecular, por lo que su taxonomía, relaciones filogenéticas o espectro de especies de importancia clínica son todavía poco conocidos en la mayoría de los géneros citados.

Mientras que las especies del género *Trichoderma* habían sido consideradas hasta recientemente como inocuas para el hombre y otros animales (Kredics *et al.* 2011), *Acrophialophora*, *Cladosporium*, *Microascus* y *Scopulariopsis* tienen una extensa historia como agentes causantes de infecciones en el hombre, pero de muy baja incidencia o de dudosa patogenicidad (de Hoog *et al.* 2011, Sandoval-Denis *et al.* 2013). Sin embargo, cada vez con mayor frecuencia se publican casos de infecciones producidas por especies de los géneros antes mencionados, así como de otros géneros raros o poco comunes en clínica, los cuales, a pesar del desarrollo de nuevas técnicas diagnósticas y terapias antifúngicas, se asocian con altas tasas de morbilidad y mortalidad (Pfaller *et al.* 2006, Puebla 2012). Ésto se ha relacionado fundamentalmente con el incremento o combinación de una serie de factores de riesgo, entre los que podemos destacar la presencia de enfermedades de base severas, la aplicación de terapias inmunosupresoras intensas y/o prolongadas, la aparición de cepas resistentes a la terapia antifúngica, pero tambien la falta de estudios clínicos robustos que avalen el desarrollo de esquemas de tratamiento efectivos (Romani 2008, Puebla 2012).

1.4.1 *Acrophialophora*

Acrophialophora es un género de hongos anamórficos termotolerantes, capaces de crecer y esporular a temperaturas superiores a 40ºC, aislados fundamentalmente de suelos, particularmente de la India o suelos áridos de Egipto o Pakistán, de raíces u hojas de plantas gramíneas o leguminosas y material de compostaje (Domsch *et al.* 2007).

Propuesto por Edward (1959), inicialmente con una sola especie, *Acrophialophora nainiana* Edward, aislada de suelo de la India, y caracterizada por tener micelio al principio hialino, volviéndose subhialino a marrón claro, desarrollar conidióforos erectos, no ramificados, septados, con paredes gruesas, verrucosas y pigmentadas que se tornan subhialinas hacia el ápice, en el cual se originan fiálides en forma de botella, con base ancha y apice delgado, las cuales dan lugar a conidios de hialinos a subhialinos, unicelulares, equinulados y agrupados en largas cadenas.

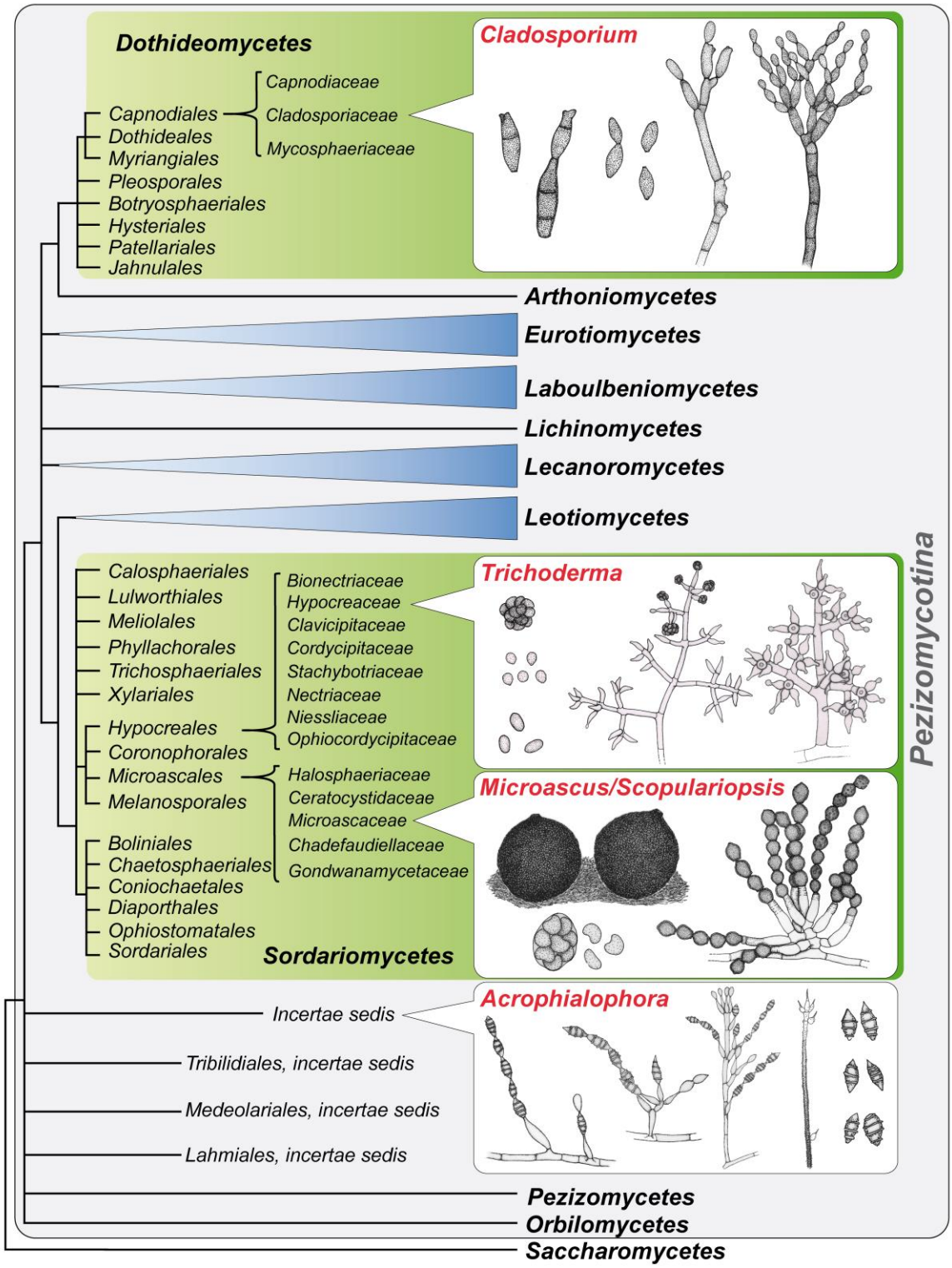

Figura 7 Arbol esquemático mostrando las ubicaciones taxonómicas y características morfológicas principales de los generos incluidos en este trabajo (en rojo). Adaptado de Hibbett *et al.* (2007) y *Catalogue of Life* (http://www.catalogueoflife.org/).

Sin embargo, dicho género no fue inmediatamente aceptado y *A. nainiana* fue transferida al género *Paecilomyces* Bainier y considerada sinónima de *P. fusisporus* Saksena (Barron 1968, Dal Vesco & Peyronel 1968). Posteriormente, el género fue revalidado por Samson & Mahmood (1970) principalmente sobre la base de las diferencias en la morfología de los conidióforos de *Acrophialophora* y *Paecilomyces*. Dichos autores rechazaron la sinonimia antes establecida restableciendo la especie tipo y propusieron la nueva combinación *Acrophialophora fusispora* (Saksena) Samson para *P. fusisporus* (sinónimo *Masoniella indica* M.A. Salam & P. Rama Rao) y describieron una nueva especie, *Acrophialophora levis* Samson & Tariq Mahmood. Sin embargo, Ellis (1971) consideró a *A. fusispora*, *A. nainiana* y *Ampullifera seudatica* Subrahamanyam como especies sinónimas, aceptando tan solo dos especies válidas para el género: *A. fusispora*, como especie tipo y *A. levis*. Posteriormente, Al-Mohsen *et al.* (2000), en una revisión basada en el estudio de cepas clínicas y ambientales del género, consideraron estas dos especies como sinónimas, debido a la ausencia de caracteres morfológicos evidentes para diferenciarlos, proponiendo *A. fusispora* como la única especie para el género. La posición taxonómica de *Acrophialophora* es aún incierta, clasificándose como un género *incertae sedis* en *Pezizomycotina*.

1.4.2 *Cladosporium*

Cladosporium agrupa a hongos dematiáceos, cosmopolitas, comúnmente aislados de restos vegetales, suelo, aire, agua, alimentos congelados, pero tambien como comensales intestinales en insectos e incluso como organismos fitopatógenos, etc (Domsch *et al.* 2007, Bensch *et al.* 2012). Morfológicamente se caracterizan por tener tanto las hifas como el aparato conidiogénico pigmentados. Los conidióforos son complejos, usualmente ramificados, con conidiogénesis holoblástica y células conidiógenas generalmente poliblásticas y simpodiales, formando largas cadenas acrópetas, a menudo ramificadas, de conidios. Los conidios suelen ser muy variables tanto en su forma, ornamentación como en número de septos transversales (Bensch *et al.* 2012). La principal característica morfológica del género es la cicatriz presente en el locus conidiógeno y en el conidio, denominada cicatriz coronada o cladosporioide, ésta se compone de un anillo periclinal protuberante que rodea a un domo convexo central (David 1997).

El género *Cladosporium* fue originalmente descrito por Link, incluyendo cuatro especies: *C. abietinum* (Pers.) Link, *C. atrum* Link, *C. aureum* Link y *C. herbarum* (Pers.) Link, siendo posteriormente lectotipificado con *C. herbarum* (Clements & Shear 1931, Bensch *et al.* 2012). Sin embargo, desde su descripción se le han sumado más de 700 especies. Según Bensch *et al.* (2012), la existencia en la literatura de

descripciones muy imprecisas del género sumado a la gran variabilidad morfológica de las especies y a la gran diversidad de sustratos que colonizan, son factores que han propiciado la descripción de esta gran diversidad de especies. Debido a que algunas de las especies de *Cladosporium* se asociaron al género teleomórfico *Mycosphaerella* Johanson, Öfvers. K. VetenskAkad, ambos géneros se clasificaron en la familia *Mycosphaerellaceae*, originalmente incluida en el orden *Dothideales* (Bensch *et al.* 2012). Sin embargo, recientes estudios de *Cladosporium* y *Mycosphaerella*, basados en filogenia molecular, demuestran que ambos géneros no constituyen un linaje monofilético. *Cladosporium* se incluye en una familia independiente (*Cladosporiaceae*) y sus teleomorfos se transferieron al nuevo género *Davidiella* Crous & U. Braun (Crous *et al.* 2007a). Actualmente tanto la familia *Cladosporiaceae* como *Mycosphaerellaceae* pertenecen al orden *Capnodiales* (*Dothideomycetes*) (Schoch *et al.* 2006, 2009a, 2009b, Crous *et al.* 2009b, Bensch *et al.* 2012). Otros estudios moleculares más recientes han permitido delimitar con mayor presición tanto al género como a sus especies. Dichos estudios pusieron de manifiesto que *Cladosporium*, en un sentido amplio, incluía un grupo heterogéneo de microorganismos que si bien presentaban afinidad morfológica, no estaban relacionados evolutivamente, estando distribuidos en diferentes géneros, familias e incluso ordenes de *Sordariomycetes* (de Hoog *et al.* 1995, Crous *et al.* 2007b, Seifert *et al.* 2007, Schubert *et al.* 2007a, Braun *et al.* 2008). Esta reorganización taxonómica afectó a muchas especies originalmente incluidas en *Cladosporium* y de interés tanto fito- como zoopatológico, incluyendo las especies termotolerantes consideradas patógenos primarios en humanos como *C. bantianum* (Sacc.) Borelli, *C. carrionii* Trejos y *C. devriesii* Padhye & Ajello, actualmente clasificadas en el género *Cladophialophora* Borelli (de Hoog *et al.* 1995). Además, se demostró que las especies más frecuentemente aisladas en el ambiente, como *C. cladosporioides* (Fresen.) G.A. de Vries, *C. herbarum* y *C. sphaerospermum* Penz. son polifiléticos y corresponden realmente a complejos de especies crípticas (Schubert *et al.* 2007b, Zalar *et al.* 2007, Dugan *et al.* 2008, Bensch *et al.* 2010).

1.4.3 *Microascus*, *Scopulariopsis* y géneros relacionados

El género *Microascus*, erigido por Zukal (1885) y tipificado por *M. longirostris* Zukal, es un género tradicionalmente referido a formas sexuales de la familia *Microascaceae* (*Microascales*, *Sordariomycetes, Ascomycetes*). Esta familia agrupa a hongos saprobios comúnmente colonizadores del suelo y material vegetal en descomposición, aunque se han aislado también del aire, papel y ambientes húmedos (Barron *et al.* 1961, Morton & Smith 1963, Samson *et al.* 2010). *Microascus* actualmente comprende 32 especies de hongos pleomórficos con formas asexuales

clasificadas en los géneros *Scopulariopsis* Bainier, *Wardomyces* Brooks & Hansf. y *Wardomycopsis* Udagawa & Furuya (Malloch 1970, Udagawa & Furuya 1978, Guarro *et al.* 2012).

Las especies de ***Microascus*** se caracterizan por la formación de ascomas periteciales, esféricos o ampuliformes, que originan ascos de ovales a esféricos, evanescentes y con 8 esporas unicelulares, las cuales son asimétricas, más o menos reniformes o triangulares y con 0-2 poros germinativos. Las ascosporas se liberan formando largos cirros o acúmulos gelatinosos en el extremo apical del cuello del ascoma (Barron *et al.* 1961, Morton & Smith 1963, Guarro *et al.* 2012).

Las especies de ***Scopulariopsis*** son hialinas o dematiáceas, caracterizadas por tener células conidiógenas anelídicas que originan conidios secos, los cuales se disponen en largas cadenas basípetas (Morton & Smith 1963, Samson *et al.* 2010).

Wardomyces incluye formas asexuales que se caracterizan por tener un micelio hialino y por producir conidios dematiáceos provistos de una línea germinativa, los cuales se forman a partir de células conidiógenas poliblásticas. Las especies *W. aggregatus* Malloch, *W. columbinus* (Demelius) Henneb., *W. dimerus* W. Gams y *W. simplex* J. Sugiy., Y. Kawas. & H. Kurata se distinguen por tener además un segundo tipo de conidiogénesis, similar al de *Scopulariopsis*, el cual origina cadenas de conidios, unicelulares o bicelulares, a partir de anélides (Gams 1968, Hennebert 1968, Sugiyama *et al.* 1968, 1969). Cabe destacar, sin embargo, que las especies que presentaban dos tipos de conidiogenesis fueron transferidas a otros géneros, *Gamsia* y *Hennebertia* (Morelet 1969), aunque este cambio taxonómico no fue aceptado por diversos autores (Domsch *et al.* 2007, Seifert *et al.* 2011, Whitton *et al.* 2012).

Las especies de ***Wardomycopsis*** se caracterizan, al igual que en *Wardomyces,* por formar conidios dematiáceos provistos con línea germinativa, pero dispuestos en cortas cadenas y naciendo a partir de anélides (Udagawa & Furuya 1978).

Malloch (1970) y Udagawa & Furuya (1978) describieron especies de *Microascus* con anamorfos pertenecientes a *Wardomyces* (*Microascus giganteus* Malloch) y *Wardomycopsis* (*Microascus inopinatus* Udagawa & Furuya), respectivamente.

La conexión entre *Microascus* y sus diversos géneros anamórficos ha sido objeto de debate a lo largo de casi cien años. La relación entre *Microascus* y *Scopulariopsis* fue originalmente establecida por Curzi (1930, 1931) tras la descripción de *Microascus cirrosus*, considerando además a los géneros *Sphaerella* Hans., *Peristomium* Lech., *Nephrospora* Loub. y *Acaulium* Sopp como sinónimos de *Microascus*. *Scopulariopsis* fue propuesto por Bainier (1907) para acomodar tres

especies: *S. brevicaulis* (Sacc.) Bainier como tipo del género, *S. rubellus* Bainier y *S. rufulus* Bainier, especies que originalmente estaban incluidas en la sección *anomala* del género *Penicillium* (Biourge 1923). Actualmente el género incluye un total de 77 especies (Fuente: Index Fungorum, Mycobank), muchas de las cuales no han sido revisadas desde su publicación original. Por otro lado, ocho de estas han sido asociadas a su correspondiente teleomorfo (*M. brevicaulis, M cinereus, M. cirrosus, M. longirostris, M.manginii, M. niger, M soppii y M. trigonosporus*).

El género *Pithoascus* Arx fue descrito por von Arx (1973a) para incluir a tres especies, *P. nidicola* (Massee & E.S. Salmon) Arx como especie tipo, *P. intermedius* (C.W. Emmons & B.O. Dodge) Arx y *P. schumacheri* (E.C. Hansen) Arx, segregadas de *Microascus* y caracterizadas por formar ascomas con ostiolos rudimentarios o ausentes, ascosporas fusiformes desprovistas de poros germinativos y ausencia de anamorfo (Skou 1973, von Arx 1973a, 1973b). Además de estas tres especies, en el género se incluyeron otras dos, *P. exsertus* (Skou) Arx *y P. stoveri* Arx (von Arx *et al.* 1988). La posterior detección de la presencia, aunque muy reducida, de anamorfos similares a *Scopulariopsis* en *P. intermedius*, *P. nidicola* y *P. schumacheri* llevó a Valmaseda *et al.* (1986) a redescribir el género (Roberts 1985, Domsch *et al.* 2007). Sin embargo, la separación de *Microascus* y *Pithoascus* no ha sido completamente aceptada, siendo considerados ambos géneros sinónimos por muchos autores (Malloch & Hubart 1987, Abbott *et al.* 2002, Guarro *et al.* 2012).

Diversos autores han sugerido la inclusión en el género *Scopulariopsis* de todas las especies anamórficas relacionadas con *Microascus*, así como las descritas en los géneros *Cephalotrichum* Link, *Doratomyces* Corda, *Kernia* Nieuwl. y *Trichurus* Clem. (Gams 1968, Malloch & Cain 1971). Sin embargo, los escasos estudios moleculares que se han realizado hasta la fecha demuestran que tanto *Microascus* como *Scopulariopsis* son géneros polifiléticos (Issakainen *et al.* 2003, Ropars *et al.* 2012, Lackner *et al.* 2014), por lo que antes de proponer cualquier cambio taxonómico es necesario profundizar en las relaciones filogenéticas de todos estos hongos.

1.4.4 *Trichoderma*

El género *Trichoderma* es un grupo muy numeroso de hongos asexuales morfológicamente muy similares entre sí y cuyos teleomorfos pertenecen al género *Hypocrea* Fr. Son especies saprobias, muchas de ellas termotolerantes, ubicuas y en su mayoría de distribución mundial. Se encuentran en una gran diversidad de sustratos, especialmente suelos de bosques o de cultivo, madera en descomposición, papel, textiles, etc (Domsch *et al.* 2007), y han sido extensivamente estudiados dadas sus propiedades ecológicas y fisiológicas. Las especies de *Trichoderma* son

importantes productores de compuestos bioactivos y algunas se usan como agentes para el biocontrol de hongos fitopatógenos (Domsch *et al.* 2007).

Se caracterizan por tener un crecimiento muy rápido y expansivo, con colonias al principio de color blanco que luego se vuelven amarillas o verdes en su superficie por la intensa producción de conidios. Producen conidióforos con múltiples ramificaciones dicotómicas o en verticilos y pueden presentar apéndices estériles. Por lo general, dichos conidióforos se agrupan en estructuras similares a esporodoquios denominadas pústulas. Las células conidiógenas son fialídicas, tienen forma de botella y se desarrollan en las ramas laterales del conidióforo. Los conidios suelen ser de subglobosos a elipsoidales, lisos o rugosos, hialinos o más comúnmente de color verde (Domsch *et al.* 2007).

Trichoderma fue descrito por Persoon (1794) con *T. viride* Pers. como especie tipo y otras tres especies; *T. caesium* Pers., *T. nigrescens* Pers. y *T. roseum* Pers., aunque más tarde Bisby (1939) lo consideró un género monotípico con *T. viride* como su única especie incluida. Rifai (1969) realizó la primera revisión taxonómica moderna del género y concluyó que *Trichoderma* constaba de al menos nueve especies, las cuales fueron agrupadas en función de su morfología; dichos grupos se trataron como "agregados" de especies (Druzhinina & Kubicek 2005, Kredics *et al.* 2011, Samuels *et al.* 2012). Posteriormente, Bisset (1984, 1991a, 1991b, 1991c) y Gams & Bissett (1998) elevaron estos "agregados" a la categoría de secciones, un sistema aún utilizado en la actualidad y en el que se consideran un total de cinco secciones morfológicas: *Longibrachiatum*, *Pachybasium*, *Trichoderma*, *Saturnisporum* e *Hypocreanum*, con aproximadamente 40 especies en total (Druzhinina & Kubicek 2005). La aplicación de herramientas de biología molecular a partir del año 2000 llevó a considerables cambios en la taxonomía de *Trichoderma*. Kullnig-Gradinger *et al.* (2002) reconocieron 46 especies filogenéticas mediante el análisis de secuencias de genes ribosómicos y fragmentos de los genes codificantes para el factor de elongación (*Tef*1) y la Endoquitinasa 42 (*chi*18-5). Más tarde, Druzhunina & Kubicek (2005) aumentaron a 88 el número de taxones en *Trichoderma*/*Hypocrea*, estandarizando un sistema de identificación mediante *barcoding* basado en los marcadores antes mencionados. Desde entonces el número de especies aceptadas de *Trichoderma* prácticamente se ha quintuplicado, comprendiendo actualmente 252 especies (Bissett *et al.* 2015). Es importante resaltar que la identificación fenotípica de todos estos hongos es muy problemática, por lo que se recomienda que cualquier identificación en *Trichoderma* debe de ser confirmada mediante secuenciación de ADN (Kredicks *et al.* 2011).

Cabe destacar que éste es uno de los pocos grupos de hongos pleomórficos en los cuales la comunidad micológica ha tomado una decisión referente a la conservación de un nombre de acuerdo al nuevo código de nomenclatura (Jaklitsch & Voglmayr 2013). Durante el año 2013, y mediante una consulta llevada a cabo por la Subcomisión Internacional sobre *Trichoderma* e *Hypocrea* (ISTH), perteneciente a la Comisión Internacional para la Taxonomía Fúngica, se propuso adoptar el nombre de *Trichoderma* para las especies incluidas en el binomio *Trichoderma*/*Hypocrea* a la espera de su aceptación por el Comité de Nomenclatura de Hongos (Jaklitsch & Voglmayr 2013, Rossman *et al.* 2013).

1.4.5 Importancia clínica

Los géneros antes mencionados son ubicuos en la naturaleza, colonizando principalmente en suelo, aire y material vegetal de todo el mundo. Sin embargo, algunas de sus especies son capaces de causar infecciones en el hombre y animales, por lo que se los considera pátogenos oportunistas emergentes, de particular importancia para la población inmunusuprimida, principalmente pacientes transplantados o con tumores linforreticulares o de tejidos hematopoyéticos (de Hoog *et al.* 2011, Kredics *et al.* 2011).

Existen relativamente pocos casos clínicos atribuidos al género *Acrophialophora*, siendo *A. fusispora* la única especie citada en clínica y asociada principalmente a cuadros de colonización o infección pulmonar, especialmente en transplantados pulmonares (Al-Mohsen *et al.* 2000, González-Escalada *et al.* 2000, Cimon *et al.* 2005, Guarro *et al.* 2007), progresando en algunos casos a infección sistémica por diseminación sanguinea (Sutton *et al.* 1997, Al-Mohsen *et al.* 2000, Guarro *et al.* 2007, Li *et al.* 2013). También es responsable de casos de queratitis (Shukla 1983, Arthur *et al.* 2001, Guarro *et al.* 2007), así como de abscesos cerebrales en humanos (Al-Mohsen *et al.* 2000, Li *et al.* 2013) y animales (Whelsh & Ely 1999, Guarro & Gené 2002, Sigler & Sutton 2002), por lo cual se le ha atribuído un cierto potencial neurotrópico (Revankar & Sutton 2010).

Las especies del género *Cladosporium* se asocian frecuentemente a casos de rinitis y sinusitis alergica (Sellart-Altissent *et al.* 2007, de Hoog *et al.* 2011), debido a su amplia y fácil dispersión por vía aerea, siendo este género considerado el segundo alergeno fúngico de mayor importancia después de *Alternaria* (Torras *et al.* 1981, de Ana *et al.* 2006, Sellart-Altisent *et al.* 2007). Las especies de *Cladosporium* a menudo se aislan de muestras clínicas, pero debido a los escasos conocimientos sobre su potencial patogénico suelen ser consideradas contaminantes de laboratorio (Schell 2003). Sin embargo, algunas especies se han descrito como agentes causantes de

feohifomicosis superficiales o subcutáneas, tanto en humanos (Drabick *et al.* 1990, Romano *et al.* 1999, Vieira *et al.* 2001, Sang *et al.* 2012, Sosa *et al.* 2012) como en animales (Ma *et al.* 2013), de queratitis (Chew *et al.* 2009) e incluso causantes de infecciones profundas e invasoras involucrando al sistema respiratorio (Yano *et al.* 2003), cerebro (Kantarcioğlu *et al.* 2002, Lalueza *et al.* 2011) y un caso de infección diseminada (Bentz & Sautter 1993). La mayoría de estos casos se han atribuido a *C. cladosporioides* y en mucha menor medida a *C. herbarum*, *C. macrocarpum* Preuss, *C. oxysporum* Berk. & M.A. Curtis y *C. sphaerospermum* (de Hoog *et al.* 2011).

A diferencia de *Acrophialophora* y *Cladosporium*, las especies pertenecientes a *Scopulariopsis* y, aunque en menor medida, a *Microascus* se reconocen como potenciales patógenos oportunistas para el hombre. La especie mas importante en clínica es *S. brevicaulis* (de Hoog *et al.* 2011, Iwen *et al.* 2012). Ésta se aisla frecuentemente como agente causal de dermatomicosis y onicomicosis en pacientes inmunocompetentes (Carrillo-Muñoz *et al.* 2004, de Hoog *et al.* 2011), siendo considerado uno de principales responsables de onicomicosis no dermatofítica en el hombre (Tosti *et al.* 1996, Cuenca-Estrella *et al.* 2003). *Scopulariopsis brevicaulis, S. acremonium* (Delacr.) Vuill., *S. brumptii* Salv.-Duval, *S. flava* (Sopp) F.J. Morton & G. Sm., *M. niger* (Sopp) Curzi, *M. cinereus* Curzi, *M. cirrosus* Curzi, *M. manginii* (Loubière) Curzi y *M. trigonosporus* C.W. Emmons & B.O. Dodge, se han aislado a partir de muestras clínicas de pacientes inmunocomprometidos, demostrándose su potencial patógeno en infecciones cutáneas (de Hoog *et al.* 2011), subcutáneas (Patel *et al.* 1993), queratitis (Ragge *et al.* 1990), otomicosis (Neglia *et al.* 1987), infecciones respiratorias incluyendo senos paranasales o localizaciones pulmonares profundas (Wheat *et al.* 1984, Kriesel *et al.* 1994), endoftalmitis (Gariano & Kalina 1997), abscesos cerebrales (Petit *et al.* 2011), endocarditis de válvula protésica (Migrino *et al.* 1995, Jain *et al.* 2011) y peritonitis (Vaidya & Levine 1992).

El género *Trichoderma* incluye especies de reciente interés en clínica como agentes de infecciones oportunistas, particularmente en pacientes transplantados o bajo peritoneodiálisis, produciendo infecciones diseminadas persistentes, las cuales por lo general no responden al tratamiento antifúngico (Guarro *et al.* 1999b, Richter *et al.* 1999, Kredics *et al.* 2003, Kantarcioğlu *et al.* 2009, Kredics *et al.* 2011). La especie de mayor incidencia en clínica es *T. longibrachiatum* Rifai (Tang *et al.* 2003), aunque también se han descrito otras ocho especies como patógenos oportunistas en humanos: *T. atroviride* P. Karst., *T. citrinoviride* Bissett, *T. harzianum* Rifai, *T. koningii* Oudem., *T. orientale* (Samuels & Petrini) Jaklitsch & Samuels, *T. pseudokoningii* Rifai, *T. reesei* E.G. Simmons y *T. viride* Pers. (Ranque *et al.* 2008, Kredics *et al.* 2011). Además de infecciones diseminadas (Kredics *et al.* 2003, 2011), las especies de

Trichoderma se han descrito como agentes causales de infecciones cutáneas y subcutáneas (Munoz *et al.* 1997), otitis externa (Hennequin *et al.* 2000) o queratitis (Venugopal *et al.* 1989), responsables de cuadros respiratorios alérgicos (Furukawa *et al.* 1998, Tang *et al.* 2003) e infecciones respiratorias profundas (de Miguel *et al.* 2005, Alanio *et al.* 2008), o asociadas a infección de drenaje ventricular (Piens *et al.* 2004), endocarditis (Bustamante-Labarta *et al.* 2000) y abscesos cerebrales (Seguin *et al.* 1995). Cabe destacar además su elevada incidencia en casos de infección peritoneal asociada a la contaminación de catéteres de peritoneodiálisis (Ragnaud *et al.* 1984, Campos-Herrero *et al.* 1996, Bren 1998, Rota *et al.* 2000, Eşel *et al.* 2003, Kredics *et al.* 2003).

1.5 Sensibilidad antifúngica

Actualmente, el tratamiento de las infecciones fúngicas se encuentra restringido a un reducido espectro de drogas antifúngicas cuyos mecanismos de acción se limitan a tres objetivos principales de la estructura y función fúngica: la membrana celular, la pared celular y la síntesis de ADN, ARN y proteínas. Entre los antifúngicos que actuan sobre la membrana celular afectando su formación y permeabilidad se encuentran las alilaminas, los azoles y los polienos. Las **alilaminas**, entre las cuales la más reconocida es la terbinafina (TRB) es un grupo de fármacos sintéticos cuya acción se basa en la inhibición de la enzima escualeno monooxigenasa, impidiendo la síntesis de ergosterol. La consiguiente acumulación intracitoplasmática de escualeno y la alteración estructural de la membrana citoplasmática llevan a la muerte celular. Los **azoles** de mayor relevancia: fluconazol (FLC), itraconazol (ITC), posaconazol (PSC) y voriconazol (VRC) son también fármacos sintéticos que actúan inhibiendo la enzima lanosterol-14α-desmetilasa, impidiendo la conversion del lanosterol a ergosterol. Los **polienos**, entre los cuales el fármaco de mayor relevancia es la anfotericina B (AMB), extraída de la bacteria *Streptomyces nodosus* actúa uniéndose directamente al ergosterol, intercalándose en la membrana citoplasmática determinando un aumento de su permeabilidad por la formación de poros. Los antifúngicos que ejercen su acción a nivel de la pared celular se limitan al grupo de las **equinocandinas**, que incluyen a la anidulafungina (AFG), caspofungina (CFG) y micafungina (MFG), que son moléculas semisintéticas con acción inhibitoria sobre la enzima (1-3) β-D-glucano sintasa, impidiendo la síntesis de (1-3) β-D-glucano, un polisacárido estructural fundamental para la integridad de la pared celular fúngica. Por último, las **pirimidinas fluoradas** como la 5-fluorocitosina (5FC) actuan mediante la inhibición de la síntesis de DNA, RNA y proteínas, impidiendo la síntesis de pirimidinas (Lewis 2011, Richardson & Warnock 2012)

El incremento constante de las infecciones fúngicas oportunistas sumado a su alta mortalidad, elevadas tasas de falla terapéutica y el limitado espectro de drogas antifúngicas, hacen necesario contar con sistemas que permitan evaluar la sensibilidad de los agentes etiológicos a los distintos fármacos disponibles en el mercado principalmente con la finalidad de optimizar el tratamiento y el desenlace de dichas infecciones (Cuenca-Estrella *et al.* 2007, Perkhofer *et al.* 2010). Actualmente existen dos metodologías para la medición de la sensibilidad antifúngica tanto de hongos levaduriformes como filamentosos estandarizadas por el *Clinical & Laboratory Standards Institute* (CLSI) y el *European Committee on Antimicrobial Susceptibility Testing* (EUCAST) (CLSI 2008, Kahlmeter *et al.* 2006). Estos métodos se han basado en la amplia experiencia en estudios de sensibilidad a antibacterianos, siendo homólogos en cuanto a las técnicas empleadas. Los resultados de los tests de sensibilidad antifungica se proporcionan en términos de concentración mínima inhibitoria (MIC) para los fármacos pertenecientes a las alilaminas, los azoles, pirimidinas y polienos, es decir, la dilución más baja de antifúngico que es capaz de inhibir en gran medida el crecimiento del hongo en estudio, y en el caso de los antifúngicos pertenecientes al grupo de las equinocandinas los resultados se expresan en términos de concentración mínima efectiva (MEC), es decir, la dilución más baja del antifúngico capaz de provocar alteraciones estructurales evidentes en el hongo en estudio. Sin embargo, pese a los altos niveles de estandarización y reproducibilidad de estas técnicas, la inexistencia de puntos de corte clínicos para hongos filamentosos distintos de *Aspergillus* spp. limita considerablemente su utilización para la predicción de la respuesta clínica frente a hongos de menor frecuencia. Esto, sumado al desconocimiento de los perfiles de sensibilidad antifúngica, condiciona en gran medida la toma de decisiones clínicas. Actualmente, la elección del tratamiento antifúngico depende fundamentalmente de la experiencia del médico tratante, por lo que puede que no sea realmente el tratamiento más eficaz para el control la infección.

Los estudios de sensibilidad antifúngica son, a su vez, de vital importancia para la vigilancia de la aparición de resistencias y la detección de especies o cepas con resistencia intrínseca o adquirida (Perkhofer *et al.* 2010).

1.5.1 Sensibilidad *in vitro* de *Acrophialophora*, *Cladosporium*, *Microascus*/*Scopulariopsis* y *Trichoderma*

Pese al notable incremento de estudios de sensibilidad *in vitro* de hongos filamentosos de alta prevalencia como *Aspergillus*, *Fusarium* y mucorales, los datos de sensibilidad *in vitro* para especies pertenecientes a los géneros estudiados en este trabajo son muy escasos, y han sido obtenidos principalmente a partir de un limitado

número de casos clínicos. Muchos de estos casos son antiguos, previos al desarrollo de protocolos estandarizados para el estudio de sensibilidad antifúngica y a los recientes cambios en la taxonomía de estos géneros. Por lo tanto, sus resultados son muy difíciles de interpretar y comparar.

En cuanto a las especies de *Acrophialophora*, los azoles, particularmente ITC, PSC y voriconazol (VRC) muestran una buena actividad *in vitro* (Al-Mohsen *et al.* 2000, Guarro *et al.* 2007). La actividad de la AMB, en cambio, es muy variable (Al-Mohsen *et al.* 2000), mientras que las equinocandinas prácticamente no presentan actividad inhibitoria (Guarro *et al.* 2007).

Para las especies de *Cladosporium*, sólo se ha evaluado la actividad *in vitro* de los azoles, AMB y TRB frente a los complejos de especies *C. cladosporioides* y *C. sphaerospermum*, obteniéndose resultados muy variables (de Hoog *et al.* 2011), Mientras que la TRB y el VRC han demostrado una buena actividad frente a ambos grupos de especies (Kantarcioğlu & Yücel 2002, de Hoog *et al.* 2011), el ITC y el ketoconazol (KTC) demuestran una acción inhibitoria sólo frente a especies del complejo *C. cladosporioides*, en cambio la AMB sólo es activa frente a miembros del complejo *C. sphaerospermum* (Guarro *et al.* 1997, de Hoog *et al.* 2011).

En el caso de los géneros *Microascus*, *Scopulariopsis* y otros géneros afines, la mayoría de datos sobre sensibilidad *in vitro* se refieren a *S. brevicaulis*, la especie más frecuentemente aislada en clínica. Del mismo modo que se ha observado en general para otros hongos patógenos pertenecientes a la familia *Microascaceae* (Gilgado *et al.* 2006, Lackner *et al.* 2012), *S. brevicaulis* presenta una baja sensibilidad *in vitro* frente a todos los antifúngicos (Cuenca-Estrella *et al.* 2003, Carrillo-Muñóz *et al.* 2004, 2007).

El género *Trichoderma* presenta una mayor disponibilidad de datos de sensibilidad *in vitro*. Las equinocandinas: AFG y CSP, y el azol VRC han mostrado de forma continuada una buena actividad inhibitoria (Alanio *et al.* 2008, Kantarcioğlu *et al.* 2009, Kredics *et al.* 2011). Por el contrario, AMB, ITC, PSC y TRB se relacionan con una baja actividad o con resultados muy variables (Seguin *et al.* 1995, Tanis *et al.* 1995, Munoz *et al.* 1997, Richter *et al.* 1999, Chouaqui *et al.* 2002, Kredics *et al.* 2011).

1.5.2 Tratamiento de las infecciones causadas por *Acrophialophora*, *Cladosporium*, *Microascus*/*Scopulariopsis* y *Trichoderma*

En los últimos años se ha progresado en la creación de guías consensuadas para el diagnóstico y tratamiento de infecciones fúngicas establecidas por comités de expertos, como la *Infectious Disease Society of America* (IDSA) y la *European Society of Clinical Microbiology and Infectious Diseases* (ESCMID), en las que se incluyen recomendaciones para el tratamiento antifúngico de aspergilosis, candidiasis,

cryptococosis, mucormicosis, fusariosis y micosis endémicas (Arendrup *et al.* 2014, Cornely *et al.* 2014), además de incluir en sus últimas ediciones comentarios sobre el tratamiento de infecciones causadas por un gran número de hongos de menor prevalencia como *Acremonium*, *Paecilomyces*, *Purpureocillium* o *Scedosporium*, entre otros hongos productores de micosis oportunistas (Chowdary *et al.* 2014, Tortorano *et al.* 2014). Sin embargo, para los géneros estudiados en este trabajo, así como para muchos otros géneros fúngicos que incluyen patógenos humanos de baja frecuencia, aún no existen esquemas de tratamiento estandarizado ni recomendaciones clínicas, principalmente debido a los escasos datos tanto tanto de estudios *in vitro* como *in vivo*. Además, los pocos datos disponibles parecen indicar que no existe una correlación entre los resultados de las pruebas de sensibilidad *in vitro* y el desenlace clínico.

Las infecciones atribuidas a especies de *Acrophialophora* han mostrado una buena respuesta al tratamiento combinado con AMB e ITC en un caso de infección pulmonar con afectación del sistema nervioso central (Al-Mohsen *et al.* 2000), por otra parte, VRC ha mostrado resultados variables, siendo efectivo en un caso de infección pulmonar (Guarro *et al.* 2007), pero ineficaz en un caso de infección cerebral (Li *et al.* 2013).

Respecto a *Cladosporium*, se ha conseguido una buena respuesta con los azoles, principalmente ITC en infecciones superficiales (Romano *et al.* 1999, Gugnani *et al.* 2000, Vieira *et al.* 2001, Sosa *et al.* 2012, Ma *et al.* 2013), subcutáneas (Duquia *et al.* 2009, Sang *et al.* 2012) y profundas (Yano *et al.* 2003), mientras que VRC ha sido eficaz en un caso de infección cerebral (Lalueza *et al.* 2011). La AMB se ha asociado a una mala respuesta clínica en casos de infección cutánea (Vieira *et al.* 2001) e intrabronquial (Yano *et al.* 2003).

Los pocos casos clínicos sobre infecciones atribuidas a *Microascus* o *Scopulariopsis* muestran que VRC ha sido efectivo para el tratamiento de un absceso pulmonar (Petit *et al.* 2011) y la infección de una válvula cardíaca protésica (Jain *et al.* 2011). Existe muy poca experiencia práctica sobre el uso de equinocandinas; la CFG resultó ineficaz en un caso de sinusitis invasora (Beltrame *et al.* 2009), así como el tratamiento combinado de AMB con MFG en un caso de infección invasora (Iwen *et al.* 2012).

La mayoría de las infecciones atribuidas a especies de *Trichoderma* con buena respuesta al tratamiento antifúngico ha sido con el uso de AMB y VRC como monoterapia, o AMB combinada con CFG ó ITC (Chouaqui *et al.* 2002, Kredics et al 2011). Sin embargo, aproximadamente un tercio de los casos publicados muestran resultados desfavorables utilizando las mismas terapias antes mencionadas (Kredics *et al.* 2011).

2. INTERÉS Y OBJETIVOS

Entre las enfermedades infecciosas, la infección fúngica ha adquirido una considerable relevancia en las últimas décadas, particularmente las micosis causadas por hongos oportunistas, un hecho debido fundamentalmente al aumento de la población inmunocomprometida pero también a la dificultad que presentan tanto su prevención como su manejo clínico. Los factores de riesgo en la población que determinan la adquisición de este tipo de infecciones son: edades extremas, enfermedades de base severas (diabetes, enfermedad granulomatosa crónica, neoplasias, estadios tardíos del síndrome de inmunodeficiencia humana (VIH), entre otras), así como factores ligados al progreso de la medicina con un aumento de pacientes bajo terapia esteroidal o quimioterapia intensa y prolongada, o trasplantados de células madres u órganos sólidos bajo inmunosupresión. Muchos hongos tradicionalmente considerados saprobios o contaminantes ambientales son aislados cada vez con mayor frecuencia a partir de muestras clínicas siendo relacionados con altas tasas de letalidad.

En esta tesis se evalúan aspectos taxonómicos, morfológicos y fisiológicos de los géneros *Acrophialophora*, *Cladosporium*, *Microascus*, *Scopulariopsis* y *Trichoderma*, los cuales incluyen especies de amplia distribución mundial y de importancia clínica creciente, siendo además relevantes en el ámbito de la fitopatología y biotecnología. Estos géneros representan un serio reto para el diagnóstico microbiológico por medio de métodos clásicos, debido en parte a la gran similitud morfológica entre sus especies, a los escasos estudios previos existentes y a su compleja taxonomía.

Los géneros *Cladosporium* y *Trichoderma* incluyen numerosas especies con caracteres macro y microscópicos muy homogéneos y, por lo tanto, muy difíciles de identificar aplicando solamente criterios morfológicos. Recientes y detalladas revisiones taxonómicas basadas en la filogenia molecular han contribuido a la correcta delimitación de estos géneros, así como de las múltiples especies que los integran (Druzhinina & Kubicek 2005, Crous *et al.* 2007b, Bensch *et al.* 2012, Jacklitsch & Volglmayr 2013). Como resultado, muchas especies descritas previamente a partir de su morfología han sido transferidas a otros géneros, mientras que otras muchas han sido descritas como nuevas especies para la ciencia. Sin embargo, estos estudios se han centrado fundamentalmente en aspectos ecológicos, fitopatológicos o biotecnológicos, mientras que la diversidad e incidencia de las especies asociadas a patologías humanas o animales todavía no se ha reevaluado de acuerdo a los criterios modernos de identificación.

Tanto *Microascus* como *Scopulariopsis* son géneros con una reconocida importancia clínica. Sin embargo, las últimas revisiones taxonómicas de estos hongos

datan como mínimo de dos décadas atrás (Barron *et al*. 1961, Morton & Smith 1963, von Arx *et al.* 1988) y, desde entonces, se han descrito un gran número de nuevas especies. Los pocos estudios moleculares realizados hasta el momento parecen indicar que estos géneros no representan grupos monofiléticos (Issakainen *et al.* 2003, Lackner *et al*. 2014), pero para demostrar este hecho hay que realizar un exhaustivo estudio de las relaciones filogenéticas del mayor número posible de especies pertenecientes a *Microascus*, *Scopulariopsis* y a otros géneros incluidos en *Microascaceae*, como *Cephalotrichum*, *Doratomyces*, *Kernia*, *Trichurus*, *Wardomyces*, *Wardomycopsis*, entre otros.

Por el contrario, las especies de *Acrophialophora* se aíslan raramente en clínica y quizás por este motivo se trata de un género que ha despertado poco interés en este ámbito. Sin embargo, algunas de sus especies parecen tener un elevado potencial patogénico como atestiguan los escasos casos clínicos publicados. Son hongos difíciles de identificar debido a que las diferencias morfológicas entre ellos son poco evidentes y muy variables según las condiciones de cultivo empleadas para su estudio. De hecho, existen escasos estudios taxonómicos sobre el género (Samson & Mahmood 1970, Al-Mohsen *et al*. 2000) y nunca se ha realizado una revisión taxonómica basada en resultados de secuenciación de ADN.

Además de la problemática taxonómica, tanto el rol patogénico de ciertas especies como la epidemiología de los géneros aquí estudiados son aún desconocidos. Las terapias de elección para el tratamiento de las infecciones producidas por estos hongos son inexistentes, principalmente debido a los escasos casos clínicos publicados y la consiguiente dificultad en la recopilación de datos.

Por todo lo expuesto, el **objetivo general** de esta tesis es **estudiar morfológica y molecularmente los géneros *Acrophialophora*, *Cladosporium*, *Microascus*, *Scopulariopsis* y *Trichoderma* para clarificar su taxonomía y filogenia, proporcionando criterios objetivos para el diagnóstico de sus especies, además de establecer los patrones de sensibilidad antifúngica *in vitro* de las especies de interés clínico.**

Para su consecución, se van a desarrollar los siguientes objetivos específicos:

1. Identificar mediante técnicas tradicionales y moleculares aislados clínicos de los géneros *Acrophialophora*, *Cladosporium*, *Microascus*, *Scopulariopsis* y *Trichoderma* con el fin de establecer la diversidad de especies asociadas a muestras clínicas, tanto humanas como de animales.

2. Establecer las relaciones filogenéticas entre las especies de los géneros arriba mencionados mediante análisis multilocus de secuencias.

3. Describir, si procede, las nuevas especies detectadas mediante el estudio polifásico.

4. Determinar los perfiles de sensibilidad antifúngica *in vitro* de las especies de relevancia clínica de cada uno de los géneros estudiados.

3. MATERIALES Y MÉTODOS

3.1 Origen de los aislados

En los distintos estudios que conforman esta tesis se incluyeron un total de 394 aislados, de los cuales 300 fueron obtenidos de especímenes clínicos (274 de origen humano y 26 de origen animal) **(Anexo, Tabla 1)** y 15 de origen ambiental **(Anexo, Tabla 2)**. También se incluyeron 79 cepas tipo o de referencia **(Anexo, Tabla 3)** obtenidas de diferentes colecciones de cultivos internacionales y que se emplearon para comparar con los aislados arriba indicados. Los aislados clínicos fueron facilitados principalmente por el *Fungus Testing Laboratory, University of Texas Health Science Center* (UTHSC, San Antonio, USA), y en menor medida por el *Laboratoire de Parasitologie-Mycologie,* (*Institut de Biologie*, CHU Nantes, Francia), el *Institut Pasteur* (*Centre National de Réfeìrence Mycoses Invasives et Antifongiques*, Paris, Francia), así como por hospitales y laboratorios de España (Hospital 12 de Octubre, Madrid; Hospital Joan XXIII, Tarragona y Laboratorio Taoro, Tenerife). Las cepas tipo y de referencia se obtuvieron de las siguientes colecciones: *Agro-Industrial Fungi and Yeasts Collection* (BCCM/MUCL, Bruselas, Bélgica), *CBS-KNAW Fungal Biodiversity Centre* (CBS-KNAW, Utrecht, Holanda), *Mycothèque de l'Université Catholique de Louvain* (MUCL, Louvain-la-Neuve, Bélgica) y *University of Alberta Microfungus Collection and Herbarium* (UAMH, Edmonton, Canadá).

3.2 Identificación de los aislados

Todos los aislados se identificaron a partir de sus caracteres morfológicos, tanto macro como microscópicos, su habilidad para crecer y esporular a diferentes temperaturas y un exhaustivo análisis de secuencias del operón ribosómico y de otros genes según el grupo de hongos que se tratara.

3.2.1 Estudios morfológicos

Para la determinación de las características morfológicas se utilizaron combinaciones de diferentes medios de cultivo, dependiendo del hongo en estudio y de las recomendaciones de estudios previos, los cuales se detallan en la sección de Resultados. Los medios de cultivo utilizados fueron los siguientes: agar bajo en nutrientes (SNA; 1 g KH_2PO_4, 1 g KNO_3, 0,5 g $MgSO_4$ x $7H_2O$, 0,5 g KCl, 0,2 g de dextrosa, 0,2 g de sacarosa, agua destilada 1000 mL), agar extracto de malta (MEA; 30 g de extracto de malta, 5 g de peptona, 15 g de agar, agua destilada 1000 mL), agar harina de avena (OA; 30 g de semillas de avena filtradas tras ebullición durante una hora, 20 g de agar, completar con agua destilada hasta volumen final de 1000 mL), agar harina de maíz dextrosa (CMD; 50 g de harina de maíz; 20 g de dextrosa, 15 g de agar; agua destilada 1000 mL), agar patata zanahoria (PCA: 20 g de patata, 20 g de

zanahorias, 20 g de agar, completar a 1000 mL con agua destilada) y agar patata dextrosa (PDA; Pronadisa, España). En términos generales, los cultivos fueron incubados a 25°C en la oscuridad, y examinados a los 3, 7, 14, 21 y 28 días registrando su diámetro, color de la colonia siguiendo la nomenclatura establecida en Kornerup & Wanscher (1978), textura, topología, reverso y cualquier otra característica importante como la presencia de pigmentos difusibles y/o exudados. El estudio de las estructuras microscópicas (tamaño, color y ornamentación del micelio vegetativo, conidióforos, células conidiógenas, conidios y estructuras de reproducción sexual) se realizó mediante la observación de preparaciones semipermanentes utilizando diversos líquidos de montaje: ácido láctico al 85%, lactofenol azul algodón (50 mg azul algodón en 100 mL de ácido láctico al 85%) o el medio de Shear's (3 g de acetato de potasio, 150 mL de agua destilada, 60 mL de glicerina, 90 mL etanol 95 %) (Crous *et al.* 2009a), o mediante preparaciones permanentes montadas en alcohol-polivinilo (Sigma, USA). Las preparaciones microscópicas se realizaron a mano alzada cogiendo un fragmento de la colonia con ayuda de una aguja estéril, realizando un *squash* para la mejor observación de las estructuras de reproducción sexual, o mediante la técnica del microcultivo descrita por Riddell (Crous *et al.* 2009a). Para el examen microscópico y las mediciones de las estructuras se utilizó un microscopio de campo claro Olympus CH-2 (Olympus Corporation, Japón). Las microfotografías se obtuvieron con un microscopio motorizado Zeiss Axio-Imager M1 (Zeiss, Alemania), provisto de una cámara digital DeltaPix Infinity X21, utilizando óptica de contraste de fases y óptica Nomarki de contraste por interferencia diferencial, captando y procesando las imágenes mediante el software DeltaPix InSight 5.0 (DeltaPix, Dinamarca).

3.2.2 Determinación de los rangos de temperaturas de crecimiento

Para determinar la capacidad de los aislados en estudio de crecer a diferentes temperaturas de incubación y el efecto de estas sobre la tasa de crecimiento y esporulación de las mismas, se utilizaron placas de Petri de 9 cm con agar PDA, y una vez inoculados se incubaban a diferentes temperaturas en un rango de 5 a 45°C, a intervalos de 5°C además de 37°C. Las placas se examinaban generalmente a intervalos de 7 días hasta los 28 días, registrándose el diámetro de las colonias y la presencia/ausencia de esporulación con ayuda de un microscopio estereoscópico SD2AL (Kyowa Optical, Japón). Finalmente se consideró como la temperatura óptima, aquella en la que se obtenía una mayor tasa de crecimiento y de esporulación.

3.2.3 Estudios moleculares

Tal y como se ha expuesto en la introducción, muchos de los taxones estudiados en la presente tesis presentan características morfológicas muy similares, por ello es imprescindible para realizar una correcta identificación de los mismos y establecer las relaciones filogenéticas entre ellos, su caracterización a nivel molecular, mediante la amplificación y secuenciación de diversos marcadores genéticos; dichas secuencias fueron comparadas con las existente del material tipo y las de cepas de referencia disponibles en las bases de datos públicas o generadas en este trabajo.

3.2.3.1 Extracción del material genético

Para la obtención del ADN genómico total, se realizaron cultivos en placas de PDA o en agar sacarosa extracto de levadura (YES: 20 g de extracto de levadura, 150 g de sacarosa, 20 g de agar, agua destilada, 1000 mL), este último se usó principalmente para los hongos dematiáceos con la finalidad de reducir el efecto inhibidor de la melanina sobre la *Taq*-polimerasa de la PCR. Transcurridos 3-7 días de incubación, se realizaba la remoción del micelio aéreo y estructuras de fructificación mediante raspado utilizando un bisturí estéril. El ADN fúngico se extrajo mediante los kits comerciales *PrepMan Ultra sample preparation reagent* (*Applied Biosystems*, USA) ó el *FastDNA*® kit (*MP Biomedicals*, USA), siguiendo las instrucciones del fabricante. El ADN obtenido se cuantificó mediante el uso de los sistemas *GeneQuant pro* (*Amersham Pharmacia Biotech*, UK) ó el *NanoDrop* 3000 (*ThermoScientific*, USA).

3.2.3.2 Amplificación y secuenciación

Mediante PCR se amplificaron diversos *loci* génicos, dependiendo del grupo fúngico a tratar, los cuales se detallan para cada estudio en las secciones **Resultados** y **Anexos, Tabla 4.**

La reacción de amplificación se realizó en un volumen total de 40 µL y sus componentes eran: 20 mM buffer Tris-HCL (pH 8,4), 50 mM KCl (10X *PCR reaction buffer*; *Invitrogen*, UK); 1,5 mM $MgCl_2$ (*Invitrogen*, UK), 125 µM desoxinucleótidos trifosfato (*GeneAmp*® dNTP mix with dTTP, *Applied Biosystems*, USA), 5% dimetilsulfóxido (DMSO; Panreac Química S.L.U, España), 1,2 µM de cada cebador y 1,25 U de *Taq* ADN Polimerasa (*Invitrogen*, UK). El programa de amplificación utilizado para todos los genes consistió en una desnaturalización inicial a 94°C durante 5 minutos, seguido de 35 ciclos de desnaturalización a 95°C durante 30 segundos, hibridación desde 1 minuto a 1 minuto y 30 segundos (el tiempo y la temperatura dependían de los cebadores utilizados, según se detalla en **Anexos, Tabla 4**), extensión a 72°C por 1 minuto y una extensión final a 72°C por 7 min. Para ello se

utilizaron los termocicladores 2720 *thermal cycler* (*Applied Biosystems*, USA) y *Biometra TProfessional Basic Gradient Thermocycler* (*AnalyticJena*, Alemania).

Para corroborar la efectividad de la amplificación, los productos se sometieron a electroforesis en geles de agarosa al 1,5 ó 2%.

Los productos de la PCR se enviaron para su purificación y secuenciación a *Macrogen Corp. Europe* (Ámsterdam, Holanda) utilizando los mismos pares de cebadores empleados para su amplificación.

3.2.3.3 Ensamblaje de secuencias

Las secuencias obtenidas con los cebadores (*forward*, *reverse* y cebadores internos cuando correspondía) se revisaron visualmente para determinar su calidad y se ensamblaron para la obtención de las secuencias consenso utilizando el programa *SeqMan*, versión 7.0.0 (*DNASTAR Lasergene*, USA). Todas las secuencias generadas en este estudio fueron depositadas en las bases de datos *GenBank* (http://www.ncbi.nlm.nih.gov/genbank) o *European Nucleotide Archive* (http://www.ebi.ac.uk/ena)

3.2.3.4 Búsquedas de identidad de secuencias nucleotídicas

La identificación preliminar de los aislados se realizó mediante la determinación del grado de similitud genética con secuencias disponibles en bases de datos públicas, concretamente la del CBS (http://www.cbs.knaw.nl), GenBank (www.ncbi.nlm.nih.gov/genbank), Mycobank (http://www.mycobank.org) y NITE (http://www.nbrc.nite.go.jp). En general, aquellas secuencias con una similitud ≥98% y una cobertura ≥99% se consideraron como un resultado altamente fiable, pero no definitivo, de una identificación correcta; siendo necesaria la confirmación de dicha identificación mediante estudios fenotípicos y/o filogenéticos. En el caso de obtener resultados porcentualmente inferiores a los mencionados, se consideró como una identificación parcial a nivel de género, familia u orden, según correspondiera. A efectos de interpretación de resultados y eliminación de resultados ambiguos, sólo se consideraron como válidos aquellos que incluían secuencias provenientes de material tipo y/o cuya identificación había sido realizada por algún especialista de aquel grupo fúngico.

3.2.3.5 Obtención de secuencias de referencia

Se descargaron y utilizaron en los análisis filogenéticos un total de 523 secuencias desde las bases de datos públicas antes mencionadas. La selección de estas secuencias se basó fundamentalmente en los resultados de búsquedas de

similitud, la disponibilidad de secuencias para cada gen y taxón (incluyendo cepas tipo y de referencia) y la calidad de estas, la cual fue evaluada tomando en consideración la longitud de las secuencias y la presencia o ausencia de indeterminaciones en las mismas.

3.2.3.6 Alineamiento de múltiples secuencias

Las secuencias de los distintos genes amplificados para cada estudio se alinearon individualmente, incluyendo las secuencias descargadas desde bases de datos públicas, utilizando el algoritmo ClustalW (Thompson *et al.* 1994) incorporado en la plataforma MEGA versión 5.05 (Tamura *et al.* 2011) o versión 6 (Tamura *et al.* 2013). Los alineamientos se revisaron y ajustaron manualmente o utilizando el algoritmo MUSCLE (Edgar 2004) implementado en las dos versiones del MEGA. En caso de ser necesario, los alineamientos fueron analizados mediante el software Gblocks versión 0.91b (Castresana 2000), para la búsqueda y eliminación de regiones ambiguas.

3.2.3.7 Concatenado de los alineamientos

Con el objetivo de obtener una mayor resolución de los resultados de los análisis filogenéticos, cada estudio incluido en esta tesis se realizó utilizando una o más combinaciones de los genes seleccionados, dependiendo de la capacidad resolutiva de cada uno de ellos, la disponibilidad de secuencias y la concordancia entre las topologías de los árboles obtenidos tras el análisis filogenético de cada gen.

Para la evaluación de la congruencia filogenética entre los diferentes loci se emplearon dos técnicas: el test de homogeneidad de particiones (*Partition-homogeinity test*) llevado a cabo usando el software PAUP* versión 4.0b10 (Sinauer, USA) (Swofford 2000), y/o comparando visualmente la posición y monofilia de los clados terminales con soporte estadístico significativo en las filogenias obtenidas para cada gen y en la observada en la combinación de los mismos (Wiens 1998).

3.2.3.8 Análisis filogenéticos

Las reconstrucciones filogenéticas para cada gen y las combinaciones de los diversos genes se llevaron a cabo utilizando diferentes sistemas de inferencia filogenética: *Neighbor-Joining* (NJ), Máxima Verosimilitud (ML) y análisis Bayesiano (BI).

Los análisis de NJ y ML se llevaron a cabo utilizando el software MEGA en sus versiones 5.05 ó 6. Tanto para ML como NJ, los gaps presentes en los alineamientos se trataron como deleciones parciales y la robustez de las ramas se determinó

mediante el método de bootstrap, utilizando 1000 iteraciones. Un valor de bootstrap ≥70% se consideró como estadísticamente significativo.

Los análisis con BI se llevaron a cabo utilizando el software MrBayes versión 3.1.2 (Huelsenbeck & Ronquist 2001). Para esto, y dependiendo del estudio, se realizaron simulaciones de entre 3.000.000 a 10.000.000 generaciones, en dos series paralelas, almacenando los árboles resultantes cada 100 ó 1000 generaciones. El análisis se detenía al obtenerse la convergencia de valores estadísticos para ambas series (varianza < 0,01). Se eliminaron el 25% de los primeros árboles obtenidos (*burnin*) para posteriormente calcular el árbol consenso final (50%).

Para la selección del modelo de sustitución nucleotídica más apropiado para cada gen se utilizó la herramienta *Find Best DNA/Protein Model* incluida en MEGA o, en su defecto, utilizando el software *MrModelTest* versión 2.3 (Nylander 2004).

3.3 Registro de novedades taxonómicas

Todas las novedades taxonómicas propuestas (nuevas combinaciones, especies o géneros) en los diferentes estudios fueron depositadas en el *MycoBank* siguiendo las recomendaciones del nuevo código de nomenclatura (Crous *et al.* 2004, McNeill *et al.* 2012).

3.4 Almacenamiento y conservación de los aislados

Una vez confirmada la pureza del cultivo, todos los aislados se depositaron en la colección de cultivos fúngicos de la *Facultat de Medicina de Reus* (FMR), y se conservaron utilizando cuatro metodologías diferentes con el fin de asegurar su supervivencia.

3.4.1 Conservación de cultivos a corto plazo

Los hongos fueron cultivados, por duplicado, en tubos de polietileno, herméticos y estériles, de agar inclinado (OA, PCA ó PDA). Una vez inoculado el hongo, dichos tubos se almacenaron a temperatura ambiente y fueron subcultivados a intervalos regulares para evitar la desecación.

3.4.2 Conservación de cultivos a largo plazo

3.4.2.1 Almacenamiento en agua: A partir de las colonias del hongo bien esporulado en placas de Petri con OA, PCA o PDA, se cortaron pequeños bloques de agar, de aproximadamente 1 cm^2, con ayuda de un bisturí estéril. Seguidamente, dichos fragmentos se introdujeron en frascos de vidrio estériles conteniendo 2-3 mL de agua

destilada estéril y se guardaron en la oscuridad a temperatura ambiente (aproximadamente 25°C)

3.4.2.2 Almacenamiento en aceite mineral: Para ello se usaron tubos de vidrio de 10 cm de longitud con agar inclinado (OA, PCA o PDA), provistos de tapón con cierre hermético. Una vez inoculada la cepa y obtenida abundante esporulación, se cubrió el cultivo con aceite mineral estéril y se guardaron los tubos en la oscuridad a temperatura ambiente (aproximadamente 25°C).

3.4.2.3 Liofilizados: Inicialmente, las cepas se cultivaron en placas de Petri con OA, PCA o PDA, y una vez crecidas las colonias y con abundante esporulación se rasparon con ayuda de un asa o bisturí estéril. La masa de elementos fúngicos obtenida se depositó en un tubo de plástico estéril conteniendo 3 mL de medio *skim milk* (Difco, USA) hasta obtener una suspensión concentrada. Ésta suspensión se homogenizó y distribuyó en fracciones de 1-1,5 mL en dos o tres frascos pequeños de vidrio estériles provistos de tapón de caucho hermético. Los frascos se liofilizaron mediante el sistema automatizado *VirTis Advantage* 2.0 ES (*SP Scientific*, USA), utilizando el siguiente protocolo. Congelación inicial a -45ºC seguido de generación de vacío a 200 mTorr, desecación por sublimación a -30ºC (240min), -10ºC (240min), 10ºC (300min) y 30ºC (300min). Una vez finalizado el proceso de liofilización, los frascos fueron cerrados herméticamente mediante tapón de caucho y sellados con un anillo de seguridad metálico. Para comprobar la viabilidad y ausencia de contaminación de las muestras, el producto liofilizado de uno o más viales seleccionados al azar se subcultivaron en un medio de cultivo apropiado, evaluándose tanto la viabilidad del hongo como la pureza del cultivo.

3.4.2.4 Almacenamiento mediante herborización: Se empleó para todo el material correspondiente a las cepas tipo (holotipos, isotipos, neotipos) de las nuevas especies caracterizadas en esta tesis. Para esto, el hongo se cultivó por duplicado en el medio de cultivo más adecuado para su correcto desarrollo y esporulación. Una vez transcurrido el tiempo de incubación, se desecó el cultivo en una estufa a 45-50ºC y posteriormente cada cepa se almacenó en sobres de papel. El material tipo desecado de las nuevas especies descritas en esta tesis se depositó en el herbario de la colección del CBS-KNAW *Fungal Biodiversity Centre*, y una copia de este material (isotipo) se guardó en el herbario de la colección FMR de nuestra universidad.

3.5 Estudios de sensibilidad antifúngica.

Se determinó el patrón de sensibilidad antifúngica para la mayoría de los aislados clínicos de las especies identificadas, siguiendo el protocolo descrito por el *Clinical and Laboratory Standards Institute* en el documento M38-A2 (CLSI 2008).

Los aislados pertenecientes a especies de *Microascus* y *Scopulariopsis* fueron evaluados en el *Fungus Testing Laboratory* (UTHSC, San Antonio, USA), mientras que los aislados del resto de géneros de interés clínico estudiados fueron evaluados en la *Unitat de Micologia* de la *Facultat de Medicina i Ciències de la Salut* (URV, Reus, España). Los antifúngicos evaluados incluyeron representantes de los principales grupos antifúngicos de uso clínico: alilaminas (TRB), azoles (ITC, PSC y VRC), equinocandinas (AFG, CFG y MFG); pirimidinas fluoradas (5FC) y polienos (AMB). El rango de concentraciones ensayadas para cada antifúngico varió entre 0,016 a 16 µg/mL.

Para la preparación de los inóculos, las cepas estudiadas se sembraron en el medio de cultivo más apropiado e incubaron durante el tiempo necesario para conseguir abundante esporulación. Posteriormente se rasparon la superficie de las colonias con la ayuda de un asa o bisturí estéril, suspendiendo la masa fúngica obtenida en solución fisiológica estéril, la cual fue posteriormente filtrada mediante gasa o algodón estéril para eliminar los restos de micelio. Las suspensiones de conidios fueron cuantificadas mediante lectura en cámara de Neubauer, ajustadas a una concentración de $4x10^5$ – $5x10^6$ conidios/mL y luego diluidas 1/50 en medio de *Roswell Park Memorial Institute* (RPMI-1640, Gibco, UK). En una microplaca de 96 pocillos, se inocularón 100 µL del inóculo 1/50 para cada dilución del antifúngico a ensayar.

Las microplacas fueron incubadas en la oscuridad, sin agitación, durante 24 a 72 horas a 35ºC a excepción de las cepas de *Cladosporium*, para las cuales la temperatura de incubación fue de 25ºC debido a los requerimientos fisiológicos de las especies de este género.

La lectura de la sensibilidad a equinocandinas se realizó a las 24 ó 48 horas de incubación, mediante la determinación de la concentración mínima efectiva (MEC), definida como la mínima concentración de antifúngico en la cual se observa un crecimiento aberrante del hongo, caracterizado por la formación de masas compactas, formadas por elementos miceliares de extremos redondeados, en comparación con el crecimiento normal aterciopelado o algodonoso observable en el pocillo control. Para la lectura de la sensibilidad al resto de antifúngicos se determinó la concentración mínima inhibitoria (MIC) a las 48 y 72 horas de incubación. Se definió la MIC como la mínima concentración de antifúngico capaz de conseguir el 100% de inhibición del

crecimiento de hongo para AMB, ITC, PSC y VRC, el 80% de inhibición para TRB y el 50% de inhibición en el caso de 5FC. Todas las pruebas se realizaron por duplicado y la lectura se llevó a cabo de forma visual, con ayuda de un espejo invertido. Para el control de calidad de las pruebas se utilizaron las cepas *Aspergillus fumigatus* ATCC MYA-3626 y *Paecilomyces variotii* ATCC MYA-3630.

4. RESULTADOS

4.1 *Acrophialophora*, a poorly known fungus with clinical significance

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Acrophialophora, a Poorly Known Fungus with Clinical Significance

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***Acrophialophora fusispora* is an emerging opportunistic fungus capable of causing human infections. The taxonomy of the genus is not yet resolved and, in order to facilitate identification of clinical specimens, we have studied a set of clinical and environmental *Acrophialophora* isolates by morphological and molecular analyses. This set included the available type strains of *Acrophialophora* species and similar fungi, some of which were considered by various authors to be synonyms of *A. fusispora*. Sequence analysis of the large subunit (LSU) and internal transcribed spacer (ITS) regions of the nuclear ribosomal DNA and a fragment of the β-tubulin (*Tub*) gene revealed that *Acrophialophora* belongs in the family *Chaetomiaceae* and comprises three different species, i.e., *A. fusispora*, *Acrophialophora levis*, and *Acrophialophora seudatica*; the latter was previously included in the genus *Ampullifera*. The most prevalent species among clinical isolates was *A. levis* (72.7%), followed by *A. fusispora* (27.3%), both of which were isolated mostly from respiratory specimens (72.7%), as well as subcutaneous and corneal tissue samples. In general, of the eight antifungal drugs tested, voriconazole had the greatest *in vitro* activity, while all other agents showed poor *in vitro* activity against these fungi.**

Acrophialophora is a thermotolerant soil fungus that is widely distributed in temperate and tropical regions. Given its capacity to produce large quantities of cellulases and xylanases, it is also commonly isolated as a decomposer of compost and other self-heating substrates (1, 2).

The genus *Acrophialophora* was erected by Edward (3) with a single species, *Acrophialophora nainiana*. This fungus forms grayish colonies with a black reverse with age. Microscopically, it produces darkly pigmented, straight, septate, unbranched, setae-like conidiophores with thick verrucose walls, which are fertile toward the apex, and flask-shaped, hyaline phialides grouped in verticils. Single flask-shaped phialides are also formed directly from the aerial hyphae. *Acrophialophora* was not fully accepted as a distinct genus, however, until the work of Samson and Mahmood (4), who, after studying a large set of isolates, demonstrated that the aforementioned morphological features were stable, which supported the differentiation of *Acrophialophora* from morphologically similar genera such as *Paecilomyces* and *Masonia*. Those authors accepted three species, based mainly on the size, pigmentation, and ornamentation of their conidia; these species were *A. nainiana* (4 to 10.5 by 2 to 5 μm, hyaline, and finely echinulate), *Acrophialophora fusispora* (5 to 12 by 3 to 6 μm, brown, and finely echinulate, forming spiral bands), which had been described earlier as *Paecilomyces fusisporus* (4), and *Acrophialophora levis* (4.5 to 8 by 2 to 3.5 μm, hyaline, and smooth to slightly roughened). However, while Ellis (5) regarded *A. nainiana* as conspecific with *A. fusispora* and the latter as the type species of the genus, Al-Mohsen et al. (1) considered the three species synonyms and conserved the single species name *A. fusispora*. In this wide concept of the species, other taxa were considered conspecific with *A. fusispora*, i.e., *Masoniella indica* and *Ampullifera seudatica* (4).

Acrophialophora fusispora is currently recognized as an emerging human opportunistic pathogen (6, 7), responsible for cases of keratitis (6, 8, 9), pulmonary colonization and infection (6, 10–12), and devastating cerebral infections requiring intensive antifungal therapy (1, 13–15). Antifungal susceptibility data for *Acrophialophora* are scarce and based mostly on a few clinical reports (1, 15).

The species delimitation for *Acrophialophora*, using a modern phylogenetic approach, has not been properly revised, and the taxonomic position and boundaries of the genus are unknown. Therefore, we carried out a phenotypic and molecular study with a set of clinical and environmental isolates, including all of the available type strains of the species historically included in the genus. In addition, *in vitro* antifungal susceptibility testing was performed with eight clinically available antifungal agents against these isolates.

MATERIALS AND METHODS

Fungal isolates and sequences. A total of 39 isolates were included in this study, i.e., 32 from human clinical samples, 1 from an animal clinical sample, and 6 from environmental sources, including all of the available type strains of the genus (Table 1). Most of the clinical isolates were from the United States and were received by the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (UTHSCSA) from different parts of the country. In addition, 39 sequences retrieved from GenBank or the National Institute of Technology and Evaluation Biological Resource Center (NBRC) (Chiba, Japan) database were included in the phylogenetic analyses.

Phenotypic studies. The isolates were grown on malt extract agar (MEA) (30 g of malt extract, 5 g of peptone, 15 g of agar, and 1 liter of distilled water) and oatmeal agar (OA) (30 g of filtered oat flakes, 20 g of agar, and 1 liter of distilled water). Colony features and growth rates were determined at 7 and 14 days of incubation at different temperatures (5, 15, 25, 35, 37, 40, 45, 50, and 52°C). Microscopic features were examined after 14 days of incubation at 25°C on both media, in wet mounts with 85% lactic acid, using light

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TABLE 1 Origin and GenBank accession numbers of the sequences of the *Acrophialophora* spp. included in this study

Species and strain[a]	Origin	GenBank accession no.		
		LSU	ITS	*Tub*
A. levis				
CBS 484.70 (type strain)	Germany, composted domestic waste	KM995840	KM995878	LN624419
FMR 6662 = CBS 120407	Spain, sputum	KM995841	KM995879	LN624420
FMR 12780	Spain, sputum	KM995842	KM995880	LN624421
UTHSCSA DI-13-134	USA, sputum	KM995843	KM995881	LN624422
UTHSCSA DI-13-137	USA, sputum	KM995844	KM995882	LN624423
UTHSCSA DI-13-138	USA, bronchoalveolar lavage fluid	KM995845	KM995883	LN624424
UTHSCSA DI-13-139	USA, bronchoalveolar lavage fluid	KM995846	KM995884	LN624425
UTHSCSA DI-13-142	USA, sputum	KM995847	KM995885	LN624426
UTHSCSA DI-13-144	USA, sputum	KM995848	KM995886	LN624427
UTHSCSA DI-13-145	USA, brain	KM995849	KM995887	LN624428
UTHSCSA DI-13-146	USA, bronchoalveolar lavage fluid	KM995850	KM995888	LN624429
UTHSCSA DI-13-147	USA, bronchoalveolar lavage fluid (canine)	KM995851	KM995889	LN624430
UTHSCSA DI-13-148	USA, lung, right upper lobe	KM995852	KM995890	LN624431
UTHSCSA DI-13-150	USA, sputum	KM995853	KM995891	LN624432
UTHSCSA DI-13-151	USA, sputum	KM995854	KM995892	LN624433
UTHSCSA DI-13-152	USA, leg tissue	KM995855	KM995893	LN624434
UTHSCSA DI-13-153	USA, tissue	KM995856	KM995894	LN624435
UTHSCSA DI-13-154	USA, bronchoalveolar lavage fluid	KM995857	KM995895	LN624436
UTHSCSA DI-13-155	USA, sputum	KM995858	KM995896	LN624437
UTHSCSA DI-13-156	USA, knee tissue	KM995859	KM995897	LN624438
UTHSCSA DI-13-157	USA, bronchoalveolar lavage fluid	KM995860	KM995898	LN624439
UTHSCSA DI-13-158	USA, sputum	KM995861	KM995899	LN624440
UTHSCSA DI-13-159	USA, bronchoalveolar lavage fluid	KM995862	KM995900	LN624441
UTHSCSA DI-13-162	USA, bronchoalveolar lavage fluid	KM995863	KM995901	LN624442
UTHSCSA DI-13-163	USA, bronchoalveolar lavage fluid	KM995864	KM995902	LN624443
A. fusispora				
CBS 100.60 (*A. nainiana* type strain)	India, farm soil	KM995865	KM995903	LN624444
CBS 149.64 (*M. indica* type strain)	India, forest soil	KM995866	KM995904	LN624445
CBS 380.55 (*P. fusisporus* type strain)	India, forest soil	KM995867	KM995905	LN624446
FMR 6258 = CBS 120406	India, soil	KM995868	KM995906	LN624447
FMR 8888 = CBS 120409	India, cornea	KM995869	KM995907	LN624448
UTHSCSA DI-13-135	USA, left sphenoid sinus	KM995870	KM995908	LN624449
UTHSCSA DI-13-136	USA, brain abscess	KM995871	KM995909	LN624450
UTHSCSA DI-13-140	USA, bronchoalveolar lavage fluid	KM995872	KM995910	LN624451
UTHSCSA DI-13-141	USA, sputum	KM995873	KM995911	LN624452
UTHSCSA DI-13-143	USA, chest mass	KM995874	KM995912	LN624453
UTHSCSA DI-13-149	USA, cornea	KM995875	KM995913	LN624454
UTHSCSA DI-13-160	USA, bronchoalveolar lavage fluid	KM995876	KM995914	LN624455
UTHSCSA DI-13-161	USA, sputum	KM995877	KM995915	LN624456
A. seudatica				
CBS 916.79 (*Ampullifera seudatica* type strain)	India, soil	LN736031	LN736030	LN736032

[a] CBS, Fungal Biodiversity Centre (Utrecht, The Netherlands) culture collection; FMR, Facultat de Medicina, Universitat Rovira i Virgili (Reus, Spain); UTHSCSA, Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (San Antonio, TX).

microscopy. All isolates were identified based on the features described by Edward (3), Samson and Mahmood (4), and Ellis (5). Photomicrographs were obtained with a Zeiss Axio-Imager M1 light microscope, using phase-contrast and Nomarski differential interference optics.

DNA extraction, amplification, and sequencing. FastPrep kits (MP Biomedicals, Santa Ana, CA) were used to extract total genomic DNA from fungal mycelia harvested from colonies grown on potato dextrose agar (PDA) for 7 days at 25°C, according to the manufacturer's protocol. DNA was quantified using a Nanodrop 3000 apparatus (Thermo Scientific, Madrid, Spain).

Three nuclear DNA targets were amplified by PCR and sequenced using the following primer pairs: ITS4 and ITS5 (16) for a region spanning internal transcribed spacer 1 (ITS1) and ITS2 and the 5.8S gene of the ribosomal DNA (rDNA), LR0R and LR5 (17, 18) for a fraction of the 5′ end of the large subunit (LSU) gene of the rDNA, and BT2a and BT2b (19) for a fragment of the β-tubulin (*Tub*) gene. The amplified products were purified with the Diffinity RapidTip purification system (Sigma-Aldrich, St. Louis, MO) and stored at −20°C until sequencing.

Sequencing was performed in both directions, with the same primer pair as used for amplification, at Macrogen Europe (Macrogen Inc., Amsterdam, The Netherlands). The consensus sequences were obtained using SeqMan software (version 7.0.0; DNAStar Lasergene, Madison, WI).

Molecular identification and phylogenetic analysis. In order to assess the taxonomic position of the genus *Acrophialophora*, a first phylogenetic analysis was carried out using partial LSU sequences of the available type strains of *Acrophialophora* species complemented with 15 sequences retrieved from public databases, selected on the basis of BLAST homology searches and representing 8 different genera from the families *Chaetomi-*

aceae and *Sordariaceae*, of the subclass *Sordariomycetidae*. A second phylogenetic analysis directed to assessing the species distribution of *Acrophialophora* was conducted using partial LSU, ITS, and *Tub* sequences and included all of the type strains of *Acrophialophora* species, the type strains of the putative synonyms *Ampullifera seudatica* and *M. indica*, and several clinical and environmental isolates morphologically identified as *Acrophialophora* spp. Multiple sequence alignments were made for each individual locus using Mega version 6.06 (20), with the ClustalW function, and were manually refined when necessary. The nucleotide substitution models for each data set (GTR+G+I for LSU, JC+G for ITS, and T92+G for *Tub*) were calculated using the Find best DNA/protein model tool in Mega 6.06. In order to compare the concordance of the different loci, individual phylogenetic analyses were carried out using the maximum likelihood (ML) algorithm in Mega; the resulting trees were compared visually using a 70% bootstrap cutoff and were complemented with the partition homogeneity test, carried out as implemented in PAUP* software (version 4.0b10; Sinauer, Sunderland, MA). Since no incongruence was found ($P = 0.180$), the three genes were combined into a single data set. The combined phylogenetic analyses were made using maximum likelihood (ML) and Bayesian inference (BI) methods in Mega and MrBayes (version 3.1.2) (21), respectively. For the ML analysis, nearest-neighbor interchange (NNI) was used as the heuristic method for tree inference. Support for the internal branches was assessed by a search of 1,000 bootstrapped sets of data. A bootstrap support (bs) value of ≥70 was considered significant. For BI analysis, two simultaneous runs of 3,000,000 generations were performed, and samples were stored every 100 generations. The 50% majority-rule consensus tree and posterior probability (pp) values were calculated after the first 25% of the samples were discarded. A pp value of ≥0.95 was considered significant.

Antifungal susceptibility testing. Antifungal susceptibility testing was performed according to the methods in the CLSI M38-A2 standard (22). The antifungal drugs tested were amphotericin B (AMB), voriconazole (VRC), itraconazole (ITC), posaconazole (PSC), terbinafine (TRB), anidulafungin (AFG), caspofungin (CFG), and micafungin (MFG). The minimal effective concentration (MEC), defined as the lowest concentration that resulted in short, stubby, abnormally branched hyphae, was determined at 24 h for the echinocandins, and the MIC was determined at 48 h for the remaining drugs. The MIC was defined as the lowest concentration exhibiting 100% visual inhibition of growth for AMB, VRC, ITC, and PSC and 80% reduction in growth for TRB. Geometric mean (GM) MICs were compared using the Mann-Whitney test in GraphPad Prism for Windows (version 6; GraphPad Software, La Jolla CA).

Nucleotide sequence accession numbers. Sequences newly generated in this study were deposited in GenBank under accession numbers KM995840 to KM995877 and LN736031 (LSU), KM995878 to KM995915 and LN736030 (ITS), and LN624419 to LN624456 and LN736032 (*Tub*) (Table 1).

RESULTS

Figure 1 shows the results of the analysis of the LSU sequences (431 bp) of *Acrophialophora* species and related fungi. The type strain of *A. nainiana* (CBS 100.60) clustered with the type strains of *A. levis* (CBS 484.70) and *A. fusispora* (CBS 380.55), being included in a fully supported clade containing several members of the family *Chaetomiaceae* of the order *Sordariales*. The species closest to *Acrophialophora* were members of *Achaetomium*, *Botryotrichum*, *Chaetomidium*, and *Chaetomium*. The latter genus was also found to be polyphyletic.

Figure 2 shows the results of the phylogenetic analysis of the species of the genus *Acrophialophora* using concatenated LSU, ITS, and *Tub* sequences. The final alignment consisted of 1,804 bp (LSU, 843 bp; ITS, 502 bp; *Tub*, 459 bp) of 41 isolates, i.e., one from an environmental source, 33 from clinical origins, and the type strains of *A. fusispora*, *A. levis*, and *A. nainiana* and the putative

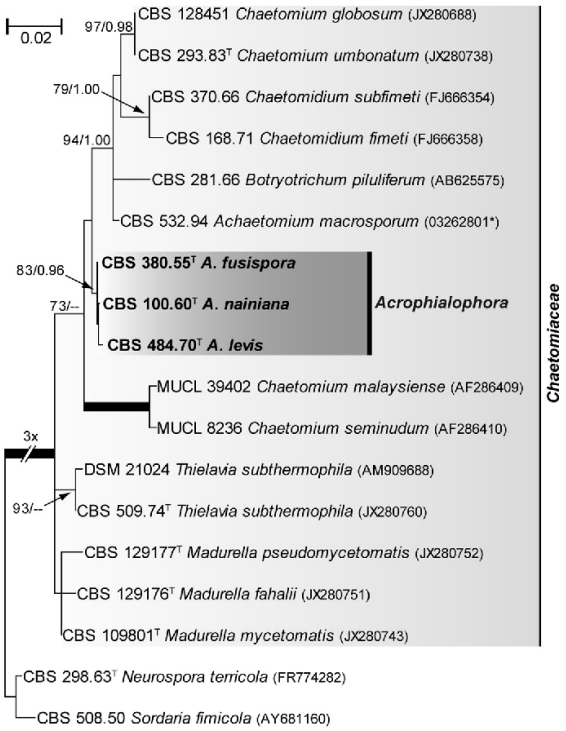

FIG 1 Maximum likelihood (ML) tree constructed with partial LSU sequences (431 bp) of *Acrophialophora* spp. and members of *Chaetomiaceae*. Branch lengths are proportional to the phylogenetic distance. ML bs and Bayesian pp values over 70% and 0.95, respectively, are shown on the nodes. Thickened branches indicate full statistical support. GenBank accession numbers are shown in parentheses. *, sequence retrieved from the National Institute of Technology and Evaluation Biological Resource Center (NBRC) (Chiba, Japan). The tree is rooted with *Neurospora terricola* and *Sordaria fimicola*. T, type strain; CBS, Fungal Biodiversity Centre (Utrecht, The Netherlands) culture collection; DSM, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany); MUCL, Mycothèque de l'Université Catholique de Louvain (Louvain-la-Neuve, Belgium).

synonyms *Ampullifera seudatica* and *M. indica*. *Chaetomium globosum* and *Chaetomium angustispirale* were used to root the tree. The tree showed two fully supported main clades, one of which included the type strain of *A. levis* and the other that of *A. fusispora*. The latter clustered in a fully supported clade with the type strains of *A. nainiana* and *M. indica*, which demonstrated them to be conspecific, since their sequences were practically identical. The type strain of *Ampullifera seudatica* formed a single lineage, basal and distant to the *A. fusispora* clade (98.5% sequence similarity with *A. fusispora* in the combined analysis), and is here considered a different species of the genus named *Acrophialophora seudatica*.

The isolates identified as *A. fusispora* and *A. levis* showed similar macroscopic features on all media tested. The colonies on MEA (Fig. 3a and h) ranged from 30 to 60 mm in diameter after 14 days at 25°C and were flat to slightly umbonate, at first white but soon becoming pale yellow to brownish gray, velvety to felty, with irregular margins and a yellow, brown, or black reverse. *Acrophialophora seudatica* grew more slowly (20 to 25 mm in diameter in 14 days), and its colonies were flat, at first white but rapidly turning

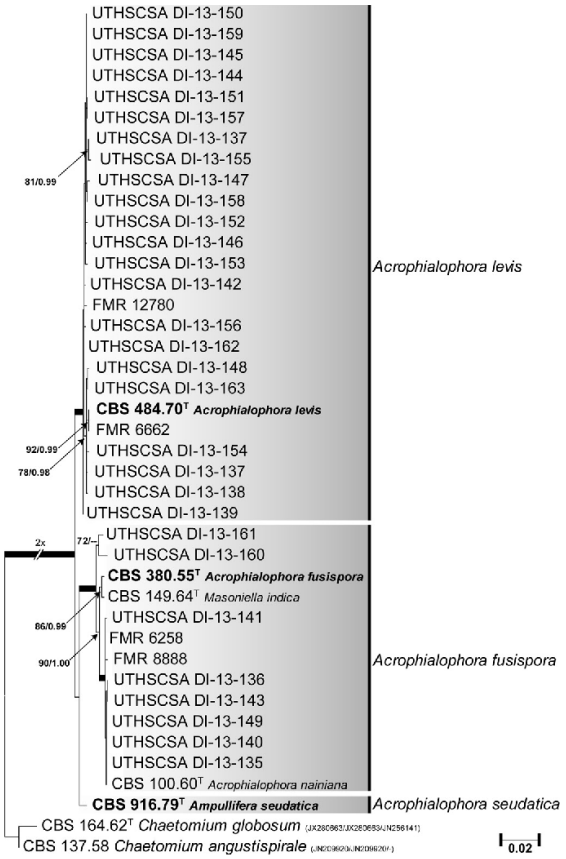

FIG 2 Maximum likelihood (ML) tree constructed with combined LSU (843 bp), ITS (502 bp), and *Tub* (459 bp) sequences of *Acrophialophora* clinical and environmental isolates. ML bs and Bayesian pp values are shown on the nodes. Thickened branches indicate full statistical support. The tree is rooted with *Chaetomium globosum* and *Chaetomium angustispirale*. GenBank accession numbers for LSU, ITS, and *Tub* sequences are shown in parentheses. T, type strain; CBS, Fungal Biodiversity Centre (Utrecht, The Netherlands) culture collection; FMR, Facultat de Medicina, Universitat Rovira i Virgili (Reus, Spain); UTHSCSA, Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (San Antonio, TX).

pale orange, velvety, with a pale orange reverse (Fig. 3p). The optimal temperature for growth was between 35 and 40°C, with a minimum of 15°C and a maximum of 50°C, for all of the species. Microscopically, the isolates of *A. fusispora* were characterized by the abundant production of flask-shaped phialides and polyphialides measuring 5 to 19 by 1.5 to 5 μm (Fig. 3d and e), swollen at the base and tapering abruptly to a narrow neck, mostly formed directly from the aerial hyphae or in the apex of well-differentiated conidiophores, which were erect, unbranched, dark-colored, and with a spiny to warted wall surface (Fig. 3c). Conidia were produced on basipetal chains and were one-celled, subhyaline to brownish, ovoid to fusiform, finely echinulate or forming spiral bands, and measuring 5 to 12 by 2 to 5 μm (Fig. 3d to g). The isolates of *A. levis* also produced abundant flask-shaped phialides and frequently polyphialides, measuring 4 to 13 by 1.5 to 5 μm (Fig. 3i and m), and hyaline to subhyaline ellipsoid to cylindrical conidia, smooth to finely echinulate and measuring 4 to 9 by 2 to 6 μm (Fig. 3m to o). The isolate of *A. seudatica* exhibited flask-shaped phialides measuring 8 to 22 by 2.5 to 4.5 μm, with long necks (Fig. 3r and s), and ovoid to fusiform conidia measuring 6 to 8 by 3 to 4 μm (Fig. 3t and u), with thick and finely verruculose walls, subhyaline or turning pale yellow when mature. This isolate was unable to produce the typical pigmented conidiophores of *Acrophialophora*. Table 2 summarizes the key morphological features that distinguish the three *Acrophialophora* species.

The majority of isolates from clinical sources belonged to *A. levis* (72.7%), while *A. fusispora* accounted for the remaining 34.3% of the isolates. The main source of isolates was the respiratory tract (72.7%), mostly from sputum and bronchoalveolar lavage (BAL) fluid specimens, followed by subcutaneous tissues (9.1%), brain tissue, and corneas (6.1% each). Other sites from which the fungi were cultured included the sphenoid sinus and a chest mass (3% each). No major differences regarding the origins of isolates were observed between *A. levis* and *A. fusispora*.

The antifungal susceptibility results for the isolates belonging to *A. levis* and *A. fusispora* are shown in Table 3. Overall, the highest MIC values were observed for AMB, with geometric mean (GM) MIC and MIC_{90} values of 5.66 μg/ml and 16 μg/ml, respectively. The azole drugs exhibited the best *in vitro* activity, with VRC being the most potent, with overall GM MIC and MIC_{90} values of 0.17 μg/ml and 0.25 μg/ml, respectively, followed by PSC and ITC. The echinocandins exhibited poor *in vitro* activity, with AFG showing the lowest GM MEC and MEC_{90} values (1.86 μg/ml and 4 μg/ml, respectively). TRB showed GM MIC and MIC_{90} values of 0.51 μg/ml and 1 μg/ml, respectively. Although the differences were subtle, the MICs for VRC, ITC, CFG, AFG, MFG, and TRB were significantly lower against *A. levis* than *A. fusispora* ($P < 0.0001$).

TAXONOMY

According to the results of our phylogenetic and morphological analyses, the following new combination is proposed: *Acrophialophora seudatica* (Subrahm.) Sandoval-Denis, Gené & Guarro comb. nov., Mycobank accession number MB811225. Basionym: *Ampullifera seudatica* Subrahmanyam, Nova Hedwigia 31:159 (1979).

DISCUSSION

To our knowledge, this is the first study involving molecular assessment of the fungal genus *Acrophialophora*, a rare opportunistic human and animal pathogen. It also includes the largest set of clinical isolates of the species studied to date. The taxonomy of the genus has been revised and the spectrum of species associated with human disease determined.

According to our results, the genus *Acrophialophora*, belonging to the sordariomycetous family *Chaetomiaceae*, comprises three species, i.e., *A. fusispora*, *A. levis*, and *A. seudatica*. This family includes mostly soilborne cellulose decomposers but also thermotolerant opportunistic pathogens, including neurotropic species such as *Achaetomium strumarium* and *Chaetomium atrobrunneum* (23, 24). Although historically the family *Chaetomiaceae* encompassed mainly ascosporulating fungi, *Acrophialophora* is not the first genus of the family showing strictly asexual reproduction. Recently, de Hoog et al. (24) demonstrated that agents of black-grain mycetomas such as *Madurella* species, which fail to produce fertile sexual morphs, also belong to *Chaetomiaceae*. Ac-

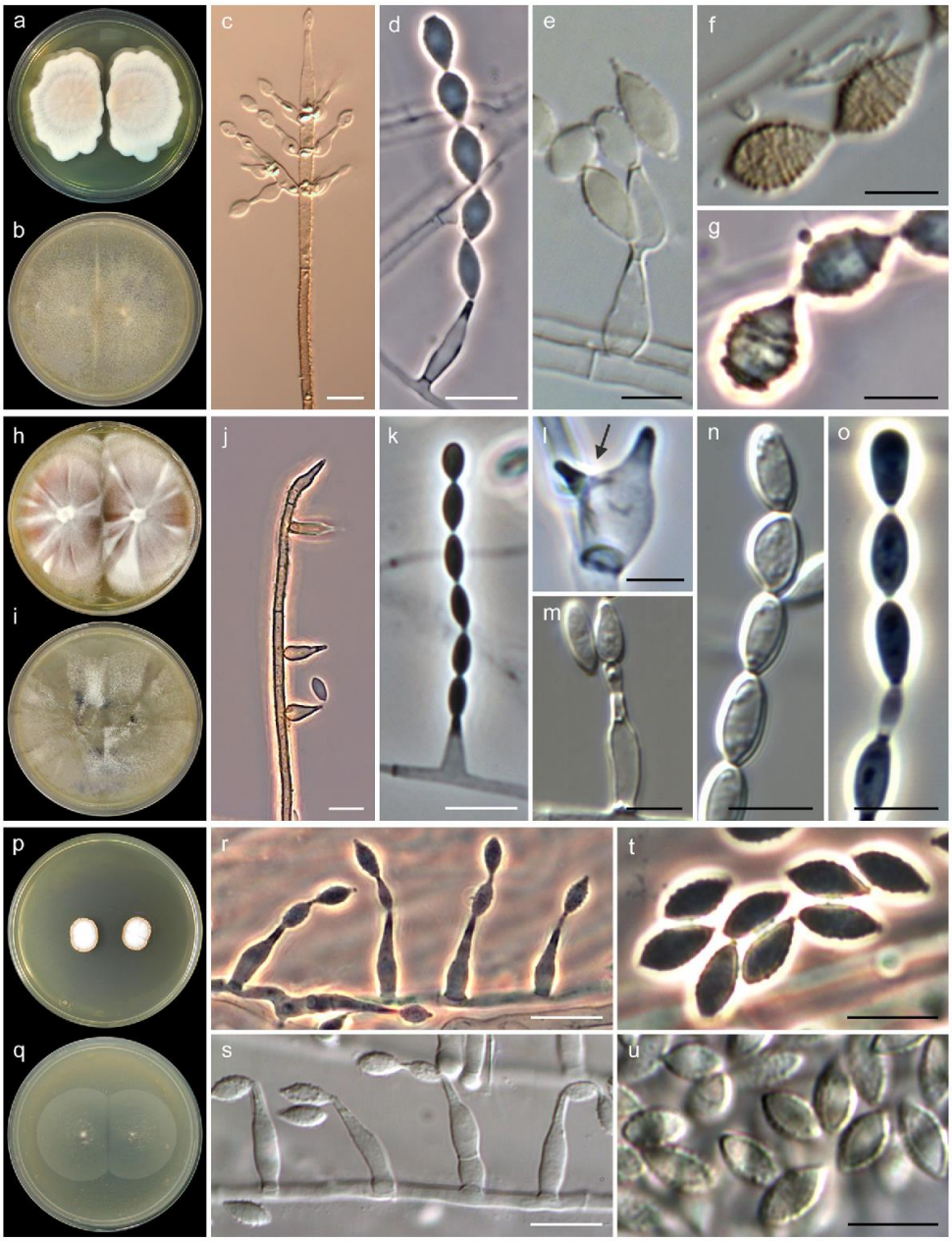

FIG 3 Key morphological features of *Acrophialophora fusispora* (a to g), *A. levis* (h to o), and *A. seudatica* (p to u). (a, h, and p) Colonies on MEA after 14 days at 25°C. (b, i, and q) Colonies on OA after 14 days at 25°C. (c and j) Conidiophores. (d, e, k, l, m, r, and s) Phialides (polyphialides indicated with an arrow). (f, g, n, o, t, and u) Conidia. White bars, 10 μm; black bars, 5 μm.

cording to our LSU phylogeny (Fig. 1), members of the genera *Acrophialophora*, *Chaetomidium*, and *Thielavia* nested unambiguously in highly supported terminal clades, while the positions of the genera *Achaetomium*, *Botryotrichum*, *Chaetomium*, and *Madurella* are unclear, with the genus *Chaetomium* forming two polyphyletic clades. The classifications of the latter genus, however, have been shown to differ significantly when molecular and conventional approaches are compared (24).

TABLE 2 Key differential features of the three species of *Acrophialophora*

Characteristic[a]	*A. fusispora*	*A. levis*	*A. seudatica*
Colony diameter (mm)	30–50	35–60	20–25
Colony color	White, pale yellow, or gray	White, pale yellow, or gray	White to pale orange
Colony texture	Velvety to felty	Velvety to felty	Velvety
Phialide size (μm)	5–19 by 1.5–5	4–13 by 1.5–5	8–22 by 2.5–4.5
Conidial size (μm)	5–12 by 2–5	4–9 by 2–6	6–8 by 3–4
Conidial shape	Ovoid to fusiform	Ellipsoid to cylindrical	Ovoid to fusiform
Conidial ornamentation	Finely echinulate to spiral sculpted	Smooth to finely echinulate	Finely verruculose
Conidial color	Subhyaline to brown	Hyaline to subhyaline	Subhyaline to pale yellow

[a] Characteristics were determined after growth on MEA for 14 days at 25°C.

Because the morphological features used to distinguish the three *Acrophialophora* species recognized by Samson and Mahmood (4) tended to overlap, Al-Mohsen et al. (1) considered them morphological variations of a single species, *A. fusispora*. However, our phylogenetic analysis of the different type strains does not support this conclusion. Our results confirm that *A. fusispora*, *A. nainiana*, and *M. indica* are conspecific, while *A. levis* and *A. seudatica* are two different species. In contrast to the observations of Al-Mohsen et al. (1), our phylogenetic results indicate that subtle morphological findings for these fungi, such as conidial size, shape, color, and ornamentation, show consistent differences to distinguish *A. fusispora*, *A. levis*, and *A. seudatica* (Table 2). The presence (mainly on OA) of erect pigmented conidiophores is typical of cultures of the two clinically relevant species of *Acrophialophora*, i.e., *A. fusispora* and *A. levis*, and can be important for the initial generic diagnosis. However, these pigmented conidiophores are absent in *A. seudatica*. This species was originally described as having simple, hyaline, straight conidiophores (25), a feature that is confirmed here. However, *A. seudatica* is known only from its type specimen isolated from a soil sample from India. Therefore, confirmation of the presence or absence of the typical conidiophores of *Acrophialophora* will be possible only by studying more isolates of this rare species.

The three species of *Acrophialophora* shared very similar LSU sequences (99.9%), but the large differences in their ITS and *Tub* sequences (<96.1% and <96.6% sequence similarity, respectively) show that both loci can discriminate between the three species, making them good candidates for barcoding targets in *Acrophialophora*.

Some authors stated that *Acrophialophora* infections may have been underdiagnosed due to the rarity of these fungi and the potential confusion with similar opportunistic molds, such as *Lomentospora prolificans* and *Scopulariopsis chartarum* (9, 13, 26, 27). Only six well-documented cases of human infections exist in the literature, most of which lacks molecular confirmation of the etiological agent. In only one case was the fungus confirmed as *A. fusispora* by sequencing of the ITS region (15). The rarity is also reflected by the scarcity of reference sequences for comparison in fungal databases, which do not include any type or correctly identified reference strains.

Since the synonymy of the species of *Acrophialophora* was formally proposed by Al-Mohsen et al. (1), *A. fusispora* has remained the only accepted species of the genus and, as such, has been cited as the causative agent in the reported clinical cases (1, 7, 14, 23). According to our results, however, *A. levis* seems to be the most common species isolated from human clinical samples. The identification of some of the isolates included in the study by Guarro et al. (6) was reassessed here by sequence comparison. One clinical isolate (FMR 8888, from a corneal infection) was confirmed as *A. fusispora*, while another (FMR 6662, isolated from sputum) was reidentified here as *A. levis*. The third clinical isolate included in that study (FMR 6404) was not available for analysis, and thus its final identification remains unknown.

Most published cases refer to pulmonary involvement, with or

TABLE 3 Results of *in vitro* antifungal susceptibility testing for the 33 clinical isolates of *Acrophialophora* spp. included in the study

	MIC or MEC (μg/ml)[a]							
Species and parameter	AMB	VRC	PSC	ITC	CFG[b]	AFG[b]	MFG[b]	TRB
A. levis (n = 24)								
GM	6.77	0.16	0.50	1.15	3.58	1.65	2.64	0.42
Range	1–32	0.06–05	0.25–1	0.5–4	0.25–32	0.25–8	0.25–32	0.125–4
MIC_{90}	32	0.25	1	2	16	4	32	1
A. fusispora (n = 9)								
GM	4.22	0.18	0.50	0.85	11.02	2.61	6.46	0.75
Range	2–32	0.125–0.25	0.25–1	0.125–1	4–32	2–8	0.125–32	0.5–1
MIC_{90}	16	0.25	1	1	16	4	32	1
Overall (n = 33)								
GM	5.66	0.17	0.49	1	4.98	1.86	3.21	0.51
Range	1–32	0.06–05	0.25–1	0.125–4	0.25–32	0.25–8	0.125–32	0.125–4
MIC_{90}	16	0.25	1	1	16	4	32	1

[a] AMB, amphotericin B; VRC, voriconazole; PSC, posaconazole; ITC, itraconazole; CFG, caspofungin; AFG, anidulafungin; MFG, micafungin; TRB, terbinafine.
[b] This column contains MEC data.

without systemic dissemination (6, 10–12). Similarly, the majority of our clinical isolates were obtained from respiratory specimens, with one-half of them being from BAL fluid samples. It was not possible, however, to distinguish between true infectious agents, colonizers, and environmental contaminants, given the nature of the samples and the absence of appropriate clinical or histopathological data. The second most common infection in clinical reports is keratitis (6, 9), while in our study it was soft tissue infection, particularly lower extremity tissue infection. Corneal and cerebral samples, in equal proportions, were the third most common sites of isolation. The lack of isolates from the central nervous system (CNS) does not allow us to confirm the potential neurotropism attributed to *Acrophialophora* (14).

The antifungal treatment of *Acrophialophora* infections has been hampered by the paucity of *in vitro* susceptibility data and the lack of specific treatment guidelines. The clinical cases have demonstrated variable results. Arthur et al. (9) reported a favorable outcome with the use of AMB and surgical debridement in a case of keratouveitis. In one report of a pulmonary infection, monotherapy with liposomal AMB (LAMB) was not effective, but the patient responded to combination therapy with LAMB and ITC (1). Guarro et al. (6) reported the use of VRC in two clinical cases, one a case of keratitis that responded favorably to the drug and one a pulmonary infection with a fatal outcome. In addition, Li et al. (15) described a negative outcome using VRC in a case of cerebral infection. The two latter cases, however, were in highly immunocompromised patients with systemic involvement. Our susceptibility results showed that, while AMB and the echinocandins have almost no activity against *Acrophialophora* species, VRC exhibits potent *in vitro* activity. This confirms the observations of Guarro et al. (6), suggesting that VRC may be a potential treatment option for *Acrophialophora* infections.

In conclusion, *Acrophialophora* includes three closely related species, *A. fusispora*, *A. levis*, and *A. seudatica*, that can be accurately identified on the basis of ITS or *Tub* sequencing and detailed morphological study. *Acrophialophora levis* appears to be the most frequent species in clinical samples. VRC shows potent *in vitro* activity against these fungi.

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4.2 *Cladosporium* species recovered from clinical samples in the United States

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Cladosporium Species Recovered from Clinical Samples in the United States

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Cladosporium **species are ubiquitous, saprobic, dematiaceous fungi, only infrequently associated with human and animal opportunistic infections. We have studied a large set of *Cladosporium* isolates recovered from clinical samples in the United States to ascertain the predominant species there in light of recent taxonomic changes in this genus and to determine whether some could possibly be rare potential pathogens. A total of 92 isolates were identified using phenotypic and molecular methods, which included sequence analysis of the internal transcribed spacer (ITS) region and a fragment of the large subunit (LSU) of the nuclear ribosomal DNA (rDNA), as well as fragments of the translation elongation factor 1 alpha (*EF-1α*) and actin (*Act*) genes. The most frequent species was *Cladosporium halotolerans* (14.8%), followed by *C. tenuissimum* (10.2%), *C. subuliforme* (5.7%), and *C. pseudocladosporioides* (4.5%). However, 39.8% of the isolates did not correspond to any known species and were deemed to comprise at least 17 new lineages for *Cladosporium*. The most frequent anatomic site of isolation was the respiratory tract (54.5%), followed by superficial (28.4%) and deep tissues and fluids (14.7%). Species of the two recently described cladosporium-like genera *Toxicocladosporium* and *Penidiella* are reported for the first time from clinical samples. *In vitro* susceptibility testing of 92 isolates against nine antifungal drugs showed a variety of results but high activity overall for the azoles, echinocandins, and terbinafine.**

Cladosporium species are among the most common fungal inhabitants worldwide, being isolated from almost any environmental source and geographic location (1). The genus is characterized by the typical form of its conidiophores, which are erect, straight or geniculate, produce abundant branched acropetal chains of smooth to roughened dry conidia, and show a distinct darkened coronate hilum, i.e., conidial scar characterized by a thick rim surrounding a central convex dome (2, 3). The relatively small conidia are easily detached and disseminated by the wind, *Cladosporium* being one of the most frequently isolated airborne fungi (2, 4).

The most common *Cladosporium* species are primarily isolated from soil and plant material, where they are frequently encountered as saprobes or secondary invaders on follicular lesions, concomitant with other plant-pathogenic fungi (1, 5, 6). However, several species are important pathogens of plants and some are also able to affect animals, including humans (7–9). *Cladosporium* is usually associated with allergic rhinitis (10) or localized superficial or deep lesions (11–14) but, rarely, can cause disseminated infections (7, 15–17).

The genus *Cladosporium* has been shown to be both morphologically and phylogenetically heterogeneous (18). On the basis of molecular data, the true human-pathogenic species *C. bantiana*, *C. carrionii*, and *C. devriesii*, characterized by their thermotolerance and the absence of conidiophores with pigmented conidial scars, were transferred to *Cladophialophora* (1, 7, 18). More recently, *Cladosporium* underwent extensive revisions based on polyphasic approaches (1, 3, 19–21), which resulted in the delimitation of 169 species currently accepted in *Cladosporium sensu stricto* (*Cladosporiaceae*, *Capnodiales*). On the other hand, a great number of taxa were excluded from that genus, now being considered doubtful species or accommodated into several related new genera, such as *Hyalodendriella*, *Ochrocladosporium*, *Rachicladosporium*, *Rhizocladosporium*, *Toxicocladosporium*, and *Verrucocladosporium* (1, 3).

The diversity of *Cladosporium* species associated with human disease is currently reduced to four, i.e., *C. cladosporioides*, *C. herbarum*, *C. oxysporum*, and *C. sphaerospermum* (7). Most of these data, however, are based on a reduced number of clinical cases with the identification of the etiological agents not confirmed by reliable methods. Moreover, three of the clinically relevant species, *C. cladosporioides*, *C. herbarum*, and *C. sphaerospermum*, have been demonstrated to be species complexes (19–21) encompassing several morphologically sibling species that can only be distinguished by means of phylogenetic analyses (1, 7). The clinical significance of these phylogenetic species, however, has yet to be evaluated (22).

The objective of this work was to assess the diversity of *Cladosporium* species associated with human and animal disease by analyzing a large set of isolates from clinical specimens by means of phenotypic and DNA sequence data analyses. In addition, the *in vitro* susceptibility of these isolates was evaluated against nine clinically available antifungal drugs.

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TABLE 1 Clinical isolates, type or reference strains, and sequences included in this study

Species	Strain/isolate no.[a]	Origin (country)[b]	GenBank nucleotide accession no. for:			
			ITS	LSU	*EF-1α*	*Act*
Cercospora beticola	CBS 116456^T	*Beta vulgaris* (Italy)	NR121315	GU214404	AY840494	AY840458
Cercospora olivascens	CBS 253.67^T	Unknown	NR111773			
Cladosporium allicinum	CBS 121624^T	*Hordeum vulgare* (Belgium)	EF679350		EF679425	EF679502
	UTHSC DI-13-173	Human, lung (USA)	LN834353		LN834449	LN834537
	UTHSC DI-13-176	Human, skin (USA)	LN834354		LN834450	LN834538
	UTHSC DI-13-266	Canine, skin (USA)	LN834355		LN834451	LN834539
Cladosporium angustisporum	CBS 125983^T	*Alloxylon wickhamii* (Australia)	HM147995		HM148236	HM148482
	UTHSC DI-13-240	Human, toe nail (USA)	LN834356		LN834452	LN834540
Cladosporium asperulatum	CBS 126339	*Eucalyptus* leaf litter (India)	HM147997		HM148238	HM148484
	CBS 126340^T	*Protea susannae* (Portugal)	HM147998		HM148239	HM148485
	UTHSC DI-13-216	Feline, nasal (USA)	LN834357		LN834453	LN834541
Cladosporium cladosporioides	CBS 101367	Soil (Brasil)	HM148002		HM148243	HM148489
	CBS 112388^T	Indoor air (Germany)	HM148003		HM148244	HM148490
	UTHSC DI-13-204	Human, abdomen (USA)	LN834358		LN834454	LN834542
	UTHSC DI-13-209	Human, pleural (USA)	LN834359		LN834455	LN834543
	UTHSC DI-13-215	Human, sputum (USA)	LN834360		LN834456	LN834544
Cladosporium colocasiae	CBS 386.64^T	*Colocasia antiquorum* (Taiwan)	HM148067		HM148310	HM148555
	CBS 119542	*Colocasia esculanta* (Taiwan)	HM148066		HM148309	HM148554
Cladosporium cucumerinum	CBS 171.52^T	Fruit of *Cucumis sativus* (Netherlands)	HM148072		HM148316	HM148561
	CBS 173.54	Fruit of *Cucumis sativus* (Netherlands)	HM148074		HM148318	HM148563
Cladosporium flabelliforme	CBS 126345^T	*Melaleuca cajuputi* (Australia)	HM148092		HM148336	HM148581
	UTHSC DI-13-267	Human, sputum (USA)	LN834361		LN834457	LN834545
Cladosporium funiculosum	CBS 122128	Unknown	HM148093		HM148337	HM148582
	CBS 122129^T	Leaf of *Vigna umbellata* (Japan)	HM148094		HM148338	HM148583
	UTHSC DI-13-175	Human, BAL fluid (USA)	LN834362		LN834458	LN834546
	UTHSC DI-13-223	Human, BAL fluid (USA)	LN834363		LN834459	LN834547
	UTHSC DI-13-242	Human, nasal wash (USA)	LN834364		LN834460	LN834548
Cladosporium halotolerans	CBS 119416^T	Hypersaline water (Namibia)	DQ780364		JN906989	EF101397
	FMR 13493	Human, unknown (Spain)	LN834365		LN834461	LN834549
	UTHSC DI-13-164	Human, bone marrow (USA)	LN834366		LN834462	LN834550
	UTHSC DI-13-182	Marine mammal, dermis (USA)	LN834367		LN834463	LN834551
	UTHSC DI-13-183	Human, bronchus (USA)	LN834368		LN834464	LN834552
	UTHSC DI-13-206	Human, BAL fluid (USA)	LN834369		LN834465	LN834553
	UTHSC DI-13-213	Human, lymph node (USA)	LN834370		LN834466	LN834554
	UTHSC DI-13-221	Human, bone marrow (USA)	LN834371		LN834467	LN834555
	UTHSC DI-13-231	Catheter tip (USA)	LN834372		LN834468	LN834556
	UTHSC DI-13-249	Human, nasal (USA)	LN834373		LN834469	LN834557
	UTHSC DI-13-250	Human, scalp (USA)	LN834374		LN834470	LN834558
	UTHSC DI-13-252	Human, toe nail (USA)	LN834375		LN834471	LN834559
	UTHSC DI-13-259	Human, BAL fluid (USA)	LN834376		LN834472	LN834560
	UTHSC DI-13-263	Human, BAL fluid (USA)	LN834377		LN834473	LN834561
Cladosporium herbaroides	CBS 121626^T	Hypersaline water (Israel)	EF679357		EF679432	EF679509
Cladosporium herbarum	CBS 121621^T	*Hordeum vulgare* (Netherlands)	EF679363		EF679440	EF679516
	UTHSC DI-13-220	Human, BAL fluid (USA)	LN834378		LN834474	LN834562
Cladosporium iranicum	CBS 126346^T	Leaf of *Citrus sinensis* (Iran)	HM148110		HM148354	HM148599
Cladosporium iridis	CBS 138.40^T	Leaf of *Iris* sp. (Netherlands)	EF679370		EF679447	EF679523
Cladosporium macrocarpum	CBS 121623^T	*Spinacia oleracea* (USA)	EF679375		EF679453	EF679529
	UTHSC DI-13-191	Human, face (USA)	LN834379		LN834475	LN834563
Cladosporium oxysporum	CBS 125991	Soil (China)	HM148118		HM148362	HM148607
	CBS 126351	Indoor air (Venezuela)	HM148119		HM148363	HM148608
Cladosporium perangustum	CBS 125996^T	*Cussonia* sp. (South Africa)	HM148121		HM148365	HM148610
	UTHSC DI-13-208	Canine, BAL fluid (USA)	LN834380		LN834476	LN834564
Cladosporium pseudocladosporioides	CBS 117153	Leaf of *Paeonia* sp. (Germany)	HM148157		HM148401	HM148646
	CBS 125993^T	Outside air (Netherlands)	HM148158		HM148402	HM148647
	UTHSC DI-13-187	Turtle, unknown (USA)	LN834381		LN834477	LN834565
	UTHSC DI-13-232	Human, shoulder (USA)	LN834382		LN834478	LN834566
	UTHSC DI-13-233	Human, BAL fluid (USA)	LN834383		LN834479	LN834567
	UTHSC DI-13-261	Human, sputum (USA)	LN834384		LN834480	LN834568

(Continued on following page)

TABLE 1 (Continued)

Species	Strain/isolate no.[a]	Origin (country)[b]	GenBank nucleotide accession no. for:			
			ITS	LSU	*EF-1α*	*Act*
Cladosporium ramotenellum	CBS 121628^T	Hypersaline water (Slovenia)	EF679384		EF679462	EF679538
	UTHSC DI-13-166	Human, nasal tissue (USA)	LN834385		LN834481	LN834569
	UTHSC DI-13-222	Animal, Nasal (USA)	LN834386		LN834482	LN834570
	UTHSC DI-13-224	Animal, Nasal (USA)	LN834387		LN834483	LN834571
Cladosporium sinuosum	CBS 121629^T	*Fuchsia excorticata* (New Zealand)	EF679386		EF679464	EF679540
Cladosporium sphaerospermum	CBS 193.54^T	Human, nail (Netherlands)	DQ780343		EU570261	EU570269
	UTHSC DI-13-184	Frog, abscess (USA)	LN834388		LN834484	LN834572
	UTHSC DI-13-229	Human, BAL fluid (USA)	LN834389		LN834485	LN834573
	UTHSC DI-13-237	Human, BAL fluid (USA)	LN834390		LN834486	LN834574
Cladosporium subinflatum	CBS 121630^T	Hypersaline water (Slovenia)	EF679389		EF679467	EF679543
	UTHSC DI-13-189	Human, toe nail (USA)	LN834391		LN834487	LN834575
Cladosporium subtilissimum	CBS 113754^T	Grape berry (USA)	EF679397		EF679475	EF679551
Cladosporium subuliforme	CBS 126500^T	*Chamaedorea metallica* (Thailand)	HM148196		HM148441	HM148686
	UTHSC DI-13-171	Human, CSF (USA)	LN834392		LN834488	LN834576
	UTHSC DI-13-180	Human, BAL fluid (USA)	LN834393		LN834489	LN834577
	UTHSC DI-13-214	Human, BAL fluid (USA)	LN834394		LN834490	LN834578
	UTHSC DI-13-254	Human, BAL fluid (USA)	LN834395		LN834491	LN834579
	UTHSC DI-13-255	Human, toe nail (USA)	LN834396		LN834492	LN834580
Cladosporium tenuissimum	CBS 125995^T	Fruits of *Lagerstroemia* sp. (USA)	HM148197		HM148442	HM148687
	UTHSC DI-13-174	Marine mammal, lung (USA)	LN834397		LN834493	LN834581
	UTHSC DI-13-177	Human, skin (USA)	LN834398		LN834494	LN834582
	UTHSC DI-13-188	Human, BAL fluid (USA)	LN834399		LN834495	LN834583
	UTHSC DI-13-205	Human, BAL fluid (USA)	LN834400		LN834496	LN834584
	UTHSC DI-13-236	Human, nasal (USA)	LN834401		LN834497	LN834585
	UTHSC DI-13-239	Human, sputum (USA)	LN834402		LN834498	LN834586
	UTHSC DI-13-253	Human, BAL fluid (USA)	LN834403		LN834499	LN834587
	UTHSC DI-13-258	Human, thorancentesis fluid (USA)	LN834404		LN834500	LN834588
	UTHSC DI-13-274	Human, toe (USA)	LN834405		LN834501	LN834589
Cladosporium sp.	UTHSC DI-13-165	Human, arm drainage (USA)	LN834406		LN834502	LN834590
	UTHSC DI-13-168	Human, BAL fluid (USA)	LN834407		LN834503	LN834591
	UTHSC DI-13-169	Human, BAL fluid (USA)	LN834408		LN834504	LN834592
	UTHSC DI-13-170	Human, toe nail (USA)	LN834409		LN834505	LN834593
	UTHSC DI-13-178	Animal, abscess (USA)	LN834410		LN834506	LN834594
	UTHSC DI-13-179	Human, hand (USA)	LN834411		LN834507	LN834595
	UTHSC DI-13-190	Human, CSF (USA)	LN834412		LN834508	LN834596
	UTHSC DI-13-207	Human, CSF (USA)	LN834413		LN834509	LN834597
	UTHSC DI-13-210	Human, skin (USA)	LN834414		LN834510	LN834598
	UTHSC DI-13-211	Human, BAL fluid (USA)	LN834415		LN834511	LN834599
	UTHSC DI-13-212	Human, ethmoid sinus (USA)	LN834416		LN834512	LN834600
	UTHSC DI-13-217	Human, nasal (USA)	LN834417		LN834513	LN834601
	UTHSC DI-13-218	Human, BAL fluid (USA)	LN834418		LN834514	LN834602
	UTHSC DI-13-219	Human, foot (USA)	LN834419		LN834515	LN834603
	UTHSC DI-13-225	Animal, BAL fluid (USA)	LN834420		LN834516	LN834604
	UTHSC DI-13-226	Human, BAL fluid (USA)	LN834421		LN834517	LN834605
	UTHSC DI-13-227	Human, sputum (USA)	LN834422		LN834518	LN834606
	UTHSC DI-13-228	Human, foot skin (USA)	LN834423		LN834519	LN834607
	UTHSC DI-13-234	Human, sputum (USA)	LN834424		LN834520	LN834608
	UTHSC DI-13-235	Human, BAL fluid (USA)	LN834425		LN834521	LN834609
	UTHSC DI-13-238	Human, leg (USA)	LN834426		LN834522	LN834610
	UTHSC DI-13-241	Human, foot (USA)	LN834427		LN834523	LN834611
	UTHSC DI-13-244	Human, BAL fluid (USA)	LN834428		LN834524	LN834612
	UTHSC DI-13-245	Human, toe (USA)	LN834429		LN834525	LN834613
	UTHSC DI-13-246	Human, BAL fluid (USA)	LN834430		LN834526	LN834614
	UTHSC DI-13-247	Human, BAL fluid (USA)	LN834431		LN834527	LN834615
	UTHSC DI-13-251	Human, BAL fluid (USA)	LN834432		LN834528	LN834616
	UTHSC DI-13-257	Human, sputum (USA)	LN834433		LN834529	LN834617
	UTHSC DI-13-262	Dolphin, bronchus (USA)	LN834434		LN834530	LN834618

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TABLE 1 (Continued)

Species	Strain/isolate no.[a]	Origin (country)[b]	GenBank nucleotide accession no. for:			
			ITS	LSU	*EF-1α*	*Act*
	UTHSC DI-13-265	Human, BAL fluid (USA)	LN834435		LN834531	LN834619
	UTHSC DI-13-268	Human, toe nail (USA)	LN834436		LN834532	LN834620
	UTHSC DI-13-269	Human, BAL fluid (USA)	LN834437		LN834533	LN834621
	UTHSC DI-13-270	Human, nail (USA)	LN834438		LN834534	LN834622
	UTHSC DI-13-271	Human, BAL fluid (USA)	LN834439		LN834535	LN834623
	UTHSC DI-13-273	Human, toe nails (USA)	LN834440		LN834536	LN834624
Cladosporium variabile	CBS 121636^T	*Spinacia oleracea* (USA)	EF679402		EF679480	EF679556
Penidiella sp.	UTHSC DI-13-256	Human, nail (USA)	LN834441	LN834445		
Toxicocladosporium banksiae	CBS 128215^T	Leaf of *Banksia emulata* (Australia)	HQ599598	HQ599599		
Toxicocladosporium chlamydosporum	CBS 124157^T	Leaf of *Eucalyptus camaldulensis* (Madagascar)	FJ790283	FJ790301		
Toxicocladosporium ficiniae	CBS 136406^T	Leaf of *Ficinia* sp. (South Africa)	KF777190	KF777241		
Toxicocladosporium irritans	CBS 185.58^T	Moldy paint (Suriname)	EU040243	EU040243		
	UTHSC DI-13-181	Human, blood (USA)	LN834442	LN834446		
	UTHSC DI-13-230	Human, finger nail (USA)	LN834443	LN834447		
Toxicocladosporium pini	CBS 138005^T	Needles of *Pinus* sp. (China)	KJ869160	KJ869217		
Toxicocladosporium posoqueriae	CBS 133583^T	Leaf of *Posoqueria latifolia* (Australia)	NR121555	KC005803		
Toxicocladosporium protearum	CBS 126499^T	Leaf of *Protea burchellii* (South Africa)	HQ599586	HQ599587		
Toxicocladosporium pseudoveloxum	CBS 128775^T	Leaf of *Phaenocoma prolifera* (South Africa)	JF499847	JF499867		
Toxicocladosporium rubrigenum	CBS 124158^T	Leaf of *Eucalyptus camaldulensis* (Madagascar)	FJ790287	FJ790305		
Toxicocladosporium sp.	UTHSC DI-13-172	Human, BAL fluid (USA)	LN834444	LN834448		
Toxicocladosporium strelitziae	CBS 132535^T	Leaf of *Strelitzia reginae* (South Africa)	NR111765	JX069858		
Toxicocladosporium veloxum	CBS 124159^T	Leaf of *Eucalyptus camaldunensis* (Madagascar)	FJ790288	FJ790306		

[a] CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; FMR, Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, TX, USA; ^{T}, ex-type strain.
[b] BAL fluid, bronchoalveolar lavage fluid specimen; CSF, cerebrospinal fluid.

MATERIALS AND METHODS

Fungal isolates. A total of 92 isolates tentatively identified as *Cladosporium* spp. were included in this study (Table 1). All of the isolates were obtained from human and animal clinical specimens, mostly from the United States, received in the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (UTHSC) from different parts of the country mainly for identification purposes.

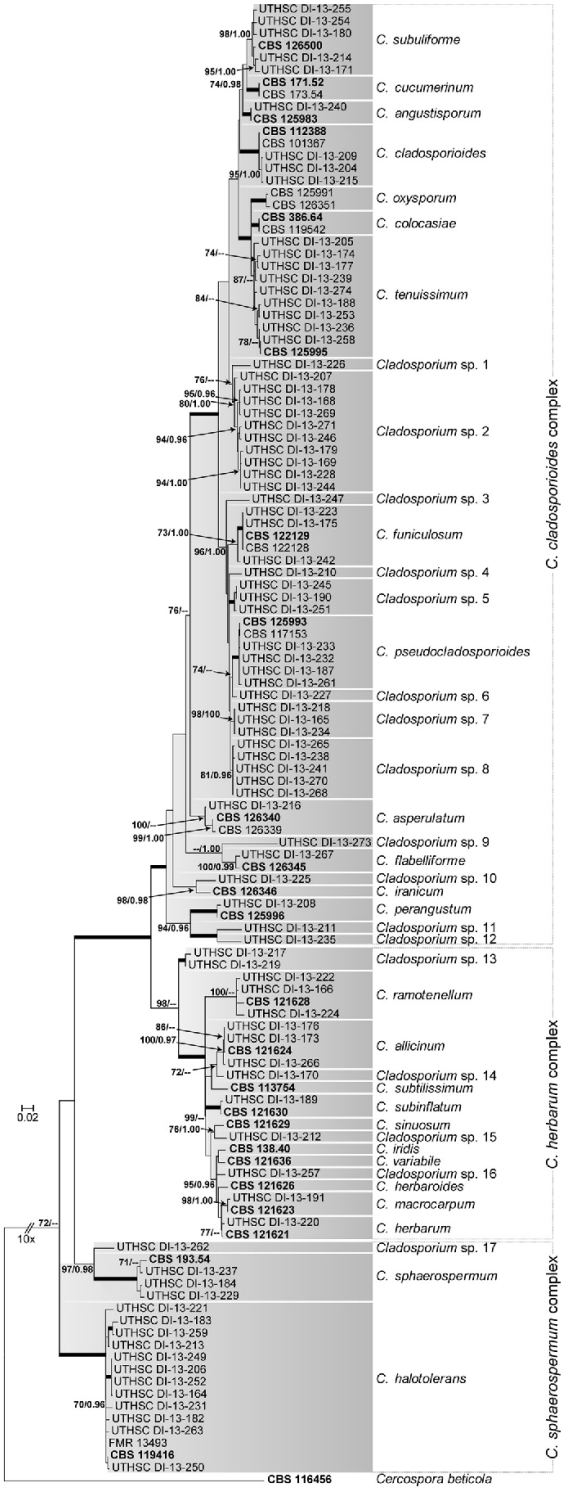
Phenotypic identification. The isolates were morphologically characterized following the procedures outlined in Bensch et al. (1), Crous et al. (23), Schubert et al. (19), and Zalar et al. (20). Briefly, all of the isolates were grown on synthetic nutrient-poor agar (SNA) (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 1 liter water) and potato dextrose agar (PDA) (Pronadisa, Spain) for 7 days at 25°C. Microscopic observations were made from cultures on SNA mounted in Shear's solution (23). Colony characteristics were recorded from cultures on SNA and PDA. For the estimation of cardinal growth temperatures, the isolates were grown on PDA agar for 14 days at temperatures ranging from 15°C to 35°C at intervals of 5°C, as well as at 32°C and 37°C.

DNA extraction, amplification, and sequencing. Total genomic DNA was extracted from mycelia obtained from colonies growing on PDA, using FastPrep (MP Biomedicals, Santa Ana, CA) according to the manufacturer's protocol. DNA was quantified using the NanoDrop 3000 (Thermo Scientific, Madrid, Spain).

The primers ITS5 and ITS4 (24) were used to amplify a region spanning internal transcribed spacer 1 (ITS1) and ITS2 and the 5.8S gene of the ribosomal DNA (rDNA); the primer pair LR0R/LR5 (25, 26) was used to amplify a fragment of the large subunit (LSU) gene of the rDNA; and the EF-728F/EF-986R and ACT-512F/ACT-783R primer pairs (27) were used for the translation elongation factor 1α gene (*EF-1α*) and the actin gene (*Act*), respectively.

Sequencing was performed in both directions using the same PCR primers at Macrogen Europe (Macrogen, Inc., Amsterdam, the Netherlands). Consensus sequences were obtained using SeqMan version 7.0.0 (DNAStar Lasergene, Madison, WI).

Molecular identification and phylogenetic analyses. An initial presumptive generic identification of the isolates was performed based on BLAST searches of ITS and LSU sequences in the GenBank (http://www.ncbi.nlm.nih.gov/) and CBS (http://www.cbs.knaw.nl/) databases. Multiple sequence alignments of each locus were performed in MEGA version 6 (28) using the ClustalW application (29), refined with MUSCLE (30), and manually adjusted if necessary. Phylogenetic reconstructions were made using maximum-likelihood (ML) and Bayesian Inference (BI) under MEGA version 6 and MrBayes version 3.1.2 (31), respectively. The best nucleotide substitution model (generalized time-reversible model with gamma distribution and a portion of invariable sites [GTR+G+I] for the three independent data sets) was estimated using MrModelTest version 2.3 (32) following the Akaike criterion. Phylogenetic analyses using ML were at first made individually for each locus and compared in order to assess for any incongruent results between nodes with high statistical support. As no incongruences were observed, the four loci were combined as follows: ITS, *EF-1α*, and *Act* for members of *Cladosporium sensu stricto* and ITS combined with LSU for members of other cladosporiumlike genera. For the ML analysis, nearest-neighbor interchange (NNI) was used as the heuristic method for tree inference. Support for the internal branches was assessed by a search of 1,000 bootstrapped sets of



data. A bootstrap support value of ≥70 was considered significant. For BI analysis, two simultaneous runs of 10,000,000 generations were performed and samples were stored every 1,000 generations. The 50% majority-rule consensus tree and posterior probability values (PP) were calculated after discarding the first 25% of the samples. A PP value of ≥0.95 was considered significant.

The first combined phylogenetic analysis with ITS, *EF-1*α, and *Act* sequences of clinical isolates belonging to *Cladosporium sensu stricto* and all the available type and reference strains was carried out following the alignments of Bensch et al. (1; data not shown). Only sequences of those species closely related (>95% similarity) to the clinical isolates tested here were included in the final analysis.

Antifungal susceptibility. The antifungal susceptibility test was performed according to the CLSI M38-A2 standard (33) with slight modifications. The incubation temperature was set to 25°C, given the optimal growth requirements of *Cladosporium* and related taxa (1, 33). Nine antifungal agents were tested: amphotericin B (AMB), 5-fluorocytosine (5FC), itraconazole (ITC), posaconazole (PSC), voriconazole (VRC), terbinafine (TRB), anidulafungin (AFG), caspofungin (CFG), and micafungin (MFG). The minimal effective concentration (MEC), defined as the lowest drug concentration at which short, stubby, highly branched hyphae were observed, was determined at 24 h for the echinocandins, and the MIC was determined at 48 h for the remaining drugs. The MIC was defined as the lowest concentration exhibiting 100% inhibition of visible growth for AMB, ITC, PSC, and VRC or 50% and 80% reduction in growth for 5FC and TRB, respectively. *Paecilomyces variotii* ATCC MYA-3630 and *Aspergillus fumigatus* ATCC MYA-3626 were used as quality control strains. Statistical analyses of the MIC/MEC data were performed using the Mann-Whitney test in Prism version 6.0 (GraphPad Software, San Diego, CA).

Nucleotide sequence accession numbers. DNA sequences determined in this study were deposited in GenBank under accession numbers LN834353 to LN834448 (rDNA), LN834449 to LN834536 (*EF-1*α), and LN834537 to LN834624 (*Act*) (Table 1).

RESULTS

Analysis of ITS and LSU sequences showed that 88 isolates (96%) belonged to *Cladosporium sensu stricto*, three isolates (3%) to the genus *Toxicocladosporium*, and one isolate (1%) to the genus *Penidiella*.

The phylogenetic analysis of *Cladosporium sensu stricto* included 121 taxa and 1,002 bp (447 bp for ITS, 337 bp for *EF-1*α, and 218 bp for *Act*), of which 485 bp were constant (347 bp for ITS, 60 bp for *EF-1*α, and 78 bp for *Act*), 496 were variable (97 bp for ITS, 260 bp for *EF-1*α, and 139 bp for *Act*) and 328 were parsimony informative (24 bp for ITS, 197 bp for *EF-1*α, and 107 bp for *Act*) (Fig. 1). The majority of isolates (57, 65%) nested into the *C. cladosporioides* complex: 28 belonged to nine species (i.e., *C. angustisporum*, *C. asperulatum*, *C. cladosporioides*, *C. flabelliforme*, *C. funiculosum*, *C. perangustum*, *C. pseudocladosporioides*, *C. subuliforme*, and *C. tenuissimum*), while 29 isolates clustered into 12 terminal subclades genetically distant from any currently known species of the genus. A total of 14 isolates were related to the *C.*

FIG 1 Maximum-likelihood (ML) tree inferred from combined ITS, *EF-1*α, and *Act* sequences of *Cladosporium* isolates. Branch lengths are proportional to phylogenetic distance. ML bootstrap support (BS) values of ≥70% and posterior probability (PP) values of ≥0.95 are shown above the branches. Thickened branches indicate BS of 100% and PP of 1.00. *Cercospora beticola* (CBS 116456) was used to root the tree. Type strains are indicated in bold font. CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; FMR, Facultat de Medicina, Reus, Spain; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, TX.

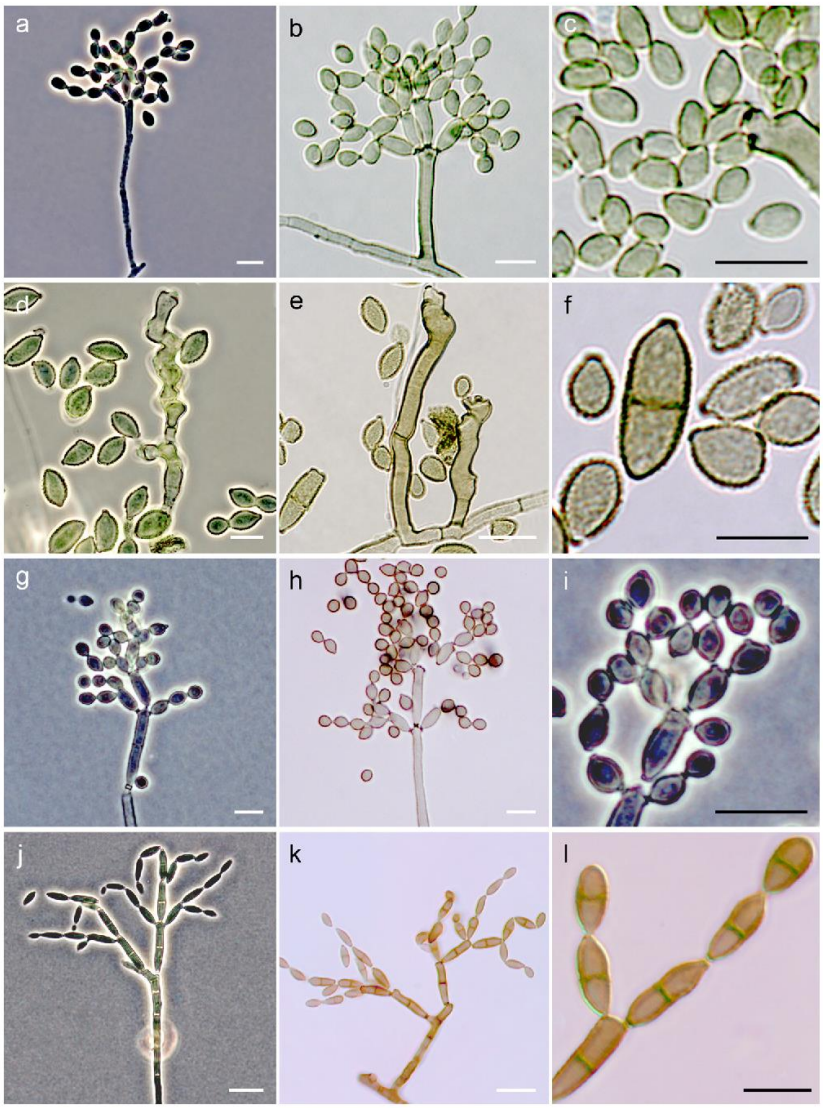


FIG 2 Conidiophores and conidia of fungi belonging to the *C. cladosporioides* complex (a to c), *C. herbarum* complex (d to f), *C. sphaerospermum* complex (g to i), and *Toxicocladosporium* spp. (j to l). White bars, 10 μm; black bars, 5 μm.

herbarum complex (16%), mostly corresponding to five species (i.e., *C. allicinum*, *C. herbarum*, *C. macrocarpum*, *C. ramotenellum*, and *C. subinflatum*), while five isolates clustered into four new lineages in the genus. Seventeen isolates were nested within the *C. sphaerospermum* complex (19%) and mostly belonged to two species (i.e., *C. halotolerans* and *C. sphaerospermum*), while a single isolate represented a new lineage.

Distinct morphological features of isolates in the *C. cladosporioides* complex included the formation of mostly unbranched, cylindrical conidiophores, bearing ovoidal to ellipsoidal intercalary and terminal conidia, smooth or rarely showing fine ornamentation (Fig. 2a to c); the maximum temperatures for growth were 32°C for *C. cladosporioides*, *C. flabelliforme*, *C. perangustum*, and *C. pseudocladosporioides* and 35°C for *C. angustisporum*, *C. funiculosum*, *C. subuliforme*, and *C. tenuissimum*. Isolates of the *C. herbarum* complex exhibited mostly nodulose conidiophores, bearing distinctly ornamented globose to subglobose terminal conidia (Fig. 2d to f); none of the isolates of this complex were able to grow at temperatures above 32°C, and *C. allicinum* exhibited a maximum growth temperature of 30°C. Isolates of the *C. sphaerospermum* complex formed cylindrical and branched conidiophores, bearing globose to subglobose conidia, smooth or

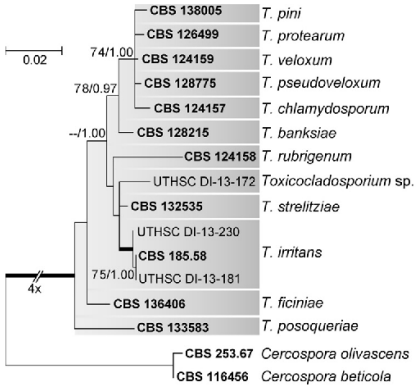


FIG 3 Maximum-likelihood (ML) tree inferred from combined ITS and LSU sequences of *Toxicocladosporium* isolates. Branch lengths are proportional to phylogenetic distance. ML bootstrap support (BS) values of ≥70% and posterior probability (PP) values of ≥0.95 are shown above the branches. Thickened branches indicate BS of 100% and PP of 1.00. *Cercospora beticola* (CBS 116456) and *Cercospora olivascens* (CBS 253.67) were used to root the tree. Type strains are indicated in bold font. CBS, CBS-KNAW Fungal Biodiversity Centre, the Netherlands; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, TX.

finely ornamented (Fig. 2g to i); the maximum temperatures for growth were 32°C for *C. sphaerospermum* and 35°C for *C. halotolerans*. None of the clinical isolates formed sexual morphs in culture.

Overall, the most commonly identified species was *C. halotolerans* (14.8%), followed by *C. tenuissimum* (10.2%) and *C. subuliforme* (5.7%). However, 39.8% of isolates did not match with any known taxa and represent at least 17 putative new *Cladosporium* species (Fig. 1). The most common anatomical site of isolation was the respiratory tract (54.5%), mainly from bronchoalveolar lavage (BAL) fluid and nasal specimens, followed by superficial sites (28.4%); these percentages were similar for all of the species and species complexes identified.

Phylogenetic analysis of the *Toxicocladosporium* isolates included 15 taxa and 984 bp (530 bp for LSU and 454 bp for ITS), of which 826 bp were constant (464 bp for LSU and 362 bp for ITS), 155 were variable (66 bp for LSU and 89 bp for ITS), and 129 were parsimony informative (56 bp for LSU and 73 bp for ITS) (Fig. 3). Two clinical isolates belonged to *Toxicocladosporium irritans*, while the isolate UTHSC DI-13-172 formed an independent lineage, genetically related to *Toxicocladosporium strelitziae* but showing distinctive morphological features and probably corresponding to a new species. The main morphological characteristics of members of *Toxicocladosporium* were the presence of nonnodulose conidiophores with dark and thickened cell walls and septa, producing conidia without the typical coronate scars of *Cladosporium* (Fig. 2j to l), and a maximum temperature for growth of 35°C.

According to the LSU sequence analysis, a single isolate (UTHSC DI-13-256), originally identified as *C. sphaerospermum*, was related to but distant (<98.2% sequence similarity) from members of the genus *Penidiella* (i.e., *Penidiella aggregata* and *Penidiella drakensbergensis*; sequence accession numbers JF499862 and KC005792, respectively) (data not shown). However, its final identification was not possible given the scarcity of DNA sequences of the latter species for comparison. This isolate was characterized by restricted growth (3 to 4 mm at 25°C for 7 days) and the production of solitary penicillate conidiophores, composed of chains of ramoconidia with slightly pigmented and thickened conidiogenous scars.

The results of the antifungal susceptibility testing are summarized in Table 2. The overall results for *Cladosporium* species showed a geometric mean (GM) MIC and MIC_{90} for AMB of 0.64 μg/ml and 2 μg/ml, respectively. Among the azoles, ITC and PSC were the most active, with both drugs having a GM MIC of 0.43 μg/ml and respective MIC_{90}s of 0.5 μg/ml and 1 μg/ml, while VRC showed a GM MIC and MIC_{90} of 1.68 μg/ml and 4 μg/ml, respectively. Flucytosine showed variable activity and had a GM MIC and MIC_{90} of 1.37 μg/ml and 4 μg/ml, respectively. TRB exhibited the most potent activity, with a GM MIC and MIC_{90} of 0.09 μg/ml and 1 μg/ml, respectively. With the exception of CFG, the echinocandins exhibited strong *in vitro* activity, with GM MIC values of 0.19 μg/ml and 0.12 μg/ml for AFG and MFG. All of the *Cladosporium* species tested showed similar susceptibility patterns except for *C. sphaerospermum*, where the three isolates tested exhibited higher MIC and MEC values, especially for the azoles, AMB, AFG, and MFG ($P < 0.001$). Comparison of antifungal susceptibility by species complex (Table 2) showed that AMB exhibited more potent activity against members of the *C. herbarum* complex, with GM MIC and MIC_{90} values of 0.18 μg/ml and 1 μg/ml ($P < 0.002$), while members of the *C. sphaerospermum* complex exhibited higher GM MIC and MIC_{90} values for AMB, PSC, ITC, and CSP ($P < 0.003$). *Toxicocladosporium* and *Penidiella* isolates exhibited similar susceptibility patterns, with mostly low GM MIC and MIC_{90} values against all antifungals tested but without statistically significant differences.

DISCUSSION

Members of *Cladosporium* are relatively easy to identify to genus and species complex based on their typical conidiogenous structures. However, morphological identification of *Cladosporium* species is difficult given the high morphological similarity between closely related species. In light of our results, it is strongly recommended that phenotypic identifications be confirmed with DNA sequencing. Several authors have demonstrated the usefulness of the *EF-1α* and *Act* loci to allow a good species delimitation in *Cladosporium* (1, 19, 21). This is especially important for members of the *C. cladosporioides* complex, which demonstrated the greatest species diversity, the highest number of species associated with clinical samples, and also, the greatest number of undescribed species. Moreover, we found that *C. cladosporioides*, the species most frequently cited as being clinically relevant, was poorly represented in our set of isolates, while *C. asperulatum*, *C. funiculosum*, *C. flabelliforme*, *C. pseudocladosporioides*, *C. subuliforme*, and *C. tenuissimum* are described for the first time from clinical samples. Similarly, in the *C. sphaerospermum* complex, most of the isolates morphologically identified as *C. sphaerospermum* were genetically reidentified as belonging to the phenotypically similar species *C. halotolerans*, which according to our data, emerged as the most common species from clinical origins. The latter species has never been associated with human infection; however, some isolates had been reported from human or animal clinical samples (1). In the case of the *C. herbarum* complex, 13 of the 14 isolates morphologically identified as *C. herbarum*, also

TABLE 2 Results of *in vitro* antifungal susceptibility testing of the 92 clinical isolates included in the study

Genus	Species (no. of isolates tested)	MIC/MEC parameter[a]	Result (μg/ml) for[b]: AMB	5FC	VRC	PSC	ITC	TBF	CFG[c]	AFG[c]	MFG[c]
Cladosporium	*C. cladosporioides* complex (57)	Range	0.06–2	0.06–>32	0.25–16	<0.03–1	<0.03–2	<0.03–4	0.125–8	0.03–0.5	0.03–0.5
		GM	0.73	1.20	1.65	0.40	0.34	0.12	2.78	0.19	0.11
		MIC_{90}	1	4	4	0.5	0.5	1	8	0.5	0.25
	C. tenuissimum (9)	Range	0.5–1	1–>16	1–4	0.25–0.5	0.25–0.5	0.06–1	4–8	0.125–0.5	0.125–0.25
		GM	0.93	2.72	1.85	0.37	0.29	0.18	4.67	0.37	0.15
		MIC_{90}	1.00	4.00	2.00	0.50	0.50	0.25	8.00	0.50	0.25
	C. subuliforme (5)	Range	1–2	0.25–2	0.25–2	0.25–0.5	0.25	0.06–1	4–8	0.06–0.5	0.06–0.25
		GM	1.15	1.00	0.66	0.29	0.25	0.28	5.28	0.16	0.12
		MIC_{90}	1.00	2.00	1.00	0.25	0.25	1.00	8.00	0.25	0.13
	C. pseudocladosporioides (4)	Range	0.5–1	0.5–1	2–4	0.25–0.5	0.5–1	0.03–2	0.25–8	0.03–0.25	0.03–0.125
		GM	0.59	0.71	2.38	0.42	0.59	0.21	2.38	0.15	0.07
		MIC_{90}									
	C. cladosporioides (3)	Range	0.5–1	1–2	0.5–16	0.25–1	0.25–0.5	0.5–2	1–4	0.125–0.25	0.125
		GM	0.79	1.26	1.59	0.40	0.31	1.00	2.00	0.16	0.13
		MIC_{90}									
	C. funiculosum (3)	Range	0.06–1	0.5–1	0.5–2	0.25–0.5	0.125–0.25	0.03–0.06	4	0.06–0.25	0.06–0.125
		GM	0.31	0.63	1.00	0.31	0.20	0.04	4.00	0.12	0.08
		MIC_{90}									
	C. angustisporum (1)	Range	1.00	1.00	4.00	0.50	0.50	2.00	4.00	0.13	0.06
		GM									
		MIC_{90}									
	C. asperulatum (1)	Range	1	0.25	2	0.25	0.5	0.03	8	0.25	0.125
		GM									
		MIC_{90}									
	C. flabelliforme (1)	Range	2	2	2	0.5	0.25	0.03	4	0.25	0.125
		GM									
		MIC_{90}									
	C. perangustum (1)	Range	0.5	4	4	0.5	0.5	0.03	4	0.125	0.06
		GM									
		MIC_{90}									
	Cladosporium sp. (29)	Range	0.125–2	0.06–>16	0.25–8	<0.03–1	<0.03–2	<0.03–4	0.125–8	0.03–0.5	0.03–0.5
		GM	0.67	1.10	1.73	0.43	0.36	0.09	2.00	0.18	0.10
		MIC_{90}	1.00	4.00	4.00	0.50	0.50	1.00	8.00	0.50	0.25
	C. herbarum complex (14)	Range	<0.03–2	0.5–>16	0.5–8	0.06–0.5	0.06–1	<0.03–0.125	0.125–8	0.06–1	0.06–0.5
		GM	0.18	2.97	1.81	0.37	0.35	0.05	0.67	0.23	0.15
		MIC_{90}	1	8	4	0.5	0.5	0.125	2	0.5	0.5
	C. allicinum (3)	Range	<0.03–0.125	2–4	2–4	0.5	0.25–0.5	0.03–0.06	1.00	0.25–0.5	0.125–0.25
		GM	0.05	2.52	3.17	0.50	0.40	0.05	1.00	0.31	0.20
		MIC_{90}									
	C. ramotenellum (3)	Range	1–2	4–>16	1–2	0.5	0.5–1	<0.03–0.125	0.5–8	0.25–1	0.125–0.5
		GM	1.26	4.00	1.59	0.50	0.63	0.05	2.00	0.40	0.20
		MIC_{90}									
	C. herbarum (1)	Range	0.06	8	2	0.5	0.5	0.03	0.5	0.125	0.125
		GM									
		MIC_{90}									
	C. macrocarpum (1)	Range	0.5	2	1	0.25	0.5	0.125	1	0.125	0.5
		GM									
		MIC_{90}									
	C. subinflatum (1)	Range	0.5	0.5	4	0.5	0.5	0.03	0.5	0.5	0.25
		GM									
		MIC_{90}									
	Cladosporium sp. (5)	Range	0.06–0.5	2–4	0.5–8	0.06–0.5	0.06–0.5	<0.03–0.125	0.125–0.5	0.06–0.5	0.06–0.125
		GM	0.11	2.30	1.32	0.25	0.19	0.05	0.29	0.14	0.08
		MIC_{90}	1.00	4.00	4.00	0.50	0.50	1.00	8.00	0.50	0.25
	C. sphaerospermum complex (17)	Range	0.125–2	0.06–4	0.5–16	0.06–4	0.25–>16	<0.03–1	0.06–4	<0.03–1	0.06–1
		GM	1.13	1.13	1.70	0.64	1.13	0.06	1.27	0.15	0.13
		MIC_{90}	2	2	8	2	32	0.5	4	0.25	0.125
	C. halotolerans (13)	Range	0.125–2	0.06–4	0.5–2	0.06–1	0.25–2	<0.03–1	0.06–4	<0.03–0.25	0.06–0.125
		GM	1.00	0.90	1.11	0.47	0.56	0.08	1.00	0.11	0.10
		MIC_{90}	2.00	2.00	2.00	1.00	2.00	1.00	4.00	0.25	0.13
	C. sphaerospermum (3)	Range	1–2	2–4	8–16	2–4	>16	<0.03–0.125	2–4	0.25–1	0.125–1
		GM	1.59	2.52	10.08	2.52	>16	0.03	3.17	0.50	0.40
		MIC_{90}									
	Cladosporium sp. (1)	Range	2	2	2	0.5	0.5	<0.03	2	0.25	0.125
		GM									
		MIC_{90}									
	Overall (88)	Range	<0.03–2	0.06–>16	0.25–16	<0.03–4	<0.03–>16	<0.03–4	0.06–8	<0.03–1	0.03–1
		GM	0.64	1.37	1.68	0.43	0.43	0.09	1.91	0.19	0.12
		MIC_{90}	2.00	4.00	4.00	0.50	1.00	1.00	8.00	0.50	0.25
Toxicocladosporium	*T. irritans* (2)	Range	0.5–1	0.25–2	0.25	0.25–1	0.5	<0.03–0.06	0.125–2	0.06–0.25	0.06–0.5
		GM	0.71	0.71	0.25	0.50	0.50	0.03	0.50	0.12	0.17
		MIC_{90}									
	Toxicocladosporium sp. (1)	Range	0.5	0.125	0.25	0.125	0.125	<0.03	1	0.125	0.06
		GM									
		MIC_{90}									

(Continued on following page)

TABLE 2 (Continued)

Genus	Species (no. of isolates tested)	MIC/MEC parameter[a]	Result (μg/ml) for[b]: AMB	5FC	VRC	PSC	ITC	TBF	CFG[c]	AFG[c]	MFG[c]
	Overall (3)	Range	0.5–1	0.125–2	0.25	0.125–1	0.125–0.5	<0.03–0.06	0.125–2	0.06–0.25	0.06–0.5
		GM	0.63	0.40	0.25	0.31	0.31	0.02	0.63	0.12	0.12
		MIC_{90}									
Penidiella	*Penidiella* sp. (1)	Range	2	0.06	0.125	0.06	>0.03	>0.03	0.25	0.25	0.25
		GM									
		MIC_{90}									

[a] GMs and MIC_{90}s are shown only for species with ≥5 isolates. GM, geometric mean.
[b] AMB, amphotericin B; 5FC, flucytosine; VRC, voriconazole; PSC, posaconazole; ITC, itraconazole; CFG, caspofungin; AFG, anidulafungin; MFG, micafungin; TRB, terbinafine.
[c] These columns include MEC data.

considered a clinically relevant species, were found to belong to other species of this complex (i.e., *C. allicinum*, *C. macrocarpum*, and *C. ramotenellum*). While *C. macrocarpum* has been identified as the causative agent of human infections (17), *C. allicinum* and *C. ramotenellum* have never been reported before in the clinical setting, although some isolates have been recorded as obtained from human samples (1). However, due to the lack of clinical histories and histopathological findings, it was impossible for us to confirm a pathogenic role of the species reported here for the first time from clinical specimens.

It is remarkable that our phylogenetic analysis was unable to provide species-level identification of a high number of *Cladosporium* isolates (39.8%) that were originally considered to belong to several common morphospecies. Instead, those unidentified isolates were grouped into 5 terminal clades and 12 monotypic lineages, representing a large variety of phylogenetic species. It is probable that many of these clades and monotypic lineages represent new species; however, further studies combining phenotypic and molecular data would be necessary to confirm these findings. We report also for the first time the isolation of *Toxicocladosporium* and *Penidiella* species from clinical specimens. Isolates of these recently proposed genera were only known from leaves of several plants and from environmental sources (3, 34). According to our data, the vast majority of isolates were obtained from respiratory specimens, including BAL fluid, nasal, and sputum samples. This is not rare, because *Cladosporium* is preponderant in the airborne mycobiota (35), being considered one of the most important respiratory allergenic fungi, after *Alternaria* (10, 34, 36).

Reports of invasive infections by *Cladosporium* are extremely rare. Bentz and Sautter (37) reported a mixed disseminated infection by *Aspergillus fumigatus* and *C. cladosporioides* in an immunocompromised patient. *Cladosporium cladosporioides* and *C. macrocarpum* have been reported from two clinical cases involving the central nervous system (15, 17), while *C. sphaerospermum* was isolated from an intrabronchial infection (38). However, in none of these cases was the etiology of the infection supported by histopathological studies. The isolation of *Cladosporium* species from deep tissues seems improbable considering the inability of these organisms to grow at temperatures exceeding 35°C, and thermotolerance being one of the most important virulence factors for invasive or disseminated infections (39). In our study, less than half of the isolates exhibited very limited growth at 35°C, while none was able to grow at 37°C. However, surprisingly, several of our isolates were obtained from deep tissue samples, including bone marrow, cerebrospinal fluid (CSF), and lung and lymphatic tissue samples, among others. Isolation of these fungi from invasive infections may have been due to environmental contamination of the samples; however, occasionally isolates that fail to grow in culture at 37°C have been reported to cause invasive disease in immunocompromised individuals (40).

There is a paucity of information regarding antifungal susceptibility patterns for *Cladosporium* species. Most data are from a few reported clinical cases (7, 13, 15, 41). Our study provides the first *in vitro* data for a large set of clinical isolates, including several species obtained from diverse anatomical sites and not previously reported from clinical samples. Case reports have shown a favorable outcome using azole-based therapies. ITC has shown efficacy in the treatment of superficial infections caused by *C. cladosporioides*, *C. sphaerospermum*, and *C. oxysporum* (8, 14, 37, 41–44), while VRC was effective against *C. macrocarpum* in a brain abscess (17). This agrees with our *in vitro* data, which demonstrated that the azoles, particularly ITC and PSC, have good activity against *Cladosporium* species, although VRC displayed variable activity. AMB has been shown to be ineffective against *C. cladosporioides* (41) and *C. sphaerospermum* (38) in cases of skin and intrabronchial infections, respectively. Our results, however, suggest that this drug might be effective, especially against members of the *C. herbarum* complex. Kantarcioğlu and Yücel (45) reported potent *in vitro* activity of TRB against a set of unidentified *Cladosporium* species. Our data confirmed the results of that study, with TRB showing significant activity against all of the species tested. Echinocandin activity against *Cladosporium* species has not been previously evaluated; however, we observed that both AFG and MFG exhibited notable *in vitro* activity against all of our isolates, indicating that they could represent an important alternative for the treatment of infections by these fungi pending further confirmatory studies.

In conclusion, our study has significantly expanded the diversity of *Cladosporium* species seen in clinical specimens as a result of the molecular characterization of these isolates. We were unable, however, to document these organisms as etiologic agents in human or animal disease due to the lack of clinical information and/or histopathological findings. It is also important to note that most reported cases of *Cladosporium* infections lack molecular confirmation, and in those cases where they have been so characterized, the strains are not available. Given that many journals require the public availability of DNA sequence data, we recommend that clinical strains be deposited in international culture collections, thereby making them available for reidentification and further study.

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4.3 *Scopulariopsis*, a poorly known opportunistic fungus: spectrum of species in clinical samples and *in vitro* responses to antifungal drugs

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Scopulariopsis, a Poorly Known Opportunistic Fungus: Spectrum of Species in Clinical Samples and *In Vitro* Responses to Antifungal Drugs

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Ninety-nine isolates of clinical origin, tentatively identified as *Scopulariopsis* or *Microascus*, were morphologically and molecularly characterized by a combined analysis of the D1/D2 domains of the 28S rRNA gene and a fragment of the elongation factor 1-α gene (EF1-α) sequences. The most prevalent species was *Scopulariopsis brevicaulis* (49.4%), followed by *Scopulariopsis gracilis* (14.4%), *Scopulariopsis brumptii* (7.2%), *Microascus cinereus* (5.2%), the *Scopulariopsis candida* species complex (3.1%), and *Microascus cirrosus* (2.1%). The most common anatomic sites of isolation were the respiratory tract (61.6%), superficial tissue (19.2%), and deep tissue or fluid samples (19.2%). The antifungal susceptibilities of the isolates to eight drugs were tested *in vitro*, with all the drugs generally showing poor activity.

The genus *Scopulariopsis* contains both hyaline and dematiaceous molds, and their teleomorphs are included in the genus *Microascus* (order *Microascales*). They are saprobes commonly isolated from soil, air, plant debris, paper, and moist indoor environments (1–3). Some species are known to be opportunistic pathogens, mainly causing superficial tissue infections, and they represent some of the principal causes of nondermatophytic onychomycoses (4, 5). Less common clinical manifestations include keratitis following eye trauma (6) and otomycosis (7). The fungi have also been involved in deep tissue infections, mainly in immunocompromised and occasionally in immunocompetent patients, causing, for example, pneumonia (8), endophthalmitis (9), subcutaneous and brain abscesses (10, 11), invasive sinusitis (12), peritonitis (13), and endocarditis (14, 15). The most frequently reported species in all clinical presentations and anatomic locations is *Scopulariopsis brevicaulis*. Other less frequent species are *Scopulariopsis acremonium*, *Scopulariopsis brumptii*, *Scopulariopsis flava*, *Microascus niger*, *Microascus cinereus*, *Microascus cirrosus*, *Microascus manginii*, and *Microascus trigonosporus* (5, 16–18).

Currently, there are close to 40 accepted species of *Scopulariopsis* and *Microascus*. For many of these species, the anamorph-teleomorph connection has already been established (1, 5, 16). However, the sexual states of some *Scopulariopsis* species are still unknown.

According to the new International Code of Nomenclature for fungi, algae, and plants, the dual nomenclature system that has been traditionally used for fungi, which includes both anamorph and teleomorph states, is no longer allowed and hence a unique name must be chosen (19). We judge that since the name *Scopulariopsis* has been used much more frequently in the literature, including in medical publications, this name should have priority over *Microascus*. However, since no formal proposal has yet been submitted, in the present paper we apply the traditional nomenclature for known *Scopulariopsis* and *Microascus* species.

Although the isolation of *Scopulariopsis* species from clinical specimens is relatively easy, as they grow well on routine laboratory media, it might be difficult to identify them morphologically down to the species level (18). Histopathology has limited significance in diagnostics since in tissue, the fungi show features similar to those of other more common pathogenic molds, such as *Aspergillus* or *Fusarium* species (18, 20). The sequencing of the ribosomal operon has been used for the identification of clinical strains of *Scopulariopsis*, although the results may not be reliable because of insufficient availability of reference sequences in the public databases (17, 18, 21, 22). Interestingly, due to the high genetic variability of the internal transcribed spacer (ITS) sequences found in a large set of *Scopulariopsis* strains isolated from cheese, Ropars et al. (23) used the combined analysis of partial sequences of the long subunit (LSU) rRNA gene, β-tubulin (TUB), and elongation factor 1-α (EF1-α) genes for the taxonomic circumscription of *Scopulariopsis* species and proposed the EF1-α gene to be the most phylogenetically informative genomic region for identifying *Scopulariopsis* species.

The high rates of resistance of these fungi to practically all currently used antifungal agents, including amphotericin B (AMB) and voriconazole (VRC), which are among the most commonly used drugs for the prophylaxis and first-line treatment of systemic mold infections, is significant. The appropriate therapy for *Scopulariopsis* infections has yet to be defined (22, 24). The effectiveness of AMB has been estimated to be only about 40% of successful treatments (24), which has resulted in high mortality

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rates and infection relapses (15, 20). *In vitro* antifungal susceptibility studies on these fungi are scarce and have involved mainly topical drugs. Several clinical reports have underlined the lack of correlation between *in vitro* susceptibility test results and clinical outcomes (21, 22, 25).

Because in most of the clinical reports of *Scopulariopsis* infections, morphological identification of the etiological agent has not been confirmed at the molecular level, the real prevalence of *Scopulariopsis* species in clinical samples, apart from those from *S. brevicaulis*, is unknown. We therefore studied a large set of clinical isolates, most of which were received at a mycology reference laboratory in the United States, in order to define the species spectrum and the relative frequencies of *Scopulariopsis* in clinical specimens. The *in vitro* antifungal susceptibilities of the most prevalent species were also determined.

MATERIALS AND METHODS

Fungal isolates and sequences. Ninety-nine clinical isolates received as *Scopulariopsis* or *Microascus* species were included in this study. In addition, 23 type and reference strains were studied. Five D1/D2 rRNA gene and six elongation factor 1-α gene (EF1-α) sequences retrieved from GenBank were also included in the phylogenetic analyses.

Morphological identification. The isolates were subcultured onto potato-dextrose agar (PDA) (Pronadisa, Spain), oatmeal agar (OA) (30 g of filtered oat flakes, 20 g of agar, 1 liter of distilled water), and potato-carrot agar (PCA) (20 g each of filtered potatoes and carrots, 20 g of agar, 1 liter of distilled water) up to 21 days at 25°C in darkness. The microscopic features were obtained from direct wet mounts and slide cultures on PDA, OA, or PCA, mounted in lactic acid or lactophenol. All isolates were morphologically identified as per Morton and Smith (2), de Hoog et al. (5), and Guarro et al. (26).

DNA extraction, amplification, and sequencing. Isolates were grown on YES agar (20 g of yeast extract, 150 g of sucrose, 20 g of agar, 1 liter of distilled water) for 5 days at 25°C. The total genomic DNA was extracted from agar cultures using the PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA), according to the manufacturer's protocol. DNA was quantified using a Nanodrop 3000 (Thermo Scientific, Madrid, Spain).

To amplify a 440-bp fragment of the D1/D2 domains of the 28S rRNA gene and a 1,200-bp fragment of the EF1-α gene, we used the primers and protocols described previously by O'Donnell (27) and Rehner and Buckley (28), respectively. The amplified products were purified with the Diffinity RapidTip purification system (Sigma-Aldrich, St. Louis, MO, USA) and stored at −20°C until sequencing.

Sequencing was made in both directions with the same primer pair used for amplification at Macrogen Europe (Macrogen Inc., Amsterdam, The Netherlands). The consensus sequences were obtained using the SeqMan software version 7.0.0 (DNAStar Lasergene, Madison, WI, USA).

Molecular identification and phylogenetic analysis. Preliminary molecular identification of the isolates was performed using BLAST searches for both amplified fragments. Only the sequences of type or reference strains deposited in the GenBank/EMBL database were considered for identification purposes. A maximal level of identity (MLI) of ≥98% was considered to allow for a species-level identification. MLI values of <98% provided identification only at genus level.

Multiple sequence alignments were made in MEGA version 5.05 (29) using the ClustalW application (30) and manually refined under the same software platform. The ambiguous areas of the alignment were removed using the Gblocks server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) (31).

Concordance of the D1/D2 and EF1-α gene data sets was evaluated with the partition-homogeneity test implemented with PAUP* version 4.0b10 (32). The selection of the best nucleotide substitution model (GTR+G+I) was made using jModelTest version 2.1.1 (33). Phylogenetic reconstruction of the combined data sets was made with the maximum likelihood (ML) analysis under MEGA version 5.05 using nearest-neighbor interchange (NNI) as a heuristic method for tree inference. Support for the internal branches was assessed by a search of 1,000 bootstrapped sets of data. A bootstrap support (BS) of ≥70 was considered significant. In addition, Metropolis-coupled Markov chain Monte Carlo (MCMCMC) sampling was performed using MrBayes version 3.2.1 with two simultaneous runs for 6 million generations. Bayesian posterior probabilities (PP) were obtained from the 50% majority-rule consensus of trees sampled every 100 generations, after removing the first 25% of the resulting trees. A PP value of ≥0.95 was considered significant. The strains *Petriella setifera* CBS 437.75 and *Parascedosporium putredinis* CBS 127.84 were used as outgroups.

Antifungal susceptibility testing. Antifungal susceptibility testing was performed according to CLSI document M38-A2 (34). The minimal effective concentration (MEC) was determined at 24 h for the echinocandins, and the MIC was determined at 48 h for the remaining drugs. The MIC was defined as the lowest concentration exhibiting 100% visual inhibition of growth for AMB, VRC, itraconazole (ITC), and posaconazole (PSC) and an 80% reduction in growth for terbinafine (TRB).

Nucleotide sequence accession numbers. The clinical isolates characterized in this study have been deposited in GenBank under accession numbers HG380346 through HG380499.

RESULTS

Of the 99 isolates studied, 97 were morphologically identified as members of the *Scopulariopsis* or *Microascus* genus. The remaining two isolates were identified as a *Scedosporium* sp. and *Phialosimplex caninus*, respectively. The *Scopulariopsis* isolates were characterized by moderately fast growth and flat, velvety or powdery, white, tan, dark brown, gray, or black colonies. Microscopically, they showed hyaline or dematiaceous septate hyphae with cylindrical or flask-shaped conidiogenous cells (annellides) formed singly or in small groups directly on the vegetative hyphae or on short and usually branched conidiophores. The conidia were one celled, hyaline, light green to dark brown, flat at the base, globose, limoniform or bullet-shaped with a smooth or rough surface, hydrophobic, and produced in long chains. When present, teleomorphs were characterized by dark, ostiolate, globose to pyriform perithecia with or without a neck, superficial or immersed in the agar. The asci were ovate and evanescent and contained eight one-celled, straw-colored, asymmetrical, reniform, lunate, or triangular ascospores.

Figure 1 shows the phylogenetic tree inferred from the ML and Bayesian analyses of the concatenated EF1-α and D1/D2 sequences of a representative number of the clinical isolates tested in this study, including the type and reference strains of clinically relevant species of the genus. Of the 48 isolates identified as *S. brevicaulis*, 8 were randomly chosen for the phylogenetic analysis, since the isolates in this group showed a high sequence similarity (>99.3%). In the tree, the clades are named according to the degree of similarity with the type or reference strains of known species. Twenty-three well-supported clades were formed, 14 of which corresponded with previously recognized species.

The *S. gracilis* clade grouped the type strain of this species together with 14 clinical isolates. All the isolates were characterized by the production of abundant conidia, usually from well-differentiated branched conidiophores. Ten of these isolates also developed ascomata and ascospores morphologically very similar to those of *M. cinereus*. However, they can be distinguished from *M. cinereus* mainly by having lunate ascospores, measuring 4.5 to 6.5 by 2 to 4 μm, and by the presence of branched conidiophores

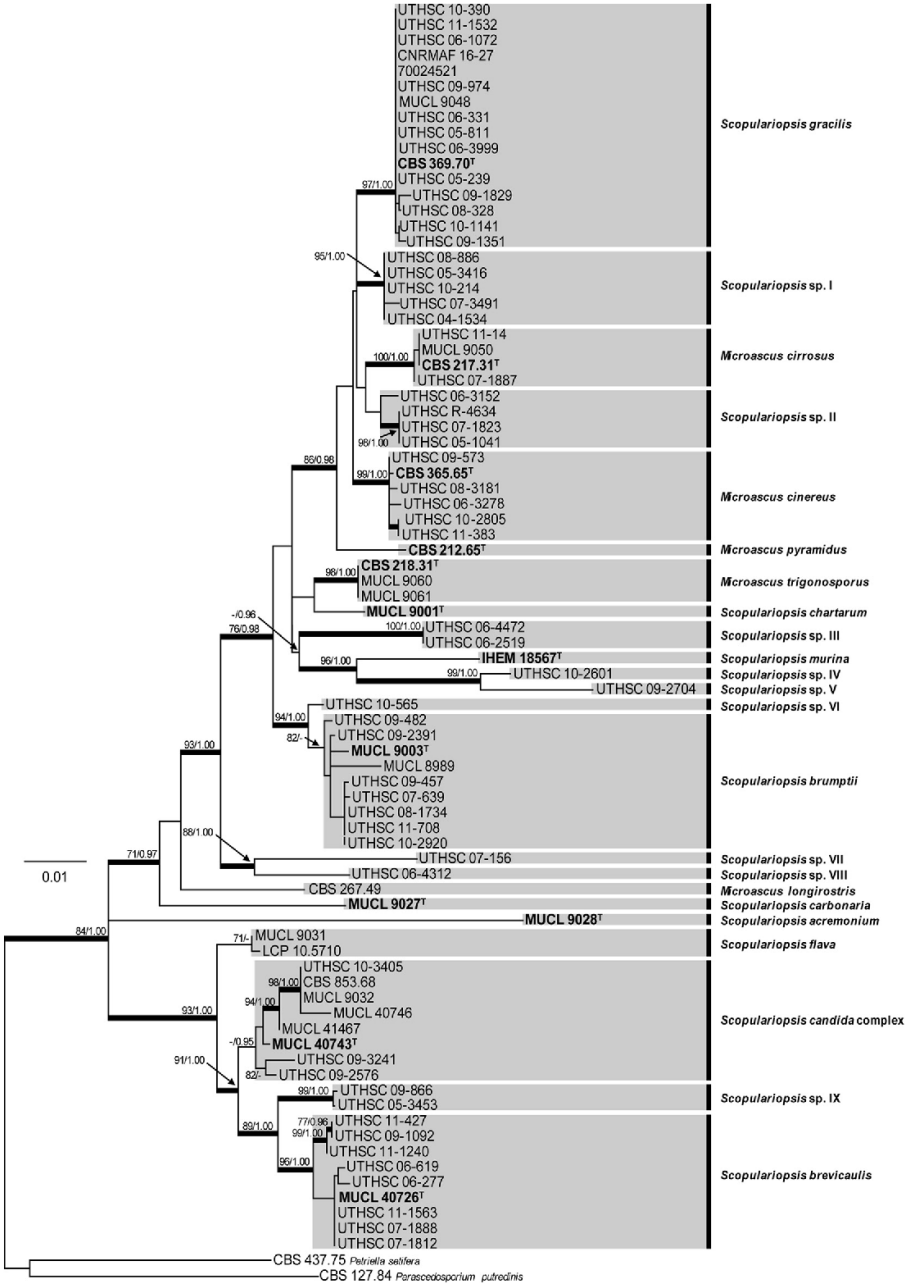

FIG 1 Maximum likelihood tree obtained from the combined EF1-α and D1/D2 sequences of representative isolates. In the tree, the branch lengths are proportional to phylogenetic distance. Bootstrap support values of ≥70/Bayesian posterior probability scores of ≥0.95 are indicated on the nodes. The supported branches and type strains are shown in bold type. T, type strain.

(Fig. 2). The isolates belonging to the clade of *M. cinereus sensu stricto* showed ascospores of variable shape (reniform, broadly lunate, or triangular) and that were slightly smaller (4 to 5.5 by 2.5 to 4 μm), and the conidiophores were mostly simple, usually reduced to a single conidiogenous cell growing directly on the vegetative hyphae (Fig. 2).

Ten isolates that morphologically resembled *M. trigonosporus* were grouped in the clades *Scopulariopsis* sp. I, II, and VI, which

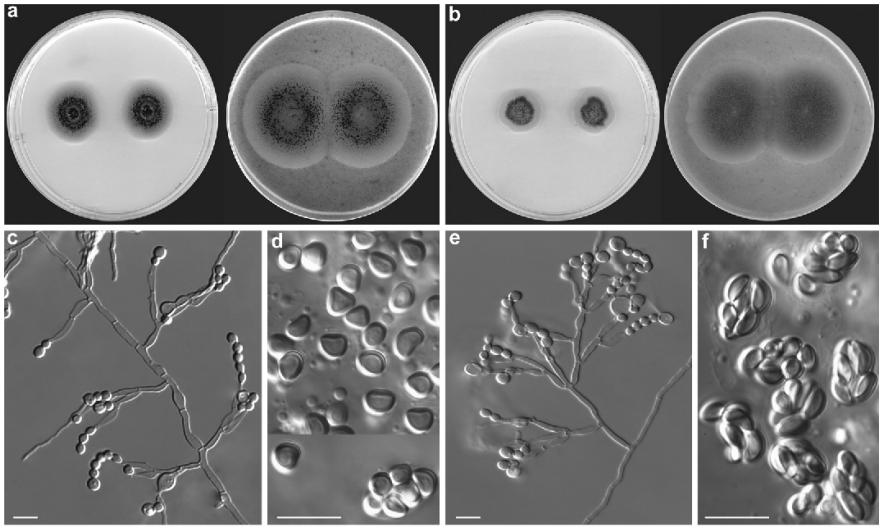

FIG 2 (a, c, and d) *Microascus cinereus*. (b, e, and f) *Scopulariopsis gracilis*. (a and b) Colonial features on PCA (left) and OA (right). (c and e) Conidiophores. (d and f) Asci and ascospores. Scale bars = 10 μm.

were phylogenetically distant from the type strain of that species (CBS 218.31), i.e., they have 97.8%, 97.6%, and 98.0% sequence similarities, respectively.

The clades *Scopulariopsis* sp. III to V, VII, and VIII comprise 6 clinical isolates that were not morphologically similar or phylogenetically related to any known species.

A large clade, named the *S. candida* complex, includes the epitype of *S. candida*, a reference strain of *M. manginii*, three reference strains of *M. niger*, and three clinical isolates, two of which had been morphologically identified as *S. candida* and one as *M. niger*. Since the different species included in this group showed high sequence similarities (>98.7%) but exhibited different morphological characteristics, all the species included in this group were treated as a complex. The two clinical isolates grouped in the clade *Scopulariopsis* sp. IX were morphologically identified as *M. manginii* but proved to be phylogenetically distant from the reference strain of this species (MUCL 41467) and from the epitype of its anamorph *S. candida* (<97.9% and <98.2% sequence similarities, respectively).

Molecular identification showed that the most common species was *S. brevicaulis* (49.4%) followed by *S. gracilis* (14.4%), *S. brumptii* (7.2%), *M. cinereus* (5.2%), the *S. candida* species complex (3.1%), and *M. cirrosus* (2.1%). Table 1 summarizes the key morphological features for distinguishing the most common species identified in this study. The correlation between morphological and molecular identifications at the species level was 67%. The remaining isolates were identified with confidence only at the genus level.

Most clinical isolates studied were of respiratory origin (61.6%), mainly obtained from bronchoalveolar lavage (BAL) fluid and sputum samples, followed by superficial tissue samples (19.2%) principally isolated from the nails and skin. The remaining 19.2% of isolates were from miscellaneous deep tissue or sterile fluid specimens (Table 2). *S. brevicaulis* was the most common species from all clinical origins. *S. gracilis* was most frequently isolated from BAL fluid and sputum samples, while *S. brumptii* and *M. cirrosus* were only recovered from lower respiratory tract samples.

The results of the antifungal susceptibility testing are summarized in Table 3. All antifungal drugs showed similar low activities. AMB showed an overall geometric mean MIC (GM) and MIC_{90} of 16.9 μg/ml and 32.0 μg/ml, respectively. The activities of the azoles were similar for all the species tested; VRC and PSC displayed GM values of 16.4 μg/ml and 14.6 μg/ml, respectively. In contrast, ITC showed almost no activity, with an MIC_{90} of ≥32 μg/ml. The echinocandins had the highest activities, with overall GMs of 4.0 μg/ml, 3.7 μg/ml, and 1.1 μg/ml for anidulafungin (AFG), caspofungin (CFG), and micafungin (MFG), respectively. TRB also showed limited activity, with an overall GM and MIC_{90} of 1.9 μg/ml and 8 μg/ml, respectively. *M. cirrosus* and the *S. candida* complex were the most susceptible species.

DISCUSSION

This study involved the highest number of isolates of *Scopulariopsis* and *Microascus* of clinical origin ever to be evaluated to date. In agreement with earlier reviews of clinical cases (17, 22), *S. brevicaulis* was the most commonly isolated species in our study. *S. gracilis* was the second most commonly isolated species in our study, which is interesting since this fungus has never been reported in human infections or isolated from clinical specimens. In numerous isolates of this species, a sexual state was present that morphologically resembled that of *M. cinereus*. The teleomorph of *S. gracilis* probably has never been described in the literature because it is often confused with *M. cinereus*. In a recent review of 33 human invasive infections from *Scopulariopsis* or *Microascus* species, *M. cinereus* was, after *S. brevicaulis*, the second most commonly isolated species (12%) (17). However, our study emphasized some difficulties in the morphological identification of this species, since almost a third of the isolates morphologically identified as *M. cinereus* were found after sequencing to be from *S. gracilis*. The inadequacy of the morphological criteria was also

TABLE 2 Anatomical sources of isolates of *Scopulariopsis* and *Microascus* spp. from clinical samples

Species	No. of isolates obtained from:				Total no. (%) of isolates
	Superficial tissue	Deep tissue/ fluids	Upper respiratory tract	Lower respiratory tract	
Scopulariopsis brevicaulis	15	10	8	15	48 (49.4)
Scopulariopsis gracilis	0	3	1	10	14 (14.4)
Scopulariopsis brumptii	0	0	0	7	7 (7.2)
Microascus cinereus	0	1	0	4	5 (5.2)
Scopulariopsis candida complex[a]	2	0	0	1	3 (3.1)
Microascus cirrosus	0	0	0	2	2 (2.1)
Other species	2	3	2	11	18 (18.6)
Total no. (%) of species	19 (19.2)	19 (19.2)	11 (11.1)	50 (50.5)	97 (100)

[a] Includes *Scopulariopsis asperula*, *S. candida*, *Scopulariopsis fusca*, *Microascus manginii*, and *Microascus niger*.

TABLE 1 Key morphological features of the most commonly identified species in this study

Species	Characteristics of:									
	Colony color	Ascomata	Ascospores		Conidiogenous cells		Conidia			
			Shape	Size (μm)	Shape	Arrangement	Shape	Color	Size (μm)	Ornamentation
Scopulariopsis brevicaulis	Tan	Absent			Cylindrical	Single or in groups (3–10), usually on well-differentiated branched conidiophores	Globose or ovoid	Light brown	5–8 by 5–7	Verrucose
Scopulariopsis gracilis	Olive gray	Present or absent	Lunate	4.5–6.5 by 2–4	Bottle-shaped	Usually in groups (3–10) in well-differentiated branched conidiophores	Obovate or clavate	Yellowish-brown	3.5–5.5 by 2–3.5	Smooth
Scopulariopsis brumptii	Greenish-gray becoming dark brown	Absent			Bottle-shaped	Single or in groups (2–4) on poorly differentiated conidiophores	Ovate	Greenish-brown	4–6 by 2.5–4.5	Smooth or rough-walled
Microascus cinereus	Gray	Present	Reniform or broadly lunate, some triangular	4–5.5 by 2.5–4	Bottle-shaped	Single or in groups (2–5) on poorly differentiated conidiophores	Obovate or clavate	Yellowish-brown	3–5 by 2–3	Smooth
Microascus cirrosus	Brownish-gray	Present	Broadly reniform	4–5.5 by 3–5	Bottle-shaped	Single or in groups (2–5) on poorly differentiated conidiophores	Broadly clavate	Yellowish-brown	4–5.5 by 3.5–4.5	Smooth

observed for the 10 isolates listed as *M. trigonosporus*, as they finally were found to correspond to three distant and probably undescribed phylogenetic species. Clarification regarding the taxonomic status of the morphospecies *M. trigonosporus* is important because some invasive infections have been attributed to this species, e.g., a pneumonia in a bone marrow recipient (35) and an endocarditis case (36). Only in the latter case was the identity of the fungus verified by ITS sequencing.

Morphological identification of clinical isolates of *Scopulariopsis* and *Microascus* is still useful since the features of conidia and sexual reproductive structures are quite characteristic for this fungal group, allowing for accurate identification at least to the genus level. Molecular tools are widely used in clinical laboratories for identification of fungi (17), with rRNA genes being the most commonly used target. However, a thorough taxonomic molecular study is still lacking. *Scopulariopsis* isolates from clinical cases were identified mostly by performing a BLAST search in GenBank (17, 21, 22). Our study demonstrated that this approach is not very useful, mainly due to the lack of reference sequences for comparison. Furthermore, the D1/D2 region, the primary target used for species identification in clinical reports, shows a low interspecific variation in this fungal group. We observed that the isolates of *S. brumptii* and many *Microascus* spp., including the type strains of *M. trigonosporus*, *M. cinereus*, and *S. gracilis*, were erroneously identified by BLAST searches as *M. cirrosus*, with MLI values of ≥98. A clinical isolate previously reported as *M. cirrosus* (22) was reidentified here as *S. gracilis*.

Other loci, such as ITS, which is the most commonly sequenced DNA fragment for the identification of clinical molds, was difficult to amplify and too variable for phylogenetic studies in *Scopulariopsis* species, proposing the EF1-α gene to be a more reliable phylogenetic marker (23). We have obtained good resolution using a two-gene phylogeny (D1/D2 and EF1-α). Only one clade, the *S. candida* species complex, could not be clearly resolved with the sequence data used. Interestingly, several isolates involved in this study could not be assigned to any of the currently accepted species and apparently might represent new species. However, further phylogenetic studies testing more genetic markers and reference strains are needed to clarify the taxonomic positions of such isolates.

TABLE 3 Results of *in vitro* antifungal susceptibility testing of *Scopulariopsis* and *Microascus* species

Data by species (no. of isolates tested)	MIC/MEC parameter[a]	Result (μg/ml) for[b]:							
		AMB	VRC	ITC	PSC	AFG	CFG	MFG	TRB
Scopulariopsis brevicaulis (48)	GM	19.0	22.3	29.5	20.9	4.6	2.5	0.5	1.7
	Range	2–32	2–32	1–32	1–32	0.25–16	1–16	0.06–16	0.5–4
	MIC_{90}	32	32	32	32	16	16	16	2
Scopulariopsis gracilis (14)	GM	19.5	26.3	32.0	20.5	4.0	5.7	4.0	3.0
	Range	4–32	8–32	32	1–32	<0.015–16	1–16	<0.015–16	1–4
	MIC_{90}	32	32	32	32	16	16	16	4
Scopulariopsis brumptii (7)	GM	19.5	11.9	32.0	10.8	1.8	3.3	0.5	1.2
	Range	4–32	2–32	32	1–32	0.25–16	2–8	0.125–16	0.5–2
	MIC_{90}	32	32	7	32	4	8	1	2
Microascus cinereus (5)	GM	13.7	13.7	23.5	21.8	12.7	9.3	9.3	2.9
	Range	2–32	1–32	2–32	1–32	4–16	2–16	0.125–16	0.5–4
	MIC_{90}	16	32	32	32	16	8	16	4
Scopulariopsis candida complex (3)[c]	GM	3.2	32.0	32.0	32.0	0.8	2.0	0.1	3.2
	Range	1–8	32	32	32	0.25–4	2	0.06–0.25	2–4
	MIC_{90}								
Microascus cirrosus (2)	GM	32.0	5.7	32.0	32.0	0.2	2.8	0.7	2.8
	Range	32	4–8	32	32	<0.015–16	1–8	0.03–16	2–4
	MIC_{90}								
Overall (97)	GM	16.9	16.4	26.7	14.6	4.0	3.7	1.1	1.9
	Range	0.25–32	1–32	1–32	0.125–32	<0.015–16	1–16	<0.015–16	0.5–4
	MIC_{90}	32	32	32	32	16	16	16	8

[a] GM, geometric mean; MIC_{90}, drug concentration that inhibited 90% of isolates, shown only for groups with ≥5 isolates.
[b] AMB, amphotericin B; VRC, voriconazole; ITC, itraconazole; PSC, posaconazole; AFG, anidulafungin; CFG, caspofungin; MFG, micafungin; TRB, terbinafine.
[c] Includes *Scopulariopsis asperula*, *Scopulariopsis candida*, *Scopulariopsis fusca*, *Microascus manginii*, and *Microascus niger*.

Interestingly, most *S. gracilis* and *M. cinereus* and all *S. brumptii* and *M. cirrosus* isolates tested in our study were from respiratory samples, which suggests a tendency toward localization at this anatomic site. Considering that most of the isolates from a respiratory origin (68%) have been obtained from BAL fluid samples rather than from proven cases, the possible role of these fungi in lung infection warrants further research.

With the exception of the study by Aguilar et al. (24), the antifungal susceptibilities of *Scopulariopsis* and *Microascus* species have been evaluated mainly in *S. brevicaulis* (25, 37–39). Although our study included newer antifungals, the results generally agree with previous data, showing resistance to practically all the available antifungal drugs. Similar data were obtained with testing of other fungi also belonging to the *Microascales*, such as *Scedosporium* spp. (40). In our study, susceptibility to AMB was rarely observed, VRC and PSC showed moderate activities against only a few isolates, and ITC and TBF showed almost no activity. The echinocandins, especially MFG and AFG, demonstrated better *in vitro* activities than the azoles; however, a high number of resistant isolates were also detected. Since no treatment guidelines are available for this group of fungi, therapies for most reported clinical cases were based on previous experience with those used for *Aspergillus* or other clinically relevant molds. Although most clinical cases reported negative outcomes regardless of the type of antifungal treatment (20, 22), VRC has shown some clinical efficacy (11, 15). Our results showed similar *in vitro* activities for VRC and PSC, although there are no clinical reports using PSC. Echinocandins have only rarely been used to treat *Scopulariopsis* infections. Beltrame et al. (41) unsuccessfully used CFG after negative results with VRC in a case of fungal sinusitis caused by *S. acremonium*. More recently, Iwen et al. (17) reported a negative outcome with a combination of liposomal AMB plus MFG against an invasive infection by *S. brevicaulis*.

In conclusion, although infrequent, systemic *Scopulariopsis* and *Microascus* infections are difficult to treat and hence are frequently fatal. Morphological species-level identification is difficult; the combined analysis of EF1-α and D1/D2 can be useful for the identification of the most common clinically relevant species. The isolates studied here showed high levels of resistance to the currently available antifungal agents.

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4.4 Redefining *Microascus*, *Scopulariopsis* and allied genera

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Redefining *Microascus, Scopulariopsis* and allied genera

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Key words

Ascomycota
Microascaceae
Microascales
multigene phylogeny
taxonomy

Abstract The genera *Microascus* and *Scopulariopsis* comprise species commonly isolated from soil, decaying plant material and indoor environments. A few species are also recognised as opportunistic pathogens of insects and animals, including humans. In the past, the taxonomy of these fungi has been based on morphology only. With the aim to clarify the taxonomy and phylogeny of these fungi, we studied a large set of clinical and environmental isolates, including the available ex-type strains of numerous species, by means of morphological, physiological and molecular analyses. Species delineation was assessed under the Genealogical Phylogenetic Species Recognition (GCPSR) criterion using DNA sequence data of four loci (ITS region, and fragments of rDNA LSU, translation elongation factor 1-α and β-tubulin). The genera *Microascus* and *Scopulariopsis* were found to be separated in two distinct lineages. The genus *Pithoascus* is reinstated and the new genus *Pseudoscopulariopsis* is erected, typified by *P. schumacheri*. Seven new species of *Microascus* and one of *Scopulariopsis* are described, namely *M. alveolaris*, *M. brunneosporus*, *M. campaniformis*, *M. expansus*, *M. intricatus*, *M. restrictus*, *M. verrucosus* and *Scopulariopsis cordiae*. *Microascus trigonosporus* var. *macrosporus* is accepted as a species distinct from *M. trigonosporus*. Nine new combinations are introduced. *Microascus cinereus*, *M. longirostris*, *P. schumacheri* and *S. flava* are neotypified. A table summarising the morphological features of the species treated and identification keys for each genus are provided.

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INTRODUCTION

Scopulariopsis was erected by Bainier (1907) for a group of fungi with asexual propagation, with *S. brevicaulis* as type species and two additional taxa, *S. rubellus* and *S. rufulus*. *Scopulariopsis brevicaulis* was originally described as *Penicillium brevicaule* by Saccardo (1882) and included in the *Penicillium* section *Anomala* (Biourge 1923). In the current sense, the distinctive features of *Scopulariopsis* are its annellidic conidiogenesis with mostly thick-walled, basally truncate conidia arranged in long, dry chains and its colony colour varying from white to brown or black, but never in bright green shades like *Penicillium* (Morton & Smith 1963, Samson et al. 2010). Some asexual genera with morphological features similar to those of *Scopulariopsis*, such as *Acaulium*, *Masoniella*, *Phaeoscopulariopsis* and *Torula* were considered to be synonymous (Curzi 1930, Morton & Smith 1963). *Scopulariopsis* currently comprises species with a worldwide distribution that are commonly isolated from soil, air, plant debris and dung (Domsch et al. 2007). In addition, some species have been described as colonisers or pathogens of mammals, including humans and insects (de Hoog et al. 2011, Iwen et al. 2012, Sandoval-Denis et al. 2013).

Several authors (Curzi 1930, 1931, Abbott et al. 1998, Abbott & Sigler 2001, Issakainen et al. 2003) have demonstrated by culturing, mating studies and molecular methods, that the sexual morphs of *Scopulariopsis* belong to the ascomycete genus *Microascus*. Abbott & Sigler (2001) confirmed the existence of both homothallic and heterothallic species. *Microascus* was included in the family *Microascaceae* (1951), order *Microascales*, together with other fungi with annellidic conidiogenesis (Lumbsch & Huhndorf 2007). *Microascus* is characterised by globose to ampulliform perithecial ascomata with cylindrical or papillate necks, and a dark peridium of *textura angularis*. The asci are ovate to globose, unitunicate, non-pedicellate, and evanescent, formed in basipetal rows and containing eight 1-celled ascospores. The ascospores are typically asymmetrical, reniform, lunate or triangular, dextrinoid when young, often with an inconspicuous germ-pore, and extruded in a long cirrhus or a gelatinous ball at the top of the ascomata (Barron et al. 1961, Morton & Smith 1963, Guarro et al. 2012).

Von Arx (1973a) erected *Pithoascus* with three species, i.e. *P. intermedius*, *P. nidicola* (type species) and *P. schumacheri*. These three species were previously included in *Microascus* and had ascomata with rudimentary or inconspicuous ostioles, navicular to fusiform ascospores without germ pores, while they lacked asexual morphs. Von Arx (1978) added *Pithoascus langeronii*, which produced an arthroconidial asexual morph. Nevertheless, species of *Pithoascus* (i.e. *P. intermedius*, *P. schumacherii*) were shown to produce a reduced, scopulariopsis-like asexual morph (Roberts 1985, Valmaseda et al. 1986). Valmaseda et al. (1986) erected the new monotypic genus *Pithoascina* for the arthroconidia-forming species *P. langeronii.* Based on these features, *P. langeronii* was later transferred to the genus *Eremomyces* (*Eremomycetaceae*, *Dothideomycetes*) by Malloch & Sigler (1988) and more recently to *Arthrographis*, being renamed as *Arthrographis arxii* (Giraldo et al. 2014).

Several authors consider *Pithoascus* s.str. as a synonym of *Microascus* (Malloch & Hubart 1987, Abbott et al. 2002, Guarro et al. 2012) since some species show intermediate morpho-

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logical characteristics. In addition, other asexual genera of the *Microascaceae* phylogenetically close to *Scopulariopsis*, i.e. *Wardomyces* and *Wardomycopsis*, also produce a *Microascus* sexual form (Malloch 1970, Udagawa & Furuya 1978); these authors maintained wider generic concepts.

Barron et al. (1961) and later Morton & Smith (1963) published comprehensive monographic reviews on *Microascus* and *Scopulariopsis* based on morphological criteria. Morphology seems to be insufficient for establishing species limits in these fungi. Although most species can be identified by detailed morphological study, phenotypic characters appear to overlap in several cases (Sandoval-Denis et al. 2013). DNA sequencing and multilocus phylogenetic analysis have considerably improved our understanding of species concepts in many fungal groups (Lackner & de Hoog 2011, Summerbell et al. 2011, Lackner et al. 2014, Samson et al. 2014), but as yet no such study has been undertaken to revise *Microascus*, *Scopulariopsis* and allied genera.

Presently, 77 species are accepted in *Scopulariopsis* and 32 in *Microascus*. In addition, many described species are of doubtful identity because their type materials are lost and their protologues are uninterpretable. A further complicating factor is that the new International Code of Nomenclature for Fungi, Algae and Plants no longer allows dual nomenclature for those fungal species that present both sexual and asexual morphs (Hawksworth et al. 2011, Hibbett & Taylor 2013). However, to resolve which name has priority, both at genus and species levels, requires understanding of relationships among species, as well as a stable and well-defined generic circumscription. In the case if *Scopulariopsis* and *Microascus* would be congruent, the former name has been recommended (Hawksworth 2012, Sandoval-Denis et al. 2013).

In a recent study on *Scopulariopsis* and *Microascus* species associated with human disease, we characterised several isolates that could not be identified (Sandoval-Denis et al. 2013). The present work aims to clarify the taxonomic position of these putative new species using the Genealogical Phylogenetic Species Recognition (GCPSR) criterion (Taylor et al. 2000). We provide a multigene phylogeny of *Scopulariopsis*, *Microascus* and related fungi based on a large set of isolates, which includes all available ex-type cultures and well-identified reference strains from international culture collections.

MATERIALS AND METHODS

Isolates

In the present study we evaluate a total of 141 fungal strains, representing 67 fungal species (Table 1). The strains were mainly obtained from different international culture collections, but also from human clinical specimens in the USA.

DNA extraction, amplification and phylogenetic analysis

All the strains were cultured on YES agar (20 g yeast extract, 150 g sucrose, 20 g agar, 1 L distilled water) for 5 d at 25 °C. Fresh mycelium was removed by scrapping the agar surface and total genomic DNA extraction was obtained using the PrepmanUltra sample preparation reagent (Applied Biosystems, Foster City, CA, USA), according to manufacturer's conditions.

Four nuclear DNA regions were amplified and sequenced. These comprised a fragment (490 bp) including the internal transcribed spacer ITS-1 and ITS-2 and the 5.8S rDNA gene (ITS), a fragment (450 bp) including the D1/D2 regions of the LSU rDNA gene, a fragment (820 bp) of the translation elongation factor 1-alpha (EF-1α) and a fragment (470 bp) of the beta-tubulin gene (TUB). The different loci were amplified using the primer pairs ITS5/ITS4 for the ITS region (White et al. 1990), NL1/NL4b for the LSU region (O'Donnell 1993), 983F/2218R for EF-1α (Rehner & Buckley 2005) and BT2a/BT2b for TUB (Glass & Donaldson 1995). PCR amplification reaction had a total volume of 40 µL and consisted in 20 mM Tris-HCl (pH 8.4), 50 mM KCl (10X PCR reaction buffer; Invitrogen, Life Technologies Ltd, Paisley, UK) 1.5 mM $MgCl_2$ (Invitrogen, Life Technologies Ltd, Paisley, UK), 125 µM of each deoxynucleoside triphosphate (GeneAmp® dNTP mix with dTTP, Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA), 5 % dimethyl sulfoxyde (DMSO; Panreac Química S.L.U, Barcelona, Spain), 1.2 µM of each primer and 1.25 U of *Taq* DNA Polymerase (Invitrogen, Life Technologies Ltd, Paisley, UK). The amplification programme consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at a suitable temperature for 1 min, extension for 1 min and 20 s at 72 °C, and a final extension for 1 min at 72 °C. Annealing temperatures for each gene were 55 °C for ITS, 51 °C for LSU and 57 °C for EF-1α and TUB. The amplified products were purified with Diffinity Rapid Tip® purification system (Sigma-Aldrich, St. Louis, MO, USA) and stored at -20 °C until sequencing.

Sequencing was conducted in both directions with the same primer pair used for amplification at Macrogen Europe (Macrogen Inc. Amsterdam, The Netherlands). Consensus sequences were obtained using SeqMan v. 7.0.0 (DNASTAR Lasergene, Madison, WI, USA). The newly generated sequences obtained in this study and their GenBank accession numbers are summarised in Table 1. Additionally, 167 relevant sequences, obtained from public databases (GenBank, NITE) and selected on the basis of BLAST homology search results, were incorporated in the phylogenetic analyses (Table 1).

Sequences were aligned individually for each locus using ClustalW (Thompson et al. 1994), under MEGA v. 5.05 (Tamura et al. 2011), refined with MUSCLE (Edgar 2004) under the same platform and manually adjusted if needed. Phylogenetic reconstructions by maximum likelihood (ML) and bayesian inference were carried out using MEGA v. 5.05 and MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001), respectively. The best nucleotide substitution model for each locus and the combined dataset (GTR+G+I) were estimated using MrModeltest v. 2.3 (Nylander 2004). ML phylogeny was first made separately for each locus (data not shown) and assessed for their concordance by comparing the phylogenetic placement and monophyly of the terminal clades and internal nodes with significant bootstrap (bs) support. Since there was no discordance, the loci were combined into two different datasets. A first analysis was carried out using sequences of both ITS and LSU loci in order to establish the boundaries of the genera with all the available ex-type strains of *Microascus / Scopulariopsis* species complemented with several sequences of related genera of the *Microascaceae* and *Graphiaceae*. To establish the species distribution among the genera, a second combined dataset was created including LSU, ITS, EF-1α and TUB sequences made up of a subset of those previously analysed strains and numerous environmental and clinical isolates morphologically identified as *Microascus* or *Scopulariopsis* species.

For ML analysis, the trees were inferred using Nearest-Neighbour-Interchange as a heuristic method and gaps were treated as partial deletion with a 95 % site coverage cut-off. The robustness of branches was assessed by a bootstrap analysis of 1 000 replicates (Felsenstein 1985). Bootstrap values over 70 % were considered significant.

The Bayesian analyses consisted of two parallel runs of four incrementally heated Markov Chains starting from a random tree topology. The analyses lasted for five million generations

Table 1 Strains and sequence accession numbers included in this study.

Current name	Original name	Strain number[1]	Source	Origin	Sequence accession number[2]			
					ITS	LSU	EF-1α	TUB
Aspergillus baarnensis	*Scopulariopsis halophilica*	CBS 380.74 (ex-type)	*Undaria pinnatifida*	Japan	**LM652376**	**LM652499**	–	–
Doratomyces purpureofuscus	*Doratomyces purpureofuscus*	CBS 139.42; NBRC 7677	Manure	The Netherlands: Limburg	00767701*	00767701*	–	–
Doratomyces stemonitis	*Doratomyces stemonitis*	CBS 127.22; MUCL 4031	Seed	The Netherlands: Wageningen	**LM652377**	DQ836907	–	–
Gamsia aggregata	*Wardomyces aggregatus*	CBS 251.69 (ex-isotype)	Dung of carnivore	USA	**LM652378**	**LM652500**	–	–
Gamsia simplex	*Wardomyces simplex*	CBS 546.69 (ex-isotype)	Milled *Oryza sativa*	Japan	**LM652379**	**LM652501**	–	–
Graphium penicillioides	*Graphium penicillioides*	CBS 102632 (ex-epitype)	Wood, *Populus nigra*	Czech Republic	AB038432	AF175961	–	–
Hypocrea atroviridis	*Hypocrea atroviridis*	CBS 110086; NBRC 101776 (ex-type)	Decorticated wood	France	11776204*	11776205*	–	–
Kernia nitida	*Magnusia nitida*	CBS 282.52; NBRC 8200	*Chrysolina sanguinolenta*	France	00820001*	00820001*	–	–
Kernia pachypleura	*Kernia pachypleura*	NBRC 30413; UAMH 8858	Soil of paddy field	Japan	03041301*	03041301*	–	–
Lophotrichus macrosporus	*Lophotrichus macrosporus*	FMR 5571; NBRC 32894	Sheep dung	Iraq	03289401*	03289401*	–	–
Lophotrichus plumbescens	*Lophotrichus plumbescens*	NBRC 30864; UAMH 8710 (ex-type)	Soil	Thailand; Bangkok	03086401*	03086401*	–	–
Microascus alveolaris sp. nov.	*Microascus* sp.	UTHSC 04-1534; FMR 12354	Human BAL	USA	**LM652380**	HG380482	HG380405	**LM652596**
	Microascus sp.	UTHSC 05-3416; FMR 12350	Human BAL	USA	**LM652381**	HG380483	HG380406	**LM652597**
	Microascus sp.	UTHSC 05-1041; FMR 12351	Human sputum	USA	**LM652382**	HG380488	HG380411	**LM652598**
	Microascus sp.	UTHSC 06-3152; FMR 12346	Human BAL	USA	**LM652383**	HG380487	HG380410	**LM652599**
	Microascus sp.	UTHSC 07-1823; FMR 12342	Human Sputum	USA	**LM652384**	HG380489	HG380412	**LM652600**
	Microascus sp.	CBS 139501; UTHSC 07-3491; FMR 12252 (ex-type)	Human BAL	USA	**LM652385**	HG380484	HG380407	**LM652601**
	Microascus sp.	UTHSC 08-886; FMR 12340	Human BAL	USA	**LM652386**	HG380485	HG380408	**LM652602**
	Microascus sp.	UTHSC 10-214; FMR 12336	Human BAL	USA	**LM652387**	HG380486	HG380409	**LM652603**
	Microascus sp.	UTHSC R-4634; FMR 12333	Human lung Tissue	USA	**LM652388**	HG380490	HG380413	**LM652604**
Microascus albonigrescens†	*Microascus albonigrescens*	IHEM 18560	Litter treated with urea	Japan: Nemuro-shi	**LM652389**	**LM652502**	–	–
Microascus brunneosporus sp. nov.	*Microascus* sp.	CBS 138276; UTHSC 06-4312; FMR 12343 (ex-type)	Human BAL	USA	**LM652390**	HG380497	HG380420	**LM652605**
Microascus campaniformis sp. nov.	*Microascus* sp.	CBS 138126; UTHSC 10-565; FMR 12334 (ex-type)	Human BAL	USA	**LM652391**	HG380495	HG380418	**LM652606**
Microascus caviariformis†	*Microascus caviariformis*	CBS 536.87: UAMH 5592 (ex-type)	Decaying meat	Belgium	**LM652392**	**LM652503**	–	–
Microascus chartarus comb. nov.	*Masonia chartarum*	CBS 294.52; MUCL 9001 (ex-type)	Mouldy wall-paper in a house	England: London.	**LM652393**	HG380463	HG380386	**LM652607**
Microascus cinereus	*Microascus cinereus*	UTHSC 06-3278; FMR 12345	BAL	USA	**LM652394**	HG380347	HG380424	**LM652608**
	Microascus cinereus	UTHSC 08-3181: FMR 12339	Human sternum tissue	USA	**LM652395**	HG380348	HG380425	**LM652609**
	Microascus cinereus	UTHSC 09-573; FMR 12239	Human BAL	USA	**LM652396**	HG380349	HG380426	**LM652610**
	Microascus cinereus	UTHSC 10-2805; FMR 12217 (ex-neotype)	Human BAL	USA	**LM652397**	HG380350	HG380427	**LM652611**
	Microascus cinereus	UTHSC 11-383; FMR 12331	Human BAL	USA	**LM652398**	HG380351	HG380428	**LM652612**
	Microascus griseus	CBS 365.65; ATCC 16204 (ex-type)	Soil	India: Maharashtra	**LM652399**	HG380346	HG380423	**LM652613**
Microascus cirrosus	*Microascus cirrosus*	CBS 217.31 (ex-type)	Leaf of *Prunus* sp.	Italy	**LM652400**	HG380429	HG380352	**LM652614**
	Microascus cirrosus	CBS 277.34; MUCL 9050	Roots of *Vitis vinifera*	Italy	**LM652401**	**LM652504**	**LM652556**	**LM652615**
	Microascus desmosporus	CBS 301.61; MUCL 9054	Unknown	UK	**LM652402**	**LM652505**	**LM652557**	**LM652616**
	Microascus cirrosus	UTHSC 07-1887: FMR 12256	Induced human sputum	USA	**LM652403**	HG380431	HG380354	**LM652617**
	Microascus cirrosus	UTHSC 11-14; FMR 12332	Human BAL	USA	**LM652404**	HG380432	HG380355	**LM652618**
Microascus croci comb. nov.	*Scopulariopsis chartarum*	FMR 3997	Aquatic sediment, Ebro river	Spain: Tarragona	**LM652405**	**LM652506**	**LM652558**	**LM652619**
	Microascus cirrosus	FMR 4004	Aquatic sediment, Besós river	Spain: Barcelona	**LM652406**	**LM652507**	**LM652559**	**LM652620**
	Scopulariopsis croci	CBS 158.44; MUCL 9002 (ex-type)	From *Crocus* sp.	The Netherlands: Lisse	**LM652407**	**LM652508**	**LM652560**	**LM652621**
	Masoniella tertia	CBS 296.61; MUCL 9005 (ex-type)	Air	Brazil: Pernambuco	**LM652408**	**LM652509**	**LM652561**	**LM652622**
Microascus expansus sp. nov.	*Microascus* sp.	UTHSC 06-2519; FMR 12267	Human pleural fluid	USA	**LM652409**	HG380491	HG380414	**LM652623**
	Microascus sp.	CBS 138127; UTHSC 06-4472; FMR 12266 (ex-type)	Human sputum	USA	**LM652410**	HG380492	HG380415	**LM652624**
Microascus giganteus†	*Microascus giganteus*	CBS 746.69 (ex-type)	Insect frass in dead log	Canada: Ontario	**LM652411**	**LM652510**	–	–
Microascus gracilis comb. nov.	*Paecilomyces fuscatus*	CBS 369.70 (ex-isotype)	Food	Japan	**LM652412**	HG380467	HG380390	**LM652625**
	Scopulariopsis gracilis	UTHSC 09-1351; FMR 12234	Human joint fluid	USA	**LM652413**	HG380476	HG380399	**LM652626**
	Scopulariopsis gracilis	UTHSC 09-1829; FMR 12231	Human BAL	USA	**LM652414**	HG380477	HG380400	**LM652627**
	Scopulariopsis gracilis	UTHSC 10-390; FMR 12335	Human BAL	USA	**LM652415**	**LM652511**	**LM652562**	**LM652628**

Table 1 (cont.).

Current name	Original name	Strain number[1]	Source	Origin	Sequence accession number[2]			
					ITS	LSU	EF-1α	TUB
	Microascus cinereus	CBS 195.61; MUCL 9048	Soil	England	**LM652416**	HG380468	HG380391	**LM652629**
	Microascus cinereus	CBS 300.61; MUCL 9049	Seed of *Zea mays*	USA: Iowa	**LM652417**	**LM652512**	**LM652563**	**LM652630**
Microascus hyalinus comb. nov.	*Kernia hyalina*	CBS 766.70 (ex-isotype)	Dung of cow	USA	**LM652418**	**LM652513**	**LM652564**	**LM652631**
Microascus intricatus sp. nov.	*Microascus* sp.	CBS 138128; UTHSC 07-156; FMR 12264 (ex-type)	Human BAL	USA	**LM652419**	HG380496	HG380419	**LM652632**
	Microascus sp.	FMR 12362	Soil	Argentina: Iguazú	**LM652420**	**LM652514**	**LM652565**	**LM652633**
Microascus longirostris	*Microascus longirostris*	CBS 196.61; MUCL 9058 (ex-neotype)	Wasp's nest	USA: Maine	**LM652421**	**LM652515**	**LM652566**	**LM652634**
	Microascus longirostris	CBS 415.64	Soil	Japan	**LM652422**	**LM652516**	**LM652567**	**LM652635**
Microascus macrosporus comb & stat. nov.	*Microascus trigonosporus* var. *macrosporus*	CBS 662.71	Soil	USA	**LM652423**	**LM652517**	**LM652568**	**LM652636**
Microascus murinus comb. nov.	*Scopulariopsis murina*	CBS 830.70; IHEM 18567 (ex-type)	Composed municipal waste	Germany: Giessen	**LM652424**	HG380481	HG380404	**LM652637**
Microascus paisii comb. nov.	*Scopulariopsis brumptii*	UTHSC 07-639; FMR 12263	Human BAL	USA	**LM652425**	HG380451	HG380374	**LM652638**
	Scopulariopsis brumptii	UTHSC 08-1734; FMR 12248	Human BAL	USA	**LM652426**	HG380452	HG380375	**LM652639**
	Scopulariopsis brumptii	UTHSC 09-2391; FMR 12229	Human sputum	USA	**LM652427**	HG380453	HG380376	**LM652640**
	Scopulariopsis brumptii	UTHSC 09-457; FMR 12241	Human sputum	USA	**LM652428**	HG380454	HG380377	**LM652641**
	Scopulariopsis brumptii	UTHSC 09-482; FMR 12240	Human BAL	USA	**LM652429**	HG380455	HG380378	**LM652642**
	Scopulariopsis brumptii	UTHSC 10-2920; FMR 12215	Human BAL	USA	**LM652430**	HG380456	HG380379	**LM652643**
	Scopulariopsis brumptii	UTHSC 11-708; FMR 12210	Human sputum	USA	**LM652431**	HG380457	HG380380	**LM652644**
	Scopulariopsis brumptii	CBS 896.68; MUCL 8989	Soil on a *Triticum sativum* field	Germany: Schleswig	**LM652432**	HG380449	HG380372	**LM652645**
	Masonia grisea	CBS 295.52; MUCL 9003 (ex-type)	Culture contaminant	England	**LM652433**	HG380450	HG380373	**LM652646**
	Torula paisii	CBS 213.27; MUCL 7915 (ex-type)	Man	Italy	**LM652434**	**LM652518**	**LM652569**	**LM652647**
	Scopulariopsis brumptii	MUCL 8990	Soil	Germany: Schleswig-Holstein	**LM652435**	**LM652519**	**LM652570**	**LM652648**
	Scopulariopsis chartarum	CBS 897.68; MUCL 8993	Soil on a wheat field	Germany	**LM652436**	**LM652520**	**LM652571**	**LM652649**
	Scopulariopsis melanospora	CBS 272.60; MUCL 9040 (ex-isotype)	Milled *Oriza sativa*	USA	**LM652437**	**LM652521**	**LM652572**	**LM652650**
	Scopulariopsis sphaerospora	CBS 402.34; MUCL 9045 (ex-type)	Unknown	Austria	**LM652438**	**LM652522**	**LM652573**	**LM652651**
Microascus pyramidus	*Microascus pyramidus*	CBS 212.65 (ex-isotype)	Desert soil	USA: California	**LM652439**	HG380435	HG380358	**LM652652**
Microascus restrictus sp. nov.	*Microascus* sp.	CBS 138277; UTHSC 09-2704; FMR 12227 (ex-type)	Human left hallux	USA	**LM652440**	HG380494	HG380417	**LM652653**
Microascus senegalensis	*Microascus senegalensis*	CBS 277.74; IHEM 18561 (ex-type)	Mangrove soil	Senegal	**LM652441**	**LM652523**	**LM652574**	**LM652654**
Microascus singularis†	*Microascus singularis*	CBS 414.64	Laboratory contaminant	Japan: Tokyo	**LM652442**	**LM652524**	–	–
Microascus trigonosporus	*Microascus trigonosporus* var. *trigonosporus*	CBS 218.31 (ex-type)	Unknown	USA	**LM652443**	HG380436	HG380359	**LM652655**
	Microascus trigonosporus	CBS 199.61; MUCL 9061	Milled rice	Burma, Japan	**LM652444**	HG380438	HG380361	**LM652656**
	Scopulariopsis coprophila	CBS 262.35; MUCL 9841	Mushroom bed	UK	**LM652445**	**LM652525**	**LM652575**	**LM652657**
Microascus verrucosus sp. nov.	*Microascus* sp.	CBS 138278; UTHSC 10-2601; FMR 12219 (ex-type)	Human BAL	USA	**LM652446**	HG380493	HG380416	**LM652658**
Parascedosporium tectonae	*Graphium tectonae*	CBS 127.84 (ex-type)	Seed	Jamaica	AY228113	EF151332	EF151409	–
Petriella setifera	*Petriella setifera*	CBS 437.75	Wood panel in coastal water	Hong Kong	–	DQ470969	DQ836911	–
	Petriella setifera	FMR 7736; NBRC 100025	Soil	Spain; Canary Islands	10002501*	10002501*	–	–
Petriella sordida	*Petriella sordida*	CBS 124169	Corner of a bathroom	The Netherlands	GQ426957	AY281099	–	–
Petriellopsis africana	*Petriellidium africanum*	CBS 311.72 (ex-type)	Brown sandy soil	Namibia	AJ888425	EF151331	–	–
Phitoascus ater comb. nov.	*Scopulariopsis atra*	CBS 400.34; IHEM 18608 (ex-type)	Unknown	Unknown	**LM652447**	**LM652526**	**LM652576**	**LM652659**
Phitoascus exsertus	*Microascus exsertus*	CBS 583.75	From *Osmia rufa*	Denmark: Sjaelland	**LM652448**	**LM652527**	**LM652577**	**LM652660**
	Microascus exsertus	CBS 819.70 (ex-type)	From *Megachile willoughbiella*	Denmark: Tastrup	**LM652449**	**LM652528**	**LM652578**	**LM652661**
Phitoascus intermedius	*Microascus intermedius*	CBS 217.32 (ex-type)	Root of *Fragaria vesca*	USA: North Carolina	**LM652450**	**LM652529**	**LM652579**	**LM652662**
Phitoascus nidicola	*Microascus nidicola*	CBS 197.61 (ex-epitype)	From *Dipodomys merriami*	USA: Utah	**LM652451**	**LM652530**	**LM652580**	**LM652663**
Pithoascus platysporus†	*Pithoascus platysporus*	CBS 419.73 (ex-type)	Agricultural soil	The Netherlands	**LM652452**	**LM652531**	–	–
Phitoascus stoveri	*Microascus stoveri*	CBS 176.71 (ex-type)	Root of *Beta vulgaris*	USA: Ohio	**LM652453**	**LM652532**	**LM652581**	**LM652664**
Pseudallescheria ellipsoidea	*Petriellidium ellipsoideum*	CBS 418.73 (ex-type)	Soil	Tajikistan	EF151323	EF151323	–	–
Pseudoscopulariopsis hibernica comb. nov.	*Scopulariopsis hibernica*	UAMH 2643; ATCC 16690	From soil	Ireland	**LM652454**	**LM652533**	**LM652582**	**LM652665**

Pseudoscopulariopsis schumacheri comb. nov.	*Microascus schumacheri*	CBS 435.86 (ex-neotype)	From soil	Spain: Puerto de la Quesera	**LM652455**	**LM652534**	**LM652583**	**LM652666**
Scedosporium aurantiacum	*Scedosporium aurantiacum*	CBS 116910	Ulcer of ankle	Spain	HQ231818	EF151326	–	–
Scedosporium boydii	*Pseudallescheria boydii*	CBS 330.93	Bronchial secretion	The Netherlands	AY863196	AY882372	–	–
Scopulariopsis acremonium†	*Scopulariopsis danica*	CBS 290.38; MUCL 9028 (ex-type)	Skin of a horse	Denmark	**LM652456**	HG380439	HG380362	–
	Scopulariopsis acremonium	MUCL 8274	Wheat field soil	Germany: Schleswig-Holstein	**LM652457**	**LM652535**	–	–
	Scopulariopsis acremonium	MUCL 8409	Soil	Germany: Schleswig-Holstein	**LM652458**	**LM652536**	–	–
Scopulariopsis asperula	*Microascus niger*	MUCL 40729; UAMH 7879	Indoor air	Canada: Alberta	**LM652459**	**LM652537**	**LM652584**	**LM652667**
	Microascus niger	MUCL 40746; UAMH 9029	Dung of *Mephitis mephitis*	Canada: Alberta	**LM652460**	HG380434	HG380357	**LM652668**
	Scopulariopsis asperula	CBS 853.68	Compost soil	Germany	**LM652461**	JQ434669	JQ434621	JQ434558
	Scopulariopsis fusca	UTHSC 10-3405; FMR 12212	Toenail	USA	**LM652462**	HG380461	HG380384	**LM652669**
	Scopulariopsis fusca	CBS 401.34; MUCL 9032 (ex-type)	Carcass of rabbit	Austria	**LM652463**	HG380465	HG380388	**LM652670**
	Torula bestae	CBS 289.38; MUCL 9012 (ex-type)	From man	Italy	**LM652464**	**LM652538**	**LM652585**	**LM652671**
Scopulariopsis brevicaulis	*Microascus brevicaulis*	MUCL 40726 (ex-type)	Indoor air	Canada: Alberta	**LM652465**	HG380440	HG380363	**LM652672**
	Scopulariopsis alboflavescens	CBS 399.34 (ex-type)	Diseased skin	Austria	**LM652539**	**LM652466**	JQ434600	JQ434537
	Scopulariopsis brevicaulis	UTHSC 06-277; FMR 12273	Human hair	USA	**LM652467**	HG380441	HG380364	**LM652674**
	Scopulariopsis brevicaulis	UTHSC 06-619; FMR 12271	Human toenail	USA	**LM652468**	HG380442	HG380365	**LM652675**
	Scopulariopsis brevicaulis	UTHSC 06-1072; FMR 12247	Human BAL	USA	**LM652469**	**LM652540**	**LM652586**	**LM652676**
	Scopulariopsis brevicaulis	UTHSC 07-1812; FMR 12257	Human toenail	USA	**LM652470**	HG380443	HG380366	**LM652677**
	Scopulariopsis brevicaulis	UTHSC 07-1888; FMR 12255	Human spine	USA	**LM652471**	HG380444	HG380367	**LM652678**
	Scopulariopsis brevicaulis	UTHSC 09-1092; FMR 12236	Human toe	USA	**LM652472**	HG380445	HG380368	**LM652679**
	Scopulariopsis brevicaulis	UTHSC 09-1373; FMR 12233	Human sputum	USA	**LM652473**	**LM652541**	**LM652587**	**LM652680**
	Scopulariopsis brevicaulis	UTHSC 11-1240; FMR 12206	Human lung mass	USA	**LM652474**	HG380446	HG380369	**LM652681**
	Scopulariopsis brevicaulis	UTHSC 11-1563; FMR 12204	Human BAL	USA	**LM652475**	HG380447	HG380370	**LM652682**
	Scopulariopsis brevicaulis	UTHSC 11-427; FMR 12211	Human sputum	USA	**LM652476**	HG380448	HG380371	**LM652683**
	Scopulariopsis insectivora	CBS 335.35; MUCL 9035	Pupa of *Pteronus pini*	The Netherlands	**LM652542**	**LM652477**	**LM652588**	**LM652684**
	Scopulariopsis koningii	CBS 208.61	Elephant	Unknown	**LM652543**	**LM652478**	**LM652589**	**LM652685**
	Scopulariopsis stercoraria	MUCL 14213	Soil	Belgium: Heverlee	**LM652544**	**LM652479**	**LM652590**	**LM652686**
Scopulariopsis canadensis†	*Scopulariopsis canadensis*	CBS 204.61 (ex-type)	Seed of *Beta vulgaris*	Canada	**LM652545**	**LM652480**	–	–
Scopulariopsis candida	*Scopulariopsis alboflavescens*	MUCL 9007	Unknown	Unknown	**LM652546**	**LM652481**	**LM652591**	**LM652687**
	Scopulariopsis brevicaulis var. *alba*	CBS 119.43; MUCL 9016	Soil	The Netherlands	**LM652547**	**LM652482**	**LM652592**	**LM652688**
	Scopulariopsis candelabrum	CBS 205.27; MUCL 9026	Unknown	France	**LM652548**	**LM652483**	**LM652593**	**LM652689**
	Scopulariopsis candida	MUCL 40743 (ex-epitype)	Indoor air	Canada	**LM652484**	HG380458	HG380381	**LM652690**
	Scopulariopsis candida	UTHSC 09-3241; FMR 12226	Scalp	USA	**LM652485**	HG380460	HG380383	**LM652691**
	Scopulariopsis candida	UTHSC 09-2576; FMR 12228	Sputum	USA	**LM652486**	HG380459	HG380382	**LM652692**
	Microascus manginii	MUCL 41467	Cheese 'Tome de Savoie'	France	**LM652487**	HG380433	HG380356	**LM652693**
	Nephrospora manginii	CBS 170.27 (ex-type)	Unknown	France	**LM652488**	**LM652549**	**LM652594**	**LM652694**
Scopulariopsis carbonaria†	*Scopulariopsis carbonaria*	MUCL 9027 (ex-type)	Soil	Panama	**LM652489**	HG380462	HG380385	**LM652695**
Scopulariopsis coprophila†	*Scopulariopsis fimicola*	CBS 206.61	Mushroom bed	UK	**LM652490**	**LM652550**	–	–
Scopulariopsis cordiae sp. nov.	*Scopulariopsis* sp.	CBS 138129; UTHSC 09-866; FMR 12338 (ex-type)	Human finger	USA	**LM652491**	HG380499	HG380422	**LM652673**
	Scopulariopsis sp.	UTHSC 05-3453; FMR 12349	Human JP Drain	USA	**LM652492**	HG380498	HG380421	**LM652696**
Scopulariopsis flava	*Scopulariopsis flava*	CBS 207.61; MUCL 9031 (ex-neotype)	Cheese	UK	**LM652493**	HG380464	HG380387	**LM652697**
Scopulariopsis parva	*Scopulariopsis parvula*	MUCL 9041 (ex-type)	Soil	Canada	**LM652494**	**LM652551**	–	–
Scopulariopsis soppii	*Microascus soppii*	UAMH 9169 (ex-type)	wood of *Populus tremuloides*	Canada: Alberta	**LM652495**	**LM652552**	**LM652595**	**LM652698**
Trichoderma asperellum	*Trichoderma asperellum*	CBS 433.97; NBRC 101777 (ex-type)	Sclerotia of *Sclerotinia minor* buried in soil	USA: Maryland	11776302*	11776301*	–	–
Trichurus spiralis	*Trichurus spiralis*	NBRC 100833	Mushroom	Japan: Kumamoto-shi	11058901*	11058901*	–	–
Trichurus terrophilus	*Trichurus terrophilus*	NBRC 7660; CBS 448.51; UAMH 8848	Timber of *Eucalyptus saligna*	South Africa	00766001*	00766001*	–	–
Wardomyces inflatus	*Wardomyces hughesii*	CBS 216.61 (ex-isotype)	Wood, *Acer* sp.	Canada: Québec	**LM652496**	**LM652553**	–	–
Wardomycopsis humicola	*Scopulariopsis humicola*	CBS 487.66 (ex-type)	Soil	Canada: Ontario	**LM652497**	**LM652554**	–	–
Wardomycopsis inopinata	*Wardomycopsis inopinata*	FMR 10305	Soil	Myanmar	**LM652498**	**LM652555**	–	–

[1] ATCC: American type culture collection, Manassas, VA, USA; CBS: CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; FMR: Facultat de Medicina i Ciències de la Salut, Reus, Spain; IHEM: Biomedical Fungi and Yeasts Collection, Scientific Institute of Public Health, Belgium; MUCL: Université Catholique de Louvain, Louvain-la-Neuve, Belgium; NBRC: National Biological Resource Centre, Japan; UAMH: University of Alberta Microfungus Collection and Herbarium, Canada; UTHSC: Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center, San Antonio, USA.

[2] ITS: Internal transcribed spacer regions of the rDNA and 5.8S region; LSU: partial large subunit of the rDNA; EF-1α: Partial translation elongation factor gene; TUB: partial beta-tubulin gene.

† Excluded or doubtful species name. * Sequences obtained from the NBRC database. Sequences newly generated in this study are indicated in **bold**.

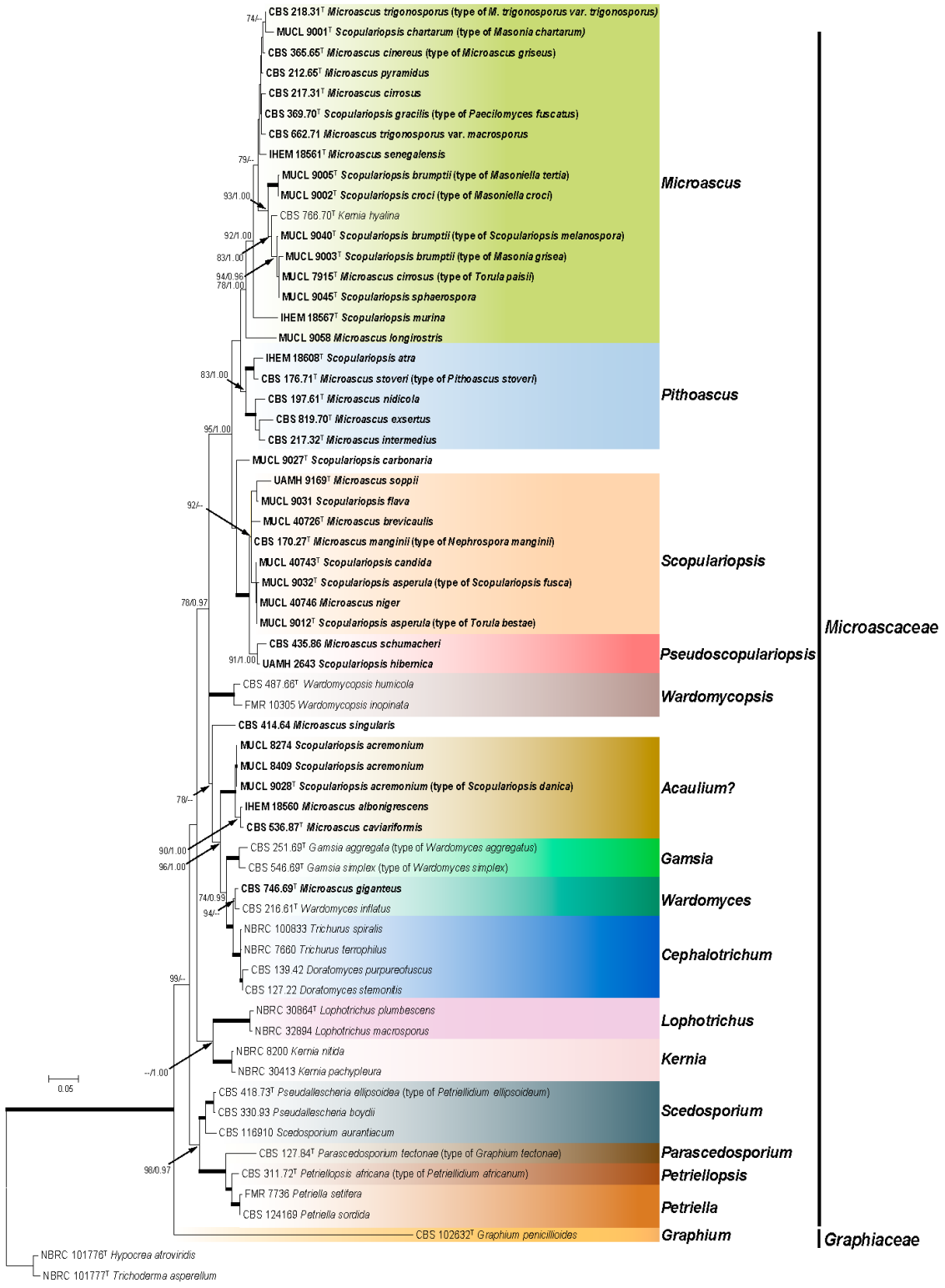

Fig. 1 Maximum likelihood (ML) tree obtained from the combined LSU and ITS sequences of 61 representative taxa of *Microascaceae* and *Graphiaceae*. Numbers on the branches are ML bootstrap values (bs) above 70 %, followed by Bayesian posterior probabilities (pp) above 0.95. Full supported branches are indicated in **bold**. Branch lengths are proportional to distance. Strains considered current members of the genera *Microascus* or *Scopulariopsis* genera are represented in **bold**. Ex-type strains are indicated with T. The original name of each strain, when applied, is given between parenthesis. The tree was rooted to *Hypocrea atroviridis* (NBRC 101776) and *Trichoderma asperellum* (NBRC 101777).

with a sampling frequency of every 100 generations. The 50 % majority rule consensus trees and posterior probabilities (pp) were calculated from 37 500 trees after discarding 12 500 trees for burn-in. Posterior probability values equal or above 0.95 were considered significant. The resulting trees were plotted using FigTree v.1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). The alignments originated in this study have been deposited in TreeBASE (http://www.treebase.org).

Morphology

All isolates were grown on oatmeal agar (OA; 30 g filtered oat flakes, 20 g agar, 1 L distilled water) and potato-carrot agar (PCA; 20 g each of filtered potatoes and carrots, 20 g agar, 1 L distilled water). They were incubated at different temperatures (5, 15, 25, 30, 35, 37, 40 and 45 °C) and examined at 7 and 14 d to determine colony growth rates. In descriptions, colour notations of the colonies were from Kornerup & Wanscher (1978). Measurements and descriptions of microscopic structures were made using an Olympus CH2 light microscope (Olympus Corporation, Tokyo, Japan) from cultures on PCA or OA at 25 °C for 14 and 21 d to ensure ascomata development. All isolates were examined on slides mounted on 85 % lactic acid. Features of the sexual morph structures were obtained from squash preparations or spore mounts. Photographs of the microscopic structures were made using a Zeiss Axio Imager M1 light microscope (Zeiss, Oberkochen, Germany) with a mounted DeltaPix Infinity X digital camera using Nomarski differential interference contrast and phase contrast optics. Nomenclatural data was deposited in MycoBank (Crous et al. 2004).

RESULTS

Generic circumscription of the Microascaceae

To delineate generic boundaries, we conducted a phylogenetic analysis using the combined LSU and ITS datasets including 54 currently accepted species belonging to 12 genera of *Microascaceae* and one species of the family *Graphiaceae*. *Trichoderma asperellum* and *Trichoderma atroviride* were selected as outgroup (Fig. 1). The final alignment consisted of 63 taxa and contained 996 characters (LSU 504, ITS 492), of which 618 were conserved and 297 were phylogenetically informative (LSU 98, ITS 199). Fig. 1 shows the ML tree including bs and pp values. The trees obtained from ML and Bayesian analyses of the individual loci and the combined analysis showed congruent topologies.

The phylogenetic inferences showed that *Microascus / Scopulariopsis* were polyphyletic, with species distributed into several distant lineages. However, most species of *Microascus / Scopulariopsis* clustered into a single large, well-supported lineage (bs = 95 % / pp = 1.00). This lineage comprised four sublineages, which we interpret as three distinct genera, *Microascus*, *Pithoascus* and *Scopulariopsis*, the fourth one representing a putative undescribed genus.

The members of a sublineage referred to as *Microascus* were characterised by dark-coloured colonies and mostly brown to green-brown mycelia, conidiogenous apparatus and conidia. The conidiogenous cells (annellides) were born singly on aerial hyphae or on penicillate conidiophores. They were ampulliform or lageniform and usually had a long and narrow cylindrical annellated zone tapering gradually to the conidiogenous locus, and produced smooth to roughened conidia. Sexual morphs were observed in 13 species. Ascomata were ostiolate, rarely non-ostiolate, mostly globose to ampulliform, glabrous or hairy, papillate or with long cylindrical necks, and had a dark brown to black peridium of *textura angularis* with exception of the unidentified strains FMR 12362 and UTHSC 07-156, which showed perithecia with peridia of *textura intricata*. The ascospores ranged from reniform to ellipsoidal, triangular or quadrangular, were straw-coloured to pale brown and exhibited a single, mostly inconspicuous germ pore (Fig. 2a–h).

Members of the *Pithoascus* sublineage showed flat, white to grey colonies without aerial mycelia. The mycelium and the conidiogenous apparatus were subhyaline and the latter consisted of solitary, short, mostly ampulliform annellides with a short-cylindrical neck. With the exception of strain IHEM 18608, all strains of the *Pithoascus* clade exhibited a sexual morph characterised by black ascomata with an inconspicuous ostiole and navicular to fusiform ascospores without germ pores (Fig. 2i–n).

The *Scopulariopsis* sublineage included fungi with white, pale grey, tan or brown colonies. The mycelium was mostly hyaline. Annellides were hyaline or pale brown, more or less cylindrical, with a wide, flat conidiogenous opening and mostly formed on densely penicillate conidiophores. Conidia were hyaline or pale brown, smooth or distinctly roughened, often showing a protruding base. A sexual morph was observed in four species and was characterised by dark, globose to subglobose perithecia with a peridium of *textura angularis* and with a papillate or a long cylindrical ostiolar neck. Ascospores were reniform to broadly lunate, hyaline or pale yellow, with a single, inconspicuous germ pore (Fig. 2s–y).

The reference strains of *Microascus schumacheri* (CBS 435.86) and *Scopulariopsis hibernica* (UAMH 2643) formed a well-supported clade (bs 91, pp 1.00), basal to the *Scopulariopsis* clade. Because the former two taxa shared several morphological features that deviated from those of *Scopulariopsis*, they were accommodated in a new genus named *Pseudoscopulariopsis*. Members of this clade were characterised by forming grey or olivaceous colonies and hyaline to subhyaline conidiogenous cells, which usually consisted of annellides arising from swollen basal cells. The annellides were short, more or less ampulliform and with a cylindrical annellated zone. The sexual morph was only observed in *P. schumacheri*, which produced black perithecia and fusiform or navicular, straw-coloured ascospores without germ pores (Fig. 2o–r).

However, this phylogenetic approach had insufficient resolution to establish the limits among the different species included in each genus. Similarly, the ex-type strain of *Scopulariopsis carbonaria* (MUCL 9027) was related to the *Scopulariopsis* clade, but its position was not resolved with this analysis.

The reference strain of *Microascus singularis* (CBS 414.64) formed a solitary branch in an *incertae sedis* position. The main morphological distinction of this isolate was the production of conidia showing longitudinal bands. A strongly-supported clade, composed by the ex-type strains of *Microascus caviariformis* (CBS 536.87) and *Scopulariopsis danica* (MUCL 9028), two reference strains of *Scopulariopsis acremonium* (MUCL 8274 and MUCL 8409) and a reference strain of *Microascus albonigrescens* (IHEM 18560), clustered apart from the genera included in the study. The ex-type strain of *Microascus giganteus* (CBS 746.69) was placed very far from the *Microascus* clade. It formed a well-supported clade with the ex-type strain of *Wardomyces inflatus* (CBS 216.61) and with another fully supported clade, which included several reference strains of the genera *Doratomyces* and *Trichurus*. Our phylogenetic analyses were concordant with the observations made by Abbott (2000), who considered *Doratomyces* and *Trichurus* as congeneric with *Cephalotrichum*, all being characterised by the formation of dry-spored synnemata and lacking sexual morphs.

Lophotrichus and *Kernia*, characterised by hairy ascomata and ellipsoidal ascospores with two germ-pores and graphium- or

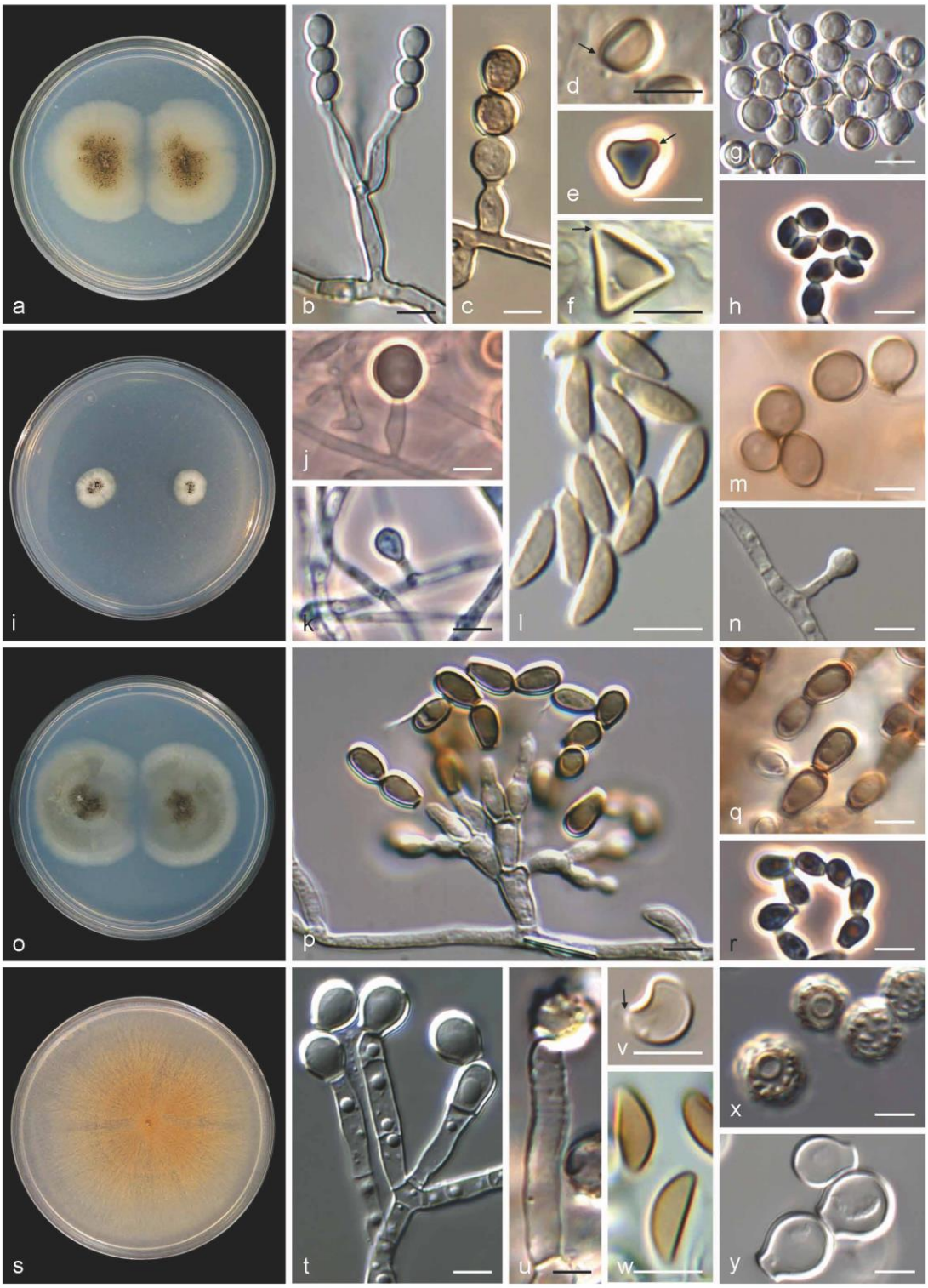

Fig. 2 Key morphological features to distinguish *Microascus* (a–h), *Pithoascus* (*Pi.*) (i–n), *Pseudoscopulariopsis* (*Ps.*) (o–r) and *Scopulariopsis* (s–y). a, i, o, s. Colonies on PCA after 21 d at 25 °C; b, c, j, k, p, t, u. conidiogenous cells; d–f, l, v, w. ascospores (germ pores indicated with arrows); g, h, m, n, q, r, x, y. conidia (a, b, d. *M. cinereus* CBS 365.65; c. *M. restrictus* CBS 138277; e. *M. trigonosporus* CBS 218.31; f. *M. pyramidus* CBS 212.65; g. *M. chartarus* MUCL 9001; h. *M. gracilis* CBS 369.70; i, k, l, n. *Pi. nidicola* CBS 197.61; j, m. *Pi. ater* IHEM 18608; o–r. *Ps. hibernica* UAMH 2643; s, u, x. *S. brevicaulis* MUCL 40726; t, v, y. *S. candida* MUCL 40743; w. *S. soppii* UAMH 9169. — Scale bars: 5 µm.

scopulariopsis-like asexual morphs, respectively, formed well-supported clades related to *Scedosporium* and allied genera (i.e., *Parascedosporium*, *Petriella* and *Petriellopsis*), characterised by scedosporium-like asexual morphs with slimy conidia.

Some species traditionally included in *Pithoascus* and *Scopulariopsis* clustered in orders different from *Microascales*. The ex-type strain of *Pithoascus platysporus* (CBS 419.73) and a reference strain of *Scopulariopsis coprophila* (CBS 206.61) were closely related to the *Hypocreales;* the ex-type strain of *Scopulariopsis canadensis* (CBS 204.61) grouped with members of the *Xylariales*; the ex-type strains of *Scopulariopsis parva* (MUCL 9041) and *Scopulariopsis halophilica* (CBS 380.74) clustered outside the *Sordariomycetes*, being related to members of the *Eurotiales* (data not shown).

Species distribution in Microascus, Pithoascus, Pseudoscopulariopsis and Scopulariopsis

The final alignment of the combined matrix included 106 strains from *Microascus*, *Pithoascus*, *Scopulariopsis* and *Pseudoscopulariopsis* species and involved 2 219 characters (LSU 437, ITS 493, EF-1α 816, TUB 473), of which 1 493 were conserved, 673 were variable and 486 were phylogenetically informative (LSU 47, ITS 112, EF-1α 176, TUB 151). *Petriella setifera* and *Parascedosporium tectonae* were selected as outgroup taxa. The resulting ML tree is shown in Fig. 3 including bs and pp values. The topology of the trees obtained from ML and Bayesian analyses from each individual locus and the combined analysis were concordant. The multilocus analysis confirmed the results obtained from phylogenetic inferences using the combined LSU and ITS dataset. In total 34 well-supported clades were resolved and were distributed among four main lineages corresponding to *Microascus*, *Pithoascus*, *Scopulariopsis* and the new genus *Pseudoscopulariopsis* proposed here. The *Microascus* lineage comprised 20 well-supported subclades, 13 of which included an ex-type strain of a known species or a strain considered to be authentic for a particular species, while seven subclades corresponded to new species, which are described here. *Pithoascus* comprised five well-supported monophyletic subclades, each of which included an ex-type strain of a known species. *Scopulariopsis* encompassed six well-supported subclades, of which five included an ex-type strain or a strain considered as authentic, while one subclade corresponded to a new species described here. The new genus *Pseudoscopulariopsis* encompassed two subclades, each one including a single reference strain of a species previously identified as *Microascus* or *Scopulariopsis*, respectively.

In the combined phylogenetic analysis, the ex-type strain of *Scopulariopsis carbonaria* (MUCL 9027) was basal to the *Microascus* and *Pithoascus* clades. According to the original description (Morton & Smith 1963), this species showed a high similarity in annellidic and conidial morphology with members of the *Microascus* lineage; however, after several attempts to induce sporulation this strain remained sterile, and thus its taxonomic position could not be resolved.

TAXONOMY

Based on the results of the above multilocus sequence analysis and a morphological analysis, the boundaries of the genera *Microascus*, *Pithoascus* and *Scopulariopsis* have been reassessed accordingly. Their current circumscription is revised and several new taxa and combinations are proposed as follows:

Microascus Zukal, Verh. Zool.-Bot. Ges. Wien 35: 339. 1885

= *Peristomium* Lechmere, Compt. Rend. Hebd. Séances Acad. Sci. 154: 178. 1912.
= *Masonia* G. Sm., Trans. Brit. Mycol. Soc. 35: 149. 1952.
≡ *Masoniella* G. Sm., Trans. Brit. Mycol. Soc. 35: 237. 1952.

Type species. *Microascus longirostris* Zukal.

Colonies restricted or spreading, pale grey, brown, olivaceous or black, velvety, floccose or fasciculate, granular and often forming concentric rings due to the production of ascomata. *Ascomata* perithecial, immersed or superficial, scattered or aggregated, globose to ampulliform, glabrous or covered with scattered hairs, ostiolate, usually with a neck of variable length and shape, sometimes with a tuft of ostiolar hairs; peridium dark brown or black, composed of thick-walled, slightly flattened cells, *textura angularis* or *textura intricata*. *Asci* unitunicate, 8-spored, obovate, barrel-shaped or nearly globose, formed in basipetal rows, evanescent. *Ascospores* 1-celled, asymmetrical, reniform, heart-shaped, triangular or quadrangular, dextrinoid when young, extruded through the ostiole into a gelatinous drop or a long cirrhus. *Conidiogenous cells* annellidic, borne singly and laterally on the vegetative hyphae, or in groups of 2–5 on short simple or little branched conidiophores, ampulliform or lageniform, subhyaline or darkening with age, smooth- or rough-walled with a distinct cylindrical annellated zone, *Conidia* 1-celled, pale yellowish to dark brown, globose to subglobose, obovate or clavate, with a truncate base and rounded or pointed at the apex, smooth- and thin-walled or finely rough- and thick-walled, produced singly or in basipetal dry chains. Solitary conidia present in some species, borne sessile or on short stalks from the vegetative hyphae.

Microascus alveolaris Sandoval-Denis, Gené & Guarro, *sp. nov.* — MycoBank MB809418, Fig. 4

Etymology. In reference to the isolation source of most isolates.

Colonies on OA and PCA at 25 °C attaining 31–36 and 18–29 mm diam, after 14 d, respectively, flat, slightly velvety, somewhat granular at the centre due to the presence of ascomata, white to grey (4B1), abundant submerged mycelium in the outer zone, with a wide white margin; reverse white to grey (4B1). *Vegetative hyphae* septate, hyaline to light brown, smooth- and thin-walled, 1.5–3 µm wide. *Ascomata* superficial or immersed, formed predominantly at the centre of the colony, globose to subglobose, 110–290 µm diam, usually with an ostiolar cylindrical neck up to 100 µm long, black, glabrous, the apice sometimes with a tuft of hyaline, septate and acicular hairs, up to 60 µm long; peridium of *textura angularis*. *Asci* irregularly ellipsoidal, 8–12 × 7.5–11 µm. *Ascospores* broadly triangular, rarely reniform, 4–6 × 3–5 µm, with a single germ pore, straw coloured, bright yellow in mass. *Conidiophores* absent or as a basal single cell of 5–12 × 2–2.5 µm, bearing groups of 2–3 annellides, rarely slightly branched up to 80 µm long, hyaline to subhyaline, smooth-walled. *Annellides* mostly sessile, single and lateral on vegetative hyphae, lageniform, 6–17 × 1.5–3.5 µm, tapering slightly towards the annellated zone 1–2 µm wide, hyaline to subhyaline, smooth- and thin-walled. *Conidia* ellipsoidal, navicular or bullet-shaped, 3–5 × 2–3.5 µm, with truncate base and rounded apex, subhyaline to pale brown, brown in mass, thin- and smooth-walled, arranged in long chains. Solitary conidia sometimes present, borne laterally from vegetative hyphae, sessile or on short stalks, unicellular, subglobose or obovoidal, 3–5 × 2.5–4 µm, subhyaline or pale brown, smooth- and more or less thick-walled.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.

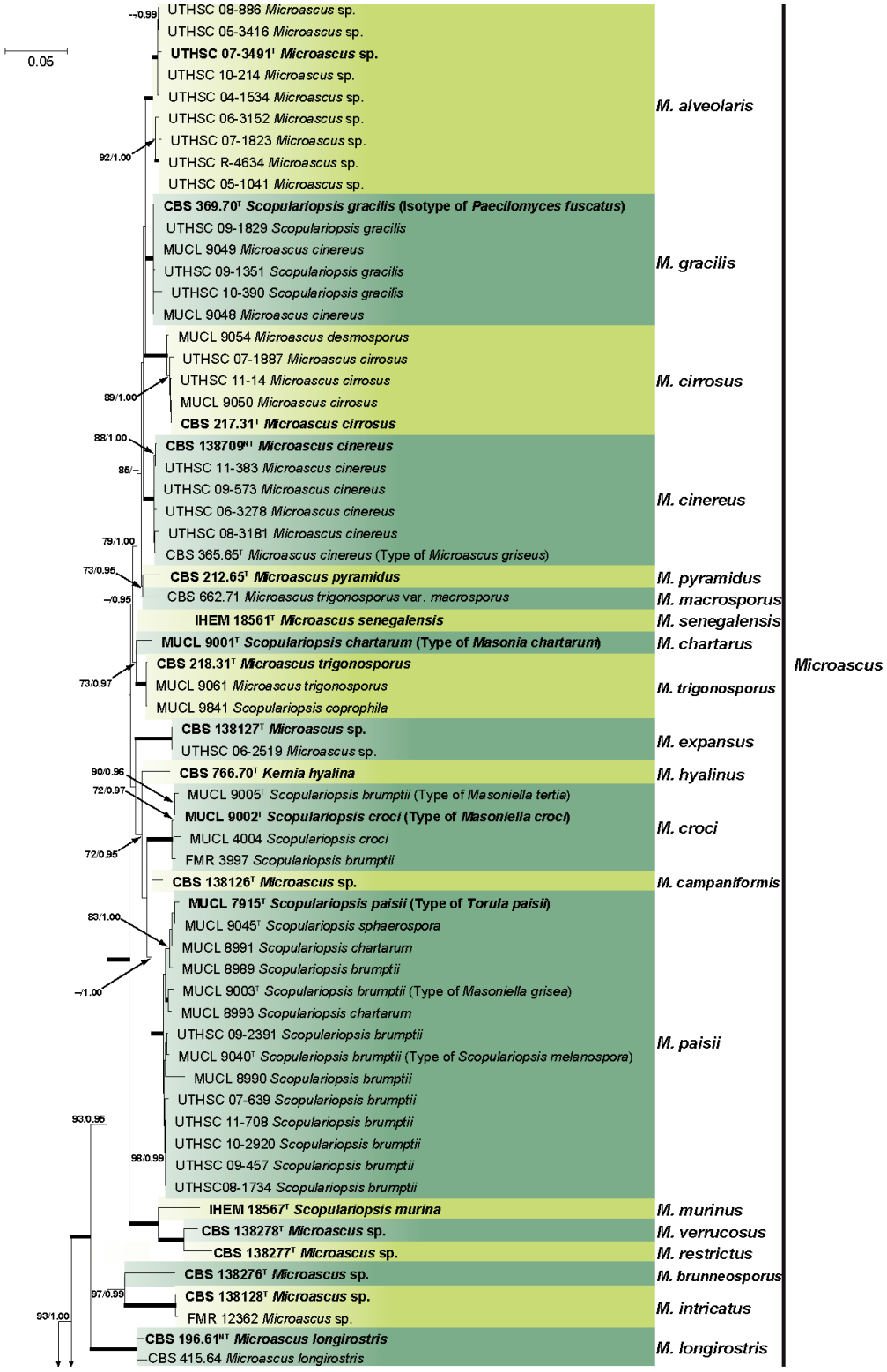

Fig. 3 Maximum likelihood (ML) tree obtained from the combined ITS, LSU, EF-1α and TUB sequences of 105 strains from *Microascus*, *Pithoascus*, *Pseudoscopulariopsis* and *Scopulariopsis* species. Numbers on the branches are ML bootstrap values (bs) above 70 %, followed by Bayesian posterior probabilities (pp) above 0.95. Full supported branches are indicated in **bold**. Branch lengths are proportional to distance. Ex-type strains are indicated with ^{T}. Ex-neotype strains are indicated with ^{NT}. The original name of each strain, when applied, is given on parenthesis. The tree was rooted to *Petriella setifera* (CBS 437.75) and *Parascedosporium tectonae* (CBS 127.84).

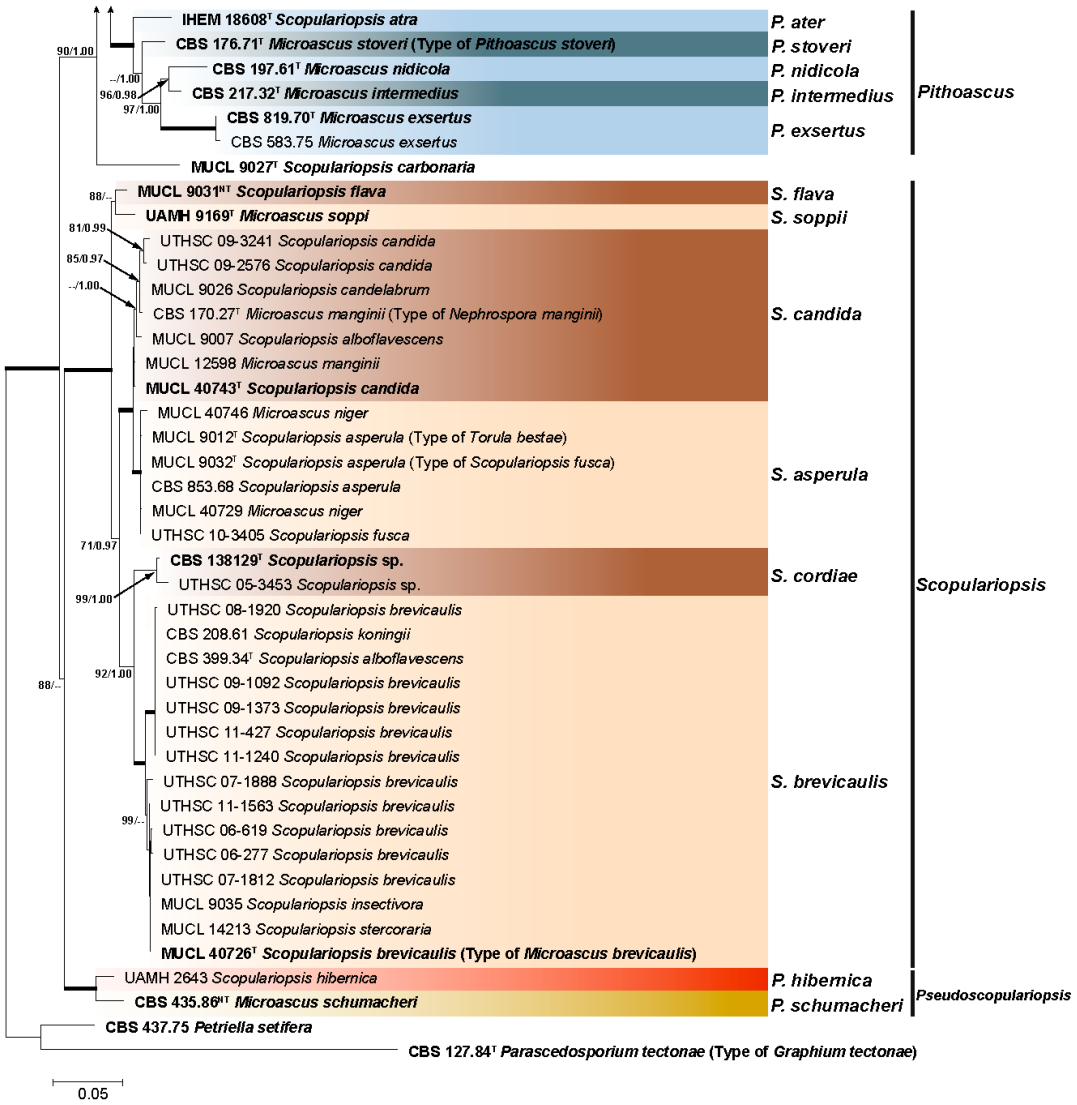

Fig. 3 (cont.)

Specimens examined. USA, from bronchoalveolar lavage fluid, 2007, *D.A. Sutton* (holotype CBS H-22111, culture ex-type CBS 139501 = UTHSC 07-3491 = FMR 12252); from sputum, 2005, *D.A. Sutton* (UTHSC 05-1041 = FMR 12351); from bronchoalveolar lavage fluid, 2005, *D.A. Sutton* (UTHSC 05-3416 = FMR 12350); from bronchoalveolar lavage fluid, 2006, *D.A. Sutton* (UTHSC 06-3152 = FMR 12346); from sputum, 2007, *D.A. Sutton* (UTHSC 07-1823 = FMR 12342); from bronchoalveolar lavage fluid, 2004, *D.A. Sutton* (UTHSC 04-1534 = FMR 12354); from bronchoalveolar lavage fluid, 2008, *D.A. Sutton* (UTHSC 08-886 = FMR 12340); from bronchoalveolar lavage fluid, 2010, *D.A. Sutton* (UTHSC 10-214 = FMR 12336); from lung tissue, *D.A. Sutton* (UTHSC R-4634 = FMR 12333).

Notes — All the strains included in this species were isolated from the respiratory tract of human patients. Morphologically, *M. alveolaris* is close to *M. campaniformis*, *M. macrosporus*, *M. pyramidus* and *M. trigonosporus*, all showing similar triangular-shaped ascospores. *Microascus alveolaris* can be differentiated by its membranous and white colonies, the smaller size of the ascospores and narrower conidia.

Microascus brunneosporus Sandoval-Denis, Gené & Guarro, *sp. nov.* — MycoBank MB809419, Fig. 5

Etymology. From the Latin *brunneus*-, brown, referring to the colour of the ascospores.

Colonies on OA at 25 °C attaining 21–25 mm diam in 14 d, flat, velvety, granular at the centre due to the presence of ascomata, dull green (30E3) to olive-brown (4F4), with submerged mycelium towards the outer zone, margin regular; reverse dark green (30F4). On PCA at 25 °C attaining 15–17 mm diam in 14 d, slightly elevated, downy, fasciculate at the centre, dull green (30E3), with a white and regular margin; reverse dull green (30D4). *Vegetative hyphae* septate, subhyaline to pale brown, smooth- and thin-walled, 1.5–3 µm wide. *Ascomata* immersed, globose, 110–205 µm diam, with a short cylindrical ostiolar neck up to 40 µm long, black, glabrous; peridium with a *textura angularis*. *Asci* irregularly ellipsoidal or ovoidal, 11–14 × 7–8 µm. *Ascospores* ellipsoidal to allantoid, 5–7 × 2–3 µm, light yellow-brown, brown in mass, with a single and inconspicuous germ pore. *Conidiophores* absent or as a basal single cell of 5–15 × 1.5–2.5 µm, bearing 1–3 annellides, rarely slightly

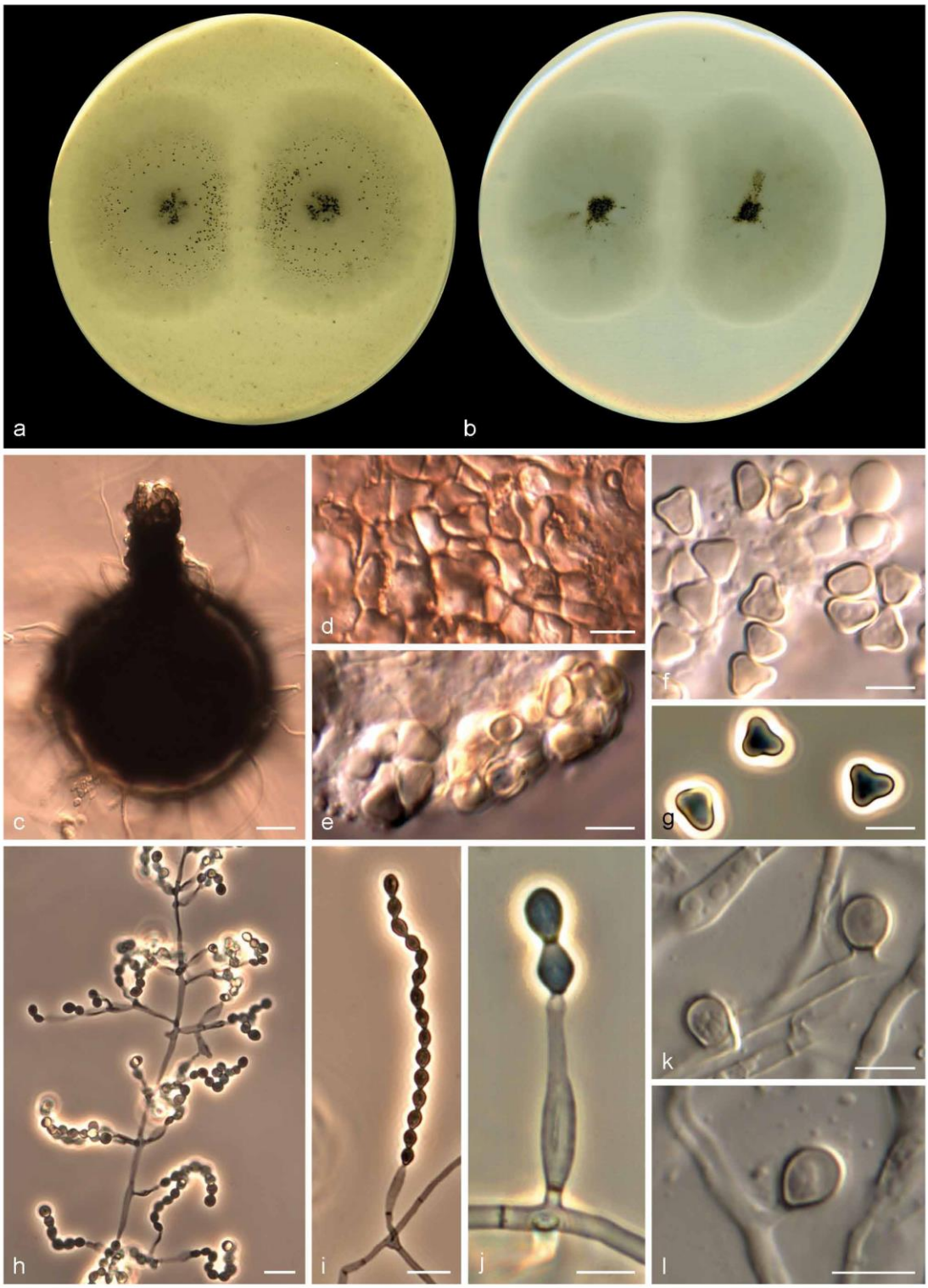

Fig. 4 *Microascus alveolaris* CBS 139501. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c. ascoma; d. peridium; e–g. asci and ascospores; h–j. conidiophores, annellides and conidia; k, l. solitary conidia. — Scale bars: c = 30 µm; h, i = 10 µm; all others = 5 µm.

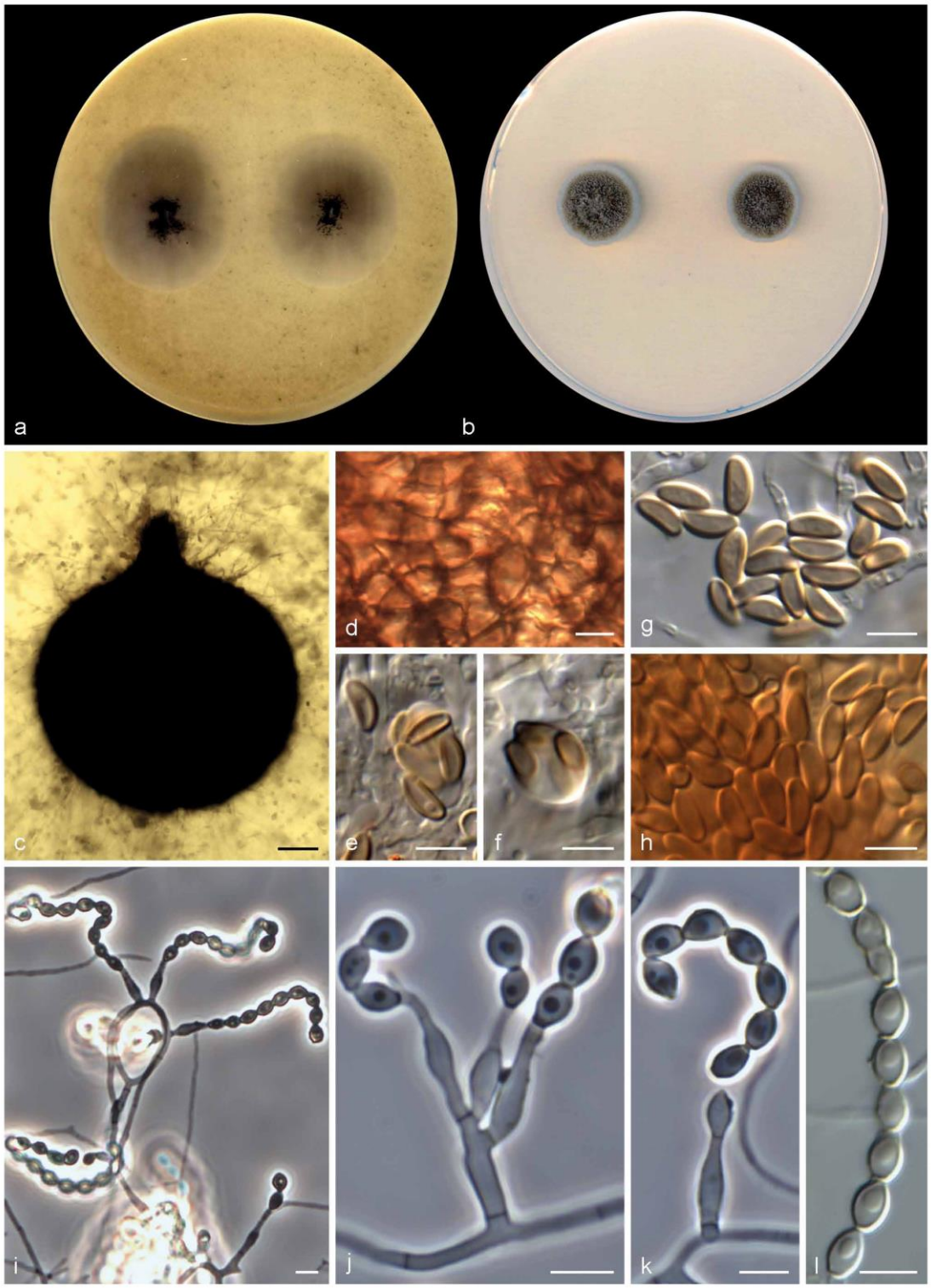

Fig. 5 *Microascus brunneosporus* CBS 138276. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c. ascoma; d. peridium; e–h. asci and ascospores; i–k. conidiophores, annellides and conidia; l. conidial chain. — Scale bars: c = 50 µm; all others = 5 µm.

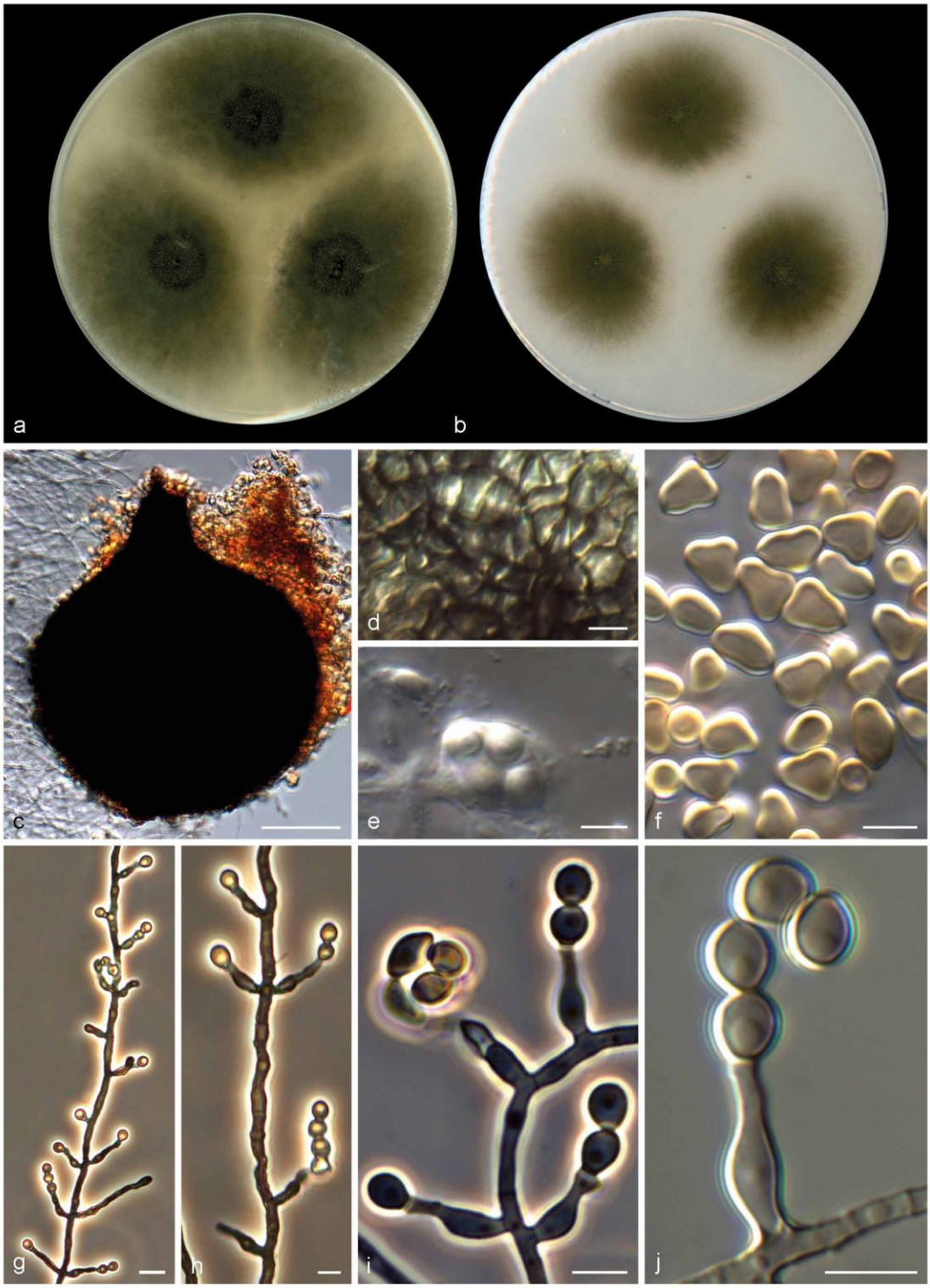

Fig. 6 *Microascus campaniformis* CBS 138126. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c. ascoma; d. peridium; e, f. asci and ascospores; g–j. conidiophores, annellides and conidia. — Scale bars: c = 50 µm; g = 10 µm; all others = 5 µm.

branched up to 30 µm long, subhyaline, smooth-walled. *Annellides* mostly sessile, single and lateral on vegetative hyphae, more or less lageniform, 9–14 × 2–2.5 µm, tapering to a cylindrical annellated zone 1–1.5 µm wide, subhyaline, smooth- or rough-walled, thin-walled. *Conidia* subglobose, ellipsoidal or navicular, 4–5 × 2.5–5 µm, with truncate base, light green-brown, thin- and smooth-walled, arranged in long chains. Solitary conidia not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 35 °C, minimum 15 °C.

Specimen examined. USA, from bronchoalveolar lavage fluid, 2006, *D.A. Sutton* (holotype CBS H-21783, culture ex-type CBS 138276 = UTHSC 06-4312 = FMR 12343).

Notes — This species is similar to *M. cinereus* and *M. gracilis*. However, the latter two species produce reniform or broadly lunate, straw coloured ascospores with an often conspicuous germ pore.

Microascus campaniformis Sandoval-Denis, Cano & Deanna A. Sutton, *sp. nov.* — MycoBank MB809205, Fig. 6

Etymology. From the Latin *campanus-*, bell, referring to the shape of the ascospores.

Colonies on OA at 25 °C attaining 27–34 mm diam in 14 d, flat, velvety to slightly granular at the centre, dull green (30E4), with an irregular margin; reverse dull green (30E4). On PCA at 25 °C, colonies attaining 14–25 mm diam in 14 d, flat, velvety, fluffy at the centre, dull green (28E4) to dark green (30E4), with a white and regular margin; reverse dark green (28F3). *Vegetative hyphae* septate, subhyaline, smooth- or rough- and thin-walled, 1.5–2.5 µm wide. *Ascomata* immersed or superficial, usually formed at the periphery of the colony, globose to subglobose, 150–220 µm diam, with a short cylindrical ostiolar neck up to 80 µm long, widening at the ostiolar opening, rarely with a tuft of hyaline, straight and septate hairs up to 50 µm long, black, glabrous; peridium with a *textura angularis*. *Asci* irregularly ellipsoidal or subglobose, 18–21 × 10–15 µm. *Ascospores* broadly triangular, 6–7 × 4–4.5 µm, often with an elongated side towards a single germ pore, straw coloured, bright yellow-orange in mass. *Conidiophores* absent or as a basal cell of 5 × 2 µm, bearing groups of 5–8 annellides, or slightly branched up to 60 µm long, hyaline to subhyaline, smooth-walled. *Annellides* somewhat lageniform, 9–14 × 2–3 µm, with a more or less swollen base and tapering abruptly to a cylindrical annellated zone, 1–1.5 µm wide. *Conidia* subglobose to broadly ellipsoidal, 4–5 × 2.5–3.5 µm, with a truncate base, light green-brown, dark brown in mass, thick-walled, arranged in long chains. Solitary conidia and chlamydospores not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.

Specimen examined. USA, from bronchoalveolar lavage fluid, 2010, *D.A. Sutton* (holotype CBS H-21784, culture ex-type CBS 138126 = UTHSC 10-565 = FMR 12334).

Notes — *Microascus campaniformis* is similar to *M. alveolaris*, *M. macrosporus*, *M. pyramidus* and *M. trigonosporus* in having distinctive triangular shaped ascospores. However, *M. campaniformis* can be differentiated by its green colonies and inequilateral ascospores that show an elongation at one side towards the germ pore. In contrast, the ascospores of *M. alveolaris*, *M. macrosporus* and *M. trigonosporus* are almost equilateral with rounded ends, while those of *M. pyramidus* have attenuated ends acquiring a nearly square shape. *Microascus campaniformis* is phylogenetically close to *M. paisii* sharing similar annellides. However, a sexual morph has not been observed in *M. paisii*.

Microascus chartarus (G. Sm.) Sandoval-Denis, Gené & Guarro, *comb. nov.* — MycoBank MB809206

Basionym. Masonia chartarum G. Sm., Trans. Brit. Mycol. Soc. 35: 150. 1952.
≡ *Masoniella chartarum* (G. Sm.) G. Sm., Trans. Brit. Mycol. Soc. 35: 237. 1952.
≡ *Scopulariopsis chartarum* (G. Sm.) F.J. Morton & G. Sm., Mycol. Pap. 86: 64. 1963.

Specimen examined. UK, London, isolated from mouldy wall-paper, 1950, *K. Maunsell* (*Masonia chartarum* ex-type culture CBS 294.52 = MUCL 9001).

Notes — *Microascus chartarus* has been reported from soil, dust and indoor-air (Domsch et al. 2007). It was originally described as a member of *Masonia* G. Sm. (1952a). However, *Masonia* is an illegitimate homonym of *Masonia* Hansford (1944), and thus the new genus *Masoniella* was erected (Smith 1952b). Most members of *Masoniella* were later transferred to *Scopulariopsis* (Morton & Smith 1963); both genera share the same conidiogenesis (annellidic, percurrent) and conidiogenous cells, distinctly narrower at the base, then swollen, and ending in a slender annellidic zone. Our phylogenetic analysis shows that *M. chartarus* is included in the *Microascus* sublineage and it is closely related to *M. trigonosporus*. *Microascus trigonosporus* can be distinguished by the production of a sexual morph, with triangular ascospores and mostly globose to subglobose and pale brown conidia. No sexual morph is known for *M. chartarus* and its conidia are ovate, often with a pointed end, green-brown (Morton & Smith 1963). *Microascus croci* and *M. paisii* resemble *M. chartarus* and also lack a sexual morph. However, these two species can be differentiated from *M. chartarus* by their conidial shape and colour, which are globose and ellipsoidal to short clavate in *M. croci* and *M. paisii*, respectively, and pale brown in both species. In addition, *M. croci* is able to grow from 5–30 °C and *M. paisii* grows from 15–37 °C, while *M. chartarus* has a narrower temperature range growing from 15–25 °C.

Microascus cinereus (Émile-Weil & Gaudin) Curzi, Boll. Staz. Patolog. Veget. Roma 11: 60. 1931.

Basionym. Scopulariopsis cinerea Émile-Weil & Gaudin, Arch. Méd. Exp. Anat. Path. 28: 452. 1919.
= *Scopulariopsis oidiospora* Zach, Oesterr. Bot. Z. 83: 182. 1934.
= *Microascus lunasporus* P.M. Jones, Mycologia 28: 503. 1936.
≡ *Scopulariopsis lunaspora* P.M. Jones, Mycologia 28: 504. 1936.
= *Microascus pedrosoi* C.A. Fuentes & F.A. Wolf, Mycologia 48: 63. 1956.
= *Microascus griseus* P.N. Matur & Thirum., Sydowia 16: 49. 1962.
= *Microascus reniformis* Orr, Persoonia 8: 194. 1975.

Specimens examined. India, Maharashtra, Poona, from soil, 1965, *M.J. Thirumalachar* (*M. griseus* ex-type culture CBS 365.65 = ATCC 16204). – USA, from bronchoalveolar lavage fluid, 2010, *D.A. Sutton* (neotype of *M. cinereus* designated here CBS H-21937, MBT198511) culture ex-neotype CBS 138709 = UTHSC 10-2805 = FMR 12217; from bronchoalveolar lavage fluid, 2006, *D.A. Sutton* (UTHSC 06-3278 = FMR 12345); from sternum tissue, 2008, *D.A. Sutton* (UTHSC 08-3181 = FMR 12339); from bronchoalveolar lavage fluid, 2009, *D.A. Sutton* (UTHSC 09-573 = FMR 12239); from bronchoalveolar lavage fluid, 2009, *D.A. Sutton* (UTHSC 11-383 = FMR 12331).

Notes — *Microascus cinereus* has a widespread distribution and a wide range of substrates. It has been isolated mainly from stored cereals, soil and dung (Barron et al. 1961, Udagawa 1962, Guarro et al. 2012), but it has also been described as an opportunistic pathogen of animals and humans (Baddley et al. 2000, de Hoog et al. 2011, Sandoval-Denis et al. 2013). Descriptions of *M. cinereus* are available in Barron et al. (1961) and Guarro et al. (2012). However, according to our observations, their measurements might have included isolates of *Microascus gracilis* from which *M. cinereus* has to be differentiated (Sandoval-Denis et al. 2013). The isolates of *M. cinereus* studied here showed asci 7–12 × 5–10 µm, ascospores 4–5.5 × 2.5–4 µm and conidia 3–5 × 2–3 µm. In addition, while

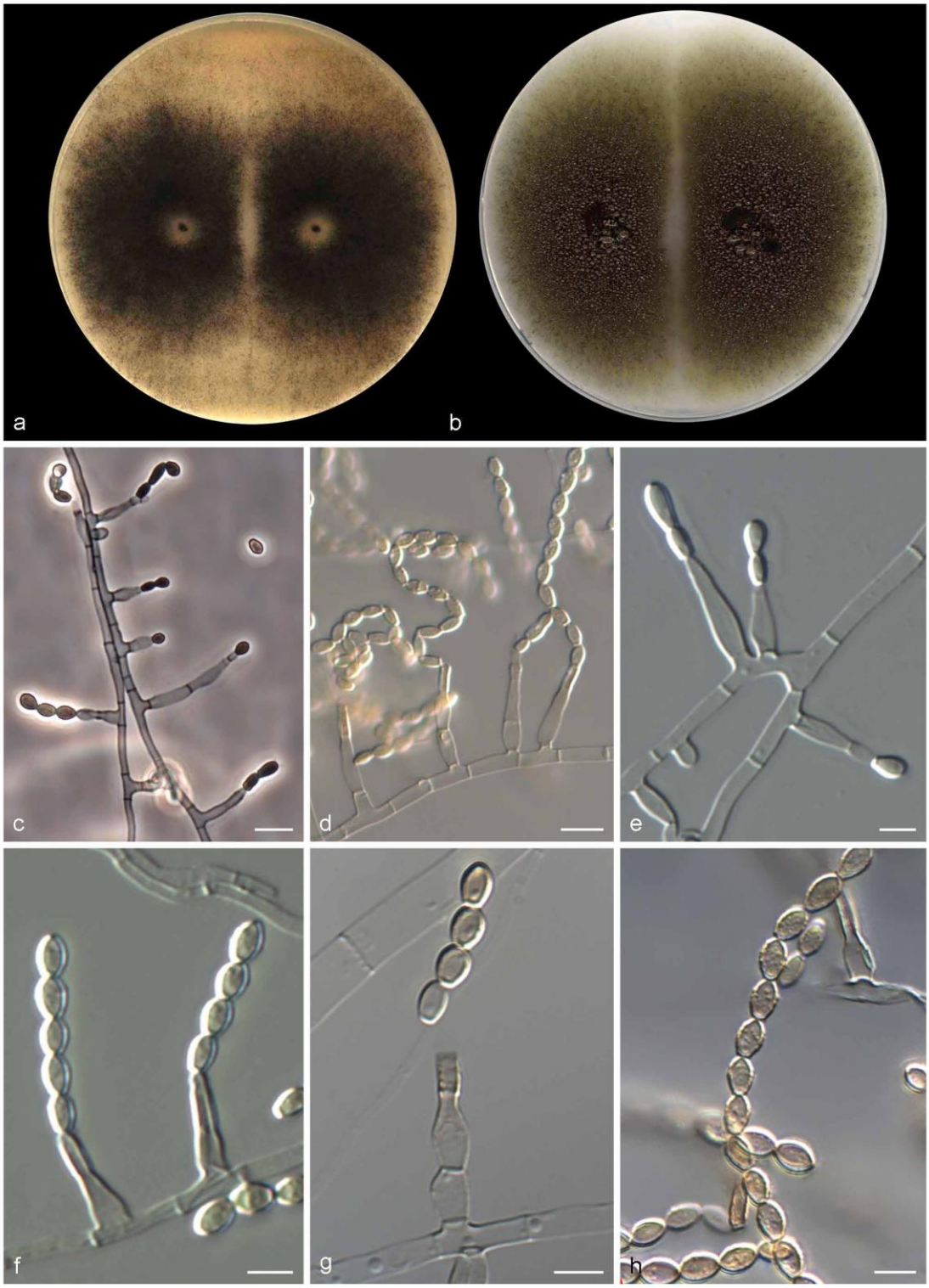

Fig. 7 *Microascus expansus* CBS 138127. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c–g. conidiophores, annellides and conidia; h. conidial chains. — Scale bars: c, d = 10 µm; all others = 5 µm.

M. cinereus produces pale to dark or black-grey colonies, at first velvety becoming slightly granular due to the presence of ascomata, *M. gracilis* produces dull green colonies, becoming olive-grey to olive-brown with ascomata mostly covered by aerial mycelium. Since no ex-type material of *M. cinereus* is available, the strain CBS 138709 (UTHSC 10-2805) is proposed here as neotype. Despite the existence of an ex-type culture of *M. griseus*, a synonym of *M. cinereus*, we consider it important to neotypify *M. cinereus* in order to conserve the oldest and most widely used epithet of this taxon. The original description of *M. cinereus* was based on an isolate obtained from a human nail but none of the isolates available share this original substrate. However, we believe that the isolate CBS 138709 (UTHSC 10-2805), obtained from human bronchoalveolar fluid agrees with the original and modern descriptions of this species (Émile-Weil & Gaudin 1919, Barron et al. 1961, Guarro et al. 2012).

Microascus cirrosus Curzi, Boll. Staz. Patol. Veg. Roma 10: 308. 1930

Specimens examined. Italy, from a leaf of *Prunus* sp., 1931, *M. Curzi* (ex-type culture CBS 217.31); from root of *Vitis vinifera*, 1934, *M. Curzi* (CBS 277.34 = MUCL 9050). – UK, from unknown substrate, 1961, *G. Smith* (CBS 301.61 = MUCL 9054). – USA, from sputum, 2007, *D.A. Sutton* (UTHSC 07-1887 = FMR 12256); from bronchoalveolar lavage fluid, 2011, *D.A. Sutton* (UTHSC 11-14 = FMR 12332).

Notes — *Microascus cirrosus* is a saprobic species with a worldwide distribution, commonly isolated from soil and dung (Barron et al. 1961, von Arx et al. 1988, Guarro et al. 2012). It has also been associated to superficial and respiratory human infections (de Hoog et al. 2011, Sandoval-Denis et al. 2013). Morton & Smith (1963) considered the asexual morph of this species to be conspecific with *Scopulariopsis paisii* (see *Microascus paisii*). However, according to our results, the ex-type strain of *Torula paisii* (MUCL 7915) was shown to be phylogenetically distant to the ex-type strain of *M. cirrosus* (CBS 217.31), and thus should be considered as a distinct species. *Microascus cirrosus* can be distinguished by having subglobose to obovate conidia measuring 4–6.5 × 4–6 µm, while those of *M. paisii* are broadly ellipsoidal to short clavate, measuring 4–6 × 2–4.5 µm. *Microascus cirrosus* is also similar to *M. cinereus*. However, *M. cirrosus* produces broadly reniform ascospores measuring 5–6 × 3–4 µm and larger conidia, while *M. cinereus* produces broadly lunate or almost triangular ascospores measuring 4–5.5 × 2.5–4 µm, and obovate to clavate conidia measuring 3–5 × 2–3 µm.

Microascus croci (J.F.H. Beyma) Sandoval-Denis, Gené & Guarro, *comb. nov.* — MycoBank MB809207

Basionym. Scopulariopsis croci J.F.H. Beyma, Antonie van Leeuwenhoek 10: 52. 1945.

≡ *Masoniella croci* (J.F.H. Beyma) G. Sm., Trans. Brit. Mycol. Soc. 37: 166. 1954.

= *Masoniella tertia* Bat., J.A. Lima & C.T. Vasconc., Publções Inst. Micol. Recife. 263: 14. 1960.

Specimens examined. Brazil, Pernambuco, Recife, isolate from air, 1952, *A. Batista* (*Masoniella tertia* ex-type culture MUCL 9005 = CBS 296.61). – Spain, Tarragona, Riumar, from aquatic sediment of the Ebro River, May 1991, *K. Ulfig* & *J. Gené* (FMR 3997); Barcelona, from aquatic sediment of the Besós river, July 1991, *J. Gené* (FMR 4004). – The Netherlands, Lisse, from *Crocus* sp. Queen of the blues, 1943, *H. Diddens* (*Scopulariopsis croci* ex-type culture MUCL 9002 = CBS 158.44).

Notes — The clade representing *M. croci* included isolates from air, aquatic sediments, soil and plants, originating from Europe and South America. *Masoniella tertia* was considered a synonym of *S. melanospora* (Udagawa 1959) and a later synonym of *S. brumptii* (Morton & Smith 1963). However, the current combined analysis showed that the ex-type cultures of *M. tertia* and *S. melanospora* are phylogenetically unrelated, which agrees with their morphological features. All the isolates included in this clade have mostly globose conidia and are able to grow from 5–30 °C. Although no sexual morph has been reported for this species, one of the strains tested here (FMR 4004) was able to produce small and sterile peritecial-like ascomata after 8 mo of incubation on OA.

Microascus expansus Sandoval-Denis, Gené & Cano, *sp. nov.* — MycoBank MB809208, Fig. 7

Etymology. From the Latin *expansio-*, expansion, referring to the quick growth of the colonies.

Colonies on OA and PCA at 25 °C growing rapidly, 65–81 and 70–75 mm diam, respectively, in 14 d, flat, velvety to powdery, more or less funiculose at the centre, olive (3F3) to grey-brown (4–5F3), with an irregular margin; reverse olive grey (2F2) or olive (2F4). *Vegetative hyphae* septate, hyaline to pale brown, smooth- and thin-walled, 1.5–3 µm wide. *Conidiophores* absent or as a basal single cell of 4–5 × 2–4 µm, bearing groups of 2–5 annellides, or slightly branched up to 20 µm long, hyaline to subhyaline, smooth-walled. *Annellides* slightly lageniform or somewhat subulate, 5–12 × 1.5–3.5 µm, tapering to a cylindrical annellated zone 1.5–2 µm wide, smooth-walled. *Conidia* bullet-shaped or broadly clavate, 4–8 × 2.5–3.5 µm, with a distinctive truncate base and rounded or slightly pointed apex, subhyaline to pale brown in mass, smooth- or finely roughened, thick-walled, arranged in long chains. *Sexual morph* not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.

Specimens examined. USA, from sputum, 2006, *D.A. Sutton* (holotype CBS H-21785, culture ex-type CBS 138127 = UTHSC 06-4472 = FMR 12266); from pleural fluid, 2006, *D.A. Sutton* (UTHSC 06-2519 = FMR 12267).

Notes — *Microascus expansus* is known thus far from clinical isolates of human origin. Both isolates are able to grow at 40 °C. Other *Microascus* species able to grow at this temperature are *M. alveolaris*, *M. campaniformis*, *M. cinereus*, *M. cirrosus*, *M. gracilis*, *M. intricatus*, *M. macrosporus*, *M. pyramidus* and *M. restrictus*. However, except *M. restrictus*, all these species produce sexual morphs, while *M. expansus* produces only the asexual morph. *Microascus expansus* can be differentiated from *M. restrictus* by a faster growth rate, reaching > 60 mm diam at 25–30 °C in 14 d.

Microascus gracilis (Samson) Sandoval-Denis, Gené & Guarro, *comb. nov.* — MycoBank MB809209

Basionym. Scopulariopsis gracilis Samson, Arch. Mikrobiol. 85: 179. 1972.

≡ *Paecilomyces fuscatus* N. Inagaki, Trans. Mycol. Soc. Japan 4: 4. 1962.

Specimens examined. Japan, from wheat flour, 1970, *N. Inagaki* (*Paecilomyces fuscatus* ex-type culture CBS 369.70). – UK, isolate from soil, 1959, *J. Mendy* (MUCL 9048 = CBS 195.61). – USA, Iowa, isolate from a seed of *Zea mays*, 1961, *G.L. Barron* (MUCL 9049 = CBS 300.61); from synovial fluid, 2009, *D.A. Sutton* (UTHSC 09-1351 = FMR 12234); from bronchoalveolar lavage fluid, 2009, *D.A. Sutton* (UTHSC 09-1829 = FMR 12231); from bronchoalveolar lavage fluid, 2010, *D.A. Sutton* (UTHSC 10-390 = FMR 12335).

Notes — *Scopulariopsis gracilis* was proposed by Samson & von Klopotek (1972) as a new name for *Paecilomyces fuscatus*, probably to avoid nomenclatural conflict with *Scopulariopsis fusca* (Zach 1934).

Microascus gracilis has been isolated mainly from food in Asia, North and South America, and from soil in Europe. Recently, this species was reported from human clinical specimens, but its pathogenicity has not been demonstrated (Sandoval-Denis

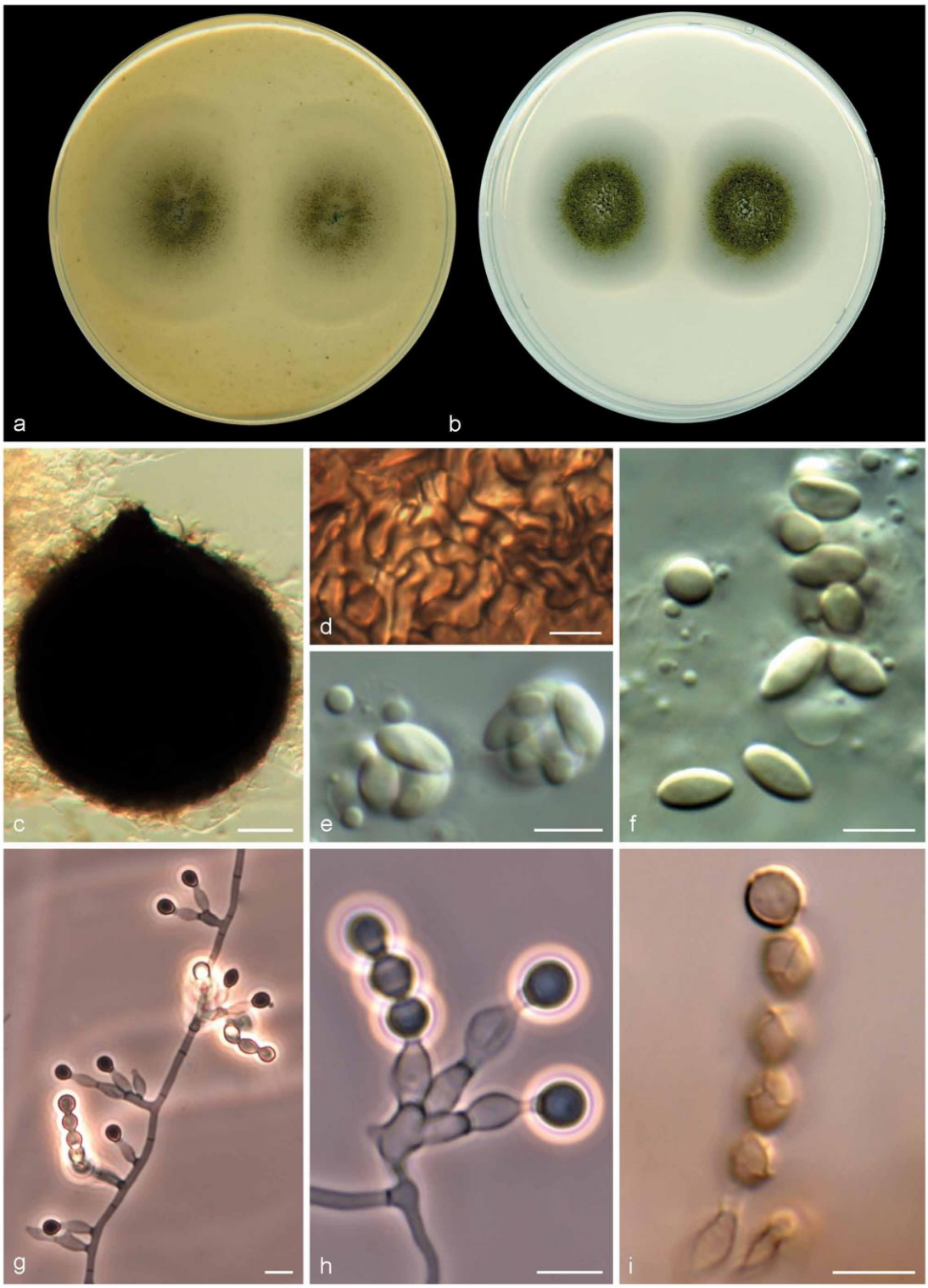

Fig. 8 *Microascus intricatus* CBS 138128. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c. ascoma; d. peridium; e, f. asci and ascospores; g, h. conidiophores, annellides and conidia; i. conidial chain. — Scale bars: c = 40 µm; all others = 5 µm.

et al. 2013). *Microascus gracilis* and *M. cinereus* are very similar making their identification difficult in the absence of the sexual morph; in fact two reference strains (MUCL 9048 and MUCL 9049) and some clinical isolates were previously identified as *M. cinereus*. However, sequence comparison revealed that these species only showed 98.1 %, 97.8 % and 97 % sequence similarity for ITS, EF-1α and TUB, respectively. Morphologically *M. gracilis* can be differ-entiated from *M. cinereus* by its lunate ascospores, measuring 4.5–6.5 × 2–4 µm (as opposed to reniform to broadly lunate ascospores measuring 4–5.5 × 2.5–4 µm in *M. cinereus*), asci measuring 8–18 × 6–10 µm (against 7–12 × 5–10 µm in *M. cinereus*), the formation of complex conidiophores and the morphology and colour of the colony. The asexual-morph of *M. gracilis* also resembles to that of *M. murinus* and *M. paisii*. However, *M. gracilis* produces annellides 5–20 × 1–2.5 µm, usually formed on well-defined and branched conidiophores, and subglobose to ellipsoidal conidia 3.5–5.5 × 2–3.5 µm; the annellides of *M. murinus* and *M. paisii* are shorter (6.5–11 × 1.7–2.5 µm and 10–14 × 2–2.5 µm, respectively) borne mostly from the aerial mycelium and producing cylindrical and broadly ellipsoidal conidia, respectively.

Microascus hyalinus (Malloch & Cain) Sandoval-Denis, Gené & Guarro, *comb. nov.* — MycoBank MB809210

Basionym. *Kernia hyalina* Malloch & Cain, Canad. J. Bot. 49: 860. 1971.

Specimen examined. USA, from cow dung, 1964, *J.C. Krug* (ex-type culture CBS 766.70).

Notes — This species has been isolated from soil and dung in Europe and North America (Malloch & Cain 1971, Guarro et al. 2012). The species was originally described in *Kernia* by Malloch & Cain (1971), although deviating considerably from the typical features of *Kernia* such as restricted growth, non-ostiolate, hairy ascomata, and ellipsoidal to reniform, orange to copper coloured ascospores with a germ pore at each end (Malloch & Cain 1971, von Arx 1978). Although several species of *Kernia* have been described with a scopulariopsis-like asexual morph, our phylogenetic analysis based on a combined LSU and ITS sequence dataset (Fig. 1) showed *Kernia* to be phylogenetically distant to both *Scopulariopsis* and *Microascus*. However, *K. hyalina* is shown to have more affinity with species of *Microascus* rather than with species of *Kernia* nested within the *Microascus* lineage, a relationship previously suggested by Issakainen et al. (2003). The lack of ascomatal appendages, the production of hyaline to yellowish ascospores with a single germ pore, the shape and colour of the annellides and conidia, and the growth rate of the colonies point toward *Microascus* rather than toward *Kernia*. Therefore, our phylogenetic and morphological data confirm this taxon as a distinct species in *Microascus*.

Microascus intricatus Sandoval-Denis, Stchigel & Deanna A. Sutton, *sp. nov.* — MycoBank MB809211, Fig. 8

Etymology. Referring to the *textura intricata* of the peridium.

Colonies on OA at 25 °C growing rather slowly, attaining 28–30 mm diam in 14 d, flat, finely granular, with scarce aerial mycelium, olive grey (2F2), with a white regular margin; reverse white to grey. On PCA at 25 °C colonies attaining 35–38 mm diam in 14 d, flat, velvety to finely granular, with a densely fasciculate centre, olive brown (4F3/4F4), with a white regular margin; reverse olive brown (4F3/4F4). *Vegetative hyphae* septate, subhyaline to light brown, smooth- and thin-walled, 2–2.5 µm wide. *Ascomata* immersed or superficial, globose to subglobose, 140–200 µm diam, with a papillate to short cylindrical ostiolar neck up to 40 µm long, black, glabrous; peridium with a *textura intricata*. *Asci* irregularly ellipsoidal or subglobose, 7.5–9.5 × 5.5–6.5 µm. *Ascospores* fusiform, 5–6 × 2.5–3.5 µm, straw coloured, yellow-orange in mass, with one inconspicuous germ pore. *Conidiophores* absent or as a basal single cell, of 2.5–3 × 3–5 µm, bearing groups of 2–3 annellides, or slightly branched up to 50 µm long, septate, subhyaline, smooth-walled. *Annellides* mostly sessile, single and lateral on vegetative hyphae, more or less ampulliform, 8–10(–11) × 2–2.5 µm, with a swollen base, tapering abruptly to a cylindrical annellated zone, 1–1.5 µm wide, subhyaline, smooth-walled. *Conidia* globose to broadly ellipsoidal, 4–5 × 3–3.5 µm, with truncate base, pale brown, dark brown in mass, and smooth- to rough-walled, thin-walled, arranged in long chains.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.

Specimens examined. Argentina, Iguazú, from soil, *Calduch, Guarro & Stchigel* (FMR 12362). – USA, from bronchoalveolar lavage fluid, 2007, *D.A. Sutton* (holotype CBS H-21786, culture ex-type CBS 138128 = UTHSC 07-156 = FMR 12264).

Notes — *Microascus intricatus* is described on the basis of two strains, isolated from a clinical (human) sample in the USA and from soil, in Argentina. This species deviates from the other congeneric species in having a perithecial peridium wall with *textura intricata* and by forming short fusiform ascospores. Nonetheless, the abundant conidiation and ascomata with straw-coloured ascospores bearing a single germ pore match with the circumscription of *Microascus*, confirming our phylogenetic results.

Microascus longirostris Zukal, Verh. Zool.-Bot. Ges. Wien 35: 33. 1885

= *Microascus variabilis* Massee & E.S. Salmon, Ann. Bot., Lond. 15: 349. 1901.

Specimens examined. Japan, Tokyo, from soil, 1962, *S. Udagawa* (CBS 415.64 = NBRC 7554). – USA, Maine, Kittery Point, from a wasp's nest, 1961, *R. Thaxter* (neotype designated here CBS H-14440, MBT198046) culture ex-neotype CBS 196.61 = MUCL 9058.

Notes — *Microascus longirostris* has been reported from many sources, mostly from dung of several mammals, soil, wood, seeds, air, as well from clinical samples in South and North America, Europe and Australia (Barron et al. 1961). The protologue of this species was made on the basis of ascomata on the natural substrata only (dog dung and rotten wood) (Zukal 1885, Barron et al. 1961). No ex-type strain or holotype material of this species was available. *Microascus longirostris* is the type of *Microascus* and, in order to stabilize the nomenclature, a neotype is here designated. Although none of the cultures studied here have the same geographical origin or host as the original specimen, the morphological characteristics of the two strains studied agree with the fungus described by Zukal in its original publication (Zukal 1885). The neotype culture selected here also corresponds with the modern descriptions of *M. longirostris* based on cultural characteristics given by Barron et al. (1961), Morton & Smith (1963) and von Arx et al. (1988), being also part of the material revised and considered as authentic by those authors.

Microascus macrosporus (G.F. Orr) Sandoval-Denis, Gené & Guarro, *comb. & stat. nov.* — MycoBank MB809212

Basionym. *Microascus trigonosporus* C.W. Emmons & B.O. Dodge var. *macrosporus* G.F. Orr, Canad. J. Bot. 39: 1617. 1961.

Specimen examined. USA, California, from soil, 1971, *G.F. Orr* (CBS 662.71 = UAMH 9336).

Notes — This species was originally described from desert soil as a variety of *M. trigonosporus*. However, while *M. macro-*

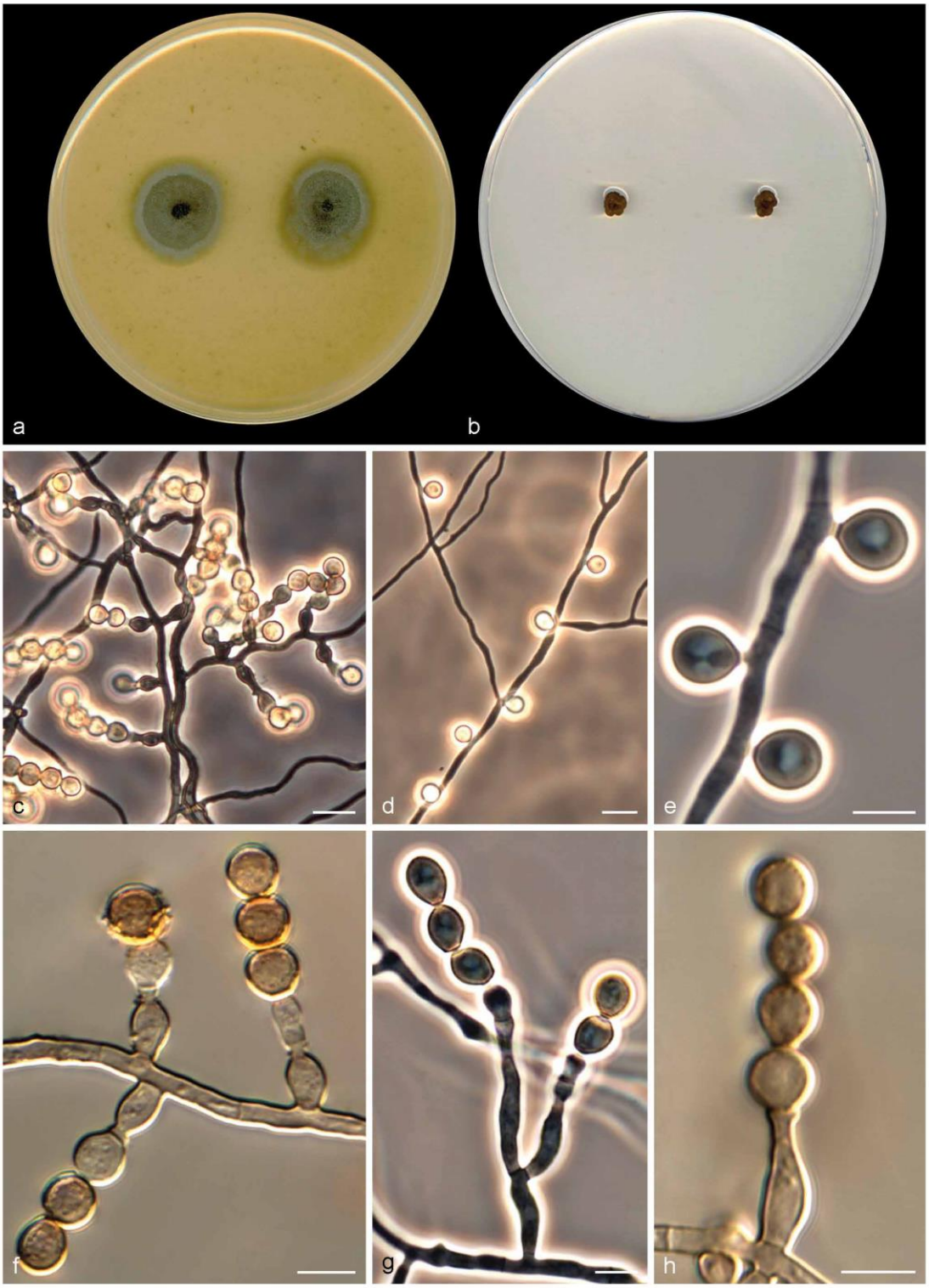

Fig. 9 *Microascus restrictus* CBS 138277. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c, f–h. conidiophores, annellides and conidia; d, e. solitary conidia. — Scale bars: c, d = 10 µm; all others = 5 µm.

sporus has ascospores measuring 5–6.5 × 5.5–7.5 µm, those of *M. trigonosporus* are distinctly smaller (3–5 × 3–4 µm). *Microascus pyramidus* is another phylogenetically closely related and morphologically similar species. However, its ascospores have distinctly attenuated ends and its conidia are 4.5–5.5 × 3–4 µm. *Microascus macrosporus* produces ascospores with rounded ends and larger conidia (5–7 × 4–5 µm).

Microascus murinus (Samson & Klopotek) Sandoval-Denis, Gené & Guarro, *comb. nov.* — MycoBank MB809218

Basionym. *Scopulariopsis murina* Samson & Klopotek, Arch. Mikrobiol. 85: 175. 1972.

Specimen examined. Germany, Giessen, from composed municipal waste, 1970, *A. von Klopotek* (ex-type culture CBS 830.70 = IHEM 18567).

Notes — This species was originally isolated from domestic waste in Germany. Although *M. murinus* shares morphological features with *M. chartarus*, *M. croci*, *M. paisii*, *M. restrictus* and *M. verrucosus*, it can be differentiated by having smaller cylindrical conidia, measuring 4–6 × 1.5–2 µm and slightly larger annellides, measuring 6.5–11 × 1.5–2.5 µm.

Microascus paisii (Pollacci) Sandoval-Denis, Gené & Guarro, *comb. nov.* — MycoBank MB809213

Basionym. *Torula paisii* Pollacci (as '*pais*'), Atti Ist. Bot. Univ. Pavia, ser. 2, 18: 130. 1921.

≡ *Phaeoscopulariopsis paisii* (Pollacci) M. Ota, Jap. J. Dermatol. Urol. 28: 5. 1928. *nom. inval.* (Seifert et al. 2011).

≡ *Scopulariopsis paisii* (Pollacci) Nannf., Repertorio sistematico dei miceti dell'uomo e degli animali 4: 259. 1934.

= *Scopulariopsis sphaerospora* Zach, Oesterr. Bot. Z. 83: 180. 1934.

= *Scopulariopsis brumptii* Salv.-Duval, Thèse Fac. Pharm. Paris. 23: 58. 1935.

= *Scopulariopsis versicolor* Salv.-Duval, Thèse Fac. Pharm. Paris. 23: 63. 1935.

= *Masoniella grisea* (G. Sm.) G. Sm., Trans. Brit. Mycol. Soc. 35: 237. 1952.

≡ *Masonia grisea* G. Sm., Trans. Brit. Mycol. Soc. 35: 149. 1952, *nom. illeg.*

= *Scopulariopsis melanospora* Udagawa, J. Agric. Sci. (Tokyo) 5: 18. 1959.

Specimens examined. Austria, from unknown substrate, 1934, *F. Zach* (*S. sphaerospora* ex-type culture MUCL 9045 = CBS 402.34). – Germany, Schleswig-Holstein, Kiel, Kitzeberg, from soil on a *Triticum sativum* field, 1966, *W. Gams* (MUCL 8989 = CBS 896.68); Schleswig-Holstein, Kiel, from soil, 1966, *W. Gams* (MUCL 8990); from soil on a wheat field, 1966, *W. Gams* (MUCL 8993 = CBS 897.68). – Italy, from human, 1927, *G. Pollacci* (*T. paisii* ex-type culture MUCL 7915 = CBS 213.27). – UK, isolated as a culture contaminant, 1946, *G. Smith* (*M. grisea* ex-type culture MUCL 9003 = CBS 295.52). – USA, from milled *Oriza sativa*, 1955, *S. Udagawa* (*S. melanospora* ex-type culture MUCL 9040 = CBS 272.60); from bronchoalveolar lavage fluid, 2007, *D.A. Sutton* (UTHSC 07-639 = FMR 12263); from bronchoalveolar lavage fluid, 2008, *D.A. Sutton* (UTHSC 08-1734 = FMR 12248); from sputum, 2009, *D.A. Sutton* (UTHSC 09-457 = FMR 12241); from bronchoalveolar lavage fluid, 2009, *D.A. Sutton* (UTHSC 09-482 = FMR 12240); from sputum, 2009, *D.A. Sutton* (UTHSC 09-2391 = FMR 12229); from bronchoalveolar lavage fluid, 2010, *D.A. Sutton* (UTHSC 10-2920 = FMR 12215); from sputum, 2011, *D.A. Sutton* (UTHSC 11-708 = FMR 12210).

Notes — *Microascus paisii* has had a confusing nomenclatural history. As *Scopulariopsis paisii*, it was erroneously considered the asexual morph of *M. desmosporus* by Morton & Smith (1963). We have also observed discrepancies concerning *T. paisii* among fungal databases. In MycoBank, *T. paisii* is considered as the asexual morph of *M. cirrosus* whereas Index Fungorum lists *T. paisii* as a synonym of *Scytalidium thermophilum*. Our phylogenetic analysis showed that the ex-type of *T. paisii* (MUCL 7915) belongs to the *Microascus* lineage. Within this lineage, it belongs to a well-supported subclade together with the ex-type strains of *Masoniella grisea*, *S. melanospora* and *S. sphaerospora* and several reference strains of *S. brumptii*. This subclade might represent the rarely opportunist species *S. brumptii*. However, since there is no type material of *S. brumptii* available, and *T. paisii* being the oldest type strain included in this subclade, the latter name has preference according to the nomenclatural principle that the correct name is the oldest legitimate one (McNeill et al. 2012). Therefore, according to our data the new combination *Microascus paisii* should be adopted.

Regarding data pertaining to *S. brumptii*, *M. paisii* has a worldwide distribution, being isolated from multiple substrates, including air, decaying wood or soil and is an opportunistic pathogen of human and warm-blooded animals (Morton & Smith 1963, de Hoog et al. 2011, Sandoval-Denis et al. 2013). This species morphologically resembles *M. chartarus* and *M. croci*, but it can be differentiated by its dark grey or black colonies and its ability to grow and sporulate well at 37 °C.

Microascus pyramidus G.L. Barron & J.C. Gilman, Canad. J. Bot. 39: 1618. 1961

Specimen examined. USA, from desert soil, 1957, *G.L. Barron* (ex-type culture CBS 212.65).

Notes — This species was originally isolated from desert soil in North America (Barron et al. 1961). Morphologically, it is similar to other *Microascus* species producing triangular ascospores as *M. alveolaris*, *M. campaniformis*, *M. macrosporus* and *M. trigonosporus*. However, ascospores of *M. pyramidus* are wider (5–6.5 × 5.5–7 µm), have attenuated ends and often acquire a nearly square shape (von Arx et al. 1988). The asexual morph of *M. pyramidus* is morphologically similar to those of *M. macrosporus* and *M. campaniformis*. *Microascus macrosporus* produces globose to ovoid conidia measuring 5–7 × 4–5 µm, while those of *M. pyramidus* are markedly narrower measuring 4.5–5.5 × 3–4 µm, and those of *M. campaniformis* are subglobose to broadly ellipsoidal measuring 4–5 × 2.5–3.5 µm.

Microascus restrictus Sandoval-Denis, Gené & Deanna A. Sutton, *sp. nov.* — MycoBank MB809420, Fig. 9

Etymology. From the Latin *restringere*-, restrict, referring to the restricted growth of the colony.

Colonies on OA at 25 °C growing rather slowly, attaining 23–25 mm diam in 14 d, flat, downy, olive grey (3F2) to brown-grey (5F2), with an irregular margin; reverse brown-grey (5F2). On PCA at 25 °C growing restrictedly, attaining 3–5 mm diam after 14 d, membranous, lobulate, with an irregular undulate margin, olive brown (4E5) to brown (5E5); reverse brown-grey (5E2). *Vegetative hyphae* septate, subhyaline becoming dark brown with age, smooth- and thin-walled, 1.5–3 µm wide. *Conidiophores* absent or as a basal single cell of 4–6 × 3–5 µm, bearing groups of 2–3 annellides, or slightly branched up to 20 µm long, subhyaline, smooth-walled. *Annellides* mostly sessile borne single and laterally on vegetative hyphae, ampulliform, 7–19 × 2–4.5 µm, with a swollen base, tapering abruptly to a cylindrical and darker annellated zone 1.5–2 µm wide, subhyaline, becoming darker with age, smooth-walled. *Conidia* globose to obovoidal, 4.5–6 × 4–5.5 µm, with truncate base, dark brown, smooth or finely roughened, thick-walled, arranged in short chains. Solitary conidia sometimes present, borne laterally from vegetative hyphae, sessile or on short stalks, globose or obovate, 5–5.5 × 4.5–5 µm, dark brown, smooth- and thick-walled. *Sexual morph* not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.

Specimen examined. USA, from human left hallux, 2009, *D.A. Sutton* (holotype CBS H-21787, culture ex-type CBS 138277= UTHSC 09-2704 = FMR 12227).

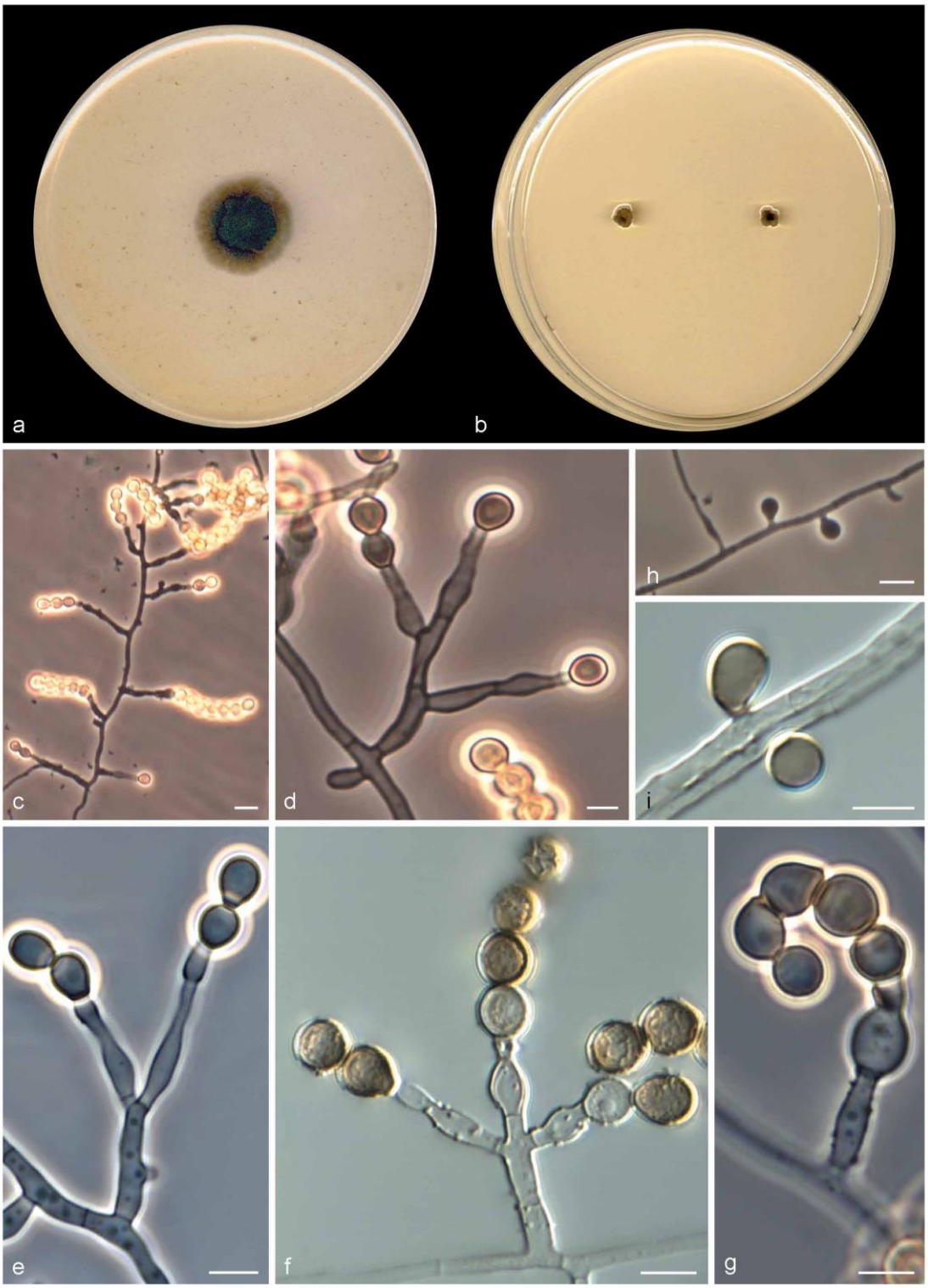

Fig. 10 *Microascus verrucosus* CBS 138278. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c–g. conidiophores, annellides and conidia; h, i. solitary conidia. — Scale bars: c, h = 10 µm; all others = 5 µm.

Notes — *Microascus restrictus* is morphologically very similar to *M. verrucosus*. However, while *M. restrictus* shows larger smooth-walled annellides measuring 7–19 × 2–4.5 µm, smaller conidia (4.5–6 × 4–5.5 µm) and is able to grow at 40 °C, *M. verrucosus* has annellides measuring 8–10 × 1–3 µm, typically warted when mature, larger conidia (5–7 × 4.5–6 µm) and is unable to growth at 40 °C.

Microascus senegalensis Arx, Persoonia 8: 194. 1975

Specimen examined. Senegal, Joel, from mangrove soil, *J.A. von Arx* (ex-type culture IHEM 18561 = CBS 277.74).

Notes — This species has been reported from soil, seeds and plant debris as well as from human skin, in Africa, India and North America (von Arx et al. 1988). The most remarkable features of *M. senegalensis* are the presence of large reniform ascospores (7–9 × 2–3 µm) with a single and often protuberant germ pore (von Arx et al. 1988, Guarro et al. 2012).

Microascus trigonosporus C.W. Emmons & B.O. Dodge, Mycologia 23: 317. 1931

≡ *Scopulariopsis trigonospora* C.W. Emmons & B.O. Dodge, Mycologia 23: 317. 1931.

?= *Microascus trigonosporus* C.W. Emmons & B.O. Dodge var. *terreus* Kamyschko, Novosti Sist. Nizsh. Rast. 76: 175. 1966.

?= *Microascus trigonosporus* C.W. Emmons & B.O. Dodge var. *macroperithecia* Sage, Steiman, Seigle-Mur. & Guiraud, Mycotaxon 55: 194. 1995, nom inval. Art. 40.5 (Melbourne).

Specimens examined. Japan, Burma, from milled rice, 1961, *S. Udagawa* (MUCL 9061 = CBS 199.61). – UK, from mushroom bed, 1935, *W.M. Ware* (MUCL 9841 = CBS 262.35). – USA, from unknown substrate, 1931, *C.W. Emmons* (*M. trigonosporus* var. *trigonosporus* ex-type culture CBS 218.31).

Notes — This is a cosmopolitan species, commonly reported from soil, seeds and dung (Barron et al. 1961). It is also considered as a human pathogen that has been associated with pneumonia in an immunocompromised patient (Mohammedi et al. 2004) and endocarditis (Wang et al. 2011). Among the species producing triangular-shaped ascospores (*M. alveolaris*, *M. campaniformis*, *M. macrosporus* and *M. pyramidus*), *M. trigonosporus* produces the smaller ones, measuring 3–5 × 3–4 µm.

Microascus verrucosus Sandoval-Denis, Gené & Cano, *sp. nov*. — MycoBank MB809421; Fig. 10

Etymology. From the Latin *verruca*-, wart, referring to the warted ornamentation of the annellides.

Colonies on OA at 25 °C growing slowly, attaining 19–22 mm diam in 14 d, flat, finely granular, olive grey (2–3F2), with an immersed and slightly undulated margin; reverse olive grey (1–3F2). On PCA at 25 °C growing restrictedly, attaining 1–5 mm diam in 14 d, adherent, membranous or slightly downy, hemispherical, cerebriform, with lobulated margin, olive (2E3/2E4); reverse olive (2E2) to olive grey (2E3). *Vegetative hyphae* septate, subhyaline becoming dark brown with age, smooth- to rough-walled, thin-walled, 1.5–2.5 µm wide. *Conidiophores* absent or as a basal single cell of 4–5 × 2.5–4 µm, bearing groups of 2–3 annellides, rarely slightly branched up to 25 µm long. *Annellides* mostly sessile and borne directly on vegetative hyphae, lageniform, 8–10 × 1–3 µm, constricted at the basal septum, followed by a slightly swollen portion and tapering to a more or less cylindrical annellated zone 1–1.5 µm wide, usually sparsely warted. *Conidia* globose to subglobose, 5–7 × 4.5–6 µm, often with an inconspicuous truncate base, dark brown, smooth or finely roughened, thick-walled, arranged in short chains. Solitary conidia sometimes present, borne laterally from vegetative hyphae, sessile or on short stalks, globose or broadly ellipsoidal, 5–6 × 4–5 µm, dark brown, smooth- and thick-walled. *Chlamydospores* not observed. *Sexual morph* not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 35 °C, minimum 15 °C.

Specimen examined. USA, from bronchoalveolar lavage fluid, 2010, *D.A. Sutton* (holotype CBS H-21788, culture ex-type CBS 138278 = UTHSC 10-2601 = FMR 12219).

Notes — *Microascus verrucosus* can be differentiated from *M. restrictus*, its closest morphological relative, by its sparsely warted annellides and its inability to grow at 40 °C. *Microascus verrucosus* is phylogenetically close to *M. murinus*; however, this latter species has cylindrical conidia with pointed apices.

Pithoascus Arx, Proc. Kon. Ned. Akad. Wetensch. 76: 295. 1973

Type species. *Pithoascus nidicola* (Massee & E.S. Salmon) Arx.

Colonies restricted, white, becoming grey or darkening due to the production of ascomata; usually with scarce aerial mycelium. *Ascomata* perithecial, immersed or somewhat superficial, gregarious, often grouped on dense crusts, globose, glabrous, often with an inconspicuous ostiolar opening or with a short cylindrical ostiolar neck; peridium black, composed of thick-walled, slightly flattened cells, *textura angularis*. *Asci* unitunicate, 8-spored, broadly clavate or barrel-shaped, evanescent. *Ascospores* 1-celled, asymmetrical, navicular, fusiform or falcate, yellow, straw- or honey-coloured, dextrinoid when young, without germ pores. *Asexual morph* present in some species. *Conidiogenous cells* annellidic, borne singly and laterally on the vegetative hyphae, short, ampulliform, hyaline, smooth-walled. *Conidia* 1-celled, globose to pyriform, with a truncate base, smooth- and thin-walled, solitary.

Pithoascus ater (Zach) Sandoval-Denis, Cano & Guarro, *comb. nov*. — MycoBank MB809214

Basionym. *Scopulariopsis atra* Zach, Oesterr. Bot. Z. 83: 184. 1934.

Specimen examined. From human nail, 1934, *F. Zach* (ex-type culture IHEM 18608 = CBS 400.34).

Notes — A single strain of this species is available. It was isolated from a human nail, but its pathogenic role was not clearly established (Zach 1934). *Pithoascus ater* is the only species of the genus for which a sexual morph is unknown and by contrast shows abundant conidial production. However, the ex-type strain of *P. ater* shows similar morphological characteristics to the known asexual morphs of *Pithoascus* species, such as *P. stoveri* and *P. intermedius*. The main distinctive feature of *P. ater* is the abundant production of solitary, globose and smooth-walled pale brown conidia measuring 4–9 × 4.5–8.5 µm. In contrast, conidia of *P. stoveri* and *P. intermedius* are rarely seen in culture and, when present, are hyaline, obovate to pyriform (5–8 × 3–4 µm) or globose to subglobose (4–8 × 4.5–7.5 µm), respectively. Other species, i.e. *P. nidicola* and *P. exsertus* produce only sexual morphs in culture.

Pithoascus exsertus (Skou) Arx, Persoonia 7: 373. 1973

Basionym. *Microascus exsertus* Skou, Antonie van Leeuwenhoek 39: 533. 1973.

Specimens examined. Denmark, Sjaelland, Bjerge Strand, from *Osmia rufa*, 1975, *J.P. Skou* (CBS 583.75); Tastrup, Hojbakkegard, Experimental Station, from *Megachile willoughbiella*, 1970, *J.P. Skou* (ex-type culture CBS 819.70).

Notes — This fungus is considered as an entomogenous species, which has been isolated from a leaf-cutting bee (*Mega-*

chile willughbiella) and from a Red Mason-bee (*Osmia rufa*), both in northern Europe. Morphologically, *P. exsertus* can be differentiated from the other species of the genus by its larger ascomata (210–450 µm diam) and its long, falcate to nearly cylindrical and yellow ascospores, 6.5–12 × 1–2.5 µm.

Pithoascus intermedius (C.W. Emmons & B.O. Dodge) Arx, Proc. Kon. Ned. Akad. Wetensch. 76: 292. 1973

Basionym. *Microascus intermedius* C.W. Emmons & B.O. Dodge, Mycologia 23: 324. 1931.

Specimen examined. USA, North Carolina, Chadbourn, from decaying root of *Fragaria vesca*, 1932, *C.W. Emmons & B.O. Dodge* (ex-type culture CBS 217.32).

Notes — This species has been reported mainly from soil in North America, Europe and Asia, and also as a potential pathogenic species isolated from human hair and nails (von Arx et al. 1988, Guarro et al. 2012). *Pithoascus intermedius* is morphologically similar to *P. nidicola* and *P. stoveri*. However, *P. intermedius* can be identified by its small, fusiform ascospores, 5–6 × 2–2.5 µm.

Pithoascus nidicola (Massee & E.S. Salmon) Arx, Proc. Kon. Ned. Akad. Wetensch. 76: 292. 1973

Basionym. *Microascus nidicola* Massee & E.S. Salmon, Ann. Bot., Lond. 15: 313. 1901.

Specimen examined. USA, Utah, from *Dipodomys merriami*, *C.W. Emmons* (ex-epitype culture CBS 197.61).

Notes — *Pithoascus nidicola* was originally isolated from a wasp's nest in England and later from soil samples in North America (Massee & Salmon 1901, Barron et al. 1961). The ex-epitype culture, however, was isolated from a kangaroo rat in the USA (von Arx 1973b, Abbott et al. 2002). This species is similar to *P. stoveri*; however, *P. nidicola* can be differentiated by having larger ascomata (90–160 µm diam) with thicker walls (6–10 µm) and navicular to nearly lunate straw coloured ascospores. In contrast, *P. stoveri* produces ascomata measuring 50–110 µm diam, with a wall 4–7 µm thick, and navicular, golden to brown coloured ascospores (von Arx 1973b, Abbott et al. 2002, Guarro et al. 2012). Although conidia had never been reported for *P. nidicola*, we observed the development of a reduced asexual morph on PCA forming globose to ampulliform hyaline conidia, 4–5 × 2.5–3.5 µm, borne on short conidiogenous cells (Fig. 2). These asexual structures resemble those of *P. intermedius*, but the conidia of the latter are globose to subglobose and larger (4–8 × 4–7.5 µm).

Pithoascus stoveri Arx, Persoonia 7: 373. 1973

≡ *Microascus stoveri* (Arx) S.P. Abbott, Mycologia 94: 368. 2002.

Specimen examined. USA, Ohio, from root of *Beta vulgaris*, *W.L. White* (ex-type culture CBS 176.71).

Notes — This species was originally isolated from a root of sugar beet in the USA. *Pithoascus stoveri* is morphologically similar to *P. nidicola*; however, the former species forms an asexual morph in culture, has a smaller ascomata (50–110 µm diam) and navicular golden yellow to brown ascospores. *Pithoascus nidicola* produces ascomata 90–160 µm diam, and navicular to nearly lunate straw-coloured ascospores.

Pseudoscopulariopsis Sandoval-Denis, Gené & Guarro, *gen. nov.* — MycoBank MB809215

Type species. *Pseudoscopulariopsis schumacheri* (Curzi) Sandoval-Denis, Gené & Guarro.

Colonies restricted, greyish, dark olive grey to olivaceous black; floccose with abundant submerged mycelium, often becoming crustose and dark. *Ascomata* black, globose or ovate, glabrous, with a short cylindrical ostiolar neck and a peridium of *textura epidermoidea*. *Asci* unitunicate, 8-spored, ovoid, evanescent. *Ascospores* 1-celled, asymmetrical, navicular to fusiform, subhyaline, pale yellow or brown, without germ pores. *Conidiogenous cells* short, annellidic, often with a swollen base, mostly borne on a short and swollen supporting cell forming short swollen conidiophores, rarely borne singly on aerial hyphae. *Conidia* 1-celled, subglobose, obovate to short clavate, with truncate base and rounded apex, smooth- and thin-walled, pale brown to brown-grey, arranged in short chains.

Pseudoscopulariopsis hibernica (A. Mangan) Sandoval-Denis, Gené & Cano, *comb. nov.* — MycoBank MB809216

Basionym. *Scopulariopsis hibernica* A. Mangan, Trans. Brit. Mycol. Soc. 48: 617. 1965.

Specimen examined. Ireland, from soil, *A. Mangan* (UAMH 2643 = ATCC 16690).

Notes — This is a species described from soil and only a few isolates are available, all of them derived from the same isolation source (Mangan 1965). The isolate studied here, although not the ex-type culture, was isolated and considered authentic by the same authors (Mangan 1965) and matched in all aspects with the protologue (Mangan 1965). Phylogenetically, *P. hibernica* clustered close to *P. schumacheri*, both species being characterised by short cylindrical annellides mostly formed in small groups on short and swollen supporting cells, commonly darkening with age. *Pseudoscopulariopsis hibernica* can be differentiated mainly by its lack of a sexual morph, the presence of shorter (9–15 × 3–5 µm) and darker annellides, and its larger (5–7 × 5–6 µm) subglobose conidia.

Pseudoscopulariopsis schumacheri (E.C. Hansen) Sandoval-Denis, Gené & Guarro, *comb. nov.* — MycoBank MB809549

Basionym. *Sphaerella schumacheri* E.C. Hansen, Vidensk. Meddel. Dansk Naturhist. Foren. Kjøbenhavn: 16. 1876.
≡ *Rosellinia schumacheri* (E.C. Hansen) Sacc., Syll. Fung. 1: 276. 1882.
≡ *Microascus schumacheri* (E.C. Hansen) Curzi, Boll. Staz. Patol. Veg. Roma, N.S. 23: 8. 1931.
≡ *Pithoascus schumacheri* (E.C. Hansen) Arx, Proc. Kon. Ned. Akad. Wetensch. 76: 292. 1973.
= *Melanospora stysanophora* Mattir., Nuovo Giorn. Bot. Ital. 18: 121. 1886.
≡ *Microascus stysanophorus* (Mattir.) Curzi, Boll. Staz. Patol. Veg. Roma, N.S. 10: 391. 1930.
≡ *Microascus stysanophorus* (Mattir.) G.L. Barron, Cain & J.C. Gilman, Canad. J. Bot. 39: 1621. 1961.
≡ *Pithoascus stysanophorus* (Mattir.) Valmaseda, A.T. Martínez & Barrasa, Canad. J. Bot. 65: 1805. 1987.

Specimen examined. Spain, Puerto de la Quesera, from soil, 1986, *A.T. Martínez* (neotype designated here MA-Fungi 16319, MBT178643), culture ex-neotype CBS 435.86.

Notes — This species was originally described from dung of rodents, in Denmark, but no ex-type strain was preserved nor herbarium material listed in the protologue. The modern descriptions of the species by Barron et al. (1961), Valmaseda et al. (1986), von Arx et al. (1988) and Guarro et al. (2012) are based on the same isolate studied here; however, a type specimen has never been designated. We agree with the observations of all these authors in that morphological features of CBS 435.86 match with those of the protologue of the species (Hansen 1876). Therefore, due to the scant live material available and the inexistence of ex-type cultures, we have selected this strain as neotype in order to fix the application of the name. Although neither the substrate nor the geographic origin correspond to

that indicated in the protologue of the species, this strain clearly represents *P. schumacheri* according to concepts maintained by subsequent authors (Valmaseda et al. 1986, von Arx 1988, Guarro et al. 2012).

Morphologically, *P. schumacheri* resembles *Pithoascus* species by its navicular to fusiform ascospores lacking germ pores, thus being included in that genus by von Arx (1973a). Nevertheless, *P. schumacheri* can be differentiated by its restricted growth and the *textura epidermoidea* of the ascoma wall. Phylogenetically, *P. schumacheri* is related to *P. hibernica*; however, the former can be differentiated by the presence of ascomata and the production of obovate to short-clavate conidia, measuring 4.5–6 × 2.5–4 µm, on mostly hyaline annellides which measure 3.5–22.5 × 1.5–2.5 µm.

Scopulariopsis Bainier, Bull. Soc. Mycol. France 23: 98. 1907

?= *Acaulium* Sopp, Skr. Vidensk.-Selsk. Christiana, Math.-Naturvidensk. Kl. I, 11: 42. 1912.
= *Phaeoscopulariopsis* M. Ota, Jap. J. Dermatol. Urol. 28: 405. 1928, *nom. inval.* Art 34 (Seifert et al. 2011).

Type species. *Scopulariopsis brevicaulis* (Sacc.) Bainier.

Colonies spreading fast, velvety, funiculose or granular, varying from white and grey-white to several shades of buff, brown or dark brown, but never in shades of green or black. *Ascomata* perithecial, immersed or superficial, developing slowly, scattered; globose to subglobose or pyriform, glabrous, ostiolate, papillate or with a cylindrical neck; peridium black, composed of thick-walled, slightly flattened cells of *textura angularis*. *Asci* unitunicate, 8-spored, subglobose, irregularly ovoidal or ellipsoidal, evanescent. *Ascospores* 1-celled, asymmetrical, short, broadly reniform or lunate, dextrinoid when young, with or without an inconspicuous germ pore. *Conidiogenous cells* annellidic, borne on branched penicillate conidiophores, occasionally singly on vegetative hyphae or in groups of 2–3 on short stalks, cylindrical, often with a slightly swollen base followed by a cylindrical annellated portion that ends in a flat and wide conidiogenous opening, hyaline, smooth- or rough-walled. *Conidia* 1-celled, hyaline, avellaneous or brown, globose to ovate, with a rounded or pointed apex and a conspicuously protuberant and truncate base, smooth- or rough- and thick-walled, arranged in long basipetal dry chains.

Scopulariopsis asperula (Sacc.) S. Hughes, Canad. J. Bot. 36: 803. 1958

Basionym. *Torula asperula* Sacc., Michelia 2: 560. 1882.
= *Acaulium nigrum* Sopp, Skr. Vidensk.-Selsk. Christiana, Math.-Naturvidensk. Kl. I 11: 47. 1912.
≡ *Penicillium nigrum* (Sopp) Biourge, Cellule 33: 1043. 1923.
≡ *Microascus niger* (Sopp) Curzi, Boll. Staz. Patol. Veg. Roma, N.S. 11: 8. 1931.
= *Scopulariopsis repens* Bainier, Bull. Soc. Mycol. France 23: 125. 1907.
≡ *Penicillium repens* (Bainier) Biourge, Cellule 33: 225. 1923.
= *Monilia arnoldii* L. Mangin & Pat., Bull. Soc. Mycol. France 24: 164. 1908.
≡ *Scopulariopsis arnoldii* (L. Mangin & Pat.) Vuill., Bull. Soc. Mycol. France 27: 148. 1911.
= *Scopulariopsis ivorensis* H. Boucher, Bull. Soc. Pathol. Exot. 11: 312. 1918.
= *Torula bestae* Pollacci, Rivista Biol. 4: 317. 1922.
≡ *Phaeoscopulariopsis bestae* (Pollacci) M. Ota, Jap. J. Dermatol. Urol. 28: 405. 1928, *nom. inval.* (Seifert et al. 2011).
≡ *Scopulariopsis bestae* (Pollacci) Nannf., Repertorio sistematico dei miceti dell'uomo e degli animale 4: 254. 1934.
= *Scopulariopsis fusca* Zach, Oesterr. Bot. Z. 83: 174. 1934.
= *Acaulium nigrum* Sopp var. *glabrum* Salv.-Duval, Thèse Fac. Pharm. Paris. 23: 55. 1935.
= *Scopulariopsis roseola* N. Inagaki, Trans. Mycol. Soc. Japan 4: 1. 1962.

Specimens examined. Austria, from a carcass of rabbit, 1934, *F. Zach* (*Scopulariopsis fusca* ex-type culture MUCL 9032 = CBS 401.34). – Canada, Alberta, Girouxville, from indoor air ex RCS strip, *Apis mellifera* overwintering facility, Jan. 1994, *S.P. Abbott* (MUCL 40729 = UAMH 7879); Alberta, 10 km south of Leduc, from dung of *Mephitis mephitis*, June 1997, *S.P. Abbott* (MUCL 40746 = UAMH 9029). – Germany, from compost soil, 1958, *K.H. Domsch* (CBS 853.68). – Italy, from human, 1938, *G. Pollacci* (*Torula bestae* ex-type culture MUCL 9012 = CBS 289.38). – USA, from toenail, 2010, *D.A. Sutton* (UTHSC 10-3405 = FMR 12212).

Notes — This species has been mostly recovered from environmental samples such as soil, air, mouldy indoor environments, food such as cheese and butter, as well as from human clinical specimens, mainly skin and nails (Ropars et al. 2012, Sandoval-Denis et al. 2013).

Torula bestae was considered by Morton & Smith (1963) to be conspecific with *S. koningii*. However, although both species show smooth conidia, *T. bestae* typically exhibits darker fuscous-black colonies and conidia. Later, Abbott & Sigler (2001), using mating experiments, established that *S. arnoldii*, *S. asperula*, *S. bestae*, *S. fusca* and *S. roseola* were all synonyms of the heterothallic species *Microascus niger*. The same authors also selected neotype and epitype cultures for *M. niger* (UAMH 9489) and *S. asperula* (UAMH 9037), respectively, which unfortunately were not available for our study. However, all the reference strains studied here were genetically related with the type cultures of *S. fusca* and *S. bestae*, both species regarded as synonyms of *S. asperula*. Recently, Ropars et al. (2012) confirmed the synonymy of these species using a multilocus analysis based on D1/D2, TUB and EF-1α sequences which results are confirmed by our phylogenetic analysis. Morphologically, *S. asperula* is close to *S. brevicaulis* and *S. flava*; however, *S. asperula* can be differentiated by having dark brown to fuscous or violaceus colonies, and globose to ovate, coarsely verrucose or smooth walled, fuscous to sepia coloured conidia, mostly with a pointed apex and measuring 5–8 × 4–6.5 µm. In contrast, *S. brevicaulis* shows tan colonies, globose and verrucose pale brown conidia, 6–9 × 5.5–9 µm, while *S. flava* exhibits white colonies and obovoidal, verrucose and hyaline conidia, 6–9.5 × 5–8.5 µm.

Scopulariopsis brevicaulis (Sacc.) Bainier, Bull. Soc. Mycol. France 23: 99. 1907

Basionym. *Penicillium brevicaule* Sacc., Fungi Ital. 893: 1881.
= *Monilia penicillioides* Delacr., Bull. Soc. Mycol. France 13: 114. 1897.
≡ *Penicillium penicillioides* (Delacr.) Vuill., Bull. Soc. Mycol. France 27: 75. 1911.
≡ *Scopulariopsis penicillioides* (Delacr.) Smith & Ramsb., Trans. Brit. Mycol. Soc. 5: 164. 1915.
= *Monilia koningii* Oudem., Arch. Neerl. Sci., sér. 2: 287. 1902.
≡ *Scopulariopsis koningii* (Oudem.) Vuill., Bull. Soc. Mycol. France 27: 143. 1911.
= *Penicillium coccophilum* Sacc., Ann. Mycol. Berl. 5: 178. 1907.
= *Scopulariopsis rufulus* Bainier, Bull. Soc. Mycol. France 23: 105. 1907.
≡ *Penicillium rufulum* (Bainier) Sacc., Syll. Fung. 22: 1275. 1913.
= *Penicillium brevicaule* Sacc. var. *hominis* Brumpt & Langeron in Brumpt, E. Précis de parasitologie, Ed. 1: 838. 1910.
≡ *Scopulariopsis brevicaulis* (Sacc.) Bainier var. *hominis* (Brumpt & Langeron) Brumpt & Langeron in Brumpt, E. Précis de parasitologie, Ed 2: 902. 1913.
= *Scopulariopsis hominis* (Brumpt & Langeron) Sartory, Champ. Parasit. Fasc. 8: 612. 1922.
= *Acaulium insectivorum* Sopp, Skr. Vidensk.-Selsk. Christiana, Math.-Naturvidensk. Kl. I, 11: 60. 1912.
≡ *Penicillium insectivorum* (Sopp) Biourge, Cellule 33: 103. 1923.
≡ *Scopulariopsis insectivora* (Sopp) Thom, The Penicillia: 532. 1930.
= *Acaulium anomalum* Sopp, Skr. Vidensk.-Selsk. Christiana, Math.-Naturvidensk. Kl. I, 11: 65. 1912.
= *Penicillium brevicaule* Sacc.var. *intermedium* Cagnetto, Sperimentale 67, Suppl. to Fasc. 4: 210. 1913.
= *Sporotrichum stercorarium* Ehrenb., Jahrb. Gewächsk. 1: 178. 1818.
≡ *Scopulariopsis stercoraria* (Ehrenb.) S. Hughes, Canad. J. Bot. 36: 803. 1958.

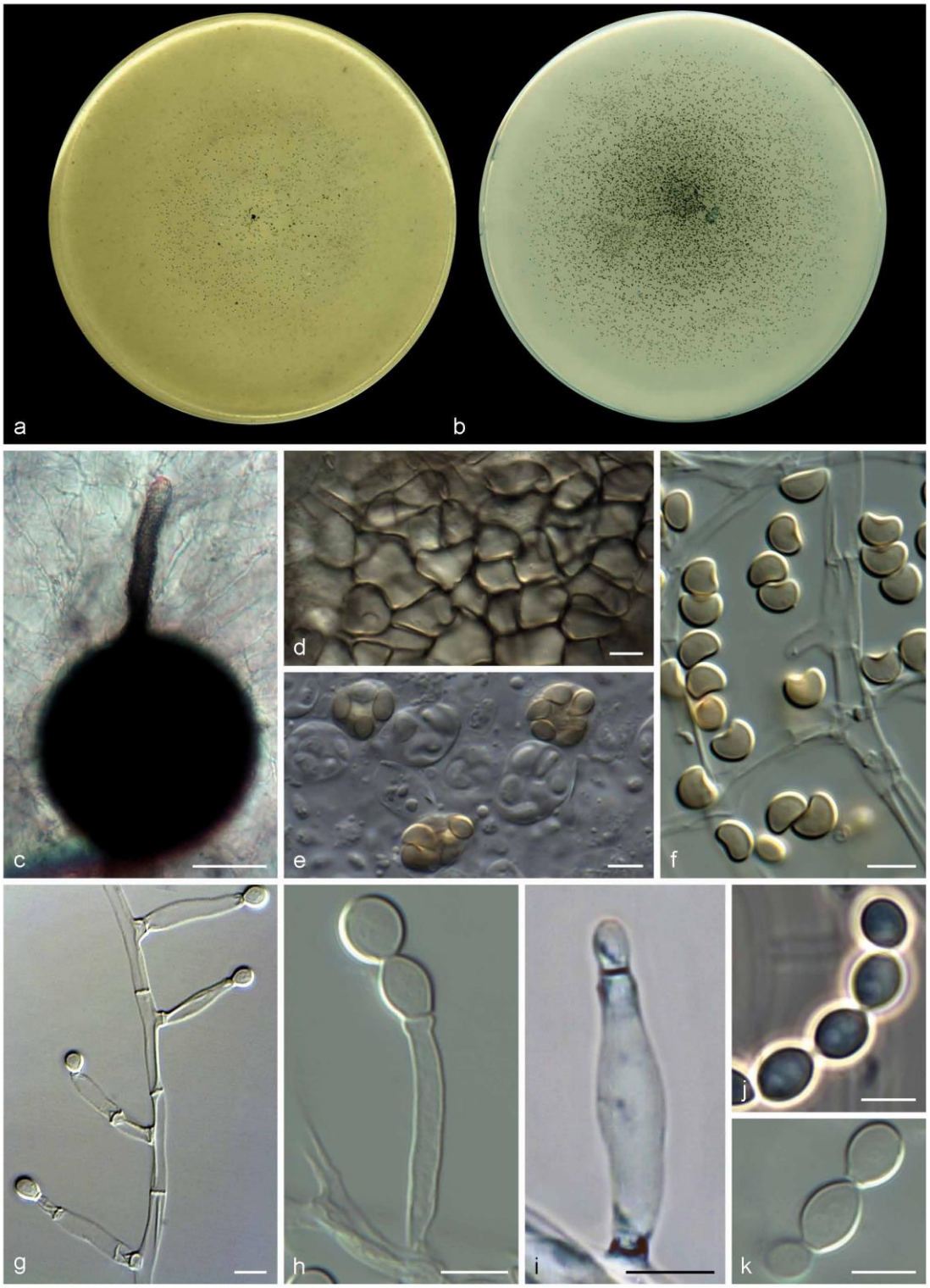

Fig. 11 *Scopulariopsis cordiae* CBS 138129. a, b. Colonies on OA and PCA, respectively, after 21 d at 25 °C; c. ascoma; d. peridium; e, f. asci and ascospores; g–i. conidiophores, annellides and conidia; j, k. conidial chains. — Scale bars: c = 50 µm; all others = 5 µm.

= *Scopulariopsis alboflavescens* Zach, Oesterr. Bot. Z. 83: 177. 1934.
= *Microascus brevicaulis* S.P. Abbott. Mycologia 90: 298. 1998.

Specimens examined. Austria, from human diseased skin, 1934, *F. Zach* (*S. alboflavescens* ex-type culture CBS 339.34). – Belgium, Heverlee, from soil, *J. Meyer* (as *S. stercoraria* MUCL 14213). – Canada, Alberta, from indoor air, March 1994, *S.P. Abbott* (*M. brevicaulis* ex-type culture UAMH 7770 = MUCL 40726). – The Netherlands, Wageningen, from pupa of *Pteronus pini*, 1935, *J. Rozsypal* (as *S. insectivora* CBS 335.35 = MUCL 9035). – Unknown geographical origin, Elephant, 1951, *I.M. Scott* (as *S. koningii* CBS 208.61). – USA, from hair, 2006, *D.A. Sutton* (UTHSC 06-277 = FMR 12273); from toenail, 2006, *D.A. Sutton* (UTHSC 06-619 = FMR 12271); from toenail, 2007, *D.A. Sutton* (UTHSC 07-1812 = FMR 12257); from human spine, 2007, *D.A. Sutton* (UTHSC 07-1888 = FMR 12255); from maxillary sinus, 2008, *D.A. Sutton* (UTHSC 08-1920 = FMR 12247); from toe, 2009, *D.A. Sutton* (UTHSC 09-1092 = FMR 12236); from sputum, 2009, *D.A. Sutton* (UTHSC 09-1373 = FMR 12233); from sputum, 2011, *D.A. Sutton* (UTHSC 11-427 = FMR 12211); from lung mass, 2011, *D.A. Sutton* (UTHSC 11-1240 = FMR 12206); from bronchoalveolar lavage fluid, 2011, *D.A. Sutton* (UTHSC 11-1563 = FMR 12204).

Notes — This is a species with a worldwide distribution. It has been isolated from a wide range of substrates and locations and also recognised as an important human opportunistic pathogen (de Hoog et al. 2011, Sandoval-Denis et al. 2013). The history of this taxon was reviewed by Morton & Smith (1963). These authors synonymised *S. insectivora* and *S. brevicaulis*, but regarded *S. stercoraria* and *S. koningii* as different species since the latter two taxa exhibited smooth conidia. Later, Abbott & Sigler (2001) based on mating studies demonstrated that *S. brevicaulis* and *S. koningii* were conspecific. The present data confirmed the synonymy of the four mentioned species. In contrast, our results showed that the ex-type strain of *S. alboflavescens* (CBS 339.34), a species currently considered a synonym of *S. candida*, is conspecific with *S. brevicaulis*. *Scopulariopsis alboflavescens* was described as having smooth conidia and colonies at first white becoming pale yellowish, features that distinguished it from *S. brevicaulis* (Zach 1934). However, our morphological study of the ex-type strain of *S. alboflavescens* revealed that despite the fact that it forms whitish to pale yellow colonies, it also produces some finely roughened conidia. Ropars et al. (2012) already suggested a relationship between the two species using a phylogenetic analysis based in LSU, TUB and EF-1α sequences, showing that the ex-type strain of *S. alboflavescens* nested in a clade closely related to *S. brevicaulis* and far from the *S. candida* clade. However, those authors concluded that *S. candida* was a polyphyletic species. Abbott & Sigler (2001) reported the formation of fertile ascomata when crossing the ex-type strain of *S. alboflavescens* (CBS 339.34) with several strains of *S. candida* and the ex-types of *S. candida* (MUCL 40743) and *Nephrospora manginii* (basionym of *M. manginii* CBS 170.27), thus supporting the synonymy of *S. alboflavescens* and *S. candida* already proposed by Morton & Smith (1963). However, considering that strains of *M. manginii* (CBS 170.27 and MUCL 12598), the ex-epitype of *S. candida* (MUCL 40743) and *S. alboflavescens* (CBS 399.34) were all self-fertile in our study, the phylogenetic results and the placement of *S. alboflavescens* as a synonym of *S. brevicaulis*, including strains showing smooth conidia and whitish to pale yellow colonies, is supported.

Scopulariopsis candida (Guég.) Vuill., Bull. Soc. Mycol. France 27: 143. 1911

Basionym. *Monilia candida* Guég., Bull. Soc. Mycol. France 15: 271. 1899.
= *Nephrospora manginii* Loubière, Compt. Rend. Hebd. Séances Acad. Sci. 177: 209. 1923.
≡ *Microascus manginii* (Loubière) Curzi, Boll. Staz. Patol. Veg. Roma 2: 60. 1931.
= *Monilia candida* auct. non Pers.: Loubière in Compt. Rend. Hebd. Séances Acad. Sci. 177: 209. 1923.
= *Scopulariopsis brevicaulis* (Sacc.) Bainier var. *glabra* (Thom) Thom sensu Raper & Thom, Manual of the Penicillia: 699. 1949.
= *Chrysosporium keratinophilum* (Frey) Carmich. var. *denticola* C. Moreau, Mycopathol. Mycol. Appl. 37: 37. 1969.
≡ *Basipetospora denticola* (C. Moreau) C. Moreau, Bull. Soc. Mycol. France 87: 43, 1971.

Specimens examined. Canada, British Columbia, Chilliwak, from indoor air, March 1997, *S.P. Abbott* (*S. candida* ex-type culture MUCL 40743 = UAMH 9004). – France, from unknown origin, 1927, *L. Mangin* (*N. manginii* ex-type culture CBS 170.27); from unknown substrate, 1927, *L. Mangin* (as *S. candelabrum* MUCL 9026 = CBS 205.27); from cheese 'tome de Savoie', Aug. 1998, *C. Decock* (as *M. manginii* MUCL 41467). – Unknown origin, 1966 (as *S. alboflavescens* MUCL 9007). – USA, from sputum, 2009, *D.A. Sutton* (UTHSC 09-2576 = FMR 12228); from scalp, 2009, *D.A. Sutton* (UTHSC 09-3241 = FMR 12226).

Notes — This species has been reported from environmental samples (air, dust and soil) generally from the Northern Hemisphere, especially in Europe and North America; and also from clinical samples, mainly from superficial tissue of humans and animals (de Hoog et al. 2011). It is morphologically close to *S. brevicaulis*, *S. asperula* and *S. flava*. However, *S. candida* has subglobose to broadly ovate, hyaline, smooth-walled conidia and white colonies. In contrast, *S. brevicaulis* and *S. asperula* produce tan or fuscous brown colonies, respectively, while *S. flava* produces white colonies and obovoidal rough-walled conidia. When the sexual morph is present, it is characterised by globose perithecia, 100–170 µm diam, and reniform to heart-shaped ascospores which are somewhat wider (4–6 × 5–6 µm) than those of its closest relatives such as *S. brevicaulis* (5–6 × 3.5–4.5 µm), *S. cordiae* (4.5–5.5 × 3.5–4 µm) and *S. soppii* (6–7 × 2.5–3 µm).

Scopulariopsis cordiae Sandoval-Denis, Gené & Cano, *sp. nov.* — MycoBank MB809217, Fig. 11

Etymology. From the Latin *cordiae*-, heart, referring to the heart-shaped ascospores.

Colonies on OA and PCA at 25 °C attaining 35–36 and 48–50 mm diam, respectively, after 14 d, flat, with scarce aerial mycelium, white to light grey (4B1) and granular due to the abundant production of ascomata, regular margin with abundant submerged mycelium; reverse whitish. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 1.5–3.5 µm wide. *Ascomata* abundant, superficial or immersed, globose or subglobose, 100–150 µm diam, with a long cylindrical ostiolar neck up to 390 µm, black, glabrous; peridium with a *textura angularis*. *Asci* irregularly ellipsoidal, 9–15.5 × 7.5–10 µm. *Ascospores* broadly lunate to reniform, 4.5–5.5 × 3.5–4 µm, straw coloured, bright yellow in mass, with a single and inconspicuous germ pore. *Conidiophores* absent. *Annellides* sessile, borne single and laterally on vegetative hyphae, hyaline, smooth and thin-walled, cylindrical, 8–15 × 1.5–3.5 µm, tapering gradually to a cylindrical annellated zone 1–1.5 µm wide. *Conidia* broadly ellipsoidal to obovoidal, 2.5–6 × 2–5 µm, with truncate base, hyaline, white in mass, smooth- and thick-walled, arranged in chains. *Chlamydospores* and solitary conidia not observed.

Cardinal temperature for growth — Optimum 25–30 °C, maximum 40 °C, minimum 15 °C.

Specimens examined. USA, from a human J.P Drain, 2005, *D.A. Sutton* (UTHSC 05-3453 = FMR 12349); from human finger, 2009, *D.A. Sutton* (holotype CBS H-21789, culture ex-type CBS 138129 = UTHSC 09-866 = FMR 12338).

Notes — *Scopulariopsis cordiae* morphologically resembles the sexual morph of *S. candida* in the shape and size of the ascomata, asci and ascospores. *Scopulariopsis cordiae* can be differentiated by its faster growth rate, the sparkled appearance of the colonies, the presence of long cylindrical necks in numerous submerged ascomata, and the slightly reduced size and shape of its ascospores and conidia.

Scopulariopsis flava (Sopp) F.J. Morton & G. Sm., Mycol. Pap. 86: 43. 1963

Basionym. *Acaulium flavum* Sopp, Skr. Vidensk.-Selsk. Christiana, Math.-Naturvidensk. Kl. I 11: 53. 1912.
= *Penicillium brevicaule* Sacc. var. *album* Thom, Bull. U.S. Bur. Anim. Ind. 118: 47. 1910.
≡ *Scopulariopsis brevicaulis* (Sacc.) Bainier var. *alba* (Thom) Thom, The Penicillia: 520. 1930.
= *Scopulariopsis aurea* Sartory, Champ. Parasit. Fasc. 9: 650. 1922.
= *Scopulariopsis casei* Loubière, Thèse Fac. Sci. Paris, Sér. 4: 62. 1924.
= *Scopulariopsis grylli* Sartory, Ann. Mycol., Berl., 30: 469. 1932.

Specimen examined. UK, from cheese, 1948, *G. Smith* (neotype designated here CBS H-21939, MBT198047) culture ex-neotype CBS 207.61 = MUCL 9031).

Notes — This species is commonly isolated from cheese and soil in Europe and North America (Ropars et al. 2012). However, Sopp's original description of *A. flavum* is based on an isolate obtained from an insect larva. There are also reports of this species as human opportunistic pathogen (de Hoog et al. 2011). *Scopulariopsis flava* is morphologically close to *S. brevicaulis*. However, while *S. brevicaulis* produces tan, powdery to granular colonies, and globose to ovoid conidia with rounded or pointed apices, becoming verrucose and pale brown when mature, *S. flava* produces white floccose to fasciculate colonies, and hyaline globose to obovoidal conidia, with rounded apices and a coarsely roughened wall.

We only could study a single strain of *S. flava* (MUCL 9031), which is morphologically identical to the asexual morph of *A. flavum* and fits with the modern concept of *S. flava* by Morton & Smith (1963). Abbott et al. (2002) considered this strain a probable poorly pigmented variant of *S. brevicaulis*. However, our analyses show that MUCL 9031 is phylogenetically and morphologically distant from *S. brevicaulis*. Considering that Morton & Smith (1963) regarded MUCL 9031 as an authentic strain of *S. brevicaulis* var. *alba*, the epithet *alba* would take priority over the younger one *flava*, however the former epithet was already used in *Scopulariopsis* (*S. alba*, currently *Doratomyces albus* according to Dominik & Majchrowicz (1970)). Thus to avoid nomenclatural confusion we prefer to maintain the epithet *flava* for the present species. Phylogenetically *S. flava* is related to *S. soppii*, but the latter differs morphologically in producing larger conidia (5.5–9 × 5–8 µm vs 6.5–7 × 5.5–6.5 µm in *S. flava*), and falcate to lunate ascospores measuring 6–7 × 2.5–3 µm. It is noteworthy that a short description of a sexual morph was included in the protologue of *A. flavum* having 'oval-round' ascospores measuring 6–7 µm (Sopp 1912). However, we were not able to induce the production of ascomata in the above-mentioned strain and according to Abbott et al. (2002) no sexual morph has been reported since the original description of the species (Sopp 1912).

Scopulariopsis soppii S.P. Abbott, Mycologia 94: 364. 2002

≡ *Microascus soppii* S.P. Abbott, Mycologia 94: 364. 2002.

Specimen examined. Canada, Alberta, Elk Island National Park, from dry, rotten wood of *Populus tremuloides*, *T. Lumley* (ex-type culture UAMH 9169).

Notes — This species has been isolated from decayed wood and sandy loam (Abbott et al. 2002). It is phylogenetically and morphologically close to *S. flava* from which it can be differentiated based on the size of the conidia and the size and shape of its ascospores (see *S. flava*).

IDENTIFICATION KEYS

According to the morphological features, identification keys were constructed for the different genera including all the phylogenetic species recognised in this study.

Key to Microascus, Scopulariopsis and allied genera

1. Colonies white, tan or brown coloured; conidiogenous cells cylindrical, hyaline or pale brown; conidia thick-walled with a protruding flat base . . . . . *Scopulariopsis*
1. Colonies grey-white, olive-green or black; conidiogenous cells ampulliform or lageniform, subhyaline or brown-green; conidia otherwise 2
2. Asexual morph absent; if present conidiophores simple, short; ascospores without germ pores 3
2. Asexual morph always present, conidiophores often branched up to 80 µm long; ascospores with a germ pore . . . . . *Microascus*
3. Ascomata peridium of *textura angularis*; asexual morph when present forming short, hyaline and single annellides . . . . . *Pithoascus*
3. Ascomata peridium of *textura epidermoidea*; asexual morph usually abundant, forming long annellides from short swollen conidiophores, darkening with time . . . . . *Pseudoscopulariopsis*

Key to Microascus species

1. Ascomata present in culture 2
1. Ascomata absent in culture 14
2. Growth at 40 °C 3
2. No growth at 40 °C 10
3. Peridium with *textura angularis* 4
3. Peridium with *textura intricata* . . . . . *M. intricatus*
4. Ascospores always triangular or quadrangular 5
4. Ascospores reniform to broadly lunate, rarely triangular . 8
5. Ascospores with rounded ends 6
5. Ascospores with attenuated (pointed) ends *M. pyramidus*
6. Ascospores 5–6.5 × 5.5–7.5 µm . . . . . *M. macrosporus*
6. Ascospores narrower 7
7. Ascospores 4–6 × 3–5 µm, yellow in mass *M. alveolaris*
7. Ascospores 6–7 × 4–4.5 µm elongated to one side, yellow to orange in mass . . . . . *M. campaniformis*
8. Colonies dull to olive-green; ascospores lunate 4.5–6.5 × 2–4 µm; conidiophores irregularly branched . . *M. gracilis*
8. Colonies light to brown-grey; ascospores reniform to broadly lunate 4–6 × 2.5–4 µm; conidiophores usually simple 9
9. Ascomata up to 300 µm diam; ascospores pale red-brown in mass; conidia 2–3 µm wide . . . . . *M. cinereus*
9. Ascomata less than 230 µm diam; ascospores straw coloured; conidia 4–6 µm wide . . . . . *M. cirrosus*
10. Ascospores triangular 4–5 × 3–4 µm . . *M. trigonosporus*
10. Ascospores otherwise 11
11. Ascospores reniform 12
11. Ascospores broadly ovoid to ellipsoidal 13
12. Ascospores 7–9 × 2–3 µm, straw coloured, yellow in mass, with a protuberant germ pore . . . . . *M. senegalensis*
12. Ascospores 3–4 × 2–3.5 µm, hyaline to subhyaline with an indistinct germ pore . . . . . *M. longirostris*
13. Ascospores ellipsoidal 5–7 × 2–3 µm, light brown to brown in mass . . . . . *M. brunneosporus*
13. Ascospores broadly ovoid 3.5–5 × 2–3.5 µm, pale yellow in mass . . . . . *M. hyalinus*
14. Growth at 35 °C 15
14. No growth at 35 °C 19
15. Colonies fast growing (> 60 mm in 14 d); conidia bullet-shaped to broadly clavate, finely roughened 4–8 × 2.5–3.5 µm . . . . . *M. expansus*
15. Colonies growing restrictedly; conidia otherwise 16

16. Colonies finely granular, olive-grey; conidiophores sparsely warted . . . . . *M. verrucosus*
16. Colonies downy to velvety, dark brown-grey; conidiophores smooth-walled 17
17. Growth at 40 °C; conidia globose to obovoidal 4.5–6 × 4–5.5 µm, thick- and rough-walled . . . . . *M. restrictus*
17. No growth at 40 °C; conidia smooth-walled 18
18. Conidia broadly ellipsoidal to short clavate 4–6 × 2–4.5 µm with rounded apex . . . . . *M. paisii*
18. Conidia cylindrical, 4–6 × 1.5–2 µm, usually with a pointed apex . . . . . *M. murinus*
19. Conidia globose, 3.5–5 × 3–4 µm, brown coloured; slow growth at 5 °C . . . . . *M. croci*
19. Conidia ovate, usually with a pointed apex, 4–5.5 × 3–4 µm, green-brown; no growth at 5 °C . . . . . *M. chartarus*

Key to Pithoascus species

1. Colonies black, velvety or powdery; sexual morph not formed in culture; asexual morph abundant showing conidia globose to pyriform, 4–9 × 4.5–8.5 µm . . . . . *P. ater*
1. Colonies light grey to black, becoming crustose by the formation of ascomata; asexual morph scarce or absent 2
2. Ascomata 210–450 µm diam; ascospores falcate with attenuated ends, up to 12 µm long . . . . . *P. exsertus*
2. Ascomata less than 170 µm diam; ascospores otherwise 3
3. Ascomata 50–110 µm diam; ascospores navicular, 6–7.5 × 2–3 µm, golden yellow to brown coloured . . . *P. stoveri*
3. Ascomata 90–160 µm diam; ascospores fusiform 4
4. Ascospores fusiform 5–6 µm long, honey coloured; asexual morph when present forming globose to subglobose hyaline conidia 4–8 × 4–7.5 µm . . . . . *P. intermedius*
4. Ascospores fusiform, nearly lunate 6–8 µm long, subhyaline to straw coloured; asexual morph when present forming globose to ampulliform hyaline conidia 4–5 × 2.5–3.5 µm on short stalks. . . . . *P. nidicola*

Key to Pseudoscopulariopsis species

1. Colonies grey-white; conidia obovate or short clavate, 4.5–6 × 2.5–4 µm; sexual morph present in culture, with pale brown fusiform or navicular ascospores, measuring 8–13 × 2.5–4 µm. . . . . *P. schumacheri*
1. Colonies olive-grey; conidia subglobose 5–7 × 5–6 µm; sexual morph absent . . . . . *P. hibernica*

Key to Scopulariopsis species

1. Colonies white, tending to light grey when ascomata are present 2
1. Colonies tan, pale brown to fuscous brown. 5
2. Conidia smooth 3
2. Conidia rough at maturity. 4
3. Conidiophores abundant; conidia 6–9.5 × 5–8.5 µm; ascospores when present hyaline, heart shaped . *S. candida*
3. Conidiophores scarce; conidia 2.5–6 × 2–5 µm; ascospores straw coloured, reniform to broadly lunate. . . . . *S. cordiae*
4. Conidia pale yellow in mass, globose to subglobose, 5.5–9 × 5–8 µm; sexual morph present; ascospores falcate to lunate. . . . . *S. soppii*
4. Conidia white in mass, globose to obovoidal, 6.5–7 × 5.5–6.5 µm; sexual morph not observed . . . . . *S. flava*
5. Conidia brown in mass, verrucose at maturity, globose to ovoid; 6–9 × 5.5–9 µm . . . . . *S. brevicaulis*
5. Conidia dark brown to fuscous, smooth or verrucose, globose to ovate usually with a pointed apex, 5–8 × 4–6.5 µm . . . . . *S. asperula*

EXCLUDED OR DOUBTFUL SPECIES

Microascus albonigrescens (Sopp) Curzi, Boll. Staz. Patol. Veg. Roma 11: 60. 1931

Specimen examined. Japan, Hokkaido, from litter, treated with urea, 1967, *S. Udagawa* (IHEM 18560 = CBS 109.69).

Notes — Our phylogenetic study demonstrates that this taxon does not belong to *Microascus* s.str. It nested in a clade related to the genera *Cephalotrichum*, *Gamsia* and *Wardomyces*, and might represent the former genus *Acaulium*, which was typified by Sopp (1912) with *Scopulariopsis albonigrescens* (sexual morph *M. albonigrescens*). Given the absence of type material, however, we prefer not to introduce any taxonomic changes until a more accurate study of the available reference material can be carried out.

Microascus caviariformis Malloch & Hubart, Canad. J. Bot. 65: 2384. 1987

Specimen examined. Belgium, Prov. de Liège, Flemalle, Cave de Ramioul, from decaying meat, 1985, *J.M. Hubart* (ex-type culture CBS 536.87).

Notes — As in the case of *M. albonigrescens*, our phylogenetic data revealed that this taxon shows affinity with members of *Cephalotrichum*, *Gamsia* and *Wardomyces* rather than *Microascus*. It could represent another species of the former genus *Acaulium*.

Microascus decorticatus C. Ram, Nova Hedwigia 21: 226. 1972

Notes — The original description stated a close morphological relationship of this species with *M. cinereus* and *M. gracilis*. *Microascus decorticatus* was described having slightly larger ascomata, while the asci and ascospores are nearly identical in size and shape to those of *M. cinereus*. Because the ex-type culture (IMUFPe 2194) was not available for study, the taxonomy of this species remains unclear.

Microascus desmosporus (Lechmere) Curzi, Boll. Staz. Patol. Veg. Roma 11: 60. 1931

≡ *Peristomium desmosporum* Lechmere, Compt. Rend. Hebd. Séances Acad. Sci. 155: 178. 1912.
?= *Peristomium desmosporum* Lechmere var. *verticillium* Lechmere, Bull. Trimestriel Soc. Mycol. France: 178. 1913.
= *Microascus desmosporus* (Lechmere) Curzi var. *macroperithecia* Sage, Steiman, Seigle-Mur. & Guiraud, Mycotaxon 55: 191. 1995, *nom inval.* Art. 40.5 (Melbourne).

Notes — This species has a controversial taxonomy. Barron et al. (1961) recognize *M. desmosporus* as a species distinct from *M. cirrosus* while Morton & Smith (1963) regarded both species as conspecific. However, the ex-type strain of *M. cirrosus* was not examined by the latter authors. Von Arx (1975) and von Arx et al. (1988) regarded *M. desmosporus* as a doubtful species considering that *Peristomium desmosporum* and *M. desmosporus* are based on two different fungi. In the absence of type cultures the taxonomy of these fungi remains unknown.

Microascus dimonatus Sage, Steiman, Seigle-Mur. & Guiraud, Mycotaxon 55: 195. 1995

Notes — This name is currently considered as invalid in fungal databases (Index Fungorum, MycoBank) following the

Art. 40.5 of the International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). As stated in the original description, an ex-type culture of this fungus exists (CMPG 1274); however, it was not available for examination. Therefore, according to Guarro et al. (2012) further studies are necessary to clarify the taxonomy of this species.

Microascus giganteus Malloch, Mycologia 62: 731. 1970

Specimen examined. Canada, Ontario, from insect frass in dead log, 1968, *D.W. Malloch* (ex-type culture CBS 746.69 = UAMH 9425).

Notes — This is a coprophilous species, originally isolated from insect dung (Malloch 1970), which resembles *Microascus* species in many morphological characteristics, particularly in those of the sexual state. However, it shows large (up to 750 µm) and hairy ascomata, the asci are formed irregularly at the centre of the perithecia and it has a *Wardomyces* asexual morph. The genus *Wardomyces*, typified by *W. anomalus*, is characterised by having swollen conidiogenous cells forming conidia in lateral or basipetal succession, the conidia are subglobose, ovoid or ellipsoid, brown to blackish and present a single longitudinal germ-slit (Brooks & Hansford 1923, Hennerbert 1962). According to our combined LSU and ITS sequence analysis (Fig. 1), *M. giganteus* is closely related to *W. inflatus*, thus the morphological features and phylogenetic evidence seem to demonstrate that *M. giganteus* is a *Wardomyces* species. However, sequence comparison with the type species of *Wardomyces* is required for taxonomical changes.

Microascus inopinatus Udagawa & Furuya, Mycotaxon 7: 91. 1978

≡ *Wardomycopsis inopinata* Udagawa & Furuya, Mycotaxon 7: 92. 1978.

Specimen examined. Myanmar, from soil, 2008, *C. Hartung* (FMR 10305).

Notes — This taxon was described as unusual among the genus *Microascus* since its asexual morph exhibits annellated conidiogenous cells producing short catenate and globose conidia with a prominent longitudinal germ-slit. Thus, the genus *Wardomycopsis* was established to accommodate its asexual state. Although the ex-type culture was not available, the reference strain included in our study fits clearly with the protologue of the species. The phylogenetic analysis of the LSU and ITS sequences showed that this taxon was located far from the *Microascus* clade forming a highly supported clade with the ex-type strain of *Wardomycopsis humicola* (basionym *Scopulariopsis humicola* CBS 487.66).

Microascus microcordiformis Matsush., Matsush. Mycol. Mem. 9: 16. 1996

Notes — The original description correlates with a *Microascus* species; being morphologically close to *M. longirostris*. However, in absence of live type material, the status of this name remains unknown.

Microascus pilosus Valldos. & Guarro, Nova Hedwigia 57: 123. 1993

Specimen examined. Spain, Burgos, from rabbit dung, 1986, *M. Hernandez* (ex-isotype FMR 2604).

Notes — No live ex-type material is available for phylogenetic analyses. An isotype specimen (FMR 2604) is preserved at the Universitat Rovira i Virgili. The morphological examination of this specimen corresponded with the original description showing that this species clearly belongs to *Microascus* (Guarro et al. 2012).

Microascus singularis (Sacc.) Malloch & Cain, Canad. J. Bot. 49: 859. 1971

Basionym. *Fairmania singularis* Sacc., Ann. Mycol. 4: 276. 1906.
= *Microascus doguetii* Moreau, Rev. Mycol. 18: 177.1953.

Specimen examined. Japan, Tokyo, laboratory contaminant, 1962, *S. Udagawa* (CBS 414.64).

Notes — The morphological features of the asexual morph, with conidia showing longitudinal bands, and the analysis of the LSU and ITS sequences of a reference isolate (CBS 414.64) showed that this taxon formed a phylogenetic lineage related to *Wardomyces* and *Wardomycopsis*. However, given the absence of an ex-type strain, a deeper phylogenetic analysis is required.

Microascus tardifaciens Y. Horie & Udagawa, Mycotaxon 17: 331. 1983

≡ *Scopulariopsis tardifaciens* Y. Horie & Udagawa, Mycotaxon 17: 331. 1983.

Notes — According to the protologue, this species is similar morphologically in colony features and its asexual morph to *M. albonigrescens* and *S. acremonium*. In this sense, our phylogenetic analysis (Fig. 1) showed that these species could probably correspond to a species of the former genus *Acaulium*. However, the ex-type culture (NHL 2912) of *M. tardifaciens* was not available for study.

Pithoascus platysporus Arx & Veenb.-Rijks, Persoonia 7: 374. 1973

Specimen examined. The Netherlands, Wageningen, from agricultural soil, date unknown, *J.W. Veenbaas-Rijks* (ex-type culture CBS 419.73).

Notes — This species was described showing reddish brown, broadly cylindrical ascospores that differs from the main characteristics of the genus *Pithoascus*. According to Abbott et al. (2002), the ascospore morphology suggests a closer affinity to *Kernia* or *Lophotrichus*. Although we were unable to obtain sporulation from the ex-type strain (CBS 419.73), the analysis of the LSU and ITS sequences showed that this taxon was phylogenetically far from the *Microascales* and was closely related with the *Hypocreales*.

Scopulariopsis acremonium (Delacr.) Vuill., Bull. Soc. Mycol. France 27: 148. 1911

Basionym. *Monilia acremonium* Delacr., Soc. Mycol. Fr. 13: 114. 1897.
= *Scopulariopsis communis* Bainier, Bull. Soc. Mycol. France 23: 125.1907.
= *Penicillium scopulariopsis* Sacc., Syll. Fung. 22: 1275. 1913.
= *Oospora glabra* Hanzawa, J. Coll. Agric. Tohuko Imper. Univ. 4: 1912.
= *Scopulariopsis candelabrum* Loubière, Rech. Struct. Mucor. (Thesis), Paris: 63. 1924.
= *Penicillium brevicaule* Sacc. var. *glabrum* Thom, Bull. U.S. Bur. Anim. Ind. 118: 48. 1910.
≡ *Scopulariopsis brevicaulis* (Sacc.) Bainier var. *glabra* (Thom) Thom in The Penicillia: 250. 1930.
= *Scopulariopsis danica* J.F.H. Beyma, Zentralbl. Bakteriol., 2 Abt. 99: 390. 1939.

Specimens examined. Denmark, from horse skin infected with *Trichophyton* sp., 1938, *C. Werdelin* (*Scopulariopsis danica* ex-type culture MUCL 9028). – Germany, from wheat field soil, 1963, *W. Gams* (MUCL 8274); from soil, collector unknown (MUCL 8409).

Notes — This species was transferred to *Scopulariopsis* from *Monilia acremonium* by Vuillemin (1911). No ex-type strain of *M. acremonium* exists. This taxon was later considered conspecific with *Scopulariopsis danica* by Morton & Smith (1963), from which an ex-type culture (MUCL 9028) was available. Our phylogenetic and morphological studies seem to confirm this

synonymy; however, this species is phylogenetically distant from *Scopulariopsis* and related to the genera *Cephalotrichum*, *Gamsia*, *Trichurus* and *Wardomyces*, clustering with a reference strain of *S. albonigrescens* and probably corresponding to a species of the former genus *Acaulium* (see *M. albonigrescens*).

Scopulariopsis argentea Szilvinyi, Zentralbl. Bakteriol., 2 Abt. 103: 173. 1941

Notes — Type material was studied by Morton & Smith (1963) and considered as 'unidentifiable'. The protologue suggest a species of *Paecilomyces*. No living ex-type material is available.

Scopulariopsis bertaccini Redaelli, Giorn. Ital. Derm. Syph. 75: 825. 1934

Notes — Type material was studied by Morton & Smith (1963), and considered not to be a *Scopulariopsis* species. No living type material is available.

Scopulariopsis canadensis F.J. Morton & G. Sm., Mycol. Pap. 86: 55. 1963

Specimen examined. Canada, British Columbia, from seed of *Beta vulgaris*, 1958, *S.J. Hughes* (ex-type culture CBS 204.61).

Notes — Our phylogenetic data showed that the ex-type culture (CBS 204.61) is related to the *Xylariales*.

Scopulariopsis carbonaria F.J. Morton & G. Sm., Mycol. Pap. 86: 59. 1963

Specimen examined. Panama, from soil, 1961, *R. Coghill* (ex-type culture MUCL 9027 = CBS 205.61).

Notes — Our phylogenetic data on LSU, ITS, EF-1α and TUB showed that the ex-type strain (MUCL 9027) formed an isolated lineage basal to the *Microascus* and *Pithoascus* clades. However, the ex-type culture was sterile, impeding further comparisons.

Scopulariopsis castellanii M. Ota & Komaya, Dermatol. Wochenschrift 78: 163. 1924

Notes — Morton & Smith (1963) considered its original description as 'unidentifiable'. Since no living type material exists, the identity of this taxon remains unknown.

Scopulariopsis coprophila (Cooke & Massee) W. Gams, Cephalosporium-artige Schimmelpilze (Stuttgart): 207. 1971

Basionym. *Monosporium coprophilum* Cooke & Massee, Grevillea 16: 10. 1887.
= *Monilia fimicola* Costantin & Matr., Rev. Gén. Bot. 6: 292. 1894.
≡ *Oospora fimicola* (Costantin & Matr.) Cub. & Megliola, C. R. Accad. Lincei: 440. 1903.
≡ *Scopulariopsis fimicola* (Costantin & Matr.) Vuill., Bull. Soc. Mycol. France 27: 143. 1911.

Specimen examined. UK, from mushroom bed, 1946, *C.J. La Touche* (as *Scopulariopsis fimicola* MUCL 9030 = CBS 206.61).

Notes — Originally described as *Monosporium coprophilum*, this species was transferred to *Scopulariopsis* by Gams (1971) who also considered it to be conspecific with *Monilia fimicola*. The latter taxon had been previously transferred to *Scopulariopsis* by Vuillemin (1911) as *Scopulariopsis fimicola*, a species that was regarded as valid by Morton & Smith (1963); however, these authors did not consider *M. coprophilum*, whose oldest epithet has priority over *fimicola*. According to their observations, *S. fimicola* shares colonial and micromorphological characteristics with *Scopulariopsis baarnensis*, both species being included in different series of *Scopulariopsis* (Morton & Smith 1963). The latter species was transferred to the genus *Gliomastix*, as *G. murorum* var. *polychroma* by Dickinson (1968), currently named *Gliomastix polychromum* (*Bionectriaceae*, *Hypocreales*) (Summerbell et al. 2011).

Although no ex-type culture of *S. coprophila* is available, two reference strains were included in this study, one of them (MUCL 9641) was reidentified here as *M. trigonosporus*. Interestingly, the second strain (CBS 206.61), according to its LSU and ITS sequences it is phylogenetically related with members of *Bionectriaceae*.

Scopulariopsis finkii Sartory & R. Sartory ex Vuill., Encycl. Mycol. 2: 65. 1931

Notes — Original description of this fungus is too vague and inadequate for recognition of the species (Morton & Smith 1963). Type material does not exist.

Scopulariopsis halophilica Tubaki, Trans. Mycol. Soc. Japan 14: 367. 1973

Specimen examined. Japan, Osaka, from *Undaria pinnatifida*, 1974, *K. Tubaki* (ex-type culture CBS 380.74).

Notes — This name was considered by Pitt & Hocking (1985) to be a synonym of *Basipetospora halophila*, a fungus formerly described as *Oospora halophila* by van Beyma (1933). Recently, Samson et al. (2014) transferred this species to the genus *Aspergillus* under the new name *A. baarnensis*. Our phylogenetic data on LSU and ITS confirmed those results, showing its relationships with the *Eurotiales*.

Scopulariopsis hanii Moustafa & Abdul-Wahid, Nova Hedwigia 51: 476. 1990

Notes — According to the protologue, this fungus is morphologically compatible with the asexual morph of *Microascus* and should be considered as member of this genus. In many aspects, this species resembles *M. restrictus* and *M. verrucosus* from which it can be differentiated by having annellides, conidia and solitary sessile conidia at least two times larger. The protologue indicates that holotype material was deposited in the Herbarium of the Royal Botanic Gardens (IMI 326933). However, this accession number corresponds to an isolate of *Scopulariopsis croci*. No living culture of *S. hanii* was available for study.

Scopulariopsis lanosa J.F.H. Beyma, Zentralbl. Bakteriol., 2 Abt. 99: 423. 1937

Notes — This species was excluded from the genus by Morton & Smith (1963). No ex-type strain is available.

Scopulariopsis lilacea Szilvinyi, Zentralbl. Bakteriol., 2 Abt. 103: 174. 1941

Notes — Morton & Smith (1963) regarded the species as 'unidentifiable'. The illustrations included in the protologue seem to represent a conidial apparatus similar to *Fusarium*, while none of the described structures fits with those of *Scopulariopsis*. No live cultures were available.

Scopulariopsis lingualis Neto bis & C. Martins, Compt. Rend. Seanc. Soc. Biol. 106: 1179. 1931

Notes — Morton & Smith (1963) regarded the species as 'unidentifiable'. No living type material was available.

Scopulariopsis longipes H.Q. Pan & T.Y. Zhang, Mycosystema 33: 2. 2014

Notes — This species was recently described based only on morphological features but no phylogenetic study was carried out. Unfortunately, the type material listed in the protologue (holotype HMAS 196252, dried culture HSAUP II $_{07}$4334) is not available for comparison. According to the authors, *S. longipes* morphologically resembles *S. fusca* (syn. *S. asperula*), but differs in having smaller conidia (3.5–5 µm wide vs 5–8 µm in *S. fusca*).

Scopulariopsis maduramycosis Q.T. Chen, Chin. Med. J. 99: 378. 1986

Notes — This name has been invalidated since it was published without an adequate description or holotype information.

Scopulariopsis mencieri C.W. Dodge, Medical Mycology. Fungous diseases of men and other mammals: 648. 1935

Notes — According to Morton & Smith (1963) the description is inadequate. No living type material is available.

Scopulariopsis minima Sartory, Hufschm. & J. Mey., Bull. Acad. Méd. Paris, sér. 3, 103: 606. 1930

Notes — This species is not a *Scopulariopsis* according to Morton & Smith (1963). No living material is available.

Scopulariopsis mottai Vuill., Encycl. Mycol. 2: 62. 1931

Notes — Considered as a doubtful species by Dodge (1935), and probably not a *Scopulariopsis* according to Morton & Smith (1963). No living material is available.

Scopulariopsis musae Matsush., Matsush. Mycol. Mem. 5: 27. 1987

Notes — The protologue of this species has morphological features that could correspond with an asexual state of *Microascus*. However, it shows asymmetrical, curved and extremely large conidia, features that do not match with the typical characteristics of the genus. No living culture is available for study.

Scopulariopsis nicotianae J.F.H. Beyma, Zentralbl. Bakteriol., 2 Abt. 91: 354. 1933

Notes — According to Morton & Smith (1963) the fungus probably belongs to a fungal genus different from *Scopulariopsis*. No living material is available.

Scopulariopsis nivea Demelius, Verh. Zool.-Bot. Ges. Wien 66: 490. 1916

Notes — According to the original description the fungus might represent a *Scopulariopsis* species; however, the description is too vague. No living specimen is available.

Scopulariopsis olivacea Szilvinyi, Zentralbl. Bakteriol., 2 Abt. 103: 174. 1941

Notes — Description and illustrations of the protologue seem to indicate that this is a *Penicillium* species. No living cultures are available.

Scopulariopsis parva (A.H.S. Br. & G. Sm.) Samson, Stud. Mycol. 6: 102. 1974

Basionym. *Paecilomyces parvus* A.H.S. Br. & G. Sm., Trans. Brit. Mycol. Soc. 40: 58. 1957.

Specimen examined. Canada, Alberta, from soil, 1961, *J.W. Carmichael* (*Scopulariopsis parvula* ex-type culture MUCL 9041 = CBS 209.61).

Notes — This species was originally described as *Scopulariopsis parvula* by Morton & Smith (1963). Samson (1974) considered this taxon as synonym of the older species *Paecilomyces parvus*, transferred to *Scopulariopsis* since it produces conidia with a truncate base, thus the new combination *S. parva* was established. However, the analysis of the LSU and ITS sequences of the ex-type culture (basionym *Scopulariopsis parvula* MUCL 9041) showed that this fungus is related with the *Eurotiomycetes*.

Scopulariopsis penicillioides H.Q. Pan, Y.L. Jiang, H.F. Wang & T.Y. Zhang, Mycosystema 33: 3. 2014

Notes — This name is an illegitimate later homonym of *Scopulariopsis penicillioides* (Delacroix) Smith & Ramsbottom (1915), a species considered a synonym of *S. brevicaulis* in Morton & Smith (1963), but not listed in the repositories for fungal names such as Index Fungorum or MycoBank. Comparing the original descriptions of these two fungi we conclude that they are different species. While *S. penicillioides* (Delacroix) Smith & Ramsbottom, a species based on *Monilia penicillioides* Delacroix (1897), shows pale-yellow, oval and echinulate conidia, the fungus described by Pan et al. (2014) has pale-brown, ellipsoidal to broadly obovoid and smooth-walled conidia. The latter fungus resembles *Pseudoscopulariopsis hibernica* but mainly differs in having narrower conidia (3–4.5 µm vs 5–6 µm in *P. hibernica*). The molecular study of these fungi has not been carried out because the type material listed in the protologue of *S. penicillioides* (holotype HMAS 196253, dried culture HSAUP II $_{07}$4299) is not available for comparison.

Scopulariopsis polychromica Szilvinyi, Zentralbl. Bakteriol., 2 Abt. 103: 175. 1941

Notes — According to Morton & Smith (1963) the fungus is unrecognisable from the original description. The original illustrations seem to represent a 'degenerated' strain. A type does not exist.

Scopulariopsis rosacea Szilvinyi, Zentralbl. Bakteriol., 2 Abt. 103: 175. 1941

Notes — 'Unidentifiable' according to Morton & Smith (1963). No living material is available.

Scopulariopsis rubellus Bainier, Bull. Soc. Mycol. France 23: 104. 1907

Notes — Inadequately described according to Morton & Smith (1963). An ex-type strain does not exist.

Scopulariopsis sehnsuchta Mello, Bull. Soc. Pathol. Exot. 25: 296. 1932

Notes — This fungus was poorly described according to Morton & Smith (1963). No living material is available.

Scopulariopsis silvatica (Oudem.) Apinis, Nova Hedwigia 5: 73. 1963

Basionym. *Spicaria silvatica* Oudem., Arch. Néerl. Sci., sér. 2, 7: 291. 1902.

Notes — The description of *S. silvatica* (Apinis 1962) and the illustrations of the basionym *Spicaria silvatica*, seem to represent a fungus not belonging to *Scopulariopsis*, showing a phialidic conidiogenesis with abundant intercalary phialides. No living material is available for examination.

Scopulariopsis spinosa E. Müll. & Pacha-Aue, Nova Hedwigia 18: 161. 1970

Notes — The original illustrations of the fungus suggest the conidial apparatus typical of a *Penicillium* species. No living culture is available.

Scopulariopsis sputicola (Galippe) C.W. Dodge, Medical Mycology. Fungous diseases of men and other mammals: 648. 1935

Basionym. *Monilia sputicola* Galippe, J. Anatomie 21: 538. 1885.

Notes — 'Unidentifiable' according to Morton & Smith (1963). No living material is available.

Scopulariopsis tritici K.B. Deshp. & K.S. Deshp., Curr. Sci. 34: 222. 1965

Notes — The original description and illustration seem to belong to a species of *Stachybotrys* or related taxa. According to the authors of the species, the holotype and an ex-type culture were deposited in the Herbarium Cryptogamae India Orientalia (New Delhi) and in the Herbarium of the Botany Department, Marathwada University (Aurangabad) from India. However, no records of this species were found in the respective catalogues.

Scopulariopsis venerei Greco, Origine des Tumeurs (Etiologie du Cancer, etc.) et Observations de Mycoses (Blastomycoses, etc.) Argentines (Buenos Aires): 716. 1916

Notes — Morton & Smith (1963) considered this fungus as a possible species of *Botrytis*. No living material is available.

Scopulariopsis verticillioides Kamyschko, Notul. Syst. Sect. Cryptog. Inst. Bot. Acad. Sci. U.S.S.R. 14: 225. 1961

Notes — Living material of this species is not available.

Scopulariopsis verrucaria ('*verrucifera*') H.F. Wang & T.Y. Zhang, Mycosystema 33: 4. 2014

Notes — This species was recently described based only on morphological features. This has not been included in our phylogenetic study because the type material listed in the protologue (holotype HMAS 196254, dried culture HSAUP II $_{06}$4334) is not available for comparison. According to the original description and illustration of the species, it is similar to *Microascus verrucosus*. However, *S. verrucaria* differs in having dark brown colonies, and conidia of 3–5 µm wide covered by a gelatinous membrane, while *M. verrucosus* has olive grey colonies and rough, somewhat wider conidia (5–7 µm).

Scopulariopsis vignolo-lutatii (Matr.) C.W. Dodge, Medical Mycology. Fungous diseases of men and other mammals: 650. 1935

Basionym. *Acaulium vignolo-lutatii* Matr. (as *Vignoli-Lutatii*), in Vignolo-Lutati, Arch. Derm. Syph., Berlin 118: 690. 1913.

Notes — Dodge (1935), although with no strong conviction due to its inadequate description, transferred *Acaulium vignoli-lutatii* to *Scopulariopsis*. Morton & Smith (1963) considered this species as a possible member of the *Scopulariopsis brumptii* series, but with an inadequate description for recognition of the fungus. The original description and illustration seem to refer to a fungus morphologically close to *M. paisii*. No living cultures are available for study.

Scopulariopsis yunnanensis Q.T. Chen & C.L. Jiang, Acta Mycol. Sin. 4: 167. 1985

Notes — The original description seems to refer to a *Scopulariopsis* species. However, it was described forming bifurcate chains of conidia. In addition, the conidia are smaller than those of the currently known *Scopulariopsis* species. According to the authors (Jiang et al. 1985), *S. yunnanensis* is similar to *S. parva*, a species that according to our results is phylogenetically related with the *Eurotiomycetes*. The ex-type strain of this species is not available.

DISCUSSION

In this study we have reviewed the taxonomic circumscription of *Microascus* and *Scopulariopsis*, traditionally referred to as sexual and asexual morphs, respectively, and related genera using a polyphasic approach based on the evaluation of molecular, physiological and morphological data. These results show that *Microascus* and *Scopulariopsis* constitute two phylogenetically distant lineages, which are clearly different from *Pithoascus*, a genus revalidated in the present work, and from the lineage proposed here as the novel genus *Pseudoscopulariopsis*. Furthermore, combining the results of a multilocus sequence analysis and phenotypic data, we were able to delineate the accepted species of the four genera, proposing several new ones.

One of the first attempts to clarify phylogenetically the relationships among the different genera of the *Microascales* by the use of partial LSU sequences was that of Issakainen et al. (2003). That study demonstrated polyphyly of several genera of *Microascaceae* and raised questions concerning correct positions of several members of the family and their generic circumscriptions, suggesting a possible subdivision of *Microascus* and *Scopulariopsis* into several smaller genera. However, the LSU fragments used were too small and poorly informative, thus no final conclusions were made. Nevertheless, our phylogenetic analysis based on combined, longer LSU and ITS sequences proved to be useful to resolve topology of the different genera in the *Microascacae*. Confirming the findings of Issakainen et al. (2003), we demonstrated that *Microascus* and *Scopulariopsis* are clearly polyphyletic and that their currently accepted species are grouped in at least seven different lineages. In addition, the combined LSU and ITS phylogeny showed that the dry-spored synnematous genera *Cephalotrichum*, *Doratomyces*, *Stysanus*, and *Trichurus* are conspecific, which agrees with Abbott (2000) who synonymised and integrated these four genera under the name *Cephalotrichum*. However, given the lack of ex-type strains for most of the species, no formal decision is made at the moment. The genus *Cephalotrichum*, as well as several other genera of the *Microascaceae*, such as *Kernia*, *Wardomyces* and *Wardomycopsis*, have been considered in the past as probably congeneric with *Scopulariopsis* or *Microascus* (Morton & Smith 1963, Abbott 2000). Our phylogeny demonstrates that, although these genera share similar morphological and ecological traits, they are in fact genetically distant. The phylogenetic data is supported by relevant morphological differences, such as the presence of germ slits, synnemata or conspicuously hairy ascomata (Abbott 2000, Issakainen et al. 2003).

The recognition of *Pithoascus* as a valid genus has always been a matter of discussion. Probably one of the strongest reasons to synonymise the genus with *Microascus* was the

Table 2 Relevant phenotypic features of members of *Microascus*, *Pithoascus*, *Pseudoscopulariopsis* and *Scopulariopsis*.

Species	Colony	Sexual morph	Asexual morph	Ascomata diam (µm)	Ascospore size (µm)	Ascospore shape	Germ pore	Conidial size (µm)	Conidial shape	Growth at (° C)				
										5	25	30	35	40
Microascus														
M. alveolaris	white to grey	+	+	110–290	4–6 × 3–5	broadly triangular	+	3–5 × 2–3.5	ellipsoidal to bullet shaped	–	+	+	+	+
M. brunneosporus	dull green to olive brown	+	+	110–205	5–7 × 2–3	ellipsoidal	+	4–5 × 2.5–5	subglobose to navicular	–	+	+	+	–
M. campaniformis	dull green	+	+	150–220	6–7 × 4–4.5	triangular with an elongated side	+	4–5 × 2.5–3.5	subglobose to broadly ellipsoidal	–	+	+	+	+
M. chartarus	grey to smoke grey	–	+					4–5.5 × 3–4	ovate	–	+	–	–	–
M. cinereus	light to dark grey	+	+	95–300	4–5.5 × 2.5–4	reniform, broadly lunate, rarely triangular	+	3–5 × 2–3	obovate or clavate	–	+	+	+	+
M. cirrosus	brown-grey	+	+	140–230	5–6 × 3–4	broadly reniform	+	4–6.5 × 4–6	subglobose to obovate	–	+	+	+	+
M. croci	grey to mouse grey	–	+					3.5–5 × 3–4	globose	+	+	+	–	–
M. expansus	olive to grey-brown	–	+					4–8 × 2.5–3.5	bullet shaped to broadly clavate	+	+	+	+	+
M. gracilis	dull to olive green	+	+	130–300	4.5–6.5 × 2–4	lunate	+	3.5–5.5 × 2–3.5	subglobose or ellipsoidal	–	+	+	+	+
M. hyalinus	dark grey	+	+	50–180	3.5–5 × 2–3.5	broadly ovoid	+	3.5–5 × 2–3.5	ovoid	–	+	+	+	–
M. intricatus	white to olive grey	+	+	140–200	5–6 × 2.5–3.5	fusiform	+	4–5 × 3–3.5	globose to broadly ellipsoidal	–	+	+	+	+
M. longirostris	grey-black to brown-black	+	+	180–300	3–4 × 2–3.5	reniform	+	4–6 × 3–4	obovate	–	+	+	–	–
M. macrosporus	grey opaque	+	+	130–250	5–6.5 × 5.5–7.5	triangular	+	5–7 × 4–5	globose to ovoid	–	+	+	+	+
M. murinus	pale to dark grey	–	+					4–6 × 1.5–2	cylindrical	–	+	+	+	–
M. paisii	grey-brown grey-black	–	+					4–6 × 2–4.5	broadly ellipsoidal to short clavate	–	+	+	+	–
M. pyramidus	grey to violaceus grey	+	+	125–250	5–6.5 × 5.5–7	triangular or quadrangular with attenuated ends	+	4.5–5.5 × 3–4	obovate	–	+	+	+	+
M. restrictus	olive to brown-grey	–	+					4.5–6 × 4–5.5	globose to obovoidal	–	+	+	+	+
M. senegalensis	pale brown	+	+	180–250	7–9 × 2–3	reniform	+	4.5–5.5 × 3.5–4	obovate	–	+	+	+	–
M. trigonosporus	grey	+	+	130–250	3–5 × 3–4	triangular	+	2.5–3.5 × 3.5–5.5	globose to ovoid	–	+	+	+	–
M. verrucosus	olive grey	–	+					5–7 × 4.5–6	globose to subglobose	–	+	+	+	–
Pithoascus														
P. ater	brown-black	–	+					4–9 × 4.5–8.5	globose to pyriform	+	+	+	+	–
P. exsertus	white	+	–	210–450	6.5–12 × 1–2.5	falcate	–			–	+	+	+	+
P. intermedius[1]	white to light grey	+	+	95–150	5–6 × 2–2.5	fusiform	–	4–8 × 4–7.5	globose to subglobose	–	+	+	+	–
P. nidicola	white to light grey	+	+	90–160	6–8 × 2–2.5	fusiform, nearly lunate	–	4–5 × 2.5–3.5	globose to ampulliform	–	+	+	+	+
P. stoveri[2]	light to dark grey	+	+	50–110	6–7.5 × 2–3	navicular	–	5–8 × 3–4	obovate to pyriform	–	+	+	–	–
Pseudoscopulariopsis														
P. hibernica	olive grey	–	+					5–7 × 5–6	subglobose	–	+	–	–	–
P. schumacheri[3]	grey-white	+	+	140–190	8–13 × 2.5–4	fusiform or navicular	–	4.5–6 × 2.5–4	obovate, short clavate	–	+	–	–	–
Scopulariopsis														
S. asperula[4]	dark brown	+	+	130–190	4.5–6.5 × 3.5–4	broadly reniform		5–8 × 4–6.5	globose to ovate	–	+	+	+	–
S. brevicaulis	tan	+	+	70–150	5–6 × 3.5–4.5	broadly reniform	+	6–9 × 5.5–9	globose to ovate	–	+	+	+	+
S. candida	white	+	+	100–170	4–6 × 5–6	reniform, heart shaped	+	6–9.5 × 5–8.5	subglobose to broadly ovate	–	+	+	+	–
S. cordiae	white to light grey	+	+	100–150	4.5–5.5 × 3.5–4	reniform	+	2.5–6 × 2–5	broadly ellipsoidal to obovoidal	–	+	+	+	+
S. flava	white	–	+					6.5–7 × 5.5–6.5	globose to obovoidal	–	+	+	+	–
S. soppii	white	+	+	130–200	6–7 × 2.5–3	lunate	+	5.5–9 × 5–8	globose to subglobose	–	+	+	+	–

[1] Asexual morph data obtained from Roberts (1985).
[2] Asexual morph data obtained from von Arx et al. (1988).
[3] Sexual morph data obtained from von Arx et al. (1988).
[4] Sexual morph data obtained from Abbott & Sigler (2001).

publication of the species *M. caviariformis* by Malloch & Hubart (1987), a fungus exhibiting intermediate characteristics between *Microascus* and *Pithoascus*. *Microascus caviariformis* showed the typical ascospores of *Pithoascus*, although with an inconspicuous germ pore, and produced abundant conidia. Our study demonstrated that both genera were clearly separated, thus confirming the original observations made by Skou (1973) and von Arx (1973a, b). Moreover, our data showed that *M. caviariformis* was located outside the main clades, which represent the genera *Microascus*, *Pithoascus*, *Scopulariopsis* and *Pseudoscopulariopsis*, forming a distant and strongly supported clade with *S. acremonium* and a reference strain of *M. albonigrescens*. The latter fungus was described as the sexual morph of the type species of *Acaulium* (Sopp 1912), and this genus was considered as congeneric with *Microascus* (Curzi 1930, Barron et al. 1961, Morton & Smith 1963). However, our phylogenetic analysis suggests that the old genus *Acaulium* might be revalidated. Considering that neither original cultures of Sopp, nor an ex-type strain of *M. albonigrescens* were available for analysis, we think it is best to not propose further taxonomic changes at the moment. Furthermore, *M. singularis* proved to be related to the putative *Acaulium* clade, which also constituted a new lineage among the *Microascaceae*. More study is needed on members of these clades and their closest phylogenetic relatives.

Although the morphological differences distinguishing the genera treated here are subtle, they correlate with the phylogenetic data, shape, size and colour of annellides and conidia, shape of ascospores and presence of germ pores being the most informative morphological characters (Table 2). Other features, such as shape and size of ascomata and number and shape of ostiolar necks, are frequently associated with environmental changes related to incubation conditions (Barron et al. 1961). On the basis of our data, we recommend using PCA or OA culture media to achieve the best growth and sporulation ratio. On PCA these fungi produce fast and abundant sporulation but some species, particularly those with little growth of their asexual morphs, might sporulate better on OA. It is most likely that variable morphological differences observed in the past might have led to incorrect identification of strains due to overlapping characteristics between closely related species. For example, two strains (MUCL 9048 and MUCL 9049) that were received as *M. cinereus* were finally reidentified as belonging to the undescribed sexual morph of *M. gracilis*, a species morphologically close to *M. cinereus* but differentiated by size and complexity of conidiophores and shape of ascospores (Table 2). Barron et al. (1961) and Udagawa (1962) mentioned a wide variation in size and shape of ascospores of isolates identified as *M. trigonosporus*, i.e. from triangular to nearly quadrangular, with some isolates showing spores distinctly longer at one side, but judging from our data this ascospore shape variation might correspond to different phylogenetic species. Most of the species of *Microascus* newly described here showed triangular ascospores of variable size. The new species *M. campaniformis* produced inequilateral spores with the longer side towards the germ pore, although the measurements do not coincide with those previously described by the authors mentioned above.

One of the main objectives of the present work was to assess the phylogenetic relationships among members of *Microascus* and *Scopulariopsis* in order to comply with the requirements of the new International Code of Nomenclature for fungi, algae and plants (Hawksworth et al. 2011). As has been discussed extensively by Hawksworth (2012), for this particular dual-name combination an option might be to retain the most 'widely-used' name. Accordingly, *Scopulariopsis* should have priority over *Microascus*, primarily because of the abundant medical literature on this genus (Hawksworth 2012, Sandoval-Denis et al. 2013). Our proposal to separate both genera is an alternative approach that maintains the names of the most relevant species of each genus, including those of the species that are significant in medicine as well as some important plant pathogens.

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4.5 Phylogeny of the clinically relevant species of the emerging fungus *Trichoderma* and their antifungal susceptibilities

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Phylogeny of the Clinically Relevant Species of the Emerging Fungus *Trichoderma* and Their Antifungal Susceptibilities

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A set of 73 isolates of the emerging fungus *Trichoderma* isolated from human and animal clinical specimens were characterized morphologically and molecularly using a multilocus sequence analysis that included the internal transcribed spacer (ITS) regions of the nuclear ribosomal DNA and fragments of the translation elongation factor 1 alpha (*Tef1*), endochitinase CHI18-5 (*Chi18-5*), and actin 1 (*Act1*) genes. The most frequent species was *Trichoderma longibrachiatum* (26%), followed by *Trichoderma citrinoviride* (18%), the *Hypocrea lixii/Trichoderma harzianum* species complex (15%), the newly described species *Trichoderma bissettii* (12%), and *Trichoderma orientale* (11%). The most common anatomical sites of isolation in human clinical specimens were the respiratory tract (40%), followed by deep tissue (30%) and superficial tissues (26%), while all the animal-associated isolates were obtained from superficial tissue samples. Susceptibilities of the isolates to eight antifungal drugs *in vitro* showed mostly high MICs, except for voriconazole and the echinocandins.

The genus *Trichoderma* of the order *Hypocreales* (*Ascomycota*) comprises a large number of saprobic species with a worldwide distribution (1). They are commonly found in soil and play an important role as decomposers of decaying plant material and insect pathogens (2). In addition, members of this genus are important biotechnologically due to their ability to produce a wide spectrum of bioactive compounds (3).

Trichoderma species are infrequent but emergent human pathogens. *Trichoderma* infections in humans have been related with several risk factors, being associated mostly with peritoneal dialysis, organ transplantation, and hematologic disorders (4). They cause severe and persistent disseminated infections that usually fail to respond to treatment with amphotericin B (AMB) or voriconazole (VRC) (5–9). Other diseases attributed to members of this genus are allergic and acute invasive sinusitis (10, 11), keratitis (12), otitis externa (13), skin and subcutaneous infections (14), peritonitis (9, 15–20), deep pulmonary infections (21–23), endocarditis (24), and brain abscess (25). Most infections are caused by *Trichoderma longibrachiatum*, which is recognized as the main human pathogen of the genus (4, 11, 26), but eight other species (i.e., *T. atroviride*, *T. citrinoviride*, *T. harzianum*, *T. koningii*, *T. orientale*, *T. pseudokoningii*, *T. reesei*, and *T. viride*) have also been reported occasionally (4, 26–28). On the other hand, data on animal infections by *Trichoderma* spp. are very limited. The few case reports available mostly involve superficial infections in cold-blooded animals (29, 30), and more recently, there were three cases of pulmonary infection by *T. pseudokoningii* (31).

Trichoderma isolates can be easily recognized by their characteristic green and rapidly growing colonies, as well as the typical branching patterns of the conidiophores. Traditionally, those species that developed the sexual morph were included in the genus *Hypocrea*. Despite the recent change in fungal nomenclature in favor of "one fungus, one name" (32), in this group of fungi both generic names are still used (33). Classically, species in the genus have been organized in "species aggregates" (34) and later in sections and clades (35–38). This latter taxonomical scheme reflects the modern phylogenetic-based classification of the genus (39). Most clinical reports have based identification of the etiological agents on their morphological characteristics (17). However, morphological identification of *Trichoderma* species can be problematic, because some species show great homoplasy in their conidial structures (39, 40). DNA sequence analysis has helped clarify the taxonomy of *Trichoderma*, and several new species have been described based on multigene phylogenies (39, 41, 42). In the clinical setting, species identification has been based mainly on ribosomal DNA (rDNA) sequences (8, 23, 28, 40), although it has been shown that the use of this locus does not provide sufficient resolution to accurately distinguish closely related species (4, 26, 41, 43). This, together with the poor *in vitro* activities of commonly used antifungals against *Trichoderma* isolates, is an impediment for the treatment of *Trichoderma* infections (11, 43) and limits our understanding of the epidemiology of the species.

The objective of this study was to assess the spectrum of *Trichoderma* species in clinical samples. Herein, a large set of clinical isolates was identified by using morphological and molecular data, via comparison of the multilocus sequences with those of reference strains. In addition, the *in vitro* antifungal susceptibilities of these isolates to eight currently used antifungal drugs were determined.

MATERIALS AND METHODS

Fungal isolates and sequences. A total of 73 isolates of *Trichoderma* were included in this study; 63 were obtained from human clinical specimens and 10 from animal clinical sources (Table 1). Most of them were from the

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TABLE 1 Origin and GenBank accession numbers of the sequences of the *Trichoderma* isolates identified in this study

Species	Isolate no.[a]	Origin[b]	GenBank accession no.			
			ITS	*Tef1*	*Chi18-5*	*Act1*
H. lixii/T. harzianum species complex	UTHSC 02-2663	Human maxillar sinus, USA	KJ174168	HG931199	HG931272	
	UTHSC 03-2105	Marine sponge, USA	KJ174171	HG931202	HG931275	
	UTHSC 05-2749	Human sputum, USA	KJ174170	HG931200	HG931274	
	UTHSC 04-134	Manatee, USA	KJ174169	HG931200	HG931273	
	UTHSC 07-2109	Human blood, USA	KJ174172	HG931203	HG931276	
	UTHSC 08-418	Sea turtle, USA	KJ174173	HG931204	HG931277	
	UTHSC 09-3558	Human cornea, USA	KJ174174	HG931205	HG931278	
	UTHSC 10-1527	Human BAL, USA	KJ174175	HG931206	HG931279	
	UTHSC 11-2939	Goat hair, USA	KJ174176	HG931207	HG931280	
	UTHSC 11-3209	Marine sponge, USA	KJ174177	HG931208	HG931281	
	UTHSC 11-3234	Human stool, USA	KJ174178	HG931209	HG931282	
T. asperelloides	UTHSC 07-2264	Human nails, USA	KJ174188	HG931219		HG931187
	UTHSC 09-1326	Marine sponge, USA	KJ174189	HG931220		HG931188
T. asperellum	UTHSC 07-1832	Human sputum, USA	KJ174187	HG931218		HG931186
T. atroviride	UTHSC 03-1690	Manatee skin, USA	KJ174190	HG931221		HG931189
	UTHSC 08-2439	Marine sponge, USA	KJ174191	HG931222		HG931190
	UTHSC 10-2682	Turtle shell, USA	KJ174192	HG931223		HG931191
	UTHSC 11-1239	Human lung mass, USA	KJ174193	HG931224		HG931192
T. bissettii sp. nov.	UTHSC 07-852	Human sinus, USA	KJ174232	HG931263	HG931323	
	UTHSC 07-2998	Human nails, USA	KJ174233	HG931264	HG931324	
	UTHSC 08-615	Human wound, USA	KJ174234	HG931265	HG931325	
	UTHSC 08-2443^T = CBS 137447	Human sinus, USA	KJ174235	HG931266	HG931326	
	UTHSC 09-2160	Human BAL, USA	KJ174236	HG931267	HG931327	
	UTHSC 11-455	Human foot, USA	KJ174237	HG931268	HG931328	
	UTHSC 12-337	Human bone, USA	KJ174238	HG931269	HG931329	
	UTHSC 12-944	Human vertebral body, USA	KJ174239	HG931270	HG931330	
	UTHSC 12-1543	Human nails, USA	KJ174240	HG931271	HG931331	
T. citrinoviride	UTHSC 03-1479	Human BAL, USA	KJ174194	HG931225	HG931291	
	UTHSC 03-3702	Human blood, USA	KJ174196	HG931227	HG931293	
	UTHSC 06-3324	Human toe nail, USA	KJ174195	HG931226	HG931292	
	UTHSC 08-1945	Human ascitic fluid, USA	KJ174197	HG931228	HG931294	
	UTHSC 09-959	Human pleural fluid, USA	KJ174198	HG931229	HG931295	
	UTHSC 09-2229	Human eye, USA	KJ174199	HG931230	HG931296	
	UTHSC 10-434	Human abdominal wound, USA	KJ174200	HG931231	HG931297	
	UTHSC 10-1704	Human BAL, USA	KJ174201	HG931232	HG931298	
	UTHSC 10-2923	Human lung, USA	KJ174202	HG931233	HG931299	
	UTHSC 11-1116	Human sputum, USA	KJ174203	HG931234	HG931300	
	UTHSC 11-1353	Human blood, USA	KJ174204	HG931235	HG931301	
	UTHSC 11-3314	Human BAL, USA	KJ174205	HG931236	HG931302	
	UTHSC 12-536	Human BAL, USA	KJ174206	HG931237	HG931303	
T. erinaceus	UTHSC 07-1088	Human nails, USA	KJ174207	HG931238		HG931193
T. gamsii	UTHSC 09-135	Human sputum, USA	KJ174208	HG931239		HG931194
T. koningiopsis	UTHSC 06-1272	Human nails, USA	KJ174209	HG931240		HG931195
	UTHSC 11-2740	Human BAL, USA	KJ174210	HG931241		HG931196
	UTHSC 11-3372	Canine footpad, USA	KJ174211	HG931242		HG931197
T. longibrachiatum	UTHSC 06-2352	Human ear, USA	KJ174212	HG931243	HG931304	
	UTHSC 06-2504	Human BAL, USA	KJ174213	HG931244	HG931305	
	UTHSC 06-3659	Human CSF, USA	KJ174214	HG931245	HG931306	
	UTHSC 07-2530	Human BAL, USA	KJ174215	HG931246	HG931307	
	UTHSC 07-3636	Human nail, USA	KJ174216	HG931247	HG931308	
	UTHSC 07-3704	Human nail, USA	KJ174217	HG931248	HG931309	
	UTHSC 07-3821	Human BAL, USA	KJ174218	HG931249	HG931310	

(Continued on following page)

TABLE 1 (Continued)

Species	Isolate no.[a]	Origin[b]	GenBank accession no.			
			ITS	*Tef1*	*Chi18-5*	*Act1*
	UTHSC 08-1222	Human lung tissue, USA	KJ174219	HG931250	HG931311	
	UTHSC 09-2900	Human sputum, USA	KJ174220	HG931251	HG931312	
	UTHSC 09-3339	Human BAL, USA	KJ174221	HG931252	HG931313	
	UTHSC 10-244	Human pleural fluid, USA	KJ174222	HG931253	HG931314	
	UTHSC 10-457	Human maxillar sinus, USA	KJ174223	HG931254	HG931315	
	UTHSC 11-589	Human BAL, USA	KJ174224	HG931255	HG931316	
	UTHSC 11-942	Human peritoneal fluid, USA	KJ174225	HG931256	HG931317	
	UTHSC 11-997	Human mediastinal mass, USA	KJ174226	HG931257	HG931318	
	UTHSC 11-3265	Human blood, USA	KJ174227	HG931258	HG931319	
	UTHSC 11-3571	Human sputum, USA	KJ174228	HG931259	HG931320	
	UTHSC 11-3808	Human sputum, USA	KJ174229	HG931260	HG931321	
	UTHSC 12-264	Human BAL, USA	KJ174230	HG931261	HG931322	
T. orientale	UTHSC 03-91	Human sputum, USA	KJ174179	HG931210	HG931283	
	UTHSC 04-373	Human sinus, USA	KJ174181	HG931212	HG931285	
	UTHSC 06-2183	Human blood, USA	KJ174180	HG931211	HG931284	
	UTHSC 07-285	Human BAL, USA	KJ174182	HG931213	HG931286	
	UTHSC 07-1541	Human peritoneal fluid, USA	KJ174183	HG931214	HG931287	
	UTHSC 09-1967	Human arm, USA	KJ174184	HG931215	HG931288	
	UTHSC 09-2386	Human blood, USA	KJ174185	HG931216	HG931289	
	FMR 12739	Human vascular protesis, Spain	KJ174186	HG931217	HG931290	
T. sinuosum	UTHSC 07-3543	Human skin, USA	KJ174231	HG931262		HG931198

[a] FMR, Facultat de Medicina i Ciències de la Salut, Reus, Spain; UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center.
[b] CSF, cerebrospinal fluid; BAL, broncheoalveolar lavage fluid.

United States and were received at the Fungus Testing Laboratory of the University of Texas Health Science Center at San Antonio (UTHSC) mainly for identification purposes. In addition, 248 sequences corresponding to type or reference strains of *Trichoderma* species or related genera retrieved from GenBank were also included in the phylogenetic analyses.

Morphological identification. The isolates were subcultured onto potato-dextrose agar (PDA; Pronadisa, Spain) and cornmeal agar (cornmeal, 50 g; agar, 15 g; water, 1 liter) with 2% glucose (CMD) (42), incubated in the dark at different temperatures (15, 25, 30, 35, 37, and 40°C), and measured daily to determine colony growth rates. Microscopic observations were made from plate and slide cultures on PDA or CMD plates incubated for 7 to 14 days at 25°C. Slides were mounted on lactic acid or 3% potassium hydroxide and examined using an Olympus CH2 light microscope (Olympus Corporation, Tokyo, Japan). All isolates were identified morphologically after the methods described by Samuels et al. (39, 42, 44) and Jacklitsch (45) and by using an interactive *Trichoderma* identification key (http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm [46]). Color standards followed those of Kornerup and Wanscher (47). Photomicrographs were made with an Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany), using Nomarski differential interference.

DNA extraction, amplification, and sequencing. PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA) was used to extract total genomic DNA from mycelia that were scraped from colonies grown on YES agar (yeast extract, 20 g; sucrose, 150 g; agar, 20 g; distilled water, 1 liter) after 3 to 5 days of incubation at 25°C. DNA was quantified using a Nanodrop 3000 apparatus (Thermo Scientific, Madrid, Spain).

Four different nuclear DNA targets were amplified by PCR and sequenced using the following primer pairs: ITS5/ITS4 for the internal transcribed spacer 1 and 2 (ITS1 and ITS2) and the 5.8S gene of the rRNA (48), EF-1H/EF-2T for a fragment of the translation elongation factor 1 alpha gene (*Tef1*) (49), Chit42-1a/Chit42-2a for a fragment of the endochitinase CHI18-5 gene (*Chi18-5*) (50), and Act-1/Act4R for a fragment of the actin 1 gene (*Act1*) (51), according to the protocols described by the respective authors. The amplified products were purified using the Diffinity rapid tip purification system (Sigma-Aldrich, St. Louis, MO) and stored at −20°C until sequencing.

Amplicons were sequenced in both directions, using the PCR primers at Macrogen Europe (Macrogen Inc., Amsterdam, The Netherlands). Consensus sequences were assembled using SeqMan version 7.0.0 (DNASTAR, Madison, WI).

Molecular identification and phylogenetic analysis. An initial identification was made by comparing ITS sequences with those in *Trich*OKEY (http://isth.info/) (52). Multiple sequence alignments were made in MEGA version 5.05 (53) using the ClustalW application (54), refined with MUSCLE (55), and manually adjusted using the same software platform. Phylogenetic reconstructions were made using the individual loci via maximum-likelihood (ML) and Bayesian inference (BI) with MEGA version 5.05 and MrBayes version 3.1.2 (56), respectively. The best substitution model for all gene matrices (GTR+I+G) was estimated using MrModelTest version 2.3 (57). For ML analyses, nearest-neighbor interchange was used as the heuristic method for tree inference. Support for internal branches was assessed by 1,000 ML bootstrapped pseudoreplicates of data. Bootstrap support (bs) of ≥70 was considered significant. For BI analyses, Markov chain Monte Carlo (MCMC) sampling was performed with two simultaneous runs for 3 million generations, with samples taken every 100 generations. The 50% majority rule consensus trees and posterior probability values (pp) were calculated after removing the first 25% of the resulting trees for burn-in. A pp value of ≥0.95 was considered significant.

The phylogenies obtained from each locus were compared in order to assess for incongruent results among the different DNA matrices, by comparing the phylogenetic placement and internal nodes with significant bs/pp support. Given that no incongruence was observed, the different matrices were combined for the final phylogenetic analyses. Given the high genetic variability observed between the different clades and the in-

ability to obtain a reliable alignment that included all of the species, the different *Trichoderma* clades were analyzed individually using different gene combinations according to previous phylogenetic studies (41, 44, 58, 59): ITS, *Tef1*, and *Chi18-5* for the clades *Longibrachiatum* and *Harzianum* and ITS, *Tef1*, and *Act1* for the clades *Viride*, *Hamatum*, and *Chlorospora*. Analyses of the combined data set were performed using the same parameters mentioned above. *Hypocrea pachybasioides* and *Trichoderma minutisporum* were selected as outgroup taxa.

Antifungal susceptibility testing. Antifungal susceptibility testing was performed according to CLSI document M38-A2 (60) with AMB, VRC, posaconazole (PSC), itraconazole (ITC), caspofungin (CFG), anidulafungin (AFG), micafungin (MFG), and terbinafine (TBF). The minimal effective concentration (MEC) was determined at 24 h for the echinocandins, and the MIC was determined at 48 h for the remaining drugs. The MIC was defined as the lowest concentration exhibiting 100% visual inhibition of growth for AMB, VRC, ITC, and PSC and an 80% reduction in growth for TRB. *Paecilomyces variotii* ATCC MYA-3630 and *Aspergillus fumigatus* ATCC MYA-3626 were used as quality control strains. Statistical analyses of the MIC/MEC data were performed using the Kruskal-Wallis test in the Prism program for Windows, version 6.0 (GraphPad Software, San Diego, CA).

Nucleotide sequence accession numbers. DNA sequences determined in this study have been deposited in GenBank; the accession numbers are reported in Table 1.

RESULTS

Analysis of the ITS sequences showed that our isolates were distributed among five clades of four different sections of the genus *Trichoderma* (data not shown). Most of them (67.1%) belonged to section *Longibrachiatum* (35, 36), clade *Longibrachiatum*; 15.1% belonged to section *Pachybasium* (37, 38), clade *Harzianum*; 12.3% and 4.1% belonged to section *Trichoderma* (37), clades *Viride* and *Hamatum*, respectively; 1.4% of the isolates belonged to section *Hypocreanum* (37), clade *Chlorospora*. Distinct morphological features of the isolates nested in clade *Longibrachiatum* included formation of yellow to green colonies on PDA, sometimes with a diffusible yellow pigment, rapid sporulation at 30 to 35°C, and abundant growth at 40°C. The conidiophores that arose mainly from the aerial hyphae were hyaline and composed of a long, thick central axis from which secondary branches emerged. The phialides were lageniform, cylindrical or with a slight swelling in the middle, hyaline, and smooth walled. They arose singly and directly from the main axis or from the branches or formed whorls on a supporting cell. Intercalary phialides were common. Conidia were oblong to ellipsoidal, green, and smooth walled.

The isolates that belonged to the *Harzianum* clade on the same medium formed flat, white, greyish or pale yellowish colonies that grew and sporulated well at 25 to 30°C, growth was restricted at 35°C, and they were unable to grow at 40°C. The conidiophores were irregularly branched and formed on the aerial hyphae. The phialides were short and ampulliform, often arranged in whorls. Intercalary phialides were not present. The conidia were globose to ovoidal, green, and smooth walled.

The isolates included in the *Viride* and *Hamatum* clades produced yellow or green colonies with different growth rates between 25 and 40°C. A strong coconut odor was present in some isolates. The conidiophores exhibited a pyramidal branching pattern or were verticillate and were formed on the aerial hyphae. The phialides were mostly ampulliform and grouped in whorls. Intercalary phialides were observed in isolates of *T. gamsii* and *T. koningiopsis*. The conidia were yellow or green, globose or ellipsoidal, smooth walled or finely roughened.

The only isolate that belonged to the *Chlorospora* clade was unable to sporulate under the culture conditions used in this study and only could be identified by using molecular methods.

The tree topologies obtained with ML and BI analyses were nearly identical, except for minor differences in internal nodes with low or insignificant statistical support. Phylogenetic analysis of the *Longibrachiatum* clade included 1,388 bp (ITS, *Tef1*, and *Chi18-5*), corresponding to 23 species of *Trichoderma* (Fig. 1). The isolates mainly belonged to three species (i.e., *T. longibrachiatum*, *T. citrinoviride*, and *T. orientale*), while 9 isolates formed a well-supported subclade (bs 100/pp 1.00), which corresponded to a new species-level lineage. Since these isolates were morphologically and phylogenetically different from any previously known species of *Trichoderma*, they are described below as a new species named *Trichoderma bissettii*. Phylogenetic analysis of the *Harzianum* clade included 1,055 bp, based on the same three loci and representing 15 *Trichoderma* species, revealed that all of the isolates were nested with low support in the *H. lixii*/*T. harzianum* species complex sensu Druzhinina et al. (58) (Fig. 2). The majority of the human clinical isolates were nested with the type strain of *H. lixii* (CBS 110080), while only two isolates (UTHSC 07-2109 and UTHSC 11-3234) grouped with the type strain of *T. harzianum* (CBS 226.95). A single isolate (UTHSC 11-3209) grouped with the reference strain *T. afroharzianum* DAOM 231421. Two isolates of animal origin (UTHSC 03-2105 and UTHSC 08-418) grouped with the reference strain of *H. lixii* DAOM 231412, while isolate UTHSC 10-1527 grouped in a different clade with the reference strain of *H. lixii* CBS 115343. Phylogenetic analysis of the *Viride* clade included 1,253 bp (ITS, *Tef1*, and *Act1*) from 22 *Trichoderma* species (Fig. 3). Four isolates grouped with a reference strain of *T. atroviride* (CBS 142.95), with only one of them from human origin. One isolate nested with the type strain of *T. gamsii* (CBS 120075) and another isolate grouped with two reference strains of *T. erinaceus* (DIS 7 and CBS 117088), while two isolates of human origin and one animal-associated isolate nested with the type strain of *T. koningiopsis* (CBS 119075). Phylogenetic analysis of the *Hamatum* clade included 1,274 bp (ITS, *Tef1*, and *Act1*) and 10 species (Fig. 4). One isolate nested with the type strain of *T. asperellum* (CBS 433.97), while two isolates (one of human and one of animal origin) grouped with a reference strain of *T. asperelloides* (CBS 125399). Phylogenetic analysis of the *Chlorospora* clade included 1,917 bp (ITS, *Tef1*, and *Act1*) and 8 species (Fig. 5). The single isolate included in this clade was from a human clinical specimen and grouped with a reference strain of *T. sinuosum* (CBS 114247).

In summary, the molecular identification showed that the isolates belonged to 11 different *Trichoderma* species (Table 2), in addition to those belonging to the *H. lixii*/*T. harzianum* complex. The correlation between the molecular and the morphological identification is shown in Table 3. An agreement between both methods was found for 49.3% of the isolates. All of the isolates within the *T. longibrachiatum* and *H. lixii*/*T. harzianum* species complex were correctly identified morphologically. In contrast, most of the isolates of *T. citrinoviride*, *T. bissettii*, and *T. orientale* were identified morphologically as *T. longibrachiatum* or *T. pseudokoningii*. Most of the isolates identified morphologically as *T. atroviride* corresponded phylogenetically to other species of the *Viride* clade; however, two isolates belonged to the *Hamatum* clade (*T. asperelloides* and *T. asperellum*).

All the *Longibrachiatum* clade isolates were recovered from hu-

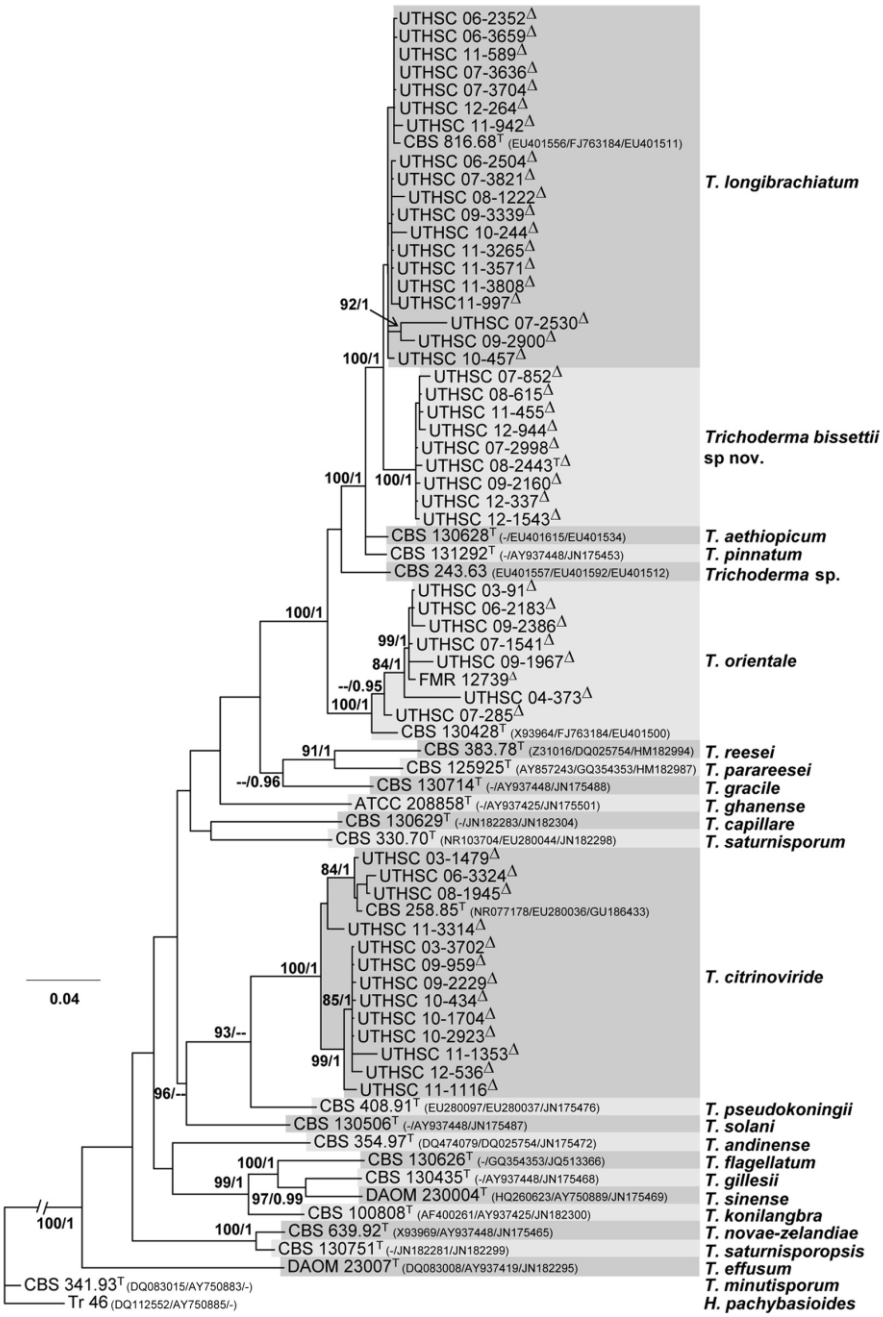

FIG 1 Bayesian tree inferred from combined ITS (456-bp), *Tef1* (466-bp), and *Chi18-5* (466-bp) sequences of *Trichoderma* and *Hypocrea* isolates belonging to the *Longibrachiatum* clade. Branch lengths are proportional to phylogenetic distance. ML bootstrap support values of >70% and posterior probability values of >0.95 are shown above the branches. The GenBank accession numbers given after some strains are those of the ITS/*Tef1*/*Chi18-5* genes. Missing sequences are indicated by a dash. *Hypocrea pachybasioides* and *T. minutisporum* were used to root the tree. Superscript T, type strain; Δ, strain of human origin. ATCC, American Type Culture Collection; CBS-KNAW, Fungal Biodiversity Centre culture collection, The Netherlands; DAOM, Agriculture and Agri-Food Canada National Mycological Culture Collection; FMR, Facultat de Medicina i Ciències de la Salut, Reus, Spain; Tr, collection of Earl Nelson maintained at the USDA-ARS Beltsville collection.

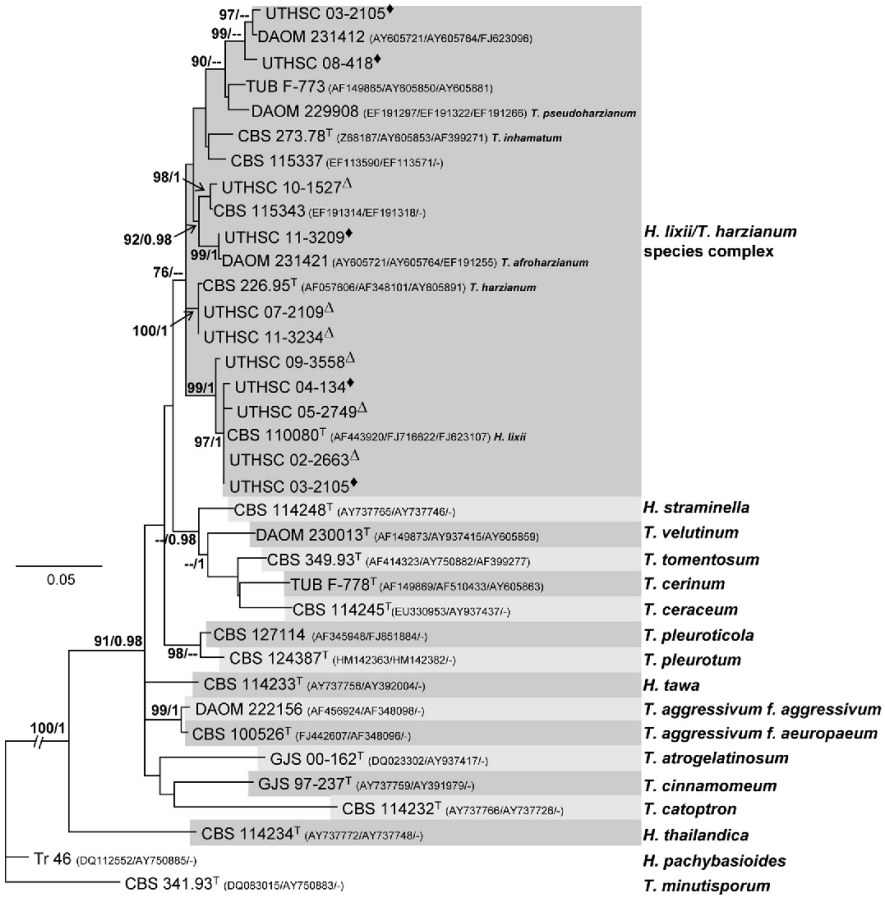

FIG 2 Bayesian tree inferred from combined ITS (407-bp), *Tef1* (238-bp), and *Chi18-5* (410-bp) sequences of *Trichoderma* and *Hypocrea* isolates belonging to the *Harzianum* clade. Branch lengths are proportional to phylogenetic distance. ML bootstrap support values of >70% and posterior probability values of >0.95 are shown above the branches. GenBank accession numbers given after some strains are those of the ITS/*Tef1*/*Chi18-5* genes. Missing sequences are indicated by a dash. *Hypocrea pachybasioides* and *T. minutisporum* were used to root the tree. Superscript T, type strain; Δ, strain of human origin; ♦, strain of animal origin. CBS-KNAW, Fungal Biodiversity Centre culture collection, The Netherlands; DAOM, Agriculture and Agri-Food Canada National Mycological Culture Collection; GJS, collection of Gary J. Samuels maintained at the USDA-ARS Beltsville collection; Tr, collection of Earl Nelson maintained at the USDA-ARS Beltsville collection; TUB, Technical University of Budapest Microbial Culture Collection.

man specimens, mainly from the respiratory tract. Isolates of human origin that belonged to the other clades were mainly from superficial tissues. All the animal-associated isolates were obtained from superficial tissue specimens.

Results of the antifungal susceptibility testing are summarized in Table 4. The geometric mean (GM) MIC and MIC_{90} values for AMB were 1.05 μg/ml and 2 μg/ml, respectively. Among the azoles, VRC was the most active, with an overall GM MIC and MIC_{90} of 1.62 μg/ml and 4 μg/ml, respectively, while PSC and ITC showed GM MIC values of 9.98 μg/ml and 16.15 μg/ml, respectively ($P < 0.0001$). The echinocandins showed the most potent *in vitro* activities, with overall GM MIC values of 0.23, 0.24, and 0.09 μg/ml for CFG, AFG, and MFG, respectively ($P < 0.05$). Terbinafine showed variable activity, with overall GM MIC and MIC_{90} values of 0.75 μg/ml and 4 μg/ml, respectively. All the *Trichoderma* species tested exhibited similar susceptibility patterns without showing statistical significance.

Taxonomy and related information. *Trichoderma bissettii* Sandoval-Denis & Guarro, sp. nov. (Fig. 6). MycoBank accession number MB807940. The etymology is in honor of John Bissett for his extensive work on *Hypocrea* and *Trichoderma*. The new species differs from *T. longibrachiatum* by having slightly longer conidia and narrower phialides borne on longer basal cells.

Colonies on PDA at 30°C to 35°C attaining 84 to 112 mm in diameter in 48 h, covering the plate (9 cm diameter) after 72 h, flat, forming concentric rings of whitish mycelium, fluffy and dense, with velvety to slightly granular zones, light yellow (2-A5) to olive-yellow (3-D7), margin irregular; reverse pale yellow (3-A3) to vivid yellow (3-A6); yellow diffusing pigment formed within 24 h at 15, 25, 30, and 35°C and within 48 h at 40°C. On CMD at 30 to

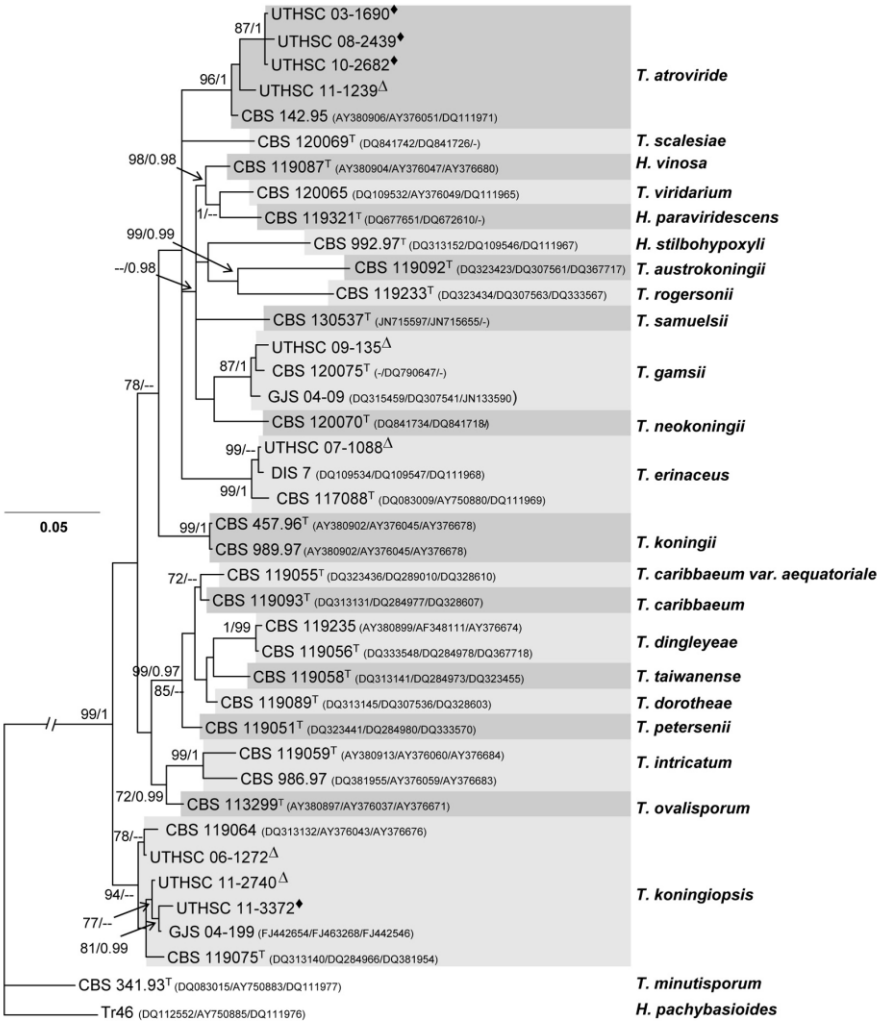

FIG 3 Bayesian tree inferred from combined ITS (389-bp), *Tef1* (304-bp), and *Act1* (560-bp) sequences of *Trichoderma* and *Hypocrea* isolates belonging to the *Viride* clade. Branch lengths are proportional to phylogenetic distance. ML bootstrap support values of >70% and posterior probability values of >0.95 are shown above the branches. GenBank accession numbers given after the some strains are those of the ITS/*Tef1*/*Act1* genes. Missing sequences are indicated by a dash. *Hypocrea pachybasioides* and *T. minutisporum* were used to root the tree. Superscript T, type strain; Δ, strain of human origin; ♦, strain of animal origin. CBS-KNAW, Fungal Biodiversity Centre culture collection, The Netherlands; DIS, CABI-Bioscience cultures held by GJS; GJS, collection of Gary J. Samuels maintained at the USDA-ARS Beltsville collection; Tr, collection of Earl Nelson maintained at the USDA-ARS Beltsville collection.

35°C attaining 70 to 100 mm in diameter in 48 h, completely filling the plate after 72 h, flat, forming concentric rings of whitish, often submerged mycelia, with granular deep green (26-D8) zones, minute deep green (26-D8) pustules (<1 mm) often formed, margin irregular; reverse deep green (26-D8); diffusible pigment not formed. Vegetative hyphae septate, hyaline, smooth and thin walled. Conidiophores usually consisting of a distinctive main axis, 3 to 5 μm wide, 2 or 3 side branches at right angles, straight or slightly bent toward upwards, up to 200 μm long, hyaline, smooth walled. Sterile hairs not formed. Phialides borne singly and laterally on the main axis or from side branches, or divergent in small whorls of 2 to 3 phialides arising from supporting cells (5.0)5.7 to 9.1(10.0) μm. Phialides cylindrical to lageniform, often curved or flexuous, (6.0)7.0 to 10.4(13.0) μm long, (2.0)2.1 to 2.9(3.5) μm at the widest point, length/width ratio (L/W) of (2.0)2.6 to 4.6(6.5), (1.4)1.5 to 1.8(2.0) μm wide at the base, hyaline, smooth walled. Intercalary phialides abundant, with lateral cylindrical conidiogenous opening 1.5 to 3 by 1 to 1.5 μm. Conidia unicellular, broadly ellipsoidal to nearly oblong, (3.5)3.9 to 5.1(6.0) by (2.0)2.3 to 2.9(4.0) μm, L/W (1.3)1.5 to 2.1(3.0), at first white, rapidly becoming yellowish-green to dark green, smooth and thin walled. Chlamydospores abundant, terminal and intercalary, subglobose or ellipsoidal, (6.5)6.8 to 8.4(9.5) by (5.5)6.6 to 8.2(8.5) μm, smooth and thick walled. Sexual morph not observed. Car-

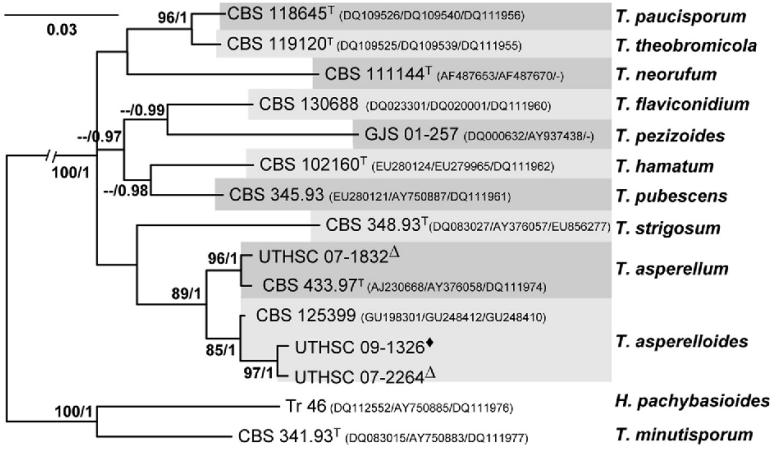

FIG 4 Bayesian tree inferred from combined ITS (405-bp), *Tef1* (310-bp), and *Act1* (559-bp) sequences of *Trichoderma* and *Hypocrea* isolates belonging to the *Hamatum* clade. Branch lengths are proportional to phylogenetic distance. ML bootstrap support values of >70% and posterior probability values of >0.95 are shown above the branches. GenBank accession numbers given after some strains are those of the ITS/*Tef1*/*Act1* genes. Missing sequences are indicated by a dash. *Hypocrea pachybasioides* and *T. minutisporum* were used to root the tree. Superscript T, type strain; Δ, strain of human origin; ◆, strain of animal origin. CBS-KNAW, Fungal Biodiversity Centre culture collection, The Netherlands; GJS, collection of Gary J. Samuels maintained at the USDA-ARS Beltsville collection; Tr, collection of Earl Nelson maintained at the USDA-ARS Beltsville collection.

dinal temperature for growth: optimum, 30 to 35°C; maximum, 40°C; minimum, 15°C.

Holotype. USA, from human sinusal cavity, 2008, D. A. Sutton (CBS H-21626; ex-type cultures CBS 137447 = UTHSC 08-2443 = FMR 12635).

DISCUSSION

In the present study, a large set of human and animal clinical isolates of *Trichoderma* was identified molecularly by using different gene combinations depending on the clade. The most common species were members of the *Longibrachiatum* clade, which is in agreement with findings reported by Kuhls et al. (40) who, by using ITS sequences, concluded that the spectrum of human pathogenic species of *Trichoderma* appeared to be restricted to this clade, particularly to *T. longibrachiatum*. Nevertheless, those authors' conclusions might have been biased, since in that study only six clinical isolates were included. More recently, several studies have emphasized the importance of this species as the causal agent of human infections (4, 26, 27). Three other species within the clade have been associated with human disease, i.e., *T. citrinoviride*, *T. orientale*, and *T. reesei* (21, 27, 40) The latter species has not been identified in this study, but the new species *T. bissettii* might be considered an opportunistic human pathogen of the *Longibrachiatum* clade. This is supported by the fact that *T. bisset-*

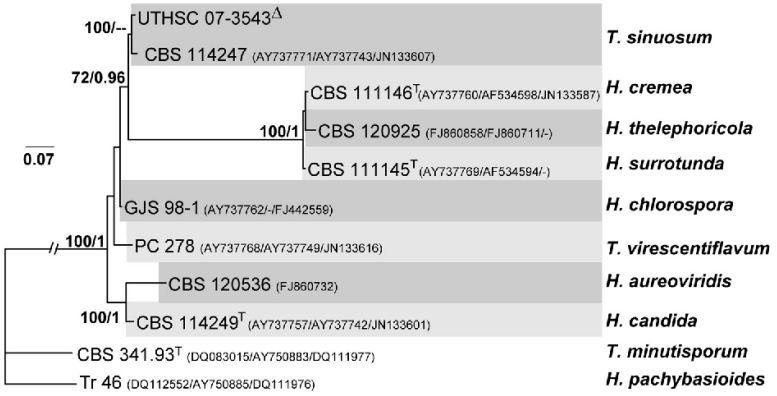

FIG 5 Bayesian tree inferred from the combined ITS (580-bp), *Tef1* (629-bp), and *Act1* (708-bp) sequences of *Trichoderma* and *Hypocrea* isolates belonging to the clade *Chlorospora*. Branch lengths are proportional to phylogenetic distance. ML bootstrap support values of >70% and posterior probability values of >0.95 are shown above the branches. GenBank accession numbers given after some strains are those of the ITS/*Tef1*/*Act1* genes. Missing sequences are indicated by a dash. *Hypocrea pachybasioides* and *T. minutisporum* were used to root the tree. Superscript T, type strain; Δ, strain of human origin. CBS-KNAW, Fungal Biodiversity Centre culture collection, The Netherlands; GJS, collection of Gary J. Samuels; PC, collection of Priscilla Chaverri; Tr, collection of Earl Nelson maintained at the USDA-ARS Beltsville collection.

TABLE 2 Numbers and sites of *Trichoderma* spp. isolated from human and animal sources

Species	No. of isolates from: Human			Animal	Total (%) no. of isolates
	Superficial	Respiratory	Deep tissue	Superficial tissue	
T. longibrachiatum	3	10	6	0	19 (26.0)
T. citrinoviride	3	5	5	0	13 (18.0)
H. lixii/T. harzianum species complex	2	3	1	5	11 (15.0)
T. bissettii	4	3	2	0	9 (12.0)
T. orientale	1	3	4	0	8 (11.0)
T. atroviride	0	0	1	3	4 (5.4)
T. koningiopsis	1	1	0	1	3 (4.0)
T. asperelloides	1	0	0	1	2 (3.0)
T. asperellum	0	1	0	0	1 (1.4)
T. erinaceum	1	0	0	0	1 (1.4)
T. gamsii	0	1	0	0	1 (1.4)
T. sinuosum	1	0	0	0	1 (1.4)
Total	17	27	19	10	73 (100)

tii was recovered from several different parts of the body, including deep tissue samples, and that it could grow at temperatures as high as 40°C, like the other clinically relevant species of the clade. Remarkably, *T. bissettii* was the fourth most common species identified. This species was probably not detected previously in clinical settings because its morphology is very similar to that of *T. longibrachiatum* and *T. orientale*. However, some subtle morphological differences were observed between them. In *T. bissettii*, the length and L/W ratio of the conidia were higher, the width of the basal portion of the phialides was narrower, and the length of the basal cell was longer than in *T. longibrachiatum* and *T. orientale*. It should also be noted that *T. bissetti* showed slightly higher MICs to AMB than *T. longibrachiatum* and *T. orientale*. In addition to these morphological differences, sequencing of the *Tef1* locus was a suitable phylogenetic marker that allowed correct discrimination of *T. bissettii* from its closest relatives.

Apart from species of the *Longibrachiatum* clade, several clinical cases have been attributed to other *Trichoderma* species (4, 8, 26, 61). Three clinical cases have been associated with *T. harzianum*, although the fungus was identified by ITS sequencing in only two of them (5, 8, 62). The *Harzianum* clade was the second most common in our study and, unlike the *Longibrachiatum* clade, it included isolates from humans and animals. Although this clade comprises more than 15 species, our clinical isolates only belonged to the *H. lixii/T. harzianum* species complex. This complex is formed by several phylogenetic species whose boundaries have not yet been delimited. Thus, the taxonomy of these species is unsettled (58, 63).

The *Viride* clade is the largest and most diverse group of species in *Trichoderma*, and it is composed of many phylogenetic species isolated from very diverse sources with a wide geographic distribution. Most of these species show high morphological homoplasy, which makes identification difficult (39). Three species of this clade have been linked to human disease. Ranque et al. (28) described an infection by *T. atroviride* in a liver transplant recipient, and morphological identification was later confirmed by molecular techniques (4). On the other hand, isolates recovered from two patients with peritonitis attributed to *T. koningii* (19, 20) were later reidentified as *T. longibrachiatum* (40, 62). Several cases have been attributed to *T. viride*, but the identification of the causal agent has never been confirmed molecularly (4). From the set of isolates studied, only *T. atroviride* was identified from both human and animal origins. *Trichoderma koningii* was identified morphologically on only one occasion, but molecularly that isolate corresponded to the closely related species *T. koningiopsis*. This latter species is commonly isolated from soil and has been described as an endophyte of *Theobroma* sp. and as a mycoparasite (44), but it has never been reported from clinical samples. The three isolates identified here as *T. koningiopsis* were from both human and animal origins. Two isolates identified morphologically as *T. atroviride* corresponded molecularly to *T. gamsii* and *T. erinaceus*. Both species are known to be soil saprophytes and endophytes (44, 45), but this is the first report of their isolation from clinical samples.

Only two species of the *Hamatum* clade were found in this study, i.e., *T. asperellum* recovered from a human sputum sample and *T. asperelloides* obtained from human nail and from a marine sponge. Both species are indistinguishable morphologically and are only known as saprophytes from agricultural soils, particularly from tropical regions (59). None of the isolates could be correctly

TABLE 3 Correlation between the morphological and molecular identification of the *Trichoderma* isolates from human and animal sources

Molecular identification	Morphological identification: *T. atroviride*	*T. citrinoviride*	*T. harzianum*	*T. koningii*	*T. longibrachiatum*	*T. pseudokoningii*	*T. strigosum*	*Trichoderma* spp.[a]	Total	% agreement
T. longibrachiatum					19				19	100
T. citrinoviride		3			7	3			13	23.1
H. lixii/T. harzianum species complex			11						11	100
T. bissettii					7	2			9	0
T. orientale					7	1			8	0
T. atroviride	3							1	4	75
T. koningiopsis	1			1				1	3	0
T. asperelloides	1						1		2	0
T. asperellum	1								1	0
T. erinaceus	1								1	0
T. gamsii	1								1	0
T. sinuosum								1	1	0
Total	8	3	11	1	40	6	1	3	73	49.3

[a] Isolates that failed to sporulate on the culture media.

TABLE 4 Results of *in vitro* antifungal susceptibility testing of the 73 clinical isolates of *Trichoderma* spp. included in the study

Species (no. of isolates tested)		MIC or MEC (μg/ml)							
		AMB	VRC	PSC	ITC	CFG	AFG	MFG	TBF
T. longibrachiatum (19)	GM	1.19	1.67	14.87	22.21	0.27	0.38	0.12	0.75
	MIC range	0.06–2	0.5–8	2–32	1–32	0.06–4	0.06–2	0.03–0.5	0.125–4
	MIC_{90}	2	4	32	32	0.5	1	0.5	2
T. citrinoviride (13)	GM	0.29	1.80	17.80	18.78	0.28	0.30	0.07	0.25
	MIC range	0.03–2	0.5–4	2–32	2–32	0.03–2	0.03–8	0.03–2	0.25–1
	MIC_{90}	1	4	32	32	1	2	0.25	1
H. lixii/T. harzianum species complex (11)	GM	1.55	1.77	3.76	9.66	0.16	0.07	0.06	0.78
	MIC range	0.5–4	0.25–16	1–32	1–32	0.06–0.5	0.03–0.25	0.03–0.25	0.25–4
	MIC_{90}	2	8	32	32	0.5	0.25	0.25	1
T. bissettii (9)	GM	2.33	1.17	17.28	14.81	0.21	0.29	0.10	1.17
	MIC range	2–4	0.5–4	4–32	1–32	0.125–0.25	0.125–2	0.06–0.25	0.25–2
	MIC_{90}	4	2	32	32	0.25	1	0.125	1
T. orientale (8)	GM	1.41	1.09	5.19	12.34	0.27	0.27	0.09	0.60
	MIC range	0.5–4	0.5–4	1–32	1–32	0.06–2	0.06–1	0.03–0.5	0.25–1
	MIC_{90}	4	4	32	32	2	1	0.125	1
T. atroviride (4)	GM	2.83	4.76	16.00	22.63	0.25	0.21	0.12	4.00
	MIC range	1–8	2–32	2–32	8–32	0.03–0.5	0.03–1	0.03–0.5	1–16
	MIC_{90}								
T. koningiopsis (3)	GM	0.25	4.00	11.31	32.00	0.16	0.08	0.08	0.79
	MIC range	0.06–2	1–4	4–32	32	0.06–0.5	0.03–0.5	0.06–0.125	0.5–1
	MIC_{90}								
T. asperelloides (2)	GM	1.41	2.00	2.00	11.31	0.06	0.35	0.04	2.00
	MIC range	1–2	2	2	8–16	0.03–0.125	0.125–1	0.03–0.06	2
	MIC_{90}								
T. asperellum (1)	GM	2.00	2.00	4.00	16.00	0.25	2.00	0.06	4.00
	MIC range								
	MIC_{90}								
T. erinaceus (1)	GM	0.50	1.00	4.00	32.00	0.25	0.25	0.06	1.00
	MIC range								
	MIC_{90}								
T. gamsii (1)	GM	1.00	1.00	32.00	32.00	0.25	1.00	0.25	4.00
	MIC range								
	MIC_{90}								
T. sinuosum (1)	GM	0.25	0.13	1.00	1.00	1.00	0.06	0.03	0.25
	MIC range								
	MIC_{90}								
Overall (73)	GM	1.05	1.62	9.98	16.15	0.23	0.24	0.09	0.75
	MIC range	0.03–8	0.125–32	1–32	1–32	0.03–4	0.03–8	0.03–2	0.125–16
	MIC_{90}	2	4	32	32	0.5	1	0.25	4

identified morphologically, because they could not be distinguished from *T. atroviride*, *T. harzianum*, and *T. strigosum*.

The *Chlorospora* clade is composed of a few species that show high morphological similarity to each other, and they are commonly found in soil or as endophytes (63). *Trichoderma sinuosum* was the only isolate from this clade, a species never reported found in a clinical setting.

Trichoderma spp. have been reported from deep tissue infections, mainly affecting the peritoneum and brain, followed by respiratory and other infection sites (4, 6, 8, 17, 61). The species studied here were mainly found in human respiratory specimens, followed by deep tissue and superficial tissue samples in similar amounts. Animal isolates were only from superficial tissue samples. While the isolates of the species of the *Longibrachiatum* clade were exclusively from human clinical specimens, most of the animal-associated isolates were found to belong to the *H. lixii/T. harzianum* species complex (Table 2). The isolation of *Trichoderma* spp. from animal sources (i.e., *T. asperel-*

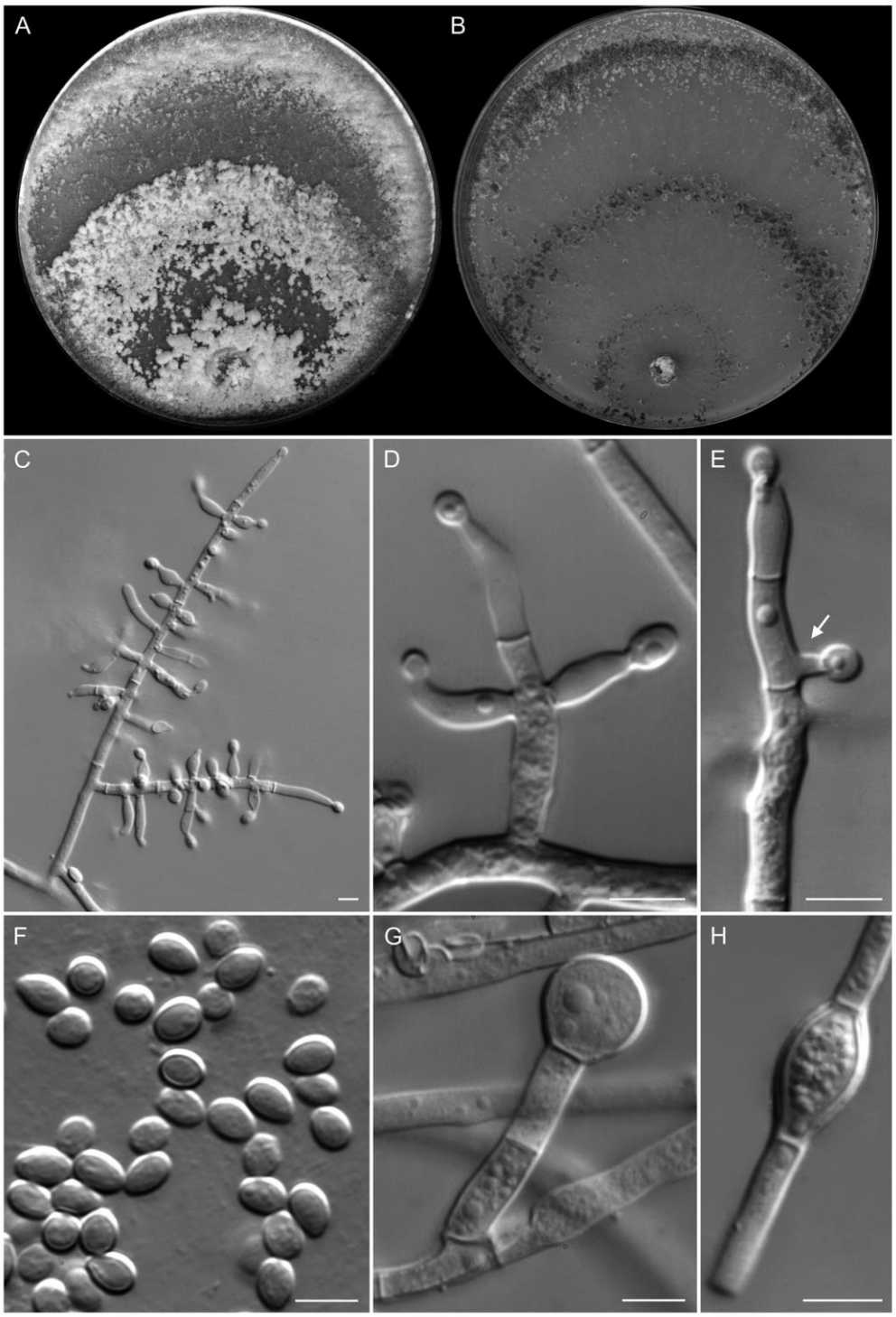

FIG 6 *Trichoderma bissettii* sp. nov. (UTHSC 08-2443). (A and B) Colonies on PDA and CMD, respectively, after 3 days at 25°C. (C) Conidiophore; (D) phialides; (E) intercalary phialide (arrow); (F) conidia; (G and H) chlamydospores. Bars, 5 μm.

loides, *T. atroviride*, *T. harzianum*, *T. longibrachiatum*, and *T. pseudokoningii*) has been previously reported (4, 31, 64), especially from marine sponges, although their role(s) have not been yet elucidated (64).

Among the human isolates, the most prevalent species identified in our study were *T. longibrachiatum* and *T. citrinoviride*, which were mainly isolated from respiratory and deep tissue samples; the other species identified were mainly from superficial tissue samples. Most of the species found here have previously been associated with opportunistic human diseases. However, *T. asperelloides*, *T. asperellum*, *T. erinaceum*, *T. gamsii*, *T. koningiopsis*, and *T. sinuosum* were found in clinical specimens for the first time. Despite the fact that these species have not been proven to be etiological agents of infections, their ability to grow at 40°C (in the first two species) and at 35 to 37°C (in the other four) and their prevalence in clinical specimens could indicate their possible role as human pathogens.

Current knowledge on the *in vitro* antifungal susceptibilities of pathogenic species of *Trichoderma* is mainly inferred from clinical cases that have shown variable results, probably due to the use of different methodologies (8, 26, 61). Our isolates showed similar antifungal patterns to those previously reported (6, 8, 23, 61); most of the antifungal agents tested showed high MICs. Although some isolates showed low MICs for AMB, especially against the less frequent species, generally the AMB MICs were considerably high. Among the azoles, VRC was the most active drug *in vitro* for most species, while PSC and ITC showed practically no clinically relevant activity; this correlates with the previous data of other authors (4). The activities of the echinocandins against *Trichoderma* have been proven before *in vitro*, especially for AFG, which has been shown to be at least four times more active than CFG (4, 65, 66). However, no data are available on the activity of MFG which, according to our study, showed the best *in vitro* activity against all the species tested. Similar to the previously published data, the activity of TBF varied, with relatively low MICs (4, 67). Clinical cases have reported unpredictable results regardless of the antifungal drug used. Most of the successfully resolved clinical cases were treated with AMB or its lipid formulations plus surgical debridement (61). The clinical experience with azole-based therapies also shows variable outcomes; VRC used mostly in combination with CFG has shown some positive results against deep infections by *T. longibrachiatum*, *T. reesei*, and *T. viride* (7, 21–23, 68). ITC has shown success alone and in combination with AMB and surgical resection against *T. longibrachiatum* (10, 11, 25), although according to our susceptibility results the apparent effectiveness of ITC might be only anecdotical. Ketoconazole was successful in a peritonitis case attributed to an unidentified *Trichoderma* species (15), while there are no reports on the use of PSC against *Trichoderma* infections. Our results suggest that this latter drug should be avoided, because most of the pathogenic species showed high MIC values. The use of echinocandins has been reported only for CFG in combination with VRC (21–23).

To date, this is the largest study involving clinical isolates of *Trichoderma*. The spectrum of *Trichoderma* species reported from clinical specimens has been expanded to 12 species, belonging to four different sections of the genus, including the new species *T. bissettii*, which was represented by nine isolates.

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5. DISCUSIÓN GENERAL

En las últimas décadas se ha observado un incremento constante y considerable de las infecciones fúngicas ocasionadas tanto por patógenos primarios como por especies oportunistas. Esto se debe principalmente al aumento de la población de riesgo como consecuencia del progreso de la medicina moderna con el uso de técnicas de monitoreo invasivas y terapias agresivas para el tratamiento de enfermedades de base severas (Richardson & Warnock 2012). Aunque los principales responsables de infecciones fúngicas en pacientes inmunocomprometidos siguen siendo especies de *Aspergillus*, *Candida*, *Cryptococcus*, mucorales y *Pneumocystis*, un buen número de hongos saprobios ambientales, considerados inocuos para la salud, están siendo aislados cada vez con mayor frecuencia como agentes causales de este tipo de infecciones, siendo asociados a una alta letalidad. Sin embargo, se estima que un buen número de infecciones causadas por hongos oportunistas pasan inadvertidas (Richardson & Warnock 2012) o no son diagnosticadas debido principalmente a las dificultades que conlleva demostrar su papel en la etiología e identificar sus agentes causales.

Acrophialophora, *Cladosporium*, *Microascus*, *Scopulariopsis* y *Trichoderma* son géneros de hongos patógenos oportunistas para el hombre de baja incidencia en clínica pero, a pesar de su rareza, algunas de sus especies son capaces de ocasionar infecciones realmente severas y con desenlace fatal (de Hoog *et al.* 2011). La mayoría de estos géneros contienen numerosas especies difíciles de distinguir debido a su gran parecido morfológico, lo que puede inducir fácilmente a una identificación errónea de sus aislados. Cabe destacar que nunca se ha llevado a cabo un extenso estudio de cada uno de los géneros mencionados con el objeto de averiguar el espectro real de especies que pueden estar asociadas a muestras clínicas. Por lo que para tal finalidad, en los trabajos incluidos en la presente memoria se han investigado numerosos aislados de origen clínico pertenecientes a dichos géneros mediante la combinación de datos moleculares, de análisis morfológicos, fisiológicos y estudios de sensibilidad antifúngica *in vitro*. Además, se han revisado las características morfológicas de las especies más relevantes en clínica, destacando las características diferenciales más importantes para facilitar su identificación en el laboratorio.

Acrophialophora es un género que se aísla principalmente de suelos y material vegetal en regiones con clima tropical y temperado (Samson & Mahmood 1970). A pesar de que inicialmente el género comprendía tres especies, el estudio de Al-Mohsen *et al.* (2000) concluyó que las diferencias morfológicas atribuidas a dichas especies eran meras variaciones morfológicas de la misma especie. Algunos autores no aceptaron dicha propuesta (Domsch *et al.* 2007, Liu 2011), pero durante más de una década *A. fusispora* fue considerada la única especie del género, siendo éste probablemente el

motivo por el que todos los casos clínicos publicados hasta la fecha de infecciones humanas por *Acrophialophora* fueron atribuidos exclusivamente a dicha especie (de Hoog *et al.* 2011, Li *et al.* 2013). Sin embargo, el estudio filogenético que hemos llevado a cabo con cepas de *Acrophialophora*, aisladas tanto de muestras clínicas como ambientales, demuestra que el género comprende tres especies: *A. fusispora*, *A. levis* y *A. seudatica*, siendo esta última la única representada por una sola cepa de origen ambiental. Las diferencias genéticas observadas entre estas especies se correlacionan con las diferencias morfológicas, principalmente referidas al tamaño, pigmentación y ornamentación de los conidios. Cabe destacar que de entre las cepas clínicas estudiadas, en contra de lo esperado, la especie identificada con mayor frecuencia ha sido *A. levis* (72,7%), seguida de *A. fusispora* con un porcentaje considerablemente menor (27,3%).

La mayoría de casos clínicos asociados a *Acrophialophora* se refieren a infecciones pulmonares (Al-Mohsen *et al.* 2000, Guarro *et al.* 2007). En individuos con fibrosis quística, *Acrophialophora* a menudo se encuentra colonizando las vías respiratorias, siendo este hecho un factor indicador del posterior deterioro progresivo de la función respiratoria (González-Escalada *et al.* 2000, Cimon *et al.* 2005). También se han descrito casos de queratitis (Arthur *et al.* 2001, Guarro *et al.* 2007), y en menor medida afectando al sistema nervioso central (Al-Mohsen *et al.* 2000, Li *et al.* 2013), por ello se le ha atribuido un cierto potencial neurotrópico (Revankar & Sutton 2010, Liu 2011). En nuestro estudio, la mayoría de los aislados clínicos procedían de muestras respiratorias, principalmente del tracto respiratorio inferior, obtenidas mediante lavado broncoalveolar, aunque la falta de datos clínicos nos impidió establecer el papel etiológico de los mismos. Considerando los antecedentes, creemos que el aislamiento de *Acrophialophora* de muestras clínicas debe de ser interpretado con cautela y, de entrada, no debería ser tratado como un mero contaminante de laboratorio. El género *Acrophialophora* ha sido continuamente destacado como patógeno en la literatura médica, llegando a afectar incluso a individuos inmunocompetentes y generalmente con un desenlace fatal (Cimon *et al.* 2005, Liu 2011).

Nuestros resultados de sensibilidad *in vitro* concuerdan con los pocos datos publicados (Al-Mohsen *et al.* 2000, Arthur *et al.* 2001, Guarro *et al.* 2007, Li *et al.* 2013). Sólo los azoles mostraron una buena actividad inhibitoria *in vitro*, especialmente el VRC. Sin embargo, pese a su aparente efectividad, los resultados clínicos obtenidos con los azoles son muy variables. El ITC fue eficaz en combinación con AMB en un caso de infección pulmonar con afectación del sistema nervioso central (Al-Mohsen *et al.* 2000), pero la misma combinación no fue efectiva en una infección pulmonar sistémica (Guarro *et al.* 2007); el tratamiento con VRC fue exitoso en un caso de infección corneal (Guarro

et al. 2007), aunque este mismo antifúngico no fue efectivo para el tratamiento de una infección cerebral (Li *et al.* 2013). Sin embargo, VRC es el antifúngico recomendado para el tratamiento de infecciones por *Acrophialophora* (Guarro *et al.* 2007), lo cual es apoyado no sólo por los resultados *in vitro* obtenidos en nuestro estudio, sino también por su farmacocinética ya que VRC es capaz de traspasar la barrera hemato-encefálica alcanzando concentraciones fungicidas en el sistema nervioso central, un hecho a tener en cuenta dado el potencial neurotrópico atribuido al género (Schwartz & Thiel 2003, Revankar & Sutton 2010, Liu 2011).

Resulta interesante destacar que los aislados pertenecientes a *A. levis* mostraron una gran homogeneidad genética, en cambio los del clado de *A. fusispora* presentaron una estructura filogenética muy diversa, con muchas ramas internas, lo que sugiere que podría tratarse de un complejo de especies. Sin embargo, preferimos mantener este clado como *A. fusispora* debido a la baja variabilidad genética entre los aislados a pesar de utilizar tres marcadores filogenéticos (<0.8% en el análisis combinado con LSU, ITS y *Tub*) y a la inexistencia de características morfológicas diferenciales entre ellos. Zhang *et al.* (2015) han obtenido resultados similares al estudiar la filogenia y taxonomía de los géneros *Acrophialophora* y *Taifanglania* Z.Q. Liang, Y.F. Han, H.L. Chu & R.T.V. Fox, mediante el uso combinado de secuencias de las regiones ITS, SSU y *Tub*. En dicho trabajo, *Taifanglania* se considera un género sinónimo de *Acrophialophora*, por lo que dichos autores propusieron seis nuevas combinaciones y tres nuevas especies de *Acrophialophora* de origen ambiental, *A. acuticonidiata* Y. Zhang & L. Cai, *A. angustiphialis* Y. Zhang & L. Cai y *A. ellipsoidea* Y. Zhang & L. Cai. El análisis filogenético demostró además que dos de las especies recientemente transferidas a *Acrophialophora*, *A. curticatenata* (Z.Q. Liang & Y.F. Han) Y. Zhang & L. Cai y *A. biformis* (Z.Q. Liang, H.L. Chu & Y.F. Han) Y. Zhang & L. Cai, forman junto con las cepas tipo de *A. fusispora* y *A. nainiana* una estructura filogenética similar a la observada en nuestros resultados referida al clado de *A. fusispora*. Sin embargo, Zhang y colaboradores consideraron a estos taxones como especies diferentes, sin discutir su similitud morfológica o genética. Lamentablemente, las secuencias empleadas en el estudio de Zhang *et al.* (2015) aún no están disponibles para ser contrastadas con nuestros resultados. No obstante, en dicho estudio se confirma que *A. levis* y *A. fusispora* son especies diferentes y que el género forma parte de la familia *Chaetomiaceae* (*Sordariales*, *Sordariomycetes*).

El género *Cladosporium* ha sido extensamente estudiado y recientemente revisado por diversos autores, tanto a nivel fenotípico como molecular (Crous *et al.* 2007b, Schubert *et al.* 2007b, Zalar *et al.* 2007, Bensch *et al.* 2010, Bensch *et al.* 2012). Dichos estudios han contribuido a clarificar la taxonomía del género, así como

comprender mejor la biología y patogenicidad de sus especies, sin embargo dichos estudios se centran principalmente en especies fitopatógenas. Hasta la fecha, no existía ningún estudio sobre la diversidad de especies de *Cladosporium* con cepas exclusivamente de origen clínico. La identificación de las especies de *Cladosporium* mediante técnicas tradicionales siempre ha resultado ser muy problemática debido a la dificultad que conlleva la interpretación de los caracteres morfológicos para distinguir las numerosas especies que integran el género. Como cabía esperar, la utilización de técnicas moleculares, y en concreto el análisis de secuencias ITS y de los genes *Tef*1 y *Act* han resultado ser de gran utilidad para el diagnóstico diferencial de especies (Crous *et al.* 2007b, Schubert *et al.* 2007b, Zalar *et al.* 2007, Bensch *et al.* 2012). Siguiendo la estrategia molecular propuesta en los trabajos antes mencionados, nuestro estudio ha puesto de manifiesto la gran diversidad de especies de *Cladosporium* presentes en muestras clínicas. Las especies identificadas con mayor frecuencia han sido *C. halotolerans* (14,8%), *C. tenuissimum* (10,2%) y *C. subuliforme* (5,7%), y con mucha menos frecuencia *C. allicinum*, *C. asperulatum*, *C. funiculosum*, *C. flabelliforme*, *C. pseudocladosporioides* y *C. ramotenellum*. Todas ellas, excepto *C. halotolerans*, han sido identificadas por primera vez a partir de muestras clínicas humanas. Por el contrario, las especies históricamente consideradas de mayor interés en clínica, *C. cladosporioides*, *C. herbarum* y *C. sphaerospermum*, se encuentran entre las menos representadas (igual o menor a 3%), incluso *C. oxysporum*, descrita como patógeno oportunista en diversas ocasiones (de Hoog *et al.* 2011), no ha sido identificada en nuestro grupo de aislados. Sin embargo, lo más sorprendente del estudio ha sido el hallazgo de un elevado porcentaje de aislados (39,8%), agrupados en 17 clados, imposibles de identificar a nivel de especie pues no concuerdan, ni genética- ni morfológicamente, con ninguna de las especies actualmente aceptadas en el género. En este sentido, consideramos tentativamente a dichos clados como representantes de 17 nuevas especies filogenéticas, cuya caracterización morfológica requiere aún de estudios posteriores. No menos interesante, ha sido la identificación, por primera vez en muestras clínicas, de especies pertenecientes a los géneros *Penidiella* y *Toxicocladosporium*. Ambos géneros, pertenecientes también al orden *Capnodiales*, incluyen especies saprobias previamente clasificadas en *Cladosporium* y recientemente segregadas en base a estudios de filogenia molecular (Crous *et al.* 2007a, 2007b). De entre los aislados clínicos estudiados, dos se han identificado como *T. irritans*, mientras otros dos corresponden a probables nuevas especies para *Penidiella* y *Toxicocladosporium*. La gran diversidad de especies observada entre los aislados clínicos y el elevado número de potenciales nuevos taxones parece indicar que la taxonomía de *Cladosporium* requiere de numerosos estudios que nos permitirán

profundizar en el conocimiento de la biología, epidemiología o patogenicidad de sus especies.

Los estudios sobre la sensibilidad antifúngica *in vitro* de especies de *Cladosporium* son prácticamente inexistentes. La mayoría de datos derivan de casos clínicos, y estos han sido obtenidos mediante métodos no estandarizados debido a su antigüedad. Nuestros resultados muestran MICs/MECs relativamente bajas para los azoles y la TRB, resultados que coinciden con los obtenidos en estudios previos (Romano *et al.* 1999, Vieira *et al.* 2001, Kantarcioğlu & Yücel 2002, Duquia *et al.* 2010, Sosa *et al.* 2012, Sang *et al.* 2012, Ma *et al.* 2013). Sin embargo, para AMB se han obtenido rangos muy variables, a excepción de las especies pertenecientes al complejo *C. herbarum* que mostraron valores significativamente menores frente a este antifúngico. Esto contrasta con los datos clínicos disponibles, en los cuales la AMB ha sido ineficaz para el tratamiento de infecciones por *C. cladosporioides* (Vieira *et al.* 2001) y *C. sphaerospermum* (Yano *et al.* 2003). Es notable destacar que, a pesar de que no existen datos sobre la utilización de equinocandinas *in vivo* frente a *Cladosporium* y que la mayoría de los hongos dematiáceos presentan resistencia a dicho grupo de antifúngicos (Odabasi *et al.* 2004), tanto la AFG como la MFG demostraron una potente actividad *in vitro* frente a las cepas de *Cladosporium*. Si bien en *Cladosporium* las estructuras reproductivas son, por lo general, muy melanizadas, el micelio vegetativo tiende a permanecer hialino o subhialino, pudiendo explicar la buena actividad *in vitro* observada para las equinocandinas.

En cuanto a los géneros *Microascus* y *Scopulariopsis*, hasta la fecha no existía ninguna revisión taxonómica basada en filogenia molecular, a pesar de ser géneros de la familia *Microascacae* muy conocidos pero de menor relevancia en clínica comparado con otros miembros de la familia como son *Lomentospora* Hennebert & Desai y *Scedosporium* (de Hoog *et al.* 2011, Lackner *et al.* 2014). En nuestro primer estudio se investigó la diversidad de especies de *Microascus* y *Scopulariopsis* en un grupo de 99 cepas obtenidas de especímenes clínicos humanos y animales, mediante un análisis polifásico que incluyó una filogenia basada en secuencias de DNA de dos loci (las regiones D1/D2 del gen LSU y un fragmento del gen *Tef*1) combinado con un análisis morfológico y fisiológico. Nuestros resultados coincidieron con los de otros autores (Miossec *et al.* 2011, Iwen *et al.* 2012) en que la especie más destacada en clínica es *S. brevicaulis*. Sin embargo, se puso de manifiesto que *S. gracilis* Samson es la segunda especie aislada con más frecuencia. Hasta antes de nuestro estudio, *S. gracilis* era conocida solamente como una especie saprobia ambiental (Inagaki 1962, Samson & von Klopotek 1972). Muchas de las cepas investigadas de *S. gracilis* desarrollaron el teleomorfo con características morfológicas muy similares a las presentes en *M.*

cinereus y *M. cirrosus*, dos especies descritas como patógenos oportunistas para el hombre (Krisher *et al.* 1995, Marques *et al.* 1995, Célard *et al.* 1999, Baddley *et al.* 2000, de Hoog *et al.* 2011). La gran similitud morfológica entre estas especies puede ser el motivo por el que *S. gracilis* no haya sido identificada en casos clínicos previos, siendo probablemente confundida con alguna de las especies de *Microascus* o *Scopulariopsis* asociadas con anterioridad a infecciones humanas. Una evidencia de lo expuesto es el elevado número de aislados recibidos como *M. cinereus*, *S. brumptii*, *S. chartarum,* además de un aislado previamente publicado como *M. cirrosus* (Miossec *et al.* 2011), y reidentificados en nuestro estudio como *S. gracilis*. Por otra parte, otras especies previamente consideradas de relevancia clínica como *M. trigonosporus* y *S. acremonium* no fueron detectadas entre nuestros aislados. A pesar de que un buen número de cepas clínicas habían sido inicialmente identificadas morfológicamente como *M. trigonosporus,* el análisis filogenético demostró que constituían linajes filogenéticos independientes y genéticamente bien diferenciados del de la cepa tipo de *M. trigonosporus*. Del total de aislados clínicos investigados en este estudio inicial, 18 no pudieron ser identificados a nivel de especie, agrupados en nueve clados terminales que consideramos como potenciales nuevas especies para los géneros *Microascus* y *Scopulariopsis*.

El estudio de sensibilidad antifúngica aplicado a dichas cepas demostró una elevada resistencia *in vitro* a la mayoría de antifúngicos disponibles para uso clínico. Los azoles, la AMB y la TRB mostraron rangos de MICs variables, aunque por lo general muy elevados. Por el contrario, las equinocandinas presentaron una buena actividad *in vitro*, principalmente la MFG seguida de CFG. Estos resultados concuerdan con estudios previos, basados principalmente en *S. brevicaulis* (Aguilar *et al.* 1999, Cuenca-Estrella *et al.* 2003, Carrillo-Muñoz *et al.* 2004, 2007). Nuestro estudio aportó nuevos datos sobre la sensibilidad de otras especies de importancia clínica tales como *S. gracilis*, *M. cinereus* y *M. cirrosus*. Recientemente, Skóra *et al.* (2015), utilizando un amplio panel de cepas obtenidas de colecciones internacionales, observaron resultados similares para otras especies de *Microascus* y *Scopulariopsis*, lo cual parece indicar que la resistencia antifúngica es un factor intrínseco muy importante a tener en cuenta frente a infecciones causadas por especies de ambos géneros. Ésta alta resistencia explicaría la baja o nula efectividad del tratamiento antifúngico observada en casos clínicos, comúnmente relacionados con fallo terapéutico y desenlaces fatales (Steinbach *et al.* 2004, Miossec *et al.* 2011).

Otra interesante observación derivada de este primer trabajo fue la gran distancia genética existente entre los diversos miembros de *Microascus* y *Scopulariopsis*, la cual concordaba con la obtenida en los escasos estudios moleculares previos sobre dichos hongos (Issakainen *et al.* 2003, Lackner *et al.* 2014). En estos estudios se sugería la

unificación de ambos géneros o bien su segregación en diferentes géneros. Por esta razón, con el fin de circunscribir la taxonomía de los géneros mencionados y caracterizar las potenciales nuevas especies detectadas, llevamos a cabo un segundo estudio basado en una filogenia más exhaustiva utilizando cuatro *loci*: LSU (regiones D1/D2), ITS, *Tef*1 y *Tub*. Esta vez incluimos todas las cepas tipo disponibles para las especies de los géneros antes mencionados y representantes de otros géneros de la familia *Microascaceae*. Los resultados obtenidos confirmaron que la delimitación genérica de *Microascus* y *Scopulariopsis*, tal y como se entendía hasta la fecha, era polifilética, y que los miembros de ambos géneros se distribuían en al menos siete linajes independientes, cada uno con características morfológicas diferenciales. En base a estos resultados, se circunscribieron *Microascus* y *Scopulariopsis* como géneros separados y diferentes al género *Pithoascus*, además de proponer a *Pseudoscopulariopsis* como un nuevo género para los *Microascaceae.* De acuerdo a esta nueva organización taxonómica, se propusieron diversas nuevas combinaciones, incluyendo dos especies de relevancia clínica: *S. gracilis*, ahora denominada *Microascus gracilis,* y *S. brumptii* sinonimizada con *Scopulariopsis paisii* (Pollacci) Nann. y ahora propuesta como *Microascus paisii* (Pollacci) Sandoval-Denis, Gené & Guarro. En esta nueva propuesta taxonómica, algunos miembros de otros géneros previamente considerados sinónimos de *Microascus* y/o *Scopulariopsis,* como *Acaulium* y *Fairmania,* formaban linajes independientes al resto de hongos estudiados. Por lo que en este segundo trabajo, éstos se consideraron sólo tentativamente como géneros diferentes, aunque pendientes de confirmar mediante un estudio filogenético más exhaustivo. Dentro de los *Microascaceae* existen diversos géneros con anamorfos similares a *Scopulariopsis*, entre ellos podemos citar a *Cephalotrichum*, *Doratomyces*, *Kernia*, *Microascus*, *Pithoascus*, *Trichurus*, *Wardomyces* y *Wardomycopsis.* La diferenciación de estos géneros basada exclusivamente en criterios morfológicos puede resultar muy compleja o ambigua y, a menudo, necesita de la observación conjunta del anamorfo y teleomorfo, y este último no siempre se desarrolla en cultivo. Por ello, el uso de herramientas moleculares constituye una mejora significativa para la identificación de las especies y géneros antes mencionados. Aún cuando la región ITS ha sido sugerida como el marcador universal para hongos (Schoch *et al.* 2012, Irinyi *et al.* 2015a, 2015b), nuestros resultados indican que presenta una baja resolución, por lo que el uso de regiones ribosómicas como ITS y LSU para la identificación de especies en *Microascus* y *Scopulariopsis* tiene una utilidad limitada. Por el contrario, los otros dos *loci*, *Tef*1 y *Tub*, demostraron una muy buena resolución, tanto para establecer la filogenia como para la identificación de las especies de este grupo de hongos.

El género *Trichoderma* ha sido motivo de numerosos estudios con aislados procedentes de sustratos naturales para conocer mejor su biología, distribución geográfica o su aplicación como agente para el biocontrol de plagas en agricultura. Sin embargo, los estudios sobre diversidad de especies en muestras clínicas, así como datos sobre su sensibilidad antifúngica son muy escasos y la mayoría procede casi exclusivamente de los casos clínicos publicados. Hasta hace relativamente poco, las especies de *Trichoderma* se distribuían en quince grupos filogenéticos o clados, agrupados en cinco secciones (*Longibrachiatum, Pachybasium, Trichoderma, Saturnisporum* e *Hypocreanum*) determinadas según el grado de similitud morfológica y genética, además de algunas especies de posición incierta denominadas "linages solitarios" (Chaverri & Samuels 2002). En un estudio reciente, se introdujeron dos nuevos clados denominados *Stipitatum* y *Phyllostachydis* (Zhu & Zhuang 2015), en base a la estrategia molecular propuesta en anteriores estudios y detallada en la Subcomisión Internacional de Taxonomía de *Trichoderma* e *Hypocrea* (http://www.isth.info/). En nuestro caso, el análisis filogenético aplicado a las cepas clínicas de *Trichoderma* ha puesto de manifiesto que éstas se distribuyen en tan solo cinco clados, concretamente y por orden de relevancia: *Longibrachiatum*, *Harzianum*, *Viride*, *Hamatum* y *Chlorospora.* Diversos autores han demostrado la preponderancia en clínica de las especies pertenecientes al clado *Longibrachiatum*, siendo *T. longibrachiatum*, *T. citrinoviride* y *T. orientale* las más destacadas en clínica, no sólo por su alta frecuencia de aislamiento sino también por ser especies termotolerantes, uno de los factores de patogenicidad más importantes de los hongos oportunistas (Seyedmousavi *et al.* 2013). En nuestro estudio confirmamos esta distribución de especies clínicas, pero además detectamos un nuevo linaje integrado por cepas morfológicamente similares a *T. longibrachiatum* y a *T. orientale*, aisladas repetidamente de muestras humanas, tanto respiratorias como de tejidos profundos. Dicho linaje ha sido descrito por nuestro grupo como una nueva especie de *Trichoderma* denominada *T. bissettii*. De entre las especies identificadas, *T. bissettii* ocupa la tercera posición en cuanto a la frecuencia de aislamiento dentro del clado *Longibrachiatum*, y la cuarta respecto al género. *Trichoderma asperelloides*, *T. asperellum*, *T. erinaceus*, *T. gamsii*, *T. koningiopsis* y *T. sinuosum* son otras especies identificadas en nuestro trabajo, aisladas por primera vez de muestras clínicas humanas y animales. Sin embargo, serán precisos más estudios para determinar si realmente son hongos capaces de ocasionar infecciones en humanos.

La gran semejanza morfológica de las especies de *Trichoderma* contrasta con su elevada diversidad genética (Kuhls *et al.* 1999, Samuels 2006) dificultado su identificación mediante técnicas tradicionales. Un ejemplo claro de ello son dos de las especies más conocidas del género, *T. longibrachiatum* y *T. orientale,* las cuales

durante mucho tiempo fueron consideradas un mismo hongo, anamorfo y teleomorfo, respectivamente. Sólo diferencias morfológicas sutiles observadas por Kuhls *et al.* (1997) y Samuels *et al.* (1998) sugerían la posibilidad de que pudieran ser consideradas especies diferentes. Cabe destacar además, que dichas especies son indistinguibles a nivel de sus secuencias ITS. Sin embargo, Druzhunina *et al.* (2008) y de Respinis *et al.* (2010), mediante análisis multilocus del ITS, *Tef*1, calmodulina (*cal)* y *chi*18-5, y análisis proteómico basado en MALDI-TOF, respectivamente, demostraron que eran especies distintas. El análisis de la región ITS es de gran utilidad para distinguir clados, sin embargo posee una resolución muy limitada para discriminar entre especies de *Trichoderma*, siendo imposible identificarlas si se tratan de especies muy cercanas como *T. orientale*, *T. longibrachiatum* o la nueva especie *T. bissettii*. Por lo que para reconocer a dichas especies también se deben analizar otros genes estructurales como *Act*, *chi*18-5 y *Tef*1. De todos modos si bien éstos otorgan una mejor resolución a nivel de especies, no permiten por si solos una identificación fiable de todas las especies de *Trichoderma* de importancia clínica. Ante esto, para la identificación de cepas clínicas de *Trichoderma* recomendamos la utilización de al menos dos marcadores diferentes, contrastando los resultados con las extensas bases de datos de secuencias tipo disponibles en GenBank y la base de datos especializada *TrichO*KEY (www.isth.info) (Druzhinina *et al.* 2005).

El clado *Harzianum*, incluye especies patógenas oportunistas y agentes de biocontrol, siendo la más conocida *T. harzianum* (Druzhinina *et al.* 2010, Chaverri *et al.* 2015). Nuestro trabajo demostró, una vez más, la importancia clínica de las especies pertenecientes a este clado en base a su alta frecuencia de aislamiento a partir de muestras clínicas humanas y animales. Sin embargo, la topología obtenida indicó una interesante distribución filogenética de los aislados morfológicamente identificados como *T. harzianum*, los cuales se agrupaban formando al menos cinco linajes independientes. Una topología similar ya había sido observada por Druzhinina *et al.* (2010), quienes demostraron que los taxones *H. lixii* y *T. harzianum,* originalmente considerados respectivamente como teleomorfo y anamorfo de una misma entidad (Chaverri & Samuels 2002), no sólo eran especies diferentes sino que otros aislados morfológicamente indistinguibles de las especies mencionadas formaban linajes independientes. Sin embargo, hasta la fecha no se había realizado ninguna propuesta taxonómica formal de las especies contenidas en este complejo, por lo que en nuestro trabajo todos estos linajes se tratan como un complejo de especies denominado "*H. lixii*/*T. harzianum* complex". Recientemente, mediante el empleo de una filogenia molecular basada en los *loci Act*, *Cal*, ITS, RNA polimerasa subunidad II (RPB2) y *Tef*1, Chaverri *et al.* (2015) establecieron los límites entre los linajes que conforman este

complejo, demostrando la existencia de 14 especies y estableciendo diferencias morfológicas relevantes para su identificación. Sin embargo, dado que estos datos aún no están disponibles no ha sido posible realizar el estudio comparativo para determinar la identidad definitiva de nuestros aislados clínicos bajo esta nueva circunscripción.

Los ensayos *in vitro* sobre sensibilidad antifúngica han demostrado una muy buena actividad de las equinocandinas frente a las especies de *Trichoderma* identificadas, particularmente de MFG y CFG, seguido de las alilaminas, representadas por la TRB. En cambio, los azoles y la AMB exhibieron una baja actividad inhibitoria. Estudio previos otorgan a estos últimos fármacos una actividad variable y sugieren la ausencia de una relación entre su actividad *in vitro* y el desenlace clínico (Piens *et al.* 2004, de Miguel *et al.* 2005, Alanio *et al.* 2008, Lagrange-Xélot *et al.* 2008). En cambio, los pocos estudios existentes concuerdan con la buena actividad *in vitro* de las equinocandinas (Pfaller *et al.* 1998, Kahn *et al.* 2006, Hatvani *et al.* 2013). Aunque contrariamente a nuestros resultados, otorgan una mejor actividad a la AFG que a la CFG. Sin embargo, sólo CFG ha sido utilizado con éxito *in vivo* en combinación con VRC (Piens *et al.* 2004, de Miguel *et al.* 2005, Alanio *et al.* 2008). Por el contrario, no existen datos acerca de la utilización clínica de MFG, que demostró la mejor actividad en nuestro estudio, siendo activa sobre todas las especies identificadas en nuestro grupo de aislados.

En general, para todos los géneros y especies estudiadas, no observamos ninguna correlación entre las especies identificadas y la localización anatómica de las muestras clínicas. La gran mayoría de aislados fueron obtenidos de muestras del tracto respiratorio, principalmente lavados broncoalveolares. Sin embargo, la falta de datos clínicos e histopatológicos, nos impidió establecer una relación de causalidad directa entre los aislados incluidos en los diversos trabajos y un cuadro infeccioso determinado. Sin embargo, el hecho de que muchas de las especies identificadas han sido aisladas reiteradamente de muestras clínicas diversas puede sugerir su implicación en distintos procesos infecciosos. Lo más evidente es que todos estos trabajos demuestran que la diversidad de especies en clínica es mucho mayor de lo que se creía anteriormente, cuando se identificaba únicamente a partir de criterios morfológicos. La lista a tener en cuenta de hongos potencialmente patógenos oportunistas para el hombre se ha incrementado considerablemente a partir del uso de técnicas moleculares para la identificación fúngica. De todos modos, es la aplicación combinada de diferentes criterios, principalmente morfología y análisis de secuencias, lo que nos va a permitir realizar una identificación fiable de las cepas fúngicas. Sólo así podremos avanzar en otros aspectos de los hongos patógenos emergentes, como conocer su epidemiología, respuesta a los antifúngicos *in vivo*, virulencia, etc.

Otra constante presente en nuestros trabajos y que parece una característica común a todos los hongos oportunistas, es la obtención de una amplia distribución de valores de MICs y MECs, no pudiendo determinar patrones de sensibilidad antifúngica *in vitro* específicos para los géneros y especies estudiadas. Ante esto y dada la imposibilidad de predecir la respuesta a los antifúngicos a pesar de disponer de una correcta identificación de los aislados, es preciso enfatizar en la necesidad e importancia de realizar estudios de sensibilidad *in vitro* para cualquier aislado de origen clínico sospechoso de un proceso infeccioso.

Por último, cabe destacar que a pesar de haber delimitado géneros, identificado e incluso descrito numerosas especies a partir tanto de aislados clínicos como ambientales, todavía queda pendiente resolver la taxonomía de muchos otros, como por ejemplo la identificación de 37 aislados, distribuidos en 19 clados y probablemente correspondientes a nuevas especies para los géneros *Cladosporium*, *Penidiella* y *Toxicocladosporium.* Pero también queda pendiente determinar la posición taxonómica de las especies excluidas de los géneros *Microascus* (*M. albonigrescens, M. caviariformis, M. giganteus, M. singularis)*, *Pithoascus* (*P. platysporus*) y *Scopulariopsis* (*S. acremonium, S. carbonaria, S. canadensis, S. coprophila* y *S. parva*), algunas de ellas relacionadas con otros géneros pobremente o nada estudiados a nivel molecular como son *Cephalotrichum*, *Doratomyces*, *Trichurus*, *Wardomyces* y *Wardomycopsis.*

6. CONCLUSIONES

En el presente trabajo se han realizado diversos estudios polifásicos, que involucraron el análisis morfológico, fisiológico y molecular de secuencias de múltiples *loci*, con la finalidad de esclarecer la taxonomía y filogenia de importantes géneros de hongos patógenos oportunistas, tales como: *Acrophialophora*, *Cladosporium*, *Microascus*, *Scopulariopsis* y *Trichoderma*. Además, se ha determinado la diversidad de especies asociadas a clínica humana y animal de dichos géneros y se ha establecido el perfil de sensibilidad antifúngica de la mayoría de las especies. En base a todo ello podemos llegar a las siguientes conclusiones para cada uno de los grupos de hongos estudiados:

Acrophialophora

1. El género *Acrophialophora*, tradicionalmente considerado monoespecífico, pertenece a la familia *Chaetomiaceae* del orden *Sordariales* (*Sordariomycetidae*) y realmente incluye tres especies: *A. fusispora*, *A. levis* y la nueva combinación *A. seudatica*.

2. La especie con mayor prevalencia entre los aislados clínicos estudiados es *A. levis,* seguida de *A. fusispora*, siendo ambas especies sensibles únicamente a los azoles, principalmente al VRC y a la TRB.

Cladosporium

3. Las especies identificadas con mayor frecuencia entre los aislados clínicos estudiados son, por este orden: *C. halotolerans*, *C. tenuissimum*, *C. subuliforme* y *C. pseudocladosporioides*.

4. Se han identificado por primera vez asociadas a muestras clínicas las siguientes especies de *Cladosporium*: *C. asperulatum*, *C. funiculosum*, *C. flabelliforme*, *C. pseudocladosporioides*, *C. subuliforme* y *C. tenuissimum*, además de algunas cepas pertenecientes a géneros relacionados tales como *Penidiella* y *Toxicocladosporium*.

5. Se han identificado un total de 19 especies filogenéticas nuevas para la ciencia, en concreto 17 en *Cladosporium* y dos pertenecientes a los géneros mencionados *Penidiella* y *Toxicocladosporium*.

6. Para este grupo de hongos se ha obtenido una elevada actividad *in vitro* de las equinocandinas, principalmente AFG y MFG, los azoles y la TRB.

Microascus*/*Scopulariopsis

7. El análisis molecular basado en la combinación de los marcadores ITS, LSU, *Tef1* y *Tub*, nos ha permitido delimitar filogenéticamente los géneros *Microascus*, *Scopulariopsis* y *Pithoascus*, y proponer el nuevo género *Pseudoscopulariopsis*.

8. Se han propuesto un total de ocho nuevas especies para la ciencia, siete para el género *Microascus* y una para *Scopulariopsis*, con los siguientes nombres: *M. alveolaris*, *M. brunneosporus*, *M. campaniformis*, *M. expansus*, *M. intricatus*, *M. restrictus*, *M. verrucosus* y *S. cordiae*. Además, se han propuesto diez nuevas combinaciones correspondientes a: *M. chartarus*, *M. croci*, *M. gracilis*, *M. hyalinus*, *M. macrosporus, M. murinus*, *M. paisii*, *Pithoascus ater, Pseudoscopulariopsis hibernica* y *Ps. schumacheri*.

9. Debido a la ausencia de material tipo se han designado neotipos para *M. longirostris, M. cinereus, Ps. schumacheri* y *S. flava.*

10. Como era de esperar, *S. brevicaulis* ha demostrado ser la especie más frecuente, representando casi la mitad de los aislados clínicos, seguida de *M. gracilis*, *M. paisii*, *M. cinereus*, *S. asperula* y *M. cirrosus*.

11. En general las especies de *Microascus* y *Scopulariopsis* muestran poca sensibilidad a los diferentes antifúngicos ensayados. Las equinocandinas, especialmente MFG y AFG y los azoles VRC y PSC mostraron la actividad más elevada *in vitro*.

Trichoderma

12. Además de las especies de *Trichoderma* tradicionalmente relacionadas con patología humana, se ha identificado y propuesto una nueva especie para la ciencia: *T. bissettii,* perteneciente al clado *Longibrachiatum*.

13. Las especies más frecuentemente identificadas entre los aislados estudiados resultaron ser *T. longibrachiatum*, *T. citrinoviride*, el grupo de especies filogenéticas del complejo *H. lixii/T. harzianum, T. bissettii* y *T. orientale*.

14. *Trichoderma asperelloides*, *T. asperellum*, *T. erinaceus*, *T. gamsii*, *T. koningiopsis* y *T. sinuosum* se han identificado por primera vez en muestras clínicas.

15. El VRC y las equinocandinas resultaron ser los antifúngicos más activos *in vitro* frente a estos hongos.

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8. ANEXOS

Tabla 1. Aislados clínicos incluidos en esta tesis

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Acrophialophora fusispora	8888	CBS 120409	India, cornea	*Acrophialophora fusispora*
	13150	UTHSCSA DI-13-135	USA, seno esfenoidal izquierdo	*Acrophialophora fusispora*
	13151	UTHSCSA DI-13-136	USA, absceso cerebral	*Acrophialophora fusispora*
	13155	UTHSCSA DI-13-140	USA, lavado broncoalveolar	*Acrophialophora fusispora*
	13156	UTHSCSA DI-13-141	USA, esputo	*Acrophialophora fusispora*
	13158	UTHSCSA DI-13-143	USA, masa pecho	*Acrophialophora fusispora*
	13164	UTHSCSA DI-13-149	USA, cornea	*Acrophialophora fusispora*
	13175	UTHSCSA DI-13-160	USA, lavado broncoalveolar	*Acrophialophora fusispora*
	13176	UTHSCSA DI-13-161	USA, esputo	*Acrophialophora fusispora*
Acrophialophora levis	6662	CBS 120407	España, esputo	*Acrophialophora fusispora*
	12780	-	España, esputo	*Acrophialophora fusispora*
	13149	UTHSCSA DI-13-134	USA, esputo	*Acrophialophora fusispora*
	13152	UTHSCSA DI-13-137	USA, esputo	*Acrophialophora fusispora*
	13153	UTHSCSA DI-13-138	USA, lavado broncoalveolar	*Acrophialophora fusispora*
	13154	UTHSCSA DI-13-139	USA, lavado broncoalveolar	*Acrophialophora fusispora*
	13157	UTHSCSA DI-13-142	USA, esputo	*Acrophialophora fusispora*
	13159	UTHSCSA DI-13-144	USA, esputo	*Acrophialophora fusispora*
	13160	UTHSCSA DI-13-145	USA, cerebro	*Acrophialophora fusispora*
	13161	UTHSCSA DI-13-146	USA, lavado broncoalveolar	*Acrophialophora fusispora*
	13162	UTHSCSA DI-13-147	USA, lavado broncoalveolar (canino)	*Acrophialophora fusispora*
	13163	UTHSCSA DI-13-148	USA, pulmón, lóbulo superior derecho	*Acrophialophora fusispora*
	13165	UTHSCSA DI-13-150	USA, esputo	*Acrophialophora fusispora*
	13166	UTHSCSA DI-13-151	USA, esputo	*Acrophialophora fusispora*

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Acrophialophora levis	13167	UTHSCSA DI-13-152	USA, tejido pierna	*Acrophialophora fusispora*
	13168	UTHSCSA DI-13-153	USA, tejido	*Acrophialophora fusispora*
	13169	UTHSCSA DI-13-154	USA, lavado broncoalveolar	*Acrophialophora fusispora*
	13170	UTHSCSA DI-13-155	USA, esputo	*Acrophialophora fusispora*
	13171	UTHSCSA DI-13-156	USA, tejido rodilla	*Acrophialophora fusispora*
	13172	UTHSCSA DI-13-157	USA, lavado broncoalveolar	*Acrophialophora fusispora*
	13173	UTHSCSA DI-13-158	USA, esputo	*Acrophialophora fusispora*
	13174	UTHSCSA DI-13-159	USA, lavado broncoalveolar	*Acrophialophora fusispora*
	13177	UTHSCSA DI-13-162	USA, lavado broncoalveolar	*Acrophialophora fusispora*
	13178	UTHSCSA DI-13-163	USA, lavado broncoalveolar	*Acrophialophora levis*
Alternaria dennissii/malorum	13361	UTHSC DI-13-248	USA, seno etmoidal	*Cladosporium elatum*
Aspergillus fumigatus	13311	UTHSC DI-13-186	USA, vertebra T-5	*Cladosporium cladosporioides*
Cladosporium allicinum	13298	UTHSC DI-13-173	USA, pulmón	*Cladosporium cladosporioides*
	13301	UTHSC DI-13-176	USA, piel	*Cladosporium* sp.
	13379	UTHSC DI-13-266	USA, piel (canino)	*Cladosporium herbarum*
Cladosporium angustisporum	13353	UTHSC DI-13-240	USA, uña pie	*Cladosporium cladosporioides*
Cladosporium asperulatum	13329	UTHSC DI-13-216	USA, nasal (felino)	*Cladosporium cladosporioides* Cplx
Cladosporium cladosporioides	13317	UTHSC DI-13-204	USA, abdomen	*Cladosporium cladosporioides* Cplx
	13322	UTHSC DI-13-209	USA, efusión pleural	*Cladosporium cladosporioides* Cplx
	13328	UTHSC DI-13-215	USA, esputo	*Cladosporium cladosporioides* Cplx
Cladosporium flabelliforme	13380	UTHSC DI-13-267	USA, esputo	*Cladosporium* sp.
Cladosporium funiculosum	13300	UTHSC DI-13-175	USA, lavado broncoalveolar/tejido pulmonar	*Cladosporium cladosporioides*
	13336	UTHSC DI-13-223	USA, lavado broncoalveolar	*Cladosporium cladosporioides* Cplx

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Cladosporium halotolerans	13307	UTHSC DI-13-182	USA, lesión dérmica (mamífero marino)	*Cladosporium sphaerospermum*
	13289	UTHSC DI-13-164	USA, médula ósea	*Cladosporium sphaerospermum*
	13319	UTHSC DI-13-206	USA, lavado broncoalveolar	*Cladosporium cladosporioides* Cplx
	13326	UTHSC DI-13-213	USA, nodo linfático	*Cladosporium sphaerospermum*
	13334	UTHSC DI-13-221	USA, médula ósea	*Cladosporium cladosporioides* Cplx
	13344	UTHSC DI-13-231	USA, punta de catéter	*Cladosporium* sp.
	13362	UTHSC DI-13-249	USA, nasal	*Cladosporium* sp.
	13363	UTHSC DI-13-250	USA, cuero cabelludo	*Cladosporium* sp.
	13365	UTHSC DI-13-252	USA, uña pie	*Cladosporium sphaerospermum*
	13376	UTHSC DI-13-263	USA, lavado broncoalveolar	*Cladosporium sphaerospermum*
	13493	-	España, desconocido	*Cladosporium sphaerospermum*
Cladosporium herbarum	13333	UTHSC DI-13-220	USA, lavado broncoalveolar	*Cladosporium herbarum* Cplx
Cladosporium macrocarpum	13316	UTHSC DI-13-191	USA, boca	*Cladosporium herbarum*
Cladosporium perangustum	13321	UTHSC DI-13-208	USA, lavado broncoalveolar (canino)	*Cladosporium cladosporioides* Cplx
Cladosporium pseudocladosporioides	13312	UTHSC DI-13-187	USA, tortuga	*Cladosporium cladosporioides*
	13345	UTHSC DI-13-232	USA, hombro	*Cladosporium* sp.
	13346	UTHSC DI-13-233	USA, lavado broncoalveolar	*Cladosporium cladosporioides* Cplx
	13374	UTHSC DI-13-261	USA, esputo	*Cladosporium cladosporioides*
	13381	UTHSC DI-13-268	USA, uña pie	*Cladosporium* sp.
Cladosporium ramotenellum	13291	UTHSC DI-13-166	USA, Tejido nasal	*Cladosporium ramotenellum*
	13335	UTHSC DI-13-222	USA, nasal (animal)	*Cladosporium* sp.
	13337	UTHSC DI-13-224	USA, nasal (animal)	*Cladosporium* sp.
Cladosporium sp. nov.	13290	UTHSC DI-13-165	USA, drenaje brazo	*Cladosporium cladosporioides*

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Cladosporium sp. nov.	13293	UTHSC DI-13-168	USA, lavado broncoalveolar	*Cladosporium cladosporioides*
	13294	UTHSC DI-13-169	USA, lavado broncoalveolar	*Cladosporium cladosporioides*
	13295	UTHSC DI-13-170	USA, uña pie	*Cladosporium sp.*
	13303	UTHSC DI-13-178	USA, absceso (animal)	*Cladosporium cladosporioides*
	13304	UTHSC DI-13-179	USA, mano	*Cladosporium cladosporioides*
	13308	UTHSC DI-13-183	USA, bronquial	*Cladosporium sphaerospermum*
	13315	UTHSC DI-13-190	USA, líquido cefalorraquídeo	*Cladosporium cladosporioides*
	13320	UTHSC DI-13-207	USA, líquido cefalorraquídeo	*Cladosporium cladosporioides* Cplx
	13323	UTHSC DI-13-210	USA, piel	*Cladosporium cladosporioides* Cplx
	13324	UTHSC DI-13-211	USA, lavado broncoalveolar	*Cladosporium cladosporioides* Cplx
	13325	UTHSC DI-13-212	USA, seno etmoidal	*Cladosporium herbarum* Cplx
	13330	UTHSC DI-13-217	USA, nasal	*Cladosporium* sp.
	13331	UTHSC DI-13-218	USA, lavado broncoalveolar	*Cladosporium cladosporioides* Cplx
	13332	UTHSC DI-13-219	USA, piel	*Cladosporium herbarum* Cplx
	13338	UTHSC DI-13-225	USA, lavado broncoalveolar (animal)	*Cladosporium cladosporioides* Cplx
	13339	UTHSC DI-13-226	USA, lavado broncoalveolar	*Cladosporium cladosporioides* Cplx
	13340	UTHSC DI-13-227	USA, esputo	*Cladosporium* sp.
	13341	UTHSC DI-13-228	USA, piel pie	*Cladosporium* sp.
	13347	UTHSC DI-13-234	USA, esputo	*Cladosporium cladosporioides* Cplx
	13348	UTHSC DI-13-235	USA, lavado broncoalveolar	*Cladosporium* sp.
	13351	UTHSC DI-13-238	USA, pierna	*Cladosporium cladosporioides*
	13354	UTHSC DI-13-241	USA, pie	*Cladosporium cladosporioides*
	13355	UTHSC DI-13-242	USA, lavado nasal	*Cladosporium cladosporioides*
	13357	UTHSC DI-13-244	USA, lavado broncoalveolar	*Cladosporium* sp.
	13358	UTHSC DI-13-245	USA, dedo pie	*Cladosporium cladosporioides*

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Cladosporium sp. nov.	13359	UTHSC DI-13-246	USA, lavado broncoalveolar	*Cladosporium* sp.
	13360	UTHSC DI-13-247	USA, lavado broncoalveolar	*Cladosporium cladosporioides*
	13364	UTHSC DI-13-251	USA, lavado broncoalveolar	*Cladosporium cladosporioides*
	13370	UTHSC DI-13-257	USA, esputo	*Cladosporium herbarum*
	13372	UTHSC DI-13-259	USA, lavado broncoalveolar	*Cladosporium sphaerospermum*
	13375	UTHSC DI-13-262	USA, bronquial (delfín)	*Cladosporium cladosporioides*
	13378	UTHSC DI-13-265	USA, lavado broncoalveolar	*Cladosporium* sp.
	13382	UTHSC DI-13-269	USA, lavado broncoalveolar	*Cladosporium cladosporioides*
	13383	UTHSC DI-13-270	USA, uña pie	*Cladosporium cladosporioides*
	13384	UTHSC DI-13-271	USA, lavado broncoalveolar	*Cladosporium cladosporioides*
	13386	UTHSC DI-13-273	USA, uña pie	*Cladosporium cladosporioides*
Cladosporium sphaerospermum	13309	UTHSC DI-13-184	USA, absceso (rana)	*Cladosporium* sp.
	13342	UTHSC DI-13-229	USA, lavado broncoalveolar	*Cladosporium sp.*
	13350	UTHSC DI-13-237	USA, lavado broncoalveolar	*Cladosporium sphaerospermum*
Cladosporium subinflatum	13314	UTHSC DI-13-189	USA, uña pie	*Cladosporium herbarum*
Cladosporium subuliforme	13296	UTHSC DI-13-171	USA, líquido cefalorraquídeo	*Cladosporium cladosporioides*
	13305	UTHSC DI-13-180	USA, lavado broncoalveolar	*Cladosporium cladosporioides*
	13327	UTHSC DI-13-214	USA, lavado broncoalveolar	*Cladosporium cladosporioides* Cplx
	13367	UTHSC DI-13-254	USA, lavado broncoalveolar	*Cladosporium cladosporioides*
	13368	UTHSC DI-13-255	USA, uña pie	*Cladosporium* sp.
Cladosporium tenuissimum	13299	UTHSC DI-13-174	USA, pulmón (mamífero marino)	*Cladosporium cladosporioides*
	13302	UTHSC DI-13-177	USA, desconocido	*Cladosporium* sp.
	13313	UTHSC DI-13-188	USA, lavado broncoalveolar	*Cladosporium* sp.
	13318	UTHSC DI-13-205	USA, lavado broncoalveolar	*Cladosporium cladosporioides* Cplx

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Cladosporium tenuissimum	13349	UTHSC DI-13-236	USA, nasal	*Cladosporium cladosporioides*
	13352	UTHSC DI-13-239	USA, esputo	*Cladosporium cladosporioides*
	13366	UTHSC DI-13-253	USA, lavado broncoalveolar	*Cladosporium* sp.
	13371	UTHSC DI-13-258	USA, líquido toracentesis	*Cladosporium* sp.
	13387	UTHSC DI-13-274	USA, pie	*Cladosporium cladosporioides*
Hypocrea lixii/Trichoderma harzianum Cplx	12485	UTHSC 02-2663	USA, seno maxilar	*Trichoderma* sp.
	12488	UTHSC 03-2105	USA, manatí	*Trichoderma harzianum*
	12489	UTHSC 05-2749	USA, esputo	*Trichoderma* sp.
	12611	UTHSC 04-134	USA, esponja marina	*Trichoderma* sp.
	12620	UTHSC 07-2109	USA, sangre	*Trichoderma* sp.
	12629	UTHSC 08-418	USA, tortuga marina	*Trichoderma* sp.
	12645	UTHSC 09-3558	USA, cornea	*Trichoderma* sp.
	12649	UTHSC 10-1527	USA, lavado broncoalveolar	*Trichoderma* sp.
	12661	UTHSC 11-2939	USA, pelo de cabra	*Trichoderma* sp.
	12662	UTHSC 11-3209	USA, esponja marina	*Trichoderma harzianum*
	12663	UTHSC 11-3234	USA, deposición	*Trichoderma* sp.
Microascus alveolaris	12346	UTHSC 06-3152	USA, lavado broncoalveolar	*Microascus trigonosporus*
	12350	UTHSC 05-3416	USA, lavado broncoalveolar	*Microascus trigonosporus*
	12252	UTHSC 07-3491=CBS 139501^{T}	USA, lavado broncoalveolar	*Scopulariopsis* sp.
	12333	UTHSC R-4634	USA, tejido pulmón	*Microascus trigonosporus*
	12336	UTHSC 10-214	USA, lavado broncoalveolar	*Microascus trigonosporus*
	12340	UTHSC 08-886	USA, lavado broncoalveolar	*Microascus* sp.
	12342	UTHSC 07-1823	USA, esputo	*Microascus* sp.
	12351	UTHSC 05-1041	USA, esputo	*Microascus trigonosporus*
	12354	UTHSC 04-1534	USA, lavado broncoalveolar	*Microascus trigonosporus*

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Microascus brunneosporus	12343	UTHSC 06-4312=CBS 138276^{T}	USA, lavado broncoalveolar	*Microascus cinereus*
Microascus campaniformis	12334	UTHSC 10-565= CBS 138126^{T}	USA, lavado broncoalveolar	*Microascus trigonosporus*
Microascus cinereus	12339	UTHSC 08-3181	USA, esternón	*Microascus trigonosporus*
	12217	UTHSC 10-2805^{NT}	USA, lavado broncoalveolar	*Scopulariopsis* sp.
	12239	UTHSC 09-573	USA, human BAL - tejido pulmón	*Scopulariopsis* sp.
	12331	UTHSC 11-383	USA, lavado broncoalveolar	*Microascus cinereus*
	12345	UTHSC 06-3278	USA, lavado broncoalveolar	*Microascus* sp.
Microascus cirrosus	12332	UTHSC 11-14	USA, lavado broncoalveolar	*Microascus cirrosus*
	12256	UTHSC 07-1887	USA, esputo	*Scopulariopsis brumptii*
Microascus expansus	12267	UTHSC 06-2519	USA, líquido pleural	*Scopulariopsis* sp.
	12266	UTHSC 06-4472=CBS 138127^{T}	USA, esputo	*Scopulariopsis brumptii*
Microascus gracilis	12205	UTHSC 11-1532	USA, lavado broncoalveolar	*Scopulariopsis* sp.
	12344	UTHSC 06-3999	USA, lavado broncoalveolar	*Microascus cinereus*
	12335	UTHSC 10-390	USA, lavado broncoalveolar	*Microascus* sp.
	12347	UTHSC 06-1072	USA, lavado broncoalveolar	*Microascus cinereus*
	12348	UTHSC 06-331	USA, lavado broncoalveolar	*Microascus cinereus*
	12337	UTHSC 09-974	USA, líquido pleural	*Microascus cinereus*
	12341	UTHSC 08-328	USA, lavado broncoalveolar	*Microascus cinereus*
	12224	UTHSC 10-1141	USA, esputo	*Scopulariopsis* sp.
	12352	UTHSC 05-811	USA, lavado broncoalveolar	*Microascus cinereus*
	12353	UTHSC 05-239	USA, lavado broncoalveolar	*Microascus cinereus*
	12231	UTHSC 09-1829	USA, lavado broncoalveolar	*Scopulariopsis chartarum*
	12234	UTHSC 09-1351	USA, líquido sinovial	*Scopulariopsis brumptii*
	12607	CNRMAF 16-27	Francia, líquido pleural	*Microascus* sp.
	12674	70024521	Francia, pulmón	*Microascus cirrosus*

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Microascus intricatus	12264	UTHSC 07-156=CBS 138128^{T}	USA, lavado broncoalveolar	*Scopulariopsis* sp.
Microascus paisii	12210	UTHSC 11-708	USA, esputo	*Scopulariopsis* sp.
	12215	UTHSC 10-2920	USA, lavado broncoalveolar	*Scopulariopsis* sp.
	12229	UTHSC 09-2391	USA, esputo	*Scopulariopsis brumptii*
	12240	UTHSC 09-482	USA, lavado broncoalveolar	*Scopulariopsis* sp.
	12241	UTHSC 09-457	USA, esputo	*Scopulariopsis brumptii*
	12248	UTHSC 08-1734	USA, lavado broncoalveolar	*Scopulariopsis brumptii*
	12263	UTHSC 07-639	USA, lavado broncoalveolar	*Scopulariopsis brumptii*
Microascus restrictus	12227	UTHSC 09-2704=CBS 138277^{T}	USA, *hallux* derecho	*Scopulariopsis* sp.
Microascus verrucosus	12219	UTHSC 10-2601=CBS 138278^{T}	USA, lavado broncoalveolar	*Scopulariopsis* sp.
Penidiella sp.	13369	UTHSC DI-13-256	USA, uña	*Cladosporium sphaerospermum*
Phialosimplex caninus	12245	UTHSC 08-3533	USA, abdominal (canino)	*Scopulariopsis* sp.
Scopulariopsis asperula	12212	UTHSC 10-3405	USA, dedo mayor pie	*Scopulariopsis* sp.
Scopulariopsis brevicaulis	3654	CBS 208.61	Desconocido, elefante	*Scopulariopsis koningii*
	12204	UTHSC 11-1563	USA, lavado broncoalveolar	*Scopulariopsis* sp.
	12206	UTHSC 11-1240	USA, masa pulmonar	*Scopulariopsis* sp.
	12207	UTHSC 11-1140	USA, pulmón	*Scopulariopsis brevicaulis*
	12208	UTHSC 11-1079	USA, tejido pulmón	*Scopulariopsis brevicaulis*
	12209	UTHSC 11-736	USA, lavado broncoalveolar	*Scopulariopsis brevicaulis*
	12211	UTHSC 11-427	USA, esputo	*Scopulariopsis* sp.
	12213	UTHSC 10-3379	USA, lavado broncoalveolar	*Scopulariopsis* sp.
	12214	UTHSC 10-3342	USA, tejido pie	*Scopulariopsis brevicaulis*
	12216	UTHSC 10-2899	USA, líquido sinovial	*Scopulariopsis brevicaulis*
	12218	UTHSC 10-2715	USA, esputo	*Scopulariopsis brevicaulis*
	12220	UTHSC 10-1746	USA, uña mano	*Scopulariopsis brevicaulis*

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Scopulariopsis brevicaulis	12221	UTHSC 10-1688	USA, seno maxilar	*Scopulariopsis* sp.
	12222	UTHSC 10-1397	USA, aspirado sinusal	*Scopulariopsis* sp.
	12225	UTHSC 10-562	USA, esputo	*Scopulariopsis brevicaulis*
	12230	UTHSC 09-1994	USA, lavado broncoalveolar	*Scopulariopsis brevicaulis*
	12232	UTHSC 09-1523	USA, tráquea	*Scopulariopsis brevicaulis*
	12233	UTHSC 09-1373	USA, esputo	*Scopulariopsis* sp.
	12235	UTHSC 09-1175	USA, pie derecho	*Scopulariopsis brevicaulis*
	12236	UTHSC 09-1092	USA, dedo pie	*Scopulariopsis* sp.
	12237	UTHSC 09-910	USA, seno	*Scopulariopsis* sp.
	12238	UTHSC 09-711	USA, dedo pie	*Scopulariopsis* sp.
	12242	UTHSC 09-316	USA, esputo	*Scopulariopsis brevicaulis*
	12243	UTHSC 08-3586	USA, seno	*Scopulariopsis* sp.
	12246	UTHSC 08-2981	USA, tejido aorta	*Scopulariopsis brevicaulis*
	12247	UTHSC 08-1920	USA, seno maxilar	*Scopulariopsis* sp.
	12249	UTHSC 08-432	USA, lavado broncoalveolar	*Scopulariopsis* sp.
	12250	UTHSC 08-294	USA, nasal	*Scopulariopsis brevicaulis*
	12251	UTHSC 07-3689	USA, esputo	*Scopulariopsis* sp.
	12253	UTHSC 07-3444	USA, muslo	*Scopulariopsis brevicaulis*
	12254	UTHSC 07-2095	USA, esputo	*Scopulariopsis* sp.
	12255	UTHSC 07-1888	USA, columna vertebral	*Scopulariopsis* sp.
	12257	UTHSC 07-1812	USA, dedo pie	*Scopulariopsis* sp.
	12258	UTHSC 07-1404	USA, tejido mano derecha	*Scopulariopsis brevicaulis*
	12259	UTHSC 07-1401	USA, piel pie	*Scopulariopsis* sp.
	12260	UTHSC 07-1278	USA, lavado broncoalveolar	*Scopulariopsis brevicaulis*
	12261	UTHSC 07-1133	USA, tejido pulmón	*Scopulariopsis* sp.

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Scopulariopsis brevicaulis	12262	UTHSC 07-1087	USA, muslo	*Scopulariopsis brevicaulis*
	12265	UTHSC 07-46	USA, tejido pulmón	*Scopulariopsis* sp.
	12268	UTHSC 06-2337	USA, seno	*Scopulariopsis* sp.
	12269	UTHSC 06-1868	USA, hueso	*Scopulariopsis* sp.
	12270	UTHSC 06-1850	USA, lavado broncoalveolar	*Scopulariopsis* sp.
	12271	UTHSC 06-619	USA, uña pie	*Scopulariopsis* sp.
	12272	UTHSC 06-481	USA, uña mano	*Scopulariopsis* sp.
	12273	UTHSC 06-277	USA, pelo	*Scopulariopsis brevicaulis*
	12274	UTHSC 06-12	USA, herida codo	*Scopulariopsis brevicaulis*
	12275	UTHSC 05-3598	USA, corazón	*Scopulariopsis* sp.
	12276	UTHSC 05-3476	USA, herida codo	*Scopulariopsis brevicaulis*
	12355	UTHSC R-4315	USA, lavado broncoalveolar	*Scopulariopsis* sp.
	12561	-	España, saco aéreo *Eos* sp.	*Scopulariopsis cf brevicaulis*
Scopulariopsis candida	12226	UTHSC 09-3241	USA, cuero cabelludo	*Scopulariopsis* sp.
	12228	UTHSC 09-2576	USA, esputo	*Scopulariopsis* sp.
Scopulariopsis cordiae	12338	UTHSC 09-866=CBS 138129^{T}	USA, dedo	*Microascus* sp.
	12349	UTHSC 05-3453	USA, drenaje JP	*Microascus manginii*
Toxicocladosporium irritans	13306	UTHSC DI-13-181	USA, sangre	*Cladosporium* sp.
	13343	UTHSC DI-13-230	USA, uña mano	*Cladosporium* sp.
Toxicocladosporium sp.	13297	UTHSC DI-13-172	USA, lavado broncoalveolar	*Toxicocladosporium* sp.
Trichoderma asperelloides	12621	UTHSC 07-2264	USA, uñas	*Trichoderma* sp.
	12638	UTHSC 09-1326	USA, esponja marina	*Trichoderma* sp.
Trichoderma asperellum	12619	UTHSC 07-1832	USA, esputo	*Trichoderma* sp.
Trichoderma atroviride	12610	UTHSC 03-1690	USA, piel (manatí)	*Trichoderma* sp.
	12634	UTHSC 08-2439	USA, esponja marina	*Trichoderma* sp.

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Trichoderma atroviride	12651	UTHSC 10-2682	USA, caparazón (tortuga)	*Trichoderma* sp.
	12658	UTHSC 11-1239	USA, masa pulmonar	*Trichoderma* sp.
Trichoderma bissettii	12616	UTHSC 07-852	USA, seno	*T. longibrachiatum* Cplx
	12623	UTHSC 07-2998	USA, uñas	*Trichoderma* sp.
	12630	UTHSC 08-615	USA, herida	*Trichoderma* sp.
	12635	UTHSC 08-2443=CBS 137447^{T}	USA, seno	*Trichoderma longibrachiatum* Cplx
	12640	UTHSC 09-2160	USA, lavado broncoalveolar	*Trichoderma longibrachiatum* Cplx
	12653	UTHSC 11-455	USA, pie	*Trichoderma longibrachiatum* Cplx
	12670	UTHSC 12-337	USA, hueso	*Trichoderma longibrachiatum* Cplx
	12672	UTHSC 12-944	USA, cuerpo vertebral	*Trichoderma* sp.
	12673	UTHSC 12-1543	USA, uña	*Trichoderma* sp.
Trichoderma citrinoviride	12487	UTHSC 03-1479	USA, lavado broncoalveolar	*Trichoderma* sp.
	12493	UTHSC 06-3324	USA, uña pie	*Trichoderma* sp.
	12612	UTHSC 03-3702	USA, sangre	*Trichoderma* sp.
	12633	UTHSC 08-1945	USA, líquido ascítico	*Trichoderma longibrachiatum* Cplx
	12637	UTHSC 09-959	USA, líquido pleural	*Trichoderma longibrachiatum* Cplx
	12641	UTHSC 09-2229	USA, ojo	*Trichoderma longibrachiatum* Cplx
	12647	UTHSC 10-434	USA, herida abdominal	*Trichoderma longibrachiatum* Cplx
	12650	UTHSC 10-1704	USA, lavado broncoalveolar	*Trichoderma* sp.
	12652	UTHSC 10-2923	USA, pulmón	*Trichoderma longibrachiatum* Cplx
	12657	UTHSC 11-1116	USA, esputo	*Trichoderma* sp.
	12659	UTHSC 11-1353	USA, sangre	*Trichoderma longibrachiatum* Cplx
	12665	UTHSC 11-3314	USA, lavado broncoalveolar	*Trichoderma* sp.
	12671	UTHSC 12-536	USA, lavado broncoalveolar	*Trichoderma longibrachiatum* Cplx
Trichoderma erinaceus	12617	UTHSC 07-1088	USA, uñas	*Trichoderma* sp.

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Trichoderma gamsii	12636	UTHSC 09-135	USA, esputo	*Trichoderma* sp.
Trichoderma koningiopsis	12614	UTHSC 06-1272	USA, uñas	*Trichoderma* sp.
	12660	UTHSC 11-2740	USA, lavado broncoalveolar	*Trichoderma* sp.
	12666	UTHSC 11-3372	USA, pata (canino)	*Trichoderma* sp.
Trichoderma longibrachiatum	12491	UTHSC 06-2352	USA, oído	*Trichoderma longibrachiatum* Cplx
	12492	UTHSC 06-2504	USA, lavado broncoalveolar	*Trichoderma longibrachiatum* Cplx
	12494	UTHSC 06-3659	USA, líquido cefalorraquídeo	*Trichoderma longibrachiatum* Cplx
	12622	UTHSC 07-2530	USA, lavado broncoalveolar	*Trichoderma longibrachiatum* Cplx
	12626	UTHSC 07-3636	USA, uña	*Trichoderma* sp.
	12627	UTHSC 07-3704	USA, uña	*Trichoderma* sp.
	12628	UTHSC 07-3821	USA, lavado broncoalveolar	*Trichoderma* sp.
	12632	UTHSC 08-1222	USA, tejido pulmón	*Trichoderma* sp.
	12643	UTHSC 09-2900	USA, esputo	*Trichoderma longibrachiatum* Cplx
	12644	UTHSC 09-3339	USA, lavado broncoalveolar	*Trichoderma* sp.
	12646	UTHSC 10-244	USA, líquido pleural	*Trichoderma* sp.
	12648	UTHSC 10-457	USA, seno maxilar	*Trichoderma longibrachiatum* Cplx
	12654	UTHSC 11-589	USA, lavado broncoalveolar	*Trichoderma longibrachiatum* Cplx
	12655	UTHSC 11-942	USA, líquido peritoneal	*Trichoderma longibrachiatum* Cplx
	12656	UTHSC 11-997	USA, masa mediastínica	*Trichoderma* sp.
	12664	UTHSC 11-3265	USA, sangre	*Trichoderma longibrachiatum* Cplx
	12667	UTHSC 11-3571	USA, esputo	*Trichoderma longibrachiatum* Cplx
	12668	UTHSC 11-3808	USA, esputo	*Trichoderma longibrachiatum* Cplx
	12669	UTHSC 12-264	USA, lavado broncoalveolar	*Trichoderma* sp.
Trichoderma orientale	12486	UTHSC 03-91	USA, esputo	*Trichoderma* sp.
	12490	UTHSC 06-2183	USA, sangre	*Trichoderma* sp.

Tabla 1. (Continuación)

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Trichoderma orientale	12613	UTHSC 04-373	USA, seno	*Trichoderma longibrachiatum* Cplx
	12615	UTHSC 07-285	USA, lavado broncoalveolar	*Trichoderma* sp.
	12618	UTHSC 07-1541	USA, líquido peritoneal	*Trichoderma* sp.
	12639	UTHSC 09-1967	USA, brazo	*Trichoderma* sp.
	12642	UTHSC 09-2386	USA, sangre	*Trichoderma longibrachiatum* Cplx
	12739	134846/v	España, prótesis vascular	*Trichoderma* sp.
Trichoderma sinuosum	12625	UTHSC 07-3543	USA, piel	*Trichoderma* sp.

CBS = *CBS-KNAW Fungal Biodiversity Centre*, Utrecht, Holanda, CNRMAF = *Centre National de Réfeìrence Mycoses Invasives et Antifongiques, Institut Pasteur,* Paris, Francia. FMR = *Facultat de Medicina de Reus*, Reus, España. UTHSC = *Fungus Testing Laboratory, University of Texas Health Science Center*, Texas, USA. ^{T} = tipo. ^{NT} = neotipo, Cplx = complejo de especies.

Tabla 2. Aislados ambientales incluidos en esta tesis

Especie	Número FMR	Número otras colecciones	Origen	Recibida como
Microascus cirrosus	4002	-	Sustrato desconocido	*Microascus cirrosus*
	4003	-	Sustrato desconocido	*Microascus cirrosus*
Microascus croci	4004	-	Sustrato desconocido	*Microascus cirrosus*
	3997	-	España, sedimentos rio Ebro	*Scopulariopsis chartarum*
Microascus intricatus	12362	-	Argentina, Iguazú, tierra	*Microascus intermedius*
Microascus trigonosporus	3905	-	Sustrato desconocido	*Microascus trigonosporus*
	7306	-	Cuba, suelo	*Microascus trigonosporus*
Scopulariopsis brevicaulis	4012	-	Sustrato desconocido	*Scopulariopsis* sp.
Wardomycopsis humicola	3993	807/805	España, sedimentos rio Ter	*Wardomycopsis humicola*
	4039	-	España, playa La Gola	*Wardomycopsis humicola*
	4041	-	España, playa El Prat	*Wardomycopsis humicola*
	4042	-	España, playa Pals	*Wardomycopsis humicola*
	4043	-	España, sedimentos ribera del Ebro	*Wardomycopsis humicola*
Wardomycopsis inopinata	10305	37F	Myanmar, suelo	*Wardomycopsis cf inopinata*
	10306	37C	Myanmar, suelo	*Wardomycopsis cf inopinata*

FMR = *Facultat de Medicina de Reus*, Reus, España.

Tabla 3. Cepas tipo y de referencia de colecciones internacionales

Especie	Número colección	Número FMR	Origen	Recibida como
Acrophialophora fusispora	CBS 120406	6258	India, suelo	*Acrophialophora fusispora*
Acrophialophora levis	CBS 484.70^{T}	13486	Alemania, compost	*Acrophialophora levis*
Acrophialophora nainiana	CBS 380.55^{T}	13483	India, suelo de bosque	*Paecilomyces fusisporus*
	CBS 149.64^{IT}	13484	India, suelo de bosque	*Masoniella indica*
	CBS 100.60^{IT}	13485	India, suelo de granja	*Acrophialophora nainiana*
Acrophialophora seudatica	CBS 916.79^{T}	13886	India, suelo	*Ampullifera seudatica*
Aspergillus baarnensis	CBS 380.74^{T}	12693	Japón, *Undaria pinnatifida*	*Scopulariopsis halophilica*
Microascus albonigrescens	IHEM 18560	12564	Japón, basura	*Microascus albonigrescens*
Microascus caviariformis	CBS 536.87^{T}	12998	Bélgica, carne en descomposición	*Microascus caviariformis*
Microascus chartarum	MUCL 9001^{T}	12581	Inglaterra, papel tapiz	*Masoniella chartarum*
Microascus cinereus	CBS 365.65^{T}	12681	India, suelo	*Microascus griseus*
Microascus cirrosus	MUCL 9050	12594	Italia, raíz de *Vitis vinifera*	*Microascus cirrosus*
	MUCL 9054	12595	Reino Unido, sustrato desconocido	*Microascus desmosporus*
	CBS 217.31^{T}	12695	Italia, hoja de *Prunus* sp.	*Microascus cirrosus*
Microascus croci	MUCL 9005^{T}	12577	Brasil, aire	*Masoniella tertia*
	MUCL 9002^{T}	12583	Holanda, *Crocus* sp.	*Masoniella croci*
Microascus giganteus	CBS 746.69^{T}	12694	Canadá, fecas de insecto	*Microascus giganteus*
Microascus gracilis	MUCL 9049	12591	USA, semilla de *Zea mays*	*Microascus cinereus*
	MUCL 9048	12592	Inglaterra, suelo	*Microascus cinereus*
	CBS 369.70^{IT}	12680	Japón, comida	*Paecilomyces fuscatus*
Microascus hyalinus	CBS 766.70^{IT}	13147	USA, fecas de vaca	*Kernia hyalina*
Microascus longirostris	MUCL 40734	12596	Canadá, aire	*Microascus longirostris*
	CBS 196.61	12733	USA, nido de avispa	*Microascus longirostris*
	CBS 415.64	13144	Japón, suelo	*Microascus longirostris*

Tabla 3. (Continuación)

Especie	Número colección	Número FMR	Origen	Recibida como
Microascus macrosporus	CBS 662.71	13145	USA, suelo	*Microascus trigonosporus* var. *macrosporus*
Microascus murina	IHEM 18567^{T}	12563	Alemania, basura	*Scopulariopsis murina*
Microascus paisii	MUCL 9040^{T}	12575	USA, arroz molido	*Scopulariopsis melanospora*
	MUCL 9003^{T}	12576	Inglaterra, contaminante de laboratorio	*Masoniella grisea*
	MUCL 8990	12578	Alemania, suelo	*Scopulariopsis brumptii*
	MUCL 8993	12582	Alemania, campo de trigo	*Scopulariopsis chartarum*
	MUCL 7915^{T}	12593	Italia, humano	*Torula paisii*
	MUCL 9045^{T}	12602	Austria, sustrato desconocido	*Scopulariopsis sphaerospora*
	MUCL 8989	12727	Alemania, campo de *Triticum sativum*	*Scopulariopsis brumptii*
	MUCL 8991	12728	Alemania, suelo	*Scopulariopsis chartarum*
Microascus pyramidus	CBS 212.65^{IT}	12682	USA, suelo	*Microascus pyramidus*
Microascus senegalensis	IHEM 18561^{T}	12565	Senegal, suelo en manglar	*Microascus senegalensis*
Microascus singularis	CBS 414.64	12684	Japón, contaminante de laboratorio	*Microascus singularis*
Microascus trigonosporus	MUCL 9061	12601	Japón, arroz molido	*Microascus trigonosporus*
	CBS 218.31^{T}	12685	USA, sustrato desconocido	*Microascus trigonosporus*
	MUCL 9060	12734	Puerto Rico, sustrato desconocido	*Microascus trigonosporus*
	MUCL 9841	12735	Reino Unido, cama de hongos	*Scopulariopsis coprophila*
Pithoascus ater	IHEM 18608^{T}	12609	Desconocido	*Scopulariopsis atra*
Pithoascus exsertus	CBS 583.75	12761	Dinamarca, *Osmia rufa*	*Pithoascus exsertus*
	CBS 819.70^{T}	12760	Dinamarca, *Megachile willoughbiella*	*Pithoascus exsertus*
Pithoascus intermedius	CBS 217.32^{T}	12762	USA, *Fragaria vesca*	*Microascus intermedius*
Pithoascus nidicola	CBS 197.61^{T}	12759	USA, *Dipodomys merriami*	*Microascus nidicola*
Pithoascus platysporus	CBS 419.73^{T}	12763	Holanda, suelo	*Pithoascus platysporus*

Tabla 3. (Continuación)

Especie	Número colección	Número FMR	Origen	Recibida como
Pithoascus schumacheri	CBS 435.86	12764	España, suelo	*Pithoascus schumacheri*
Pithoascus stoveri	CBS 176.71^{T}	12765	USA, raíz de *Beta vulgaris*	*Pithoascus stoveri*
Scopulariopsis acremonium	MUCL 9028^{T}	12573	Dinamarca, piel de caballo	*Scopulariopsis danica*
	MUCL 8274	12725	Alemania, suelo	*Scopulariopsis acremonium*
	MUCL 8409	12726	Alemania, suelo	*Scopulariopsis acremonium*
Scopulariopsis asperula	CBS 373.76	3989	Holanda, envoltura de queso	*Scopulariopsis asperula*
	MUCL 9032^{T}	12587	Austria, carcasa de conejo	*Scopulariopsis fusca*
	MUCL 9012^{T}	12588	Italia, humano	*Torula bestae*
	MUCL 40729	12599	Canadá, aire	*Microascus niger*
	MUCL 40746	12600	Canadá, feca de *Mephitis mephitis*	*Microascus niger*
	CBS 373.76	3989	Holanda, queso	*Scopulariopsis asperula*
Scopulariopsis brevicaulis	MUCL 9035	12586	Holanda, pupa de *Pteronus pini*	*Scopulariopsis insectivora*
	MUCL 14213	12589	Bélgica, suelo	*Scopulariopsis stercoraria*
	MUCL 40726^{T}	12590	Canadá, aire	*Microascus brevicaulis*
	CBS 399.34^{T}	12766	Austria, piel humana	*Scopulariopsis alboflavescens*
Scopulariopsis canadensis	CBS 204.61^{T}	12729	Canadá, semilla de *Beta vulgaris*	*Scopulariopsis canadensis*
Scopulariopsis candida	MUCL 9026	12574	Francia, sustrato desconocido	*Scopulariopsis candelabrum*
	MUCL 9007	12579	Desconocido	*Scopulariopsis alboflavescens*
	MUCL 9016	12584	Holanda, suelo	*Scopulariopsis brevicaulis* var. *alba*
	MUCL 40732	12597	Canadá, aire	*Microascus manginii*
	MUCL 41467	12598	Francia, queso	*Microascus manginii*
	CBS 170.27^{T}	13146	Francia, sustrato desconocido	*Nephrospora manginii*
	MUCL 40743^{ET}	12770	Canadá, aire	*Scopulariopsis candida*
Scopulariopsis carbonaria	MUCL 9027^{T}	12580	Panamá, suelo	*Scopulariopsis carbonaria*

Tabla 3. (Continuación)

Especie	Número colección	Número FMR	Origen	Recibida como
Scopulariopsis coprophila	CBS 206.61	12730	Reino Unido, cama de hongos	*Scopulariopsis coprophila*
Scopulariopsis flava	MUCL 9031	12585	Reino Unido, queso	*Scopulariopsis flava*
Scopulariopsis hibernica	UAMH 2643	12997	Irlanda, suelo	*Scopulariopsis hibernica*
Scopulariopsis parvula	MUCL 9041^{T}	12732	Canada, suelo	*Scopulariopsis parvula*
Scopulariopsis soppii	UAMH 9169^{IT}	12999	Canada, madera en descomposición	*Microascus soppii*
Wardomyces humicola	CBS 369.62^{T}	6311	Canadá, suelo	*Wardomyces humicola*
Wardomyces inflatus	CBS 216.61^{IT}	4161	Canadá, madera en descomposición	*Wardomyces inflatus*
Wardomycopsis humicola	CBS 487.66^{IT}	4160	Canadá, suelo	*Wardomycopsis humicola*

CBS = *CBS-KNAW Fungal Biodiversity Centre*, Utrecht, Holanda. FMR = *Facultat de Medicina de Reus*, Reus, España. IHEM = *Agro-Industrial Fungi and Yeasts Collection*, BCCM/MUCL, Bruselas, Bélgica. MUCL = *Mycothèque de l'Université Catholique de Louvain*, Louvain-la-Neuve, Bélgica. UAMH = *University of Alberta Microfungus Collection and Herbarium*, Edmonton, Canadá. ^{T} = tipo, ^{ET} = epitipo, ^{IT} = isotipo

Tabla 4. Cebadores y condiciones utilizados en esta tesis

Locus		Cebador			Hibridación		Capítulo
		Dirección	Nombre	Secuencia (5'-3')*	Temp. (ºC)	Tiempo (s)	
Actina (*Act*)		*Forward*	Act-1	TGGGACGATATGGAIAAIATCTGGCA	56	60	4.5
		Reverse	Act4R	TCITCGTATTCTTGCTTIGAIATCCACAT			
		Forward	ACT-512F	ATGTGCAAGGCCGGTTTCGC	57	60	4.2
		Reverse	ACT-783R	TACGAGTCCTTCTGGCCCAT			
Endoquitinasa CHI18-5 (*chi18-5*)		*Forward*	Chit42-1a	GCTYTCCATCGGTGGCTGGAC	63	60	4.5
		Reverse	Chit42-2a	GGAGTTGGGGTAGCTCAGC			
Factor de elongación (*Tef*1/EF-1α/EF1-α)		*Forward*	EF-1H	ATGGGTAAGGAAGACAAGAC	57	60	4.5
		Reverse	EF-2T	GGAAGTACCAGTGATCATGTT			
		Forward	EF-728F	CATCGAGAAGTTCGAGAAGG	57	60	4.2
		Reverse	EF-986R	TACTTGAAGGAACCCTTACC			
		Forward	EF-983F	GCYCCYGGHCAYCGTGAYTTYAT	57	80	4.3, 4.4
		Reverse	EF-2218R	ATGACACCRACRGCRACRGTYTG			
ITS		*Forward*	ITS4	TCCTCCGCTTATTGATATGC	55	60	4.1, 4.2, 4.4, 4.5
		Reverse	ITS5	GGAAGTAAAAGTCGTAACAAGG			
Tubulina (*Tub*)		*Forward*	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	57	60	4.1, 4.4
		Reverse	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC			
28S (LSU)	Regiones D1/D2	*Forward*	NL1	GCATATCAATAAGCGGAGGAAAA	51	60	4.3, 4.4
		Reverse	NL4b	GGTCCGTGTTTCAAGACGG			
	Regiones D1/D3	*Forward*	LR0R	ACCCGCTGAACTTAAGC	53	80	4.1, 4.2
		Reverse	LR5	TCCTGAGGGAAACTTCG			

*, secuencias nucleotídicas de acuerdo a la nomenclatura IUPAC (Johnson 2010).