TAXONOMÍA DE HONGOS CELOMICETOS DE INTERÉS CLÍNICO

Nicomedes Miguel Antonio Valenzuela López

UNIVERSITAT ROVIRA i VIRGILI

Taxonomía de hongos celomicetos de interés clínico

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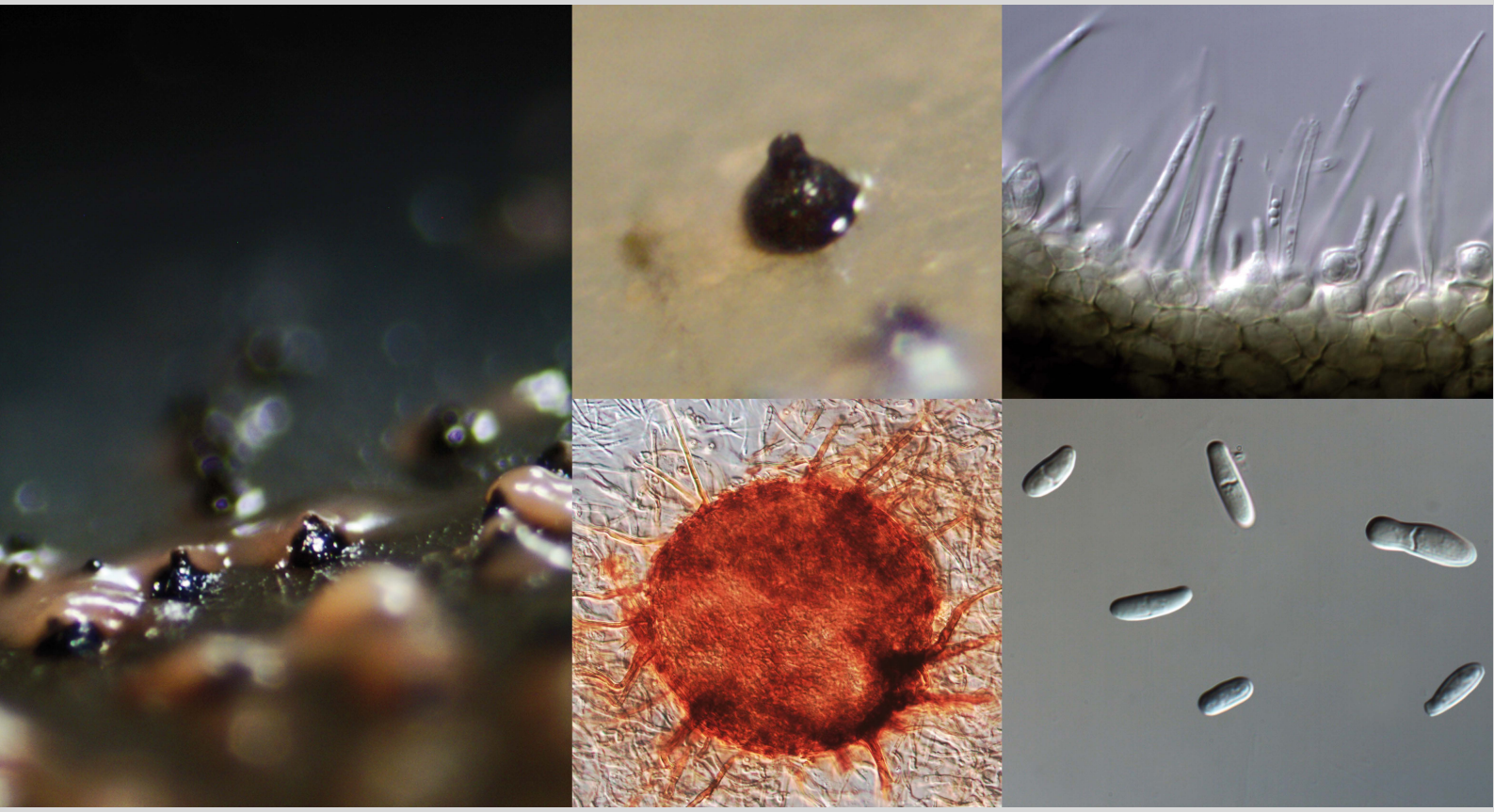

TESIS DOCTORAL
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Dirigida por los Doctores: José Francisco Cano Lira,
Alberto Miguel Stchigel Glikman y Josep Guarro Artigas

Departament de Ciències Mèdiques Bàsiques
Facultat de Medicina i Ciències de la Salut
Universitat Rovira i Virgili

Reus
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HAGO CONSTAR que el presente trabajo, titulado "**Taxonomía de hongos celomicetos de interés clínico**", que presenta **Nicomedes Miguel Antonio Valenzuela López** para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de Ciencias Médicas Básicas de esta universidad.

Reus, 08 de Junio de 2018

Los directores de la tesis doctoral

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AGRADECIMIENTOS

En primer lugar, agradecer a Dios, quien me ha mantenido firme en el camino y porque me ayuda en todo momento. A mi amada esposa Dámaris, quien apareció en el momento justo en mi vida, quien me da la alegría y las energías cuando me siento cansado y me anima a superarme cada día. A mí querida familia, pilar fundamental que me ha hecho lo que soy hoy en día; a mis padres y a mis hermanos, los amo con todo mi corazón, siempre estando ahí conmigo en todo para apoyarme. También expresar mi gratitud a mis directores de doctorado, por su confianza puesta en mí, por enseñarme lo importante que es ser meticuloso y dedicado, que el tiempo vuela y que hay que estar constantemente estudiando. Al Dr. Stchigel, por su paciencia y enseñanzas en la micología clásica, instruyéndome en el maravilloso mundo de los hongos. Al Dr. Cano, por recibirme en el programa, por las cervecitas tras publicar un trabajo, por su paciencia y cercanía, y por instruirme en la excelencia y en la jerga española. Al Dr. “Profesor” Josep Guarro, el jefe del grupo, con quien crecí en la redacción de artículos científicos, por guiarme y ayudarme en cada momento que lo necesitaba, por sus gestiones de “jefe” consiguiendo cepas en el CBS. Así como también a los restantes miembros del grupo que de alguna u otra forma me han ayudado a terminar este camino en la micología, los Doctores Josepa Gené, Dania García, Javier Capilla, Loida López, María José Figueras, Emilio Mayayo y Beli.

Agracader especialmente a las entidades colaboradoras como el *Fungal Testing Laboratory*, en especial a los doctores Wiederhold y a la memoria de Deanna Sutton, ya que muchos de los logros alcanzados en este trabajo se deben a ellos; también al Dr. Pedro Crous, quien con gran entusiasmo y amabilidad nos facilito un gran número de cepas del CBS y colobaró en importantes artículos con nosotros; también agradecer a Ana y Olga del *Instituto Carlos III*, a Dea García y su grupo de micología del *Institut Pasteur*, por sus colaboraciones y la importante participación que se ha forjado en los trabajos en conjunto.

A los TMs Dr. Luis Zaror y Dr. Patricio Godoy, con quienes tuve los primeros encuentros en cursos de micología y quienes fueron importantes nexos para poder venir a España a realizar el doctorado.

A mi grupo de Microbiología de la Universidad de Antofagasta, quienes depositaron su confianza en mí para poder perfeccionarme en esta materia, con especial cariño a Joselyne Gahona y Juan Silva, mis profes de micro.

Durante estos cuatro años han sido decenas las personas que han pasado por la Unidad y que han aportado con su granito de arena en mi desarrollo como doctorando y como persona, a todos ellos y mis compañeros (una larga lista por nombrar), los que han pasado, los que están y los recientes, a todos les agradezco su apoyo y amistad.

A todos, ¡muchas GRACIAS!

ÍNDICE

I. Índice de abreviaturas

act	Actina
ADN	Ácido desoxirribonucleico
AFG	Anidulafungina
AMB	Anfotericina B
ARN	Ácido ribonucleico
ATCC	*American Type Culture Collection* (EE.UU.)
BI	Inferencia Bayesiana
BLAST	Basic Local Alignment Tool
BS	Soporte de *bootstrap*
CBS	*Westerdijk Fungal Biodiversity Institute* (Utrecht, The Netherlands)
CFG	Caspofungina
CLSI	*Clinical Laboratory and Standards Institute* (EE.UU.)
CME	Concentración mínima eficaz
CMI	Concentración mínima inhibitoria
CNM-CM	*Centro Nacional Español de Microbiología del Instituto de Salud Carlos III* (Madrid, España).
CNRMA	*Centre National de Réfeìrence Mycoses Invasives et Antifongiques, Institut Pasteur* (Paris, Francia).
comb. nov.	Nueva combinación
D1-D2	Dominios D1 y D2 del gen 28S del rARN
d	Días
diam	Diámetro
dNTP	Desoxinucleótido trifosfato
***et al*.**	Y co-autores
EUCAST	*European Committee on Antimicrobial Suceptibility Testing*
***fam. nov*.**	Nueva familia
Fig.	Figura
FLC	Fluconazol
FMR	*Facultat de Medicina, Reus*
g	Gramo
***gen. nov*.**	Nuevo género

GM	Media geométrica
ITC	Itraconazol
ITS	Región espaciadora intergénica transcrita del ARNr
MCMC	*Markov chain Monte Carlo*
MEA	Agar extracto de malta
MEC	Concentración mínima efectiva
MEGA	*Molecular Evolutionary Genetic Analysis*
MFG	Micafungina
MIC	Concentración mínima inhibitoria
ML	Máxima verosimilitud
mL	Mililitro
mM	Mili molar
MP	Máxima parsimonia
mTorr	Mili Torr
MUSCLE	*Multiple Sequence Comparison by Log-Expectation*
NCBI	*National Center for Biotechnology Information* (EE.UU.)
NJ	*Neighbor-Joining*
NNI	*Nearest-Neighbor-Interchange*
OA	Agar harina de avena
PAUP	*Phylogenetic Analysis Using Parsimony*
pb	Pares de bases
PCA	Agar patata-zanahoria
PCR	Reacción en cadena de la polimerasa
PDA	Agar patata-glucosa
pp	Probabilidad posterior
PSC	Posaconazol
rpb2	ARN polimerasa subunidad II
RPMI	Medio de *Roswell Park Memorial Institute*
RNAr	ARN ribosomal
sp. nov.	Nueva especie
tef1	Factor de elongación 1-alfa
TRB	Terbinafina

***tub*2**	Beta-tubulina
U	Unidades
UTHSC	*Fungus Testing Laboratory of the University of Texas Health Science Center* (San Antonio, EE.UU.)
var.	Variedad
VIH	Síndrome de inmunodeficiencia humana
VRC	Voriconazol
µg	Microgramo
µL	Micro litro
µm	Micrómetro
µM	Micro molar
5FC	5-Fluorcitosina
ºC	Grados Celsius

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1. INTRODUCCIÓN

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1.1. Generalidades sobre los hongos

Los hongos son un grupo monofilético (con un ancestro común) de organismos, eucariotas, morfológicamente heterogéneo, uni- o pluricelulares (Fig. 1), con núcleos haploides o diploides, que se reproducen mediante esporas de origen mitótico (asexuales; mitosporas) y/o de origen meiótico (sexuales; meiosporas), y que a diferencia de las plantas y algas carecen de cloroplastos, a pesar de que al igual que ellas son mayoritariamente inmóviles (salvo los taxones menos evolucionados) y sus células están delimitadas por una pared cuyo componente fundamental es la quitina. Debido a que los hongos son incapaces de realizar la fotosíntesis como mecanismo de obtención de energía, y a que no pueden sintetizar sus estructuras celulares a partir de moléculas inorgánicas, estos se ven obligados a utilizar un mecanismo de digestión externa (ya que no poseen un sistema digestivo) de la materia orgánica preformada (organismos quimioheterotróficos), mediante la secreción de exoenzimas que hidrolizan macromoléculas (mayoritariamente proteínas, polisacáridos y lípidos), permitiéndole así obtener los nutrientes esenciales destinados a la generación de energía química y biosíntesis de sus estructuras celulares (Brock 2006). Por dicho motivo, los hongos son conocidos como organismos osmótrofos, o de nutrición absortiva. La mayoría de los hongos son organismos aerobios (obtienen su energía interna mediante el catabolismo oxígeno-dependiente de moléculas orgánicas), pudiendo comportarse como saprobios (nutriéndose a partir de la materia orgánica muerta; necrótrofos), como parásitos (obteniendo los nutrientes esenciales de un organismo vivo) o simbiontes (estableciendo una relación mutualista con otro organismo, con el cual intercambian nutrientes), tanto en ambientes acuáticos como en terrestres.

Los hongos pueden presentar dos tipos de talo (organización somática o vegetativa básica): unicelular (hongos levaduriformes; levaduras) y pluricelular (hongos filamentosos o miceliares). Los hongos filamentosos están formados por una sucesión de células de forma tubular que forman un todo: las hifas (Brock 2006, Brandt & Warnock 2015). Estas células pueden estar delimitadas (hifas septadas ó tabicadas) o no (hifas aseptadas o cenocíticas) entre sí por septos (invaginaciones de la pared celular que separa la hifa en compartimentos intercomunicados o no entre sí). El conjunto de hifas que forman un individuo se denomina micelio. Existen algunos hongos capaces de producir ambos tipos de talo dependiendo de las condiciones ambientales. Cuando el parámetro determinante es la temperatura, estos se denominan hongos térmicamente dimórficos (usualmente presentan una forma filamentosa a 25ºC y una levaduriforme a 37ºC), muchos de los cuales son patógenos animales (el hombre incluido) y pertenecen a un grupo taxonómico concreto (la familia *Ajellomycetaceae*, del orden *Onygenales*) (Harris 2008, de Hoog *et al.* 2011).

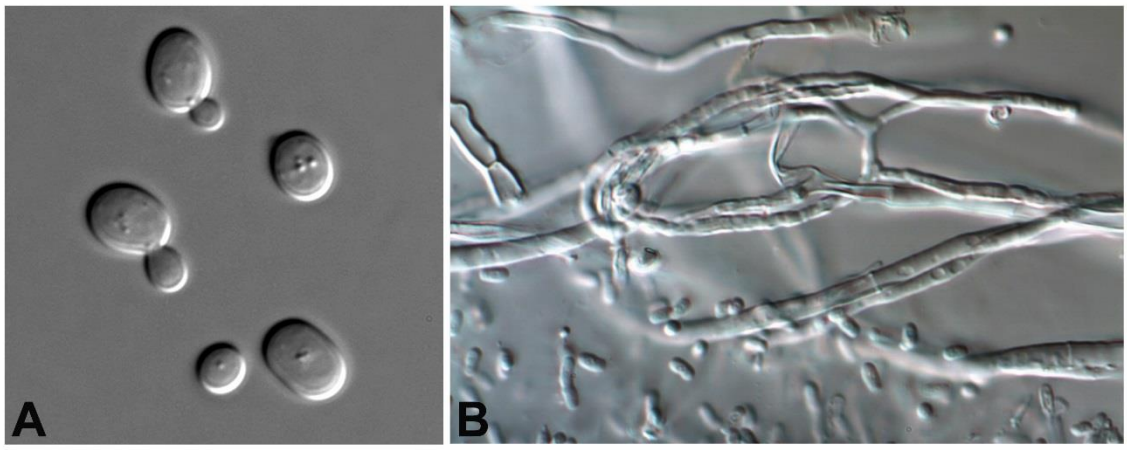


Figura 1. Talo fúngico. A. Unicelular. B. Pluricelular (filamentoso).

La estrategia reproductiva de los hongos puede incluir una fase de reproducción asexual (anamorfo o forma asexual) caracterizada por la producción de esporas cuyos núcleos provienen de la división mitótica de un núcleo preexistente, y/o una fase de reproducción sexual (teleomorfo ó forma sexual) caracterizada por la división meiótica (y posteriormente mitótica) de un núcleo diploide (producto de la fusión de dos núcleos haploides de individuos sexualmente compatibles). El hongo que es capaz de reproducirse mediante ambas estrategias de forma simultánea se denomina holomorfo (Fig. 2). También es posible que un individuo presente dos estrategias de reproducción asexual (morfológica y estructuralmente) diferentes, las que son denominadas sinanamorfos, como es el caso del hongo celomiceto *Neoscytalidium dimidiatum* (capaz de formar esporas asexuales a partir de la fragmentación de las hifas vegetativas y también en el interior de un cuerpo fructífero con diferenciación tisular).

Originalmente los hongos fueron clasificados dentro del reino *Plantae* (Haeckel 1866), en el subreino *Talobionta* (talofitas, o plantas con talo) y se creía que descendían de las algas rojas (rodofíceas o rodífitas) (Scagel *et al*. 1980, Carlile & Watkinson 1994). Sin embargo, Whittaker (1969) propuso un sistema de clasificación de los seres vivos en cinco reinos, dos para la clasificación de los organismos más simples como las bacterias y protozoos (*Monera* y Protista), y los otros tres para los organismos más complejos (*Animalia*, *Fungi*, *Plantae*), en el cual los hongos se agrupan dentro del reino *Fungi* hasta el día de hoy (Fig. 3) (Spatafora *et al*. 2017).

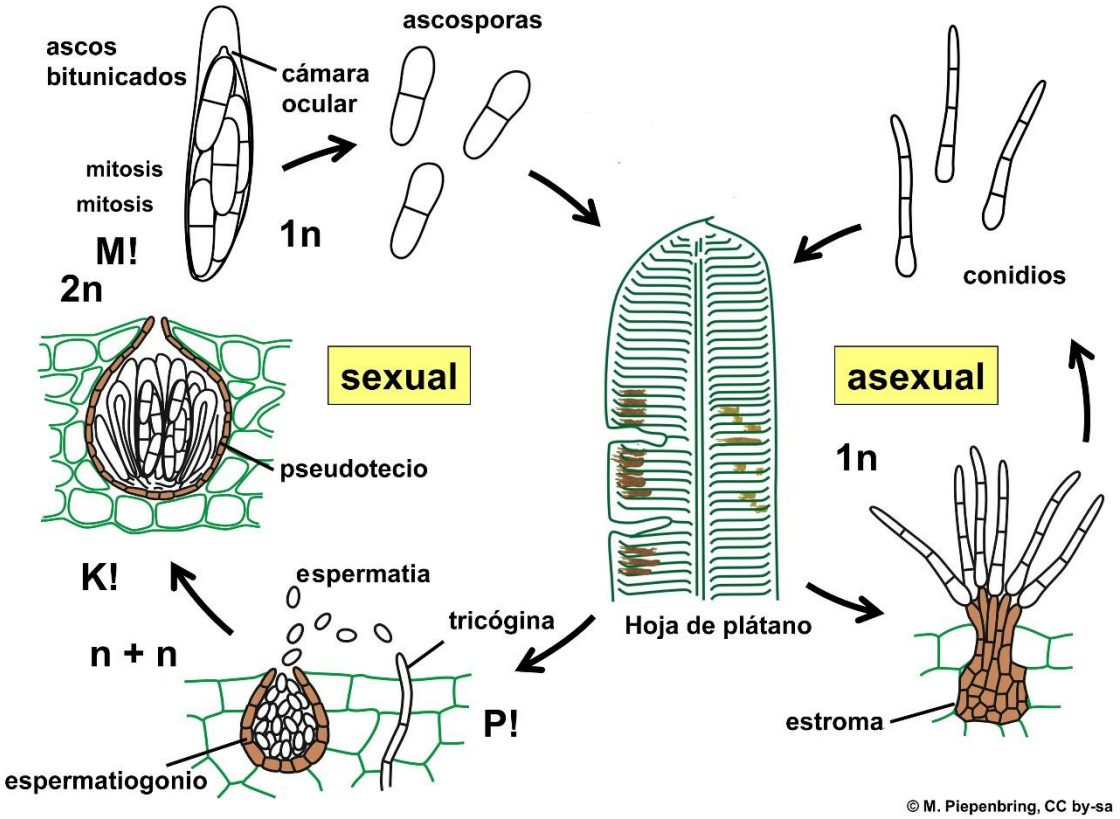


Figura 2. Ciclo de vida de *Mycosphaerella* sp. con su forma asexual *Pseudocercospora* sp. (*Mycosphaerellales*, *Ascomycota*); Abreviaciones: 1n = haploide, P! = plasmogamia, n+n = dicariótico, K! = cariogamia, 2n = diploide, M! = meiosis. Adaptado de M. Piepenbring (http://species-id.net/openmedia/Mycological_teaching_diagrams_by_Meike_Piepenbring).

1.2. Los hongos celomicetos

1.2.1. Historia

El término “celomiceto” fue introducido por Grove (1919) para acomodar un grupo de hongos morfológicamente caracterizados por producir sus conidios (esporas asexuales) dentro de una cavidad piriforme o una matriz fúngica en forma de cojín, pertenecientes a los géneros *Phloeospora*, *Phomopsis* y *Phyllosticta*. A partir de aquí, el término se fue expandiendo para agrupar a todos los hongos con esas características, proponiéndose posteriormente como una clase, *Coelomyctetes*, dentro de la subdivisión *Deuteromycotina* (que agrupaba a todos los hongos con tan solo el mecanismo de reproducción asexual, llamados por dicho motivo hongos “imperfectos”) (Ainswoth 1966). Sin embargo, actualmente este término está obsoleto debido a que posteriores estudios filogenéticos han demostrado su naturaleza polifilética (la existencia de más de un ancestro) del taxón, y solo se mantiene

su uso por fines prácticos, especialmente en fitopatología y en clínica (Taylor 1995, Kendrick 2000).

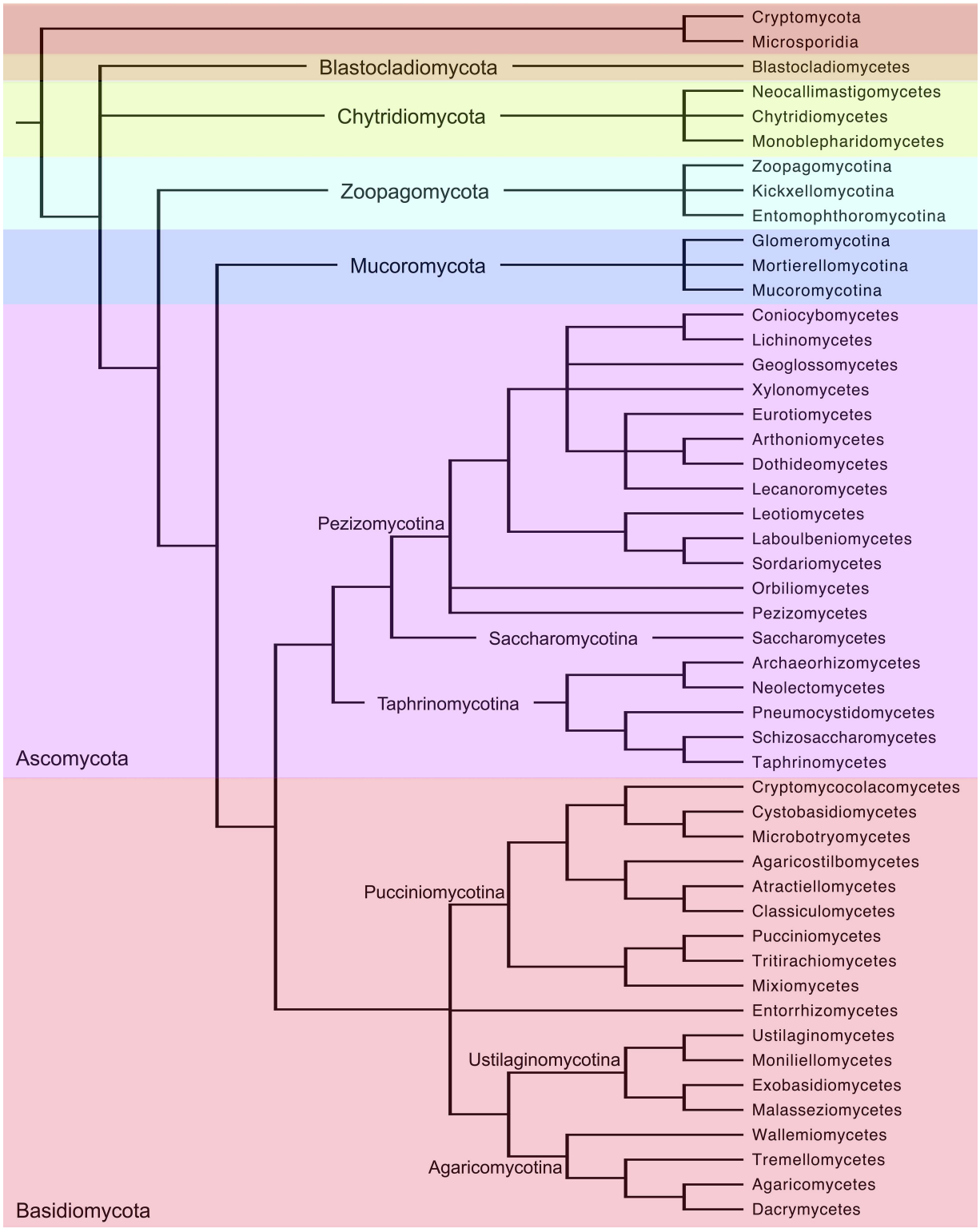


Figura 3. Clasificación taxonómica vigente del reino *Fungi*, representando por los actuales 8 filos, 12 subfilos y 46 clases. Adaptado de Spatafora *et al*. (2017).

1.2.2. Ecología

Los hongos celomicetos (cómo grupo morfológico) habitan una gran gama de nichos ecológicos, estando presentes mayoritariamente en ambientes terrestres como saprobios o como patógenos de plantas (Cortinas *et al*. 2006, Wikee *et al*. 2011, Udayanga *et al*. 2011, Maharachchikumbura *et al*. 2011, 2013; Hyde *et al*. 2014), pero también se pueden encontrar libres en el suelo (Someya *et al*. 1997), o como endófitos (un tipo de asociación mutualista) de plantas (Rajagopal *et al*. 2012). En ambientes acuáticos también se ha reportado el hallazgo de varias especies, principalmente en plantas acuáticas de agua dulce y saladas, e incluso en aguas residuales (Aveskamp *et al*. 2008, Al-Saadoon & Al-Dossary 2014). También pueden encontrarse como simbiontes en la formación de líquenes o micorrizas (Diederich *et al*. 2001, 2012; Lawrey *et al*. 2011, Oliveira *et al*. 2014). Algunos pueden comportarse como parásitos de otros hongos (hiperparásitos), y un reducido número son patógenos de insectos y vertebrados (seres humanos incluidos) (El-Bassam *et al*. 2002, Krockenberger 2010, de Hoog *et al*. 2011, Stchigel & Sutton 2013, Valenzuela-Lopez *et al*. 2017).

1.2.3. Morfología

Los hongos celomicetos se caracterizan por la producción de un número limitado de estructuras morfológicas. Los conidiomas son sus estructuras reproductivas más distintivas, siendo su naturaleza la de cuerpos fructíferos asexuales, de pared (peridio) delgada (pseudoparenquimatosa, compuesta por células que forman un “tejido” discreto) o gruesa (escleroparenquimatosa, compuesto por una masa de células de paredes engrosadas, de consistencia muy dura que en sus fases iniciales recuerda a esclerocios, y en cuyo interior se abre con el tiempo una cavidad donde se desarrollan las células conidiógenas productoras de conidios), la que se encuentra, por lo general, en combinación o sobre el tejido del huésped (generalmente vegetal). Dichos conidiomas se pueden clasificar en cuatro tipos básicos: acervular (forma de platillo, unido íntimamente al tejido huésped, Fig. 4.A), picnidial (usualmente piriforme y formado de “tejido” fúngico, o en conjunto con el tejido del huésped, Fig. 4.B), estromático (masa de células o de hifas vegetativas unidas o no al tejido huésped, Fig. 4.C) y picnotirial (forma de escudo, en el tejido superficial del huésped, Fig. 4.D), siendo los más frecuentes los conidiomas acervulares y picnidiales. Los conidiomas pueden presentar ciertas ornamentaciones externas, tales como setas, o estar cubiertos de hifas. Su color, forma, textura de la pared, producción (solitarios o agrupados), y su localización en el tejido del huésped o cuando se desarrollan en medios de cultivo *in vitro* (superficiales o

inmersos) son empleados como criterios taxonómicos secundarios para su clasificación infragenética (en la metodología "clásica" de identificación) (Sutton 1980, Boerema *et al*. 2004, Kirk *et al.* 2008). Los conidióforos se encuentran dentro del conidioma, y estas hifas especializadas contienen o soportan a las células conidiógenas. Estas estructuras se caracterizan en base a su longitud, número de ramificaciones, presencia de septos y color (Fig. 5).

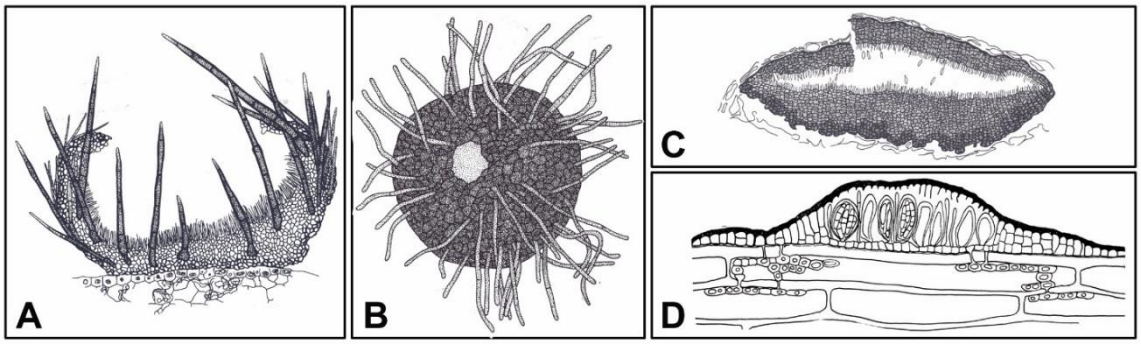


Figura 4. Tipos de conidiomas: A, acervular (*Dinemasporium strigosum*); B, picnidial (*Chaetodiplodia caulina*); C, estromático (*Cryptomycella pteridis*); D, picnotirial (*Pycnothyrium* sp.). A-C. Adaptado de Sutton (1980), D. Adaptado de von Arx & Müller (1975).

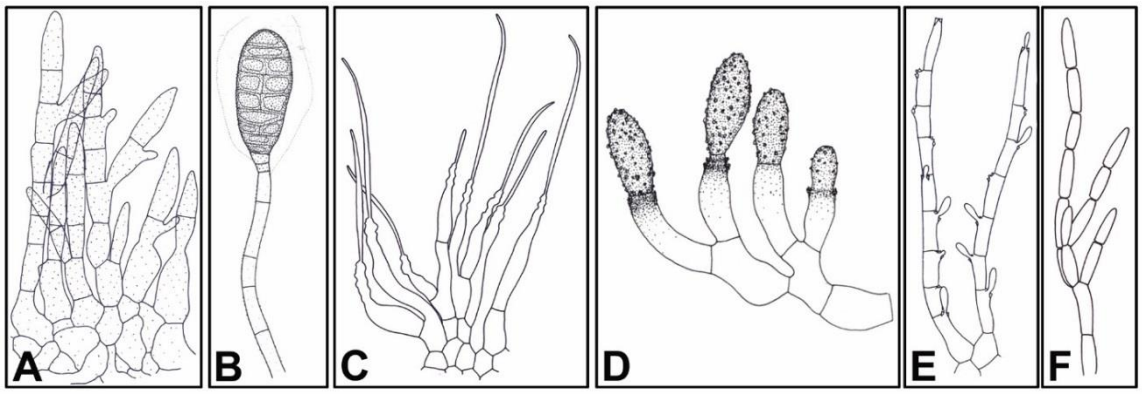


Figura 5. Diferentes tipos de conidióforos de hongos celomicetos. A, *Digitosporium piniphilum*; B, *Myxocyclus polycistis*; C, *Polystigmina rubra*; D, *Leptomelanconium piceae*; E, *Pleurophoma pleurospora*; F, *Phacidiella salicina*. Adaptado de Sutton (1980).

Sin embargo, es más habitual que en los hongos celomicetos los conidióforos estén reducidos a tan solo una célula conidiógena, estructura reproductiva asexual donde los conidios son producidos mediante un proceso denominado conidiogénesis, por diferentes mecanismos: enteroblásticos (a partir de una célula conidiógena fialídica o anelídica; de neoformación, estando involucradas las capas más internas de la pared de la célula conidiógena en la producción del conidio); holoblásticos (de neoformación, estando involucradas todas las capas de la pared de la célula conidiógena en la producción del

conidio) o tálicos (a partir de la diferenciación de una célula preexistente). Con fines taxonómicos, se documentan su color, grosor y ornamentación de la pared, forma y tamaño (Fig. 6).

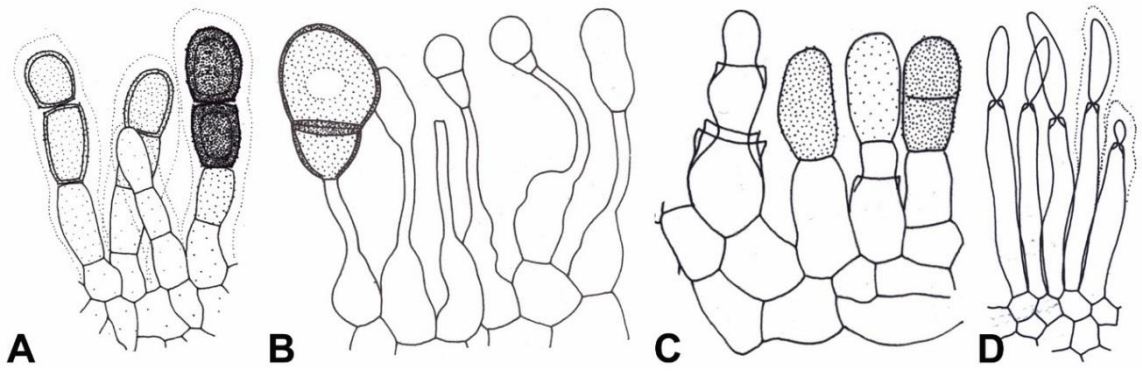
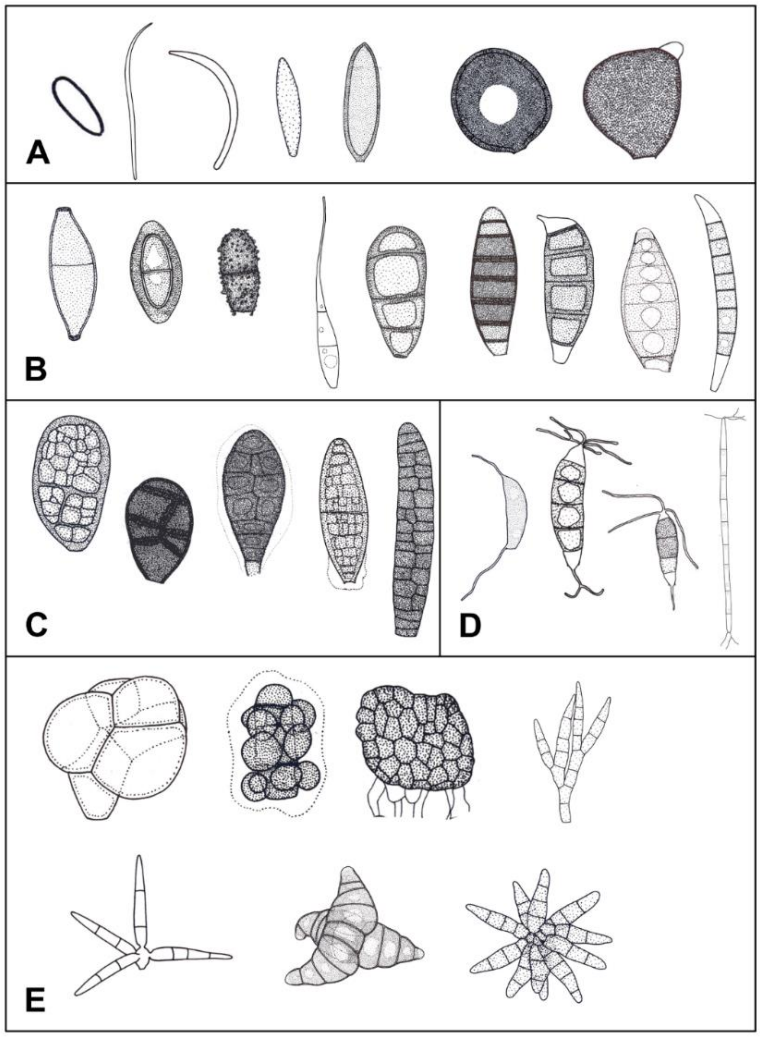


Figura 6. Células conidiógenas y sus diferentes tipos de conidiogénesis: A, Tálica-holoártrica (*Staninwardia breviuscula*); B, Holobástica (*Kamatella apiospora*); C, Enteroblástica-anelídica (*Coniothyrium palmarum*); D, Enteroblástica-fialídica (*Amerosporium polynematoides*). Adaptado de Sutton (1980).



Los conidios, son probablemente las estructuras morfológicamente más diversas capaces de ser formadas por los celomicetos, pudiendo presentar o no apéndices celulares y acelulares, masas gelatinosas alrededor de ellos, una gran variedad de colores, grosor y ornamentación de su pared, y número variable de septos y tamaños (Fig. 7).

Figura 7. Diversidad de conidios en los hongos celomicetos. A, sin septos; B, con septos transversales; C, muriformes (septos transversales y longitudinales); D, con apéndices; E, de formas variables. Adaptado de Sutton (1980).

Además de las estructuras anteriormente mencionadas, algunos celomicetos pueden presentar setas alrededor del conidioma, clamidosporas en el micelio superficial y/o sumergido y células apresorias (apresorios). Las setas son hifas modificadas de pared gruesa y de color oscuro, que pueden presentar septos, tener una pared lisa u ornamentada, y presentar diferentes (usualmente aguzadas) terminaciones (Fig. 8). Las clamidosporas corresponden a células de resistencia, presentan una pared mucho más gruesa que el resto de las estructuras formadas por estos hongos, y están caracterizadas por su ubicación sobre la hifa que las produce (intercalar o terminal), su forma, coloración y si presentan septos (Fig. 9). Las células apresorias se encuentran principalmente en el género fitopatógeno *Colletotrichum*, y tienen como función la adhesión al huésped en estados tempranos de la infección, sirviendo en la identificación de algunas de las especies del mencionado género (Fig. 10).

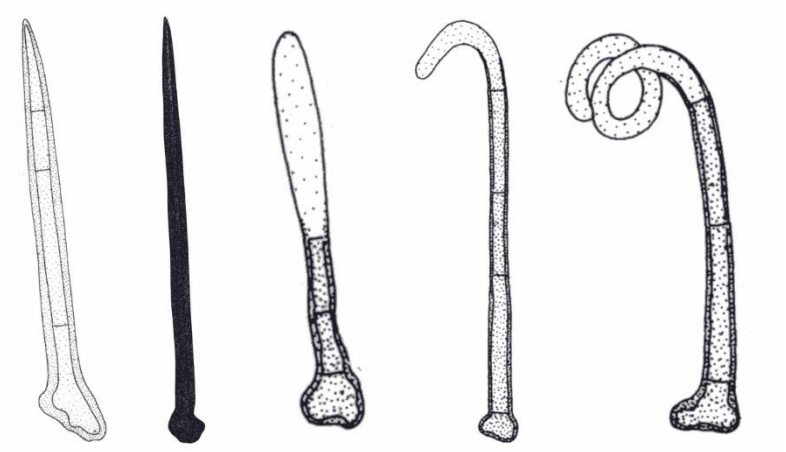


Figura 8. Diferentes tipos de setas presentes en los hongos celomicetos. Adaptado de Sutton (1980).

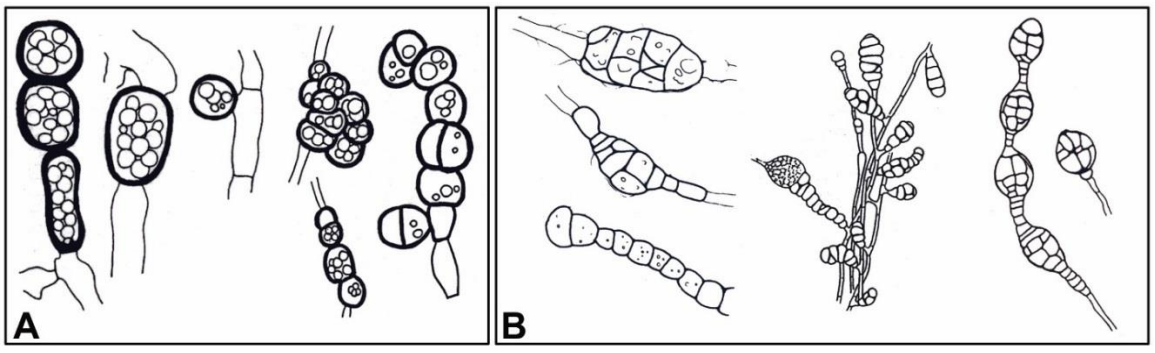


Figura 9. Tipos de clamidosporas: A, unicelulares, intercalares o terminales; B, multicelulares, botrioides o alternariodes, intercalares o terminales. Adaptado de Boerema *et al*. (2004).

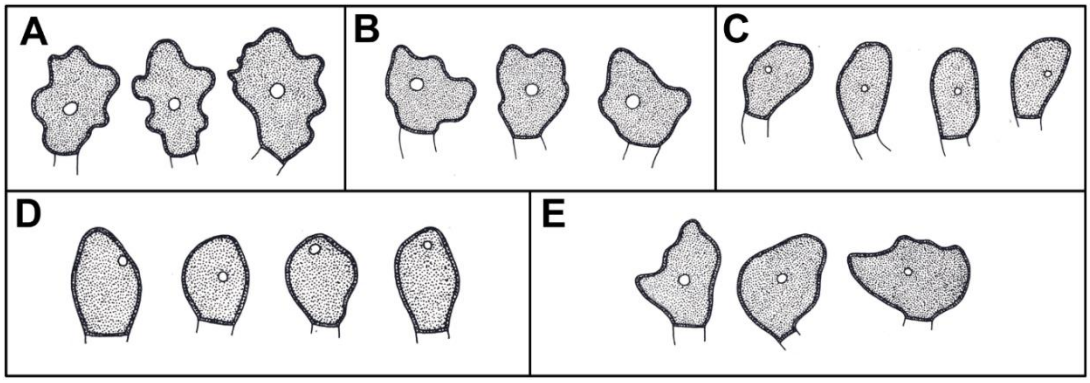


Figura 10. Apresorias: A, *Colletotrichum crassipes*; B, *C. musae*; C, *C. orbiculare*; D, *C. coffeanum*; E, *C. gloeosporioides*. Adaptado de Sutton (1980).

1.2.4. Clasificación taxonómica

La taxonomía de los hongos históricamente se ha basado en la clasificación sistemática establecida por Linneo (1753), la cual consiste en la comparación de sus caracteres morfológicos, ubicando organismos similares en un mismo grupo. Estos grupos se denominan taxones y están ordenados jerárquicamente (Fig. 11).

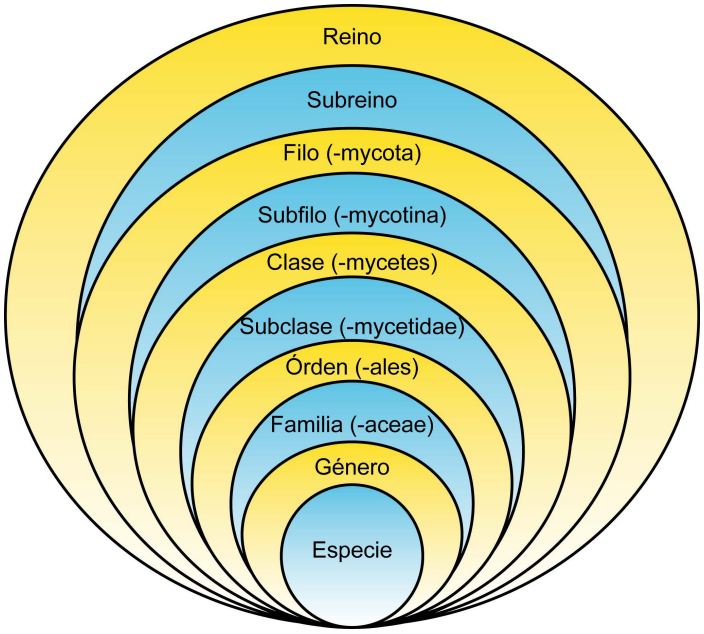


Figura 11. Rangos taxonómicos y sus terminaciones en la clasificación de los hongos (Hibbett *et al*. 2007).

Originalmente, los hongos celomicetos fueron agrupados en la clase *Coelomycetes* de la subdivisión *Deuteromycotina* (hongos exclusivamente con reproducción asexual). La clase *Coelomycetes* estaba divida en tres órdenes: *Melanconiales* (hongos que producen

acérvulos), *Sphaeropsidales* (hongos que producen picnidios) y *Pycnothyriales* (hongos que producen picnotirios) (Ainsworth 1966). Posteriormente, Sutton (1980) en base principalmente al tipo de conidiogénesis, mantuvo dichos hongos dentro de la clase *Coelomycetes*, distribuyéndolos en cinco órdenes: *Blastales*, *Enterothallales*, *Phialidales*, *Thallales* y *Tretales*. Sin embargo, actualmente el sistema de clasificación precedente ya no es aceptado, debido a que en base a los estudios de filogenia molecular se ha demostrado de que no es un grupo monofilético (de Gruyter *et al*. 2009, 2013; Aveskamp *et al*. 2010). Actualmente, el término "coelomycete" se sigue empleando en base a criterios prácticos en patología vegetal y animal, pero se ha demostrado mediante estudios moleculares que este grupo de hongos se encuentra distribuido en, al menos, tres clases diferentes: *Dothideomycetes*, *Leotiomycetes* y *Sordariomycetes*, del filo *Ascomycota* (Schoch *et al*. 2009, Maharachchikumbura *et al*. 2014, Wijayawardene *et al*. 2016, Valenzuela-Lopez *et al*. 2017).

1.3. Generalidades sobre los géneros de hongos celomicetos aislados de especímenes clínicos incluidos en la presente tesis

En la presente tesis se han estudiado una gran diversidad de hongos celomicetos aislados de muestras clínicas procedentes de los Estados Unidos de Norteamérica así como de Europa (España y Francia), los cuales correspondieron a los géneros *Colletotrichum*, *Diaporthe*, *Didymella*, *Epicoccum*, *Lasiodiplodia*, *Medicopsis*, *Neocucurbitaria*, *Neoscytalidium*, *Nigrograna*, *Paraconiothyrium*, *Parathyridaria*, *Phoma*, *Pseudochaetosphaeronema*, *Tintelnotia* y *Trematosphaeria*, los cuales se distribuyen en diferentes órdenes (Fig. 12). Estos organismos, involucrados mayoritariamente en la producción de infecciones oportunistas, son por lo general hongos ubicuos de bajo potencial patogénico, lo que sumado a su relativa baja incidencia en clínica humana contribuye a la falta de datos relevantes sobre su epidemiología y tratamiento antifúngico efectivo (Stchigel & Sutton 2013, Guégan *et al*. 2016, Valenzuela-Lopez *et al*. 2017).

Las patologías que producen en el hombre se pueden clasificar de acuerdo al tipo de infecciones que son capaces de producir, y en la mayoría de los casos éstas suceden por traumatismo de la piel con materiales contaminados por esporas fúngicas. Las infecciones superficiales pueden afectar la piel, el pelo, los ojos y las uñas (Arenas 2011). Las micosis subcutáneas, pueden presentarse en forma de quistes, pero la forma prevalente de presentación de infecciones por los celomicetos es el eumicetoma de grano negro, esta afección está definida como un síndrome anátomo-patológico de tipo inflamatorio crónico, ésta puede ser causada tanto por bacterias (conocida como actinomicetoma) o por hongos

(eumicetoma) o la mezcla de ambos. Generalmente afecta al pie, pero puedo ocurrir en otras zonas anatómicas, caracterizándose por un aumento del volumen de la zona infectada, con la deformación y aparición de fístulas con contenido purulento donde se encuentra el agente causante de la infección, formando gránulos que pueden ser de color blanco o marrón oscuro (Arenas 2011, van de Sande 2013, van de Sande *et al*. 2017).

Las micosis sistémicas causadas por celomicetos son muy raras, y se suelen reportar en pacientes inmunocomprometidos, los que por lo general han recibido tratamiento antineoplásico o han sido receptores de transplantes de órganos (Benne *et al*. 1993, Balis *et al*. 2006, Tan *et al*. 2008, Woo *et al*. 2008, Kindo *et al*. 2010, Arora *et al*. 2012, Guégan *et al*. 2016).

Por otro lado, la identificación de estos hongos en el laboratorio clínico no siempre es sencillo, debido principalmente a que no se emplea el medio de cultivo apropiado para producir los cuerpos de fructificación típicos, o bien porque ellos no son capaces de desarrollarlos en cultivo. Sin embargo, hoy en día ya no es tan difícil realizar una identificación molecular en los laboratorios clínicos, pero su dificultad radica en que tan solo se emplea un único marcador filogenético para tal finalidad (como p. ej. la región espaciadora intergénica [ITS]), dando como resultado la identificación de varias especies a la vez o géneros incluso (Stchigel & Sutton 2013, Valenzuela-Lopez *et al*. 2017, Valenzuela-Lopez *et al*. 2018b). Los exámenes histopatológicos siguen siendo importantes a la hora de confirmar un diagnóstico positivo de una infección causada por un hongo. Los elementos fúngicos de los celomicetos en los tejidos son bastante pleomórficos: pueden presentar hifas moniliformes (forma de collar), estructuras levaduriformes o hifas con o sin septos, en su mayoría pigmentados (Revankar & Sutton 2011, Guarner & Brandt 2011). Además, la observación directa de estructuras fúngicas utilizando KOH al 10 ó 20% en una proporción 1:1 con el tejido potecialmente infectado, es útil. Para la identificación histopatológica de infecciones causadas por celomicetos se recomienda las tinciones de hematoxilina-eosina (H&E), de Gomori- Groccot (GMS) y el ácido peryódico de Schiff (PAS), y en caso de que el hongo no contenga suficiente melanina en sus estructuras se recomienda utilizar la tinción de Fontana-Masson (Guarner & Brandt 2011).

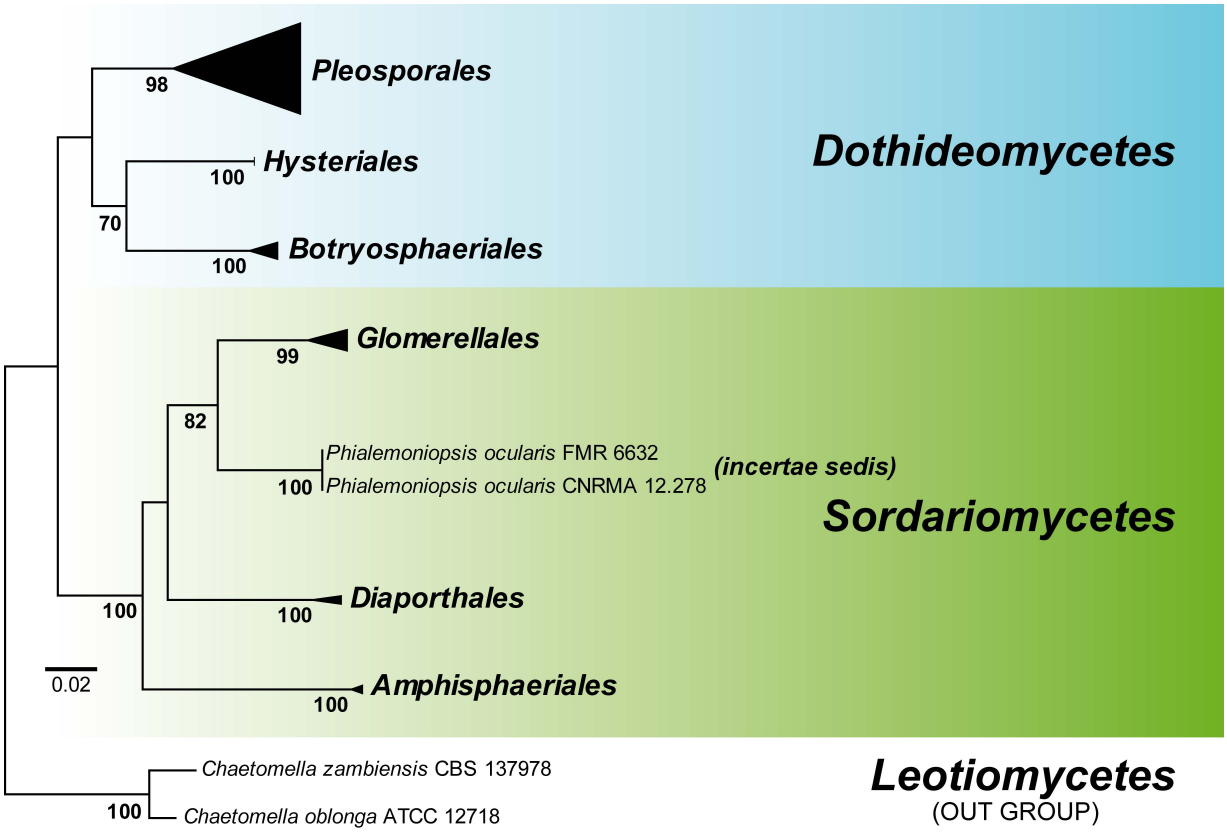


Figura 12. Clasificación taxonómica de los hongos celomicetos aislados de especímenes clínicos y reportados en la literatura como causantes de micosis humana.

En los siguientes apartados se describirán las principales características de los grupos de hongos celomicetos que han sido estudiados en la presente tesis.

1.3.1. Celomicetos del orden *Botryosphaeriales* (*Dothideomycetes*)

Este orden fue introducido por Schoch y co-autores (2006), basados en el análisis filogenético de cuatro marcadores moleculares. Este grupo de hongos se encuentra caracterizado por producir ascostromas (masas de tejido parecidas a un cojín, dentro de los cuales se abren una o más cavidades [lóculos] dentro de los cuales se producen ascos y ascosporas) de pared oscura y gruesa. Ascos bitunicados, con un número par de ascosporas uni- o bicelulares, incoloras cuando están inmaduras y marrones cuando son maduras (Schoch *et al*. 2006, 2009).

1.3.1.1. *Lasiodiplodia theobromae*

Propuesto inicialmente dentro del género *Botryodiplodia* (Patouillard 1892) fue posteriormente introducido con el nombre que se le conoce actualmente por Griffon y Maublanc (1909). Es un hongo cosmopolita presente en el suelo, material vegetal en descomposición y/o en plantas vivas, en este último sustrato como fitopatógeno (Alves *et al*. 2008). Para el hombre es un patógeno oportunista que infecta tejidos superficiales por traumatismo o por contacto con material (suelo o restos vegetales) contaminado (Summerbell *et al*. 2004, Saha *et al*. 2012. Gu *et al*. 2016).

Morfológicamente está caracterizado por producir conidiomas picnidiales, en cuyo interior presenta paráfisis (hifas estériles) hialinas, cilíndricas y septadas, células conidiógenas holoblásticas, hialinas, de pared lisa y delgada, y conidios inicialmente incoloros que se vuelven de color marrón oscuro y con una superficie estriada con el tiempo, subovoides a elipsoidales, con un extremo redondeado y una base trunca, de pared gruesa, presentando un septo transversal cuando el conidio está maduro (Fig. 13).

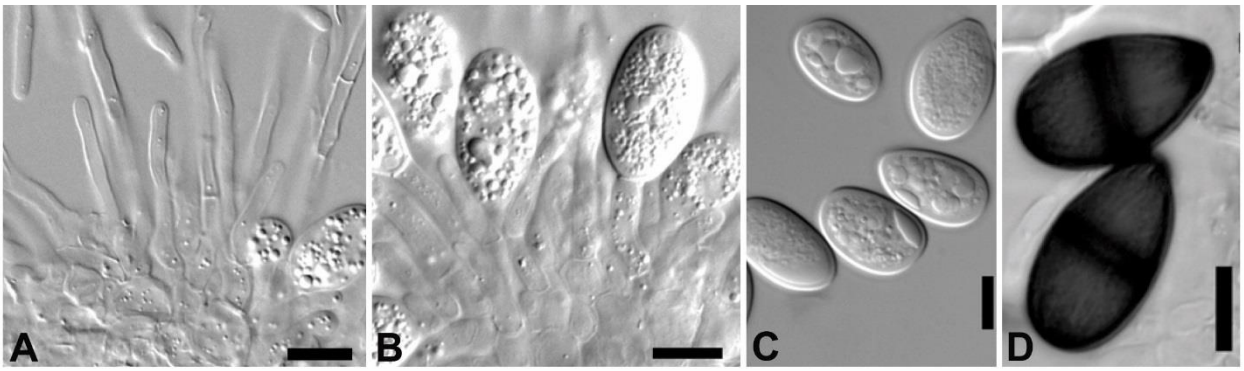


Figura 13. *Lasiodiplodia theobromae* (CBS164.96). A. Paráfisis. B. Células conidiógenas. C-D. Conidios. Escala = 10 µm. Adaptado de Alves *et al*. (2008).

1.3.1.2. *Neoscytalidium dimidiatum*

Este hongo cuyo nombre original era el de *Torula dimidiata* (Penzig 1887), ha ido cambiando sucesivamente de género hasta el actual *Neoscytalidium* (Crous *et al*. 2006) debido a la confusión creada con respecto a si pertenecía o no al género *Fusicoccum*, o si su sinanamorfo celomiceto *Hendersonula toruloidea* (Natrass 1933) correspondía filogenéticamente al mencionado género. El análisis molecular y morfológico de estos géneros y los fenotípicamente relacionados evidenció que *Fusicoccum* era polifilético, por lo

que no era factible ubicar dentro de *Fusicoccum* a *Scytalidium dimidiatum*. Además se demostró filogenéticamente que *Scytalidium sensu stricto* no pertenecía a la familia *Botryosphaeriaceae*, hecho que reforzó la propuesta del nuevo género *Neoscytalidium* (Crous *et al*. 2006). Algunos aislados clínicos considerados como una variante hialina (*N*. *dimidiatum* var. *hialinum*) (Madrid *et al*. 2009), resultaron posteriormente ser genéticamente idénticos al fenotipo pigmentado, descartando así la nueva variante y manteniendo una única especie. *Neoscytalidium dimidiatum* es un hongo dematiáceo y queratinofílico, distribuido mundialmente, aunque su hallazgo es más frecuente en zonas tropicales y subtropicales, conocido principalmente como fitopatógeno, pero capaz de producir micosis en el hombre por contacto directo con sus propágulos de dispersión presentes en plantas colonizadas (Hay 2002). *In vitro*, este hongo forma estructuras reproductivas similares a las de un hongo hifomiceto (es decir, que tan solo produce estructuras conidiógenas sencillas, estando ausentes cualquier tipo de cuerpo fructífero), y solo en algunas ocaciones es posible encontrar su sinanamorfo celomiceto *Hendersonula toruloidea* en cultivos viejos (Madrid *et al*. 2009). En el hombre este hongo produce desde infecciones superficiales (piel, uñas, cornea) (Elewski 1996, Barua *et al*. 2007, Godoy *et al*. 2009, Cursi *et al*. 2013) hasta profundas, y menos frecuentemente infecciones sistémicas (Benne *et al*. 1993, Mani *et al*. 2008, Ikram *et al*. 2009).

Morfológicamente, *N*. *dimidiatum* desarrolla colonias de crecimiento rápido, con abundante micelio aéreo de color gris oscuro a negras. Sin embargo, existe un fenotipo con colonias hialinas. Microscópicamente, forma hifas hialinas a marrones, de las cuales se producen cadenas de artroconidios (Fig. 14).

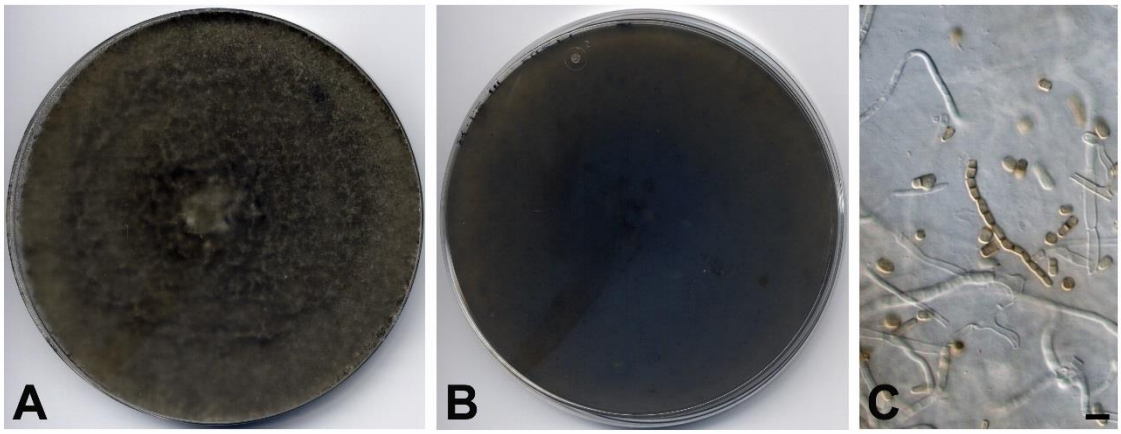


Figura 14. *Neoscytalidium dimidiatum* (FMR 13640). A-B. Colonia en PDA (anverso y reverso). C. Artroconidios. Escala = 10 µm.

1.3.2. Celomicetos del orden *Diaporthales* (*Sordariomycetes*)

Este orden fue descrito por Nannfeldt (1932), y contiene un grupo bien conocido de hongos ascomicetos patógenos de plantas, endófitos o saprobios (Castlebury *et al*. 2002, Rossman *et al*. 2007, Maharachchikumbura *et al*. 2016). Sus miembros se caracterizan por producir ascomas periteciales de color marrón oscuro a negro, inmersos en un estroma dentro de los tejidos del huesped, sin paráfisis o con un número muy reducido, los ascos son unitunicados, las ascosporas son desde pequeñas hasta grandes, aseptadas o septadas, incoloras o de pared pigmentada (Senanayake *et al*. 2017).

1.3.2.1. *Diaporthe*

Hasta hace algunos años *Phomopsis* (anamorfo) era considerado un género distinto del ascomiceto conocido con el nombre de *Diaporthe* (teleomorfo). Sin embargo, con el advenimiento de las técnicas moleculares y los análisis filogenéticos se demostró que ambos géneros estaban relacionados (Udayanga *et al*. 2012). Más recientemente, Rossman y co-autores (2015) propusieron recomendaciones con respecto al uso de nombres dentro del orden *Diaporthales*, teniendo *Diaporthe* prioridad sobre *Phomopsis*, debido a que el primero fue descrito con anterioridad (Nitschke 1870) del segundo (Bubák 1905). Estos cambios en la taxonomía generan confusión en el ámbito clínico y es importante ir introduciendo paulatinamente los nuevos nombres científicos en la rutina de la identificación de los hongos de interés médico.

Actualmente, existe un limitado conocimiento sobre las especies de *Diaporthe* involucrados en infecciones, sobre todo debido a que muchos aislados han sido identificados como *Phomopsis* sp. y, en el mejor de los casos, se ha realizado un análisis filogenético limitado, basado tan solo en el estudio de un único marcador filogenético como el ITS, con lo cual resulta imposible poder llegar a identificar el hongo a nivel de especie.

Las especies del género *Diaporthe* que han sido reportadas como responsables de micosis humanas son *Diaporthe bougainvilleicola*, *D*. *phaseolorum* y *D*. *phoenicicola*, causando principalmente infecciones superficiales (queratitis, dermatomicosis) y de tejidos subcutáneos (micetomas) (Iriart *et al*. 2001, Gajjar *et al*. 2011, Cariello *et al*. 2013).

La fase asexual de *Diaporthe* tiene un crecimiento muy rápido en los medios de cultivo empleados habitualmente en el laboratorio microbiológico, pudiendo formar conidiomas

picnidiales o estromáticos, en cuyo interior se hayan células conidiógenas fialídicas, hialinas y cilíndricas, presentando dos tipos de conidios: forma *alfa*, caracterizados por ser de tamaño pequeño, incoloros, unicelulares, de ovoides a elipsoidales; y la forma *beta*, más grandes y alargados, incoloros, unicelulares y filiformes (Fig. 15).

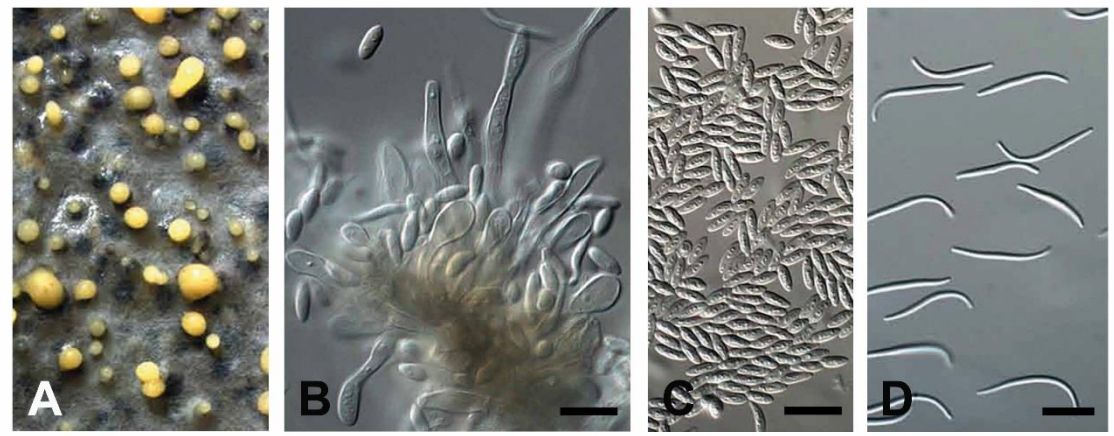


Figura 15. *Diaporthe hongkongensis* (CBS 115448). A. conidiomas a la lupa. B. Células conidiógenas. C. Alfa conidios. D. Beta conidios. Escala = 10 µm. Adaptado de Gomes *et al*. (2013).

1.3.3. Celomicetos del orden *Glomerellales* (*Sordariomycetes*)

Orden propuesto por primera vez por Chadefaud (1960) como “Glomérellales”, y que posteriormente Réblová y co-autores (2011) lo introdujeron formalmente basados en el análisis filogenético de las subunidades mayor (28S) y menor (18S) del ARNr (también conocidos como LSU y SSU). Se caracteriza por producir ascomas periteciales de color marrón oscuro, cuyo ostiolo está cubierto de perífisis, el tejido interascal tapizado de paráfisis, los ascos son unitunicados, las ascosporas de medidas y formas variadas, aseptadas o septadas, incoloras o pigmentadas (Réblová *et al*. 2011).

1.3.3.1. *Colletotrichum*

Este género agrupa importantes especies fitopatógenas, distribuidas mundialmente, pero más frecuentemente aisladas en regiones tropicales y subtropicales (Cannon *et al*. 2012). A pesar de que no es un género especialmente patógeno para el hombre, un reducido número de especies del mismo, es capaz de producir micosis, principalmente queratitis y

menos frecuentemente infecciones subcutáneas (Liao *et al*. 1983, Guarro *et al*. 1998, Castro *et al*. 2001, O'Quinn *et al*. 2001, Mendiratta *et al*. 2005, Potea *et al*. 2017). Cano y co-autores (2004) realizaron una revisión sistemática sobre las especies de este género productoras de infecciones en el hombre, en base a la caracterización de sus estructuras vegetativas y reproductivas y en la reconstrucción de su filogenia basada en las secuencias de la región ITS y de los dominios D1-D2 del gen LSU del ARNr. Sin embargo, debido a la complejidad taxonómica de este género, actualmente se necesitan por lo menos entre cinco y seis marcadores filogenéticos para llegar a la correcta identificación a nivel de especie. *Colletotrichum* se caracteriza por producir conidiomas acervulares, los cuales pueden estar o no ornamentados con setas, sus células conidiógenas son fialídicas, hialinas y cilíndricas, sus conidios son incoloros, unicelulares, mayoritariamente cilíndricos y de grandes dimensiones, redondos o aguzados en los extremos. Además, muchas especies son capaces de desarrollar células apresorias (Fig.16).

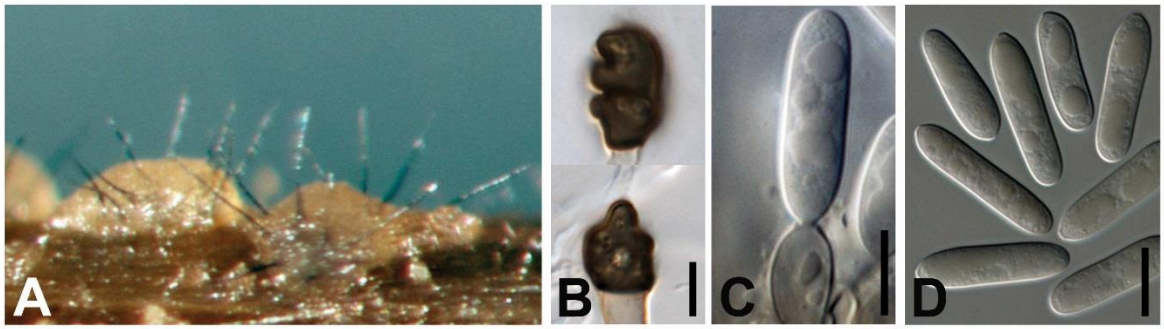


Figura 16. *Colletotrichum dracaenophilum* (CBS 118199). A. Conidiomas acervulares a la lupa. B. Células apresorias. C. Célula conidiógena. D. Conidios. Escala = 10 µm. Adaptado de Damm *et al*. (2019).

1.3.4. Celomicetos del orden *Pleosporales* (*Dothideomycetes*)

Este orden fue propuesto por Luttrell (1955), y posteriormente reintroducido formalmente por Barr (1987), basado en las características generales de los miembros de la familia *Pleosporaceae*. Actualmente, es uno de los órdenes más extensos de la clase *Dothideomycetes* (Hyde *et al*. 2013, Liu *et al*. 2017, Valenzuela-Lopez *et al*. 2018a). Los miembros de este orden se caracterizan por desarrollar ascomas periteciales de color marrón oscuro, los que presentan pseudoparáfisis, ascos por lo general cilíndricos y ascosporas de forma variada, uniseptadas o multiseptadas, incoloras o pigmentadas.

1.3.4.1. Géneros *Didymella*, *Epicoccum* y *Phoma*

Estos géneros, pertenecientes a la familia *Didymellaceae*, guardan una estrecha relación morfológica y filogenética entre sí (de Gruyter *et al*. 2009, Aveskamp *et al*. 2010). Previo a los estudios filogenéticos, basados en el análisis de las secuencias nucleotídicas de diferentes genes estructurales, ya se tenía conocimiento sobre la estrecha relación entre el género *Didymella* como el teleomorfo del género *Phoma*, y que el género *Epicoccum* era un sinanamorfo de algunas de las especies de *Phoma* (Boerema *et al*. 2004). Estos hongos son cosmopolitas, presentes tanto en ambientes terrestres como en acuáticos, muchos de ellos importantes fitopatógenos (Aveskamp *et al*. 2008), y a pesar de que las infecciones fúngicas causadas en humanos por especies pertenecientes a la familia *Didymellaceae* son poco frecuentes, existen reportes tanto de micosis superficiales como profundas (Punithalingam 1979, Bakerspigel *et al*. 1981, Rai 1989, Balis *et al*. 2006). Morfológicamente, los anamorfos de *Didymella* y los sinanamorfos de *Epicoccum* presentan las características morfológicas típicas del género *Phoma*: conidiomas picnidiales conteniendo en su interior células conidiógenas fialídicas, incoloras, de cilíndricas a subglobosas, que producen conidios mayoritariamente incoloros (ocasionalmente de color marrón claro), de pared delgada, y principalmente unicelulares (en algunos casos con un septo transversal), de ovoides a cilíndricos. Algunas especies son capaces de producir clamidosporas, uni- o pluricelulares (Fig. 17).

Figura 17. *Didymella glomerata* (UTHSC DI16-205). A. Conidiomas picnidiales a la lupa. B. Picnidios. C. Clamidosporas alternaroides. D. Células conidiógenas. E. Conidios. Escala: B = 100 µm. C-E = 10 µm.

1.3.4.2. *Medicopsis romeroi*

Esta especie fue por primera vez descrita por Borelli (1959) a partir de un caso de micetoma humano procedente de Venezuela. Posteriormente, ha sido reportada con cierta frecuencia produciendo infecciones cutáneas y subcutáneas (Girard *et al*. 2004, Ocampo *et*

al. 2012, Ahmed *et al*. 2014b, Guégan *et al*. 2016). También ha sido reportada en material vegetal, pero como saprobia (de Gruyter *et al*. 2013). Este hongo se caracteriza por producir conidiomas picnidiales cubiertos con setas de color marrón oscuro a negras, con células conidiógenas fialídicas en su interior, incoloras, de cilíndricas a subglobosas, produciendo conidios incoloros, de pared delgada, unicelulares y de forma cilíndrica a elipsoidal (Fig. 18).

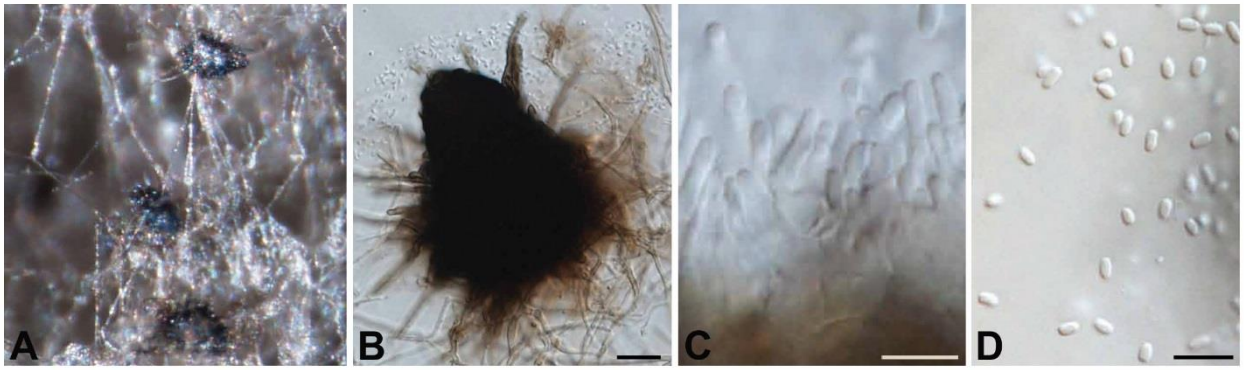


Figura 18. *Medicopsis romeroi* (CBS 252.60). A. Conidiomas picnidiales a la lupa. B. Picnidio. C. Células conidiógenas. D. Conidios. Escala = 10 µm. Adaptado de Ahmed *et al*. (2014b).

1.3.4.3. *Neocucurbitaria*

Varias especies del género *Neocucurbitaria*, previamente clasificadas como pertenecientes al género *Pyrenochaeta* (de Gruyter *et al*. 2013, Ahmed *et al*. 2014b, Jaklitsch & Voglmayr 2016, Wanasinghe *et al*. 2017, Valenzuela-Lopez *et al*. 2018a), han sido reportadas produciendo micosis en humanos, principalmente infecciones de tejidos superficiales (tales como piel y uñas; *Neocucurbitaria* cava y *Neocucurbitaria unguis-hominis*) (Stchigel & Sutton 2013, Valenzuela-Lopez *et al*. 2017, 2018b). *Neocucurbitaria cava* y *N. unguis-hominis* se caracterizan por producir conidiomas picnidiales, cubiertos o no por setas, en cuyo interior se desarrollan conidióforos con células conidiógenas fialídicas, hialinas, cilíndricas o subglobosas; los conidios son incoloros, de pared delgada, unicelulares, pequeños y de forma ovoide a elipsoidal (Fig. 19).

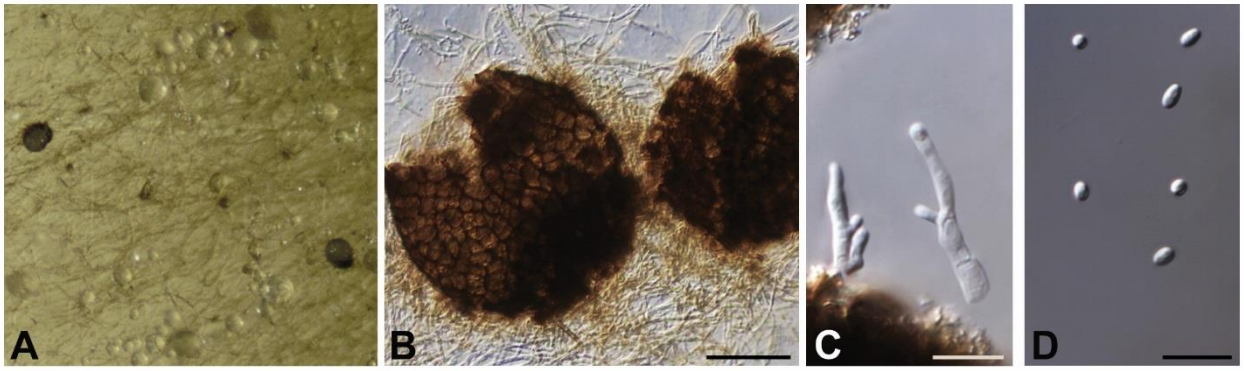


Figura 19. *Neocucurbitaria unguis-hominis* (CBS 112.79). A. Conidiomas picnidiales a la lupa. B. Picnidios. C. Conidióforos y células conidiógenas. D. Conidios. Escala: B = 50 µm. C-D = 10 µm.

1.3.4.4. *Nigrograna mackinnonii*

Esta especie ubicada originalmente en el género *Pyrenochaeta* por sus características morfológicas, fue aislada a partir de un caso de micetoma humano en Venezuela (Borelli 1976). Posteriormente, dicho taxón ha sufrido varios cambios nomenclaturales debido a su reubicación taxonómica, hasta que finalmente de Gruyter y co-autores (2013), basándose en un análisis filogenético, propusieron el nuevo género *Nigrograna* (nombre que refiere la manifestación clínica de producir granos negros en el micetoma) para acomodar este hongo, el cual carecía hasta ese momento de una asociación a nivel de familia. En un estudio posterior sobre todas las especies causantes de micetoma de grano negro, se propuso una nueva combinación para esta especie dentro del género *Biatriospora*, basado en un análisis filogenético multi-locus (Ahmed *et al*. 2014b). Sin embargo, el análisis multi-locus y el estudio morfológico de aislados provenientes de material vegetal desarrollado por Jaklitsch y Voglmayr (2016), descartó *N. mackinnonii* como perteneciente al género *Biatriospora*, proponiendo la nueva familia *Nigrograna*ceae.

Nigrograna mackinnonii, está morfológicamente caracterizado por producir conidiomas picnidiales cubiertos por hifas de color marrón oscuro, en cuyo interior se hayan células conidiógenas fialídicas, hialinas, de cilíndricas a subglobosas, las que generan conidios de incoloros a marrón claro, de pared delgada, unicelulares y de forma elipsoidal. A diferencia de las otras especies del género, ésta especie no produce conidióforos diferenciados (Jaklitsch & Voglmayr 2016). La figura 20 muestra los caracteres morfológicos de otra especie del género *Nigrograna*, concretamente *N. fuscidula*.

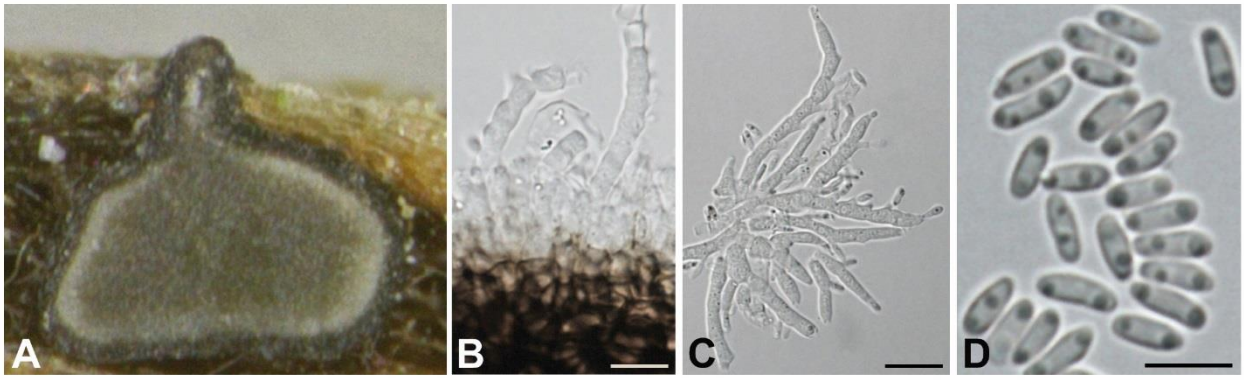


Figura 20. *Nigrograna fuscidula* (CBS 141476). A. Corte desde el material vegetal del conidioma picnidial. B. Pared del conidioma y conidióforos. C. Conidióforos y células conidiógenas. D. Conidios. Escala: B-C = 10 µm. D = 5 µm. Adaptado de Jaklitsch & Voglmayr (2016).

1.3.4.5. *Paraconiothyrium*

Este género fue propuesto por Verkley y co-autores (2004) para acomodar aquellos hongos morfológicamente similares a *Coniothyrium* o *Microsphaeropsis*, siendo éste un taxón cosmopolita y con gran potencial biotecnológico (Fukami *et al*. 2000, da Silva *et al*. 2003, Tsuda *et al*. 2003). Actualmente, solo dos especies del género han sido involucradas en infecciones humanas: *Paraconiothyrium fuckelii* y *Paraconiothyrium cyclothyrioides*, causando infecciones superficiales y profundas (Kiehn *et al*. 1987, Gordon *et al*. 2012, Guégan *et al*. 2016). Este género está morfológicamente caracterizado por producir conidiomas picnidiales o estromáticos, en cuyo interior se localizan células conidiógenas fialídicas, hialinas, de cilíndricas a subglobosas; los conidios inicialmente son incoloros pero al madurar se vuelven de color marrón claro, de pared delgada, unicelulares y de forma cilíndrica u ovoide (Fig. 21).

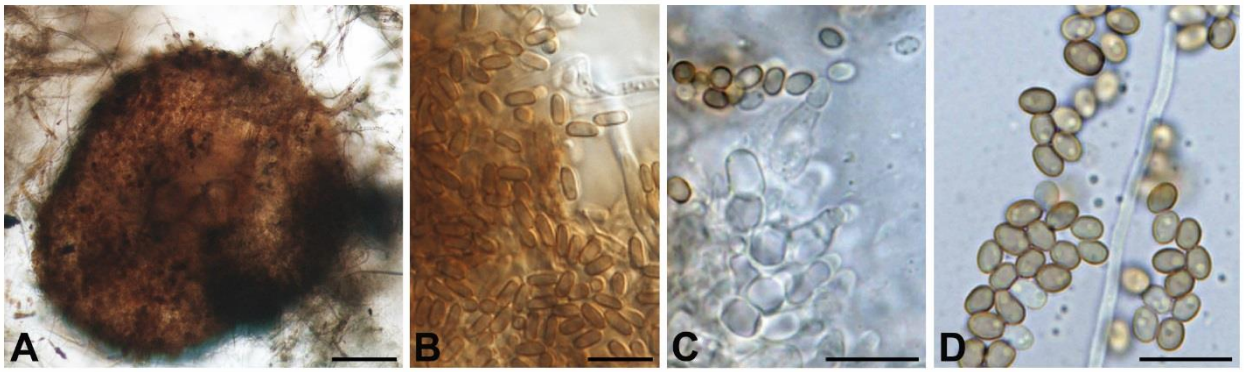


Figura 21. *Paraconiothyrium maculicutis* (CBS 101461). A. Picnidio. B. Conidios. *Paraconiothyrium fuckelii* (CBS 797.95). C. Células conidiógenas. D. Conidios. Escala: A = 20 µm. B-D = 10 µm. Adaptado de de Gruyter *et al*. (2013) y Verkley *et al*. (2014).

1.3.4.6. *Parathyridaria percutanea*

Esta especie fue descrita por primera vez por Ahmed y co-autores (2014a) con el nombre de *Roussoella percutanea*, siendo aislada de una lesión subcutánea del pie en un hombre. Actualmente, en base a un estudio filogenético, esta especie fue reubicada en la familia *Thyridariaceae*, como una combinación nueva dentro del género *Parathyridaria* (Jaklitsch & Voglmayr 2016). Este hongo produce conidiomas picnidiales, células conidiógenas fialídicas, hialinas, de globosas a subglobosas, y conidios incoloros a marrón claro, con paredes delgadas, unicelulares y elipsoidales (Fig. 22).

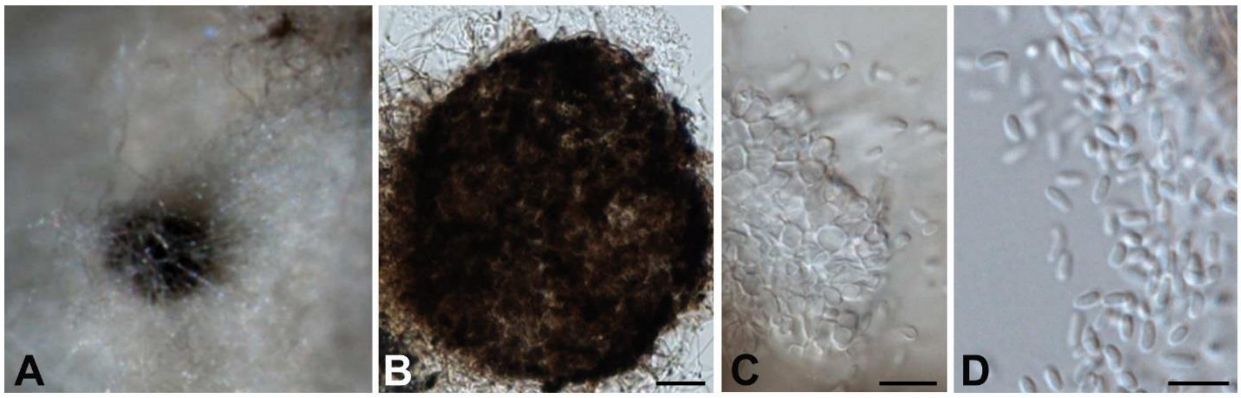


Figura 22. *Parathyridaria percutanea* (CBS 868.95). A. Conidioma picnidial a la lupa. B. Picnidio. C. Células conidiógenas. D. Conidios. Escala: B = 20 µm. C-D = 10 µm. Adaptado de Ahmed *et al*. (2014a).

1.3.4.7. *Pseudochaetosphaeronema*

Este género fue introducido por Punithalingam (1979) para reacomodar *Chaetosphaeronema larense*. Previamente, *C. larense* fue introducido por Borelli y Zamora (1973), el cual se aisló de un caso de micetoma en un trabajador de la agricultura venezolano. El aislado fue capaz de producir picnidios *in vitro*, lo que llevó a Punithalingam a comparar las características morfológicas entre *C. larense* y la cepa tipo del género *Chaetosphaeronema* (*C. hispidulum*), llegando a la conclusión de que eran especies distintas incluso a nivel genérico basado únicamente en caracteres morfológicos (Punithalingam 1979).

Hasta hace poco, *P. larense* era la única especie del género, pero recientemente se han introducido dos especies más: *Pseudochaetosphaeronema ginkgonis* y *Pseudochaetosphaeronema martinelli*, la primera de ellas aislada de material vegetal, y la segunda de origen clínico (una lesión subcutánea). Sin embargo, ambas especies no son

capaces de fructificar *in vitro*, y su pertenencia al género ha sido propuesta en base al análisis filogenético (Ahmed *et al*. 2015b, Zhang *et al*. 2016). *Pseudochaetosphaeronema larense* se caracteriza por producir conidiomas picnidiales de cuello largo; células conidiógenas fialídicas, hialinas, de cilíndricas a subglobosas y conidios incoloros, de pared delgada, unicelulares, y de forma ovoide a cilíndrica (Fig. 23).

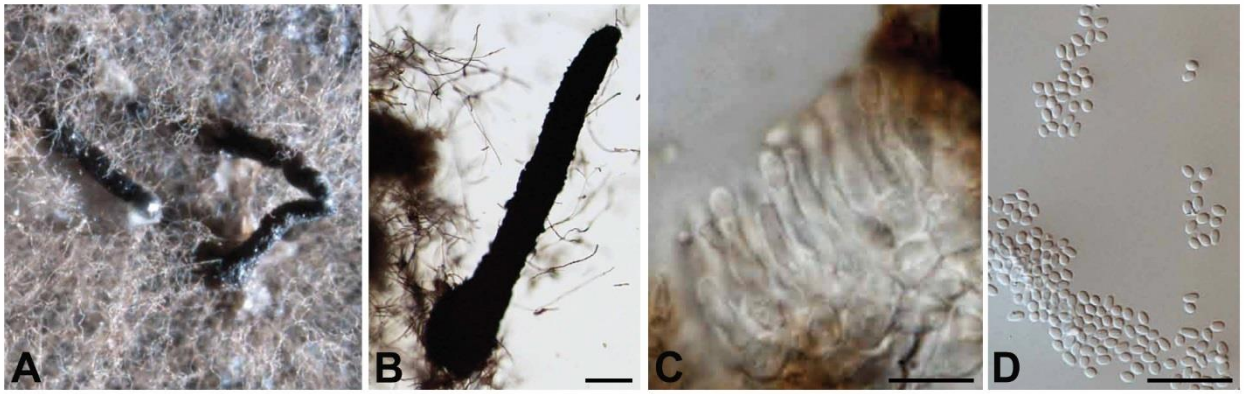


Figura 23. *Pseudochaetosphaeronema larense* (CBS 640.73). A. Conidiomas picnidiales a la lupa. B. Picnidio. C. Conidióforos y células conidiógenas. D. Conidios. Escala = 10 µm. Adaptado de Ahmed *et al*. (2014b).

1.3.4.8. *Trematosphaeria grisea*

Trematosphaeria grisea fue previamente clasificada como *Madurella grisea*, uno de los principales agentes productores de eumicetoma (Mackinnon *et al*. 1949), y sistemáticamente incapaz de fructificar *in vitro* (de Hoog *et al*. 2011). Sin embargo, esta especie ha sido reubicada en el género *Trematosphaeria* como una combinación nueva, después del análisis filogenético empleando varios marcadores moleculares (Ahmed *et al*. 2014b). Su distribución está todavía en entredicho, aunque parece ser más frecuente en zonas tropicales, habiendo sido colectada en Latinoamérica, Asia y Europa, tanto de origen clínico como de ambientes acuáticos.

Trematosphaeria grisea se caracteriza morfológicamente por desarrollar conidiomas picnidiales con setas de color marrón oscuro, en cuyo interior desarrollan conidióforos cortos; células conidiógenas fialídicas, hialinas, cilíndricas y conidios incoloros, de pared delgada, unicelulares, de forma cilíndrica a elipsoidal. Solo se ha observado fructificación en los aislados ambientales (Fig. 24).

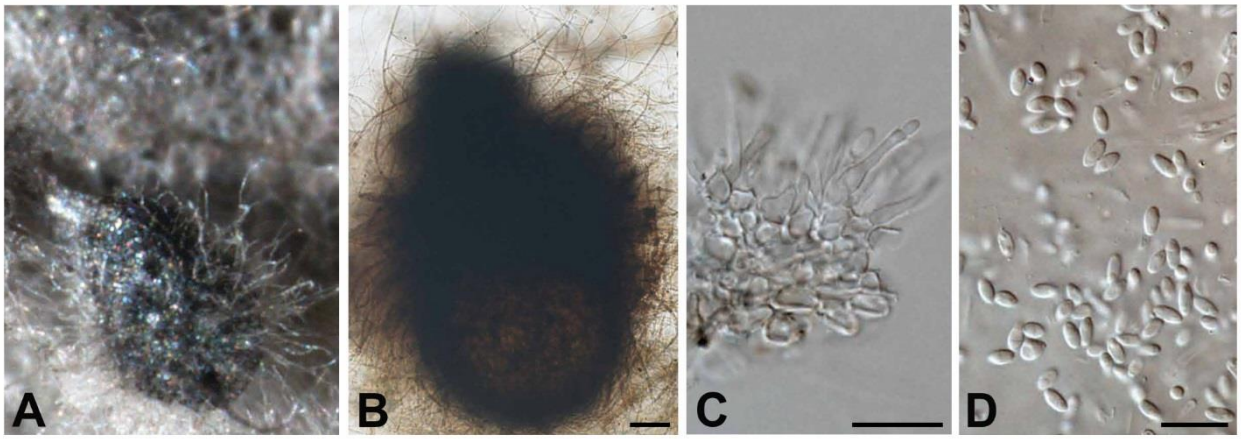


Figura 24. *Trematosphaeria grisea* (CBS 120271). A. conidioma picnidial a la lupa. B. Picnidio. C. Conidióforos y células conidiógenas. D. Conidios. Escala: B = 20 µm. C-D = 10 µm. Adaptado de Ahmed *et al*. (2014b).

1.3.4.9. *Tintelnotia destructans*

Esta especie fue reportada en patología recientemente, habiendo sido aislada de muestras superficiales (uña y ojo). Su distribución está aparentemente restringida a Europa, con especímenes provenientes de Alemania, Finlandia, Holanda e Italia. Como características destacables del taxón, esta especie es capaz de crecer hasta los 40ºC y formar picnidios dentro de la uña infectada (Ahmed *et al*. 2017). Morfológicamente está caracterizada por presentar conidiomas picnidiales con ostiolos (perforaciones apicales de la pared peridial) anchos; células conidiógenas fialídicas, hialinas y subglobosas y conidios incoloros inicialmente y marrón claro al madurar, de pared delgada, unicelulares y de forma elipsoidal (Fig. 25).

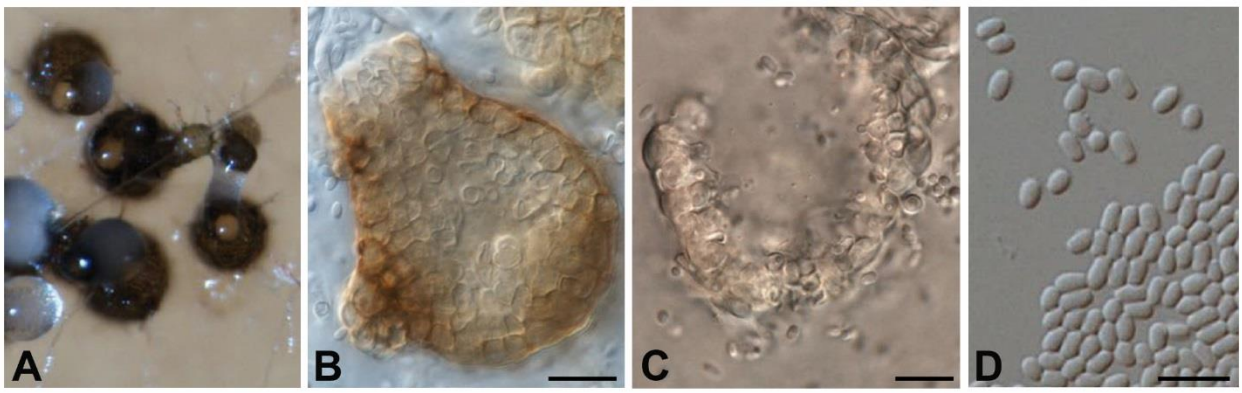


Figura 25. *Tintelnotia destructans* (CBS 127737). A. Conidiomas picnidiales a la lupa. B. Picnidio. C. Células conidiógenas. D. Conidios. Escala = 10 µm. Adaptado de Ahmed *et al*. (2017).

1.4. Sensibilidad antifúngica y tratamiento de las infecciones causadas por hongos celomicetos

El conocimiento sobre el tratamiento terapéutico de las infecciones causadas por los hongos celomicetos es aún muy limitado, debido principalmente a las características fisiológicas de estos hongos, tales como su lento crecimiento y su frecuente incapacidad de esporular *in vitro*, lo que hace inviable el poder evaluar su grado de sensibilidad frente a las diferentes drogas antifúngicas acorde a técnicas estándar (CLSI 2008, EUCAST 2008). El mecanismo de acción de los antifúngicos está enfocado en interaccionar con la integridad estructural o interferir en la biosíntesis de la membrana plasmática (alilaminas, azoles y polienos), de la pared celular (equinocandinas) y de la síntesis de ADN, ARN y proteínas (pirimidinas fluoradas) (Lewis 2011, Richardson & Warnock 2012).

Los antifúngicos poliénicos, aunque no son históricamente los primeros, son uno de los más antiguos, habiendo sido introducidos para tratar micosis entre los años 1950 y 1960. El más conocido y empleado de ellos es la anfotericina B, antifúngico de amplio espectro y de elección para el tratamiento de infecciones profundas o sistémicas. Sin embargo, es altamente nefrotóxico. La aparición en 1990 de una presentación liposomal redujo significativamente su toxicidad (Laniado-Laborín 2009, Al-Nakeeb *et al*. 2015). Para el tratamiento de infecciones superficiales puede emplearse otro polieno, la nistatina, que se aplica por vía tópica. La acción antifúngica de dichas substancias se basa en su capacidad de unión al ergosterol (principal esterol de la membrana plasmática de los hongos), lo que genera canales que alteran la permeabilidad de la membrana citoplasmática induciendo la muerte celular (Laniado-Laborín 2009, Ruiz-Camps & Cuenca-Estrella 2009).

Los antifúngicos azólicos son un grupo de fármacos sintéticos los cuales se han introducido en el mercado entre los años 1980 al 2015, se clasifican por el número de anillos azólicos (de uno a tres: azoles, imidazoles y triazoles). El mecanismo de acción se basa en la inhibición de la desmetilación del lanosterol de la membrana fúngica por medio del bloqueo de la enzima C14-alfa-desmetilasa (enzima responsable de la transformación del lanosterol en ergosterol) (Ananda-Rajah *et al*. 2012). Los azoles se encuentran indicados en el tratamiento tanto de las micosis superficiales como de las sistémicas. Los más utilizados en clínica son el fluconazol, itraconazol, voriconazol, posaconazol y el isavuconazol (Allen *et al*. 2015).

Las equinocandinas (lipopéptidos) fueron introducidos para el uso humano por primera vez en el 2001 (a pesar de que se desarrollaron a partir de 1974) con la caspofungina, al que posteriormente se le ha sumado la micafungina y la anidulafungina. El mecanismo de

acción de estos antifúngicos se basa en inhibir la 1,3-β-D-glucano sintasa, la cual tiene por función formar polímeros de glucano, uno de los componentes principales de la pared celular del hongo, motivo por el cual estas drogas tienen un efecto fungicida, promoviendo la destrucción celular (Ferrer *et al*. 2013).

La flucitosina (una pirimidina fluorada) introducida en 1964, es un fármaco fungistático que penetra en el interior de la célula fúngica siendo metabolizado hasta producir ácido 5-fluoruradílico, el cual se incorpora en la cadena del ARN, ocasionando la consecuente inhibición del proceso de traducción a proteínas. Actualmente este antifúngico no se recomienda a menudo debido a que solo es efectivo contra las levaduras, es tóxico y tiende a seleccionar cepas resistentes (Gavalda & Ruiz 2003, Perfect *et al*. 2010). Otros antifúngicos, como la terbinafina (alilamina) presentan un efecto antifúngico debido al bloqueo de la ruta biosintética del ergosterol por inhibición de la escualeno epoxidasa, tiene un efecto fungicida (Carrillo-Muñoz *et al*. 1999).

De todos los antifúngicos antes mencionados, muy pocos estudios existen de ellos comprobando su efectividad frente a los hongos celomicetos, y en la mayoría de casos son solo refractarios a algunos de ellos y con un número reducido de aislados de una especie determinada. También esto se puede deber a que los reportes de casos son esporádicos, y porque son difíciles de trabajar en el laboratorio de rutina. Uno de los escasos trabajos sobre la sensibilidad *in vitro* de los celomicetos frente a los antifúngicos corresponden a Sutton (1999), en el que se demuestra que la mayoría de los celomicetos eran susceptibles a la anfotericina B, y que existían algunas cepas resistentes. Posteriormente, el estudio realizado por Stchigel y Sutton (2013) llegaron a una conclusión similar, indicando que la mayoría de los celomicetos son sensibles a la mayoría de los antifúngicos actualmente utilizados en clínica y que puntualmente algunas cepas de los géneros *Paraconiothyrium*, *Phoma* y *Pyrenochaeta* fueron resistentes a la anfotericina B, que la sensibilidad frente a los azoles fue variable, y que la mayor resistencia fue observada frente a las equinocandinas. Sin embargo, no se puede extrapolar estos resultados a todos los celomicetos, debido a que existe una gran diversidad de especies.

Finalmente, a pesar del escaso número de trabajos sobre la sensibilidad antifúngica y de que no existen valores de corte epidemiológicos para los hongos celomicetos, algunos autores han tratado de establecer guías para el tratamiento de sus infecciones, el cual consiste principalmente en administrar antifúngicos del tipo triazoles (como el voriconazol), y en menor medida la anfotericina B, junto a la remoción del tejido infectado mediante desbridamiento quirúrgico (Chowdhary *et al*. 2014, Guégan *et al*. 2016).

2. INTERÉS Y OBJETIVOS

UNIVERSITAT ROVIRA I VIRGILI
TAXONOMÍA DE HONGOS CELOMICETOS DE INTERÉS CLÍNICO
Nicomedes Miguel Antonio Valenzuela López

En las últimas tres décadas la micología médica ha dedicado un especial interés a los hongos patógenos oportunistas, los cuales ocasionan un amplio rango de patologías infecciosas, principalmente en individuos inmunocomprometidos, lo cual determina que dichas infecciones sean a menudo difíciles de tratar y, por dicho motivo, tengan una alta mortalidad asociada. Dichos hongos, tradicionalmente considerados meros contaminantes de laboratorio e inofensivos para el hombre, constituyen actualmente un nuevo motivo de preocupación para la salud pública. A pesar de que los hongos celomicetos se aíslan con cierta frecuencia de especímenes clínicos, se desconoce la incidencia real de sus especies cómo agentes infecciosos para el hombre. Solo un estudio pormenorizado de un gran número de aislados, su correcta identificación y el establecimiento de patrones de sensibilidad antifúngica nos permitirá en el futuro realizar un diagnóstico más preciso de las infecciones causadas por este grupo de hongos y, a su vez, aplicar un tratamiento antifúngico eficaz, el cuál será decisivo para la supervivencia del paciente. Los hongos celomicetos representan un serio reto en cuanto a su identificación en el laboratorio microbiológico clínico, debido (en parte) a la similitud morfológica entre ellos, a su lento crecimiento en los medios de cultivo, y porque muchos de los aislados permanecen estériles por un largo período de tiempo o de forma permanente, siendo necesario recurrir a técnicas moleculares para poder identificarlos y, eventualmente, emitir un diagnóstico.

Las especies de hongos celomicetos implicados en infecciones humanas pertenecen mayoritariamente a los géneros *Colletotrichum*, *Lasiodiplodia*, *Medicopsis* y *Neoscytalidium*. Sin embargo, el número de taxones reportados como patógenos oportunistas crece año tras año, de forma paralela a la incorporación de las técnicas moleculares en el laboratorio clínico empleadas para su identificación.

Por lo antes expuesto, el objetivo general de la presente tesis doctoral es:

- **Determinar el espectro de especies y géneros de hongos celomicetos que producen infecciones oportunistas en humanos, contribuyendo al esclarecimiento de su taxonomía y filogenia, así como determinar sus patrones de sensibilidad antifúngica *in vitro*, con la finalidad de poder instaurar un tratamiento terapéutico eficaz.**

Por dicho motivo, los objetivos específicos son:

1. Caracterizar fenotípicamente un gran número de aislados clínicos de los hongos de interés.

2. Obtener las secuencias nucleotídicas de la región ITS y de los dominios D1 y D3 del gen 28S del ARN ribosómico nuclear, y de fragmentos de otros genes estructurales (β-tubulina, factor de elongación 1-α, *rpb*2, etc.) de los aislados clínicos en estudio.

3. Comparar los caracteres fenotípicos y genotípicos de los aislados clínicos con aquellos correspondientes a las cepas tipo o de referencia de hongos celomicetos previamente descritos, para establecer sus relaciones filogenéticas y su ubicación taxonómica, proponiendo una metodología eficaz para su correcta identificación.

4. Determinar la sensibilidad *in vitro* de los aislados identificados frente a los antifúngicos existentes actualmente en el mercado.

3. MATERIALES Y MÉTODOS

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3.1. Origen de los aislados

En la presente tesis se han estudiado un total de 452 aislados, de los cuales 330 fueron obtenidos a partir de especímenes clínicos (Anexo, Tabla 1) y 122 eran de origen ambiental (Anexo, Tabla 2), incluyendo un importante número de cepas tipo y de referencia obtenidas de colecciones de cultivos internacionales. Los aislados clínicos fueron facilitados mayoritariamente por el *Fungus Testing Laboratory*, *University of Texas Health Science Center* (UTHSC, San Antonio, Estados Unidos de Norteamérica), el *Institut Pasteur* (CNRMA, *Centre National de Réfeìrence Mycoses Invasives et Antifongiques*, Paris, Francia) y la colección del *Centro Nacional de Microbiología del Instituto de Salud Carlos III* (CNM-CM, Madrid, España). Las cepas tipo y de referencia se obtuvieron principalmente de la colección del *Westerdijk Fungal Biodiversity Institute* (antiguo CBS-KNAW, Utrecht, Países Bajos), la *International Collection of Microorganisms from Plants* (ICMP, Auckland, Nueva Zelanda) y la *American Type Culture Collection* (ATCC, Virginia, Estados Unidos de Norteamérica). Todas ellas son instituciones de referencia e importancia internacional, con las que la Unidad de Micología y Microbiología Ambiental viene colaborando desde hace más de tres décadas.

3.2. Identificación

Los aislados se identificaron a partir de la caracterización morfológica de sus colonias y de sus estructuras vegetativas y reproductivas, tanto a nivel macro- como microscópico, su habilidad para crecer a diferentes temperaturas y mediante el análisis de secuencias de diferentes marcadores moleculares.

3.2.1. Estudio morfológico

Para la determinación de las características morfológicas se utilizaron diferentes medios de cultivo, basados principalmente en estudios previos realizados por Boerema y co-autores (2004). Los medios de cultivo utilizados y su composición o procedencia se detallan a continuación: agar con extracto de malta (MEA; 40 g de extracto de malta, 15 g de agar-agar, 1000 mL de agua destilada), agar con harina de avena (OA; 30 g de copos de avena hervidos en 500 mL de agua durante 15 a 20 minutos, y filtrados tras la ebullición, 6.5 g de agar-agar; completar con agua del grifo hasta volumen final de 1000 mL), agar con extracto de patata y glucosa (PDA; *Pronadisa*, Madrid, España). Para inducir la producción de conidiomas y la esporulación, las cepas fúngicas se sembraron en agar agua con hojas

de clavel (CLA; trozos de hojas de clavel esterilizados en autoclave [en condiciones estándar] tres veces en días alternos, y posteriormente mezclados con 15 g de agar-agar disueltos en 1000 mL de agua destillada) bajo la exposición a la luz ultravioleta (12 horas luz, 12 horas oscuridad) (Fisher *et al*. 1982, Crous *et al*. 2009, Su *et al*. 2012). Los cultivos fueron examinados a los 7 y 14 días, midiendo el diámetro de las colonias, describiendo su textura y topología, así como documentando el color de la superficie y el reverso y, de existir, del pigmento difusible acorde a los patrones descritos en Kornerup y Wanscher (1978). En los casos en que las cepas eran incapaces de producir conidiomas fértiles dentro del período de las dos semanas, sus colonias fueron examinadas semanalmente durante un período total de 2 a 3 meses hasta corroborar (o no) la aparición de dichas estructuras. Para documentar el tamaño, el color, la ornamentación, la presencia y número de septos, etc., de las estructuras vegetativas (hifas, apresorios, clamidosporas) y reproductivas (conidiomas, conidióforos y/o células conidiógenas, y conidios) se realizaron preparaciones semipermanentes utilizando diversos líquidos de montaje (ácido láctico al 85%; medio de Shear: 3 g de acetato de potasio, 150 mL de agua destilada, 60 mL de glicerina, 90 mL etanol 95 %), colocando sobre una gota de los mismos el material fúngico de interés, entre porta- y cubreobjetos con la ayuda de jeringuillas tipo tuberculina con su correspondiente aguja (Crous *et al*. 2009). Los cortes histológicos de los conidiomas, con la finalidad de poder caracterizar y documentar gráficamente su estructura y la relación anatómica entre conidióforos/células conidiógenas y conidios, se realizaron a “mano alzada” cogiendo uno o varios cuerpos fructíferos (picnidios) de la colonia con ayuda de dos agujas estériles y cortándolo con una hoja de bisturí médico clásico tipo Bard-Parker número 3 con hojas Nº 10 ó 11 de preferencia. El examen microscópico y la medición de las mencionadas estructuras se utilizó mediante un microscopio de campo claro Olympus CH-2 (*Olympus Corporation*, Japón). Las microfotografías se obtuvieron con un microscopio Zeiss Axio-Imager M1 (*Zeiss*, Alemania), provisto de una cámara digital DeltaPix Infinity X21, utilizando condensador de contraste de fases y de contraste por interferencia diferencial de Nomarski, 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 las cepas en estudio de crecer a diferentes temperaturas de incubación y el efecto de estas sobre la tasa de crecimiento, se utilizaron placas de Petri de 9-10 cm de diámetro con medio PDA, y una vez inoculadas en su parte central con la cepa de interés se incubaron a diferentes temperaturas en un rango de 5 a 37°C, a intervalos de 5°C (con excepción de los 37°C). Las placas se examinaban a los 7

días de incubación por un máximo de 14 días. Finalmente se consideraba como óptima, aquella temperatura en la que se obtenía una mayor tasa de crecimiento (como diámetro colonial medido en milímetros).

3.3. Identificación molecular

Debido a que muchos de los taxones estudiados en la presente tesis eran morfológicamente similares entre sí, fue imprescindible realizar estudios filogenéticos para su correcta identificación. Para ello, las cepas de interés fueron caracterizadas a nivel molecular mediante la amplificación por PCR y secuenciación de diversos marcadores filogenéticamente informativos. Las secuencias nucleotídicas generadas fueron comparadas con las pertenecientes a cepas tipo y/o de referencia de las especies filogenéticamente próximas disponibles en las bases de datos públicas.

3.3.1. Extracción del ADN

Para la obtención del ADN genómico total, las cepas de interés fueron sembradas en placas de Petri de 9-10 cm de diámetro conteniendo PDA, o en algunos casos OA. Transcurridos 5-14 días de incubación en las condiciones ambientales previamente mencionadas, se extraía el micelio aéreo y las estructuras de fructificación mediante raspado con hoja de bisturí estéril. El ADN fúngico se obtuvo mediante el kit FastDNA® kit (*MP Biomedicals,* EE.UU.), siguiendo las instrucciones del fabricante. El ADN obtenido se cuantificó mediante el uso de NanoDrop 3000 (*ThermoScientific,* EE.UU.).

3.3.2. Amplificación y secuenciación

Mediante PCR se amplificaron diversos genes o fragmentos de los mismos, en dependencia del tipo de taxón a caracterizar, los cuales se detallan en las secciones Resultados y Anexos (Tabla 3).

La reacción de amplificación se realizó en un volumen total de 25 µL, los que contenían 5 µL de 10× PCR Buffer, 0.2mM dNTPs, 0.5 µM de cada cebador, 1 U *Taq* ADN Polimerasa (*Invitrogen,* California*,* EE.UU.) y 1–10 ng de ADN genómico. El programa de amplificación utilizado para todos los genes consistió en una desnaturalización inicial a 94°C durante 5

minutos, seguido de 30–35 ciclos de desnaturalización a 95°C durante 30 segundos, hibridación entre 53–57°C desde 45 segundos a 1 minuto y 20 segundos (el tiempo y la temperatura dependían de los cebadores utilizados, según se detalla en Anexos, Tabla 3), extensión a 72°C por 1 minuto a 1 minuto y 30 segundos, y una extensión final a 72°C por 4–7 minutos. Para ello se utilizaron los termocicladores 2720 thermal cycler (*Applied Biosystems,* EE.UU.) y Biometra TProfessional Basic Gradient Thermocycler (*Analytic Jena,* Alemania). Para corroborar la efectividad de la amplificación, los productos se sometieron a electroforesis en geles de agarosa al 1 ó 1,2% p/v. Los productos (amplicones) se enviaron para su purificación y secuenciación a Macrogen Corp. Europe (Ámsterdam, Holanda) utilizando los mismos pares de cebadores empleados para su amplificación.

3.3.3. Ensamblaje de las secuencias

Las secuencias nucleotídicas obtenidas se revisaron visualmente para determinar su calidad y fueron ensambladas para obtener la secuencia consenso utilizando el programa SeqMan versión 7.0.0 (*DNASTAR Lasergene,* EE.UU.). Las secuencias generadas en la presente tesis fueron depositadas en las bases de datos European Nucleotide Archive (http://www.ebi.ac.uk/ena) y GenBank (http://www.ncbi.nlm.nih.gov/genbank).

3.3.4. Búsquedas de identidad mediante 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, tales como la del CBS (www.westerdijkinstitute.nl), del GenBank (www.ncbi.nlm.nih.gov/genbank), y la del Q-Bank (www.q-bank.eu). Para la identificación presuntiva de las cepas de interés a nivel de especie se consideraron aquellas provenientes de cepas tipo o de referencias de colecciones internacionales cuyas secuencias mostraran una identidad ≥98% y una cobertura ≥99%. En el caso de obtener resultados porcentualmente inferiores a los previamente mencionados, se consideró como una identificación parcial, a nivel de género, familia u orden, según correspondiera.

3.3.5. Alineamiento de las secuencias

Las secuencias nucleotídicas de los diferentes genes amplificados se alinearon individualmente, incluyendo aquellas secuencias descargadas desde bases de datos públicas, utilizando los programas ClustalW (Thompson *et al*. 1994) o MUSCLE (Edgar 2004) incorporados en la plataforma MEGA versión 6.06 (Tamura *et al*. 2013), finalmente se procedió a una verificación visual de los mismos para detectar posibles errores de alineamiento. Para la obtención de una mayor resolución en los resultados de los análisis filogenéticos, cada estudio se realizó utilizando una o más combinaciones de los genes seleccionados (p. ej. LSU-ITS-*tub*2-*rpb*2), 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 individual o concatenado.

Para evaluar la congruencia filogenética entre los diferentes genes de una cepa, se empleó el test de homogeneidad de particiones (*partition-homogeinity test*) mediante el software PAUP* versión 4.0b10 (*Sinauer,* EE.UU.) (Swofford 2000), o basados en filogenias previas de otros autores (Aveskamp *et al*. 2010, de Gruyter *et al*. 2013, Chen *et al*. 2015).

3.3.6. Análisis filogenéticos

La reconstrucción filogenética para cada gen, y las combinaciones de los diversos genes, se llevaron a cabo utilizando principalmente dos tipos de inferencia filogenética: máxima verosimilitud (ML) y análisis bayesiano (BI).

El análisis de ML se llevó a cabo utilizando el software MEGA 6 o la plataforma CIPRES (Miller *et al*. 2012). Los espacios presentes en los alineamientos se trataron como deleciones parciales y la robustez de las ramas se determinó mediante el método de *bootstrap*, utilizando 1.000 iteraciones. Un valor de *bootstrap* ≥70% se consideró como estadísticamente significativo.

Los análisis de BI se llevaron a cabo utilizando el software MrBayes versión 3.2.6 (Ronquist *et al*. 2012). Para ello, se realizaron simulaciones de entre 5.000.000 a 40.000.000 generaciones, en dos series paralelas, almacenando los árboles resultantes cada 100 ó 1.000 generaciones. El análisis se detenía al obtenerse la convergencia de valores estadísticos para ambas series (varianza < 0,01). Se eliminó 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 6, y para las filogenias más extensas se utilizó el software MrModelTest versión 2.3 (Nylander 2004).

3.4. Registro de novedades taxonómicas

Todas las novedades taxonómicas propuestas (nuevas combinaciones, especies, géneros y familias) en los diferentes estudios fueron depositadas en el MycoBank (www.mycobank.org; Crous *et al*. 2004).

3.5. Almacenamiento y conservación de las cepas en colecciones de cultivos microbianos

Todos los aislados recibidos de los diferentes laboratorios de referencia o de las colecciones internacionales de cultivos microbianos se depositaron en la colección de cultivos fúngicos de la Facultad de Medicina de Reus (FMR), y se conservaron utilizando tres metodologías diferentes:

1.- Almacenamiento en agua: a partir de las colonias del hongo crecidas en placas de Petri con OA, se cortaron de 3–4 bloques del medio de cultivo de aproximadamente 1 cm^2, con ayuda de un bisturí estéril. Posteriormente, los fragmentos se introdujeron en dos frascos de vidrio estériles conteniendo 2–3 mL de agua destilada estéril c/u, y se guardaron en la oscuridad a temperatura ambiente (aproximadamente 20 ± 1 °C)

2.- Almacenamiento en aceite mineral: se usaron tres tubos de vidrio de 10 cm de longitud con agar inclinado en “pico de flauta” (dos de OA y uno de PDA), provistos de un tapón con cierre hermético. Una vez inoculada la cepa y obtenido suficiente crecimiento, se cubrió con aceite mineral estéril y se guardaron los tubos en oscuridad a temperatura ambiente.

3.- Almacenamiento liofilizado: las cepas se cultivaron en placas de Petri con OA, y una vez crecidas las colonias se retiró material de las mismas con la ayuda de un asa o bisturí estéril. La masa de elementos fúngicos así obtenida se depositó en un tubo de plástico estéril con 3 mL de *skim milk* (Difco, EE.UU.). Ésta suspensión se homogenizó mediante agitación y se distribuyó en fracciones de 1–1,5 mL en tres frascos pequeños de vidrio estériles provistos de tapón de caucho de cierre hermético. Los frascos se liofilizaron mediante el

sistema automatizado VirTis Advantage 2.0 ES (*SP Scientific,* EE.UU.), utilizando el siguiente protocolo: congelación inicial a -45ºC seguido de generación de vacío a 200 mTorr, desecación por sublimación a -30ºC (240 minutos), -10ºC (240 minutos), 10ºC (300 minutos) y 30ºC (300 minutos). 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 la ausencia de contaminación de las muestras, el producto liofilizado de uno o más viales seleccionados al azar se resembraron en un medio de cultivo apropiado, evaluándose macro- y microscópicamente tanto la viabilidad del hongo como la pureza del cultivo.

4.- Almacenamiento en herbario: para ello, las colonias del aislamiento primario de los nuevos taxones fúngicos que evidenciaron fructificación/esporulación fueron seleccionadas como material tipo. Dicho material fue desecado en una estufa de cultivo a 45–50 ºC, y posteriormente fue depositado en el herbario de la colección del CBS.

3.6. Estudios de sensibilidad antifúngica

Se determinó el patrón de sensibilidad antifúngica para un gran número de los aislados clínicos de las especies identificadas (85 cepas), siguiendo el protocolo descrito por el *Clinical & Laboratory Standards Institute* en el documento M38-A2 (CLSI 2008).

La sensibilidad de varias cepas pertenecientes a los géneros *Colletotrichum*, *Diaporthe*, *Didymella*, *Epicoccum*, *Neoascochyta*, *Neoscytalidium*, *Paraconiothyrium*, *Phoma* y *Pyrenochaetopsis* fue evaluada frente a la acción de los antifúngicos terbinafina (TRB), itraconazol (ITC), posaconazol (PSC), voriconazol (VRC), anidulafungina (AFG), caspofungina (CFG), micafungina (MFG), 5-fluorcitosina (5FC) y anfotericina B (AMB). El rango de concentraciones ensayadas para cada antifúngico varió entre 0,016 a 16 µg/mL.

Las cepas estudiadas se sembraron en OA en condiciones estándar hasta conseguir esporulación. Sin embargo, en algunos casos no se obtuvieron conidios, por lo cual se siguió un protocolo para hongos filamentosos estériles, para la obtención de un inóculo por suspensión de fragmentos de sus hifas (Chowdhary *et al*. 2013). En estos casos, la superficie de las colonias se raspó con la ayuda de un asa o bisturí estéril, suspendiendo la masa fúngica obtenida en solución fisiológica estéril, la cual fue posteriormente filtrada mediante una 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 4 x 10^5 – 5 x 10^6 conidios/mL y luego diluidas 1:50 en medio de Roswell Park Memorial Institute

(RPMI-1640, Gibco, Reino Unido). En una microplaca de 96 pocillos, se inocularon 100 µL del inóculo para cada dilución del antifúngico a ensayar. Las microplacas fueron incubadas en la oscuridad, sin agitación, durante 24 a 72 horas a 30ºC.

La lectura de la sensibilidad frente a equinocandinas se realizó a las 24 ó 48 horas de incubación, determinando la concentración mínima eficaz (CME), definida como la mínima concentración de antifúngico en la cual se observa un crecimiento aberrante de las hifas del hongo, caracterizado por masas compactas, formadas por elementos miceliares estrellados. Para la lectura de la sensibilidad frente al resto de antifúngicos, se determinó la concentración mínima inhibitoria (CMI) a las 48 y 72 horas de incubación. Se definió la CMI 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 de *Aspergillus fumigatus* ATCC MYA-3626 y *Paecilomyces variotii* ATCC MYA-3630.

4. RESULTADOS

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Estudios de los aislados clínicos provenientes de los Estados Unidos de Norteamérica

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4.1. Coelomycetous fungi in the clinical setting: Morphological convergence and cryptic diversity

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Publicado en: Journal of Clinical Microbiology 2017; 55: 552–567.

UNIVERSITAT ROVIRA I VIRGILI
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Coelomycetous Fungi in the Clinical Setting: Morphological Convergence and Cryptic Diversity

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ABSTRACT Human infections by coelomycetous fungi are becoming more frequent and range from superficial to systemic dissemination. Traumatic implantation of contaminated plant material is the most common cause. The typical morphological feature of these fungi is the production of asexual spores (conidia) within fruiting bodies called conidiomata. This study aimed to determine the distribution of the coelomycetes in clinical samples by a phenotypic and molecular study of a large set of isolates received from a U.S. reference mycological institution and by obtaining the *in vitro* antifungal susceptibility pattern of nine antifungals against a selected group of isolates. A total of 230 isolates were identified by sequencing the D1 and D2 domains of the large subunit (LSU) nuclear ribosomal RNA (nrRNA) gene and by morphological characterization. Eleven orders of the phylum *Ascomycota* were identified: *Pleosporales* (the largest group; 66.1%), *Botryosphaeriales* (19.57%), *Glomerellales* (4.35%), *Diaporthales* (3.48%), *Xylariales* (2.17%), *Hysteriales* and *Valsariales* (0.87%), and *Capnodiales*, *Helotiales*, *Hypocreales* and *Magnaporthales* (0.43% each). The most prevalent species were *Neoscytalidium dimidiatum*, *Paraconiothyrium* spp., *Phoma herbarum*, *Didymella heteroderae*, and *Epicoccum sorghinum*. The most common anatomical site of isolation was superficial tissue (66.5%), followed by the respiratory tract (17.4%). Most of the isolates tested were susceptible to the majority of antifungals, and only flucytosine showed poor antifungal activity.

KEYWORDS *Colletotrichum*, coelomycetous fungi, coelomycetes, mycosis, *Neoscytalidium*, *Phoma*, *Pyrenochaeta*, antifungal susceptibility

Received 2 November 2016 **Returned for modification** 21 November 2016 **Accepted** 29 November 2016

Accepted manuscript posted online 7 December 2016

Citation Valenzuela-Lopez N, Sutton DA, Cano-Lira JF, Paredes K, Wiederhold N, Guarro J, Stchigel AM. 2017. Coelomycetous fungi in the clinical setting: morphological convergence and cryptic diversity. J Clin Microbiol 55:552–567. https://doi.org/10.1128/JCM.02221-16.

Editor David W. Warnock, University of Manchester

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The coelomycetous fungi constitute a large number of taxa characterized by the production of conidia (asexual propagules) within a cavity lined by fungal or host tissue, called conidiomata (1), and although the majority of the human-opportunistic infections are caused by fungi producing conidia on conidiophores (modified hyphae, with one or more conidiogenous cells, which develop free on the substrate), a significant number of mycoses are produced by coelomycetous fungi (2–4). Coelomycetous fungi are mostly saprobic and parasites of terrestrial vascular plants, but they can also infect vertebrates and other fungi. They are ubiquitous in soil, in salty and freshwater environments, and in sewage (4). Although the term *Coelomycetes* is still occasionally used to refer to these fungi, this name is obsolete and is currently considered to refer to an artificial fungal class. The class *Coelomycetes* is defined in terms of the morphological characterization of the asexual reproductive structures and considers the type and the shape of their conidiomata and the ontogeny of their conidia as the most useful characteristics (5, 6); the class has traditionally been divided into the orders *Melanconiales* and *Sphaeropsidales*, depending upon the production of either acervular

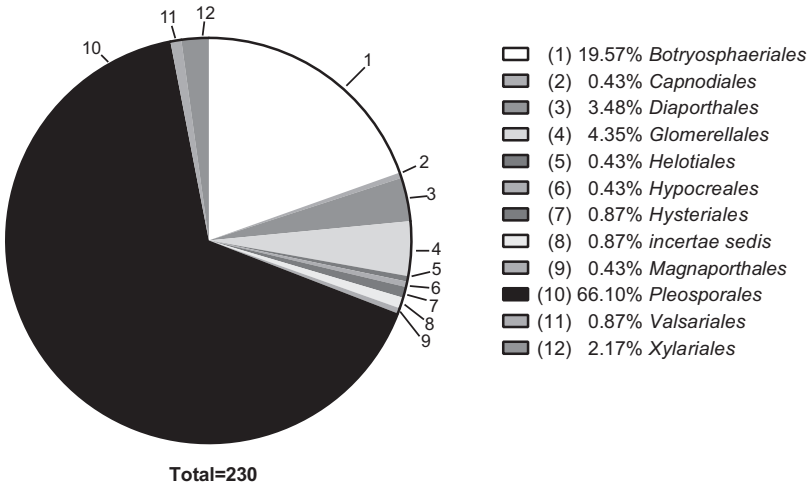

FIG 1 Distribution, by orders, of coelomycetous fungus isolates from clinical samples.

(cup-shaped) and pycnidial (globose to pyriform) conidiomata, respectively, and the *Pycnothyriales*, characterized by the production of pycnothyrial (shield-shaped, flattened, or hemispherical) conidiomata (5, 6). However, molecular studies have demonstrated that the taxonomy of the *Coelomycetes*, represented by nearly 1,000 genera and 7,000 species (1), is artificial. Recent studies, have distributed the coelomycetes into at least three classes of the phylum *Ascomycota*, i.e., *Dothideomycetes*, *Leotiomycetes*, and *Sordariomycetes* (7–9).

Infections by coelomycetous fungi are mostly acquired by traumatic implantation of plant/woody material or soil particles contaminated by their conidia rather than by inhalation of air-dispersed propagules (2, 4). The coelomycetes are responsible for a large variety of clinical entities, such as dermatitis, onychomycosis, keratitis, endophthalmitis, subcutaneous phaeohyphomycosis, cysts, mycetoma, sinusitis, osteomyelitis, bursitis, brain abscesses, and disseminated infections (4). The appropriate treatment of the infections produced by these fungi is unknown, mainly due to the wide spectrum of taxa involved and to the difficulties in their identification when the typical reproductive structures are not produced. However, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the European Conference of Medical Mycology (ECMM) have provided joint clinical guidelines for the management of phaeohyphomycosis, with some recommendations for the treatment of infections due to the most usual genera of coelomycetes, such as *Neoscytalidium*, *Phoma*, and *Pyrenochaeta*, mainly based on the use of amphotericin B and triazoles (10).

For the reasons mentioned above, the spectrum of species of these fungi in the clinical setting is practically unknown (4, 11). Therefore, the objective of this study has been to determine the distribution pattern of the coelomycetous fungi isolated from clinical specimens from the United States using molecular identification of a large set of isolates based on the sequencing of the D1 and D2 (D1-D2) domains of the large subunit (LSU) of the nuclear ribosomal RNA (nrRNA) gene. In addition, we have characterized those isolates morphologically and determined the antifungal susceptibility of a representative number of them to nine antifungal drugs.

RESULTS

A total of 86 (38%) isolates of the 230 studied were able to produce pycnidial conidiomata; 10 (4%) developed acervuli, and 35 (15%) produced the typical anamorphs of *Neoscytalidium*. The other 99 isolates (43%) remained sterile. The most common species was *Neoscytalidium dimidiatum*, representing 15% (35/230) of the isolates, followed by *Paraconiothyrium cyclothyrioides* with 7% (16/230), and both were isolated mostly from superficial tissues. The third most common taxon recovered was

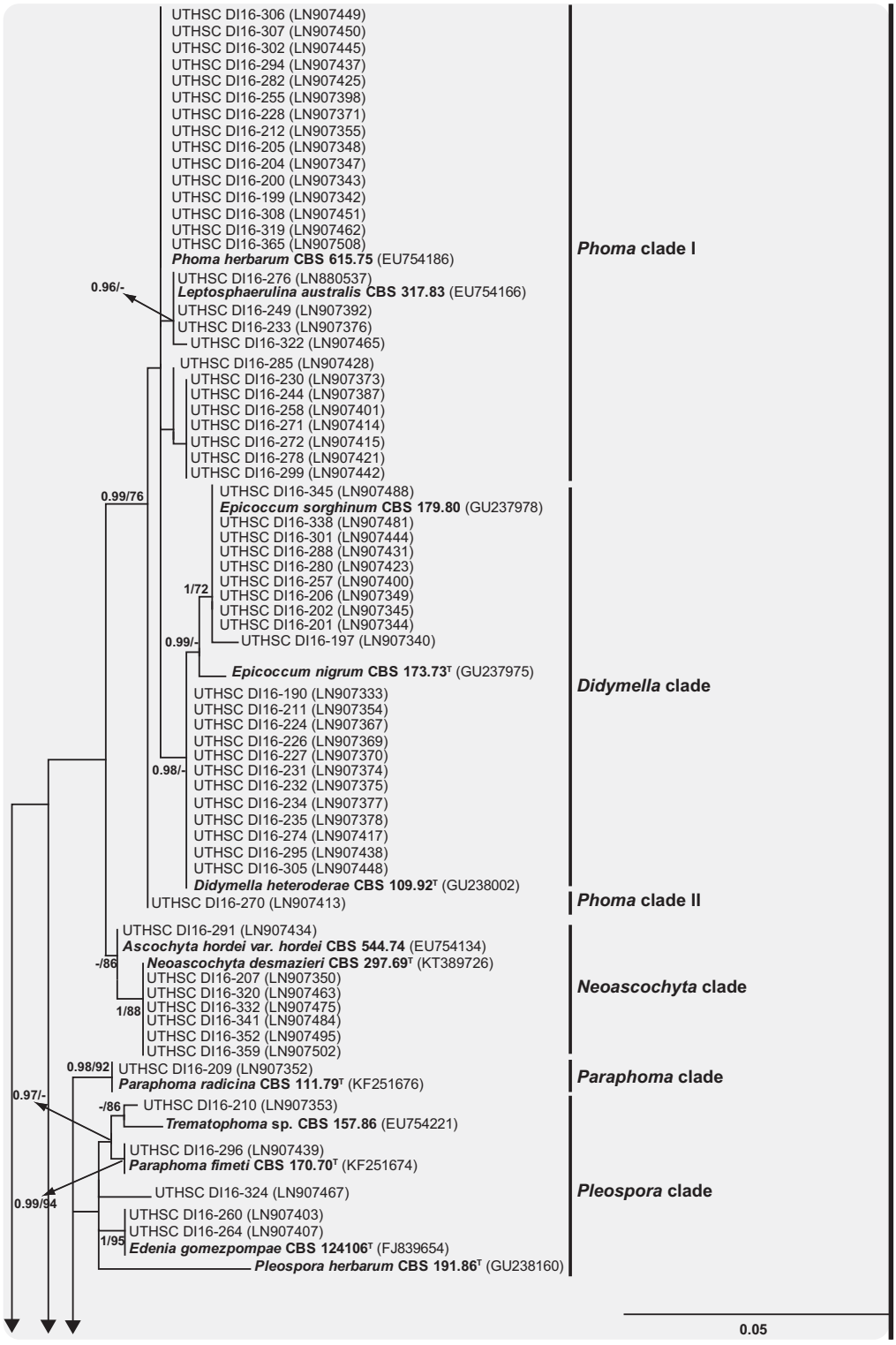

FIG 2 Maximum-likelihood tree obtained from the D1-D2 of LSU (555 bp) sequences of the 322 strains, where 92 strains are type or reference strains. In the tree, the branch lengths are proportional to phylogenetic distance. Bayesian posterior probability scores of ≥0.95 and bootstrap support values of ≥70 are indicated on the nodes. The GenBank accession numbers are given in parentheses. *Saccharomyces castellii* and *S. cerevisiae* were used to root the tree. The type (indicated by a superscript T) and reference strains are shown in bold type.

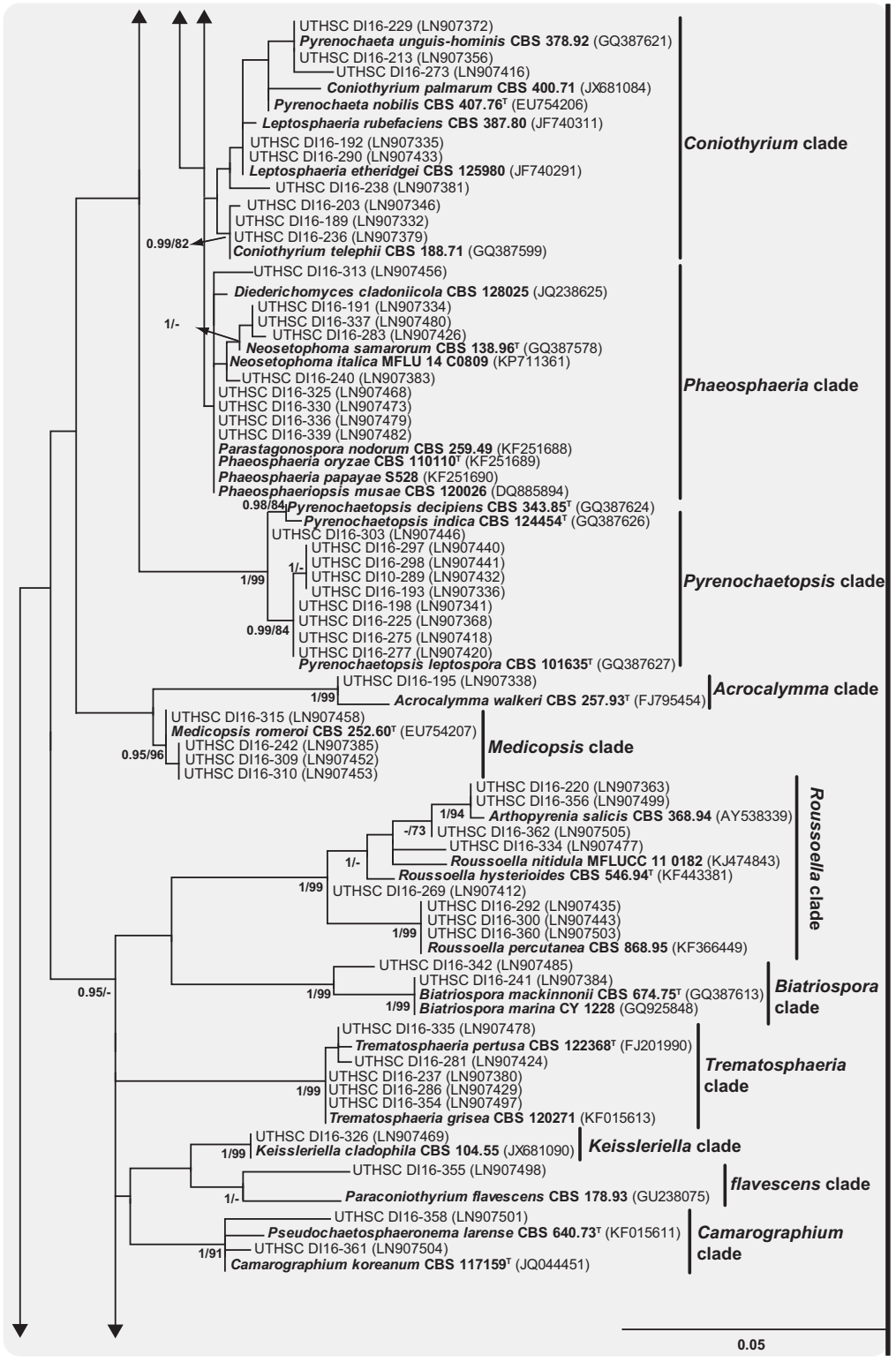

FIG 2 (Continued)

Phoma herbarum (6.5%, 15/230) from superficial and respiratory tract specimens, followed by *Didymella heteroderae* (5%, 12/230) and *Epicoccum sorghinum* (4%, 10/230), which were isolated from superficial tissues.

In the D1-D2 phylogenetic analysis, the isolates were distributed into 11 orders (Fig. 1), most of which belonged to the *Pleosporales* (66.1%) and the *Botryosphaeriales*

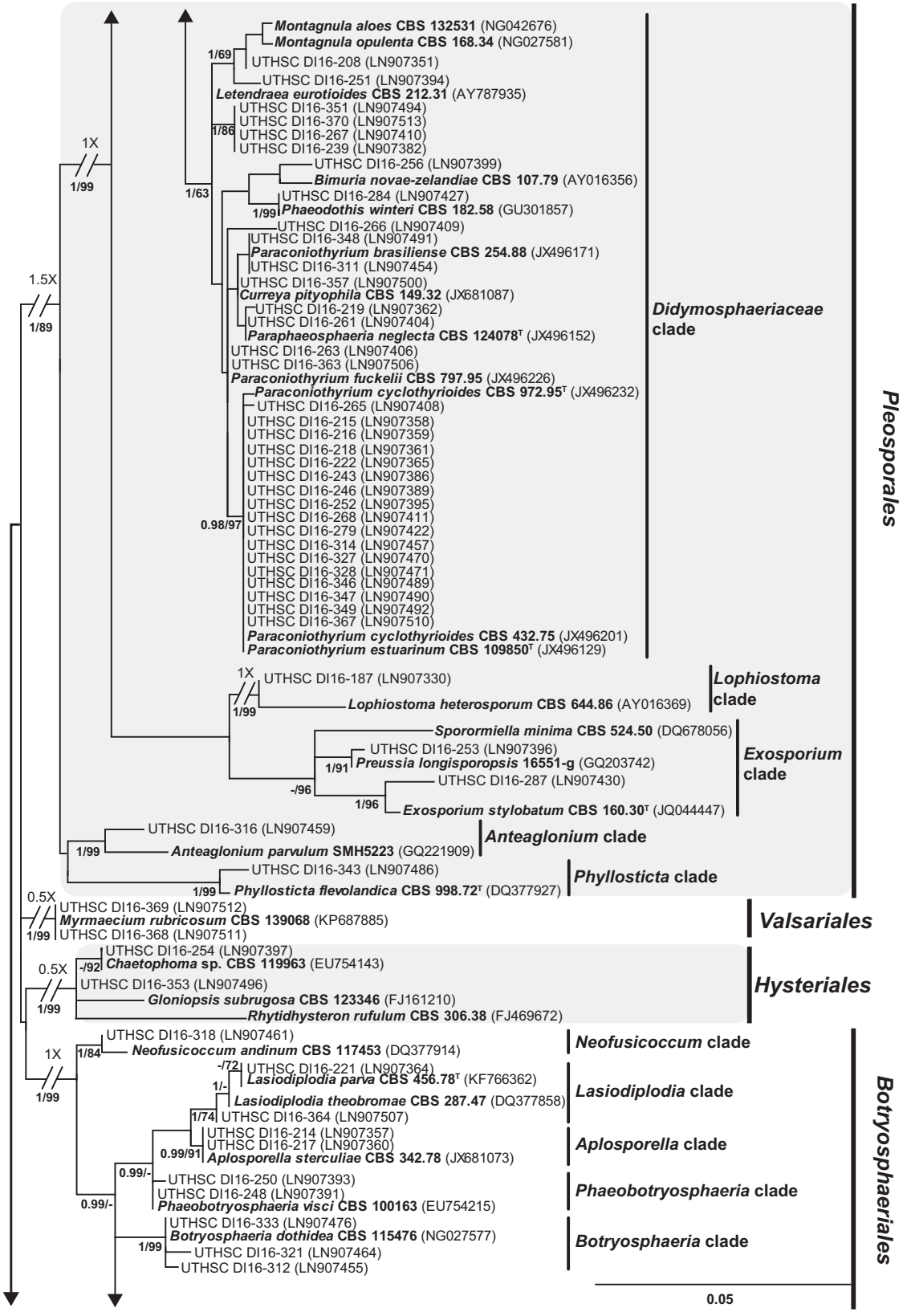

FIG 2 (Continued)

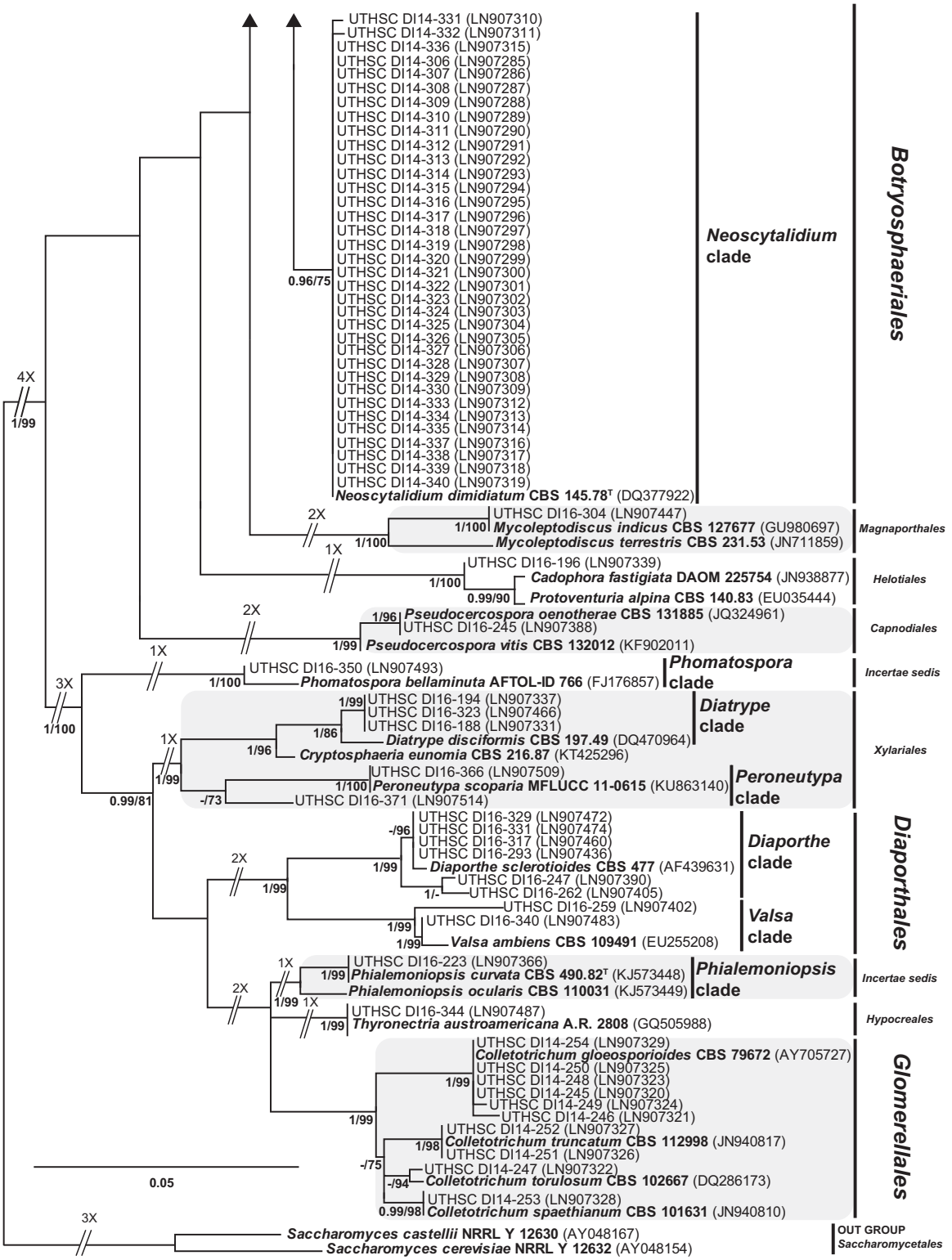

FIG 2 (Continued)

(19.57%), followed by the *Glomerellales* (4.35%), *Diaporthales* (3.48%), *Xylariales* (2.17%), and *Hysteriales* and *Valsariales* (0.87% each). The orders *Capnodiales*, *Helotiales*, *Hypocreales*, and *Magnaporthales* were represented by only one isolate each (0.43%), and the other isolates (0.87%) were *incertae sedis* (of uncertain taxonomic position).

Figure 2 shows the phylogenetic tree inferred from the analysis of 322 D1-D2 sequences corresponding to our set of isolates and numerous selected type or reference strains phylogenetically related to them. As mentioned above, the *Pleosporales* contained the largest number of isolates ($n = 152$), which were distributed into 22 clades and belonged, probably, to 61 species of 44 different genera. These clades have been named according to the first taxon historically described.

Within the *Pleosporales*, *Phoma* clade I (phylogenetically not supported) included 27 isolates, distributed mainly in two genera: *Leptosphaerulina*, with four isolates characterized by a phoma-like asexual morph, which clustered with a reference strain of *Leptosphaerulina australis*, and *Phoma*, with 15 isolates placed close to a reference strain of *Phoma herbarum* and morphologically characterized by producing pycnidia and hyaline aseptate conidia. The taxonomic position of the other eight isolates of this clade was unresolved; in fact, they formed a separate, unsupported sister clade and displayed a phoma-like asexual morph. The University of Texas Health Science Center (UTHSC) isolate DI16-270 also showed the typical morphology of *Phoma* (*Phoma* clade II) but has been placed phylogenetically distant from the mentioned genera and probably belongs to a new genus.

The *Didymella* clade included 22 isolates, 10 of which clustered with a reference strain of *Epicoccum sorghinum*; unfortunately, the morphological features of these isolates could not be studied because they produced only sterile mycelia in all the culture media tested. Twelve isolates grouped with the type strain of *Didymella heteroderae*, producing a phoma-like asexual morph, but were particularly characterized by the production of chlamydospores in long chains.

The *Neoascochyta* clade included seven isolates, six clustering with the type strain of *Neascochyta desmazieri* and another one placed together with a reference strain of *Ascochyta hordei* var. *hordei*. Morphologically, the species of this clade are mainly characterized by the production of one-septate conidia that vary in size.

The *Paraphoma* clade contained only one isolate, which showed an identical sequence to the type strain of *Paraphoma radicina* and morphologically was characterized by setose (covered with bristle-like structures) pycnidia and hyaline aseptate conidia.

The *Pleospora* clade was made up of five isolates and, with the exception of one of them, was distributed into three well-supported sister clades corresponding to the genera *Edenia*, *Paraphoma*, and *Trematophoma*. The isolate that clustered with the type strain of *Paraphoma fimeti* was separate from the type species of *Paraphoma* (*Paraphoma radicina*) and showed glabrous pycnidia instead the setose pycnidia produced by the rest of the species. Interestingly, instead of the ellipsoidal, subhyaline conidia typical of *Edenia* spp., the isolate UTHSC DI16-324 produced fusiform, hyaline, two- to three-septate conidia that are probably indicative of a new genus. The other isolates of this clade remained sterile.

The *Coniothyrium* clade included nine isolates, and its topology shows that the genera *Coniothyrium*, *Leptosphaeria*, and *Pyrenochaeta* are clearly polyphyletic using this conserved marker. Three of these isolates formed a well-supported sister clade together with a reference strain of *Coniothyrium telephii*, which is characterized by setose pycnidia. The other six isolates were distributed into the genera *Leptosphaeria* and *Pyrenochaeta*. These had a pyrenochaeta-like anamorph, producing conidiophores within pycnidia and hyaline aseptate conidia.

The *Phaeosphaeria* clade grouped nine isolates, with four of them clustering with *Neosetophoma* and producing confluent pycnidia and small hyaline conidia. The other five isolates were associated with the genera *Diederichomyces*, *Parastagonospora*, *Phaeosphaeria*, and *Phaeosphaeriopsis*. Only one isolate (UTHSC DI16-325), morphologically

resembling *Phaeosphaeriopsis* spp., was able to sporulate, displaying small conidiophores within pycnidia and one-septate, pigmented, variable-in-shape conidia.

The *Pyrenochaetopsis* clade included nine isolates, with four of them matching the type strain of *Pyrenochaetopsis leptospora*, another four isolates forming a supported sister clade separate from *P. leptospora*, and one not clustering to any of the type strains included in the analysis. All of the isolates displayed the typical phoma-like morphology, i.e., glabrous pycnidia and hyaline aseptate conidia, instead of setose pycnidia of the genus *Pyrenochaetopsis*.

The five isolates assigned to the *Acrocalymma* and *Medicopsis* clades were grouped with the type strains of *Acrocalymma walkeri* and *Medicopsis romeroi*, respectively, but differed in 4.5% of the nucleotide sequences of the respective strains of reference. These isolates remained sterile throughout.

The *Roussoella* clade was made up of eight isolates, two of which were associated with a supposed reference strain of *Arthopyrenia salicis* (CBS 368.94), whose correct identification was questioned by Liu et al. (12), and the remaining ones were associated with *Roussoella* spp.; only three isolates were able to sporulate and had a morphology similar to that of this genus, i.e., production of glabrous pycnidia and pigmented aseptate conidia.

Two isolates nested in the *Biatriospora* clade but remained sterile. The *Trematosphaeria* clade comprised five sterile isolates, two of which were phylogenetically related with the type strain of *Trematosphaeria pertusa* and the rest of which were associated with a reference strain of *Trematosphaeria grisea*.

The *Keissleriella* clade had only one isolate, which showed a phoma-like morphology and clustered with a reference strain of *Keissleriella cladophila*. Another isolate was associated with a reference strain of *Paraconiothyrium flavescens* and displayed a morphology similar to that of *Paraconiothyrium* (pycnidia, phialidic conidiogenous cells, and pigmented aseptate conidia); however, the taxonomic placement of that isolate remains doubtful because it grouped phylogenetically distant from the type species of the genus (*Paraconiothyrium estuarinum*). In the *Camarographium* clade, two sterile isolates were located that were related to the genera *Camarographium* and *Pseudochaetosphaeronema*.

The *Didymosphaeriaceae* clade comprised 33 isolates, of which 22 were phylogenetically related to *Paraconiothyrium* spp., 2 were related to *Montagnula* spp., and 2 were related to the type strain of *Paraphaeosphaeria neglecta*. Three isolates were distributed into each of the genera *Bimuria*, *Curreya*, and *Phaeodothis*, and four isolates formed a well-supported monophyletic sister clade separated from any known taxa of the family. Only three isolates (UTHSC DI16-261, UTHSC DI16-266, and UTHSC DI16-363) were able to sporulate, showing glabrous pycnidia and pale brown conidia displaying morphological features similar to those of *Paraconiothyrium* spp. The *Exosporium* clade comprised only two sterile isolates, one of which was related to the genus *Preussia* while the other was related to *Exosporium*. The *Anteaglonium*, *Lophiostoma*, and *Phyllosticta* clades comprised only one sterile isolate each one.

In the *Valsariales* clade, two isolates matched a reference strain of *Myrmaecium rubricosum*. These isolates were characterized by producing free, well-differentiated conidiophores instead of simply conidiogenous cells (phialides) inside the pycnidia.

The *Hysteriales* clade contained two sterile isolates, one related to an unidentified strain of *Chaetophoma* and one of uncertain taxonomical placement but phylogenetically related to *Chaetophoma*, *Gloniopsis*, and *Rhytidhysteron*.

The second largest clade, corresponding to the order *Botryosphaeriales*, included 45 isolates distributed in six clades but mostly concentrated into the *Neoscytalidium* clade. The fungi included in these clades were characterized by the production of stromatic conidiomata (a hard, compact mass of cells or of vegetative hyphae), holoblastic instead of phialidic conidiogenous cells, and aseptate, hyaline to brown, thick-walled conidia. The *Botryosphaeriales* included the genera *Botryosphaeria* (three isolates), *Lasiodiplodia* (two isolates), *Neofusicoccum* (one isolate), *Aplosporella* (two isolates), and *Phaeobotryosphaeria* (two isolates). Additionally, 35 isolates of *Neoscytalidium dimidia-*

tum were also placed in this order. This fungus is characterized typically by the production of holoarthric conidia (formed by disarticulation of the preexisting hyphae) in chains.

The *Capnodiales*, *Helotiales*, and *Magnaporthales* clades each included only one sterile isolate. Only the isolate of the *Helotiales* was not phylogenetically related to any previously known described species. The isolate of the *Capnodiales* was closely related to a reference strain of *Pseudocercospora oenotherae*. This genus is characterized by producing stromata in the (plant) host, subhyaline to brown conidiophores, and small or large, subhyaline to brown conidia; unfortunately, our isolate failed to sporulate. In the *Magnaporthales*, the isolate matched a reference strain of *Mycoleptodiscus indicus*. This genus is characterized by producing sporodochia (a cushion-like, densely aggregated group of conidiophores) and curved conidia; in this case, the morphological study was not possible due to the absence of sporulation of the isolate.

The *Xylariales* clade included five sterile isolates, three of which were related to the genus *Diatrype* but phylogenetically distant from a reference strain of *Diatrype disciformis*. The remaining two isolates were associated with the *Peroneutypa* clade, with one of them matching a reference strain of *Peroneutypa scoparia* and the other uncertainly placed taxonomically.

The *Diaporthales* clade grouped eight isolates, six of which belonged to the *Diaporthe* clade and were characterized by the production of hyaline conidiophores within pycnidial conidiomata, phialidic conidiogenous cells, and small conidia. The other two sterile isolates were located in the *Valsa* clade.

The *Hypocreales* clade included only a single sterile isolate that matched a reference strain of *Thyronectria austroamericana*.

The *Glomerellales* clade comprised 10 isolates, all of which belonged to the genus *Colletotrichum* and were characterized by the production of acervular conidiomata, phialidic conidiogenous cells, conidia variable in shape, and the presence of appressoria. Six of the isolates were identified as *Colletotrichum gloeosporioides*, two were identified as *Colletotrichum truncatum*, and one was identified as *Colletotrichum spaethianum*. One isolate (UTHSC DI14-247) was molecularly closely related to a reference strain of *Colletotrichum torulosum*.

Two isolates (UTHSC DI16-350 and UTHSC DI16-223) were not located in any of the previously known orders and consequently were treated as *incertae sedis*. The first one was assigned to the *Phomatospora* clade and the other, characterized by the production of sporodochia and hyaline conidia, was identified as *Phialemoniopsis curvata*.

From a total of 224 clinical isolates, 153 were recovered mainly from superficial tissues (epidermis and dermis) (66.5%), followed by 40 from the respiratory tract (17.4), 22 from miscellaneous deep tissues or fluids (9.6%), and 9 isolates from subcutaneous tissues (3.9%) (Table 1).

Approximately half of all the fungi tested (44%; 101/230) were able to grow at 37°C (Table 1); they were distributed within the orders at the following percentages: 100% (10/10) of the *Glomerellales*, 100% (2/2) of *Hysteriales*, 100% (2/2) of the *Valsariales*, 98% (44/45) of the *Botryosphaeriales*, 50% (1/2) of the isolates *incertae sedis*, and 28% (42/152) of the *Pleosporales*.

Table 2 summarizes the results of the antifungal susceptibility testing. In general, all the drugs tested, but especially terbinafine and amphotericin B, showed good activity against the coelomycetous fungi, with terbinafine being the most active (geometric mean [GM] of 0.04 μg/ml; MIC_{90} of 0.03 μg/ml). Among the triazoles, itraconazole was the least active, with an overall GM of 1 μg/ml and a MIC_{90} of 16 μg/ml. *Colletotrichum gloeosporioides*, *Neoscytalidium dimidiatum*, and *Didymella heteroderae* showed high MICs for all the antifungals tested. Posaconazole and voriconazole demonstrated similar *in vitro* potencies, with the only exceptions being activity against *Colletotrichum gloeosporioides* and *Neoascochyta desmazieri*, for which the voriconazole GMs were 2.64 and 2 μg/ml, respectively, and against *Neoscytalidium dimidiatum*, for which the posaconazole GM was 2.26 μg/ml. All the echinocandins showed good *in vitro* activity

TABLE 1 Anatomical sites of coelomycetous fungus isolates from clinical specimens

Order	Clade	No. of isolates obtained from:					37°C growth	Total no. of isolates
		Superficial tissue	Subcutaneous tissue	Deep tissue/fluids	Respiratory tract	Environment and animal		
Botryosphaeriales	*Aplosporella*	2					+	2
	Botryosphaeria	3					+	3
	Lasiodiplodia	2					+	2
	Neofusicoccum	1					−	1
	Neoscytalidium	27		3	5		+	35
	Phaeobotryosphaeria	2					+	2
Capnodiales		1					−	1
Diaporthales	*Diaporthe*	3		2	1		+	6
	Valsa	1			1		+	2
Glomerellales		7	1	1	1		+	10
Helotiales		1					−	1
Hypocreales		1					+	1
Hysteriales			1		1		+	2
incertae sedis	*Phialemoniopsis*	1					+	1
	Phomatospora			1			−	1
Magnaporthales		1					+	1
Pleosporales	*Acrocalymma*	1					−	1
	Anteaglonium	1					−	1
	Biatriospora	1			1		−	2
	Camarographium	2					−	2
	Coniothyrium	7	1	1			−	9
	Didymella	17		1	4		+	22
	Didymosphaeriaceae	26	1	2	3	1	−	33
	Exosporium	1				1	−	2
	flavescens	1					−	1
	Keissleriella		1				−	1
	Lophiostoma		1				−	1
	Medicopsis	3		1			+	4
	Neoascochyta	6			1		−	7
	Paraphoma	1					−	1
	Phaeosphaeria	2	1	1	4	1	−	9
	Phoma I	13		3	10	1	−	27
	Phoma II	1					−	1
	Phyllosticta	1					+	1
	Pleospora	1		1	3		−	5
	Pyrenochaetopsis	6		1	2		−	9
	Roussoella	5		2	1		+	8
	Trematosphaeria	4		1			+	5
Valsariales						2	+	2
Xylariales	*Diatrype*		1	1	1		−	3
	Peroneutypa		1		1		−	2
Total no. of isolates (%)		153 (66.5)	9 (3.9)	22 (9.6)	40 (17.4)	6 (2.6)		230 (100)

against these fungi, with a GM of 0.06 μg/ml. Flucytosine was the least active antifungal tested, with elevated MICs against all isolates.

DISCUSSION

This is, to our knowledge, the largest taxonomic study on coelomycetous fungi of clinical origin. It has demonstrated, based on DNA sequencing, a wider diversity of taxa than previously reported. Although two recent reviews have reported approximately 35 species of coelomycetes involved in human infections (3, 4), the present study identifies 88 species; unfortunately, the role of many of them as pathogens for human still remains uncertain because the clinical data of the patients are not allowed to be

TABLE 2 Results of *in vitro* antifungal susceptibility testing of coelomycetous fungi

Taxon (no. of isolates)	Parameter[a]	Value for the drug (μg/ml)[b]								
		AMB	VRC	ITC	PSC	AFG	CFG	MFG	TRB	5FC
Neoascochyta desmazieri (5)	GM	0.44	2	0.57	0.21	0.03	0.03	0.03	0.03	1.15
	Range	0.25–1	1–4	0.25–1	0.06–0.5	0.03–0.06	≤0.03	≤0.03	≤0.03	0.5–2
	MIC_{90}	0.5	2	1	0.5	0.03	0.03	0.03	0.03	2
Colletotrichum gloeosporioides (5)	GM	0.57	2.64	8	0.87	0.03	0.03	0.03	0.03	16
	Range	0.03–2	0.5–4	1–16	0.5–1	≤0.03	≤0.03	≤0.03	≤0.03	≥16
	MIC_{90}	2	4	16	1	0.03	0.03	0.03	0.03	16
Epicoccum sorghinum (8)	GM	0.25	0.92	0.59	0.30	0.03	0.04	0.03	0.03	2.97
	Range	0.12–1	0.5–2	0.5–1	0.12–0.5	0.03–0.06	0.03–0.5	≤0.03	≤0.03	1–8
	MIC_{90}	0.5	1	1	0.5	0.03	0.03	0.03	0.03	4
Neoscytalidium dimidiatum (16)	GM	0.22	0.59	2.56	2.26	0.13	0.2	0.47	0.08	2.83
	Range	0.06–1	0.03–16	0.06–16	0.03–16	0.03–0.5	0.03–1	0.06–8	0.03–2	0.25–16
	MIC_{90}	0.5	4	16	16	0.25	0.5	4	0.03	8
Paraconiothyrium cyclothyrioides (15)	GM	0.25	0.25	0.3	0.15	0.03	0.03	0.03	0.03	2.61
	Range	0.03–8	0.06–0.5	0.06–0.5	0.03–0.5	≤0.03	≤0.03	≤0.03	≤0.03	1–16
	MIC_{90}	0.5	0.5	0.5	0.25	0.03	0.03	0.03	0.03	4
Didymella heteroderae (11)	GM	1.76	1.87	3.31	1.07	0.34	0.13	0.14	0.03	4
	Range	0.5–8	0.06–16	0.5–16	0.5–2	0.03–8	0.03–4	0.03–2	≤0.03	1–16
	MIC_{90}	4	16	16	2	8	4	2	0.03	16
Phoma herbarum (10)	GM	0.43	0.57	0.81	0.40	0.04	0.04	0.03	0.03	2
	Range	0.12–2	0.06–4	0.25–4	0.12–1	0.03–0.12	0.03–0.12	0.03–0.06	≤0.03	0.5–16
	MIC_{90}	1	1	1	1	0.06	0.12	0.06	0.03	16
Phoma sp. (7)	GM	0.1	0.17	0.17	0.14	0.03	0.03	0.03	0.03	1.78
	Range	0.03–4	0.03–2	0.03–2	0.03–1	≤0.03	≤0.03	≤0.03	≤0.03	0.5–16
	MIC_{90}	0.25	1	0.5	0.5	0.03	0.03	0.03	0.03	4
Diaporthe sclerotioides (4)	GM	0.06	0.21	2	0.5	0.04	0.03	0.03	0.03	4
	Range	0.03–0.12	0.12–0.25	1–4	0.5	0.03–0.06	≤0.03	≤0.03	≤0.03	0.5–16
	MIC_{90}	0.12	0.25	2	0.5	0.03	0.03	0.03	0.03	8
Pyrenochaetopsis leptospora (4)	GM	0.7	0.59	0.7	0.21	0.03	0.03	0.03	0.03	4
	Range	0.03–4	0.25–2	0.06–16	0.03–1	≤0.03	≤0.03	≤0.03	≤0.03	0.5–16
	MIC_{90}	2	1	1	0.5	0.03	0.03	0.03	0.03	16
Overall (85)	GM	0.33	0.61	1	0.46	0.06	0.06	0.06	0.04	2.9
	Range	0.03–8	0.03–16	0.03–16	0.03–16	0.03–8	0.03–4	0.03–8	0.03–2	0.25–32
	MIC_{90}	2	4	16	16	0.25	0.5	2	0.03	16

[a]GM, geometric mean; MIC_{90}, drug concentration that inhibited 90% of isolates.
[b]AMB, amphotericin B; VRC, voriconazole; ITC, itraconazole; PSC, posaconazole; AFG, anidulafungin; CFG, caspofungin; MFG, micafungin; TRB, terbinafine; 5FC, flucytosine.

published. In general, the coelomycetous fungi are involved in many kinds of mycoses, with superficial to deep infections, onychomycosis, cutaneous infections, keratitis, and endophthalmitis being relatively frequent. In general, the most commonly reported species clinically are *Colletotrichum* spp. (13–20), *Neoscytalidium dimidiatum* (21–25), and *Phoma* spp. (11, 26–35). Our study partly confirms the data from previous studies in which *Neoscytalidium dimidiatum* (approximately 15%), *Paraconiothyrium cyclothyrioides* (approximately 7%), and *Phoma herbarum* (approximately 6.5%) were the most common species, having been recovered mainly from superficial tissue and respiratory tract specimens. However, of these fungi, the only species that is relatively easy to identify by phenotypic criteria is *N. dimidiatum*, which is the best known coelomycetous fungus found clinically (22, 23, 36). The identification of the other fungi mentioned above generally requires the use of molecular tools due to the difficulty of achieving *in vitro* sporulation. Although *Paraconiothyrium cyclothyrioides* was relatively common in our studied samples, there are only two clinical reports that refer to this species. Both

cases are from immunocompromised patients; in one case *P. cyclothyrioides* caused skin lesions of the lower extremities, and in the second case it produced a systemic coinfection together with *Phaeoacremonium parasiticum* (37, 38). Even though *Phoma* sporulates easily, it is commonly misidentified as other related genera, such as *Ascochyta*, because the genera have similar morphologies, physiologies, and nucleotide sequences (39, 40). Boerema et al. carried out one of the most comprehensive revisions of the taxonomy of the genus *Phoma*. Using systematic criteria that predominated then, approximately 220 species were accepted, distributed into nine sections (41). In a recent multilocus study based on the sequence data of the 18S nrRNA (SSU) and LSU genes, other authors demonstrated that such classification was totally artificial (42). Currently, *Phoma sensu stricto* is included in the family *Didymellaceae*, and the other *Phoma*-like fungi belong to other phylogenetic families, i.e., *Cucurbitariaceae*, *Leptosphaeriaceae*, *Phaeosphaeriaceae*, etc. (39, 40, 42, 43).

It is of note that one of the frequently isolated species in our study, *Didymella heteroderae* (5.2% of isolation frequency), has never been mentioned as an etiologic agent of human infections even though our results reveal its ability to grow and to sporulate at 37°C, which is uncommon in that genus and suggests its potential pathogenicity.

An important clinical presentation of the coelomycetous fungi is eumycetoma, which is restricted to a specific group of pleosporalean species of fungi, namely, *Medicopsis romeroi* (formerly, *Pyrenochaeta romeroi*) (44–46), *Biatriospora mackinnonii* (formerly *Pyrenochaeta mackinnonii*) (46), and *Trematosphaeria grisea* (formerly, *Madurella grisea*) (47–49), among others. However, in the present study only nine of the isolates that were isolated from superficial and, less frequently, from deep tissues belonged to these genera. This might be explained by the fact that the habitat of these fungi is usually restricted to arid zones of East Africa and India and, occasionally, South America (46, 50, 51).

Despite several studies in recent years devoted to infections by coelomycetous fungi, little clinical data exist. The first well-documented review of human infections caused by these fungi was carried out by Punithalingham (11), who referenced a total of 12 species belonging primarily to the genera *Botryodiplodia*, *Dothiorella*, *Hendersonula*, *Phoma*, *Phyllosticta*, *Pseudochaetosphaeronema*, and *Pyrenochaeta*. In that work, a morphological description of these taxa and their clinical origin was provided, together with a dichotomous key for their identification. However, in our study, just under 12% of the total isolates identified belonged to such genera. In a recent study, Stchigel and Sutton (4) provided detailed information about the species of these fungi isolated from clinical samples, described useful tools for their isolation and identification, and gave general guidelines for infection management and treatment. These authors concluded that these organisms are easy to isolate but that it was difficult to induce *in vitro* fructification and sporulation. Our results are in agreement with theirs because 43% of our isolates failed to sporulate, and it was only possible to identify them and to determine their phylogenetic relationships by DNA sequencing.

The prevalence of coelomycetous fungi found in these clinical specimens—more than 200 isolates recovered in a 9-year period—goes against the fact that so few studies have described infections by them. This highlights the difficulty in conducting a comprehensive study of these fungi and in establishing their real occurrence in clinical settings. The taxonomy of these fungi is very complex because numerous isolates are usually unable to sporulate *in vitro* or to produce different synanamorphs, which sometimes predominate over the traditional coelomycete structures, making their phenotypic recognition difficult; reliable identification can be done, therefore, only by gene sequencing (9, 46, 52). However, even in this case, there are a very high number of genera and species of coelomycetous fungi, and the phylogenetic boundaries of numerous taxa are still unresolved. Therefore, we carried out a phylogenetic analysis of a large set of coelomycetous fungi using LSU sequences. This marker proved useful for solving the phylogeny of most of the isolates included in the study, identi-

fying them, at least at genus level, and showing, in front of the internal transcribed spacer (ITS), the advantage of an easy alignment of sequences.

The increasing use of molecular tools in fungal taxonomy has allowed the recognition of numerous new taxa that are impossible to detect by traditional methods. Recently, several new species of coelomycetous fungi, namely, *Roussoella percutanea*, *Truncatella angustata*, *Hongkongmyces pedis*, *Rhytidhysteron* spp., *Pseudochaetosphaeronema martinelli*, and *Emarellia* spp., have been involved in cases of subcutaneous infections and eumycetoma (53–58), and some of our *Pleosporales* isolates, having failed to sporulate, could represent new taxa.

Although clinical breakpoints for coelomycetous fungi have not been defined and although *in vitro* antifungal susceptibility studies on these fungi are scarce, most of the species seem to be inhibited by amphotericin B (4). Our results show that posaconazole is the most active of the triazoles tested, and results for amphotericin B are similar *in vitro* to those reported by Chowdhary et al. (10). Currently, only disseminated infections due to *N. dimidiatum* have been conducted in animal models, and amphotericin B, voriconazole, and posaconazole have been shown to be effective in the treatment of this experimental mycosis (36). Guidelines for the management of infections due to coelomycetous fungi include only a small group of taxa (*Neoscytalidium*, *Phoma*, and *Pyrenochaeta* spp.) (10) although our study supports those protocols. A recent study by Guégan et al. (59) analyzed several coelomycetous fungi that were implicated in human mycosis and concluded that the surgical resection of infected tissues is advisable for treating well-delimited lesions and that surgery together with new triazoles could be used if lesions are extensive.

In conclusion, this study demonstrates that a wide variety of fungal taxa, identified through their morphology as coelomycetous fungi, are involved in human infections in the United States. However, more studies are necessary to understand the real prevalence of coelomycete infections throughout the world. The most active antifungal drugs to treat them seem to be terbinafine, echinocandins, and amphotericin B, while results for the azoles varied. Although the LSU gene sequence is useful for preliminary identification and for establishing phylogenetic relationships between the majority of coelomycetous fungi, future molecular studies testing a higher number genes are essential to properly identify doubtful isolates at the species level.

MATERIALS AND METHODS

Fungal isolates and sequences. A total of 230 isolates of coelomycetous fungi were included in this study, consisting of 224 from human clinical specimens, 3 from animal sources, and 3 from environmental samples. All of the isolates were provided by the Fungus Testing Laboratory of the University of Texas Health Science Center at San Antonio (UTHSC; San Antonio, Texas, USA). In addition, 92 D1-D2 sequences corresponding to type or reference strains were retrieved from GenBank and CBS databases and included in the phylogenetic analysis.

Morphological and physiological characterization. For cultural characterization, the isolates were grown on oatmeal agar (OA; 30 g of filtered oat flakes, 15 g of agar-agar, 1 liter of tap water) and malt extract agar (MEA; 40 g of malt extract, 15 g of agar-agar, 1 liter of distilled water) at 20 ± 1°C for 14 days in darkness. The ability of the isolates to grow at 37°C was determined on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) after 7 days of incubation in darkness. The morphological features of the vegetative and reproductive structures were studied using an Olympus CH2 light-field microscope (Olympus Corporation, Tokyo, Japan) in wet mounts (on water and lactic acid) and slide cultures (isolates grown on OA and MEA). The isolates were characterized phenotypically according to traditional criteria (4, 5, 41, 60). Color standards are from Kornerup and Wanscher (61). Photomicrographs were taken with an Axio-Imager M1 light-field microscope (Zeiss, Oberkochen, Germany).

DNA extraction, amplification, and sequencing. The total genomic DNA was extracted from colonies grown on PDA after 7 days of incubation at 20 ± 1°C, using a FastDNA kit protocol (Bio101; Vista, CA) with a FastPrep FP120 instrument (Thermo Savant, Holbrook, NY) according to the manufacturer's protocol. DNA was quantified using a NanoDrop 2000 instrument (Thermo Scientific, Madrid, Spain). The D1-D2 domains were amplified with the primer pair LR0R and LR5 (62). The amplicons were sequenced 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 SeqMan software, version 7.0.0 (DNAStar Lasergene, Madison, WI, USA).

Molecular identification and phylogenetic analysis. Preliminary molecular identification of the isolates was made using the D1-D2 nucleotide sequences in blastn searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the CBS database (www.cbs.knaw.nl). Only the sequences of type or reference strains

deposited in CBS/GenBank databases were considered for identification purposes. A level of identity of ≥98% was considered for species-level identification.

For the phylogenetic study, the sequences were aligned using the ClustalW application (63) of the MEGA, version 6.06 (64), computer program, refined with MUSCLE (65), and manually adjusted using the same software platform. Phylogenetic reconstructions were made by maximum-likelihood (ML) and Bayesian inference (BI) with MEGA, version 6.06, and MrBayes, version 3.2.4 (66), respectively. The best substitution model for the gene matrix (general time-reversal model incorporating invariable sites and a discrete gamma distribution [GTR+I+G]) was estimated using MrModelTest, version 2.3 (67). For ML analyses, a nearest-neighbor interchange was used as the heuristic method for tree inference. Support for internal branches was assessed by 1,000 ML bootstrapped pseudoreplicates. Bootstrap support (BS) of ≥70 was considered significant. For BI analyses, Markov chain Monte Carlo (MCMC) sampling was carried out with 23 million generations, with samples taken every 1,000 generations. The 50% majority rule consensus trees and posterior probability values (PP) were calculated after the first 25% of the resulting trees was removed for burn-in. A PP value of ≥0.95 was considered significant. *Saccharomyces castellii* (NRRL Y-12630; GenBank accession number AY048167) and *Saccharomyces cerevisiae* (NRRL Y-12632; GenBank accession number AY048154) were used as outgroups.

Antifungal susceptibility testing. Using a broth microdilution reference method (68), the *in vitro* antifungal susceptibilities of 85 isolates were determined of selected species of the genera *Colletotrichum*, *Diaporthe*, *Didymella*, *Epicoccum*, *Neoascochyta*, *Neoscytalidium*, *Paraconiothyrium*, *Phoma* sp., and *Pyrenochaetopsis*. The following antifungals were tested: amphotericin B, voriconazole, posaconazole, itraconazole, caspofungin, anidulafungin, micafungin, terbinafine, and flucytosine. The minimal effective concentration (MEC) was determined after 48 h for the echinocandins, and the MIC was determined after 48 h and 72 h for the other drugs. *Candida parapsilosis* ATCC 22019 and *Paecilomyces variotii* ATCC MYA-3630 were used as controls. The inocula for the coelomycetous fungi that did not sporulate were prepared according to the method of Chowdhary et al. (69).

Accession number(s). The DNA sequences determined in this study have been deposited in GenBank under accession numbers LN907285 to LN907514.

ACKNOWLEDGMENTS

This work was supported by the Spanish Ministerio de Economía y Competitividad, grant CGL2013-43789-P.

We have no conflicts of interest to declare.

REFERENCES

1. Kirk PM, Cannon PF, Stalpers JA, Minter DW. 2008. Ainsworth & Bisby's dictionary of the fungi, 10th ed. CAB International, Wallingford, United Kingdom.
2. Sutton DA. 1999. Coelomycetous fungi in human disease. A review: clinical entities, pathogenesis, identification and therapy. Rev Iberoam Micol 16:171–179.
3. Revankar SG, Sutton DA. 2010. Melanized fungi in human disease. Clin Microbiol Rev 23:884–928. https://doi.org/10.1128/CMR.00019-10.
4. Stchigel AM, Sutton DA. 2013. Coelomycete fungi in the clinical lab. Curr Fungal Infect Rep 7:171–191. https://doi.org/10.1007/s12281-013-0139-9.
5. Sutton BC. 1980. The Coelomycetes. Fungi Imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological, Kew, England.
6. Nag Raj TR. 1993. Coelomycetous anamorphs with appendage-bearing conidia. Mycologue Publications, Waterloo, Ontario, Canada.
7. Schoch CL, Crous PW, Groenewald JZ, Boehm EWA, Burgess TI, de Gruyter J, de Hoog GS, Dixon LJ, Grube M, Gueidan C, Harada Y, Hatakeyama S, Hirayama K, Hosoya T, Huhndorf SM, Hyde KD, Jones EBG, Kohlmeyer J, Kruys A, Li YM, Lücking R, Lumbsch HT, Marvanová L, Mbatchou JS, McVay AH, Miller AN, Mugambi GK, Muggia L, Nelsen MP, Nelson P, Owensby CA, Phillips AJL, Phongpaichit S, Pointing SB, Pujade-Renaud V, Raja HA, Plata ER, Robbertse B, Ruibal C, Sakayaroj J, Sano T, Selbmann L, Shearer CA, Shirouzu T, Slippers B, Suetrong S, Tanaka K, Volkmann-Kohlmeyer B, Wingfield MJ, Wood AR, Woudenberg JHC, Yonezawa H, Zhang Y, Spatafora JW. 2009. A class-wide phylogenetic assessment of *Dothideomycetes*. Stud Mycol 64:1–15. https://doi.org/10.3114/sim.2009.64.01.
8. Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW. 2014. *Pestalotiopsis* revisited. Stud Mycol 79:121–186. https://doi.org/10.1016/j.simyco.2014.09.005.
9. Wijayawardene NN, Hyde KD, Wanasinghe DN, Papizadeh M, Goonasekara ID, Camporesi E, Bhat DJ, McKenzie EHC, Phillips AJL, Diederich P, Tanaka K, Li WJ, Tangthirasunun N, Phookamsak R, Dai D-Q, Dissanayake AJ, Weerakoon G, Maharachchikumbura SSN, Hashimoto A, Matsumura M, Bahkali AH, Wang Y. 2016. Taxonomy and phylogeny of dematiaceous coelomycetes. Fungal Divers 77:1–316. https://doi.org/10.1007/s13225-016-0360-2.
10. Chowdhary A, Meis JF, Guarro J, de Hoog GS, Kathuria S, Arendrup MC, Arikan-Akdagli S, Akova M, Boekhout T, Caira M, Guinea J, Chakrabarti A, Dannaoui E, van Diepeningen A, Freiberger T, Groll AH, Hope WW, Johnson E, Lackner M, Lagrou K, Lanternier F, Lass-Flörl C, Lortholary O, Meletiadis J, Muñoz P, Pagano L, Petrikkos G, Richardson MD, Roilides E, Skiada A, Tortorano AM, Ullmann AJ, Verweij PE, Cornely OA, Cuenca-Estrella M. 2014. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of systemic phaeohyphomycosis: diseases caused by black fungi. Clin Microbiol Infect 20:47–75. https://doi.org/10.1111/1469-0691.12515.
11. Punithalingam E. 1979. Sphaeropsidales in culture from humans. Nova Hedwigia 31:119–158.
12. Liu JK, Phookamsak R, Dai DQ, Tanaka K, Jones EG, Xu JC, Chukeatirote E, Hyde K. 2014. *Roussoellaceae*, a new pleosporalean family to accommodate the genera *Neoroussoella* gen. nov., *Roussoella* and *Roussoellopsis*. Phytotaxa 181:1–33. https://doi.org/10.11646/phytotaxa.181.1.1.
13. Guarro J, Svidzinski TE, Zaror L, Forjaz MH, Gené J, Fischman O. 1998. Subcutaneous hyalohyphomycosis caused by *Colletotrichum gloeosporioides*. J Clin Microbiol 36:3060–3065.
14. Cano J, Guarro J, Gené J. 2004. Molecular and morphological identification of *Colletotrichum* species of clinical interest. J Clin Microbiol 42:2450–2454. https://doi.org/10.1128/JCM.42.6.2450-2454.2004.
15. Castro LG, Da Silva Lacaz C, Guarro J, Gené J, Heins-Vaccari EM, de Freitas Leite RS, Arriagada GL, Reguera MM, Ito EM, Valente NY, Nunes RS. 2001. Phaeohyphomycotic cyst caused by *Colletotrichum crassipes*. J Clin Microbiol 39:2321–2324. https://doi.org/10.1128/JCM.39.6.2321-2324.2001.
16. Fernandez V, Dursun D, Miller D, Alfonso EC. 2002. *Colletotrichum* keratitis. Am J Ophthalmol 134:435–438. https://doi.org/10.1016/S0002-9394(02)01576-3.
17. Chakrabarti A, Shivaprakash MR, Singh R, Tarai B, George VK, Fomda BA, Gupta A. 2008. Fungal endophthalmitis: fourteen years' experience from a center in India. Retina 28:1400–1407. https://doi.org/10.1097/IAE.0b013e318185e943.

18. Shivaprakash MR, Appannanavar SB, Dhaliwal M, Gupta A, Gupta S, Gupta A, Chakrabarti A. 2011. *Colletotrichum truncatum*: An unusual pathogen causing mycotic keratitis and endophthalmitis. J Clin Microbiol 49:2894–2898. https://doi.org/10.1128/JCM.00151-11.
19. Shiraishi A, Araki-Sasaki K, Mitani A, Miyamoto H, Sunada A, Ueda A, Asari S, Zheng X, Yamamoto Y, Hara Y, Ohashi Y. 2011. Clinical characteristics of keratitis due to *Colletotrichum gloeosporioides*. J Ocul Pharmacol Ther 27:487–491. https://doi.org/10.1089/jop.2011.0011.
20. Figtree M, Weeks K, Chan L, Leyton A, Bowes A, Giuffre B, Sullivan M, Hudson BJ. 2013. *Colletotrichum gloeosporioides* sensu lato causing deep soft tissue mycosis following a penetrating injury. Med Mycol Case Rep 2:40–43. https://doi.org/10.1016/j.mmcr.2013.01.003.
21. al-Rajhi AA, Awad AH, Al-Hedaithy SS, Forster RK, Caldwell KC. 1993. *Scytalidium dimidiatum* fungal endophthalmitis. Br J Ophthalmol 77:388–390. https://doi.org/10.1136/bjo.77.6.388.
22. Elewski BE. 1996. Onychomycosis caused by *Scytalidium dimidiatum*. J Am Acad Dermatol 35:336–338. https://doi.org/10.1016/S0190-9622(96)90664-7.
23. Madrid H, Ruíz-Cendoya M, Cano J, Stchigel A, Orofino R, Guarro J. 2009. Genotyping and in vitro antifungal susceptibility of *Neoscytalidium dimidiatum* isolates from different origins. Int J Antimicrob Agents 34:351–354. https://doi.org/10.1016/j.ijantimicag.2009.05.006.
24. Machouart M, Menir P, Helenon R, Quist D, Desbois N. 2013. *Scytalidium* and scytalidiosis: what's new in 2012? J Mycol Med 23:40–46. https://doi.org/10.1016/j.mycmed.2013.01.002.
25. Bakhshizadeh M, Hashemian HR, Najafzadeh MJ, Dolatabadi S, Zarrinfar H. 2014. First report of rhinosinusitis caused by *Neoscytalidium dimidiatum* in Iran. J Med Microbiol 63:1017–1019. https://doi.org/10.1099/jmm.0.065292-0.
26. Bakerspigel A. 1970. The isolation of *Phoma hibernica* from lesions on a leg. Sabouraudia 7:261–264.
27. Punithalingam E. 1976. *Phoma oculo hominis* sp. nov. from corneal ulcer. Trans Br Mycol Soc 67:142–143. https://doi.org/10.1016/S0007-1536(76)80022-8.
28. Bakerspigel A, Lowe D, Rostas A. 1981. The isolation of *Phoma eupyrena* from a human lesion. Arch Dermatol 117:362–363. https://doi.org/10.1001/archderm.1981.01650060052024.
29. Shukla NP, Rajak RK, Agarwasl GP, Gupta D. 1984. *Phoma minutispora* as a human pathogen. Mykosen 27:255–258.
30. Baker JG, Salkin IF, Forgacs P, Haines JH, Kemna ME. 1987. First report of subcutaneous phaeohyphomycosis of the foot caused by *Phoma minutella*. J Clin Microbiol 25:2395–2397.
31. Rai MK. 1989. *Phoma sorghina* infection in human being. Mycopathologia 105:167–170. https://doi.org/10.1007/BF00437250.
32. Rosen T, Rinaldi MJ, Tschen JA, Stern JK, Cernoch P. 1996. Cutaneous lesions due to *Pleurophoma* (*Phoma*) complex. South Med J 89:431–434. https://doi.org/10.1097/00007611-199604000-00018.
33. Hirsh AH, Schiff TA. 1996. Subcutaneous phaeohyphomycosis caused by an unusual pathogen: *Phoma* species. J Am Acad Dermatol 34:679–680. https://doi.org/10.1016/S0190-9622(96)80083-1.
34. Tullio V, Banche G, Allizond V, Roana J, Mandras N, Scalas D, Panzone M, Cervetti O, Valle S, Carlone N, Cuffini AM. 2010. Non-dermatophyte moulds as skin and nail foot mycosis agents: *Phoma herbarum*, *Chaetomium globosum* and *Microascus cinereus*. Fungal Biol 114:345–349. https://doi.org/10.1016/j.funbio.2010.02.003.
35. Roehm CE, Salazar JC, Hagstrom N, Valdez TA. 2012. *Phoma* and *Acremonium* invasive fungal rhinosinusitis in congenital acute lymphocytic leukemia and literature review. Int J Pediatr Otorhinolaryngol 76:1387–1391. https://doi.org/10.1016/j.ijporl.2012.06.026.
36. Ruíz-Cendoya M, Madrid H, Pastor J, Guarro J. 2010. Evaluation of antifungal therapy in a neutropenic murine model of *Neoscytalidium dimidiatum* infection. Int J Antimicrob Agents 35:152–155. https://doi.org/10.1016/j.ijantimicag.2009.09.028.
37. Gordon RA, Sutton DA, Thompson EH, Shrikanth V, Verkley GJ, Stielow JB, Mays R, Oleske D, Morrison LK, Lapolla WJ, Galfione S, Tyring S, Samathanam CA, Fu J, Wickes BL, Mulanovich V, Wanger A, Arias CA. 2012. Cutaneous phaeohyphomycosis caused by *Paraconiothyrium cyclothyrioides*. J Clin Microbiol 50:3795–3798. https://doi.org/10.1128/JCM.01943-12.
38. Colombier MA, Alanio A, Denis B, Melica G, Garcia-Hermoso D, Levy B, Peraldi MN, Glotz D, Bretagne S, Gallien S. 2015. Dual invasive infection with *Phaeoacremonium parasiticum* and *Paraconiothyrium cyclothyrioides* in a renal transplant recipient: Case report and comprehensive review of the literature of *Phaeoacremonium* phaeohyphomycosis. J Clin Microbiol 53:2084–2094. https://doi.org/10.1128/JCM.00295-15.
39. Aveskamp MM, de Gruyter J, Woudenberg JH, Verkley GJ, Crous PW. 2010. Highlights of the *Didymellaceae*: a polyphasic approach to characterise *Phoma* and related pleosporalean genera. Stud Mycol 65:1–60. https://doi.org/10.3114/sim.2010.65.01.
40. Chen Q, Jiang JR, Zhang GZ, Cai L, Crous PW. 2015. Resolving the *Phoma* enigma. Stud Mycol 82:137–217. https://doi.org/10.1016/j.simyco.2015.10.003.
41. Boerema GH, de Gruyter J, Noordeloos ME, Hamers M. 2004. *Phoma* identification manual. Differentiation of specific and infraspecific taxa in culture. CABI Publishing, Cambridge, United Kingdom.
42. de Gruyter J, Aveskamp MM, Woudenberg JH, Verkley GJ, Groenewald JZ, Crous PW. 2009. Molecular phylogeny of *Phoma* and allied anamorph genera: towards a reclassification of the *Phoma* complex. Mycol Res 113:508–519. https://doi.org/10.1016/j.mycres.2009.01.002.
43. de Gruyter J, Woudenberg JH, Aveskamp MM, Verkley GJ, Groenewald JZ, Crous PW. 2013. Redisposition of *Phoma*-like anamorphs in *Pleosporales*. Stud Mycol 75:1–36. https://doi.org/10.3114/sim0004.
44. Borelli D. 1979. Opportunistic fungi as producers of gray colonies and mycetomata. Dermatologica 159:168–174. https://doi.org/10.1159/000250685.
45. Mathuram Thiyagarajan U, Bagul A, Nicholson ML. 2011. A nodulo-cystic eumycetoma caused by *Pyrenochaeta romeroi* in a renal transplant recipient: a case report. J Med Case Rep 5:460. https://doi.org/10.1186/1752-1947-5-460.
46. Ahmed SA, Van De Sande WW, Stevens DA, Fahal A, Van Diepeningen AD, Menken SB, de Hoog GS. 2014. Revision of agents of black-grain eumycetoma in the order *Pleosporales*. Persoonia 33:141–154. https://doi.org/10.3767/003158514X684744.
47. Butz WC, Ajello L. 1971. Black grain mycetoma: a case due to *Madurella grisea*. Arch Dermatol 104:197–201. https://doi.org/10.1001/archderm.1971.04000200085015.
48. Gulati V, Bakare S, Tibrewal S, Ismail N, Sayani J, Baghla DPS. 2012. A rare presentation of concurrent *Scedosporium apiospermum* and *Madurella grisea* eumycetoma in an immunocompetent host. Case Rep Pathol 2012:154201. https://doi.org/10.1155/2012/154201.
49. de Hoog GS, Van Diepeningen AD, Mahgoub ES, Van de Sande WWJ. 2012. New species of *Madurella*, causative agents of black-grain mycetoma. J Clin Microbiol 50:988–994. https://doi.org/10.1128/JCM.05477-11.
50. Mackinnon JE, Ferrada-Urzúa LV, Montemayor L. 1943. *Madurella grisea* n. sp. Mycopathologia 4:384–393. https://doi.org/10.1007/BF01237166.
51. McGinnis MR. 1996. Mycetoma. Dermatol Clin 41:97–104.
52. Borman AM, Desnos-Ollivier M, Campbell CK, Bridge PD, Dannaoui E, Johnson EM. 2016. Novel Taxa Associated with Human Fungal Black-Grain Mycetomas: *Emarellia grisea* gen. nov., sp. nov., and *Emarellia paragrisea* sp. nov. J Clin Microbiol 54:1738–1745. https://doi.org/10.1128/JCM.00477-16.
53. Ahmed SA, Stevens DA, Van de Sande WWJ, Meis JF, de Hoog GS. 2014. *Roussoella percutanea*, a novel opportunistic pathogen causing subcutaneous mycoses. Med Mycol 52:689–698. https://doi.org/10.1093/mmy/myu035.
54. Jagielski T, Zak I, Tyrak J, Bryk A. 2015. First probable case of subcutaneous infection due to *Truncatella angustata*: a new fungal pathogen of humans? J Clin Microbiol 53:1961–1964. https://doi.org/10.1128/JCM.00400-15.
55. Tsang CC, Chan JF, Trendell-Smith NJ, Ngan AH, Ling IW, Lau SK, Woo PC. 2014. Subcutaneous phaeohyphomycosis in a patient with IgG4-related sclerosing disease caused by a novel ascomycete, *Hongkongmyces pedis* gen. et sp. nov.: first report of human infection associated with the family *Lindgomycetaceae*. Med Mycol 52:736–747. https://doi.org/10.1093/mmy/myu043.
56. Mahajan VK, Sharma V, Prabha N, Thakur K, Sharma NL, Rudramurthy SM, Chauhan PS, Mehta KS, Abhinav C. 2014. A rare case of subcutaneous phaeohyphomycosis caused by a *Rhytidhysteron* species: a clinico-therapeutic experience. Int J Dermatol 53:1485–1489. https://doi.org/10.1111/ijd.12529.
57. Mishra K, Das S, Goyal S, Gupta C, Rai G, Ansari MA, Saha R, Singal A. 2014. Subcutaneous mycoses caused by *Rhytidhysteron* species in an immunocompetent patient. Med Mycol Case Rep 5:32–34. https://doi.org/10.1016/j.mmcr.2014.07.002.
58. Ahmed SA, Desbois N, Quist D, Miossec C, Atoche C, Bonifaz A, de Hoog GS. 2015. Phaeohyphomycosis caused by a novel species, *Pseudochaeto-*

sphaeronema martinelli. J Clin Microbiol 53:2927–2934. https://doi.org/10.1128/JCM.01456-15.
59. Guégan S, Garcia-Hermoso D, Sitbon K, Ahmed S, Moguelet P, Dromer F, Lortholary O, French Mycosis Study Group. 2016. Ten-year experience of cutaneous and/or subcutaneous infections due to coelomycetes in France. Open Forum Infect Dis 3:ofw106. https://doi.org/10.1093/ofid/ofw106.
60. de Hoog GS, Guarro J, Gené J, Figueras MJ. 2000. Atlas of clinical fungi, 2nd ed. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
61. Kornerup A, Wanscher JH. 1978. Methuen handbook of colour, 3rd ed. Methuen, London, England.
62. Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990.
63. Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680. https://doi.org/10.1093/nar/22.22.4673.
64. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197.
65. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340.
66. Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755. https://doi.org/10.1093/bioinformatics/17.8.754.
67. Nylander JA. 2004. MrModeltest v2. Uppsala University, Uppsala, Sweden.
68. Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, 2nd ed. Document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
69. Chowdhary A, Kathuria S, Singh PK, Agarwal K, Gaur SN, Roy P, Randhawa HS, Meis JF. 2013. Molecular characterization and in vitro antifungal susceptibility profile of *Schizophyllum commune*, an emerging basidiomycete in bronchopulmonary mycoses. Antimicrob Agents Chemother 57:2845–2848. https://doi.org/10.1128/AAC.02619-12.

4.2. Coelomycetous *Dothideomycetes* with emphasis on the families *Cucurbitariaceae* and *Didymellaceae*

N. Valenzuela-Lopez[1,2], J.F. Cano-Lira[1], J. Guarro[1], D.A. Sutton[3], N. Wiederhold[3], P.W. Crous[4,5], and A.M. Stchigel[1]

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Publicado en: Studies in Mycology 2018; 90: 1–69.

UNIVERSITAT ROVIRA I VIRGILI
TAXONOMÍA DE HONGOS CELOMICETOS DE INTERÉS CLÍNICO
Nicomedes Miguel Antonio Valenzuela López

available online at www.studiesinmycology.org

STUDIES IN MYCOLOGY 90: 1–69 (2018).

WESTERDIJK FUNGAL BIODIVERSITY INSTITUTE

Coelomycetous *Dothideomycetes* with emphasis on the families *Cucurbitariaceae* and *Didymellaceae*

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Abstract: The taxonomy of the coelomycetes has undergone dramatic changes in recent years, but remains controversial due to the high number of taxa involved, their poor morphological differentiation, the rare occurrence of the sexual morphs, and rapid loss of fertility *in vitro*. In the present study, we revisited the families *Cucurbitariaceae* and *Didymellaceae* (*Pleosporales, Dothideomycetes*), which include numerous plant pathogens, endophytic species associated with a wide host range, and saprobes. The taxonomy of two of the most relevant genera, i.e. *Phoma* and *Pyrenochaeta*, remains ambiguous after several phylogenetic studies, and needs further revision. We have studied a total of 143 strains of coelomycetes from clinical or environmental origin, by combining the LSU, ITS, *tub*2 and *rpb*2 sequences for a multilocus analysis and a detailed morphological comparison. The resulting phylogenetic tree revealed that some fungi previously considered as members of *Cucurbitariaceae* represented five different families, and four of them, *Neopyrenochaetaceae*, *Parapyrenochaetaceae*, *Pseudopyrenochaetaceae* and *Pyrenochaetopsidaceae*, are proposed here as new. Furthermore, 13 new genera, 28 new species, and 20 new combinations are proposed within the *Pleosporineae*. Moreover, four new typifications are introduced to stabilise the taxonomy of these fungi.

Key words: *Cucurbitariaceae*, *Didymellaceae*, Multigene phylogeny, New taxa, *Phoma*, *Pleosporineae*, *Pleosporales*, *Pyrenochaeta*, *Pyrenochaetopsis*, Taxonomy.
Taxonomic novelties: New families: *Neopyrenochaetaceae* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Parapyrenochaetaceae* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaetaceae* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pyrenochaetopsidaceae* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel; **New genera:** *Allocucurbitaria* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Cumuliphoma* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ectophoma* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Juxtiphoma* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Neopyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neopyrenochaetopsis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Paracucurbitaria* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Parapyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Remotididymella* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Similiphoma* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Vacuiphoma* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Xenopyrenochaetopsis* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano; **New species:** *Allocucurbitaria botulispora* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Allophoma cylindrispora* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Cumuliphoma indica* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Cu. pneumoniae* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Didymella brunneospora* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *D. keratinophila* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Epicoccum catenisporum* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ep. keratinophilum* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Ep. ovisporum* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ep. pneumoniae* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Neoascochyta cylindrispora* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Neoa. tardicrescens* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Neocucurbitaria aquatica* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neocu. irregularis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Neopyrenochaeta fragariae* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neopyrenochaetopsis hominis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Nothophoma variabilis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Paracucurbitaria italica* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaeta terrestris* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Pyrenochaetopsis americana* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py. botulispora* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py. confluens* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py. globosa* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py. paucisetosa* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py. setosissima* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Py. uberiformis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Remotididymella anthropophila* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Vacuiphoma oculihominis* Valenzuela-Lopez, Stchigel, Guarro & Cano; **New combinations:** *Cumuliphoma omnivirens* (Aveskamp *et al.*) Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ectophoma multirostrata* (P.N. Mathur *et al.*) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Ec. pomi* (Horne) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Epicoccum proteae* (Crous) Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Juxtiphoma eupyrena* (Sacc.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Neocucurbitaria cava* (Schulzer) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neocu. hakeae* (Crous) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neocu. keratinophila* (Verkley *et al.*) Valenzuela-Lopez, Stchigel, Guarro & Cano, *Neopyrenochaeta acicola* (Moug. & Lév.) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neopy. inflorescentiae* (Crous *et al.*) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neopy. telephoni* (Rohit Sharma *et al.*) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Paracucurbitaria corni* (Bat. & A.F. Vital) Valenzuela-Lopez, Stchigel, Guarro & Cano, *Parapyrenochaeta acaciae* (Crous *et al.*) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Parapy. protearum* (Crous) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaeta lycopersici* (R.W. Schneid. & Gerlach) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Remotididymella destructiva* (Plowr.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Similiphoma crystallifera* (Gruyter *et al.*) Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Vacuiphoma bulgarica* (Aveskamp *et al.*) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Xenodidymella saxea* (Aveskamp *et al.*) Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Xenopyrenochaetopsis pratorum* (P.R. Johnst. & Boerema) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano.

Available online 21 November 2017; https://doi.org/10.1016/j.simyco.2017.11.003.

Peer review under responsibility of Westerdijk Fungal Biodiversity Institute.

Studies in Mycology

INTRODUCTION

The *Pleosporales* is the largest order of the class *Dothideomycetes* (phylum *Ascomycota*), encompassing more than 4 700 species distributed over 332 genera, and 53 families (Kirk *et al.* 2008, Zhang *et al.* 2009, 2012, Ariyawansa *et al.* 2013, Hyde *et al.* 2013, Amaradasa *et al.* 2014, Trakunyingcharoen *et al.* 2014, Wijayawardene *et al.* 2014, Crous *et al.* 2015a, Sharma *et al.* 2015, Tanaka *et al.* 2015, Jaklitsch *et al.* 2016, Jaklitsch & Voglmayr 2016, Wanasinghe *et al.* 2016, Crous & Groenewald 2017, Hashimoto *et al.* 2017, Hernández-Restrepo *et al.* 2017). These fungi are characterised by the production of pseudothecial ascomata (mostly globose and usually papillate) consisting of a peridial wall composed by several layers of cells, within which the fissitunicate (bitunicate) asci are produced amidst a persistent hamathecium (the vegetative structures inside an ascoma) (Jaklitsch & Voglmayr 2016, Jaklitsch *et al.* 2018, Zhang *et al.* 2009, 2012) and ascospores, which are mostly septate but variable in shape and pigmentation. The asexual morphs of the *Pleosporales* are characterised by conidia produced within discrete sporocarps (conidiomata), and sometimes conidia are generated on conidiophores produced on mycelium. *Phoma* and its relatives are the most common pleosporalean asexual morphs and are characterised by the presence of pycnidia (globose to pyriform conidiomata from which the conidia arise throughout an apical opening) (de Gruyter *et al.* 2009, 2010, Aveskamp *et al.* 2010, Chen *et al.* 2015). *Pleosporales* are mainly saprobic on plant debris, epiphytic, endophytic or parasitic of living plants, fungi and insects, or mycobionts in lichens (Kruys *et al.* 2006, Aveskamp *et al.* 2008, 2010, de Gruyter *et al.* 2009, Zhang *et al.* 2009, 2012, Lawrey *et al.* 2012, Kocakaya *et al.* 2015). These fungi can also infect humans (Punithalingam 1979, Ahmed *et al.* 2014, 2015, 2017, Borman *et al.* 2016, Valenzuela-Lopez *et al.* 2016).

Modern phylogenetic studies support the division of the *Pleosporales* into the suborders *Pleosporineae* and *Massarineae* (Zhang *et al.* 2009, 2012, Hyde *et al.* 2013, Tanaka *et al.* 2015). The former includes nine families, i.e. *Coniothyriaceae*, *Cucurbitariaceae*, *Didymellaceae*, *Dothidotthiaceae*, *Halojulellaceae*, *Leptosphaeriaceae*, *Neophaeosphaeriaceae*, *Phaeosphaeriaceae*, *Pleosporaceae* and *Shiraiaceae* (Zhang *et al.* 2012, de Gruyter *et al.* 2013, Ariyawansa *et al.* 2013, 2015b, Liu *et al.* 2013), which encompass plant pathogens of economic importance including the well-known genera such as *Alternaria*, *Ascochyta*, *Bipolaris*, *Didymella* and *Leptosphaeria* (Zhang *et al.* 2012, Ariyawansa *et al.* 2013, de Gruyter *et al.* 2013, Liu *et al.* 2013, Woudenberg *et al.* 2013). Recently, Tanaka *et al.* (2015) revised the suborder *Massarineae* and accepted 12 families; however, more studies are needed for a better understanding of their phylogenetic relationships. Numerous species of *Pleosporales* are relatively common in clinical samples, most of which belong to the families *Cucurbitariaceae* and *Didymellaceae* (Valenzuela-Lopez *et al.* 2016). *Cucurbitariaceae* is still a poorly known family, which was erected by Winter (1885) with *Cucurbitaria* as the type genus, and characterised by ostiolate ascomata aggregated on a basal pseudostromatic structure, hamathecium composed of wide persistent filaments, fissitunicate, cylindrical to cylindrical-clavate asci and dark, phragmosporous or muriform ascospores. In the last revision of *Cucurbitariaceae*, four sexual genera (*Cucurbitaria*, *Curreya*, *Rhytidiella* and *Syncarpella*) and two asexual genera (*Pyrenochaeta* and *Pyrenochaetopsis*) were accepted (Doilom *et al.* 2013). The latter two genera are characterised by phoma-like, setose pycnidia, and hyaline, aseptate conidia (de Gruyter *et al.* 2010, 2013). Recently, Jaklitsch & Voglmayr (2017) demonstrated that some species of *Cucurbitaria*, such as *C. obducens*, *C. piceae* (both producing muriform ascospores) and *C. rhododendri* (with phragmospores), belong to three different genera of *Melanommataceae*. Wanasinghe *et al.* (2017b) proposed *Neocucurbitaria*, characterised by solitary ascomata, the presence of periphyses and muriform ascospores, as a new genus of *Cucurbitariaceae*. However, the current members of this family need to be re-evaluated, including their asexual morphs.

The family *Didymellaceae* also includes economically important plant pathogens, such as the causal agents of blackleg and ascochyta blight (Rouxel & Balesdent 2005, McDonald & Peck 2009, Salam *et al.* 2011, de Gruyter *et al.* 2013), but also diverse endophytic, fungicolous and lichenicolous taxa belong to this fungal group (Aveskamp *et al.* 2010), whereas a few members are known as pathogens of humans (de Hoog *et al.* 2011). This family was established by de Gruyter *et al.* (2009) and embraces the species traditionally classified in the genera *Ascochyta*, *Didymella* and *Phoma*. However, *Phoma* is one of the largest and most polyphyletic fungal genera (with more than 3 000 names recorded) with species occurring in more than 25 families (http://www.indexfungorum.org).

Zhang *et al.* (2009), included *Didymellaceae* in their study and accepted the sexual genera *Didymella*, *Leptosphaerulina*, *Macroventuria*, *Monascostroma* and *Platychora*. In general, these genera are characterised by dark pseudothecial ascomata, filamentous pseudoparaphyses, 8-spored, fissitunicate, clavate to saccate asci, and hyaline, 1-septate, fusiform to biconical ascospores; with the only exception being *Leptosphaerulina*, which has hyaline to brown, ellipsoid, cylindrical or oblong, phragmosporous or muriformly septate ascospores, which also lack pseudoparaphyses. Several studies have tried to resolve the taxonomy of the asexual morphs of the *Didymellaceae*, especially *Phoma* and its relatives, with more or less success. Subsequently, de Gruyter *et al.* (2010) transferred several species of *Phoma* to *Pyrenochaetopsis* (*Cucurbitariaceae*), *Neosetophoma* and *Setophoma* (*Phaeosphaeriaceae*), and resurrected the genus *Paraphoma* (*Phaeosphaeriaceae*). The study by Aveskamp *et al.* (2010), based on the sequences of four loci, revealed that the subdivision of *Phoma* in sections (Boerema *et al.* 2004) was phylogenetically inconsistent, and they thus proposed *Boeremia* to accommodate species morphologically close to *Phoma exigua*, while species of *Phoma* section *Sclerophomella* were transferred to *Epicoccum* and *Peyronellaea*. Furthermore, de Gruyter *et al.* (2013) transferred some species of *Phoma* sections *Plenodomus* and *Heterospora* to the *Leptosphaeriaceae* and some from *Phoma* section *Pilosa* and *Ascochyta* to *Pleosporaceae*. Recently, Chen *et al.* (2015) proposed nine genera (*Allophoma*, *Calophoma*, *Heterophoma*, *Neoascochyta*, *Neodidymelliopsis*, *Nothophoma*, *Paraboeremia*, *Phomatodes* and *Xenodidymella*) in *Didymellaceae*, transferred *Microsphaeropsis* (*Didymellaceae*) to the family *Microsphaeropsidaceae*, and restricted *Phoma* to *Phoma herbarum* (Chen *et al.* 2017). Other authors have added the genera *Briansuttonomyces*, *Didymellocamarosporium*, *Heracleicola*, *Neodidymella*, *Neomicrosphaeropsis* and *Pseudoascochyta* to *Didymellaceae* (Ariyawansa *et al.* 2015a, Crous & Groenewald 2016, Crous *et al.* 2016a, Thambugala *et al.* 2016, Wijayawardene *et al.* 2016). However, the genera *Didymellocamarosporium*, *Heracleicola* and *Neodidymella* were studied by

Chen *et al.* (2017) and revealed as probable synonyms of older genera within *Didymellaceae*.

To resolve the taxonomy of the *Cucurbitariaceae* and the *Didymellaceae* we have tried to delineate the phylogenetic relationships within these families performing a multi-locus analysis including ex-type and reference strains of most of the phoma-like and pyrenochaeta-like taxa available in the culture collection of Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands; formerly CBS-KNAW), and numerous isolates of clinical origin from the USA.

MATERIALS AND METHODS

Isolates and reference fungal strains

This study comprised 70 clinical isolates previously identified as belonging to the *Pleosporales* (Valenzuela-Lopez *et al.* 2016), provided by the Fungus Testing Laboratory of the University of Texas Health Science Center at San Antonio (UTHSC; San Antonio, Texas, USA), two environmental strains from Spain (CBS 141688) and New Zealand (CBS 141689) respectively, and 71 reference and ex-type strains belonging to the *Cucurbitariaceae* and *Didymellaceae* provided by the CBS culture collection (Table 1).

Phenotypic study

For cultural characterisation, isolates were grown on oatmeal agar (OA; 30 g of filtered oat flakes, 15 g of agar-agar, 1 L tap water) and malt extract agar (MEA; 40 g of malt extract, 15 g of agar-agar, 1 L distilled water), at 25 ± 1 °C for 14 d in darkness (recipes according to Boerema *et al.* 2004 and Crous *et al.* 2009). Some of the cultures were incubated under near-ultraviolet (UV) light (12 h light, 12 h dark) or on carnation leaf agar (CLA) to induce sporulation if necessary (Fisher *et al.* 1982, Su *et al.* 2012). Colony diameters were measured after 7 d at 25 ± 1 °C, and colony characterisation was performed 14 d after inoculation on the culture media. Colours were according to Kornerup & Wanscher (1978). The ability of the isolates to grow at cardinal temperatures were determined on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) after 7 d in darkness, ranging from 5 to 35 °C at 5 °C intervals, and including 37 °C.

Micromorphological characterisation was performed by examining at least 30 individuals of each structure (Aveskamp *et al.* 2010, Chen *et al.* 2015). Wet mounts (in Shear's mounting medium and in water) of structures were examined by using an Olympus CH2 compound microscope (Olympus Corporation, Tokyo, Japan). Photo micrographs were captured using a Zeiss Axio-Imager M1 microscope (Oberkochen, Germany) with a DeltaPix Infinity X digital camera using Nomarski differential interference contrast. The production of metabolite E+ (NaOH spot test) was carried out by the application of a droplet of 1N NaOH on a colony grown on MEA (Dorenbosch 1970, Noordeloos *et al.* 1993).

DNA isolation, PCR amplification and sequencing

The total genomic DNA was extracted from colonies grown on PDA after 7 d incubation at 20 ± 1 °C, using the FastDNA kit protocol (Bio101, Vista, CA), with a FastPrep FP120 instrument (Thermo Savant, Holbrook, NY) according to the manufacturer's protocol. DNA was quantified by using Nanodrop 2000 (Thermo Scientific, Madrid, Spain). The following loci were amplified and sequenced: a fragment of the 28S nrRNA gene (LSU) with the primer pair LR0R (Rehner & Samuels 1994) and LR5 (Vilgalys & Hester 1990), internal transcribed spacer region (ITS1-5.8S-ITS2) with the primer pair ITS5 and ITS4 (White *et al.* 1990), a fragment of the beta-tubulin gene (*tub*2) with the primers TUB2Fw and TUB4Rd (Woudenberg *et al.* 2009) and a fragment of the RNA polymerase II subunit 2 gene (*rpb*2) with RPB2-5F2 (Sung *et al.* 2007) and fRPB2-7cR primers (Liu *et al.* 1999). The PCR amplifications were performed in a total volume of 25 µL containing 5 µL 10× PCR Buffer (Invitrogen, California, USA), 0.2 mM dNTPs, 0.5 µM of each primer, 1 U Taq DNA polymerase and 1–10 ng genomic DNA. PCR conditions for LSU, ITS and *tub*2 were set as follows: an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation, annealing and extension, and a final extension step at 72 °C for 10 min. For the LSU and ITS amplification, the 35 cycles consisted of 45 s at 95 °C, 45 s at 53 °C and 2 min at 72 °C; and for the *tub*2 region 30 s at 94 °C, 45 s at 56 °C and 1 min at 72 °C. The PCR program for *rpb2* amplification consisted of 5 cycles of 45 s at 94 °C, 45 s at 60 °C and 2 min at 72 °C, then 5 cycles with a 58 °C annealing temperature and 30 cycles with a 54 °C annealing temperature (Woudenberg *et al.* 2013). Sequencing of the amplicons 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 v. 7 (DNAStar Lasergene, Madison, WI, USA).

Phylogenetic analyses

Sequences of related species described in previous studies were obtained from GenBank (Aveskamp *et al.* 2009, 2010, de Gruyter *et al.* 2010, 2013, Wijayawardene *et al.* 2014, Chen *et al.* 2015, 2017, Thambugala *et al.* 2016), and listed in Table 1. For the phylogenetic study, the alignments of the sequences were performed using MEGA v. 6.06 (Tamura *et al.* 2013), using the ClustalW application (Thompson *et al.* 1994), refined with MUSCLE (Edgar 2004) and manually adjusted using the same software platform. The ambiguous regions were excluded from the analyses. Phylogenetic reconstructions were made by maximum-likelihood (ML) and Bayesian inference (BI) with RAxML v. 8.2.10 (Stamatakis 2014) and MrBayes v. 3.2.6 (Ronquist *et al.* 2012), respectively. The best substitution model for each gene matrix correspond to GTR+I+G, and was estimated using MrModelTest v. 2.3 (Nylander 2004). For ML analyses, nearest-neighbour interchange was used as the heuristic method for tree inference. Support for internal branches was assessed by 1 000 ML bootstrapped pseudoreplicates. Bootstrap support (BS) $\geq$70 was considered significant. For BI analyses, Markov chain Monte Carlo (MCMC) sampling was performed with 46 M generations, with samples taken every 1 000 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 $\geq$0.95 was considered as significant. Both ML and BS analyses were run in CIPRES (Miller *et al.* 2012). *Preussia terricola* (AFTOL-ID 282) and *Sporormiella minima* (CBS 524.50) served as outgroup taxa. Sequences generated in this study were deposited in GenBank

Table 1. Isolates used in this study and their GenBank accession numbers. Numbers of new taxa, combinations and sequences generated are indicated in bold.

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
Allocucurbitaria botulispora	*Pyrenochaeta* sp.	**CBS 142452**	**UTHSC:DI16-273; FMR 13764**	T	Human superficial tissue	USA	LN907416	**LT592932**	**LT593001**	**LT593070**
Allophoma cylindrispora	*Phoma* sp.	**CBS 142453**	**UTHSC:DI16-233; FMR 13723**	T	Human superficial tissue	USA	LN907376	**LT592920**	**LT592989**	**LT593058**
A. labilis		CBS 124.93			*Solanum lycopersicum*	The Netherlands	GU238091	GU237765	GU237619	KT389552
A. minor		CBS 325.82	FMR 14905	T	*Syzygium aromaticum*	Indonesia	GU238107	GU237831	GU237632	KT389553
A. nicaraguensis		CBS 506.91	FMR 14904	T	*Coffea arabica*	Nicaragua	GU238058	GU237876	GU237596	KT389551
A. oligotrophica	*Phoma costarricencis*	CBS 497.91	FMR 14902		*Coffea arabica*	Unknown	GU238059	GU237870	GU237597	**LT623247**
			CGMCC 3.18114	T	Air sample	China	KY742194	KY742040	KY742282	KY742128
A. piperis		CBS 268.93		T	*Peperomia pereskiifolia*	Netherlands	GU238129	GU237816	GU237644	KT389554
A. tropica		CBS 436.75	FMR 14903	T	*Saintpaulia ionantha*	Germany	GU238149	GU237864	GU237663	KT389556
A. zantedeschiae		CBS 131.93			*Calla* sp.	The Netherlands	GU238159	FJ427084	FJ427188	KT389557
		CBS 229.32			*Cicer arietinum*	Romania	KT389690	KT389473	KT389558	KT389767
Altemariaster bidentis		CBS 134021	CPC 19479	T	*Bidens sulphurea*	Brazil	KC609341	KC609333	–	KC609347
A. helianthi		CBS 327.69	IFO 9089		*Helianthus annuus*	Unknown	KC584369	KC609335	–	KC584494
Ascochyta herbicola		CBS 629.97			Water	USA	GU238083	GU237898	GU237614	KP330421
A. pisi		CBS 126.54	AFTOL-ID 1583		*Pisum sativum*	The Netherlands	DQ678070	GU237772	GU237531	DQ677967
A. rabiei		CBS 206.30			Unknown	Unknown	KT389695	KT389478	KT389772	KT389559
A. versabilis		CBS 876.97			*Silene* sp.	The Netherlands	GU238152	GU237909	GU237664	KT389561
A. viciae		CBS 451.68			*Vicia sepium*	The Netherlands	KT389701	KT389484	KT389778	KT389562
Boeremia exigua		CBS 118.38			*Cheiranthus cheiri*	Denmark	KT389706	KT389489	KT389783	KT389582
		CBS 119.38			*Nicotiana tabacum*	Unknown	KT389707	KT389490	KT389784	KT389583
B. lycopersici		CBS 378.67			*Solanum lycopersicum*	The Netherlands	GU237950	GU237848	GU237512	KT389580
Briansuttonomyces eucalypti		CBS 114879	CPC 362	T	*Eucalyptus* sp.	South Africa	KU728519	KU728479	KU728595	–
		CBS 114887	CPC 363		*Eucalyptus* sp.	South Africa	KU728520	KU728480	KU728596	–
Calophoma aquilegiicola		CBS 107.96			*Aconitum pyramidale*	The Netherlands	GU238041	GU237735	GU237581	KT389586
		CBS 108.96			*Aquilegia* sp.	The Netherlands	GU238042	GU237736	GU237582	–
C. clematidina		CBS 102.66			*Clematis* sp.	UK	FJ515630	FJ426988	FJ427099	KT389587
		CBS 108.79		T	*Clematis* sp.	The Netherlands	FJ515632	FJ426989	FJ427100	KT389588
C. clematidis-rectae		CBS 507.63		T	*Clematis* sp.	The Netherlands	FJ515647	FJ515606	FJ515624	KT389589
C. rosae			CGMCC 3.18347	T	*Rosa* sp.	China	KY742203	KY742049	KY742291	KY742135
			LC 8119		*Rosa* sp.	China	KY742204	KY742050	KY742292	KY742136

Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
Camarosporidiella aborescentis	*Camarosporium aborescentis*		MFLUCC 14-0604	T	*Colutea arborescens*	Russia	KP711378	KP711377	–	–
	Camarosporium arezzoensis		MFLUCC 14-0238	T	*Cytisus* sp.	Italy	KP120927	KP120926	–	–
C. aureum	*Camarosporium aureum*		MFLUCC 14-0620	T	*Cotinus coggygria*	Russia	KP744478	KP744436	–	–
C. clematidis	*Camarosporium clematidis*		MFLUCC 13-0336	T	*Clematis vitalba*	Italy	KJ562188	KJ562213	–	–
C. elongata	*Cucurbitaria elongata*		MFLUCC 14-0260		*Cytisus scoparius*	Italy	KJ724249	–	–	–
	Cucurbitaria elongata	CBS 171.55	AFTOL-ID 1568		*Cytisus sessilifolius*	France	DQ678061	–	–	DQ677957
C. robiniicola	*Camarosporium robiniicola*		MFLUCC 13-0527		*Robinia pseudacacia*	Italy	KJ589412	KJ562214	–	–
C. spartii	*Camarosporium spartii*		MFLUCC 13-0548		*Cytisus* sp.	Italy	KJ589413	KJ562215	–	–
Camarosporium quaternatum		CBS 142617	CPC 23216		*Daphne mezereum*	Germany	KY929170	KY929135	–	–
		CBS 142616	CPC 31081	T	*Lycium barbarum*	Hungary	KY929136	KY929171	–	–
			CPC 31518		*Lycium barbarum*	Hungary	KY929172	KY929137	–	–
Camarosporomyces flavigenus		CBS 314.80		T	Water	Romania	GU238076	KY929138	–	–
Coniothyrium palmarum		CBS 758.73	CMW 5283		*Phoenix dactylifera*	Israel	JX681085	–	–	–
		CBS 400.71			*Chamaerops humilis*	Italy	EU754153	AY720708	KT389792	KT389592
C. telephii		CBS 188.71			Air sample	Finland	GQ387599	JF740188	KT389793	KT389593
		CBS 856.97			Mineral wool	Finland	GQ387600	JF740189		
Cucurbitaria berberidis			MFLUCC 11-0387		*Berberis vulgaris*	Austria	KC506796	–	–	–
		CBS 130007	FMR 15751; MFLUCC 11-0384; CB1	T	*Berberis vulgaris*	Austria	KC506793	**LT717673**	**LT717676**	**LT854936**
Cumuliphoma indica	*Phoma omnivirens*	**CBS 654.77**	**FMR 15341**	T	Unknown	India	GU238122	FJ427043	FJ427153	**LT623261**
	Phoma omnivirens	**CBS 991.95**	**FMR 15331**		Soil	Papua New Guinea	GU238121	FJ427044	FJ427154	**LT623262**
C. omnivirens	*Phoma omnivirens*	**CBS 341.86**	**FMR 14915**	T	*Phaseolus vulgaris*	Belgium	**LT623214**	FJ427042	FJ427152	**LT623260**
C. pneumoniae	*Phoma* sp.	**CBS 142454**	**UTHSC:DI16-249; FMR 13739**	T	Human respiratory tract	USA	LN907392	**LT592925**	**LT592994**	**LT593063**
Cucurbidothis pityophila		CBS 149.32	FMR 15744		Unknown	The Netherlands	JX681087	GQ203756	**LT854934**	**LT854935**
Didymella aeria			LC 8120		Air sample	China	KY742206	KY742052	KY742294	KY742138
			CGMCC 3.18353	T	Air sample	China	KY742205	KY742051	KY742137	KY742293
D. aliena		CBS 379.93			*Berberis* sp.	The Netherlands	GU238037	GU237851	GU237578	KP330416
D. americana		CBS 185.85			*Zea mays*	USA	GU237990	FJ426972	FJ427088	KT389594
D. anserina		CBS 253.80			Unknown	Germany	KT389715	KT389498	KT389795	KT389595
	Peyronellaea sp.		UTHSC:DI16-255; FMR 13745		Human respiratory tract	USA	LN907398	**LT592926**	**LT592995**	**LT593064**

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Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
D. aquatica			CGMCC 3.18349	T	Water	China	KY742209	KY742055	KY742297	KY742140
			LC 5555		Water	China	KY742210	KY742056	KY742298	KY742141
D. arachidicola		CBS 333.75		T	*Arachis hypogaea*	South Africa	GU237996	GU237833	GU237554	KT389598
D. aurea		CBS 269.93		T	*Medicago polymorpha*	New Zealand	GU237999	GU237818	GU237557	KT389599
D. bellidis		CBS 714.85			*Bellis perennis*	The Netherlands	GU238046	GU237904	GU237586	KP330417
D. boeremae		CBS 109942		T	*Medicago littoralis* cv. Harbinger	Australia	GU238048	FJ426982	FJ427097	KT389600
D. brunneospora	*Didymella* sp.	**CBS 115.58**	**FMR 15745**	T	*Chrysanthemum roseum*	Germany	KT389723	KT389505	KT389802	KT389625
D. chenopodii		CBS 128.93			*Chenopodium quinoa* cv. Sajana	Peru	GU238055	GU237775	GU237591	KT389602
D. chloroguttulata			CGMCC 3.18351	T	Air sample	China	KY742211	KY742057	KY742299	KY742142
			LC 8122		Air sample	China	KY742212	KY742058	KY742300	KY742143
D. coffeae-arabicae		CBS 123380	PD 84/1013	T	*Coffea arabica*	Ethiopia	GU238005	FJ426993	FJ427104	KT389603
D. curtisii			PD 92/1460		*Sprekelia* sp.	The Netherlands	GU238012	FJ427041	FJ427151	KT389604
D. ellipsoidea			CGMCC 3.18350	T	Air sample	China	KY742214	KY742060	KY742302	KY742145
			LC 8123		Air sample	China	KY742215	KY742061	KY742303	KY742146
D. eucalyptica		CBS 377.91			*Eucalyptus* sp.	Australia	GU238007	GU237846	GU237562	KT389605
D. exigua		CBS 183.55		T	*Rumex arifolius*	France	EU754155	GU237794	GU237525	EU874850
D. gardeniae		CBS 626.68	IMI 108771; FMR 14901	T	*Gardenia jasminoides*	India	GQ387595	FJ427003	FJ427114	KT389606
	Peyronellaea sp.		UTHSC:DI16-211; FMR 13701		Human superficial tissue	USA	LN907354	**LT592908**	**LT592977**	**LT593046**
	Peyronellaea calorpreferens		UTHSC:DI16-226; FMR 13716		Human superficial tissue	USA	LN907369	**LT592913**	**LT592982**	**LT593051**
	Peyronellaea sp.		UTHSC:DI16-274; FMR 13765		Human superficial tissue	USA	LN907417	**LT592933**	**LT593002**	**LT593071**
	Peyronellaea sp.		UTHSC:DI16-295; FMR 13788		Human superficial tissue	USA	LN907438	**LT592944**	**LT593013**	**LT593083**
D. glomerata		CBS 528.66		T	*Chrysanthemum* sp.	The Netherlands	JX681105	FJ427013	FJ427124	GU371781
	Peyronellaea glomerata		UTHSC:DI16-205; FMR 13695		Human superficial tissue	USA	LN907348	**LT592905**	**LT592974**	**LT593043**
D. heteroderae		CBS 109.92	PD 73/1405	T	Undefined food material	The Netherlands	GU238002	FJ426983	FJ427098	KT389601
	Peyronellaea calorpreferens		UTHSC:DI16-190; FMR 13680		Human superficial tissue	USA	LN907333	**LT592896**	**LT592965**	**LT593034**

Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
	Peyronellaea calorpreferens		UTHSC:DI16-224; FMR 13714		Human superficial tissue	USA	LN907367	**LT592911**	**LT592980**	**LT593049**
	Peyronellaea calorpreferens		UTHSC:DI16-227; FMR 13717		Human superficial tissue	USA	LN907370	**LT592914**	**LT592983**	**LT593052**
	Peyronellaea calorpreferens		UTHSC:DI16-231; FMR 13721		Human superficial tissue	USA	LN907374	**LT592918**	**LT592987**	**LT593056**
	Peyronellaea calorpreferens		UTHSC:DI16-232; FMR 13722		Human deep tissue/ fluids	USA	LN907375	**LT592919**	**LT592988**	**LT593057**
	Peyronellaea calorpreferens		UTHSC:DI16-234; FMR 13724		Human superficial tissue	USA	LN907377	**LT592921**	**LT592990**	**LT593059**
	Peyronellaea calorpreferens		UTHSC:DI16-235; FMR 13725		Human superficial tissue	USA	LN907378	**LT592922**	**LT592991**	**LT593060**
	Peyronellaea calorpreferens		UTHSC:DI16-305; FMR 13798		Human respiratory tract	USA	LN907448	**LT592951**	**LT593020**	**LT593090**
D. ilicicola			CGMCC 3.18355	T	*Ilex chinensis*	Italy	KY742219	KY742065	KY742307	KY742150
			LC 8127		*Ilex chinensis*	Italy	KY742220	KY742066	KY742308	KY742151
D. infuscatispora			CGMCC 3.18356	T	*Chrysanthemum indicum*	China	KY742221	KY742067	KY742309	KY742152
			LC 8129		*Chrysanthemum indicum*	China	KY742222	KY742068	KY742310	–
D. keratinophila	*Peyronellaea* sp.	**CBS 143032**	**UTHSC:DI16-200; FMR 13690**	T	Human superficial tissue	USA	LN907343	**LT592901**	**LT592970**	**LT593039**
	Peyronellaea sp.		**UTHSC:DI16-228; FMR 13718**		Human superficial tissue	USA	LN907371	**LT592915**	**LT592984**	**LT593053**
	Phoma sp.		**UTHSC:DI16-282; FMR 13774**		Human superficial tissue	USA	LN907425	**LT592938**	**LT593007**	**LT593077**
D. lethalis		CBS 103.25			Unknown	Unknown	GU238010	GU237729	GU237564	KT389607
D. macrophylla			CGMCC 3.18357	T	*Hydrangea macrophylla*	Italy	KY742224	KY742070	KY742312	KY742154
			LC 8132		*Hydrangea macrophylla*	Italy	KY742225	KY742071	KY742313	KY742155
D. macrostoma		CBS 223.69			*Acer pseudoplatanus*	Switzerland	GU238096	GU237801	GU237623	KT389608
D. maydis		CBS 588.69		T	*Zea mays*	USA	EU754192	FJ427086	FJ427190	GU371782
D. microchlamydospora		CBS 105.95		T	*Eucalyptus* sp.	UK	GU238104	FJ427028	FJ427138	KP330424
	Phoma sp.		UTHSC:DI16-199; FMR 13689		Human superficial tissue	USA	LN907342	**LT592900**	**LT592969**	**LT593038**

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Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
	Peyronellaea sp.		UTHSC:DI16-365; FMR 13858		Human superficial tissue	USA	LN907508	**LT592964**	**LT593033**	**LT593103**
D. molleriana		CBS 229.79			*Digitalis purpurea*	New Zealand	GU238067	GU237802	GU237605	KP330418
D. musae	*Phoma* sp.		UTHSC:DI16-230; FMR 13720		Human superficial tissue	USA	LN907373	**LT592917**	**LT592986**	**LT593055**
		CBS 463.69	FMR 15339		*Mangifera indica*	India	GU238011	FJ427026	FJ427136	**LT623248**
D. negriana		CBS 358.71			*Vitis vinifera*	Germany	GU238116	GU237838	GU237635	KT389610
			ICMP 10845; LC 5249		*Vitis vinifera*	former Yugoslavia	KY742227	KY742073	KY742315	–
D. nigricans		CBS 444.81	PD 77/919		*Actinidea chinensis*	New Zealand	GU238001	GU237915	GU237559	KT389611
D. ocimicola			CGMCC 3.18358	T	*Ocimum* sp.	China	KY742232	KY742078	KY742320	–
			LC 8138		*Ocimum* sp.	China	KY742233	KY742079	KY742321	–
D. pedeiae		CBS 124517		T	*Schefflera elegantissima*	The Netherlands	GU238127	GU237770	GU237642	KT389612
D. pinodella		CBS 531.66			*Trifolium pretense*	USA	GU238017	FJ427052	FJ427162	KT389613
D. pinodes		CBS 525.77		T	*Pisum sativum*	Belgium	GU238023	GU237883	GU237572	KT389614
		CBS 374.84	FMR 15345		*Pisum sativum*	The Netherlands	EU754135	JF810523	**LT623229**	**LT623249**
D. pomorum		CBS 285.76		T	*Heracleum dissectum*	Russia	GU238025	FJ427053	FJ427163	KT389615
D. protuberans		CBS 381.96	FMR 14899	T	*Lycium halifolium*	The Netherlands	GU238029	GU237853	GU237574	KT389620
	Peyronellaea sp.		UTHSC:DI16-302; FMR 13795		Environmental	USA	LN907445	**LT592949**	**LT593018**	**LT593088**
D. pteridis		CBS 379.96	FMR 15750		*Pteris* sp.	The Netherlands	KT389722	KT389504	KT389801	KT389624
D. rhei		CBS 109177			*Rheum rhaponticum*	New Zealand	GU238139	GU237743	GU237653	KP330428
D. rumicicola	*Didymella acetosellae*	CBS 179.97			*Rumex hydrolapathum*	The Netherlands	GU238034	GU237793	GU237575	KP330415
		CBS 683.79		T	*Rumex obtusifolius*	New Zealand	KT389721	KT389503	KT389800	KT389622
D. sancta		CBS 281.83		T	*Ailanthus altissima*	South Africa	GU238030	FJ427063	FJ427170	KT389623
		CBS 644.97	FMR 15351		Opuntia ficus-indica	Argentina	**LT623211**	FJ427064	FJ427171	**LT623250**
D. segeticola			CGMCC 3.17489	T	*Cirsium segetum*	China	KP330455	KP330443	KP330399	KP330414
			CGMCC 3.17498		*Cirsium segetum*	China	KP330454	KP330442	KP330398	KP330413
D. sinensis			LC 8142		*Dendrobium officinale*	China	KY742241	KY742087	KY742329	KY742166
			LC 8143		*Dendrobium officinale*	China	KY742242	KY742088	KY742330	KY742167
Didymella sp.	*Didymella segeticola*		LC 8141		*Camellia sasanqua*	Japan	KY742238	KY742084	KY742326	KY742164
D. subglomerata		CBS 110.92			*Triticum* sp.	USA	GU238032	FJ427080	FJ427186	KT389626

Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
D. suiyangensis			CGMCC 3.18352	T	Air sample	China	KY742243	KY742089	KY742330	KY742168
			LC 8144		Air sample	China	KY742244	KY742090	KY742332	KY742169
D. viburnicola		CBS 523.73			*Viburnum cassioides*	The Netherlands	GU238155	GU237879	GU237667	KP330430
Dothidotthia aspera		CBS 119688	CPC 12932		*Acer negundo*	USA	EU673275	–	–	–
D. symphoricarpi		CBS 119687	CPC 12929	T	*Symphoricarpos rotundifolius*	USA	EU673273	–	–	–
Ectophoma multirostrata	*Phoma multirostrata*	**CBS 110.79**	**FMR 15342**		*Cucumis sativus*	The Netherlands	GU238110	FJ427030	FJ427140	**LT623264**
	Phoma multirostrata	**CBS 274.60**	**FMR 15335**	T	Soil	Maharashtra	GU238111	FJ427031	FJ427141	**LT623265**
	Phoma multirostrata	**CBS 368.65**	**FMR 15336**		Unknown	India	GU238112	FJ427033	FJ427143	**LT623266**
E. pomi	*Phoma pereupyrena*	**CBS 267.92**	**FMR 15346**	T	*Coffea arabica*	India	GU238128	GU237814	GU237643	**LT623263**
Epicoccum brasiliense		CBS 120105	FMR 14907	T	*Amaranthus* sp.	Brazil	GU238049	GU237760	GU237588	KT389627
E. camelliae			CGMCC 3.18343	T	*Camellia sinensis*	China	KY742245	KY742091	KY742333	KY742170
	Epicoccum sorghinum		UTHSC:DI16-201; FMR 13691		Human respiratory tract	USA	LN907344	**LT592902**	**LT592971**	**LT593040**
	Epicoccum sorghinum		UTHSC:DI16-202; FMR 13692		Human superficial tissue	USA	LN907345	**LT592903**	**LT592972**	**LT593041**
	Epicoccum sorghinum		UTHSC:DI16-206; FMR 13696		Human superficial tissue	USA	LN907349	**LT592906**	**LT592975**	**LT593044**
	Epicoccum sorghinum		UTHSC:DI16-280; FMR 13772		Human superficial tissue	USA	LN907423	**LT592937**	**LT593006**	**LT593076**
	Epicoccum sorghinum		UTHSC:DI16-338; FMR 13831		Human superficial tissue	USA	LN907481	**LT592959**	**LT593028**	**LT593098**
	Epicoccum sorghinum		UTHSC:DI16-345; FMR 13838		Human subcutaneous tissue	USA	LN907488	**LT592961**	**LT593030**	**LT593100**
			LC 4862		*Camellia sinensis*	China	KY742246	KY742092	KY742334	KY742171
E. catenisporum	*Epicoccum sorghinum*	**CBS 181.80**	**FMR 14911**	T	*Oryza sativa*	Guinea-Bissau	**LT623213**	FJ427069	FJ427175	**LT623253**
E. dendrobii			CGMCC 3.18359	T	*Dendrobium fimbriatum*	China	KY742247	KY742093	KY742335	–
			LC 8146		*Dendrobium fimbriatum*	China	KY742248	KY74209	KY742336	-
E. draconis		CBS 186.83	FMR 14908		*Dracaena* sp.	Rwanda	GU238070	GU237795	GU237607	KT389628
E. duchesneae			LC 8147		*Duchesnea indica*	China	KY742250	KY742096	KY742338	–
			CGMCC 3.18345	T	*Duchesnea indica*	China	KY742249	KY742095	KY742337	–
E. henningsii		CBS 104.80			*Acacia mearnsii*	Kenya	GU238081	GU237731	GU237612	KT389629
E. hordei			CGMCC 3.18360	T	*Hordeum vulgare*	Australia	KY742251	KY742097	KY742339	–

(*continued on next page*)

Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
			LC 8149		*Hordeum vulgare*	Australia	KY742252	KY742098	KY742340	–
E. huancayense		CBS 105.80		T	*Solanum* sp.	Peru	GU238084	GU237732	GU237615	KT389630
E. italicum			CGMCC 3.18361	T	*Acca sellowiana*	Italy	KY742253	KY742099	KY742341	KY742172
			LC 8151		*Acca sellowiana*	Italy	KY74225	KY742100	KY742342	KY742173
E. keratinophilum	*Phoma* sp.		**UTHSC:DI16-244; FMR 13734**		Human superficial tissue	USA	LN907387	**LT592924**	**LT592993**	**LT593062**
	Phoma sp.		**UTHSC:DI16-258; FMR 13748**		Human respiratory tract	USA	LN907401	**LT592928**	**LT592997**	**LT593066**
	Phoma sp.	**CBS 142455**	**UTHSC:DI16-271; FMR 13762**	T	Human superficial tissue	USA	LN907414	**LT592930**	**LT592999**	**LT593068**
	Phoma sp.		**UTHSC:DI16-272; FMR 13763**		Human superficial tissue	USA	LN907415	**LT592931**	**LT593000**	**LT593069**
	Phoma sp.		**UTHSC:DI16-299; FMR 13792**		Human deep tissue/ fluids	USA	LN907442	**LT592947**	**LT593016**	**LT593086**
E. latusicollum	*Epicoccum sorghinum*		UTHSC:DI16-197; FMR 13687		Human superficial tissue	USA	LN907340	**LT592898**	**LT592967**	**LT593036**
			CGMCC 3.18346	T	*Sorghum bicolor*	China	KY742255	KY742101	KY742343	KY742174
			LC 4859		*Camellia sinensis*	China	KY742256	KY742102	KY742344	KY742175
E. layuense			CGMCC 3.18362	T	*Perilla* sp.	China	KY742261	KY742107	KY742349	–
			LC 8156		*Perilla* sp.	China	KY742262	KY742108	KY742350	–
E. nigrum		CBS 125.82			Human toe nail	The Netherlands	GU237974	FJ426995	FJ427106	KT389631
		CBS 173.73		T	*Dactylis glomerata*	USA	GU237975	FJ426996	FJ427107	KT389632
E. ovisporum	*Epicoccum sorghinum*	**CBS 180.80**	**FMR 14910**	T	*Zea mays*	South Africa	**LT623212**	FJ427068	FJ427174	**LT623252**
E. pimprinum			PD 77/1028		Soil	India	GU237977	FJ427050	FJ427160	KT389633
E. plurivorum		CBS 558.81	FMR 14909	T	*Setaria* sp.	New Zealand	GU238132	GU237888	GU237647	KT389634
E. pneumoniae	*Epicoccum sorghinum*		**UTHSC:DI16-257; FMR 13747**	T	Human respiratory tract	USA	LN907400	**LT592927**	**LT592996**	**LT593065**
E. poae			LC 8161		*Poa annua*	USA	KY742268	KY742114	KY742356	KY742183
			CGMCC 3.18363	T	*Poa annua*	USA	KY742267	KY742113	KY742355	KY742182
			LC 8162		*Poa annua*	USA	KY742269	KY742115	KY742357	KY742184
E. proteae	*Phoma proteae*	**CBS 114179**	**CPC 1854; FMR 15332**	T	*Protea* cv. *carnival*	South Africa	JQ044452	JQ044433	**LT623230**	**LT623251**
E. sorghinum		CBS 179.80			*Sorghum vulgare*	Puerto Rico	GU237978	FJ427067	FJ427173	KT389635
		CBS 627.68			*Citrus* sp.	France	GU237979	FJ427072	FJ427178	KT389636
						USA	LN907431	**LT592940**	**LT593009**	**LT593079**

Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
			UTHSC:DI16-288; FMR 13780		Human superficial tissue					
			UTHSC:DI16-301; FMR 13794		Human respiratory tract	USA	LN907444	**LT592948**	**LT593017**	**LT593087**
E. viticis			BRIP 29294; LC 5257		*Andropogon gayanus*	Australia	KY742271	KY742117	KY742359	–
			CGMCC 3.18344	T	*Vitex negundo*	China	KY742272	KY742118	KY742360	KY742186
Foliophoma fallens		CBS 161.78			*Olea europaea*	New Zealand	GU238074	KY929147	–	KC584502
		CBS 284.70			*Nerium oleander*	Italy	GU238078	KY929148	–	–
Halojulella avicenniae			BCC 18422		Mangrove wood	Thailand	GU371823	–	–	GU371787
			BCC 20173		Mangrove wood	Thailand	GU371822	–	–	GU371786
Heterophoma adonidis		CBS 114309			*Adonis vernalis*	Sweden	KT389724	KT389506	KT389803	KT389637
H. nobilis		CBS 507.91			*Dictamnus albus*	The Netherlands	GU238065	GU237877	GU237603	KT389638
H. verbascicola			CGMCC 3.18364	T	*Verbascum thapsus*	China	KY742273	KY742119	KY742361	KY742187
			LC 8164		*Verbascum thapsus*	China	KY742274	KY742120	KY742362	KY742188
Juxtiphoma eupyrena	*Phoma eupyrena*	**CBS 374.91**	**FMR 15329**		*Solanum tuberosum*	The Netherlands	GU238072	FJ426999	FJ427110	**LT623268**
	Phoma eupyrena	**CBS 527.66**	**FMR 15337**		Wheat field soil	Germany	GU238073	FJ427000	FJ427111	**LT623269**
Leptosphaeria conoidea		CBS 616.75			*Lunaria annua*	The Netherlands	JF740279	JF740201	KT389804	KT389639
L. doliolum		CBS 505.75		T	*Urtica dioica*	The Netherlands	GQ387576	JF740205	JF740144	KT389640
Leptosphaerulina americana		CBS 213.55			*Trifolium pratense*	USA	GU237981	GU237799	GU237539	KT389641
L. australis		CBS 317.83			*Eugenia aromatica*	Indonesia	EU754166	GU237829	GU237540	GU371790
Libertasomyces myopori		CBS 141302	CPC 27354	T	*Myoporum serratum*	South Africa	KX228332	NR_145200	–	–
L. platani		CBS 142112	CPC 29609	T	*Platanus* sp.	New Zealand	KY173507	KY173416	KY173604	KY173585
L. quercus		CBS 134.97	INIFAT C96/108	T	*Quercus ilex*	Spain	DQ377883	–	–	–
Macroventuria anomochaeta		CBS 525.71		T	Decayed canvas	South Africa	GU237984	GU237881	GU237544	GU456346
M. wentii		CBS 526.71		T	Plant litter	USA	GU237986	GU237884	GU237546	KT389642
Microsphaeriopsis olivacea		CBS 233.77			Pinus laricio	France	GU237988	GU237803	GU237549	KT389643
M. proteae		CBS 111319	CPC 1425		*Protea nitida*	South Africa	JN712563	JN712497	–	JN712650
Neoascochyta argentina		CBS 112524		T	*Triticum aestivum*	Argentina	KT389742	KT389524	KT389822	–
N. cylindrispora	*Ascochyta* sp.		**UTHSC:DI16-352; FMR 13845**		Human superficial tissue	USA	LN907495	**LT592962**	**LT593031**	**LT593101**
	Ascochyta sp.	**CBS 142456**	**UTHSC:DI16-359; FMR 13852**	T	Human superficial tissue	USA	LN907502	**LT592963**	**LT593032**	**LT593102**
N. desmazieri		CBS 297.69		T	*Lolium perenne*	Germany	KT389726	KT389508	KT389807	KT389644

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Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
	Ascochyta sp.		UTHSC:DI16-207; FMR 13697		Human respiratory tract	USA	LN907350	**LT592907**	**LT592976**	**LT593045**
	Ascochyta sp.		UTHSC:DI16-320; FMR 13813		Unknown	USA	LN907463	**LT592956**	**LT593025**	**LT593095**
	Ascochyta sp.		UTHSC:DI16-332; FMR 13825		Human superficial tissue	USA	LN907475	**LT592958**	**LT593027**	**LT593097**
	Ascochyta sp.		UTHSC:DI16-341; FMR 13834		Human superficial tissue	USA	LN907484	**LT592960**	**LT593029**	**LT593099**
N. europaea		CBS 820.84		T	*Hordeum vulgare*	Germany	KT389729	KT389511	KT389809	KT389646
N. exitialis		CBS 118.40			Unknown	Unknown	KT389732	KT389514	KT389812	KT389647
		CBS 389.86			*Triticum aestivum*	Switzerland	KT389733	KT389515	KT389813	KT389648
N. graminicola		CBS 301.69			*Lolium multiflorum*	Germany	KT389737	KT389519	KT389817	KT389650
N. graminicola		CBS 816.84			*Hordeum vulgare*	Germany	KT389741	KT389523	KT389821	KT389651
N. paspali		CBS 560.81		T	*Paspalum dilatatum*	New Zealand	GU238124	FJ427048	FJ427158	KP330426
N. soli			LC 8166		Soil	China	KY742276	KY742122	KY742364	–
			CGMCC 3.18365	T	Soil	China	KY742275	KY742121	KY742363	–
N. tardicrescens	*Neoascochyta* sp.	**CBS 689.97**	**FMR 15352**	T	Hay	Norway	KT389744	KT389526	KT389824	KT389654
	Ascochyta sp.		**UTHSC:DI16-291; FMR 13783**		Human superficial tissue	USA	LN907434	**LT592942**	**LT593011**	**LT593081**
N. triticicola		CBS 544.74		T	*Triticum aestivum*	South Africa	EU754134	GU237887	GU237488	KT389652
Neocamarosporium betae		CBS 109410	PD 77/113		*Beta vulgaris*	Unknown	EU754178	KY940790	–	GU371774
		CBS 523.66	IHEM 3915; PD 66/270		*Beta vulgaris*	The Netherlands	EU754179	FJ426981	KT389842	KT389670
N. calvescens		CBS 246.79	PD 77/655		*Atriplex hastata*	Germany	EU754131	KY940774	–	KC584500
N. goegapense		CBS 138008	CPC 23676	T	*Mesembryanthemum* sp.	South Africa	KJ869220	KJ869163	–	–
Neocucurbitaria aquatica	*Pyrenochaeta quercina*	**CBS 297.74**	**FMR 14867**	T	Sea water	Montenegro	EU754177	**LT623221**	**LT623238**	**LT623278**
N. cava	*Pyrenochaeta cava*	**CBS 115979**	**FMR 15333**		Unknown	The Netherlands	EU754198	AY853248	**LT623234**	**LT623273**
	Pyrenochaeta cava	**CBS 257.68**	**FMR 15747; IMI 331911**	T	Wheat-field soil	Germany	EU754199	JF740260	KT389844	**LT717681**
N. hakeae	*Pyrenochaeta hakeae*	**CBS 142109**	**CPC 28920**	T	*Hakea* sp.	Australia	KY173526	KY173436	KY173613	KY173593
N. irregularis	*Pyrenochaeta unguis-hominis*	**CBS 142791**	**UTHSC:DI16-229; FMR 13719**	T	Human subcutaneous tissue	USA	LN907372	**LT592916**	**LT592985**	**LT593054**
N. keratinophila	*Pyrenochaeta keratinophila*	**CBS 121759**	**FMR 9444**	T	Man corneal scrapings	Spain	**LT623215**	EU885415	**LT623236**	**LT623275**
N. quercina	*Pyrenochaeta quercina*	CBS 115095	FMR 14868	T	*Quercus robur*	Italy	GQ387619	**LT623220**	**LT623237**	**LT623277**

Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
N. unguis-hominis	*Pyrenochaeta unguis-hominis*		UTHSC:DI16-213; FMR 13703		Unknown	USA	LN907356	**LT592910**	**LT592979**	**LT593048**
	Pyrenochaeta unguis-hominis	CBS 111112	FMR 14866		*Agapornis* sp. Lung	The Netherlands	GQ387623	**LT623222**	**LT623239**	**LT623279**
	Pyrenochaeta unguis-hominis	CBS 112.79	FMR 15748		Air sample	Wales	GQ387622	**LT717672**	**LT717675**	**LT717682**
Neodidymelliopsis achlydis		CBS 256 77		T	*Achlys triphylla*	Canada	KT389749	KT389531	KT389829	–
N. cannabis		CBS 234.37			*Cannabis sativa*	Unknown	GU237961	GU237804	GU237523	KP330403
N. longicolla	*Phoma* sp.		UTHSC:DI16-322; FMR 13815		Human respiratory tract	USA	LN907465	**LT592957**	**LT593026**	**LT593096**
		CBS 382 96		T	Soil in desert	Israel	KT389750	KT389532	KT389830	–
N. polemonii		CBS 109181		T	*Polemonium caeruleum*	The Netherlands	GU238133	GU237746	KT389828	KP330427
N. xanthina		CBS 383.68		T	*Delphinium* sp.	The Netherlands	GU238157	GU237855	KT389831	KP330431
Neomicrosphaeriopsis italica			MFLUCC 15-0485	T	*Tamarix* sp.	Italy	KU729854	KU900318	–	KU674820
			MFLUCC 15-0484		*Tamarix* sp.	Italy	KU729853	KU900319	KX453298	KU695539
Neophaeosphaeria agaves		CBS 136429	CPC 21264	T	*Agave tequilana* var. *azul*	Mexico	KF777227	NR_137833	–	–
N. filamentosa		CBS 102202			*Yucca rostrata*	Mexico	GQ387577	JF740259	–	GU371773
Neoplatysporoides aloicola		CBS 139901	CPC 24435	T	*Aloe* sp.	Tanzania	KR476754	KR476719	–	–
Neopyrenochaeta acicola	*Pyrenochaeta acicola*	**CBS 812.95**	**FMR 14872**	T	Waterpipe	The Netherlands	GQ387602	**LT623218**	**LT623232**	**LT623271**
N. fragariae	*Pyrenochaeta acicola*	**CBS 101634**	**FMR 14871**	T	*Fragaria ananassa*	The Netherlands	GQ387603	**LT623217**	**LT623231**	**LT623270**
N. inflorescentiae	*Pyrenochaeta inflorescentiae*	**CBS 119222**	**FMR 15334**	T	*Protea neriifolia*	South Africa	EU552153	EU552153	**LT623233**	**LT623272**
N. telephoni	*Pyrenochaeta telephoni*	**CBS 139022**	**FMR 15754**	T	Screen of a mobile phone	India	KM516290	KM516291	**LT717678**	**LT717685**
Neopyrenochaetopsis hominis	*Pyrenochaeta* sp.	**CBS 143033**	**UTHSC:DI16-238; FMR 13728**	T	Human superficial tissue	USA	LN907381	**LT592923**	**LT592992**	**LT593061**
Nothophoma anigozanthi		CBS 381.91	FMR 14914	T	*Anigozanthus maugleisii*	The Netherlands	GU238039	GU237852	GU237580	KT389655
N. arachidis-hypogaeae		CBS 125.93			*Arachis hypogaea*	India	GU238043	GU237771	GU237583	KT389656
N. gossypiicola		CBS 377.67	FMR 14912		*Gossypium* sp.	USA	GU238079	GU237845	GU237611	KT389658
	Phoma sp.		UTHSC:DI16-294; FMR 13787		Human deep tissue/ fluids	USA	LN907437	**LT592943**	**LT593012**	**LT593082**
N. infossa		CBS 123395		T	*Fraxinus pennsylvanica*	Argentina	GU238089	FJ427025	FJ427135	KT389659
N. macrospora		CBS 140674	UTHSC:DI16-276; FMR 13767	T	Human respiratory tract	USA	LN880537	LN880536	LN880539	**LT593073**

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Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
N. quercina		CBS 633.92	FMR 14913		*Quercus* sp.	Ukraine	EU754127	GU237900	GU237609	KT389657
	Leptosphaerulina sp.		UTHSC:DI16-270; FMR 13761		Human superficial tissue	USA	LN907413	**LT592929**	**LT592998**	**LT593067**
N. variabilis	*Phoma* sp.	**CBS 142457**	**UTHSC:DI16-285; FMR 13777**	T	Human respiratory tract	USA	LN907428	**LT592939**	**LT593008**	**LT593078**
Ochrocladosporium elatum		CBS 146.33	IMI 049629; ATCC 11280		Wood pulp	Sweden	EU040233	EU040233	–	–
O. frigidarii		CBS 103.81		T	Cooled room	Germany	EU040234	EU040234	–	–
Ophiosphaerella herpotricha		CBS 620.86	AFTOL-ID 1569		*Bromus erectus*	Switzerland	DQ678062	KF498728	–	DQ677958
Paraboeremia adianticola		CBS 187.83	FMR 15344		*Polystichum adiantiforme*	USA	GU238035	GU237796	GU237576	KP330401
P. camelliae			CGMCC 3.18106	T	*Camellia* sp.	China	KX829042	KX829034	KX829058	KX829050
			CGMCC 3.18107		*Camellia* sp.	China	KX829043	KX829035	KX829059	KX829051
			CGMCC 3.18108		*Camellia* sp.	China	KX829044	KX829036	KX829060	KX829052
P. litseae			CGMCC 3.18109	T	*Litsea* sp.	China	KX829037	KX829029	KX829053	KX829045
			CGMCC 3.18110		*Litsea* sp.	China	KX829038	KX829030	KX829054	KX829046
P. oligotrophica			CGMCC 3.18111	T	Limestone	China	KX829039	KX829031	KX829055	KX829047
			CGMCC 3.18112		Limestone	China	KX829040	KX829032	KX829056	KX829048
P. putaminum		CBS 130.69	FMR 15338		*Malus sylvestris*	Denmark	GU238138	GU237777	GU237652	**LT623254**
P. selaginellae		CBS 122.93	FMR 15348	T	*Selaginella* sp.	The Netherlands	GU238142	GU237762	GU237656	**LT623255**
Paraconiothyrium estuarinum		CBS 109850	FMR 14887	T	Sediment from estuarine	Brazil	JX496129	JX496016	JX496355	**LT854937**
Paracucurbitaria italica	*Pyrenochaeta corni*	**CBS 234.92**	FMR 14869	T	*Olea europaea*	Italy	EU754176	**LT623219**	**LT623235**	**LT623274**
P. corni	*Pyrenochaeta corni*	**CBS 248.79**	**FMR 16593**		*Fraxinus excelsior*	The Netherlands	GQ387608	**LT903672**	**LT900365**	**LT903673**
Paraepicoccum amazonense			MFLUCC 15-0493		*Tamarix* sp.	Italy	KU900294	KU752190	–	KU820871
			MFLUCC 15-0491		*Tamarix* sp.	Italy	KU900295	KU752191	–	KU820872
Paraleptosphaeria dryadis		CBS 643.86	ETH 9446		*Dryas octopetala*	Switzerland	GU301828	JF740213	–	GU371733
Parapyrenochaeta acaciae	*Pyrenochaeta acaciae*	**CBS 141291**	**FMR 15755; CPC 25527**	T	*Acacia* sp.	Australia	KX228316	KX228265	**LT717679**	**LT717686**
P. protearum	*Pyrenochaeta protearum*	**CBS 131315**	**FMR 15752; CPC 18322**	T	*Protea mundii*	South Africa	JQ044453	JQ044434	**LT717677**	**LT717683**
	Pyrenochaeta pinicola	**CBS 137997**	**FMR 15753; CPC 23455**		*Pinus* sp.	France	KJ869209	KJ869152	KJ869249	**LT717684**

Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
Phaeomycocentrospora cantuariensis		CBS 132014	CPC 11694		*Humulus scandens*	South Korea	GU253716	GU269668	–	–
			CPC 10157		*Humulus scandens*	South Korea	GU253712	GU269664	–	–
Phaeosphaeria oryzae		CBS 110110		T	*Oryza sativa*	Korea	KF251689	KF251186	KF252680	–
Phoma herbarum		CBS 502.91			*Nerium* sp.	The Netherlands	GU238082	GU237874	GU237613	KP330419
		CBS 615.75	FMR 15340		*Rosa multiflora* cv. *Cathayensis*	The Netherlands	KF251715	FJ427022	KF252703	KP330420
		CBS 377.92			Human leg	UK	KT389756	KT389536	KT389837	KT389663
			UTHSC:DI16-319; FMR 13812		Human superficial tissue	USA	LN907462	**LT592955**	**LT593024**	**LT593024**
			UTHSC:DI16-204; FMR 13694		Human deep tissue/ fluids	USA	LN907347	**LT592904**	**LT592973**	**LT593042**
			UTHSC:DI16-212; FMR 13702		Human respiratory tract	USA	LN907355	**LT592909**	**LT592978**	**LT593047**
			UTHSC:DI16-306; FMR 13799		Human respiratory tract	USA	LN907449	**LT592952**	**LT593021**	**LT593091**
			UTHSC:DI16-307; FMR 13800		Human respiratory tract	USA	LN907450	**LT592953**	**LT593022**	**LT593092**
Phomatodes aubrietiae		CBS 627.97		T	*Aubrietia* sp.	The Netherlands	GU238045	GU237895	GU237585	KT389665
P. nebulosa		CBS 100191			*Thlaspi arvense*	Poland	KP330446	KP330434	KP330390	KT389666
		CBS 740.96			*Armoracia rusticana*	The Netherlands	KT389758	KT389540	KT389839	KT389667
Pleiochaeta ghindensis		CBS 552.92			*Acacia mellifera*	Namibia	EU167561	EU167561	–	–
P. setosa		CBS 496.63	MUCL 8091		*Cytisus racemosus*	Germany	EU167563	EU167563	–	–
Pleospora herbarum		CBS 191.86		T	*Medicago sativa*	Uttar Pradesh	JX681120	NR_111243	–	KC584471
P. typhicola		CBS 132.69			*Typha angustifolia*	The Netherlands	JF740325	–	KT389843	KC584505
Preussia terricola			AFTOL-ID 282; DAOM 230091		Unknown	Unknown	AY544686	KT225529	–	DQ470895
Pseudoascochyta novae-zelandiea		CBS 141689	FMR 15110; ICMP 10493	T	*Cordyline australis*	New Zealand	LT592893	LT592892	LT592894	LT592895
P. pratensis		CBS 141688	FMR 14524	T	Soil	Spain	LT223131	LT223130	LT223132	LT223133
Pseudopyrenochaeta lycopersici	*Pyrenochaeta lycopersici*	**CBS 306.65**	**FMR 15746**	T	*Lycopersicon esculentum*	Germany	EU754205	NR_103581	**LT717674**	**LT717680**
P. terretris	*Pyrenochaeta lycopersici*	**CBS 282.72**	**FMR 15327**	T	Soil	The Netherlands	**LT623216**	**LT623228**	**LT623246**	**LT623287**
Pyrenochaeta nobilis		CBS 407.76	FMR 14870	T	*Laurus nobilis*	Italy	EU754206	EU930011	KT389845	**LT623276**
Pyrenochaetopsis americana	*Pyrenochaetopsis* sp.				Unknown	USA	LN907368	**LT592912**	**LT592981**	**LT593050**

(*continued on next page*)

Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
			UTHSC:DI16-225; FMR 13715							
P. botulispora	*Pyrenochaetopsis* sp.		**UTHSC:DI16-289; FMR 13781**		Human respiratory tract	USA	LN907432	**LT592941**	**LT593010**	**LT593080**
	Pyrenochaetopsis sp.		**UTHSC:DI16-297; FMR 13790**		Human superficial tissue	USA	LN907440	**LT592945**	**LT593014**	**LT593084**
	Pyrenochaetopsis sp.	**CBS 142458**	**UTHSC:DI16-298; FMR 13791**	T	Human respiratory tract	USA	LN907441	**LT592946**	**LT593015**	**LT593085**
P. confluens	*Pyrenochaetopsis* sp.	**CBS 142459**	**UTHSC:DI16-303; FMR 13796**	T	Human deep tissue/ fluids	USA	LN907446	**LT592950**	**LT593019**	**LT593089**
P. decipiens		CBS 343.85	FMR 14880	T	*Globodera pallida*	The Netherlands	GQ387624	**LT623223**	**LT623240**	**LT623280**
P. globosa	*Pyrenochaetopsis* sp.	**CBS 143034**	**UTHSC:DI16-275; FMR 13766**	T	Human superficial tissue	USA	LN907418	**LT592934**	**LT593003**	**LT593072**
P. indica		CBS 124454	FMR 14879	T	*Saccharum officinarum*	India	GQ387626	**LT623224**	**LT623241**	**LT623281**
P. leptospora		CBS 101635	FMR 14877	T	*Secale cereale*	Unknow	GQ387627	JF740262	**LT623242**	**LT623282**
	Coniothyrium cereale	CBS 122787	FMR 14873		Unknown	Germany	EU754151	**LT623225**	**LT623243**	**LT623283**
P. microspora	*Pyrenochaetopsis* sp.		UTHSC:DI16-198; FMR 13688		Human superficial tissue	USA	LN907341	**LT592899**	**LT592968**	**LT593037**
		CBS 102876	FMR 14874	T	Water	Montenegro	GQ387631	**LT623226**	**LT623244**	**LT623284**
P. paucisetosa	*Pyrenochaetopsis* sp.	**CBS 142460**	**UTHSC:DI16-193; FMR 13683**	T	Human superficial tissue	USA	LN907336	**LT592897**	**LT592966**	**LT593035**
P. poae		CBS 136769	FMR 14876	T	*Poa* sp.	The Netherlands	KJ869175	KJ869117	KJ869243	**LT623286**
P. setosissima	*Pyrenochaetopsis microspora*	**CBS 119739**	**FMR 14875**	T	*Coffea arabica*	Brazil	GQ387632	**LT623227**	**LT623245**	**LT623285**
P. tabarestanensis		CBS 139506	IBRC-M 30051	T	Soil	Iran	KF803343	KF730241	KX789523	–
P. uberiformis	*Pyrenochaetopsis* sp.	**CBS 142461**	**UTHSC:DI16-277; FMR 13769**	T	Human superficial tissue	USA	LN907420	**LT592935**	**LT593004**	**LT593074**
Querciphoma carteri		CBS 105.91			*Quercus robur*	Germany	KF251712	JF740181	KF252700	KT389591
Remotididymella anthropophila	*Phoma* sp.	**CBS 142462**	**UTHSC:DI16-278; FMR 13770**	T	Human respiratory tract	USA	LN907421	**LT592936**	**LT593005**	**LT593075**
R. destructiva	*Phoma destructiva* var. *destructiva*	**CBS 133.93**	**FMR 15349**		*Solanum lycopersicon*	Guadeloupe	GU238064	GU237779	GU237602	**LT623257**
	Phoma destructiva var. *destructiva*	**CBS 378.73**	**FMR 15328**	T	*Lycopersicon esculentum*	Tonga	GU238063	GU237849	GU237601	**LT623258**
	Phoma destructiva var. *diversispora*	**CBS 162.78**	**FMR 14906**		*Lycopersicon esculentum*	The Netherlands	GU238062	GU237788	GU237600	**LT623259**

Table 1. (Continued).

Species	Old name	CBS strain[1] no.	Other strain[1] no.	Status[2]	Host, substrate	Country	GenBank accession numbers[3]			
							LSU	ITS	*TUB*	*RPB2*
Shiraia bambusicola			NBRC 30754		*Phyllostachys* sp.	Japan	AB354969	AB354988	AB355003	–
			NBRC 30771		*Phyllostachys* sp.	Japan	AB354971	AB354990	AB355005	–
			NBRC 30753		*Phyllostachys* sp.	Japan	AB354968	AB354987	AB355002	–
			NBRC 30772		*Phyllostachys* sp.	Japan	AB354972	AB354991	AB355006	–
Similiphoma crystallifera	*Phoma crystallifera*	**CBS 193.82**	**FMR 15343**	T	*Chamaespartium sagittale*	Austria	GU238060	GU237797	GU237598	**LT623267**
Sporormiella minima		CBS 524.50	AFTOL-ID 1256		Dung of goat	Panama	DQ678056	KT389543	–	DQ677950
Stagonosporopsis dorenboschii		CBS 426.90		T	*Physostegia virginiana*	The Netherlands	GU238185	GU237862	GU237690	KT389678
S. hortensis		CBS 572.85			*Phaseolus vulgaris*	The Netherlands	GU238199	GU237893	GU237704	KT389681
Staurosphaeria aloes	*Hazslinszkyomyces aloes*	CBS 136437	CPC 21572	T	*Aloe dichotoma*	South Africa	KF777198	NR_137821	–	–
S. aptrootii	*Hazslinszkyomyces aptrootii*	CBS 483.95		T	*Lycium* sp.	The Netherlands	GU301806	KY929149	–	–
S. lyciicola	*Hazslinszkyomyces lycii*	CBS 142619	CPC 30998	T	*Lycium barbarum*	Hungary	KY929180	KY929150	–	–
	Hazslinszkyomyces lycii		CPC 31014		*Lycium barbarum*	Hungary	KY929181	KY929151	–	–
Vacuiphoma bulgarica	*Phoma bulgarica*	**CBS 357.84**	**FMR 14917**	T	*Trachystemon orientale*	Bulgaria	GU238050	GU237837	GU237589	**LT623256**
V. oculihominis	*Phoma* sp.		**UTHSC:DI16-308; FMR 13801**	T	Human superficial tissue	USA	LN907451	**LT592954**	**LT593023**	**LT593093**
Xenodidymella applanata		CBS 205.63			*Rubus idaeus*	The Netherlands	GU237998	GU237798	GU237556	KP330402
		CBS 115577			*Rubus idaeus*	Sweden	KT389762	KT389546	KT389850	KT389688
X. asphodeli		CBS 375.62		T	*Asphodelus albus*	France	KT389765	KT389549	KT389853	KT389689
X. catariae		CBS 102635			*Nepeta catenaria*	The Netherlands	GU237962	GU237727	GU237524	KP330404
X. humicola		CBS 220.85			*Franseria* sp.	USA	GU238086	GU237800	GU237617	KP330422
X. saxea	*Phoma saxea*	**CBS 419.92**	**FMR 15347**	T	Corroded mediterranean marble	Unknown	GU238141	GU237860	GU237655	KP330429
Xenopyrenochaetopsis pratorum	*Pyrenochaetopsis pratorum*	**CBS 445.81**	**FMR 14878**	T	*Lolium perenne*	New Zealand	GU238136	JF740263	KT389846	KT389671

[1] **AFTOL**: Assembling the Fungal Tree of Life; **ATCC**: American Type Culture Collection, Virginia, USA; **BCC**: Biotec Culture Collection, Pathum Thani, Thailand; **BCCM/IHEM**: Biomedical Fungi and Yeasts Collection, Louvain-la-Neuve, Belgium; **BCCM/MUCL**: Mycothèque de l'Université catholique de Louvain, Louvain-la-Neuve, Belgium; **BRIP**: Plant Pathology Herbarium, Department of Employment, Economic, Development and Innovation, Queensland, Australia; **CBS**: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CGMCC**: China General Microbiological Culture Collection, Beijing, China; **CMW**: Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; **CPC**: Culture collection of Pedro Crous, housed at CBS; **DAOM**: Canadian Collection of Fungal Cultures, Ottawa, Canada; **ETH**: Herbaria of the department of Environmental Systems Science. Institute of Integrative Biology, Zürich, Switzerland; **FMR**, Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; **IBRC**: Iranian Biological Resources Center, Tehran, Iran; **ICMP**: International Collection of Microorganisms from Plants, Auckland, New Zealand; **IFO**: Institute for Fermentation, Osaka, Japan, now NBRC; **IMI**: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; **INIFAT**: Instituto de Investigaciones Fundamentales en Agricultura Tropical "Alejandro de Humboldt", Santiago de las Vegas, Cuba; **LC**: Corresponding author's personal collection deposited in laboratory, housed at CAS, China; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NBRC**: Biological Resource Center, National Institute of Technology and Evaluation, Chiba, Japan; PD: Plant Protection Service, Wageningen, the Netherlands; **UTHSC**, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, Texas, USA.

[2] T: ex-type strain

[3] ITS: internal transcibed spacer regions 1 & 2 including 5.8S nrDNA gene; LSU: 28S large subunit of the nrRNA gene; *RPB2*: RNA polymerase II second subunit; *TUB*: ß-tubulin.

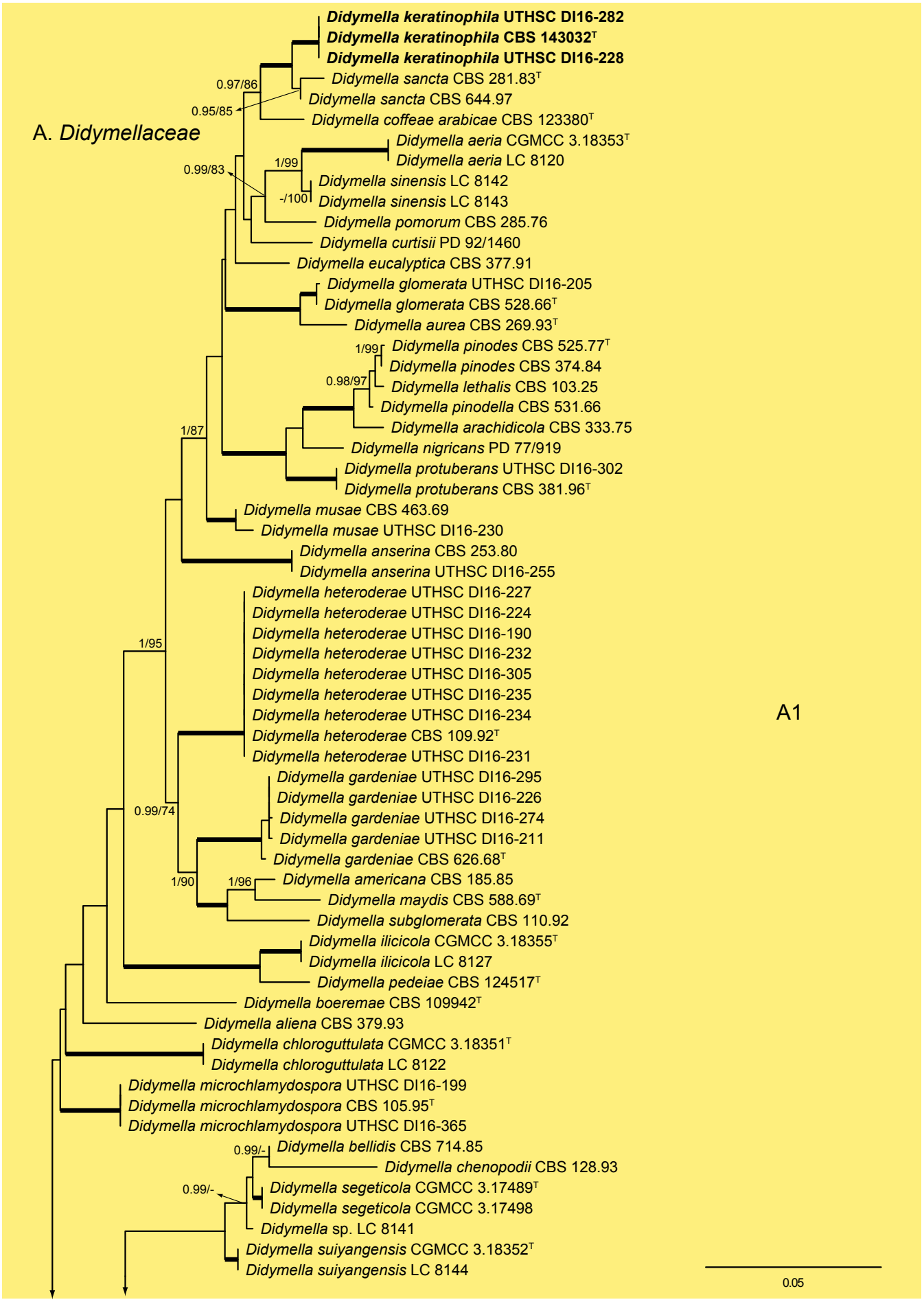

Fig. 1. Phylogenetic tree inferred from a Maximum likelihood analysis based on a concatenated alignment of LSU, ITS, *tub*2 and *rpb*2 sequences of 357 strains representing species in *Cucurbitariaceae*, *Didymellaceae* and allied families within *Pleosporales*. The Bayesian posterior probabilities (PP) above 0.95 and the RAxML bootstrap support

(see Table 1), the final matrices used for phylogenetic analyses in TreeBASE (www.treebase.org; accession number: S21115) and the novel taxonomic descriptions and nomenclature in MycoBank (www.mycobank.org; Crous *et al.* 2004).

RESULTS

Phylogenetic analyses

The final concatenated dataset obtained with both ML and Bayesian analysis contained 357 ingroup strains with a total of 1 888 characters including gaps (519 for LSU, 336 for ITS, 434 for *tub*2 and 599 for *rpb*2), of which 742 are parsimony informative (132 for LSU, 111 for ITS, 149 for *tub2* and 350 for *rpb2*). The sequence datasets did not show conflict in the tree topologies for the 70 % reciprocal bootstrap trees, which allowed to combine the four genes for the multi-locus analysis.

The ML analysis showed similar tree topology and was congruent with that obtained in the Bayesian analysis. For the BI multi-locus analysis, a total of 34 677 trees were sampled after the burn-in with a stop value of 0.01. The support values were slightly different with the two analysis methods; with BI, posterior probabilities being higher than the ML bootstrap support values (Fig. 1).

The phylogenetic tree distinguished two main supported clades corresponding to the suborders *Massarineae* (1 PP / 100 % BS), with only the family *Didymosphaeriaceae* (clade T) here included, and *Pleosporineae* (1 PP / 74 % BS), emcompassing over 19 families (clades A–S), respectively. Four of the families of the latter suborder are proposed here as new, i.e., *Pseudopyrenochaetaceae* (clade D), *Neopyrenochaetaceae* (clade E), *Pyrenochaetopsidaceae* (clade F) and *Parapyrenochaetaceae* (clade N). The main clade of the *Pleosporineae* corresponded to the *Didymellaceae* (clade A) showing 25 well-supported terminal clades with the only exception being *Epicoccum* (A2). Twenty terminal clades corresponded to known genera and six are proposed here as new: *Ectophoma* (A7), *Remotididymella* (A8), *Similiphoma* (A9), *Cumuliphoma* (A12), *Juxtiphoma* (A13) and *Vacuiphoma* (A14). The genus *Didymella* (A1; 1 PP / 90 % BS), comprised 48 species and one undescribed, including two proposed here as new: *D. keratinophila* sp. nov. (the type strain CBS 143032, UTHSC DI16-228 and UTHSC DI16-282), which is phylogenetically close to *D. sancta* and *D. coffeae-arabicae*, and *D. brunneospora* sp. nov. (CBS 115.58). Several of the clinical strains included in *Didymella* were distributed among seven known species, i.e., *D. heteroderae* (nine strains), *D. gardeniae* (four strains), *D. microchlamydospora* (two strains), and *D. anserina*, *D. glomerata*, *D. musae* and *D. protuberans* with only one strain for each. *Epicoccum* (A2; unsupported) was represented by 17 previously described species (including the type species *E. nigrum*), the new species *E. catenisporum* sp. nov., *E. ovisporum* sp. nov., *E. pneumoniae* sp. nov. (phylogenetically related with *E. camelliae*, *E. latusicollum*, *E. sorghinum* and *E. viticis*), and *E. keratinophilum* sp. nov. (phylogenetically related with *E. brasiliense* and *E. draconis*). Finally, *E. proteae* (basionym *Phoma proteae*), which clustered with *E. huancayense*, is here combined in *Epicoccum*. *Allophoma* (clade A3; 1 PP / 96 % BS) is enlarged with *A. cylindrispora* sp. nov., previously identified as *Phoma* sp. (Valenzuela-Lopez *et al.* 2016), clustering with *A. minor* and *A. piperis*. The clades from A4 to A6 encompassed three genera i.e. *Heterophoma* (A4; 1 PP / 98 % BS), *Stagonosporopsis* (A5; 1 PP / 75 % BS) and *Boeremia* (A6; 1 PP / 100 % BS). The new genus *Ectophoma* (clade A7; 1 PP / 100 % BS) comprise two new combinations previously included in *Phoma*, i.e. the generic type *E. multirostrata* (syn. *P. multirostrata*) and *E. pomi* (syn. *P. pereupyrena*). The new genus *Remotididymella* (A8; 0.97 PP / 91 % BS) comprised *R. destructiva* comb. nov. (basionym *Phoma destructiva*), the type species, and the new species *R. anthropophila*. For *Phoma crystallifera* the new monotypic genus *Similiphoma* (clade A9) and the new combination *S. crystallifera* are proposed. The clade corresponding to the genus *Paraboeremia* (clade A10; 1 PP / 99 % BS) included the six accepted species. *Macroventuria* formed a well-supported clade (A11; 1 PP / 100 % BS) and included the ex-type strains of *M. anomochaeta* and *M. wentii*. *Cumuliphoma* gen. nov. (clade A12; 1 PP / 94 % BS) included *C. omnivirens* comb. nov. (syn. *Phoma omnivirens*), *C. indica* sp. nov. (with two strains previously identified as *P. omnivirens*) and *C. pneumoniae* sp. nov., the latter represented by a clinical strain. The proposed new monotypic genus *Juxtiphoma* (clade A13; 1 PP / 100 % BS), includes two strains of *J. eupyrena* comb. nov. (basionym *Phoma eupryrena*). The new genus *Vacuiphoma* (clade A14; 1 PP / 100 % BS), included the type species *V. bulgarica* comb. nov. (basionym *Phoma bulgarica*) and the new species *V. oculihominis* described from a sterile clinical strain (UTHSC DI16-308). The genus *Nothophoma* (clade A15; 1 PP / 95 % BS) comprised seven species, including the generic type, *N. infossa*, and *N. variabilis* sp. nov., which is based on a clinical strain phylogenetically related with the ex-type strain of *N. anigozanthi*. The clade corresponding to *Ascochyta* (clade A16; 1 PP / 92 % BS), grouped five species, including the type species *A. pisi*. Clade A17 (1 PP / 100 % BS) included the type species of *Phomatodes* (*P. aubrietiae*) and two strains of *P. nebulosa*, the other species of the genus. The *Briansuttonomyces* clade (A18, 1 PP / 100 % BS), included two strains of the only species of the genus, *B. eucalypti*. The clade A19 (1 PP / 100 % BS) encompassed the ex-type strains of the two species of *Pseudoascochyta*, *P. novaezelandiae* and *P. pratensis*. The *Neomicrosphaeropsis* clade (A20; 1 PP / 100 % BS), contained the type species of the genus, *N. italica*. In *Phoma* (A21; 1 PP / 100 % BS) eight strains were grouped, all of them identified as *P. herbarum* (five from clinical origin and three reference strains). The genus *Calophoma* (A22; 1 PP / 93 % BS) comprised four species: *C. aquilegiicola*, *C. clematidis-rectae*, *C. clematidina* (type species of the genus) and *C. rosae*. The clade corresponding to *Leptosphaerulina* (A23; 1 PP / 100 % BS) contained the two known species, *L. americana* and *L. australis*. *Xenodidymella* (A24; - PP / 74 % BS), grouped the four species of this genus and the new combination *Xenodidymella saxea* (basionym *Phoma saxea*), which forms a basal clade with a strain of *X. humicola*. The clade A25 (1 PP / 100 % BS) included five species of *Neodidymelliopsis*. *Neoascochyta* (A26; 1 PP / 98 % BS) represented a basal clade of the

values (BS) above 70 % are given at the nodes (PP/BS). Fully supported branched (1 PP/100 BS) are indicated in bold. Some branches were shortened to fit them to the page, these are indicated by two diagonal lines with the number of times a branch was shortened. Newly proposed taxa are given in bold. Type strains are indicated by a superscript T. The tree was rooted with *Preussia terricola* (AFTOL-ID 828) and *Sporormiella minima* (CBS 524.50).

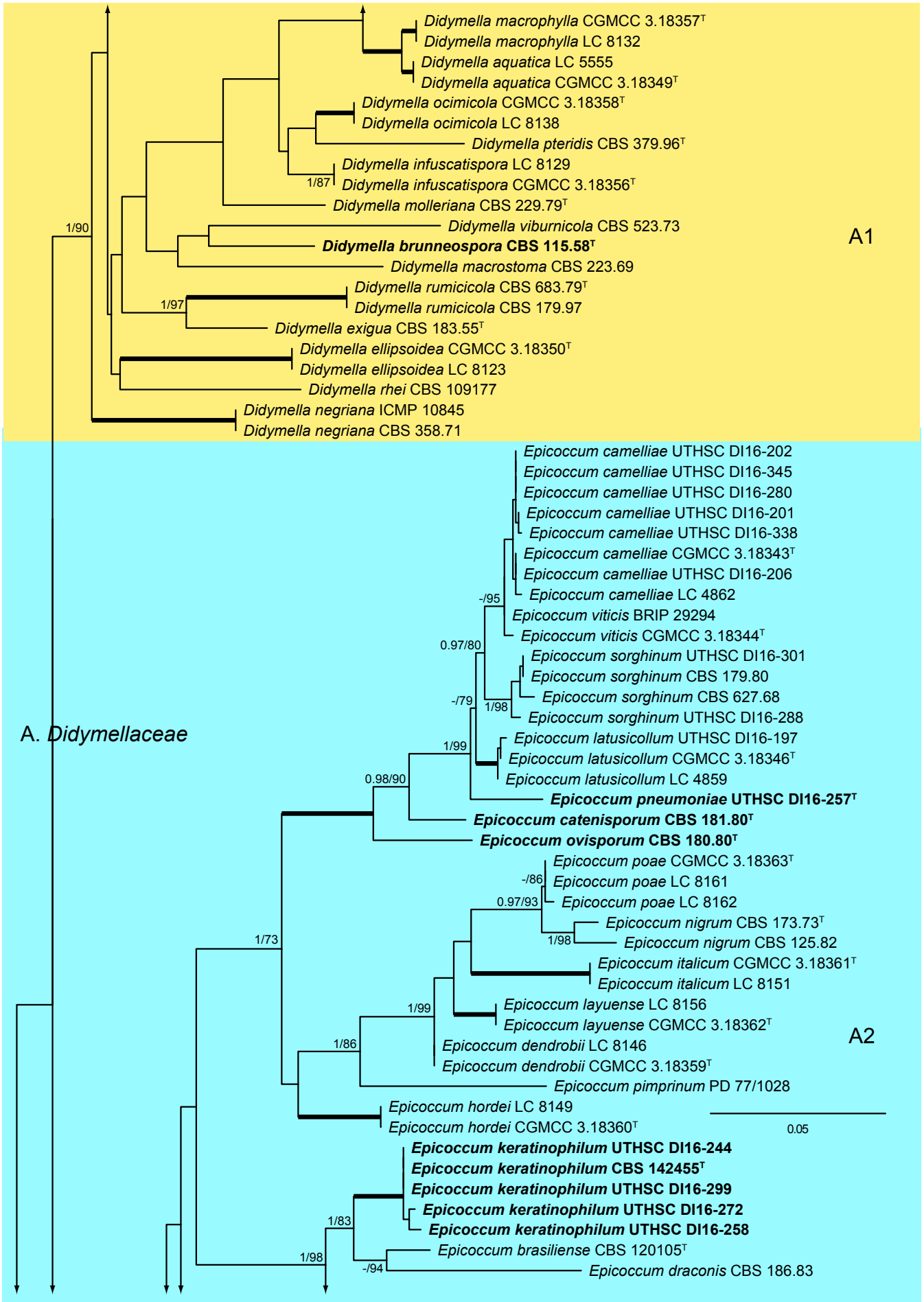

Fig. 1. (Continued).

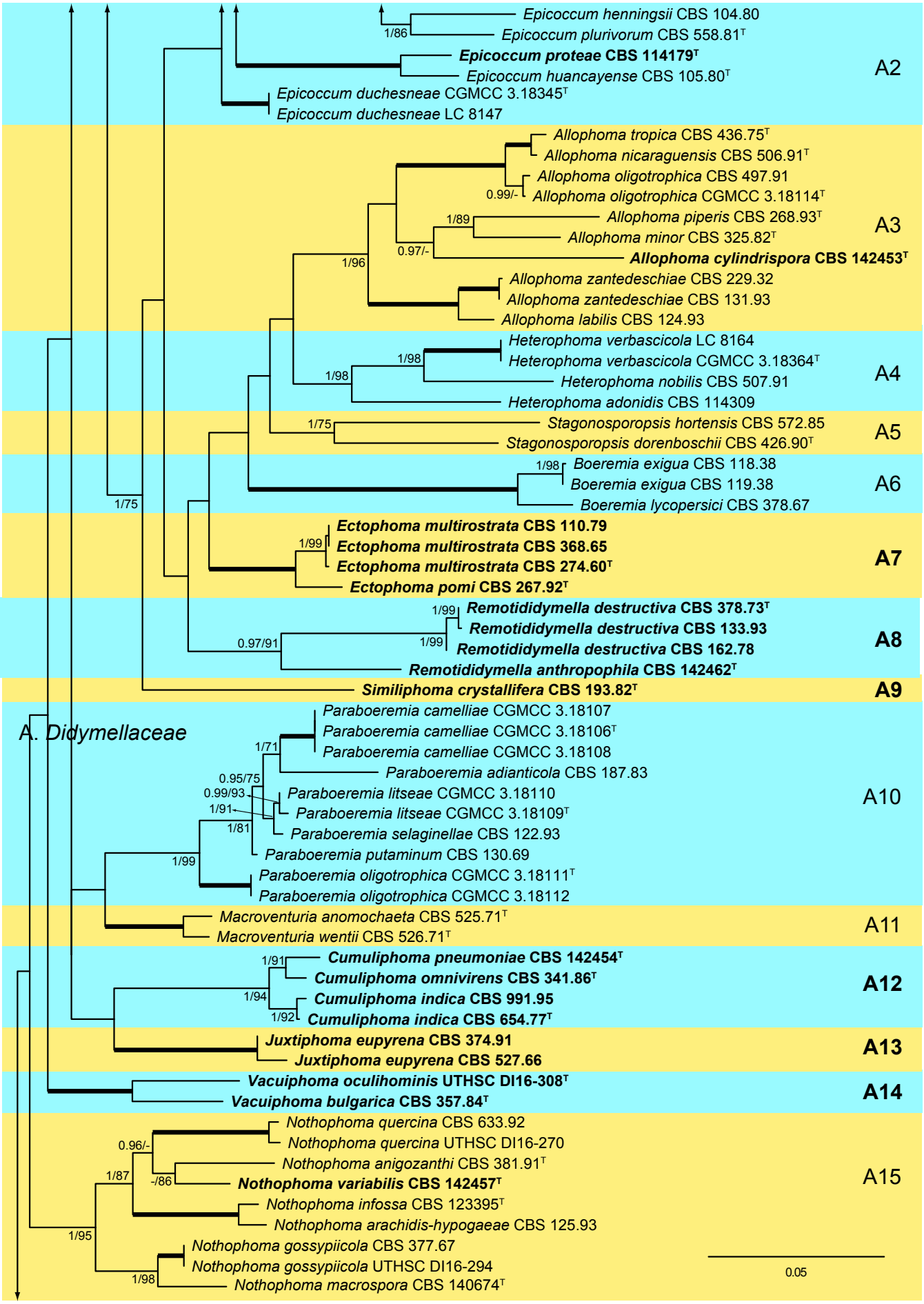

Fig. 1. (Continued).

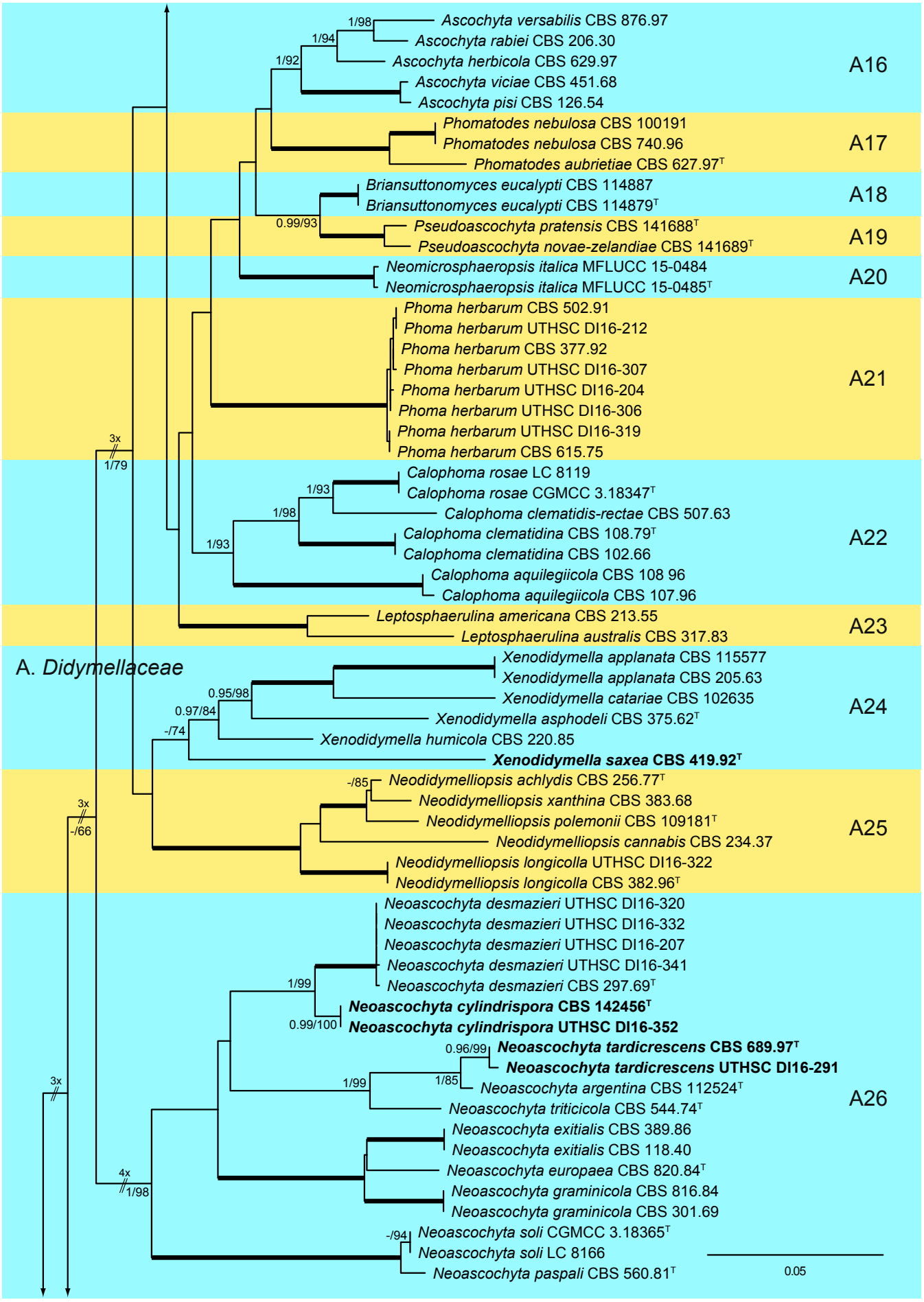

Fig. 1. (Continued).

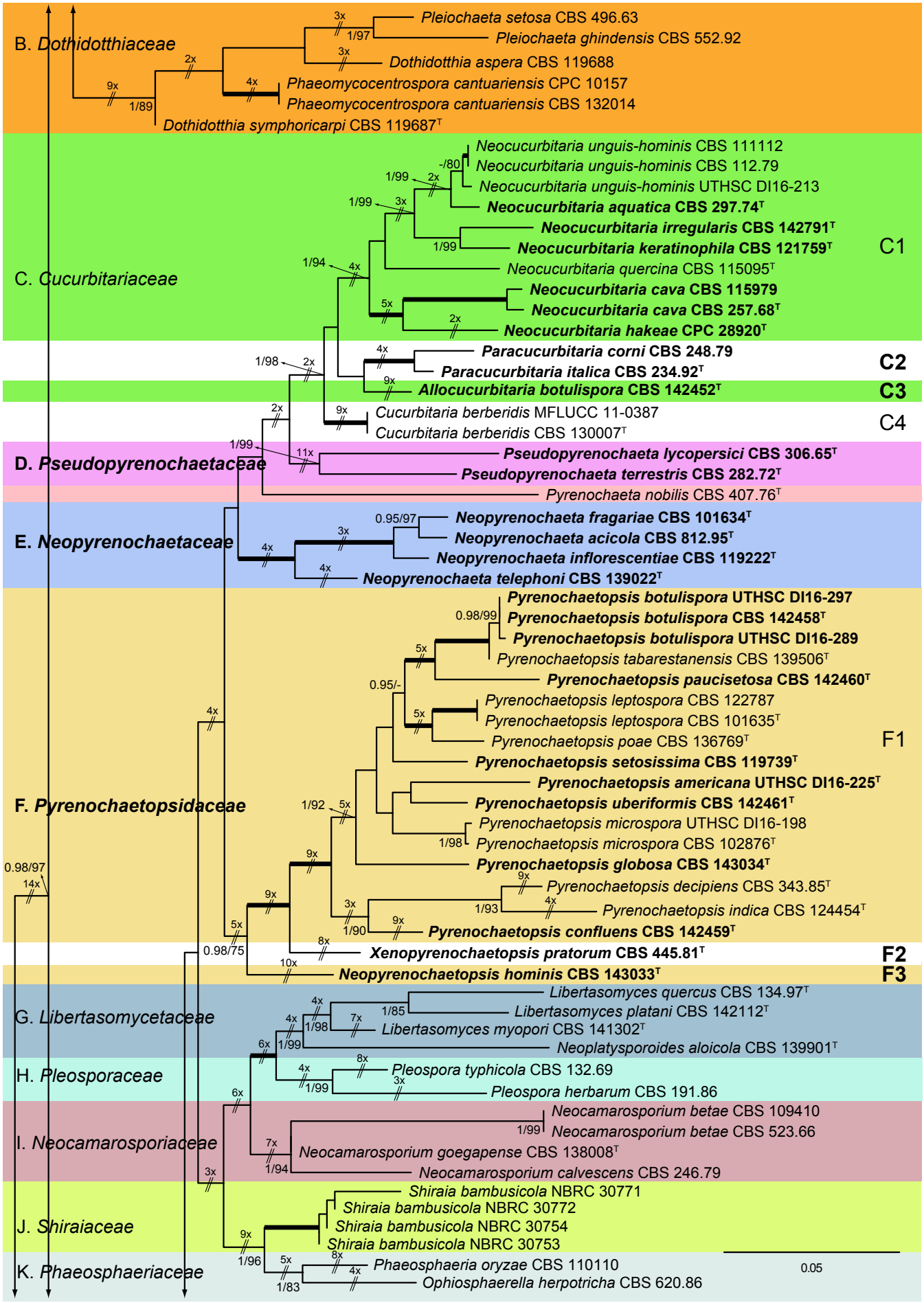

Fig. 1. (Continued).

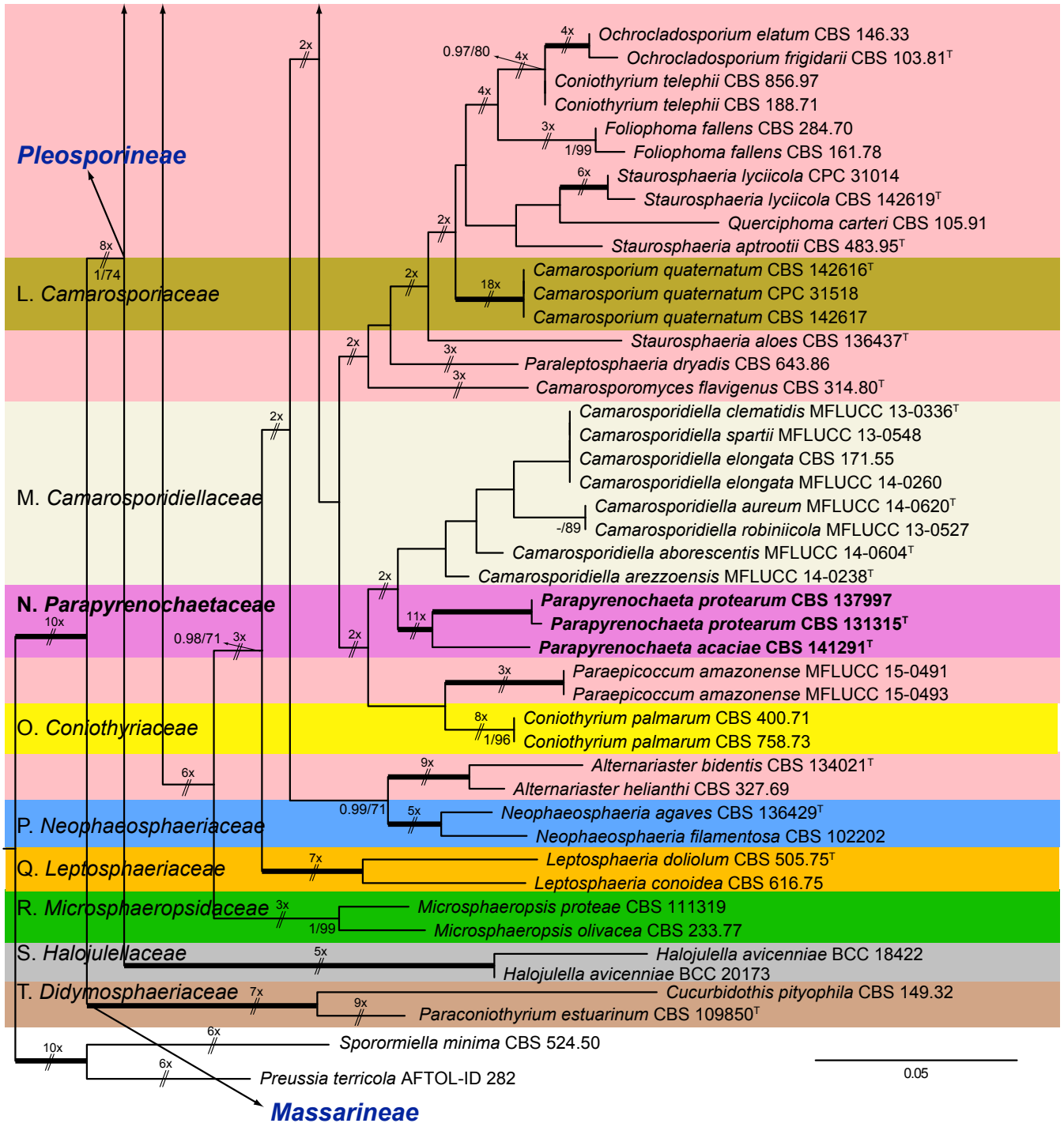

Fig. 1. (Continued).

Didymellaceae, very distant from the other genera of that family, and grouped 10 species, two of which are here proposed as new: *Neoascochyta cylindrispora* sp. nov. and *Neoascochyta tardicrescens* sp. nov.

In the family *Cucurbitariaceae* (clade C; 1 PP / 98 % BS) analyses resulted in four clades, which we recognise as genera. *Neocucurbitaria* (C1; 1 PP / 94 % BS), which included two new species, *N. irregularis* (CBS 142791) and *N. aquatica* (CBS 297.74), and the three new combinations, *N. cava* (syn. *Pyrenochaeta cava*), *N. hakeae* (basionym *Pyrenochaeta hakeae*) and *N. keratinophila* (basionym *Pyrenochaeta keratinophila*); the new genus *Paracucurbitaria* (C2; 1 PP / 100 % BS), with two species *P. corni* comb. nov. (syn. *Pyrenochaeta corni*) and *P. italica* sp. nov.; the new genus *Allocucurbitaria* (clade C3), with the type species *A. botulispora* sp. nov. Finally, the genus *Cucurbitaria* (clade C4; 1 PP / 100 % BS) including only the type species, *C. berberidis*.

Pseudopyrenochaetaceae fam. nov. (clade D; 1 PP / 99 % BS) is introduced to accommodate *Pyrenochaeta lycopersici* and *P. terrestris* in the new genus *Pseudopyrenochaeta*.

The generic type of *Pyrenochaeta*, *Pyrenochaeta nobilis*, was phylogenetically distant from the *Cucurbitariaceae* in our phylogeny, and therefore we consider this species as *incertae sedis*.

The proposed new family *Neopyrenochaetaceae* (clade E; 1 PP / 100 % BS) encompassed several taxa previously included in *Pyrenochaeta*. However, since they were located outside from *Cucurbitariaceae s. str.* we propose the new genus *Neopyrenochaeta*, with the new combinations: *N. acicola* (syn.

Pyrenochaeta acicola), *N. inflorescentiae* (basionym. *Pyrenochaeta inflorescentiae*) and *N. telephoni* (basionym *Pyrenochaeta telephoni*), and the new species *N. fragariae*.

The new family *Pyrenochaetopsidaceae* (clade F; 0.98 PP / 75 % BS) grouped three clades, which correspond to the genera *Pyrenochaetopsis*, the type genus (type species, *P. leptospora*) (F1; 1 PP / 100 % BS), *Xenopyrenochaetopsis* (type species, *X. pratorum* comb. nov.) (F2) and *Neopyrenochaetopsis* (type species, *N. hominis* sp. nov.) (F3). *Pyrenochaetopsis* encompassed seven new species: *P. americana*, *P. botulispora*, *P. confluens*, *P. globosa*, *P. pauciseptata, P. setosissima* and *P. uberiformis*.

The Clade N (1 PP / 100 % BS), which consists of several isolates previously recognised in *Pyrenochaeta*, is proposed as the new family *Parapyrenochaetaceae*. Accordingly, the new genus *Parapyrenochaeta* is proposed for *P. acaciae* comb. nov. (basionym *Pyrenochaeta acaciae*), and the type species *Parapyrenochaeta protearum* comb. nov. (basionym *Pyrenochaeta protearum*). The strain CBS 137997, previously identified as *Pyrenochaeta pinicola*, was re-identified as *Parapyrenochaeta protearum*.

The monospecific genus *Paraepicoccum* was introduced by Matsushima (1993), later epitypified as *Paraepicoccum amazonense* by Thambugala *et al.* (2016) and considered as *incertae sedis* in *Pleosporineae*, which is supported by our phylogenetic results.

Taxonomy

After multi-locus sequence analysis of 357 strains distributed among several families within *Pleosporineae* and the morphological study of 143 strains, in the present paper we propose: four new families, 13 new genera, 28 new species, 20 new combinations, and four typifications. Novel taxa are described and illustrated. Six species proved to be sterile in culture, and therefore are described based on DNA sequence data, following the approach of Chen *et al.* (2017). Clades and genera are given as they appear in the phylogenetic tree, and species are listed in alphabetical order.

Clade A: *Didymellaceae* Gruyter *et al.*, Mycol. Res. 113: 516. 2009.

Type genus: *Didymella* Sacc.

Clade A1: *Didymella*

Didymella Sacc. ex Sacc., Syll. Fung. 1: 545. 1882. emend. Chen *et al.*, Stud. Mycol. 82: 173. 2015.
Synonym: *Peyronellaea* Goid. ex Togliani, Ann. Sperim. Agrar. II 6: 93. 1952.

Type species: *Didymella exigua* (Niessl) Sacc.

Didymella anserina (Marchal) Q. Chen & L. Cai, Stud. Mycol. 82: 173. 2015.
Basionym: *Phoma anserina* Marchal, Champignon Copr. 11: 1891.
Synonyms: *Peyronellaea anserina* (Marchal) Aveskamp *et al.*, Stud. Mycol. 65: 31. 2010.
Phoma radicis-callunae R.W. Rayner, Bot. Gaz. 73: 231. 1922.
Phoma suecica J.F.H. Beyma, Antonie van Leeuwenhoek 8: 110. 1942.

Description: de Gruyter & Noordeloos (1992).

Materials examined: **Germany**, Giessen, Dec. 1979, R. Hadlok, living culture CBS 253.80. **USA**, from human sputum sample, 2008, D.A. Sutton, living cultures UTHSC DI16-255 = FMR 13745.

Notes: *Didymella anserina* is a ubiquitous soil fungus that has been found in Africa, Europe and North America. Although frequently present on herbaceous or woody plants, it has been recorded from many other substrates. Our strain UTHSC DI16-255 is the first report from a human clinical specimen, and it is morphologically similar to the reference strain of *D. anserina* (CBS 253.80).

Didymella brunneospora Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **sp. nov.** MycoBank MB820815. Fig. 2.

Etymology: From Latin *brunneus*-, brown, and *-spora*, spore, because of the conidial pigmentation.

Description: *Hyphae* hyaline to pale brown, smooth- and thin-walled, septate, 2–5 µm wide. *Conidiomata* pycnidial, pale brown to dark brown, mostly solitary, occasionally confluent, superficial on OA, glabrous, globose, 140–250 µm diam, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–5 layered, 25–70 µm thick, composed of pale brown to brown, flattened polygonal cells of 8–15 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform, 7–10 × 6.5–8 µm. *Conidia* aseptate, hyaline to pale brown, smooth- and thin-walled, obovoid to cylindrical, 4.5–7 × 3–3.5 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 26 mm diam after 7 d at 25 ± 1 °C, flattened, with abundant production of pycnidia, olive brown (M. 4E6); reverse yellowish brown (M. 5E4). Colonies on MEA reaching 28 mm diam after 7 d at 25 ± 1 °C, flattened, orange melon (M. 5A6) to orange-white (M. 5A2); reverse orange melon (M. 5A6) to orange white (M. 5A2). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined: **Germany**, isolated from flower-stalk of *Chrysanthemum roseum*, R. Schneider (**holotype** CBS H-23199, ex-holotype living cultures IMB 8675 = DSM 62044 = CBS 115.58 = FMR 15745).

Notes: *Ascochyta pyrethri* (Brunaud 1887), reported on decaying stems of *Pyrethri sinensis* in Saintes (France), was originally described (very briefly and lacking of measurements of their reproductive structures) in French, but later Latinised by Saccardo (Saccardo 1892), changing the order of the authors. The description of that fungus by Saccardo was based on the original diagnosis: pycnidia conical-globose, sparse to arrange in linear series, erumpent, black; conidia numerous, ovoid, ellipsoidal or long ellipsoidal, somewhat obtuse at both ends, straight or slightly curved, subhyaline. However, Saccardo described the conidia as not being constricted at the septum, which was not mentioned in the original description. Moreover, the protologue lacks illustrations and references to herbarium material, which makes this taxon doubtful. The strain CBS 115.58, previously identified as *Ascochyta pyrethri*; clusters distant from *Ascochyta* and produces pale brown, aseptate conidia, features not seen in that genus, and thus being considered herein as a new species of the genus *Didymella*.

Didymella gardeniae (S. Chandra & Tandon) Q. Chen & L. Cai, Stud. Mycol. 82: 176. 2015.

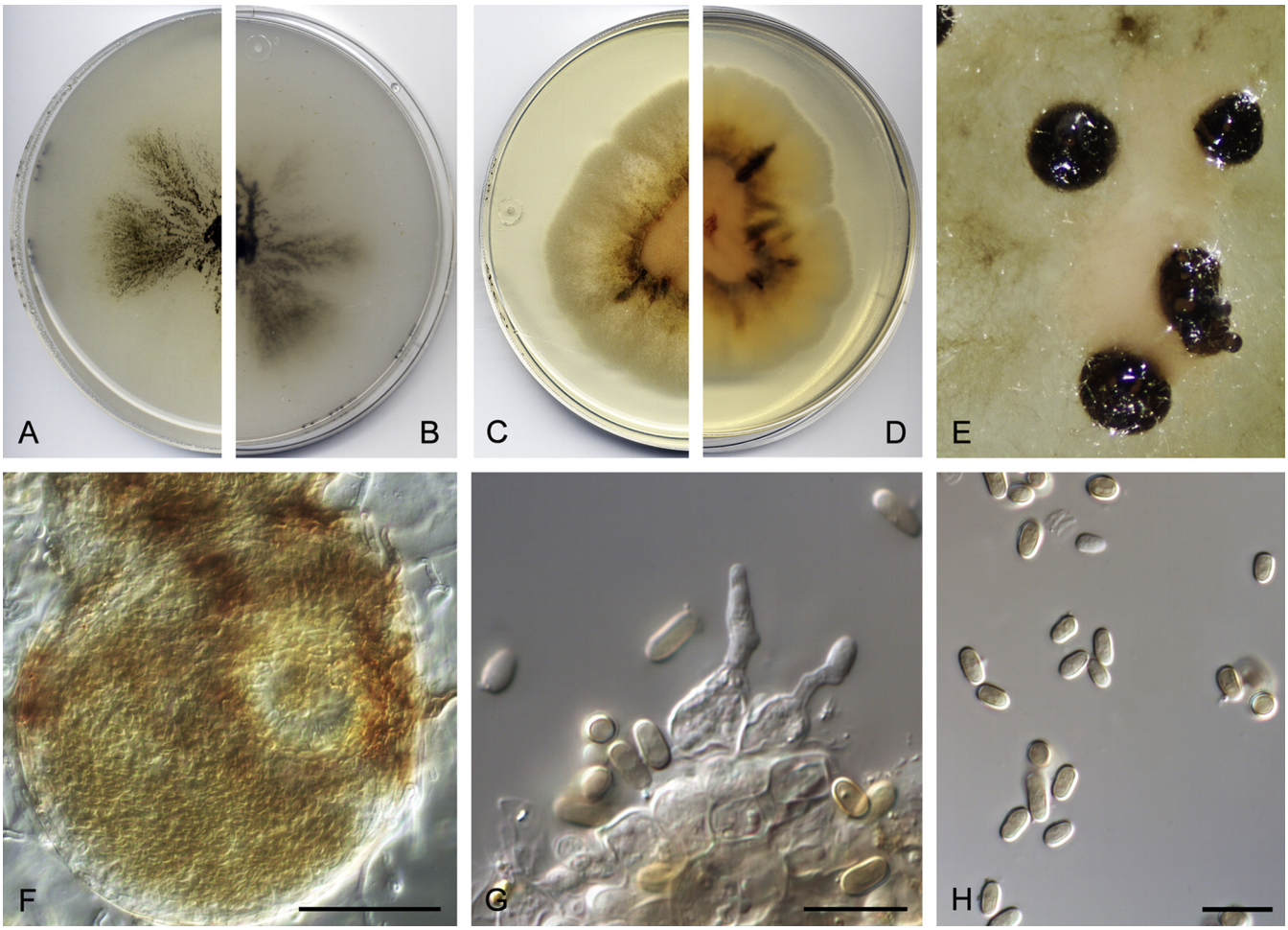

Fig. 2. *Didymella brunneospora* (CBS 115.58). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

Basionym: *Pyrenochaeta gardeniae* S. Chandra & Tandon, Mycopathol. Mycol. Appl. 29: 274. 1966.
Synonyms: *Phoma gardeniae* (S. Chandra & Tandon) Boerema, Verslagen Meded. Plantenziektenk. Dienst Wageningen 156: 27. 1980.
Peyronellaea gardeniae (S. Chandra & Tandon) Aveskamp *et al.*, Stud. Mycol. 65: 32. 2010.

Description: de Gruyter & Boerema (2002).

Materials examined: **India**, Allahabad, from the leaf of *Gardenia jasminoides*, 1966, S. Chandra & R.N. Tandon (**isotype** CBS H-7605, ex-isotype living cultures CBS 626.68 = IMI 108771 = FMR 14901). **USA**, from human nail distrophy, 2006, D.A. Sutton, living cultures UTHSC DI16-211 = FMR 13701; from human toe nail, 2007, D.A. Sutton, living cultures UTHSC DI16-226 = FMR 13716; from human toe nails, 2009, D.A. Sutton, living cultures UTHSC DI16-274 = FMR 13765; from human wound neck, 2010, D.A. Sutton, living cultures UTHSC DI16-295 = FMR 13788.

Notes: *Didymella gardeniae* was first isolated from a leaf of *Gardenia jasminoides* in India (Chandra & Tandon, 1966), and it seems to be a common soil- and air-borne fungus recovered also from Netherlands Antilles. Here, it is for first time associated with human clinical specimens from North America. Morphologically our strains resemble *D. gardeniae*, but have setose pycnidia, which are more characteristic of *Pyrenochaeta* than phoma-like taxa. Also remarkable is the fact that our strains are capable of growing at 37 °C.

Didymella glomerata (Corda) Q. Chen & L. Cai, Stud. Mycol. 82: 176. 2015. Fig. 3.
Basionym: *Coniothyrium glomeratum* Corda, Icon. Fung. (Prague) 4: 39. 1840.
Synonyms: *Phoma glomerata* (Corda) Wollenw. & Hochapfel, Z. Parasitenk. 3: 592. 1936.
Peyronellaea glomerata (Corda) Goid. ex Togliani, Ann. Sperim. Agrar. III 6: 93. 1952.

Description: Boerema *et al.* (2004).

Materials examined: **Lectotype** designated here (MBT 377971): plate 8, fig. 108, in Corda, AKJ. 1840. Icones fungorum hucusque cognitorum. Tomus IV, Praga (http://bibdigital.rjb.csic.es/ing/Libro.php?Libro=1812). **The Netherlands**, from *Chrysanthemum* sp., 1963 (**epitype designated here** CBS H-16351, MBT377905, ex-epitype living cultures CBS 528.66 = PD 63/590). **USA**, from human superficial tissue sample, 2006, D.A. Sutton, living culture UTHSC DI16-205 = FMR 13695.

Notes: *Coniothyrium glomeratum* was introduced by Corda (1840). The description of this fungus is brief, and the illustrations are not very detailed. The natural source has been mentioned as dry greyed wood chips, but without any geographic location. No original material of the basionym exists. Therefore, we designate the illustration by Corda here as lectotype and CBS H-16351 as epitype of *Coniothyrium glomeratum*. Other authors placed this fungus in other genera, such as *Aphosphaeria*, *Ascochyta*, *Peyronella* and *Phoma*, but also in *Alternaria*, because the production of alternarioid chains of chlamydospores *in vitro*. For a complete discussion about synonymies of this fungus see Boerema *et al.* (1965), who gave an exhaustive morphological description *in vitro* of this fungus. *Didymella glomerata* is characterised by the production of subhyaline to carbonaceous, small to large, glabrous pycnidia bearing one (to two or three) ostioles, aseptate, hyaline to dark-coloured, ovoid

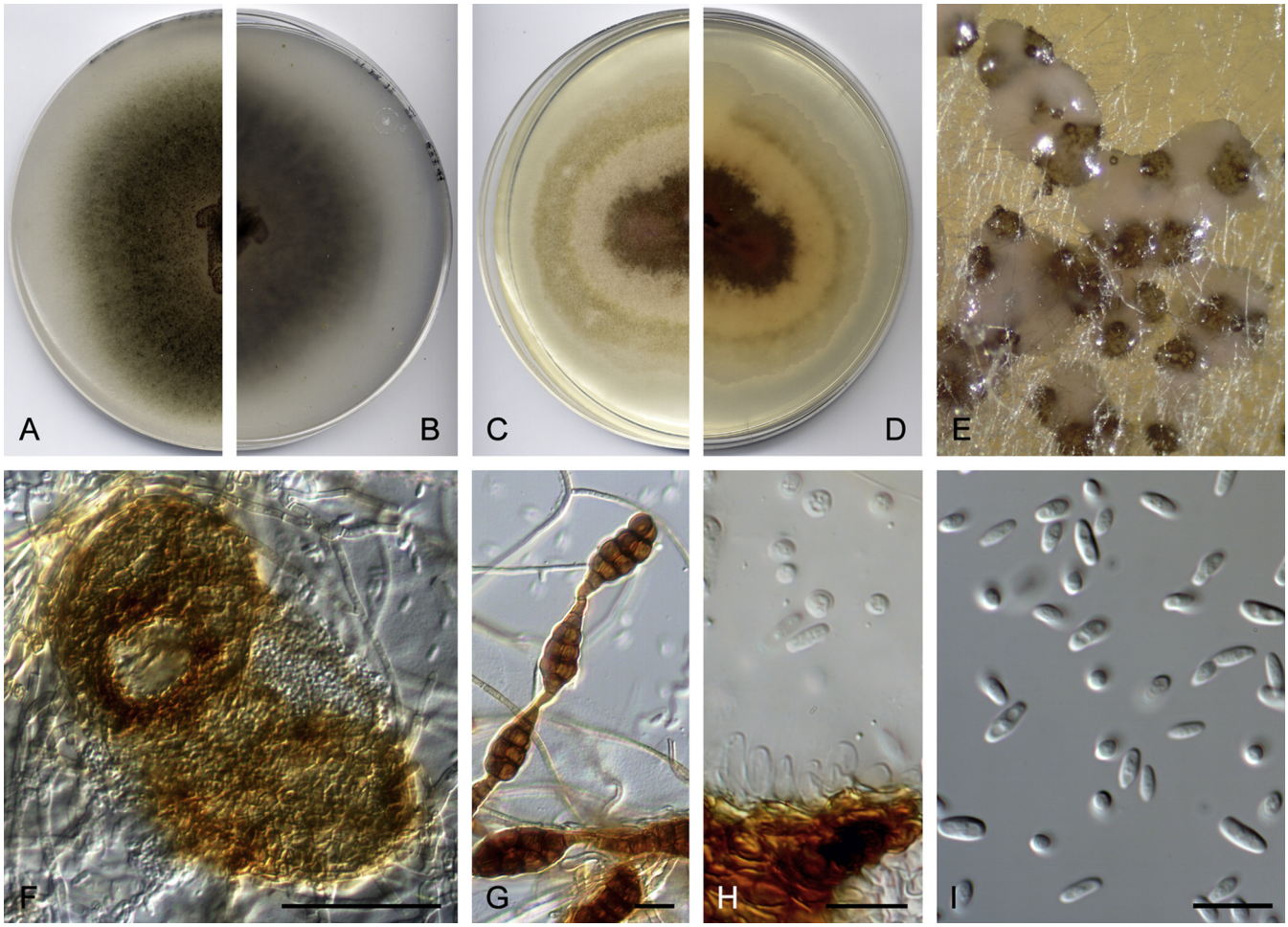

Fig. 3. *Didymella glomerata* (UTHSC DI16-205). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Alternaroid chlamydospores. **H.** Conidiogenous cells. **I.** Conidia. Scale bars: F = 100 µm. G–I = 10 µm.

to ellipsoidal conidia measuring mostly 6–7.5 × 3–3.5 µm, and alternaroid chlamydospores in chains. The fungus is distributed worldwide, and has been recovered from soil, different kinds of living and dead plants, and inorganic materials, and it can also infect humans (Punithalingam 1979, de Hoog *et al.* 2011). The strain UTHSC DI16-205, that phylogenetically clusters with the reference strain CBS 528.66 of *Didymella glomerata*, is morphologically indistinguishable from it.

Didymella heteroderae (Sen. Y. Chen *et al.*) Q. Chen & L. Cai, Stud. Mycol. 82: 176. 2015.
Basionym: *Phoma heteroderae* Sen Y. Chen *et al.*, Mycologia 88: 885. 1996 (1997).
Synonyms: *Peyronellaea heteroderae* (Sen Y. Chen *et al.*) Crous, Persoonia 32: 223. 2014.
Phoma pomorum var. *calorpreferens* Boerema *et al.*, Persoonia 15: 207. 1993.
Phoma calorpreferens (Boerema *et al.*) Aveskamp *et al.*, Mycologia 101: 370. 2009.
Peyronellaea calorpreferens (Boerema *et al.*) Aveskamp *et al.*, Stud. Mycol. 65: 31. 2010.

Description: Boerema (1993).

Materials examined: **The Netherlands**, from undefined food material, 1973, G.H. Boerema (**holotype** L 990.290.418, ex-holotype living cultures CBS 109.92 = PD 73/1405). **USA**, from human left plantar foot, 2005, D.A. Sutton, living cultures UTHSC DI16-190 = FMR 13680; from human nail, 2007, D.A. Sutton, living cultures UTHSC DI16-224 = FMR 13714; from human nail, 2007, D.A. Sutton, living cultures UTHSC DI16-227 = FMR 13717; from human fingernail, 2007, D.A. Sutton, living cultures UTHSC DI16-231 = FMR 13721; from human urine, 2007, D.A. Sutton, living cultures UTHSC DI16-232 = FMR 13722; from human nail, 2007, D.A. Sutton, living cultures UTHSC DI16-234 = FMR 13724; from human scalp, 2007, D.A. Sutton, living cultures UTHSC DI16-235 = FMR 13725; from human sputum sample, 2011, D.A. Sutton, living culturse UTHSC DI16-305 = FMR 13798.

Notes: Our strains are morphologically similar to the ex-type strain of *D. heteroderae*, and also show an identical DNA nucleotide sequence dataset. However, we proved that our strains are able to grow and sporulate at 37 °C (Valenzuela-Lopez *et al.* 2016), a higher temperature than that given in the original species description (Boerema *et al.* 2004).

Didymella keratinophila Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB820813. Fig. 4.

Etymology: From Greg κερατινων-, keratin, and – φίλος, friend of, because the source from which the fungus was isolated.

Description: *Hyphae* pale brown, smooth- and thin-walled, septate, 2.5–8 µm wide. *Conidiomata* pycnidial, brown, solitary, superficial on OA, glabrous, broadly ellipsoidal, 250–270 × 200–230 µm, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–5 layered, 15–35 µm thick, composed of brown, flattened polygonal cells of 5–10 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform or globose, 4.5–6 × 3–4.5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, guttulate, ovoid to cylindrical, 4–6 × 2.5–3 µm. *Chlamydospores* absent.

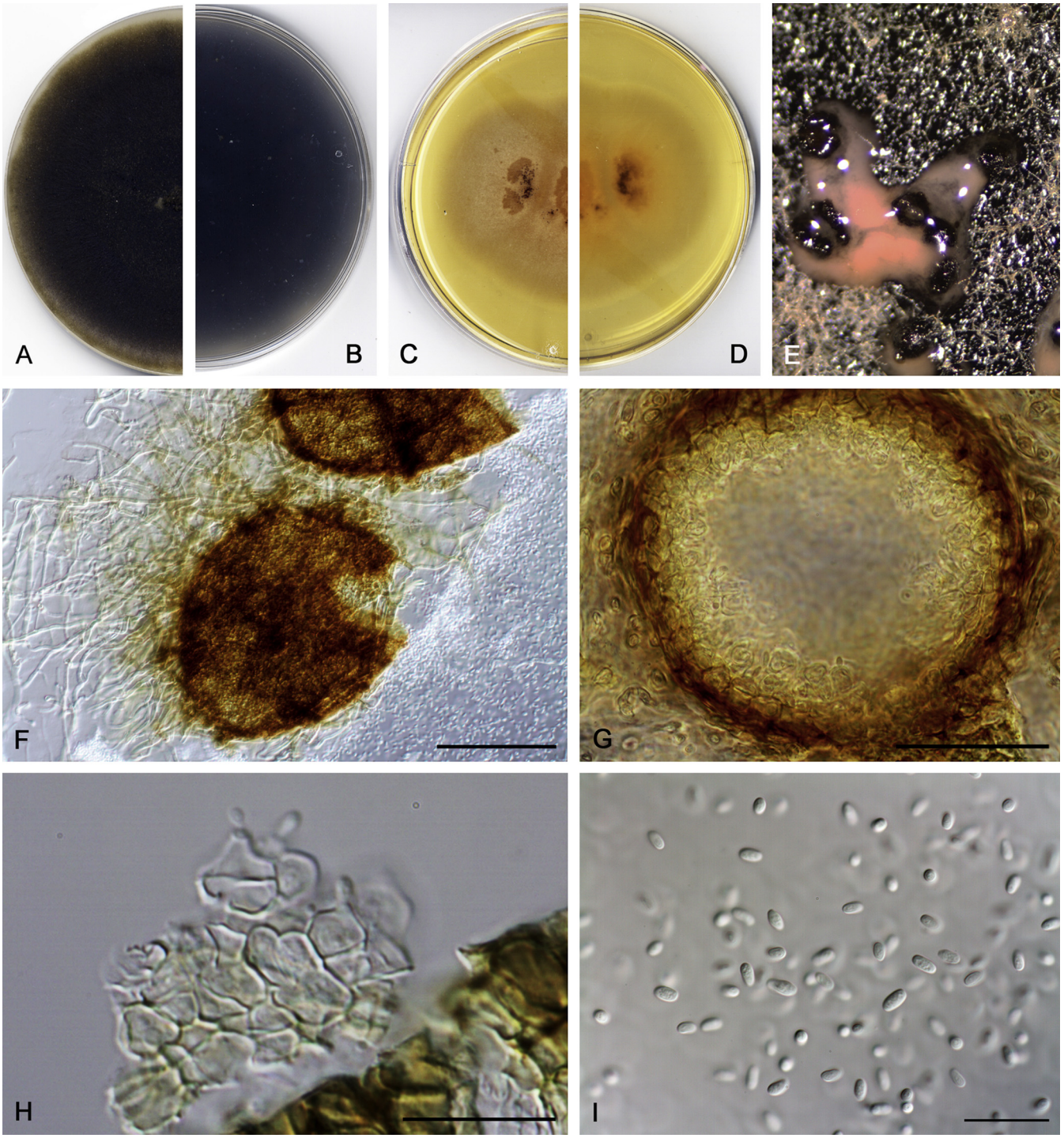

Fig. 4. *Didymella keratinophila* (CBS 143032). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Section of pycnidium. **H.** Conidiogenous cells. **I.** Conidia. Scale bars: F, G = 100 µm. H, I = 10 µm.

Culture characteristics: Colonies on OA reaching 54 mm diam after 7 d at 25 ± 1 °C, flattened, greyish brown (M. 5F3); reverse greyish brown (M. 5F3). Colonies on MEA reaching 57–67 mm after 7 d at 25 ± 1 °C, flattened, brownish orange (M. 5C3); reverse brownish grey (M. 5C2). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 37 °C.

Materials examined: **USA**, from human finger-hand lesion, 2006, D.A. Sutton (**holotype** CBS H-23200, ex-type living cultures CBS 143032 = UTHSC DI16-200 = FMR 13690); from human toe nail, 2007, D.A. Sutton, living cultures UTHSC DI16-228 = FMR 13718; from human nail, 2009, D.A. Sutton, living cultures UTHSC DI16-282 = FMR 13774.

Notes: *Didymella keratinophila* was recovered from a human superficial tissue specimen in the USA, and forms a well-supported sister clade with *D. sancta*. *Didymella keratinophila* differs phenotypically from *D. sancta* (and related species, such as *D. glomerata*, *D. musae* and *D. pomorum*) by the absence of chlamydospores *in vitro* (brown, alternarioid, phragmosporous and dyctiosporous, singly and terminally produced in *D. sancta*), smaller conidia (4–6 × 2.5–3 µm *vs.* 5–7 (–7.5) × 2.5–4 (–4.5) µm in *D. sancta*) and a negative NaOH spot test.

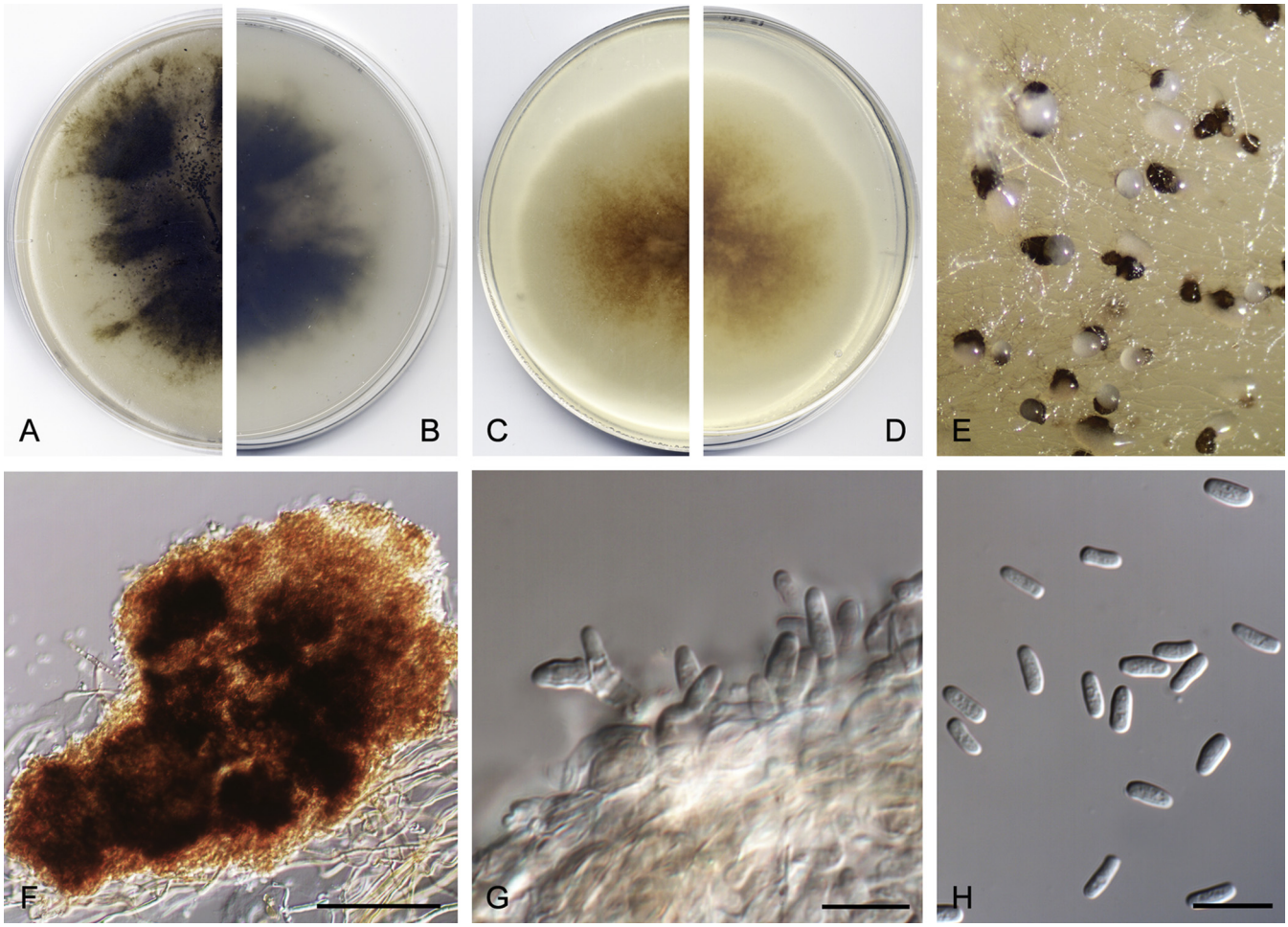

Fig. 5. *Didymella musae* (CBS 463.69). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

Didymella microchlamydospora (Aveskamp & Verkley) Q. Chen & L. Cai, Stud. Mycol. 82: 178. 2015.
Basionym: *Phoma microchlamydospora* Aveskamp & Verkley, Mycologia 101: 374. 2009.

Description: Aveskamp *et al.* (2009).

Materials examined: **UK**, from leaves of *Eucalyptus* sp., 1994, A.M. Ainsworth (**holotype** CBS H-20147, ex-holotype living culture CBS 105.95). **USA**, from human skin leg, 2006, D.A. Sutton, living cultures UTHSC DI16-199 = FMR 13689; from human corneal lesion, 2014, D.A. Sutton, living cultures UTHSC DI16-365 = FMR 13858.

Notes: *Phoma microchlamydospora* was described as a new species by Aveskamp *et al.* (2009) from leaves of *Eucalyptus* sp. in Great Britain, being subsequently transferred to the genus *Didymella* by Chen *et al.* (2015) after a phylogenetic study. Our two strains of this species differ in the geographic origin (USA) and in substrate (isolated from human clinical specimens), but they are morphologically and genetically similar to the ex-type living culture of *D. microchlamydospora*, being characterised by the production of abundant micropycnidia, globose pycnidia with 1–3 papillate ostioles, frequently on a neck, hyaline, one-celled, globose to ellipsoidal conidia, and relatively small, one-celled to multi-celled chlamydospores arranged in chains.

Didymella musae (P. Joly) Q. Chen & L. Cai, Stud. Mycol. 82: 178. 2015. Fig. 5.
Basionym: *Peyronellaea musae* P. Joly, Rev. Mycol. 26: 97. 1961.

Synonym: *Phoma jolyana* Piroz. & Morgan-Jones, Trans. Brit. Mycol. Soc. 51: 200. 1968.

Description: Boerema (1993).

Materials examined: **India**, from fruit of *Mangifera indica*, May 1969, living cultures CBS 463.69 = FMR 15339. **USA**, from human cornea lesion, 2007, D.A. Sutton, living cultures UTHSC DI16-230 = FMR 13720.

Notes: The strain UTHSC DI16-230, which is morphologically similar to the reference strain CBS 463.69, only differs genetically in a few nucleotides of the *tub*2 gene.

Didymella protuberans (Lév.) Q. Chen & L. Cai, Stud. Mycol. 82: 180. 2015.
Basionym: *Phoma protuberans* Lév., Ann. Sci. Nat. Bot. III 5: 281. 1846.
Synonyms: *Peyronellaea protuberans* (Lév.) Aveskamp *et al.*, Stud. Mycol. 65: 33. 2010.
Didymella alectorolophi Rehm, Hedwigia 64: 294. 1923.
Peyronellaea alectorolophi (Rehm.) Aveskamp *et al.*, Stud. Mycol. 65: 31. 2010.
Phoma alecotorolophi Boerema *et al.*, Persoonia 16: 366. 1997.
Phoma obtusa Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 378. 1870.
Peyronellaea obtusa (Fuckel) Aveskamp *et al.*, Stud. Mycol. 65: 33. 2010.

Description: Chen *et al.* (2015).

Materials examined: **The Netherlands**, from a leaf of *Lycium halimifolium*, 1971 (**neotype** HMAS 246694, ex-neotype living cultures CBS 381.96 = PD 71/706). **USA**, from chocolate, 2011, D.A. Sutton, living cultures UTHSC DI16-302 = FMR 13795.

Notes: The strain UTHSC DI16-302 isolated from the USA clusters with the ex-neotype strain of *Didymella protuberans*, being morphologically similar.

Didymella rumicicola (Boerema & Loer.) Q. Chen & L. Cai, Stud. Mycol. 82: 181. 2015.
Basionym: *Phoma rumicicola* Boerema & Loer., New Zealand J. Bot. 18: 473. 1980.

Description: Chen *et al.* (2015).

Materials examined: **New Zealand**, Levin, from *Rumex obtusifolius*, Jun. 1979, G.F. Laundon (**holotype** PDD 50667, **isotype** CBS H-7627, ex-isotype living cultures CBS 683.79 = LEV 15094). **The Netherlands**, Baarn, from a stem of *Rumex hydrolapathum*, Mar. 1996, H.A. van der Aa, living culture CBS 179.97.

Notes: The strain CBS 179.97 was initially identified as *Didymella acetosellae*; this strain is genetically identical to the ex-type strain of *D. rumicicola* (CBS 683.79), and the host pertains to the same genus of plants (*Rumex*). Consequently, we assigned this strain to *D. rumicicola*.

***Didymella* sp.**

Material examined: **Japan**, from *Camellia sasanqua*, living culture LC 8141.

Notes: This strain was considered by Chen *et al.* (2017) as a reference strain of *Didymella segeticola*. However, in our phylogenetic study, this strain is distinct from the ex-type strain of *Didymella segeticola*, with strain LC 8141 differing in 7 bp in *rpb2*. It was also isolated from a different host and country, and therefore we maintain this strain as *Didymella* sp.

Clade A2: *Epicoccum*

Epicoccum Link, Mag. Neuesten Entdeck. Gesammten Naturk. Ges. Naturf. Freunde Berlin 7: 32. 1815, emend. Q. Chen & L. Cai, Stud. Mycol. 82: 171. 2015.

Type species: *Epicoccum nigrum* Link.

Epicoccum camelliae Q. Chen *et al.*, Stud. Mycol. 87: 140. 2017.

Description: Chen *et al.* (2017).

Materials examined: **China**, Jiangxi, Ganzhou, leaves of *Camellia sinensis*, 7 Sep. 2013, Y. Zhang (**holotype** HMAS 247159, ex-holotype culture CGMCC 3.18343 = LC 4858); *ibid.* LC4862. **USA**, from human respiratory tract, 2006, D.A. Sutton, living cultures UTHSC DI16-201 = FMR 13691; from human nail, 2006, D.A. Sutton, living cultures UTHSC DI16-202 = FMR 13692; from human toe nail, 2006, D.A. Sutton, living cultures UTHSC DI16-206 = FMR 13696; from human toe nail, 2009, D.A. Sutton, living cultures UTHSC DI16-280 = FMR 13772; from human nail, 2011, D.A. Sutton, living cultures UTHSC DI16-338 = FMR 13831; from human abscess, 2012, D.A. Sutton, living cultures UTHSC DI16-345 = FMR 13838.

Notes: A total of six isolates molecularly identified as *E. camelliae* clustered together with *E. viticis* forming a low-supported clade. Our isolates, as well as those of Chen *et al.* (2017), remained sterile. Consequently, further studies will be needed to fully characterise this species.

Epicoccum catenisporum Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, **sp. nov.**, MycoBank MB819762. Fig. 6.

Etymology: From Latin *catena*-, chain, and *-spora*, spore, because of the disposition of the chlamydospores in chains.

Description: *Hyphae* pale brown, smooth- and thin-walled, septate, 2.5–5 µm wide. *Conidiomata* pycnidial, brown to dark brown, solitary, superficial and immersed (OA), glabrous, subglobose, 170–190 × 140–160 µm, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–5 layered, 15–50 µm thick, composed of brown to dark brown, flattened polygonal cells of 5–10 µm diam, *Conidiogenous cells* phialidic, hyaline, smooth-walled, doliiform or ampulliform, 4–6 × 2–4 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, ovoid or ellipsoidal, 4–5 × 2–3 µm, guttulate. *Chlamydospores* aseptate, dark brown, smooth- and thick-walled, in chains or singly, then intercalary disposed, ellipsoidal to ovoid, 9.5–12 × 4.5–8.5 µm.

Culture characteristics: Colonies on OA reaching 53 mm diam after 7 d at 25 ± 1 °C, flattened, powdery due to the production of abundant pycnidia, orange grey (M. 5B1) to yellowish brown (M. 5F5); reverse pale brown (M. 5D4) to brownish grey (M. 5F2). Colonies on MEA reaching 36 mm after 7 d at 25 ± 1 °C, flattened to floccose, white (M. 5A1) to orange white (M. 5A2); reverse white (M. 5A1) to pale orange (M. 5A4). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 15 °C; maximum temperature of growth 35 °C.

Material examined: **Guinea-Bissau**, Gacheu, from a leaf spot of *Oryza sativa*, Oct. 1978, deposited by G.H. Boerema (**holotype** CBS H-23203, ex-holotype living cultures CBS 181.80 = PD 78/974 = FMR 14911).

Notes: The strain CBS 181.80 was previously identified as *Phoma sorghina* (currently *E. sorghinum*) by Aveskamp *et al.* (2009). However, it is phylogenetically different from that species. *Epicoccum catenisporum* is morphologically characterised by the production of pycnidia as observed in several other members of *Epicoccum*.

Epicoccum keratinophilum Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.**, MycoBank MB819758. Fig. 7.

Etymology: From Greek κεράτινη-, keratin, and -φίλος, friend, linked to the origin of the fungus.

Description: *Hyphae* pale brown, smooth- and thin-walled, septate, 2.5–5 µm wide. *Conidiomata* pycnidial, brown, solitary, superficial or immersed (OA), glabrous, subglobose, 200–300 × 180–240 µm, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–4 layered, 15–45 µm thick, composed of brown to dark brown, flattened polygonal cells of 5–20 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform to globose, 4–5 µm diam. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical, 4–6 × 1.5–2 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 30–35 mm diam after 7 d at 25 ± 1 °C, flattened, entire edge, yellowish brown (M. 5E7) to brownish grey (M. 5F2); reverse brownish grey (M.5F2). Colonies on MEA reaching 30–37 mm diam after 7 d at 25 ± 1 °C, flattened, entire edge, white (M. 2A1) to yellowish white (M. 3A2); reverse dull yellow (M. 3B3). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Materials examined: **USA**, Texas, from animal skin lesion, 2009, D.A. Sutton (**holotype** CBS H-23032, ex-holotype living cultures CBS 142455 = UTHSC DI16-271 = FMR 13762); Texas, from human superficial tissue, 2007, D.A. Sutton, living cultures UTHSC DI16-244 = FMR 13734; from human bronchial

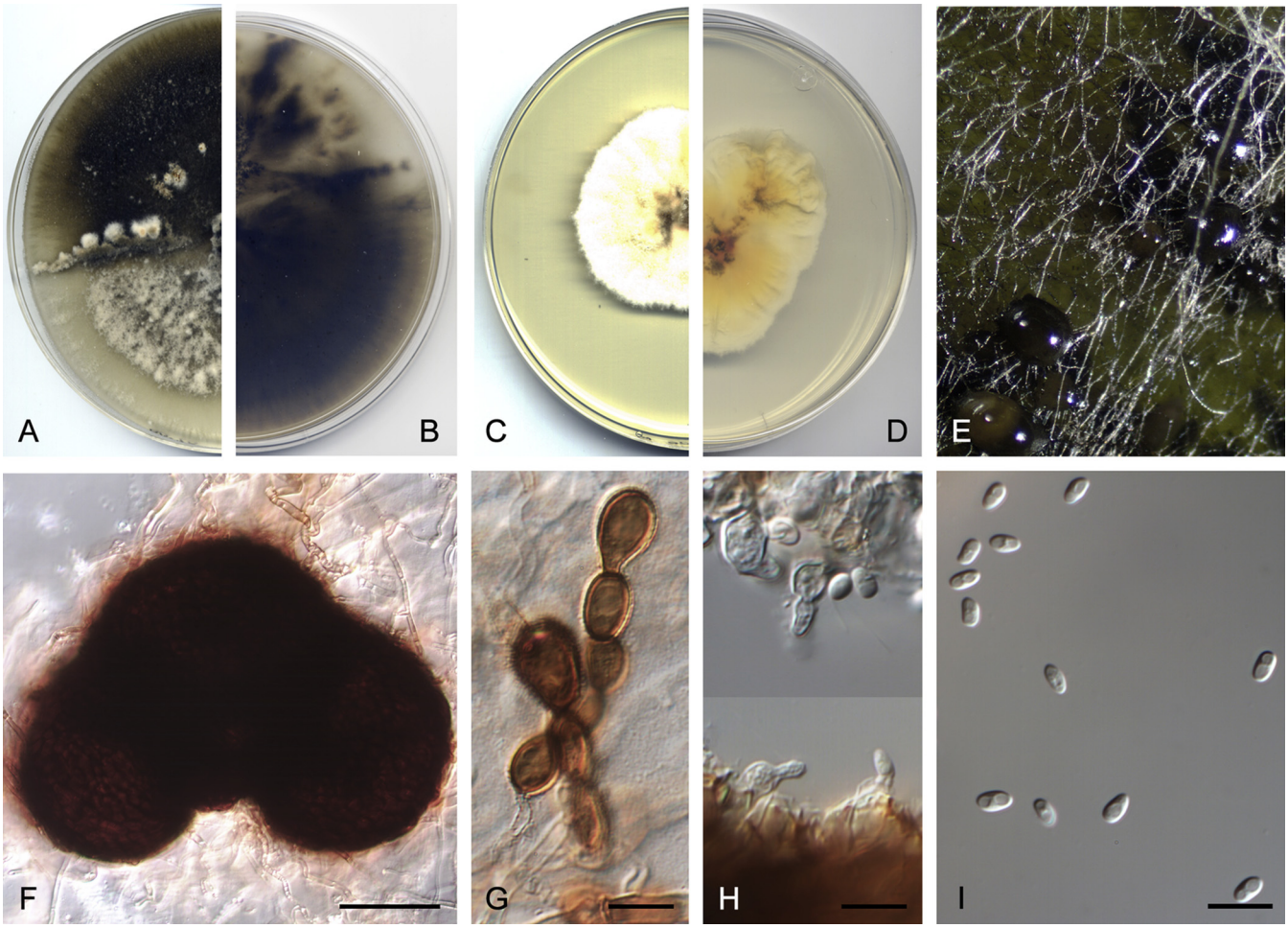

Fig. 6. *Epicoccum catenisporum* (CBS 181.80). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Chlamydospores in chains. **H.** Conidiogenous cells. **I.** Conidia. Scale bars: F = 100 µm. G–I = 10 µm.

wash sample, 2008, D.A. Sutton, living cultures UTHSC DI16-258 = FMR 13748; from human toe nail, 2009, D.A. Sutton, living cultures UTHSC DI16-272 = FMR 13763; from human biopsy tissue, 2011, D.A. Sutton, living culture UTHSC DI16-299 = FMR 13792.

Notes: In our phylogenetic tree *E. keratinophilum* forms a well-supported clade distant from its morphological relatives *E. brasiliense* and *E. draconis*. All *E. keratinophilum* strains have been recovered from clinical samples, and morphologically differ from *E. brasiliense* in producing smaller pycnidia and conidia, and from both *E. brasiliense* and *E. draconis* by a negative NaOH spot test reaction.

Epicoccum latusicollum Q. Chen *et al.*, Stud. Mycol. 87: 144. 2017.

Description: Chen *et al.* (2017).

Materials examined: **China**, Jiangxi, Ganzhou, endophyte of *Camellia sinensis*, 7 Sep. 2013, Y. Zhang, living culture LC 4859; Shandong, Jining, on leaves of *Sorghum bicolor*, 3 Aug. 2013, N. Zhou (**holotype** HMAS 247164, ex-holotype living culture CGMCC 3.18346 = LC 5158). **USA**, from human eye, 2005, D.A. Sutton, living cultures UTHSC DI16-197 = FMR 13687.

Notes: The strain UTHSC DI16-197 that was isolated from a human eye sample clustered with the ex-type strain of *E. latusicollum* that was recently introduced by Chen *et al.* (2017), being characterised by the production of pycnidial conidiomata. Unfortunately, our strain was sterile, and morphological comparison was not possible, but genetically it is identical to the latter species.

Epicoccum ovisporum Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, **sp. nov.**, MycoBank MB819761. Fig. 8.

Etymology: From Latin *ovum*-, egg, and *-spora*, spore, due to the shape of the conidia.

Description: *Hyphae* hyaline to pale brown, smooth- and thin-walled, septate, 2.5–5 µm wide. *Conidiomata* pycnidial, brown, solitary, mostly superficial on OA and immersed into MEA, glabrous, subglobose to globose, 100–190 × 85–180 µm, with short papillate ostiolar neck; pycnidial wall of *textura angularis*, 3–4 layered, 12.5–35 µm thick, composed of brown, flattened polygonal cells of 5–20 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, doliiform to ampulliform, 5–6 × 2–3 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, guttulate, ovoid, ellipsoidal to cylindrical, 5–7 × 2–3 µm. *Chlamydospores* multi-celled, brown to dark brown, smooth-walled, disposed in chains or singly, then intercalary and terminally, globose to subglobose, 10–22.5 × 10–20 µm.

Culture characteristics: Colonies on OA reaching 36 mm diam after 7 d at 25 ± 1 °C, flattened, with abundant production of pycnidia, greenish grey (M. 29B2); reverse orange grey (M.5B2). Colonies on MEA reaching 38 mm after 7 d at 25 ± 1 °C, floccose, orange grey (M. 5B2) to grey (M. 5C1), producing a hyaline exudate; reverse yellowish brown (M. 5D8). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 15 °C; maximum temperature of growth 30 °C.

Material examined: **South Africa**, Potchefstroom, from a leaf of *Zea mays*, Nov. 1978, isolated by W.J. Jooste, deposited by G.H. Boerema (**holotype** CBS H-23204, ex-holotype living cultures CBS 180.80 = PD 78/1100 = FMR 14910).

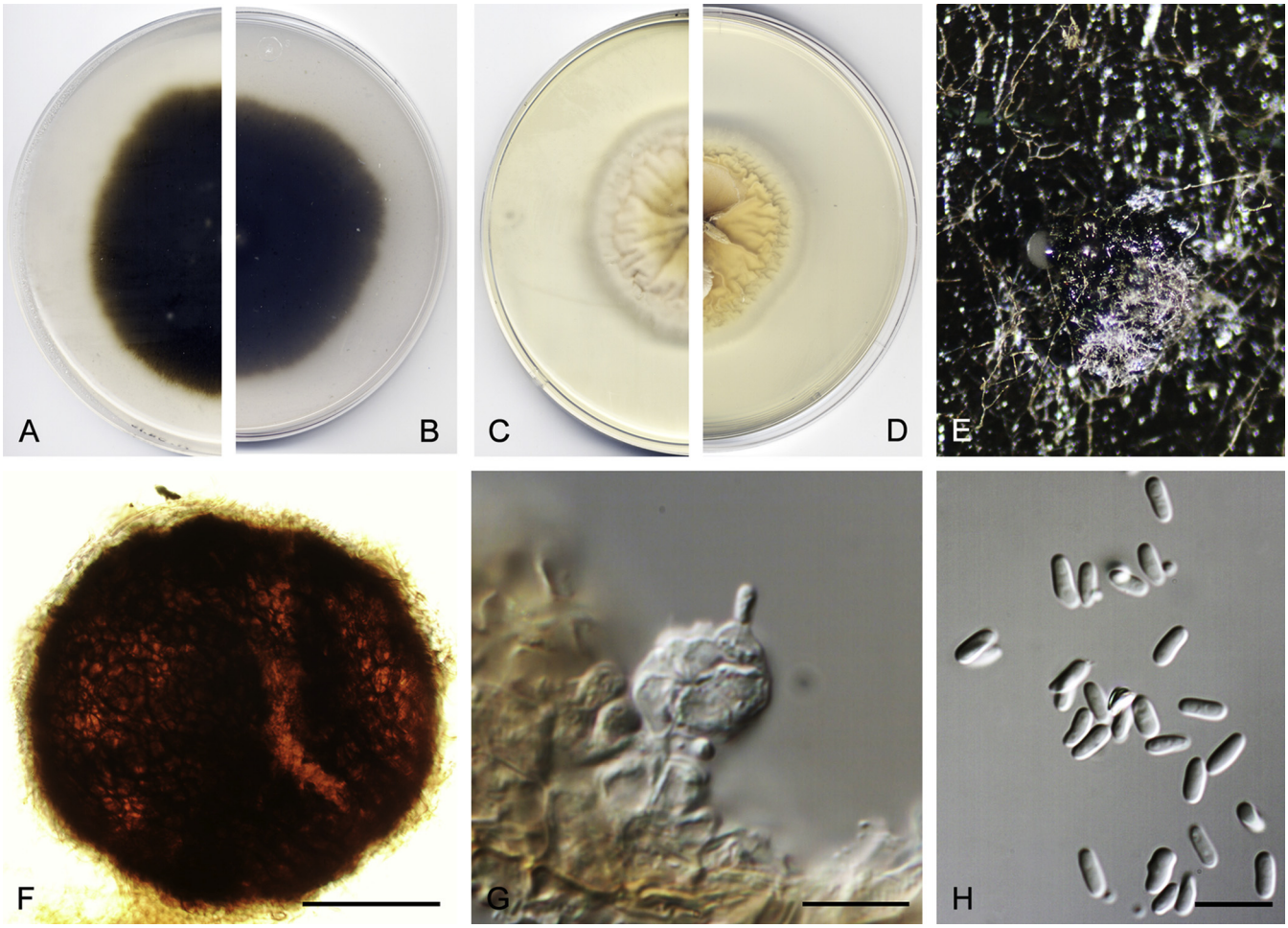

Fig. 7. *Epicoccum keratinophilum* (CBS 142455). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cell. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

Notes: The strain CBS 180.80 was previously assigned to *E. sorghinum* (Aveskamp *et al.* 2009, 2010); however, in our phylogenetic tree it represents a new species, forming a basal clade together with *E. catenisporum* and *E. sorghinum*, being distant from the rest of the species of the genus. The above-mentioned species are morphologically similar to *E. ovisporum* by producing pycnidia instead of sporodochia.

Epicoccum pneumoniae Valenzuela-Lopez, Stchigel, Guarro & Cano, **sp. nov.** MycoBank MB822112.

Etymology: The species name refers to the infection associated with this specimen.

Culture sterile. *Epicoccum pneumoniae* differs from its closest phylogenetic species *Epicoccum latusicollum* based on alignment of the concatenated four loci deposited in TreeBASE (S21115): LSU deletion in position: 382; ITS positions: 587 (C); *tub*2 positions: 1075 (T), 1102 (T), 1152 (T), 1159 (T), 1161 (G), 1207 (G), 1209 (T), 1210 (A), 1212 (G), 1213 (T), 1254 (T), 1260 (C), 1284 (C); *rpb*2 positions: 1312 (A), 1336 (A), 1339 (G), 1351 (C), 1354 (T), 1384 (C), 1453 (T), 1456 (C), 1495 (T), 1553 (C), 1609 (T), 1757 (T), 1769 (C), 1813 (C), 1816 (C), 1843 (C), 1873 (C), 1897 (C).

Culture characteristics: Colonies on OA reaching 29 mm diam after 7 d at 25 ± 1 °C, flattened, reddish grey (M. 9B2) to white (M. 9A1); reverse white (M. 9A1). Colonies on MEA reaching 31 mm after 7 d at 25 ± 1 °C, flattened to floccose, pinkish white (M. 9A2) to white (M. 9A1); reverse white (M. 9A1). NaOH spot test negative. Crystals absent.

Material examined: **USA**, from human sputum sample, 2008, D.A. Sutton (**holotype** FMR H-13747, ex-holotype living cultures UTHSC DI16-257 = FMR 13747).

Notes: The strain UTHSC DI16-257, which remained sterile, forms a basal clade with *E. latusicollum*; however, this strain clearly differs phylogenetically from the latter species mainly in the loci *tub*2 and *rpb*2. Therefore it is proposed here as a new species, *Epicoccum pneumoniae*.

Epicoccum proteae (Crous) Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, **comb. nov.** MycoBank MB820830.
Basionym: *Phoma proteae* Crous, Persoonia 27: 151. 2011.

Description: Crous *et al.* (2011).

Material examined: **South Africa**, Western Cape Province, Somerset West, Karibia Farm, from leaves of *Protea* cv. Carnival (*P. compacta* × *P. neriifolia*), 21 July 1998, J.E. Taylor & S. Denman (**holotype** CBS H-20771, ex-holotype living cultures CPC 1854 = CBS 114179 = FMR 15332).

Notes: This species was first proposed by Crous *et al.* (2011) within *Phoma*, which is characterised by producing brown, globose pycnidia and hyaline, aseptate conidia. However, our phylogenetic study showed that the ex-type strain of this species clustered in *Epicoccum*. Therefore, we propose a new combination for this species.

Epicoccum sorghinum (Sacc.) Aveskamp *et al.*, Stud. Mycol. 65: 36. 2010.
Basionym: *Phyllosticta sorghina* Sacc., Michelia 1: 140. 1878.
Synonym: *Phoma sorghina* (Sacc.) Boerema *et al.*, Persoonia 7: 134. 1973.

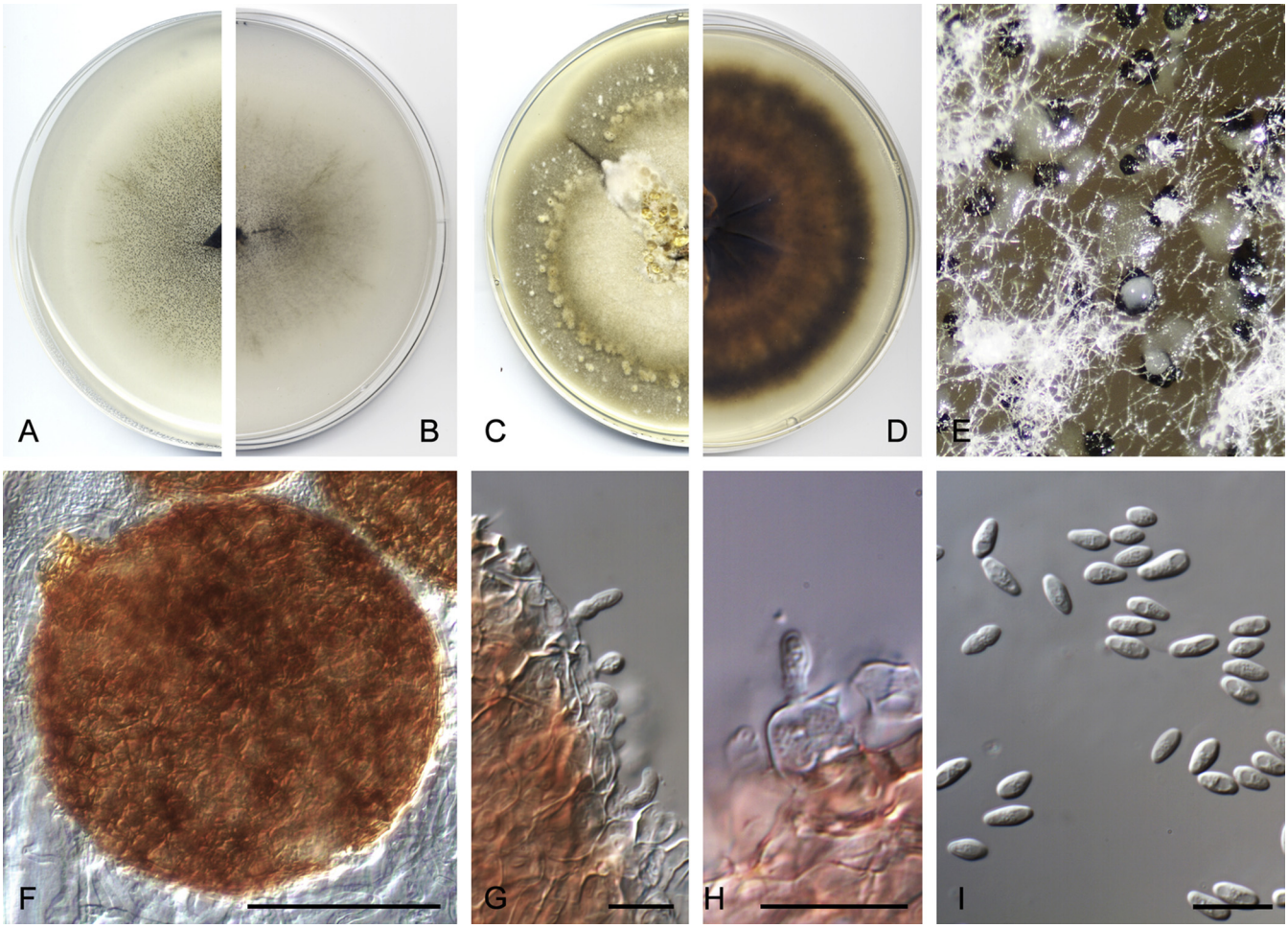

Fig. 8. *Epicoccum ovisporum* (CBS 180.80). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G, H.** Conidiogenous cells. **I.** Conidia. Scale bars: F = 100 µm. G–I = 10 µm.

Description: Boerema *et al.* (2004).

Materials examined: **France**, Antibes, from a twig of *Citrus* sp., 1966, living cultures CBS 627.68 = PD 66/926. **Puerto Rico**, Mayaguez, from *Sorghum vulgare*, Apr. 1976, R. Alconera, living cultures CBS 179.80 = PD 76/1018; **USA**, from human foot, 2010, D.A. Sutton, living cultures UTHSC DI16-288 = FMR 13692; from human bronchial wash sample, 2011, D.A. Sutton, living cultures UTHSC DI16-301 = FMR 13794.

Notes: Two strains (UTHSC DI16-288 and UTHSC DI16-301) isolated from human clinical specimens in the USA clustered with the reference strains CBS 179.80 and CBS 627.68 of *E. sorghinum*. The latter species had been reported from several different substrates mainly from vegetal materials and it seems to be a widely distributed fungus, also having been associated with human infections (Punithalingam 1985, Rai 1989). Morphologically *E. sorghinum* was described producing mainly pycnidial conidiomata. Unfortunately, our strains were sterile, and further studies are needed to resolve the taxonomy of this species.

Clade A3: *Allophoma*

Allophoma Q. Chen & L. Cai, Stud. Mycol. 82: 162. 2015.

Type species: *Allophoma tropica* (R. Schneid. & Boerema) Q. Chen & L. Cai, Stud. Mycol. 82: 162. 2015.

Allophoma cylindrispora Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB819625. Fig. 9.

Etymology: From Latin *cylindricus*-, of cylindrical shape, and *-spora*, spore.

Description: *Hyphae* brown, septate, smooth- and thin-walled, 2.5–5 µm wide. *Conidiomata* pycnidial, brown to dark brown, confluent, superficial and immersed (OA), glabrous, ovoid, 120–210 × 90–140 µm, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–4-layered, 15–30 µm thick, composed of brown to dark brown, flattened polygonal cells of 5–12.5 µm diam,. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform, 3.5–4 × 4.5–5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical, 3–4 × 2 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 36 mm diam after 7 d at 25 ± 1 °C, flattened, beige (M. 4C3) to olive brown (M. 4F3); reverse blond (M. 4C4) to olive brown (M. 4F3). Colonies on MEA reaching 25–27 mm after 7 d at 25 ± 1 °C, flattened, white (M. 4A1); reverse pale yellow (M. 4A4) to yellowish orange (M. 4B7). NaOH spot test negative. Crystals absent. Optimal temperature of growth and of sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined: **USA**, from a human eye lesion, 2007, D.A. Sutton (**holotype** CBS H-23030, ex-holotype living cultures CBS 142453 = UTHSC DI16-233 = FMR 13723).

Notes: This species forms a clade which is distinct from the closest relatives, *A. minor* and *A. piperis*. Unfortunately, the morphological distinction between these three species is difficult. Although these species differ in geography and substrate,

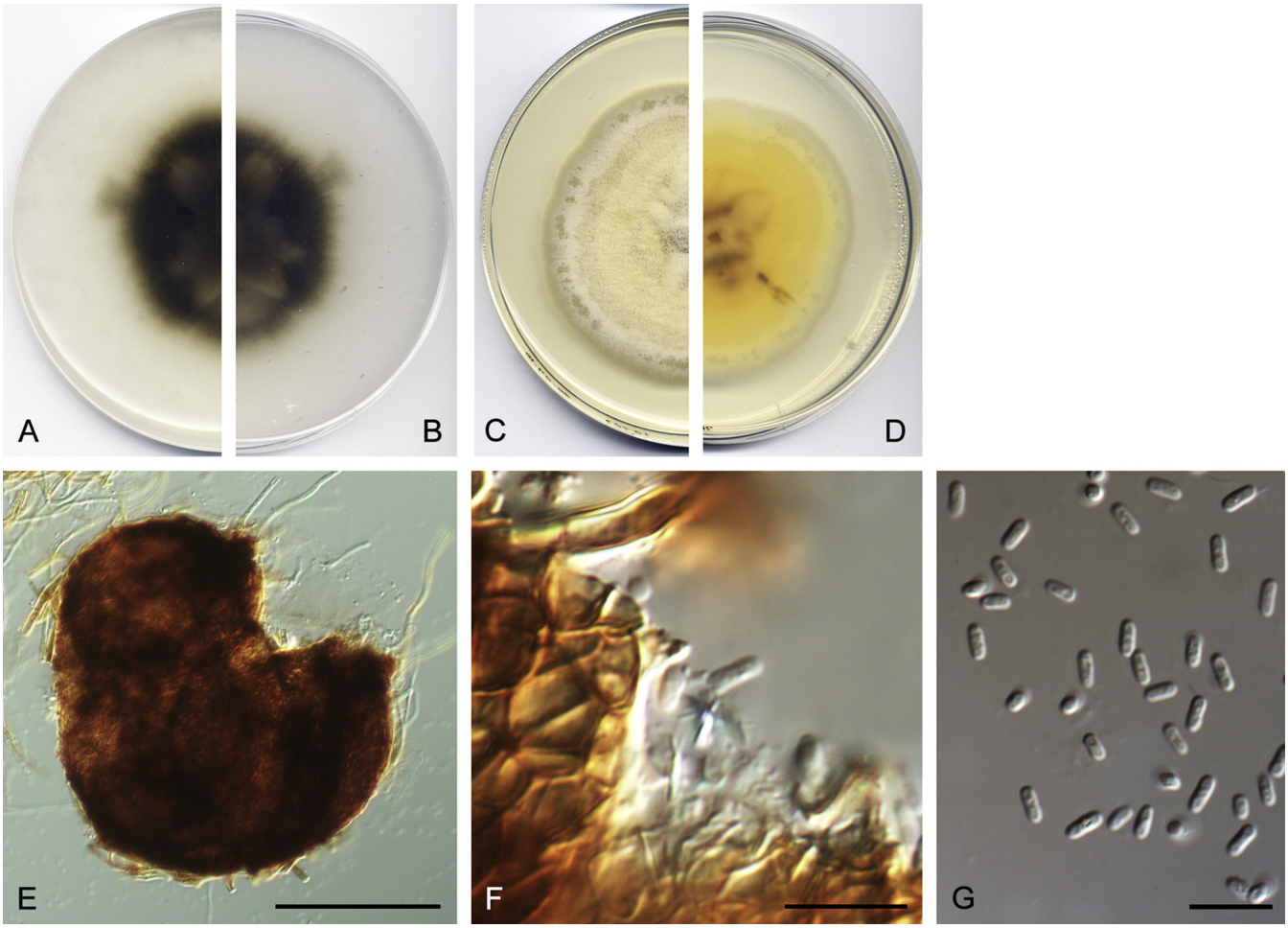

Fig. 9. *Allophoma cylindrispora* (CBS 142453). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidium. **F.** Conidiogenous cells. **G.** Conidia. Scale bars: E = 100 µm. F, G = 10 µm.

molecular data is required for species identification. *Allophoma cylindrispora* sporulates poorly in culture.

Clade A7: ***Ectophoma***

Ectophoma Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **gen. nov.** MycoBank MB819952.

Etymology: From the Greek εκτος, outside, because it is phylogenetically far from *Phoma*.

Conidiomata pycnidial, brown to dark brown, solitary or confluent, pycnidial wall of *textura angularis*, glabrous, globose to subglobose or irregular, ostiolate, with one or more short necks. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform to globose. *Conidia* aseptate, hyaline, smooth- and thin-walled, oblong to ellipsoidal, guttulate.

Type species: *Ectophoma multirostrata* (P.N. Mathur *et al.*) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel.

Ectophoma multirostrata (P.N. Mathur *et al.*) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **comb. nov.**, MycoBank MB819953. Fig. 10.
Basionym: *Sphaeronaema multirostratum* P.N. Mathur *et al.*, Sydowia 13: 146. 1959.
Synonym: *Phoma multirostrata* (P.N. Mathur *et al.*) Dorenb. & Boerema, Mycopathol. Mycol. Appl. 50: 256. 1973.
Description: Boerema *et al.* (2004).

Materials examined: **India**, Maharashtra, Poona, Talegaon, from poultry farm soil, Mar. 1959, M. J. Thirumalachar (**isotype** CBS H-7616, ex-isotype living cultures CBS 274.60 = IMI 081598 = FMR 15335); Maharashtra, Poona, Talegaon, from soil, Mar. 1959, M.J. Thirumalachar, living cultures CBS 368.65 = PD 92/1757 = FMR 15336. **The Netherlands**, Hoorn, greenhouse, from the stem of *Cucumis sativus*, Aug. 1967, G.H. Boerema, living cultures CBS 110.79 = PD 65/8875 = FMR 15342.

Notes: Aveskamp *et al.* (2009) transferred this species from *Sphaeronaema* to *Phoma*. In our study, *P. multirostrata* forms a distinct clade, separated from all genera previously described in the *Didymellaceae*. Therefore, we propose a new genus to accommodate this species.

Ectophoma pomi (A.S. Horne) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **comb. nov.** MycoBank MB819954. Fig. 11.
Basionym: *Polyopeus pomi* A.S. Horne, J. Bot., Lond. 58: 240. 1920.
Synonym: *Phoma pereupyrena* Gruyter *et al.*, Persoonia 15: 398. 1993.

Description: Boerema *et al.* (2004).

Material examined: **India**, from a leaf spot of *Coffea arabica*, 1976, deposited by J. de Gruyter (**neotype designated here** CBS H-23202, MBT377913, ex-neotype living cultures CBS 267.92 = PD 76/1014 = FMR 15346).

Notes: *Polyopeus pomi*, introduced by Horne (1920) was validly described growing on potato mush agar, and was isolated from the fruits of *Malus domestica* “Cox's Orange Pippin”, in the UK, where it produced dark spots. No illustration is available, and no type material is mentioned in the publication. Therefore, based on the original description, we propose CBS H-23202 as neotype. The fungus produces black, subglobose to irregularly

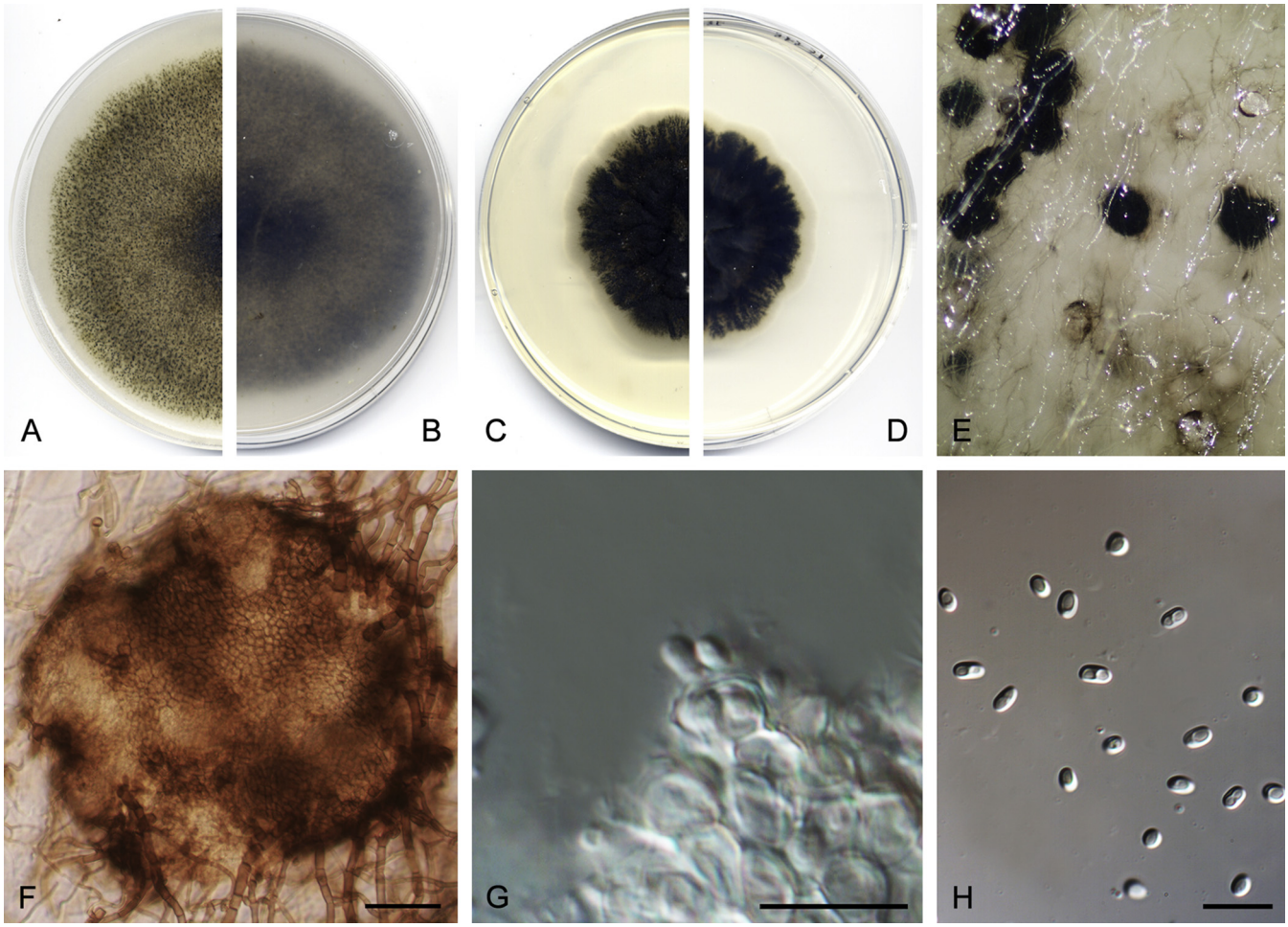

Fig. 10. *Ectophoma multirostrata* (CBS 274.60). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

shaped pycnidia with a blackish neck, and hyaline, ellipsoidal conidia, 5–9 × 2–3 µm.

Clade A8: ***Remotididymella***

Remotididymella Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, **gen. nov.** MycoBank MB819990.

Etymology: From Latin *remotus*-, distant, because it is phylogenetic far removed from the similar genus *Didymella*.

Conidiomata pycnidial, brown to dark brown, mostly confluent; pycnidial wall of *textura angularis*, mostly glabrous, globose or irregularly-shaped, with a single ostiole. *Conidiogenous cells* phialidic, hyaline, smooth-walled, globose or ampulliform. *Conidia* aseptate, hyaline, smooth- and thin-walled, allantoid or cylindrical, guttulate.

Type species: *Remotididymella destructiva* (Plowr.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel.

Remotididymella anthropophila Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB819991. Fig. 12.

Etymology: From Greek ανθρώπος-, human, and –φίλος, friend, because that fungus has been isolated from a human sample.

Description: *Hyphae* brown, smooth- and thin-walled, septate, 2.5–8 µm wide. *Conidiomata* pycnidial, apricot to pale brown, translucent, solitary or confluent, superficial (OA), glabrous, globose to subglobose, 300–400 × 250–400 µm, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–5 layered, 30–40 µm thick, composed of subhyaline to pale brown flattened polygonal cells of 5–20 µm diam,. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform to globose, 5–6 µm diam. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical, 5.5–7.5 × 1.5–2.5 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 60 mm diam after 7 d at 25 ± 1 °C, flattened, yellowish brown (M. 5E3) to greyish brown (M. 5F3); reverse greyish brown (M. 5F3). Colonies on MEA reaching 35–36 mm diam after 7 d at 25 ± 1 °C, flattened, greyishorange (M. 5B3) to pale brown (M. 5D6); reverse orange white (M. 5A2) to brownish yellow (M. 5C7). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 35 °C.

Material examined: **USA**, Texas, from human bronchial secretion, D.A. Sutton (**holotype** CBS H-23039, ex-holotype living cultures CBS 142462 = UTHSC DI16-278 = FMR 13770).

Notes: The new species *Remotididymella anthropophila* is genetically distinct from its nearest neighbour *R. destructiva*. Morphologically it is the only species of the genus that produces pale-brown pycnidia, which is unusual in phoma-like species, and it differs in substrate and location with the latter species.

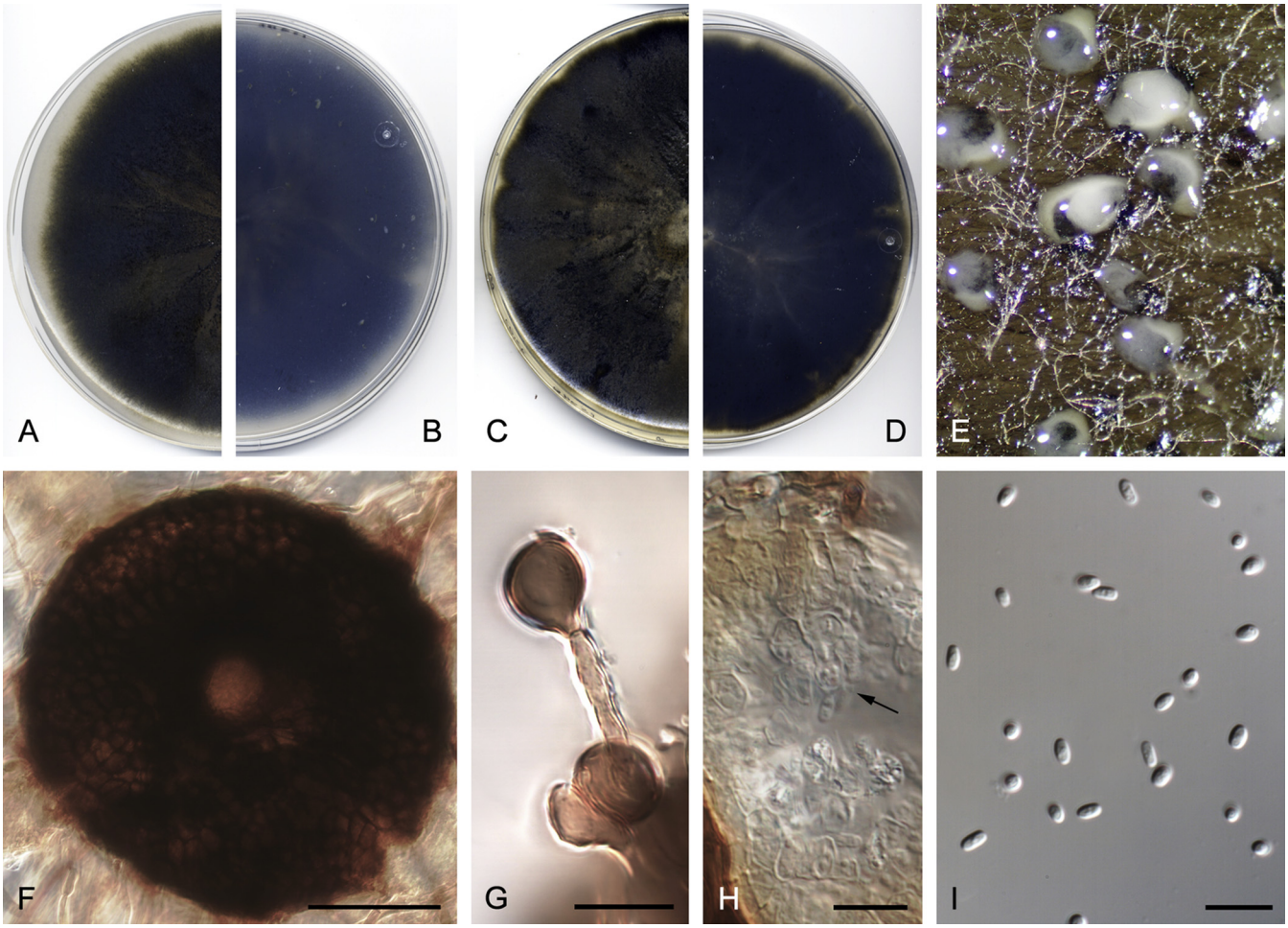

Fig. 11. *Ectophoma pomi* (CBS 267.92). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Chlamydospores. **H.** Arrow indicate the conidiogenous cell. **I.** Conidia. Scale bars: F = 50 µm. G–I = 10 µm.

Remotididymella destructiva (Plowr.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **comb. nov.** MycoBank MB819992. Fig. 13.

Basionym: *Phoma destructiva* Plowr., Gard. Chron. II 16: 621. 1881.

Synonyms: *Diplodina destructiva* (Plowr.) Petr., Annls mycol. 19(1/2): 19. 1921.

Phoma destructiva var. diversispora Gruyter *et al.*, Persoonia 18: 28. 2002.

Description from ex-epitype (CBS 378.73): *Hyphae* brown, smooth- and thin-walled, septate, 2.5–6 µm wide. *Conidiomata* pycnidial, dark brown, mostly confluent, rarely solitary, superficial or immersed (OA), glabrous, ovoid to irregularly-shaped, 120–250 × 90–180 µm, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–4 layered, 12.5–50 µm thick, composed of brown, flattened polygonal cells of 5–10 µm diam,. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform, 10–12 × 5–6 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, variable in shape, mostly allantoid to cylindrical, 3.5–8 × 2–2.5 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 21 mm diam after 7 d at 25 ± 1 °C, flattened, front and reverse dark grey (M. 4F1). Colonies on MEA reaching 10 mm diam after 7 d at 25 ± 1 °C, flattened, front and reverse olive brown to dark grey (M. 5F2). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 15 °C; maximum temperature of growth 30 °C.

Materials examined: **Lectotype** designated here (MBT378116): fig. 123, in Plowright. 1881. The Gardeners' chronicle: a weekly illustrated journal of horticulture and allied subjects (http://www.biodiversitylibrary.org/item/84372#page/639/mode/1up). **Guadeloupe**, from fruit of *Lycopersicon esculentum*, 1987, living cultures CBS 133.93 = PD 88/961 = IMI 173142. **The Netherlands**, Berkel en Rodenrijs, from a leaf of *Lycopersicon esculentum*, Oct. 1977, G.H. Boerema, living culture CBS 162.78 = PD 77/725. **Tonga**, Friendly Islands, from decaying fruit of *Lycopersicon esculentum*, 1967, G.F. Laundon (**epitype designated here** CBS H-16200, MBT377914, ex-epitype living cultures CBS 378.73 = FMR 15328 = CECT 2877).

Notes: *Phoma destructiva* was originally described by Plowright (1881), infecting fruits of *Lycopersicon esculentum* in King's Lynn, UK. Later, many representative specimens were collected from the similar hosts in other countries of Europe, and in North and South America (de Gruyter *et al.* 2002, Boerema *et al.* 2004). *Phoma destructiva* is characterised by the production of olivaceous black, globose, glabrous pycnidia with up to three papillate ostioles, hyaline, aseptate, subglobose to ellipsoidal conidia of σ = 5.8 × 2.2 µm, scarce and larger 1-septate conidia, and by the absence of chlamydospores. De Gruyter *et al.* (2002), based on morphological differences of the conidia, recognized two varieties, *destructiva* and *diversispora*. However, the isolates CBS 378.73 and CBS 133.93, representative strains of "*Phoma destructiva* var. *destructiva*", and CBS 162.78, representative of "*Phoma destructiva* var. *diversispora*", were phylogenetically and

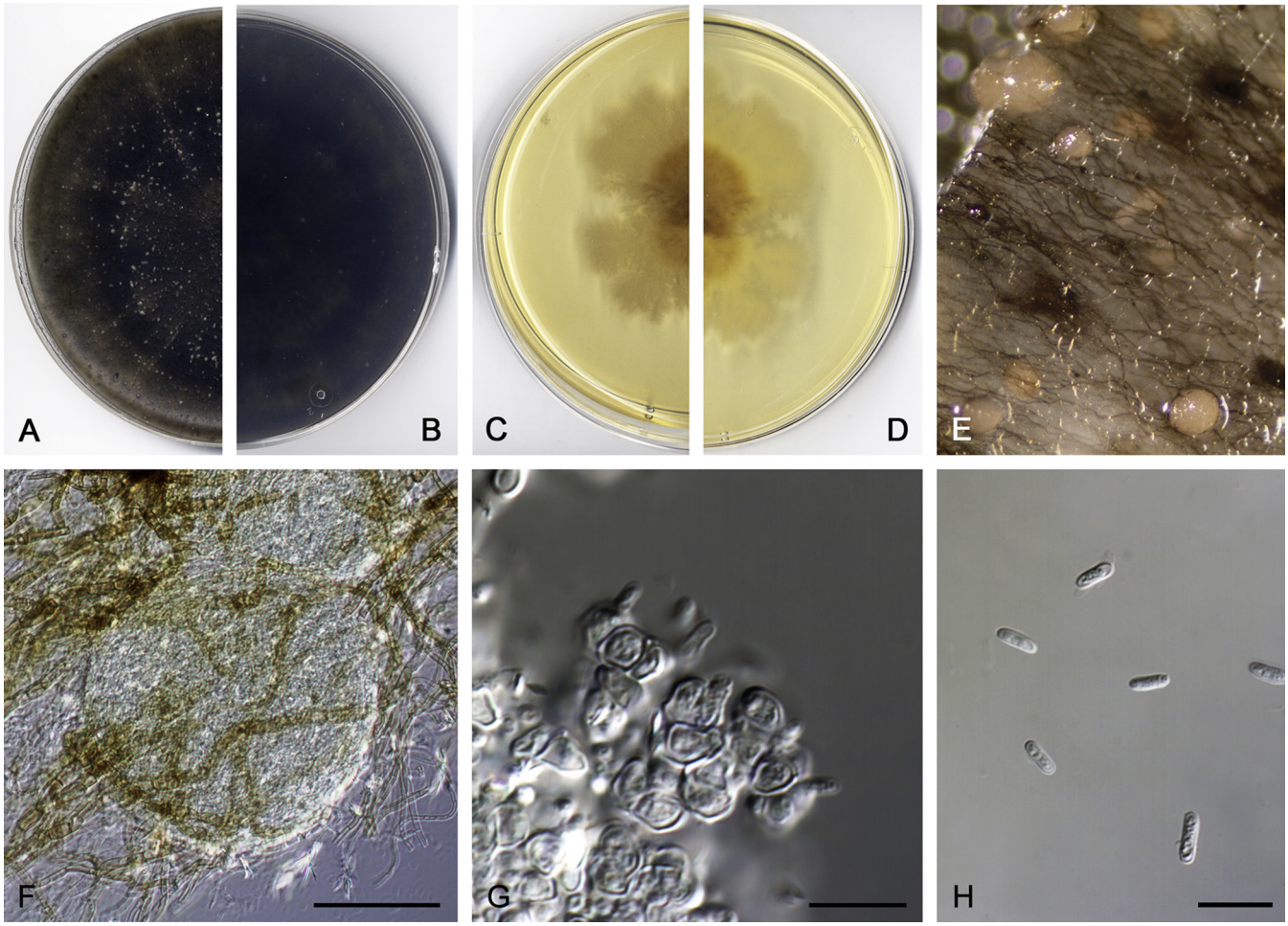

Fig. 12. *Remotididymella anthropophila* (CBS 142462). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

morphologically very similar in our study. Therefore, we did not accept these varieties, and propose CBS H-16200 as the epitype of *Remotididymella destructiva*.

Clade A9: *Similiphoma*

Similiphoma Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, **gen. nov.**, MycoBank MB820847.

Etymology: From Latin *similis*-, similar to, due to the morphological similarity with *Phoma*.

Conidiomata pycnidial, brown, confluent or solitary; pycnidium wall of *textura angularis*, glabrous or with short hyphal outgrowths, globose to subglobose, with one or two papillate ostioles. *Conidiogenous cells* phialidic, hyaline, smooth-walled, globose or ampulliform. *Conidia* aseptate, hyaline, smooth- and thin-walled, ellipsoidal to cylindrical, guttulate.

Type species: *Similiphoma crystallifera* (de Gruyter *et al.*) Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel.

Similiphoma crystallifera (Gruyter *et al.*) Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, **comb. nov.** MycoBank MB820848. Fig. 14.
Basionym: *Phoma crystallifera* Gruyter *et al.*, Persoonia 15: 393. 1993.

Description: Boerema *et al.* (2004).

Material examined: **Austria**, Kärnten, Wallersberg near Völkermarkt, from *Chamaespartium sagittale*, 1982, H.A. van der Aa (**holotype** L 992.177-456, ex-holotype living cultures CBS 193.82 = FMR 15343).

Notes: *Similiphoma crystallifera* CBS 193.82 clustered phylogenetically distant from the closest morphologically related genera *Ectophoma*, *Epicoccum* and *Phoma*. Consequently, we designated this strain as the type species of the new genus *Similiphoma*.

Clade A10: *Paraboeremia*

Paraboeremia Q. Chen & L. Cai, Stud. Mycol. 82: 183. 2015.

Type species: *Paraboeremia selaginellae* (Sacc.) Q. Chen & L. Cai.

Paraboeremia putaminum (Speg.) Q. Chen & L. Cai, Stud. Mycol. 82: 184. 2015. Fig. 15.
Basionym: *Phoma putaminum* Speg., Atti Soc. Crittog. Ital. 3: 66. 1881.

Description: de Gruyter & Noordeloos (1992).

Material examined: **Denmark**, from the rhizosphere of *Malus sylvestris*, Mar. 1968, E. Sønderhousen, living cultures CBS 130.69 = CECT 20054 = IMI 331916 = FMR 15338.

Notes: This species was introduced by Spegazzini in 1881, isolated from pine wood in Sweden, and in the last study of this species by Chen *et al.* (2015) from two reference strains (CBS 130.69 and CBS 372.91) was placed within the genus *Paraboeremia*. However, without an illustration and *rpb2* sequences, in our study, the *rpb2* sequence and the illustration were provided of the reference strain CBS 130.69, which it resembles morphologically (de Gruyter & Noordeloos 1992), but further studies are needed to clarify its typification.

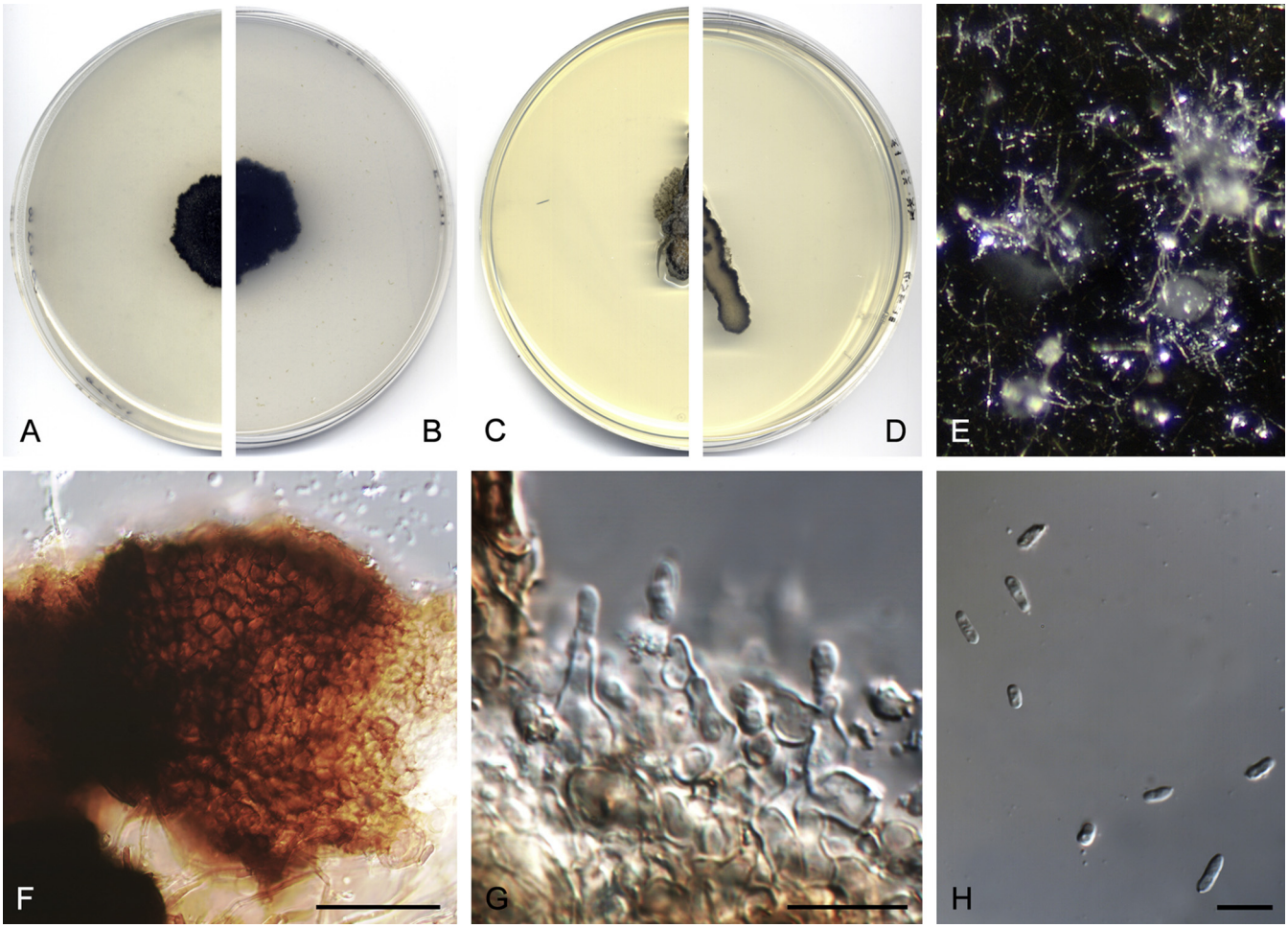

Fig. 13. *Remotididymella destructiva* (CBS 378.73). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

Paraboeremia selaginellae (Sacc.) Q. Chen & L. Cai, Stud. Mycol. 82: 184. 2015.
Basionym: *Phyllosticta selaginellae* Sacc., Malpighia 11: 304. 1897.
Synonym: *Phoma selaginellicola* Gruyter *et al.*, Persoonia 15: 399. 1993.

Description: Chen *et al.* (2015).

Material examined: **The Netherlands**, from a leaf of *Selaginella* sp., 1977, G.H. Boerema (**neotype** HMAS 246693, MBT202501, ex-neotype living cultures CBS 122.93 = PD 77/1049 = FMR 15348).

Notes: This species was already typified by Chen *et al.* (2015) providing DNA sequence data and illustrations. However, the *rpb2* sequence was not given, and therefore in the present study the *rpb2* sequence of the ex-type strain CBS 122.93 is added.

Clade A12: ***Cumuliphoma***

Cumuliphoma Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, **gen. nov.** MycoBank MB819878.

Etymology: From Latin *cumulus*-, heap or pile, in reference to the aggregated pycnidia.

Conidiomata pycnidial, brown, mostly confluent, pycnidial wall of *textura angularis*, mostly glabrous, globose or nearly so, with a single ostiole. *Conidiogenous cells* phialidic, hyaline, smooth-walled, globose to ampulliform. *Conidia* aseptate, hyaline, smooth- and thin-walled, ellipsoidal to cylindrical, guttulate. *Chlamydospores* mostly absent.

Type species: *Cumuliphoma omnivirens* (Aveskamp *et al.*) Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano.

Cumuliphoma indica Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **sp. nov.** MycoBank MB819880. Fig. 16.

Etymology: The name refers to the geographic origin of the fungus, India.

Description: *Hyphae* pale brown to brown, smooth- and thin-walled, septate, 2.5–8 µm wide. *Conidiomata* pycnidial, brown to dark brown, mostly confluent, rarely solitary, immersed (OA and MEA), glabrous, ovoid to irregularly-shaped, 150–180(–520) × 140–150(–490) µm, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 3–5-layered, 25–60 µm thick, composed of brown, flattened polygonal cells of 7–23 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, globose to ampulliform, 5–6 × 4–5.5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, ellipsoidal to cylindrical, 4–5.5 × 2–2.5 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 42 mm diam after 7 d at 25 ± 1 °C, flattened, olive brown (M. 4F3); reverse dark grey (M. 4F1). Colonies on MEA reaching 37 mm diam after 7 d at 25 ± 1 °C, flattened, brownish grey (M. 5F2) to pale grey (M. 5C2); reverse brownish grey (M. 5F2). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 15 °C; maximum temperature of growth 30 °C.

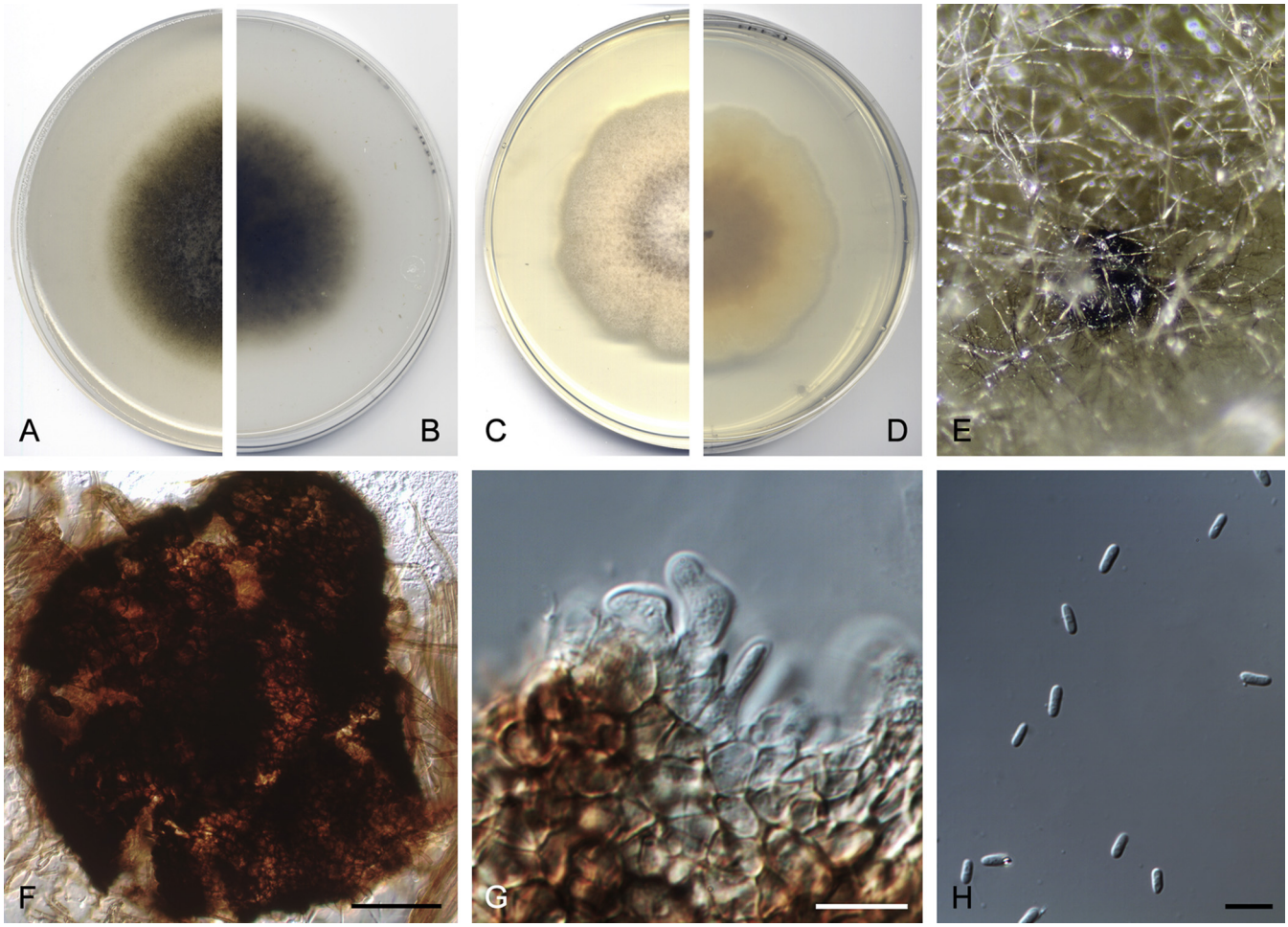

Fig. 14. *Similiphoma crystallifera* (CBS 193.82). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

Materials examined: **India**, Jabalpur, from an unknown substrate, 1977, isolated by D.P. Tiwari (**holotype** CBS H-20152, ex-holotype living cultures CBS 654.77 = FMR 15341). **Papua New Guinea**, Varirata National Park, from soil, Aug. 1995, A. Aptroot, living cultures CBS 991.95 = FMR 15331.

Notes: The isolates CBS 654.77 and CBS 991.95 were received as "*Phoma omnivirens*". However, these isolates were phylogenetically distant from the ex-type strain of *C. omnivirens* (CBS 341.86), and also both differ morphologically from the latter due to the absence of chlamydospores and micropycnidia.

Cumuliphoma omnivirens (Aveskamp *et al.*) Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, **comb. nov.** MycoBank MB819882.
Basionym: *Phoma omnivirens* Aveskamp *et al.*, Mycologia 101: 375. 2009.

Description: Aveskamp *et al.* (2009).

Material examined: **Belgium**, Gembloux, from *Phaseolus vulgaris*, 1968, isolated by L. Obando (**holotype** CBS H-20151, ex-holotype living cultures CBS 341.86 = FMR 14915).

Notes: *Cumuliphoma omnivirens* is the only species of the genus that produces chlamydospores. Phylogenetically, it is closely related to *C. pneumoniae*, but is distinct from this species in both *rpb2* and *tub2* sequences by 9 bp.

Cumuliphoma pneumoniae Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, **sp. nov.** MycoBank MB819881. Fig. 17.

Etymology: From Greek πνευμονικός-, pulmonary, due to the origin of the ex-type strain.

Description: *Hyphae* hyaline to brown, smooth- and thin-walled, septate, 2.5–6 µm wide. *Conidiomata* pycnidial, brown to dark brown, confluent, superficial (OA), glabrous, globose to subglobose, 200–240 × 200 µm, with a short papillate ostiolar neck; pycnidial wall of *textura angularis*, 3–5 layered, 25–35 µm thick, composed of brown to dark brown, flattened polygonal cells of 5–12 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform to globose, 5–6 × 5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, ovoid to cylindrical, 2.5–5 × 2 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 28 mm diam after 7 d at 25 ± 1 °C, flattened, yellowish brown (M. 5F5); reverse brownish grey (M. 5F3). Colonies on MEA reaching 27–29 mm after 7 d at 25 ± 1 °C, flattened, grey (M. 6C1), producing a diffusible greyish orange pigment; reverse dark brown (M. 6F6). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined: **USA**, from human sputum sample, D.A. Sutton (**holotype** CBS H-23031, ex-holotype living cultures CBS 142454 = UTHSC DI16-249 = FMR 13739).

Notes: *Cumuliphoma pneumoniae* was isolated from a clinical sample of the respiratory tract. This species is morphologically closely related to *C. omnivirens*, which is also the phylogenetically nearest species. However, *C. pneumoniae* does not produce chlamydospores.

Clade A13: *Juxtiphoma*

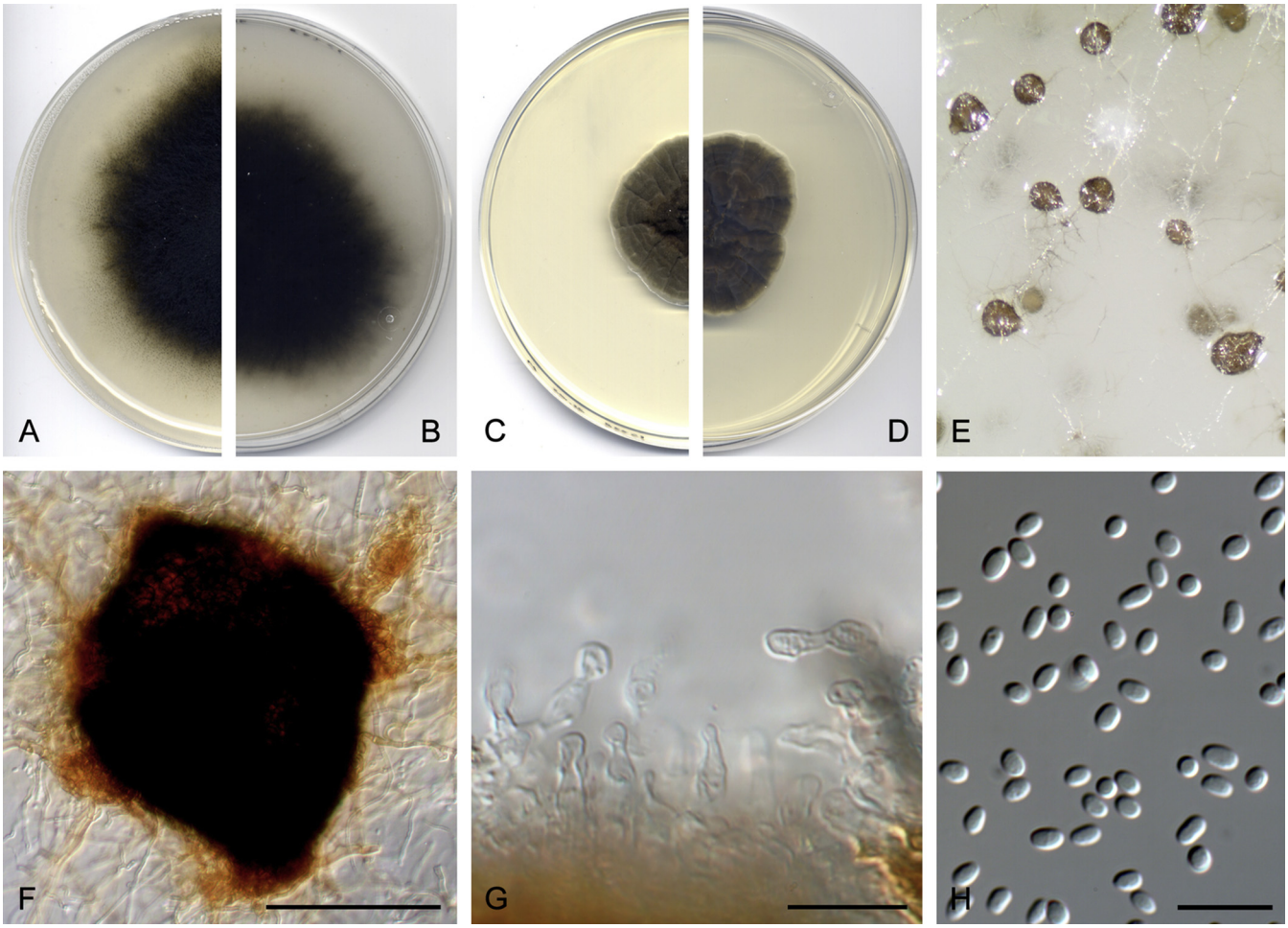

Fig. 15. *Paraboeremia putaminum* (CBS 130.69). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

Juxtiphoma Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **gen. nov.** MycoBank MB821111.

Etymology: From Latin *juxta*, next to, due to the morphological and phylogenetic similarity with *Phoma*.

Conidiomata pycnidial, brown, mostly solitary, sometimes confluent, pycnidial wall of *textura angularis*, glabrous, subglobose to conical, papillate, ostiolate. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform. *Conidia* aseptate, hyaline, smooth- and thin-walled, ovoid, ellipsoidal or cylindrical, biguttulate. *Chlamydospores* aseptate, ochraceous-brown, single or in chains, subglobose, barrel-shaped or ellipsoidal.

Type species: *Juxtiphoma eupyrena* (Sacc.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel.

Juxtiphoma eupyrena (Sacc.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **comb. nov.** MycoBank MB821112.
Basionym: *Phoma eupyrena* Sacc., Michelia 1: 525. 1879.

Description: Boerema *et al.* (2004).

Materials examined: **Germany**, Kiel-Kitzeberg, from wheat field soil, 1966, W. Gams, living cultures CBS 527.66 = FMR 15337 = ATCC 22238. **The Netherlands**, from the tuber of *Solanum tuberosum*, 1991, J. de Gruyter, living cultures CBS 374.91 = PD 78/391 = FMR 15329.

Notes: *Phoma eupyrena*, introduced by Saccardo (1879) and reported on stems of *Solanum tuberosum* (geographic origin not cited), has been revised by several authors. The description from Saccardo is minimal: blackish, depressed conical, ostiolate pycnidia with hyaline, ovoid conidia, 4 × 1.5 µm. Boerema *et al.* (2004) characterised this species morphologically and placed it in the section *Phoma*. Aveskamp *et al.* (2009) considered it phylogenetically close to "*Phoma omnivirens*", and later Aveskamp *et al.* (2010) regarded *P. eupyrena* closely related to *Microsphaeropsis*. However, in our phylogenetic tree this species formed a well-supported monophyletic clade, separate from the other genera of *Didymellaceae*. Therefore, we propose the new genus *Juxtiphoma* to accommodate this species.

Clade A14: *Vacuiphoma*

Vacuiphoma Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **gen. nov.** MycoBank MB821451.

Etymology: Based on the occurrence of empty pycnidial structures.

Conidiomata pycnidial, brown to dark brown, solitary, glabrous, subglobose or obpyriform; pycnidial wall of *textura angularis*, non-papillate.

Type species: *Vacuiphoma bulgarica* (Aveskamp *et al.*) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel.

Vacuiphoma bulgarica (Aveskamp *et al.*) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **comb. nov.** MycoBank MB821452.
Basionym: *Phoma bulgarica* Aveskamp *et al.*, Stud. Mycol. 65: 47. 2010.

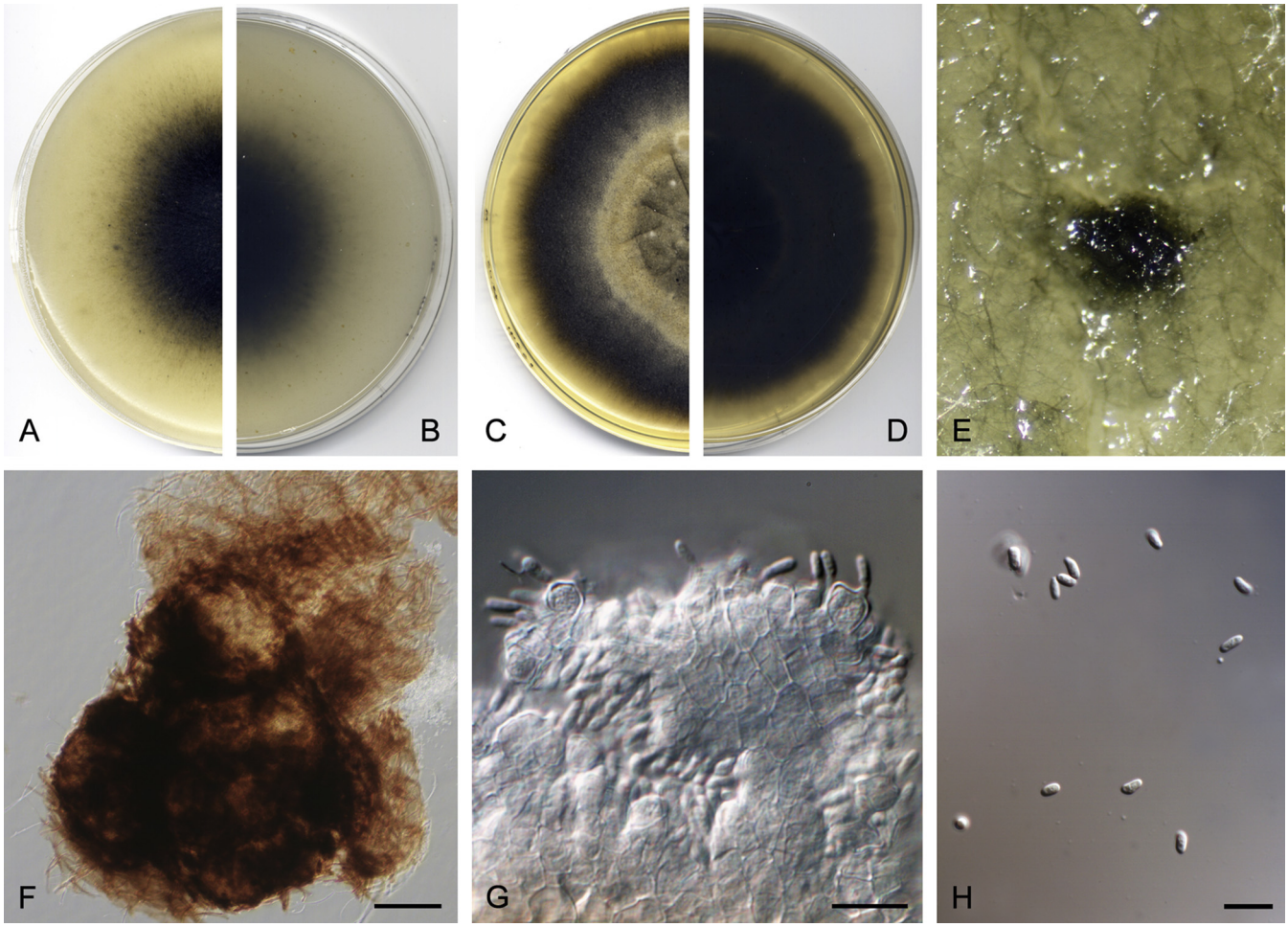

Fig. 16. *Cumuliphoma indica* (CBS 654.77). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

Description: Aveskamp *et al.* (2010).

Material examined: **Bulgaria**, Silkossia, Strandga Mountain, from leafs of *Trachystemon orientale*, 20 Jun. 1980, S. Vanev (**holotype** CBS H-20242, ex-holotype living cultures CBS 357.84 = FMR 14917).

Notes: This species was introduced by Aveskamp *et al.* (2010) within the genus *Phoma* due to the production of pycnidial conidiomata. However, this species was not able to produce conidia and remains poorly characterised. Genetically this species along with *V. oculihominis* form a distinct clade within *Didymellaceae*, thus we treat these species within the new genus *Vacuiphoma*.

Vacuiphoma oculihominis Valenzuela-Lopez, Stchigel, Guarro & Cano, **sp. nov.** MycoBank MB822113.

Etymology: The epithet refers to the human eye clinical sample, from which the fungus was isolated.

Culture sterile. *Vacuiphoma oculihominis* differs from its closest phylogenetic species, *Vacuiphoma bulgarica,* in two bp of the ITS nucleotide sequence, 12 bp of *tub*2 and 44 bp of *rpb*2, based on alignment of the concatenated four loci deposited in TreeBASE (S21115).

Culture characteristics: Colonies on OA reaching 30–34 mm diam after 7 d at 25 ± 1 °C, flattened, yellowish grey (M. 2B2) to olive grey (M. 2E2); reverse white (M. 2A1) to olive grey (M. 2E2). Colonies on MEA reaching 33 mm diam after 7 d at 25 ± 1 °C, slightly floccose, white (M. 5A1) to light orange (M. 5A4); reverse light orange (M. 5A4). NaOH spot test negative. Crystals absent. Optimal temperature of growth 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined: **USA**, Illinois, from human eye secretion, 2011, D.A. Sutton (**holotype** FMR H-13801, ex-holotype living cultures UTHSC DI16-308 = FMR 13801).

Notes: The strain UTHSC DI16-308 was recovered from a human eye clinical specimen, and remained sterile despite being cultured on different types of media. Because this strain is phylogenetically related with *V. bulgarica*, but distant from that species, it is proposed here as a new taxon.

Clade A15: *Nothophoma*

Nothophoma Q. Chen & L. Cai, Stud. Mycol. 82: 212. 2015.

Type species: *Nothophoma infossa* (Ellis & Everh.) Q. Chen & L. Cai, Stud. Mycol. 82: 213. 2015.

Nothophoma gossypiicola (Gruyter) Q. Chen & L. Cai, Stud. Mycol. 82: 213. 2015.
Basionym: *Phoma gossypiicola* Gruyter, Persoonia 18: 96. 2002.

Description: de Gruyter (2002).

Materials examined: **USA**, Texas, from a leaf of *Gossypium* sp., 1963, L.S. Bird, living cultures CBS 377.67 = FMR 14912; from human ethmoid sinus lesion, 2010, D.A. Sutton, living cultures UTHSC DI16-294 = FMR 13787.

Notes: This species was recently placed within the genus *Nothophoma* by Chen *et al.* (2015). In our study, one isolate from human clinical specimen was identified as *N. gossypiicola*, which

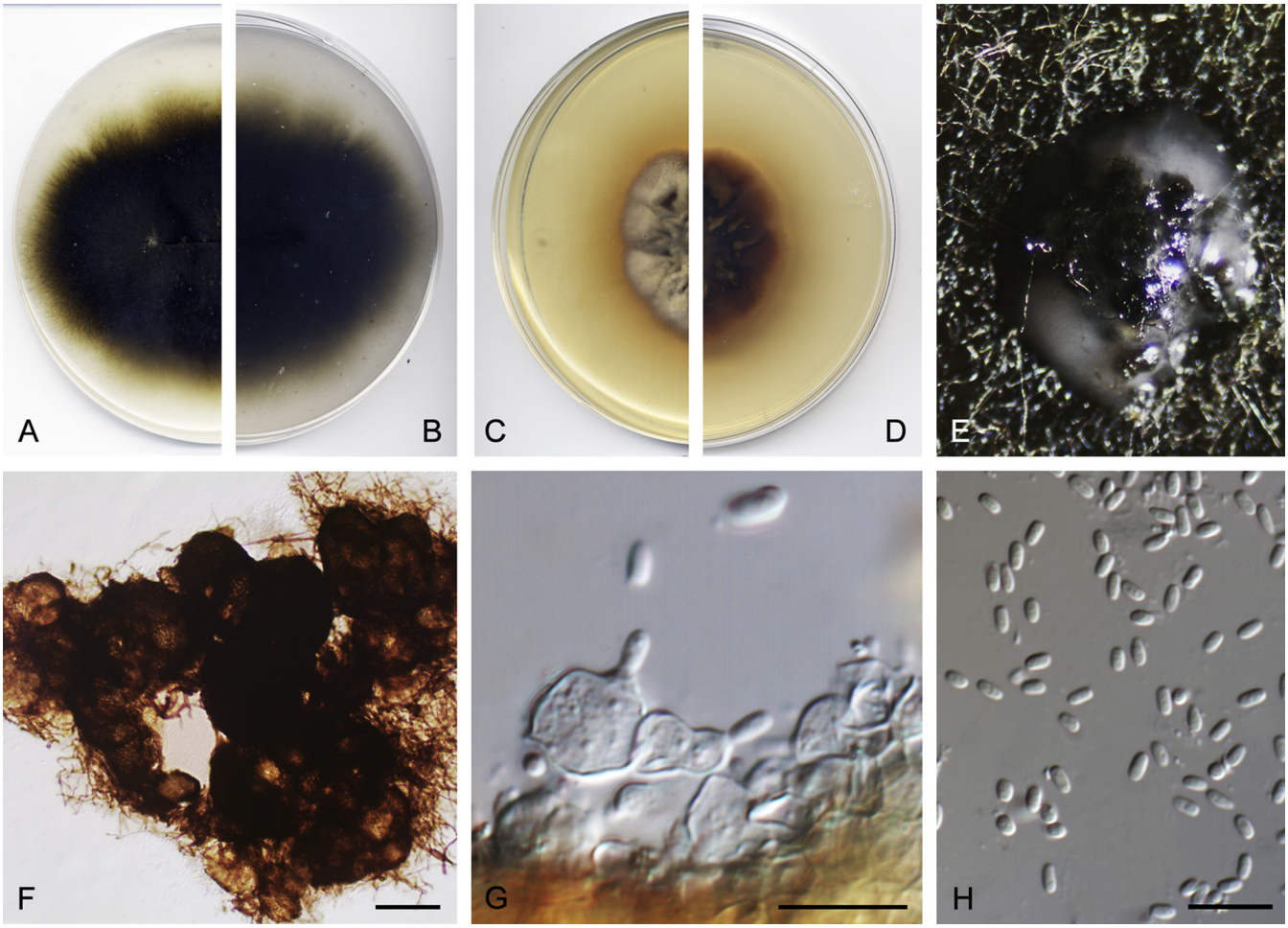

Fig. 17. *Cumuliphoma pneumoniae* (CBS 142454). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

it resembles in both morphology and DNA sequences from the reference strain CBS 377.67, isolated from the same country. This species is morphologically characterised by producing longer conidia (10–12.5 × 2.5–3.5 µm) and chlamydospores arranged in chains. However, further studies are needed to resolve its typification.

Nothophoma macrospora Valenzuela-Lopez *et al.*, Persoonia 36: 431. 2016.

Description: Crous *et al.* (2016b).

Material examined: **USA**, Arizona, Phoenix, from human respiratory secretion of a patient with pneumonia, 1 Apr. 2009, D.A. Sutton (**holotype** CBS H-22377, ex-holotype living cultures CBS 140674 = UTHSC DI16-276 = FMR 13767).

Notes: This species was recently proposed by Valenzuela-Lopez *et al.* (2016), which is phylogenetically related with *N. gossypiicola*, but differs morphologically from the latter species in pycnidial shape, conidia (up to 2 *vs* non-septate) and the absence of chlamydospores (see Crous *et al.* 2016b). Furthermore, here the sequence of *rpb2* is provided and differs in 13 bp from *N. gossypiicola*, and therefore *N. macrospora* is also phylogenetically distinct from *N. gossypiicola*.

Nothophoma quercina (Syd.) Q. Chen & L. Cai, Stud. Mycol. 82: 213. 2015.
Basionym: *Cicinobolus quercinus* Syd., Ann. Mycol. 13: 42. 1915.
Synonyms: *Ampelomyces quercinus* (Syd.) Rudakov, Mikol. Fitopatol. 13: 109. 1979.
Phoma fungicola Aveskamp *et al.*, Stud. Mycol. 65: 26. 2010.

Description: Aveskamp *et al.* (2010).

Materials examined: **Ukraine**, Crimea, in the vicinity of Feodosiya, on *Microsphaera alphitoides* from *Quercus* sp., 1979, O.L. Rudakov living cultures CBS 633.92 = ATCC 36786, VKM MF-325 = FMR 14913. **USA**, from human superficial foot lesion, 2009, D.A. Sutton, living cultures UTHSC DI16-270 = FMR 13761.

Notes: This species was already accommodated by Chen *et al.* (2015) within *Nothophoma*, and is characterised by producing globose to suboblate, glabrous, solitary pycnidia and hyaline, aseptate conidia (see Aveskamp *et al.* 2010). In our study, one human clinical strain isolated in the USA clustered with the reference strain CBS 633.92 of *N. quercina*. Morphologically it resembles the latter strain, and only a few differences in bp were genetically noted. However, both strains form a well-supported clade and were identified as the same species.

Nothophoma variabilis Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB819624. Fig. 18.

Etymology: From Latin *variabilis*, due to the variable shape of the conidia.

Description: *Hyphae* pale brown, septate, smooth- and thin-walled, 2.5–6 µm wide. *Conidiomata* pycnidial, brown, confluent, superficial (OA), glabrous, subglobose, 150–350 × 130–270 µm, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 3–6-layered, 25–35 µm thick, composed of brown to dark brown, flattened polygonal cells of

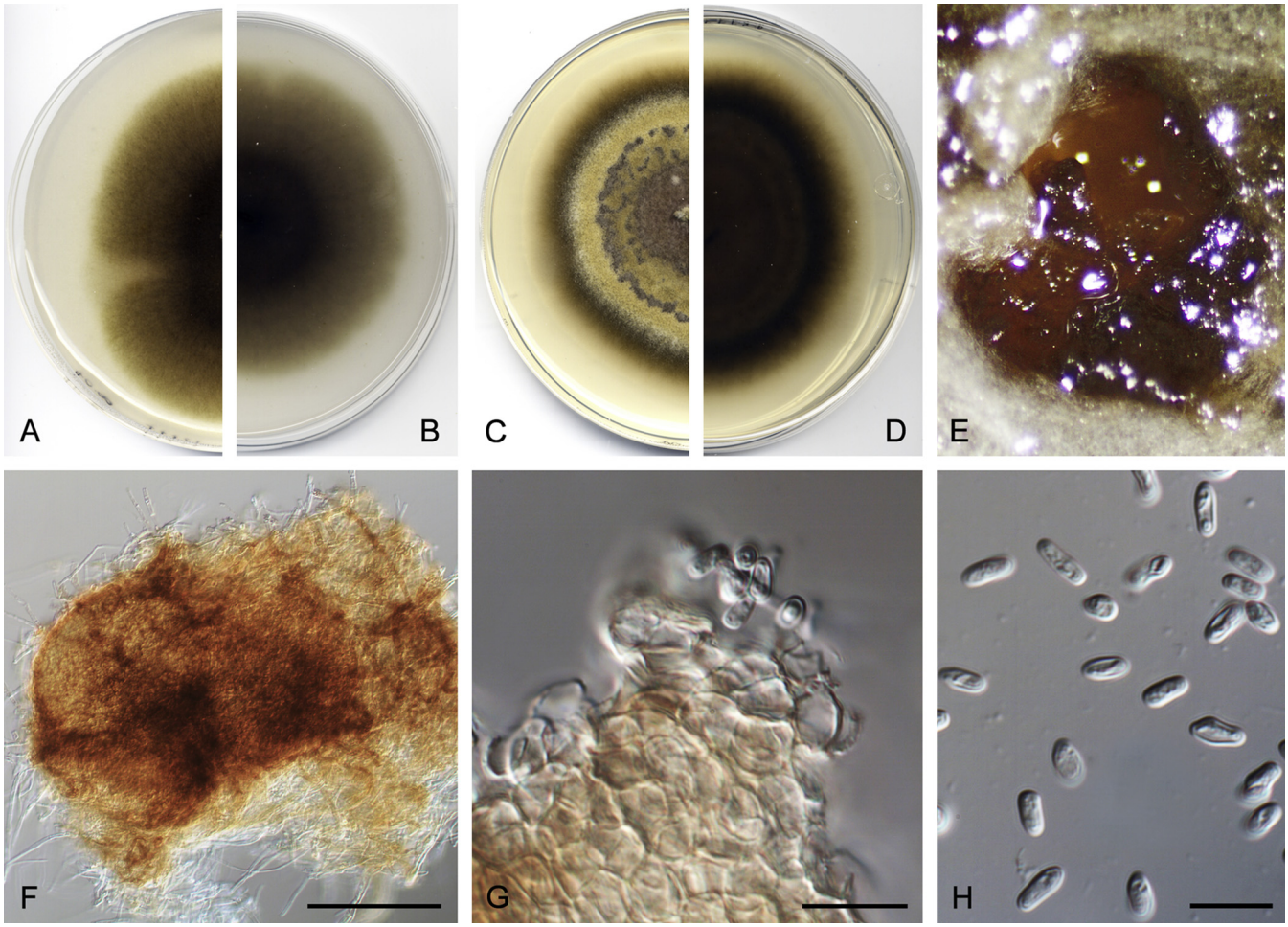

Fig. 18. *Nothophoma variabilis* (CBS 142457). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

5–20 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform, 6 × 5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, ellipsoidal to cylindrical or irregularly shaped, 4–7 × 3–3.5 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 31 mm diam after 7 d at 25 ± 1 °C, flattened, greyish yellow (M. 4B4) to olive brown (M. 4F3); reverse olive brown (M. 4F3). Colonies on MEA reaching 36 mm after 7 d at 25 ± 1 °C, flattened, olive brown (M. 4F3) to greyish yellow (M. 4C5); reverse olive brown (M. 4F3) to brownish grey (M. 4F2). NaOH spot test negative. Crystals absent. Optimal temperature of growth and of sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 35 °C.

Material examined: **USA**, from human bronchial wash sample, 2009, D.A. Sutton (**holotype** CBS H-23034, ex-holotype living cultures CBS 142457 = UTHSC DI16-285 = FMR 13777).

Notes: This species was recovered from a clinical specimen of the respiratory tract, and it is closely related to *N. anigozanthi*. Both species can be differentiated by the presence of a single pycnidial ostiole (*vs.* 1–4 in *N. anigozanthi*), absence of a neck and production of wider conidia (3–3.5 µm *vs.* 1.5–2.5 µm) in *N. variabilis*. The NaOH spot test was negative, whereas it produces a dull green to vinaceous black pigmentation in *N. anigozanthi*.

Clade A21: *Phoma*

Phoma Sacc., Michelia 2: 4. 1880. emend. Q. Chen & L. Cai, Stud. Mycol. 82: 194. 2015.
Synonym: *Atradidymella* M.L. Davey & Currah, Amer. J. Bot. 96: 1283. 2009.

Type species: *Phoma herbarum* Westend.

Phoma herbarum Westend., Bull. Acad. Roy. Sci. Belgique, Cl. Sci. 19: 118. 1852. emend. Chen *et al.*, Stud. Mycol. 82: 195. 2015.
Synonyms: *Atradidymella muscivora* M.L. Davey & Currah, Amer. J. Bot. 96: 1283. 2009.
Phoma muscivora M.L. Davey & Currah, Amer. J. Bot. 96: 1283. 2009.
Phoma cruris-hominis Punith., Nova Hedwigia 31: 135. 1979.

Description: Chen *et al.* (2015).

Materials examined: **The Netherlands**, Emmeloord, from the stem of *Rosa multiflora* cv. Cathayensis, Apr. 1965, G.H. Boerema, living cultures CBS 615.75 = PD 73/665 = IMI 199779 = FMR 15340; Naaldwijk, from a stem base of *Nerium* sp., 1986, J. de Gruyter, living cultures CBS 502.91 = PD 82/276. **UK**, from a leg of woman, Apr. 1977, Y.M. Clayton, holotype of "*Phoma cruris-hominis*" IMI 213845, living cultures CBS 377.92 = IMI 213845. **USA**, from human urine catheter, 2006, D.A. Sutton, living cultures UTHSC DI16-204 = FMR 13694; from human bronchial wash sample, 2006, D.A. Sutton, living cultures UTHSC DI16-212 = FMR 13702; from human sputum sample, 2011, D.A. Sutton, living cultures UTHSC DI16-306 = FMR 13799; from human bronchial sample, 2011, D.A. Sutton, living cultures UTHSC DI16-307 = FMR 13800; from human nail, 2010, D.A. Sutton, living cultures UTHSC DI16-319 = FMR 13812.

Notes: In this study five strains from human clinical specimens were identified as *Phoma herbarum*, all of them corresponding in morphology and genetically with the reference strains CBS 377.92, CBS 502.91 and CBS 615.75. This species was already described as an opportunistic human pathogenic fungus by Punithalingam (1979), and this fact is confirmed in our study.

Clade A24: *Xenodidymella*

Xenodidymella Q Chen *et al.*, Stud. Mycol. 82: 205. 2015.

Type species: *Xenodidymella applanata* (Niessl) Q. Chen & L. Cai.

Xenodidymella saxea (Aveskamp *et al.*) Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, **comb. nov.** MycoBank MB820831.
Basionym: *Phoma saxea* Aveskamp *et al.*, Stud. Mycol. 65: 23. 2010.

Description: Aveskamp *et al.* (2010).

Material examined: **Germany**, Oldenburg, from corroded Mediterranean marble, June 1992, J. Kuroczkin (**holotype** CBS H-20240, ex-holotype cultures CBS 419.92 = FMR 15347).

Notes: This species was introduced by Aveskamp *et al.* (2010) and placed together with "*Phoma humicola*" (currently *Xenodidymella humicola*). In our phylogenetic tree, this species was related to the *Xenodidymella* clade. Despite that this species could represent another genus based on its low phylogenetic support and morphology, more studies are needed to resolve its taxonomic placement in the *Didymellaceae*. Thus, a new combination is proposed for this species. Morphologically *X. saxea* is characterised by producing dimorphic conidia: I) aseptate, hyaline, smooth- and thin-walled, (sub-) globose, (3–)3.5–5.5 µm diam, guttulate; and II) aseptate, hyaline, smooth- and thin-walled, cylindrical to ellipsoidal, (3.5–)4.5–7(–7.5) × 2.5–3.5 (–4) µm.

Clade A25: ***Neodidymelliopsis***

Neodidymelliopsis Q. Chen *et al.*, Stud. Mycol. 82: 207. 2015.

Type species: *Neodidymelliopsis cannabis* (G. Winter) Q. Chen & L. Cai.

Neodidymelliopsis longicolla L.W. Hou *et al.*, Stud. Mycol. 87: 153. 2017.

Description: Chen *et al.* (2017).

Materials examined: **Israel**, En Avdat, Negev desert, from soil, Feb. 1996, A. van Iperen (**holotype** CBS H-23016, ex-holotype living culture CBS 382.96). **USA**, from human bronchial wash sample, 2011, D.A. Sutton, living cultures UTHSC DI16-322 = FMR 13815.

Notes: This species was recently proposed by Chen *et al.* (2017), and is characterised by producing globose to flask-shaped, glabrous or pycnidia with hyphal outgrowths. The most characteristic features include its elongated neck, the conidia that are initially hyaline and aseptate, but became pale-brown and septate with age. In our study, the strain UTHSC DI16-322 clustered with the ex-type strain of *N. longicolla.* However, no morphological comparison was possible because our strain remained sterile.

Clade A26: *Neoascochyta*

Neoascochyta Q. Chen& L. Cai, Stud. Mycol. 82: 198. 2015.

Type species: *Neoascochyta exitialis* (Morini) Q. Chen & L. Cai, Stud. Mycol. 82: 199. 2015.

Neoascochyta cylindrispora Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB819691. Fig 19.

Etymology: From Latin *cylindricus*-, of cylindrical shape, and -*spora*, spore, due to the conidial morphology.

Description: *Hyphae* pale to dark brown, septate, smooth- and thin- to thick-walled, 4–6 µm wide. *Conidiomata* pycnidial, brown to dark brown, solitary or confluent, superficial on natural substrate (palm leaf), immersed in culture (OA), glabrous, subglobose, 150–300 × 130–160 µm, bearing a single ostiolar neck; pycnidial wall of *textura angularis*, composed of brown to dark brown, flattened polygonal cells of 4.5–11.5 µm diam, 2–4 layered, 15–60 µm thick,. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform or globose, 5 × 6 µm wide. *Conidia* 0–1-septate, hyaline, smooth- and thick-walled, mostly cylindrical or slightly allantoid, 11–11.5 × 3.5–4 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 30–34 mm diam after 7 d at 25 ± 1 °C, flattened, with an entire edge, dark green (M. 28F6); reverse dark green (M. 28F6) to greenish grey (M. 28F2). Colonies on MEA reaching 25–28 mm 7 d at 25 ± 1 °C, flattened, with an entire edge, white (M. 2A1) to olive grey (M. 2E2); reverse white (M. 2A1) to dark green (M. 27F3). NaOH spot test negative. Crystals absent. Optimal temperature for sporulation, 15 °C; optimal temperature of growth 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Materials examined: **USA**, from human corneal secretion, 2013, D.A. Sutton (**holotype** CBS H-23033, ex-holotype cultures CBS 142456 = UTHSC DI16-359 = FMR 13852); from human eye secretion, 2012, D.A. Sutton, culture UTHSC DI16-352 = FMR 13845.

Notes: *Neoascochyta cylindrispora* is phylogenetically distinct from *N. desmazieri*. It differs from the latter also morphologically by its glabrous pycnidia (covered by hyphal outgrowths in *N. desmazieri*), its smaller conidiogenous cells (5–6 µm wide *vs.* 7.5–11 µm wide in *N. desmazieri*) and shorter conidia (11–11.5 µm *vs.* 8.5–18 µm in *N. desmazieri*).

Neoascochyta desmazieri (Cavara) Q. Chen & L. Cai, Stud. Mycol. 82: 198. 2015.
Basionym: *Ascochyta desmazieri* Cavara, Z. Pflanzenkrankh 3: 21. 1893 (as "*desmazieresii*").

Description: Chen *et al.* (2015).

Materials examined: **Germany**, Hohenlieth, from *Lolium perenne*, Apr. 1967, U.G. Schlösser (**neotype** HMAS 246690, ex-neotype living culture CBS 297.69). **USA**, from human respiratory tract, 2006, D.A. Sutton, living cultures UTHSC DI16-207 = FMR 13697; from unknow source of clinical sample, 2010, D.A. Sutton, living cultures UTHSC DI16-320 = FMR 13813; from human head superficial tissue sample, 2011, D.A. Sutton, living cultures UTHSC DI16-332 = FMR 13825; from human toe nail, 2011, D.A. Sutton, living cultures UTHSC DI16-341 = FMR 13834.

Notes: In this study four strains from human clinical specimens clustered with the ex-type strain of *N. desmazieri*, and those strains were morphologically and genetically identical with the type, only differing in location and substrate of isolation.

Neoascochyta tardicrescens Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **sp. nov.**, MycoBank MB819693. Fig 20.

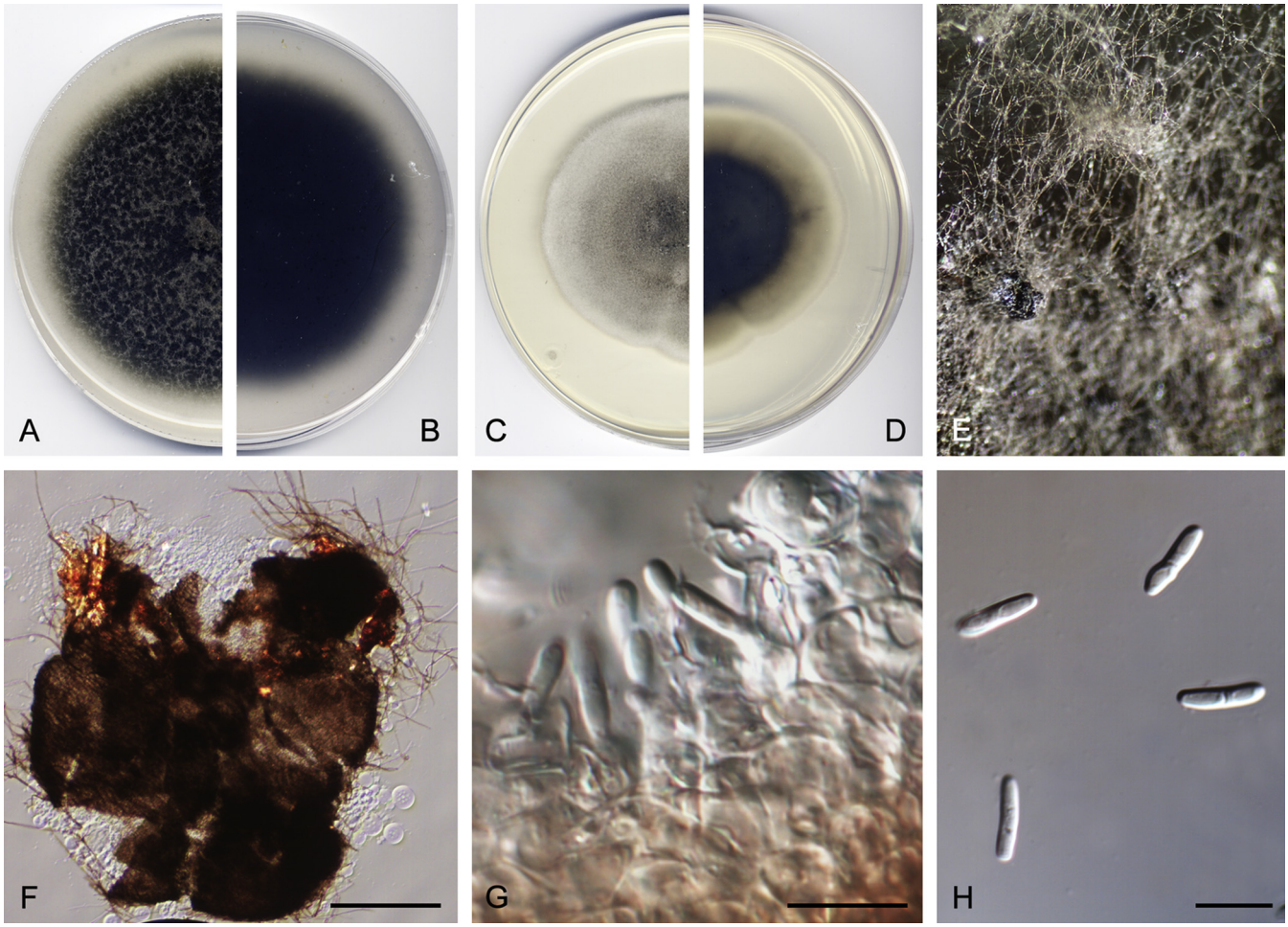

Fig. 19. *Neoascochyta cylindrispora* (CBS 142456). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

Etymology: From Latin *tarde*-, slowly, and *-crescens*, growing, in reference to the slow growing colonies.

Description: *Hyphae* pale to dark brown, septate, smooth- and thin- to thick-walled, 4–6 µm wide. *Conidiomata* pycnidial, brown to dark brown, solitary, superficial and immersed (OA), glabrous or covered with hyphal outgrows, globose to subglobose, 100–120 × 100–170 µm, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–4 layered, composed of brown to dark brown, flattened polygonal cells of 12.5–25 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform, 5–10.5 × 5–8.5 µm. *Conidia* 1-septate, hyaline, smooth- and thick-walled, cylindrical to allantoid, 10–13.5 × 3–4 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 6 mm diam after 7 d at 25 ± 1 °C, flattened, undulate, dark green (M. 27F3); reverse olive brown (M. 4F3) to brownish grey (M. 4F2). Colonies on MEA reaching 7 mm diam after 7 d at 25 ± 1 °C, flattened, undulate, yellowish grey (M. 2B2); reverse yellowish-brown (M. 5E8) to greenish grey (M. 28F2). NaOH spot test negative. Crystals absent. Optimal temperature for sporulation 15 °C; optimal temperature of growth 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Materials examined: **Norway**, Oslo, from hay, Apr. 1997, M. Torp (**holotype** CBS H-9005, ex-holotype living cultures CBS 689.97 = FMR 15352). **USA**, from human feet, 2010, D.A. Sutton, living cultures UTHSC DI16-291 = FMR 13783.

Notes: The strains CBS 689.97 and UTHSC DI16-291 grow and sporulate better at lower temperatures (around 15 °C) than at room temperature, and clearly differ morphologically from *N. argentina* in producing smaller conidiomata (100–120 × 100–170 µm *vs.* 210–390 × 140–270 ìm), in the presence of necks (absent *vs.* present), in the number of ostioles (1 *vs.* 1–3), and in their smaller conidiogenous cells (5–10.5 × 5–8.5 µm *vs.* 7.5–14.5 × 6–13.5 µm). Nonetheless, these strains formed a sister clade to *N. argentina*.

Clade C: *Cucurbitariaceae* G. Winter, Rabenhorst's Kryptogamen-Flora, Pilze-Ascomyceten 1.2: 308. 1885.

Type genus: *Cucurbitaria* Gray, Nat. Arr. Brit. Pl. (London) 1: 519. 1821.

Clade C1: *Neocucurbitaria* Wanas., E.B.G. Jones & K.D. Hyde, Mycosphere 8: 408. 2017.

Type species: *Neocucurbitaria unguis-hominis* (Punith. & M.P. English) Wanas. *et al.*

Neocucurbitaria aquatica Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **sp. nov.** MycoBank MB822114.

Etymology: The species name refers to the habitat from which the fungus was recovered (sea water).

Culture sterile. *Neocucurbitaria aquatica* differs from its phylogenetically closest species *N. unguis-hominis*, based on the

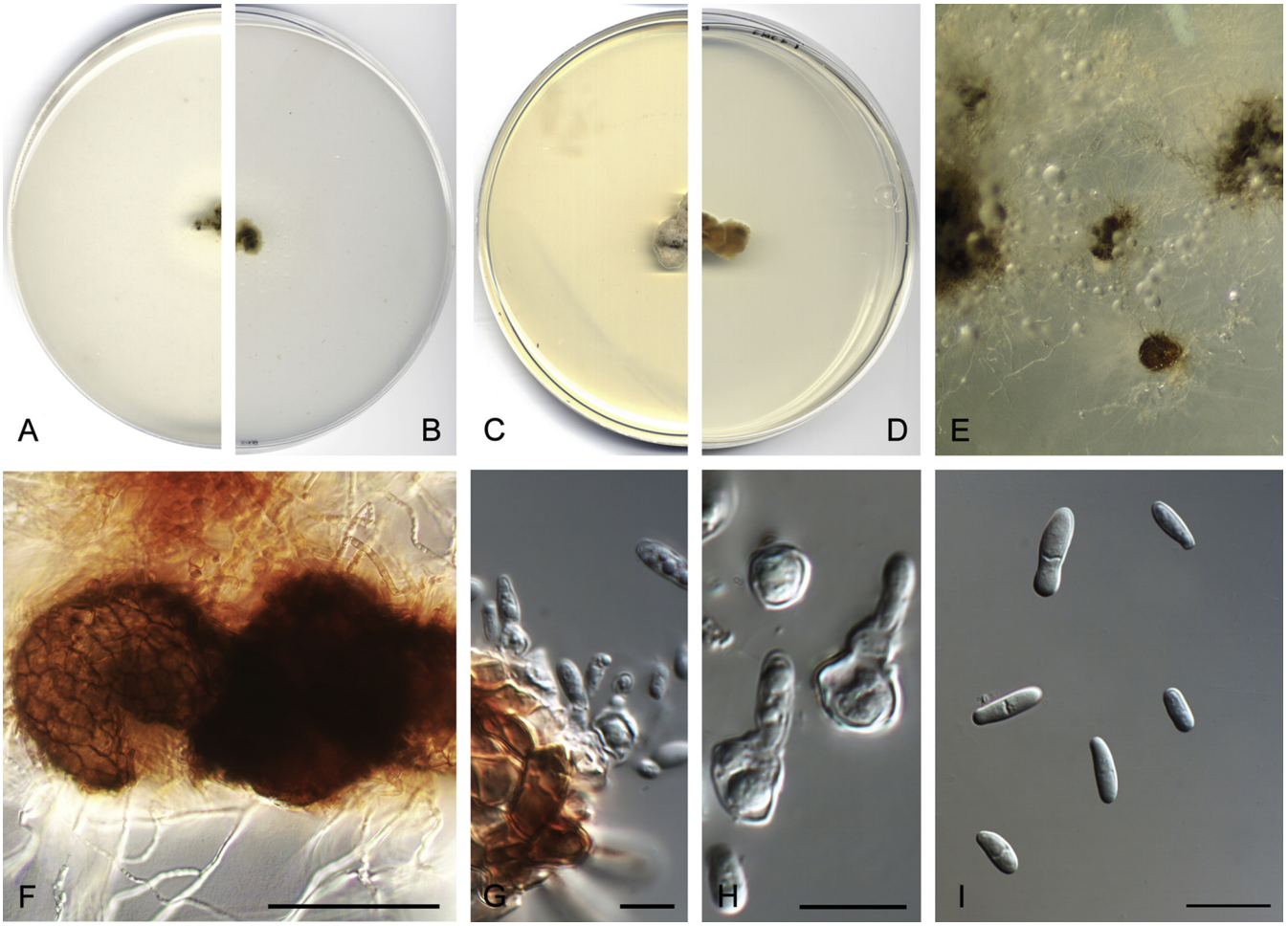

Fig. 20. *Neoascochyta tardicrescens* (CBS 689.97). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G, H.** Conidiogenous cells. **I.** Conidia. Scale bars: F = 100 µm. G–I = 10 µm.

alignment of the concatenated four loci deposited in TreeBASE (S21115): LSU position, 412 (C); ITS positions, 539 (C), 595 (A); *tub*2 positions, 1121 (G), 1170 (G), 1257 (T); *rpb*2 positions, 1351 (A), 1387 (T), 1439 (T), 1801 (C), and 1816 (C).

Culture characteristics: Colonies on OA reaching 21–24 mm diam after 7 d at 25 ± 1 °C, flattened, olive (M. 3F4); reverse olive (M. 3F4) to dark grey (M. 3F1). Colonies on MEA reaching 16–17 mm diam after 7 d at 25 ± 1 °C, flattened, yellowish grey (M. 3B2); reverse grey (M. 3B1). NaOH spot test negative. Crystals absent. Optimal temperature of growth 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 35 °C.

Material examined: **Montenegro**, Kotor bay, from sea water, Oct. 1973, M. Muntañola-Cvetkovic, (**holotype** CBS H-16102, ex-holotype living culture CBS 297.74 = FMR 14867).

Notes: *Neocucurbitaria aquatica* was previously identified as "*Pyrenochaeta quercina*" based on LSU and SSU loci sequencing (de Gruyter *et al.* 2010). However, in our phylogenetic analysis using four loci, *N. aquatica* was closely related to *Neocucurbitaria unguis-hominis*. As *N. aquatica* was recovered from sea water, and is phylogenetically unrelated to the ex-type strain of *Neocucurbitaria quercina* (CBS 115095), we propose it as a new species.

Neocucurbitaria cava (Schulzer) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **comb. nov.** MycoBank MB821491. Fig. 21.

Basionym: *Phoma cava* Schulzer, Verh. Zool.-bot. Ges. Wien 21:1248. 1871.
Synonyms: *Aposphaeria cava* (Schulzer) Sacc. & Schulzer, Syll. fung. (Abellini) 3: 174. 1884.
Coniothyrium cavum (Schulzer) Kuntze, Revis. gen. pl. (Leipzig) 3(2): 459. 1898.
Pleurophoma cava (Schulzer) Boerema *et al.*, Persoonia 16: 172. 1996.
Pyrenochaeta cava (Schulzer) Gruyter *et al.*, Mycologia 102: 1076. 2010.

Description from ex-epitype culture (CBS 257.68): *Hyphae* hyaline to brown, smooth- and thin-walled, septate, 2.5–3.5 µm wide. *Conidiomata* pycnidial, brown, solitary or confluent, semi-immersed or immersed (OA), glabrous, subglobose, 140–200 × 100–140 µm, with one ostiolar neck; pycnidial wall of *textura angularis*, composed of brown, flattened polygonal cells of 2.5–5 µm diam. *Conidiophores* hyaline, smooth-walled, straight or sinuous to slightly curved, slightly tapering towards the apex, branched at the base, 10–22 × 1.5–2.5 µm. *Conidiogenous cells* integrated to the conidiophore, phialidic, hyaline, smooth-walled, doliiform, with a more or less cylindrical collarette, up to 3 per conidiophore. *Conidia* aseptate, hyaline, smooth- and thin-walled, mostly cylindrical to slightly allantoid, 2.5–3.5 × 1–1.5 µm, guttulate.

Culture characteristics: Colonies on OA reaching 16 mm diam after 7 d at 25 ± 1 °C, flattened, olive (M. 3F4); reverse dark grey

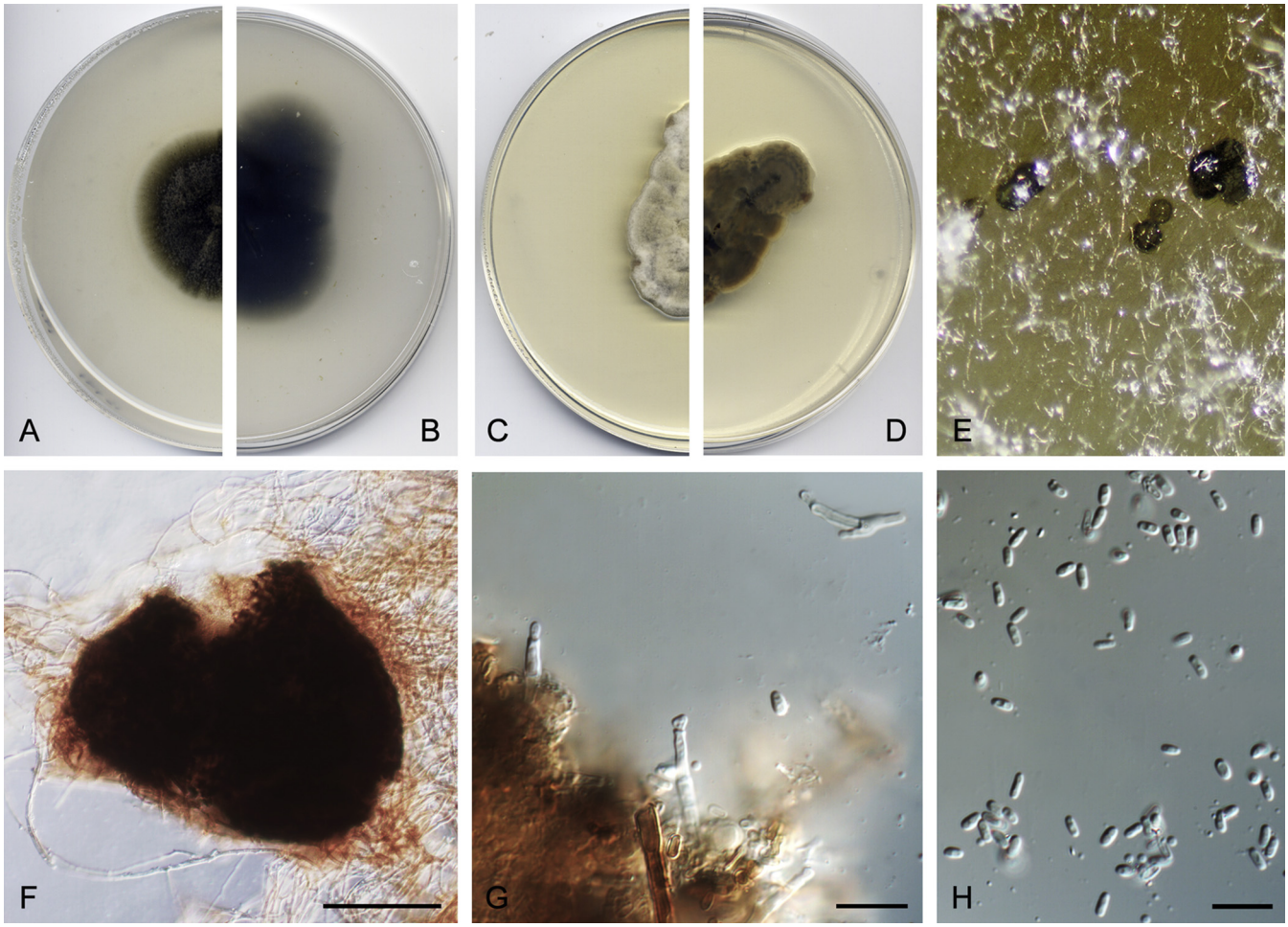

Fig. 21. *Neocucurbitaria cava* (CBS 257.68). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Conidiophores. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

(M. 3F1). Colonies on MEA reaching 14 mm after 7 d at 25 ± 1 °C, flattened, yellowish grey (M. 3B2); reverse olive brown (M. 4E4). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 35 °C.

Materials examined: **Germany**, Kiel-Kitzeberg, from wheat field soil, 1965, W. Gams (**epitype** CBS H-20320, ex-epitype living cultures CBS 257.68 = IMI 331911 = FMR 15747). **Italy**, on branch of *Quercus cerris*, M. Farras, living cultures CBS 115953 = FMR 15333.

Notes: *Pyrenochaeta cava* was epitypified by de Gruyter *et al.* (2010). In our phylogenetic analysis this species clustered in *Neocucurbitaria*, a genus recently introduced by Wanasinghe *et al.* (2017b). Therefore, we propose the new combination *N. cava*.

Neocucurbitaria hakeae (Crous) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **comb. nov.** MycoBank MB821492.
Basionym: *Pyrenochaeta hakeae* Crous, Persoonia 37: 353. 2016.

Description: Crous *et al.* (2016a).

Material examined: **Australia**, Western Australia, Denmark, Lights Beach, on leaves of *Hakea* sp., 19 Sep. 2015, P.W. Crous (**holotype** CBS H-22894, ex-holotype living cultures CBS 142109 = CPC 28920).

Notes: In our phylogenetic tree, this species forms a sister clade to *N. cava*. Therefore, we propose a new combination to accommodate this species in the genus *Neocucurbitaria*. Morphologically, *N. hakeae* resembles *N. unguis-hominis*, but the former species is the only species of the genus that produces pale brown conidiophores.

Neocucurbitaria irregularis Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB819769. Fig. 22.

Etymology: From Latin *irregularis*, irregular, referring to the shape of its conidia.

Description: *Hyphae* brown, smooth- and thin-walled, septate, 2–5 µm wide. *Conidiomata* pycnidial, brown, solitary or confluent, superficial (OA), glabrous, subglobose to ovoid, 75–130 × 65–120 µm, with 3–4 papillate ostiolar necks, pycnidial wall of *textura angularis*, 2–5 layered, 10–35 µm thick, composed of brown, flattened polygonal cells of 3–12 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, doliiform, 2.5 × 3.5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, ellipsoidal to cylindrical, 2.5–4 × 1.5–2. µm, guttulate.

Culture characteristics: Colonies on OA reaching 17–18 mm diam after 7 d at 25 ± 1 °C, flattened, olive brown (M. 4F6); reverse brownish grey (M. 4F2). Colonies on MEA reaching 11 mm after 7 d at 25 ± 1 °C, flattened, pale yellow (M. 4A3); reverse pale yellow (M. 4A4) to greyish yellow (M. 4C6). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 35 °C.

Material examined: **USA**, from human arm injury, 2000, D.A. Sutton (**holotype** CBS H-23029, ex-holotype living cultures CBS 142791 = UTHSC DI16-229 = FMR 13719).

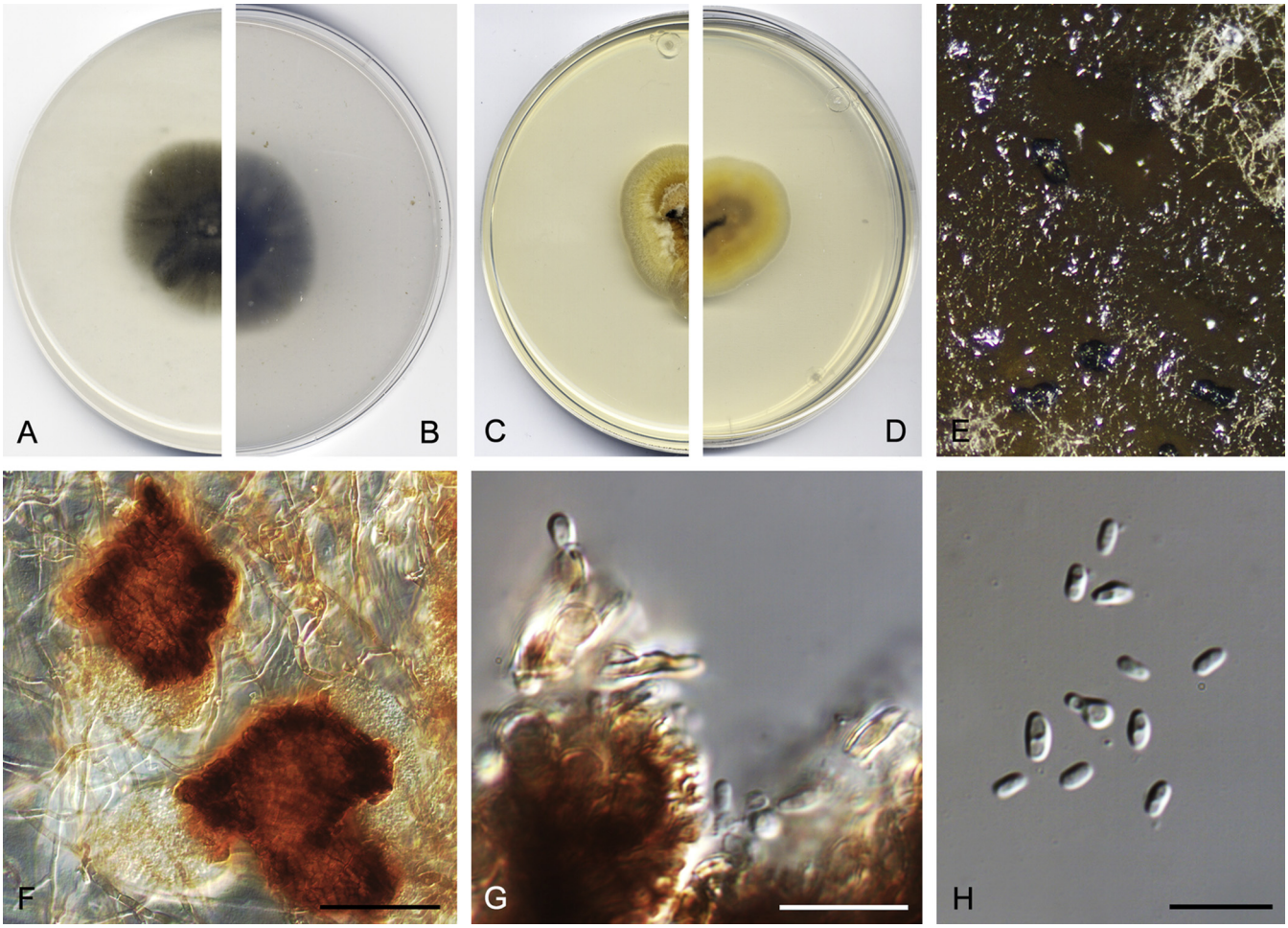

Fig. 22. *Neocucurbitaria irregularis* (CBS 142791). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

Notes: *Neocucurbitaria irregularis* is proposed to accommodate a clinical isolate previously identified as "*Pyrenochaeta unguis-hominis*" (Valenzuela-Lopez *et al.* 2016). This isolate forms a basal clade together with *N. keratinophila* and *N. unguis-hominis*. However, it is morphologically well-differentiated from the latter two species, by having small, simple conidiogenous cells instead of filiform conidiophores.

Neocucurbitaria keratinophila (Verkley *et al.*) Valenzuela-Lopez, Stchigel, Guarro & Cano, **comb. nov.** MycoBank MB821494.
Basionym: *Pyrenochaeta keratinophila* Verkley *et al.*, Revta Iberoamer. Micol. 27: 24. 2010.

Description: Verkley *et al.* (2010).

Material examined: **Spain**, Alicante, from human corneal scrapings (keratitis), Mar. 2007, A. Rodriguez & J. Guarro (**holotype** CBS H-20122, ex-holotype living CBS 121759 = FMR 9444).

Notes: This species described by Verkley *et al.* (2010) was isolated from a human corneal specimen with a case of keratitis. Morphologically it resembles *Pyrenochaeta*. However, in our phylogenetic analyses this species clustered close to *N. irregularis*. Therefore, we propose a new combination for this fungus in *Neocucurbitaria*.

Neocucurbitaria quercina (Kabát & Bubák) Wanas. *et al.*, Mycosphere 8: 412. 2017. Fig. 23.
Basionym: *Pyrenochaeta quercina* Kabát & Bubák, Hedwigia 52: 342. 1912.

Description taken from Bubák & Kabát (1912), which is based on the holotype: *Conidiomata* pycnidial dark brown, solitary or confluent, setose, globose, 150–220 µm diam. *Setae* dark brown, tapered towards the apex, erect or decumbent, smooth- and thick-walled, up to 65 µm long, 5 µm broad at the base. *Conidiophores* cylindrical, tapered toward the apex, erect or slightly curved, hyaline, 25 × 3–3.5 µm. *Conidia* aseptate, hyaline, bacilliform, 2–3 × 1.5 µm.

Description from the ex-neotype culture (CBS 115095): *Hyphae* brown, smooth- and thin-walled, septate, 2.5–5 µm wide. *Conidiomata* pycnidial brown, solitary or confluent, superficial (OA), mostly glabrous or covered with somewhat shortest setae, globose, 70–90 µm diam, 100–230 × 90–130 µm when ovoid, with 1–2 papillate ostiolar necks; pycnidial wall of *textura angularis*, composed of brown, flattened polygonal cells of 3–12 µm diam; setae brown, erect, rounded at the top, septate, thin-walled, 7–10 × 2.5–3.5 µm,. *Conidiophores* hyaline, smooth-walled, straight or sinuous to slightly curved, slightly tapering towards the apex, branched at the base, 6.5–14 × 2–3 µm. *Conidiogenous cells* terminal and lateral on the conidiophore, phialidic, hyaline, smooth-walled, ampulliform when terminal, with a more or less cylindrical collarette, up to 4 per conidiophore. *Conidia* aseptate, hyaline, smooth- and thin-walled, ovoid to cylindrical, 1.5–3 × 1.2–1.5 µm, guttulate.

Culture characteristics: Colonies on OA reaching 21 mm diam after 7 d at 25 ± 1 °C, flattened, olive (M. 3F4); reverse dark grey (M. 3F1). Colonies on MEA reaching 12 mm after 7 d at 25 ± 1 °C,

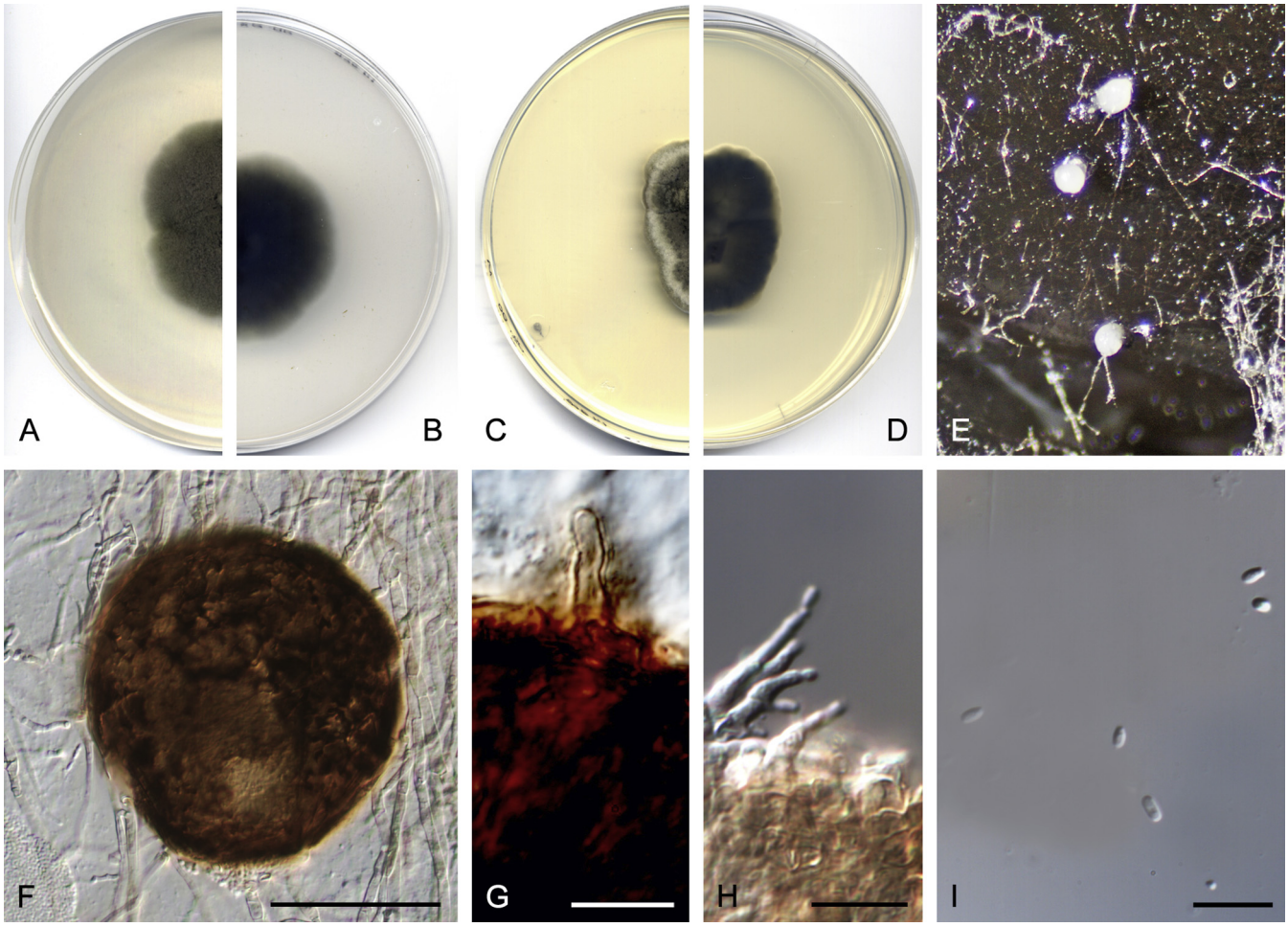

Fig. 23. *Neocucurbitaria quercina* (CBS 115095). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Setae. **H.** Conidiophores. **I.** Conidia. Scale bars: F = 50 µm. G–I = 10 µm.

flattened, olive (M. 3F4) to pale grey (M. 3B1); reverse dark grey (M. 3F1). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 15 °C; maximum temperature of growth 35 °C.

Material examined: **Italy**, from *Quercus robur*, Nov 1971, S. Mutto Accordi (**neotype designated here** CBS H-23205, MBT377969, ex-neotype living cultures CBS 115095 = FMR 14868).

Notes: Bubák & Kabát (1912) described *Pyrenochaeta quercina* from *Quercus cerris* leaves, in Bukovina forest, Moldavia. The holotype is apparently missing. We studied the isolate CBS 115095, identified previously as *P. quercina* by de Gruyter *et al.* (2010), which has been recovered from *Quercus robur* in Italy. Recently, Wanasinghe *et al.* (2017b) transferred *P. quercina* to *Neocucurbitaria*. In our phylogenetic tree this strain clustered with *N. cava* and *N. hakeae*, confirming the right placement into *Neocucurbitaria*. Because the strain CBS 115095 was isolated from a related host to that of the basionym (both are different species of oaks), we designated this strain as the neotype for *Pyrenochaeta quercina*, in order to stabilize the taxonomy of the species.

Neocucurbitaria unguis-hominis (Punith. & M.P. English) Wanas. *et al.*, Mycosphere 8: 412. 2017. Fig. 24.
Basionym: *Pyrenochaeta unguis-hominis* Punith. & M.P. English, Trans. Br. mycol. Soc. 64: 539. 1975.

Description: Punithalingam & English (1975).

Materials examined: **The Netherlands**, Utrecht, from lung sample of *Agapornis* sp., C. Hoek, living cultures CBS 111112 = FMR 14866. **USA**, unknown substrate, 2006, D.A. Sutton, living cultures UTHSC DI16-213 = FMR 13703. **Wales**, Cardiff, from air sample, Apr. 1974, G.H. Boerema, living cultures CBS 112.79 = IMI 386095 = PD 74/1018 = FMR 15748.

Notes: *Pyrenochaeta unguis-hominis* was established by Punithalingam & English (1975) for a fungus recovered from a human toe-nail. Later, Wanasinghe *et al.* (2017b) considered this the type species of *Neocucurbitaria*. Interestingly, the three strains studied by us were able to grow and sporulate at 37 °C, being the only species of the genus that displays such abilities.

Clade C2: *Paracucurbitaria*

Paracucurbitaria Valenzuela-Lopez, Stchigel, Guarro & Cano, **gen. nov.** MycoBank MB821453.

Etymology: From Greek παρα-, beside, referring to the morphological similarity with the asexual morph of *Cucurbitaria*.

Conidiomata pycnidial, pale brown to brown, solitary or confluent, superficial or semi-immersed, pycnidial wall of *textura angularis*, 2–4 layered, glabrous or ornamented, subglobose to ovoid, ostiolate. *Conidiophores* if present, septate, hyaline, straight or sinuous to slightly curved, slightly tapering towards the apex. *Conidiogenous cells* integrated in the conidiophore, phialidic, hyaline, smooth-walled, ampulliform when terminal, with a more or less cylindrical collarette, several per conidiophore. *Conidia* aseptate, hyaline, smooth- and thin-walled, ellipsoidal to cylindrical, guttulate.

Type species: *Paracucurbitaria corni* (Bat. & A.F. Vital) Valenzuela-Lopez, Stchigel, Guarro & Cano.

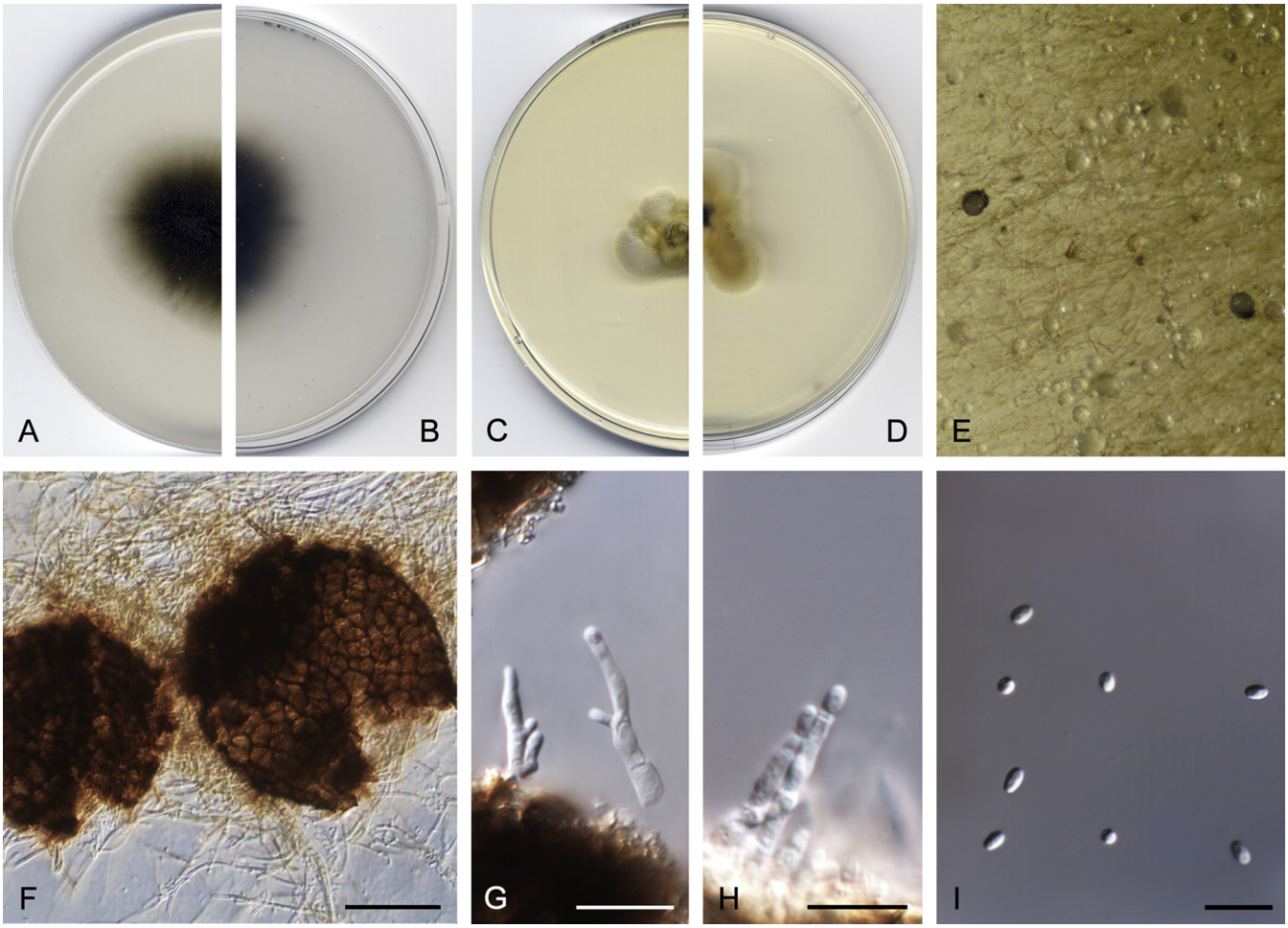

Fig. 24. *Neocucurbitaria unguis-hominis* (CBS 112.79). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G, H.** Conidiophores. **I.** Conidia. Scale bars: F = 50 µm. G–I = 10 µm.

Paracucurbitaria corni (Bat. & A.F. Vital) Valenzuela-Lopez, Stchigel, Guarro & Cano, **comb. nov.** MycoBank MB821454. Fig. 25.

Basionym: *Plenodomus corni* Bat. & A.F. Vital, Anais Soc. Biol. Pernambuco 15: 420. 1957.

Synonyms: *Phoma riggenbachii* Boerema & J.D. Janse, Eur. J. For. Path. 11: 428. 1981.

Pyrenochaeta corni (Bat. & A.F. Vital) Boerema, Loer. & Hamers, Persoonia 16: 158. 1996.

Description from reference strain (CBS 248.79): *Hyphae* hyaline to pale brown, smooth- and thin-walled, septate, 2.5–4 µm wide. *Conidiomata* pycnidial, pale brown to brown, solitary or confluent, superficial or semi-immersed (OA), glabrous, globose to subglobose, 110–210 × 110–190 µm diam, with 2–5 ostiolar necks; pycnidial wall of *textura angularis*, initially pseudoparenchymatous, scleroplectenchymatous with the age (mainly on MEA), 3–4 layered, 15–30 µm thick, composed of brown to dark brown, flattened polygonal cells of 3–6 µm diam. *Conidiophores* branched at the base, septate, hyaline, straight or sinuous to slightly curved, slightly tapering towards the apex, 6.5–18 µm long. *Conidiogenous cells* integrated in the conidiophore, phialidic, hyaline, smooth-walled, doliiform or ampulliform, 3.5–7.5 × 1.3–3.5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, mostly cylindrical or rarely ovoid, 1.8–4 × 1.2–1.6 µm, guttulate.

Culture characteristics: Colonies on OA reaching 14 mm diam after 7 d at 25 ± 1 °C, flattened, olive (M. 2E6); reverse olive (M. 2E6) to dark grey (M. 2F1). Colonies on MEA reaching 10 mm after 7 d at 25 ± 1 °C, flattened, olive brown (M. 4D6) to dark grey (M. 4F1); reverse olive brown (M. 4D6) to dark grey (M. 4F1). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined **The Netherlands**, Scheerwolde, from *Fraxinus excelsior* with bacterial canker (also from *Prays fraxinella*), 1978, deposited by G. H. Boerema, living cultures CBS 248.79 = PD 78/1092 = FMR 16593.

Notes: *Plenodomus corni* was erected by Batista & Vital (1957) as a new species on branches of *Cornus sanguinea* from Hungary, and it was characterised by producing brown to black, solitary or clustered, mostly immersed, glabrous, globose to subglobose, pycnidial conidiomata of 115–135 µm diam, with a pseudoparenchymatous wall 12.5–20 µm thick, composed of polygonal to subglobose cells of 2.5–4 µm diam, with phialidic, hyaline, filiform or flask-shaped conidiogenous cells, 3.5–6 × 1–2 µm, and hyaline, bacilliform, 1.5–3 × 1 µm conidia. Later, Janse (1981) isolated a similar fungus from *Fraxinus excelsior* with a bacterial canker, and also from dead, discoloured tissue surrounding galleries and holes of *Prays fraxinella* (ash bud moth). This fungus (living culture CBS 248.79) was considered by Boerema *et al.* (1981) as the same taxon as *Plenodomus corni*. However, a new name was necessary to transfer *Plenodomus corni* to the genus *Phoma* because the species name was occupied (*Phoma corni* Fuckel ex Sacc.). The strain CBS 248.79 was characterised by the production of

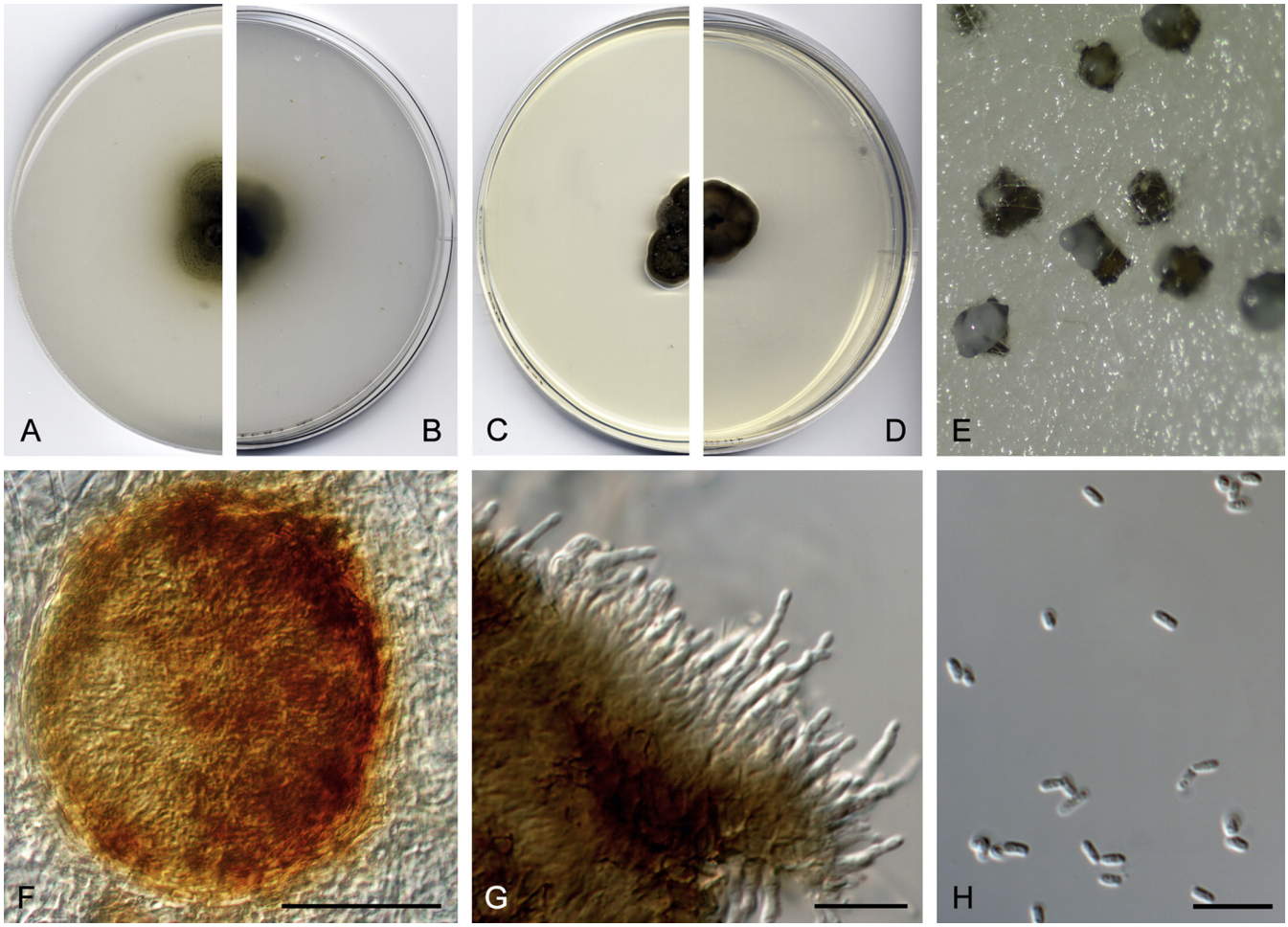

Fig. 25. *Paracucurbitaria corni* (CBS 248.79). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium **G.** Conidiophores. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

pycnidial conidiomata with a scleroplectenchymatous wall, variable in size and in shape, 100–200 µm diam, and of aseptate conidia (measuring 2.1–2.6 × 0.8–1.2 µm), produced on elongated conidiogenous cells. However, CBS 248.79 shows some morphological variation depending of the culture media employed: on MEA it shows a scleroplectenchymatous wall as given in the original description by Janse, but on OA it resembles the description given by Batista & Vital (1957), but it does not produce setose pycnidia as mentioned by Boerema *et al.* (1996). The strain CBS 248.79 forms a distinct monophyletic clade within the *Cucurbitariaceae*. Therefore, we propose the new combination, *Paracucurbitaria corni*.

Paracucurbitaria italica Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **sp. nov.** MycoBank MB822116. Fig. 26.

Etymology: The name of the species refers to the country of origin of the fungus, Italy.

Description: *Hyphae* hyaline to pale brown, smooth- and thin-walled, septate, 2.5–4 µm wide. *Conidiomata* pycnidial, brown, solitary or confluent, superficial or semi-immersed (OA), covered by hyphal outgrowths, subglobose to ovoid, 190–240 × 170–190 µm diam, with 1–2 ostiolar necks; pycnidial wall of *textura angularis*, 2–4 layered, 10–15 µm thick, composed of brown to dark brown, flattened polygonal cells of 5–13 µm diam. *Conidiophores* septate, hyaline, straight or sinuous to slightly curved, slightly tapering towards the apex, 15–20 µm long. *Conidiogenous cells* phialidic, hyaline, smooth-walled, filiform or flask-shaped, 4–9 × 2–3.5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, ellipsoidal to cylindrical, 2.5–3 × 1–1.5 µm, guttulate.

Culture characteristics: Colonies on OA reaching 13 mm diam after 7 d at 25 ± 1 °C, flattened, olive (M. 2E6); reverse olive (M. 2E6) to dark grey (M. 2F1). Colonies on MEA reaching 11 mm after 7 d at 25 ± 1 °C, flattened, white (M. 2A1); reverse white (M. 2A1). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined: **Italy**, Rende, from *Olea europaea* leaves, 26 Feb. 1992, C. Candiano (**holotype** CBS H-16104, ex-holotype living cultures CBS 234.92 = FMR 14869).

Notes: The strain CBS 234.92 was previously identified as "*Pyrenochaeta corni*" by de Gruyter *et al.* (2010). However, this strain is phylogenetically distinct from its closest relative, *Paracucurbitaria corni*, and differs morphologically by the production of ornamented conidiomata (covered with hyphal outgrowths *vs.* glabrous). Consequently, we propose CBS 234.92 as the ex-type strain of *Paracucurbitaria italica* sp. nov.

Clade C3: *Allocucurbitaria*

Allocucurbitaria Valenzuela-Lopez, Stchigel, Guarro & Cano, **gen. nov.** MycoBank MB821455.

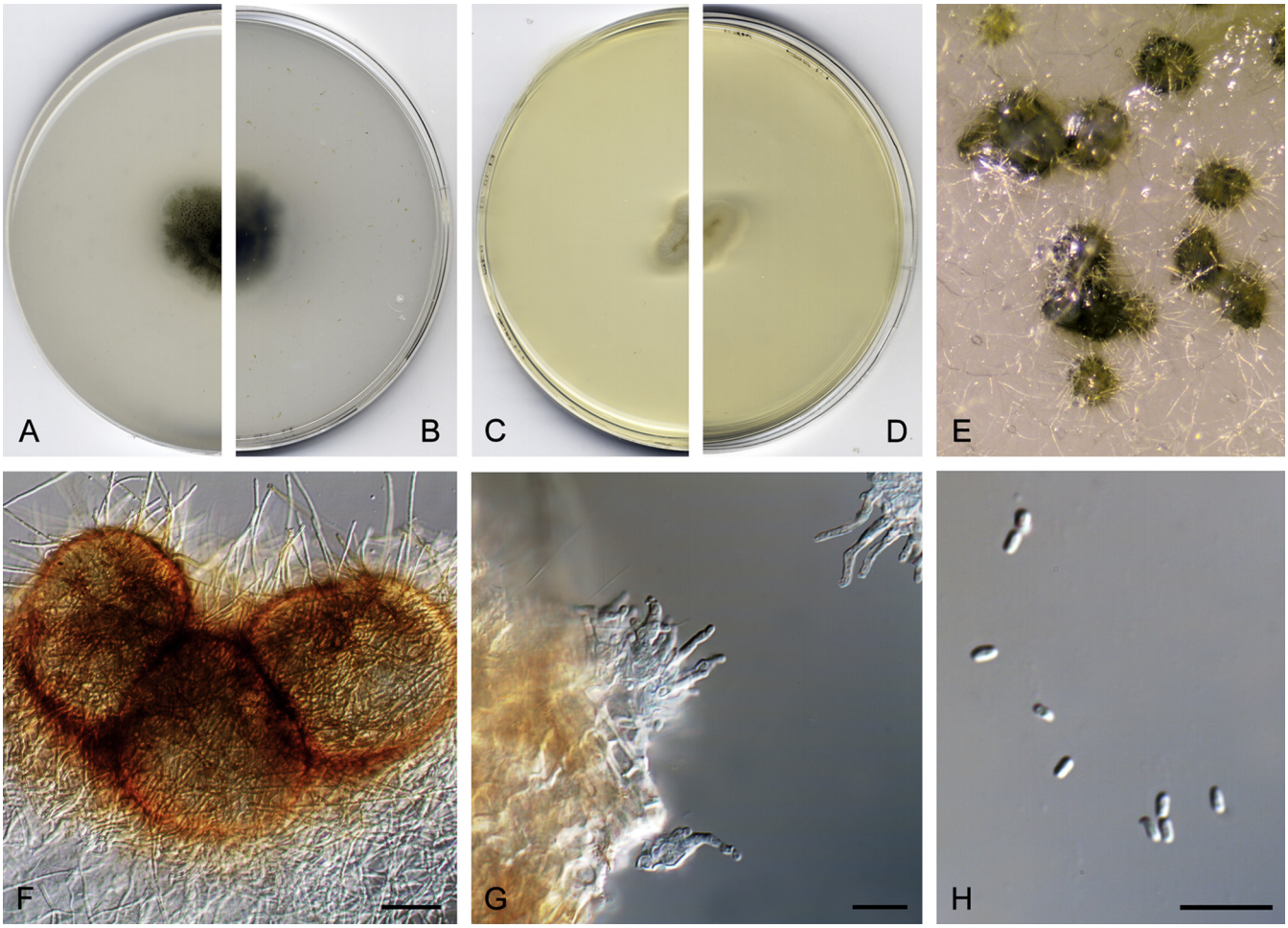

Fig. 26. *Paracucurbitaria italica* (CBS 234.92). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Conidiophores. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

Etymology: From Greek *àλλο-*, different, due to is related but phylogenetically and morphologically different to the genus *Cucurbitaria*.

Conidiomata pycnidial, brown, solitary or confluent, superficial, pycnidial wall of *textura angularis*, glabrous, subglobose to ovoid, ostiolate. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical to allantoid, guttulate.

Type species: *Allocucurbitaria botulispora* Valenzuela-Lopez, Stchigel, Guarro & Cano.

Allocucurbitaria botulispora Valenzuela-Lopez, Stchigel, Guarro & Cano, **sp. nov.** MycoBank MB819770. Fig. 27.

Etymology: From Latin *botulus-*, sausage, and *-spora*, spores, due to the shape of the conidia.

Description: *Hyphae* pale brown, smooth- and thin-walled, septate, 1.5–2.5 µm wide. *Conidiomata* pycnidial, brown, confluent, superficial (OA), glabrous, subglobose to ovoid, 60–160 × 60–120 µm diam, with 1–2 papillate ostiolar necks; pycnidial wall of *textura angularis*, 2–4 layered, 10–30 µm thick, composed of pale brown to brown, flattened polygonal cells of 3–10 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform, 5–8 × 2–2.5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical to allantoid, 3–5 × 1–1.5 µm, guttulate.

Culture characteristics: Colonies on OA reaching 26–29 mm diam after 7 d at 25 ± 1 °C, flattened, greyish yellow (M. 4C6); reverse olive brown (M. 4D5). Colonies on MEA reaching 22 mm after 7 d at 25 ± 1 °C, slightly floccose, yellowish white (M. 4A2); reverse pale orange (M. 5A3) to deep orange (M. 5A8). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 15 °C; maximum temperature of growth 37 °C.

Material examined: **USA**, from human scab on leg, 2009, D.A. Sutton (**holotype** CBS H-23028, ex-holotype living cultures CBS 142452 = UTHSC DI16-273 = FMR 13764).

Notes: The strain CBS 142452 (= UTHSC DI16-273) was originally assigned to *Pyrenochaeta* (Valenzuela-Lopez *et al.* 2016). Morphologically, this strain displays a morphology more similar to phoma-like taxa (with glabrous pycnidia) than to species of *Pyrenochaeta* (because of its setose conidiomata). In our phylogenetic analysis, this fungus was placed in an uncertain taxonomic position within *Cucurbitariaceae*. Therefore, we proposed to accommodate CBS 142452 as a new species of the new genus *Allocucurbitaria*.

Clade D: *Pseudopyrenochaetaceae* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **fam. nov.** MycoBank MB820426.

Etymology: From Latin *pseudo-*, resembling but not equalling, because the morphological similarity to *Pyrenochaeta*.

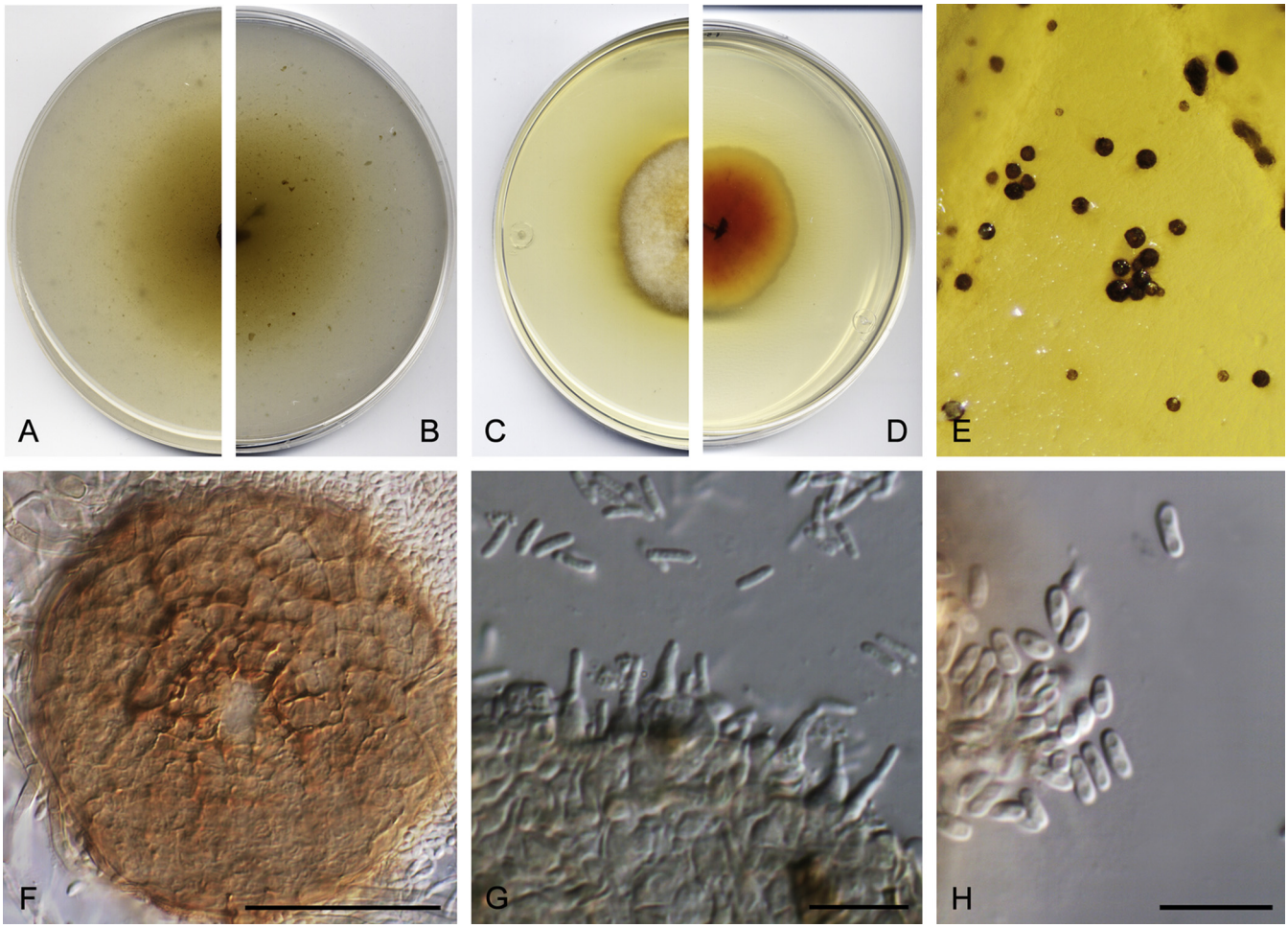

Fig. 27. *Allocucurbitaria botulispora* (CBS 142452). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiophores. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

Conidiomata pycnidial, brown to dark brown, solitary, setose, globose to subglobose, papillate, ostiolate. *Conidiophores* simple, filiform, septate. *Conidiogenous cells* phialidic, intercalary, disposed along the conidiophores as short side projections. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical to allantoid.

Type genus: *Pseudopyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano.

Pseudopyrenochaeta Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **gen. nov.** MycoBank MB820427.

Etymology: The name refers to the morphological similarity with the genus *Pyrenochaeta*.

Conidiomata pycnidial, brown to dark brown, solitary, setose, globose to subglobose, with a papillate ostiolar neck. *Conidiophores* hyaline, simple, filiform, septate. *Conidiogenous cells* phialidic, hyaline, intercalary along the conidiophore, arising as very short lateral projections immediately below the transverse septa. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical to allantoid.

Type species: *Pseudopyrenochaeta lycopersici* (R.W. Schneid. & Gerlach) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano.

Pseudopyrenochaeta lycopersici (R.W. Schneid. & Gerlach) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **comb. nov.** MycoBank MB820431.
Basionym: *Pyrenochaeta lycopersici* R.W. Schneid. & Gerlach, Phytopath. Z. 56: 121. 1966.

Description: Schneider & Gerlach (1966).

Material examined: **Germany**, Berlin, from *Lycopersicon esculentum* root, Nov. 1971, R. Schneider & G.H. Boerema (**isotype** CBS H-17628, ex-isotype culture CBS 306.65 = FMR 15746 = BBA 9911 = DSM 62931).

Notes: In previous studies, the ex-isotype strain of *Pyrenochaeta lycopersici* (CBS 306.65) was phylogenetically located in the *Cucurbitariaceae* (de Gruyter *et al.* 2010, Wanasinghe *et al.* 2017b). However, de Gruyter *et al.* (2013) placed it as *incertae sedis*. According to our results, *P. lycopersici* falls phylogenetically outside the family *Cucurbitariaceae* and represents a new genus, *Pseudopyrenochaeta*, in the new family, *Pseudopyrenochaetaceae*.

Pseudopyrenochaeta terrestris Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB822117.

Etymology: The species name refers to soil, the substrate from which the fungus was recovered.

Culture sterile. *Pseudopyrenochaeta terrestris* differs from its closest phylogenetic species, *P. lycopersici*, based on the alignment of the concatenated four loci deposited in TreeBASE (S21115), by six bp of LSU, 20 bp of ITS, 16 bp of *tub*2, and 47 bp of *rpb*2.

Culture characteristics: Colonies on OA reaching 22 mm diam after 7 d at 25 ± 1 °C, flattened, olive grey (M. 3E3); reverse olive grey (M. 3E3) to dark grey (M. 3F1). Colonies on MEA reaching 11 mm after 7 d at 25 ± 1 °C, slightly flattened, white (M. 3A1); reverse yellowish grey (M. 3C2). NaOH spot test negative. Crystals absent.

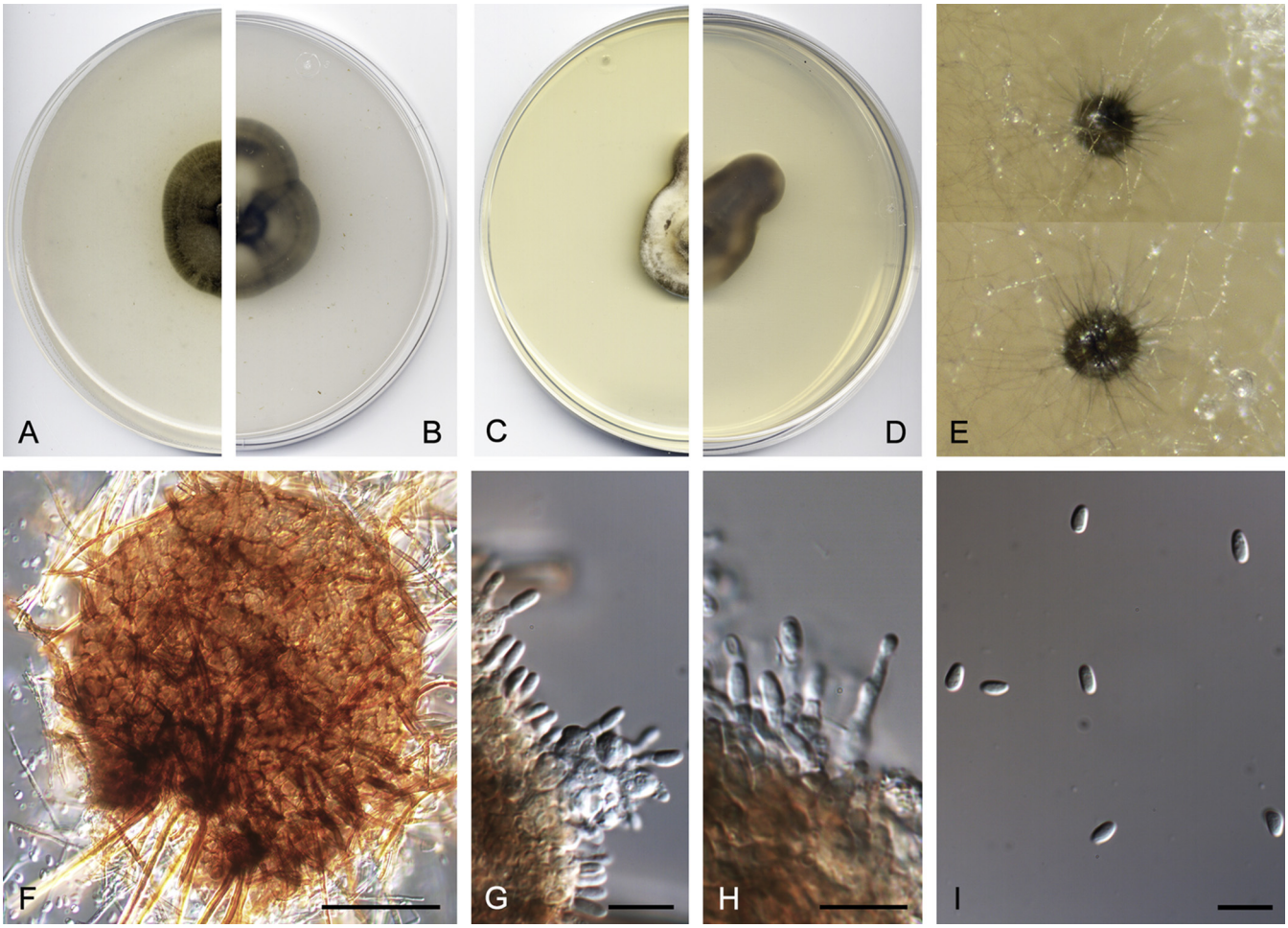

Fig. 28. *Neopyrenochaeta acicola* (CBS 812.95). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G, H.** Conidiogenous cells. **I.** Conidia. Scale bars: F = 50 µm. G–I = 10 µm.

Material examined: **The Netherlands**, Naaldwijk, from greenhouse soil, Feb. 1972, L.H. Kaastra-Höweler (**holotype** FMR H-15327, ex-holotype living cultures CBS 282.72 = FMR 15327).

Notes: The strain CBS 282.72, deposited as "*Pyrenochaeta lycopersici*", clustered with the ex-type strain of *Pseudopyrenochaeta lycopersici*. However, both strains differ significantly in all nucleotide sequences of the phylogenetic markers used in the present study. Therefore, strain CBS 282.72 is proposed here as the new species *Pseudopyrenochaeta terrestris*.

Clade E: *Neopyrenochaetaceae* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, **fam. nov.** MycoBank MB820416.

Etymology: Relating to the distinct phenotypic and genetic relationship to the genus *Pyrenochaeta* and its relatives.

Conidiomata pycnidial, pale brown to brown, solitary, pycnidial wall of *textura angularis*, setose, ovoid to globose, with a non-papillate or papillate ostiolar neck. *Conidiogenous cells* phialidic, ampulliform or lageniform. *Conidia* aseptate, hyaline, smooth- and thin-walled, ovoid to subcylindrical.

Type genus: *Neopyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano.

Neopyrenochaeta Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **gen. nov.** MycoBank MB820313.

Etymology: Referring to its morphological similarity to the genus *Pyrenochaeta*.

Conidiomata pycnidial, pale brown to brown, solitary, pycnidial wall of *textura angularis*, setose, ovoid to globose, ostiolate. *Conidiogenous cells* phialidic, ampulliform or lageniform. *Conidia* aseptate, hyaline, smooth- and thin-walled, ovoid to subcylindrical.

Type species: *Neopyrenochaeta acicola* (Moug. & Lév.) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano.

Neopyrenochaeta acicola (Moug. & Lév.) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **comb. nov.** MycoBank MB820314. Fig. 28.
Basionym: *Vermicularia acicola* Moug. & Lév. apud Léveillé, Annls Sci. nat. (Bot.) III, 9:259. 1848 (as "Moug. Lév."; non *Phoma acicola* sensu Saccardo), Syll. Fung. 3:100. 1884 [as "(Lév.) Sacc."; = *Sclerophoma pythiophila* (Corda) Höhn.].
Synonym: *Phoma leveillei* var. *leveillei* Boerema & G.J. Bollen, Persoonia 8: 115. 1975.

Description and synonymy: Boerema *et al.* (2004).

Material examined: **The Netherlands**, from water pipe sample, 1995, Y. Driessen (**neotype** CBS H-20314, ex-neotype living cultures CBS 812.95 = FMR 14872).

Notes: *Pyrenochaeta acicola* was neotypified and relegated to the *Cucurbitariaceae* by de Gruyter *et al.* (2010). Although *Neopyrenochaeta acicola* morphologically resembles a *Pyrenochaeta* species, our phylogenetic analyses revealed that this taxon is distant from the type species of *Pyrenochaeta*, *P. nobilis*, and therefore we proposed the new genus *Neopyrenochaeta* for this and a few related species.

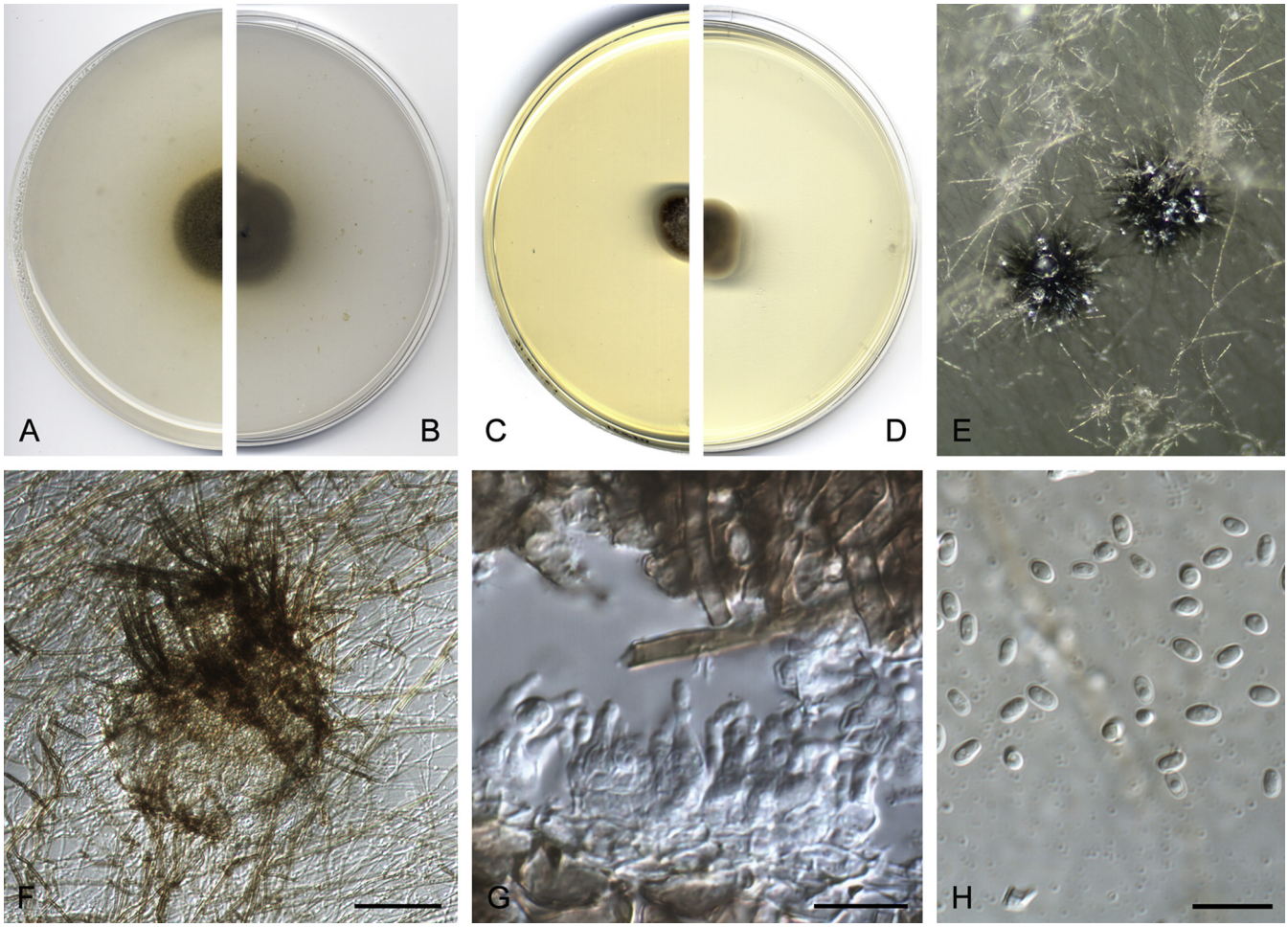

Fig. 29. *Neopyrenochaeta fragariae* (CBS 101634). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

Neopyrenochaeta fragariae Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **sp. nov.** MycoBank MB820316. Fig. 29.

Etymology: Relating to the host from the fungus was isolated, *Fragaria* (strawberry).

Description: *Hyphae* pale brown, smooth- and thin-walled, septate, 2.5–3 µm wide. *Conidiomata* pycnidial, pale brown to brown, solitary, superficial (OA), ovoid to globose, 170–220 × 160–210 µm, covered with brown to dark brown, septate, erect, smooth- and thick-walled setae tapering towards the apex, 110–120 × 2.5–5.5 µm, mainly disposed around the ostiole, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–5 layered, 20–60 µm thick, composed of brown, flattened polygonal cells of 5–15 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform, 4.5–7 × 3.5–4 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, ovoid to ellipsoidal, 3.5–5 × 2–3 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 14 mm diam after 7 d at 25 ± 1 °C, flattened, olive brown (M. 4E8); reverse olive brown (M. 4F2). Colonies on MEA reaching 11 mm after 7 d at 25 ± 1 °C, flattened, yellowish-brown (M. 5F4); reverse yellowish brown (M. 5E4). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined: **The Netherlands**, Arnhem, from *Fragaria* sp., 1976, M.M.J. Dorenbosch (**holotype** CBS H-23206, ex-holotype living cultures CBS 101634 = PD 76/416 = FMR 14871).

Notes: The strain CBS 101634 was previously named *Pyrenochaeta acicola*. Although it is morphologically similar to the latter mentioned species (now in *Neopyrenochaeta*), these fungi differ in 23 and 11 nucleotides for *rpb2* and *tub2*, respectively. Therefore, a new species name is proposed for CBS 101634.

Neopyrenochaeta inflorescentiae (Crous *et al.*) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **comb. nov.** MycoBank MB820317.
Basionym: *Pyrenochaeta inflorescentiae* Crous *et al.*, CBS Diversity Ser. (Utrecht) 7: 115. 2008.

Description: Marincowitz *et al.* (2008).

Material examined: **South Africa**, Western Cape Province, from *Protea neriifolia*, 6 Jun. 2000, S. Marincowitz (**holotype** PREM 58657, ex-holotype living cultures CBS 119222 = CPC 13163 = FMR 15334).

Notes: In our phylogenetic analysis, the ex-type strain of *Pyrenochaeta inflorescentiae* (CBS 119222) clustered with *N. acicola* and *N. fragariae* in a terminal clade distant from the type species of the genus *Pyrenochaeta*, *P. nobilis*, and outside the family *Cucurbitariaceae*, where that fungus was previously placed. For that reason, we accommodate *P. inflorescentiae* in the new genus *Neopyrenochaeta* (*Neopyrenochaetaceae*).

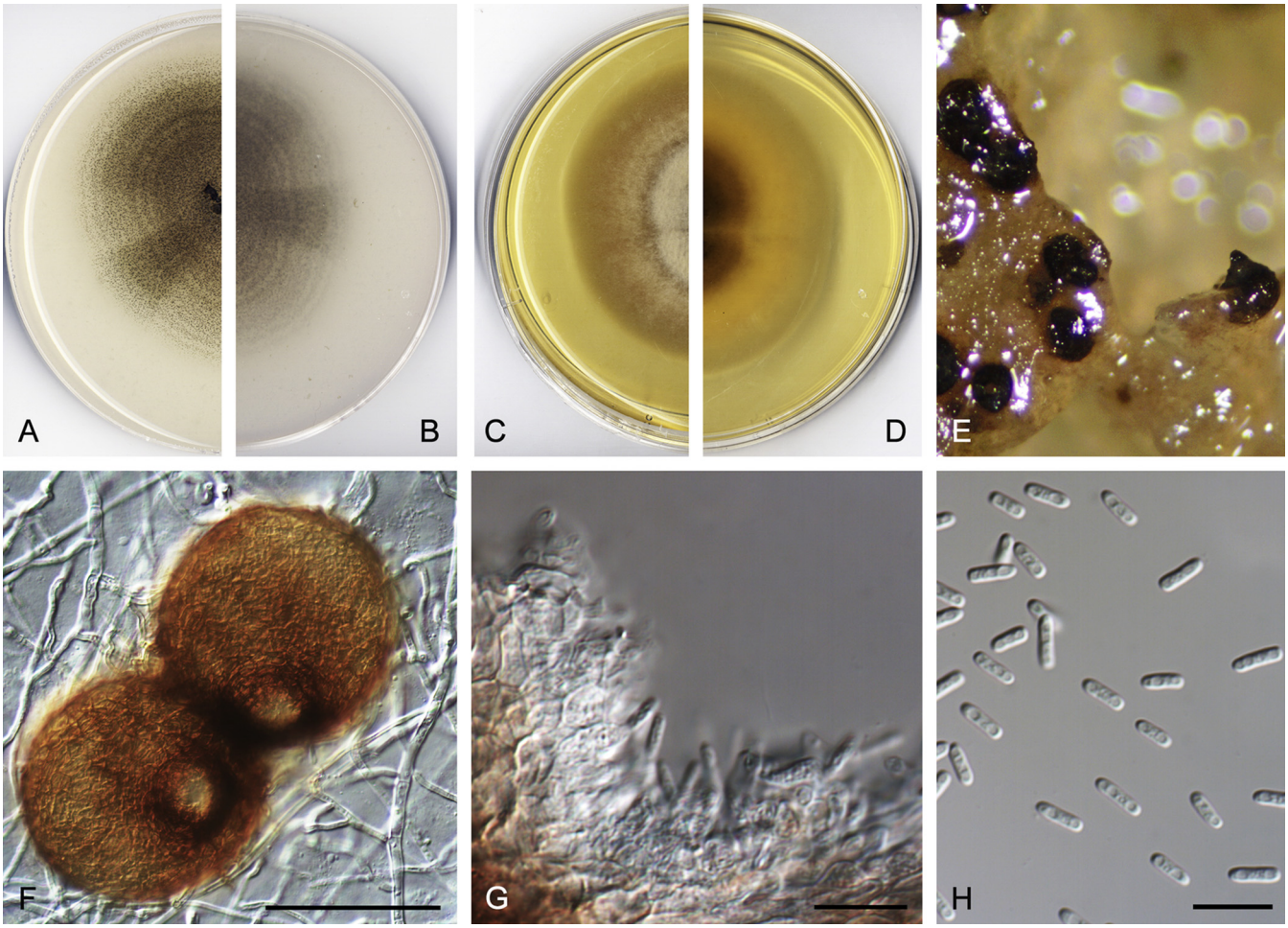

Fig. 30. *Pyrenochaetopsis botulispora* (CBS 142458). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

Neopyrenochaeta telephoni (Rohit Sharma *et al.*) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **comb. nov.** MycoBank MB820318.
Basionym: *Pyrenochaeta telephoni* Rohit Sharma *et al.*, Persoonia 35: 321. 2015.

Description: Crous *et al.* (2015b).

Material examined: **India**, Maharashtra, Pune, from screen of mobile phone, 2013, R. Kurli & P. Rahi (**holotype** MCC H1001, ex-holotype living cultures MCC 1159 = CBS 119222 = FMR 15754).

Notes: Recently, Sharma *et al.* (in Crous *et al.* 2015b) proposed the new species *Pyrenochaeta telephoni*, recovered from a mobile phone. Morphologically, it resembles other species of *Pyrenochaeta*; however, in our phylogenetic analysis this fungus forms a basal terminal clade in *Neopyrenochaeta*, which is distant from *Cucurbitariaceae s. str.*

Clade F: *Pyrenochaetopsidaceae* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, **fam. nov.** MycoBank MB820308.

Conidiomata pycnidial, pale brown to brown, solitary or confluent; pycnidial wall of *textura angularis*, glabrous or setose, subglobose to ovoid, with a non-papillate or papillate ostiolar neck. *Conidiogenous cells* phialidic, hyaline, discrete or integrated in septate, acropleurogenous conidiophores. *Conidia* aseptate, hyaline, smooth- and thin-walled, ovoid, cylindrical to allantoid, guttulate.

Type genus: *Pyrenochaetopsis* Gruyter, Aveskamp & Verkley.

Clade F1: *Pyrenochaetopsis*

Pyrenochaetopsis Gruyter, Aveskamp & Verkley, Mycologia 102: 1076. 2010.

Conidiomata pycnidial, honey to citrine or olivaceous to olivaceous black, solitary to confluent, superficial or submerged, with a non-papillate or papillate ostiolar neck; pycnidial wall pseudoparenchymatous, setose, globose to subglobose. *Conidiogenous cells* phialidic, hyaline, discrete and integrated in septate, acropleurogenous conidiophores. *Conidia* aseptate, cylindrical to allantoid, guttulate (de Gruyter *et al.* 2010).

Type species: *Pyrenochaetopsis leptospora* (Sacc. & Briard) Gruyter *et al.*

Pyrenochaetopsis americana Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB822115.

Etymology: The species name denotes the geographic area where the fungus is from.

Culture sterile. *Pyrenochaetopsis americana* differs from its closest phylogenetic species, *Pyrenochaetopsis uberiformis*, in five nucleotides for ITS, 19 for *tub*2 and 34 for *rpb*2, based on alignment of the concatenated four loci deposited in TreeBASE (S21115).

Culture characteristics: Colonies on OA reaching 30 mm diam after 7 d at 25 ± 1 °C, flattened, dark olive (M. 3F3); reverse olive

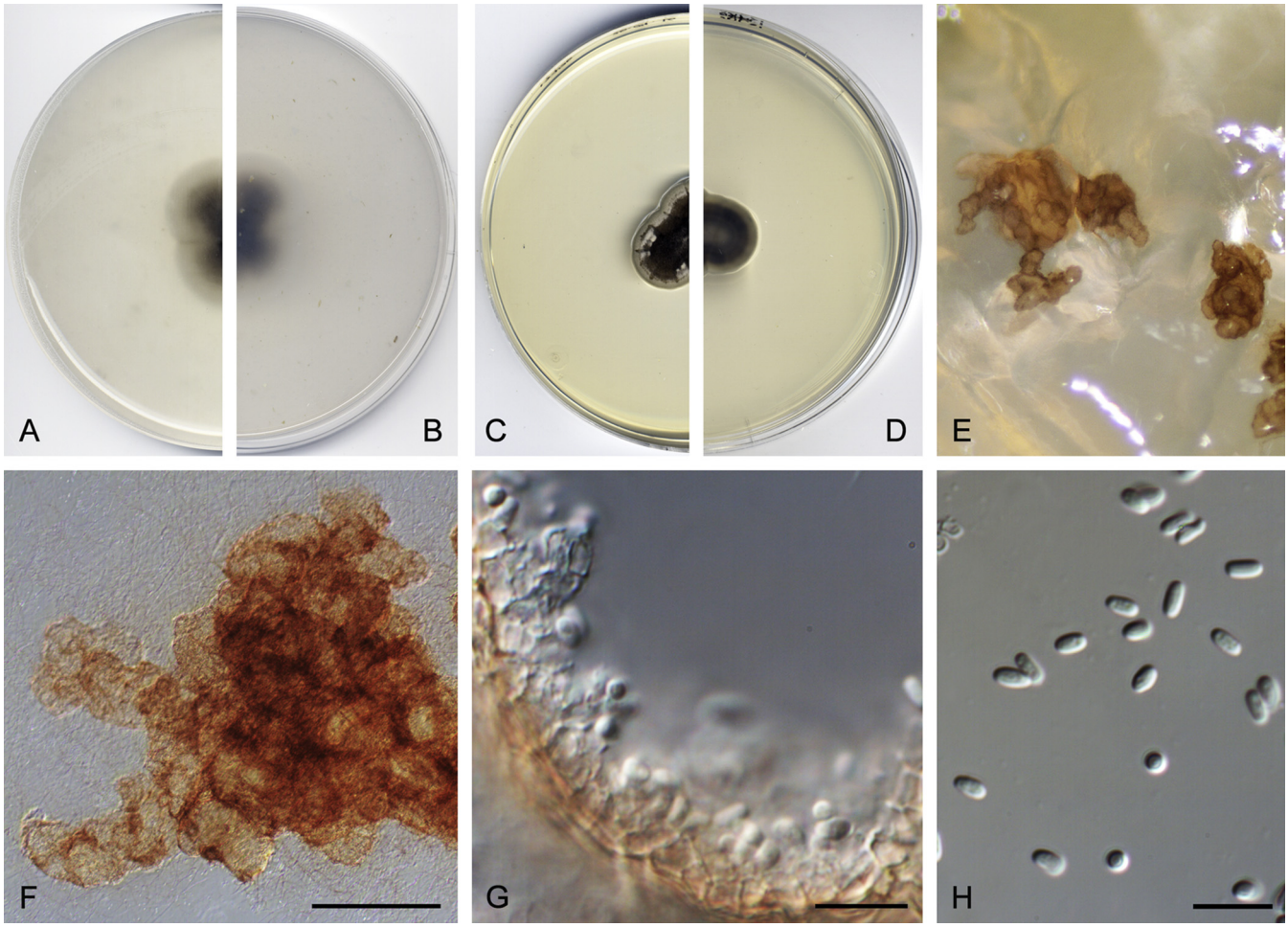

Fig. 31. *Pyrenochaetopsis confluens* (CBS 142459). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

grey (M. 3D2). Colonies on MEA reaching 19 mm diam after 7 d at 25 ± 1 °C, flattened, olive grey (M. 3D2) to white (M. 3A1); reverse white (M. 3A1). NaOH spot test negative. Crystals absent.

Material examined: **USA**, substrate unknown, 2007, D.A. Sutton (**holotype** FMR H-13715, ex-holotype living cultures UTHSC DI16-225 = FMR 13715).

Notes: The strain UTHSC DI16-225, which remained sterile in all culture media tested in this study, forms an unsupported sister clade with *P. uberiformis*, from which it is phylogenetically distant. Therefore, UTHSC DI16-225 is proposed here as a new species different from *P. uberiformis*.

Pyrenochaetopsis botulispora Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB819764. Fig. 30.

Etymology: From Latin *botulus*-, sausage, and *-spora*, spore, relating to the morphology of the conidia.

Description: *Hyphae* brown, smooth- and thin-walled, septate, 2–7 µm wide. *Conidiomata* pycnidial, brown, solitary or confluent, superficial (OA), glabrous, subglobose or globose, 140–190 × 130–160 µm, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–3 layered, 15–35 µm thick, composed of brown, flattened polygonal cells of 5–8 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, subglobose, ca. 4 × 5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical, 4.5–6 × 2–2.5 µm, guttulate.

Culture characteristics: Colonies on OA reaching 25–30 mm diam after 7 d at 25 ± 1 °C, flattened, with abundant production of pycnidia, yellowish brown (M. 5E8); reverse yellowish-brown (M. 5F6). Colonies on MEA reaching 30 mm diam after 7 d at 25 ± 1 °C, flattened, orange grey (M. 5B2) to brownish orange (M. 5C5); reverse yellowish brown (M. 5E7) to greyish orange (M. 5B5). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 15 °C; maximum temperature of growth 30 °C.

Material examined: **USA**, from human sputum sample, 2011, D.A. Sutton (**holotype** CBS H-23035, ex-holotype living cultures CBS 142458 = UTHSC DI16-298 = FMR 13791); from human bronchial wash sample, 2010, D.A. Sutton, living culture UTHSC DI16-289 = FMR 13781; from human foot skin, 2011, D.A. Sutton, living culture UTHSC DI16-297 = FMR 13790.

Notes: *Pyrenochaetopsis botulispora* is proposed to accommodate three isolates from clinical specimens, which form a sister clade to *P. paucisetosa*, being well differentiated phylogenetically from their closest relatives. Morphologically, *P. botulispora* is characterised by producing glabrous pycnidia, which are setose in *P. paucisetosa*, and by its slightly longer conidia (4.5–6 × 2–2.5 µm *vs.* 3–4 × 2–2.5 µm in in *P. paucisetosa*).

Pyrenochaetopsis confluens Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB819763. Fig. 31.

Etymology: From Latin *confluens*, confluent, due to the production of tightly aggregated conidiomata.

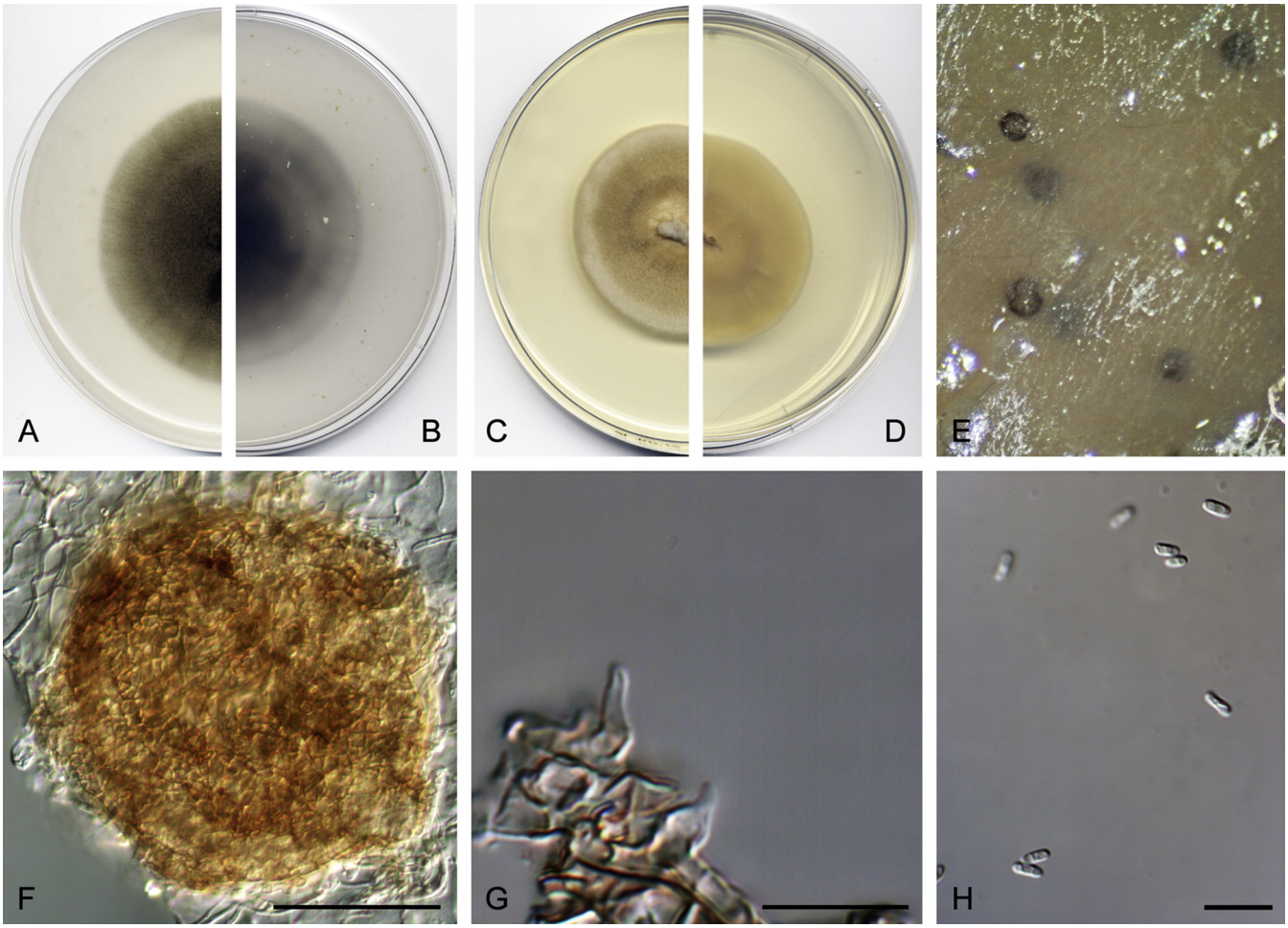

Fig. 32. *Pyrenochaetopsis globosa* (CBS 143034). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

Description: *Hyphae* pale brown, smooth- and thin-walled, septate, 2–5 µm wide. *Conidiomata* pycnidial, pale brown, translucent, aggregated, immersed (MEA), subglobose or globose, 80–140 × 70–90 µm, with 1–2 papillate ostiolar necks, covered by brown setae around the ostiole; setae erect, smooth- and thick- walled, septate, 15–22.5(–35) × 2.5–4.5 µm; pycnidial wall of *textura angularis*, 2–3 layered, 13–20 µm thick, composed of brown, flattened polygonal cells of 5–8 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, subglobose, 4.5–7.5 × 6.5–7.5 µm. *Conidia* aseptate, hyaline, aseptate, smooth- and thin-walled, guttulate, ovoid to cylindrical, 2–4 × 2–2.5 µm.

Culture characteristics: Colonies on OA reaching 15 mm diam after 7 d at 25 ± 1 °C, flattened, white (M. 4A1) to olive brown (M. 4E4); reverse olive brown (M. 4F3). Colonies on MEA reaching 10 mm diam after 7 d at 25 ± 1 °C, flattened, white (M. 4A1) to brownish-grey (M. 4F2); reverse brownish-grey (M. 4F2). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined: **USA**, from human blood sample, 2011, D.A. Sutton (**holotype** CBS H-23036, ex-holotype living cultures CBS 142459 = UTHSC DI16-303 = FMR = 13796).

Notes: The strain CBS 142459 forms a distinct clade phylogenetically distant from *P. decipiens* and *P. indica*. This new species grows slowly on all culture media tested and produces aggregated conidiomata.

Pyrenochaetopsis decipiens (Marchal) Gruyter *et al.*, Mycologia 102: 1077. 2010.
Basionym: *Pyrenochaeta decipiens* Marchal, Bull. Soc. Roy. Bot. Belg. 30:139. 1891.
Synonym: *Phoma terricola* Boerema, Versl. Meded. plziektenk. Dienst Wageningen 163 (Jaarb. 1984): 38. 1985.

Material examined: **The Netherlands**, Hoofddorp, on cyst of *Globodera pallida*, May 1985, D. Hugo, No. 727 (**neotype** CBS H-20315, ex-neotype living cultures CBS 343.85 = IMI 386097 = FMR 14880).

Notes: In this study, newer genomic sequences data from the ex-type strain of *Pyrenochaetopsis decipiens* are provided. Unfortunately, we have not been able to induce this fungus to sporulate.

Pyrenochaetopsis globosa Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB821496. Fig. 32.

Etymology: From Latin *globosus*, globose, due to the production of globose conidiomata.

Description: *Hyphae* hyaline to pale brown, smooth- and thin-walled, septate, 2–4 µm wide. *Conidiomata* pycnidial, pale olivaceus-brown to brown, solitary or aggregated, semi-immersed or immersed, mainly globose (70–200 µm diam), sometimes ovoid (150–220 × 140–190 µm), glabrous or covered by hyphal outgrowths, with 1–2 papillate ostiolar necks; pycnidial wall of *textura angularis*, 3–5 layered, 25–35 µm thick, composed of pale olive-brown to brown, flattened polygonal cells of 3–10 µm diam. *Conidiogenous cells* phialidic, hyaline,

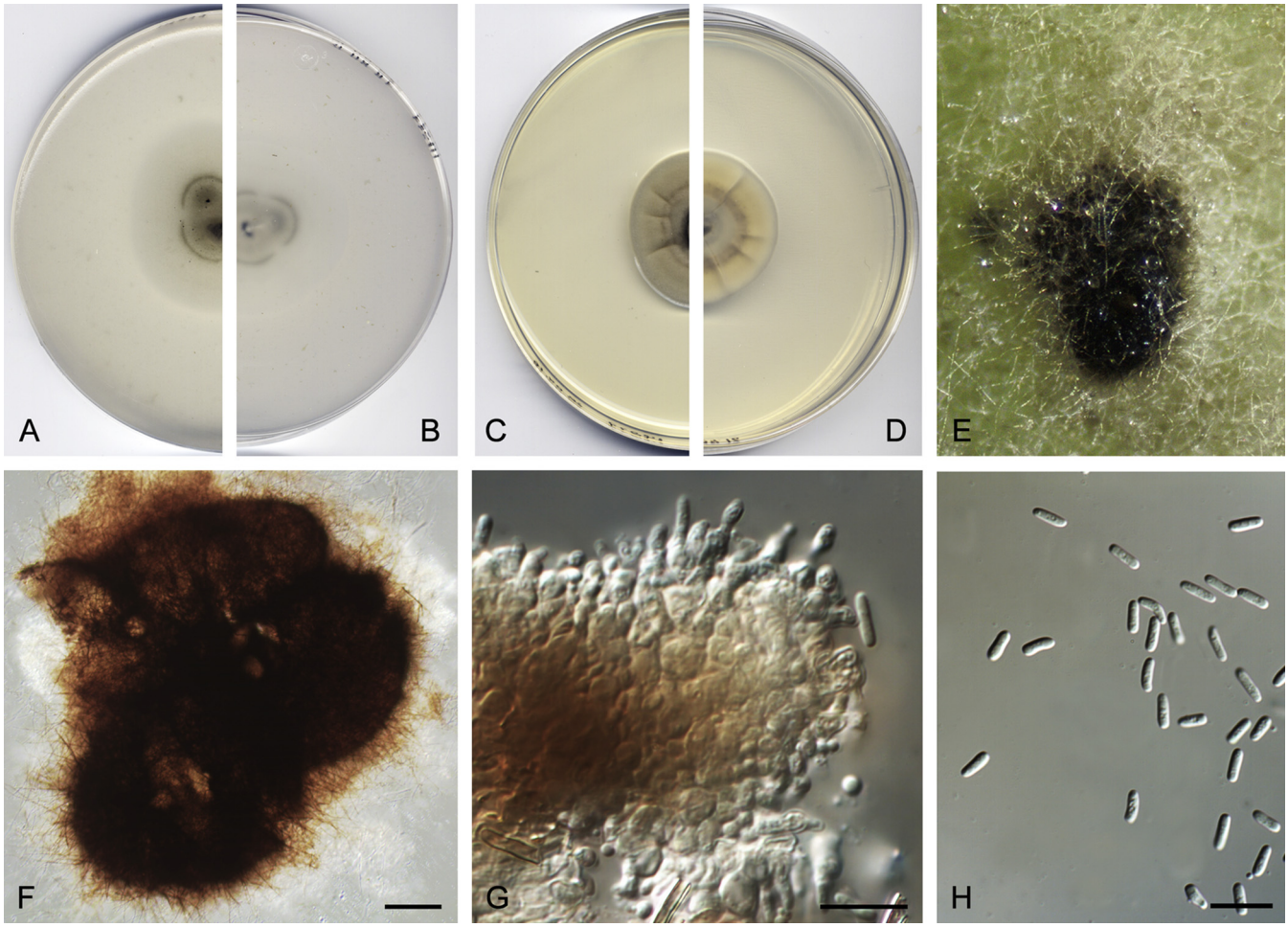

Fig. 33. *Pyrenochaetopsis leptospora* (CBS 101635). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

smooth-walled, lageniform to ampulliform, 3.5–5 × 2.5–3 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, ovoid to cylindrical, 3–5.5 × 1.5–2 µm, guttulate.

Culture characteristics: Colonies on OA reaching 27 mm diam after 7 d at 25 ± 1 °C, flattened, yellowish brown (M. 5E5); reverse greyish brown (M. 5F3). Colonies on MEA reaching 20 mm diam after 7 d at 25 ± 1 °C, flattened, brownish-orange (M. 5C3); reverse pale brown (M. 5D4). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined: **USA**, from human dermatitis sample, 2009, D.A. Sutton (**holotype** CBS H-23208 ex-holotype living cultures CBS 143034 = UTHSC DI16-275 = FMR 13766).

Notes: The strain CBS 143034, which is morphologically similar to *P. uberiformis* (slightly different in pycnidial and conidial size), forms a large clade wherein there is *P. uberiformis* and several other species of the genus *Pyrenochaetopsis*. Because the nucleotide sequences of both fungi differ in 19 bp for *rpb2* and 13 bp for *tub2*, *P. globosa* is proposed as a new species for the genus.

Pyrenochaetopsis indica (T.S. Viswan.) Gruyter *et al.*, Mycologia 102: 1077. 2010.
Basionym: *Pyrenochaeta indica* T.S. Viswan., Curr. Sci. 26:118. 1957.
Synonym: *Phoma indica* (T.S. Viswan.) Gruyter & Boerema, Persoonia 17: 556. 2002.

Description: Boerema *et al.* (2004).

Material examined: **India**, Poona, on leaf spot of *Saccharum officinarum* (**holotype** AMH-11, ex-holotype living cultures IMI 062569 = CBS 124454 = FMR 14879).

Notes: We studied the ex-type strain of *Pyrenochaetopsis indica*, providing new genomic sequence data. It is morphologically characterised by its setose pycnidia, and the production of globose to subglobose, olivaceous chlamydospores solitary or in chains. Morphologically it is difficult to differentiate this species from *P. decipiens*. However, *Pyrenochaetopsis indica* clearly differs genetically from the latter in its *tub2* and *rpb2* sequences. Unfortunately, all cultures remained sterile.

Pyrenochaetopsis leptospora (Sacc. & Briard) Gruyter *et al.*, Mycologia 102: 1076. 2010. Fig. 33.
Basionym: *Pyrenochaeta leptospora* Sacc. & Briard, Revue Mycol. 11: 16. 1889.
Synonyms: *Pyrenochaeta spegazziniana* Trotter, Syll. Fung. 25: 190. 1931.
Phoma briardii Gruyter & Boerema, Persoonia 17: 555. 2002.

Description: Boerema *et al.* (2004).

Material examined: **Germany**, subtrate unknown, J.W. Veenbaas, living cultures CBS 122787 = FMR 14873. **The Netherlands**, on *Secale cereal* (**epitype** CBS H-20313, ex-epitype living cultures CBS 101635 = PD 71/1027 = FMR 14877).

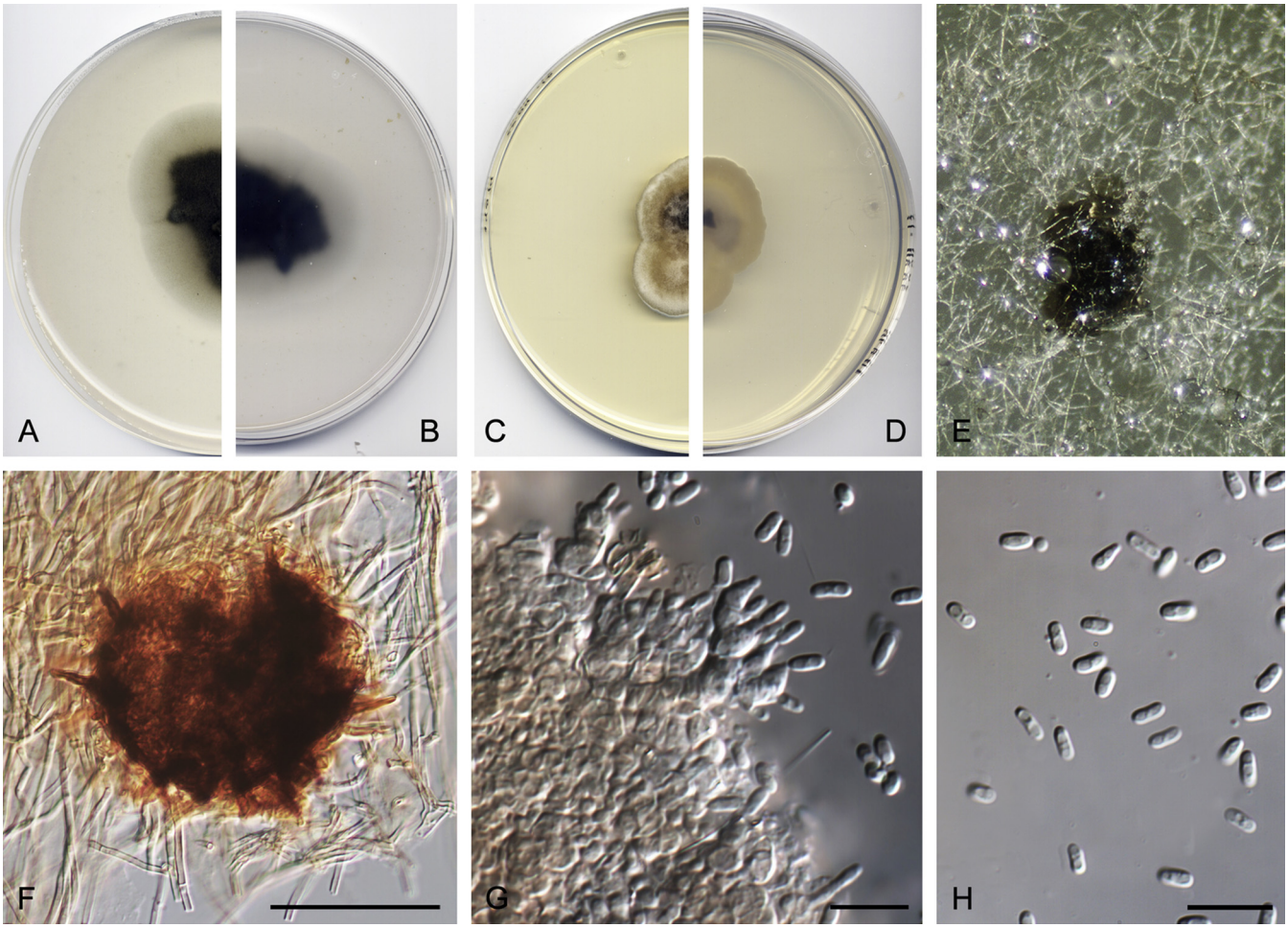

Fig. 34. *Pyrenochaetopsis microspora* (CBS 102876). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

Notes: We received isolate CBS 122787 as "*Coniothyrium cerealis*", but it was identified as *P. leptospora* in our phylogenetic study.

Pyrenochaetopsis microspora (Gruyter & Boerema) Gruyter *et al.*, Mycologia 102: 1077. 2010. Fig. 34.
Basionym: *Phoma leveillei* var. *microspora* Gruyter & Boerema, Persoonia 17: 553. 2002.

Description: Boerema *et al.* (2004).

Materials examined: **Montenegro**, Lake of Skadar, from water, 1975 (**holotype** HLB 999-242399, ex-holotype living cultures CBS 102876 = PD 75/911 = FMR 14874). **USA**, from human sinusitis sample, 2006, D.A. Sutton, living cultures UTHSC DI16-193 = FMR 13688.

Notes: In this paper, the ex-type strain of *Pyrenochaetopsis microspora* was examined, and new genomic sequence data and illustrations are provided. Furthermore, one human clinical specimen clustered with the ex-type living culture, being morphologically and genetically very closely related.

Pyrenochaetopsis paucisetosa Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB819766. Fig. 35.

Etymology: From Latin *paucus*, few, and *-setosus*, setose, because the conidiomata are covered by a few setae.

Description: *Hyphae* brown, smooth- and thin-walled, septate, 2–3 µm wide. *Conidiomata* pycnidial, brown, solitary, superficial or immersed (OA), setose, globose to ovoid, 150–190 × 140–160 µm, with a papillate ostiolar neck, covered by a few, brown, erect or slightly curved, smooth- and thick-walled, septate setae, (50–)63–68(–83) × 2–3.5 µm; pycnidial wall of *textura angularis*, 2–5 layered, 20–50 µm thick, composed of brown, flattened polygonal cells of 4–13 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform, 3.5–4 × 3–3.5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical, 3–4 × 2–2.5 µm, guttulate.

Culture characteristics: Colonies on OA reaching 25 mm diam after 7 d at 25 ± 1 °C, flattened, olive brown (M. 4F5); reverse brownish grey (M. 4F2). Colonies on MEA reaching 21 mm diam after 7 d at 25 ± 1 °C, floccose, pale grey (M. 4C1); reverse medium-grey (M. 4E1). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 15 °C; maximum temperature of growth 35 °C.

Material examined: **USA**, from human toe nail, 2005, D.A. Sutton (**holotype** CBS H-23037, ex-holotype living cultures CBS 142460 = UTHSC DI16-193 = FMR 13683).

Notes: *Pyrenochaetopsis paucisetosa*, recovered from a specimen of superficial human tissue, produces pycnidia covered by a few setae, and conidia smaller than in other species of the genus. Phylogenetically, *P. paucisetosa* is well-separated from *P. botulispora*.

Pyrenochaetopsis poae Crous & Quaedvlieg, Persoonia 32: 197. 2014.

Description: Crous *et al.* (2014).

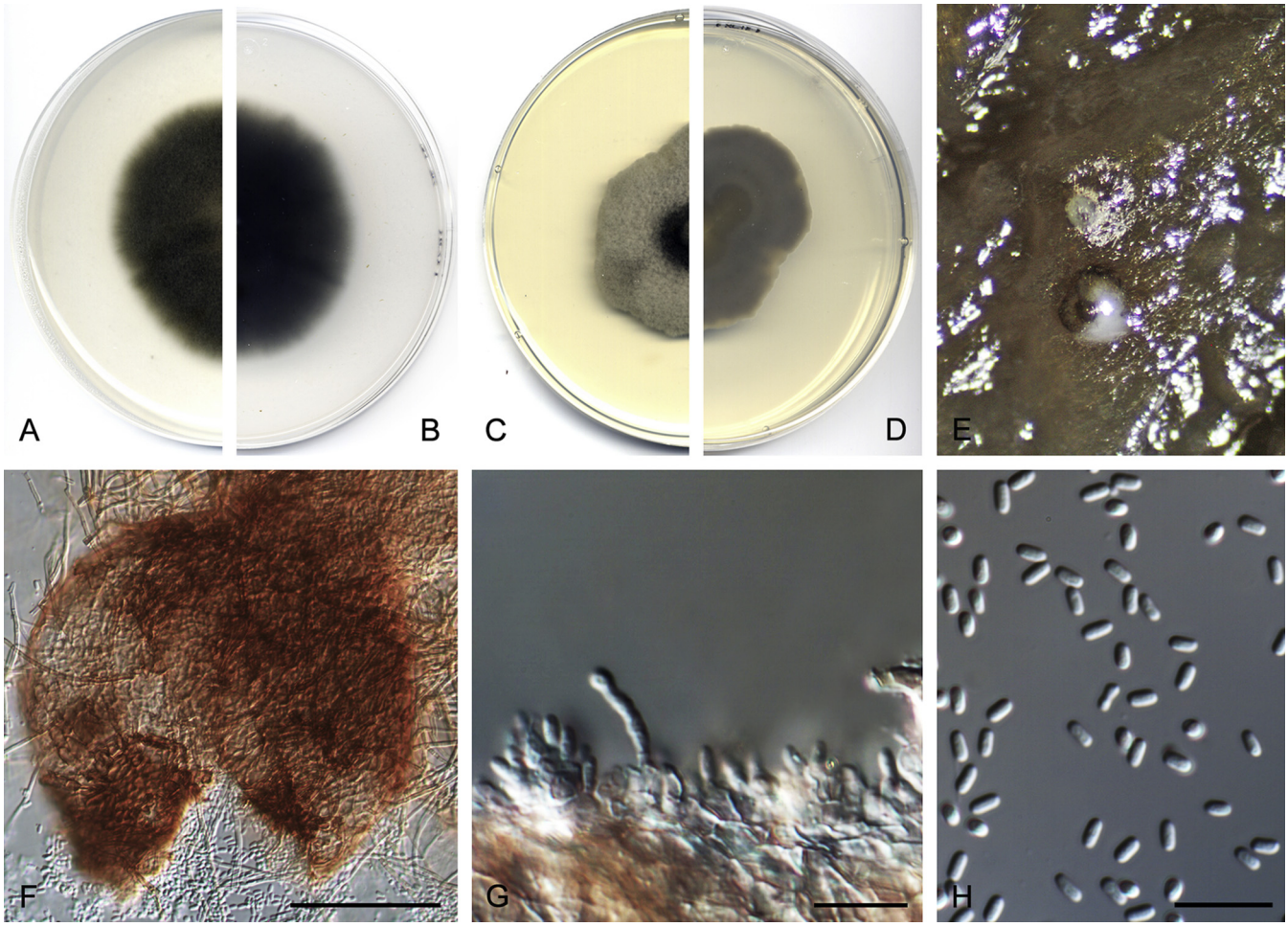

Fig. 35. *Pyrenochaetopsis paucisetosa* (CBS 142460). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

Material examined: **Netherlands**, Raalte, on *Poa* sp. (*Poaceae*), 2013, W. Quaedvlieg (**holotype** CBS H-21677, ex-holotype living cultures CBS 136769 = D779 = FMR 14876).

Notes: We studied the ex-type strain of *Pyrenochaetopsis poae*, which is morphologically similar to the generic type of *P. leptospora*. In this paper, we provide *rpb2* sequence that, together with *tub2*, are useful to differentiate these taxa.

Pyrenochaetopsis setosissima Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **sp. nov.** MycoBank MB819767. Fig. 36.

Etymology: From Latin –*setosissimus*, bearing many setae, relating to the ornamentation of the pycnidia.

Description: *Hyphae* brown, smooth- and thin-walled, septate, 2–5 µm wide. *Conidiomata* pycnidial, brown, solitary or confluent, superficial (OA), subglobose to ovoid, 150–230 × 150–200 µm, with a papillate ostiolar neck, covered by many dark brown, erect, smooth- and thick-walled, septate setae, 33–83 × 2–4 µm; pycnidial wall of *textura angularis*, 2–4 layered, 20–50 µm thick, composed of brown, flattened polygonal cells of 5–15 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform, 5–4.5 × 4–4.5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical, 4–5 × 2–2.5 µm, guttulate.

Culture characteristics: Colonies on OA reaching 25 mm diam after 7 d at 25 ± 1 °C, flattened, olive brown (M. 4F4); reverse brownish grey (M. 4F2). Colonies on MEA reaching 18 mm diam after 7 d at 25 ± 1 °C, flattened, light orange (M. 5A4); reverse orange white (M. 5A2). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined: **Brazil**, Minas Gerais, Lavras, from *Coffea arabica* leaf, Jun. 1999, L.H. Pfenning (**holotype** CBS H-23209, ex-holotype living cultures CBS 119739 = FMR 14875).

Notes: The isolate CBS 119739 was identified as *P. microspora* by de Gruyter *et al.* (2010) using SSU and LSU sequences as phylogenetic markers. However, in our phylogenetic study employing more markers, it clusters distant from the latter species. *Pyrenochaetopsis setosissima* is morphologically very similar to *P. microspora*, and can only be distinguished based on molecular data (differing in 19 bp for *tub2* and 31 bp for *rpb2*).

Pyrenochaetopsis uberiformis Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB819765. Fig. 37.

Etymology: From Latin –*ubera*, mammaries, and -*forma*, shape, relating to the anatomy of its pycnidia.

Description: *Hyphae* brown, smooth- and thin-walled, septate, 2–3 µm wide. *Conidiomata* pycnidial, brown, solitary or confluent, superficial or immersed (OA), glabrous, globose or ovoid, 200–440 × 130–410 µm, with a papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–4 layered, 15–30 µm thick, composed of pale brown to brown, flattened polygonal cells of

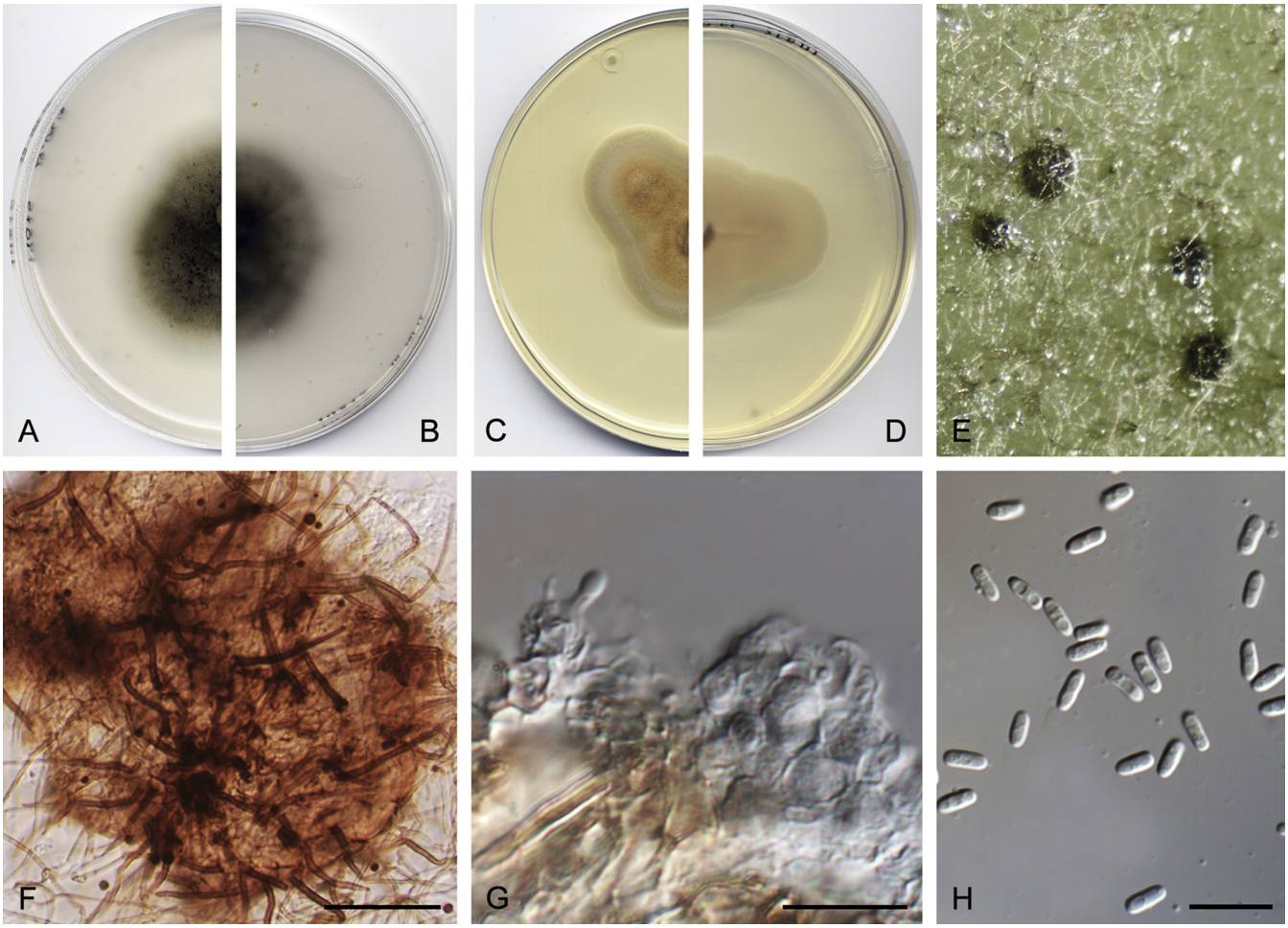

Fig. 36. *Pyrenochaetopsis setosissima* (CBS 119739). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

5–10 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform, 3–4 × 4–5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical, 4–6 × 2–2.5 µm, guttulate.

Culture characteristics: Colonies on OA reaching 27 mm diam after 7 d at 25 ± 1 °C, flattened, yellowish brown (M. 5E5); reverse greyish brown (M. 5F3). Colonies on MEA reaching 20 mm diam after 7 d at 25 ± 1 °C, flattened, brownish orange (M. 5C3); reverse pale brown (M. 5D4). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined: **USA**, from human ear lesion, 2009, D.A. Sutton (**holotype** CBS H-23038, ex-holotype living cultures CBS 142461 = UTHSC DI16-277 = FMR 13769).

Notes: The strain CBS 142461 clustered within the *Pyrenochaetopsis* clade, distant from other species of the genus, with the exception of *P. americana*, which forms a sister clade. Both strains differ in their *rpb2* and *tub2* sequences. Therefore, we propose strain CBS 142461 as representative of the new species *P. uberiformis*.

Clade F2: *Xenopyrenochaetopsis*

Xenopyrenochaetopsis Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **gen. nov.** MycoBank MB820311.

Etymology: From Greek ξένος-, strange, alien, because it is phylogenetically distinct from the genus *Pyrenochaetopsis*.

Conidiomata pycnidial, pale brown to brown, solitary or confluent; pycnidial wall of *textura angularis*, glabrous, globose, ostiolate. *Conidiogenous cells* phialidic, hyaline. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical, guttulate.

Type species: *Xenopyrenochaetopsis pratorum* (P.R. Johnst. & Boerema) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano.

Xenopyrenochaetopsis pratorum (P.R. Johnst. & Boerema) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **comb. nov.** MycoBank MB820312. Fig. 38.
Basionym: *Phoma pratorum* P.R. Johnst. & Boerema, New Zealand J. Bot. 19: 395. 1981.
Synonym: *Pyrenochaetopsis pratorum* (P.R. Johnst. & Boerema) Gruyter *et al.*, Stud. Mycol. 75: 24. 2012.

Description from ex-isotype (CBS 445.81): *Hyphae* pale brown to brown, smooth- and thin-walled, septate, 2.5–5 µm wide. *Conidiomata* pycnidial, pale brown to brown, solitary or confluent, semi-immersed or immersed (OA), glabrous, globose to irregular, (88–)160–270 × (80–)100–250 µm, with 1–3 papillate ostiolar necks; pycnidial wall of *textura angularis*, 2–4 layered, 10–30 µm thick, composed of pale brown to brown, flattened polygonal cells of 2.5–8 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform, 3–3.5 × 1.5–2 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, subreniform to oblong or cylindrical, 4–5 × 1.5–2 µm, guttulate.

Culture characteristics: Colonies on OA reaching 5 mm diam after 7 d at 25 ± 1 °C, flattened, olive (M. 3D4) to olive grey (M.3F2);

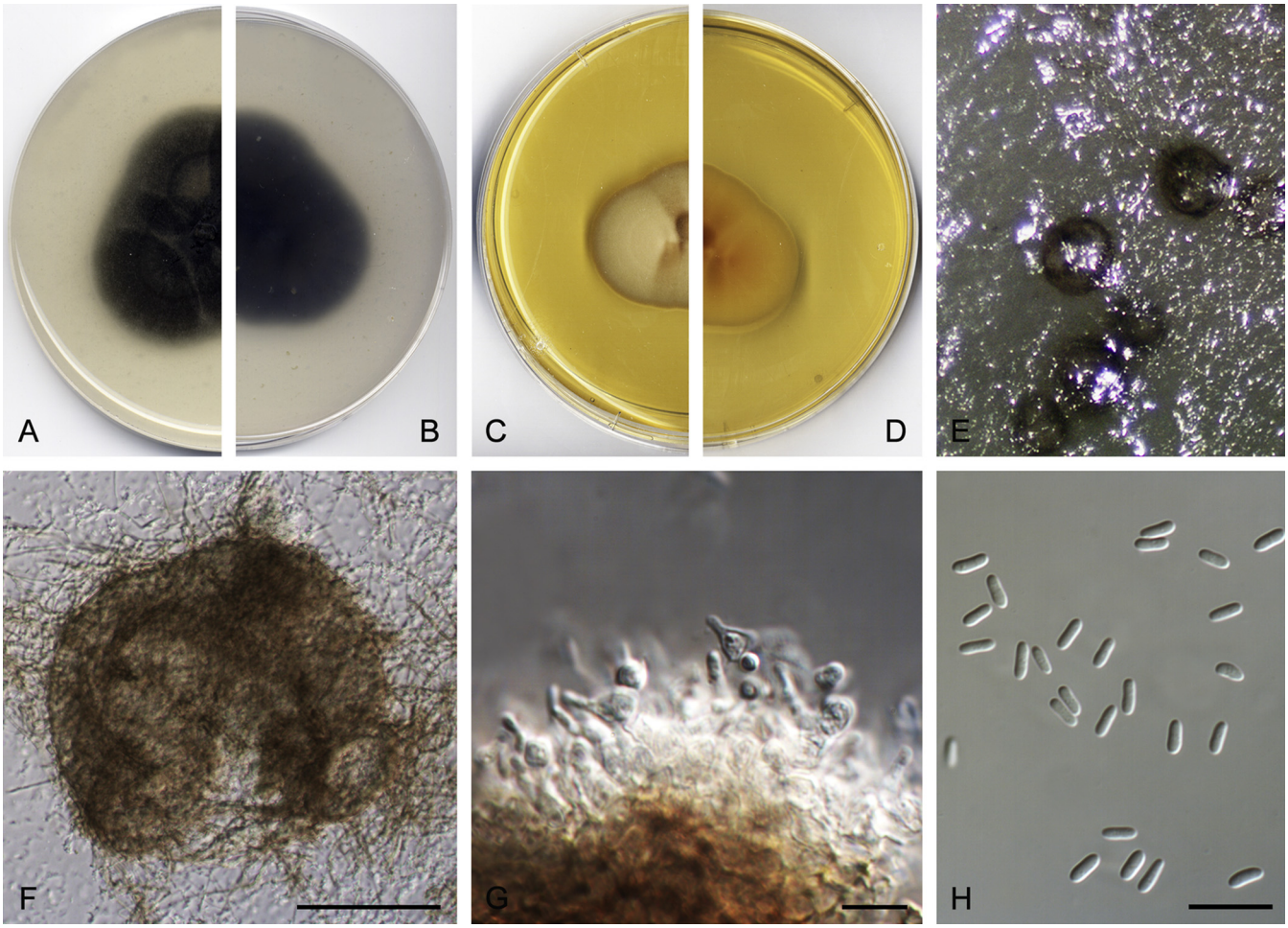

Fig. 37. *Pyrenochaetopsis uberiformis* (CBS 142461). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

reverse olive (M.3F4). Colonies on MEA reaching 4 mm diam after 7 d at 25 ± 1 °C, flattened, yellowish white (M. 3A2); reverse ash blonde (M. 3C3). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 15 °C; maximum temperature of growth 25 °C.

Material examined: **New Zealand**, Rakura, near Hamilton, from a leaf of *Lolium perenne* (*Poaceae*), 1980, P.R. Johnston (**isotype** CBS H-7625, CBS H-7626, ex-isotype living cultures CBS 445.81 = PDDCC 7049 = PD 80/1254 = FMR 14878).

Notes: *Pyrenochaetopsis pratorum* was proposed as a new combination for *Phoma pratorum* by de Gruyter *et al.* (2013). In that study, it clustered with *Pyrenochaetopsis* but was situated phylogenetically distinct from *P. leptospora*. However, in our phylogenetic analysis this species clustered outside *Pyrenochaetopsis s. str.* Moreover, *Phoma pratorum* differs in the main distinctive morphological feature of the genus *Pyrenochaetopsis*, the production of setose pycnidia (glabrous in *P. pratorum*). Therefore, we accommodate this species in the new genus *Xenopyrenochaetopsis.*

Clade F3: *Neopyrenochaetopsis*

Neopyrenochaetopsis Valenzuela-Lopez, Cano, Guarro & Stchigel, **gen. nov.** MycoBank MB820309.

Etymology: Referring to its close phylogenetic relationship with the genus *Pyrenochaetopsis*.

Conidiomata pycnidial, brown, solitary or confluent, pycnidial wall of *textura angularis*, glabrous, subglobose to ovoid, ostiolate. *Conidiogenous cells* phialidic, ampulliform to globose. *Conidia* aseptate, hyaline, smooth- and thin-walled, ovoid to cylindrical.

Type species: *Neopyrenochaetopsis hominis* Valenzuela-Lopez, Cano, Guarro & Stchigel.

Neopyrenochaetopsis hominis Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MB820310. Fig. 39.

Etymology: Relating to its isolation from a human specimen.

Description: *Hyphae* pale yellow to pale brown, smooth- and thin-walled, septate, 2–3 µm wide. *Conidiomata* pycnidial, brown, solitary or confluent, superficial or immersed (OA), glabrous, subglobose to ovoid, 160–170 × 140–160 µm, with a single papillate ostiolar neck; pycnidial wall of *textura angularis*, 2–4 layered, 15–40 µm thick, composed of brown, flattened polygonal cells of 2.5–8 µm diam. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform to globose, 4–5 µm diam wide. *Conidia* aseptate, hyaline, smooth- and thin-walled, ovoid to narrowly ellipsoidal, 3–3.5 × 1.5–2 µm, guttulate. *Chlamydospores* absent.

Culture characteristics: Colonies on OA reaching 31 mm diam after 7 d at 25 ± 1 °C, flattened, greyish yellow (M. 3B4); reverse greyish yellow (M. 3C4); yellow pigment diffusing into the agar. Colonies on MEA reaching 29 mm diam after 7 d at 25 ± 1 °C, floccose, dull yellow (M. 3B3) to white (M. 3A1); reverse brownish yellow (M. 5C8); diffusible pigment yellowish. NaOH spot test negative. Crystals absent. Optimal temperature of

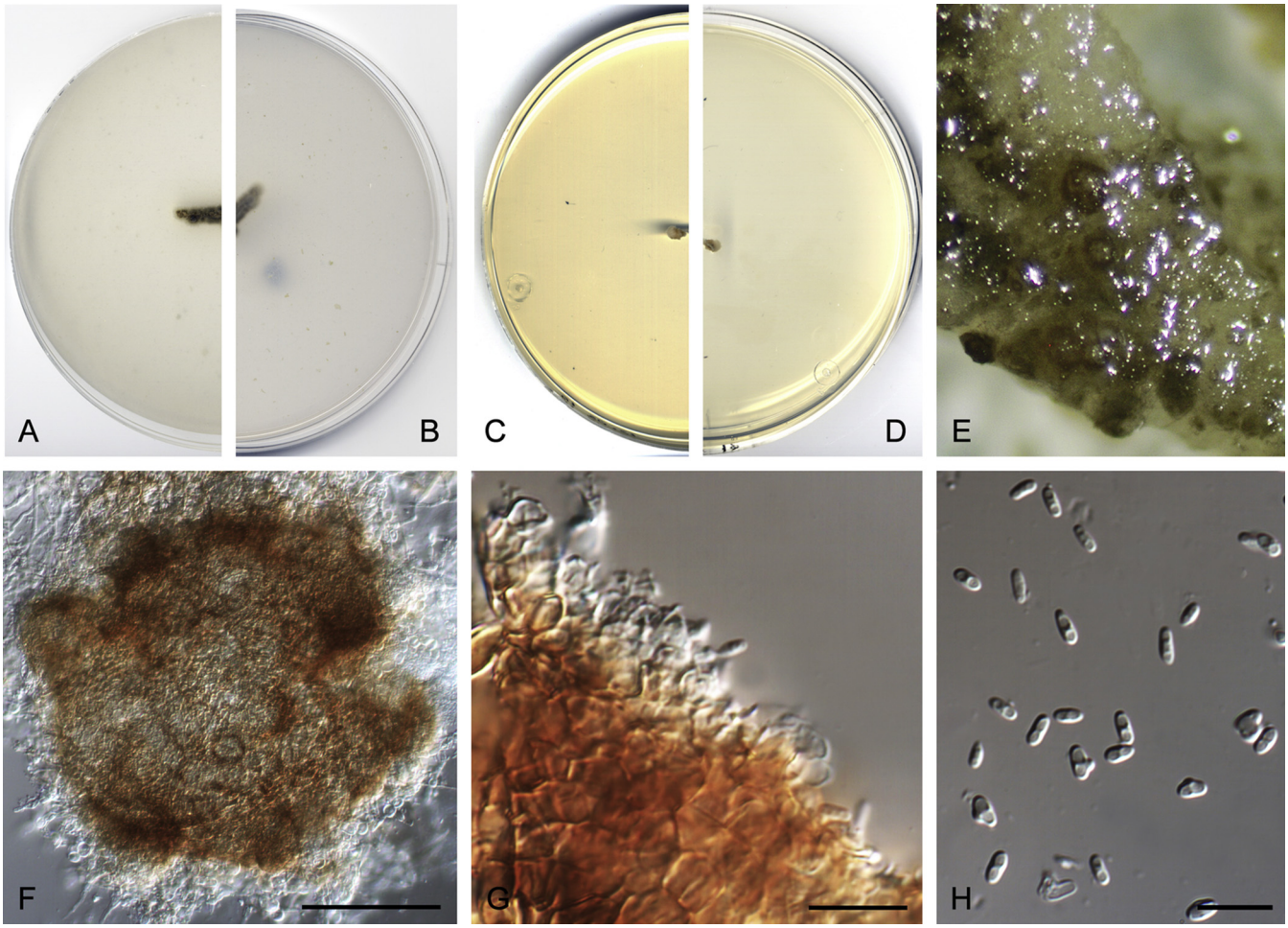

Fig. 38. *Xenopyrenochaetopsis pratorum* (CBS 445.81). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 30 °C.

Material examined: **USA**, from human skin tissue, 2007, D. A. Sutton (**holotype** CBS H-23207, culture ex-holotype living cultures CBS 143033 = UTHSC DI16-238 = FMR 13728).

Notes: The strain CBS 143033, recovered from a clinical sample, forms a distinct basal clade within the *Pyrenochaetopsidaceae*. Morphologically, *N. hominis* can be differentiated from the other taxa mainly by the production of smaller-sized conidia, and a yellow diffusing pigment on MEA and OA.

Clade N: *Parapyrenochaetaceae* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **fam. nov.** MycoBank MB820418.

Etymology: Named after its close morphological relationship with *Pyrenochaeta*.

Conidiomata pycnidial, brown, solitary, pycnidial wall of *textura angularis*, setose, globose, ostiolate. *Conidiogenous cells* phialidic, ampulliform or lageniform. *Conidia* aseptate, hyaline, smooth- and thin-walled, allantoid or ellipsoidal.

Type genus: *Parapyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano.

Parapyrenochaeta Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **gen. nov.** MycoBank MB820319.

Etymology: Based on its close morphological relationship to *Pyrenochaeta*.

Conidiomata pycnidial, pale brown to brown, solitary, setose, globose, ostiolate; pycnidial wall of *textura angularis*. *Conidiogenous cells* phialidic, ampulliform or lageniform. *Conidia* aseptate, hyaline, smooth- and thin-walled, allantoid or ellipsoidal.

Type species: *Parapyrenochaeta protearum* (Crous) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano.

Parapyrenochaeta acaciae (Crous *et al.*) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **comb. nov.** MycoBank MB820321. Fig. 40.
Basionym: *Pyrenochaeta acaciae* Crous *et al.*, Persoonia 36: 349. 2016.

Description: Crous *et al.* (2016b).

Material examined: **Australia**, Victoria, on leaf of *Acacia* sp. (*Fabaceae*), 7 Nov. 2014, J. Edwards, I.G. Pascoe & P.W. Crous (**holotype** CBS H-22601, ex-holotype living cultures CPC 25527 = CBS 141291 = FMR 15755).

Notes: *Pyrenochaeta acaciae* was described by Crous *et al.* (2016b) based on morphological and nucleotide sequence data, highlighting the close relationship with *P. protearum*. In our phylogenetic study, *P. acaciae* clustered distant from the *Cucurbitariaceae* s. str., forming a distinct clade related to *P. protearum*.

Parapyrenochaeta protearum (Crous) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, **comb. nov.** MycoBank MB820320. Fig. 41.

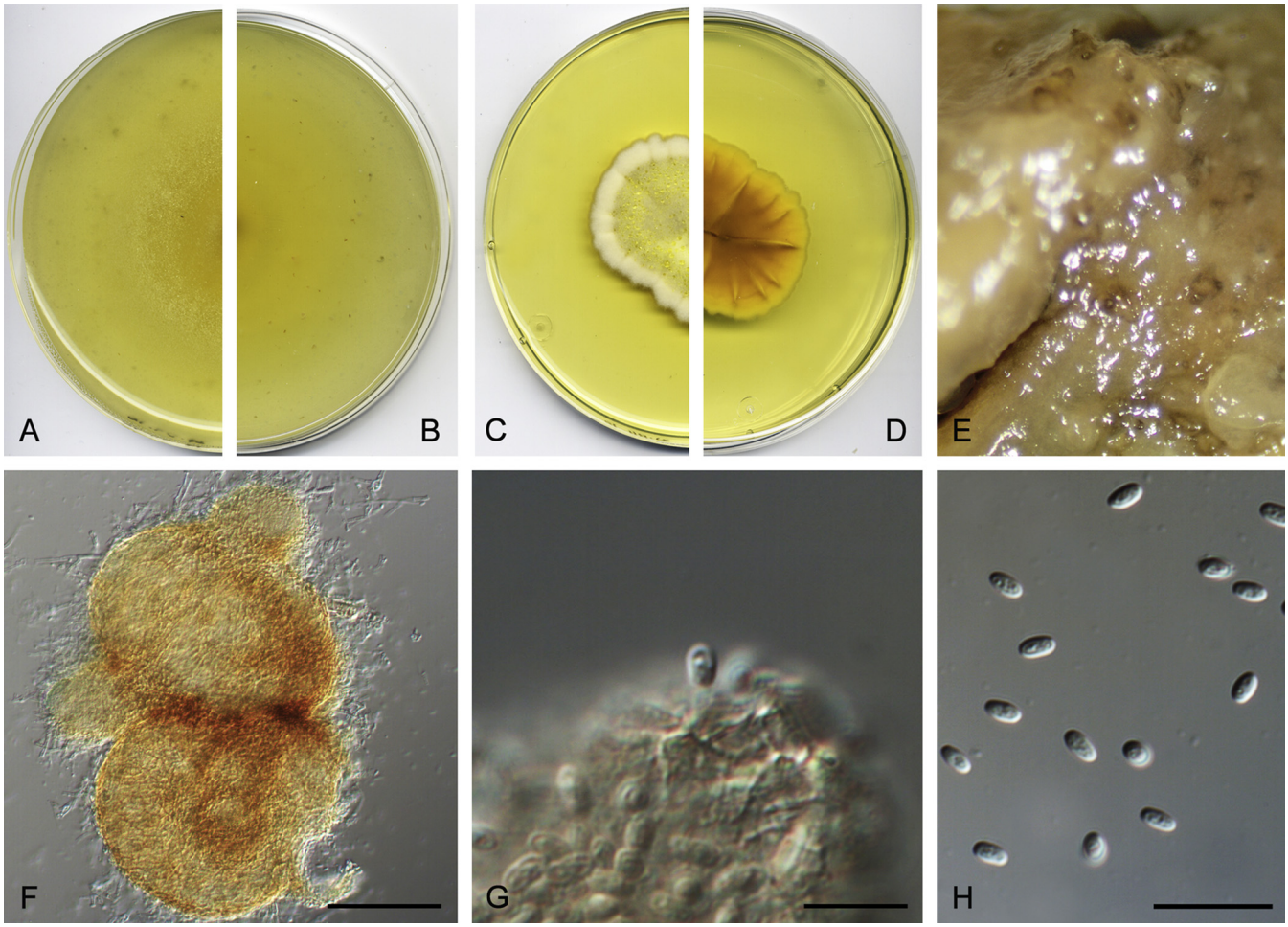

Fig. 39. *Neopyrenochaetopsis hominis* (CBS 143033). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Conidiogenous cell. **H.** Conidia. Scale bars: F = 100 µm. G, H = 10 µm.

Basionym: *Pyrenochaeta protearum* Crous, Persoonia 27: 153. 2011.
Synonym: *Pyrenochaeta pinicola* Crous, Persoonia 32: 255. 2014.

Description: Crous *et al.* (2011).

Materials examined: **France**, Nice, L'aire d'Esterel petrol filling station, on needles of *Pinus* sp., 20 Jul. 2013, P.W. Crous, living cultures ex-type of *P. pinicola*, CPC 23455 = CBS 137997 = FMR 15753. **South Africa**, Western Cape Province, on leaves of *Protea mundii*, 4 May 2010, P.W. Crous (**holotype** of *P. protearum*, CBS H-20772, ex-holotype living cultures CPC 18322 = CBS 131315 = FMR 15752).

Notes: *Pyrenochaeta protearum* morphologically resembles phoma-like taxa in producing single phialides covering the inner source of the pycnidia, and having small, ((3–)4–5(–6) × (2–)2.5(–3) µm), aseptate, hyaline conidia, but also resembles pyrenochaeta-like species due to its setose pycnidia (Crous *et al.* 2011). Based on ITS and LSU nucleotide sequences, this fungus has been related to *Leptosphaeria*, *Pyrenochaeta* and *Pyrenochaetopsis*, and was included in the genus *Pyrenochaeta* (Crous *et al.* 2011). However, our results revealed that this fungus is phylogenetically distant from *Pyrenochaeta* spp., and from the members of the *Cucurbitariaceae*, and therefore we accommodated it in the new genus *Parapyrenochaeta*. We also studied the ex-type strain of *Pyrenochaeta pinicola* (Crous *et al.* 2014), which was morphologically and genetically very closely related to *Pa. protearum.* Therefore, we reduce *Py. pinicola* to synonymy under *Pa. protearum*.

DISCUSSION

The taxonomy of the coelomycetes has undergone major changes in recent years, mainly due to the extensive use of molecular techniques, which has resulted in a more natural classification of these fungi. In this regard, the taxonomic circumscription of the genera *Phoma* (*Didymellaceae*) and *Pyrenochaeta* (*Cucurbitariaceae*) have proven to be especially complex. In recent studies on *Didymellaceae,* Chen *et al.* (2015, 2017) restricted *Phoma* to *P. herbarum*, accepting 17 genera in the family *Didymellaceae*. They demonstrated that by combining four loci, but especially by using the *rpb2* marker, it was possible to resolve the phylogeny of the *Didymellaceae*. However, in recent studies, several *Phoma* species accepted by Aveskamp *et al.* (2010) such as *P. bulgarica*, *P. crystallifera*, *P. destructiva*, *P. eupyrena*, *P. multirostrata*, *P. omnivirens*, *P. pereupyrena* and *P. saxea*, were not included. Currently, several genera such as *Didymellocamarosporium*, *Endocoryneum*, *Heracleicola*, *Neodidymella*, *Platychora* and *Pseudohendersonia* have been added to the *Didymellaceae* based mainly on the ribosomal gene analyses (Ariyawansa *et al.* 2015a, Hyde *et al.* 2016, Wijayawardene *et al.* 2016). However, recently, Chen *et al.* (2017) demonstrated that the genera mentioned above are simple synonyms of previous genera of that family such as *Ascochyta*, *Boeremia*, *Stagonosporopsis* and *Neomicrosphaeropsis*. Therefore, sequences of those taxa need to be verified with proper genes to resolve their taxonomic

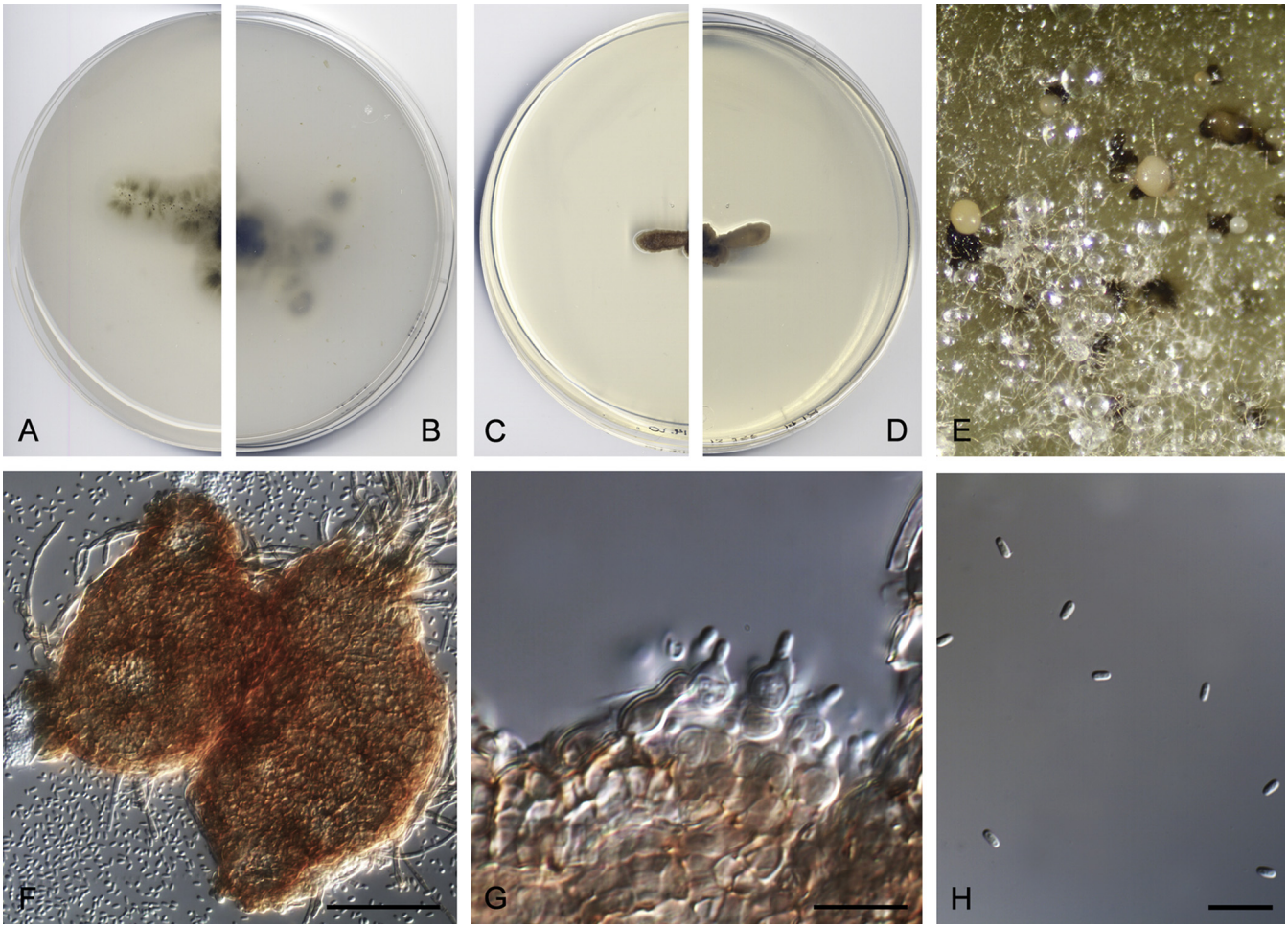

Fig. 40. *Parapyrenochaeta acaciae* (CBS 141291). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidia. **G.** Conidiogenous cells. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

placement within the *Didymellaceae*. For this reason, our proposal was to revise this family testing a large set of coelomycetous fungi recently isolated from clinical specimens (Valenzuela-Lopez *et al.* 2016), but also including several reference species of *Phoma* from the prior study of Aveskamp *et al.* (2010). This resulted in the proposal of six new genera, *viz*. *Cumuliphoma*, *Ectophoma*, *Juxtiphoma*, *Remotididymella*, *Similiphoma* and *Vacuiphoma*, 14 new species, and nine new combinations.

The taxonomic placement of *Pyrenochaeta* continues to be a topic of discussion, as this genus accommodates at least 163 epithets (www.indexfungorum.org). It is currently related to the *Cucurbitariaceae*, but an earlier phylogenetic study involving *Pyrenochaeta* species performed by Schoch *et al.* (2006), showed that *P. nobilis*, its type species, occupied an unclear taxonomic placement within the *Pleosporales*. Subsequently, this genus occupied an intermediate position as *incertae sedis* between the *Leptosphaeriaceae* and *Didymellaceae* (de Gruyter *et al.* 2009), or belonging to the *Leptosphaeriaceae* (Zhang *et al.* 2009). Later, de Gruyter *et al.* (2010) placed *Pyrenochaeta* in *Cucurbitariaceae*, and several species of *Phoma* in the new genus *Pyrenochaetopsis*. However, by employing additional gene *loci* in our phylogeny, the type species *P. nobilis* clustered distant from *Cucurbitariaceae s. str.*, being placed as *incertae sedis* in the *Pleosporineae*. Moreover, several species previously identified as *Pyrenochaeta* have proved to be phylogenetically scattered within the *Pleosporineae*. Therefore, we introduced four new families with several new genera to accommodate all *Pyrenochaeta* species which clustered outside the *Cucurbitariaceae,* i.e. *Neopyrenochaetaceae* (which includes *Neopyrenochaeta* gen. nov.), *Parapyrenochaetaceae* (within *Parapyrenochaeta* gen. nov.), *Pseudopyrenochaetaceae* (including *Pseudopyrenochaeta* gen. nov.) and *Pyrenochaetopsidaceae* (including the two new genera, *Neopyrenochaetopsis* and *Xenopyrenochaetopsis*).

In the revision of *Cucurbitariaceae* by Doilom *et al.* (2013), the authors accepted six genera in the family, although *Curreya*, *Rhytidiella* and *Syncarpella* were not sequenced. This family was recently enlarged by Wanasinghe *et al.* (2017b) proposing the new genus *Neocucurbitaria* to accommodate *N. acerina*, *N. unguis-hominis* (syn. *Pyrenochaeta unguis-hominis*, the type species of that genus) and *N. quercina* (syn. *Pyrenochaeta quercina*) and considering the genus *Fenestella* as belonging to this family; however, the type species and more species of this genus should be studied to clarify its taxonomy. *Neocucurbitaria* has been also modified in our study to include *N. cava* (syn. *Pyrenochaeta cava*), *N. hakeae* (syn. *Pyrenochaeta hakeae*), *N. keratinophila* (syn. *Pyrenochaeta keratinophila*), and the new species *N. aquatica* and *N. irregularis*. Here, we have also enlarged the current concept of *Cucurbitariaceae* with the proposal of the new genera *Allocucurbitaria* (with the only species *A. botulispora*), which is closely related to *Cucurbitaria* and *Paracucurbitaria*, with *P. corni* and the new species *P. italica* forming a clade distinct from *Neocucurbitaria*. In fact, *Cucurbitariaceae* is currently circumscribed with four genera, i.e. the three mentioned above, and *Cucurbitaria*. In contrast, *Camarosporium*, which was included in *Cucurbitariaceae* by Doilom

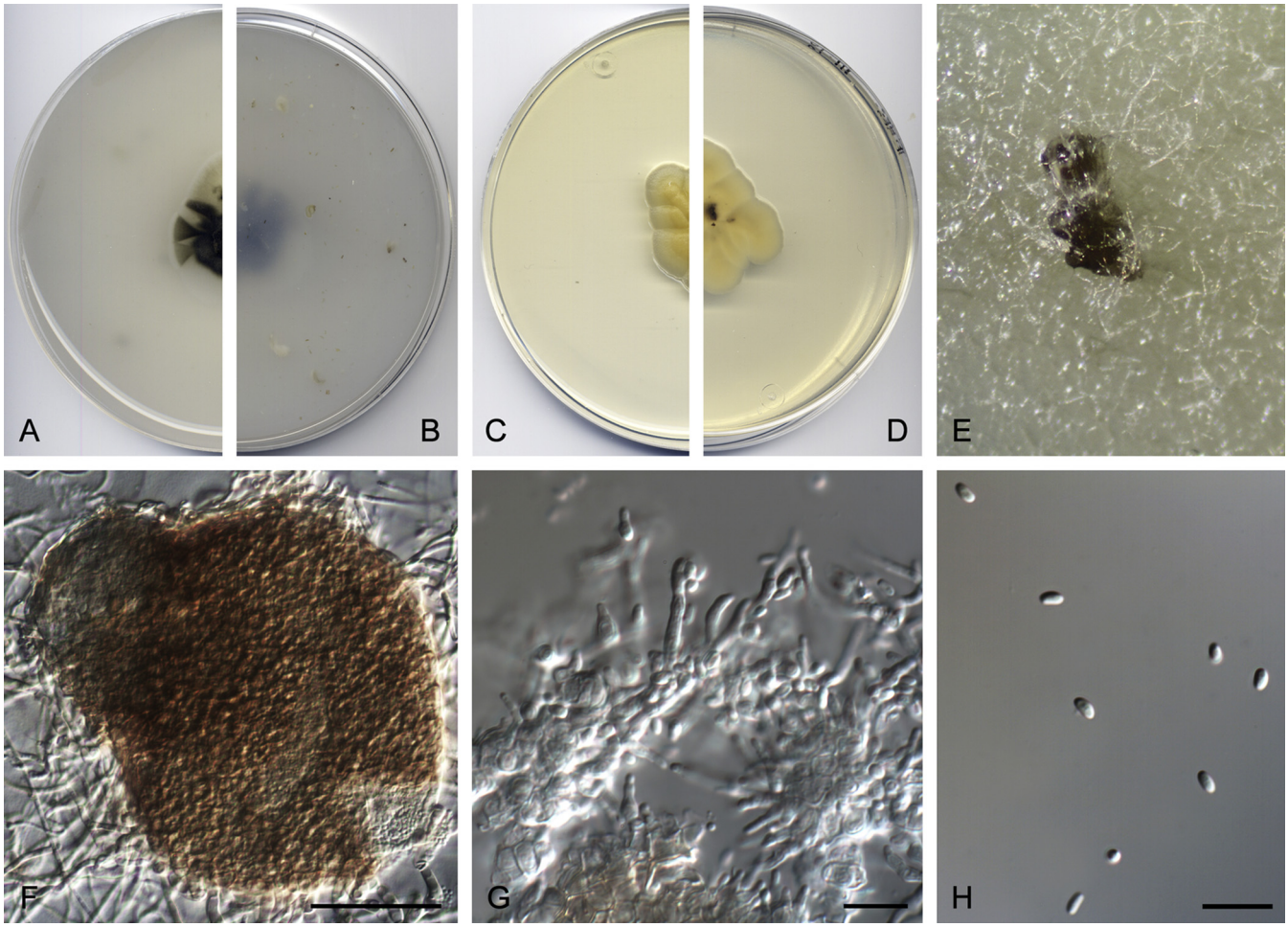

Fig. 41. *Parapyrenochaeta protearum* (CBS 131315). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E.** Pycnidia forming on OA. **F.** Pycnidium **G.** Conidiophores. **H.** Conidia. Scale bars: F = 50 µm. G, H = 10 µm.

et al. (2013), has been recently placed in *Coniothyriaceae* by Crous & Groenewald (2017), who studied and epitypified the generic type of *Camarosporium* and several phoma-like species, proposing the new family *Libertasomycetaceae* within *Pleosporineae*. In the same year, Wanasinghe *et al.* (2017a) have studied a large set of camarosporium-like fungi proposing the new families *Camarosporidiellaceae* and *Neocamarosporiaceae* and resurrected the family *Camarosporiaceae.* However, in our phylogeny, several members of *Coniothyriaceae* and *Leptosphaeriaceae* remain in an ambiguous taxonomic position within *Pleosporineae*. Furthermore, in our study the family *Camarosporidiellaceae* was phylogenetically unsupported, which is probably caused by the lack of *rpb2* or *tub2* sequences; therefore further studies are needed to understand the relationships of this family with the other members of this suborder.

At the present study, we have clarified the generic concept of two of the largest genera of coelomycetes (*Phoma* and *Pyrenochaeta*) through a polyphasic approach that included the analysis of four phylogenetic markers of 143 additional isolates. This approach allowed a better delimitation of members of *Cucurbitariaceae* and *Didymellaceae* of the suborder *Pleosporineae* that currently encompasses the following 19 families: *Camarosporiaceae*, *Camarosporidiellaceae*, *Coniothyriaceae*, *Cucurbitariaceae*, *Didymellaceae*, *Dothidotthiaceae*, *Halojulellaceae*, *Leptosphaeriaceae*, *Libertasomycetaceae*, *Microsphaeropsidaceae*, *Neocamarosporiaceae*, *Neophaeosphaeriaceae*, *Neopyrenochaetaceae*, *Parapyrenochaetaceae*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Pseudopyrenochaetaceae*, *Pyrenochaetopsidaceae* and *Shiraiaceae*.

ACKNOWLEDGEMENTS

The authors are very grateful to the following researchers or institutions: Markéta Šandová (Curator of the Natural History Museum, Czech Republic, PRM), Fátima Sales (Curator of the Herbarium of the Botanical Garden of the University of Coimbra, Portugal), Marc Jeanson and Simon Chagnoux (Muséum National d'Histoire Naturelle, Paris, France), Begoña Aguirre-Hudson (Curator of the Royal Botanic Gardens, Kew, UK), Rossella Marcucci (Curator of the Saccardo's Herbarium, Università degli Studi di Padova, Italy) and Jamie Minnaert-Grote and Andrew Miller (The Illinois Natural History Survey Fungarium, Illinois, USA). We also thank to reviewers for their valuable comments and suggestions to improve the content of the manuscript. This study was supported by the Spanish Ministerio de Economía y Competitividad, grant CGL2013-43789-P.

REFERENCES

Ahmed SA, Desbois N, Quist D, *et al.* (2015). Phaeohyphomycosis caused by a novel species, *Pseudochaetosphaeronema martinelli*. *Journal of Clinical Microbiology* **53**: 2927–2934.

Ahmed SA, Hofmüller W, Seibold M, *et al.* (2017). *Tintelnotia*, a new genus in *Phaeosphaeriaceae* harbouring agents of cornea and nail infections in humans. *Mycoses* **60**: 244–253.

Ahmed SA, Van De Sande WW, Stevens DA, *et al.* (2014). Revision of agents of black-grain eumycetoma in the order *Pleosporales*. *Persoonia* **33**: 141–154.

Amaradasa BS, Madrid H, Groenewald JZ, *et al.* (2014). *Porocercospora seminalis* gen. et comb. nov., the causal organism of buffalograss false smut. *Mycologia* **106**: 77–85.

Ariyawansa HA, Hyde KD, Jayasiri SC, *et al.* (2015a). Fungal diversity notes 111–252–taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* **75**: 27–274.

Ariyawansa HA, Jones EBG, Suetrong S, *et al.* (2013). *Halojulellaceae* a new family of the order *Pleosporales*. *Phytotaxa* **130**: 14–24.

Ariyawansa HA, Phukhamsakda C, Thambugala KM, *et al.* (2015b). Revision and phylogeny of *Leptosphaeriaceae*. *Fungal Diversity* **74**: 19–51.

Aveskamp MM, de Gruyter J, Crous PW (2008). Biology and recent developments in the systematics of *Phoma*, a complex genus of major quarantine significance. *Fungal Diversity* **31**: 1–18.

Aveskamp MM, de Gruyter J, Woudenberg JHC, *et al.* (2010). Highlights of the *Didymellaceae*: a polyphasic approach to characterise *Phoma* and related pleosporalean genera. *Studies in Mycology* **65**: 1–60.

Aveskamp MM, Verkley GJM, de Gruyter J, *et al.* (2009). DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties. *Mycologia* **101**: 363–382.

Batista AC, Vital AF (1957). Contribuiçao ao Estudo dos Fungos *Sphaeropsidales*. *Anais da Sociedade de Biologia de Pernambuco* **15**: 413–427.

Boerema GH (1993). Contributions towards a monograph of *Phoma* (Coelomycetes) – II. Section *Peyronellaea*. *Persoonia* **15**: 197–221.

Boerema GH, Bollen GJ (1975). Conidiogenesis and conidial septation as differentiating criteria between *Phoma* and *Ascochyta*. *Persoonia* **8**: 111–444.

Boerema GH, de Gruyter J, Noordeloos ME (1997). Contributions towards a monograph of *Phoma* (Coelomycetes) – IV. Section *Heterospora*: taxa with large sized conidial dimorphs, in vivo sometimes as *Stagonosporopsis* synanamorphs. *Persoonia* **16**: 335–371.

Boerema GH, de Gruyer J, Noordeloos ME, *et al.* (2004). *Phoma identification manual. Differentiation of specific and infra-specific taxa in culture*. CABI Publishing, Wallingford, UK.

Boerema GH, Dorenbosch MMJ, Kesteren HA van (1965). Remarks on species of *Phoma* referred to *Peryonellaea*. *Persoonia* **4**: 47–68.

Boerema GH, Kesteren HA van, Loerakker WM (1981). Notes on *Phoma*. *Transactions of the British Mycological Society* **77**: 61–74.

Boerema GH, Loerakker WM, Hamers ME (1996). Contributions towards a monograph of *Phoma* (Coelomycetes) – III. 2. Misapplications of the type species name and the generic synonyms of section *Plenodomus* (Excluded species). *Persoonia* **16**: 141–189.

Borman AM, Desnos-Ollivier M, Campbell CK, *et al.* (2016). Novel taxa associated with human fungal black-grain mycetomas: *Emarellia grisea* gen. nov., sp. nov., and *Emarellia paragrisea* sp. nov. *Journal of Clinical Microbiology* **54**: 1738–1745.

Brunaud P (1887). Supplément à la liste des Sphaeroidées trouvées à Saintes. *Revue Mycologique Toulouse* **9**: 13–17.

Bubák F, Kabát JE (1912). Mykologische Beiträge. *VII. Hedwigia* **52**: 340–363.

Chandra S, Tandon RN (1966). Three new leaf-infecting fungi from Allahabad. *Mycopathologia et Mycologia Applicata* **29**: 273–276.

Chen Q, Hou LW, Duan WJ, *et al.* (2017). *Didymellaceae* revisited. *Studies in Mycology* **87**: 105–159.

Chen Q, Jiang JR, Zhang GZ, *et al.* (2015). Resolving the *Phoma* enigma. *Studies in Mycology* **82**: 137–217.

Corda ACJ (1840). *Icones fungorum hucusque cognitorum*: **4**: 1–53.

Crous PW, Carris LM, Giraldo A, *et al.* (2015a). The Genera of Fungi – fixing the application of the type species of generic names – G 2: *Allantophomopsis, Latorua, Macrodiplodiopsis, Macrohilum, Milospium, Protostegia, Pyricularia, Robillarda, Rotula, Septoriella, Torula*, and *Wojnowicia*. *IMA Fungus* **6**: 163–198.

Crous PW, Gams W, Stalpers JA, *et al.* (2004). MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**: 19–22.

Crous PW, Groenewald JZ (2016). They seldom occur alone. *Fungal Biology* **120**: 1392–1415.

Crous PW, Groenewald JZ (2017). The Genera of Fungi – G 4: *Camarosporium* and *Dothiora*. *IMA Fungus* **8**: 131–152.

Crous PW, Shivas RG, Quaedvlieg W, *et al.* (2014). Fungal Planet description sheets: 214–280. *Persoonia* **32**: 184–306.

Crous PW, Summerell BA, Shivas RG, *et al.* (2011). Fungal Planet description sheets: 92–106. *Persoonia* **27**: 130–162.

Crous PW, Verkley GJM, Groenewald JZ, *et al.* (2009). *Fungal Biodiversity. CBS Laboratory Manual Series*. Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

Crous PW, Wingfield MJ, Burgess TI, *et al.* (2016a). Fungal Planet description sheets: 469–557. *Persoonia* **37**: 218–403.

Crous PW, Wingfield MJ, Le Roux JJ, *et al.* (2015b). Fungal Planet description sheets: 371–399. *Persoonia* **35**: 264–327.

Crous PW, Wingfield MJ, Richardson DM, *et al.* (2016b). Fungal Planet description sheets: 400–468. *Persoonia* **36**: 316–458.

Davey ML, Currah RS (2009). *Atradidymella muscivora* gen. et sp. nov. (*Pleosporales*) and its anamorph *Phoma muscivora* sp. nov.: a new pleomorphic pathogen of boreal bryophytes. *American Journal of Botany* **96**: 1281–1288.

De Gruyter J (2002). Contributions towards a monograph of *Phoma* (Coelomycetes) – IX Section *Macrospora*. *Persoonia* **18**: 85–102.

De Gruyter J, Aveskamp MM, Woudenberg JHC, *et al.* (2009). Molecular phylogeny of *Phoma* and allied anamorph genera: towards a reclassification of the *Phoma* complex. *Mycological Research* **113**: 508–519.

De Gruyter J, Boerema GH (2002). Contributions towards a monograph of *Phoma* (Coelomycetes) – VIII. Section *Paraphoma*: taxa with setose pycnidia. *Persoonia* **17**: 541–561.

De Gruyter J, Boerema GH, van der Aa HA (2002). Contributions towards a monograph of *Phoma* (Coelomycetes) VI – 2. Section *Phyllostictoides*: outline of its taxa. *Persoonia* **18**: 1–53.

De Gruyter J, Noordeloos ME (1992). Contributions towards a monograph of *Phoma* (Coelomycetes) – I. 1. Section *Phoma*: taxa with very small conidia in vitro. *Persoonia* **15**: 71–92.

De Gruyter J, Woudenberg JH, Aveskamp MM, *et al.* (2010). Systematic reappraisal of species in *Phoma* section *Paraphoma, Pyrenochaeta* and *Pleurophoma*. *Mycologia* **102**: 1066–1081.

De Gruyter J, Woudenberg JHC, Aveskamp MM, *et al.* (2013). Redisposition of *Phoma*-like anamorphs in *Pleosporales*. *Studies in Mycology* **75**: 1–36.

De Hoog GS, Guarro J, Gené J, Figueras MJ (2011). *Atlas of clinical fungi. CD-ROM v. 3.1*. Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

Doilom M, Liu JK, Jaklitsch WM, *et al.* (2013). An outline of the family *Cucurbitariaceae*. *Sydowia* **65**: 167–192.

Dorenbosch MMJ (1970). Key to nine ubiquitous soil-borne *Phoma*-like fungi. *Persoonia* **6**: 1–14.

Edgar RC (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.

Fisher NL, Burgess LW, Toussoun TA, Nelson PE (1982). Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* **72**: 151–153.

Hashimoto A, Matsumura M, Hirayama K, *et al.* (2017). *Pseudodidymellaceae* fam. nov.: phylogenetic affiliations of mycopappus-like genera in Dothideomycetes. *Studies in Mycology* **87**: 187–206.

Hernández-Restrepo M, Gené J, Castañeda-Ruiz RF, *et al.* (2017). Phylogeny of saprobic microfungi from Southern Europe. *Studies in Mycology* **86**: 53–97.

Horne AS (1920). Diagnoses of fungi from "spotted" apples. *Journal of Botany, British and Foreign* **58**: 238–242.

Hyde KD, Hongsanan S, Jeewon R, *et al.* (2016). Fungal diversity notes 367–490: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* **80**: 1–270.

Hyde KD, Jones EBG, Liu JK, *et al.* (2013). Families of *Dothideomycetes*. *Fungal Diversity* **63**: 1–313.

Jaklitsch WM, Checa J, Blanco MN, *et al.* (2018). A preliminary account of the *Cucurbitariaceae*. *Studies in Mycology* **90**: 71–118.

Jaklitsch WM, Olariaga I, Voglmayr H (2016). *Teichospora* and the *Teichosporaceae*. *Mycological Progress* **15**: 31.

Jaklitsch WM, Voglmayr H (2016). Hidden diversity in *Thyridaria* and a new circumscription of the *Thyridariaceae*. *Studies in Mycology* **85**: 35–64.

Jaklitsch WM, Voglmayr H (2017). Three former taxa of *Cucurbitaria* and considerations on *Petrakia* in the *Melanommataceae*. *Sydowia* **69**: 81–95.

Janse JD (1981). The bacterial disease of ash (*Fraxinus excelsior*), caused by *Pseudomonas syringae* subsp. *savastanoi* pv. *Fraxini* II. Etiology and taxonomic considerations. *European Journal of Forest Pathology* **11**: 425–438.

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). *Ainsworth and Bisby's Dictionary of the Fungi*, 10th ed. CAB International, Wallingford, UK.

Kocakaya Z, Halici MG, Kocakaya M (2015). *Phoma candelariellae* sp. nov., a lichenicolous fungus from Turkey. *Mycotaxon* **130**: 1185–1189.

Kornerup A, Wanscher JH (1978). *Methuen Handbook of Colour*, 3rd ed. Methuen, London, England.

Kruys A, Eriksson OE, Wedin M (2006). Phylogenetic relationships of coprophilous *Pleosporales* (Dothideomycetes, Ascomycota), and the classification of some bitunicate taxa of unknown position. *Mycological Research* **110**: 527–536.

Lawrey JD, Diederich P, Nelsen MP, *et al.* (2012). Phylogenetic placement of lichenicolous *Phoma* species in the *Phaeosphaeriaceae* (*Pleosporales*, Dothideomycetes). *Fungal Diversity* **55**: 195–213.

Liu YJ, Whelen S, Hall BD (1999). Phylogenetic relationships among ascomycetes evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* **16**: 1799–1808.

Liu YX, Hyde KD, Ariyawansa HA, *et al*. (2013). *Shiraiaceae*, new family of *Pleosporales* (Dothideomycetes, Ascomycota). *Phytotaxa* **103**: 51–60.

Marincowitz S, Crous PW, Groenewald JZ, Wingfield MJ (2008). *Microfungi Occurring on Proteaceae in the Fynbos. [CBS Biodiversity Series No. 7]*. Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

Matsushima T (1993). Matsushima mycological memoirs 7. *Matsushima Mycological Memoirs* **7**: 1–141.

McDonald GK, Peck D (2009). Effects of crop rotation, residue retention and sowing time on the incidence and survival of ascochyta blight and its effect on grain yield of field peas (*Pisum sativum* L.). *Field Crops Research* **111**: 11–21.

Miller MA, Pfeiffer W, Schwartz T (2012). The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. In: *Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the Extreme to the Campus and Beyond: 1–8*. Association for Computing Machinery, USA.

Noordeloos ME, de Gruyter J, van Eijk GW, Roeijmans HJ (1993). Production of dendritic crystals in pure cultures of *Phoma* and *Ascochyta* and its value as a taxonomic character relative to morphology, pathology and cultural characteristics. *Mycological Research* **97**: 1343–1350.

Nylander JAA (2004). *MrModeltest v2. Program Distributed by the Author*. Evolutionary Biology Centre, Uppsala University, Sweden.

Plowright CB (1881). On the fungoid diseases of the tomato. *Gardeners' Chronicle* **16**: 620–622.

Punithalingam E (1979). Sphaeropsidales in culture from humans. *Nova Hedwigia* **31**: 119–158.

Punithalingam E (1985). *Phoma sorghina*. CMI Descriptions of Pathogenic Fungi and Bacteria No. 825.

Punithalingam E, English MP (1975). *Pyrenochaeta unguis-hominis* sp. nov. on human toe-nails. *Transactions of the British Mycological Society* **64**: 539–541.

Rai MK (1989). *Phoma sorghina* infection in human being. *Mycopathologia* **105**: 167–170.

Rehner SA, Samuels GJ (1994). Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98**: 625–634.

Ronquist F, Teslenko M, van der Mark P, *et al*. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.

Rouxel T, Balesdent MH (2005). The stem canker (blackleg) fungus, *Leptosphaeria maculans*, enters the genomic era. *Molecular Plant Pathology* **6**: 225–241.

Saccardo PA (1879). Fungi Gallici lecti a cl. viris P. Brunaud, C.C. Gillet et Abb. Letendre. *Michelia* **1**: 500–538.

Saccardo PA (1892). *Sylloge Fungorum omnium hucusque cognitorum*: **10**. Supplementum Universale, Pars II, Italy, Padova: 1–964.

Salam MU, MacLeod WJ, Maling T, *et al*. (2011). A meta-analysis of severity and yield loss from ascochyta blight on field pea in Western Australia. *Australasian Plant Pathology* **40**: 591–600.

Schneider R, Gerlach W (1966). *Pyrenochaeta lycopersici* nov. spec., der Erreger der Korkwurzelkrankheit der Tomate. *Journal of Phytopathology* **56**: 117–122.

Schoch CL, Shoemaker RA, Seifert KA, *et al*. (2006). A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* **98**: 1041–1052.

Sharma R, Sharma R, Crous PW (2015). *Matsushimamyces*, a new genus of keratinophilic fungi from soil in central India. *IMA Fungus* **6**: 337–343.

Spegazzini C (1881). Nova addenda ad mycologiam venetam. *Atti della Società Crittogamologica Italiana* **3**: 42–71.

Stamatakis A (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Su YY, Qi YL, Cai L (2012). Induction of sporulation in plant pathogenic fungi. *Mycology* **3**: 195–200.

Sung G-H, Sung J-M, Hywel-Jones NL, *et al*. (2007). A multi-gene phylogeny of *Clavicipitaceae* (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution* **44**: 1204–1223.

Tamura K, Stecher G, Peterson D, *et al*. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolutionary* **31**: 2725–2729.

Tanaka K, Hirayama K, Yonezawa H, *et al*. (2015). Revision of the *Massarineae* (*Pleosporales*, Dothideomycetes). *Studies in Mycology* **82**: 75–136.

Thambugala KM, Daranagama DA, Phillips AJL, *et al*. (2016). Microfungi on *Tamarix*. *Fungal Diversity* **82**: 239–306.

Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673–4680.

Trakunyingcharoen T, Lombard L, Groenewald JZ, *et al*. (2014). Mycoparasitic species of *Sphaerellopsis*, and allied lichenicolous and other genera. *IMA Fungus* **5**: 391–414.

Valenzuela-Lopez N, Sutton DA, Cano-Lira JF, *et al*. (2016). Coelomycetous fungi in the clinical setting: morphological convergence and cryptic diversity. *Journal of Clinical Microbiology* **55**: 552–567.

Verkley GJ, Gené J, Guarro J, *et al*. (2010). *Pyrenochaeta keratinophila* sp. nov., isolated from an ocular infection in Spain. *Revista Iberoamericana de Micología* **27**: 22–24.

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

Wanasinghe DN, Hyde KD, Crous PW, *et al*. (2017a). Phylogenetic revision of *Camarosporium* (*Pleosporineae*, *Dothideomycetes*) and allied genera. *Studies in Mycology* **87**: 207–256.

Wanasinghe DN, Jones EB, Camporesi E, *et al*. (2016). Taxonomy and phylogeny of *Laburnicola* gen. nov. and *Paramassariosphaeria* gen. nov. (*Didymosphaeriaceae*, *Massarineae*, *Pleosporales*). *Fungal Biology* **120**: 1354–1373.

Wanasinghe DN, Phookamsak R, Jeewon R, *et al*. (2017b). *Fenestellaceae* with descriptions of new *Fenestella* species and *Neocucurbitaria* gen. nov. *Mycosphere* **8**: 397–414.

White TJ, Bruns T, Lee S, *et al*. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). Academic Press, San Diego, California, USA: 315–322.

Wijayawardene NN, Crous PW, Kirk PM, *et al*. (2014). Naming and outline of Dothideomycetes–2014 including proposals for the protection or suppression of generic names. *Fungal Diversity* **69**: 1–55.

Wijayawardene NN, Hyde KD, Wanasinghe DN, *et al*. (2016). Taxonomy and phylogeny of dematiaceous coelomycetes. *Fungal Diversity* **77**: 1–316.

Winter G (1885). Pilze–Ascomyceten. In: *GL Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz*, **1**: 65–528.

Woudenberg JHC, Aveskamp MM, de Gruyter J, *et al*. (2009). Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia* **22**: 56–62.

Woudenberg JHC, Groenewald JZ, Binder M, *et al*. (2013). *Alternaria* redefined. *Studies in Mycology* **75**: 171–212.

Zhang Y, Crous PW, Schoch CL, Hyde KD (2012). Pleosporales. *Fungal Diversity* **53**: 1–221.

Zhang Y, Schoch CL, Fournier J, *et al*. (2009). Multi-locus phylogeny of *Pleosporales*: a taxonomic, ecological and evolutionary re–evaluation. *Studies in Mycology* **64**: 85–102.

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4.3. Pleosporalean fungi from USA: family structure

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Pleosporalean coelomycetes from USA: family structure.

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Abstract:

A high number of fungi are characterized by the production of conidia within a conidioma, traditionally being named coelomycetes. These fungi can be found on the most diverse habitats including human infections. Recently, the taxonomy of the coelomycetes has drastically changed, remaining still controversial. Most of these organisms belong to *Pleosporales*, one of the largest order in *Dothideomycetes* that includes relevant plant pathogens and numerous species. We have revisited the families of the *Pleosporales* proposing a modern restructuration of most of them, based on the molecular and morphological study of a wide set of fresh or well preserved strains from clinical or environmental origin. We have studied a total of 106 isolates by combining the LSU, ITS, *tub*2, *rpb*2 and *tef*1 sequences including numerous types and reference strains for comparison purposes. The resulting phylogenetic tree revealed that the *Pleosporales* are included in 70 families, represented into *Pleosporales*, including the new family *Medicopsidaceae* proposed here. Our strains were distributed in 15 families and 12 genera, 30 species, and five new combinations are proposed as new.

Key words: Coelomycetes, *Didymosphaeriaceae*, Multigene phylogeny, *Pleosporales*, *Phaeosphaeriaceae*, taxonomy, *Thyridariaceae*.

Taxonomic novelties: New family: *Medicopsidaceae* Valenzuela-Lopez, Cano, Guarro & Stchigel; **New genera:** *Deannamyces*, *Dictyophoma*, *Didymosphaeomyces*, *Neodictyophoma*, *Neothyridaria*, *Parachaetomella*, *Paranigrograna*, *Pararoussoella*, *Setosamyces*, *Sphaeriamyces*, *Xenoleptosphaeria*, *Xenoroussoella*; **New species:** *Anteaglonium oculorum*, *Deannamyces macrospora*, *Dictyophoma flavescens*, *Didymosphaeomyces unguis*, *Edenia oculi*, *Keissleriella profunda*, *Montagnula cylindrispora*, *Neodictyophoma brunneospora*, *Neosetophoma americana*, *Neothyridaria solani*, *Nigrograna cutanea*, *Parachaetomella ligni-putridi*, *Paranigrograna pneumonia*, *Paraphaeosphaeria ellipsospora*, *Paraphaeosphaeria suttonii*, *Pararoussoella pulmonaris*, *Parathyridaria hominis*, *Parathyridaria naris*, *Parathyridaria ovina*, *Phaeodothis diversispora*, *Roussoella oculi-hominis*, *Setosamyces obispora*, *Sphaeriamyces fuckelii*, *Trematophoma pneumonia*, *Trematosphaeria hominis*, *Trematosphaeria setosa*, *Xenoleptosphaeria confluens*, *Xenoroussoella coprophila*, *Xenoroussoella papuae*, *Xenoroussoella profunda*; **New combinations:** *Neothyridaria solani*, *Setosamyces glycines*, *Setosamyces telephii*, *Thyridaria mukdahanensis*, *Xenoroussoella mexicana*.

INTRODUCTION

A high number of fungi are characterized by the production of conidia within a cavity lined by fungal tissue, host tissue, or the combination of both, called conidioma (Sutton 1980).These organisms were traditionally considered coelomycetes and most of them being saprobic or parasites of terrestrial vascular plants, and commonly found in soil or salty or freshwater environments; although with a lesser occurrence they can also infect vertebrates, including humans and other fungi (Aveskamp *et al*. 2008, de Hoog *et al*. 2000, Hyde et al. 2013, Valenzuela-López *et al*. 2017). In spite of the fact that the term coelomycetes is still used to refer to these fungi is an obsolete name, however, it is considered an artificial fungal class (Taylor 1995). The conidioma structure is usual acervular (cup-shaped and open), pycnidial (globose to pyriform and closed), or showing what seems more or less intermediate structure as sporodochium-like or stroma (Sutton 1980, Nag Raj 1993, Kirk *et al*. 2008). In this wide group there are included a huge number of fungi representing nearly 1,000 genera and 7,000 species (Kirk *et al*. 2008), which, modern molecular studies, have demonstrated that belong to at least three classes of the phylum *Ascomycota*, i.e.s *Dothideomycetes*, *Leotiomycetes*, and *Sordariomycetes* (Schoch *et al*. 2009, Maharachchikumbura *et al*. 2014, Wijayawardene *et al*. 2016).

In a previous study, we have demonstrated that a high variety of coelomycetes are present in clinical samples and that most of these fungi show a phoma-like or paraconiothyrium-like morphology and belong to several families of the *Pleosporales* (Valenzuela-Lopez *et al*. 2017). The order *Pleosporales* encompasses more than 4,700 species included in more than 230 genera, and 39 families (Kirk *et al*. 2008, Zhang *et al*. 2009, 2012, Ariyawansa *et al*. 2013, Hyde *et al*. 2013, Wijayawardene *et al*. 2014) and is probably the largest order of the class *Dothideomycetes*. The asexual morphs of the *Pleosporales* produce mainly their conidia within discrete conidiomata, or can arise from single conidiophores produced on the mycelia. Most of them are true plant pathogens including numerous species of *Alternaria*, *Ascochyta*, *Bipolaris*, *Didymella*, *Leptosphaeria*, *Parastagonospora* and *Phoma* (Zhang *et al*. 2009, 2012, Ariyawansa *et al*. 2013, de Gruyter *et al*. 2013, Liu *et al*. 2013, Quaedvlieg *et al*. 2013, Woudenberg *et al*. 2013, Hyde *et al*. 2014). Of all these coelomycetes, the most commonly recognized, due to their globose to pyriform pycnidia and one-celled hyaline conidia are the phoma-like fungi. However, this common morphology in fact corresponds to a complex taxonomy. For instance the former genus *Phoma* is highly polyphyletic, its species being distributed in the families *Cucurbitariaceae*, *Didymellaceae*, *Leptosphaeriaceae* and *Phaeosphaeriaceae* (Aveskamp *et al*. 2010, de Gruyter *et al*. 2013). The paraconiothyrium-like fungi which are characterized by pycnidial or

stromatic conidiomata producing mostly relatively small, subhyaline to pigmented, one or two-celled conidia, and previously classified in *Coniothyrium* or *Microsphaeropsis*, have also resulted to be polyphyletic and forming different lineages within the *Pleosporales* (Verkley *et al*. 2004, 2013, 2014, Schoch *et al*. 2009, Zhang *et al*. 2009, 2012, Aveskamp *et al*. 2010, de Gruyter *et al*. 2013, Quaedvlieg *et al*. 2013). The type species of *Microsphaeropsis, M. olivacea,* was recently placed by Chen *et al*. (2015) in the new family *Microsphaeropsidaceae* outside of *Didymellaceae*, and the type species of *Coniothyrium*, *C. palmarum*, forms a distinct clade outside of *Leptosphaeriaceae*, on which has been based the resurrection of the family *Coniothyriaceae* in the *Pleosporales* (de Gruyter *et al*. 2012). Since several species of *Coniothyrium* were genetically distant from *Coniothyrium s. str*. (Verkley *et al*. 2004) the genus *Paraconiothyrium* was introduced to accommodate them, this latter has been accepted in *Didymosphaeriaceae* (Ariyawansa *et al*. 2014). In general the traditional pleosporalean coelomycetes are difficult to identify morphologically because the lack or presence of reduced characteristic under *in vitro* conditions, which is worsened by the fact that most of these fungi remain sterile under the usual experimental conditions. Therefore, molecular analysis are required in most cases to achieve an accurate identification, being necessary to perform a multi-locus analysis to reproduce a reliable phylogenetic lineage (Schoch *et al*. 2006, 2009, Zhang *et al*. 2009, 2012, Valenzuela-Lopez *et al*. 2018).

In light of the background presented above the aim of this study is to resolve the taxonomic placement of 80 coelomycetous strains isolated from USA that was previously identified as members of *Pleosporales*, as well as 24 reference and ex-type strains provided by the CBS culture collection. For that reason, we have tried to delineate the phylogenetic relationship within the pleosporalean families performing a multi-locus analysis with at least three of the five phylogenetic markers sequenced: a fragment of the 28S nrRNA (LSU), the internal transcribed spacer region (ITS), a fragment of the beta-tubulin (*tub*2), a fragment of the RNA polymerase II subunit 2 (*rpb*2) and a fragment of translation elongation factor 1-alpha (*tef*1) genes, and revising their taxonomy.

MATERIALS AND METHODS

Isolates and reference fungal strains

The study comprises 80 clinical isolates previously identified as belonging to the *Pleosporales* (Valenzuela-Lopez *et al*. 2017), provided by the Fungus Testing Laboratory of the University of Texas Health Science Center at San Antonio (UTHSC; San Antonio, Texas, USA) (Table 1). For comparative purposes 24 reference and ex-type strains provided by the CBS culture

collection and two environmental strains from Spain (FMR 15573 and FMR 15906), were also included (Table 2).

Phenotypic study

For cultural characterisation, isolates were incubated on oatmeal agar (OA), malt extract agar (MEA) and carnation leaf agar (CLA), following the protocols of Valenzuela-Lopez *et al*. (2018). Colony diameters were measured after 7 d at 25 ± 1 ºC, and colony characterisation was performed 14 d after inoculation on the culture media. Colours were according to Kornerup & Wanscher (1978). The ability of the isolates to grow at cardinal temperatures were determined on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) after 7 d in darkness, from 5 to 35 °C ,at 5 °C intervals, and 37 °C.

Digital images of fruiting bodies were captured with an Olympus CH2 (Olympus Corporation, Tokyo, Japan), and micro-morphological structures with a Zeiss Axio-Imager M1 (Oberkochen, Germany) with a DeltaPix Infinity X digital camera using Nomarski differential interference contrast. Measurements were carried out by examining at least 30 individuals of each structure mounting in Shear's medium and in water (Aveskamp *et al.* 2010, Chen *et al.* 2015, Valenzuela-Lopez *et al*. 2018). The production of metabolite E+ (NaOH spot test) was carried out by the application of a droplet of 1N NaOH on a colony grown on MEA (Dorenbosch 1970, Noordeloos *et al*. 1993).

DNA extration, PCR and sequencing

The total genomic DNA was extracted from colonies grown on PDA after 7 d incubation at 25 ± 1 ºC, using the FastDNA kit protocol (Bio101, Vista, CA), with a FastPrep FP120 instrument (Thermo Savant, Holbrook, NY) according to the manufacturer's protocol. DNA was quantified by using Nanodrop 2000 (Thermo Scientific, Madrid, Spain). The following loci were amplified and sequenced: LSU with the primers LR0R (Rehner & Samuels 1994),d LR5 (Vilgalys & Hester 1990) and ITS with the primers ITS5 and ITS4 (White *et al.* 1990), *tub*2 with the primers TUB2Fw and TUB4Rd (Woudenberg *et al.* 2009), *rpb*2 with the primers RPB2-5F2 (Sung *et al.* 2007) and fRPB2-7cR (Liu *et al.* 1999), and *tef*1 with the primers TEF1-983F and TEF1-2218R (Schoch *et al*. 2006). The PCR amplifications were performed in a total volume of 25 µL containing 5 µL 10× PCR Buffer (Invitrogen, California, USA), 0.2 mM dNTPs, 0.5 µM of each primer, 1 U Taq DNA polymerase and 1–10 ng genomic DNA. PCR conditions for LSU, ITS, *tub*2 and *tef*1 were set as follows: an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation, annealing and extension, and a final extension step at 72 °C for 10 min. For the LSU and ITS amplification, the 35 cycles consisted of 45 s at 95 °C, 45 s at 53 °C and 2 min at 72 °C; for the *tub*2 region 30 s at 94 °C, 45 s at 56 °C and 1 min at 72 °C; and for *tef*1

region 30 s at 94 °C, 1 min 20 s at 57 °C and 1 min 30 s at 72 °C. The PCR program for *rpb2* amplification was performed following the protocol of Woudenberg *et al.* (2013). Sequencing of the amplicons 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 v. 7 (DNAStar Lasergene, Madison, WI, USA).

Phylogenetic analyses

Sequences of species used in the phylogenetic analysis were obtained from GenBank and listed in Table 1. For the phylogenetic study, the alignments of the sequences were performed using MEGA v. 6.06 (Tamura *et al*. 2013), with the ClustalW application (Thompson 1994), refined with MUSCLE (Edgar 2004) and manually adjusted using the same software platform. The ambiguous regions were excluded from the analyses. Phylogenetic reconstructions were made by maximum-likelihood (ML) and Bayesian inference (BI) with RAxML v. 8.2.10 (Stamatakis 2014) and MrBayes v. 3.2.6 (Ronquist *et al*. 2012), respectively. The best substitution model for each gene matrix correspond to GTR+I+G, and was estimated using MrModelTest v. 2.3 (Nylander 2004). For ML analyses, nearest-neighbour interchange was used as the heuristic method for tree inference. Support for internal branches was assessed by 1 000 ML bootstrapped pseudoreplicates. Bootstrap support (BS) ≥70 was considered significant. For BI analyses, Markov chain Monte Carlo (MCMC) sampling was performed with 10 M generations, with samples taken every 1 000 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 ≥0.95 was considered as significant. Both ML and BS analyses were run in CIPRES (Miller *et al*. 2010). Sequences generated in this study were deposited in GenBank (see Table 1 and 2), the final matrices used for phylogenetic analyses in TreeBASE (www.treebase.org; accession number: SXXXX) and the novel taxonomic descriptions and nomenclature in MycoBank (www.mycobank.org; Crous *et al.* 2004).

RESULTS AND DISCUSION

Phylogeny of pleosporalean families

The fungi included in the study were analyzed performing a multi-locus alignment of the LSU, *rpb*2 and *tef*1 sequences of our strains and others from types or reference strains retrieved from Genbank (see Table 2) to confirm and determine their exact placement within the

Pleosporales; genera of the order *Helotiales* as outgroup. Since the topologies of the consensus trees inferred with ML and BI analyses coincided, only the consensus tree of the first is shown (Fig. 1).The resulting tree showed a total of 75 clades that corresponded to the majority of accepted pleosporalean families. The strains included in the study were accommodated in fourteen of them, i.e. *Acrocalymmaceae*, *Amorosiaceae*, *Anteagloniaceae*, *Coniothyriaceae*, *Dictyosporiaceae*, *Didymosphaeriaceae*, *Leptosphaeriaceae*, *Lindgomycetaceae*, *Lophiostomataceae*, *Macrodiplodiopsidaceae*, *Nigrograna*ceae, *Phaeosphaeriaceae*, *Thyridariaceae*, *Trematosphaeriaceae*, and the new family ***Medicopsidaceae,*** proposed here for the monotypic genus *Medicopsis*. Furthermore, the type species of *Pyrenochaeta ligni-putridi* was relocated within the order *Helotiales* and proposed here in the new genus ***Parachaetomella***. The most relevant clades of each family and the new proposed taxa were highlighted and discussed below.

In spite of the numerous recent studies on the *Pleosporales* taxa the boundaries of the different families of the *Pleosporales* are not yet well defined. The first attempts to circumscribe on a molecular basis the different families of *Pleosporales* were carried out by Zhang *et al*. (2009) and Hyde *et al*. (2013) who performing multi-locus analyses accepted 15 and 41 families, respectively. However, more recently Liu *et al*. (2017) enlarged the number of families of *Pleosporales* up to 55. In the present study the taxonomy of the *Pleosporales* has been revised and a total of 71 families have been accepted in this order. Thus, for each family involved where new taxa are propose was constructed its phylogenetic tree, and the number of taxa, characters and information generated from the BI and ML for each dataset is commented in alphabetic order as follows:

Amorosiaceae

The family *Amorisiaceae* currently includes the genera *Amorosia* and *Angustimassarina*. The former only includes the dematiaceous hyphomycete *A. littoralis* that was isolated from marine sediment and originally phylogenetically placed into *Sporormiaceae* (Mantle *et al*. 2006). The latter genus is characterised by narrowly fusiform ascospores, and can produce an asexual morph, and also is considered parasitic of other ascomycetes (Thambugala *et al*. 2015). However, the phylogenetic relationships between both genera are unclear, resulting clearly separated phylogenetically; while *Amorosia* formed a sister clade with the genus *Teichospora* (*Teichosporaceae*) our strain (FMR 13779 joined with *Angustimassarina*, therefore, in a second phylogenetic analysis on Angustimassarina revealed that previous species in this genus remains doubtful due to their ITS and *tef*1 sequences are quite similar among them and more phylogenetic markers should be tested to recognize these species. The Fig. 1 showed a multi-locus tree based in the analysis of three markers (LSU, ITS, *tef*1) that included our

mentioned strain of *Angustimassarina,* the nine accepted species of the genus and *Amorosia littoralis*, while that the type strain of *Westerdykella cylindrica* (CBS 454.72) was used as out group. These data suggested that *Angustimassarina* should be limited to two species; the three markers providing very similar results, i.e., (443 conserved base pairs of 446 bp in ITS and 738 bp of 739 in LSU), and only six species have *tef*1 sequences, in which 17 bp of 628 bp are variable among them. These results demonstrated the polyphyletic characteristic of *Angustimassarina* which should be re-evaluated with more phylogenetic markers to confirm the correct boundaries of this genus. Since the strain FMR 13779 is phylogenetically different from to the species accepted in this genus, here is proposed as the new species ***A***. ***marina***.

Anteagloniaceae

The family *Anteagloniaceae* was introduced by Hyde *et al*. (2013) to accommodate the genus *Anteaglonium,* morphologically characterized by producing hysterothecial ascomata and related with the genera *Anteaglonium* and *Flammeascoma* (Liu *et al*. 2015). In a recent study of this family by Jayasiri *et al*. (2016), it was included a coelomycetous morph associated to *Anteaglonium*, and characterized by producing pycnidia, with long, unbranched, hyaline conidiophores and hyaline conidiogenous cells formed in the innermost layer of the wall, and hyaline, oval to globose, aseptate conidia. In our first phylogenetic tree (Fig. 1) the strain UTHSC DI16-316 (FMR 13809) formed a well-supported sister clade with *A*. *latirostrum* although both species were phylogenetically distinct. Therefore, we proposed this strain as the new species ***A. oculorum***.

Coniothyriaceae

The family *Coniothyriaceae* was proposed by Cooke (1983) with *Coniothyrium* as the generic type. These fungiare characterized by pycnidia, annellidic conidiogenous cells and brown, thick-walled, aseptate or 1-septate, verrucose conidia with *C*. *palmarum* as its type species. Previously it was associated with *Leptosphaeriaceae* (de Gruyter *et al*. 2009), but currently both have demonstrated to be phylogenetically separated within the *Pleosporales* (de Gruyter *et al*. 2013, Hyde *et al*. 2013, Wanasinghe *et al*. 2017). In their study on camarosporium-like species, Wanasinghe *et al*. (2017) showed the phylogenetic relationship of *Coniothyriaceae* with *Camarosporidiellaceae*, and placed the genus *Staurosphaeria* in the first family. However, in this study this family shows a polyphyletic relationship within the suborder *Pleosporineae*, revealing that *Coniothyrium* species were placed in a different clade, also confirms that the type species *C*. *palmarum* is close related with *Camarosporidiella* species. In addition, this study shows that *Staurosphaeria* forms a distinc clade outside of *Coniothyriaceae sensu stricto*. On the other hand, strains previously identified as *C*. *telephii* were studied here, and

demonstrated to be phylogenetically distinc from strains of *C*. *palmarum* (CBS 400.71 and 758.73), therefore, it has been erected here the new genus *Setosamyces* to accommodate it. *Coniothyrium telephii* was proposed by de Gruyter *et al*. (2013) as a new combination for *Phoma septicidalis* (Boerema 1979), its basionym *Pyrenochaeta telephii* (Allescher 1896) is morphologically characterised by producing setose pycnidial conidiomata and small, hyaline, aseptate conidia, this demonstrated the morphological difference with the description of the type species of *Coniothyrium*. Thus, here is introduced the new combination *Setosamyces telephii*, and for the strain CBS 101636 previously identified as *C*. *telephii* is proposed as the new species *S*. *glycines* based on the phylogenetic analysis and by morphological features (see Taxonomy section). Further studies trying to collect additional fresh material are needed to clarify the taxonomy of this complex family.

Dictyosporiaceae

This family was formally introduced by Boonmee *et al*. (2016) to accommodate those species with cheiroid, digitate and palmate, dictyosporous conidia and their sexual morphs. Currently, it comprises eleven genera, *Dictyosporium* being the generic type. In the first phylogenetic analysis of this study (Fig. 1), the strain UTHSC DI16-355 and CBS 178.93 both previously identified as *Paraconiothyrium* spp. (de Gruyter *et al*. 2012, Valenzuela-Lopez *et al*. 2017) clustered within this family.

Families which have been revisited and pending of writing are: ***Didymosphaeriaceae, Leptosphaeriaceae, Lindgomycetaceae Lophiostomataceae, Macrodiplodiopsidaceae, Medicopsidaceae, Nigrogranaceae, Phaeosphaeriaceae, Thyridariaceae, Trematosphaeriaceae.***

Taxonomy

Nigrogranaceae Jaklitsch & Voglmayr, Stud. Mycol. 85: 54. 2016.

Type genus: *Nigrograna* Gruyter, Verkley & Crous.

Nigrograna Gruyter *et al*., Stud. Mycol. 75: 31. 2012, emend. Jaklitsch & Voglmayr, Stud. Mycol. 85: 54. 2016.

Type species: *Nigrograna mackinnonii* (Borelli) Gruyter, Verkley & Crous.

Nigrograna cutanea Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MBXXX.

Etymology: The species name refers to the sort of clinical specimen (skin), from which the fungus was isolated.

Culture sterile. This species differs from its closest species, *Nigrograna mackinnonii* in the sequence of the sequences of ITS, *rpb*2 and *tef*1, being this latter the most informative (see TreeBASE SXXXX).

Culture characteristics: Colonies on OA reaching 20 mm diam after 7 d at 25±1 ºC, flattened, olive grey (M. 3F2); reverse dark grey (M. 3F1). Colonies on MEA reaching 12 mm after 7 d at 25±1 ºC, convex with papillate surface, olive grey (M. 3F2); reverse dark grey (M. 3F1). NaOH spot test negative. Crystals absent. Optimal, minimum and maximum temperatures were 25, 15 and 35º C, respectively.
Material examined: **USA**, from human skin, 2007, D.A. Sutton (**holotype** CBS H-XXX, ex-holotype living cultures CBS XXX = UTHSC DI16-241 = FMR 13731).

Notes: This species is phylogenetically easy to distinguish from the other species of the genus, but shares along with *N*. *mackinnonii* the human origin of the clinical specimens, the latter species being associated to the production of black grain mycetoma (Borelli 1976, Ahmed *et al*. 2014). Unfortunately, morphological comparison was not possible since this strain remains sterile.

Paranigrograna Valenzuela-Lopez, Cano, Guarro & Stchigel, **gen. nov.** MycoBank MBXXXX.

Etymology: It refers to its close phylogenetic relationship with *Nigrograna*.

Culture sterile. This fungus typically produces a greenish-yellow diffusible pigment on MEA.

Type species: *Paranigrograna pneumoniae* Valenzuela-Lopez, Cano, Guarro & Stchigel.

Paranigrograna pneumoniae Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MBXXX.

Etymology: It refers to the respiratory sample, from which the fungus was.

Culture remaining sterile. This strain differs phylogenetically from the closest species of *Nigrograna* (see Fig. X "tree"), particularly *N. obliqua* (XX% identity (ITS) and XX% identity (EF) and forms a distinct basal branch within *Nigrograna*ceae.

Culture characteristics: Colonies on OA reaching 13 mm diam after 7 d at 25±1 ºC, felted, olive brown (M. 4F4); reverse dark grey (M 4F1). Colonies on MEA reaching 12 mm after 7 d at 25±1 ºC, slightly floccose, greyish-beige (M. 4C2); reverse dark grey (M. 4F1). NaOH spot test negative. Crystals absent. Maximum, optimal and minimum temperature of growth 30, 25and 15 ºC, respectively. Optimal temperature for sporulation 25 ºC.

Material examined: **USA**, from human bronchial washing, 2011, D.A. Sutton (**holotype** CBS H-XXX, ex-type living cultures CBS XXX = UTHSC DI16-342 = FMR 13835).

Notes: This strain, forms a distinct basal branch outside from *Nigrograna s. str*, but close to that genus. Thus is considered a new within the *Nigrograna*ceae. Unfortunately, this strain remains sterile but produces a characteristic greenish-yellow pigment on MEA.

Thyridariaceae Q. Tian & K.D. Hyde, Fungal Diversity 63: 254. 2013. emend. Jaklitsch & Voglmayr, Stud. Mycol. 85: 44. 2016.

Synonym: *Roussoellaceae* J.K. Liu *et al*., Phytotaxa 181: 7. 2014.

Type genus: *Thyridaria* Sacc.

Neothyridaria Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **gen. nov.** MycoBank MBXXXX.

Etymology: Because its phylogenetic relationship with *Thyridaria*.

Conidiomata pycnidial, brown, immersed to erumpent, solitary, globose, peridium of *textura angularis*, with a single papillate ostiolar neck. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform to doliiform. *Conidia* aseptate, pale brown, smooth- and thick-walled, subcylindrical, guttulate.

Type species: *Neothyridaria solani* (Crous & M.J. Wingf.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel.

Neothyridaria solani (Crous & M.J. Wingf.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **comb. nov.** MycoBank MBXXX.
Basionym: *Roussoella solani* Crous & M.J. Wingf., Persoonia 36: 341. 2016.
Description: Crous *et al*. (2016).

Material examined: **France**, La Reunión, on stems of *Solanum mauritianum* (*Solanaceae*), 13 Mar 2015, P.W. Crous & M.J. Wingfield (**holotype** CBS H-22597, ex-type living cultures CBS 141288 = CPC 26331).

Notes: This species, based on DNA sequences, was recently proposed by Crous & M.J. Wingfield (2016) in *Roussoella*. However, in our phylogenetic study this species was located into a sister clade related with *Neoroussoella*, *Parathyridaria* and *Thyridaria*. Morphologically, this species differs considerably from the asexual morph of *Neoroussoella bambusae*, the phylogenetically closer, that produces conidiophores (absents in *Nt. solani*), anellidic conidiogenous cells (phialidic in *Nt. solani*) and hyaline conidia (pale brown in *Nt. solani*) (Liu JK *et al*. 2014). Genetically, *Nt. solani* differs from *Nr. bambusae* in 14 and 33 bp in their LSU and ITS sequences, respectively. Therefore, because these differences the new combination XXXXX is proposed.

Pararoussoella Valenzuela-Lopez, Cano, Guarro & Stchigel, **gen. nov.** MycoBank MBXXXX.

Etymology: Based on the phylogenetic relationship with *Roussoella*.

Sexual or asexual reproductive structures absent. Phenotypically this fungus is capable of produce a reddish pigment on culture. No known sexual-morph.

Type species: *Pararoussoella pulmonaris* Valenzuela-Lopez, Cano, Guarro & Stchigel.

Pararoussoella pulmonaris Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MBXXX.

Etymology: The name refers to the respiratory tract where the fungus was isolated.

Culture sterile. *Pararoussoella pulmonaris* differs phenotypically from its closest phylogenetic genus *Xenoroussoella* by the production of a reddish soluble pigment on OA, and genetically in 15 bp of the LSU sequence, 38 bp of ITS and 163 bp of *rpb*2, based on alignment of the

concatenated four loci deposited in TreeBASE (SXXXX).

Culture characteristics: Colonies on OA reaching 17–20 mm diam after 7 d at 25 ± 1 ºC, flattened, front and reverse dark brown (M. 7F5). Colonies on MEA reaching 8–12 mm diam after 7 d at 25 ± 1 ºC, slightly flattened, grey (M. 4C1); reverse olive brown (M. 4E3). NaOH spot test negative. Crystals absent. Optimal temperature of growth 30ºC; minimum temperature of growth 15ºC; maximum temperature of growth 35ºC.
Material examined: **USA**, from human bronchial washing, 2009, D.A. Sutton (**holotype** CBS H-XXX, ex-holotype living cultures CBS XXX = UTHSC DI16-269 = FMR 13760).

Notes: This strain is phylogenetic distinguishable from its most close relative, *Xenoroussoella*, being clearly a different taxon. Unfortunately, our strain was not able to sporulate, and further studies are needed for a more complete characterization of this fungus.

Parathyridaria Jaklitsch & Voglmayr, Stud. Mycol. 85: 48. 2016.

Type species: *Parathyridaria ramulicola* Jaklitsch, Fourn. & Voglmayr.

Parathyridaria brachi Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MBXXX.

Etymology: The name refers to the clinical specimen (human arm), from which the fungus was isolated.

Culture sterile. This species differs from the closest phylogenetic species, *Parathyridaria percutanea*, in 14 bp of ITS, 7 bp of *rpb*2 and 36 bp of *tef*1, based on alignment of the concatenated four loci deposited in TreeBASE (SXXXX).

Culture characteristics: Colonies on OA reaching 23 mm diam after 7 d at 25±1 ºC, flattened, olive (M. 3E5); reverse smoke grey (M 3C2) to olive (M 3F4). Colonies on MEA reaching 18 mm diam after 7 d at 25±1 ºC, slightly floccose, white (M. 4A1); reverse dark yellow (M. 4C8). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25ºC; minimum temperature of growth 5ºC; maximum temperature of growth 37ºC.

Material examined: **USA**, from human arm sample, 2010, D.A. Sutton (**holotype** CBS H-XXX, ex-holotype living cultures CBS XXX = UTHSC DI16-292 = FMR 13784).

Notes: This strain was recovered from a human clinical specimen in USA, as well as *Parathyridaria percutanea,* but unfortunatrly since our strain is sterile the morphological comparison between both fungi was not possible. However, our strain differs considerably in the ITS, *rpb*2 and *tef*1nucleotide sequences to be considered as a different species, being the last marker the most informative.

Parathyridaria naris Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MBXXX.

Etymology: Referring to the nose where the fungus was isolated.

Culture sterile. This species forms a distinct phylogenetic branch from the other species of the genus, differing from its close relative *P. ovina* in 20 bp (LSU), 2 bp (ITS), 143 bp (*rpb*2) and 6 bp (*tef*1).SXXXX).

Culture characteristics: Colonies on OA reaching 19–22 mm diam after 7 d at 25±1 ºC, flattened, white (M. 1A1) to olive (M. 1E4); reverse olive (M. 1E4). Colonies on MEA reaching 16–20 mm diam after 7 d at 25±1 ºC, floccose, white (M. 1A1) to olive (M. 1E4); reverse pale-grey (M. 1B1) to dark-grey (M. 1F1). NaOH spot test negative. Crystals absent. Optimal temperature of growth 25ºC; minimum temperature of growth 15ºC; maximum temperature of growth 35ºC.

Material examined: **USA**, from human nose, 2011, D.A. Sutton (**holotype** CBS H-XXX, ex-holotype living cultures CBS XXX = UTHSC DI16-334 = FMR 13827).

Notes: This strain is phylogenetically well-delimitate, being *rpb*2 sequences the most informative marker, however, since this culture is sterile further studies are needed to characterise morphologically this species.

Parathyridaria ovina Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MBXXX. Fig. X.

Etymology: From Latin *ovina*, sheep, because the aspect of the pycnidia under reflected light. *Description*: *Hyphae* pale brown, 2.5–5 µm wide, smooth- and thin-walled, septate. *Conidiomata* pycnidial, brown to dark brown, solitary, superficial, glabrous (but covered by abundant hyaline hyphae under reflected light), globose to subglobose, 260–290 × 250–260 µm diam, with a single papillate otiolar neck, pycnidial wall of *textura angularis*, 2-4 layered, 15–25 µm thick, composed of brown to dark brown, flattened polygonal cells of 5–15 µm diam.

Conidiogenous cells phialidic, doliiform to ampulliform, hyaline, 3–5 × 3–3.5 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, cylindrical, 2.5–3.5 × 2 µm, guttulate.

Culture characteristics: Colonies on OA reaching 20 mm diam after 7 d at 25±1 ºC, flattened, white (M. 4A1) to beige (M. 4C3); reverse white (M. 4A1) to olive brown (M. 4F8). Colonies on MEA reaching 17 mm diam after 7 d at 25±1 ºC, flattened to floccose, white (M. 4A1); reverse pale yellow (M. 4A3) to olive brown (M. 4F8). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25ºC; minimum temperature of growth 5ºC; maximum temperature of growth 37ºC.

Material examined: **USA**, from joint fluid , 2013, D.A. Sutton (**holotype** CBS H-XXX, ex-holotype living cultures CBS XXX = UTHSC DI16-360 = FMR 13853).

Notes: The proposal of *Parathyridaria ovina* as a new species is mainly based on its phylogenetic relationships, morphologically, it is easy to distinguishs from its closest relative, *P*. *percutanea*, in the colony features on OA (flattened *vs* floccose), and the largest dimensions of the pycnidia (260–290 × 250–260 µm *vs* 59–102 × 54–96 µm) and conidia (2.5–3.5 × 2 µm *vs* 1.2–2.0 × 0.7–0.9 µm).

Roussoella Sacc., Atti Inst. Veneto Sci. lett. 6: 410. 1888.

Type species: *Roussoella nitidula* Sacc. & Paol.

Roussoella oculorum Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, **sp. nov.** MycoBank MBXXX. Fig. X.

Etymology: From Latin *oculorum*, referring to the eyes from which the fungus was isolated.

Description: *Hyphae* pale brown to brown, 2–3 µm wide, smooth- and thin-walled, septate. *Conidiomata* pycnidial, dark brown, solitary, superficial on carnation leaf agar (CLA), covered by short hyphal outgrowths, globose to subglobose, 140–200 × 140–170 µm diam, with a single papillate otiolar neck, pycnidial wall of *textura angularis*, 2–4 layered, 15–25 µm thick, composed of brown to dark brown, flattened polygonal cells of 5–13 µm diam. *Conidiogenous cells* phialidic, doliiform, hyaline, 3–3.5 × 2–2.5 µm. *Conidia* aseptate, initially hyaline becoming pale brown with the age, smooth- and thin-walled, ellipsoidal, 3–3.5 × 2.5–3 µm, aguttulate.

Culture characteristics: Colonies on OA reaching 20–23 mm diam after 7 d at 25±1 ºC, flattened,

brownish-grey (M. 4F2); reverse dark grey (M 4F1). Colonies on MEA reaching 20–24 mm diam after 7 d at 25±1 ºC, floccose, white (M. 4A1); reverse dark grey (M. 4F1). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25ºC; minimum temperature of growth 5ºC; maximum temperature of growth 37ºC.

Material examined: **USA**, from human left eye sample, 2014, D.A. Sutton (**holotype** CBS H-XXX, ex-holotype living cultures CBS XXX = UTHSC DI16-362 = FMR 13855).

Notes: This strain formed a basal clade to the genus *Roussoella*, although it is only supported by the Bayesian posterior probability. Morphologically, this fungus only produces an asexual-morph that resembles the pycnidia described in other species of *Roussoella*. We proposed this fungus as the type strain of a new species of *Roussoella*, mostly due to its phylogenetical relationships with the other species of the genus.

Thyridaria Sacc., Grevillea 4: 21. 1875.

Type species: *Thyridaria broussonetiae* (Sacc.) Traverso.

Thyridaria mukdahanensis (Phook., D.Q. Dai & K.D. Hyde) Valenzuela-Lopez, Cano, Guarro & Stchigel, **comb. nov.** MycoBank MBXXX.
Basionym: *Roussoella mukdahanensis* Phook., D.Q. Dai & K.D. Hyde, Fungal Diversity 82: 32. 2016.
Description: Dai *et al*. (2016).

Material examined: **Thailand**, Mukdahan Province, Nongsung District, Wang Hai village, on dead culms of bamboo, 13 April 2011, R. Phookamsak (**holotype** MFLU 11–0237, ex-holotype living cultures MFLUCC 11–0201 = KUMCC).

Notes: This species was proposed by Dai *et al*. (2016) a saprobic fungus on dead bamboo culms in Thailand. Morphologically, this fungus produces a sexual morph compatible with *Roussoella*. In our phylogenetic study, this species clustered with *Thyridaria*, therefore, we propose the new combination including this species in that genus.

Xenoroussoella Valenzuela-Lopez, Cano, Guarro & Stchigel, **gen. nov.** MycoBank MBXXXX.

Etymology: From Greek ξένος-, strange, rare, because it is phylogenetically distinct from the genus *Roussoella*.

Conidiomata pycnidial, brown, immersed to erumpent, solitary to aggregated, globose, pycnidial wall of *textura angularis*, with a single papillate ostiolar neck. *Conidiogenous cells* phialidic, subhyaline, smooth-walled, globose to ampulliform. *Conidia* aseptate, brown, smooth- and thick-walled, ellipsoid, guttulate. Some species can produce crystals on culture.

Type species: *Xenoroussoella mexicana* (Crous & Yáñez-Morales) Valenzuela-Lopez, Cano, Guarro & Stchigel.

Xenoroussoella coprophila Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MBXXX. Fig. X.

Etymology: The name refers to dung, from which the fungus was isolated.

Description: *Hyphae* brown, 2–4 µm wide, smooth- and thin-walled, septate. *Conidiomata* pycnidial, brown to dark brown, solitary, semi-immersed, covered by hyphal outgrowths, globose, 200–260 µm diam, with a single papillate ostiolar neck, pycnidial wall of *textura angularis*, 2–3 layered, 10–20 µm thick, composed of brown to dark brown, flattened polygonal cells of 3–8 µm diam. *Conidiogenous cells* phialidic, ampulliform, hyaline, smooth, 4.5–6.5 × 3–4.5 µm. *Conidia* aseptate, pale brown, smooth- and thin-walled, guttulate, ellipsoidal, 3.5–4.5 × 2.5–3 µm.

Culture characteristics: Colonies on OA reaching 24–28 mm diam after 7 d at 25±1 ºC, flattened, olive (M. 3F4); reverse dark-grey (M 3F1). Colonies on MEA reaching 22–25 mm after 7 d at 25±1 ºC, slightly floccose, white (M. 3A1) to grey (M. 3C1); reverse olive grey (M. 3D2). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25ºC; minimum temperature of growth 15ºC; maximum temperature of growth 35ºC.
Material examined: **Spain**, Burgos, Vizcaínos, from an herbivorous animal dung sample, Jul 2016, J. Guarro, M. Guevara-Suarez & J.P.Z. Siqueira (**holotype** CBS H-XXX, ex-holotype living cultures CBS XXX = FMR 15573).

Notes: The strain FMR 15573 resembles morphologically the asexual-morphs of *Thyridariaceae*. However, due to that this strain was recovered from dung and it is phylogenetically distinct within *Xenoroussoella*, we proposed as a novel taxon.

Xenoroussoella mexicana (Crous & Yáñez-Morales) Valenzuela-Lopez, Cano, Guarro & Stchigel, **comb. nov.** MycoBank MBXXX.

Basionym: *Roussoella mexicana* Crous & Yáñez-Morales, Persoonia 35: 273. 2015.
Description: Crous *et al*. (2015).

Material examined: **Mexico**, Pozo del Tigre, Mpio. de Jalpan, Puebla State, on leaf spots of *Coffea arabica* (*Rubiaceae*), Caturra Rojo variety plantations, 23 Oct 2014, M. de Jesús Yáñez-Morales (**holotype** CMPH, isotype CBS H-22402, ex-isotype living culture CPC 25355). *Notes*: The new combination of *Xenoroussoella mexicana* is proposed here based on our phylogenetic study, in which Morphologically, this species producies brown, solitary or aggregated, glabrous, globose pycnidia with globose to ampulliform conidiogenous cells and brown and aseptate conidia while that their LSU and ITS sequences demonstrated that it is close to *Roussoella* but enough differently to justifie the proposal of a new genus.

Xenoroussoella papuae Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MBXXX. Fig. X.

Etymology: The name refers to Papua New Guinea the geographic region where this fungus was isolated.

Description: *Hyphae* pale brown to brown, 2–4 µm wide, smooth- and thin-walled, septate. *Conidiomata* pycnidial, brown to dark brown, solitary, superficial (OA), glabrous or covered by somewhat hyphal outgrowths, globose to subglobose, 190–200 × 150–210 µm diam, with a single papillate otiolar neck, pycnidial wall of *textura angularis*, 2–3 layered, 10–20 µm thick, composed of brown to dark brown, flattened polygonal cells of 3–10 µm diam. *Conidiogenous cells* phialidic, globose to ampulliform rarely doliform, hyaline, smooth, 4.5–8.5 × 5–5.5 µm. *Conidia* aseptate, initially to pale brown, smooth- and thick-walled, guttulate, ellipsoidal, 5–5.5 × 2.5–3.5 µm.

Culture characteristics: Colonies on OA reaching 24 mm diam after 7 d at 25±1 ºC, flattened, olive brown (M. 4F6); reverse olive brown (M. 4F8). Colonies on MEA reaching 21 mm after 7 d at 25±1 ºC, slightly floccose, beige (M 4C3) to olive brown (M 4D3); reverse olive brown (M. 3F5). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation 25ºC; minimum temperature of growth 15ºC; maximum temperature of growth 30ºC.

Material examined: **Papua New Guinea**, Madang Prov., Balek Wildlife Sanctuary, c. 15 km S of Madang along road to Lae, from bamboo, 3 Nov 1995, A. Aptroot (**holotype** CBS H-6391; CBS H-6392, ex-holotype living cultures CBS 170.96 = FMR 16827). **USA**, from human wrist sample, 2012, D.A. Sutton, living cultures UTHSC DI16-356 = FMR 13849.

Notes: The strain CBS 170.96 was initially identified as *Roussoella intermedia* (Ahmed *et al*. 2014), but in the last study by Jaklitsch & Voglmayr (2016) this strain was considered an unidentified species of that genus due to that the reference strain NBRC 106245 fix better as *R*. *intermedia* and is phylogenetically related to *R*. *pustulans*, which is reinforced by the morphological characteristic among them. The present study revealed this strain phylogenetically distant from *Roussoella* and for this reason has been placed in the new genus *Xenoroussoella*. Morphologically the strain shows the generic characteristics of a roussoella-like fungus.

Xenoroussoella profunda Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MBXXX.

Etymology: The name refers to a human deep fluid sample, from which the fungus was isolated. Culture sterile. This species forms a distinct phylogenetic branch together with *Xenoroussoella mexicana* and *X*. *papuae*. It differs from *X*. *papuae* mainly in the sequences of ITS and *tef*1, being *rpb*2 sequence less informative.

Culture characteristics: Colonies on OA reaching 20 mm diam after 7 d at 25±1 ºC, flattened, white (M. 1A1); reverse white (M. 1A1). Colonies on MEA reaching 21 mm after 7 d at 25±1 ºC, flattened, white (M. 1A1); reverse white (M. 1A1). NaOH spot test negative. Crystals absent. Optimal temperature of growth 25ºC; minimum temperature of growth 15ºC; maximum temperature of growth 30ºC.

Material examined: **USA**, from human cerebrospinal fluid, 2012, D.A. Sutton (**holotype** CBS H-XXX, ex-holotype living cultures CBS XXX = UTHSC DI16-220 = FMR 13710).

Notes: The strain UTHSC DI16-220, unfortunately, remains sterile and is only well-differentiated by phylogenetic analysis, being ITS and *tef*1 the genes most informative. However, further studies are needed to characterise morphologically this species.

***Xenoroussoella* sp.**

Material examined: **The Netherlands**, Flevoland, Roggebotzand, from *Salix* sp. bark, 4 May 1994, A. Aptroot, living culture CBS 368.94.

Notes: This strain was previously identified as *Arthopyrenia salicis*; however, no herbarium

material or studies of this strain was provided. In our study this strain clustered close to the species of *Xenoroussoella*.

Didymosphaeriaceae Munk, Dansk bot. Ark. 15(no. 2): 128. 1953.

Synonym: *Montagnulaceae* M.E. Barr, Mycotaxon 77: 194. 2001.

Type genus: *Didymosphaeria* Fuckel.

Didymosphaeria Fuckel, Jb. nassau. Ver. Naturk. 23-24: 140. 1870.
Type species: *Didymosphaeria futilis* (Berk. & Broome) Rehm.

Montagnula Berl., Icon. fung. (Abellini) 2: 68. 1896.
Type species: *Montagnula infernalis* (Niessl) Berl.

Paraconiothyrium Verkley, Stud. Mycol. 50: 327. 2004.
Type species: *Paraconiothyrium estuarinum* Verkley & M. da Silva.

Paraphaeosphaeria O.E. Erikss., Arkiv før Botanik 6 (4-5): 405. 1967.
Type species: *Paraphaeosphaeria michotii* (Westend.) O.E. Erikss.

Paraphaeosphaeria ellipsospora Valenzuela-Lopez, Stchigel, Guarro & Cano, **sp. nov.**, MycoBank MBXXX. Fig. X.

Etymology: xxx.

Description: *Hyphae* hyaline to pale brown to brown, 2.5–3 µm wide, smooth- and thin-walled, septate. *Pycnidia* solitary or confluent, superficial or immersed (OA), pale brown to dark brown, globose to subglobose, glabrous, 210–250 × 180–220 µm. diam., pycnidial wall of *textura angularis*, 2–5 layered, 15–60 µm. thick, composed of pale brown to brown, flattened polygonal cells of 5–12 µm diam., neck absent, with a single ostiolum. *Conidiogenous cells* holoblastic, phialidic, doliform or ampulliform, hyaline, smooth, 9–12 × 4–5 µm. *Conidia* ellipsoidal, pale brown to brown, smooth, thin walled, aseptate, aguttulate, 4.5–7 × 3–3.5 µm.

Culture characteristics: Colonies on OA reaching 25 mm diam. 7 d at 25±1 ºC, flattened, greyish-yellow (M. 4C5) to yellowish-orange (M. 4A7); reverse greyish-yellow (M. 4C5).

Colonies on MEA reaching 27 mm 7 d at 25±1 ºC, flattened, brown (M. 6E7) to brownish grey (M. 6C2); reverse dark-brown (M. 6F7) to light-brown (M. 6D8). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation, 25ºC; minimum temperature of growth, 5ºC; maximum temperature of growth, 30ºC.

Material examined: **USA**, from bronchial wash*,* 2008, D.A. Sutton (**holotype** CBS H-XXX, ex-holotype living cultures CBS XXX = UTHSC DI16-261 = FMR = 13751).

Notes: xxx.

Trematosphaeriaceae K.D. Hyde, Y. Zhang ter, Suetrong & E.B.G. Jones, Cryptog. Mycol., 32: 347. 2011.

Type genus: *Trematosphaeria* Fuckel.

Trematosphaeria Fuckel, Jb. nassau. Ver. Naturk. 23-24: 161. 1870.

Type species: *Trematosphaeria pertusa* Fuckel.

Trematosphaeria setosa Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.**, MycoBank MBXXXX. Fig. X.

Etymology: xxx.

Description: *Hyphae* brown, smooth- and thin-walled, septate, 2–5 µm wide. *Conidiomata* pycnidial, brown to dark brown, solitary, superficial and immersed (OA), covered by hyphal outgrowths, globose to subglobose, 100–130 × 80–110 µm, with a single papillate otiolar neck, pycnidial wall of *textura angularis*, 2–4-layered, 10–38 µm thick, composed of brown to dark brown, flattened polygonal cells of 3–5 µm diam. *Conidiogenous cells* phialidic, acicular or cylindrical, hyaline, smooth, 5–10 × 2–3 µm. *Conidia* aseptate, hyaline, smooth- and thin-walled, guttulate, cylindrical and slightly curved to ovoid, 3–4.5 × 2–2.5 µm.

Culture characteristics: Colonies on OA reaching 12 mm diam after 7 d at 25±1 ºC, flattened,

olive brown (M. 4E3) to brownish-grey (M. 4F2); reverse greyish-beige (M 4C2) to brownish-grey (M. 4F2). Colonies on MEA reaching 10 mm after 7 d at 25±1 ºC, flattened, olive brown (M. 4F4); reverse grey (M. 4F1). NaOH spot test negative. Crystals absent. Optimal temperature of growth and sporulation, 25ºC; minimum temperature of growth, 5ºC; maximum temperature of growth, 37ºC.

Material examined: **USA**, from human wound on tissue arm, 2011, D.A. Sutton, living cultures (**holotype** CBS H-XXX, ex-holotype living cultures CBS XXX = UTHSC DI16-335 = FMR = 13828).

Notes: xxx.
Trematosphaeria hominis Valenzuela-Lopez, Cano, Guarro & Stchigel, **sp. nov.** MycoBank MBXXX.

Etymology: xxx.

Culture sterile. This species is phylogenetical related with *Trematosphaeria asexualis* but it differs from that species in 13, 66, 30 and 24 bp of the LSU, ITS, *rpb*2 and *tef*1 sequences.
Culture characteristics: Colonies on OA reaching 20 mm diam after 7 d at 25±1 ºC, flattened, white (M. 1A1); reverse white (M. 1A1). Colonies on MEA reaching 21 mm after 7 d at 25±1 ºC, flattened, white (M. 1A1); reverse white (M. 1A1). NaOH spot test negative. Crystals absent. Optimal temperature of growth 25ºC; minimum temperature of growth 15ºC; maximum temperature of growth 30ºC.

Material examined: **USA**, from human tissue, 2009, D.A. Sutton (**holotype** CBS H-XXX, ex-holotype living cultures CBS XXX = UTHSC DI16-281 = FMR 13773).

Notes: xxx.

REFERENCES

Ahmed SA, Desbois N, Quist D, et al. (2015). Phaeohyphomycosis caused by a novel species, Pseudochaetosphaeronema martinelli. Journal of Clinical Microbiology 53: 2927–2934.

Ahmed SA, Hofmüller W, Seibold M, et al. (2017). Tintelnotia, a new genus in Phaeosphaeriaceae harbouring agents of cornea and nail infections in humans. Mycoses 60: 244–253.

Ahmed SA, Van De Sande WW, Stevens DA, et al. (2014). Revision of agents of black-grain eumycetoma in the order Pleosporales. Persoonia 33: 141–154.

Ariyawansa HA, Hyde KD, Jayasiri SC, et al. (2015a). Fungal diversity notes 111–252–taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 75: 27–274.

Ariyawansa HA, Jones EBG, Suetrong S, et al. (2013). Halojulellaceae a new family of the order Pleosporales. Phytotaxa 130: 14–24.

Ariyawansa HA, Phukhamsakda C, Thambugala KM, et al. (2015b). Revision and phylogeny of Leptosphaeriaceae. Fungal Diversity. 74:19–51.

Aveskamp MM, de Gruyter J, Crous PW (2008). Biology and recent developments in the systematics of Phoma, a complex genus of major quarantine significance. Fungal Diversity 31: 1–18.

Aveskamp MM, de Gruyter J, Woudenberg JHC, et al. (2010). Highlights of the Didymellaceae: A polyphasic approach to characterise Phoma and related pleosporalean genera. Studies in Mycology 65: 1–60.

Boerema GH, de Gruyer J, Noordeloos ME, et al. (2004). Phoma identification manual. Differentiation of specific and infra-specific taxa in culture. CABI publishing, Wallingford, U.K.

Borman AM, Desnos-Ollivier M, Campbell CK, et al. (2016). Novel taxa associated with human fungal black-grain mycetomas: Emarellia grisea gen. nov., sp. nov., and Emarellia paragrisea sp. nov. Journal of Clinical Microbiology 54: 1738–1745.

Chen Q, Jiang JR, Zhang GZ, et al. (2015a). Resolving the Phoma enigma. Studies in Mycology 82: 137–217.

Crous PW, Gams W, Stalpers JA, et al. (2004). MycoBank: an online initiative to launch mycology into the 21st century. Studies in Mycology 50: 19–22.

Crous PW, Groenewald JZ (2016). They seldom occur alone. Fungal Biology 120: 1392–1415.

Crous PW, Groenewald JZ (2017). The Genera of Fungi – G 4: Camarosporium and Dothiora. IMA Fungus 8: 131–152.

Crous PW, Verkley GJM, Groenewald JZ, et al. (2009). Fungal Biodiversity. CBS Laboratory Manual Series. Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

De Gruyter J, Woudenberg JH, Aveskamp MM, et al. (2010). Systematic reappraisal of species

in Phoma section Paraphoma, Pyrenochaeta and Pleurophoma. Mycologia 102: 1066–1081.

De Gruyter J, Woudenberg JHC, Aveskamp MM, et al. (2013). Redisposition of Phoma-like anamorphs in Pleosporales. Studies in Mycology 75: 1–36.

De Hoog GS, Guarro J, Gené J, Figueras MJ (2011). Atlas of clinical fungi. CD-ROM v. 3.1. Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

Edgar RC (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797.

Fisher NL, Burgess LW, Toussoun TA, Nelson PE (1982). Carnation leaves as a substrate and for preserving cultures of Fusarium species. Phytopathology 72: 151–153.

Hashimoto A, Matsumura M, Hirayama K, et al. (2017). Pseudodidymellaceae fam. nov.: Phylogenetic affiliations of mycopappus-like genera in Dothideomycetes. Studies in Mycolology 87: 187–206.

Hernández-Restrepo M, Gené J, Castañeda-Ruiz RF, et al. (2017). Phylogeny of saprobic microfungi from Southern Europe. Studies in Mycology 86: 53–97.

Hyde KD, Jones EBG, Liu JK, et al. (2013). Families of Dothideomycetes. Fungal Diversity 63: 1–313.

Hyde KD, Hongsanan S, Jeewon R, et al. (2016). Fungal diversity notes 367–490: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 80: 1–270.

Jaklitsch WM, Olariaga I, Voglmayr H (2016). Teichospora and the Teichosporaceae. Mycological Progress 15: 31.

Jaklitsch WM, Voglmayr H (2016). Hidden diversity in Thyridaria and a new circumscription of the Thyridariaceae. Studies in Mycology 85: 35–64.

Jaklitsch, WM, Voglmayr H (2017). Three former taxa of Cucurbitaria and considerations on Petrakia in the Melanommataceae. Sydowia 69: 81–95.

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). Ainsworth and Bisby's Dictionary of the Fungi, 10th edn. CAB International., Wallingford, UK.

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

Kruys A, Eriksson OE, Wedin M (2006). Phylogenetic relationships of coprophilous Pleosporales (Dothideomycetes, Ascomycota), and the classification of some bitunicate taxa of unknown position. Mycological Research 110: 527–536.

Lawrey JD, Diederich P, Nelsen MP, et al. (2012). Phylogenetic placement of lichenicolous Phoma species in the Phaeosphaeriaceae (Pleosporales, Dothideomycetes). Fungal Diversity 55: 195–213.

Liu YX, Hyde KD, Ariyawansa HA, et al. (2013). Shiraiaceae, new family of Pleosporales (Dothideomycetes, Ascomycota). Phytotaxa 103: 51–60.

Liu YJ, Whelen S, Hall BD (1999). Phylogenetic relationships among ascomycetes evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16: 1799–1808.

Miller MA, Pfeiffer W, Schwartz T. (2012). The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. In: Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the extreme to the campus and beyond: 1–8. Association for Computing Machinery, USA.

Nylander JAA (2004). MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Sweden.

Punithalingam E (1979). Sphaeropsidales in culture from humans. Nova Hedwigia 31: 119–158.

Ronquist F, Teslenko M, van der Mark P, et al. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.

Rouxel T, Balesdent MH (2005). The stem canker (blackleg) fungus, Leptosphaeria maculans, enters the genomic era. Molecular Plant Pathology 6: 225–241.

Salam MU, MacLeod WJ, Maling T, et al. (2011). A meta-analysis of severity and yield loss from ascochyta blight on field pea in Western Australia. Australasian Plant Pathology 40: 591–600.

Schoch CL, Shoemaker RA, Seifert KA, et al. (2006). A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98: 1041–1052.

Stamatakis A (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313.

Su YY, Qi YL, Cai L (2012). Induction of sporulation in plant pathogenic fungi. Mycology 3: 195–200.

Sutton BC (1980). The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stromata, 1st edn. Commonwealth Mycological Institute, UK.

Tamura K, Stecher G, Peterson D, et al. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolutionary 31: 2725–2729.

Tanaka K, Hirayama K, Yonezawa H, et al. (2015). Revision of the Massarineae (Pleosporales, Dothideomycetes). Studies in Mycology 82: 75–136.

Thambugala KM, Daranagama DA, Phillips AJL, et al. (2016). Microfungi on Tamarix. Fungal Diversity 82: 239–306.

Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680.

Valenzuela-Lopez N, Sutton DA, Cano-Lira JF, et al. (2016). Coelomycetous fungi in the clinical

setting: morphological convergence and cryptic diversity. Journal of Clinical Microbiology 55: 552–567.

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4238–4246.

Wanasinghe DN, Jones EB, Camporesi E, et al. (2016). Taxonomy and phylogeny of Laburnicola gen. nov. and Paramassariosphaeria gen. nov. (Didymosphaeriaceae, Massarineae, Pleosporales). Fungal Biology 120: 1354–1373.

Wanasinghe DN, Hyde KD, Crous PW, *et al*. (2017). Phylogenetic revision of *Camarosporium* (Pleosporineae, Dothideomycetes) and allied genera. Studies in Mycology 87: 207–256.

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

Wijayawardene NN, Crous PW, Kirk PM, et al. (2014). Naming and outline of Dothideomycetes–2014 including proposals for the protection or suppression of generic names. Fungal Diversity 69: 1–55.

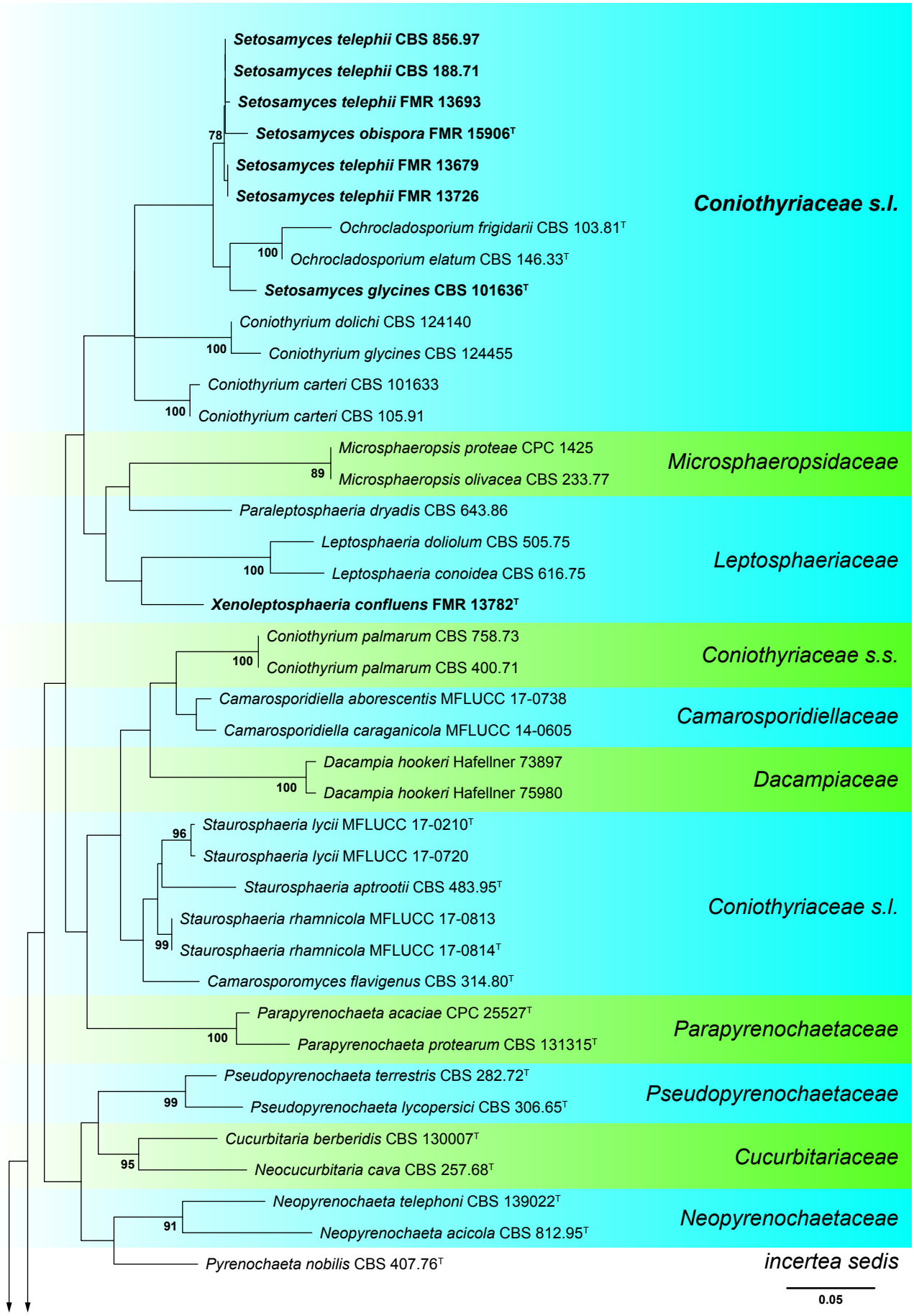
Wijayawardene NN, Hyde KD, Wanasinghe DN, et al. (2016). Taxonomy and phylogeny of dematiaceous coelomycetes. Fungal Diversity 77: 1–316.

Woudenberg JHC, Aveskamp MM, de Gruyter J, et al. (2009). Multiple Didymella teleomorphs are linked to the Phoma clematidina morphotype. Persoonia 22: 56–62.

Woudenberg JHC, Groenewald JZ, Binder M, et al. (2013). Alternaria redefined. Studies in Mycology 75: 171–212.

Zhang Y, Schoch CL, Fournier J, *et al*. (2009). Multi-locus phylogeny of *Pleosporales*: a taxonomic, ecological and evolutionary re–evaluation. *Studies in Mycology* **64**: 85–102.

Zhang Y, Crous PW, Schoch CL, Hyde KD (2012). Pleosporales. Fungal Diversity 53: 1–221.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Ochrocladosporium frigidarii CBS 103.81T
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Ochrocladosporium elatum CBS 146.33T
Setosamyces glycines CBS 101636T
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Microsphaeropsis proteae CPC 1425
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Camarosporomyces flavigenus CBS 314.80T
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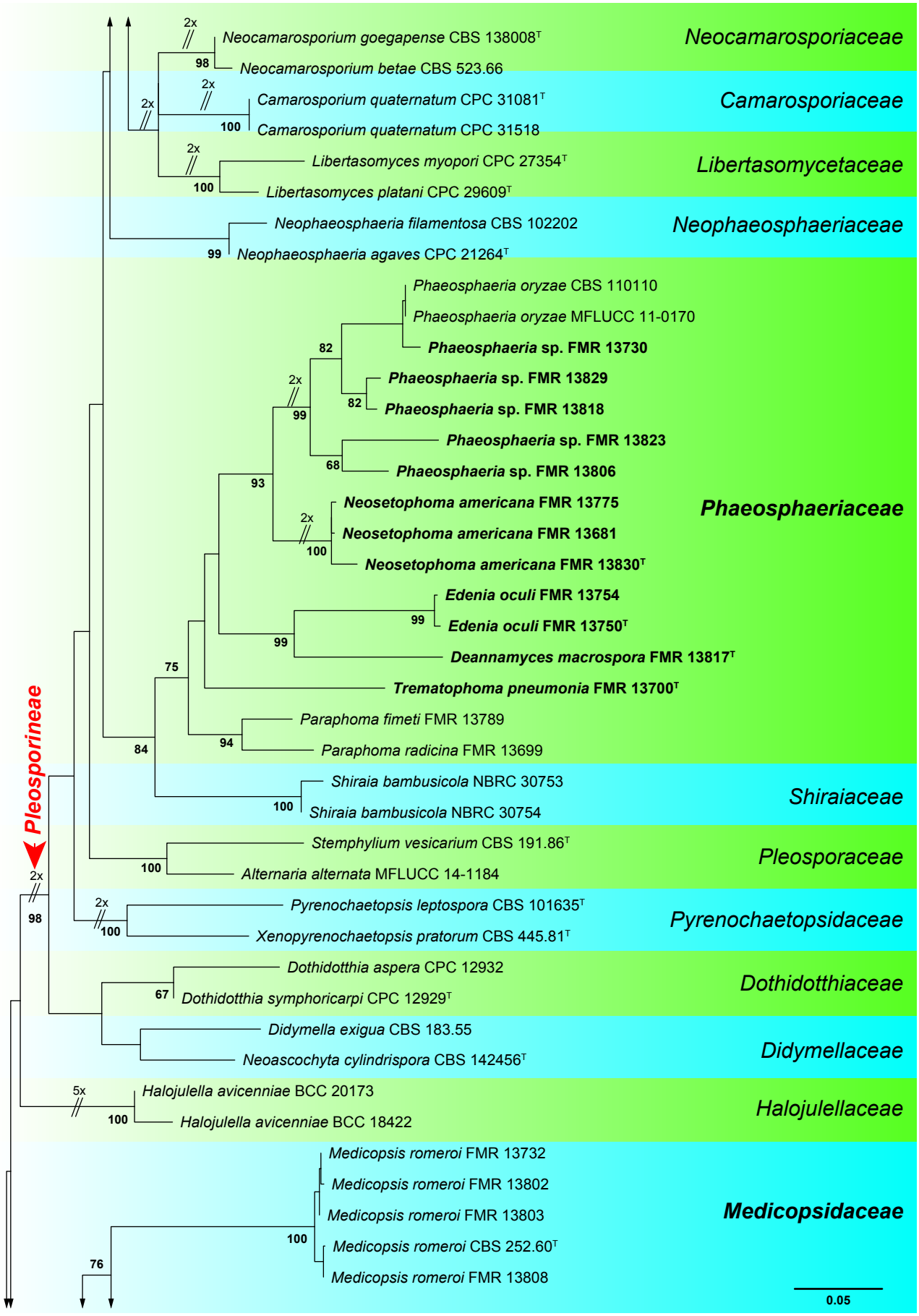
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Trematophoma pneumonia FMR 13700T
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Paraphoma radicina FMR 13699
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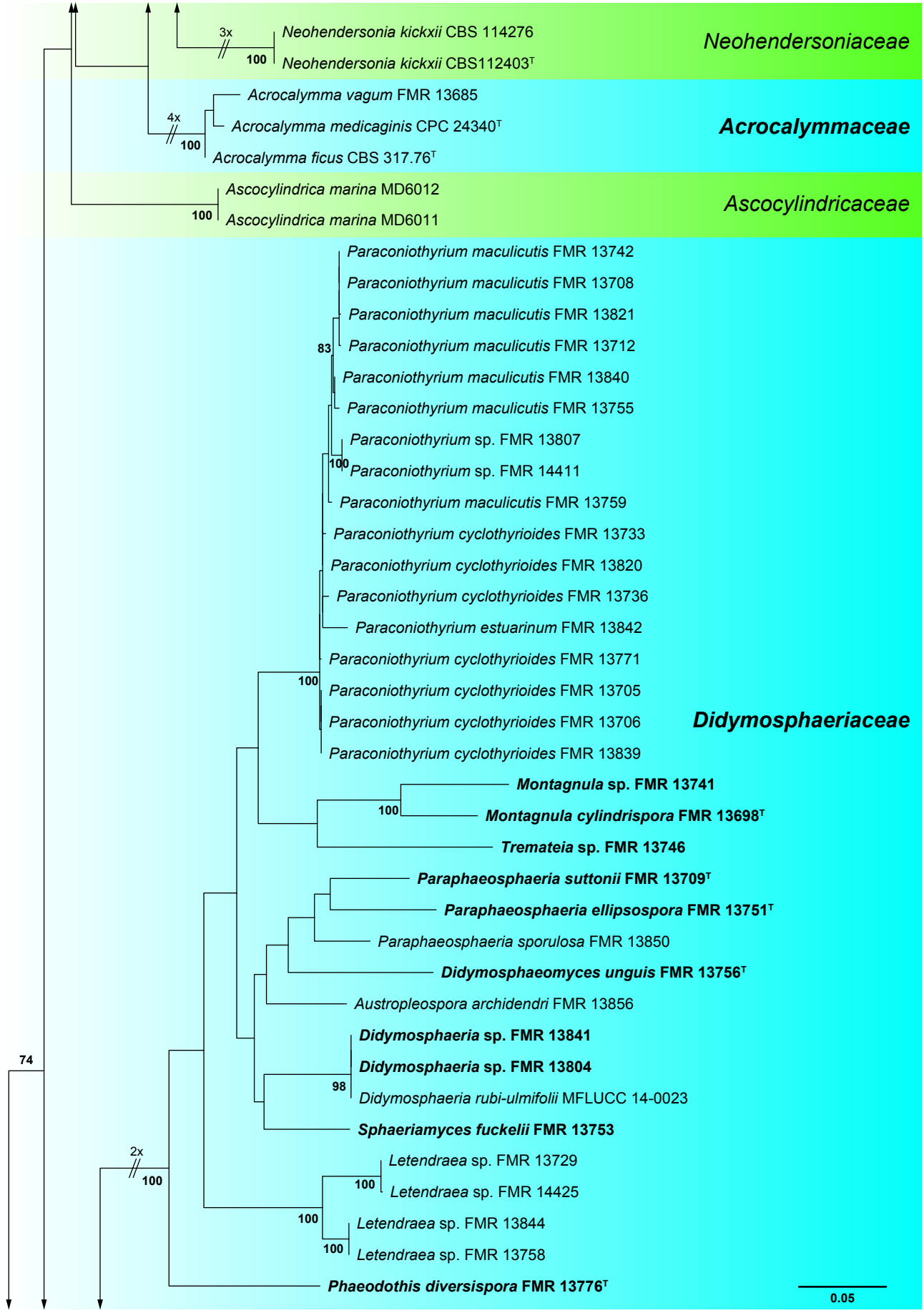
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Ascocylindrica marina MD6011
Paraconiothyrium maculicutis FMR 13742
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Paraconiothyrium maculicutis FMR 13821
83
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Paraconiothyrium maculicutis FMR 13755
Paraconiothyrium sp. FMR 13807
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Paraconiothyrium cyclothyrioides FMR 13736
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Paraconiothyrium cyclothyrioides FMR 13839
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Trematea sp. FMR 13746
Paraphaeosphaeria suttonii FMR 13709T
Paraphaeosphaeria ellipsospora FMR 13751T
Paraphaeosphaeria sporulosa FMR 13850
Didymosphaeomyces unguis FMR 13756T
Austropleospora archidendri FMR 13856
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Didymosphaeria sp. FMR 13804
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Didymosphaeria rubi-ulmifolii MFLUCC 14-0023
Sphaeriamyces fuckelii FMR 13753
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Letendraea sp. FMR 13844
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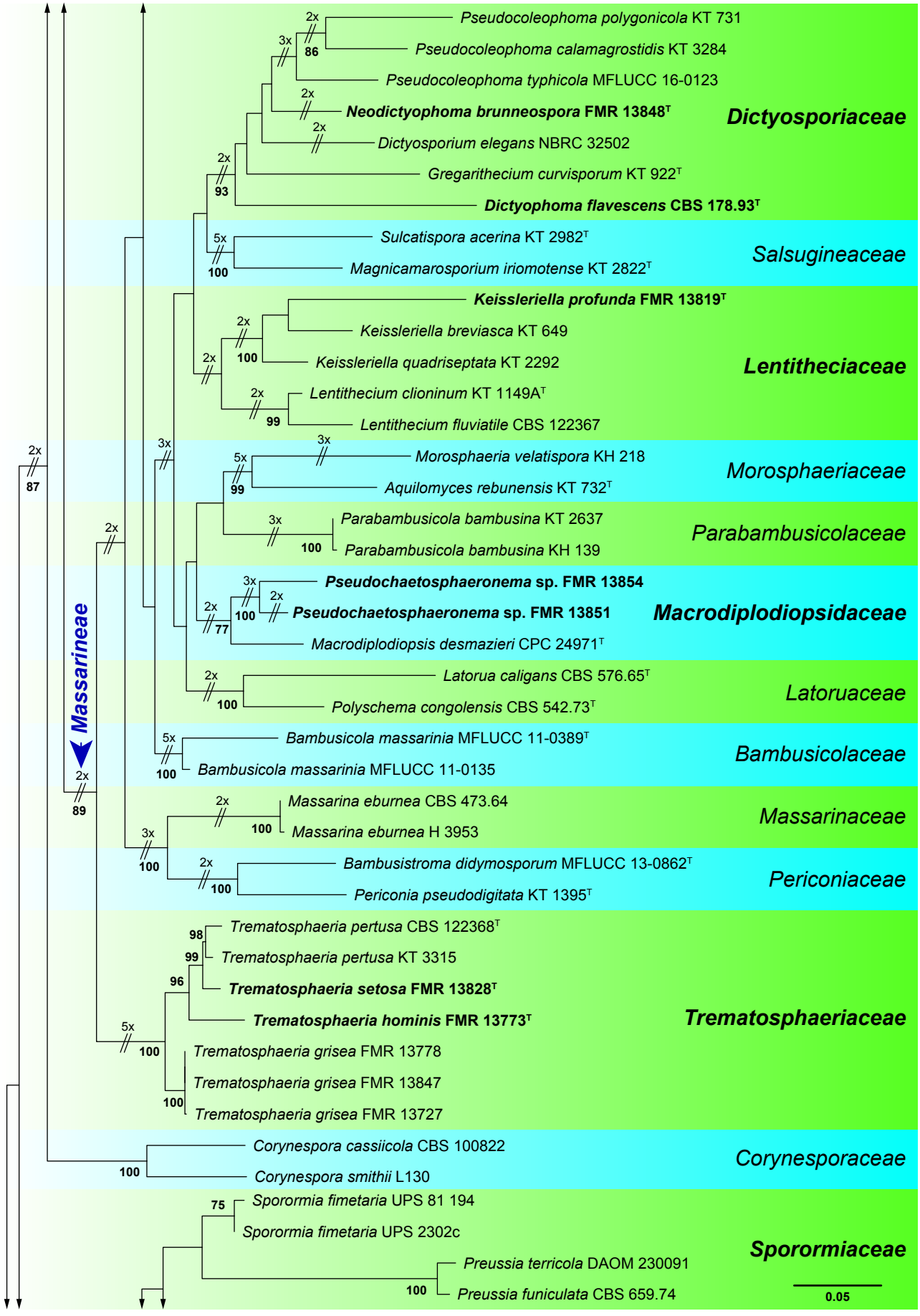
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Neodictyophoma brunneospora FMR 13848T
Dictyosporium elegans NBRC 32502
Gregarithecium curvisporum KT 922T
Dictyophoma flavescens CBS 178.93T
Dictyosporiaceae
Sulcatispora acerina KT 2982T
Magnicamarosporium iriomotense KT 2822T
Salsugineaceae
Keissleriella profunda FMR 13819T
Keissleriella breviasca KT 649
Keissleriella quadriseptata KT 2292
Lentithecium clioninum KT 1149AT
Lentithecium fluviatile CBS 122367
Lentitheciaceae
Morosphaeria velatispora KH 218
Aquilomyces rebunensis KT 732T
Morosphaeriaceae
Parabambusicola bambusina KT 2637
Parabambusicola bambusina KH 139
Parabambusicolaceae
Pseudochaetosphaeronema sp. FMR 13854
Pseudochaetosphaeronema sp. FMR 13851
Macrodiplodiopsis desmazieri CPC 24971T
Macrodiplodiopsidaceae
Latorua caligans CBS 576.65T
Polyschema congolensis CBS 542.73T
Latoruaceae
Bambusicola massarinia MFLUCC 11-0389T
Bambusicola massarinia MFLUCC 11-0135
Bambusicolaceae
Massarina eburnea CBS 473.64
Massarina eburnea H 3953
Massarinaceae
Bambusistroma didymosporum MFLUCC 13-0862T
Periconia pseudodigitata KT 1395T
Periconiaceae
Massarineae
Trematosphaeria pertusa CBS 122368T
Trematosphaeria pertusa KT 3315
Trematosphaeria setosa FMR 13828T
Trematosphaeria hominis FMR 13773T
Trematosphaeria grisea FMR 13778
Trematosphaeria grisea FMR 13847
Trematosphaeria grisea FMR 13727
Trematosphaeriaceae
Corynespora cassiicola CBS 100822
Corynespora smithii L130
Corynesporaceae
Sporormia fimetaria UPS 81 194
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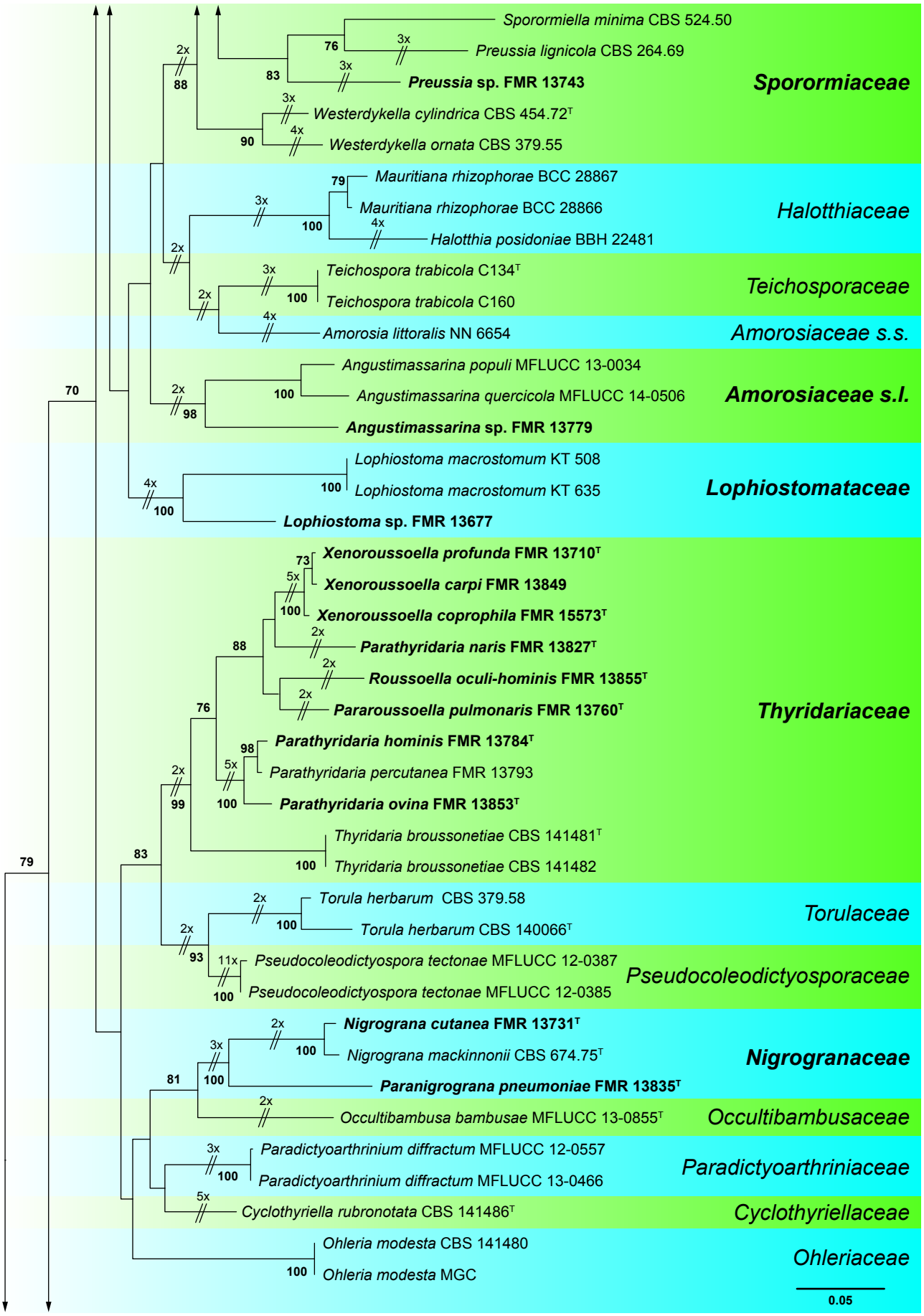
Preussia terricola DAOM 230091
Preussia funiculata CBS 659.74
Sporormiaceae
0.05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Preussia lignicola CBS 264.69
Preussia sp. FMR 13743
Westerdykella cylindrica CBS 454.72T
Westerdykella ornata CBS 379.55
Sporormiaceae
Mauritiana rhizophorae BCC 28867
Mauritiana rhizophorae BCC 28866
Halotthia posidoniae BBH 22481
Halotthiaceae
Teichospora trabicola C134T
Teichospora trabicola C160
Teichosporaceae
Amorosia littoralis NN 6654
Amorosiaceae s.s.
Angustimassarina populi MFLUCC 13-0034
Angustimassarina quercicola MFLUCC 14-0506
Angustimassarina sp. FMR 13779
Amorosiaceae s.l.
Lophiostoma macrostomum KT 508
Lophiostoma macrostomum KT 635
Lophiostoma sp. FMR 13677
Lophiostomataceae
Xenoroussoella profunda FMR 13710T
Xenoroussoella carpi FMR 13849
Xenoroussoella coprophila FMR 15573T
Parathyridaria naris FMR 13827T
Roussoella oculi-hominis FMR 13855T
Pararoussoella pulmonaris FMR 13760T
Parathyridaria hominis FMR 13784T
Parathyridaria percutanea FMR 13793
Parathyridaria ovina FMR 13853T
Thyridaria broussonetiae CBS 141481T
Thyridaria broussonetiae CBS 141482
Thyridariaceae
Torula herbarum CBS 379.58
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Torulaceae
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Pseudocoleodictyosporaceae
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Paranigrograna pneumoniae FMR 13835T
Nigrograna cutanea
Occultibambusa bambusae MFLUCC 13-0855T
Occultibambusaceae
Paradictyoarthrinium diffractum MFLUCC 12-0557
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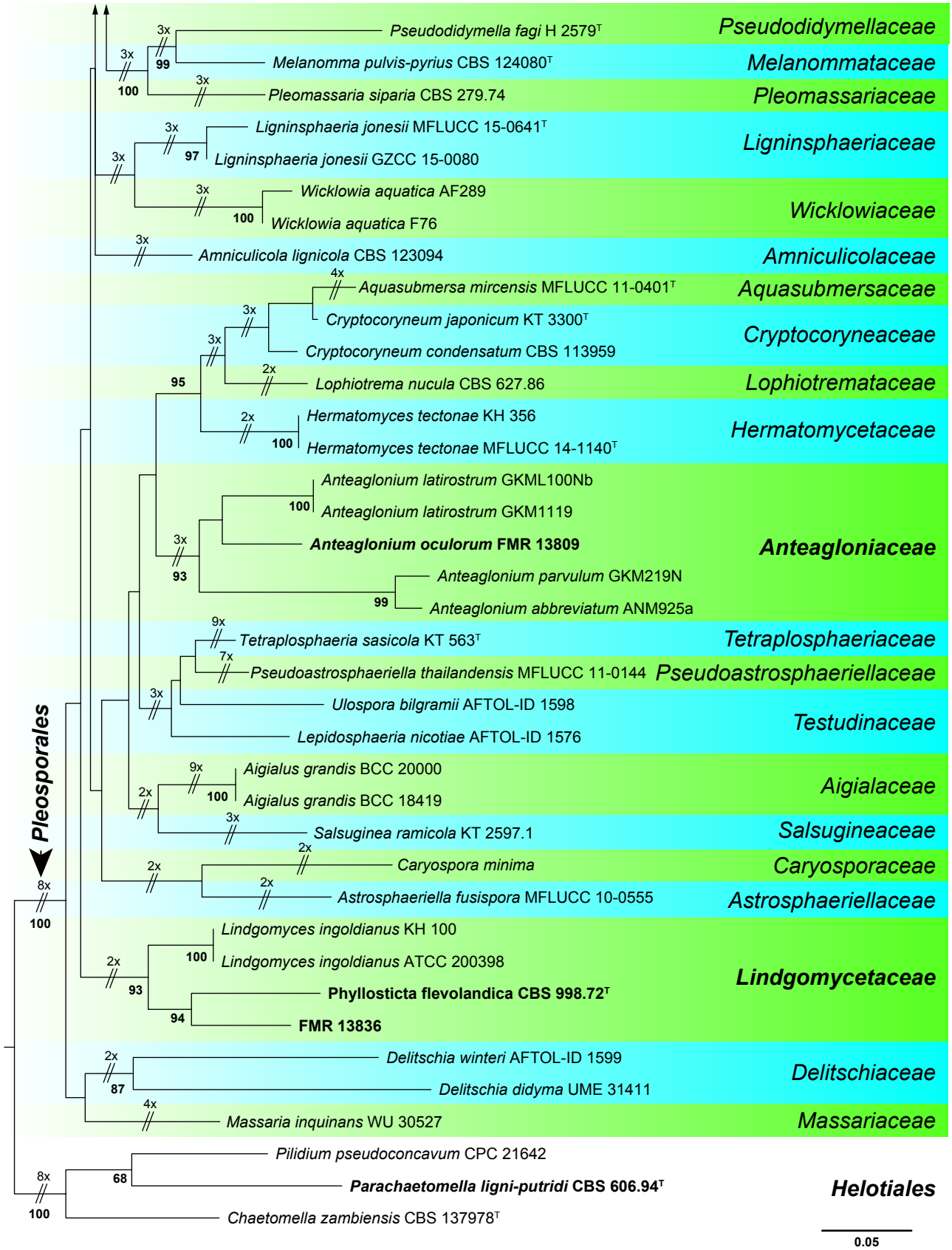
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Pleomassaria siparia CBS 279.74
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Ligninsphaeria jonesii GZCC 15-0080
Wicklowia aquatica AF289
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Amniculicola lignicola CBS 123094
Aquasubmersa mircensis MFLUCC 11-0401T
Cryptocoryneum japonicum KT 3300T
Cryptocoryneum condensatum CBS 113959
Lophiotrema nucula CBS 627.86
Hermatomyces tectonae KH 356
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Anteaglonium latirostrum GKML100Nb
Anteaglonium latirostrum GKM1119
Anteaglonium oculorum FMR 13809
Anteaglonium parvulum GKM219N
Anteaglonium abbreviatum ANM925a
Tetraplosphaeria sasicola KT 563T
Pseudoastrosphaeriella thailandensis MFLUCC 11-0144
Ulospora bilgramii AFTOL-ID 1598
Lepidosphaeria nicotiae AFTOL-ID 1576
Aigialus grandis BCC 20000
Aigialus grandis BCC 18419
Salsuginea ramicola KT 2597.1
Caryospora minima
Astrosphaeriella fusispora MFLUCC 10-0555
Lindgomyces ingoldianus KH 100
Lindgomyces ingoldianus ATCC 200398
Phyllosticta flevolandica CBS 998.72T
FMR 13836
Delitschia winteri AFTOL-ID 1599
Delitschia didyma UME 31411
Massaria inquinans WU 30527
Pilidium pseudoconcavum CPC 21642
Parachaetomella ligni-putridi CBS 606.94T
Chaetomella zambiensis CBS 137978T
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Melanommataceae
Pleomassariaceae
Ligninsphaeriaceae
Wicklowiaceae
Amniculicolaceae
Aquasubmersaceae
Cryptocoryneaceae
Lophiotremataceae
Hermatomycetaceae
Anteagloniaceae
Tetraplosphaeriaceae
Pseudoastrosphaeriellaceae
Testudinaceae
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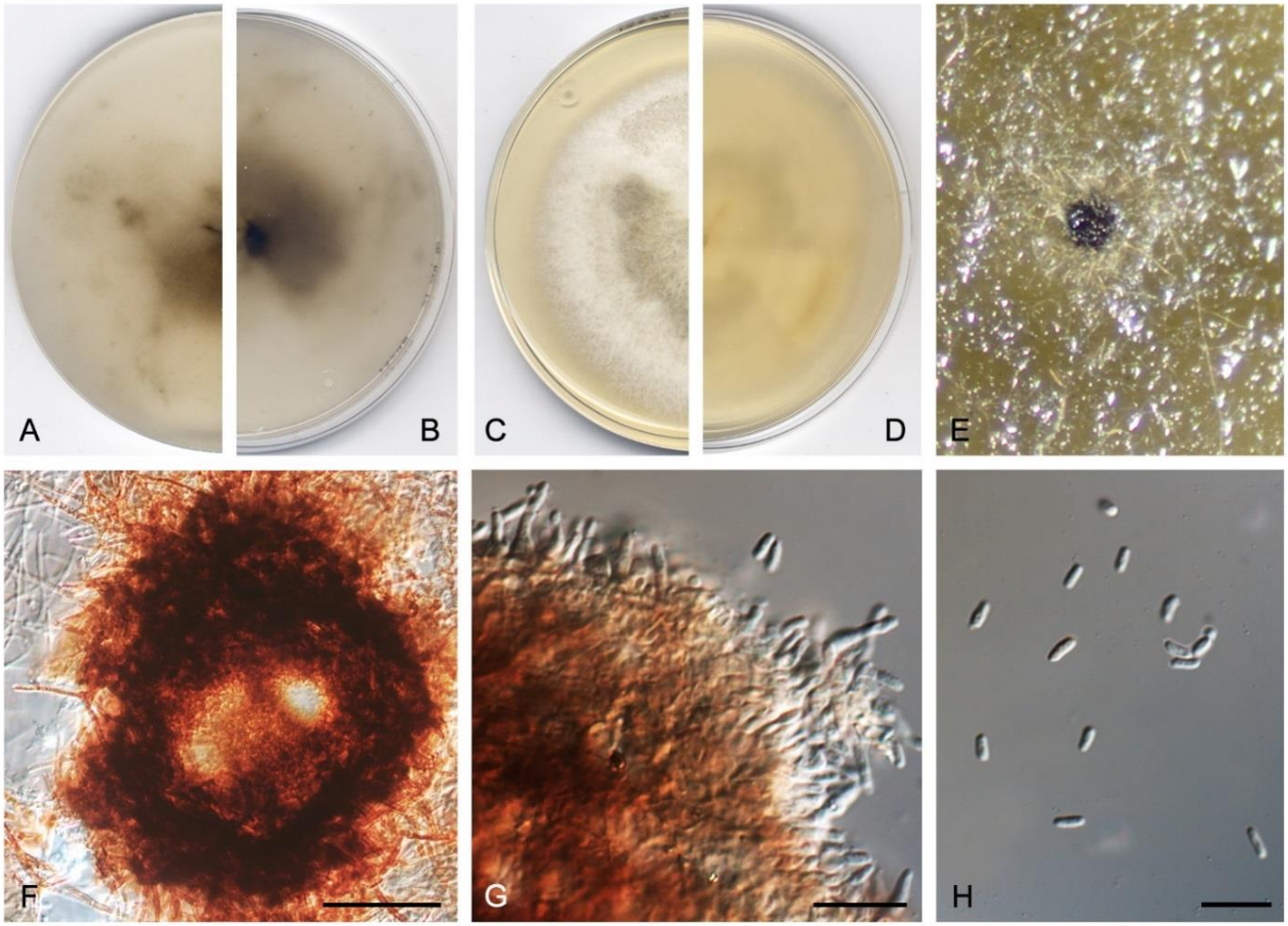
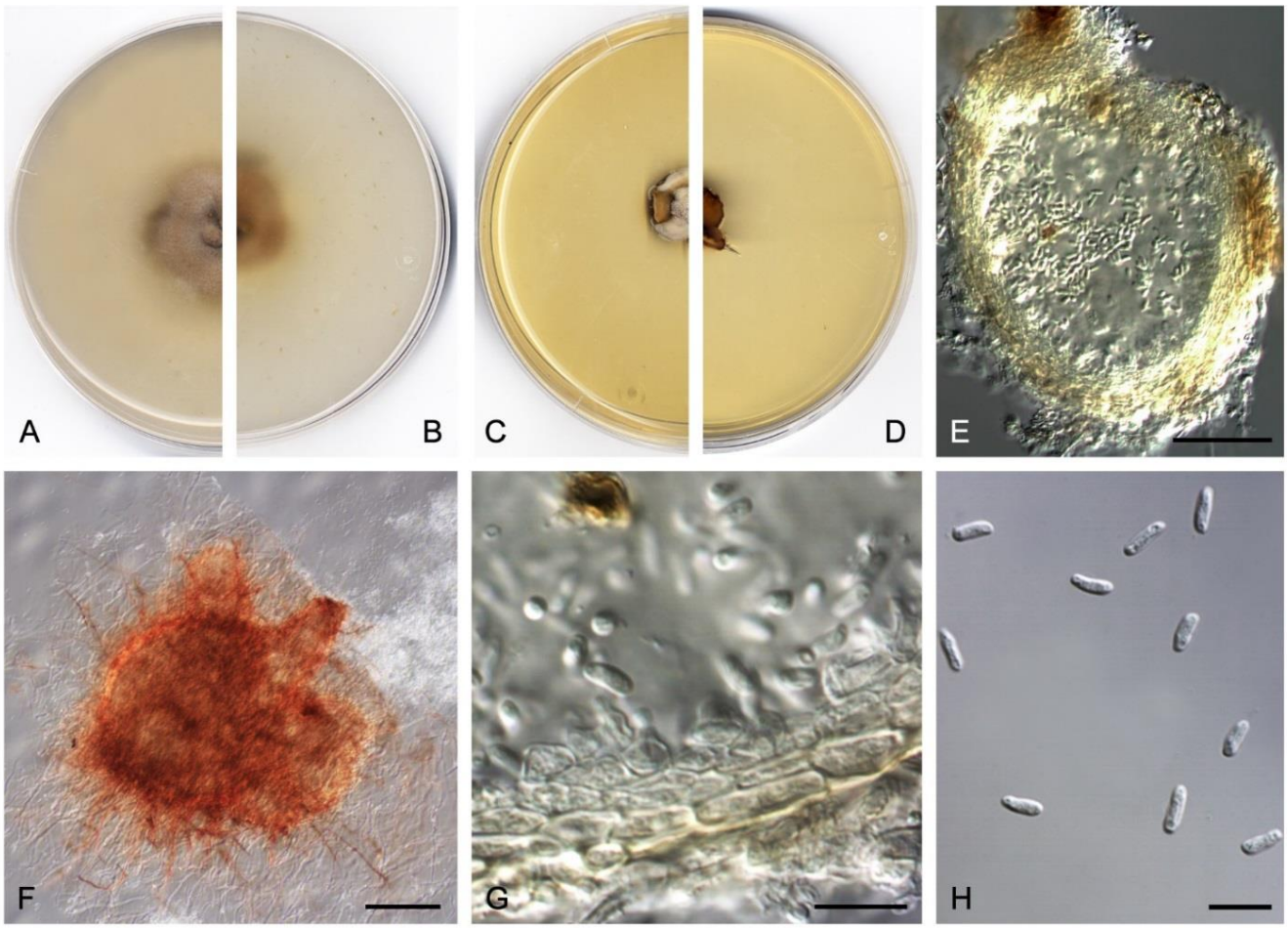
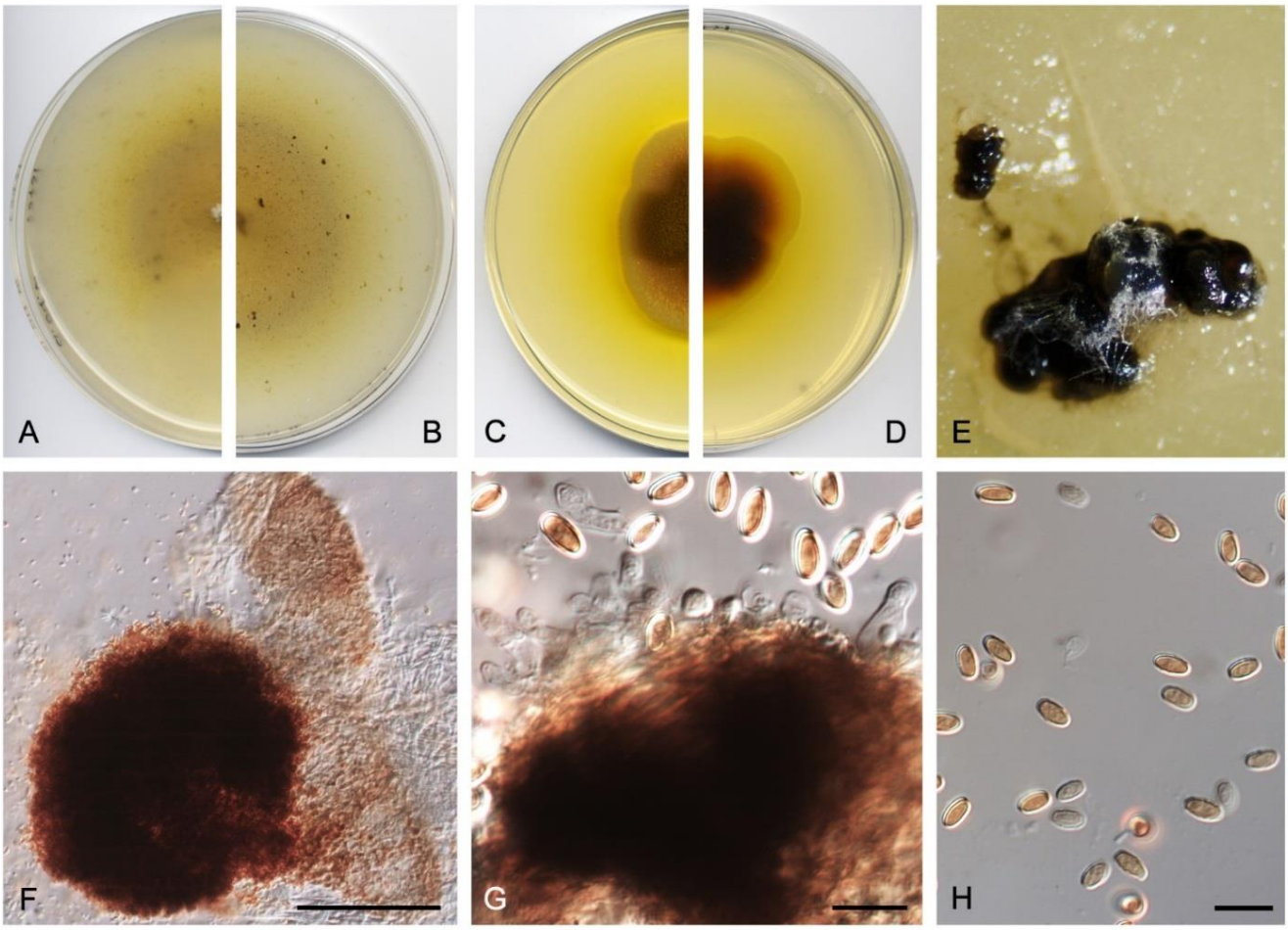


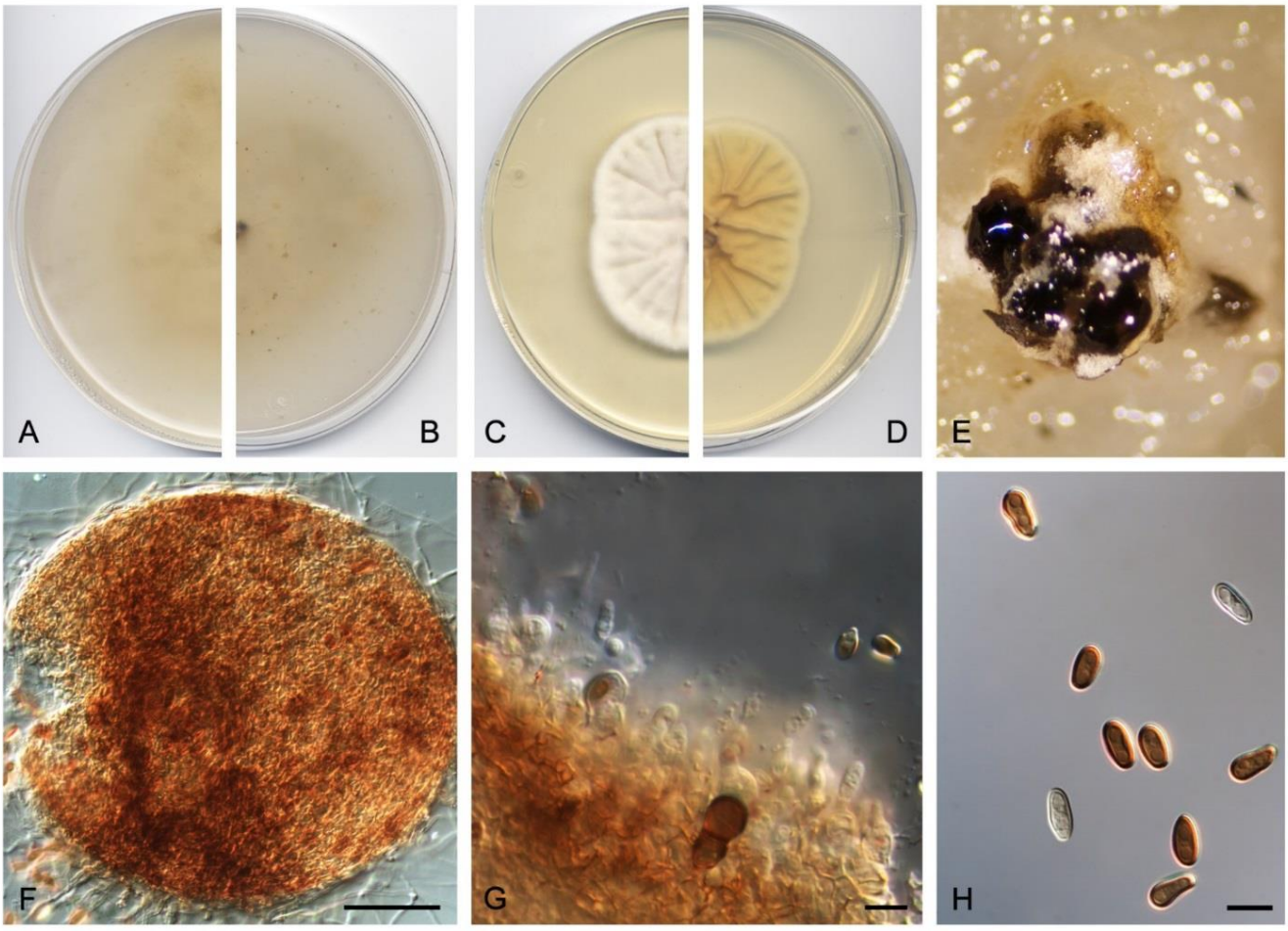
13698 *Montagnula cylindrispora*



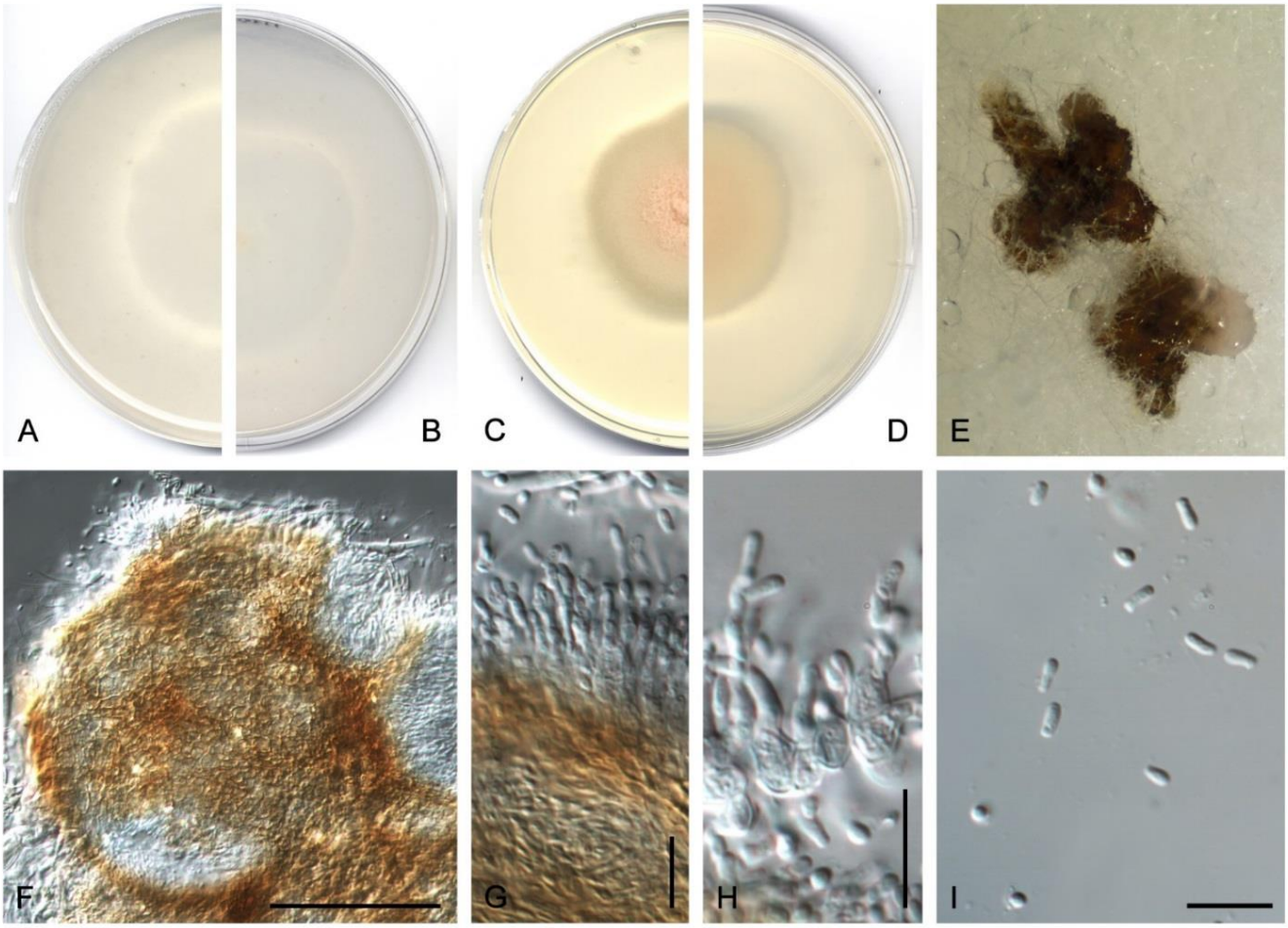
13750 *Edenia oculi*



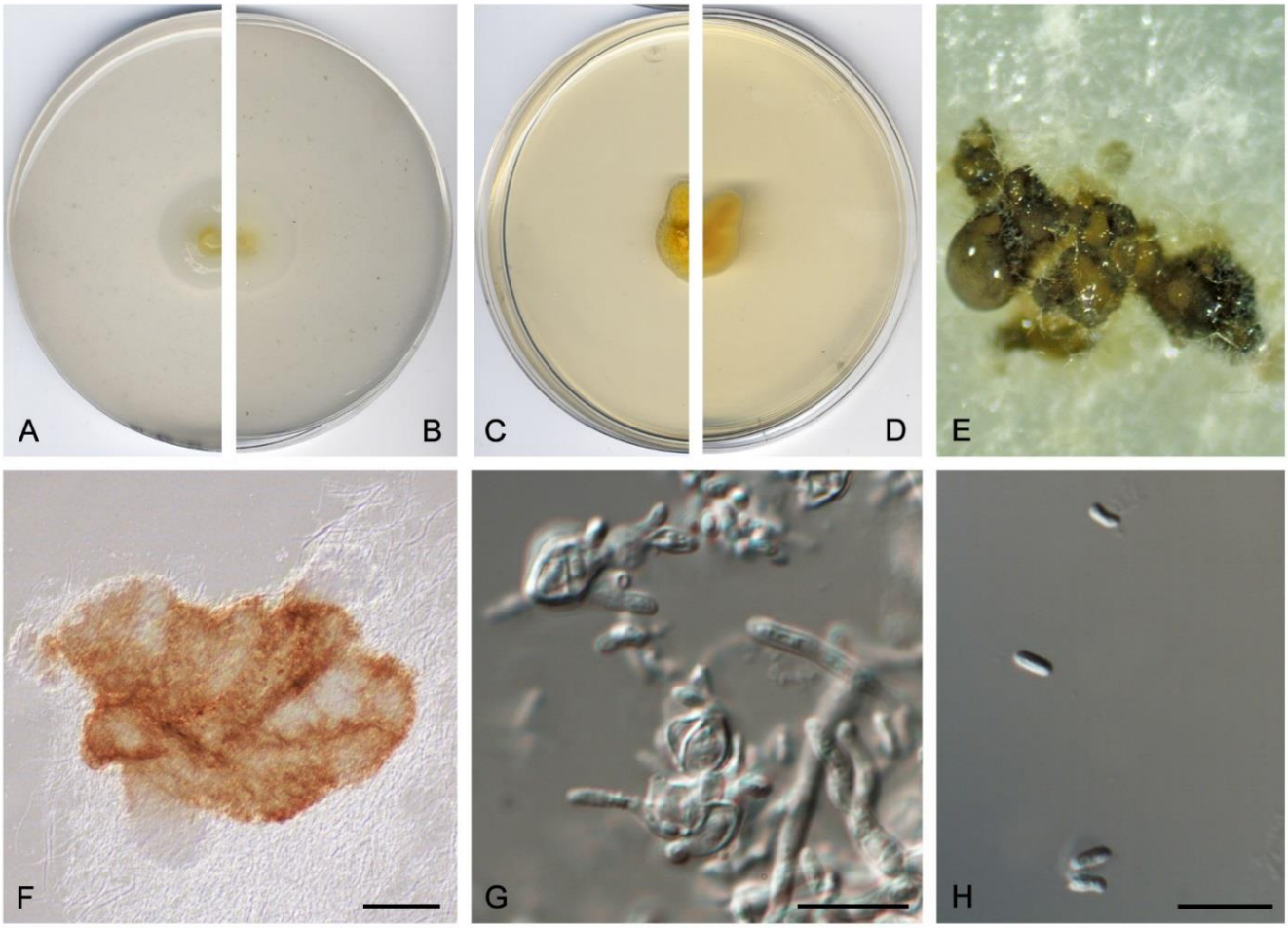
13751 *Paraphaeosphaeria ellipsospora*



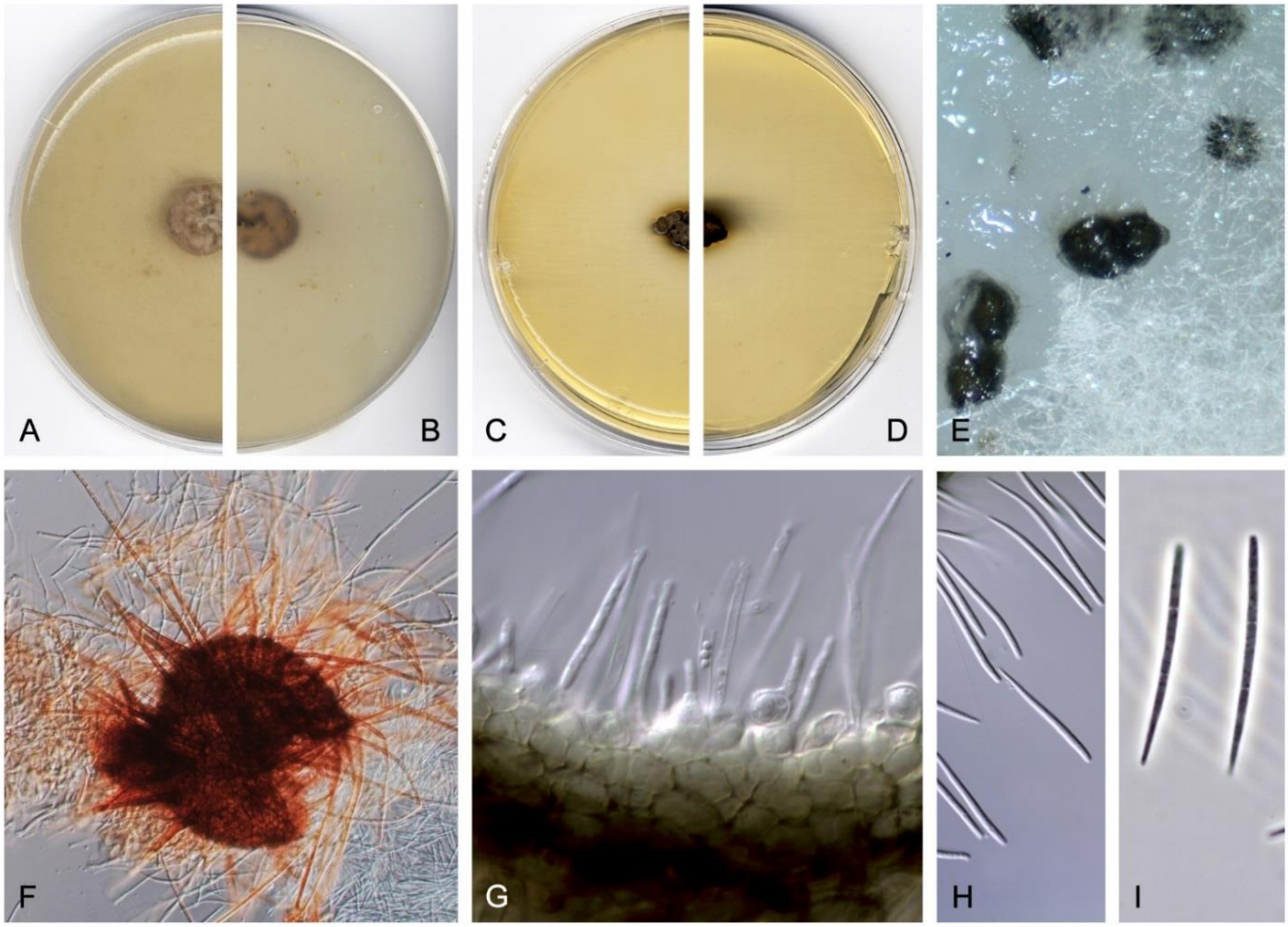
13756 *Didymosphaeomyces unguis*



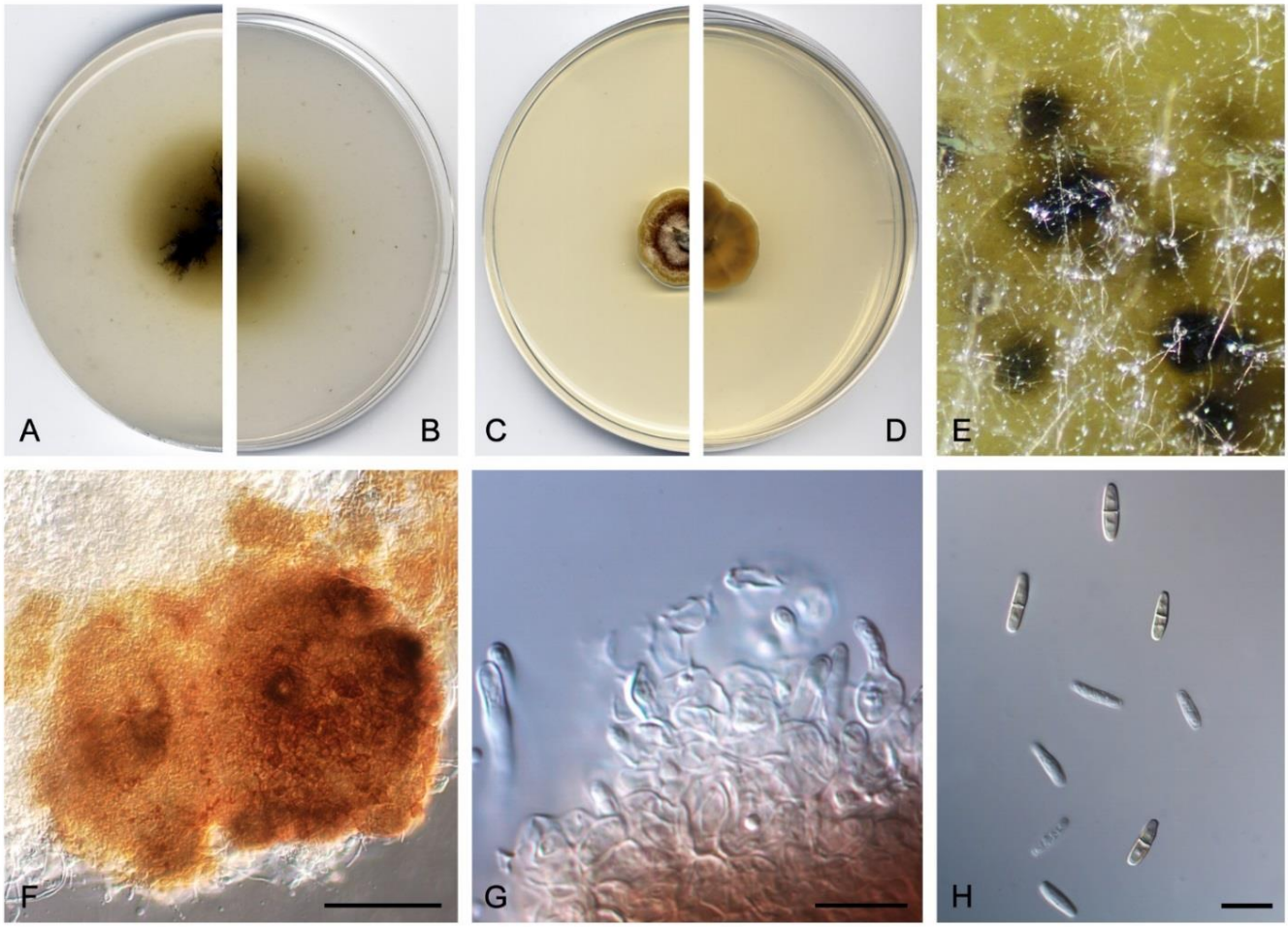
13776 *Phaeodothis diversispora*



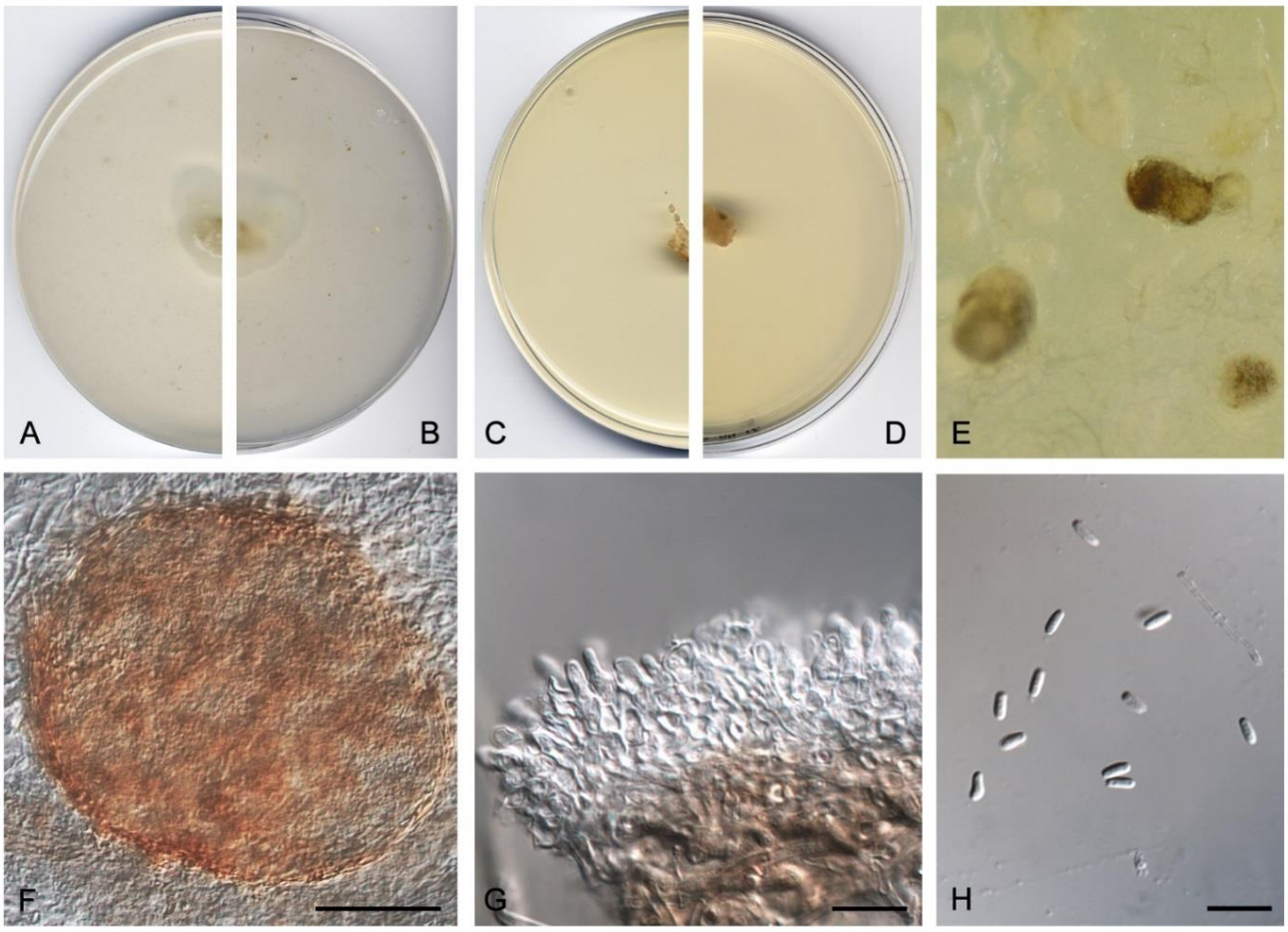
13782 *Xenoleptosphaeria confluens*



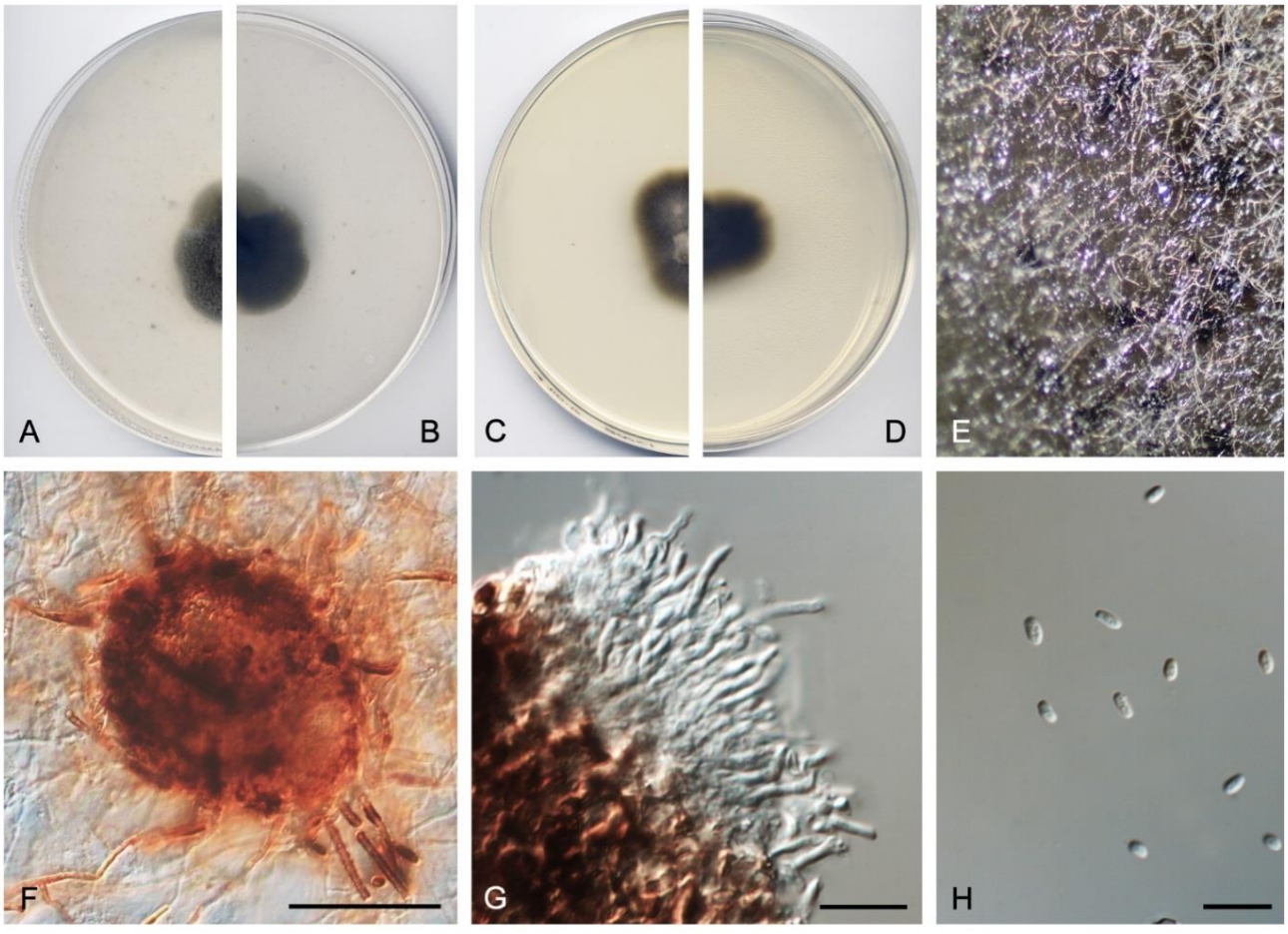
13817 *Deannamyces macrospora*



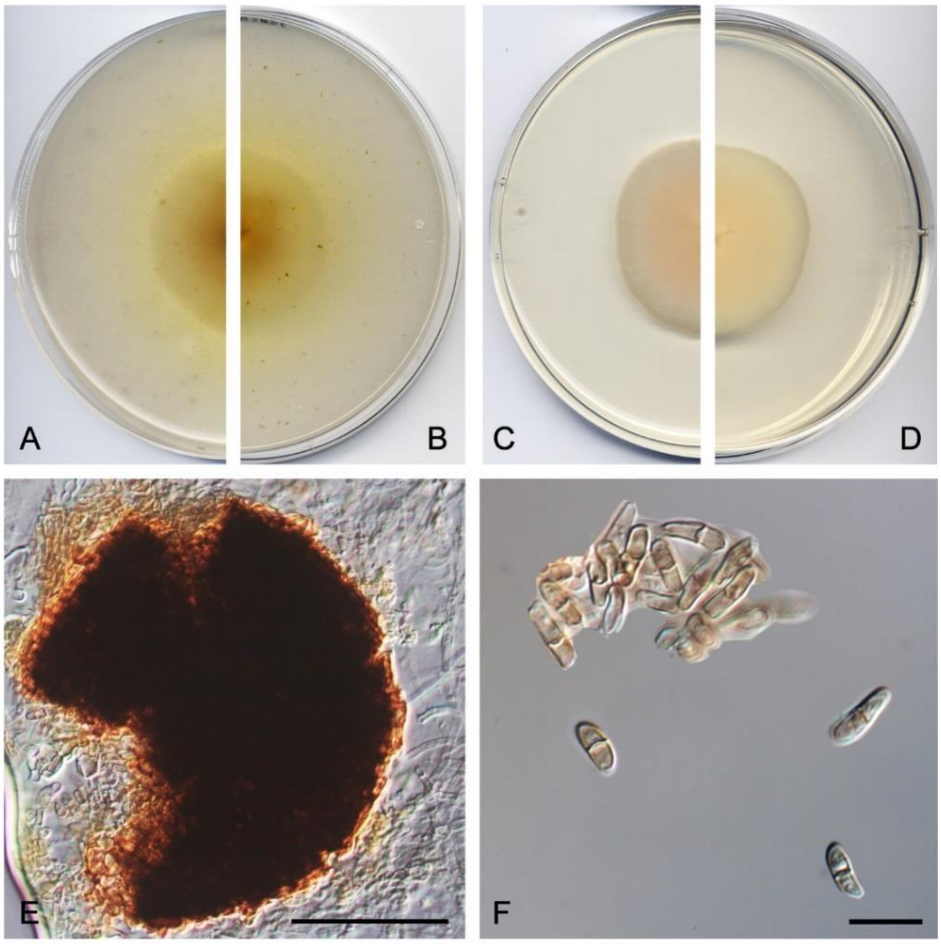
13818 *Phaeosphaeria* sp nov



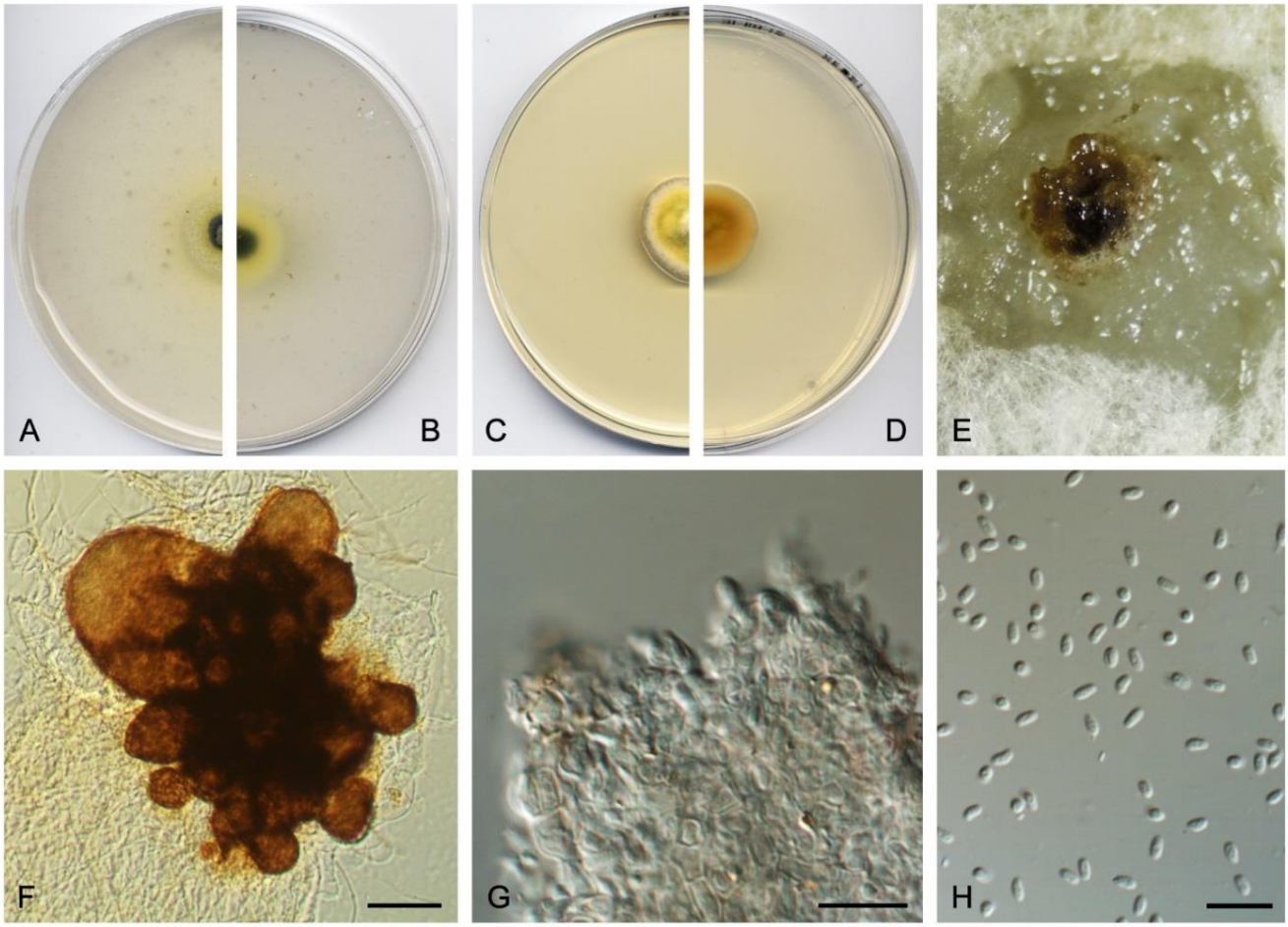
13819 *Keissleriella profunda*



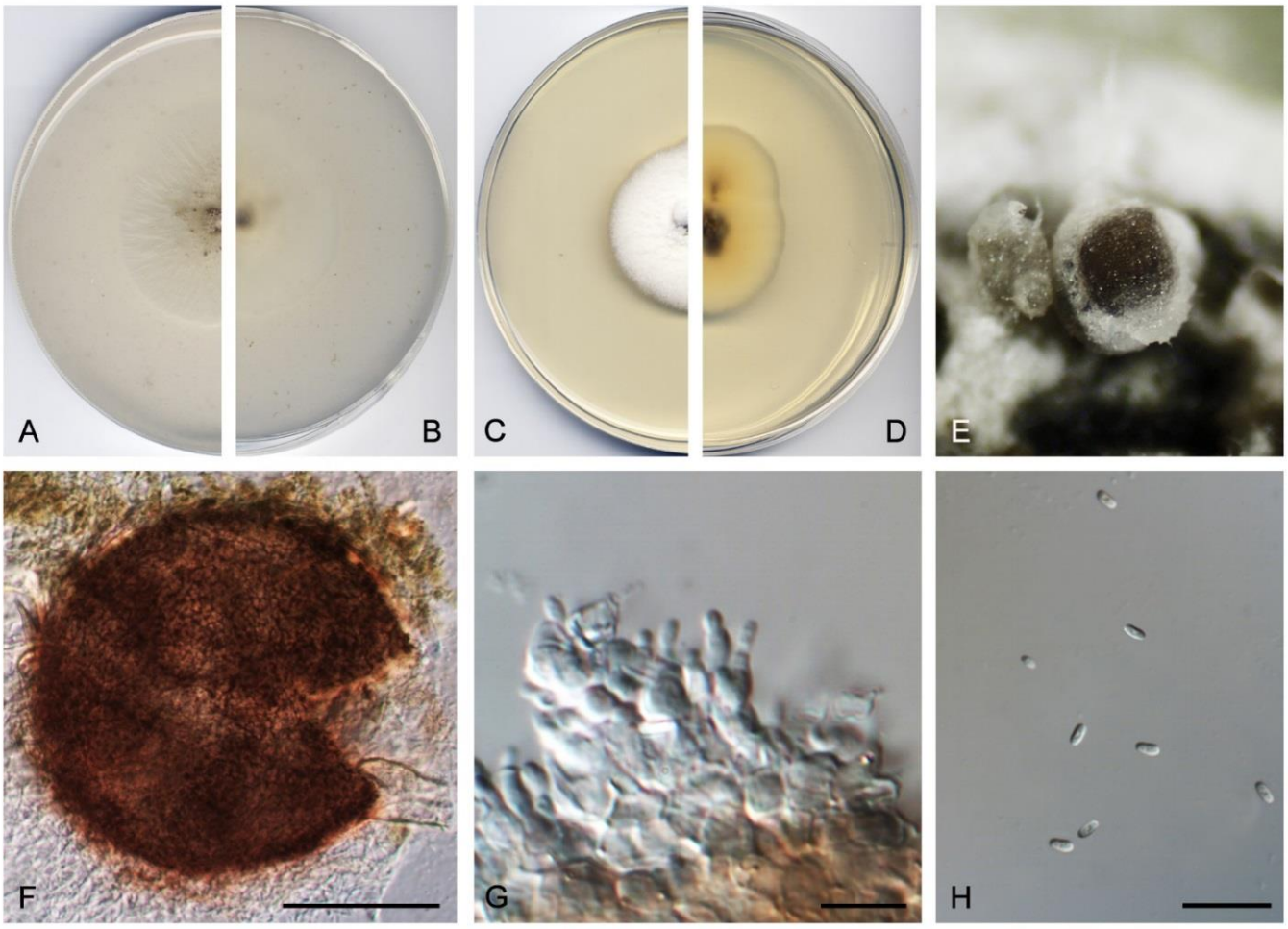
13828 *Trematosphaeria setosa*



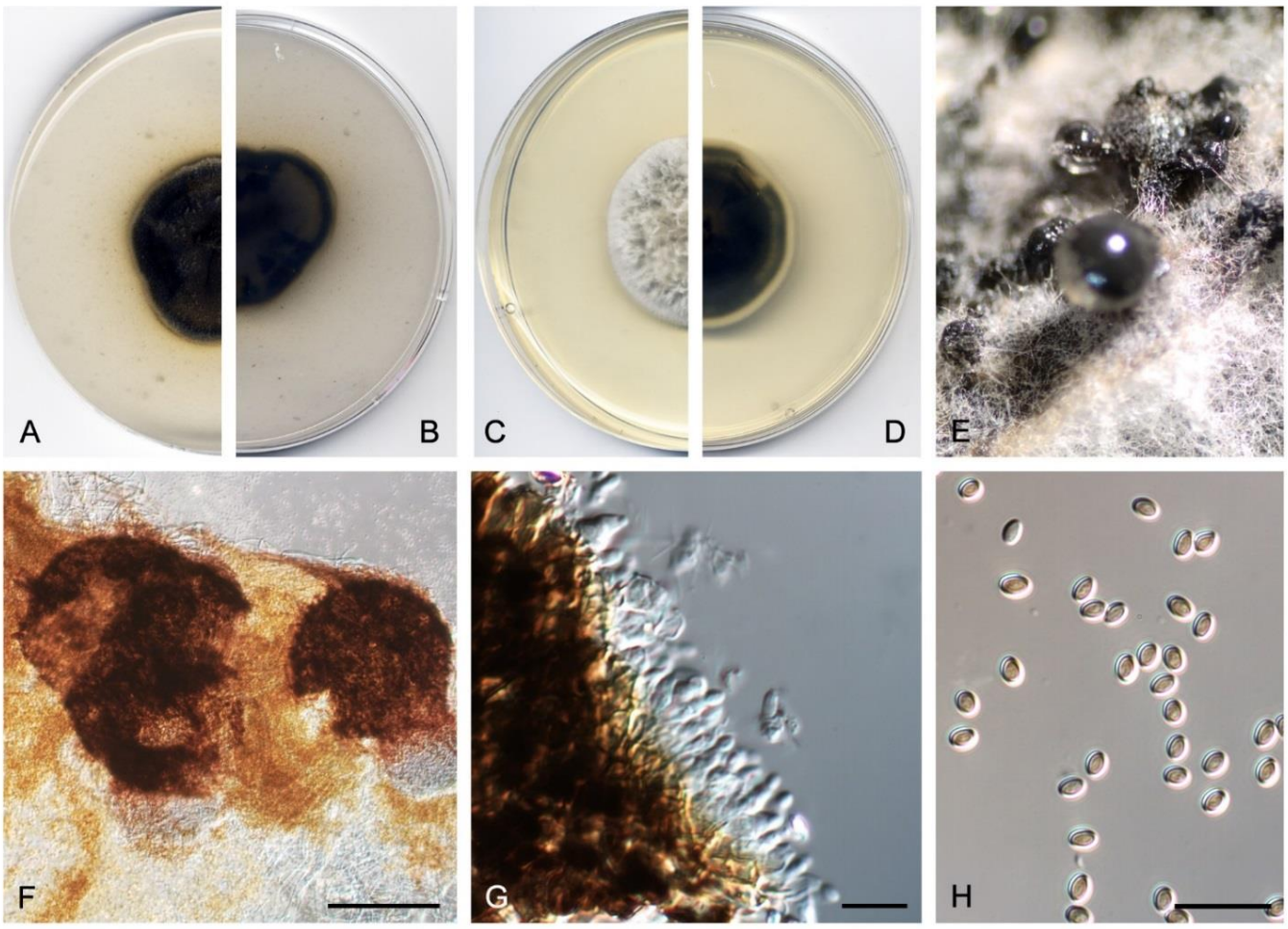
13829 *Phaeosphaeria* sp nov



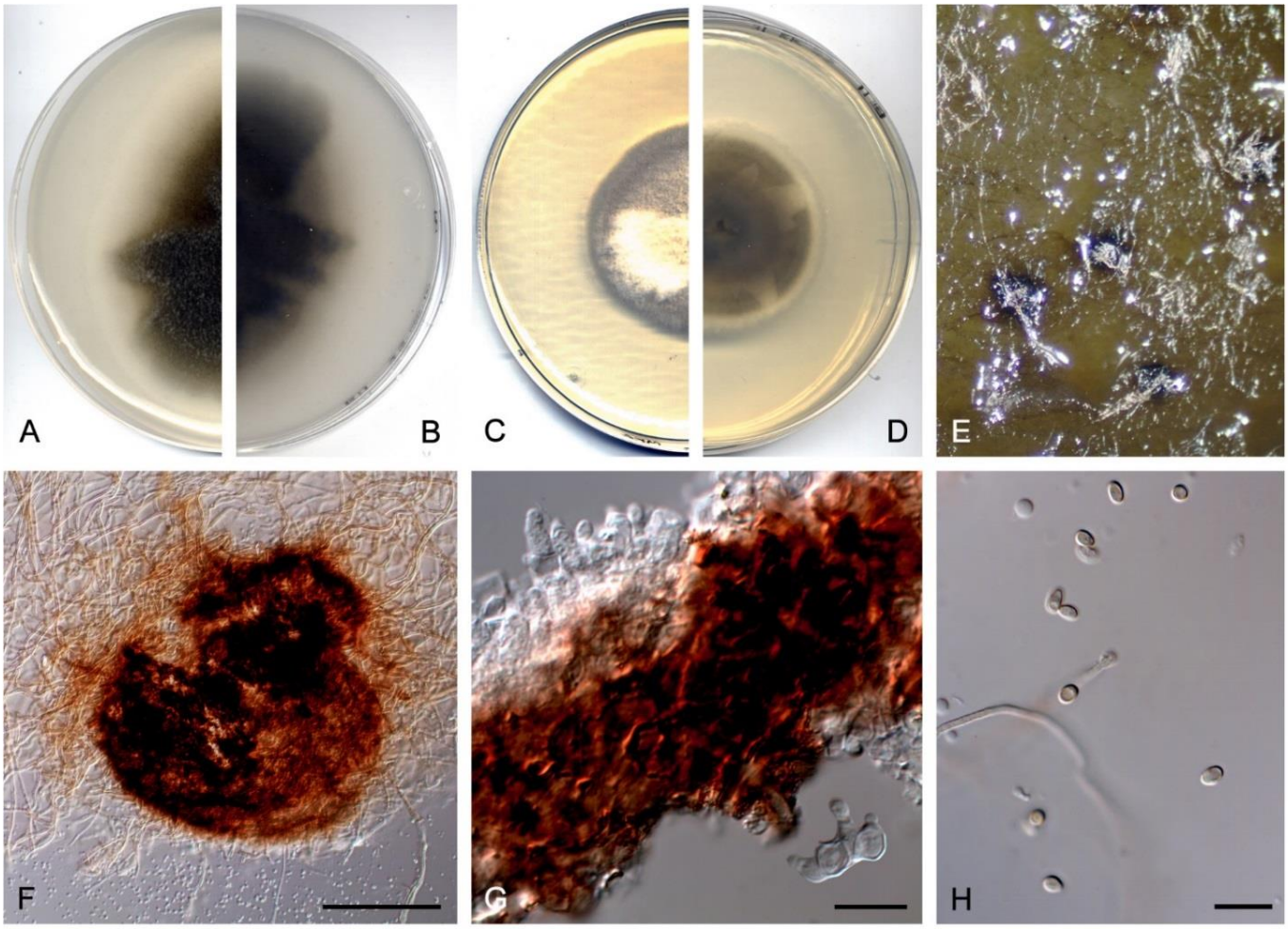
13830 *Neosetophoma americana*



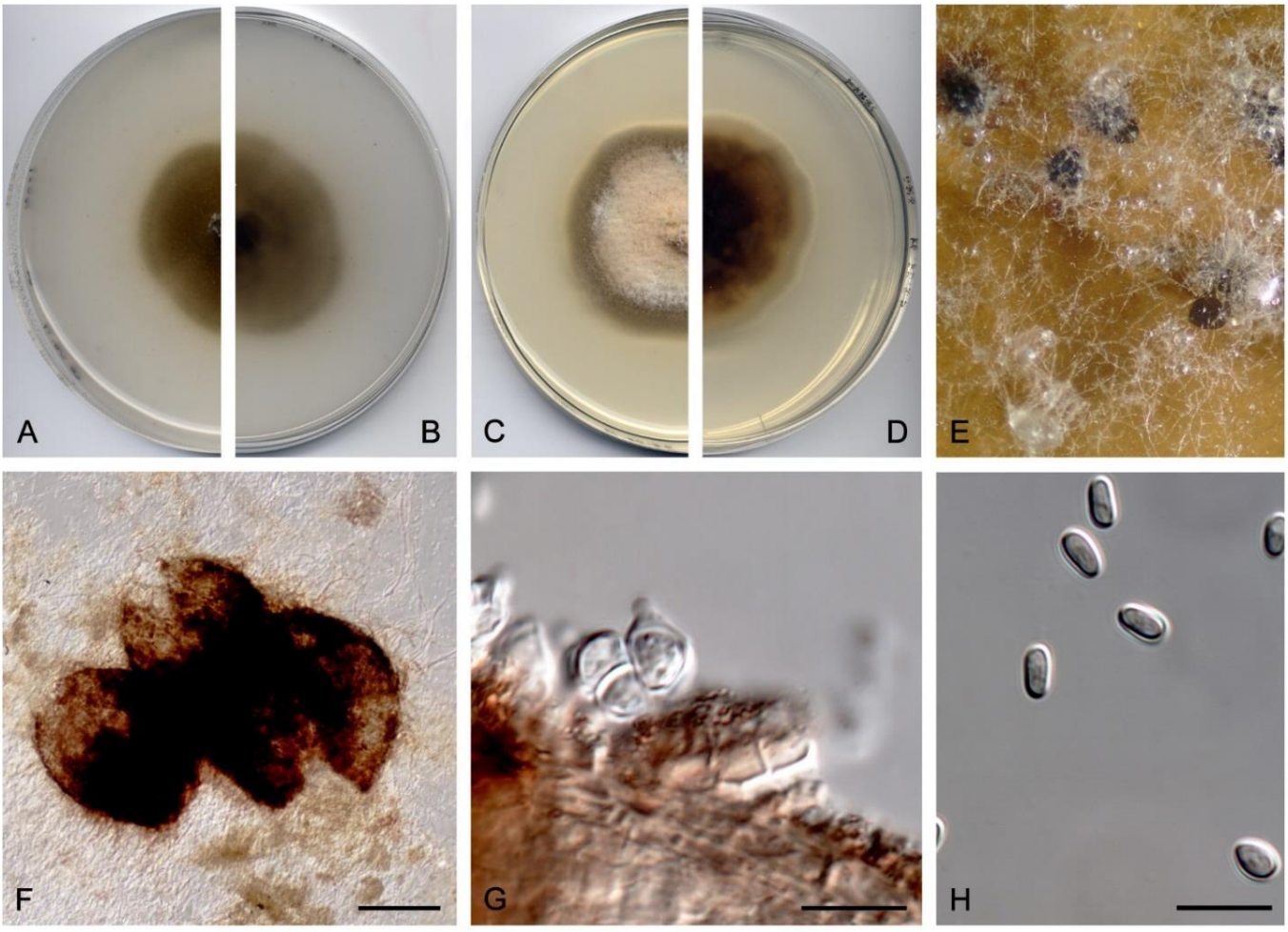
13853 *Parathyridaria ovina*



13855 *Roussoella oculi-hominis*



15573 *Xenoroussoella coprophila*



16827 *Xenoroussoella papuae*

Table 2. Isolates from USA used in this study and their GenBank accession numbers. Newly generated sequences are indicated in bold.

Family	Species	Old name	Strain number[1]	Status[2]	Host, substrate	GenBank accession numbers[3]				
						LSU	ITS	TUB	RPB2	TEF-1α
Acrocalymmaceae		Acrocalymma walkeri	UTHSC DI16-195; FMR 13685		Human scalp	LN907338	**LT796832**	**LT796912**	**LT796992**	**LT797072**
Amorosiaceae		Exosporium sp.	**UTHSC DI16-287; FMR 13779**		Marine environment	LN907430	**LT796869**	**LT796949**	**LT797029**	**LT797109**
Anteagloniaceae		**Anteaglonium sp.**	**UTHSC DI16-316; FMR 13809**	T	Human eye	LN907459	**LT796880**	**LT796960**	**LT797040**	**LT797120**
Dictyosporiaceae		Paraconiothyrium sp.	UTHSC DI16-355; FMR 13848		Human skin	LN907498	**LT796899**	**LT796979**	**LT797059**	**LT797139**
Didymosphaeriaceae		**Montagnula opulenta**	**UTHSC DI16-208; FMR 13698**	T	Unknown	LN907351	**LT796834**	**LT796914**	**LT796994**	**LT797074**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-215; FMR 13705		Human leg	LN907358	**LT796837**	**LT796917**	**LT796997**	**LT797077**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-216; FMR 13706		Human skin	LN907359	**LT796838**	**LT796918**	**LT796998**	**LT797078**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-218; FMR 13708		Human eye	LN907361	**LT796839**	**LT796919**	**LT796999**	**LT797079**
Didymosphaeriaceae		**Paraphaeosphaeria neglecta**	**UTHSC DI16-219; FMR 13709**	T	Unknown	LN907362	**LT796840**	**LT796920**	**LT797000**	**LT797080**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-222; FMR 13712		Human scalp	LN907365	**LT796842**	**LT796922**	**LT797002**	**LT797082**
Didymosphaeriaceae		**Letendraea sp.**	**UTHSC DI16-239; FMR 13729**		Human arm	LN907382	**LT796845**	**LT796925**	**LT797005**	**LT797085**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-243; FMR 13733		Human nail	LN907386	**LT796849**	**LT796929**	**LT797009**	**LT797089**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-246; FMR 13736		Human finger	LN907389	**LT796850**	**LT796930**	**LT797010**	**LT797090**
Didymosphaeriaceae		Montagnula sp.	UTHSC DI16-251; FMR 13741		Human scalp	LN907394	**LT796851**	**LT796931**	**LT797011**	**LT797091**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-252; FMR 13742		Human biopsy	LN907395	**LT796852**	**LT796932**	**LT797012**	**LT797092**
Didymosphaeriaceae		**Kalmusia sp.**	**UTHSC DI16-256; FMR 13746**	T	Human eye	LN907399	**LT796854**	**LT796934**	**LT797014**	**LT797094**
Didymosphaeriaceae		**Paraphaeosphaeria neglecta**	**UTHSC DI16-261; FMR 13751**	T	Human bronch wash	LN907404	**LT796856**	**LT796936**	**LT797016**	**LT797096**
Didymosphaeriaceae		**Paraconiothyrium fuckelii**	**UTHSC DI16-263; FMR 13753**		Human bronchoalveolar lavage	LN907406	**LT796857**	**LT796937**	**LT797017**	**LT797097**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-265; FMR 13755		Human hand	LN907408	**LT796859**	**LT796939**	**LT797019**	**LT797099**
Didymosphaeriaceae		**Paraconiothyrium sp.**	**UTHSC DI16-266; FMR 13756**	T	Human nail	LN907409	**LT796860**	**LT796940**	**LT797020**	**LT797100**
Didymosphaeriaceae		**Letendraea sp.**	**UTHSC DI16-267; FMR 13758**		Human arm	LN907410	**LT796861**	**LT796941**	**LT797021**	**LT797101**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-268; FMR 13759		Human toe nail	LN907411	**LT796862**	**LT796942**	**LT797022**	**LT797102**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-279; FMR 13771		Human bronchoalveolar lavage	LN907422	**LT796864**	**LT796944**	**LT797024**	**LT797104**
Didymosphaeriaceae		**Phaeodothis sp.**	**UTHSC DI16-284; FMR 13776**	T	Human right upper lobe	LN907427	**LT796867**	**LT796947**	**LT797027**	**LT797107**
Didymosphaeriaceae		Paraconiothyrium brasiliense	UTHSC DI16-311; FMR 13804		Unknown	LN907454	**LT796876**	**LT796956**	**LT797036**	**LT797116**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-314; FMR 13807		Human finger	LN907457	**LT796878**	**LT796958**	**LT797038**	**LT797118**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-327; FMR 13820		Human biopsy	LN907470	**LT796884**	**LT796964**	**LT797044**	**LT797124**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-328; FMR 13821		Human knee lesion	LN907471	**LT796885**	**LT796965**	**LT797045**	**LT797125**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-346; FMR 13839		Unknown	LN907489	**LT796893**	**LT796973**	**LT797053**	**LT797133**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-347; FMR 13840		Unknown	LN907490	**LT796894**	**LT796974**	**LT797054**	**LT797134**
Didymosphaeriaceae		Paraconiothyrium brasiliense	UTHSC DI16-348; FMR 13841		Human scalp	LN907491	**LT796895**	**LT796975**	**LT797055**	**LT797135**
Didymosphaeriaceae		Paraconiothyrium sp.	UTHSC DI16-349; FMR 13842		Human nail	LN907492	**LT796896**	**LT796976**	**LT797056**	**LT797136**
Didymosphaeriaceae		**Letendraea sp.**	**UTHSC DI16-351; FMR 13844**		Human pleural fluid	LN907494	**LT796897**	**LT796977**	**LT797057**	**LT797137**
Didymosphaeriaceae		Curreya pityophila	UTHSC DI16-357; FMR 13850		Human bronchoalveolar lavage	LN907500	**LT796901**	**LT796981**	**LT797061**	**LT797141**
Didymosphaeriaceae		Paraconiothyrium sp.	UTHSC DI16-363; FMR 13856		Human joint fluid	LN907506	**LT796906**	**LT796986**	**LT797066**	**LT797146**
Didymosphaeriaceae		Paraconiothyrium cyclothyrioides	UTHSC DI16-367; FMR 14411		Human nail	LN907510	**LT796907**	**LT796987**	**LT797067**	**LT797147**

Didymosphaeriaceae	**Letendraea sp.**	**UTHSC DI16-370; FMR 14425**		Human neck	LN907513	**LT796908**	**LT796988**	**LT797068**	**LT797148**
Incerta sedis	Pyrenochaeta sp.	UTHSC DI16-290; FMR 13782		Human shoulder	LN907433	**LT796870**	**LT796950**	**LT797030**	**LT797110**
Incertae sedis	Medicopsis romeroi	UTHSC DI16-242; FMR 13732		Human hand	LN907385	**LT796848**	**LT796928**	**LT797008**	**LT797088**
Incertae sedis	Preussia sp.	UTHSC DI16-253; FMR 13743		Human toe nail	LN907396	**LT796853**	**LT796933**	**LT797013**	**LT797093**
Incertae sedis	Medicopsis romeroi	UTHSC DI16-309; FMR 13802		Human ankle	LN907452	**LT796874**	**LT796954**	**LT797034**	**LT797114**
Incertae sedis	Medicopsis romeroi	UTHSC DI16-310; FMR 13803		Human foot	LN907453	**LT796875**	**LT796955**	**LT797035**	**LT797115**
Incertae sedis	Medicopsis romeroi	UTHSC DI16-315; FMR 13808		Human pleural tissue	LN907458	**LT796879**	**LT796959**	**LT797039**	**LT797119**
Insertae sedis	Coniothyrium telephii	UTHSC DI16-189; FMR 13679		Human maxillary sinus	LN907332	**LT796830**	**LT796910**	**LT796990**	**LT797070**
Insertae sedis	Coniothyrium telephii	UTHSC DI16-203; FMR 13693		Unknown	LN907346	**LT796833**	**LT796913**	**LT796993**	**LT797073**
Insertae sedis	Coniothyrium telephii	UTHSC DI16-236; FMR 13726		Human blood	LN907379	**LT796843**	**LT796923**	**LT797003**	**LT797083**
Lentitheciaceae	**Keissleriella cladophila**	**UTHSC DI16-326; FMR 13819**		Animal biopsy	LN907469	**LT796883**	**LT796963**	**LT797043**	**LT797123**
Lindgomycetaceae	**Phyllosticta sp.**	**UTHSC DI16-343; FMR 13836**		Human thumb	LN907486	**LT796892**	**LT796972**	**LT797052**	**LT797132**
Lophiostomataceae	Trematosphaeria sp.	UTHSC DI16-187; FMR 13677		Human biopsy	LN907330	**LT796829**	**LT796909**	**LT796989**	**LT797069**
Macrodiplodiopsidaceae	**Pseudochaetosphaeronema sp.**	**UTHSC DI16-358; FMR 13851**		Human arm	LN907501	**LT796902**	**LT796982**	**LT797062**	**LT797142**
Macrodiplodiopsidaceae	**Camarographium sp.**	**UTHSC DI16-361; FMR 13854**		Human skin	LN907504	**LT796904**	**LT796984**	**LT797064**	**LT797144**
Nigrogranaceae	**Biatriospora mackinnonii**	**UTHSC DI16-241; FMR 13731**	T	Human skin	LN907384	**LT796847**	**LT796927**	**LT797007**	**LT797087**
Nigrogranaceae	**Biatriospora sp.**	**UTHSC DI16-342; FMR 13835**	T	Human bronch wash	LN907485	**LT796891**	**LT796971**	**LT797051**	**LT797131**
Phaeosphaeriaceae	**Neosetophoma sp.**	**UTHSC DI16-191; FMR 13681**		Human sputum	LN907334	**LT796831**	**LT796911**	**LT796991**	**LT797071**
Phaeosphaeriaceae	Paraphoma radicina	UTHSC DI16-209; FMR 13699		Human leg	LN907352	**LT796835**	**LT796915**	**LT796995**	**LT797075**
Phaeosphaeriaceae	**Trematophoma sp.**	**UTHSC DI16-210; FMR 13700**		Human bronchoalveolar lavage	LN907353	**LT796836**	**LT796916**	**LT796996**	**LT797076**
Phaeosphaeriaceae	**Parastagonospora nodorum**	**UTHSC DI16-240; FMR 13730**		Marine environment	LN907383	**LT796846**	**LT796926**	**LT797006**	**LT797086**
Phaeosphaeriaceae	**Edenia sp.**	**UTHSC DI16-260; FMR 13750**		Human eye	LN907403	**LT796855**	**LT796935**	**LT797015**	**LT797095**
Phaeosphaeriaceae	**Edenia sp.**	**UTHSC DI16-264; FMR 13754**		Human eye	LN907407	**LT796858**	**LT796938**	**LT797018**	**LT797098**
Phaeosphaeriaceae	**Neosetophoma sp.**	**UTHSC DI16-283; FMR 13775**		Human maxillary sinus	LN907426	**LT796866**	**LT796946**	**LT797026**	**LT797106**
Phaeosphaeriaceae	Paraphoma fimeti	UTHSC DI16-296; FMR 13789		Human lung	LN907439	**LT796872**	**LT796952**	**LT797032**	**LT797112**
Phaeosphaeriaceae	**Diederichomyces cladoniicola**	**UTHSC DI16-313; FMR 13806**		Human lung tissue	LN907456	**LT796877**	**LT796957**	**LT797037**	**LT797117**
Phaeosphaeriaceae	**Edenia sp.**	**UTHSC DI16-324; FMR 13817**	T	Human foot bone tissue	LN907467	**LT796881**	**LT796961**	**LT797041**	**LT797121**
Phaeosphaeriaceae	**Phaeosphaeria sp.**	**UTHSC DI16-325; FMR 13818**		Human liver	LN907468	**LT796882**	**LT796962**	**LT797042**	**LT797122**
Phaeosphaeriaceae	**Diederichomyces cladoniicola**	**UTHSC DI16-330; FMR 13823**		Human bronch wash	LN907473	**LT796886**	**LT796966**	**LT797046**	**LT797126**
Phaeosphaeriaceae	**Phaeosphaeria sp.**	**UTHSC DI16-336; FMR 13829**		Human maxillary sinus	LN907479	**LT796889**	**LT796969**	**LT797049**	**LT797129**
Phaeosphaeriaceae	**Neosetophoma sp.**	**UTHSC DI16-337; FMR 13830**	T	Human sputum	LN907480	**LT796890**	**LT796970**	**LT797050**	**LT797130**
Thyridariaceae	**Arthopyrenia salicis**	**UTHSC DI16-220; FMR 13710**	T	Human cerebrospinal fluid	LN907363	**LT796841**	**LT796921**	**LT797001**	**LT797081**
Thyridariaceae	**Roussoella sp.**	**UTHSC DI16-269; FMR 13760**	T	Human bronch wash	LN907412	**LT796863**	**LT796943**	**LT797023**	**LT797103**
Thyridariaceae	**Roussoella percutanea**	**UTHSC DI16-292; FMR 13784**	T	Human arm	LN907435	**LT796871**	**LT796951**	**LT797031**	**LT797111**
Thyridariaceae	Roussoella percutanea	UTHSC DI16-300; FMR 13793		Human foot	LN907443	**LT796873**	**LT796953**	**LT797033**	**LT797113**
Thyridariaceae	**Arthopyrenia sp.**	**UTHSC DI16-334; FMR 13827**	T	Human nose	LN907477	**LT796887**	**LT796967**	**LT797047**	**LT797127**
Thyridariaceae	**Arthopyrenia salicis**	**UTHSC DI16-356; FMR 13849**		Human wrist	LN907499	**LT796900**	**LT796980**	**LT797060**	**LT797140**
Thyridariaceae	**Roussoella percutanea**	**UTHSC DI16-360; FMR 13853**	T	Human joint fluid	LN907503	**LT796903**	**LT796983**	**LT797063**	**LT797143**
Thyridariaceae	**Arthopyrenia sp.**	**UTHSC DI16-362; FMR 13855**	T	Human eye	LN907505	**LT796905**	**LT796985**	**LT797065**	**LT797145**
Trematosphaeriaceae	Trematosphaeria grisea	UTHSC DI16-237; FMR 13727		Human elbow	LN907380	**LT796844**	**LT796924**	**LT797004**	**LT797084**
Trematosphaeriaceae	**Trematosphaeria grisea**	**UTHSC DI16-281; FMR 13773**	T	Human skin	LN907424	**LT796865**	**LT796945**	**LT797025**	**LT797105**
Trematosphaeriaceae	Trematosphaeria grisea	UTHSC DI16-286; FMR 13778		Human leg	LN907429	**LT796868**	**LT796948**	**LT797028**	**LT797108**

4.4. *Nothophoma macrospora* sp. nov.

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Publicado en: Fungal Planet sheets, Persoonia 2016; 36: 430–431.

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TAXONOMÍA DE HONGOS CELOMICETOS DE INTERÉS CLÍNICO
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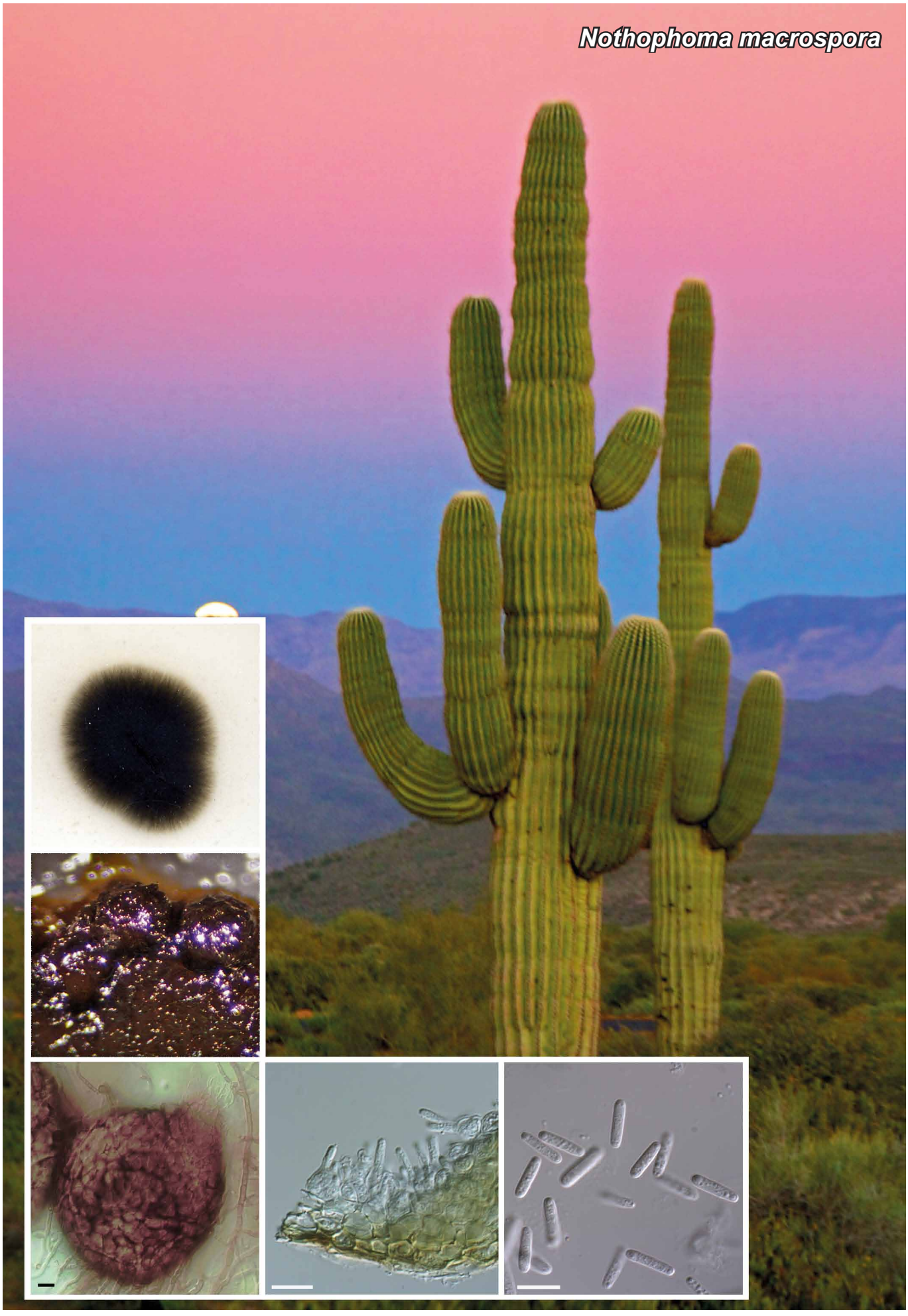


Fungal Planet 456 – 4 July 2016

Nothophoma macrospora Valenzuela-Lopez, Stchigel, Cano & Deanna A. Sutton, *sp. nov.*

Etymology. G. *μακρό*-, large, and *-σπορά*, spore, referring to the big size of the conidia.

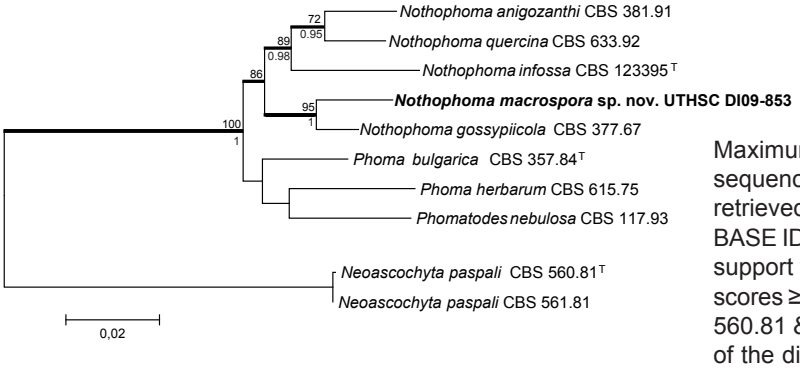
Classification — *Didymellaceae*, *Pleosporales*, *Dothideomycetes*.

Hyphae pale to dark brown, 3–10 µm wide, thin- to thick-walled, smooth to granulose due to the production of dark granules, septate, anastomosing. *Conidiomata* pycnidial dark brown, pyriform to heart-shaped by the occasional production of 2–3(–4) necks, rarely globose, 100–300 × 100–300 µm; peridium 3–5-layered, 15–25 µm thick, peridial cells globose to polygonal, pale to dark brown, 5–10 µm diam, thick-walled; neck usually present, paler than the peridial wall, cylindrical to conical, (50–)90–150 × (50–)80–110 µm, papillate, ornamented with a crown of short, subhyaline, conical to digitiform projections around the ostiolum, ostiolum of late opening; exuded conidial masses not observed; conidiogenous cells enteroblastic, phialidic, globose to flask-shaped, hyaline, thin-walled, 5–10 µm diam; conidia (9–)10–15 × 2.5–3(–3.5) µm, hyaline, cylindrical to slightly clavate at one or both ends, 0(–2)-septate, narrowing slightly at the septa, guttulate, sometimes producing a similar conidia on a lateral bulge, then forming irregular chains. *Chlamydospores* absent, but some hyphae cells become darker, thicker and barrel-shaped.

Culture characteristics — Colonies on OA reaching 30 mm diam in 7 d at 25 °C, olive brown (M.4F3), flattened, granulose due to the production of numerous pycnidia; reverse concolorous. Colonies on MEA attaining 37–41 mm in 7 d at 25 °C, yellowish white (M.4A2) to light brown (M.6D8), flattened, compact, reverse concolorous. NaOH spot test: negative. Crystals absent.

Typus. USA, Arizona, Phoenix, from respiratory secretion of a patient with pneumonia, 1 Apr. 2009, *D.A. Sutton* (holotype CBS H-22377, cultures ex-type UTHSC DI09-853 = FMR 13767 = CBS 140674, ITS sequence GenBank LN880536, LSU sequence GenBank LN880537, *actA* sequence GenBank LN880538, *tub2* sequence GenBank LN880539, MycoBank MB815051).

Notes — This fungus was isolated from a human clinical specimen. Morphologically, *Nothophoma macrospora* resembles the species previously classified into *Phoma* section *Macrospora* (Boerema et al. 2004), i.e. *Phoma andropogonivora*, *P. boeremae*, *P. chenopodii*, *P. commelinicola*, *P. gossypiicola*, *P. necator*, *P. rabiei*, *P. xanthina* and *P. zeae-maydis*. These species produce the largest conidia of the genus. Based on a megablast search of NCBIs GenBank nucleotide database, the closest hits using the LSU sequence are *Peyronellaea combreti* (GenBank KJ869191; Identities = 887/889 (99 %), no gaps) and *Peyronellaea prosopidis* (GenBank KF777232; Identities = 887/889 (99 %), no gaps). Closest hits using ITS sequence are *Leptosphaerulina australis* (GenBank KF293970; Identities = 493/497 (99 %), gaps 1/497 (0 %)), *Didymella glomerata* (GenBank AB369471; Identities = 493/497 (99 %), gaps 1/497 (0 %)) and *Nothophoma quercina* (GenBank AB369461; Identities = 493/497 (99 %), gaps 1/497 (0 %)). In a similar search in the Q-Bank fungal nucleotide database (www.q-bank.eu), the closest hit is *Nothophoma anigozanthi* CBS 381.91 (Identities = 468/473 (99 %), gaps = 1/473 (0 %)). The closest hit using the beta-tubulin (*tub2*) sequence is *Nothophoma gossypiicola* (GenBank GU237611; Identities = 323/335 (99 %), no gaps), as well was using the actin (*actA*) sequence against Q-Bank (*Nothophoma gossypiicola* CBS 377.67; Identities = 214/224 (96 %), no gaps). Our phylogenetic tree, built by using the ITS, LSU, *tub2* and *actA* sequences, corroborated that our fungus represents a new species of the genus *Nothophoma*, *N. gossypiicola* being the most phylogenetically and morphologically related species. *Nothophoma macrospora* differs from *N. gossypiicola* by its lower growing rate on OA, the shape (pyriform to hearth-shaped vs globose), the number of necks (up to 4 vs 0–1) and the ornamentation (papillate vs non-papillate) of the pycnidia, and the presence of conidial septa (up to 2 vs non-septate).



Maximum likelihood tree obtained from the combined DNA sequences dataset from four loci of our isolate and sequences retrieved from the GenBank and the Q-Bank databases (TreeBASE ID 18137). Above the nodes are presented the bootstrap support values ≥ 70 %, and the Bayesian posterior probability scores ≥ 0.95 are indicated below. *Neoascochyta paspali* (CBS 560.81 & CBS 561.81) was used as outgroup. Ex-type strains of the different species are indicated with ^{T}. The new species proposed in this study is indicated in **bold**. The alignment was performed by MEGA v. 6.06 (Tamura et al. 2013), and the tree building by MEGA v. 6.06 and by MrBayes v. 3.2.4 (Huelsenbeck & Ronquist 2001).

Colour illustrations. USA, Arizona, Phoenix, McDowell mountain park (image credit: Hector Lopez and Brenda, www.hmlopezphoto.com); colony on OA after 7 d at 25 °C, conidiomata under stereomicroscope, pycnidia, conidiogenous cells, conidia. Scale bars = 10 µm.

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Revisión bibliográfica de los hongos celomicetos de interés clínico

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4.5. DNA sequencing to clarify the taxonomical conundrum of the clinical coelomycetes

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Aceptado en: Mycoses 2018; doi: 10.1111/myc.12785.

UNIVERSITAT ROVIRA I VIRGILI
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Article type : Review Article

DNA sequencing to clarify the taxonomical conundrum of the clinical coelomycetes

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Running head: Coelomycetes of clinical interest.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/myc.12785

Summary

The taxonomy of the fungi that produce human infections and that develop asexual fruiting bodies in culture has become very complex. Recent molecular studies have produced dramatic changes in their classification. Currently, the coelomycetes traditionally included in *Sphaeropsidales* and *Melanconiales* are in fact distributed across at least three different classes of the Phylum Ascomycota. Approximately 1,000 genera and 7,000 species have been grouped in the classes *Dothideomycetes*, *Leotiomycetes* and *Sordariomycetes* and their proper identification can only be made by analysing their DNA sequences and comparing them with those corresponding to type strains available in the adequate databases. To facilitate this task for scientists and clinicians involved in the study of these complex, and every day more numerous taxa, we have updated the knowledge about the taxonomy of the commonest coelomycetes of clinical interest with the aim of improving their identification and antifungal treatment.

Keywords: Coelomycetes, *Medicopsis*, mycosis, *Neocucurbitaria*, *Phoma*, *Pyrenochaeta*.

INTRODUCTION

The number of species of coelomycetes involved in human infections has increased enormously in recent years[1-4]. This has been a consequence of the current use of molecular biology approaches in taxonomy that have allowed more stable systematic criteria to be established and a redefinition of modern concepts of genera and species. On that basis, numerous taxa have recently been delimited. For instance, two of the most relevant genera of coelomycetes such as *Phoma* and *Pyrenochaeta* have undergone major changes. The former

has been reduced to only one species within the family *Didymellaceae*; while *Pyrenochaeta* has been excluded from *Cucurbitariaceae* and maintained as *incertae sedis*, its species mainly being distributed into the genus *Neocucurbitaria*.[5,6] The coelomycetes have been classified, traditionally, by their morphological features.[3,4] However, its classification turned out to be obsolete, and these fungi have been considered, based on phylogenetic analyses in three different classes, i.e. *Dothideomycetes* (bitunicate ascostromatic-like fruiting bodies), *Leotiomycetes* (apothecium-like fruiting bodies), and *Sordariomycetes* (unitunicate ascomata-like fruiting bodies) of the phylum Ascomycota.[4,7-9] Nevertheless, in the clinical setting the term "Coelomycete" is still used to refer to fungi morphologically characterized by producing conidia within fruiting bodies (= conidiomata) of two types, acervular (cup-shaped) or pycnidial (globose to pyriform) conidiomata.[10,11] Taxonomically, species with clinical prevalence are distributed mainly within the two first classes mentioned above, as was shown by Valenzuela-Lopez *et al.* [4], in a study of a large number of coelomycetous isolates from the USA, which demonstrated that the majority of them were distributed across at least eleven orders, being the *Pleosporales* the most prevalent.

With an ability to cause human infections, the coelomycetes have been involved in numerous opportunistic mycoses ranging from superficial to deep infections, most of them acquired by traumatic implantation of plant material or soil particles mainly in subtropical and tropical areas.[1,3-4,12-14] The most frequent are reduced to a specific group of taxa that includes *Colletotrichum* spp., *Medicopsis romeroi* and *Neoscytalidum dimidiatum*, although apart from these a huge number of species are occasionally also involved in human pathologies (see Table 1). Approximately 50 species have been reported in human mycoses, mainly causing subcutaneous infections.

Identification of the coelomycetes in the clinical laboratory is not easy because of their difficulty in sporulating. Recognizing and characterizing the most representative morphological structures of these fungi requires some expertise and even then these microorganisms remain sterile in many cases.[3] The use of molecular techniques based on the amplification and sequencing of appropriate phylogenetic markers is very important in the identification in this group of fungi.

Treatments are still not established due to the lack of clinical breakpoints for these fungi and to the difficulties in performing antifungal susceptibility testing against these fungi that hardly sporulate. Only few studies on coelomycetes have demonstrated that the use of surgical resection and a few drugs such as amphotericin B and triazoles have shown some efficacy.[14-15]

The aim of this paper is to update the current knowledge of the taxonomy of these taxa involved in human infections.

LABORATORY IDENTIFICATION

Isolation and morphological identification of clinical coelomycetous isolates

For cultural isolation and characterization the isolates should be inoculated on the following culture media: malt extract agar (MEA; 40 g of malt extract, 15 g of agar-agar, 1 L distilled water), oatmeal agar (OA; 30 g of filtered oat flakes, 15 g of agar-agar, 1 L tap water) and potato dextrose agar (PDA, 4 g of potato infusion, 20 g dextrose, 15 g of agar-agar, 1 L tap water) at 25 ± 1°C for 14 days in darkness. If the isolate does not sporulate, its incubation under near ultraviolet (UV) light (12 hours light, 12 hours dark) or on carnation leaf agar (CLA) to induce sporulation can be useful.[16-17] For micromorphological characterization, the use of wet mounts prepared in Shear's mounting medium (potassium acetate 3 g, distilled

water 150 mL, glycerin 60 mL, ethanol (95 %) 90 mL) or 85% lactic acid are recommended. Table 1 summarizes the most relevant morphological features of the most prevalent coelomycetes in the clinical setting.

DNA extraction, amplification, and sequencing

For identification purposes the fungal genomic DNA should be extracted from colonies grown on PDA after 7 days of incubation at 25 ± 1°C, following the protocols by Valenzuela-Lopez *et al*. [4].

Molecular identification of coelomycetes

To this purpose, the fragment of the 28S nrRNA gene (LSU) and internal transcribed spacer region (ITS) should be amplified, and depending on the fungus secondary phylogenetic markers such as beta-tubulin gene (*tub*2) and/or RNA polymerase II subunit 2 gene (*rpb*2) should be additionally tested (see Table 2).

Preliminary identification of coelomycetes can be carried out using the BLAST nucleotide tool of the NCBI (https://blast.ncbi.nlm.nih.gov/Blast/), the Westerdijk Fungal Biodiversity Institute (former CBS) (http://www.westerdijkinstitute.nl/Collections/) or the Q-Bank (http://www.q-bank.eu/Fungi/) databases. For accurate identification of the isolates, the sequences must be compared with those of type or reference strains. It is also recommended to perform an alignment and its phylogenetic analysis using appropriate softwares such as MrBayes, RAxML, MEGA or several other useful programs.[18-20]

We suggest, depending on the fungi in order to identify, to follow the scheme of figure 1 and the primers listed in Table 2. In general, the genes ITS, LSU, *tub*2 and translation elongation factor 1-alpha (*tef*1) are easy to amplify; and also the *rpb*2, which is a bit more difficult to amplify; however, it is one of the most informative marker. [5-6] Nowadays, most of ITS and LSU genes of these species are sequenced and available in the public databases. Recently,

additional phylogenetic markers have been sequenced, which increase the possibility of a better taxonomic classification and identification of the fungi.

In figure 2 we provide an example of phylogenetic analysis with a tree performed by using the MEGA software with a set of LSU sequences of clinical coelomycetes available. However, the precise identification always depends on the phylogenetic markers used (see above). Unfortunately, most of the coelomycetes information in public databases it is not updated and in many cases is necessary an exhaustive literature search for a correct taxonomic placement of the fungus.

Recently, the use of matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry for identifying microorganisms in the clinical laboratory has quickly become important.[21-23] This technique was used previously mostly for yeasts, but more recently it has been also adapted for identifying molds. A recent study by Fraser *et al*. [23] also demonstrated the usefulness of this technique for coelomycetes that produce black grain mycetoma.

Conclusions

The taxonomy of the coelomycetes that have produced human infections is confusing because they are currently distributed across a high number of genera and species. They can only be properly identified by DNA sequencing and comparison with reference strains. However, their identity is crucial for their correct antifungal management, which is still little known.

ACKNOWLEDGMENTS

This work was supported by the Spanish Ministerio de Economía y Competitividad,

grant CGL2017-88094-P.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. Sutton DA. Coelomycetous fungi in human disease. A review: clinical entities, pathogenesis, identification and therapy. *Rev Iberoam Micol*. 1999;16:171–179.
2. de Hoog, Guarro J, Gene J, Figueras MJ. Atlas of clinical fungi. Utrecht/Reus: Centraalbureau voor Schimmelcultures/Universitat Rovira i Virgili, 2000.
3. Stchigel AM, Sutton DA. Coelomycete fungi in the clinical lab. *Curr Fungal Infect Rep*. 2013;7:171–191.
4. Valenzuela-Lopez N, Sutton DA, Cano-Lira JF, *et al*. Coelomycetous fungi in the clinical setting: morphological convergence and cryptic diversity. *J Clin Microbiol*. 2017;55:552–567.
5. Chen Q, Jiang JR, Zhang GZ, Cai L, Crous PW. Resolving the *Phoma* enigma. *Stud Mycol*. 2015;82:137–217.
6. Valenzuela-Lopez N, Cano-Lira JF, Guarro J, *et al*. Coelomycetous Dothideomycetes with emphasis on the families *Cucurbitariaceae* and *Didymellaceae*. *Stud Mycol*. 2018;90:1–69.
7. Schoch CL, Crous PW, Groenewald JZ, *et al*. A class-wide phylogenetic assessment of Dothideomycetes. *Stud Mycol*. 2009;64:1–15.
8. Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW. *Pestalotiopsis* revisited. *Stud Mycol*. 2014;79:121–186.
9. Wijayawardene NN, Hyde KD, Wanasinghe DN, *et al*. Taxonomy and phylogeny of dematiaceous coelomycetes. *Fungal Divers*. 2016;77:1–316.

10. Sutton BC. *The Coelomycetes: Fungi Imperfecti with pycnidia, acervuli and stromata*. Kew, Surrey, England, Commonwealth Mycological Institute; 1980.
11. Kirk PM, Cannon PF, Stalpers JA, Minter DW. *Ainsworth & Bisby's dictionary of the fungi*. 10th ed. Wallingford, United Kingdom: CAB International; 2008.
12. Ahmed SA, Van De Sande WW, Stevens DA, *et al*. Revision of agents of black-grain eumycetoma in the order Pleosporales. *Persoonia*. 2014;33:141–154.
13. Vasant JA, Maggiani F, Borman AM. Subcutaneous mycotic cyst caused by *Roussoella percutanea* in a UK renal transplant patient. *Mycopathologia*. 2017;182:721–725.
14. Guégan S, Garcia-Hermoso D, Sitbon K, *et al*. Ten-year experience of cutaneous and/or subcutaneous infections due to coelomycetes in France. *Open Forum Infect Dis*. 2016;3:ofw106.
15. Chowdhary A, Meis JF, Guarro J, *et al*. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of systemic phaeohyphomycosis: diseases caused by black fungi. *Clin Microbiol Infect*. 2014;20:47–75.
16. Fisher NL, Burgess LW, Toussoun TA, Nelson PE. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology*. 1982;72:151–153.
17. Su YY, Qi YL, Cai L. Induction of sporulation in plant pathogenic fungi. *Mycology*. 2012;3:195–200.
18. Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*. 2001;17:754–755.
19. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 2014;30:1312–1313.
20. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol*. 2013;30:2725–2729.

21. Havlicek V, Lemr K, Schug KA. Current trends in microbial diagnostics based on mass spectrometry. *Anal Chem*. 2013;85:790–797.
22. Sanguinetti M, Posteraro B. Identification of molds by matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J Clin Microbiol*. 2017;55:369–379.
23. Fraser M, Borman AM, Johnson EM. Rapid and robust identification of the agents of black-grain mycetoma by matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J Clin Microbiol*. 2017;55:2521–2528.
24. Campbell CK, Mulder JL. Skin and nail infection by Scytalidium hyalinum sp. nov. *Sabouraudia*. 1977;15:161–166.
25. Moore MK. Morphological and physiological studies of isolates of *Hendersonula toruloidea* Nattrass cultured from human skin and nail samples. *J Med Vet Mycol*. 1988;26:25–39.
26. Kombila M, Martz M, Gomez de Diaz M, de Bievre C, Richard-Lenoble D. *Hendersonula toruloidea* as an agent of mycotic foot infection in Gabon. *J Med Vet Mycol*. 1990;28:215–223.
27. Al-Rajhi AA, Awad AH, Al-Hedaithy SS, Forster RK, Caldwell KC. *Scytalidium dimidiatum* fungal endophthalmitis. *Br J Ophthalmol*. 1993;77:388–390.
28. Benne CA, Neeleman C, Bruin M, de Hoog G, Fleer A. Disseminating infection with *Scytalidium dimidiatum* in a granulocytopenic child. *Eur J Clin Microbiol Infect Dis*. 1993;12:118–121.
29. Geramishoar M, Zomorodian K, Zaini F, *et al*. First case of cerebral phaeohyphomycosis caused by *Nattrassia mangiferae* in Iran. *Jpn J Infect Dis*. 2004;57:285–286.

30. Jabbarvand M, Hashemian MR, Abedinifar Z, Amini A. *Nattrassia mangiferae* keratitis after laser in situ keratomileusis. *J Cataract Refract Surg*. 2004;30:268–272.
31. Padhi S, Uppin SG, Uppin MS, *et al*. Mycetoma in South India: retrospective analysis of 13 cases and description of two cases caused by unusual pathogens: *Neoscytalidium dimidiatum* and *Aspergillus flavus*. *Int J Dermatol*. 2010;49:1289–1296.
32. Jagielski T, Zak I, Tyrak J, Bryk A. First probable case of subcutaneous infection due to *Truncatella angustata*: a new fungal pathogen of Humans?. *J Clin Microbiol*. 2015;53:1961–1964.
33. Rebell G, Forster RK. *Lasiodiplodia theobromae* as a cause of keratomycoses. *Sabouraudia*. 1976;14:155–170.
34. Restrepo A, Arango M, Velez H, Uribe L. The isolation of *Botryodiplodia theobromae* from a nail lesion. *Sabouraudia*. 1976;14:1–4.
35. Slomovic AR, Forster RK, Gelender H. *Lasiodiplodia theobromae* panophthalmitis. *Can J Ophthalmol*. 1985;20:225–228.
36. Maslen MM, Collis T, Stuart R. *Lasiodiplodia theobromae* isolated from a subcutaneous abscess in a Cambodian immigrant to Australia. *J Med Vet Mycol*. 1996;34:279–283.
37. Woo PC, Lau SK, Ngan AH, Tse H, Tung ET, Yuen KY. *Lasiodiplodia theobromae* pneumonia in a liver transplant recipient. *J Clin Microbiol*. 2008;46:380–384.
38. Kindo AJ, Pramod C, Anita S, Mohanty S. Maxillary sinusitis caused by *Lasiodiplodia theobromae*. *Indian J Med Microbiol*. 2010;28:167–169.
39. Papacostas LJ, Henderson A, Choong K, Sowden D. An unusual skin lesion caused by *Lasiodiplodia theobromae*. *Med Mycol Case Rep*. 2015;8: 44–46.

40. Tan DH, Sigler L, Gibas CF, Fong IW. Disseminated fungal infection in a renal transplant recipient involving *Macrophomina phaseolina* and *Scytalidium dimidiatum*: case report and review of taxonomic changes among medically important members of the *Botryosphaeriaceae*. *Med Mycol*. 2008;46:285–292.
41. Srinivasan A, Wickes BL, Romanelli AM, et al. Cutaneous infection caused by Macrophomina phaseolina in a child with acute myeloid leukemia. J Clin Microbiol. 2009;47:1969–72.
42. Premamalini T, Ambujavalli BT, Vijayakumar R, Rajyoganandh SV, Kalpana S, Kindo AJ. Fungal keratitis caused by *Macrophomina phaseolina* - A case report. *Med Mycol Case Rep*. 2012;1:123-126.
43. Kirkness CM, Seal DV, Clayton YM, Punithalingam E. *Sphaeropsis subglobosa* keratomycosis - First reported case. *Cornea*. 1991;10:85–89.
44. Cariello PF, Wickes BL, Sutton DA, *et al*. *Phomopsis bougainvilleicola* prepatellar bursitis in a renal transplant recipient. *J Clin Microbiol*. 2013;51:692–695.
45. Iriart X, Binois R, Fior A, *et al*. Eumycetoma caused by *Diaporthe phaseolorum* (*Phomopsis phaseoli*): a case report and a mini-review of *Diaporthe*/*Phomopsis* spp invasive infections in humans. *Clin Microbiol Infect*. 2001;17:1492–1494.
46. Gajjar DU, Pal AK, Parmar TJ, *et al*. Fungal scleral keratitis caused by *Phomopsis phoenicicola*. *J Clin Microbiol*. 2011;49:2365–2368.
47. Liesegang TJ, Forster RK. Spectrum of microbial keratitis in South Africa. *Am J Ophthalmol*. 1980;90:38–47.
48. O'Quinn RP, Hoffmann JL, Boyd AS. *Colletotrichum* species as emerging opportunistic fungal pathogens: a report of 3 cases of phaeohyphomycosis and review. *J Am Acad Dermatol*. 2001;45:56–61.

49. Liao WQ, Shao JZ, Li SQ, *et al*. Colletotrichum dematium caused keratitis. *Chin Med J*. 1983;96:391–394.
50. Mendiratta DK, Thamke D, Shukla AK, Narang P. Keratitis due to *Colletotrichum dematium* - A case report. *Indian J Med Microbiol*. 2005;23:56–58.
51. Singh R, Gupta V, Chakarborty A, Nijhawan R, Arora S, Gupta A. Fungal endophthalmitis complicating subconjunctival injection of triamcinolone acetonide in anterior scleritis. *Ann Ophthalmol* (Skokie). 2006;38:253–256.
52. Castro LG, da Silva LC, Guarro J, *et al*. Phaeohyphomycotic cyst caused by *Colletotrichum crassipes*. *J Clin Microbiol*. 2001;39:2321–2324.
53. Shukla PK, Khan ZA, Lal B, Agrawal PK, Srivastava OP. Clinical and experimental keratitis caused by *Colletotrichum* state of *Glomerella cingulata* and *Acrophialophora fusispora*. *Sabouraudia*. 1983;21:137–147.
54. Guarro J, Svidzinski TE, Zaror L, Forjaz MH, Gené J, Fischman O. Subcutaneous hyalohyphomycosis caused by *Colletotrichum gloeosporioides*. *J Clin Microbiol*. 1998;36:3060–3065.
55. Potea ST, Chakraborty A, Lahiri KK, Patolea MS, Deshmukhc RA, Shah SR. Keratitis by a rare pathogen *Colletotrichum gloeosporioides*: A case report. *J Mycol Med*. 2017;27:407–411.
56. Ritterband DC, Mahendra S, Seedor JA. *Colletotrichum graminicola*: A new corneal pathogen. *Cornea*. 1997;16:362–364.
57. Shivaprakash MR, Appannanavar SB, Dhaliwal M, *et al*. *Colletotrichum truncatum*: an unusual pathogen causing mycotic keratitis and endophthalmitis. *J Clin Microbiol*. 2011;49:2894–2898.

58. Chowdhary A, Guarro J, Randhawa HS, *et al*. A rare case of chromoblastomycosis in a renal transplant recipient caused by a non-sporulating species of *Rhytidhysteron*. *Med Mycol*. 2008;46:163–166.
59. Mahajan VK, Sharma V, Prabha N, *et al*. A rare case of subcutaneous phaeohyphomycosis caused by a *Rhytidhysteron* species: a clinico-therapeutic experience. *Int J Dermatol*. 2014;53:1485–1489.
60. Mishra K, Das S, Goyal S, *et al*. Subcutaneous mycoses caused by *Rhytidhysteron* species in an immunocompetent patient. *Med Mycol Case Rep*. 2014;5:32–34.
61. Guarro J, Höfling-Lima AL, Gené J, *et al*. Corneal ulcer caused by the new fungal species *Sarcopodium oculorum*. *J Clin Microbiol*. 2002;40:3071–3075.
62. Perdomo H, García D, Gené J, *et al*. *Phialemoniopsis*, a new genus of Sordariomycetes, and new species of *Phialemonium* and *Lecythophora*. *Mycologia*. 2013;105:398–421.
63. Zaitz C, Heins-Vaccari EM, Freitas RS, *et al*. Subcutaneous phaeohyphomycosis caused by *Phoma cava*. Report of a case and review of the literature. *Rev Inst Med trop S Paulo*. 1997;39:43–48.
64. Ferrer C, Pérez-Santonja JJ, Rodríguez AE, *et al*. New Pyrenochaeta species causing keratitis. *J Clin Microbiol*. 2009;47:1596–1598.
65. Verkley GJ, Gené J, Guarro J, *et al*. *Pyrenochaeta keratinophila* sp. nov., isolated from an ocular infection in Spain. *Rev Iberoam Micol*. 2010;27:22–24.
66. English MP, Atkinson R. Onychomycosis in elderly chiropody patients. *Br J Dermatol*. 1974;91:67–72.
67. Punithalingam E, English MP. *Pyrenochaeta unguis-hominis* sp.nov. on human toe-nails. *Trans Br Mycol Soc*. 1975;64:539–541.

68. Toh YF, Yew SM, Chan CL, *et al*. Genome anatomy of *Pyrenochaeta unguis-hominis* UM 256, a multidrug resistant strain isolated from skin scraping. *PLoS One*. 2016;11:e0162095.

69. Balis E, Velegraki A, Fragou A, Pefanis A, Kalabokas T, Mountokalakis T. Lung mass caused by Phoma exigua. *Scand J Infect Dis*. 2006;38:552–555.

70. Punithalingam E. Sphaeropsidales in culture from humans. *Nova Hedwigia*. 1979;31:119–158.

71. Rai MK. *Phoma sorghina* infection in human being. *Mycopathologia*. 1989;105:167–170.

72. Bakerspigel A, Lowe D, Rostas A. The isolation of *Phoma eupyrena* from a human lesion. *Arch Dermatol*. 1981;117:362–363.

73. Punithalingam E. *Phoma oculo-hominis* sp.nov. from corneal ulcer. *Trans Br Mycol Soc*. 1976;67:142–143.

74. Colombier MA, Alanio A, Denis B, *et al*. Dual Invasive Infection with *Phaeoacremonium parasiticum* and *Paraconiothyrium cyclothyrioides* in a Renal Transplant Recipient: Case Report and Comprehensive Review of the Literature of *Phaeoacremonium* Phaeohyphomycosis. *J Clin Microbiol*. 2015;53:2084–2094.

75. Kiehn TE, Polsky B, Punithalingam E, Edwards FF, Brown AE, Armstrong D. Liver infection caused by Coniothyrium fuckelii in a patient with acute myelogenous leukemia. *J Clin Microbiol*. 1987;25:2410–2412.

76. Borelli D. *Pyrenochaeta romeroi* nova especie. *Dermatol Venez*. 1959;1:325–326.

77. Girard C, Dereure O, Rispail P, Durand L, Guilhou JJ. Subcutaneous phaeohyphomycosis due to Pyrenochaeta romeroi in a patient with leprosy. *Acta Derm Venereol*. 2004;84:154–155.

78. Ocampo MA, Kanitakis J, Bienvenu AL, Chauvet C, Euvrard S. Phaeohyphomycosis caused by *Pyrenochaeta romeroi* mimicking a plantar wart in a kidney transplant recipient. *Transpl Infect Dis*. 2012;14:173–174.
79. Borelli D, Zamora R, Senabre G. *Chaetosphaeronema larense* nova species agente de micetoma. *Gaceta Med Caracas*. 1976;84:307–318.
80. Ahmed SA, Desbois N, Quist D, *et al*. Phaeohyphomycosis caused by a novel species, *Pseudochaetosphaeronema martinelli*. *J Clin Microbiol*. 2015;53:2927–2934.
81. Chabasse D, de Bievre C, Legrand E, *et al*. Subcutaneous abscess caused by *Pleurophomopsis lignicola* Petr: first case. *J Med Vet Mycol*. 1995;33:415–417.
82. Padhye AA, Karpati A, Rosenthal SA, Punithalingam E. Subcutaneous phaeohyphomycotic abscess caused by *Pleurophomopsis lignicola*. *Med Mycol*. 2004;42:129–134.
83. Borelli D. *Pyrenochaeta mackinnonii* nova species agente de micetoma. *Castellania*. 1976;4:227–234.
84. Ahmed SA, Hofmüller W, Seibold M, *et al*. *Tintelnotia*, a new genus in *Phaeosphaeriaceae* harbouring agents of cornea and nail infections in humans. *Mycoses*. 2017;60:244–253.
85. Shukla NP, Rajak RK, Agarwal GP, Gupta DK. *Phoma minutispora* as a Human Pathogen. *Mycoses*. 1984;27:255–258.
86. Ahmed SA, Stevens DA, van de Sande WW, Meis JF, de Hoog GS. *Roussoella percutanea*, a novel opportunistic pathogen causing subcutaneous mycoses. *Med Mycol*. 2014;52:689–698.
87. Borman AM, Desnos-Ollivier M, Campbella CK, Bridge PD, Dannaoui E, Johnson EM. Novel taxa associated with Human fungal black-grain Mycetomas: *Emarellia*

grisea gen. nov., sp. nov., and *Emarellia paragrisea* sp. nov. *J Clin Microbiol*. 2016;54:1738–1745.

88. Segretain G, Destombes P. Mycetomas caused by *Madurella grisea* and *Pyrenochaeta romeroi*. *Sabouraudia*. 1969;7:51–61.
89. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR Protocols: A Guide to Methods and Applications. Orlando, Florida: Academic Press; 1990:315–322.
90. Rehner SA, Samuels GJ. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycol Res*. 1994;98:625–634.
91. Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *J Bacteriol*. 1990;172:4238–4246.
92. Carbone I, Kohn L. A method for designing primer sets for speciation studies in filamentous Ascomycetes. *Mycologia*. 1999;91:553–556.
93. O'Donnell K, Cigelnik E. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Mol Phylogenet Evol*. 1997;7:103–116.
94. Glass NL, Donaldson G. Development of primer sets designed for use with PCR to amplify conserved genes from filamentous Ascomycetes. *Appl Environ Microb*. 1995;61:1323–1330.
95. Woudenberg JHC, Aveskamp MM, de Gruyter J, Spiers AG, Crous PW. Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia*. 2009;22:56–62.

96. Guerber JC, Liu B, Correll JC, Johnston PR. Characterization of diversity in *Colletotrichum acutatum* sensu lato by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. *Mycologia*. 2003;95:872–895.
97. Crous PW, Groenewald JZ, Risede JM, Hywel-Jones NL. *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Stud Mycol*. 2004;50:415–430.
98. Liu YJ, Whelen S, Hall BD. Phylogenetic relationships among ascomycetes evidence from an RNA polymerase II subunit. *Mol Biol Evol*. 1999;16:1799–1808.
99. Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW. A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia*. 2006;98:1041–1052.

Figure legend

Figure 1. Flow scheme showing the phylogenetic markers for coelomycete identification ("?" means that ITS not always it's an useful tool to resolve the identification at species level). Data referred to genes are included in Table 2.

Figure 2. Maximum likelihood tree obtained from the D1-D2 of LSU (588 bp) sequences of 40 coelomycetous isolates. Bootstrap support values of ≥70 are indicated on the nodes. The GenBank accession numbers are given for each isolate. *Chaetomella oblonga* ATCC 12718 and *C. zambiensis* CBS 137978 were used to root the tree.

Table 1 Species of coelomycetes of clinical interest and their morphological characteristics

Order & Family	Species (former name)	Human infection	Reference	Conidiomata	Conidia	Other features
Amphisphaeriales						
Bartaliniaceae	*Truncatella angustata*	Subcutaneous	23	Acervular to pycnidial	Fusiform, 3-septate, 15–23 × 6–8 µm, with apical appendages; basal cell hyaline, obconic, thin-walled; mediun cells brown, doliiform to subcylindrical, thick-walled	
Botryosphaeriales						
Botryosphaeriaceae	*Hendersonula toruloidea* synanamorph: *Neoscytalidium dimidiatum* (*Scytalidium hyalinum*)	Cutaneous, keratitis, onychomycosis, black grain eumycetoma, endophthalmitis, subcutaneous, Cerebral, systemic	24-31	Pycnidial	Initially hyaline, aseptate, becoming dark brown and septate with age, 12–20 × 4–8 µm	The synanamorph *N. dimidiatum* produces arthroconidia
	Lasiodiplodia theobromae (*Botryodiplodia theobromae*)	Keratitis, onychomycosis, endophthalmitis, subcutaneous, pneumonia, sinusitus	32-38	Pycnidial	Initially hyaline, becoming dark brown with longitudinal striations , ellipsoidal, thick-walled, 1-septate, 20–30 × 10–15 µm	Sexual morph on vegetal material. Paraphyses hyaline, cylindrical, septate
	Macrophomina phaseolina	Cutaneous, keratitis, systemic	39-41	Pycnidial	Hyaline, ellipsoid to obovoid, 14–30 × 5–10	Sclerotia on vegetal material

					µm	
	Neodeightonia subglobosa (*Sphaeropsis subglobosa*)	Keratitis	42	Stromatic	Brown, obovoidal or subspherical to spherical, with truncate base, thick-walled, aseptate, 9–12 × 6–9 µm	
Diaporthales						
Diaporthaceae	*Diaporthe bougainvilleicola* (*Phomopsis bougainvilleicola*)	Bursitis	43	Stromatic	Hyaline, of two types: alpha, ellipsoidal or fusiform, 5–8.4 × 1.2–2 µm, and beta, filiform, curved, 16–31 × 0.5–0.9 µm	
	Diaporthe phaseolorum (*Phomopsis phaseoli*)	Black grain eumycetoma	44	Pycnidial	Hyaline, of two types: alpha, ovoid, 6.7–7 × 2.4 µm, and beta, filiform, 13.3–22.5 × 0.5–0.9 µm	
	Diaporthe phoenicicola (*Phomopsis phoenicicola*)	Keratitis	45	Pycnidial	Hyaline, only alpha-conidia, ovoid to fusiform, 8–12 × 2–2.5 µm	
Glomerellales						
Glomerellaceae	*Colletotrichum coccodes* (*C. atramentarium*)	Keratitis, subcutaneous	46-47	Acervular	Hyaline, straight, fusiform, 16–22 × 3–4 µm	Apressoria brown, clavate, 11–16.5 × 6–9.5 µm

	Colletotrichum dematium	Keratitis, endophthalmitis	48-50	Acervular	Hyaline, falcate, acute apex, 20–30 × 3–5 µm	Apressoria brown, clavate to circular, 8–11.5 × 6.5–8 µm
	Colletotrichum gigasporum (*C. crassipes*)	Subcutaneous	51	Acervular	Hyaline, cylindrical with rounded ends, 27–30 × 9–10 µm	Apressoria brown to dark brown, variable in shape including ovoid, clavate or slightly irregular, 15–30 × 7–14 µm
	Colletotrichum gloeosporioides	Keratitis, subcutaneous	52-54	Acervular	Hyaline, straight, cylindrical, obtuse at the apex, 9–24 × 3–4.5 µm	Apressoria brown, clavate or irregular, 6–20 × 4–12 µm
	Colletotrichum graminicola	Keratitis	55	Acervular	Hyaline, fusiform to falcate, 23–29 × 3.5–5 µm	Apressoria brown, irregular, 17–20 × 12–14 µm
	Colletotrichum truncatum	Keratitis	56	Acervular	Hyaline, fusiform, 20–23.5 × 3.5–4 µm	Appressoria light brown, solitary or in groups, ellipsoidal or clavate, 6.5–13 × 5.5–7.5 µm
Hysteriales						
Hysteriaceae	*Rhytidhysteron rufulum*	Subcutaneous	57-59	Pycnidial	Hyaline, globose to subglobose, smooth- and thin-walled	Sexual morph on vegetal material

Incertae sedis	*Phialemoniopsis ocularis* (*Sarcopodium oculorum*)	Keratitis	60-61	Pycnidial or sporodochial	Hyaline, ellipsoidal, smooth-walled, aseptate, 2–3.5 × 1–1.5 µm	Chlamydospores, brown, thick- and rough-walled, intercallary, solitary or in chains, globose to pyriform, 5–9 × 3–6 µm
Pleosporales						
Cucurbitariaceae	*Neocucurbitaria cava* (*Pyrenochaeta cava*)	Subcutaneous	62	Pycnidial	Hyaline, cylindrical to slightly allantoid, smooth- and thin-walled, aseptate, 2.5–3.5 × 1–1.5 µm	Growth at 37ºC[a]
	Neocucurbitaria keratinophila (*Pyrenochaeta keratinophila*)	Keratitis	63-64	Pycnidial	Hyaline, ellipsoidal, smooth- and thin-walled, aseptate, 2.5–3 × 1–2 µm	Growth at 37ºC[a]
	Neocucurbitaria unguis-hominis (*Pyrenochaeta unguis-hominis*)	Onychomycosis	65-67	Pycnidial	Hyaline, cylindrical, smooth- and thin-walled, aseptate, 2–3.5 × 1–1.5 µm	Growth at 37ºC[a]
Didymellaceae	*Boeremia exigua* var. *exigua* (*Phoma exigua*)	Lung mass	68	Pycnidial	Hyaline, smooth- and thin-walled, mainly aseptate or 1(–2)-septate, 4.5–8 × 2.5–4 µm	

Didymella glomerata (*Phoma glomerata*)	Subcutaneous	69	Pycnidial	Hyaline, variable in shape, smooth- and thin-walled, aseptate, 4–8.5 × 1.5–3 µm	Chlamydospores dark brown, solitary or in chains, multicellular-dictyosporous, 30–65 × 15–25 µm
Epicoccum sorghinum (*Phoma sorghina*)	Subcutaneous	70	Pycnidial	Hyaline, variable in shape mostly ovoid-ellipsoidal, smooth- and thin-walled, aseptate, 4.5–7 × 2–3 µm	Chlamydospores dark brown, solitary or in chains, irregular, dictyosporous, 8–35 µm diam.
Juxtiphoma eupyrena (*Phoma eupyrena*)	Subcutaneous	71	Pycnidial	Hyaline, ellipsoidal, smooth- and thin-walled, aseptate, 4–5.5 × 2–2.5 µm	Chlamydospores dark brown, barrel shaped, 4–15 µm diam.
Phoma herbarum (*Phoma cruris-hominis*)	Subcutaneous	69	Pycnidial	Hyaline, ellipsoidal to ovoid, smooth- and thin-walled, aseptate, 4.5–6 × 2–3 µm	
Stagonosporopsis oculi-hominis (*Phoma oculi-homini*)	Keratitis	72	Pycnidial	Hyaline to brown, cylindrical, smooth- and thin-walled, aseptate (3–7 × 1–2 µm) or 1-septate(9–16 × 3–4.5 µm)	

Didymosphaeriaceae	*Paraconiothyrium cyclothyrioides*	Systemic	73	Stromatic	Initially hyaline, yellowish brown with age, cylindrical, thin-walled, aseptate, 3–4.2 × 1.2–1.5 µm	
	Paraconiothyrium fuckelii (*Coniothyrium fuckelii*)	Systemic	74	Pycnidial	Pale brown, cylindrical, smooth- and thin-walled, aseptate, 2.5–4 × 1.5–2 µm	
Incertae sedis	*Medicopsis romeroi* (*Pyrenochaeta romeroi*)	Cutaneous, black grain eumycetoma, subcutaneous	11,75-77	Pycnidial	Hyaline, cylindrical to ellipsoidal, smooth- and thin-walled, aseptate, 2–2.8 × 1.2–1.5 µm	Growth at 37ºC[a]
Macrodiplodiopsidaceae	*Pseudochaetosphaeronema larense* (*Chaetosphaeronema larense*)	Black grain eumycetoma	78	Pycnidial	Hyaline, cylindrical, smooth- and thin-walled, aseptate, 2–3 × 1–1.8 µm	
	Pseudochaetosphaeronema martinelli	Subcutaneous	79			Culture sterile
Melanommataceae	*Pleurophomopsis lignicola*	Subcutaneous, sinusitus	80-81	Pycnidial	Hyaline, ellipsoidal, smooth- and thin-walled, aseptate, 2–3 × 0.5–1 µm	
Nigrograna ceae	*Nigrograna mackinnonii* (*Pyrenochaeta mackinnonii*)	Black grain eumycetoma	82	Pycnidial	Subhyaline, ellipsoidal, smooth, aseptate, 2.5–3 × 1.5–2 µm	

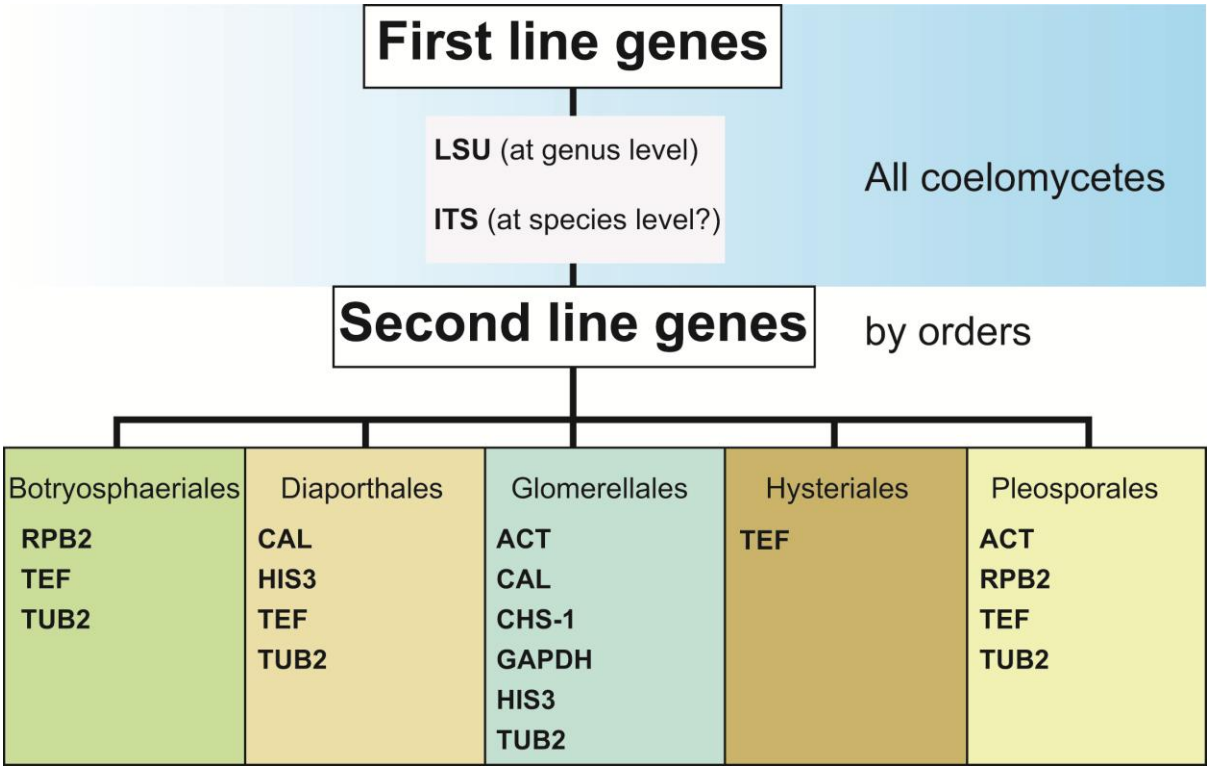
Phaeosphaeriaceae	*Tintelnotia destructans*	Onychomycosis	83	Pycnidial	Hyaline to pale brown, ellipsoidal, smooth- and thin-walled, aseptate, 2–3.2 × 1–2 µm	Growth at 37ºC[a]
Sporormiaceae	*Westerdykella minutispora* (*Phoma minutispora*)	Subcutaneous	84	Pycnidial	Hyaline, subglobose to ellipsoidal, smooth- and thin-walled, aseptate, 2–2.5 × 1.5–2 µm	Chlamydospores dark brown, subglobose or irregular, solitary mostly terminally on hyphae, 6–15 µm diam. Can produce sexual morph
Thyridariaceae	*Parathyridaria percutanea* (*Roussoella percutanea*)	Subcutaneous	85	Pycnidial	Hyaline to pale brown, ellipsoidal, smooth- and thin-walled, aseptate, 1.2–2 × 0.7–0.9 µm	Growth at 37ºC[a]
Trematosphaeriaceae	*Emarellia grisea*	Black grain eumycetoma	86			Culture sterile
	Emarellia paragrisea	Black grain eumycetoma	86			Culture sterile
	Trematosphaeria grisea (*Madurella grisea*)	Black grain eumycetoma	11,87-88	Pycnidial	Hyaline to pale brown, clavate to ellipsoidal, smooth- and thin-walled, aseptate, 4–5.4 × 2–2.4 µm	Growth at 37ºC[a]

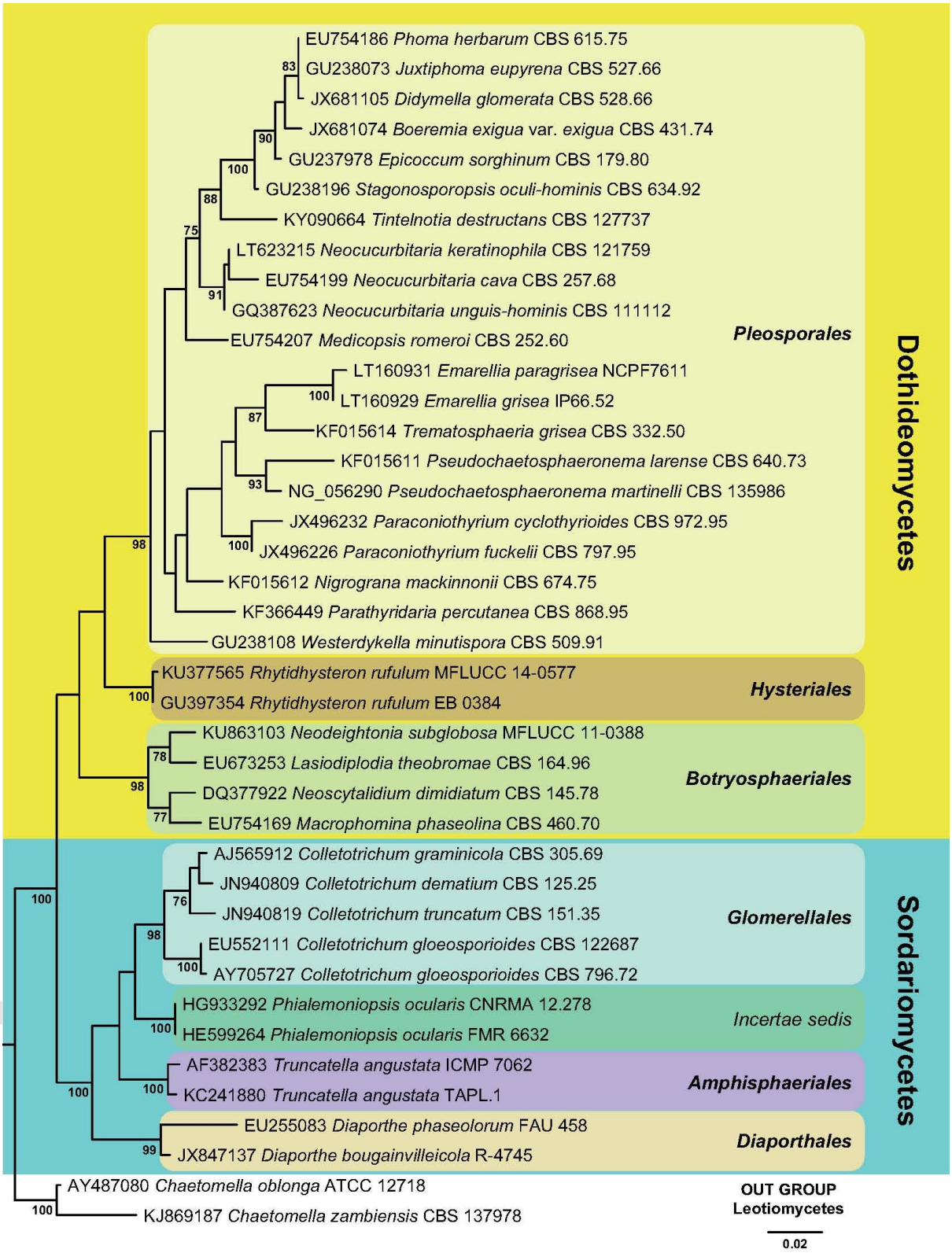
[a] species that was previously tested at different temperature conditions

Table 2 Primers used for coelomycetes identification

Gene	Product name	Primer	Direction	Sequence (5'–3')	Reference	Used in
Internal transcribed spacer (complete)	ITS	ITS-5	Forward	GGA AGT AAA AGT CGT AAC AAG G	White *et al.*[89]	All coelomycetes
		ITS-4	Reverse	TCC TCC GCT TAT TGA TAT GC	White *et al.*[89]	
28S ribosomal RNA	LSU	LR0R	Forward	GTA CCC GCT GAA CTT AAG C	Rehner & Samuels[90]	All coelomycetes
		LR5	Reverse	TCC TGA GGG AAA CTT CG	Vilgalys & Hester[91]	
Actin	ACT	ACT-512F	Forward	ATG TGC AAG GCC GGT TTC GC	Carbone & Kohn[92]	*Colletotrichum*, *Didymella*, *Phoma*
		ACT-783R	Reverse	TAC GAG TCC TTC TGG CCC AT	Carbone & Kohn[92]	
Beta-tubulin	TUB2	T1	Forward	AAC ATG CGT GAG ATT GTA AGT	O'Donnell & Cigelnik[93]	*Colletotrichum*, *Diaporthe*
		Bt-2b	Reverse	ACC CTC AGT GTA GTG ACC CTT GGC	Glass & Donaldson[94]	
		TUB2Fd	Forward	GTB CAC CTY CAR ACC GGY CAR TG	Woudenberg *et al.*[95]	*Didymellaceae*, Pleosporalean coelomycetes
		TUB4Rd	Reverse	CCR GAY TGR CCR AAR ACR AAG TTG TC	Woudenberg *et al.*[95]	
Calmodulin	CAL	CAL-228F	Forward	GAG TTC AAG GAG GCC TTC TCC C	Carbone & Kohn[92]	*Colletotrichum*, *Diaporthe*
		CAL-737R	Reverse	CAT CTT TCT GGC CAT CAT GG	Carbone & Kohn[92]	
Chitin synthase 1	CHS-1	CHS-79F	Forward	TGG GGC AAG GAT GCT TGG AAG AAG	Carbone & Kohn[92]	*Colletotrichum*

		CHS-354R	Reverse	TGG AAG AAC CAT CTG TGA GAG TTG	Carbone & Kohn[92]	
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	GDF1	Forward	GCC GTC AAC GAC CCC TTC ATT GA	Guerber *et al*.[96]	*Colletotrichum*
		GDR1	Reverse	GGG TGG AGT CGT ACT TGA GCA TGT	Guerber *et al*.[96]	
Histone H3	HIS3	CYLH3F	Forward	AGG TCC ACT GGT GGC AAG	Crous *et al*.[97]	*Colletotrichum*
		CYLH3R	Reverse	AGC TGG ATG TCC TTG GAC TG	Crous *et al*.[97]	
RNA polymerase II second largest subunit	RPB2	fRPB2-5F	Forward	GAY GAY MGW GAT CAY TTY GG	Liu *et al*.[98]	All coelomycetes
		fRPB2-7R	Reverse	CCC ATW GCY TGC TTM CCC AT	Liu *et al*.[98]	
Translation elongation factor 1-alpha	TEF	EF1-728F	Forward	CAT CGA GAA GTT CGA GAA GG	Carbone & Kohn[92]	*Diaporthe*
		EF1-986R	Reverse	TAC TTG AAG GAA CCC TTA CC	Carbone & Kohn[92]	
		TEF1-983F	Forward	GCY CCY GGH CAY CGT GAY TTY AT	Schoch *et al*.[99]	All coelomycetes
		TEF1-2218R	Reverse	AT GAC ACC RAC RGC RAC RGT YTG	Schoch *et al*.[99]	





Estudios de los aislados clínicos provenientes de dos laboratorios de referencia europeos

UNIVERSITAT ROVIRA I VIRGILI
TAXONOMÍA DE HONGOS CELOMICETOS DE INTERÉS CLÍNICO
Nicomedes Miguel Antonio Valenzuela López

4.6. Diversity of coelomycetes in human infections: a 10-year experience from two European Reference Centers.

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Enviado: Journal of Clinical Microbiology 2018.

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Diversity of coelomycetous fungi in human infections: a 10-year experience of two European reference centres.

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Running title: Coelomycetous fungi of clinical interest in Europe.

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No conflict of interest declared.

Word count: abstract = 129; text = 3184 (without ref).

ABSTRACT

The coelomycetous fungi are difficult to properly identify from their phenotypic characterization and their role as etiologic agents of human infections is not clear. We studied the species distribution of these fungi among clinical isolates that had been collected and stored over a ten-year period in two European reference laboratories (France and Spain). We identified phenotypically and molecularly 97 isolates by sequencing the D1-D2 fragment of the 28S nrRNA (LSU) gene. Species of the orders *Pleosporales* and *Glomerellales* were present in both collections, and *Botryosphaeriales* and *Diaporthales* only in the French one. The most prevalent species were *Medicopsis romeroi*, *Neocucurbitaria keratinophila*, *Neocucurbitaria unguis-hominis* and *Paraconiothyrium cyclothyrioides*, which had been recovered primarily from superficial tissues. The *Didymellaceae* was the most common family represented, with 27 isolates distributed into five genera.

Keywords: *Colletotrichum*, coelomycetes, coelomycetous fungi, *Didymella*, *Medicopsis*, mycosis, *Neocucurbitaria*, *Paraconiothyrium*, *Phoma*.

INTRODUCTION

Human infections by coelomycetous fungi are rare and poorly characterized due to the difficulty in identifying these fungi using only phenotypic tools. The coelomycetous fungi are characterized by the production of conidia into fruiting bodies (= conidiomata), and were originally included in the orders *Sphaeropsidales* and *Melanconiales* of the class Coelomycetes, taxa which today lack scientific validity due to the demonstrated polyphyletic character of this sort of fungus (1–3). They cause superficial or subcutaneous infections, mostly following a traumatic inoculation of contaminated plant material or soil particles during agricultural work in tropical and subtropical areas (4–6). The most common coelomycetous fungi involved in these infections are the etiologic agents of black-grain eumycetoma, such as *Biatriospora mackinnonii; Falciformispora* spp., *Medicopsis romeroi*, and *Pseudochaetosphaeronema larense*. Other common coelomycetous fungi include *Lasiodiplodia theobromae* and *Neoscytalidium dimidiatum* (synanamorph of *Hendersonula toruloidea*) (7–11), which typically cause onychomycosis, subcutaneous phaeohyphomycosis (12–15), and eumycetoma (16). In addition, many species of *Phoma* and *Pyrenochaeta* have been reported as occasional agents of localized and systemic infections in humans (9, 17–20). The taxonomy of several coelomycetous genera mentioned before have been revised recently but they still constitute a group of highly polyphyletic taxa that are usually difficult to identify phenotypically (2, 21–24).

In a recent study conducted in the USA, Valenzuela-Lopez *et al*. (6) identified 230 fungal strains by sequencing the D1-D2 domains of the 28S rRNA gene (LSU), from which 152 (66.1%) strains belonged to the order *Pleosporales,* the rest being distributed in several orders of the phylum Ascomycota. Most of these strains were recovered from superficial tissue. *Neoscytalidium dimidiatum, Paraconiothyrium*

cyclothyrioides and members of the family *Didymellaceae* were the most prevalent taxa. In addition, those authors demonstrated the usefulness of the LSU as a good molecular marker for a preliminary identification of coelomycetous fungi at genus level. In fact, such locus is easily amplified and many sequences are available in the GenBank database. However, the nucleotide sequences of more phylogenetically informative genes need analysing in order to identify the fungi at species level. Genes such as the RNA polymerase II subunit 2 (*rpb*2), translation elongation factor 1-alpha (*tef*1), beta-tubulin (*tub*2) and the ribosomal internal transcribed spacer region (ITS), combined in a multi-locus analysis, have all been recommended for this purpose (25)

Until now, the coelomycetous fungi involved in invasive fungal infections (IFIs) are poorly known in Europe, probably due to the infrequency of these fungi and the complexity of their identification in the absence of characteristic fruiting bodies when grown on culture media used in the clinical lab. In a recent French study, eighteen proven cases of cutaneous and subcutaneous primary infections by coelomycetous fungi were reported and analysed in patients from tropical and subtropical regions (26).

For a better knowledge of the diversity of coelomycetous fungi involved in human infections, we studied a large set of clinical isolates that had been identified in two mycology reference centres in France and Spain, and determined their *in vitro* antifungal susceptibility pattern.

RESULTS

Locations of infections

The majority of the isolates were recovered from superficial tissue, mainly skin (44%; 43/97), eyes (27%; 26/97), nails/hairs (18%; 17/97) and mouth/sinus (2%; 2/97). A few were recovered from deeper sites: bones (4%, 4/97), blood (2%, 2/97), cerebrospinal fluid (n=1), bone marrow (n=1) and lung (n=1) (Table 1 & 2).

Phylogenetic analyses

The maximum-likelihood (ML) phylogenetic analysis of the LSU sequences (approximately 584 pb) demonstrated that the 97 isolates were distributed into four orders, but scattered into fourteen clades (Fig. 1). Most of the isolates (81%; 78/97) belonged to the order *Pleosporales*, which were distributed into nine clades corresponding to 23 species of twelve genera, followed by those of the *Botryosphaeriales* (8%; 8/97), the *Diaporthales* (6%; 6/97) and the *Glomerellales* (5%; 5/97).

The most common species identified was *Medicopsis romeroi* (11%; 11/97), followed by *Paraconiothyrium cyclothyrioides*, *Neocucurbitaria keratinophila* and *N. unguis-hominis* (8% each; 8/97). These species were mostly isolated from cutaneous lesions (Table 2).

Clade 1 of the *Pleosporales* corresponded to the family *Didymellaceae*, which included 27 isolates distributed into five genera, morphologically characterized by their production of pycnidial conidiomata and hyaline, aseptate conidia. The five genera were *Didymella*, *Epicoccum*, *Neoascochyta*, *Phoma* and *Xenodidymella*. *Didymella* was represented by 13 isolates, six of them clustering with the type strain of *D. gardeniae* (CBS 626.68), and the other seven clustered with a reference strain of *D. glomerata*

(CBS 528.66). The genus *Epicoccum* grouped five of the isolates, three of them clustering with a reference strain of *E. sorghinum* (CBS 179.80) and the other two with the type strain of the type species of the genus, *E. nigrum* (CBS 173.73). The genus *Phoma* was represented by seven clinical isolates and a reference strain of *Phoma herbarum* (CBS 615.75). Two additional isolates included in this clade (CNRMA 16.76 and CNM-CM 6201) grouped with the type strains of *Xenodidymella saxea* (CBS 419.92) and *Neoascochyta desmazieri* (CBS 297.69), respectively.

Clade 2 had two species of *Preussia*: CNM-CM 7335 grouped with a reference strain of *P. typharum* (CBS 107.69), while CNM-CM 7343 represented an unknown species forming a sister clade with the type strain of *P. terricola* (CBS 317.65).

Clade 3 grouped three isolates of *Paraphoma*, one of them (CNM-CM 8075) clustered with the type strain of *P. fimeti* (CBS 170.70), and the remaining two (CNRMA 15.665 and CNRMA 9.467) representing unidentified phoma-like species.

Clade 4 had two sister clades of the genus *Tintelnotia*, which produced pycnidia and hyaline, aseptate conidia. The isolate CNM-CM 7430 was identified as *T. destructans*. However, the other two isolates (CNM-CM 7080 and CNM-CM 7981) did not cluster with any known species of the genus and might represent new species.

Clade 5 had 20 isolates of *Neocucurbitaria*. *Neocucurbitaria keratinophila* and *N. unguis-hominis* were the most common species, both with eight isolates each. *Neocucurbitaria cava*, with a single isolate (CNRMA 15.708), was also included in this clade. Three Spanish isolates, CNM-CM 6489, CNM-CM 7025 and CNM-CM 7132 were identified as *Neocucurbitaria* sp. due to being phylogenetically different from the other isolates and, again, might be a new species of the genus. *Neocucurbitaria* spp.

produces pycnidia, ornamented or not, with bristle-like setose structures, and hyaline, aseptate conidia.

Clade 6 had eleven isolates of *Medicopsis romeroi* (syn. *Pyrenochaeta romeroi)*, which produces pycnidia and hyaline, aseptate conidia.

Clade 7 is represented by a single isolate (CNRMA 11.1115), phylogenetically distinct from the known pleosporalean fungi, possibly representing a novel taxon.

Clades 8 and 9 belonged to the family *Didymosphaeriaceae*. Clade 8 included a single isolate (CNM-CM 6000) phylogenetically related to a reference strain of *Paraphaeosphaeria michotii* (MFLUCC 13-0349). Clade 9 grouped ten isolates, two related to a reference strain of *Paraconiothyrium fuckelii* (CBS 797.95) and eight with the type strain of *Paraconiothyrium cyclothyrioides* (CBS 972.95). Members of the *Didymosphaeriaceae* form pycnidia and pale brown, 0-1 septate conidia.

The order *Botryosphaeriales* are present in Clades 10 to 12. Clade 10 had only one isolate (CNRMA 12.597) which clustered with a reference strain of *Neofusicoccum luteum* (CBS 110299); Clade 11 also had a single isolate (CNRMA 6.1007) that clustered with the type strain of *Diplodia seriata* (CBS 112555), and Clade 12 grouped six isolates, five of them clustering with the type strain of *Lasiodiplodia theobromae*, and CNRMA 15.383 identified as *Lasiodiplodia* sp. These fungi produce stromatic conidiomata and aseptate, hyaline to brown, thick-walled conidia.

Clade 13 included the type strain of *Diaporthe sclerotioides* (CBS 296.67) and six isolates corresponding to unidentified species of the genus *Diaporthe* (*Diaporthales*), none of them able to be morphologically distinguished since they produce pycnidia and small hyaline conidia.

Clade 14, corresponding to the *Glomerellales*, was used as outgroup. Five isolates nested in the *Colletotrichum* clade, two clustering with reference strains of *C. gigasporum* (CBS 159.75) and *C. gloeosporioides* (CBS 122687), respectively; and the other three, could not be identified. All the isolates showed the typical morphology of *Colletotrichum,* i.e., acervuli, conidia variable in shape, flattened with thickened tip branches (appressoria).

Antifungal susceptibility testing

The minimum inhibitory concentration (MIC) was determined for 46 of the isolates included here (16 from Spain and 30 from France) (Table 3, Table S1). Globally, the geometric mean (GM) and MIC_{50} values of itraconazole and caspofungin were the highest (Table 3). The MIC of amphotericin B (0.06-1 mg/L) was generally low among the *Pleosporales* with the exception of one isolate of *M. romeroi* and one of *D. gardeniae*, with MICs of 8 and 32 mg/L, respectively. The azole MIC ranged between 0.03 and 1 mg/L for isolates belonging to the genera *Paraconiothyrium*, *Paraphoma*, *Tintelnotia* and *Neocucurbitaria*, with the exception of two isolates of *N. unguis-hominis,* which showed higher values (16 mg/L). The terbinafine MIC was low except for *Diaporthe* spp. and a few isolates of *Colletotrichum* spp. and *M. romeroi*.

DISCUSSION

The present study is the largest on this taxonomically complex group of fungi from clinical origin, with almost a hundred isolates morphologically and molecularly characterized from two southern European countries (France and Spain). Most of these coelomycetous fungi belonged to the order *Pleosporales* and were most commonly recovered from superficial infections. Similar results were observed in a previous work that focused on coelomycetous fungi collected at a North American reference centre (6). However, the diversity of the fungi identified in that study was higher, i.e. eleven orders were represented against four here.

In the present study, *Medicopsis romeroi* was the most frequently isolated species whereas the most common taxon in the American study was *Neoscytalidium dimidiatum*. Interestingly, while *M. romeroi* is usually reported as an etiologic agent of black grain eumycetoma (4, 11, 26–29), our isolates were mainly recovered from eye and non-mycetoma subcutaneous infections.

The second most frequently isolated species were *Paraconiothyrium cyclothyrioides*, *Neocucurbitaria unguis-hominis* and *N. keratinophila*. *Paraconiothyrium cyclothyrioides* is an emerging pathogen (6, 26, 30, 31) and was represented by eight isolates recovered from skin or superficial locations and mainly from tropical regions. *Neocucurbitaria unguis-hominis*, initially described as an agent of human onychomycosis (17), was equally distributed across both centres (n=8 isolates). Regarding *N. keratinophyla*, this species was reported for the first time from a corneal infection in Spain (18, 19). Interestingly, as well as being the first case reported for this species, all the isolates of *N. keratinophyla* were recovered in Spain from superficial tissue.

Other coelomycetous fungi we identified in the present work were *Didymella glomerata* and *Phoma herbarum*. Although *Phoma* spp. are commonly reported as a coelomycete involved in human infections (9, 20, 32–39), recent extensive changes in taxonomy and nomenclature have spread all but one of the species into different genera of the *Didymellaceae*, *Phoma herbarum* remaining as the unique species of the genus (22–24). Interestingly, *Didymella gardeniae* was commonly found in our study (five isolates from Spain and one from France).

Recently, Ahmed *et al*. (40) proposed *Tintelnotia destructans*, a new phoma-like fungus belonging to the *Phaeosphaeriaceae* able to cause eye and nail infections. They reported the successful use of terbinafine against a case of keratitis by this species. Two of the Spanish isolates recovered from superficial specimens (one cutaneous exudate and one nail sample) were molecularly related to the above-mentioned species but phylogenetically different and might represent a new taxon.

Lasiodiplodia theobromae (order *Botryosphaeriales*) is the only species of this genus involved in human opportunistic infections (41–46). Valenzuela-Lopez *et al*. (6) found a higher species diversity in the North American study than we report here, since five of the French isolates were identified as *L. theobromae*. The other three isolates of the *Botryosphaeriales* we found were related, one to a different species of *Lasiodiplodia* and the other two to other genera, specifically *Neofusicoccum* and *Diplodia*.

Four species of the genus *Diaporthe* (formerly *Phomopsis*; order *Diaporthales*), i.e. *D. bougainvilleicola*, *D. longicolla*, *D. phaseolorum* and *D. phoenicicola*, are considered opportunistic pathogens that cause mycoses that range from superficial to deep infections (47–51). Six isolates from France were phylogenetically placed into the latter genus. However, our results are only preliminary since only one phylogenetic

marker was analysed. Similar was observed in several polyphyletic genera of the coelomycetes (52, 53).

We also report the finding of five clinical isolates of *Colletotrichum*. Two of the isolates corresponded to *C. gigasporum* (formerly *C. crassipes*) and *C. gloeosporioides*, taxa that have previously been reported as agents of keratitis, endophthalmitis and phaeohyphomicotic cyst; the other three isolates could not be identified at species level. This genus encompasses numerous plant pathogens that are found worldwide, although mainly in tropical and subtropical regions (54). The taxonomy of *Colletotrichum* is complicated and the genus is organized in species-complexes (55–59). Species such as *C. coccodes*, *C. crassipes*, *C. dematium*, *C. gloeosporioides*, *C. graminicola* and *C. truncatum* cause superficial and deep infections (endophthalmitis, keratitis, subcutaneous cyst or more rarely arthritis) (60–65). Further studies, including different phylogenetic markers, are needed to delimit the different species and clarify their pathogenic role.

The antifungal susceptibility of coelomycetous fungi involved in human infections is poorly known, mainly because they do not easily sporulate. In spite of the limited number of isolates tested here, amphotericin B seemed the most active drug *in vitro* together with terbinafine, in agreement with Valenzuela-Lopez *et al*. (6). Until more *in vitro* data is available, the antifungal treatment of the infection by this sort of fungus remains purely empirical. In a recent study, Guégan *et al*. (26) recommended extensive surgical resection of affected tissues as a first-line treatment for solitary subcutaneous lesions by coelomycetous fungi, followed by an antifungal therapy (posaconazole or voriconazole) in the case of relapse or amphotericin B in refractory cases.

Since our study is based on isolates from the two reference centres, we cannot comment on the incidence of infections due to coelomycetes nor compare their epidemiology between France and Spain. However, we still provide a good picture of the great diversity of coelomycetous fungi in the clinical context, and the basis for future studies on this interesting but neglected group of fungi.

MATERIAL AND METHODS

Fungal isolates

We studied 97 isolates of coelomycetous fungi recovered from clinical specimens, 51 of which were provided by the French National Reference Centre for Invasive Mycoses and Antifungals (NRCMA) at the *Institut Pasteur*, Paris (CNRMA isolates, n=51). The NRCMA offers expertise on difficult-to-identify fungi and the epidemiological surveillance of all cases of IFIs, which are notified on a voluntary basis either through active or passive surveillance programmes. The Spanish National Centre of Microbiology at the *Instituto de Salud Carlos III*, Madrid provided 46 isolates (CNM-CM isolates, n=46). This mycology reference laboratory receives isolates from the National Health System on a voluntary basis, the main aim of which is to support it by identifying and profiling the antifungal susceptibility of fungal isolates. The isolates were collected between 2005 and 2015. Table 1 gives information about the country of isolation and the location of the infection in the body.

Morphological and physiological characterization

For morphology studies, the isolates were cultured on oatmeal agar (OA; 30 g of filtered oat flakes, 15 g of agar-agar, 1 L tap water) and malt extract agar (MEA; 40 g of malt extract, 15 g of agar-agar, 1 L distilled water) at 20 ± 1ºC for 14 days in darkness.

The morphological features of the vegetative and reproductive structures were studied using an Olympus CH2 bright-field microscope (Olympus Corporation, Tokyo, Japan) in wet mounts (on water and lactic acid) and slide cultures (by growing the isolates on OA and MEA) of the fungal isolates, following Valenzuela-Lopez *et al*. (6). Colour standards by Kornerup & Wanscher (66) were used in colony description. Photomicrographs were taken with an Axio-Imager M1 microscope (Zeiss, Oberkochen, Germany).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from colonies grown on potato dextrose agar (PDA; 4 g of potato infusion, 20 g dextrose, 15 g of agar-agar, 1 L tap water) after seven days of incubation at 20 ± 1ºC, using the FastDNA kit protocol (Bio101, Vista, CA), with a FastPrep FP120 instrument (Thermo Savant, Holbrook, NY) following the manufacturer's protocol. DNA was quantified using the Nanodrop 2000 (Thermo Scientific, Madrid, Spain). LSU was amplified with the primer pair LR0R and LR5 (67). The amplicons were sequenced 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 made using LSU nucleotide sequences in $BLAST_N$ searches. Twenty-eight LSU sequences of type or reference strains deposited in the GenBank database by the Westerdijk Fungal Biodiversity Institute (CBS) and the Mae Fah Luang University (MFLUCC) culture collections were used for identification and phylogenetic purposes. DNA sequences

294 generated in this study were deposited in GenBank (accession numbers are given in
295 Table 1).

296 For the phylogenetic study, sequences were aligned using the ClustalW
297 application (68) of the MEGA 6.06 (69) computer program, and manually adjusted
298 using the same software platform. Phylogenetic reconstructions were made by
299 maximum-likelihood (ML) and Bayesian inference (BI) with MEGA 6.06 and MrBayes
300 3.2.4 (70), respectively. The best substitution model for the gene matrix (TN93+G) was
301 estimated using MEGA 6.06. For ML analyses, nearest-neighbour interchange was used
302 as the heuristic method for tree inference. Support for internal branches was assessed by
303 1,000 ML bootstrapped pseudoreplicates. Bootstrap support (BS) of ≥70 was considered
304 significant. For BI analyses, Markov chain Monte Carlo (MCMC) sampling was carried
305 out with four million generations, with samples taken every 1,000 generations. The 50%
306 majority rule consensus trees and posterior probability values (PP) were calculated after
307 removing the first 25% of the resulting trees for burn-in. A PP value of ≥0.95 was
308 considered significant. Reference strains of *Colletotrichum gigasporum* (CBS 159.75),
309 *C. gloeosporioides* (CBS 122687) and *C. hippeastri* (CBS 241.78) were used as
310 outgroup.

311 **Antifungal susceptibility testing**

312 The *in vitro* susceptibility testing in both reference centres (n= 46 isolates)
313 followed the European Committee on Antimicrobial Susceptibility Testing (EUCAST)
314 procedure (71, 72). The antifungals used were amphotericin B (Sigma-Aldrich Química,
315 Madrid, Spain), itraconazole (Sigma-Aldrich Química, Madrid, Spain), posaconazole
316 (Schering-Plough Research Institute, Kenilworth, N.J.), voriconazole (Pfizer S.A.,
317 Madrid, Spain), caspofungin (Merck & Co., Inc., Rahway, N.J.), micafungin (Astellas

Pharma Inc, Tokyo, Japan) and terbinafine (Novartis, Basel, Switzerland). For the NCRMA, all antifungal drugs were obtained from ALSACHIM, Strasbourg, France.

The isolates were cultured on potato carrot agar (PCA; 20 g each of filtered potatoes and carrots, 20 g of agar, 1 L of distilled water) or OA for seven to 30 days at 25°C and 30°C to obtain sporulation. Conidia were then collected in sterile water containing 0.01% (v/v) Tween 80 (Sigma-Aldrich, St. Louis, MO, USA), and the suspension was adjusted to 2–5 x 10^5 conidia/mL. The minimal effective concentration (MEC) was determined for each echinocandin and the minimal inhibitory concentration (MIC) for the other drugs (90% inhibition for amphotericin B and 80% for the azoles) after 24 h and 48 h of incubation at 35°C. *Aspergillus flavus* ATCC 204304 and *Aspergillus fumigatus* ATCC 204305 were used as quality control strains in all tests carried out. Susceptibility profiles were determined for 46 isolates since non-sporulating isolates were excluded at the NRCMA.

ACKNOWLEDGMENTS

We thank Cécile Gautier (National Reference Center for invasive Mycoses and Antifungals [NRCMA]) for technical assistance. This work was supported by the Spanish *Ministerio de Economía y Competitividad*, grant CGL2017-88094-P.

Olga Rivero-Menendez holds a fellowship from the *Fondo de Investigaciones Sanitarias* (grant FI14CIII/00025). Ana Alastruey-Izquierdo is supported by a research project from the *Fondo de Investigación Sanitaria* (FIS) (project PI16CIII/00035).

Members of the French Mycoses Study Group who contributed their clinical isolates to this study are as follows: Annecy (S. Bland), Bichat (C. Chochillon), Boulogne-Billancourt (N.Ait-Ammar), Caen (C. Duhamel), Clermont Ferrand (P.Poirier), Cochin (A. Paugam), Corbeil-Essones (D. Kubab), Guadeloupe (M. Nicolas), La Réunion (S. Picot), Lyon (A.L. Bienvenu), Martinique (N. Desbois), Nantes (F. Morio), Necker (M.E. Bougnoux), Nouvelle Calédonie (R. Goursaud), Pitié (A. Fekkar), Poitiers (C. Kauffmann), Quinze-Vingts (L. Merabet), Rouen (L. Favennec), Saint Etienne (H. Raberin), Saint-Louis (A. Alanio), Toulouse (S. Cassaing).

REFERENCES

1. Sutton BC. 1980. The Coelomycetes. Fungi Imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological, Kew, England.

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

3. Wijayawardene NN, Hyde KD, Wanasinghe DN, Papizadeh M, Goonasekara ID, Camporesi E, Bhat DJ, McKenzie EHC, Phillips AJL, Diederich P, Tanaka K, Li WJ, Tangthirasunun N, Phookamsak R, Dai D-Q, Dissanayake AJ, Weerakoon G, Maharachchikumbura SSN, Hashimoto A, Matsumura M, Bahkali AH, Wang Y. 2016. Taxonomy and phylogeny of dematiaceous coelomycetes. Fungal Divers 77: 1–316.

4. Sutton DA. 1999. Coelomycetous fungi in human disease. A review: Clinical entities, pathogenesis, identification and therapy. Rev Iberoam Micol 16:171–179.

5. Stchigel AM, Sutton DA. 2013. Coelomycete fungi in the clinical lab. Curr Fungal Infect Rep 7:171–191.

6. Valenzuela-Lopez N, Sutton DA, Cano-Lira JF, Paredes K, Wiederhold N, Guarro J, Stchigel AM. 2017. Coelomycetous fungi in the clinical setting: Morphological convergence and cryptic diversity. J Clin Microbiol 55:552–567.

7. André M, Brumpt V, Destombes P, Segretain G. 1968. Fungal mycetoma with black grains due to *Pyrenochaeta romeroi* in Cambodia. Bull Soc Pathol Exot Filiales 61:108–12.

8. Borelli D, Zamora R, Senabre G. 1976. *Chaetosphaeronema larense* nova species agente de micetoma. *Gaceta Med Caracas* 84:307–318.

9. Punithalingam E. 1979. *Sphaeropsidales* in culture from humans. Nova Hedwigia 31:119–158.

10. de Hoog GS, Guarro J, Figueras MJ, Gené J. 2000. Atlas of clinical fungi, 2nd ed Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

11. Ahmed SA, van de Sande WW, Stevens DA, Fahal A, van Diepeningen AD, Menken SB, de Hoog GS. 2014. Revision of agents of black-grain eumycetoma in the order Pleosporales. Persoonia 33:141–154.

12. Gentles JC, Evans EG. 1970. Infection of the feet and nails with *Hendersonula toruloidea*. Sabouraudia 8:72–75.

13. Gugnani HC, Oyeka CA. 1989. Foot infections due to *Hendersonula toruloidea* and *Scytalidium hyalinum* in coal miners. J Med Vet Mycol 27:167–179.

14. Kombila M, Martz M, Gómez de Díaz M, de Bievre C, Richard-Lenoble D. 1990. *Hendersonula toruloidea* and an agent of mycotic foot infection in Gabon. J Med Vet Mycol 28:215–223.

15. Madrid H, Ruíz-Cendoya M, Cano J, Stchigel A, Orofino R, Guarro J. 2009. Genotyping and in vitro antifungal susceptibility of *Neoscytalidium dimidiatum* isolates from different origins. Int J Antimicrob Agents 34:351–354.

16. Restrepo A, Arango M, Velez H, Uribe L. 1976. The isolation of *Botryodiplodia theobromae* from a nail lesion. Sabouraudia 14:1–4.

17. Punithalingam E, English MP. 1975. *Pyrenochaeta unguis-hominis* sp. nov. on human toe-nails. Trans Br Mycol Soc 64:539–541.

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

19. Verkley GJ, Gené J, Guarro J, Pérez-Santonja JJ, Rodríguez AE, Colom MF, Alio JL, Ferrer C. 2010. *Pyrenochaeta keratinophila* sp. nov., isolated from an ocular infection in Spain. Rev Iberoam Micol 27:22–24.

20. Roehm CE, Salazar JC, Hagstrom N, Valdez TA. 2012. *Phoma* and *Acremonium* invasive fungal rhinosinusitis in congenital acute lymphocytic leukemia and literature review. Int J Pediatr Otorhinolaryngol 76:1387–1391.

21. de Gruyter J, Woudenberg JH, Aveskamp MM, Verkley GJ, Groenewald JZ, Crous PW. 2013. Redisposition of Phoma-like anamorphs in Pleosporales. Stud Mycol 75:1–36.

22. Chen Q, Jiang JR, Zhang GZ, Cai L, Crous PW. 2015. Resolving the *Phoma* enigma. Stud Mycol 82:137–217.

23. Chen Q, Hou LW, Duan WJ, Crous PW, Cai L. 2017. *Didymellaceae* revisited. Stud Mycol 87:105–159.

24. Valenzuela-Lopez N, Cano-Lira JF, Guarro J, Sutton DA, Wiederhold N, Crous PW, Stchigel AM. 2018. Coelomycetous Dothideomycetes with emphasis on the families *Cucurbitariaceae* and *Didymellaceae*. Stud Mycol 90:1–69.

25. Valenzuela-Lopez N, Cano-Lira JF, Stchigel AM, Guarro J. 2018. DNA sequencing to clarify the taxonomical conundrum of the clinical coelomycetes. Mycoses. (IN PRESS) DOI:10.1111/myc.12785.

26. Guégan S, Garcia-Hermoso D, Sitbon K, Ahmed S, Moguelet P, Dromer F, Lortholary O. 2016. Ten-Year experience of cutaneous and/or subcutaneous infections due to Coelomycetes in France. Open Forum Infect Dis doi:10.1093/ofid/ofw106.

27. Khan Z, Ahmad S, Kapila K, Ramaswamy NV, Alath P, Joseph L, Chandy R. 2011. *Pyrenochaeta romeroi*: a causative agent of phaeohyphomycotic cyst. J Med Microbiol 60:842–846.

28. van de Sande WW. 2013. Global burden of human mycetoma: a systematic review and meta-analysis. PLoS Negl Trop Dis 7: e2550. doi: 10.1371/journal.pntd.0002550.

29. Borman AM, Desnos-Ollivier M, Campbell CK, Bridge PD, Dannaoui E, Johnson EM. 2016. Novel taxa associated with human fungal black-grain mycetomas: *Emarellia grisea* gen. nov., sp. nov., and *Emarellia paragrisea* sp. nov. J Clin Microbiol 54:1738–1745.

30. Gordon RA, Sutton DA, Thompson EH, Shrikanth V, Verkley GJM, Stielow JB, Mays R, Oleske D, Morrison LK, Lapolla WJ, Galfione S, Tyring S, Samathanam CA, Fu J, Wickes BL, Mulanovich V, Wanger A, Arias CA. 2012. Cutaneous phaeohyphomycosis caused by *Paraconiothyrium cyclothyrioides*. J Clin Microbiol 50:3795–3798.

31. Colombier MA, Alanio A, Denis B, Melica G, Garcia-Hermoso D, Levy B, Peraldi MN, Glotz D, Bretagne S, Gallien S. 2015. Dual invasive infection with *Phaeoacremonium parasiticum* and *Paraconiothyrium cyclothyrioides* in a renal transplant recipient: Case report and comprehensive review of the literature of *Phaeoacremonium* phaeohyphomycosis. J Clin Microbiol 53:2084–2094.

32. Bakerspigel A. 1970. The isolation of *Phoma hibernica* from lesions on a leg. Sabouraudia 7:261–264.

33. Bakerspigel A, Lowe D, Rostas A. 1981. The isolation of *Phoma eupyrena* from a human lesion. Arch Dermatol 117:362–363.

34. Shukla NP, Rajak RK, Agarwasl GP, Gupta D. 1984. *Phoma minutispora* as a human pathogen. Mykosen 27:255–258.

448 35. Baker JG, Salkin IF, Forgacs P, Haines JH, Kemna ME. 1987. First report of
449 subcutaneous phaeohyphomycosis of the foot caused by *Phoma minutella*. J Clin
450 Microbiol 25:2395–2397.
451 36. Rai M. 1989. *Phoma sorghina* infection in human being. Mycopathologia
452 105:167–170.
453 37. Rosen T, Rinaldi MJ, Tschen JA, Stern JK, Cernoch P. 1996. Cutaneous lesions
454 due to *Pleurophoma* (*Phoma*) Complex. South Med J 89:431–434.
455 38. Hirsh AH, Schiff TA. 1996. Subcutaneous phaeohyphomycosis caused by an
456 unusual pathogen: *Phoma* species. J Am Acad Dermatol 34:679–680.
457 39. Tullio V, Banche G, Allizond V, Roana J, Mandras N, Scalas D, Panzone M,
458 Cervetti O, Valle S, Carlone N, Cuffini AM. 2010. Non-dermatophyte moulds as
459 skin and nail foot mycosis agents: *Phoma herbarum*, *Chaetomium globosum* and
460 *Microascus cinereus*. Fungal Biol 114:345–349.
461 40. Ahmed SA, Hofmüller W, Seibold M, de Hoog GS, Harak H, Tammer I, van
462 Diepeningen AD, Behrens-Baumann W. 2017. *Tintelnotia*, a new genus in
463 *Phaeosphaeriaceae* harbouring agents of cornea and nail infections in humans.
464 Mycoses 60:244–253.
465 41. Summerbell RC, Krajden S, Levine R, Fuksa M. 2004. Subcutaneous
466 phaeohyphomycosis caused by *Lasiodiplodia theobromae* and successfully treated
467 surgically. Med Mycol 42:543–547.
468 42. Woo PC, Lau SK, Ngan AH, Tse H, Tung ET, Yuen KY. 2008. *Lasiodiplodia*
469 *theobromae* pneumonia in a liver transplant recipient. J Clin Microbiol 46:380–
470 384.
471 43. Kindo AJ, Pramod C, Anita S, Mohanty S. 2010. Maxillary sinusitis caused by
472 *Lasiodiplodia theobromae*. Indian J Med Microbiol 28:167–169.

44. Saha S, Sengupta J, Banerjee D, Khetan A. 2012. *Lasiodiplodia theobromae* keratitis: a case report and review of literature. Mycopathologia 174:335–339.

45. Papacostas LJ, Henderson A, Choong K, Sowden D. 2015. An unusual skin lesion caused by *Lasiodiplodia theobromae*. Med Mycol Case Rep 8:44–46.

46. Gu HJ, Kim YJ, Lee HJ, Dong SH, Kim SW, Huh HJ, Ki CS. 2016. Invasive fungal sinusitis by *Lasiodiplodia theobromae* in an patient with aplastic anemia: An extremely rare case report and literature review. Mycopathologia 181:901–908.

47. Sutton DA, Timm WD, Morgan-Jones G, Rinaldi MG. 1999. Human phaeohyphomycotic osteomyelitis caused by the Coelomycete *Phomopsis* Saccardo 1905: Criteria for identification, case history, and therapy. J Clin Microbiol 37:807–811.

48. Gajjar DU, Pal AK, Parmar TJ, Arora AI, Ganatra DA, Kayastha FB, Ghodadra BK, Vasavada AR. 2011. Fungal scleral keratitis caused by *Phomopsis phoenicicola*. J Clin Microbiol 49:2365–2368.

49. Garcia-Reyne A, López-Medrano F, Morales JM, García Esteban C, Martín I, Eraña I, Meije Y, Lalueza A, Alastruey-Izquierdo A, Rodríguez-Tudela JL, Aguado JM. 2011. Cutaneous infection by *Phomopsis longicolla* in a renal transplant recipient from Guinea: first report of human infection by this fungus. Transpl Infect Dis 13:204–207.

50. Iriart X, Binois R, Fior A, Blanchet D, Berry A, Cassaing S, Amazan E, Papot E, Carme B, Aznar C, Couppié P. 2011. Eumycetoma caused by *Diaporthe phaseolorum* (*Phomopsis phaseoli*): a case report and a mini-review of *Diaporthe*/*Phomopsis* spp invasive infections in humans. Clin Microbiol Infect 17:1492–1494.

498 51. Cariello PF, Wickes BL, Sutton DA, Castlebury LA, Levitz SM, Finberg RW,
499 Thompson EH, Daly JS. 2013. *Phomopsis bougainvilleicola* prepatellar bursitis in
500 a renal transplant recipient. J Clin Microbiol 51:692–695.
501 52. Udayanga D, Liu X, Crous PW, McKenzie EHC, Chukeatirote E, Hyde KD.
502 2012. A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). Fungal
503 Divers 56:157–171.
504 53. Gomes RR, Glienke C, Videira SI, Lombard L, Groenewald JZ, Crous PW. 2013.
505 *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia
506 31:1–41.
507 54. Cannon PF, Damm U, Johnston PR, Weir BS. 2012. *Colletotrichum* - current
508 status and future directions. Stud Mycol 73:181–213.
509 55. Damm U, Cannon PF, Woudenberg JH, Johnston PR, Weir BS, Tan YP, Shivas
510 RG, Crous PW. 2012. The *Colletotrichum boninense* species complex. Stud
511 Mycol 73:1–36.
512 56. Damm U, Cannon PF, Woudenberg JH, Crous PW. 2012. The *Colletotrichum*
513 *acutatum* species complex. Stud Mycol 73:37–113.
514 57. Weir BS, Johnston PR, Damm U. 2012. The *Colletotrichum gloeosporioides*
515 species complex. Stud Mycol 73:115–180.
516 58. Damm U, O'Connell RJ, Groenewald JZ, Crous PW. 2014. The *Colletotrichum*
517 *destructivum* species complex - hemibiotrophic pathogens of forage and field
518 crops. Stud Mycol 79:49–84.
519 59. Liu F, Cai L, Crous PW, Damm U. 2014. The *Colletotrichum gigasporum* species
520 complex. Persoonia 33:83–97.

60. Guarro J, Svidzinski TE, Zaror L, Forjaz MH, Gené J, Fischman O. 1998. Subcutaneous hyalohyphomycosis caused by *Colletotrichum gloeosporioides*. J Clin Microbiol 36:3060–3065.

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

62. Yegneswaran PP, Pai V, Bairy I, Bhandary S. 2010. *Colletotrichum graminicola* keratitis: first case report from India. Indian J Ophthalmol 58:415–417.

63. Shivaprakash MR, Appannanavar SB, Dhaliwal M, Gupta A, Gupta S, Gupta A, Chakrabarti A. 2011. *Colletotrichum truncatum*: An unusual pathogen causing mycotic keratitis and endophthalmitis. J Clin Microbiol 49:2894–2898.

64. Figtree M, Weeks K, Chan L, Leyton A, Bowes A, Giuffre B, Sullivan M, Hudson BJ. 2013. *Colletotrichum gloeosporioides* sensu lato causing deep soft tissue mycosis following a penetrating injury. Med Mycol Case Rep 2:40–43.

65. Cho JC, Sharma RS, Sutton DA, Wiederhold NP, Sanders C, Wickes BL, Estrada SJ. 2015. Fungal arthritis secondary to *Colletotrichum gloeosporioides*. JMM Case Rep 2 doi: 10.1099/jmmcr.0.000012.

66. Kornerup A, Wanscher JH. 1978. Methuen handbook of colour 3rd ed. Methuen, London, England.

67. Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptoccocus* species. J Bacteriol 172:4238–4246.

68. Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680.

546 69. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6:
547 Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–
548 2729.
549 70. Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of
550 phylogenetic trees. Bioinformatics 17:754–755.
551 71. Subcommittee on Antifungal Susceptibility Testing of the ESCMID European
552 Committee for Antimicrobial Susceptibility Testing (EUCAST). 2008. EUCAST
553 Technical Note on the method for the determination of broth dilution minimum
554 inhibitory concentrations of antifungal agents for conidia-forming moulds. Clin
555 Microbiol Infect 14:982–984.
556 72. Garcia-Hermoso D, Hoinard D, Gantier JC, Grenouillet F, Dromer F, Dannaoui E.
557 2009. Molecular and phenotypic evaluation of *Lichtheimia corymbifera* (formerly
558 *Absidia corymbifera*) complex isolates associated with human mucormycosis:
559 rehabilitation of *L. ramosa*. J Clin Microbiol 47:3862–3870.

560

561 **FIGURE LEGEND**

562 **FIG 1** Maximum likelihood tree obtained from the D1-D2 of LSU (584 bp) sequences
563 of the 125 strains, where 28 belong to type or reference strains. The branch lengths are
564 proportional to phylogenetic distance. Bayesian posterior probability scores ≥ 0.95 and
565 Bootstrap support values ≥ 70% are indicated on the nodes. Some branches were
566 shortened to fit them to the page, these are indicated by two diagonal lines with the
567 number of times a branch was shortened. The species of the genus *Colletotrichum* were
568 used to root the tree. Superscript ^{T} indicated the type strains.

569

570

TABLE 1 Taxonomical identification of the isolates studied, origin and GenBank accession numbers. New sequences generated are indicate in bold.

Order	Species	Strain no. [a]	Origin	Country	GenBank accesion no. [b]
Botryosphaeriales	***Diplodia seriata***	CBS 112555^{T}	***Vitis vinifera*** dead plant	Portugal	KF766327
		CNRMA 6.1007	bone	France	**LT965964**
	Lasiodiplodia sp.	CNRMA 15.383	eye	France (West Indies, Guadeloupe)	**LT965965**
	Lasiodiplodia theobromae	CBS 164.96^{T}	fruit along coral reef coast	Papua New Guinea	NG_042460
		CNRMA 10.1369	skin	France (West Indies, Martinique)	**LT965966**
		CNRMA 10.813	eye	France (West Indies, Martinique)	**LT965967**
		CNRMA 11.360	eye	France (West Indies, Martinique)	**LT965968**
		CNRMA 13.891	skin	France	**LT965969**
		CNRMA 14.708	eye	France (West Indies, Guadeloupe)	**LT965970**
	Neofusicoccum luteum	CBS 110299	***Vitis vinifera*** cane	Portugal	AY928043
		CNRMA 12.597	eye	France	**LT965971**
Diaporthales	***Diaporthe sclerotioides***	CBS 296.67^{T}	***Cucumis sativus*** root	The Netherlands	AF439628
	Diaporthe sp.	CBS 477	***Cucumis sativus***	USA	AF439631
		CNRMA 8.522	eye	France	**LT965972**
		CNRMA 9.205	eye	France (West Indies, Guadeloupe)	**LT965973**
		CNRMA 11.385	eye	France (West Indies, Martinique)	**LT965974**
		CNRMA 12.311	blood	France	**LT965975**
		CNRMA 13.515	skin	France	**LT965976**
		CNRMA 14.198	skin	France	**LT965977**

Glomerellales	***Colletotrichum gigasporum***	CBS 159.75	air and stored grains	India	DQ286206
		CNRMA 16.553	skin	France (West Indies, Guadeloupe)	**LT965978**
	Colletotrichum gloeosporioides	CBS 122687	***Leucospermum*** sp. leaf litter	South Africa	EU552111
		CNRMA 15.504	eye	France (West Indies, Martinique)	**LT965979**
	Colletotrichum hippeastri	CBS 241.78	***Hippeastrum*** sp.	The Netherlands	DQ286167
	Colletotrichum sp.	CNM-CM4760	corneal swab	Spain	**LT965980**
		CNM-CM 6116	conjuntival	Spain	**LT965981**
		CNM-CM 7345	humor acuosus	Spain	**LT965982**
Pleosporales	***Didymella gardeniae***	CBS 626.68^{T}	***Gardenia jasminoides*** leaf	India	GQ387595
		CNM-CM 3697	nail	Spain	**LT965983**
		CNM-CM 3895	nail	Spain	**LT965984**
		CNM-CM 5036	scales	Spain	**LT965985**
		CNM-CM 5814	conjunctival exudate	Spain	**LT965986**
		CNM-CM 7499	conjunctival exudate	Spain	**LT965987**
		CNRMA 11.794	skin	France	**LT965988**
	Didymella glomerata	CBS 528.66	***Chrysanthemum*** sp. cutting	The Netherlands	EU754184
		CNM-CM 3356	toenail	Spain	**LT965989**
		CNM-CM 3546	nail	Spain	**LT965990**
		CNM-CM 4675	nail	Spain	**LT965991**
		CNM-CM 7099	cutaneous exudate	Spain	**LT965992**
		CNRMA 9.1046	skin	France	**LT965993**

	CNRMA 10.867	skin	France	**LT965994**
	CNRMA 15.6	mouth/sinus	France	**LT965995**
Epicoccum nigrum	CBS 173.73^{T}	***Dactylis glomerata*** seed	USA	GU237975
	CNM-CM 5281	skin	Spain	**LT965996**
	CNM-CM 5724	vitreous humor	Spain	**LT965997**
Epicoccum sorghinum	CBS 179.80	***Sorghum vulgare***	Puerto Rico	GU237978
	CNRMA 7.167	bone	France (New Caledonia)	**LT965998**
	CNRMA 10.947	skin	France (New Caledonia)	**LT965999**
	CNRMA 10.948	skin	France (New Caledonia)	**LT966000**
Medicopsis romeroi	CBS 252.60^{T}	maduromycosis	Venezuela	EU754207
	CNM-CM 3387	knee ulcer	Spain	**LT966001**
	CNM-CM 7645	cutaneous exudate	Spain	**LT966002**
	CNRMA 4.200	eye	France	**LT966003**
	CNRMA 5.321	skin	France	**LT966005**
	CNRMA 7.1225	skin	France	**LT966007**
	CNRMA 8.1363	skin	France	**LT966008**
	CNRMA 11.680	skin	France	**LT966010**
	CNRMA 11.949	bone	France	**LT966011**
	CNRMA 14.407	skin	France	**LT966013**
	CNRMA 15.461	bone	France	**LT966014**
	CNRMA 15.7	skin	France	**LT966015**
Neoascochyta desmazieri	CBS 297.69^{T}	***Lolium perenne***	Germany	KT389726
	CNM-CM 6201	nail	Spain	**LT966016**

Neocucurbitaria cava	CBS 257.68^{T}	wheat-field soil	Germany	EU754199
	CNRMA 15.708	mouth/sinus	France	**LT966017**
Neocucurbitaria keratinophila	CBS 121759^{T}	corneal scrapings	Spain	LT623215
	CNM-CM 5882	cutaneous exudate	Spain	**LT966018**
	CNM-CM 6401	fingernail	Spain	**LT966019**
	CNM-CM 6455	cutaneous exudate	Spain	**LT966020**
	CNM-CM 7013	cutaneous exudate	Spain	**LT966021**
	CNM-CM 7457	cutaneous exudate	Spain	**LT966022**
	CNM-CM 7731	cutaneous exudate	Spain	**LT966023**
	CNM-CM 8010	conjunctival exudate	Spain	**LT966024**
	CNM-CM 8674	toenail	Spain	**LT966025**
Neocucurbitaria unguis-hominis	CBS 112.79	airborn	Wales	GQ387622
	CNM-CM 7037	nail	Spain	**LT966026**
	CNM-CM 7089	cutaneous lession	Spain	**LT966027**
	CNM-CM 8717	urine	Spain	**LT966028**
	CNM-CM 8743	toenail	Spain	**LT966029**
	CNRMA 4.1112	eye	France	**LT966030**
	CNRMA 6.243	eye	France	**LT966031**
	CNRMA 16.153	eye	France	**LT966032**
	CNRMA 16.19	lung	France	**LT966033**
Neocucurbitaria sp.	CNM-CM 6489	wound exudate	Spain	**LT966034**
	CNM-CM 7025	hair	Spain	**LT966035**
	CNM-CM 7132	toenail	Spain	**LT966036**

Paraconiothyrium cyclothyrioides	CBS 972.95^{T}	soil	Papua New Guinea	JX496232
	CNM-CM 6313	conjunctival exudate	Spain	**LT966037**
	CNM-CM 6513	nail	Spain	**LT966038**
	CNM-CM 4767	abscess	Spain	**LT966039**
	CNRMA 11.383	skin	France (West Indies, Martinique)	**LT966041**
	CNRMA 11.855	skin	France	**LT966042**
	CNRMA 13.245	skin	France	**LT966043**
	CNRMA 16.374	skin	France (West Indies, Guadeloupe)	**LT966044**
	CNRMA 16.556	skin	France (West Indies, Guadeloupe)	**LT966045**
Paraconiothyrium fuckelii	CBS 797.95	***Rubus*** sp. dead stem	Denmark	JX496226
	CNRMA 3.240	eye	France	**LT966046**
	CNRMA 4.493	eye	France	**LT966047**
Paraphaeosphaeria michotii	MFLUCC 13-0349	***Poaceae*** dead leaves	Italy	KJ939282
	CNM-CM 6000	skin	Spain	**LT966048**
Paraphoma fimeti	CBS 170.70^{T}	***Apium graveolens*** seeds	The Netherlands	GQ387584
	CNM-CM 8075	wound exudate	Spain	**LT966049**
Paraphoma sp.	CNRMA 9.467	skin	France	**LT966050**
	CNRMA 15.665	skin	France	**LT966051**
Phaeosphaeriopsis obtusispora	CBS 246.64	***Aloe arborescens*** dead leaf	Portugal	JX681119
Phoma herbarum	CBS 615.75	***Rosa multiflora*** dead stem	The Netherlands	EU754186

	CNM-CM 2132	right toe	Spain	**LT966052**
	CNM-CM 3526	bone marrow	Spain	**LT966053**
	CNM-CM 3597	blood culture	Spain	**LT966054**
	CNM-CM 8031	nail	Spain	**LT966055**
	CNRMA 9.1095	skin	France	**LT966056**
	CNRMA 11.1097	eye	France	**LT966057**
	CNRMA 12.1227	eye	France	**LT966058**
pleosporelean fungus	CNRMA 11.1115	skin	France	**LT966059**
Preussia sp.	CNM-CM 7343	nail	Spain	**LT966060**
Preussia terricola	CBS 317.65^{T}	***Musa sapientum*** rhizosphere	Honduras	GQ203725
Preussia typharum	CBS 107.69	Dung of deer	Japan	GQ203726
	CNM-CM 7335	nail	Spain	**LT966061**
Pseudophaeosphaeria rubi	MFLUCC 14-0259	***Rubus idaeus*** dead branch	Italy	KX765299
Tintelnotia destructans	CBS 127737^{T}	anterior eye chamber cornea	Germany	KY090664
	CNM-CM 7430	Unknown	Spain	**LT966062**
Tintelnotia sp.	CNM-CM 7080	nail	Spain	**LT966063**
	CNM-CM 7981	cutaneous exudate	Spain	**LT966064**
Xenodidymella saxea	CBS 419.92^{T}	Corroded mediterranean marble	Unknown	GU238141

	CNRMA 16.76	Cerebrospinal fluid	France	**LT966065**

[a] **CBS**: Strains from Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CNM-CM**: Isolates from the National Centre for Microbiology, Instituto Carlos III, Madrid, Spain; **CNRMA**: Isolates from the National Reference Center for Invasive Mycoses and Antifungals; Institut Pasteur, Paris, France; **MFLUCC**: Strains from Mae Fah Luang University Culture Collection, Chiang Rai, Thailand. Type strains are indicated by a superscript [T]

[b] LSU, large subunit ribosomal DNA sequences

TABLE 2 Localization of infections due to coelomycetous fungi isolates

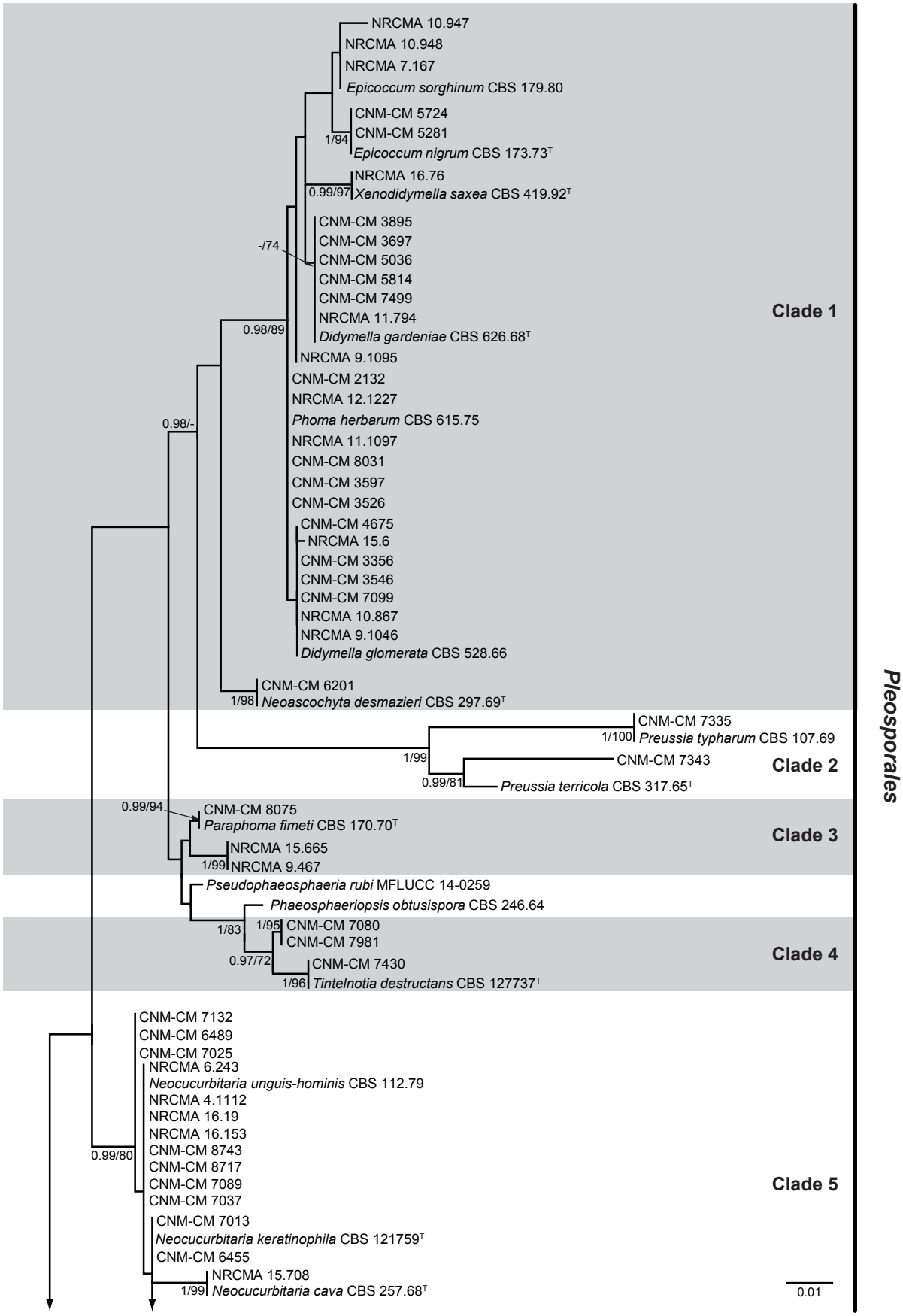
	no. of isolates obtained from:		
Orders	**Superficial infection**	**Deep infection**	**Total no. of isolates**
Botryosphaeriales	7	1	8
Diaporthales	5	1	6
Glomerellales	5		5
Pleosporales	71	7	78
Total no. of isolates (%)	**88 (91)**	**9 (9)**	**97 (100)**

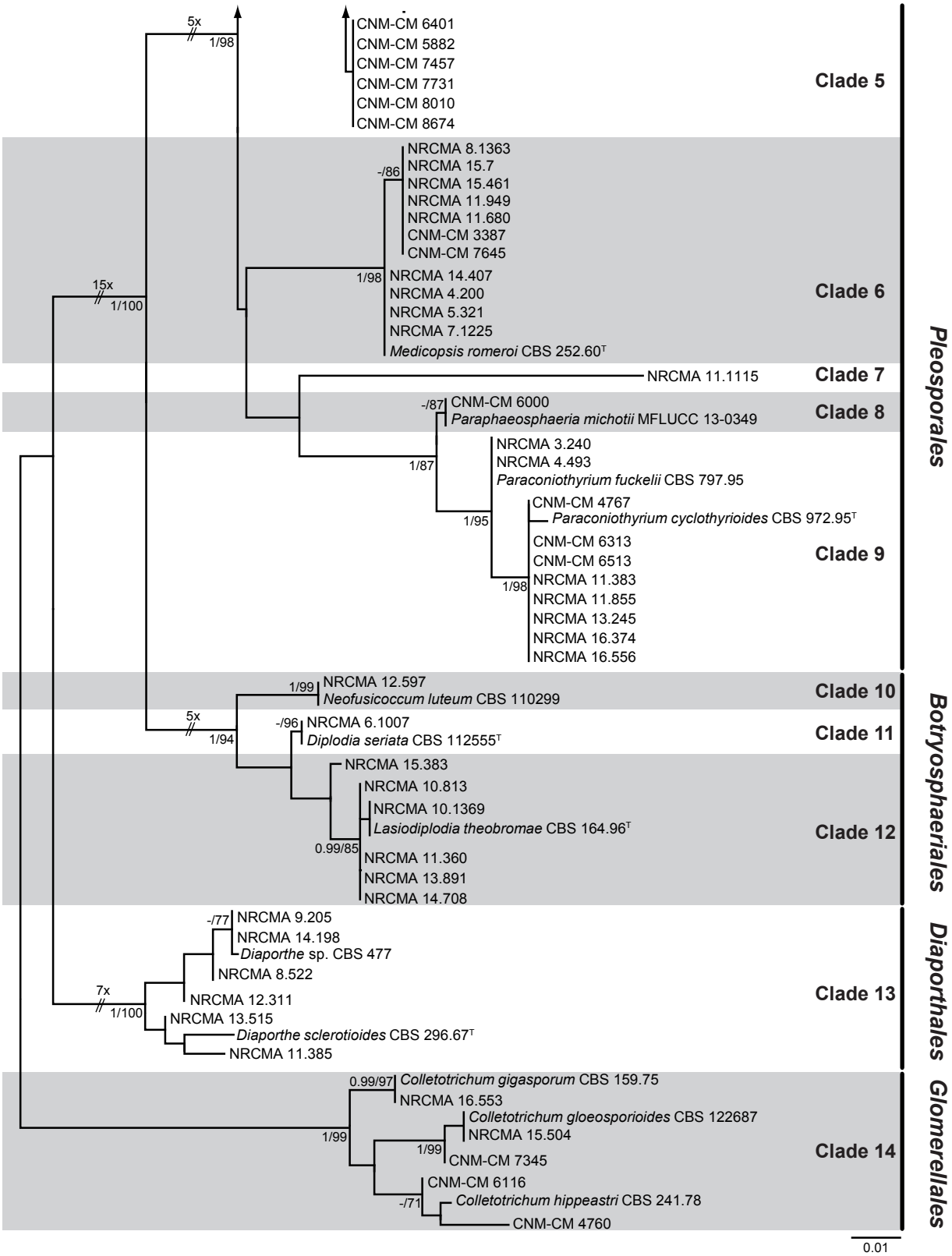
TABLE 3 Overall i*n vitro* antifungal activity against the 46 coelomycetous isolates as determined by EUCAST[a] methodology

Antifungal agent	MIC/MEC values (mg/L)[b]				
	range	median	GM	MIC_{50}	MIC_{90}
Amphotericin B	0.03 -16	0.5	0.41	0.25	1
Itraconazole	0.014 -16	2	1.72	0.5	16
Voriconazole	0.03 -16	0.5	0.70	0.6	4
Posaconazole	0.014 -16	0.5	0.58	0.25	8
Caspofungin	0.125- 16	2	2.17	1	8
Micafungin	0.015- 16	0.5	0.53	0.125	8
Terbinafin	0.014- 16	0.25	0.39	0.25	2

[a]EUCAST, European Committee on Antimicrobial Susceptibility Testing procedure (71);

[b]MIC, minimum inhibitory concentration; MEC, minimal effective concentration; MIC_{50} and MIC_{90}, MIC encompassing 50 and 90% of isolates tested, respectively.


NRCMA 10.947
NRCMA 10.948
NRCMA 7.167
Epicoccum sorghinum CBS 179.80
CNM-CM 5724
CNM-CM 5281
Epicoccum nigrum CBS 173.73T
NRCMA 16.76
Xenodidymella saxea CBS 419.92T
CNM-CM 3895
CNM-CM 3697
CNM-CM 5036
CNM-CM 5814
CNM-CM 7499
NRCMA 11.794
Didymella gardeniae CBS 626.68T
NRCMA 9.1095
CNM-CM 2132
NRCMA 12.1227
Phoma herbarum CBS 615.75
NRCMA 11.1097
CNM-CM 8031
CNM-CM 3597
CNM-CM 3526
CNM-CM 4675
NRCMA 15.6
CNM-CM 3356
CNM-CM 3546
CNM-CM 7099
NRCMA 10.867
NRCMA 9.1046
Didymella glomerata CBS 528.66
CNM-CM 6201
Neoascochyta desmazieri CBS 297.69T
CNM-CM 7335
Preussia typharum CBS 107.69
CNM-CM 7343
Preussia terricola CBS 317.65T
CNM-CM 8075
Paraphoma fimeti CBS 170.70T
NRCMA 15.665
NRCMA 9.467
Pseudophaeosphaeria rubi MFLUCC 14-0259
Phaeosphaeriopsis obtusispora CBS 246.64
CNM-CM 7080
CNM-CM 7981
CNM-CM 7430
Tintelnotia destructans CBS 127737T
CNM-CM 7132
CNM-CM 6489
CNM-CM 7025
NRCMA 6.243
Neocucurbitaria unguis-hominis CBS 112.79
NRCMA 4.1112
NRCMA 16.19
NRCMA 16.153
CNM-CM 8743
CNM-CM 8717
CNM-CM 7089
CNM-CM 7037
CNM-CM 7013
Neocucurbitaria keratinophila CBS 121759T
CNM-CM 6455
NRCMA 15.708
Neocucurbitaria cava CBS 257.68T
1/94
0.99/97
-/74
0.98/89
0.98/-
1/98
1/100
1/99
0.99/81
0.99/94
1/99
1/83
1/95
0.97/72
1/96
0.99/80
1/99
Clade 1
Clade 2
Clade 3
Clade 4
Clade 5
Pleosporales
0.01


CNM-CM 6401
CNM-CM 5882
CNM-CM 7457
CNM-CM 7731
CNM-CM 8010
CNM-CM 8674
Clade 5
NRCMA 8.1363
NRCMA 15.7
NRCMA 15.461
NRCMA 11.949
NRCMA 11.680
CNM-CM 3387
CNM-CM 7645
NRCMA 14.407
NRCMA 4.200
NRCMA 5.321
NRCMA 7.1225
Medicopsis romeroi CBS 252.60T
Clade 6
NRCMA 11.1115
Clade 7
CNM-CM 6000
Paraphaeosphaeria michotii MFLUCC 13-0349
Clade 8
NRCMA 3.240
NRCMA 4.493
Paraconiothyrium fuckelii CBS 797.95
CNM-CM 4767
Paraconiothyrium cyclothyrioides CBS 972.95T
CNM-CM 6313
CNM-CM 6513
NRCMA 11.383
NRCMA 11.855
NRCMA 13.245
NRCMA 16.374
NRCMA 16.556
Clade 9
Pleosporales
NRCMA 12.597
Neofusicoccum luteum CBS 110299
Clade 10
NRCMA 6.1007
Diplodia seriata CBS 112555T
Clade 11
NRCMA 15.383
NRCMA 10.813
NRCMA 10.1369
Lasiodiplodia theobromae CBS 164.96T
NRCMA 11.360
NRCMA 13.891
NRCMA 14.708
Clade 12
Botryosphaeriales
NRCMA 9.205
NRCMA 14.198
Diaporthe sp. CBS 477
NRCMA 8.522
NRCMA 12.311
NRCMA 13.515
Diaporthe sclerotioides CBS 296.67T
NRCMA 11.385
Clade 13
Diaporthales
Colletotrichum gigasporum CBS 159.75
NRCMA 16.553
Colletotrichum gloeosporioides CBS 122687
NRCMA 15.504
CNM-CM 7345
CNM-CM 6116
Colletotrichum hippeastri CBS 241.78
CNM-CM 4760
Clade 14
Glomerellales
5x
1/98
15x
1/100
-/86
1/98
1/87
-/87
1/95
1/98
1/99
5x
1/94
-/96
0.99/85
-/77
7x
1/100
0.99/97
1/99
1/99
-/71
0.01

UNIVERSITAT ROVIRA I VIRGILI
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4.7. *Neocucurbitaria keratinophila* an emerging opportunistic fungus causing superficial mycosis in Spain.

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Enviado: Medical Mycology 2018.

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Neocucurbitaria keratinophila: an emerging opportunistic fungus causing superficial mycosis in Spain.

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Running title: *Neocucurbitaria keratinophila*, a coelomycetous fungus of clinical interest in Spain.

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ABSTRACT

Although there have been few reports of opportunistic infections (superficial and systemic) caused by coelomycetous fungi, they are becoming more frequent. *Neocucurbitaria keratinophila* (formerly *Pyrenochaeta keratinophila*), characterized by producing pycnidial conidiomata and small hyaline conidia, seems to be an emergent opportunistic pathogen in Spain. Since this fungus was first reported from human keratitis, eight strains have been isolates from clinical cases in Spain. This is a retrospective study of these fungal strains, including phenotypic and molecular characterizations, and *in vitro* antifungal susceptibility assays. These clinical strains were identified by multi-locus analysis, by sequencing the internal transcribed spacer region (ITS1-5.8S-ITS2) and fragments of the 28S nrRNA (LSU), beta-tubulin (*tub*2) and RNA polymerase II subunit 2 (*rpb*2) genes. All the strains tested were susceptible to the majority of antifungals, being isavuconazole the only drug that showed a poor antifungal activity.

Keywords: Antifungal susceptibility, coelomycetes, superficial mycosis, *Neocucurbitaria*, *Pyrenochaeta*.

Introduction

Coelomycetous fungi are commonly found as saprobic or parasites of terrestrial vascular plants, although they can also be found in diverse environments such as soil, freshwater, salty water, sewage and even inorganic materials, and more rarely as human opportunistic pathogens.[1,2] Although the class Coelomycetes is now obsolete, it is still used in clinics to refer fungi that are morphologically characterized by producing asexual fruiting bodies called conidiomata inside which numerous conidia are produced on conidiophores and/or from conidiogenous cells. Although human infections by coelomycetous fungi are relatively scarce in comparison with other fungi, they are becoming more frequently reported, producing superficial and systemic infections.[1–5] Currently, identifying coelomycetous fungi is still complex because of the small number of discriminative morphological features and the high number of taxa involved.[1,6] For that reason a multi-locus analysis is necessary, using three or more phylogenetic markers for accurate identification.[7–9]

In a recent study on coelomycetous fungi from clinical origin [D. Garcia-Hermoso, N. Valenzuela-Lopez, O. Rivero-Menendez, *et al*. Journal Clinical Microbiology. Send manuscript to publisher][9bis], we found that a relatively high number of isolates of *Neocucurbitaria keratinophila* (formerly *Pyrenochaeta keratinophila*) had been deposited between 2005 and 2015 at the Mycology Reference Laboratory of the Spanish National Center for Microbiology, all of them recovered from cases of superficial mycosis. We recently carried out studies on the biodiversity of coelomycetous fungi from clinical origin in different countries, such as France and the United States of America.[2,9bis] Although we identified more than 300 isolates from numerous specimens, *Neocucurbitaria keratinophila* was not found. To our knowledge, this species has only been isolated from clinical samples in Spain[9bis]. Prior to our finding, *N. keratinophila*

has only been reported from a human case of keratitis in a diabetic Spanish woman.[10] Since this fungus is morphologically similar to a species of *Pyrenochaeta*, it was proposed by Verkley and co-workers as a new species of the genus as *P. keratinophila*.[11] However, in a recent revision of the genus, this species was described as phylogenetically different from *Pyrenochaeta sensu stricto*, being placed in the genus *Neocucurbitaria*.[8]

The objective of this study was to carry out accurate identification of *N. keratinophila,* studying all those known isolates. A multi-locus analysis was developed using four phylogenetic markers, including a fragment of the 28S nrRNA (LSU), the internal transcribed spacer region (ITS1-5.8S-ITS2), and fragments of the beta-tubulin (*tub*2) and RNA polymerase II subunit 2 (*rpb*2) genes. Another objective was to characterize their morphology. In addition, we determined their antifungal susceptibility to nine antifungal drugs.

Material and Methods

Fungal isolates

We processed eight isolates from clinical specimens deposited at the Mould Collection of the Spanish National Center of Microbiology (CNM-CM) (Table 1).

Morphological and physiological characterization

For cultural characterization, the isolates were grown on oatmeal agar (OA; 30 g of filtered oat flakes, 15 g of agar-agar, 1 L tap water) and malt extract agar (MEA; 40 g of malt extract, 15 g of agar-agar, 1 L distilled water), at 20 ± 1ºC for 14 days in darkness.

Colour notations of the colonies were according to Kornerup and Wanscher.[12]. The ability of the isolates to grow at cardinal temperatures was determined on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) in 7 d of darkness, ranging from 5 to 35°C at intervals of 5°C, and also at 37°C. Metabolite E+ (NaOH spot test) was produced by applying a droplet of 1N NaOH onto a colony grown on MEA.[13,14]

The structures were assessed under an Olympus CH2 microscope on wet mounts (using the Shear's medium) and slide cultures on OA and MEA, following Valenzuela-Lopez *et al*. (2017)[8] Photomicrographs were taken with an Axio-Imager M1 microscope (Zeiss, Oberkochen, Germany).

DNA extraction, amplification and sequencing

The total genomic DNA was extracted from colonies grown on PDA after 7 d of incubation at 20 ± 1ºC, using the FastDNA kit protocol (Bio101, Vista, CA), with a FastPrep FP120 instrument (Thermo Savant, Holbrook, NY) following the manufacturer's protocol. DNA was quantified using the Nanodrop 2000 (Thermo Scientific, Madrid, Spain). Each gene was amplified with the primer pair listed in Table 2.[15–19] The amplicons were sequenced 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

Molecular identification of the isolates was carried out by multi-locus analysis using the ITS, LSU, *rpb*2 and *tub*2 sequences of 29 type and reference strains from previous

studies.[8,9,20] DNA sequences generated in this study have been deposited in the GenBank and the accession numbers are given in Table 1.

For the phylogenetic study, the sequences were aligned using the ClustalW application of MEGA 6.06 and manually adjusted using the same software platform.[21,22] Phylogenetic reconstructions were made by maximum-likelihood (ML) and Bayesian inference (BI) in CIPRES.[23] The best substitution model for each gene matrix was estimated using MrModelTest v. 2.3, shown in Table 3.[24] For ML analyses, nearest-neighbour interchange was used as the heuristic method for tree inference. Support for internal branches was assessed by 1,000 ML bootstrapped pseudoreplicates. Bootstrap support (BS) of ≥70 was considered significant. For BI analyses, Markov chain Monte Carlo (MCMC) sampling was carried out with five million generations, 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 species *Cucurbitaria berberidis* was used as outgroup. The final matrices used for phylogenetic analysis was submitted in TreeBASE (www.treebase.org; accession number: S22786).

Antifungal susceptibility testing

The *in vitro* susceptibility testing was carried out following the procedure laid down by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[25], including the following nine antifungals: amphotericin B (AMB; range 0.03–16 mg/L; Sigma-Aldrich Quimica, Madrid, Spain), isavuconazole (ISV, range 0.015–8 mg/L; Basilea Pharmaceutica, Basel, Switzerland), itraconazole (ITC, range 0.015–8 mg/L; Sigma-Aldrich Quimica, Madrid, Spain), posaconazole ((PCZ; range 0.015–8 mg/L;

Sigma-Aldrich Quimica, Madrid, Spain), voriconazole (VCZ; range 0.015–8 mg/L; Sigma-Aldrich Quimica, Madrid, Spain), anidulafungin (AND, range 0.007–4 mg/L; Pfizer, Madrid, Spain), caspofungin (CPF, range 0.03–16 mg/L; Merck, Madrid, Spain), micafungin (MCF; range 0.004–2 mg/L; Astellas Pharma Inc, Tokyo, Japan) and terbinafine (TRB, range 0.03–16 mg/L; Sigma-Aldrich Quimica, Madrid, Spain). The strains were cultured on PDA for 7 to 30 d at 25°C and 30°C to obtain sporulation. Conidia were then collected in water and the suspension was adjusted to 2–5 × 10^5 colony-forming units (CFU)/mL. Minimal effective concentrations (MEC) were determined for echinocandins, and minimal inhibitory concentrations (MIC) for the other drugs after 48 h and 72 h of incubation at 35°C. *Aspergillus flavus* ATCC 204304 and *Aspergillus fumigatus* ATCC 204305 were used for quality control.

Results

Phenotypic characterization

Five of the eight isolates tested produced fertile structures under *in vitro* conditions and were characterized by the production of colonies of 8–10 mm diameter after 7 d on MEA at 20 ± 1ºC, flattened, margin entire edge or lobate, immersed mycelium initially white (M. 3A1) and becoming greyish yellow (M. 3C3); colonies on OA reached 15–16 mm diameter, flattened, margin entire edge, immersed mycelium initially olive (M. 3D3) becoming olive grey (M. 3F3), with felty grey aerial mycelium; pycnidial conidiomata were solitary, superficial, brown to dark brown, mostly glabrous or covered with somewhat short setae, globose (100–180 µm diameter) to subglobose (180–200 × 130–150 µm), only seen on OA; conidiogenous cells were ampulliform to doliform, 8–16 × 2–4 µm, integrated to acropleurogenous, branched at the base, conidiophores as terminal and lateral openings; conidia were aseptate, hyaline, smooth-

and thin-walled, subglobose to ellipsoid, 3.5–4.5 × 1.5–3.5 µm, with few guttules (Fig. 1).

The NaOH spot test was negative. Crystals were absent. Optimal temperature for growth and sporulation 25°C; minimum temperature of growth 15°C; maximum temperature of growth 35°C.

Phylogenetic analysis

The final concatenated dataset obtained with both ML and BI analysis contained 37 ingroup strains with a total of 2,503 characters including gaps (815 for LSU, 531 for ITS, 319 for *tub*2 and 838 for *rpb*2), 435 of which were parsimony informative (35 for LSU, 85 for ITS, 94 for *tub*2 and 221 for *rpb*2). The sequence datasets did not show conflict in any of the tree topologies for the 70% reciprocal bootstrap trees, which allowed us to combine the four genes in the multi-locus analysis. The ML showed similar tree topology and was congruent with that obtained in the BI. For the BI multi-locus analysis, a total of 2,816 trees were sampled after the burn-in with a stop value of 0.01. The support values were highly similar with the two methods (Fig. 2).

In the phylogenetic tree, all (n=19) of the currently accepted species of *Neocucurbitaria* have been separated appropriately from all (n=9) *N. keratinophila* strains (including the ex-type strain CBS 121759) isolated from Spanish clinical samples, which formed a clade with 1 PP / 100 % BS.

Table 4 summarizes the results of the antifungal susceptibility testing. In general, all the drugs assayed showed good activity against *N. keratinophila*, with anidulafungin as the most active, having a geometric mean (GM) of 0.01 µg/mL and a MIC_{90} (causing inhibition of 90% of the isolates) of 0.015 µg/mL. Posaconazole was the most active

azole, with an overall GM of 0.1 µg/mL and a MIC_{90} of 0.12 µg/mL; while isavuconazole showed higher MICs (GM of 2 µg/mL and; MIC_{90} of 2 µg/mL).

Discussion

Although all isolates of *N. keratinophila* in this study could not be proven as agents of mycoses, all of them were isolated from human superficial specimens, which reinforces its clinical importance as a potential emergent opportunistic pathogen.

The genus *Neocucurbitaria* was recently introduced by Wanasinghe *et al*. (2017)[20] to accommodate the sexual morph of *N. acerina*, and to propose the coelomycetous fungus *N. unguis-hominis* (formerly *Pyrenochaeta unguis-hominis*) as the type species for the genus. *Neocucurbitaria* is characterized morphologically by producing brown to black ascomata, scattered or aggregated, globose to pyriform or turbinate, asci bitunicate, fissitunicate, cylindrical and containing 4–8 muriform ascospores, while the asexual morph is pycnidial, producing hyaline and aseptate conidia. Subsequently, recent studies enlarged the genus to a total of 19 species.[8,9] There is a small number of pyrenochaeta-like species involved in human mycoses, but they are a group of species that produce well-characterized mycoses, such as eumycetoma by *Medicopsis romeroi* (syn. *Pyrenochaeta romeroi*) and *Nigrograna mackinnonii* (syn. *P. mackinnonii*).[4] *Neocucurbitaria unguis-hominis* was one of the former coelomycetous fungi of this genus reported as able to cause human infections, i.e. toe-nail infection,[26] while the second most frequent species to cause opportunistic superficial mycoses was *N. keratinophila*, reported as producing keratitis.[10] Interestingly, all eight strains in this study are clinical isolates from Spain, which would suggest an abnormal geographical distribution of this fungus. As the first report of *N. keratinophila* was also from Spain, this might also suggest that this fungus is endemic in our country. However, further studies are needed to clarify what sort of ecological niches this fungus occupies and

what kind of role it has, as well as whether its geographical distribution is really as limited as has been found so far.

There have been few antifungal studies on coelomycetous fungi, and clinical breakpoints are not defined. However, some authors recommend treatments that include surgical resection and antifungal treatment. Amphotericin B and triazoles have shown some efficacy in the limited data reported so far.[2,27–29] In this study, the *in vitro* antifungal susceptibility pattern for *N. keratinophila* showed low MICs ($\leq$ 2 µg/mL) to all antifungals tested although more data are needed to establish protocols for antifungal treatment of the infections caused by coelomycetous fungi.

Acknowledgments

This work was supported by the Spanish *Ministerio de Economía y Competitividad*, grant CGL2017-88094-P.

Olga Rivero-Menendez holds a predoctoral fellowship from Fondo de Investigacion Sanitaria (FI14CIII/00025). Ana Alastruey-Izquierdo is supported by research project from Fondo de Investigación Sanitaria (PI16CIII/00035)

Declaration of interest: Ana Alastruey-Izquierdo has received research grants or honoraria as a speaker or advisor from Gilead Sciences, Pfizer, F2G and Scynexis outside the submitted work. The other authors report no conflicts of interest.

References

1. Stchigel AM, Sutton DA. Coelomycete fungi in the clinical lab. *Curr Fungal Infect Rep*. 2013; 7: 171–191.
2. Valenzuela-Lopez N, Sutton DA, Cano-Lira JF, et al. Coelomycetous fungi in the clinical setting: Morphological convergence and cryptic diversity. *J Clin Microbiol*. 2017; 55: 552–567.
3. Sutton DA. Coelomycetous fungi in human disease. A review: Clinical entities, pathogenesis, identification and therapy. *Rev Iberoam Micol*. 1999; 16: 171–179.
4. Ahmed SA, van de Sande WWJ, Stevens DA, et al. Revision of agents of black-grain eumycetoma in the order *Pleosporales*. *Persoonia*. 2014; 33: 141–154.
5. Vasant JA, Maggiani F, Borman AM. Subcutaneous mycotic cyst caused by *Roussoella percutanea* in a UK renal transplant patient. *Mycopathologia*. 2017; 182: 721–725.
6. Wijayawardene NN, Hyde KD, Wanasinghe DN, et al. Taxonomy and phylogeny of dematiaceous coelomycetes. *Fungal Divers*. 2016; 77: 1–316.
7. Chen Q, Jiang JR, Zhang GZ, Cai L, Crous PW. Resolving the *Phoma* enigma. *Stud Mycol*. 2015; 82: 137–217.
8. Valenzuela-Lopez N, Cano-Lira JF, Guarro J, et al. Coelomycetous Dothideomycetes with emphasis on the families *Cucurbitariaceae* and *Didymellaceae*. *Stud Mycol*. 2018; 90: 1–69.
9. Jaklitsch WM, Checa J, Blanco MN, Olariaga I, Tello S, Voglmayr H. A preliminary account of the *Cucurbitariaceae*. *Stud Mycol*. 2018; 90: 71–118.
10. Ferrer C, Pérez-Santonja JJ, Rodríguez AE, et al. New *Pyrenochaeta* species causing keratitis. *J Clin Microbiol*. 2009; 47: 1596–1598.

11. Verkley GJ, Gené J, Guarro J, et al. *Pyrenochaeta keratinophila* sp. nov., isolated from an ocular infection in Spain. *Rev Iberoam Micol*. 2010; 27: 22–24.

12. Kornerup A, Wanscher JH. Methuen handbook of colour 3rd ed. Methuen, London, England, 1978.

13. Dorenbosch MMJ. Key to nine ubiquitous soil-borne *Phoma*-like fungi. *Persoonia*. 1970; 6: 1–14.

14. Noordeloos ME, de Gruyter J, van Eijk GW, Roeijmans HJ. Production of dendritic crystals in pure cultures of *Phoma* and *Ascochyta* and its value as a taxonomic character relative to morphology, pathology and cultural characteristics. *Mycol Res*. 1993; 97: 1343–1350.

15. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR Protocols: A Guide to Methods and Applications. Orlando, Florida: Academic Press, 1990: 315–322.

16. Rehner SA, Samuels GJ. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycol Res*. 1994; 98: 625–634.

17. Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol*. 1990; 172: 4238–4246.

18. Woudenberg JHC, Aveskamp MM, de Gruyter J, Spiers AG, Crous PW. Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia*. 2009; 22: 56–62.

19. Liu YJ, Whelen S, Hall BD. Phylogenetic relationships among ascomycetes evidence from an RNA polymerase II subunit. *Mol Biol Evol*. 1999; 16: 1799–1808.

20. Wanasinghe DN, Phookamsak R, Jeewon R, et al. A family level rDNA based phylogeny of *Cucurbitariaceae* and *Fenestellaceae* with descriptions of new *Fenestella* species and *Neocucurbitaria* gen. nov. *Mycosphere*. 2017; 8: 397–414.

21. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*. 1994; 22: 4673–4680.

22. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*. 2013; 30: 2725–2729.

23. Miller MA, Pfeiffer W, Schwartz T. The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. In: Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the Extreme to the Campus and Beyond: 1–8. Association for Computing Machinery, USA, 2012.

24. Nylander JAA. MrModeltest v2. Program Distributed by the Author. Evolutionary Biology Centre, Uppsala University, Sweden, 2004.

25. Subcommittee on Antifungal Susceptibility Testing of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). EUCAST Technical Note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds. *Clin Microbiol Infect*. 2008; 14: 982–984.

26. English MP, Atkinson R. Onychomycosis in elderly chiropody patients. *Br J Dermatol*. 1974; 91: 67–72.

27. Chowdhary A, Meis JF, Guarro J, et al. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of systemic phaeohyphomycosis: diseases caused by black fungi. *Clin Microbiol Infect*. 2014; 20: 47–75.

28. Ahmed SA, de Hoog GS, Stevens DA, Fahal AH, van de Sande WW. In vitro antifungal susceptibility of coelomycete agents of black grain eumycetoma to eight antifungals. *Med Mycol*. 2015; 53: 295–301.

29. Guégan S, Garcia-Hermoso D, Sitbon K, et al. Ten-Year experience of cutaneous and/or subcutaneous infections due to Coelomycetes in France. *Open Forum Infect Dis*. 2016. doi:10.1093/ofid/ofw106.

Figure legend

Figure 1. *Neocucurbitaria keratinophila* CBS 121759 (ex-type strain) (A-G). (A), (B). Colonies on MEA and OA (surface). (C). Pycnidia on OA (indicated by arrows). (D). Pycnidium. (E), (F). Conidiogenous cells. (G). Conidia. *Neocucurbitaria keratinophila* CNM-CM 6401 (H-N). (H), (I). Colonies on MEA and OA (surface). (J). Pycnidia on OA (indicated by arrows). (K). Pycnidium. (L), (M). Conidiogenous cells and conidiophores. (N). Conidia. Scale bars: (D), (K) = 50 µm. (E-G), (L-N) = 10 µm.

Figure 2. Maximum likelihood tree based on a concatenated alignment of LSU, ITS, *tub*2 and *rpb*2 sequences of 37 strains representing a total of 19 species of *Neocucurbitaria*. The Bayesian posterior probabilities (PP) above 0.95 and the RAxML bootstrap support values of ⩾70 are indicated on the nodes. Fully supported branched (1 PP/100 BS) are indicated in bold. Some branches were shortened to fit them to the page, these are indicated by two diagonal lines with the number of times a branch was shortened. Type strains are indicated by a superscript ^{T}. Species involved in the clinical setting are indicated with a cross. The tree was rooted with *Cucurbitaria berberidis* (CBS 130007 and CBS 142401).

Table 1. Origin and GenBank accession numbers of the sequences of the isolates used in this study. New sequences generated are indicated in bold.

Species	Isolate no.[a]	Source	Country	GenBank accession no. LSU	ITS	*tub*2	*rpb*2
Cucurbitaria berberidis	CBS 130007[T]	*Berberis vulgaris*	Austria	KC506793	LT717673	LT717676	MF795800
	CBS 142401	*Berberis* sp.	Austria	MF795756	MF795756	MF795886	MF795798
Neocucurbitaria acanthocladae	CBS 142398[T]	*Genista acanthoclada*	Greece	MF795766	MF795766	MF795894	MF795808
Neocucurbitaria acerina	CBS 142403	*Acer pseudoplatanus*	Austria	MF795768	MF795768	MF795896	MF795810
	MFLUCC 16-1450[T]	*Acer campestre*	Italy	KY563076	KY563073	-	
Neocucurbitaria aetnensis	CBS 142404[T]	*Genista aetnensis*	Italy	MF795769	MF795769	MF795897	MF795811
	WU 36930	*Genista aetnensis*	Italy	MF795770	MF795770	MF795898	MF795812
Neocucurbitaria aquatica	CBS 297.74[T]	Sea water	Montenegro	EU754177	LT623221	LT623238	LT623278
Neocucurbitaria cava	CBS 115979	Unknown	The Netherlands	EU754198	AY853248	LT623234	LT623273
	CBS 257.68[T]	Wheat-field soil	Germany	EU754199	JF740260	KT389844	LT717681
Neocucurbitaria cinereae	CBS 142406[T]	*Genista cinerea*	Spain	MF795771	MF795771	MF795899	MF795813
Neocucurbitaria cisticola	CBS 142402[T]	*Cistus monspeliensis*	Spain	MF795772	MF795772	MF795900	MF795814
Neocucurbitaria hakeae	CBS 142109[T]	*Hakea* sp.	Australia	KY173526	KY173436	KY173613	KY173593
Neocucurbitaria irregularis	CBS 142791[T]	Human arm injury	USA	LN907372	LT592916	LT592985	LT593054
Neocucurbitaria juglandicola	CBS 142390[T]	*Juglans regia*	Austria	MF795773	MF795773	MF795901	MF795815

Neocucurbitaria keratinophila	CBS 121759^{T}, FMR 9444	Human corneal scrapings	Spain	LT623215	EU885415	LT623236	LT623275
	CNM-CM 5882, FMR 17319	Human cutaneous exudate	Spain	LT966018	**LT992276**	**LT992260**	**LT992268**
	CNM-CM 6401, FMR 17320	Human fingernail	Spain	LT966019	**LT992277**	**LT992261**	**LT992269**
	CNM-CM 6455, FMR 17321	Human cutaneous exudate	Spain	LT966020	**LT992278**	**LT992262**	**LT992270**
	CNM-CM 7013, FMR 17322	Human cutaneous exudate	Spain	LT966021	**LT992279**	**LT992263**	**LT992271**
	CNM-CM 7457, FMR 17323	Human cutaneous exudate	Spain	LT966022	**LT992280**	**LT992264**	**LT992272**
	CNM-CM 7731, FMR 17324	Human cutaneous exudate	Spain	LT966023	**LT992281**	**LT992265**	**LT992273**
	CNM-CM 8010, FMR 17325	Human conjunctival exudate	Spain	LT966024	**LT992282**	**LT992266**	**LT992274**
	CNM-CM 8674, FMR 17326	Human toenail	Spain	LT966025	**LT992283**	**LT992267**	**LT992275**
Neocucurbitaria populi	CBS 142393^{T}	*Populus* sp.	Sweden	MF795774	MF795774	MF795902	MF795816
Neocucurbitaria quercina	CBS 115095^{T}	*Quercus robur*	Italy	GQ387619	LT623220	LT623237	LT623277
Neocucurbitaria rhamni	CBS 142391^{T}	*Rhamnus frangula*	Austria	MF795775	MF795775	-	MF795817
	WU 36936	*Rhamnus frangula*	Austria	MF795776	MF795776	MF795903	MF795818
Neocucurbitaria rhamnicola	CBS 142396^{T}	*Rhamnus lycioides*	Spain	MF795780	MF795780	MF795906	MF795822
	WU 36947	*Rhamnus alaternus*	Spain	MF795781	MF795781	MF795907	MF795823
Neocucurbitaria rhamnioides	CBS 142395^{T}	*Rhamnus myrtifolius*	Spain	MF795782	MF795782	MF795908	MF795824
	WU 36949	*Rhamnus saxatilis* ssp. *prunifolius*	Greece	MF795783	MF795783	MF795909	MF795825

Neocucurbitaria ribicola	CBS 142394[T]	*Ribes rubrum*	Austria	MF795785	MF795785	MF795911	MF795827
	WU 36952	*Ribes rubrum*	Austria	MF795786	MF795786	MF795912	MF795828
Neocucurbitaria unguis-hominis	CBS 111112	*Agapornis* sp. Lung	The Netherlands	GQ387623	LT623222	LT623239	LT623279
	UTHSC DI16-213	Unknown	USA	LN907356	LT592910	LT592979	LT593048
Neocucurbitaria vachelliae	CBS 142397[T]	*Vachellia gummifera*	Morocco	MF795787	MF795787	MF795913	MF795829

[a] CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CNM-CM, Center of Microbiology Mould Collection of the Instituto de Salud Carlos III, Madrid, Spain; FMR, Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, Texas, USA; WU, Fungarium of the Department of Botany and Biodiversity Research, University of Vienna, Austria.
[T] Type strain.

Table 2. Primers used in this study, with sequences and sources.

Gene	Product name	Primer	Direction	Sequence (5'–3')	Reference
Internal transcribed spacer (complete)	ITS	ITS-5	Forward	GGA AGT AAA AGT CGT AAC AAG G	15
		ITS-4	Reverse	TCC TCC GCT TAT TGA TAT GC	15
28S ribosomal RNA	LSU	LR0R	Forward	GTA CCC GCT GAA CTT AAG C	16
		LR5	Reverse	TCC TGA GGG AAA CTT CG	17
Beta-tubulin	*tub*2	TUB2Fd	Forward*	GTB CAC CTY CAR ACC GGY CAR TG	18
		TUB4Rd	Reverse	CCR GAY TGR CCR AAR ACR AAG TTG TC	18
RNA polymerase II second largest subunit	*rpb*2	RPB2-5F2	Forward	GAY GAY MGW GAT CAY TTY GG	19
		fRPB2-7cR	Reverse	CCC ATW GCY TGC TTM CCC AT	19

***B**: C or G or T; **Y**: C or T; **R**: A or G; **M:** A or C; **W:** A or T

Table 3. Nucleotide substitution models used in phylogenetic analysis.

Gene	Substitution model[a]
LSU	GTR + I
ITS	SYM + I + G
*tub*2	HKY + G
*rpb*2	GTR + I + G

[a] GTR, General time-reversible model; HKY, Kishino and Yano model; SYM, symmetrical model. G, gamma distribution; I, proportion of invariable sites.

Table 4. Results of *in vitro* antifungal susceptibility testing.

Taxon (no. of isolates)	Parameter[a]	Values for the drug (μg/mL)[b]								
		AMB	ISV	ITC	PCZ	VCZ	AND	CPF	MCF	TRB
N. keratinophila (8)	GM	0.29	2	0.5	0.1	1	0.01	0.39	0.03	0,25
	Range	0.12-1	1-4	0.25-1	0.06-0.25	0.25-2	≤0.015	0.25-1	≤0.03	≤0.025
	MIC_{90}	0.5	2	0.5	0.12	2	0.015	0.25	0.03	0,25

[a] GM, geometric mean; MIC_{90}, drug concentration that inhibited 90% of isolates.

[b] AMB, amphotericin B; ISV, isavuconazole; ITC, itraconazole; PCZ, posaconazole; VCZ, voriconazole; AND, anidulafungin; CPF, caspofungin; MCF, micafungin; TRB, terbinafine.

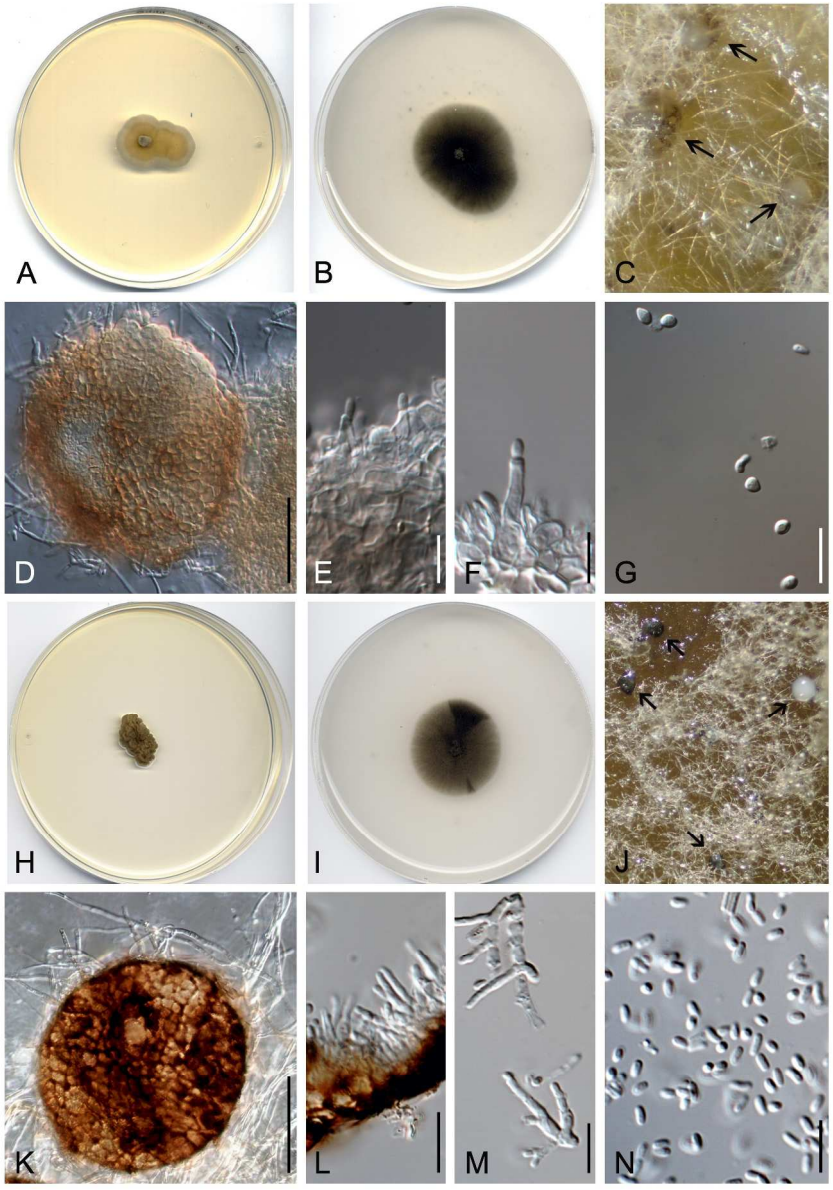

Figure 1. Neocucurbitaria keratinophila CBS 121759 (ex-type strain) (A-G). (A), (B). Colonies on MEA and OA (surface). (C). Pycnidia on OA (indicated by arrows). (D). Pycnidium. (E), (F). Conidiogenous cells. (G). Conidia. Neocucurbitaria keratinophila CNM-CM 6401 (H-N). (H), (I). Colonies on MEA and OA (surface). (J). Pycnidia on OA (indicated by arrows). (K). Pycnidium. (L), (M). Conidiogenous cells and conidiophores. (N). Conidia. Scale bars: (D), (K) = 50 µm. (E-G), (L-N) = 10 µm.

240x341mm (300 x 300 DPI)

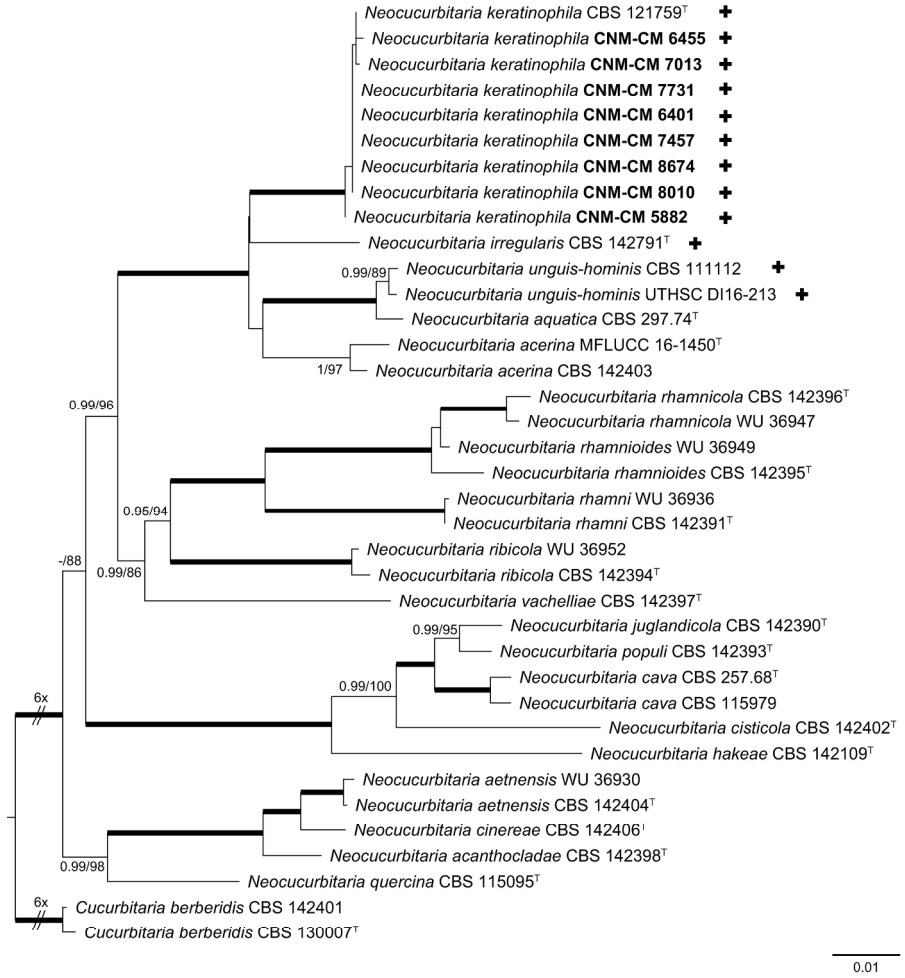

Figure 2. Maximum likelihood tree based on a concatenated alignment of LSU, ITS, tub2 and rpb2 sequences of 37 strains representing a total of 19 species of Neocucurbitaria. The Bayesian posterior probabilities (PP) above 0.95 and the RAxML bootstrap support values of ≥70 are indicated on the nodes. Fully supported branched (1 PP/100 BS) are indicated in bold. Some branches were shortened to fit them to the page, these are indicated by two diagonal lines with the number of times a branch was shortened. Type strains are indicated by a superscript T. Species involved in the clinical setting are indicated with a cross. The tree was rooted with Cucurbitaria berberidis (CBS 130007 and CBS 142401).

218x226mm (300 x 300 DPI)

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4.8. *Pseudoascochyta* gen. nov., *P. novae-zelandiae* sp. nov. y *P. pratensis* sp. nov.

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Publicado en: Fungal Planet sheets, Persoonia 2016; 37: 254–257.

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Pseudoascochyta Valenzuela-Lopez, Stchigel, Cano-Canals, Guarro & Cano, *gen. nov.*

Etymology. Name reflects the morphological similarity with the genus *Ascochyta*, but from which it is distinct.

Classification — *Didymellaceae*, *Pleosporales*, *Dothideomycetes*.

Hyphae pale to dark brown, smooth- and thin- to thick-walled, septate. *Pycnidia* brown to dark brown, globose, solitary, pycnidial wall of *textura angularis*, neck absent, ostiolated or not. *Conidiogenous cells* enteroblastic, phialidic, globose to flask-shaped, hyaline, thin-walled. *Conidia* hyaline, cylindrical, 1-septate, guttulate. *Chlamydospores* absent.

Type species. *Pseudoascochyta pratensis* Valenzuela-Lopez, Cano-Canals, Stchigel, Guarro & Cano.

MycoBank MB817646.

Pseudoascochyta pratensis Valenzuela-Lopez, Cano-Canals, Stchigel, Guarro & Cano, *sp. nov.*

Etymology. From Latin *pratum*, prairie, referring to the toponymy of the place where the specimen was collected.

Hyphae pale to dark brown, 2–2.5 mm wide, smooth- and thin- to thick-walled, septate. *Pycnidia* dark brown, globose, with hyphal outgrowths, mostly immersed, solitary, 250–330 mm diam, pycnidial wall of *textura angularis* (*textura epidermoidea* on sterile carnation leaves), 30–40 mm thick, outer wall 2–3-layered, composed of dark brown, flattened polygonal cells of 5–25 mm diam, inner wall 4–6-layered, composed of hyaline to subhyaline, flattened polygonal cells, neck absent, ostiole absent (formed very late when the fungus grow on sterile carnation leaves, of 25–35 mm diam). *Conidiogenous cells* enteroblastic, phialidic, globose to flask-shaped, hyaline, thin-walled, 5–8 mm diam. *Conidia* hyaline, cylindrical, 1-septate, (8–)10–12 × 2.5–3 mm, narrowing slightly at the septa, smooth- and thin-walled, guttulate.

Culture characteristics — Colonies on OA reaching 12 mm diam in 7 d at 25 ± 1 °C, flattened, granulose due to the production of pycnidia, dark green (M.30F3); reverse olive brown (M.4F3) to brownish grey (M.4F2). Colonies on MEA reaching 15 mm in 7 d at 25 ± 1 °C, flattened, compact, greyish brown (M.7F3); reverse dark brown (M.8F5). NaOH spot test negative. Crystals absent. Optimal temperature for sporulation, 15 °C; optimal temperature of growth, 25 °C; minimum temperature of growth, 5 °C; maximum temperature of growth, 30 °C.

Typus. Spain, Tarragona, Prades, from soil, 13 Apr. 2015, *J. Cano-Canals* (holotype CBS H-22735, cultures ex-type FMR 14524 = CBS 141688, ITS sequence GenBank LT223130, LSU sequence GenBank LT223131, *tub2* sequence GenBank LT223132, *rpb2* sequence GenBank LT223133, MycoBank MB817647).

Notes — The fungus was isolated from a soil sample. Morphologically, *Pseudoascochyta pratensis* resembles species of the genus *Ascochyta* (Chen et al. 2015). Based on a megablast search of NCBIs GenBank nucleotide database, the closest hits using the LSU sequence are *Ascochyta phacae* (GenBank EU167570; Identities = 841/841 (100 %), no gaps) and *Microsphaeropsis olivacea* (GenBank JX681101; Identities = 840/841 (99 %), no gaps). Closest hits using the ITS sequence are *Ascochyta medicaginicola* (GenBank EU167575; Identities = 550/558 (99 %), gaps 3/558), *Leptosphaerulina australis* (GenBank JN712494; Identities = 537/542 (99 %), gaps 1/542). The closest hit using the *tub2* sequence is *Phoma* sp. (GenBank KT309385; Identities = 330/332 (99 %), no gaps). The closest hit using the *rpb2* (*RPB2*) sequence is *Ascochyta pisi* (GenBank EU874867; Identities = 844/923 (91 %), gaps 2/923). Our phylogenetic tree (see FP484), built by using the combined LSU, ITS, *tub2* and *rpb2* sequence alignment, corroborated that our fungus represents a new genus and a new species. *Pseudoascochyta pratensis* differs from *A. medicaginicola* var. *macrospora* by its lower growth rate on OA, the absence of crystal production, its larger conidia (28 × 6 mm vs (8–)10–12 × 2.5–3 mm) and the number of septa (1–3 vs 1).

Colour illustrations. Prades, Tarragona, Spain; colony on OA after 7 d at 25 ± 1 °C, conidiomata (pycnidia) under the stereomicroscope, pycnidia, conidiogenous cells, conidia. Scale bars = 10 µm.

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Fungal Planet 484 – 21 December 2016

Pseudoascochyta novae-zelandiae Valenzuela-Lopez, Stchigel, Guarro & Cano, *sp. nov.*

Etymology. Referring to the geographical origin of the fungus.

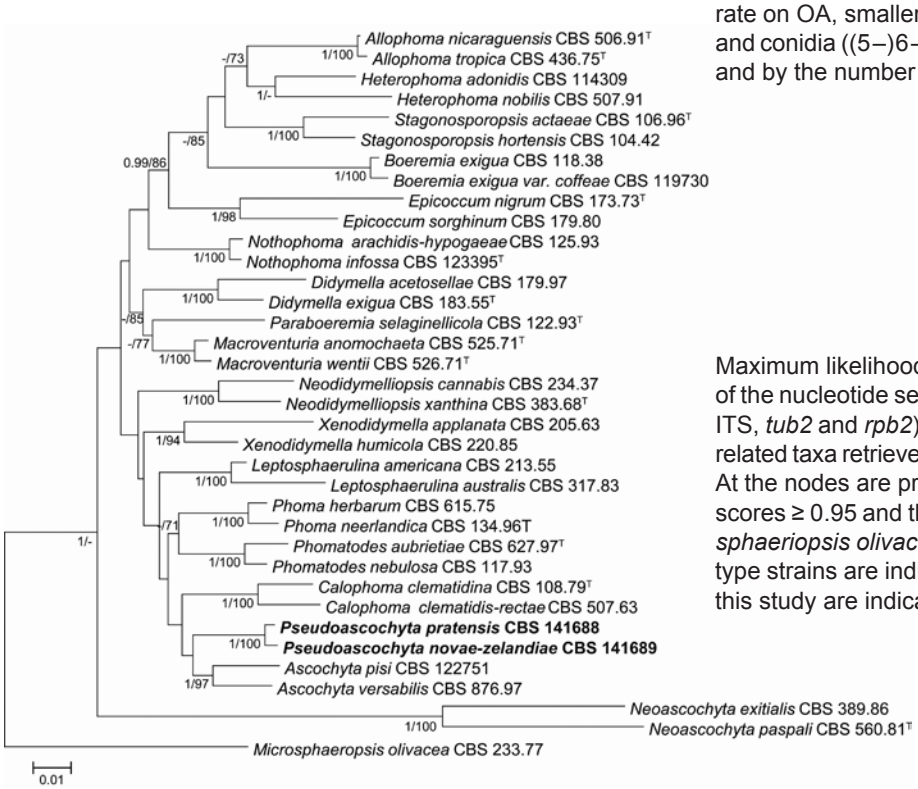
Classification — *Didymellaceae*, *Pleosporales*, *Dothideomycetes*.

Hyphae brown, 2.5–3 mm wide, smooth- and thin- to thick-walled, septate. *Pycnidia* brown to dark brown, globose, immersed, solitary, 120–250 mm diam, pycnidial wall of *textura angularis*, 3-layered, 10–15 mm thick, composed of dark brown polygonal cells of 5–25 mm diam, neck absent, non-ostiolated. *Conidiogenous cells* enteroblastic, phialidic, globose to flask-shaped, hyaline, thin-walled, 5–6 mm diam. *Conidia* hyaline, cylindrical, aseptate, (5–)6–7 × 2–2.5 mm, guttulate.

Culture characteristics — Colonies on OA reaching 50 mm diam in 7 d at 25 ± 1 °C, flattened, granulose due to the production of pycnidia, olive (M.3F2) to olive grey (M.3F4); reverse dark grey (M.1F1). Colonies on MEA reaching 48 mm diam in 7 d at 25 ± 1 °C, flattened, compact, olive (M.2E5) to yellowish white (M.2A2); reverse dark brown (M.6F6) to olive brown (M.4D4). NaOH spot test negative. Crystals absent. Optimal temperature for sporulation, 15 °C; optimal temperature of growth, 25 °C; minimum temperature of growth, 5 °C; maximum temperature of growth, 30 °C.

Typus. New Zealand, Wellington, Titahi Bay, *Cordyline australis* (*Agavaceae*), 1 May 1990, *P.R. Johnston* (holotype CBS H-22734, cultures ex-type FMR 15110 = CBS 141689, ITS sequence GenBank LT592892, LSU sequence GenBank LT592893, *tub2* sequence GenBank LT592894, *rpb2* sequence GenBank LT592895, MycoBank MB817648).

Notes — The fungus was isolated from a cabbage tree, endemic to New Zealand. Based on a megablast search of NCBIs GenBank nucleotide database, the closest hits using the LSU sequence are *Ascochyta phacae* (GenBank EU167570; Identities = 842/842 (100 %), no gaps) and *Microsphaeropsis olivacea* (GenBank JX681101; Identities = 841/842 (99 %), no gaps). Closest hits using the ITS sequence are *Ascochyta medicaginicola* (GenBank EU167575; Identities = 540/546 (99 %), gaps 2/546), *Leptosphaerulina australis* (GenBank JN712494; Identities = 537/542 (99 %), gaps 1/542). The closest hit using the *tub2* sequence is *Phoma* sp. (GenBank KT309385; Identities = 332/332 (100 %), no gaps). The closest hit using the *rpb2* sequence is *Ascochyta pisi* (GenBank EU874867; Identities = 760/835 (91 %), gaps 1/835 (0%)). Our phylogenetic tree, built by using the concatenated LSU, ITS, *tub2* and *rpb2* sequence alignment, corroborated that our fungus represents a new species. *Pseudoascochyta novae-zelandiae* differs from *P. pratensis* (the type species of the genus) by its faster growth rate on OA, smaller pycnidia (130–250 mm vs 250–330 mm) and conidia ((5–)6–7 × 2–2.5 mm vs (8–)10–12 × 2.5–3 mm), and by the number of conidial septa (aseptate vs 1-septate).



Maximum likelihood tree obtained from the combined dataset of the nucleotide sequences of four different nuclear *loci* (LSU, ITS, *tub2* and *rpb2*) of the new proposed species and those of related taxa retrieved from the GenBank (TreeBASE ID 19426). At the nodes are presented the Bayesian posterior probability scores ≥ 0.95 and the bootstrap support values ≥ 70 %. *Microsphaeriopsis olivacea* CBS 233.77 was used as outgroup. Ex-type strains are indicated with T. The new species proposed in this study are indicated in **bold** face.

Colour illustrations. Titahi Bay, Wellington, New Zealand (image credit: Graeme Simpson, www.graemesimpsonimages.com); colony on OA after 7 d at 25 °C, conidiomata under the stereomicroscope, pycnidia, conidiogenous cells, conidia. Scale bars = 10 µm.

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4.9. *Coniella heterospora* sp. nov.

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Publicado en: Fungal Planet sheets, Persoonia 2017; 39: 290–291.

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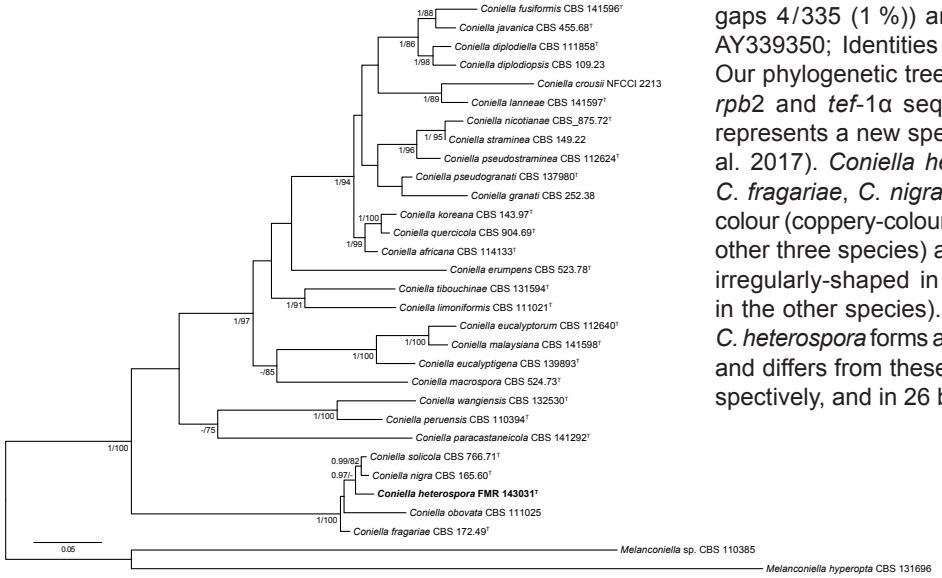
Fungal Planet 629 – 20 December 2017

Coniella heterospora Valenzuela-Lopez, Cano, Guarro & Stchigel, *sp. nov.*

Etymology. Named after the variable shape of the conidia.

Classification — *Schizoparmaceae*, *Diaporthales*, *Sordariomycetes*.

Hyphae hyaline to pale brown, smooth- and thin- to thick-walled, septate, 2–5 µm wide. *Pycnidia* initially hyaline, becoming dark brown with age due to the production of conidia, glabrous, semi-immersed or superficial (OA), solitary, confluent with age, globose, (320–)370–500(–800) µm diam, without neck, ostiolate, pycnidial wall of *textura angularis*, 50–65 µm thick, 5–6-layered, composed of hyaline to pale brown or brown, flattened polygonal cells of 5–15 µm diam, on the inside of the pycnidium there is a basal central cushion-like structure composed of hyaline cells from which the conidiophores arise. *Conidiophores* densely aggregated, hyaline, branched at the base and with 2–3 supporting cells, or reduced to a single conidiogenous cell. *Conidiogenous cells* hyaline, determinate, smooth- and thin-walled, lageniform, 6.5–12(–16) × 1.5–2 (–2.5) µm, 1–1.5 µm wide at apex. *Conidia* hyaline at first, becoming coppery-coloured when mature, aseptate, smooth- and thin- to thick-walled, mostly with a large guttula, sometimes biguttulate, variable in shape, mostly ellipsoidal, sometimes naviculate, limoniform, subsphaerical or irregularly-shaped, mostly laterally compressed, apex acute to nearly rounded, truncate at the base, with a longitudinal germ slit in older conidia, with a minute basal appendage formed by rests of the conidiogenous cell, (4.5–)5.5–8(–9.5) × (3–)4.5–6(–6.5) × 4–4.5(–5.5) µm.



Maximum likelihood tree obtained from the combined DNA sequences dataset from four loci (LSU, ITS, *rpb2* and *tef*-1α) of our isolate and sequences retrieved from the GenBank database. Ex-type strains of the different species are indicated with ^{T}. The new species proposed in this study is indicated in **bold**. The Bayesian posterior probabilities (≥ 0.95) and RAxML bootstrap support values (≥ 70 %) are provided at the nodes. *Melanconiella hyperopta* CBS 131696 and *Melanconiella* sp. CBS 110385 were used as outgroup.

Culture characteristics — Colonies on OA reaching 79 mm diam after 7 d at 25 ± 1 °C, flattened, white (M. 4A1); reverse white (M. 4A1). Colonies on MEA reaching 86 mm diam after 7 d at 25 ± 1 °C, floccose, brownish grey (M. 4D2) to dark grey (M. 4F1); reverse dark grey (M. 4F1). NaOH spot test negative. Crystals absent. Optimal temperature of sporulation and growth, 25 °C; minimum temperature of growth, 15 °C; maximum temperature of growth, 30 °C.

Typus. Spain, Huelva, Almonte, road HF6245 from Los Cabezudos village to Los Bodegones village, from herbivorous dung, Mar. 2016, coll. *C. González-García* and *G. Sisó*, isol. *N. Valenzuela-Lopez* (holotype CBS H-23198, cultures ex-type FMR 15231 = CBS 143031, ITS, LSU, *tef*-1α and *rpb2* sequences GenBank LT800501, LT800500, LT800503 and LT800502, MycoBank MB820451).

Notes — *Coniella heterospora* is characterised by the production of coppery-coloured conidia that are variable in shape. Based on a megablast search of NCBIs GenBank nucleotide database, the closest hit using the LSU sequence is *C. fragariae* CBS 183.52 (GenBank KJ710442; Identities = 835/838 (99 %), no gaps). Closest hits using the ITS sequence are *C. fragariae* CBS 198.82 (GenBank KJ710465; Identities = 600/601 (99 %), no gaps) and *C. solicola* CPC 17308 (GenBank KX833598; Identities = 589/591 (99 %), no gaps). The closest hits using the *rpb2* sequence are *C. solicola* CBS 114007 (GenBank KX833504; Identities = 756/767 (99 %), no gaps) and *C. fragariae* CBS 454.68 (GenBank KX833477; Identities = 751/767 (98 %), no gaps). The closest hits using the *tef*-1α sequence are *C. solicola* CPC 17308 (GenBank KX833702; Identities = 311/335 (93 %), gaps 4/335 (1 %)) and *C. fragariae* STE-U 3713 (GenBank AY339350; Identities = 327/359 (91 %), gaps 9/359 (2 %)). Our phylogenetic tree, built by using concatenated LSU, ITS, *rpb2* and *tef*-1α sequences, corroborated that our isolate represents a new species (Alvarez et al. 2016, Marin-Felix et al. 2017). *Coniella heterospora* is morphologically similar to *C. fragariae*, *C. nigra* and *C. solicola*, but differs in conidium colour (coppery-coloured in *C. heterospora* vs dark brown in the other three species) and shape (very variable and sometimes irregularly-shaped in *C. heterospora*, and scarcely variable in the other species). The phylogenetic analysis showed that *C. heterospora* forms a basal branch with *C. solicola* and *C. nigra*, and differs from these species in 8 bp and 10 bp for *rpb2*, respectively, and in 26 bp for both *tef*-1α nucleotide sequences.

Colour illustrations. Los Cabezudos-Los Bodegones, Huelva, Spain; colony on MEA and OA after 14 d at 25 ± 1 °C; pycnidia under the stereomicroscope; conidiophores, conidiogenous cells and conidia; conidia, some of them showing minute basal cellular appendage (indicated by arrows). Scale bars: conidiophores = 20 µm, conidiogenous cells and conidia = 10 µm..

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4.10. *Alfaria dactylis* sp. nov.

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Aceptado en: Fungal Planet sheets, Persoonia 2018.

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Alfaria dactylis

Fungal Planet XXX – date

Alfaria dactylis Valenzuela-Lopez, Cano, Guarro & Stchigel *sp. nov.*

Classification — *Stachybotryaceae, Hypocreales, Sordariomycetes.*

Etymology. From Latin *dactylus*, date, due to the nature of the substrate (date, the fruit of *Phoenix dactylifera*) from which the fungus was isolated.

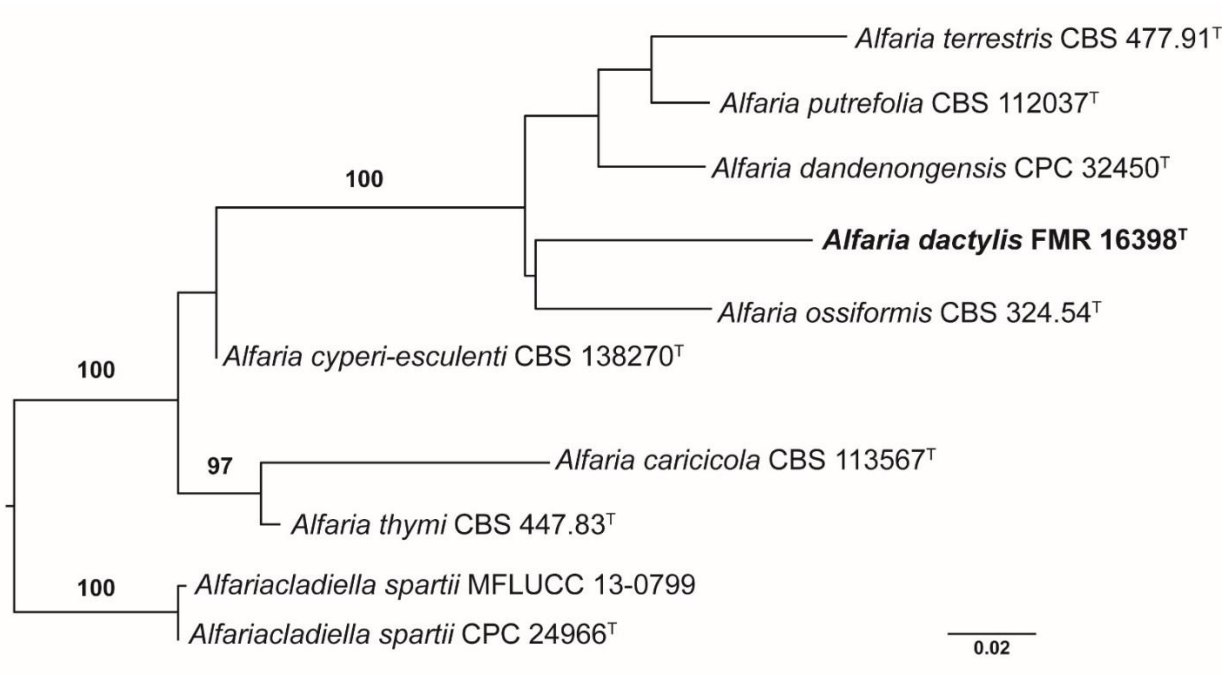
Hyphae hyaline to pale green, smooth- and thin- to thick-walled, septate, 2.5–5 μm wide. *Conidiomata* discrete, cupulate, stromatic, unilocular, non-ostiolate, superficial, solitary or confluent, greenish-black, covered by setae, broadly lenticular, 177–275 μm × 133–242 μm, filled with black mass of slimy conidia; *conidioma wall* 10–27 μm broad, pseudoparenchymatous, of *textura globulosa* and *textura angularis*, composed of 2 to 4 layers of pale green to dark green, globose to flattened polygonal cells of 5–7.5 μm diam.; *setae* greenish-black, smooth- and thick-walled, multiseptate, unbranched, straight, narrowing towards the sharp apices, 60–200 μm long, 4–8 μm wide at the base. *Conidiophores* densely aggregated, arising from the basal part of the locule, unbranched or branched at the base with 2–4 supporting cells, pale green, smooth-walled, up to 47 μm long, bearing 1–3 conidiogenous cells. *Conidiogenous cells* phialidic, cylindrical, elongate, hyaline to pale green, smooth-walled, 7–16 × 1.5–2.5 μm. *Conidia* hyaline to pale green, aseptate, smooth- and thin-walled, guttulate, lanceolate, 8.5–11.5 × 2–2.5 μm, with an obtuse apex and truncate at the base.

Culture characteristics — Colonies on OA reaching 19–21 mm diam. after 7 d at 25±1 ºC, margin regular, flattened, with sparse aerial mycelium, surface white (M. 4A1); reverse white (M. 4A1). Colonies on MEA reaching 18–20 mm diam. after 7 d at 25±1 ºC, margin regular, flattened, covered by dense white felty aerial mycelia, surface white (M. 4A1) to pale yellow (M. 4A3); reverse white (M. 4A1) to yellowish-orange (M. 4A6). NaOH test negative.

Typus. SPAIN, Tarragona, from palm fruit of *Phoenix dactylifera*, February 2017, coll. *I.A. Iturrieta-González*, isol. *N. Valenzuela-Lopez*, holotype CBS H-XXX, cultures ex-type FMR 16398 = CBS XXX, ITS sequence GenBank LT984556, LSU sequence GenBank LT984557, *btub* sequence GenBank LT984555, and *tef-1α* sequence GenBank LT984553, MycoBank MB 824149.

Notes — *Alfaria dactylis* is characterized by the production of large, lanceolate, pale green conidia and discrete, cupulate, stromatic condiomata covered by abundant setae, being morphologically similar to *A*. *dandenongensis* but differing in aspect of their conidia (cylindrical, granular and verrucolose in *A*. *dandenongensis*) and setae (smooth-walled in *A*. *dactylis* vs verruculose in *A*. *dandenongensis*) (Crous *et al*. 2017). Despite the fact of *A*. *dactylis* is phylogenetically close related to *A*. *ossiformis*, it is morphologically distinct from the latter species by its setose conidiomata (lacking of setae in *A*. *ossiformis*) (Lombard *et al*. 2016).

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hit using the LSU sequence is *A*. *ossiformis* CBS 324.54 (GenBank KU845993; Identities = 810/810 (100 %), no gaps). Closest hits using ITS sequence are *A*. *putrefolia* CBS 112037 (GenBank KU845985; Identities = 533/544 (98 %), 6 gaps (1%)) and *A*. *ossiformis* CBS 324.54 (GenBank NR_145068; Identities = 534/547 (98 %), 7 gaps (1%)). The closest hits using *tub*2 sequence are *C*. *terrestris* CBS 477.91 (GenBank KU846019; Identities = 288/308 (94 %), 4 gaps (1%)) and *C*. *putrefolia* CBS 112038 (GenBank KU846017; Identities = 285/307 (93 %), 2 gaps (0%)). The closest hits using *tef*-1α sequence are *A*. *terrestris* CBS 127305 (GenBank KU846012; Identities = 315/362 (87 %), 14 gaps (3 %)) and *A*. *ossiformis* CBS 324.54 (GenBank KU846009; Identities = 313/360 (87 %), 17 gaps (4 %)).



Maximum likelihood tree obtained from the combined DNA sequences dataset from six loci (ITS, LSU, *tef*-1α, *cmdA*, β-*tub* and *rpb*2) of our isolate and sequences retrieved from the GenBank database. Ex-type strains of the different species are indicated with ^{T}. The new species proposed in this study is indicated in bold. The RAxML bootstrap support values (≥ 70 %) are provided at the nodes. *Alfariacladiella spartii* CPC 24966 and MFLUCC 13-0799 were used as outgroup.

Colour illustrations. Tarragona, Spain; colony on MEA and OA after 14d at 25±1 ºC; conidiomata under the stereomicroscope; cupulate stromatic conidiomata, conidiophores, conidiogenous cells and conidia. Scale bars: conidiomata = 50 µm, conidiophores and conidia = 10 µm.

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References

Crous PW, Shivas RG, Quaedvlieg W, *et al*. (2014). Fungal Planet Description Sheets: 214–280. *Persoonia*, 32:184–306.

Lombard L, Houbraken J, Decock C, *et al*. (2016). Generic hyper-diversity in *Stachybotriaceae*. *Persoonia*, 36:156–246.

Crous PW, Wingfield MJ, Burgess TI, *et al*. (2017). Fungal Planet Description Sheets: 625–715. *Persoonia*, 39:270–467.

5. DISCUSIÓN GENERAL

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En las últimas décadas se ha producido un cambio dramático en la clasificación de los hongos celomicetos, debido mayoritariamente al conocimiento más profundo de su filogenia y ecología (Sutton BC 1980, Sutton DA 1999, Wijayawardene *et al*. 2016, Valenzuela-Lopez *et al*. 2018a). Sin embargo, la taxonomía de este grupo de hongos es aún compleja, considerándose su identificación un verdadero desafío para la mayoría de los micólogos y, más aún, para los profesionales del laboratorio clínico (Stchigel & Sutton 2013, Valenzuela-Lopez *et al*. 2017). Cada nueva propuesta taxonómica, por lo general, necesita de una cuidadosa revisión de todos los taxones ubicados en la antigua clase Coelomycetes, muchos de los cuales han sido instituidos (hace ya un siglo y medio) en base a sus caracteres morfológicos y tipo de hospedador (mayoritariamente plantas vasculares terrestres) (Sutton BC 1980, Boerema *et al*. 2004). Sin embargo, la propuesta de nuevas especies de hongos celomicetos para la ciencia no siempre ha implicado la necesaria comparación con los taxones previamente descritos, realizándose tales propuestas únicamente en base a los análisis filogenéticos de secuencias nucleotídicas (correspondientes a varios genes o regiones genómicas, en el mejor de los casos), los cuales no siempre han sido adecuadamente seleccionados por su bajo poder resolutivo. Debido a ello, a pesar de las grandes ventajas taxonómicas que ofrecen los métodos de identificación basados en el empleo de criterios moleculares (en comparación con el estudio de las características fenotípicas), algunas de las propuestas taxonómicas recientes resultan, como mínimo, controvertidas.

A pesar de que Kirk y co-autores (2008) mencionan que el número de especies de hongos celomicetos es de aproximadamente 7.000, es probable que su número sea significativamente mayor, si nos basamos en el aumento (durante la última década) de la descripción de nuevas especies de este grupo de hongos (Aveskamp *et al*. 2010, de Gruyter *et al*. 2013, Hyde *et al*. 2013, Chen *et al*. 2015, Wijayawardene *et al*. 2016, Valenzuela-Lopez *et al*. 2018a). A pesar de ello, el número de especies de celomicetos capaces de producir infecciones (mayoritariamente oportunistas) en el hombre es comparativamente bajo si lo referimos a la totalidad de taxones descritos para este grupo de hongos (Sutton DA 1999, Stchigel & Sutton 2013). Durante el desarrollo de la presente tesis doctoral, se ha realizado una extensa revisión bibliográfica, documentándose un total de 38 especies distribuidas en 25 géneros (pertenecientes a tres clases de hongos diferentes: *Dothideomycetes*, *Leotiomycetes* y *Sordariomicetes*), como causantes de micosis superficiales y subcutáneas, y, en menor medida, de infecciones profundas o sistémicas. A pesar de esta aparente escasa diversidad de especies patógenas para el hombre, el número real de taxones implicados en infecciones humanas es considerablemente mayor a lo descrito en la literatura, tal se ha podido demostrar durante el desarrollo de la presente tesis doctoral.

Nuestro estudio abarca la caracterización fenotípica y molecular de 452 cepas, de las cuales 322 provenían de muestras clínicas. De estas últimas, 224 procedían del *Fungus Testing Laboratory* (UTHSC, San Antonio, EE.UU.), 51 fueron remitidas por el *Institut Pasteur* (CNRMA, París, Francia) y otras 46 por el *Instituto de Salud Carlos III* (CNM-CM, Madrid, España). Respecto al origen anatómico de las mismas, más de la mitad de ellas (≈ 53%) fueron aisladas a partir de tejidos superficiales. Este hecho no es fortuito, debido a que está probado que la vía de adquisición más frecuente de las infecciones por este grupo de hongos es el contacto de lesiones de la piel con material contaminado, principalmente restos vegetales o suelo (Stchigel & Sutton 2013). Sin embargo, algunos de estos hongos han sido aislados de zonas anatómicas más profundas, produciendo quistes o eumicetomas, e incluso a nivel sistémico (cultivos de líquido céfalo-raquídeo y sangre, entre otros), pero con escasa frecuencia.

La mayoría de las cepas (> 50%) estudiadas en la presente tesis se ubicaron taxonómicamente en el orden *Pleosporales*, tanto las provenientes de los Estados Unidos de Norteamérica como las de dos laboratorios de referencia europeos (España y Francia) (Valenzuela-Lopez *et al*. 2017, Garcia-Hermoso *et al*. 2018{sometido}). Esto se debe a que, probablemente, el orden *Pleosporales* es uno de los más diversos dentro de la clase *Dothideomycetes*, con más de 50 familias (Hyde *et al*. 2013, Liu *et al*. 2017). El resto de cepas se distribuyó ponderadamente dentro de los órdenes *Botryosphaeriales*, *Diaporthales* y *Glomerellales* (de la clase *Dothideomycetes*), independientemente de su origen geográfico. Sin embargo, las cepas norteamericanas mostraron una mayor diversidad taxonómica, con una distribución en once órdenes, mientras que las procedentes de Francia se ubicaron en cuatro órdenes y las de España únicamente en dos (Valenzuela-Lopez *et al*. 2017, Garcia-Hermoso *et al*. 2018{sometido}, Valenzuela-Lopez *et al*. 2018b). Los géneros más frecuentemente reportados en el presente trabajo fueron *Didymella* (38 cepas), *Neoscytalidium* (35), *Paraconiothyrium* (28), *Neocucurbitaria* (21), *Epicoccum* (20), *Colletotrichum* y *Medicopsis* (16). Por lo tanto, la mayoría de los hongos aislados de especímenes clínicos pertenecieron a especies de los géneros *Didymella* y *Epicoccum*, de la familia *Didymellaceae*; los cuales anteriormente estaban asimilados al género *Phoma* (Boerema *et al*. 2004, de Gruyter *et al*. 2009, 2013; Aveskamp *et al*. 2010, Chen *et al*. 2015, 2017). Ochenta y cinco de las cepas de origen clínico habían sido identificadas en origen cómo pertenecientes al género *Phoma*; sin embargo, su estudio taxonómico polifásico (que implica la caracterización fisiológica, morfológico-estructural y molecular de las cepas con fines taxonómicos) permitió reubicarlas en distintos géneros de la familia *Didymellaceae*, algunos de los cuales resultaron ser nuevos para la ciencia, mientras que otras fueron finalmente identificadas como especies no descritas anteriormente (Valenzuela-Lopez *et al*. 2018a).

La gran mayoría de las especies del género *Pyrenochaeta* han sido descritas entre principios de los siglos XIX y XX, alcanzando un número de 163 epítetos (www.indexfungorum.org). Previamente, este género constituía una sección dentro del género *Phoma*, pero los estudios realizados por de Gruyter y co-autores (2010, 2013) han permitido redistribuir sus especies entre los géneros *Pyrenochaeta* y *Pyrenochaetopsis*, corroborando que ambos pertenecían a la familia *Cucurbitariaceae* (en tanto que *Phoma* se adscribía a la familia *Didymellaceae*). Estudios filogenéticos posteriores (Wanasinghe *et al*. 2017), permitieron proponer el nuevo género *Neocucurbitaria* (familia *Cucurbitariaceae*), dentro del cual fueron reubicadas varias especies de *Pyrenochaeta* (Wanasinghe *et al*. 2017). En la presente tesis doctoral, se intentó delimitar con precisión la familia *Cucurbitariaceae*, proponiéndose nuevos géneros dentro de ella y separando el género *Pyrenochaetopsis*, considerado previamente dentro de esta familia. En consecuencia, todos nuestros aislados inicialmente identificados dentro del género *Phoma* fueron segregados tanto en *Cucurbitariaceae* como en *Didymellaceae*; otros aislados, considerados inicialmente como phoma-like se reubicaron en el género *Pyrenochaetopsis* y otros resultaron ser nuevos géneros. Estos resultados nos permiten concluir que la filogenia de *Pyrenochaeta sensu stricto* aún no está resuelta, por lo que hemos propuesto de mantenerla como un género *incertae sedis* (Valenzuela-Lopez *et al*. 2018a). Además, en paralelo con nuestro trabajo, Jaklitsch y co-autores (2018) han introducido nuevos géneros en la familia *Cucurbitariaceae*; estas inclusiones han ratificado nuestras propuestas taxonómicas dentro de la familia, y han reforzado la necesidad de realizar una revisión en profundidad de *Pyrenochaeta sensu stricto*.

Desde el punto de vista clínico tanto especies del género *Phoma* como *Pyrenochaeta* han sido relacionadas con micosis oportunistas, en su mayoría a nivel de tejido superficial o subcutáneo, sin embargo, a pesar de que casos clínicos asociados al género *Phoma* son raros o escasos, tuvo una relativa frecuencia entre los años 1970 y 1990 (Bakerspigel A 1970, Punithalingam E 1976, 1979, Bakerspigel *et al*. 1981, Shukla *et al*. 1984, Baker *et al*. 1987, Rai MK 1989, Hirsh AH & Schiff TA 1996, Rosen *et al*. 1996). Actualmente, el número de casos debidos a este género ha disminuido (Tullio *et al*. 2010, Roehm *et al*. 2012), lo que quizás se deba a que esté subestimada su incidencia al necesitarse personal altamente especializado para identificación, o que se considere como un mero contaminante. Nuestro estudio de cepas clínicas, podría considerarse como un muy buen ejemplo de lo expuesto anteriormente, ya que un gran número de aislados clínicos inicialmente constaban como phoma-like; en base a ello, consideramos que no debemos de prescindir de los aislados de este grupo de hongos, si queremos evaluar adecuadamente la incidencia de los celomicetos en clínica. Por otro lado, dos especies previamente pertenecientes a *Pyrenochaeta* actualmente se conocen como *Neocucurbitaria keratinophila* y *N. unguis-hominis*

(Wanasinghe *et al*. 2017, Valenzuela-Lopez *et al*. 2018a); estas dos especies son las de mayor incidencia en clínica para dicho género; es de destacar que *N*. *keratinophila* en particular fue aislada únicamente a partir de infecciones fúngicas superficiales en España, concretamente nueve, lo cual indicaría una distribución muy restringida de la misma, si tenemos en consideración el elevado número de cepas clínicas estudiadas tanto a partir de Estados Unidos como de Francia. Todos nuestros aislados de las dos especies de *Neocucurbitaria* fueron aislados a partir de muestras de tejidos superficiales como piel, uñas o del ojo; ambas especies, ya habían sido previamente reportadas de casos clínicos humanos (Punithalingam E & English MP 1975, Ferrer *et al*. 2009, Verkley *et al*. 2010).

Otro hongo celomiceto con un número importante de aislados correspondió a *Neoscytalidium dimidiatum*, aunque se lo trate en el laboratorio clínico como un hifomiceto debido a su rápido crecimiento en medios artificiales y la producción de artroconidios. Los casos clínicos reportados para este hongo son muy variados, causando micosis oportunistas mayoritariamente en tejidos superficiales, o incluso siendo responsables de otras micosis más profundas como quistes, llegando a estar presente en sangre o en líquido cefalorraquídeo entre otros (Elewski *et al*. 1996, Madrid *et al*. 2009, Machouart *et al*. 2013, Bakhshizadeh *et al*. 2014, da Silva *et al*. 2016, James *et al*. 2017).

Otro de los géneros más frecuentemente aislado y al mismo tiempo prevalente en el presente estudio correspondió a *Paraconiothyrium*. Este género fue introducido en un principio como relacionado con *Coniothyrium*, sin embargo, desde el punto de vista de su filogenia están muy distantes uno del otro (Verkley *et al*. 2014). En nuestro trabajo hemos obtenido 28 aislados a partir de muestras clínicas correspondientes a dicho taxón, con especial prevalencia de *P*. *cyclothyrioides* (13 aislados). Este resultado es destacable, ya que actualmente los casos clínicos asociados a este género son muy escasos (Gordon *et al*. 2012, Colombier *et al*. 2015, Guégan *et al*. 2016). Sin embargo, este bajo número de casos reportados puede deberse a una incorrecta identificación debido a la semejanza morfológica con *Microsphaeropsis arundinis*, el cual también ha sido reportado como agente responsable de micosis oportunistas (Sutton DA 1999, Stchigel & Sutton 2013). Actualmente y mediante estudios de filogenia molecular, el género *Microsphaeropsis* ha sido reubicado y propuesto dentro de su propia familia, *Microsphaeropsidaceae*, muy cercana a *Didymellaceae* (Chen *et al*. 2015). En base a lo expuesto, podríamos considerar *Paraconiothyrium* spp. como un patógeno emergente causante de micosis superficiales.

El género *Colletotrichum* fue otro de los celomicetos con un número importante de aislados. En la actualidad, las especies reportadas en clínica son seis, *Colletotrichum coccodes*, *C*. *crassipes*, *C*. *dematium*, *C*. *gloeosporioides*, *C*. *graminicola* y *C*. *truncatum*, las que principalmente producen micosis oculares (p. ej. endoftalmitis, queratitis) y raramente micosis profundas (p. ej. quistes) (Guarro *et al*. 1998, Cano *et al*. 2004, Yegneswaran *et al*. 2010, Shivaprakash *et al*. 2011, Figtree *et al*. 2013, Cho *et al*. 2015). Por otra parte, en base a los estudios de filogenia molecular se está comprobando que la taxonomía de este género es muy compleja y que probablemente las especies actuales sean en realidad complejos de especies (Cannon *et al*. 2012, Damm *et al*. 2012, Weir *et al*. 2012, Liu *et al*. 2014), lo que implicaría que todos los aislados clínicos deberán ser reevaluados en vistas a su correcta identificación, ya que en la mayoría de los casos solo se han tomado en consideración caracteres morfológicos y como máximo uno o dos marcadores filogenéticos. Actualmente, se ha comprobado que para realizar una correcta identificación a nivel de especie se necesita secuenciar al menos seis marcadores filogenéticos. Por lo tanto, será necesario el realizar un gran esfuerzo para determinar el papel de las diferentes especies del género en clínica.

Dentro de las manifestaciones clínicas más importantes causadas por los hongos celomicetos está el eumicetoma, enfermedad más frecuente en zonas tropicales o subtropicales y que se adquiere principalmente por abrasiones o heridas causadas por espinas de plantas, las que están contaminadas con las esporas de los agentes etiológicos. La infección se desarrolla de forma local, crónica y progresiva, afectando principalmente al tejido subcutáneo, produciendo una tumefacción desfigurante de la cual drena pus y gránulos que pueden ser de color claro u oscuros, dependiendo del agente causante de la infección. Además, ésta se presenta en su mayoría en las extremidades del huesped, principalmente en los pies o en las manos, pero puede ocurrir en cualquier zona anatómica (van de Sande 2013, van de Sande *et al*. 2017). Esta patología es producida por un diverso grupo de agentes que van desde bacterias como *Nocardia* o *Streptomyces* (actinomicetoma) así como por hongos filamentosos como *Madurella* o *Trematosphaeria* (eumicetoma) (van de Sande 2013). Sin embargo, en los últimos años se ha incrementado el número de reportes de eumicetoma de grano negro debido a *Medicopsis romeroi* y *Nigrograna mackinnonii* (antiguamente incluidos dentro del género *Pyrenochaeta*), así como a *Trematosphaeria grisea* (Ahmed *et al*. 2014b). Para el tratamiento de las infecciones debidas a estas últimas especies fúngicas, se recomienda una combinación de itraconazol o ketoconazol junto con la remisión quirúrgica del tejido infectado (Welsh O *et al*. 2014). Hasta la fecha, son escasos los trabajos científicos que evalúen la sensibilidad frente a los antifúngicos contra los agentes etiológicos de eumicetoma, y el tratamiento con dichos azoles está tan solo indicado frente a la infección causada por *Madurela mycetomatis*. Sin embargo, la especie *M*. *romeroi* parece demostrar

un patrón de susceptibilidad diferente, presentando elevados rangos de CMI contra el grupo de los azoles (Ahmed *et al*. 2015a). En esta tesis, 25 aislados fueron identificados como pertenecientes a los géneros antes mencionados, siendo *M*. *romeroi* el más prevalente (16/25) seguido por *Trematosphaeria* spp. (7/25), y en menor medida *Nigrograna* spp. (2/25), todas provenientes de muestras de origen clínico procedentes de los tres países estudiados. Desafortunadamente no se obtuvieron datos clínicos de relevancia, pero la mayoría de las cepas fueron aisladas de especímines clínicos procedentes de infecciones de tejidos superficiales o en menor medida de tejidos subcutáneos. Por otra parte, solo un reducido número de aislados fue capaz de esporular, por lo que solo seis aislados de *M*. *romeroi* se sometieron a ensayos de sensibilidad antifúngica presentando una gran variabilidad en cuanto a la CMI, con algunos aislados cuya CMIs eran elevadas para la mayoría de los antifúngicos probados (Garcia-Hermoso *et al*. 2018{sometido}).

El tratamiento contra las infecciones causadas por hongos celomicetos sigue representando un gran desafío, principalmente porque no existen puntos de corte clínicos, y porque muchas veces el hongo no es capaz de esporular, dificultando los ensayos *in vitro*. Existen pocos estudios que evalúen la sensibilidad de los hongos celomicetos más prevalentes en micosis humanas frente a los diferentes antifúngicos. Uno de estos estudios es una guía elaborada por Chowdhary y co-autores (2014), donde se resume todos los estudios clínicos realizados hasta el momento sobre los géneros *Neoscytalidium*, *Phoma* y *Pyrenochaeta*; en el mismo, y dependiendo del género, se proporciona información con respecto a qué antifúngicos y procedimientos clínicos se consideran más adecuados para tratar sus infecciones. En el caso de *Neoscytalidium*, se menciona que la anfotericina B y la terbinafina son más activas *in vitro* que los azoles, y a pesar de que no se ha determinado un tratamiento para las infecciones diseminadas, se menciona que tanto la anfotericina B como el voriconazol y posaconazol son los compuestos más activos. En el caso de *Phoma*, la intervención quirúrgica sin administración de antifúngicos puede llegar a ser suficiente, pero para casos más complicados se recomienda además el uso de la anfotericina B junto con azoles como voriconazol o itraconazol, dependiendo del tipo de la lesión. En el caso de *Pyrenochaeta* no existe un tratamiento protocolizado consensuado (Chowdhary *et al*. 2014).

En los últimos años autores como Ahmed y co-autores (2015a) han realizado un esfuerzo para determinar la sensibilidad frente a los antifúngicos de los agentes etiológicos de eumicetoma (*Medicopsis romeroi*, *Nigrograna mackinnonii* y *Trematosphaeria grisea*), ello ha facilitado el abordar el tratamiento de este tipo de infecciones (Ahmed *et al*. 2015a). Recientemente, Guegán y co-autores (2016) han proporcionado más información sobre la sensibilidad *in vitro* y las opciones terapéuticas frente a las infecciones ocasionadas por

algunos celomicetos como por ejemplo *Didymella*, *Medicopsis* y *Paraconiothyrium*, indicando como primera línea la resección quirúrgica seguido en función de los casos de un tratamiento antifúngico, inicialmente con voriconazol o posaconazol y en el caso de que la infección sea refractaria con anfotericina B. Sin embargo, todavía quedan un gran número de hongos celomicetos por evaluar, en este trabajo doctoral se ha realizado el esfuerzo de probar la sensibilidad *in vitro* frente a diversos antifúngicos de un gran número de aislados. De los 85 aislados ensayados provenientes de los Estados Unidos de Norteamérica, estos estaban distribuidos en nueve géneros, y los 46 aislados provenientes de Europa (España y Francia) pertenecían a trece géneros. Además, se evaluó la sensibilidad de los aislados de *Neocucurbitaria keratinophila* que fueron capaces de fructifivar *in vitro*. A partir de estos estudios se puede concluir que la mayoría de los celomicetos fueron sensibles a los antifúngicos ensayados, con excepción de los géneros *Colletotrichum* y *Neoascochyta*, los que presentaron CMIs relativamente elevadas frente al voriconazol y el itraconazol; también se ha observado que los aislados de *Medicopsis* no eran sensibles al itraconazol y a la caspofungina, corroborando los resultados obtenidos por Ahmed y co-autores (2015a).

Finalmente, se considera importante el reseñar que a pesar de las apreciables aportaciones realizadas por la presente tesis doctoral sobre la taxonomía, biodiversidad, clínica y de sensibilidad antifúngica de los hongos celomicetos en clínica, creemos que todavía queda un largo camino por recorrer. Los hongos celomicetos continúan siendo un grupo de hongos filogenéticamente diverso y complejo, el cual necesita de estudios taxonómicos profundos para poner en evidencia las relaciones evolutivas existentes entre sus miembros. También, por otra parte, poner en evidencia todas las especies que pueden actuar como patógenos oportunistas del hombre, identificándolos apropiadamente y evaluando su patrón de sensibilidad frente a los diferentes antifúngicos.

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6. CONCLUSIONES

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En el presente trabajo se ha realizado un estudio polifásico (morfológico, fisiológico y molecular) de un gran número de aislados de hongos celomicetos de origen clínico, así como de un cierto número de aislados de origen ambiental provenientes de colecciones internacionales de cultivos microbianos, y de muestras de suelo y heces de animales herbívoros (aisladas durante el presente estudio), con la finalidad de clarificar su taxonomía y posición filogenética. Los resultados obtenidos, los que también incluyen el perfil de sensibilidad antifúngica de aquellas especies de hongos celomicetos más frecuentemente recuperadas de especímenes clínicos, se exponen a continuación:

Del estudio de los aislados clínicos provenientes de los Estados Unidos de Norteamérica, así como de los aislados ambientales:

1. Los 230 aislados procedentes del *Fungus Testing Laboratory, University of Texas Health Science Center* (UTHSC, San Antonio, EE.UU.) se distribuyeron en once órdenes diferentes: *Botryosphaeriales*, *Capnodiales*, *Diaporthales*, *Glomerellales*, *Helotiales*, *Hypocreales*, *Hysteriales*, *Magnaporthales*, *Pleosporales*, *Valsariales* y *Xylariales*, siendo el orden *Pleosporales* el que agrupó el mayor número de hongos celomicetos (n = 152, 66.1 %).

2. A partir del estudio polifásico de 70 aislados del UTHSC (identificados previamente como *Phoma* spp. o *Pyrenochaeta* spp.), 71 cepas de referencia o tipo del *Westerdijk Fungal Biodiversity Institute* (CBS, Utrecht, Países Bajos), un aislado del *International Collection of Microorganisms from Plants* (ICMP, Nueva Zelanda) y un aislado de la colección de microorganismos Facultad de Medicina de Reus (FMR, España), se propusieron cuatro nuevas familias, 13 nuevos géneros y 29 nuevas especies, con un total de 20 nuevas combinaciones y cuatro tipificaciones, las cuales se mencionan a continuación:
   Nuevas familias: *Neopyrenochaetaceae* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Parapyrenochaetaceae* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaetaceae* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pyrenochaetopsidaceae* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel; Nuevos géneros: *Allocucurbitaria* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Cumuliphoma* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ectophoma* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Juxtiphoma* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Neopyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano,

Neopyrenochaetopsis Valenzuela-Lopez, Cano, Guarro & Stchigel, *Paracucurbitaria* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Parapyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Remotididymella* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Similiphoma* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Vacuiphoma* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Xenopyrenochaetopsis* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano; Nuevas especies: *Allocucurbitaria botulispora* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Allophoma cylindrispora* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Cumuliphoma indica* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Cu*. *pneumoniae* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Didymella brunneospora* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *D*. *keratinophila* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Epicoccum catenisporum* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ep*. *keratinophilum* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Ep*. *ovisporum* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ep*. *pneumoniae* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Neoascochyta cylindrispora* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Neoa*. *tardicrescens* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Neocucurbitaria aquatica* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neocu*. *irregularis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Neopyrenochaeta fragariae* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neopyrenochaetopsis hominis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Nothophoma macrospora* Valenzuela-Lopez, Stchigel, Cano & Deanna A. Sutton, *No. variabilis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Paracucurbitaria italica* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaeta terrestris* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Pyrenochaetopsis americana* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py*. *botulispora* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py*. *confluens* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py*. *globosa* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py*. *paucisetosa* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py*. *setosissima* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Py*. *uberiformis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Remotididymella anthropophila* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Vacuiphoma oculihominis* Valenzuela-Lopez, Stchigel, Guarro & Cano; Nuevas combinaciones: *Cumuliphoma omnivirens* (Aveskamp *et al*.) Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ectophoma multirostrata* (P.N. Mathur et al.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Ec*. *pomi* (Horne) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Epicoccum proteae* (Crous) Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Juxtiphoma eupyrena* (Sacc.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Neocucurbitaria cava* (Schulzer) Valenzuela-

Lopez, Crous, Stchigel, Guarro & Cano, *Neocu*. *hakeae* (Crous) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neocu*. *keratinophila* (Verkley *et al*.) Valenzuela-Lopez, Stchigel, Guarro & Cano, *Neopyrenochaeta acicola* (Moug. & Lév.) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neopy*. *inflorescentiae* (Crous *et al*.) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neopy*. *telephoni* (Rohit Sharma *et al*.) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Paracucurbitaria corni* (Bat. & A.F. Vital) Valenzuela-Lopez, Stchigel, Guarro & Cano, *Parapyrenochaeta acaciae* (Crous *et al*.) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Parapy*. *protearum* (Crous) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaeta lycopersici* (R.W. Schneid. & Gerlach) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Remotididymella destructiva* (Plowr.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Similiphoma crystallifera* (Gruyter *et al*.) Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Vacuiphoma bulgarica* (Aveskamp *et al*.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Xenodidymella saxea* (Aveskamp *et al*.) Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Xenopyrenochaetopsis pratorum* (P.R. Johnst. & Boerema) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano.

3. El resto de los aislados del UTHSC (n = 82), CBS (n = 24) y FMR (n = 2) se distribuyeron en 15 familias del orden *Pleosporales*, se proponen como potenciales nuevos taxones y que corresponden a un total de una nueva familia, 12 nuevos géneros y 30 nuevas especies, más cinco combinaciones nuevas, las cuales se mencionan a continuación:
   Nueva familia: *Medicopsidaceae*; Nuevos géneros: *Deannamyces*, *Dictyophoma*, *Didymosphaeomyces*, *Neodictyophoma*, *Neothyridaria*, *Parachaetomella*, *Paranigrograna*, *Pararoussoella*, *Setosamyces*, *Sphaeriamyces*, *Xenoleptosphaeria*, *Xenoroussoella*; Nuevas especies: *Anteaglonium oculorum*, *Deannamyces macrospora*, *Dictyophoma flavescens*, *Didymosphaeomyces unguis*, *Edenia oculi*, *Keissleriella profunda*, *Montagnula cylindrispora*, *Neodictyophoma brunneospora*, *Neosetophoma americana*, *Neothyridaria solani*, *Nigrograna cutanea*, *Parachaetomella ligniputridi*, *Paranigrograna pneumonia*, *Paraphaeosphaeria ellipsospora*, *Paraphaeos*. *suttonii*, *Pararoussoella pulmonaris*, *Parathyridaria hominis*, *Parathy*. *naris*, *Parathy*. *ovina*, *Phaeodothis diversispora*, *Roussoella oculi-hominis*, *Setosamyces obispora*, *Sphaeriamyces fuckelii*, *Trematophoma pneumonia*, *Trematosphaeria hominis*, *Trematosphae*. *setosa*, *Xenoleptosphaeria confluens*, *Xenoroussoella coprophila*, *Xenoro*. *papuae*, *Xenoro*.*profunda*; Nuevas combinaciones: *Neothyridaria solani*, *Setosamyces glycines*, *Se*. *telephii*, *Thyridaria mukdahanensis*, *Xenoroussoella mexicana*.

Del estudio de los aislados clínicos provenientes de dos laboratorios de referencia europeos (*Instituto Carlos III*, Madrid, España, y el *Institut Pasteur*, París, Francia):

1. Los 97 aislados clínicos provenientes de España (n = 46) y Francia (n = 51) se distribuyeron en cuatro órdenes diferentes: *Botryosphaeriales*, *Diaporthales*, *Glomerellales* y *Pleosporales*, siendo este último el más prevalente (n = 78; 81 %).

2. Ocho aislados pertenecieron a la especie *Neocucurbitaria keratinophila*, motivo por el cual se sospecha que es un patógeno emergente de distribución geográfica limitada (a España), motivo por el cual fueron caracterizadas tanto morfológica como molecularmente (mediante las secuencias nucleotídicas de los marcadores LSU, ITS, *tub*2 y *rpb*2), además de determinar su patrón de sensibilidad antifúngica *in vitro*.

Del estudio de los aislados ambientales provenientes de España y otro aislado ambiental procedente de Nueva Zelanda se concluye lo siguiente:

1. De un total de 20 aislados ambientales colectados en España y uno de Nueva Zelanda, se obtuvieron un género nuevo y cuatro nuevas especies para la ciencia: *Alfaria dactylis*, *Coniella heterospora*, *Pseudoascochyta novae-zelandiae* y *Pseudoascochyta pratensis*.

Del estudio de sensibilidad *in vitro* de diferentes géneros de hongos celomicetos frente a antifúngicos:

1. La mayoría de los 139 aislados presentaron CMIs bajas frente a los antifúngicos ensayados.

2. Los aislados de *Colletotrichum* spp. presentaron CMIs elevadas frente al itraconazol y el voriconazol, y en el caso de un aislado frente a la caspofungina y la micafungina.

3. Los aislados de *Didymella* spp. presentaron CMIs elevadas frente a la anfotericina B, itraconazol, voriconazol, anidulafungina y caspofungina.

4. Los aislados de *Neoascochyta desmazieri* presentaron CMIs elevadas solo frente al voriconazol.

5. Los aislados de *Neoscytalidium dimidiatum* presentaron CMIs elevadas frente al voriconazol, itraconazol, caspofungina y micafungina.

6. Los aislados de *Medicopsis romeroi* presentaron CMIs elevadas frente a la anfotericina B, itraconazol, caspofungina y micafungina.

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7. BIBLIOGRAFÍA

UNIVERSITAT ROVIRA I VIRGILI
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Abd-elsalam KA, Tibpromma S, Wanasinghe DN, Camporesi E, Hyde KD. *Equiseticola* gen. nov. (*Phaeosphaeriaceae*), from *Equisetum* sp. in Italy. *Phytotaxa*. 2016;284:169–180.

Ahmed SA, Stevens DA, Van de Sande WWJ, Meis JF, de Hoog GS. *Roussoella percutanea*, a novel opportunistic pathogen causing subcutaneous mycoses. *Med Mycol*. 2014a;52:689–698.

Ahmed SA, Van De Sande WW, Stevens DA, Fahal A, Van Diepeningen AD, Menken SB, de Hoog GS. Revision of agents of black-grain eumycetoma in the order *Pleosporales*. *Persoonia*. 2014b;33:141–154.

Ahmed SA, de Hoog GS, Stevens DA, Fahal AH, van de Sande WW. In vitro antifungal susceptibility of coelomycete agents of black grain eumycetoma to eight antifungals. *Med Mycol*. 2015a;53:295–301.

Ahmed SA, Desbois N, Quist D, *et al*. Phaeohyphomycosis caused by a novel Species, *Pseudochaetosphaeronema martinelli*. *J Clin Microbiol*. 2015b;53:2927–2934.

Ahmed SA, Hofmüller W, Seibold M, *et al*. *Tintelnotia*, a new genus in *Phaeosphaeriaceae* harbouring agents of cornea and nail infections in humans. *Mycoses*. 2017;60:244–253.

Ainsworth GC. A general purpose classification of fungi. *Bibliography of Systematic Mycology* 1966;1:1–4.

Allen D, Wilson D, Drew R, Perfect J. Azole antifungals: 35 years of invasive fungal infection management. *Expert Rev Anti Infect Ther*. 2015;13:787–798.

Al-Nakeeb Z, Petraitis V, Goodwin J, Petraitiene R, Walsh TJ, Hope WW. Pharmacodynamics of amphotericin B deoxycholate, amphotericin B lipid complex, and liposomal amphotericin B against *Aspergillus fumigatus*. *Antimicrob Agents Chemother*. 2015;59:2735–2345.

Al-Saadoon AH, Al-Dossary MN. Fungi from submerged plant debris in aquatic habitats in Iraq. *Int J Biodivers Conserv*. 2014;6:468–487.

Alves A, Crous PW, Correia A and Phillips AJL. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Divers*. 2008;28:1–13.

Ameen M. Epidemiology of superficial fungal infections. *Clin Dermatol* 2010;28:197–201.

Ananda-Rajah MR, Grigg A, Downey MT, *et al*. Comparative clinical effectiveness of prophylactic voriconazole/posaconazole to fluconazole/itraconazole in patients with acute myeloid leukemia/myelodysplastic syndrome undergoing cytotoxic chemotherapy over a 12-year period. *Haematologica* 2012;97:459–463.

Arenas R. Micología médica ilustrada. 4ª ed. España, Interamericana McGraw-Hill; 2011.

Ariyawansa, HA, Phukhamsakda, C, Thambugala, KM, *et al*. *Fungal Divers*. 2015;74:19–51.

Arora P, Dilbaghi N, Chaudhury A. Opportunistic invasive fungal pathogen *Macrophomina phaseolina* prognosis from immunocompromised humans to potential mitogenic RBL with an exceptional and novel antitumor and cytotoxic effect. *Eur J Clin Microbiol Infect Dis*. 2012;31:101–7.

Aveskamp MM, Gruyter J de, Crous PW. Biology and recent developments in the systematics of *Phoma*, a complex genus of major quarantine significance. *Fungal Divers*. 2008;31:1–18.

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

Baker JG, Salkin IF, Forgacs P, Haines JH, Kemna ME. First report of subcutaneous phaeohyphomycosis of the foot caused by *Phoma minutella*. *J Clin Microbiol*. 1987;25:2395–2397.

Bakerspigel A, Lowe D, Rostas A. The isolation of *Phoma eupyrena* from a human lesion. *Arch Dermatol*. 1981;117:362–363.

Bakerspigel A. The isolation of *Phoma hibernica* from lesions on a leg. *Sabouraudia*. 1970;7:261–264.

Bakhshizadeh M, Hashemian HR, Najafzadeh MJ, Dolatabadi S, Zarrinfar H. First report of rhinosinusitis caused by *Neoscytalidium dimidiatum* in Iran. *J Med Microbiol*. 2014;63:1017–1019.

Balis E, Velegraki A, Fragou A, *et al*. Lung mass caused by *Phoma exigua*. *Scand J Infect Dis*. 2006;38:552–555.

Barr ME. Prodomus to class *Loculoascomycetes*. Amherst, Massachusetts; 1987.

Barua P, Barua S, Borkakoty B, Mahanta J. Onychomycosis by *Scytalidium dimidiatum* in green tea leaf pluckers: report of two cases. *Mycopathologia*. 2007;164:193–195.

Benne CA, Neeleman C, Bruin M, *et al*. Disseminating infection with *Scytalidium dimidiatum* in a granulocytopenic child. *Eur J Clin Microbiol Infect Dis*. 1993;12:118–121.

Boerema GH, de Gruyter J, Noordeloos ME, Hamers M. *Phoma* identification manual. Differentiation of specific and infraspecific taxa in culture. CABI Publishing, Cambridge, United Kingdom; 2004.

Borelli D. *Pyrenochaeta romeroi* nova especie. *Dermay Venez*. 1959;1:325–326.

Borelli D, Zamora R, Senabre G. *Chaetosphaeronema larense nova* species agente demicetoma.*Gaceta Med Caracas*. 1976;84:307–318.

Borelli D. *Pyrenochaeta mackinnonii nova* species agente de micetoma. *Castellania*. 1976;4:227–234.

Brandt ME, Warnock DW. Taxonomy and classification of fungi. Manual of clinical microbiology, 11ª ed, pp. 1935–1943. ASM Press, Washington, USA; 2015.

Brock D. Infectious Fungi. Chelsea House Publishers, New York, USA; 2006.

Bubák. Oesterreichische botanische Zeitschrift. 1905;55: 78.

Cannon PF, Damm U, Johnston PR, Weir BS. *Colletotrichum* – current status and future directions. *Stud Mycol*. 2004;73:181–213.

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

Cariello PF, Wickes BL, Sutton DA, *et al*. *Phomopsis bougainvilleicola* prepatellar bursitis in a renal transplant recipient. *J Clin Microbiol*. 2013;51:692–695.

Carlile MJ, Watkinson SC. The Fungi. 2ª ed. Academic Press Inc, Londres, UK; 1994.

Carrillo-Muñoz AJ, Pemán J, Gobernado M. New antifungals drugs. Present and future. *Rev Esp Quimioter*. 1999;12:181–204.

Castlebury LA, Rossman AY, Jaklitsch WJ, *et al*. A phylogeny overview of the *Diaporthales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia*. 2002;94:1017–1031.

Castro LG, da Silva LC, Guarro J, *et al*. *Phaeohyphomycotic* cyst caused by *Colletotrichum crassipes*. *J Clin Microbiol*. 2001;39:2321–2324.

Chadefaud M. Les végétaux non vasculaires (Cryptogamie). In: Traité de botanique systématique. Tome I (Chadefaud M, Emberger L, eds), Masson et Cie, Paris, France;1960.

Chen Q, Jiang JR, Zhang GZ, *et al*. Resolving the *Phoma* enigma. *Stud Mycol*. 2015;82:137–217.

Chen Q, Hou LW, Duan WJ, *et al*. *Didymellaceae* revisited. *Stud Mycol*. 2017;87:105–159.

Cho JC, Sharma RS, Sutton DA, *et al*. Fungal arthritis secondary to *Colletotrichum gloeosporioides*. *JMM Case Rep 2*. 2015. doi: 10.1099/jmmcr.0.000012.

Chowdhary A, Kathuria S, Singh PK, *et al*. Molecular characterization and in vitro antifungal susceptibility profile of *Schizophyllum commune*, an emerging basidiomycete in bronchopulmonary mycoses. *Antimicrob Agents Chemother*. 2013;57:2845–2848.

Chowdhary A, Meis JF, Guarro J, *et al*. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of systemic phaeohyphomycosis: diseases caused by black fungi. *Clin Microbiol Infect*. 2014;20:47–75.

Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard. 2ª ed. Documento M38-A2. Clinical and Laboratory Standards Institute, Wayne, USA; 2008.

Colombier MA, Alanio A, Denis B, *et al*. Dual invasive infection with *Phaeoacremonium parasiticum* and *Paraconiothyrium cyclothyrioides* in a renal transplant recipient: Case report and comprehensive review of the literature of *Phaeoacremonium phaeohyphomycosis*. *J Clin Microbiol*. 2015;53:2084–2094.

Cortinas MN, Burgess T, Dell B, *et al*. First record of *Colletogloeopsis zuluense* comb. nov., causing a stem canker of *Eucalyptus* in China. *Mycol Res*. 2006;110:229–236.

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G. MycoBank: an online initiative to launch mycology into the 21st century. *Stud Mycol*. 2004;50:19–22.

Crous PW, Slippers B, Wingfield MJ. Phylogenetic lineages in the *Botryosphaeriaceae*. *Stud Mycol*. 2006;55:235–254.

Crous PW, Verkley GJM, Groenewald JZ, *et al*. Fungal Biodiversity. CBS Laboratory Manual Series. Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; 2009.

Crous PW, Wingfield MJ, Burgess TI, *et al*. Fungal Planet description sheets: 558–624. *Persoonia*. 2017;38:240–384.

Cursi IB, Silva RT, Succi IB, *et al*. Onychomycosis due to *Neoscytalidium* treated with oral terbinafine, ciclopirox nail lacquer and nail abrasion: A pilot study of 25 patients. *Mycopathologia*. 2013;175:75–82.

Damm U, Cannon PF, Woudenberg JH, *et al*. The *Colletotrichum boninense* species complex. *Stud Mycol*. 2012;73:1–36.

de Gruyter J, Aveskamp MM, Woudenberg JHC, *et al*. Molecular phylogeny of *Phoma* and allied anamorph genera: towards a reclassification of the *Phoma* complex. *Mycol Res*. 2009;113:508–519.

de Gruyter J, Woudenberg JH, Aveskamp MM, *et al*. Redisposition of *Phoma*-like anamorphs in *Pleosporales*. *Stud Mycol*. 2013;75:1–36.

de Hoog GS, Guarro J, Gené J, Figueras MJ. 2011. Atlas of clinical fungi, electronic version 3.1. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Países Bajos; 2011.

da Silva M, Cerniglia CE, Pothuluri JV, Canhos VP, Esposito E. Screening filamentous fungi isolated from estuarine sediments for the ability to oxidize polycyclic aromatic hydrocarbons. *World J Microbiol Biotechnol*. 2003;19:399–405.

da Silva RT, Guimarães DA, Camargo ZP, *et al*. Cutaneous murine model of infection caused by *Neoscytalidium dimidiatum*: a preliminary study of an emerging human pathogen. *Med Mycol*. 2016;54:890–898.

Diederich P, van den Boom PPG, Aptroot A. *Cladoniicola staurospora* gen. et sp. nov., a new lichenicolous coelomycete from Western Europe. *Belgian J Bot*. 2001;134:127–130.

Diederich P, Lawrey JD, Sikaroodi M, Van Den Boom PPG, Ertz D. *Briancoppinsia*, a new coelomycetous genus of *Arthoniaceae* (*Arthoniales*) for the lichenicolous *Phoma cytospora*, with a key to this and similar taxa. *Fungal Divers*. 2012;52:1–12.

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

El-Bassam S, Benhamou N, Carisse O. The role of melanin in the antagonistic interaction between the apple scab pathogen *Venturia inaequalis* and *Microsphaeropsis ochracea*. *Can J Microbiol*. 2002;48:349–358.

Elewski BE. Onychomycosis caused by *Scytalidium dimidiatum*. *J Am Acad Dermatol*. 1996;35:336–337.

Ferrer C, Pérez-Santonja JJ, Rodríguez AE, *et al*. New *Pyrenochaeta* species causing keratitis. *J Clin Microbiol*. 2009;47:1596–1598.

Ferrer R, Gómez de la Cámara A, Jarque I, Quindós G, Tejerina T. Estudio comparativo multidisciplinar de las equinocandinas, anidulafungina, caspofungina y micafungina. *Actualidad en Farmacología y Terapéutica*. 2013;11:146–159.

Figtree M, Weeks K, Chan L, *et al*. *Colletotrichum gloeosporioides sensu lato* causing deep soft tissue mycosis following a penetrating injury. *Med Mycol Case Rep*. 2013;2:40–43.

Fisher NL, Burgess LW, Toussoun TA, Nelson PE. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology*. 1982;72:151–153.

Fukami A, Nakamura T, Kim YP, *et al*. A new anti-influenza virus antibiotic, 10-norparvulenone from *Microsphaeropsis* sp FO-5050. *J Antibiot*. 2000;53:1215–1218.

Gajjar DU, Pal AK, Parmar TJ, *et al*. Fungal scleral keratitis caused by *Phomopsis phoenicicola*. *J Clin Microbiol*. 2011;49:2365–2368.

Gavalda J,Ruiz I. Guidelines for the treatment of infection due to *Aspergillus* spp. *Enferm Infecc Microbiol Clin*. 2003;21:571–578

Girard C, Dereure O, Rispail P, *et al*. Subcutaneous phaeohyphomycosis due to *Pyrenochaeta romeroi* in a patient with leprosy. *Acta Derm Venereol*. 2004;84:154–155.

Godoy P, Nunes FG, Tomimori-Yamashita J, *et al*. Onychomycosis in São Paulo, Brazil. *Mycopathologia*. 2009;168:111–116.

Gomes RR, Glienke C, Videira SI, *et al*. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia*. 2013;31:1–41.

Gordon RA, Sutton DA, Thompson EH, *et al*. Cutaneous phaeohyphomycosis caused by *Paraconiothyrium cyclothyrioides*. *J Clin Microbiol*. 2012;50:3795–3798.

Griffon E, Maublanc A. Sur une maladie du cacaoyer. *Bulletin de la Société Mycologique de France*. 1909;25:51–58.

Grove WB. Mycological notes IV. *J Bot Lond*. 1919;57:206–210.

Gu HJ, Kim YJ, Lee HJ, *et al*. Invasive fungal sinusitis by *Lasiodiplodia theobromae* in an patient with aplastic anemia: An extremely rare case report and literature review. *Mycopathologia*. 2016;181:901–908.

Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clin Microbiol Rev*. 2011;24:247–280.

Guarro J, Svidzinski TE, Zaror L, *et al*. Subcutaneous hyalohyphomycosis caused by *Colletotrichum gloeosporioides*. *J Clin Microbiol*. 1998;36:3060–3065.

Guégan S, Garcia-Hermoso D, Sitbon K, *et al*. Ten-Year experience of cutaneous and/or subcutaneous infections due to Coelomycetes in France. *Open Forum Infect Dis*. 2016 doi:10.1093/ofid/ofw106.

Haeckel E. Generelle Morphologie der Organismen: allgemeine Grundzüge der organischen Formen-Wissenschaft, mechanisch begründet durch die von C. Darwin reformirte Decendenz-Theorie. Berlin; 1866.

Harris SD. Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems. *Mycologia*. 2008;100:823–832.

Hay RJ. *Scytalidium* infections. *Curr Op Infect Dis*. 2002;15:99–100.

Hibbett DS, Binder M, Bischoff JF, *et al*. A higher-level phylogenetic classification of the Fungi. *Mycol Res*. 2007;111:509−547.

Hirsh AH, Schiff TA. Subcutaneous phaeohyphomycosis caused by an unusual pathogen: *Phoma* species. *J Am Acad Dermatol*. 1996;34:679–680.

Hyde KD, Jones EBG, Liu JK, *et al*. Families of *Dothideomycetes*. *Fungal Divers*. 2013;63:1–313.

Hyde KD, Nilsson RH, Alias SA, *et al*. One stop shop: backbones trees for important pytopathogenic genera: I. *Fungal Divers*. 2014;67:21–125.

Ikram A, HussainW, Satti ML,Wiqar MA. Invasive infection in a young immunocompetent soldier caused by *Scytalidium dimidiatum*. *J Coll Physicians Surg Pak*. 2009;19:64–66.

Iriart X, Binois R, Fior A, *et al*. Eumycetoma caused by *Diaporthe phaseolorum* (*Phomopsis phaseoli*): a case report and a mini-review of *Diaporthe*/*Phomopsis* spp invasive infections in humans. *Clin Microbiol Infect*. 2011;17:1492–1494.

Jaklitsch WM, Voglmayr H. Hidden diversity in *Thyridaria* and a new circumscription of the *Thyridariaceae*. *Stud Mycol*. 2016;85:35–64.

Jaklitsch WM, Checa J, Blanco MN, *et al*. A preliminary account of the *Cucurbitariaceae*. *Stud Mycol*. 2018;90:71–118.

James JE, Santhanam J, Lee MC, *et al*. In vitro antifungal susceptibility of *Neoscytalidium dimidiatum* clinical isolates from Malaysia. *Mycopathologia*. 2017;182:305–313.

Kendrick WB. The Fifth Kingdom, 3ª ed. Focus Publishing, Newbury;2000.

Kiehn TE, Polsky B, Punithalingam E, *et al*. Liver infection caused by *Coniothyrium fuckelii* in a patient with acute myelogenous leukemia. *J Clin Microbiol*. 1987;25:2410–2412.

Kindo AJ, Pramod C, Anita S, Mohanty S. Maxillary sinusitis caused by *Lasiodiplodia theobromae*. *Indian J Med Microbiol*. 2010;28:167–169.

Kirk PM, Cannon PF, Stalpers JA, Minter DW. Ainsworth & Bisby's dictionary of the fungi, 10th ed. CAB International, Wallingford, United Kingdom; 2008.

Kornerup A, Wanscher JH. Methuen Handbook of Colour, 3ª ed. Methuen, London, England; 1978.

Krockenberger MB. Localised *Microsphaeropsis arundinis* infection of the subcutis of a cat. *J Feline Med Surg*. 2010;12:3231–3236.

Laniado-Laborín R, Cabrales-Vargas MN. Amphotericin B: side effects and toxicity. *Rev Iberoam Micol*. 2009;26:223–227.

Lawrey JD, Diederich P, Nelsen MP, *et al*. The first molecular phylogeny of the lichenicolous genus *Lichenoconium* (*Ascomycota*, *Dothideomycetes*), including a new species from Europe. *Fungal Biol*. 2011;115:176–187.

Lewis RE. Current concepts in antifungal pharmacology. *Mayo Clin Proc*. 2011;86:805–817.

Liao WQ, Shao JZ, Li SQ, *et al*. *Colletotrichum dematium* caused keratitis. *Chin Med J*. 1983;96:391–394.

Linneo C. Species plantarum: exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas. Holmiae: Impensis Laurentii Salvii, Estocolmo. Suecia; 1753.

Liu F, Cai L, Crous PW, Damm U. The *Colletotrichum gigasporum* species complex. *Persoonia*. 2014;33:83–97.

Liu JK, Hyde KD, Jeewon R, *et al*. Ranking higher taxa using divergence times: a case study in *Dothideomycetes*. *Fungal Divers*. 2017;84:75–99.

Luttrell ES. The ascostromatic *Ascomycetes*. *Mycologia*. 1955;47:511–532.

Machouart M, Menir P, Helenon R, Quist D, Desbois N. *Scytalidium* and *scytalidiosis*: what's new in 2012? *J Mycol Med*. 2013;23:40–46.

Mackinnon JE, Ferrada-Urzúa LV, Montemayor L. *Madurella grisea* n. sp. *Mycopathologia*. 1949;4:384–393.

Madrid H, Ruíz-Cendoya M, Cano J, *et al*. Genotyping and in vitro antifungal susceptibility of *Neoscytalidium dimidiatum* isolates from different origins. *Int J Antimicrob Agents*. 2009;34:351–354.

Maharachchikumbura SSN, Guo LD, Chukeatirote E, Bahkali AH, Hyde KD. *Pestalotiopsis*-morphology, phylogeny, biochemistry and diversity. *Fungal Divers*. 2011;50:167–187.

Maharachchikumbura SSN, Guo LD, Chukeatirote E, McKenzie EHC, Hyde KD. A destructive new disease of *Syzygium samarangense* in Thailand caused by the new species *Pestalotiopsis samarangensis*. *Trop Plant Pathol*. 2013;38:227–235.

Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW. *Pestalotiopsis* revisited. *Stud Mycol*. 2014;79:121–186.

Maharachchikumbura SSN, Hyde KD, Jones EBG, *et al*. Families of *Sordariomycetes*. *Fungal Divers*. 2016;79:1–317.

Mani RS, Chickabasaviah YT, Nagarathna S, *et al*. Cerebral phaeohyphomycosis caused by *Scytalidium dimidiatum*: a case report from India. *Med Mycol*. 2008;46:705–711.

Mendiratta DK, Thamke D, Shukla AK, Narang P. Keratitis due to *Colletotrichum dematium*-A case report. *Indian J Med Microbiol*. 2005;23:56–58.

Miller MA, Pfeiffer W, Schwartz T. The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. In: Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the Extreme to the Campus and Beyond: 1–8. Association for Computing Machinery, USA; 2012.

Nannfeldt JA. Studien über die Morphologie und Systematik der nichtlichenisierten inoperculaten *Discomyceten*. *Nova Acta Regiae Societatis Scientiarum Upsaliensis*. 1932;8:1–368.

Nattrass RM. A new species of *Hendersonula* (*H. toruloidea*) on deciduous trees in Egypt. *Trans Br Mycol Soc*. 1933;18:189–198.

Nitschke T. Pyrenomycetes Germanici. 1870;2:161–320.

Nylander JAA. MrModeltest v2. Program Distributed by the Author. Evolutionary Biology Centre, Uppsala University, Sweden; 2004.

Ocampo MA, Kanitakis J, Bienvenu AL, *et al*. Phaeohyphomycosis caused by *Pyrenochaeta romeroi* mimicking a plantar wart in a kidney transplant recipient. *Transpl Infect Dis*. 2012;14:173–174.

Oliveira RJV, Lima TEF, Cunha IB, *et al*. *Corniculariella brasiliensis*, a new species of *coelomycetes* in the *rhizosphere* of *Caesalpinia echinata* (*Fabaceae*, *Caesalpinioideae*) in Brazil. *Phytotaxa*. 2014;178:197–204.

O'Quinn RP, Hoffmann JL, Boyd AS. *Colletotrichum* species as emerging opportunistic fungal pathogens: a report of 3 cases of phaeohyphomycosis and review. *J Am Acad Dermatol*. 2001;45:56–61.

Patouillard NT, Lagerheim G, de. Champignons de l'Equateur (Pugillus II). *Bull trimest soc mycol Fr*. 1892;8:113–140.

Penzig O. Funghi agrumicoli. Contribuzione allo studio dei funghi parassiti degli agrumi. *Michelia*. 1882;2:385–508.

Perfect JR, Dismukes WE, Dromer F, *et al*. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of América. *Clin Infect Dis*. 2010;1:291–322.

Potea ST, Chakraborty A, Lahiri KK, *et al*. Keratitis by a rare pathogen *Colletotrichum gloeosporioides*: A case report. *J Mycol Med*. 2017;27:407–411.

Punithalingam E, English MP. *Pyrenochaeta unguis-hominis* sp. nov. on human toe-nails. *Trans Br Mycol Soc*. 1975;64:539–541.

Punithalingam E. *Phoma oculo hominis* sp. nov. from corneal ulcer. *Trans Br Mycol Soc*. 1976;67:142–143.

Punithalingam E. *Sphaeropsidales* in culture from humans. *Nova Hedwigia*. 1979;31:119–158.

Rai MK. *Phoma sorghina* infection in human being. *Mycopathologia*. 1989;105:167–170.

Rajagopal K, Maheswari S, Kathiravan G. Diversity of endophytic fungi in some tropical medicinal plants – A report. *Afr J Micro Res*. 2012;6:2822–2827.

Réblová M, Gams W, Seifert KA. *Monilochaetes* and allied genera of the *Glomerellales*, and a reconsideration of families in the *Microascales*. *Stud Mycol*. 2011;68:163–191.

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

Richardson MD, Warnock DW. Fungal Infection: Diagnosis and Management, 4ª ed. Wiley-Blackwell, Nueva York, USA; 2012.

Roehm CE, Salazar JC, Hagstrom N, Valdez TA. *Phoma* and *Acremonium* invasive fungal rhinosinusitis in congenital acute lymphocytic leukemia and literature review. *Int J Pediatr Otorhinolaryngol*. 2012;76:1387–1391.

Ronquist F, Teslenko M, van der Mark P, *et al*. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol*. 2012;61: 539–542.

Rosen T, Rinaldi MJ, Tschen JA, Stern JK, Cernoch P. Cutaneous lesions due to *Pleurophoma* (*Phoma*) complex. *South Med J*. 1996;89:431–434.

Rossman AY, Farr DF, Castlebury LA. A review of the phylogeny and biology of the *Diaporthales*. *Mycoscience*. 2007;48:135–144.

Rossman AY, Adams GC, Cannon PF, *et al*. Recommendations of generic names in *Diaporthales* competing for protection or use. *IMA Fungus*. 2015;6:145–154.

Ruiz-Camps I, Cuenca-Estrella M. Antifungal for systemic use. *Enferm Infecc Microbiol Clin*. 2009;27:353–362.

Saha S, Sengupta J, Banerjee D, Khetan A. *Lasiodiplodia theobromae* keratitis: a case report and review of literature. *Mycopathologia*. 2012;174:335–339.

Scagel RF, Bandoni RJ, Rouse GE, Schofield WE, Stein JR, Taylor TMC. El reino vegetal. Omega, Barcelona, España; 1980.

Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW. A multigene phylogeny of the *Dothideomycetes* using four nuclear loci. *Mycologia*. 2006;98:1041–1052.

Schoch CL, Crous PW, Groenewald JZ, *et al*. 2009. A class-wide phylogenetic assessment of *Dothideomycetes*. *Stud Mycol*. 2009;64:1–15.

Senanayake IC, Crous PW, Groenewald JZ. Families of *Diaporthales* based on morphological and phylogenetic evidence. *Stud Mycol*. 2017;86:217–296.

Shivaprakash MR, Appannanavar SB, Dhaliwal M, *et al*. *Colletotrichum truncatum*: An unusual pathogen causing mycotic keratitis and endophthalmitis. *J Clin Microbiol*. 2011;49:2894–2898.

Shukla NP, Rajak RK, Agarwasl GP, Gupta D. *Phoma minutispora* as a human pathogen. *Mykosen*. 1984;27:255–258.

Someya A, Yaguchi T, Udagawa S. *Microsphaeropsis rugospora*, a new species from Japanese soil. *Mycoscience*. 1997;38:429–431.

Spatafora JW, Aime MC, Grigoriev IV, Martin F, Stajich JE, Blackwell M. The Fungal Tree of Life: from Molecular Systematics to Genome-Scale Phylogenies. *Microbiol Spectr*. 2017. doi: 10.1128/microbiolspec.FUNK-0053-2016.

Stchigel AM, Sutton DA. Coelomycete fungi in the clinical lab. *Curr Fungal Infect Rep*. 2013;7:171–191.

Su YY, Qi YL, Cai L. Induction of sporulation in plant pathogenic fungi. *Mycology*. 2012 3:195–200.

Subcommittee on Antifungal Susceptibility Testing of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). EUCAST Technical Note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds. *Clin Microbiol Infect*. 2008;14:982–984.

Summerbell RC, Krajden S, Levine R, Fuksa M. Subcutaneous phaeohyphomycosis caused by *Lasiodiplodia theobromae* and successfully treated surgically. Med Mycol. 2004;42:543–547.

Sutton BC. The Coelomycetes. Fungi Imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological, Kew, England; 1980.

Sutton DA. Coelomycetous fungi in human disease. A review: clinical entities, pathogenesis, identification and therapy. *Rev Iberoam Micol*. 1999;16:171–179.

Swofford DL. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer, Sunderland, USA; 2000.

Tamura K, Stecher G, Peterson D, *et al*. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*. 2013;31:2725–2729.

Tan DH, Sigler L, Gibas CF, Fong IW. Disseminated fungal infection in a renal transplant recipient involving *Macrophomina phaseolina* and *Scytalidium dimidiatum*: case report and review of taxonomic changes among medically important members of the *Botryosphaeriaceae*. *Med Mycol*. 2008;46:285–92.

Taylor JW. Making the *Deuteromycota* redundant: a practical integration of mitosporic and meiosporic fungi. *Can J Bot*. 1995;73:754–759.

Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680.

Tibpromma S, Liu JK, Promputtha I, *et al*. *Ophiosimulans tanaceti* gen. et sp. nov. (*Phaeosphaeriaceae*) on *Tanacetum* sp. (*Asteraceae*) from Italy. *Mycol Progress*. 2016;15:46.

Tsuda M, Mugishima T, Komatsu K, *et al*. Modiolides A and B, two new 10-membered macrolides from a marine derived fungus. *J Nat Prod*. 2003;66:412–415.

Tullio V, Banche G, Allizond V, *et al*. Non-dermatophyte moulds as skin and nail foot mycosis agents: *Phoma herbarum*, *Chaetomium globosum* and *Microascus cinereus*. *Fungal Biol*. 2010;114:345–349.

Damm U, Sato T, Alizadeh A, Groenewald JZ, Crous PW. The *Colletotrichum dracaenophilum*, *C*. *magnum* and *C*. *orchidearum* species complexes. *Stud Mycol*. 2019;92:1–46.

Udayanga D, Liu X, McKenzie EHC, Chukeatirote E, Bahkali AHA, Hyde KD. The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. *Fungal Divers*. 2011;50:189–225.

Udayanga D, Liu X, Crous PW, McKenzie EHC, Chukeatirote E, Hyde KD. A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). *Fungal Divers*. 2012;56:157–171.

Valenzuela-Lopez N, Sutton DA, Cano-Lira JF, *et al*. Coelomycetous fungi in the clinical setting: Morphological convergence and cryptic diversity. *J Clin Microbiol*. 2017;55:552–567.

Valenzuela-Lopez N, Cano-Lira JF, Guarro J, *et al*. Coelomycetous *Dothideomycetes* with emphasis on the families *Cucurbitariaceae* and *Didymellaceae*. *Stud Mycol*. 2018a;90:1–69.

Valenzuela-Lopez N, Cano-Lira JF, Stchigel AM, Guarro J. DNA sequencing to clarify the taxonomical conundrum of the clinical coelomycetes. *Mycoses*. 2018b (IN PRESS). DOI:10.1111/myc.12785.

van de Sande WWJ. Global burden of human mycetoma: a systematic review and meta-analysis. *PLoS Negl Trop Dis*. 2013;7: e2550. https://doi.org/10.1371/journal.pntd.0002550.

van de Sande W, Fahal A, Ahmed SA, *et al*. Closing the mycetoma knowledge gap. *Med Mycol*. 2018;56:153–164.

Verkley GJM, da Silva M, Wicklow DT, Crous PW. *Paraconiothyrium*, a new genus to accommodate the mycoparasite *Coniothyrium minitans*, anamorphs of *Paraphaeosphaeria*, and four new species. *Stud Mycol*. 2004;50:323–336.

Verkley GJ, Gené J, Guarro J, *et al*. *Pyrenochaeta keratinophila* sp. nov., isolated from an ocular infection in Spain. *Rev Iberoam Micol*. 2010;27:22–24.

Verkley GJ, Dukik K, Renfurm R, Göker M, Stielow JB. Novel genera and species of coniothyrium-like fungi in *Montagnulaceae* (*Ascomycota*). *Persoonia*. 2014;32:25–51.

von Arx JA, Müller E. A reevaluation of the bitunicate *Ascomycetes* with keys to families and genera. *Stud Mycol*. 1975;9:1–159.

Wanasinghe DN, Phookamsak R, Jeewon R, *et al*. A family level rDNA based phylogeny of *Cucurbitariaceae* and *Fenestellaceae* with descriptions of new *Fenestella* species and *Neocucurbitaria* gen. nov. *Mycosphere*. 2017;8:397–414.

Weir BS, Johnston PR, Damm U. The *Colletotrichum gloeosporioides* species complex. *Stud Mycol*. 2012;73:115–180.

Welsh O, Al-Abdely HM, Salinas-Carmona MC, Fahal AH. Mycetoma medical therapy. *PLoS Negl Trop Dis*. 2014;8:e3218. doi:10.1371/journal.pntd.0003218.

Whittaker RH. New concepts of kingdoms of organisms. *Science*. 1969;163:150–160.

Wijayawardene NN, Hyde KD, Wanasinghe DN, *et al*. Taxonomy and phylogeny of dematiaceous coelomycetes. *Fungal Divers*. 2016;77:1–316.

Wikee S, Udayanga D, Crous PW, *et al*. *Phyllosticta* an overview of current status of species recognition. *Fungal Divers*. 2011;51:43–61.

Woo PC, Lau SK, Ngan AH, *et al*. *Lasiodiplodia theobromae* pneumonia in a liver transplant recipient. *J Clin Microbiol*. 2008;46:380–384.

Yegneswaran PP, Pai V, Bairy I, Bhandary S. *Colletotrichum graminicola* keratitis: first case report from India. *Indian J Ophthalmol*. 2010;58:415–417.

Zhang T, Deng X, Yu Y, Zhang M, Zhang Y. *Pseudochaetosphaeronema ginkgonis* sp. nov., an endophyte isolated from *Ginkgo biloba*. *Int J Syst Evol Microbiol*. 2016;66:4377–4381.

8. ANEXOS

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TAXONOMÍA DE HONGOS CELOMICETOS DE INTERÉS CLÍNICO
Nicomedes Miguel Antonio Valenzuela López

Tabla 1. Aislados principalmente clínicos procedentes de tres laboratorios de referencia incluidos en esta Tesis, nuevas especies están indicados en negrita.

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
1	13629	UTHSC:DI14-306	*Neoscytalidium dimidiatum*	EE.UU.		Abceso
2	13630	UTHSC:DI14-307	*Neoscytalidium dimidiatum*	EE.UU.		Uña del dedo del pie
3	13631	UTHSC:DI14-308	*Neoscytalidium dimidiatum*	EE.UU.		Talón
4	13632	UTHSC:DI14-309	*Neoscytalidium dimidiatum*	EE.UU.		Pie
5	13633	UTHSC:DI14-310	*Neoscytalidium dimidiatum*	EE.UU.		Uña
6	13634	UTHSC:DI14-311	*Neoscytalidium dimidiatum*	EE.UU.		Herida del pie
7	13635	UTHSC:DI14-312	*Neoscytalidium dimidiatum*	EE.UU.		Uña del dedo del pie
8	13636	UTHSC:DI14-313	*Neoscytalidium dimidiatum*	EE.UU.		Uña
9	13637	UTHSC:DI14-314	*Neoscytalidium dimidiatum*	EE.UU.		Herida del tobillo
10	13638	UTHSC:DI14-315	*Neoscytalidium dimidiatum*	EE.UU.		Uña
11	13639	UTHSC:DI14-316	*Neoscytalidium dimidiatum*	EE.UU.		Herida del talón
12	13640	UTHSC:DI14-317	*Neoscytalidium dimidiatum*	EE.UU.		Dedo del pie
13	13641	UTHSC:DI14-318	*Neoscytalidium dimidiatum*	EE.UU.		Uña del dedo del pie
14	13642	UTHSC:DI14-319	*Neoscytalidium dimidiatum*	EE.UU.		Piel
15	13643	UTHSC:DI14-320	*Neoscytalidium dimidiatum*	EE.UU.		Uña del dedo del pie
16	13644	UTHSC:DI14-321	*Neoscytalidium dimidiatum*	EE.UU.		Lavado broncoalveolear
17	13645	UTHSC:DI14-322	*Neoscytalidium dimidiatum*	EE.UU.		Uña
18	13646	UTHSC:DI14-323	*Neoscytalidium dimidiatum*	EE.UU.		Piel de los dedos del pie
19	13647	UTHSC:DI14-324	*Neoscytalidium dimidiatum*	EE.UU.		Uña
20	13648	UTHSC:DI14-325	*Neoscytalidium dimidiatum*	EE.UU.		Herida

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
21	13649	UTHSC:DI14-326	*Neoscytalidium dimidiatum*	EE.UU.		Seno
22	13650	UTHSC:DI14-327	*Neoscytalidium dimidiatum*	EE.UU.		Lavado broncoalveolear
23	13651	UTHSC:DI14-328	*Neoscytalidium dimidiatum*	EE.UU.		Pie
24	13652	UTHSC:DI14-329	*Neoscytalidium dimidiatum*	EE.UU.		Pie
25	13653	UTHSC:DI14-330	*Neoscytalidium dimidiatum*	EE.UU.		Lavado broncoalveolear
26	13654	UTHSC:DI14-331	*Neoscytalidium dimidiatum*	EE.UU.		Dedo del pie
27	13655	UTHSC:DI14-332	*Neoscytalidium dimidiatum*	EE.UU.		Dedo del pie
28	13656	UTHSC:DI14-333	*Neoscytalidium dimidiatum*	EE.UU.		Piel de pie
29	13657	UTHSC:DI14-334	*Neoscytalidium dimidiatum*	EE.UU.		Dedo del pie
30	13658	UTHSC:DI14-335	*Neoscytalidium dimidiatum*	EE.UU.		Esputo
31	13659	UTHSC:DI14-336	*Neoscytalidium dimidiatum*	EE.UU.		Herida del pie
32	13660	UTHSC:DI14-337	*Neoscytalidium dimidiatum*	EE.UU.		Tejido de cerebro
33	13661	UTHSC:DI14-338	*Neoscytalidium dimidiatum*	EE.UU.		Líquido cerebroespinal
34	13662	UTHSC:DI14-339	*Neoscytalidium dimidiatum*	EE.UU.		Cerebro
35	13663	UTHSC:DI14-340	*Neoscytalidium dimidiatum*	EE.UU.		Lavado broncoalveolear
36	13667	UTHSC:DI14-245	*Colletotrichum gloeosporioides*	EE.UU.		Dedo de la mano
37	13668	UTHSC:DI14-246	*Colletotrichum* sp.	EE.UU.		Esputo
38	13669	UTHSC:DI14-247	*Colletotrichum gloeosporioides*	EE.UU.		Piel
39	13670	UTHSC:DI14-248	*Colletotrichum gloeosporioides*	EE.UU.		Masa del codo
40	13671	UTHSC:DI14-249	*Colletotrichum gloeosporioides*	EE.UU.		Mano

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
41	13672	UTHSC:DI14-250	*Colletotrichum gloeosporioides*	EE.UU.		Cornea
42	13673	UTHSC:DI14-251	*Colletotrichum capsici*	EE.UU.		Cornea
43	13674	UTHSC:DI14-252	*Colletotrichum truncatum*	EE.UU.		Tejido del pie
44	13675	UTHSC:DI14-253	*Colletotrichum spaethianum*	EE.UU.		Muñeca
45	13676	UTHSC:DI14-254	*Colletotrichum gloeosporioides*	EE.UU.		Líquido sinovial de la rodilla
46	13677	UTHSC:DI16-187	*Trematosphaeria* sp.	EE.UU.		Biopsia del talón
47	13678	UTHSC:DI16-188	*Diatrype* sp.	EE.UU.		Hisopado de la rodilla
48	13679	UTHSC:DI16-189	*Coniothyrium telephii*	EE.UU.		Seno maxilar derecho
49	13680	UTHSC:DI16-190	*Didymella heteroderae*	EE.UU.		Pie
50	13681	UTHSC:DI16-191	*Neosetophoma* sp.	EE.UU.		Esputo
51	13682	UTHSC:DI16-192	*Leptosphaeria* sp.	EE.UU.		Piel
52	**13683**	**UTHSC:DI16-193; CBS 142460**	***Pyrenochaetopsis paucisetosa***	EE.UU.	T	Dedo del pie
53	13684	UTHSC:DI16-194	*Diatrype* sp.	EE.UU.		Osteomielitis
54	13685	UTHSC:DI16-195	*Massarina walkeri*	EE.UU.		Cuero cabelludo
55	13686	UTHSC:DI16-196	*Cadophora* sp.	EE.UU.		Abdomen
56	13687	UTHSC:DI16-197	*Epicoccum latusicollum*	EE.UU.		Cornea
57	13688	UTHSC:DI16-198	*Pyrenochaetopsis microspora*	EE.UU.		Sinusitis crónica
58	13689	UTHSC:DI16-199	*Didymella microchlamydospora*	EE.UU.		Pierna
59	**13690**	**UTHSC:DI16-200; CBS 143032**	***Didymella keratinophila***	EE.UU.	T	Dedo de la mano
60	13691	UTHSC:DI16-201	*Epicoccum camelliae*	EE.UU.		Pulmón

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
61	13692	UTHSC:DI16-202	*Epicoccum camelliae*	EE.UU.		Uña
62	13693	UTHSC:DI16-203	*Coniothyrium telephii*	EE.UU.		Piel
63	13694	UTHSC:DI16-204	*Phoma herbarum*	EE.UU.		Cateter urinario
64	13695	UTHSC:DI16-205	*Didymella glomerata*	EE.UU.		Hombro
65	13696	UTHSC:DI16-206	*Epicoccum camelliae*	EE.UU.		Uña del dedo del pie
66	13697	UTHSC:DI16-207	*Neoascochyta desmazieri*	EE.UU.		Pulmón
67	13698	UTHSC:DI16-208	*Montagnula* sp.	EE.UU.		Piel
68	13699	UTHSC:DI16-209	*Paraphoma radicina*	EE.UU.		Pierna
69	13700	UTHSC:DI16-210	*Trematophoma* sp.	EE.UU.		Lavado broncoalveolear
70	13701	UTHSC:DI16-211	*Didymella gardeniae*	EE.UU.		Distrofia de la uña
71	13702	UTHSC:DI16-212	*Phoma herbarum*	EE.UU.		Lavado broncoalveolear
72	13703	UTHSC:DI16-213	*Neocucurbitaria unguis-hominis*	EE.UU.		Ambiental
73	13704	UTHSC:DI16-214	*Aplosporella sterculiae*	EE.UU.		Tobillo
74	13705	UTHSC:DI16-215	*Paraconiothyrium cyclothyrioides*	EE.UU.		Pierna
75	13706	UTHSC:DI16-216	*Paraconiothyrium cyclothyrioides*	EE.UU.		Herida de la piel
76	13707	UTHSC:DI16-217	*Aplosporella sterculiae*	EE.UU.		Tejido del cráneo
77	13708	UTHSC:DI16-218	*Paraconiothyrium maculicutis*	EE.UU.		Cornea
78	13709	UTHSC:DI16-219	*Didymosphaeriaceae* sp.	EE.UU.		Piel
79	13710	UTHSC:DI16-220	*Roussoella sp.*	EE.UU.		Líquido cerebroespinal
80	13711	UTHSC:DI16-221	*Lasiodiplodia parva*	EE.UU.		Uña del dedo del pie

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
81	13712	UTHSC:DI16-222	*Paraconiothyrium maculicutis*	EE.UU.		Herida del cuero cabelludo
82	13713	UTHSC:DI16-223	*Phialemoniopsis curvata*	EE.UU.		Planta del pie
83	13714	UTHSC:DI16-224	*Didymella heteroderae*	EE.UU.		Uña
84	**13715**	**UTHSC:DI16-225**	***Pyrenochaetopsis americana***	EE.UU.	T	Ambiental
85	13716	UTHSC:DI16-226	*Didymella gardeniae*	EE.UU.		Uña del dedo del pie
86	13717	UTHSC:DI16-227	*Didymella heteroderae*	EE.UU.		Uña
87	**13718**	**UTHSC:DI16-228**	***Didymella keratinophila***	EE.UU.		Uña del dedo del pie
88	**13719**	**UTHSC:DI16-229; CBS 142791**	***Neocucurbitaria irregularis***	EE.UU.	T	Herida del brazo
89	13720	UTHSC:DI16-230	*Didymella musae*	EE.UU.		Cornea
90	13721	UTHSC:DI16-231	*Didymella heteroderae*	EE.UU.		Uña del dedo del pie
91	13722	UTHSC:DI16-232	*Didymella heteroderae*	EE.UU.		Orina
92	**13723**	**UTHSC:DI16-233; CBS 142453**	***Allophoma cylindrispora***	EE.UU.	T	Ojo
93	13724	UTHSC:DI16-234	*Didymella heteroderae*	EE.UU.		Uña del dedo del pie
94	13725	UTHSC:DI16-235	*Didymella heteroderae*	EE.UU.		Cuero cabelludo
95	13726	UTHSC:DI16-236	*Coniothyrium telephii*	EE.UU.		Sangre
96	13727	UTHSC:DI16-237	*Trematosphaeria grisea*	EE.UU.		Codo
97	**13728**	**UTHSC:DI16-238; CBS 143033**	***Neopyrenochaetopsis hominis***	EE.UU.	T	Piel
98	13729	UTHSC:DI16-239	*Letendraea* sp.	EE.UU.		Brazo
99	13730	UTHSC:DI16-240	*Parastagonospora nodorum*	EE.UU.		Ambiental
100	13731	UTHSC:DI16-241	*Nigrograna* sp.	EE.UU.		Piel

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
101	13732	UTHSC:DI16-242	*Medicopsis romeroi*	EE.UU.		Palma de la mano
102	13733	UTHSC:DI16-243	*Paraconiothyrium cyclothyrioides*	EE.UU.		Uña
103	**13734**	**UTHSC:DI16-244**	***Epicoccum keratinophilum***	EE.UU.		Dígito (canino)
104	13735	UTHSC:DI16-245	*Pseudocercospora* sp.	EE.UU.		Mano
105	13736	UTHSC:DI16-246	*Paraconiothyrium cyclothyrioides*	EE.UU.		Dedo de la mano
106	13737	UTHSC:DI16-247	*Diaporthe* sp.	EE.UU.		Ojo
107	13738	UTHSC:DI16-248	*Phaeobotryosphaeria* sp.	EE.UU.		Párpado
108	**13739**	**UTHSC:DI16-249; CBS 142454**	***Cumuliphoma pneumoniae***	EE.UU.	T	Esputo
109	13740	UTHSC:DI16-250	*Phaeobotryosphaeria* sp.	EE.UU.		Cuero cabelludo
110	13741	UTHSC:DI16-251	*Montagnula* sp.	EE.UU.		Cuero cabelludo
111	13742	UTHSC:DI16-252	*Paraconiothyrium maculicutis*	EE.UU.		Biopsia
112	13743	UTHSC:DI16-253	*Preussia* sp.	EE.UU.		Dedo del pie
113	13744	UTHSC:DI16-254	*Chaetophoma* sp.	EE.UU.		Aspirado
114	13745	UTHSC:DI16-255	*Didymella anserina*	EE.UU.		Esputo
115	13746	UTHSC:DI16-256	*Trematheia* sp.	EE.UU.		Ojo
116	**13747**	**UTHSC:DI16-257**	***Epicoccum pneumoniae***	EE.UU.		Esputo
117	**13748**	**UTHSC:DI16-258**	***Epicoccum keratinophilum***	EE.UU.		Lavado broncoalveolear
118	13749	UTHSC:DI16-259	Valsa sp	EE.UU.		Lavado broncoalveolear
119	13750	UTHSC:DI16-260	Edenia sp	EE.UU.		Ojo
120	13751	UTHSC:DI16-261	*Paraphaeosphaeria* sp.	EE.UU.		Lavado broncoalveolear

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
121	13752	UTHSC:DI16-262	*Diaporthe* sp.	EE.UU.		Esputo
122	13753	UTHSC:DI16-263	*Paraconiothyrium fuckelii*	EE.UU.		Lavado broncoalveolear
123	13754	UTHSC:DI16-264	*Edenia* sp.	EE.UU.		Úlcera de la cornea
124	13755	UTHSC:DI16-265	*Paraconiothyrium maculicutis*	EE.UU.		Manos
125	13756	UTHSC:DI16-266	*Didymosphaeriaceae* sp.	EE.UU.		Uña
126	13758	UTHSC:DI16-267	*Letendraea* sp.	EE.UU.		Brazo
127	13759	UTHSC:DI16-268	*Paraconiothyrium maculicutis*	EE.UU.		Uña del dedo del pie
128	13760	UTHSC:DI16-269	*Roussoella* sp.	EE.UU.		Lavado broncoalveolear
129	13761	UTHSC:DI16-270	*Nothophoma quercina*	EE.UU.		Pie
130	**13762**	**UTHSC:DI16-271; CBS 142455**	***Epicoccum keratinophilum***	EE.UU.	T	Piel
131	**13763**	**UTHSC:DI16-272**	***Epicoccum keratinophilum***	EE.UU.		Uña del dedo del pie
132	**13764**	**UTHSC:DI16-273; CBS 142452**	***Allocucurbitaria botulispora***	EE.UU.	T	Costra en la pierna
133	13765	UTHSC:DI16-274	*Didymella gardeniae*	EE.UU.		Uña del dedo del pie
134	**13766**	**UTHSC:DI16-275; CBS 142459**	***Pyrenochaetopsis globosa***	EE.UU.	T	Piel
135	**13767**	**UTHSC:DI16-276; CBS 140674**	***Nothophoma macrospora***	EE.UU.	T	Pulmón
136	**13769**	**UTHSC:DI16-277; CBS 142461**	***Pyrenochaetopsis uberiformis***	EE.UU.	T	Oreja
137	**13770**	**UTHSC:DI16-278; CBS 142462**	***Remotididymella anthropophila***	EE.UU.	T	Lavado broncoalveolear
138	13771	UTHSC:DI16-279	*Paraconiothyrium cyclothyrioides*	EE.UU.		Lavado broncoalveolear
139	13772	UTHSC:DI16-280	*Epicoccum camelliae*	EE.UU.		Uña del dedo del pie
140	13773	UTHSC:DI16-281	*Trematosphaeria* sp.	EE.UU.		Piel

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
141	**13774**	**UTHSC:DI16-282**	***Didymella keratinophila***	EE.UU.		Uña
142	13775	UTHSC:DI16-283	*Neosetophoma* sp.	EE.UU.		Seno maxilar izquierdo
143	13776	UTHSC:DI16-284	*Phaeodothis* sp.	EE.UU.		Piel
144	**13777**	**UTHSC:DI16-285; CBS 142457**	***Nothophoma variabilis***	EE.UU.	T	Lavado broncoalveolear
145	13778	UTHSC:DI16-286	*Trematosphaeria grisea*	EE.UU.		Pierna
146	13779	UTHSC:DI16-287	*Exosporium* sp.	EE.UU.		Ambiental
147	13780	UTHSC:DI16-288	*Epicoccum sorghinum*	EE.UU.		Pie
148	**13781**	**UTHSC:DI16-289**	***Pyrenochaetopsis botulispora***	EE.UU.		Lavado broncoalveolear
149	13782	UTHSC:DI16-290	*Leptosphaeria etheridgei*	EE.UU.		Hombro
150	**13783**	**UTHSC:DI16-291**	***Neoascochyta tardicrescens***	EE.UU.		Pie
151	13784	UTHSC:DI16-292	*Parathyridaria* sp.	EE.UU.		Brazo
152	13785	UTHSC:DI16-293	*Diaporthe sclerotioides*	EE.UU.		Cornea
153	13786	UTHSC:DI16-372	*Pleosporales* sp.	EE.UU.		Ojo
154	13787	UTHSC:DI16-294	*Nothophoma gossypiicola*	EE.UU.		Seno etmoidal
155	13788	UTHSC:DI16-295	*Didymella gardeniae*	EE.UU.		Herida del cuello
156	13789	UTHSC:DI16-296	*Paraphoma fimeti*	EE.UU.		Pulmón
157	**13790**	**UTHSC:DI16-297**	***Pyrenochaetopsis botulispora***	EE.UU.		Pie
158	**13791**	**UTHSC:DI16-298; CBS 142458**	***Pyrenochaetopsis botulispora***	EE.UU.	T	Esputo
159	**13792**	**UTHSC:DI16-299**	***Epicoccum keratinophilum***	EE.UU.		Biopsia
160	13793	UTHSC:DI16-300	*Parathyridaria percutanea*	EE.UU.		Pie

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
161	13794	UTHSC:DI16-301	*Epicoccum sorghinum*	EE.UU.		Lavado broncoalveolear
162	13795	UTHSC:DI16-302	*Didymella protuberans*	EE.UU.		Ambiental (chocolate)
163	**13796**	**UTHSC:DI16-303; CBS 142459**	***Pyrenochaetopsis confluens***	EE.UU.	T	Sangre
164	13797	UTHSC:DI16-304	*Mycoleptodiscus* sp.	EE.UU.		Piel
165	13798	UTHSC:DI16-305	*Didymella heteroderae*	EE.UU.		Esputo
166	13799	UTHSC:DI16-306	*Phoma herbarum*	EE.UU.		Esputo
167	13800	UTHSC:DI16-307	*Phoma herbarum*	EE.UU.		Lavado broncoalveolear
168	**13801**	**UTHSC:DI16-308**	***Vacuiphoma oculihominis***	EE.UU.	T	Ojo
169	13802	UTHSC:DI16-309	*Medicopsis romeroi*	EE.UU.		Tobillo
170	13803	UTHSC:DI16-310	*Medicopsis romeroi*	EE.UU.		Pie
171	13804	UTHSC:DI16-311	*Didymosphaeria* sp.	EE.UU.		Piel
172	13805	UTHSC:DI16-312	*Botryosphaeria dothidea*	EE.UU.		Pie
173	13806	UTHSC:DI16-313	*Diederichomyces cladoniicola*	EE.UU.		Tejido de pulmón
174	13807	UTHSC:DI16-314	*Paraconiothyrium* sp.	EE.UU.		Herida del dedo de la mano
175	13808	UTHSC:DI16-315	*Medicopsis romeroi*	EE.UU.		Tejido de pulmón
176	13809	UTHSC:DI16-316	*Anteaglonium* sp.	EE.UU.		Ojo
177	13810	UTHSC:DI16-317	*Diaporthe sclerotioides*	EE.UU.		Raspado del ojo
178	13811	UTHSC:DI16-318	*Neofusicoccum* sp.	EE.UU.		Raspado de cornea
179	13812	UTHSC:DI16-319	*Phoma herbarum*	EE.UU.		Uña
180	13813	UTHSC:DI16-320	*Neoascochyta desmazieri*	EE.UU.		Piel

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
181	13814	UTHSC:DI16-321	*Botryosphaeria dothidea*	EE.UU.		Piel
182	13815	UTHSC:DI16-322	*Neodidymelliopsis longicolla*	EE.UU.		Lavado broncoalveolear
183	13816	UTHSC:DI16-323	*Diatrype* sp.	EE.UU.		Lavado broncoalveolear
184	13817	UTHSC:DI16-324	*Edenia* sp.	EE.UU.		Hueso del pie
185	13818	UTHSC:DI16-325	*Phaeosphaeria* sp.	EE.UU.		Hígado
186	13819	UTHSC:DI16-326	*Keissleriella cladophila*	EE.UU.		Biopsia nasal (de perro)
187	13820	UTHSC:DI16-327	*Paraconiothyrium cyclothyrioides*	EE.UU.		Biopsia de la rodilla
188	13821	UTHSC:DI16-328	*Paraconiothyrium maculicutis*	EE.UU.		Rodilla
189	13822	UTHSC:DI16-329	*Diaporthe sclerotioides*	EE.UU.		Líquido sinovial
190	13823	UTHSC:DI16-330	*Diederichomyces cladoniicola*	EE.UU.		Lavado broncoalveolear
191	13824	UTHSC:DI16-331	*Diaporthe sclerotioides*	EE.UU.		Líquido sinovial
192	13825	UTHSC:DI16-332	*Neoascochyta desmazieri*	EE.UU.		Cabeza
193	13826	UTHSC:DI16-333	*Botryosphaeria dothidea*	EE.UU.		Úlcera de la cornea
194	13827	UTHSC:DI16-334	*Parathyridaria sp.*	EE.UU.		Nariz
195	13828	UTHSC:DI16-335	*Trematosphaeria sp.*	EE.UU.		Herida del brazo
196	13829	UTHSC:DI16-336	*Phaeosphaeria* sp.	EE.UU.		Seno maxilar derecho
197	13830	UTHSC:DI16-337	*Neosetophoma* sp.	EE.UU.		Esputo
198	13831	UTHSC:DI16-338	*Epicoccum camelliae*	EE.UU.		Uña
199	13832	UTHSC:DI16-339	*Diederichomyces cladoniicola*	EE.UU.		Cornea
200	13833	UTHSC:DI16-340	*Valsa ambiens*	EE.UU.		Lavado broncoalveolear

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
201	13834	UTHSC:DI16-341	*Neoascochyta desmazieri*	EE.UU.		Uña del dedo del pie
202	13835	UTHSC:DI16-342	*Nigrograna* sp.	EE.UU.		Lavado broncoalveolear
203	13836	UTHSC:DI16-343	Pleosporales sp.	EE.UU.		Pulgar
204	13837	UTHSC:DI16-344	*Nectria austroamericana*	EE.UU.		Pie
205	13838	UTHSC:DI16-345	*Epicoccum camelliae*	EE.UU.		Abceso
206	13839	UTHSC:DI16-346	*Paraconiothyrium cyclothyrioides*	EE.UU.		Dedo anular
207	13840	UTHSC:DI16-347	*Paraconiothyrium maculicutis*	EE.UU.		Piel
208	13841	UTHSC:DI16-348	*Didymosphaeria* sp.	EE.UU.		Herida del cuero cabelludo
209	13842	UTHSC:DI16-349	*Paraconiothyrium estuarinum*	EE.UU.		Uña
210	13843	UTHSC:DI16-350	*Phomatospora* sp.	EE.UU.		Hueso
211	13844	UTHSC:DI16-351	*Letendraea* sp.	EE.UU.		Líquido pleural
212	**13845**	**UTHSC:DI16-352**	***Neoascochyta cylindrispora***	EE.UU.		Ojo
213	13846	UTHSC:DI16-353	*Chaetophoma* sp.	EE.UU.		Pulmón
214	13847	UTHSC:DI16-354	*Trematosphaeria grisea*	EE.UU.		Líquido sinovial
215	13848	UTHSC:DI16-355	*Paraconiothyrium* sp.	EE.UU.		Esternón
216	13849	UTHSC:DI16-356	*Roussoella sp.*	EE.UU.		Muñeca
217	13850	UTHSC:DI16-357	*Paraphaeosphaeria sporulosa*	EE.UU.		Lavado broncoalveolear
218	13851	UTHSC:DI16-358	*Pseudochaetosphaeronema* sp.	EE.UU.		Brazo
219	**13852**	**UTHSC:DI16-359; CBS 142456**	***Neoascochyta cylindrispora***	EE.UU.	T	Cornea
220	13853	UTHSC:DI16-360	*Parathyridaria sp.*	EE.UU.		Líquido articular

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
221	13854	UTHSC:DI16-361	*Camarographium* sp.	EE.UU.		Piel
222	13855	UTHSC:DI16-362	*Roussoella* sp.	EE.UU.		Ojo
223	13856	UTHSC:DI16-363	*Austropleospora archidendri*	EE.UU.		Líquido articular
224	13857	UTHSC:DI16-364	*Lasiodiplodia theobromae*	EE.UU.		Ojo
225	13858	UTHSC:DI16-365	*Didymella microchlamydospora*	EE.UU.		Cornea
226	13859	UTHSC:DI16-366	*Eutypella* sp.	EE.UU.		Abceso
227	14411	UTHSC:DI16-367	*Paraconiothyrium* sp.	EE.UU.		Uña
228	14412	UTHSC:DI16-368	*Myrmaecium* sp.	EE.UU.		Ambiental
229	14413	UTHSC:DI16-369	*Myrmaecium* sp.	EE.UU.		Ambiental
230	14425	UTHSC:DI16-370	*Letendraea* sp.	EE.UU.		Cuello
231	14433	UTHSC:DI16-371	*Eutypa* sp.	EE.UU.		Lavado broncoalveolear
232	16704	CNRMA 15.416	*Kirschsteiniothelia tectonae*	Francia		Desconocido
233	16705	CNRMA 16.553	*Colletotrichum gigasporum*	Francia		Piel
234	16706	CNRMA 15.504	*Colletotrichum gloeosporioides*	Francia		Ojo
235	16707	CNRMA 13.515	*Diaporthe* sp.	Francia		Piel
236	16708	CNRMA 11.385	*Diaporthe* sp.	Francia		Ojo
237	16709	CNRMA 8.522	*Diaporthe* sp.	Francia		Ojo
238	16710	CNRMA 9.205	*Diaporthe* sp.	Francia		Ojo
239	16711	CNRMA 9.1095	*Phoma* sp.	Francia		Piel
240	16712	CNRMA 6.1007	*Diplodia seriata*	Francia		Hueso

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
241	16713	CNRMA 10.947	*Epicoccum* sp.	Francia		Piel
242	16714	CNRMA 10.948	*Epicoccum* sp.	Francia		Piel
243	16715	CNRMA 7.167	*Epicoccum sorghinum*	Francia		Hueso
244	16716	CNRMA 14.708	*Lasiodiplodia theobromae*	Francia		Ojo
245	16717	CNRMA 15.383	*Lasiodiplodia* sp.	Francia		Ojo
246	16718	CNRMA 11.360	*Lasiodiplodia theobromae*	Francia		Ojo
247	16719	CNRMA 10.1369	*Lasiodiplodia theobromae*	Francia		Piel
248	16720	CNRMA 13.891	*Lasiodiplodia theobromae*	Francia		Piel
249	16721	CNRMA 10.813	*Lasiodiplodia theobromae*	Francia		Ojo
250	16722	CNRMA 11.680	*Medicopsis romeroi*	Francia		Piel
251	16723	CNRMA 14.407	*Medicopsis romeroi*	Francia		Piel
252	16724	CNRMA 4.200	*Medicopsis romeroi*	Francia		Ojo
253	16725	CNRMA 4.556	*Medicopsis romeroi*	Francia		Ojo
254	16726	CNRMA 5.321	*Medicopsis romeroi*	Francia		Piel
255	16727	CNRMA 5.666	*Medicopsis romeroi*	Francia		Piel
256	16728	CNRMA 7.1225	*Medicopsis romeroi*	Francia		Piel
257	16729	CNRMA 8.1363	*Medicopsis romeroi*	Francia		Piel
258	16730	CNRMA 11.949	*Medicopsis romeroi*	Francia		Hueso
259	16731	CNRMA 11.950	*Medicopsis romeroi*	Francia		Hueso
260	16732	CNRMA 9.467	*Paraphoma* sp.	Francia		Piel

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
261	16733	CNRMA 12.597	*Neofusicoccum luteum*	Francia		Ojo
262	16734	CNRMA 11.382	*Paraconiothyrium cyclothyrioides*	Francia		Piel
263	16735	CNRMA 11.383	*Paraconiothyrium cyclothyrioides*	Francia		Piel
264	16736	CNRMA 11.855	*Paraconiothyrium cyclothyrioides*	Francia		Piel
265	16737	CNRMA 13.245	*Paraconiothyrium cyclothyrioides*	Francia		Piel
266	16738	CNRMA 16.374	*Paraconiothyrium cyclothyrioides*	Francia		Piel
267	16739	CNRMA 16.556	*Paraconiothyrium cyclothyrioides*	Francia		Piel
268	16740	CNRMA 4.493	*Paraconiothyrium fuckelii*	Francia		Ojo
269		CNRMA 3.240	*Paraconiothyrium fuckelii*	Francia		Ojo
270	16741	CNRMA 10.867	*Didymella* sp.	Francia		Piel
271	16742	CNRMA 9.1046	*Epicoccum* sp.	Francia		Piel
272	16743	CNRMA 11.794	*Didymella* sp.	Francia		Piel
273	16744	CNRMA 11.1097	*Phoma herbarum*	Francia		Ojo
274	16745	CNRMA 12.1227	*Phoma herbarum*	Francia		Ojo
275	16746	CNRMA 15.6	*Didymella microchlamydospora*	Francia		Seno maxilar
276	16747	CNRMA 16.76	*Xenodidymella saxea*	Francia		Líquido cerebroespinal
277	16748	CNRMA 14.198	*Diaporthe* sp.	Francia		Piel
278	16749	CNRMA 12.311	*Diaporthe* sp.	Francia		Sangre
279	16750	CNRMA 15.665	*Phaeosphaeriaceae* sp.	Francia		Piel
280	16751	CNRMA 15.708	*Neocucurbitaria* sp.	Francia		Seno maxilar

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
281	16752	CNRMA 16.153	*Neocucurbitaria unguis-hominis*	Francia		Ojo
282	16753	CNRMA 4.1112	*Neocucurbitaria unguis-hominis*	Francia		Ojo
283	16754	CNRMA 6.243	*Neocucurbitaria unguis-hominis*	Francia		Ojo
284		CNM-CM 2132	*Didymella glomerata*	España		Pie
285		CNM-CM 3356	*Didymella microchlamydospora*	España		Uña del dedo del pie
286		CNM-CM 3387	*Medicopsis romeroi*	España		Úlcera de la rodilla
287		CNM-CM 3526	*Didymella glomerata*	España		Médula ósea
288	16909	CNM-CM 3546	*Didymella microchlamydospora*	España		Uña
289		CNM-CM 3597	*Didymella glomerata*	España		Sangre
290		CNM-CM 3697	*Didymella heteroderae*	España		Uña
291	16910	CNM-CM 3895	*Didymella heteroderae*	España		Uña
292	16911	CNM-CM 4675	*Didymella sp.*	España		Uña
293	16912	CNM-CM 4760	*Colletotrichum boninense*	España		Raspado de cornea
294		CNM-CM 4767	*Paraconiothyrium* sp.	España		Abceso
295		CNM-CM 5036	*Didymella heteroderae*	España		Descamación de piel
296	16913	CNM-CM 5281	*Epicoccum* sp.	España		Piel
297	16914	CNM-CM 5724	*Epicoccum* sp.	España		Humor vítreo
298		CNM-CM 5814	*Didymella heteroderae*	España		Exudado de la conjuntiva
299		CNM-CM 5882	*Neocucurbitaria keratinophila*	España		Exudado cutáneo
300	16915	CNM-CM 6000	*Paraphaeosphaeria michotii*	España		Piel

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
301	16916	CNM-CM 6116	*Colletotrichum boninense*	España		Conjuntiva
302		CNM-CM 6201	*Neoascochta desmazieri*	España		Uña
303		CNM-CM 6313	*Paraconiothyrium* sp.	España		Exudado de la conjuntiva
304		CNM-CM 6401	*Neocucurbitaria keratinophila*	España		Uña
305		CNM-CM 6455	*Neocucurbitaria keratinophila*	España		Exudado cutáneo
306	16917	CNM-CM 6489	*Neocucurbitaria* sp.	España		Exudado de herida
307		CNM-CM 6513	*Paraconiothyrium sp.*	España		Uña
308		CNM-CM 7013	*Neocucurbitaria keratinophila*	España		Exudado cutáneo
309	16918	CNM-CM 7025	*Neocucurbitaria* sp.	España		Pelo
310	16919	CNM-CM 7037	*Neocucurbitaria unguis-hominis*	España		Uña
311	16920	CNM-CM 7080	*Phaeosphaeriopsis obtusispora*	España		Uña
312		CNM-CM 7089	*Neocucurbitaria unguis-hominis*	España		Lesión cutánea
313		CNM-CM 7099	*Didymella microchlamydospora*	España		Exudado cutáneo
314	16921	CNM-CM 7132	*Neocucurbitaria* sp.	España		Uña del dedo del pie
315	16922	CNM-CM 7335	*Preussia* sp.	España		Uña
316	16923	CNM-CM 7343	*Preussia* sp.	España		Uña
317	16924	CNM-CM 7345	*Colletotrichum gloeosporioides*	España		Humor acuoso
318	16925	CNM-CM 7430	*Tintelnotia destructans*	España		Desconocido
319		CNM-CM 7457	*Neocucurbitaria keratinophila*	España		Exudado cutáneo
320		CNM-CM 7499	*Didymella heteroderae*	España		Exudado de la conjuntiva

Tabla 1. (continuación)

No.	Nº FMR[a]	Otra colección[b]	Taxón	País de origen	Estatus[c]	Fuente
321		CNM-CM 7645	*Medicopsis romeroi*	España		Exudado cutáneo
322		CNM-CM 7731	*Neocucurbitaria keratinophila*	España		Exudado cutáneo
323	16926	CNM-CM 7981	*Tintelnotia* sp.	España		Exudado cutáneo
324		CNM-CM 8010	*Neocucurbitaria keratinophila*	España		Exudado de la conjuntiva
325		CNM-CM 8031	*Phoma herbarum*	España		Uña
326		CNM-CM 8075	*Paraphoma fimeti*	España		Exudado de herida
327		CNM-CM 8674	*Neocucurbitaria keratinophila*	España		Uña del dedo del pie
328	16927	CNM-CM 8695	*Didymella* sp.	España		Exudado de la conjuntiva
329		CNM-CM 8717	*Neocucurbitaria unguis-hominis*	España		Orina
330		CNM-CM 8743	*Neocucurbitaria unguis-hominis*	España		Uña del dedo del pie

[a] **FMR**, Número de la colección de los aislados de la *Facultad de Medicina de Reus*, Tarragona, España.

[b] **CBS**, aislados de la colección del *Westerdijk Fungal Biodiversity Institute*, Utrecht, Países Bajos; **CNM-CM**, aislados de la colección del *Centro Nacional de Microbiología del Instituto de Salud Carlos III*, Madrid, España; **CNRMA**, aislados de la colección del *National Reference Center for Invasive Mycoses and Antifungals*, Institut Pasteur, Paris, Francia; **UTHSC**, aislados de la colección del *Fungus Testing Laboratory, University of Texas Health Science Center*, San Antonio, Estados Unidos de Norteamérica.

[c] **T**, indica que corresponde a una cepa tipo.

Tabla 2. Aislados ambientales y cepas tipo o referencia incluidas en esta Tesis, nuevos especies o cambinaciones estan indicados en negrita.

No.	Nº FMR[a]	Otra colección[b]	taxón	País de origen	Estatus[c]	Fuente
1	9444	CBS 121759	*Neocucurbitaria keratinophila*	España	T	Raspado de cornea
2	14461	CBS 179.80	*Epicoccum sorghinum*	Puerto Rico		*Sorghum vulgare*
3	14861	CBS 856.97	*Coniothyrium telephii*	Finlandia		Lana mineral
4	14862	CBS 188.71	*Coniothyrium telephii*	Finlandia		Aire
5	14863	CBS 101636	*Coniothyrium telephii*	Zimbabue		*Glycine max*
6	14864	CBS 138.96	*Neosetophoma samarorum*	Países Bajos	T	*Phlox paniculata*
7	14865	CBS 568.94	*Neosetophoma samarorum*	Países Bajos		*Urtica dioica*
8	14866	CBS 111112	*Neocucurbitaria unguis-hominis*	Países Bajos		Pulmón de *Agapornis* sp.
9	**14867**	**CBS 297.74**	***Neocucurbitaria aquatica***	Montenegro	T	Agua de mar
10	14868	CBS 115095	*Neocucurbitaria quercina*	Italia		*Quercus robur*
11	**14869**	**CBS 234.92**	***Paracucurbitaria italica***	Italia	T	*Olea europaea*
12	14870	CBS 407.76	*Pyrenochaeta nobilis*	Italia	T	*Laurus nobilis*
13	**14871**	**CBS 101634**	***Neopyrenochaeta fragariae***	Países Bajos	T	*Fragaria ananassa*
14	**14872**	**CBS 812.95**	***Neopyrenochaeta acicola***	Países Bajos	T	Tubería de agua
15	14873	CBS 122787	*Pyrenochaetopsis leptospora*	Alemania		Desconocido
16	14874	CBS 102876	*Pyrenochaetopsis microspora*	Montenegro	T	Agua
17	**14875**	**CBS 119739**	***Pyrenochaetopsis setosissima***	Brasil	T	*Coffea arabica*
18	14876	CBS 136769	*Pyrenochaetopsis poae*	Países Bajos	T	*Poa* sp.
19	14877	CBS 101635	*Pyrenochaetopsis leptospora*	Desconocido	T	*Secale cereale*
20	**14878**	**CBS 445.81**	***Xenopyrenochaetopsis pratorum***	Nueva Zelanda	T	*Lolium perenne*

Tabla 2. (continuación)

No.	Nº FMR[a]	Otra colección[b]	taxón	País de origen	Estatus[c]	Fuente
21	14879	CBS 124454	*Pyrenochaetopsis indica*	India	T	*Saccharum officinarum*
22	14880	CBS 343.85	*Pyrenochaetopsis decipiens*	Países Bajos	T	*Globodera pallida*
23	14882	CBS 132531	*Montagnula aloes*	Sudáfrica	T	*Aloe* sp.
24	14883	CBS 998.72	*Phyllosticta flevolandica*	Países Bajos	T	Suelo
25	14884	CBS 101461	*Paraconiothyrium maculicutis*	EE.UU.	T	Lesión cutánea humana
26	14885	CBS 972.95	*Paraconiothyrium cyclothyrioides*	Papúa Nueva Guinea	T	Suelo
27	14886	CBS 432.75	*Paraconiothyrium cyclothyrioides*	Sri Lanka		Suelo debajo de *Hevea brasiliensis*
28	14887	CBS 109850	*Paraconiothyrium estuarinum*	Brasil	T	Sedimento de un estuario
29	14888	CBS 653.85	*Paraconiothyrium fuckelii*	Alemania		*Picea abies*
30	14889	CBS 797.95	*Paraconiothyrium fuckelii*	Dinamarca		*Rubus* sp.
31	14890	CBS 168.77	*Paraconiothyrium archidendri*	Burma	T	*Pithecellobium bigeminum*
32	14891	CBS 253.92	*Paraconiothyrium lini*	Países Bajos		Tanque
33	14892	CBS 112.72	*Didymosphaeria variabile*	Italia		*Dianthus* sp.
34	14893	CBS 121164	*Didymosphaeria variabile*	Sudáfrica		*Prunus persica*
35	14894	CBS 100299	*Didymosphaeria rubi-ulmifolii*	Brasil		*Coffea arabica*
36	14895	CBS 115.92	*Didymosphaeria sp.*	Italia		*Olea europaea*
37	14896	CBS 178.93	*Paraconiothyrium flavescens*	Países Bajos	T	Suelo
38	14897	CBS 123380	*Didymella coffeae-arabicae*	Etiopia		*Coffea arabica*
39	14898	CBS 391.93	*Didymella protuberans*	Países Bajos		*Spinacia oleracea*
40	14899	CBS 381.96	*Didymella protuberans*	Países Bajos	T	*Lycium halifolium*

Tabla 2. (continuación)

No.	Nº FMR[a]	Otra colección[b]	taxón	País de origen	Estatus[c]	Fuente
41	14900	CBS 132.96	*Didymella protuberans*	Países Bajos		*Rhinanthus major*
42	14901	CBS 626.68	*Didymella gardeniae*	India	T	*Gardenia jasminoides*
43	14902	CBS 497.91	*Allophoma oligotrophica*	Desconocido		*Coffea arabica*
44	14903	CBS 436.75	*Allophoma tropica*	Alemania	T	*Saintpaulia ionantha*
45	14904	CBS 506.91	*Allophoma nicaraguensis*	Nicaragua	T	*Coffea arabica*
46	14905	CBS 325.82	*Allophoma minor*	Indonesia	T	*Syzygium aromaticum*
47	**14906**	**CBS 162.78**	***Remotididymella destructiva***	Países Bajos		*Lycopersicon esculentum*
48	14907	CBS 120105	*Epicoccum brasiliense*	Brasil	T	*Amaranthus* sp.
49	14908	CBS 186.83	*Epicoccum draconis*	Ruanda		*Dracaena* sp.
50	14909	CBS 558.81	*Epicoccum plurivorum*	Nueva Zelanda	T	*Setaria* sp.
51	**14910**	**CBS 180.80**	***Epicoccum ovisporum***	Sudáfrica	T	*Zea mays*
52	**14911**	**CBS 181.80**	***Epicoccum catenisporum***	Guinea-Bisáu	T	*Oryza sativa*
53	14912	CBS 377.67	*Nothophoma gossypiicola*	EE.UU.		*Gossypium* sp.
54	14913	CBS 633.92	*Nothophoma quercina*	Ucrania		*Quercus* sp.
55	14914	CBS 381.91	*Nothophoma anigozanthi*	Países Bajos		*Anigozanthus maugleisii*
56	**14915**	**CBS 341.86**	***Cumuliphoma omnivirens***	Bélgica	T	*Phaseolus vulgaris*
57	14916	CBS 124106	*Edenia gomezpompae*	Filipinas	T	*Senna alata*
58	**14917**	**CBS 357.84**	***Vacuiphoma bulgarica***	Bulgaria	T	*Trachystemon orientale*
59	**15327**	**CBS 282.72**	***Pseudopyrenochaeta terretris***	Países Bajos	T	Suelo
60	**15328**	**CBS 378.73**	***Remotididymella destructiva***	Tonga	T	*Lycopersicon esculentum*

Tabla 2. (continuación)

No.	Nº FMR[a]	Otra colección[b]	taxón	País de origen	Estatus[c]	Fuente
61	**15329**	**CBS 374.91**	***Juxtiphoma eupyrena***	Países Bajos		*Solanum tuberosum*
62	**15331**	**CBS 991.95**	***Cumuliphoma indica***	Papúa Nueva Guinea		Suelo
63	**15332**	**CBS 114179**	***Epicoccum proteae***	Sudáfrica	T	*Protea cv. carnival*
64	**15333**	**CBS 115979**	***Neocucurbitaria cava***	Países Bajos		Desconocido
65	**15334**	**CBS 119222**	***Neopyrenochaeta inflorescentiae***	Sudáfrica	T	*Protea neriifolia*
66	**15335**	**CBS 274.60**	***Ectophoma multirostrata***	Maharashtra	T	Suelo
67	**15336**	**CBS 368.65**	***Ectophoma multirostrata***	India		Desconocido
68	**15337**	**CBS 527.66**	***Juxtiphoma eupyrena***	Alemania		Suelo de un campo de trigo
69	15338	CBS 130.69	*Paraboeremia putaminum*	Dinamarca		*Malus sylvestris*
70	15339	CBS 463.69	*Didymella musae*	India		*Mangifera indica*
71	15340	CBS 615.75	*Phoma herbarum*	Países Bajos		*Rosa multiflora cv. cathayensis*
72	**15341**	**CBS 654.77**	***Cumuliphoma indica***	India	T	Desconocido
73	**15342**	**CBS 110.79**	***Ectophoma multirostrata***	Países Bajos		*Cucumis sativus*
74	**15343**	**CBS 193.82**	***Similiphoma crystallifera***	Austria	T	*Chamaespartium sagittale*
75	15344	CBS 187.83	*Paraboeremia adianticola*	EE.UU.		*Polystichum adiantiforme*
76	15345	CBS 374.84	*Didymella pinodes*	Bélgica		*Pisum sativum*
77	**15346**	**CBS 267.92**	***Ectophoma pomi***	India	T	*Coffea arabica*
78	**15347**	**CBS 419.92**	***Xenodidymella saxea***	Desconocido	T	Mármol corroído
79	15348	CBS 122.93	*Paraboeremia selaginellae*	Países Bajos	T	*Selaginella* sp.
80	**15349**	**CBS 133.93**	***Remotididymella destructiva***	Guadeloupe		*Solanum lycopersicon*

Tabla 2. (continuación)

No.	Nº FMR[a]	Otra colección[b]	taxón	País de origen	Estatus[c]	Fuente
81	15350	CBS 134.96	*Phoma herbarum*	Países Bajos		*Delphinium* sp.
82	15351	CBS 644.97	*Didymella sancta*	Argentina		*Opuntia ficus-indica*
83	**15352**	**CBS 689.97**	***Neoascochyta tardicrescens***	Noruega	T	Heno
84	15744	CBS 149.32	*Cucurbidothis pityophila*	Países Bajos		Desconocido
85	**15745**	**CBS 115.58**	***Didymella brunneospora***	Alemania	T	*Chrysanthemum roseum*
86	**15746**	**CBS 306.65**	***Pseudopyrenochaeta lycopersici***	Alemania	T	*Lycopersicon esculentum*
87	**15747**	**CBS 257.68**	***Neocucurbitaria cava***	Alemania	T	Suelo de un campo de trigo
88	15748	CBS 112.79	*Neocucurbitaria unguis-hominis*	Gales		Muestra de aire
89	15749	CBS 606.94	*Pyrenochaeta ligni-putridi*	Suiza	T	*Picea abies*
90	15750	CBS 379.96	*Didymella pteridis*	Países Bajos		*Pteris* sp.
91	15751	CBS 130007	*Cucurbitaria berberidis*	Austria	T	*Berberis vulgaris*
92	**15752**	**CBS 131315**	***Parapyrenochaeta protearum***	Sudáfrica	T	*Protea mundii*
93	**15753**	**CBS 137997**	***Parapyrenochaeta protearum***	Francia		*Pinus* sp.
94	**15754**	**CBS 139022**	***Neopyrenochaeta telephoni***	India	T	Pantalla de un teléfono móbil
95	**15755**	**CBS 141291**	***Parapyrenochaeta acaciae***	Australia	T	*Acacia* sp.
96	**16593**	**CBS 248.79**	***Paracucurbitaria corni***	Países Bajos		*Fraxinus excelsior*
97	16827	CBS 170.96	*Roussoella sp.*	Papúa Nueva Guinea		Bambú
98	16828	CBS 119687	*Dothidotthia symphoricarpi*	EE.UU.	T	*Symphoricarpos rotundifolius*
99	16829	CBS 119688	*Dothidotthia aspera*	EE.UU.		*Acer negundo*
100	16848	CBS 134.97	*Libertasomyces quercus*	España	T	*Quercus ilex*

Tabla 2. (continuación)

No.	Nº FMR[a]	Otra colección[b]	taxón	País de origen	Estatus[c]	Fuente
101	16849	CBS 139506	*Pyrenochaetopsis tabarestanensis*	Irán	T	Suelo
102	**14524**	**CBS 141688**	***Pseudoascochyta pratensis***	España	T	Suelo
103	15109		*Libertasomyces* sp.	España		Heces de pájaro
104	**15110**	**CBS 141689; ICMP 10493**	***Pseudoascochyta novae-zelandiea***	Nueva Zelanda	T	*Cordyline australis*
105	15230		*Preussia intermedia*	España		Heces de hervíboro
106	**15231**	**CBS 143031**	***Coniella heterospora***	España	T	Heces de hervíboro
107	15232		*Preussia intermedia*	España		Heces de hervíboro
108	15247		*Westerdykella sp.*	España		Heces de hervíboro
109	15572		*Neocucurbitaria cava*	España		Heces de hervíboro
110	15573		*Roussoella sp.*	España		Heces de hervíboro
111	15652		*Roussoella sp.*	España		Heces de hervíboro
112	15653		*Allophoma labilis*	Argentina		Suelo
113	15846		*Nothophoma gossypiicola*	España		Heces de hervíboro
114	15847		*Preussia sp.*	España		Heces de hervíboro
115	15848		*Preussia sp.*	España		Heces de hervíboro
116	15906		*Coniothyrium sp.*	España		Heces de hervíboro
117	15907		*Allophoma labilis*	México		Suelo
118	15908		*Didymella subglomerata*	México		Suelo
119	16396		*Didymella glomerata*	España		Suelo
120	16397		*Volutella ciliata*	España		Suelo

Tabla 2. (continuación)

No.	Nº FMR[a]	Otra colección[b]	taxón	País de origen	Estatus[c]	Fuente
121	**16398**	**CBS 144249**	***Alfaria dactylis***	España	T	Phoenix dactylifera
122	16399		*Volutella ciliata*	España		Suelo

[a] **FMR**, Número de la colección de los aislados de la *Facultad de Medicina de Reus*, Tarragona, España.

[b] **CBS**, aislados de la colección del *Westerdijk Fungal Biodiversity Institute*, Utrecht, Países Bajos; **ICMP**, aislado de la colección del *International Collection of Microorganisms from Plants*, Auckland, Nueva Zelanda.

[c] **T**, indica que corresponde a una cepa tipo.

Tabla 3. Cebadores y condiciones de hibridación en la PCR utilizadas en esta tesis.

Locus	Cebador	Dirección	Secuencia (5'–3')	Tº Hibridación (Cº)	Tiempo (s)	Referencia
Actina (*act*)	ACT-512F	Forward	ATG TGC AAG GCC GGT TTC GC	56	60	Carbone & Kohn
	ACT-783R	Reverse	TAC GAG TCC TTC TGG CCC AT			Carbone & Kohn
Beta-tubulina (*tub*2)	TUB2Fd	Forward	GTB CAC CTY CAR ACC GGY CAR TG	56	45	Woudenberg *et al*.
	TUB4Rd	Reverse	CCR GAY TGR CCR AAR ACR AAG TTG TC			Woudenberg *et al*.
Factor de elongación (*tef*1)	TEF1-983F	Forward	GCY CCY GGH CAY CGT GAY TTY AT	57	80	Schoch *et al*.
	TEF1-2218R	Reverse	AT GAC ACC RAC RGC RAC RGT YTG			Schoch *et al*.
Región espaciadora intergénica del ARNr (ITS)	ITS-5	Forward	GGA AGT AAA AGT CGT AAC AAG G	53	60	White *et al*.
	ITS-4	Reverse	TCC TCC GCT TAT TGA TAT GC			White *et al*.
Subunidad mayor de la ARN polimerasa II (*rpb*2)	fRPB2-5F	Forward	GAY GAY MGW GAT CAY TTY GG	56	60	Liu *et al*.
	fRPB2-7R	Reverse	CCC ATW GCY TGC TTM CCC AT			Liu *et al*.
Subunidad mayor 28S del ARNr (LSU)	LR0R	Forward	GTA CCC GCT GAA CTT AAG C	53	60	Rehner & Samuels
	LR5	Reverse	TCC TGA GGG AAA CTT CG			Vilgalys & Hester

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